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Soil properties that influence grassland production: their identification, production-indicator potentials and explanatory mechanisms

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UNIVERSITY *of* LIMERICK

**Soil properties that influence grassland production:
their identification, production-indicator potentials and
explanatory mechanisms**

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ABSTRACT

Soil properties that drive grassland production in the absence of mineral nitrogen applications and explanatory mechanisms thereof were sought in this study. Sixteen grassland farms varying in nitrogen inputs were selected in Counties Limerick and Clare, Ireland. Soil was sampled in 1995 and physical, chemical and biological properties analysed in the laboratory. Management data describing lime and fertiliser applications, grazing practices, soil disturbance, livestock and fodder production were obtained by interview. Grassland production for the year 1996/97 was measured as: stocking density; gross margin; and total energy required for livestock production, including maintenance, growth, offspring and milk and fodder sales, taking non-grassland feed energy into account. The botanical composition was determined in 1998.

Data were not normally distributed therefore the research was conducted in an exploratory manner. Descriptive scatter graphs and statistics, bivariate nonparametric Spearman's rank correlation, multiple regression analyses, principle components analysis (PCA) and canonical correspondence analysis (CCA) were implemented.

Four farms were in conversion to organic management, seven were organically managed and one was conventionally managed. Four farms had not had fertiliser applications. Microbial biomass, bacterial populations, and arginine ammonification activity that was interpreted as N-mineralisation and N-immobilisation activities *in vitro*, were not extreme in productive farms. Production measured as grassland energy correlated ($P < 0.05$) with: fertiliser inputs and mechanical disturbance; soil pH; depth to bedrock; soil plant-available magnesium and calcium contents, influences of which were direct. Root mass and N-mineralisation *in vitro* correlated inversely ($P < 0.05$) with production. Influential factors were highly correlated with each other ($P < 0.05$). Each influential factor, ordinated with variables correlated to it, gave rise to a production-related gradient of farms in PCA. A suite of N-mineralisation *in vitro* and its related variables reliably indicated grassland farm production in ordination space.

Fertiliser and lime applications, vegetation, grazing, soil depth and metabiosis influenced bacterial populations and activities. Root mass and N-mineralisation rates *in vitro* were least in disturbed and mineral nitrogen fertilised soils. N-mineralisation *in vitro* was greatest in extensive farms and N-immobilisation *in vitro* indicated carbon abundance in botanically diverse swards. Soil microbial biomass, bacterial populations and activities behaved individualistically along a possible succession in grazed grassland. Based on observations, and on allosteric and catabolite repression of amino acid use, it was hypothesised that 'available carbon, AC, is used before organic nitrogen, ON' is ammonified. However, ammonification *in vitro* was uncorrelated with soil organic carbon or soil nitrogen contents or C:N ratio, because soil C and N analyses applied in the study may not have accounted for microbe-available resources. Soil organic carbon content was relatively high, but least in the conventional farm. In contrast to other studies in the literature, soil organic carbon content was not correlated with microbial biomass and arginine ammonification but may be above threshold values needed to facilitate biological function in the soils examined.

Available forms of C and N ought to be considered as interdependent determinants of soil organic matter turnover rates subject to fertiliser use and grazing in grassland soils.

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Table of contents

Chapter 1	
Introduction	
Soil properties in relation to management and production in low input, organic and conventional grassland: the case for biological assessment	1
Chapter 2	
Selection of study farms and a description of their key farm management features	3
2.1 Introduction	3
2.2 Review of literature: key farm management features in relation to soil properties	3
2.3 Materials and methods	5
2.3.1 Selection of study farms	5
2.3.2 Compilation of data on farm management features	5
2.3.3 Measurement of key farm management features	6
2.3.3.1 Soil pH	6
2.3.3.2 Nutrient and lime applications	7
2.3.3.3 Grazing practices and likely excreta	7
2.3.3.4 Soil disturbance and reseeding interventions	11
2.3.4 Management systems operational at the time of sampling	11
2.3.5 Statistical analyses	12
2.3.5.1 Descriptive statistics	12
2.3.5.2 Normality of the data and data transformation	12
2.3.5.2.1 Data distribution and choice of statistical analyses procedures	13
2.3.5.3 Spearman's rank correlation analysis	14
2.4 Results and discussion	14
2.4.1 Soil pH	14
2.4.2 Nutrient and lime applications	15
2.4.3 Grazing practices and likely excreta	17
2.4.4 Soil disturbance and reseeding interventions on the study farms	21
2.4.5 Summary of the farm management systems operational on the study farms at the time of soil sampling	22
2.4.6 Summary description of grassland management features in the study farms	22
Chapter 3	
Site related and soil physical and chemical properties of the study farms in relation to farm management features	23
3.1 Introduction	23
3.2 Site related properties of the study farm soils in relation to farm management features	
3.2.1 Introduction	25
3.2.2 Review of literature	25
3.2.3 Materials and Methods	28
3.2.3.1 Site-related variables measured in situ	28
3.2.3.1.1 Altitude	28
3.2.3.1.2 Slope	29

Table of contents

Chapter 1	
Introduction	
Soil properties in relation to management and production in low input, organic and conventional grassland: the case for biological assessment	1
Chapter 2	
Selection of study farms and a description of their key farm management features	3
2.1 Introduction	3
2.2 Review of literature: key farm management features in relation to soil properties	3
2.3 Materials and methods	5
2.3.1 Selection of study farms	5
2.3.2 Compilation of data on farm management features	5
2.3.3 Measurement of key farm management features	6
2.3.3.1 Soil pH	6
2.3.3.2 Nutrient and lime applications	7
2.3.3.3 Grazing practices and likely excreta	7
2.3.3.4 Soil disturbance and reseeding interventions	11
2.3.4 Management systems operational at the time of sampling	11
2.3.5 Statistical analyses	12
2.3.5.1 Descriptive statistics	12
2.3.5.2 Normality of the data and data transformation	12
2.3.5.2.1 Data distribution and choice of statistical analyses procedures	13
2.3.5.3 Spearman's rank correlation analysis	14
2.4 Results and discussion	14
2.4.1 Soil pH	14
2.4.2 Nutrient and lime applications	15
2.4.3 Grazing practices and likely excreta	17
2.4.4 Soil disturbance and reseeding interventions on the study farms	21
2.4.5 Summary of the farm management systems operational on the study farms at the time of soil sampling	22
2.4.6 Summary description of grassland management features in the study farms	22
Chapter 3	
Site related and soil physical and chemical properties of the study farms in relation to farm management features	23
3.1 Introduction	23
3.2 Site related properties of the study farm soils in relation to farm management features	
3.2.1 Introduction	25
3.2.2 Review of literature	25
3.2.3 Materials and Methods	28
3.2.3.1 Site-related variables measured in situ	28
3.2.3.1.1 Altitude	28
3.2.3.1.2 Slope	29

3.2.3.1.3 Aspect	29
3.2.3.2 Map-derived soil parameters	29
3.2.3.2.1 Soil depth	29
3.2.3.2.2 Depth of the A-horizon	29
3.2.3.2.3 Percent clay	29
3.2.3.2.4. Silt: clay ratio	29
3.2.3.2.5 Soil series represented by the study farms	30
3.2.3.2.6 Natural drainage	30
3.2.3.3 Statistical Analysis	30
3.2.4 Results and discussion	30
3.2.4.1 Site-related parameters measured in situ	31
3.2.4.1.1 Altitude	31
3.2.4.1.2 Slope	31
3.2.4.1.3 Aspect	32
3.2.4.2 Map-derived soil parameters	32
3.2.4.2.1 Soil depth and depth of the A horizon	32
3.2.4.2.2 Per cent clay	34
3.2.4.2.3 Silt:clay ratio	35
3.2.4.2.4 Soil series represented by the study farms	35
3.2.4.2.5 Natural drainage	37
3.3 Physical properties of soils on the study farms in relation to farm management features	
3.3.1 Introduction	38
3.3.2 Review of literature	38
3.3.3 Materials and methods	42
3.3.3.1. Soil sampling	42
3.3.3.2 Parameters measured	42
3.3.3.2.1 Root mass	42
3.3.3.2.2 Bulk density	43
3.3.3.2.3 Particle density	43
3.3.3.2.4 Total porosity (pore space)	43
3.3.3.2.5 Aggregate ratio	43
3.3.3.2.6 Aggregate stability	43
3.3.3.3 Statistical analysis	44
3.3.4 Results and discussion	45
3.3.4.1 Root mass	45
3.3.4.2 Bulk density	46
3.3.4.3. Particle density	46
3.3.4.4 Total porosity (pore space)	48
3.3.4.5 Aggregate ratio	49
3.3.4.6 Aggregate stability	
3.4 Chemical properties of soils on the study farms in relation to farm management features	
3.4.1 Introduction	50
3.4.2 Review of Literature	50
3.4.3 Materials and methods	55
3.4.3.1. Soil sampling	55
3.4.3.2 Parameters measured	55

3.4.3.2.1 Organic carbon content	55
3.4.3.2.2 Soil nitrogen content	55
3.4.3.2.3 Carbon: nitrogen ratio	55
3.4.3.2.4 Plant-available soil phosphorus	55
3.4.3.2.5 Plant-available soil potassium	55
3.4.3.2.6 Plant-available soil calcium	56
3.4.3.2.7 Plant-available soil magnesium	56
3.4.3.2.8 Cation exchange capacity	56
3.4.3.2.9 Polysaccharide content	56
3.4.3.3 Statistical analysis	56
3.4.4 Results and discussion	57
3.4.4.1 Organic carbon content	57
3.4.4.2 Soil nitrogen content	58
3.4.4.3 Carbon:nitrogen ratio	59
3.4.4.4 Plant-available soil phosphorus	61
3.4.4.5 Plant-available soil potassium	62
3.4.4.6 Plant-available soil calcium	63
3.4.4.7 Plant-available soil magnesium	64
3.4.4.8 Cation exchange capacity	65
3.4.4.9 Soil polysaccharide content	65

Chapter 4

Botanical composition of the study farms in relation to farm management practices, site-related and soil properties

4.1 Introduction	68
4.2 Review of literature	68
4.3 Materials and methods	70
4.3.1 Sampling dates	70
4.3.2 Field sampling	70
4.3.3 Sample storage	70
4.3.4 Preparation of samples for analysis	70
4.3.5 Sub-sampling for botanical composition analysis	70
4.3.6 Identification of species in vegetation samples	71
4.3.7 Vegetation mass, dry weight, and botanical species abundance measurements	71
4.3.8 Statistical analysis	72
4.4 Results and discussion	72
4.4.1 Introduction	72
4.4.2 Botanical composition of the study farms	73
4.4.2.1 <i>Trifolium</i> species	73
4.4.2.2 <i>Lolium perenne</i>	73
4.4.2.3 Grasses other than <i>Lolium perenne</i>	76
4.4.2.4 Less-desirable species	78
4.4.3 Grass content and botanical diversity	80
4.4.4 Vegetation mass and dry weight	82
4.4.5 Comparison of plant communities	82

Chapter 5	
Soil carbon and nitrogen transformation biology in relation to influential grassland factors on the study farms	86
5.1 Introduction	
5.2. Review of literature: organic matter transformations	88
5.2.1. Mineralisation of carbon and nitrogen	88
5.2.1.1 Sources of substrates for microbial mineralisation and immobilisation	90
5.2.1.2 Substrate transformations by soil microbial activity	92
5.2.2 Analysis of soil biology	93
5.2.2.1 Overcoming obstacles to the analysis of soil biology	93
5.2.2.2 Biological components responsible for carbon and nitrogen transformations, their analyses, and their interpretation in agriculture	95
5.2.2.2.1 Microbial biomass	95
5.2.2.2.1.1 Laboratory analysis and interpretation of soil microbial biomass	96
5.2.2.2.2 Living organisms and their functional capabilities	97
5.2.2.2.2.1 Enumeration of functional populations in soil	98
5.2.2.2.3 Microbial activities in the transformation of carbon and nitrogen	100
5.2.2.2.3.1 Regulation of microbial transformation of carbon and nitrogen	101
5.2.2.2.3.2 Intracellular regulation of carbon and nitrogen metabolism	102
5.2.2.2.3.3 Laboratory measurement of microbial activity	104
5.2.2.2.3.4 Arginine ammonification represents mineralisation, and is under intracellular control	106
5.2.2.2.4 Enzyme activity in soil	109
5.2.2.2.4.1 Laboratory analysis of soil enzymes	110
5.2.2.3 Sampling, treatment and environmental factors influencing soil biology	110
5.2.2.3.1 Spatial variation	110
5.2.2.3.2 Temporal variation	111
5.2.2.3.3 Sample treatment variation	112
5.2.2.4 Interpreting in-vitro soil biology data for agricultural purposes	114
5.2.2.5 Conclusions	116
5.2.3 Environmental and management effects on C and N mineralisation	117
5.2.3.1 Substrate availability and C:N ratio	117
5.2.3.2 Complex effects of some environmental factors on soil carbon and nitrogen transformations	120
5.2.3.3 Influence of nutrient inputs, liming and grazing on carbon and nitrogen transformations in managed grassland	122
5.2.3.3.1 Comparison of basic management systems	122
5.2.3.3.2 Specific effects of management history	124
5.2.3.3.2.1 Tillage, pasture and soil organic matter issues	124
5.2.3.3.2.2 Lime application	126
5.2.3.3.2.3 Mineral nitrogen	126
5.2.3.3.2.4 Differences in effect between organic and mineral nitrogen sources	127
5.2.3.3.2.5 Effects of phosphate fertilisers	130
5.2.3.3.2.6 Effects of grazing	130
5.3 Materials and Methods	133
5.3.1 Introduction	133
5.3.2 Farm selection	134
5.3.3 Soil sampling for biological analysis	134

5.3.4 Storage of soil samples for biological analysis	134
5.3.5 Preparation of soil samples for biological analysis	134
5.3.6. Estimation of soil dry weight equivalent	134
5.3.7 Parameters measured	135
5.3.7.1 Microbial biomass	135
5.3.7.1.1 Ninhydrin reactive nitrogen	135
5.3.7.1.2 Substrate-induced respiration	136
5.3.7.2 Laboratory estimation of viable bacterial populations using the plate count method	137
5.3.7.2.1 Numbers of bacterial cfu	137
5.3.7.2.2 Numbers of bacterial cfu with protease activity	138
5.3.7.2.3 Number of bacterial cfu releasing ammonia from arginine	138
5.3.7.2.4 Numbers of bacterial cfu with urease activity	138
5.3.7.2.5 Numbers of bacterial cfu with exopolysaccharide activity	138
5.3.7.2.6 Numbers of bacterial cfu with cellulase activity	139
5.3.7.2.7 Numbers of bacterial cfu with starch hydrolysis activity	139
5.3.7.3 Microbial activity as arginine ammonification	139
5.3.7.4 Urease enzyme activity	140
5.3.8 Statistical analysis	141
5.4 Results and discussion	141
5.4.1 Microbial biomass	141
5.4.2 Bacterial populations, and their catabolic and biosynthetic capabilities	150
5.4.3 Microbial activity as arginine ammonification	158
5.4.4 Urease enzyme activity in soils	166
5.4.5 A summary of the soil biology of the sixteen study farms: analyses, influential factors, and recommendations	167
5.4.6 Underlying principles	167
5.4.7 Defined biological parameters assessed	169
5.4.8 Recommendations for biological analyses of grassland soils	172
5.4.9 Overall conclusions regarding the biological analysis of grassland soils	173

Chapter 6

Grassland production on the study farms in relation to influential factors

6.1 Introduction	175
6.2 Review of literature	176
6.3 Materials and methods	181
6.3.1 Introduction	181
6.3.2 Parameters measured	182
6.3.2.1 Energy from grassland	182
6.3.2.2 Gross margin per hectare	183
6.3.2.3 Livestock units per hectare	184
6.3.3 Statistical analysis	184
6.4 Results and discussion	185
6.4.1 Energy outputs per hectare	185
6.4.2 Gross margin per hectare	190
6.4.3 Livestock units per hectare	192
6.4.4 The search for interactive production determinants	194
6.4.4.1 Selection of a suitable dependent variable	194
6.4.4.2 Multiple regression models	195

6.4.4.3 Constraints of multiple regression analysis	197
6.4.4.4 Further linear multiple regression analysis, bearing constraints in mind	199
6.4.4.5 Identification of production indicators from amongst soil properties	201
6.5 Conclusions	203

Chapter 7

Explanatory mechanisms, general discussion, recommendations and conclusions

7.1 Introduction	205
7.2. Ordination analyses	206
7.2.1 Principal components analysis	207
7.2.2 Canonical correspondence analysis	208
7.2.3 Applicability of ordination procedures	209
7.2.4 Implementation of ordination procedures	210
7.2.4.1 Ordination methods	210
7.2.4.2 Treatment of data prior to ordination	210
7.2.4.3 Interpretation of ordination diagrams	210
7.3 Results of ordination analysis	211
7.3.1 What drives production, and how?	211
7.3.2 How do key factors interact?	212
7.3.2.1 Soil pH	215
7.3.2.1.1 How does soil pH 'work' in relation to the production environment?	215
7.3.2.1.2 Is soil pH influential or influenced?	217
7.3.2.1.3 Does soil pH influence soil biology, and if so, in what way?	219
7.3.2.1.4 Making sense of soil pH	220
7.3.2.2 Exploring N- mineralisation in vitro as a production indicator	220
7.3.3 How does the soil biology 'work'?	224
7.3.3.1 Environmental factors and soil biology	224
7.3.3.2 Gradients suggest an ecological succession in grazed soil	226
7.3.3.3 Substrate gain and loss factors influence heterotrophic bacteria	227
7.3.4 Patterns emerging in CCA	233
7.3.4.1 Production-related clusters	235
7.3.4.2 N-function-related linear diagrams	238
7.3.4.3 Conclusions drawn from CCA ordinations: production	241
7.3.4.4 Conclusions drawn from CCA ordinations: relative independence	241
7.4 General discussion	244
7.4.1 Introduction	
7.4.2 Soil biology, soil organic matter quality, and sustainability	244
7.4.3 'AC is used before ON', in theory	246
7.4.4 Bioindicator searches: findings and prospects	248
7.4.4.1 A suitable gauge may have helped to identify indicators in this study	248
7.4.4.2 Acknowledging definitions and constraints may have been of value	249
7.4.5. Uniqueness of this study	250
7.4.6 Limitations of this study	251
7.4.6.1. Use of working grassland farms	251
7.4.6.2. Analytical shortfalls	251
7.4.6.3 Unanswered questions	252

7.4.7 Further work	253
7.4.7.1 Work prior to hypothesis testing	254
7.4.7.2 Does arginine ammonification in vitro reflect N-function in the field?	254
7.4.7.3 Is it true that 'AC is used before ON'?	254
7.4.7.4 Substrate gain and loss factors should be explored	256
7.4.7.5 Thresholds for soil organic matter and biology should be identified	256
7.4.7.6 Application of analyses to field conditions	257
7.4.7.7 Developing an understanding of soil pH	258
7.4.8 Bringing this information to bear in agriculture	258
7.4.8.1 Bioassay of ammonification, labile C and mineral N	258
7.4.8.2 How might N-mineralisation be monitored and controlled in practice?	259
7.4.8.3 Conclusions in regard to agricultural application	261
7.5 Recommendations	261
7.6 Conclusions	263
Bibliography	266
Appendices	
Appendix A - Grassland management and production questionnaire	A1-36
Appendix B - LU conversion, excreta nutrient, and drainage score information	B1-2
Appendix C - Variable key	C1-5
Appendix D - Data spreadsheet	D1-15
Appendix E - Correlation tables	E1-13
Appendix F - Additional ordination diagrams	F1-6

List of tables		Page
Table 2.1	Soil pH values summarised for the sixteen study farms	15
Table 2.2	Nutrient and lime applications to the sampled fields for the four-year period prior to soil sampling for 16 farms. Nutrient sources are categorised as mineral, organic and total	16
Table 2.3	The number of years elapsed since mineral nitrogen and lime had been applied, prior to soil sampling, on the sixteen study farms	17
Table 2.4	Grazing continuity described as days grazed and rested in a rotation, and days grazed in a year. Intensity described both as the ratios between days grazed to days in a rotation, and as the ratio between rested to grazed days in a rotation	18
Table 2.5	Frequency of grazing described as the number of animals present multiplied by days in rotation and year	19
Table 2.6	Treading in terms of animal bulk, rotation and year	19
Table 2.7	Total estimated nutrients excreted by grazing animals in their dung and urine over the sampled area during the year prior to sampling	20
Table 2.8	Intervals since mechanical disturbance likely to aerate the soil had occurred and since reseeding practices were carried out, summarised for the sixteen farms	21
Table 2.9	Farm management systems operational in the sixteen study farms at the time of sampling	22
Table 3.1	Elevation above sea level summarised for the sixteen study farms	31
Table 3.2	Slope, summarised for the sixteen study farms	31
Table 3.3	Aspect, or variation from south, summarised for the sixteen farms	32
Table 3.4	Depth of soil in the uppermost A horizon and to the parent material	33
Table 3.5	Per cent clay in the topmost soil horizon	34
Table 3.6	The ratio of silt to clay, summarised for the sixteen study farms	35
Table 3.7	Soil series represented by the sixteen study farms	36
Table 3.8	Natural drainage and liability to drought scores summarised for the sixteen study farms	37
Table 3.9	Root mass values for the sixteen study farms, summarised	44
Table 3.10	Bulk density values, summarised for the sixteen farms	46
Table 3.11	Particle density summarised for the sixteen study farms	47
Table 3.12	Pore space values summarised for the sixteen study farms	48
Table 3.13	Ratio of small (<1mm) to large (>1mm) aggregates	49
Table 3.14	Aggregate stability described as percentage water stable aggregates, summarised for the sixteen study farms	49
Table 3.15	Organic carbon content summarised for the sixteen study farms	57
Table 3.16	Soil nitrogen content, summarised for the sixteen study farms	58
Table 3.17	Soil carbon to nitrogen ratios, summarised for the sixteen study farms	59
Table 3.18	Plant-available soil phosphorus summarised for the sixteen study farms.	61

Table 3.19	Plant-available soil potassium, summarised for the sixteen study farms	62
Table 3.20	Calcium content of the study farm soils, summarised	63
Table 3.21	Plant-available soil magnesium, summarised for the sixteen study farms	64
Table 3.22	Cation exchange capacity of the soil summarised for the sixteen study farms	65
Table 3.23	Soil polysaccharide content, summarised for the sixteen study farms	65
Table 4.1	Abundance of botanical species in the sward as percentage dry-weight of the vegetation sampled from each selected study farm field	74
Table 4.2	The percentage dry weights of botanical species found on the study farms and the numbers of farms on which each plant type was found.	75
Table 5.1	Microbial biomass estimated indirectly as the ninhydrin-reactive nitrogen released following chloroform fumigation NRN and as the rate of carbon dioxide released during substrate-induced respiration SIR.	142
Table 5.2	Total culturable bacteria and colony forming units displaying cellulase, amylase, protease production, urease and arginine ammonification and extracellular polysaccharide production activities in vitro.	150
Table 5.3	The rate of ammonia-nitrogen released per hour following arginine amendment, per gram moist soil per hour. Positive values have been interpreted as arginine mineralisation, and negative values as nitrogen immobilisation from soil reserves.	159
Table 5.4	Soil urease enzyme activity measured as ammonia released from urea-amended soil in vitro.	166
Table 6.1	Grassland energy outputs: annual energy required for livestock and their production and for fodder sold from the land, taking bought-in and non-grassland farm produced feeds into account	185
Table 6.2	Gross margin per hectare estimated from income, including sales, premia, grants and headage payments, exclusive of farm costs, per hectare of grassland	190
Table 6.3	Livestock units per hectare of grassland summarised for the sixteen study farms	192
Table 6.4	Equation 1. Built using energy outputs GJ ha^{-1} as the dependent variable with the majority of putative independent management, soil property and botanical composition variables as possible predictors	195
Table 6.5	Equation 2. Built using energy outputs GJ ha^{-1} as the dependent variable together with each soil property for which data were complete in 14 cases	199
Table 6.6	Equation 2. Built using energy outputs GJ ha^{-1} as the dependent variable each soil property variable for which data were complete for each of the 16 study farms	200
Table 7.1	PCA ordinations of key production-related factors with variables significantly related to them	214
Table 7.2	Environmental variables related to soil biological components	225

List of figures		Page
Fig. 2.1	Location of the study farms in Counties Clare and Limerick, Ireland	6
Fig. 7.1.	PCA ordination diagram implemented with all farms and as many variables as practicable	212
Fig. 7.2	Principal components analysis of soil pH together with variables significantly related to it for the sixteen study farms.	215
Fig. 7.3	Scatter graph of soil pH (<i>x</i> axis) and farm production (<i>y</i> axis).	216
Fig. 7.4	Scatter graph of bacterial populations (<i>x</i> axis) and soil pH (<i>y</i> axis)	218
Fig. 7.5	Scatter graph of arginine ammonification (<i>x</i> axis) against soil pH (<i>y</i> axis)	219
Fig. 7.6	PCA ordination of N mineralisation <i>in vitro</i> with factors related to it, implemented with those ten farms with positive values for arginine ammonification	221
Fig. 7.7	PCA ordination of N-mineralisation <i>in vitro</i> with factors related to it, implemented with fifteen farms for which either positive or negative arginine ammonification values were available	222
Fig. 7.8	PCA ordination of all sixteen farms with variables significantly related to N-mineralisation <i>in vitro</i> , implemented without arginine ammonification as a variable	223
Fig. 7.9	PCA ordination of bacterial populations, N mineralisation and N immobilisation <i>in vitro</i> for sixteen study farms	226
Fig. 7.10.	PCA ordination of bacterial populations, N-mineralisation and N-immobilisation <i>in vitro</i> , together with significantly-related factors that might influence microbial substrate availability	228
Fig. 7.11 a	Production related clusters found in CCA implemented with six-year nutrient input data preceding production- related data collation entered as 'environmental variables' and soil pH, organic carbon and root mass as 'species' type variables	235
Fig. 7.11 b	Production related clusters found in CCA implemented with selected grazing and botanical variables as environmental vectors, and soil pH, organic carbon and root mass as 'species' type variables	236
Fig. 7.11 c	Production related clusters found in CCA implemented with bacterial populations as environmental vectors and soil pH, organic carbon and root mass as 'species' type variables	237
Fig. 7.12	Linear CCA outcome when variables describing arginine use were implemented with soil pH, depth ratio and root mass as environmental variables	238
Fig. 7.13	Angular CCA outcome when variables describing arginine use were implemented with a catabolic population and soil pH, depth ratio and root mass as environmental variables	240

Chapter 1

Chapter 1

Introduction

Soil properties in relation to management and production in low input, organic and conventional grassland: the case for biological assessment

The objectives of this thesis are to examine soil properties in the context of low input and organic grassland. Physical, chemical, topographical and biological characteristics of soil will be analysed on sixteen farms in which grassland production is the principle enterprise. The interdependent nature of soil properties will be acknowledged and each of the soil characteristics will be statistically and numerically analysed in relation to all other measured parameters to disclose inherent relationships between them, which may, in some cases, be causal. The possible basis for relevant interaction and relationship between factors will be discussed.

Having collected information by questionnaire and interview from participating farmers, the nutrient management, grazing practices and other farming practices that affect the soil will be examined in relation to each of the measured soil parameters. The intention is to understand each of the soil characteristics in the context of the way in which the soil managed, in particular to understand the impact of added fertiliser on soil biological properties, and to understand how farm management affects the functioning of the soil itself.

Underlying this thesis is an ongoing search for soil properties that could ultimately be used to predict or indicate fertility. The search is prompted in part by changes in agricultural practice in which dependence on added mineral fertiliser and intensive cultivation has been reduced. There is once again a greater reliance on the soil itself, rather than added mineral fertiliser, as a plant nutrient resource. This will require a different approach to soil assessment in which we look beyond the available N, P and K towards other soil factors that together determine plant growth. It is known that all soil properties can contribute to a productive soil in a very complex and dynamic way. Biological activity is presumed key to fertility in organically managed soil (Lampkin,

1990) where indeed greater biological activity has been reported (Bolton et al., 1985). However, attempts to scientifically formulate an index linking soil properties to fertility have been unsuccessful. To explain why the search has been fruitless, Skinner (1976) suggested some time ago that perhaps we were not asking the right questions of nature. Since then, other authors (Nannipieri et al., 1990) suggested that, for example, biological methods shown to be inadequate were still in use, and that what was being measured should be more clearly defined. Clearly defined methods, based on established biological principles, will be applied here.

The present study intends to be as comprehensive as possible in its view of the soil and factors that impact on it. Grassland lime, nutrient and grazing practice management; soil physical and chemical properties; botanical composition; soil biology; and grassland production will be described separately, each chapter having pertinent literature review and materials and methods sections. How each of the recorded farm management and nutrient-related factors, measured soil parameters and botanical composition interact with each other and with farm production will be considered progressively as the thesis develops.

By studying soil from low input, organic and conventionally farmed grassland, against the clear background of nutrient and grazing management practices, it is hoped that relationships between soil properties and fertility can be clarified. If any properties should emerge as possible indicators of soil fertility, these will be considered in the context of their interdependence with management practices and production in low input and organic grassland. Limitations of this research, future research needs, and possible application of research results will be discussed.

Chapter 2

Chapter 2

Selection of study farms and a description of their key farm management features

2.1 Introduction

This thesis investigates soil properties in the context of low input and organic grassland. In addition, specific key farm management features are measured so that their effects on grassland soil may be taken into account. The selection of the study farms is outlined here in Chapter 2, and key farm management features, namely soil pH, lime applications, fertiliser nutrient inputs, grazing practices, as well as the soil disturbance and reseeded history are introduced, measured and described for the sixteen study farms.

Prerequisite to statistical analysis, the normality of the dataset and choice of appropriate statistical procedures are also considered here.

2.2 Review of literature: key farm management features in relation to soil properties

In grassland farming, the manipulation of soil pH, nutrient applications, grazing management and tillage or reseeded interventions are intended to optimise grassland production. However, farm management practices also affect the soil, the botanical composition of the grassland, and the farm environment.

Soil pH is a critical factor in soil fertility. Together with cation exchange capacity, pH determines the availability of plant nutrients either adhering to the clay particles or present in complex organic or inorganic forms or the soil solution (Brady, 1990). Soil pH is also a determinant of soil biological activity, because soil organisms and enzymes each function best under particular soil pH conditions (Coyne, 1999).

The existence of a relationship between added nutrients and grassland yield is unquestioned, such that conventional grassland systems rely on the addition of soluble ‘artificial’ fertilisers to maximise yield (Lee and Diamond, 1972). However, nitrogen fertilisers affect the species content of the grassland sward. The positive response of

ryegrass to fertiliser nitrogen is well documented (Whitehead, 1995). In contrast, fertiliser nitrogen is inhibitory to nitrogen fixation by legumes such as clover and reduces the size of root nodules. These combined responses of increased grass growth and inhibition of nitrogen fixation reduce the amount of clover in the sward (Zuberer, 1990; Brockman, 1995a; Whitehead, 1995).

Other relationships between fertiliser inputs and soil behaviour are being clarified in the literature. Higher levels of biological activity found in organic farming systems (for example Bolton et al., 1985; Reganold, 1995) are considered pivotal to soil fertility and productivity in those systems (Lampkin, 1990). The effects of soluble and organic fertilisers differ, in that increased nitrous oxide emissions are associated with applications of farmyard manure (Mogge et al., 1999). From an environmental perspective, there is a growing awareness of the health and pollution risks associated with loss of nitrates and phosphates to water (Sadler and Turner, 1994). Recent studies also suggest that methane emission (Singh et al., 1999) and nitrogen loss to the atmosphere (Mogge et al., 1999) are enhanced by certain fertiliser inputs.

Grazing directly affects the structure of soil, and that of the sward, through treading and defoliation (Frame, 1992). The physical impact of grazing on soil and plants, and the concomitant nutrient changes brought about through herbivory and excreta, influence soil chemical and biological properties as well (Bardgett et al., 1997 & 1998a & b; Luo et al., 1999a). Grazing events may also stimulate denitrification temporarily, and to different degrees, depending on soil conditions and season (Luo et al., 1999a). Other farming procedures that disrupt both the soil and the botanical composition are tillage practices and the reseeded of grassland. That tillage practices affect soil structure and soil organic matter content is well known (Tivy, 1990; Frame, 1992).

The soil pH, nutrient, grazing, tillage and reseeded manipulations and interventions on the sixteen study farms are summarised in Chapter 2. Possible effects of these grassland management features are considered in the context of soil structure and properties, botanical composition of the grassland, management systems and production later in the thesis.

2.3 Materials and methods

2.3.1 Selection of study farms

Counties Limerick and Clare comprised the study area (Figure 2.1). Criteria for selection of each farm were that grassland be the major farm enterprise; that sustainable agricultural practices such as low-input, organic farming, or more extensive than intensive management should prevail; and that farm records be reliable and accessible. The participant farmers should also be willing and available to impart details of farming practice and production in interview during which a detailed questionnaire would be completed by the researcher.

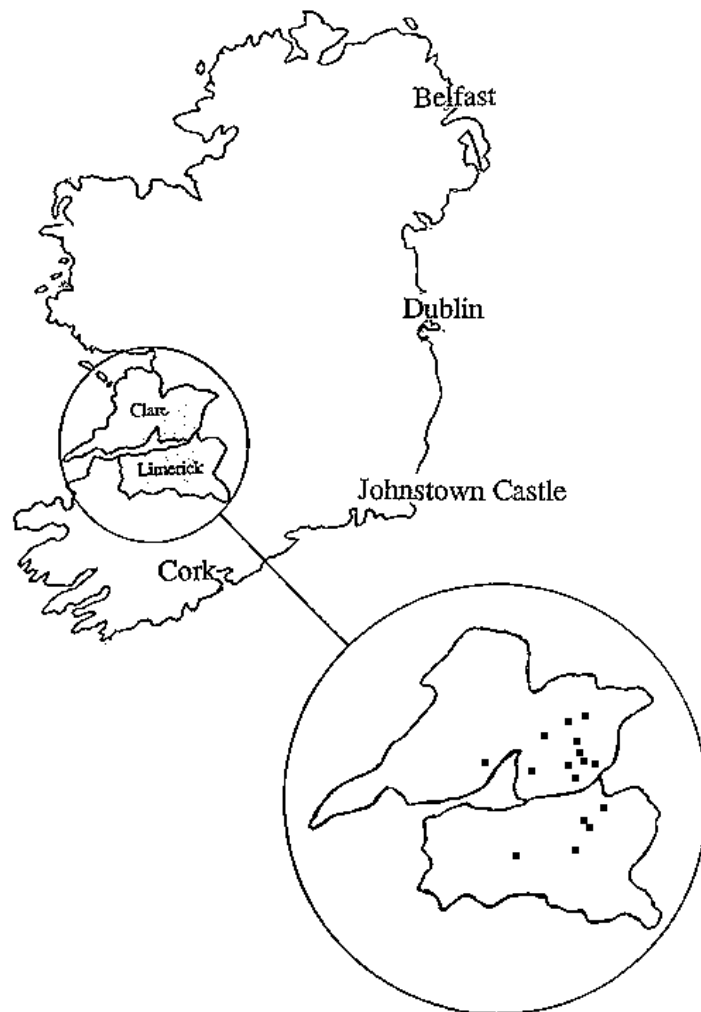
Study farms were selected primarily on the advice of a free-lance Rural Environmental Protection Scheme (REPS) advisor who furnished a list of potential study farms (P. Kelly, Kelly Consultants, personal communication, 1994). Following telephone calls, sixteen farms compatible with the selection criteria were chosen. For reasons of confidentiality, farms were encoded alphabetically. Each farm was visited in summer 1995 and, following discussion with the farmer, a single field was selected for soil sampling on the basis that its productivity was considered representative of the farm as a whole. The field was sampled and soil variables and botanical composition were analysed as reported in Chapters 3-5. Important farm management features were measured as described in following sections.

The location of each of the study farms is indicated in Figure 2.1. The identity of each farm is not disclosed because farm information was given in confidence.

2.3.2 Compilation of data on farm management features

A questionnaire was devised to collect data on farm management features. A copy of the questionnaire is given in Appendix A. The questionnaire was completed for each of the sixteen farms during an audiotaped interview with the farmer responsible in each case.

Fig. 2.1 Location of the study farms in Counties Clare and Limerick, Ireland



2.3.3 Measurement of key farm management features

The following text describes measurement of soil pH, and calculation of nutrient and lime applications, grazing events and extents, and soil mechanical disturbance history.

2.3.3.1 Soil pH

Clod samples were removed using a trowel, from the selected field, from the upper 10 cm of the soil profile at randomly chosen locations in a W pathway, avoiding gateways and paths through the sampled field. The soil was dried in the laboratory and sieved (2 mm). The sieved soil was used in determining soil pH according to Rowell (1994).

2.3.3.2 Nutrient and lime applications

The total amounts of fertilisers and lime applied on a per hectare basis on the sampled field and on the entire farm were recorded in the questionnaire. Data were recorded for the four-year period prior to 1995 when soil was sampled for physical, chemical and biological analyses, and for the six-year period up to 1997, roughly preceding farm production and botanical composition analyses. The numbers of years elapsed since applications of lime and mineral nitrogen prior to sampling were calculated. Figures for the inputs of N, P and K were calculated and expressed in kg ha^{-1} with reference to Frame (1992) and Brockman (1995b) for production and nutrient content of slurries, manures and basic slag.

2.3.3.3 Grazing practices and likely excreta

In the soil biology literature to date, the effects of grazing ruminants on the soil biota and on the nutrient cycling have been researched either by comparison of differing grazing intensities or by the simulation of grazing events. For example, grassland management systems have been compared in biodynamic and conventional farms by Reganold et al., (1993). The effects of different ruminant grazing intensities (Northup et al., 1999) as well as seasons have been compared by Bardgett et al. in 1997 and again by Bardgett et al in 1999. The effects of simulated excreta events have been studied by Bardgett et al., (1998a) and by Bronson et al., (1999).

As the present study is based on diverse grassland farm types amongst which farm management features differed considerably in terms of the duration, intensity and frequency of grazing events, methods for the comparison or for simulation of grazing events were not applicable. Although methods to describe grazing in detail can presumably be found in a grassland management context, few if any enumerative methods are documented in soil biology literature because of the general rather than itemised way in which grazing has been described there to date.

Data describing animal types and numbers and their temporal and spatial distribution on grazed land had already been collected in questionnaires. It was thought that each aspect

of grazing might affect soil in a measurable way. For example, the degree of continuity of grazing presence might affect soil biota because its opposite, recorded as the absence of grazing animals for some time, brought change elsewhere (Bardgett et al., 1997). In another example, incorporation of dung into soil had had far more immediate effect than surface distribution (Lovell and Jarvis, 1996). Could treading influence soil for example by maceration of herbage underfoot? This has not been determined elsewhere. As this 'experiment' was uncontrolled and its outcome unknown, it was thought that by trying out different enumerative approaches in an exploratory rather than deterministic manner that at least some devices would help connect grazing events with soil biology later in the study. Suitable enumerative devices had not been found elsewhere, therefore new methods (outlined below) to describe grazing in terms of (1) continuity, (2) intensity, and (3) frequency were initiated.

Methods to estimate treading (4, below) and excreta nutrients (5, below) are well established in the literature (Frame, 1992) and were adapted for study purposes. For example, estimates for treading based on live weight, hoof area, stocking rate and so on are tabulated in Frame (1992), and estimates of dung and urine NPK contents for different animal types are also available (Frame, 1992; REPS, 1993).

Grazing histories of the farms were obtained from data available in the questionnaires where grazing practices had been recorded in terms of the types and numbers of animals to which the sampled area had been exposed during the year prior to soil sampling. In order to represent what had taken place on the sampled soil, grazing measurements per hectare were based on the discrete grazed area as had been described by the farmer. To illustrate, whether the field was set stocked as part of a larger area; grazed as a discrete field unit; or in smaller paddocks, was ascertained. Where necessary, paddock sizes were calculated from field division and or rotation information. Records were made of dates when grazing commenced on the sampled area and when grazing finished due for example to either sale or housing of animals. To facilitate calculation, start and finish dates of the grazing season were allocated values according to days listed from 1-365, beginning January 1. Silage or hay growth, harvesting and grass growth were presumed to incur field closure averaging 70 days between grazing rotations in any case, as fields are normally closed for 5-6 weeks both before and after silage in the general study area (Davoren, M., University of Limerick, personal communication, 1999).

These data were then used to calculate the grazing continuity, intensity, treading and excreta variables, as described in the following paragraphs using farm A as an example. Each farm was similarly treated.

1. Grazing continuity: The number of days grazed and the number of days rested during a single grazing rotation were enumerated to describe grazing continuity in the short term. For example, the sampled field in farm A was grazed for fourteen days, and then rested for fourteen before cattle, the predominant type of animal, were returned. The number of days grazed in the year prior to soil sampling described continuity in the longer term. Cattle were first placed on the field in that year on March 1, day 60, which was subtracted from the date of either winter housing or sale as occurred on October 31, that is on day 301, on farm A. The sampled field in farm A was not closed for fodder production. The number of rotations was calculated by dividing possible grazing days, $(301-60=241)$ by the duration of the single rotation, 28. Multiplying days grazed in the rotation, 14, by 8.6, which was the approximate number of grazing rotations that had occurred, a value of 120.4 was arrived at as the number of days grazed in the year. This value pertains to the presence of cattle aged 1-2 years and over 2 years for farm A. On farm A, the same field had been grazed by a small number of horses on a year round basis, however continuity was thought best described by the predominant animal type(s) in each case.

2. Grazing intensity was represented by two ratios: the ratio between days grazed and the total number of days in a unique grazing rotation, and the number of rested to grazed days per rotation. The ratios were devised to show how in some farms grazing occurred in short sharp bursts, whereas in others the grazing was more lackadaisical. For example, when land is grazed for 3 days and rested for 21 as in the more intense paddock grazing system on farm Q, a ratio of $3/24$ or 0.13 is found, whereas the value 1 typifies set-stocked conditions, e.g. 120/120 days as in the summer grazed farm J. The rested to grazed ratio arrives at values of $21/3$, that is seven for farm Q which was managed more intensively, and $14/14$, or one, for the more extensively grazed farm A.

3. Grazing frequency was estimated in order to represent how often an area might be exposed to grazing animals and their associated activities. It was thought that frequency

would have both spatial and temporal implications. All animal types present at any one time were included in frequency value determinations. For farm A, short term frequency was arrived at by adding the numbers of bullocks aged 1-2 years (20) and 2 year olds (27), to the numbers of horses present (2). Frequency per hectare ($49/32.39 = 1.51$) and overall frequency (49) were expressed. While freedom of animal movement might somehow be represented by 'overall frequency', no attempt was made to distinguish the 'camp' ground where animals congregate and studied by Haynes and Williams (1999). However, the random soil sampling strategy and avoidance of pathways probably ensured that more generally grazed areas had been sampled overall. Continuous frequency was calculated as the total number of animals present together per hectare multiplied by the number of days on the land in a unique rotation, arriving at a value of 21.18 for farm A. Long-term frequency values resulted from multiplying the number of animals together per hectare in a normal rotation by the number of days grazed in the year prior to sampling, giving a value of 182.3 for farm A.

4. Treading was best represented by the estimated livestock equivalent bulk of animals on the land because animal weight data were not available. Animal bulk was calculated and expressed in livestock-unit equivalents per hectare, or LU ha^{-1} , based on animal number, type and age data, and on reference figures from IOFGA (1996), included in Appendix B. For example, farm A had twenty cattle aged 1-2 years each representing 0.54 LU; there were also twenty seven cattle over two years old at 0.65 LU each, and two horses equivalent to 1.2 LU, totalling 30.75 LU which on c. 32 hectares was equivalent to 0.95 LU ha^{-1} . Continuous treading was represented by livestock-unit equivalents per hectare multiplied by 14, the number of days on the land in a unique rotation, giving a value of 13.29. Long-term treading was represented by livestock-unit equivalents per hectare multiplied by the number of days grazed in the year, 120.5, resulting in a value of 114.4 for farm A.

5. Excreta N, P and K deposited in the year prior to sampling were estimated from the number of days spent per hectare by each animal type multiplied by their likely daily excreta nutrients, totalled for each type of grazing animal recorded. Reference values for likely daily excreta N, P and K were either obtained or extrapolated from Frame (1992) and REPS (1993), and are included in Appendix B. Values were determined as follows: two horses grazing year round totalled approximately 700 'horse days', which when

divided by 32.39 (the number of hectares) gave a figure of 23 horse days per hectare. Similarly, for 120.5 days grazing, twenty cattle aged 1-2y were estimated as spending 74 'young cattle days' per hectare, and twenty-seven cattle over 2y spent approximately 100 'older cattle days' per hectare. Multiplying N, P, and K values from Appendix B for both dung and urine for each animal type, then totalling these figures, gave likely excreta N (39.0), P (7.2) and K (34.3) values respectively in kg ha⁻¹ for farm A.

2.3.3.4 Soil disturbance and reseeding interventions

Soil disturbance is known to affect soil organic matter (Tivy, 1990), and therefore its disturbance history was ascertained. The number of known years since the soil in the sampled field had been tilled, and if arable crops were sown, was recorded during the interview. If the field had been reseeded and if so how long ago were recorded. Whether the seed had been surface sown, stitched in or spread following tilling operations was also clarified and recorded, so as to correctly describe any manipulation of the soil on the sampled field.

2.3.4 Management systems operational at the time of sampling

The management system operational at sampling was ascertained. One of the first questions in the interview determined whether the farm had been in conversion to organic management and if so in what conversion year or if the farm had been awarded the organic symbol, at the time of sampling. Whether the farm was managed by other than organic means was ascertained by default. Management regimen data were entered in the dataset as the number of months for which the farm had been organically managed prior to soil sampling. Zero represented both the conventional farm and those without a particularly organic regimen. Conversion to organic farming was represented as either 12 or 24 months for 1 or 2-year conversion status, respectively. Where the organic symbol had been awarded, 24 months were added to the number of months since the date of the award, to take account of management continuity during and after conversion.

No attempt was made to equate management type with fertiliser inputs at this preliminary stage because it was thought that to do so might introduce bias, and because the detail of nutrient applications was to be ascertained later in the interview (see Sections 1 and 7 of the questionnaire in Appendix A).

2.3.5 Statistical analyses

Statistical procedures allow researchers to gather, display and summarise data, to consider the probability of events, to make statistical inferences, and to draw conclusions based on evidence that observations within the population sampled are the result of a real effect rather than purely of chance (Gonick and Smith, 1993).

All of the statistical analyses of data from the sixteen study farms were carried out using the software package ‘Statistical Package for the Social Sciences’ SPSS, version 10.0 designed for Windows applications (Norušis, 1992; SPSS, 1995 & 1999). Specific statistical procedures are described in the chapter in which they are first applied.

2.3.5.1 Descriptive statistics

For Chapter 2, descriptive statistics were obtained for the minimum (Min.), the maximum (Max.), the mean and the standard deviation of the mean (SD) of values describing soil pH, fertiliser inputs and grazing events using the software package SPSS, (Norušis, 1992; SPSS, 1995 & 1999). Unless otherwise stated, the number of measurements on which the means are based is sixteen, throughout this thesis. Where fewer than sixteen measurements are available, the number of cases, *n*, is given. User-defined missing values were treated as missing and acknowledged as such in the SPSS results output from SPSS, version 10.0 (SPSS, 1999).

2.3.5.2 Normality of the data and data transformation

Hypothesis-testing procedures often require that data should be normally distributed about the population mean (Zar, 1996), an assumption that, according to Kovach (1998), is not often met. Other methods, termed ‘nonparametric’, are free from such

assumptions (Zar, 1996). Indeed the use of non-parametric or distribution-free statistical methods such as ranking is advised for the type of microbiological field data, explored later in this study, as they frequently fail to satisfy classical statistical analysis criteria (Mathes and Ries, 1995).

In order to establish which statistical and numerical methods would be appropriate, the entire study dataset was examined for distribution characteristics at the outset. Whether the data was normally distributed was visually established for each variable by plotting the frequency distribution (y) of values (x) in histograms via descriptive statistical and graphic analysis procedures implemented through SPSS (1999).

Transformation may help data conform to assumptions and reduce skewness in the data, dampening the effect of outliers, making samples and species more evenly spread out, and may be appropriate for that reason (Mathes and Ries, 1995; Kovach, 1998).

Distribution of all square root and log-transformed variables was also checked for normality in histograms of transformed values (x) vs. frequencies (y) using SPSS, as for raw data. Logarithmic or square root transformations could then be applied as appropriate in MVSP (Kovach, 1998) to those variables for which transformation reduced skewness and so improved the conformity of the data to statistical assumptions, or where transformation could enhance biological meaning of the ordination analysis. Log transformations were carried out on $x+1$ values rather than x , because zero values would be undefined when log transformed (Kovach, 1998).

2.3.5.2.1 Data distribution and choice of statistical analyses procedures

Few variables, if any, conformed to the normal distribution curve. Those variables most closely approximating normal distribution were: altitude; bulk density; total nitrogen content of the study farm soils; C:N ratio, and the numbers of botanical species.

Otherwise, distributions appeared distorted either because interim values were not represented or because extremes were absent. Lack of conformity to normal distribution seems attributable to the relatively small sample size, encompassing just sixteen farms. In particular, botanical species were absent or their individuals were found in low numbers. Furthermore, the range of values studied in working farms could not

realistically be expected to include extremes either of pH or of other key soil conditions upon which grassland production might depend, and therefore environmental gradients were relatively short.

Generally, frequency distribution of the data was not improved by either their square root or logarithmic transformation.

In conclusion, because the data did not conform to normal distribution assumptions, non-parametric, or distribution free statistical methods such as Spearman's rank correlation have been employed throughout to determine bivariate correlation coefficients amongst variables, and to estimate their statistical significance. Similarly, principle components and canonical correspondence ordination analyses that did not need data to conform to the normal distribution were applied in Chapter 7.

2.3.5.3 Spearman's rank correlation analysis

Bivariate rank correlation was established via the nonparametric Spearman's rank correlation coefficient, r . Spearman's rank correlation coefficient value is reported in its positive or negative form, $r = 0.000$ or $r = - 0.000$, to indicate direct or inverse correlation. The associated probability $P < 0.05$ or $P < 0.01$ is also given to indicate the significance of the relationship, as recorded in the results output (SPSS 1995 & 1999). Significant relationships ($P < 0.05$) between study variables are reported in the thesis.

2.4 Results and discussion

A description of the management features of the sixteen study farms is given in the following tables, and each management feature is commented on briefly.

2.4.1 Soil pH

Soil pH data are summarised in Table 2.1. The soil pH value obtained for each farm is included in the dataset in Appendix D.

Table 2.1

Soil pH values summarised for the sixteen study farms

	Min.	Max.	Mean	SD
Soil pH	4.9	7.3	6.1	0.7

Frame (1992) suggests that the optimal grassland pH range is 5.5 to 6, depending on soil type. Soil pH is not optimal on those farms where the minimum and maximum values were obtained; however, the mean value is acceptable for grassland.

2.4.2 Nutrient and lime applications

Nutrient and lime applications on the sampled fields prior to soil analyses are summarised in Tables 2.2 and 2.3. Details for four- and six- year applications for each sampled field and farm are available in the data spreadsheet, in Appendix D.

Additions that are termed ‘organic’ came from different sources including slurry, manure and from organically acceptable basic slag. Fertiliser applications ranged from basic slag and low levels of farmyard manures on farms L and H under organic management, to applications of more concentrated fertiliser nitrogen such as urea and calcium ammonium nitrate in the conventionally managed farm Q. Details of additions made to each farm and sampled field may be found in the data spreadsheet in Appendix D. Because application rates differed slightly, the spreadsheet contains N, P K and lime application values for the four-year period prior to soil analyses, summarised here, and for the six-year period prior to botanical and production analyses, and in relation to sampled fields and entire farms.

Table 2.2

Nutrient and lime applications to the sampled fields for the four-year period prior to soil sampling for 16 farms. Nutrient sources are categorised as mineral, organic and total

Nutrient source	Element	Min.	Max.	Mean	SD
Mineral fertilisers (kg ha ⁻¹)	N	0	920.0	59.3	229.6
	P	0	150.0	11.7	38.0
	K	0	300.0	23.4	76.1
Organic fertilisers (kg ha ⁻¹)	N	0	111.4	13.1	31.2
	P	0	135.0	30.3	37.4
	K	0	263.9	30.9	73.5
Total fertilisers (kg ha ⁻¹)	N	0	925.0	72.4	230.4
	P	0	153.6	42.0	46.9
	K	0	311.6	54.3	103.1
Lime (t ha ⁻¹)		0	13.6	3.5	3.8

Data are summarised in Table 2.2 for four years overall because applications were not necessarily made on a per annum basis to each of the farms, and it can be seen in Table 2.3 that many of the farms had not had mineral nitrogen applications in the years immediately preceding sampling, for example. Nutrient application rates varied considerably as would be expected in this type of study where a cross section of low input farms, farms in conversion to organic farming, fully involved in organic farming, and a conventionally managed farm, was sampled. Some farms such as farm E had been conventionally managed before the four-year period in question. This period may be regarded as transitional because many farms had recently opted for organic management (See Table 2.9, here, and the data spreadsheet in Appendix D.)

Nevertheless, the data shown in Table 2.2 are in line with fertiliser recommendations for grassland (MAFF, 1994). The maximum applications were representative of conventional farming and did not exceed recommendations. Similarly, mean additions were much lower than found in intensive grazing (MAFF, 1994), and N, P and K additions estimated on a per annum basis would have approximated 18, 10 and 13 kg ha⁻¹, respectively. Fertiliser additions are similar to those reported in other studies of the effects of fertilisers and land use on the soil biota and nutrient cycling (Mogge et al., 1999), and therefore facilitate comparisons. Lime application rates were unremarkable

but had probably been added advisedly, as they were positively correlated with soil pH values that were measured later. A Spearman’s rank correlation coefficient value $r = .435$ was obtained and the correlation was not significant ($P > 0.05$).

The numbers of years elapsed since mineral nitrogen fertilisers and since lime had been applied on the study farms is summarised in Table 2.3. Nutrient and lime application histories of each of the sixteen farms are included in the dataset in Appendix D.

Table 2.3

The number of years elapsed since mineral nitrogen and lime had been applied, prior to soil sampling, on the sixteen study farms

Years since application	Min.	Max.	Mean	SD
Mineral Nitrogen (y), n =14	0	8.0	6.7	2.6
Lime (y), n = 10	0.5	3.5	1.5	1.1

The numbers of cases reported here can be explained on the basis that only ten farms had applied lime, and information in regard to fertiliser additions was available for fourteen farms, and not for farms F and M, which were recent acquisitions. Farmers were asked about fertiliser use since 1987, which represents a maximum of eight years data prior to soil analyses. It should be noted that where “eight years since mineral nitrogen was applied” is recorded, this does not imply that applications were made prior to that time. A trend towards reduced fertiliser inputs is also reflected in the data in Table 2.3, as in many cases at least five years had elapsed since nitrogen had been applied. In farm E, for example, regular mineral fertiliser applications came to an abrupt halt just prior 1992, figures for which are therefore not included in the time-period considered on this occasion. Furthermore, eight of the study farms had not applied mineral nitrogen since at least 1987, and only one farm, Q, had already applied mineral nitrogen in the year of soil sampling.

2.4.3 Grazing practices and likely excreta

Grazing practices are summarised in Tables 2.4 - 2.6 inclusive. Excreta nutrients deposited in the year prior to sampling are summarised in Table 2.7. The full results of

grazing practices and excreta for each study farm are included in the data spreadsheet in Appendix D, and values can be accessed using the variable key given in Appendix C.

Table 2.4 summarises grazing continuity and intensity on the sixteen study farms.

Table 2.4

Grazing continuity described as days grazed and rested in a rotation, and days grazed in a year. Intensity described both as the ratios between days grazed to days in a rotation, and as the ratio between rested to grazed days in a rotation.

Grazing continuity	Min.	Max.	Mean	SD
Days grazed in a rotation (d)	3.0	365.0	48.8	96.8
Days rested in a rotation (d)	0	72.0	21.0	17.0
Days grazed in the year (d)	11.0	365.0	119.1	90.8
Grazing intensity				
Days grazed/rotation ^a (ratio)	0.13	1.00	0.45	0.27
Days rested/grazed (ratio)	0	7.00	2.25	2.16

a. Low values for the ratio between grazed / rotation days describe intensive grazing, high values extensive grazing.

On some farms, grazing occurs on extensive set stocked land giving a grazing intensity figure of 1.0 as seen in Table 2.4. These are seen to be farms P and J, in Appendix D. On other farms, larger numbers of animals are grazed together for brief periods on small paddocks that are then rested for relatively longer intervals, giving grazing intensity figures of 0.13 and 7.0. This more intensive grazing was found on the organically managed dairy farm L, and on the conventionally managed farm Q (Appendix D.)

Grazing frequency is summarised in Table 2.5.

Table 2.5

Frequency of grazing described as the number of animals present multiplied by days in rotation and year

Frequency of grazing	Min.	Max.	Mean	SD
Number of animals per hectare ($n \text{ ha}^{-1}$)	1.4	74.1	25.6	24.6
Total number present together (n)	10.0	140.0	59.0	40.4
Frequency in rotation ($n \text{ ha}^{-1} \times$ days grazed in rotation)	21.2	2712.1	431.0	640.0
Frequency in year ($n \text{ ha}^{-1} \times$ days grazed in year)	151.5	5882.1	1779.0	1641.1

The frequency of grazing events varies considerably from farm to farm. In addition, this variation reflects differences in the types of animals on the study farms, smaller animals such as goats and sheep being more numerous than beef and dairy cattle. The types of animals on each of the farms are detailed in Appendix D.

Farm P had the lowest and farm Q the highest grazing intensities (Table 2.4), numbers of animals per hectare (Table 2.5), and livestock units per hectare (Table 2.6), respectively. The least frequent grazing in the short and longer terms was recorded on farm A.

Table 2.6 describes the bulk and likely treading of animals in the short and long term.

Table 2.6

Treading in terms of animal bulk, rotation and year

	Min.	Max.	Mean	SD
Bulk of animals on field (LU ha^{-1})	0.79	74.10	12.35	20.07
Treading in rotation, ($\text{LU ha}^{-1} \times$ days grazed in rotation)	13.3	1017.0	165.0	244.4
Treading in year, ($\text{LU ha}^{-1} \times$ days grazed in year)	98.5	1715.2	605.5	486.9

The least treading in the short term was recorded on farm A. Greatest amounts of treading, both in a rotation and in the year prior to soil sampling, were recorded in farm N.

Likely excreta deposited during the grazing process are summarised in Table 2.7.

Table 2.7

Total estimated nutrients excreted by grazing animals in their dung and urine over the sampled area during the year prior to sampling

Excreta nutrients	Min.	Max.	Mean	SD
Excreta N (kg ha ⁻¹)	33.5	482.5	145.7	130.8
Excreta P (kg ha ⁻¹)	6.0	85.8	26.7	23.1
Excreta K (kg ha ⁻¹)	12.6	410.9	113.3	116.3

Values obtained in this study for likely excreta N, P and K are comparable with figures in the literature (Hynds and Carton, 1994). From Table 2.2, approximate per annum N, P and K applied by the farmer as fertiliser nutrients could be estimated as 18, 10, and 16 kg ha⁻¹ per year, respectively. In contrast, mean N, P and K deposited by grazing animals in a single year were estimated as 186, 27, and 113 kg ha⁻¹, respectively. Contrast between fertiliser and excreta nutrients is an expected feature of grazed grassland, but is made more remarkable by the current lower fertiliser input backdrop. Excreta nutrients are not a net addition to the soil fertility cycle (Frame, 1992) because they represent existent nutrients being returned from grazed herbage or from additional feedstuffs. Excreta contribute nonetheless to the nutrient milieu in soil and influence biological behaviour there, being mineralised and presented again to the animal in the grazed herbage perhaps many times in the growing season (Frame, 1992). Moreover, nutrients present in dung and urine are in a predominantly organic form (Whitehead, 1995), and based on studies to date (for example Mogge et al., 1999) organic nutrient effects will probably differ from the effects of soluble mineral fertilisers.

2.4.4 Soil disturbance and reseeded interventions on the study farms

The number of years since soil in the sampled field had been mechanically disturbed in any way was investigated during interview. The intention was to ascertain whether soil had been aerated, which may have influenced soil organic matter content for example Tivy, (1990). Whether the grassland had been reseeded was also ascertained. Table 2.8 summarises such interventions that had taken place prior to soil sampling.

Table 2.8

Intervals since mechanical disturbance likely to aerate the soil had occurred and since reseeded practices were carried out, summarised for the sixteen farms

Interval since	Min.	Max.	Mean	SD
Mechanical disturbance (y), n = 8	3.0	150.0	64.4	71.2
Reseeded (y), n = 6	2.0	20.0	11.8	7.5

Some of the farms had been in undisturbed pasture for many years. The maximum value suggested for statistical purposes was 150 years, roughly in line with farmers' suggestion that land had been tilled in some farms prior to the potato famine. Disturbance occurrence was established for only eight of the farms, and it was likely that remaining farms had been in permanent pasture for many years. It was noted that outcrops of rock combined with shallow spots would have precluded tillage operations on farm A, for example. Arable crops had been sown on five of the farms, B, H, L, S and most recently on farm Q as part of its reseeded programme. Farm B was unique in that it had been exposed to chain harrow use on a regular basis. It had also been reseeded recently, but seed was spread from the back of a tractor without prior tillage operations. Ferns were dug in on farm E, and farm O had been reclaimed with some soil disruption. Five farms, B, E, G, Q, and S had been reseeded relatively recently, while farm L may have been reseeded over 40 years prior to soil sampling. Both soil and sward may have been affected by aerating activities or by their absence from the older permanent pastures that characterised many of the farms.

2.4.5 Summary of the farm management systems operational on the study farms at the time of soil sampling

Table 2.9 describes management systems found on the farms at the time of sampling. Because it had been intended to study only organically managed farms, initial questions reflected this however it was found that farm management systems differed considerably. One farm was under conventional grassland management. Other farms were in a two-year period of conversion to organic management, or had been farmed organically for more than two years.

Table 2.9

Farm management systems operational in the sixteen study farms at the time of sampling

Management system at sampling	On farms	Number of farms
Neither organic nor conventional	J N P T	4
In conversion to organic farming	A F H M	4
Organically managed for some time	B E G L O R S	7
Conventionally managed	Q	1

Four non-organically and non-conventionally managed farms appeared so by default. Nutrient inputs to each farm were queried later in Section 7 of the questionnaire (see Appendix A). Values for the duration under which farms were organically managed and their N, P and K inputs over four and six years can be found in the spreadsheet in Appendix D.

2.4.6 Summary description of grassland management features in the study farms

Soil pH, nutrient applications, grazing practices and excreta nutrients differ considerably amongst the sixteen study farms. Pasture age varies also, some reseeded fields having been ploughed recently while others have been in continuous pasture for more than one hundred and fifty years. This variation in key farm management features provides a diverse background against which soil properties, nutrient cycling activities and grassland production may be examined in Chapters 3-7.

Chapter 3

Chapter 3

Site-related and soil physical and chemical properties of the study farms in relation to farm management features

3.1 Introduction

In Chapter 3, soil properties are described for the sixteen study farms and relationships with farm management features are discussed, in the light of comparable literature.

Soil is a complex material, yet it may be described in terms of its location, and its physical and chemical properties. Location is a major determinant of soil characteristics. Parent rock materials are physically and chemically weathered and may be geologically transported, notably via glaciations, to an alternative site. The type of parent materials, climate, vegetation, living organisms, site topography, and the time that the parent materials are subjected to soil forming processes then influence soil formation (Brady, 1990). Nevertheless, each resultant soil contains similar mineral particles such as clay, silt and sand particles in different proportions. The particular mix of mineral particles gives soil its texture.

Vegetation, detritus and biological activities contribute to soil organic matter, which helps bind the mineral particles together in a structure with altered moisture holding capacity and soil fertility. The chemical constituents in soil are determined variously by minerals from the parent materials and by soil organic matter, and are replenished by chemical and biochemical processes acting on inorganic materials from soil solids, or by added fertiliser nutrients (Brady, 1990). Soil chemistry is also affected by soil texture, structure, pH, ion exchange processes and by soil drainage. Soil minerals, organic matter, water and air are interdependent factors, and together these four major soil components interact as determinants of the plant nutrient supply in a given soil (Brady, 1990).

On a planetary scale, about half of the total land area is suitable for farming. Only a quarter of the land is suitable for cultivation (Brady, 1990), and livestock can be raised on much of the remainder (Tivy, 1990). In Ireland, the livestock carrying capacity of land has been established in field experiments, and in the knowledge of particular limitations of each soil type (Lee and Diamond, 1972). In the study by Lee and Diamond (1972), approximately 60% of lowland mineral soils in Ireland were rated as of average grazing capacity, with relatively low (2.17 LU ha^{-1}) and high (2.72 LU ha^{-1}) livestock unit per hectare potentials based on lower and higher (N) nitrogen inputs of 48 kg ha^{-1} and 230 kg ha^{-1} respectively. In 1970, there were 5.4 million livestock units on Irish grassland, and researchers concluded that livestock units in Ireland could be expected to rise substantially to an estimated 8.0 million with lower levels of N, or to 9.4 million with higher N application rates (Lee and Diamond, 1972). Increased livestock carrying potential could be achieved with existing fertiliser technology, and through the development of higher-yielding grass varieties and appropriate management systems to maximise pasture utilisation (Lee and Diamond, 1972). Production levels and N inputs increased accordingly, and by 1995, N fertilisers were used in dairying at a mean rate of 226 kg ha^{-1} , and at $67 \text{ kg ha}^{-1} \text{ y}^{-1}$ for sheep at stocking rates of 1.5 - 2 LU ha^{-1} . Nitrogen fertiliser usage on grazing land was, on average, $93 \text{ kg ha}^{-1} \text{ y}^{-1}$, varying from county to county. Stocking rate was directly related to N usage, which was broadly in line with recommendations (Murphy, et al., 1997). From 1947 to 1998, sheep numbers in Ireland increased from 2.1 to 8.3 million whilst cattle numbers increased from 4.0 to 7.7 million, (Central Statistics Office, 1999), and a large increase in capacity had been achieved, as predicted.

However, recent research has shown that the modern farming activities required to boost yield influence many important characteristics of soil, including its physical characteristics, nutrient status and organic matter content (Tivy, 1990; Reganold, 1995). Atmospheric pollution arising from the effects of current farm management practices on N mineralisation in soils (Burket and Dick, 1998; Civerolo and Dickerson, 1998), and on the storage of carbon in soils (Janzen et al., 1998) is an additional concern.

The deviation from traditional and proven agricultural practices since World War II demands deeper enquiry into the nature of soil and of crop requirements (Davies et al., 1982). Additionally, an understanding of functional mechanisms driving change in the

chemistry and botanical composition of agricultural ecosystems is lacking (Bardgett et al., 1997). Soil response to farm management features is a concern of this study.

As a basis, the site-related characteristics of the farms, and the physical and chemical properties of the soils are measured and described in Chapter 3. Statistically significant correlations ($P > 0.05$) between the soil properties and the grassland management features of the study farms described in Chapter 2 are also commented on. Comparable research is reviewed briefly.

3.2 Site-related properties of the study farms in relation to farm management features

3.2.1 Introduction

The sixteen selected study farms are clustered within a 40 km radius around Limerick City in Counties Limerick and Clare (Figure 2.1). Each farm is unique in terms of the combination of features at its particular site. Farms differ in altitude, slope and degree to which the farm is southern facing. Whether the soil is of a predominantly silt or clay nature, and its liability to drought and natural drainage characteristics, are also determined by soil location. Of additional relevance for agricultural crops is the depth of soil available for plant root development (Brady, 1990; Newton, 1993). Although Frame (1992) refers to an anonymous source as stating that “man owes his very existence to a six inch layer of top soil and the fact that it rains”, shallow soil depth is regarded along with higher altitude and poorer drainage as limitations for land capability purposes (Gardiner and Radford, 1980). Each of these site-related features of soil in the sixteen study farms is considered here.

3.2.2 Review of literature

Location often dictates farm activities. ‘Land capability’ maps classify areas according to the limitations for agriculture imposed by climate, altitude, gradient, soil type, and natural drainage (Gardiner and Radford, 1980; Frame, 1992). Of additional relevance in this study is the fact that any management practices applied on a particular site affect the soil properties and the botanical composition of that site (Frame, 1992; Bardgett et al., 1997).

Altitude is rarely mentioned in comparable soil biology research. An exception is a recent study of the effects of differing intensities of sheep grazing at altitudes of over 330 meters (Bardgett et al., 1997 & 1999) in response to agricultural intensification of hill sites. Another soil biology study examined site related factors influencing loss of nitrogen in compacted grassland at sites located at 20 m above sea level (Abbasi and Adams, 1998). Significant ($P > 0.05$) relationships between nutrient turnover processes and altitude have not been found in studies of soil response to farming practices.

Although sloped soil presents greater difficulty for farm machinery, a southerly-facing slope is likely to absorb more solar energy, increasing soil temperature relative to neighbouring level or northerly slopes (Brady, 1990). Sloped grassland may therefore be more productive, depending on conditions. Surface runoff and consequent loss of fertiliser nutrients from sloped grassland is a management concern, where greater losses may occur through storm events soon after slurry or fertiliser applications (Coulter and Tunney, 1996). Sloping soil is more prone to erosion (Fitzpatrick, 1986). Tillage operations are correlated with soil erosion in intensive management systems (Reganold et al., 1987), and erosion is reduced where more sustainable practices are applied. In comparable research studies, soils of similar slope (Reganold, et al., 1987; Abbasi and Adams, 1998) and or adjacent sites have been compared (Bolton et al., 1985; Reganold et al., 1987; Bardgett et al., 1997; Mogge et al., 1999) and generally, slope is not included as a factor in the analysis. On sloped Welsh land, management effects on denitrification were related to compaction of the soil and slope was not a separate issue (Abbasi and Adams, 1998).

Although the majority of grass roots develop within the top 10 cm of soil, and the maximal rooting depth of pasture is close to 30 cm, the depth of grassland soil is regarded as a critical productivity factor (Lockhart and Wiseman, 1988; Newton, 1993; Whitehead, 1995). A shallow A-horizon may limit rooting depth. Soil characteristics determine how water flows through to the layers beneath (Brady, 1990) and blockages or alterations in the profile may lead to impeded drainage (Brady 1990). Greater soil depth can protect land from the effects of poaching (Tivy, 1990). Management influences soil depth. For example, Reganold et al. (1987) compared two adjacent arable farms, one that had been organically managed, the other conventionally managed,

for many years. The organic farm relied on regular incorporation of green manures, crop rotations, and native soil fertility, and had undergone fewer tillage operations when growing wheat as part of its rotation, in contrast to the conventional farm that had had fertiliser inputs and more tillage interventions (Reganold et al., 1987). As a greater depth of soil in both the A- and B-horizons was found in the organically managed farm, Reganold et al. (1987) postulated that incorporation of organic material had replenished the soil in the organic farm, whereas management and tillage practices had led to soil erosion in the conventional farm. Erosion is unlikely to have influenced soil depth in the present study, where fields have been undisturbed, in permanent pasture, on most farms for many years (Section 2.4.4).

The proportions of relatively fine clay and progressively coarser silt and sand minerals define the texture of a soil and together with gravel and stones are determinants of soil drainage characteristics and influence soil organic matter content. Generally, soil organic matter content is protected in finer-textured soils, and is oxidised more rapidly in coarser sandy soils (Brady, 1990; Franzluebbers et al., 1996a). Clay content is regarded as protective, whereas sand and silt are less so, therefore measures of the percentage clay, and of the ratio of finer clay particles to coarser sand and silt particles, have been regarded as useful indicators of soil textural qualities and of associated organic matter behaviour. Furthermore, if the texture is such that grass can establish dense rooting, this provides material for humus formation, a desirable porous crumb and granular structure suitable for productive grassland (Frame, 1992).

That farming practice influences soil organic matter is seen in arable soils, where the organic carbon content is lower than that found in permanent pasture (Tivy, 1990). The effect of tillage is due partly to the aeration of the soil, because of which more microbial activity and hence more oxidation of organic matter, occurs. Plant debris mixed into the soil profile is also oxidised more rapidly than that left as ground cover. In a recent study, tillage modified the distribution of organic matter not only in the soil profile but also in clay, silt and sand fractions, influencing the inherent organic matter protection capacity of the soil (Kandeler et al., 1999b). Burket and Dick (1998) have found that contrasting management systems significantly affect mineralisation in soils of similar genesis, while soils of different genesis, or textures, responded differently to management treatments. While it is acknowledged that organic matter varies amongst

soils of differing texture, as soil texture was simply noted rather than quantified by Burket and Dick (1998), an analysis of the responses of soils of different textures to management systems was not presented in their study.

Different soil types will have natural drainage characteristics, a sandy soil being relatively free draining. Drainage improves with a more porous granular or crumb structure. Key to successful grassland production is the rapid removal of excess water to optimise the balance between soil air and soil water, and to avoid either erosion or nutrient losses through surface run-off (Frame, 1992). Soil moisture content has been recognised as an important factor regulating denitrification in pasture as it contributes to anaerobic conditions (Kaiser et al., 1991), however it also appears that factors other than oxygen availability may be involved (Luo et al., 1999a & b).

The soils in a cartographic location may be grouped in two or more soil types to form what is called a soil series. Soil series in the study area in Counties Limerick and Clare have been mapped, and their livestock carrying potential has been estimated based on existing grassland research and knowledge of the limitations of each soil type (Finch and Ryan, 1966; Finch, 1971; Lee and Diamond, 1972). In County Clare alone, over forty mineral soil series have been identified (Lee and Diamond, 1972). Some of the soil series found in County Clare are common to County Limerick, where other series have also been noted. On a global scale, soil series are correspondingly diverse.

3.2.3 Materials and Methods

Site-related parameters were derived from measurements *in situ* (3.2.3.1) and from soil maps (3.2.3.2), as described.

3.2.3.1 Site-related variables measured *in situ*

Altitude and slope were measured on site while visiting each of the study farms.

3.2.3.1.1 Altitude

An altimeter was first calibrated to zero between the high and low tide marks on the river Shannon. The altitude of each farm was measured, in meters, using the calibrated altimeter sited in the sampled field.

3.2.3.1.2 Slope

The slope of the sampled field was measured using a theodolite, and measured in degrees and minutes (0°00'). For the purposes of statistical analysis, slope values were converted to minutes (00').

3.2.3.1.3 Aspect

The aspect was estimated using a compass at the same point in the sampled field at which slope had been measured (Section 3.2.3.1.2). For the purposes of statistical analysis, aspect values were converted to degrees variation from south. If the field had more than one aspect, the value that corresponded most with the farm in general was chosen.

3.2.3.2 Map-derived soil parameters

Soil depth, depth of the A horizon, percentage clay, silt to clay ratio, and natural drainage information were abstracted directly from the published soil maps of Counties Limerick (Finch and Ryan, 1966) and Clare (Finch, 1971).

A description of the soil series represented by each study farm was also abstracted from these soil maps. Each map-derived parameter is described briefly below.

3.2.3.2.1 Soil depth

Soil depth refers to the total soil depth from the surface to the beginning of the parent material.

3.2.3.2.2 Depth of the A-horizon

The depth of the A-horizon is given as the soil depth of the topmost mineral horizons from their upper surface to the beginning of the B-horizon. The A-horizons contain partially humified organic matter and tend to be a darker colour (Brady, 1990).

3.2.3.2.3 Percent clay

Clay percent refers to the percentage clay in the topmost soil horizon.

3.2.3.2.4. Silt: clay ratio

The silt: clay ratio refers to the ratio of silt to clay in the topmost soil horizon.

3.2.3.2.5 Soil series represented by the study farms

The location of each farm was pinpointed on an ordinance survey map in order to identify the soil series represented by the farm in published soil maps of County Limerick (Finch and Ryan, 1966), and County Clare (Finch, 1971).

3.2.3.2.6 Natural drainage

The natural drainage characteristics of the study farms were deduced from the profile descriptions for the relevant soil series (Finch and Ryan, 1966; Finch, 1971), identified as in 3.2.3.2.5 above. Based on the soil drainage classes assigned to the soil series and described in Appendix B, a value was assigned to each soil.

3.2.3.3 Statistical Analysis

Descriptive statistics were determined as described in Chapter 2, Section 2.3.5.

Relationships between properties were determined by calculating Spearman's Rho correlation coefficients using SPSS Statistical Package for the Social Sciences, Release 7.0 Network Version (Norušis, 1992; SPSS, 1995). Positive correlation is reported as $r = 0.000$, and negative correlation as $r = -0.000$, together with an associated probability $P < 0.05$ or $P < 0.01$, as appropriate.

3.2 4 Results and discussion

Each of the site-related properties of the study farms was measured and the results for all site of these related parameters of the sixteen study farms are summarised in Table 3.1-3.10, below. The site related properties of individual farms are given in detail in the data spreadsheet in Appendix D, accessed using the variable key in Appendix C.

3.2.4.1 Site-related parameters measured *in situ*

3.2.4.1.1 Altitude

The elevation of the sixteen study farms is summarised in Table 3.1. The elevation of each farm is given in Appendix D, in the data spreadsheet.

Table 3.1

Elevation above sea level summarised for the sixteen study farms

Altitude	Min.	Max.	Mean	SD
Elevation above sea level (m), n = 15	25.0	177.5	86.2	42.1

The study farms include low lying to upland farms at elevations of 100 m above sea level, although none of the sixteen farms could be classified as hill farms (Frame, 1992). Farm management features described in Chapter 2 were not significantly ($P < 0.05$) correlated with altitude, possibly reflecting the generally moderate elevations of the study farms.

3.2.4.1.2 Slope

The slope of the sixteen study farms is summarised in Table 3.2. Originally in degrees and minutes, each slope value was converted to percentage incline so that comparison could be made with the literature. Detailed slope measurements may be found in Appendix D, where they are presented in minutes for statistical analysis purposes.

Table 3.2

Slope, summarised for the sixteen study farms

Slope of the sampled field	Min.	Max.	Mean	SD
Slope (percentage incline), n = 15	0.13	11.34	3.61	3.39

The results show that the majority of farms are close to being level, or are gently sloping, with less than 10% incline. Only farms E, P and R could be described as having moderately sloping land, at inclines closer to 20% (Brady, 1990). Slope is not generally mentioned in comparable research, as sites of similar topography are usually compared.

Abassi and Adams (1998) however, mention gently sloped fields with 10% incline in a study in Wales. Relationships between slope and management ($P > 0.05$) were not recorded in this study and have not been noted in comparable research.

3.2.4.1.3 Aspect

Table 3.3 summarises the variation from south, where low to moderate values represent more southerly facing fields. Details are available for each study farm in the data spreadsheet in Appendix D.

Table 3.3

Aspect, or variation from south, summarised for the sixteen farms

Variation from south	Min.	Max.	Mean	SD
Aspect (0°), n = 15	5.0	145.0	65.6	46.5

The mean of 65.6 implies that south-easterly and or south westerly aspects predominated in the study. Greater variation from due south correlated significantly with grazing continuity measured as the number of days grazed in a rotation ($r = 0.583$, $P < 0.05$); and with the number of days grazed in the year prior to sampling ($r = 0.579$, $P < 0.05$). Greater grass growth rates would be expected in fields facing due south, with growth rates declining at greater variations from due south. The significant correlations imply that grass is grazed more extensively in more north facing fields, which may be explained on the basis that slower grass growth occurs there.

3.2.4.2 Map-derived soil parameters

3.2.4.2.1 Soil depth and depth of the A horizon

The depth of soil on the study farms from the surface to the parent material, and the depth of the A-horizon are summarised in Table 3.4. The value for each farm is included in the data spreadsheet in Appendix D.

Table 3.4

Depth of soil in the uppermost A horizon and to the parent material

Depth of soil	Min.	Max.	Mean	SD
A horizon (cm)	12.7	64.0	30.4	13.2
To parent material (cm)	30.5	101.6	75.1	22.6

Some of the soils in the study farms are particularly shallow. Depending on the grass species in the sward, roots develop mostly in the top 10 cm (Whitehead, 1995). As regards the A- horizon of the study farms, the minimum depth of soil (12.5 cm) is adequate for grass production, and the mean soil depth (64.0 cm) could accommodate maximal grass rooting to 30 cm (Lockhart and Wiseman, 1988; Frame, 1992).

Altitude in the study region is probably a determinant of the depth of soil at a particular site, and altitude is negatively correlated with the depth of the A horizon, ($r = - 0.640$, $P < 0.05$). Soil depth is also negatively correlated with slope ($r = - 0.707$, $P < 0.01$). Such relationships are interesting but not unexpected, as the amounts of mineral soil found at higher elevations are usually less than those found at lower elevations due to a lesser amount of soil formation and possibly more erosion occurring (Fitzpatrick, 1986).

Grazing practice appears to relate to soil depth. At first glance, the greater the depth of soil, the greater rest interval in a rotation ($r = 0.510$, $P < 0.05$). From a different perspective, the greater the depth, the more intense the grazing appears to be ($r = - 0.616$, $P < 0.05$). This correlation may be explained in terms of the reduced poaching risk associated with deeper soil (Tivy, 1990).

In comparable research, the depth of soil available at a particular site is generally not mentioned as a separate factor. Reganold et al. (1987) mention that the soils in their study area have a depth of 20 – 46 cm in the A1 horizon, which is greater than those found here. Although Reganold et al. (1987) found differences in depth, which they attributed to management over the preceding forty years, their research was carried out on paired arable farms on similar soils, rather than across mainly grassland farms differing in soil type and depth as is the case in this study. Reliable management history data extends for eight to ten years in this study, however it would be interesting to examine management effects on grassland soil depth over a more extended time period.

The depth of soil at which a soil property is measured is of relevance, because organic matter, nutrient status and associated soil biological activity, decline with depth (Brady, 1990). In this study, all soil factors were measured in the topmost 10 cm of soil where the highest biological activities and organic matter contents are expected. It should be noted that soil property measurements were not made at different soil depths, and that references to soil depth in this study allude solely to the available depth of soil in the A-horizon or to the depth of soil to the parent material on the study farms. The relevance of ‘available soil depth’ will be made apparent in discussion in Chapters 5 and 7 of plant debris distribution in soil, which in deeper soil becomes more dilute, affecting substrate availability for microbial turnover.

3.2.4.2.2 Per cent clay

The clay content of the soils in the study farms is summarised in the Table 3.5. The value for each farm is given in the data spreadsheet in Appendix D.

Table 3.5

Per cent clay in the topmost soil horizon

Clay content	Min.	Max.	Mean	SD
Per cent clay (%)	12.0	34.0	21.6	4.8

The percentage clay is relatively low in the study farm soils, none of which could be classified as a clay soil (Brady, 1990).

Soil clay content is negatively correlated with southern facing study farms ($r = -0.574$, $P < 0.05$), and is positively linked with livestock units per hectare of grassland on the study farms ($r = 0.567$, $P < 0.05$). In other words, more northerly farms have higher clay content and carry greater livestock numbers. As previously noted, grazing duration appears to increase with more northerly farms. These correlations may indicate that soils with higher clay content have superior livestock carrying potential which is not surprising, as clay content is normally associated with greater inherent fertility.

Franzluebbbers et al., (1996a) carried out research on the active fractions of organic matter in soils with different texture by in Texas soils with from 7-45% clay content,

which extend beyond the clay content range in this study. Nitrogen immobilisation was analysed in soils of different clay contents by Trehan (1996), who concluded that the differing organic matter contents of the soils was a relevant factor. Soil organic matter content and nitrogen immobilisation are considered in Section 3.4, and in Chapters 5 and 7.

3.2.4.2.3 Silt:clay ratio

The ratio of silt to clay contents is described here in summary for the sixteen study farms. Values for individual farms are given in Appendix D, in the data spreadsheet.

Table 3.6

The ratio of silt to clay, summarised for the sixteen study farms

Ratio of silt to clay	Min.	Max.	Mean	SD
Silt:clay (ratio)	1.25	1.80	1.55	0.17

The study farm soils are described as loamy soils (Table 3.7), which contain roughly equal proportions of sand, silt and clay particles. However, they contain slightly higher proportions of the larger silt particles than clay. This is an important distinction, because clay presents a much larger surface area for aggregate formation, a crucial determinant of soil stability (Brady, 1990). The silt: clay content is looked at in relation to aggregate ratio and stability in Section 3.3.

The use of phosphate fertilisers ($r = 0.585$, $P < 0.05$), and organic phosphate additions, ($r = 0.687$, $P < 0.01$) correlated with silt:clay ratio. No explanation is proposed for these.

3.2.4.2.4 Soil series represented by the study farms

The soil series represented by each study farm has been identified, and is given for each farm in Table 3.7.

Table 3.7

Soil series represented by the sixteen study farms

Soil series	Farm	Great Soil Group	Textural class
Elton	A B L M Q T	Grey brown podzolic	Gravelly loam/ gravelly sandy loam over Silt
Ballylanders	E	Brown earth	Loam/ Shaly loam
Patrickswell	F G H S	Grey brown podzolic	Loam/Clay Loam
Baggotstown	J	Brown earth	Gravelly sandy loam
Gortaclareen	N O	Gley	Loam /clay loam Blg
Cooga	P	Brown podzolic	Sandy Loam
Kilfergus	R	Brown earth	Organic clay Loam to silty clay loam/ clay loam/shaly loam (B)

The study farms represent the principal soil series, namely Elton, found in Counties Limerick and Clare. Other major soil series such as Ballylanders, Gortaclareen and Kilfergus are also represented in the study.

Potential livestock carrying capacities at nitrogen inputs of 46 kg/ha for the soil series in the study farms have been estimated 2.2 LU ha⁻¹ for Elton, Patrickswell, Baggottstown, Cooga and Kilfergus soils, while the Ballylanders soil series potential is given as 2.05 LU ha⁻¹, and that of the Gortaclareen series is estimated as 1.48 LU ha⁻¹ (Lee and Diamond, 1972). In this study, the LU ha⁻¹ value recorded on the sampled field was lower in almost every case than the potential capacity for the particular soil series represented by the farm in question. This is expected in a study of farms in which even lower levels of nitrogen (approximately 27.5 kg ha⁻¹) were applied than the 48 kg ha⁻¹ suggested as a basis for livestock carrying potentials by Lee and Diamond (1972). Even the conventional farm, Q, at 1.58 LU ha⁻¹, was carrying less than the potential LU ha⁻¹, although apparently much higher rates of fertiliser nitrogen (347 kg ha⁻¹) were applied than the 230 kg ha⁻¹ suggested by Lee and Diamond (1972). Nevertheless, livestock densities were higher than had been recorded for Counties Limerick and Clare in a 1958 study (Lee and Diamond, 1972), indicating increased livestock numbers per hectare overall relative to earlier farming conditions. Exceptions were the two farms in which goats were the main enterprise, farm O carrying 4.35 LU ha⁻¹, and farm R at 2.82 LU ha⁻¹. Tivy (1990) describes the goat as one of the most versatile feeders of all ruminants,

browsing low trees and shrubs, and withstanding poor herbage conditions. Goats are also regarded as environmentally beneficial agents in improving hill grazing (Cooper, 1995), but are implicated in Mediterranean deforestation (Tivy, 1990). A combination of slope and soil depth at farms O and R, both being somewhat shallow and sloped, R having the shallowest soil amongst the study farms, may influence the type of animal chosen as the main enterprise on those farms. Livestock numbers are considered in the light of both market forces and environmental concerns in Chapter 6.

No resemblance was found between the classification of soils in this study and of those in earlier research on soil management effects, soil biological properties and nutrient turnover (Bolton et al., 1985; Reganold et al., 1987). Because comparison of management effects on different soil classes or series is not possible, this study considers existing research data in relation to particular measured soil properties in this and other studies.

3.2.4.2.5 Natural drainage

Table 3.8 summarises the natural drainage characteristics of the study farms, details of which may be found in Appendix D.

Table 3.8

Natural drainage and liability to drought scores summarised for the sixteen study farms

Drainage	Min.	Max.	Mean	SD
^a Drainage (score)	0	4.0	2.7	1.1

^a Drainage scores are explained in Appendix B.

Having been abstracted from soil map data, the natural drainage and liability to drought figures (Table 3.8) coincide with soil type, in that the farms with Gortaclareen type soil (N and O), which is poorly drained (0), and farm P is rated as excessively drained (4.0), and is a sandy loam soil. All other farms are classified as well drained (3.0).

The drainage status of a soil is almost inevitably described in field studies of the nitrogen cycle, or can be manipulated for laboratory research purposes.

3.3 Physical properties of soils on the study farms in relation to farm management features

3.3.1 Introduction

Properties of the soil that are examined here are: root mass, bulk density, particle density, total porosity, aggregate ratio and aggregate stability. Each of these factors contributes to or reflects the arrangement of sand, silt and clay particles in what is termed soil 'structure'. Soil structure determines the movement of water, air and nutrients in soil, and thereby influences nutrient turnover. Structure influences root development through soil as well (Brady, 1990). Soil structure is strongly influenced by farm management practices (Mullen, 1973; Parkinson, 1995).

3.3.2 Review of literature

During the 1960s and 1970s it became evident that modern agriculture modifies soil physical properties in ways that had previously been unheeded (Parkinson, 1995). Soil structure is not a permanent feature, and can be changed by cultivation and by the intensive use of soils (Parkinson, 1995). Intensive tillage may compress the soil, which decreases its penetrability and prevents root extension (Briggs and Courtney, 1989). In grassland, root development occurs largely in the top 10 cm, and although the shallow roots may hold the soil in place, they offer little protection compared to deeper-rooted crop systems (Lockhart and Wiseman, 1998; Tivy, 1990). If excessive treading damages its surface structure, grassland soil is especially vulnerable to poaching (Mullen, 1973; Tivy, 1990). Long-term physical damage is associated with relatively reduced biological activity and is known to occur in soils under conventional management (Reganold et al., 1987). However, many of the management-induced changes in soil physical properties may be reversed (Briggs and Courtney 1989). For example, tines were adopted in the late nineteenth century to break the plough-pan left from years of tillage (Tivy 1990). If good agricultural practice such as shallow infrequent tillage is adopted, the fabric of a soil may be conserved rather than depleted (Parkinson, 1995).

From the perspective of grassland productivity, greater attention is often paid to above ground plant growth, measured as sward height, leaf area index, or herbage dry matter

(Frame, 1992), than to the below ground root mass. Although inherent difficulties are associated with the study of grass root development and senescence (Whitehead, 1995), these processes are partially understood. Generally, root development occurs as part of overall plant growth. Root development is influenced by environmental factors such as available rooting depth, presence of obstacles, temperature, water and nutrient concentration (Brady, 1990; Drew, 1990). Whilst the majority of crops respond with increased root growth (Tisdale, et al., 1993), grass shoot growth in response to fertiliser nitrogen exceeds that of the root system, and is reflected in a decreased root: shoot ratio (Whitehead, 1995). Whitehead (1995) also suggested that plants maximise photosynthate distribution towards root exploration of soil in response to nitrogen deficiency, and so an increased root:shoot ratio may indicate nutrient stress.

Root development may also be facilitated or impeded by the relative penetrability of soil. When root development is possible, it disrupts and loosens soil, thus changing soil penetrability itself, and affecting soil structure. Distribution of the photosynthate and the synthesis of plant proteins as material for the developing root will eventually contribute organic matter to the soil. Meanwhile, the associated rhizosphere effects of root presence magnify the biological activity in the immediate soil environment, affecting organic matter content and nutrient turnover there (Lynch, 1991; Coyne, 1999).

Particle density refers to the particles making up the soil. Particle density is reduced by the lighter organic matter present. Bulk density is the overall density of soil in relation to volume, and includes both particles and pore space. Higher pore space implies lower bulk density. Bulk density values give useful information about soil structure and condition, because it reflects the degree to which soil has been compacted by agricultural practices. However, because of the differing carbon contents of soils from different sites, bulk density has a limited value as a measure of soil compaction when different soils are compared (Rasiah and Kay, 1998). Differences in bulk densities have been reported in comparison of organically and conventionally managed soil. An organically farmed soil was more friable, and its bulk density was slightly lower than in its conventional counterpart (Reganold et al., 1987; Reganold, 1988).

Soil structure may be considered in terms of the mass of soil particles and as the channels between soil particles referred to as soil pore space (Lampkin, 1990). Air,

water, the soil solution containing plant nutrients, plant roots, soil microorganisms, and soil invertebrates move through these pores. Pore space may be divided into two categories, namely micropores and macropores. Visible macropores or textural pores, which exist between the larger soil particles, are easily drained, allowing air access within an hour of saturation (Lampkin, 1990; Parry et al., 1999). The smaller micropores exist between the smaller silt and clay particles and within soil aggregates. Micropores within soil aggregates are also termed structural pores. Although structural pores represent a very small volume fraction of soil, they can be regarded as partially air-filled networks, which modify the aeration status of the clods (Parry et al., 1999). Micropores also tend to retain water so that the soil solution and its associated nutrients are conserved rather than leached, while providing moisture for root growth and biological activity. Plant roots, earthworms and burrowing organisms not only help to create macropore channels but also alter the organic matter content of the spaces through which they move, influencing soil structure even further (Lynch, 1991; Coyne, 1999). Micropore size controls the entry and colonisation of aggregates by microorganisms (Coyne, 1999). Soil porosity is therefore a determinant of many important soil characteristics, namely drainage, airflow, biological activity, organic matter turnover and nutrient status.

The overall effects of farm management problems such as compaction, waterlogging and poaching on soil porespace, and therefore water retention and aeration, are well documented (Mullen, 1973; Frame, 1992). Pore space in relation to microbial activity and nutrient turnover is of increasing interest, particularly as denitrification is regarded as an anaerobic activity, influenced by the relative contents of air and water in soil (Coyne, 1999; Luo et al., 1999 b). Useful nitrate is lost from soil through denitrification, a process that is always accompanied by the release of nitric oxide and nitrous oxide, potential atmospheric pollutants (Coyne, 1999) therefore soil conditions influencing denitrification are a concern. Recently, while it was found that contrasting pasture and cropped soil management influenced the structure of air-filled pore space, management did not influence the potential denitrification rates (Parry et al., 1999). Instead, denitrification differences were attributed to different organic matter distributions, soluble organic carbon being higher in the cropped soil. Another study (Luo et al, 1999 b) substantiates the influence of carbon content, but found that the availability of soil nitrate, carbon, and water interdependently influence denitrification.

Soil aggregates consist largely of organically bound soil particles, which contribute structure, resilience and water stability to the soil. Biological activities contributing to aggregate formation and stabilisation include: the binding effects of fungal hyphae; the formation of extracellular gummy polysaccharides by bacteria; and earthworm activities (Coyne, 1999). Cation bridges form between organic polymers and clay surfaces, and finer textured soils such as clay and silt and silt or clay loams form aggregates with organic matter more readily than heavier textured sand (Brady 1990; Coyne 1999). Soil aggregate formation is influenced to a lesser extent by inorganic factors, such as the presence of the polyvalent magnesium, calcium, and aluminium. The resultant aggregate has a certain strength and resilience. Crusts may form in an unstable soil surface, where crop emergence is subsequently inhibited, and the unstable soil is prone to raindrop damage and erosion (Brady, 1990). Furthermore, movement of soil material downwards with rain may clog the pore spaces, reducing soil permeability in the process (Mullen, 1973). Soil aggregate stability on exposure to water is, therefore, a laboratory measure of soil structural quality.

Aggregate ratio is an additional measure of soil stability because it reflects the relative proportions of small (< 1 mm) to large (> 1 mm) aggregates in soil that has been passed through a 2 mm sieve. An increasing aggregate ratio is an indication of decreasing soil quality, as larger aggregates are more stable, and less prone to erosion (Brady, 1990). Furthermore, aggregate size, ratio and stability are affected by farm management features, such as the use of heavy machinery in tillage operations or trampling by grazing animals, which breaks aggregates leading to poorer soil conditions (Mullen, 1973; Frame 1992).

Cultivation of soil for crop production reduces both the organic matter content (Rowell, 1994) and associated aggregate stability (Mullen, 1973). Conversely, extensification, reduced tillage operations and conservation of crop residues help to maintain aggregate stability. Permanent undisturbed pasture has generally higher aggregate stability and better soil structure than soil that has been repeatedly tilled and cropped (Mullen, 1973). Other farming practices, such as liming, encourage aggregate formation and so help to improve soil structure (Brady, 1990). In the 1950s, synthetic chemicals were added to stabilise soil aggregates but their high cost was prohibitive. Since then effective

substitutes have been found, which may ensure continued interest in use of soil conditioners, according to Brady (1990). Microbial inoculants such as microalgae have also been advocated as a means of improving aggregate stability (Rogers et al., 1991), but because of their photosynthetic habit, algal additives effects are limited to exposed soils, and would not necessarily benefit where soil is covered, as in grassland. However, improved aggregate stability can be achieved through appropriate grassland management practices particularly in regard to soil organic matter inclusion and retention, and without the use of expensive interventions. For example, in a twenty-year comparison of organic and conventional farming, fields under organic management contained a higher proportion of humus, which increased the moisture retention of the soils making them less likely to flood, thus reducing the risk of poaching (Newton, 1993).

3.3.3 Materials and methods

3.3.3.1. Soil sampling

Farms were visited in the summer of 1995 and samples were removed in a random manner from the selected field using two methods. A core sampler removed ten cylindrical soil cores 12.2 cm long and 1.52 cm in diameter from the upper 12.2 cm of the soil profile. These were used in determining root mass. Five clod samples were removed from the upper 8-10 cm of the soil profile at randomly chosen locations using a trowel. Three of these were removed very carefully and bagged to ensure that the samples were undisturbed and were used in determining bulk density. Two were used for drying and sieving and the sieved soil was used in determining particle density, aggregate ratio and aggregate stability.

3.3.3.2 Parameters measured

3.3.3.2.1 Root mass

Root mass was established from soil cores extracted using a core sampler, and approximately 22 cm³ of soil from a core of 12.2 cm depth and 1.52 cm diameter was gently broken up in water and placed on a 2 mm sieve. Soil was washed from the roots and roots were separated from stones, dried at 105^oC, and weighed. Based on the area sampled by the corer, the number of cores possible per hectare was calculated. This

figure was multiplied by grams of root mass per core, and root mass then calculated and expressed in kg ha^{-1} , for comparison purposes.

3.3.3.2.2 Bulk density

Bulk density was measured as the volume displacement of wax-sealed clods in liquid (Blake, 1965 a).

3.3.3.2.3 Particle density

Particle density was measured according to Blake (1965 b).

3.3.3.2.4 Total porosity (pore space)

Total porosity was calculated as: $100 (1 - \text{Bulk density} / \text{Particle density})$.

3.3.3.2.5 Aggregate ratio

Sieved soil (2 mm) was separated into small (< 1 mm) and large (> 1 mm) aggregates and weighed as x_g and y_g , respectively. Aggregate ratio is calculated as the ratio of small to large aggregates, or x_g/y_g .

3.3.3.2.6 Aggregate stability

Aggregates, 1-2mm in size, were soaked in water for 30min and wet-sieved for 3min. Aggregates larger than 0.212mm were considered stable (Kemper, 1965). Results were corrected for amounts of sand larger than 0.212 mm.

3.3.3.3 Statistical analysis

Descriptive statistical and Spearman's rank correlation analyses were carried out as described in Chapter 2, Section 2.3.5.

3.3.4 Results and discussion

Measurements for each physical property of the sixteen study farm soils are combined in Appendix D, in the data spreadsheet provided, and can be accessed using the key to variables in Appendix C.

3.3.4.1 Root mass

Root mass measurements for the sixteen study farms are summarised here in Table 3.11, and are included in detail for each of the study farms in Appendix D.

Table 3.9

Root mass values for the sixteen study farms, summarised

Root mass	Min.	Max.	Mean	SD
Root mass (kg ha ⁻¹)	36941	122404	78294	28119

All root mass values found in the study farms are substantially higher than those quoted from studies elsewhere. In a newly sown sward, the quantities of root-mass produced are quoted as 3000 kg ha⁻¹ within 6 months and up to 10000 kg ha⁻¹ after 3 - 4 years (Garwood, 1967, cited in Whitehead, 1995). Established swards may have 10000 - 20000 kg ha⁻¹ root mass under long-term grassland and up to 25000 kg ha⁻¹ under prairie, measured for swards in Canada, USA, and the Netherlands (Black and Wright, cited in Whitehead 1995). In the present study, the minimum root mass was found on farm E, which was also the newest sward. About three years prior to root mass analysis, the sampled field on farm E had been ploughed to dig in ferns and reseeded some months later. Maximum root mass values were associated with farms that had not been mechanically disturbed within living memory, in some instances at least since the potato famine. The relatively undisturbed nature of the study farm soils (Section 2.4.4) eight of which had been in permanent pasture since perhaps before the potato famine, may

explain why root mass values exceeded those in the literature. General root mass values for Irish grasslands are not available.

Errors may have occurred in the calculation of root mass values per hectare that were derived using a conversion factor calculated for that purpose. The conversion factor by which the number of possible sampled cores per hectare was taken into account was relatively large, and would have amplified original values considerably. If soil remained after washing, this too would have been amplified, and may have distorted the absolute values obtained. However in the comparable analysis conditions under which soils were examined in the present study, root mass measurements serve to differentiate study farm soils from each other. Whether absolute values can be compared with those found elsewhere is debatable. However, many soils in this study have been under permanent pasture for many years and have not been subjected to conventional levels of nitrogen fertiliser additions, both of which may account for the comparatively greater root mass content observed in the present study.

In accordance with the lower root mass values found in newer swards, root mass was also strongly correlated with whether the farms had been reseeded or not ($r = -0.659$, $P < 0.01$). In further agreement with the premise that tillage operations renew pasture and disrupt root mass development, a positive correlation was found with the number of years elapsed since the field had been mechanically disturbed and aerated ($r = 0.805$, $P < 0.05$), root mass values being higher in older swards.

A strongly negative correlation was found between root mass values and soil pH ($r = -0.680$, $P < 0.01$). Soil pH ranges from 4.91 to 7.15 in the sixteen study farms. According to Frame (1992), most grasses survive well in slightly acidic conditions, so complete neutrality is not required, pH 6 being preferable as a target in mineral soils. Liming is designed to counteract soil acidity, and whilst liming improves its physical structure, it enhances the chemical and biological conditions needed for the uptake of nutrients by plant roots (Frame, 1992).

Factors influencing root mass in the sixteen study farms may include fertiliser nutrient provision. Lower root mass values were significantly correlated with added potassium ($r = -0.561$, $P < 0.05$) and with added nitrogen ($r = -0.566$, $P < 0.05$). Negative

correlation was found with sources of both artificial nitrogen ($r = - 0.549$, $P < 0.05$) and organic nitrogen ($r = - 0.502$, $P < 0.05$). Insignificant negative correlations were also found between root mass values and all artificial and organic fertiliser sources of potassium and phosphate. Whitehead (1995) suggests that fertiliser nitrogen has little effect on the production of grass roots, but that increased levels of fertiliser increase the rate of root decomposition. Whether this explains the negative root mass correlation with fertiliser nitrogen in this study has not been established. Whitehead (1995) has also cited studies in which increases in nitrogen supply above a moderate provision tended to reduce root weight (Boote, 1976; Cunningham, 1968; Toomre, 1966, each of which have been cited in Whitehead, 1995). Whitehead added that when nitrogen is deficient, plants maximise root exploration of soil in search of this essential element. The more likely explanation for negative correlation in this study may be that the root elongation occurred as a foraging response in relatively deficient soils.

3.3.4.2 Bulk density

Bulk density measurements of soils for the sixteen study farms are summarised here in Table 3.12, and are included in detail for each of the study farms in the data spreadsheet in Appendix D.

Table 3.10

Bulk density values, summarised for the sixteen farms

Bulk density	Min.	Max.	Mean	SD
Bulk density (g cm ⁻³)	0.98	1.27	1.10	8.767E-02

Rowell (1994) quotes typical grassland bulk density as 0.8-1.2 g cm⁻³ and soils in the study farms fall within this range. However, the mean bulk density value is relatively high. This may suggest that soils are relatively compacted. However, Rasiah and Kay (1998) have expressed concern about using bulk density as a measure of soil compaction because of the differing soil organic matter contents at different sites. Soil organic matter in the sixteen study farms is considered separately in Section 3.4.

The bulk density values obtained for the sixteen study farms were significantly correlated with application of mineral nitrogen fertilisers ($r = 0.680$, $P < 0.01$), with

reseeding ($r = 0.632$, $P < 0.01$) and with years since mechanical disturbance ($r = -0.878$, $P < 0.01$). These issues are considered in Section 3.3.4.4 in the context of pore space, which is an aspect inherent in soil bulk density. For now, it is proposed that compaction may have occurred during any trafficking on the land, thus increasing bulk density. Mechanical disturbance would also have exposed the soil to organic matter oxidation and loss, thus increasing bulk density values. Soil organic matter is considered further in relation to soil structure in Section 3.4.

3.3.4.3. Particle density

Particle density measurements for the soils from the sixteen study farms are summarised here in Table 3.11, and are included in detail for each of the study farms in Appendix D, in the data spreadsheet.

Table 3.11

Particle density summarised for the sixteen study farms

Particle density	Min.	Max.	Mean	SD
Particle density (Mg m^{-3})	2.13	2.26	2.35	8.434E-02

Brady (1990) states that for most mineral soils particle density ranges from 2.6 to 2.75 Mg m^{-3} , however organic matter weighs considerably less than the mineral particles in soil and influence particle density values. Grassland has typical particle density values around 2.4 according to Rowell (1994). The inference is that in the sixteen study farms, organic matter content is relatively higher than in soils in the literature, for example the study farm organic matter content is higher than in farms studied by Reganold et al. (1987), leading to lower particle densities. Organic matter content in relation to soil structure is considered in Section 3.4.

No correlation was found between farm management features and particle density measurements, however significant correlations found with soil chemistry, organic matter content, and soil biological activities, which will be discussed in Section 3.4, and in Chapter 5.

3.3.4.4 Total porosity (pore space)

Total porosity (pore space) of soil from the sixteen study farms is summarised here in Table 3.12, and measurements are given in detail for each of the study farms in Appendix D, in the data spreadsheet.

Table 3.12

Pore space values summarised for the sixteen study farms

Pore space	Min.	Max.	Mean	SD
Pore space (%)	47.0	60.0	53.3	3.7

Typically, grassland soil porosity ranges from 50-67% (Rowell, 1994). Farms in this study appear to fall within a slightly lower range.

In the sixteen study farms, porosity is strongly negatively correlated with bulk density ($r = -0.879$, $P < 0.01$), and positively correlated with root mass ($r = 0.517$, $P < 0.05$). The former correlation is to be expected, since pore space is calculated from bulk density and particle density data. The latter may be due to the creation of channels by roots as they infiltrate the soil mass, contributing to soil porosity on the way. A negative correlation is seen here between mineral nitrogen additions and porosity ($r = -0.639$, $P < 0.01$). Rather than infer that nitrogen influences porosity and bulk density directly, these effects may have been mediated by the reduced root mass resulting from added fertiliser nitrogen (see Section 3.3.4.1). Root mass appears lower with added nitrogen, and with relatively fewer channels, may explain the associations found between root mass, nitrogen, porosity and bulk density.

Pore space is also negatively associated with reseeding, also a determining factor in root mass development (see Section 3.3.4.1), root mass being lowest in the newest sward on farm E. The porosity of the soil is further linked in a positive way with years since mechanical disruption likely to have aerated the sward ($r = 0.736$, $P < 0.05$), suggesting perhaps that, with root mass, it is also greater in the older established sward.

3.3.4.5 Aggregate ratio

The ratio of small to large aggregates in soil from the sixteen study farms is summarised here in Table 3.13, and measurements are given in detail for each of the study farms in Appendix D.

Table 3.13

Ratio of small (<1mm) to large (>1mm) aggregates

Aggregate ratio	Min.	Max.	Mean	SD
Aggregate ratio	1.29	10.95	3.95	2.41

The mean aggregate ratio in this study suggests relatively poor soil structural conditions overall, because smaller aggregates predominate. The aggregate ratio in the study farms resembles that of corn in a rotation rather than that of continuous meadow, in which the ratio is closer to 0.77 (Wilson, et al., 1947, cited in Brady, 1990). Aggregate ratio significantly correlated with silt: clay ratio ($r = 0.502$, $P < 0.05$), and is strongly associated with organic carbon content, discussed in Section 3.4.

3.3.4.6 Aggregate stability

The percentage water stable aggregate measurements for the sixteen study farms are summarised here in Table 3.14, and are given in detail for each of the study farms in Appendix D.

Table 3.14

Aggregate stability described as percentage water stable aggregates, summarised for the sixteen study farms

Aggregate stability	Min.	Max.	Mean	SD
Water stable aggregates (%)	63.2	89.0	81.4	6.5

The percentage water stable aggregates reported for the sixteen study farms is lower over all than those reported in Irish pasture soils (Mullen, 1973), although the mean water stable aggregate content is quite good (Brady, 1990; Mullen et al., 1974).

Aggregate stability is affected by farm management features, such as the use of heavy machinery in tillage operations or trampling by grazing animals, which breaks aggregates leading to poorer soil conditions (Frame 1992). In this study, treading in the short term was negatively associated with water stable aggregates ($r = - 0.549$, $P < 0.05$), and long-term treading effects were negative but insignificant.

The ratio of small (< 1 mm) to large (> 1 mm) aggregates correlated negatively with percentage water stable aggregates ($r = - 0.632$, $P < 0.05$) which is to be expected, because larger aggregates contribute more stability (Brady, 1990). Aggregate ratio correlated positively with silt to clay ratio ($r = 0.502$, $P < 0.05$). The percentage clay is positively, although not significantly, associated with aggregate stability. The formation and stabilisation of aggregates is determined principally by organic and microbial compounds in soil, and to a lesser extent by divalent cations. Significant relationships found in this study between soil organic matter content, soil cations and soil aggregates are considered in Section 3.4. Relationships of aggregate stability and soil organic matter with soil biology are considered in Chapter 5.

3.4 Chemical properties of soils on the study farms in relation to farm management features

3.4.1 Introduction

Soil chemistry is a crucial determinant of plant nutrient supply and of soil structural qualities.

3.4.2 Review of Literature

Mineral and organic matter constitute the chemical contents of soil. Minerals are derived from the parent rock material, from the mineralisation of organic matter, and from added fertilisers. Soil organic matter arises from plant, animal and microbial detritus resulting from above and below ground biological activity. The chemical behaviour of soil is particularly influenced by the tendency of the soil to adsorb molecules such as polymers, ions and soil organisms. Soil pH is an overriding factor because it influences the cation exchange processes, the biological activity involved in organic matter turnover, and the uptake of nutrients by plant roots (Brady, 1990).

Soil organic matter is largely derived from plant debris, animal remains and excreta, and is biologically processed by a wide range of soil organisms. In its most degraded yet most complex form as humic substances, soil organic matter contributes to the lasting fertility of the soil and to soil structure (Brady, 1990; Hayes, 1991). Although soil organic matter comprises usually less than 10% of topsoil, it helps to bind mineral particles together into a more manageable crumb structure (Briggs and Courtney, 1989; Brady 1990). The organic matter is a critical determinant of soil moisture retention, as it acts as a sponge, allowing soil to hold water. It also is the major source of soil nutrients, particularly nitrogen, and contributes to phosphorus and sulphur required for plant growth (Brady, 1990; Tivy, 1990). Soil organic carbon provides energy for those heterotrophic organisms involved in nutrient turnover processes in soil (Tivy, 1990; Parkinson, 1995).

The effects of farm management practices on soil organic matter are recognised. Tillage practices aerate the soil temporarily, mixing organic matter with different particle fractions in the process, and increase the rate of oxidation of the organic matter by the microbial population (Tivy, 1990). Soil organic matter content ranges from 5-10% in pasture soils, and ranges from 1 - 2% in cropped soils (Briggs and Courtney, 1989). Soil organic carbon constitutes approximately 50% of soil organic matter. That arable soils generally have lower levels of soil organic matter attests to the oxidising effects and loss of organic carbon in respiration in response to aeration during tillage. The removal of harvest in arable and grazed land is also a source of organic matter loss (Briggs and Courtney, 1989).

Soil organic matter may be conserved by good agricultural practices such as the maximal return of crop residue to the soil, use of green manures, and quick manure incorporation to avoid oxidation losses (Parkinson, 1995). The long-term soil structural benefits of organic management have been described (Newton, 1993).

Of the elements essential for plant growth, nitrogen is a major constituent of plant protein, such that nitrogen controls grassland yield (Brockman, 1995b; Parkinson, 1995). As soil nitrogen is not derived in any form from the soil parent material, fixation of atmospheric nitrogen by microorganisms and mineral nitrogen fertiliser additions, are

the main sources of nitrogen in agriculture. Nitrogen applications are not recommended for grass clover swards where fertiliser nitrogen would be inhibitory, and counterproductive. Conventional agriculture has increasingly relied on fertiliser nitrogen inputs: for example, maximal per annum applications of 420 kg ha⁻¹ are recommended to compensate for low soil nitrogen supply (MAFF, 1994). Soil nitrogen arises secondarily through the mineralisation of soil organic matter by soil microorganisms.

There is close correlation between the organic matter of soils and soil nitrogen content (Brady, 1990). Although soil nitrogen accounts for only 5% of soil organic matter, about 95% of soil nitrogen is present in the organic matter whilst inorganic forms such as ammonia and nitrate make up the remainder (Whitehead, 1995). Nitrogen tends to accumulate in grassland soils compared to soils under tillage, because the soil is not routinely exposed to organic matter oxidation (Whitehead, 1995). In contrast to the immediate aeration effects of tillage, soil compaction from trafficking and treading induces anaerobic soil conditions. For example, a three to four-fold higher rate of denitrification has been reported in compacted soil than in a control (Kaiser et al., 1991). Management-induced changes in the quality and content of soil organic matter and the loss of nitrogen to the environment have serious long-term consequences.

The ratio of carbon to nitrogen (C:N ratio) in soil organic matter balances nitrogen immobilisation and mineralisation, key microbial processes that occur simultaneously in soils. The C:N ratio of plant detritus entering the grassland soil ecosystem varies from species to species, and is influenced also by the age, condition, and fertilisation of the decaying herbage and root material. Dead ryegrass has a C:N ratio of approximately 44:1, and that of dead leaf clover was approximately 17:1 (Whitehead, 1995). If an organic substrate has a C:N ratio of for example 40:1, supplemental nitrogen is needed from the microbe's environment in order to synthesise biomass from the available carbon (Richards, 1987). In that situation, supplemental nitrogen is taken from the soil environment into microbial cells and is then temporarily unavailable to plants. More moderate C:N ratio materials tend not to deplete inorganic nitrogen reserves, while not contributing to net nitrogen soil stores. Below the threshold C:N ratio of 20:1, sufficient nitrogen is available in the substrate for microbial synthesis, and mineralisation tends to occur immediately (Richards, 1987).

Phosphorus is also a macronutrient, universally required for energy transformations in cellular processes (Zubay, 1993). In grassland, it is particularly vital for young developing plants (Brockman, 1995b). Phosphorus is present in soils in different forms, some being part of organic material such as in microbial biomass, while some is present in inorganic forms associated with aluminium, iron and calcium. Although there may be sufficient phosphorus in soil, because of the low solubility of phosphorus minerals, relatively little may be available to plants via the soil solution, and as a result, phosphorus deficiencies may occur (Rowell, 1994). In Ireland, phosphatic fertiliser use had increased substantially from 16000 t yr⁻¹ since the 1950s, reaching a peak of over 90000 t yr⁻¹ in 1972, and by the 1990s, levelling off at just over 60,000 t yr⁻¹. During that time, soil phosphorus levels have increased from grossly deficient levels (< 1 mg l⁻¹) to over 9 mg l⁻¹, levelling off at over 7 mg l⁻¹ in Irish soils (Coulter and Tunney, 1996). The evidence now suggests that, on average, phosphate inputs in Irish agriculture are higher than removals (Carton et al., 1996). The implication is that phosphorus should be used advisedly to achieve the required crop response, requirements being tailored to match soil conditions in what is termed a sufficiency approach (Lee, 1996). Because agricultural phosphorus exacerbates eutrophication in surface waters, phosphorus use in agriculture is being questioned (Tivy, 1990).

Potassium, with sodium, is a vital component of the transmembrane pumps that regulate water uptake systems and intracellular ion and solute concentrations (Zubay, 1993). The translocation of water by plants, and carbohydrate transport in microorganisms, plants and animals are therefore governed by potassium availability (Brockman, 1995a). Soils high in potassium may exhibit magnesium deficiency in the plant, as potassium ions inhibit magnesium uptake (Brockman, 1995a).

Calcium is an essential nutrient as it is a component of plant cell walls and biological membranes (Brockman, 1995a). Calcium is a major element involved in animal skeletal structure and in muscle activity (Zubay, 1993). It is also a key coenzyme in many microbial, plant and ruminant enzyme systems. Calcium is naturally present, but to varying extents, in soils, and may be added as a constituent of many liming materials (Brady, 1990). Calcium is also a component of the mineral fertiliser, calcium ammonium nitrate (CAN).

Soil magnesium is required primarily as part of plant chlorophyll, and is therefore of vital importance in photosynthesis and primary production (Brockman, 1995a). Magnesium is also an essential element for ruminant growth, as it is a coenzyme in movement of anions, particularly phosphates, across cell membranes (Brockman, 1995a). Magnesium is naturally present in soils, and, like calcium, is added as part of common liming materials.

The calcium and magnesium contents of soils are related to soil pH. In liming, the carbonates, oxides, or hydroxides of calcium or magnesium are generally added to decrease soil acidity (Brady, 1990). Regardless of type, each liming material reacts with carbon dioxide and water in the soil to form its respective bicarbonate. As the bicarbonate dissolves, it undergoes hydrolysis and develops alkalinity, thus raising the soil pH. The extent to which lime additions affect soil depends on the buffering capacity of the soil, clay soils and organic matter having greater capacity to buffer against pH changes than sandy soils (Brady, 1990).

Negative charges on the clay minerals in soil are balanced by opposing cations attracted to the clay surfaces. A similar phenomenon occurs with colloidal soil organic matter. Predominant soil cations are aluminium, calcium, magnesium, potassium and hydrogen. The ions are exchangeable, being released from the clay surface or bound to it depending on electrostatic forces. The cation exchange capacity is the tendency of the soil colloids to hold cations, and it is measured as the amount of charged cations that the clay in a given soil can absorb (Brady, 1990; Rowell, 1994). Absorption of cations applies also to ionised organic molecules such as amino acids therefore cation exchange capacity influences soil organic matter storage directly.

3.4.3 Materials and methods

3.4.3.1. Soil sampling

Soil was sampled and prepared as described in Section 3.3.3.1, above. Except for polysaccharide content, chemical properties of the soils were measured on this soil. For the determination of soil polysaccharide content, a further five clod samples were sealed in Ziplok bags, and frozen at -16°C on return to the laboratory for storage until analysis.

3.4.3.2 Parameters measured

3.4.3.2.1 Organic carbon content

Wet oxidation with potassium dichromate was employed to measure organic carbon content according to Rowell (1994). Organic carbon content of soil was expressed as a percentage.

3.4.3.2.2 Soil nitrogen content

Soil nitrogen content was measured using according to Rowell (1994). Soil nitrogen content of soil was expressed as a percentage.

3.4.3.2.3 Carbon: nitrogen ratio

Carbon: nitrogen ratio was calculated as organic carbon content/percentage soil nitrogen.

3.4.3.2.4 Plant-available soil phosphorus

Plant-available soil phosphorus was measured using UV spectrophotometry following soil extraction with Morgan's solution (Byrne, 1979).

3.4.3.2.5 Plant-available soil potassium

Plant-available potassium was measured using atomic absorption spectrophotometry following soil extraction with Morgan's solution (Byrne, 1979).

3.4.3.2.6 Plant-available soil calcium

The concentration of plant available soil calcium was determined by extraction with Morgan's solution followed by analysis of filtrates using an atomic absorption spectrophotometer (Varian Spectra AA 400 Plus), according to Prendergast (1997).

3.4.3.2.7 Plant-available soil magnesium

The concentration of plant available soil magnesium was also determined by extraction with Morgan's solution followed by analysis of filtrates using an atomic absorption spectrophotometer (Varian Spectra AA 400 Plus), according to Prendergast (1997).

3.4.3.2.8 Cation exchange capacity

Cation exchange capacity was measured according to Killion A., University College Dublin (personal communication, 1996).

3.4.3.2.9 Polysaccharide content

Frozen clods were allowed to defrost in their sealed bags at room temperature for 4-24 h, when soil from a depth of 30-50 mm from each of the five clods was bulked and homogenised in a Waring blender for five minutes to achieve homogeneity prior to sub-sampling. Polysaccharide content was analysed according to Brink et al. (1960).

3.4.3.3 Statistical analysis

As before, descriptive statistical and Spearman's rank correlation analyses were carried out as described in Chapter 2, Section 2.3.5, and in Chapter 3, Section 3.2.3.3.

3.4.4 Results and discussion

3.4.4.1 Organic carbon content

Table 3.15 summarises the percentage organic carbon measured in the sixteen study farm soils. Details for each farm are included in the data spreadsheet in Appendix D.

Table 3.15

Organic carbon content summarised for the sixteen study farms

Organic carbon content	Min.	Max.	Mean	SD
Organic carbon (%)	3.19	9.61	6.00	1.58

The organic carbon content of the study soils compares favourably with five Elton soils that ranged from 5.1% to 9.2% in 1966 (Brogan, 1966; McGrath, 1973), and with levels of 4.7% reported generally for Irish pastures around that time (Mullen, 1973). The mean value found in this study is slightly higher than five pasture soils that averaged 4.91% as reported more recently in the UK (Rowell, 1994). In contrast, paired arable soils in the USA that were managed organically and conventionally had 2.7% and 1.7% respectively (Reganold, 1988).

Organic matter in soils is dependent upon returns of crop residues to the soil, which is a natural feature of grassland. Generally, grassland contains higher levels of organic matter than comparable arable soils (Tivy, 1990). Similarities with Irish pasture values, and differences between values here and elsewhere may be attributed to similarities and differences in fertiliser inputs and in soil disturbance histories. To illustrate, the majority of fields in the present study have been in permanent pasture for more than 10 years, some without interruption for much longer than that, in contrast to the arable soils studied by Reganold (1988).

A strongly negative link occurs between organic carbon content and particle density ($r = -0.730$, $P < 0.01$). This effect is very likely to be the natural result of the differences between the particle density of mineral soil particles, $2.5 - 2.6 \text{ g cm}^{-3}$, and that of organic particles, usually about 1.0 g cm^{-3} (Brady, 1990). Soil organic carbon is strongly associated also with increased percent water stable aggregates ($r = 0.806$, $P < 0.01$), which is not surprising as organic carbon in moderate amounts is known to increase soil structural development and improve structural stability (Rowell, 1994).

Soil organic carbon is positively linked with cation exchange capacity ($r = 0.559$, $P < 0.05$), to which organic matter contributes (Brady, 1990).

The lowest concentration of organic carbon was found on farm Q, which was conventionally managed, with per annum applications of over 300 kg ha⁻¹ nitrogen, in largely mineral form. This concurs with evidence from the Reganold (1988).

Furthermore, the concentration of soil organic carbon was positively correlated with the number of years that had elapsed since mineral nitrogen had been applied on the farms ($r = 0.581$, $P < 0.05$). These results suggest that the application of mineral nitrogen has an immediate negative effect on soil organic carbon content. Fertiliser nitrogen is known to stimulate microorganisms to oxidise existing organic matter in the soil (Whitehead, 1995). The tendency for grassland to recover and to gain organic matter over time is supported by these results.

3.4.4.2 Soil nitrogen content

Table 3.16 summarises the soil nitrogen measurements for the sixteen study farm soils. Details for each farm are included in Appendix D, in the data spreadsheet. The term soil nitrogen has been used in this study in preference to either organic or total nitrogen because the measurement made according to Rowell (1994) takes ammonium-N into account as well as organic nitrogen content. Nitrate is essentially lost in the process, however the loss is negligible (Rowell, 1994). The method excludes most of the nitrate-N however, and according to Rowell (1994) to deem the value as referring to organic nitrogen is not strictly correct. However, as mineral nitrogen content is relatively small, errors in the interpretation of this value are deemed negligible for most purposes (Rowell, 1994).

Table 3.16

Soil nitrogen content, summarised for the sixteen study farms

	Min.	Max.	Mean	SD
Soil nitrogen (%), n = 15	0.31	0.72	0.52	0.11

Amongst the study farms, soil nitrogen content was higher than the 0.25% values obtained on average for long-term grassland on comparable soils in the UK (Johnston, 1991). Soil percent nitrogen varies with soil conditions, ranging under long-term pasture from 0.08% in sandy soils to 0.4-0.5% in clay soils, (Whitehead, 1995). The highest nitrogen content was found in soil from farm H, while the lowest was found in farm O. The bulk of soil nitrogen is associated with organic carbon as substantiated in the highly significant correlation between nitrogen and organic carbon content ($r = 0.873$, $P < 0.01$) of the study soils (Rowell, 1994; Whitehead, 1995). The organic carbon contents of soils from the study farms are considerably higher than those found elsewhere, as already pointed out. Soil organic carbon and soil nitrogen are almost synonymous, both being constituents of soil organic matter. Similar correlations are found between both soil organic carbon and soil nitrogen with variables such as particle density ($r = 0.730$, $P < 0.01$ and $r = 0.558$, $P < 0.05$) and percent water stable aggregates ($r = 0.806$, $P < 0.01$ and $r = 0.701$, $P < 0.01$ respectively), although in the case of soil nitrogen content the relationships are slightly less significant.

Applications of lime and fertilisers did not correlate significantly ($P < 0.05$) with the total nitrogen contents of the study soils. This apparent lack of effect is common in soils with good organic matter content (Whitehead, 1995), as is the case in the study farms.

3.4.4.3 Carbon:nitrogen ratio

In Table 3.17, the carbon to nitrogen ratio (C:N ratio) values for the sixteen study farms are summarised. Individual figures for the farms are included in Appendix D.

Table 3.17

Soil carbon to nitrogen ratios, summarised for the sixteen study farms

	Min.	Max.	Mean	SD
C:N ratio, n = 15	9.4	17.2	12.1	1.8

Soil C:N ratio depends on the type and quantities of contributing organic materials and on soil conditions (Tisdale et al., 1993). It may be inferred from soil organic matter and herbage residue data (Whitehead, 1995) and microbiological information (Coyne, 1999) that once organic residues are added to soil, their C:N ratio is subject to change because

of their use in microbial respiration, mineralisation and or immobilisation of material in the microbial biomass. The minimum C:N ratio found here is close to that of soil microbial biomass, normally in the range of 5:1 to 8:1 (Coyne, 1999). The maximum C:N ratio is closer to levels expected in legume residues, and normally associated with larger particle size fractions in grassland (Coyne, 1999; Rowell, 1994; Whitehead, 1995). Being between the extreme C:N ratio values possible for microbial biomass and herbage residues, C:N ratios of the study soils can be said to fall within the range expected in grassland soil organic matter.

The C:N ratio is a crucial determinant of soil mineral nitrogen content, because for example, if carbon is readily available, microorganisms will tend to avail of mineral nitrogen to facilitate carbon accrual in their biomass, leading to a plant-available nitrogen deficit (Coyne, 1999). Rowell (1994) suggests that in pastures which are not well supplied with nitrogen, plant residues with high C:N ratios accumulate. The C:N ratio is highly correlated with the number of years since mineral nitrogen was applied ($r = 0.737$, $P < 0.01$). Two or more factors may be at play here. In the absence of easily utilised soluble nitrogen, soil carbon is less prone to oxidation by microorganisms. The correlation between organic carbon content and the number of years since mineral nitrogen applications corroborates this (Section 3.4.4.1). Furthermore, without fertiliser nitrogen, the nitrogen content of soil organic matter may decline over time.

Soil aeration may be involved, as the values for natural drainage which were derived from soil series data and describe the soils in terms of increasing penetrability (see Section 3.2.4.2.6) are negatively associated with C:N ratio ($r = - 0.633$, $P < 0.05$). Less nitrogen is needed when soil organic matter is broken down under anaerobic conditions, because less cell synthesis takes place per unit of glucose used (Tivy, 1990). This shifts C:N ratio upward under anaerobic conditions.

A significant negative correlation was also found with the number of years since lime was applied ($r = - 0.693$, $P < 0.05$). As no correlation between soil pH and C:N ratio was found, it could be assumed that, in the past, lime additions altered soil pH and thus favoured organic matter transformations by the soil microorganisms. Narrowing of the C:N ratio is a common feature of organic matter oxidation by microorganisms (Whitehead, 1995).

3.4.4.4 Plant-available soil phosphorus

In Table 3.18, plant-available soil phosphorus data for the sixteen study farms is summarised. Individual soil phosphorus figures for the farms are included in Appendix D, in the data spreadsheet.

Table 3.18

Plant-available soil phosphorus summarised for the sixteen study farms.

	Min.	Max.	Mean	SD
Plant available soil phosphorus, (mg l ⁻¹)	1.4	12.9	5.4	3.7

In 1995, P levels in the study farms ranged from what was then regarded as index 0 (0-9 mg l⁻¹) to index 1, (10-15 mg l⁻¹), the mean value being within the index 0 range (MAFF, 1994). This relatively low rating of the soils is to be expected in farms where inputs of fertilisers have ranged from negligible to approximating conventional levels in just one farm (Chapter 2). For Irish agriculture, P indices have been redefined by Teagasc (Herlihy et al., 1996), and the minimum value is within the index 1 range (0-3.0 mg l⁻¹). Farms in the study would now be categorised as index 2 (3.1-6.0 mg l⁻¹), on average, with the maximum value being at index 4 (>10 mg l⁻¹).

Significant correlations were found between the total added fertiliser N, P and K (Spearman correlation coefficients $r = 0.642, 0.703, \text{ and } 0.654$, respectively, and associated probability being $P < 0.01$ in each case) and with the organic forms of N, P and K fertiliser inputs of ($r = 0.573, 0.519, \text{ and } 0.573$ respectively, and associated probability being $P < 0.05$ in each case) favoured on many of the farms. With the exceptions of the urea and CAN applied on farms Q and B respectively, mineral formulations such as 0:10:10 were applied, which included phosphate. The omnipresence of phosphate in organic matter together with nitrogen and potassium explains correlations found with organic N and K additions. In this study, forms of phosphate fertilisers such as rock phosphate and basic slag, being less soluble than their mineral counterparts, were included in the organic fertiliser category. The lowest concentration of plant-available phosphorus was found in farm T, which received no fertiliser inputs of any kind for a number of years. In contrast, farms applying phosphate prior to 1995 seem to have done so at a maximum total of 38 kg ha⁻¹ y⁻¹, almost twice

the rate now recommended. Current recommendations are 20 kg ha⁻¹ y⁻¹ for either dairying or dry stock systems on the more deficient index 1 soils and at stocking rates of 2 LU ha⁻¹ (Culleton et al., 1996). However, stocking levels have not reached 2 LU ha⁻¹ on the majority of study farms, suggesting that excessive phosphorus is being used, with probable environmental and monetary consequences.

The concentration of plant-available phosphorus in the study farm soils was significantly correlated with soil pH ($r = 0.613$, $P < 0.05$). Soil pH has a profound effect on the quantity of phosphorus absorbed and precipitated in soils, and phosphorus is more readily available to plants at higher soil pH values, reaching a maximum in the pH range 5.5 to 6.5 (Tisdale et al., 1993).

3.4.4.5 Plant-available soil potassium

In Table 3.19, plant-available soil potassium measured in the study farms is summarised. Individual figures for the potassium available in the farms are included in Appendix D, in the data spreadsheet.

Table 3.19

Plant-available soil potassium, summarised for the sixteen study farms

	Min.	Max.	Mean	SD
Plant-available potassium, mg l ⁻¹	10.0	250.0	93.6	71.8

Plant-available soil potassium content is variable among study farm soils. According to MAFF (1994), many farms are gauged as index level 1 or below, having 61-120 mg l⁻¹ available potassium. The highest level of potassium was measured in farm L, and lowest levels in farms O, Q and T. Farm Q is conventionally farmed, and T has had essentially no nutrient input for many years. No correlation was found between potassium content and fertiliser additions on the sixteen farms. Potassium removal by crops is high (Brady, 1990), and it may be that additional potassium dressings are required in many of the farms.

Soil nitrogen content is significantly correlated with plant-available potassium ($r = 0.551, P < 0.05$). In certain soils where ammonium ions and potassium ions are of a particular size small enough to get trapped within the soil crystal structure, the potassium is then in a slowly available form (Brady, 1990). While this has not been confirmed in the present analysis, it may explain the connection found here between soil nitrogen and potassium.

A significant negative correlation occurred between the length of time animals stayed off the land in a grazing rotation ($r = - 0.516, P < 0.05$) and the amount of available potassium in the soil. It may be that the longer time spent off the land, the less potassium overall is returned to the soil by the grazing animal in the excreta.

3.4.4.6 Plant-available soil calcium

The available calcium data for the soil in the sixteen study farms is summarised in Table 3.20. Individual farm details are included in the data spreadsheet, Appendix D.

Table 3.20

Calcium content of the study farm soils, summarised

Plant available soil calcium	Min.	Max.	Mean	SD
Calcium (mg kg ⁻¹)	310.0	13500.0	3989.1	3733.4

Calcium is an essential plant nutrient, and its presence and availability in soil are strongly correlated with pH ($r = - 0.773, P < 0.01$), both factors probably impacting on root growth. Correlation between calcium and root mass is negative ($r = - 0.501, P < 0.05$), and may be explained by reduced foraging in an adequate soil when calcium is more readily available.

The available calcium in the soil was significantly correlated with total fertiliser additions of potassium ($r = 0.548, P < 0.05$) and nitrogen additions ($r = 0.554, P < 0.05$) and with organic amendments of potassium ($r = 0.520, P < 0.05$) and nitrogen ($r = 0.520, P < 0.05$). Reasons for these associations are not obvious, but may be due to the

depressing effect that ammonium and potassium have on plant uptake of calcium (Tisdale et al., 1993), or to the presence of calcium in CAN formulations.

3.4.4.7 Plant-available soil magnesium

Magnesium content of the study farm soils is summarised in Table 3.21. Each farm's value for magnesium content is included in Appendix D, in the data spreadsheet.

Table 3.21

Plant-available soil magnesium, summarised for the sixteen study farms

Plant-available soil magnesium	Min.	Max.	Mean	SD
Magnesium content (mg kg ⁻¹)	60.0	350.0	214.4	80.7

A highly significant correlation exists between available magnesium and soil pH ($r = 0.668$, $P < 0.01$). This corresponds with the tendency for magnesium to form bicarbonates, which hydrolyse reducing soil acidity. A significant correlation exists between water stable aggregates and soil magnesium content ($r = 0.560$, $P < 0.05$), reflecting the contribution of magnesium to soil aggregate formation.

The root mass found in study farm soils correlated strongly with plant-available soil magnesium ($r = -0.711$, $P < 0.01$). This negative correlation suggests that when magnesium is abundant, root mass does not accumulate. Correspondingly, root elongation occurs perhaps as a foraging response in a nutrient depleted zone, where magnesium concentration is low. A strongly negative correlation was also recorded between root mass values and soil pH ($r = -0.680$, $P < 0.01$), and interdependent factors governing nutrient uptake by plant roots may be involved in this instance.

Calcium and magnesium contents of the study farm soils were significantly correlated ($r = 0.585$, $P < 0.05$). The lowest concentrations of both calcium and magnesium were found in farm N. Like magnesium, calcium was significantly correlated with soil pH ($r = 0.773$, $P < 0.01$), and with root mass ($r = -0.501$, $P < 0.05$).

3.4.4.8 Cation exchange capacity

The cation exchange capacity in the study farms is summarised in Table 3.22. The Cation exchange capacity value for soil in each farm is included in the data spreadsheet in Appendix D.

Table 3.22

Cation exchange capacity of the soil summarised for the sixteen study farms

Cation exchange capacity	Min.	Max.	Mean	SD
CEC (cmol (+) kg ⁻¹)	5.9	18.7	12.1	3.7

The cation exchange capacity varies in a range from 2-60 cmol (+) kg⁻¹ according to soil type, ranging from 5-15 cmol (+) kg⁻¹ in sandy loam soil to 30-50 in clay soils (Rowell, 1994; Parkinson, 1995). The negative correlation with particle size ($r = -0.520$, $P < 0.05$) in the study farm soils is therefore as expected. CEC was also significantly correlated with soil organic carbon content ($r = 0.559$, $P < 0.05$). Organic carbon contributes cation exchange capacity to soil (Parkinson, 1995). Correlation with the ratio of small to large aggregates ($r = 0.676$, $P < 0.01$) was also found. Higher CEC values were significantly correlated with LU ha⁻¹ ($r = 0.503$, $P < 0.05$), which would concur with the assumption that higher CEC soils should have higher fertility, at least when farmed conventionally.

3.4.4.9 Soil polysaccharide content

Table 3.23 summarises data for polysaccharide content of soils from the sixteen study farms. Polysaccharide content for each farm is included in Appendix D.

Table 3.23

Soil polysaccharide content, summarised for the sixteen study farms

	Min.	Max.	Mean	SD
Polysaccharide content (mg ml ⁻¹)	7.5	45.0	25.0	12.1

Soil polysaccharides are presumed to be largely of microbial origin, but also originate from plant materials. Polysaccharides form a relatively accessible energy store for use by soil by microorganisms. The polysaccharide contents of the study farms' soils are much higher than that reported by Reganold (1998).

The relationship between altitude and polysaccharide content ($r = 0.576$, $P < 0.05$) is unexplained, and may reflect colder soils, with less substrate utilisation. Clay soils protect soil organic matter, as is reflected in the positive correlation with the percentage clay and polysaccharide content of the study farm soils ($r = 0.516$, $P < 0.05$).

No correlation was found, however, between soil polysaccharide content and soil organic matter content. Differing methodologies and soil storage conditions prior to the analysis of soil polysaccharides and prior to organic carbon analysis may be the reason for lack of correlation. Soil samples had been frozen to prevent hydrolysis of polysaccharides, whereas soil organic matter was measured in soils, which had been spread out and left to dry (being aerated in the process), and then sieved, during which time oxidation of polysaccharides may have occurred.

Polysaccharide content of the study soils is related to the number of years since reseeded ($r = 0.812$, $P < 0.05$) and to the interval since the soil had been mechanically disturbed ($r = 0.903$, $P < 0.01$), whereas the organic carbon content of the soil is not significantly related to either. It is likely that polysaccharides are oxidised when soil is disturbed because of soil aeration, much as may have happened in the air-dried soil stored for organic matter analysis. Without disturbance, polysaccharide content may be conserved, leading to the positive correlation found here. In contrast, regular tillage of the farms during an arable ley may explain the lower polysaccharide content found elsewhere by Reganold (1998). Farms described by Reganold were arable rather than grassland, and crop rotations with tillage operations to various soil depths occurred, probably causing polysaccharide oxidation.

In summary, the soil physical, chemical and site related properties of the study farms suggest that soil property values are within the appropriate limits for grassland function in the farms examined. However, soil organic carbon and root mass contents are

considerably higher than reported in similar research, and are likely to affect both the soil biological function and its comparison with soil biology literature.

Chapter 4

Chapter 4

Botanical composition of the study farms in relation to farm management practices, site-related and soil properties

4.1 Introduction

In Chapter 4, the botanical composition of each of the sixteen study farms is measured and described. The botanical composition and diversity is examined statistically in relation to farm management and soil conditions, described in Chapters 2 and 3. A comparison is made with the botanical composition as recorded previously by O'Sullivan in 1968, in his study of the lowland grasslands of County Limerick.

4.2 Review of literature

Whether in the presence or absence of fertiliser nitrogen, primary grassland production is substantially determined by the botanical composition of the grassland, and by soil conditions (O'Sullivan, 1968). Species normally associated with managed grassland include desirable grasses, legumes, herbs, and less favourable rush and moss species (Frame, 1992).

Historically, the choice of grass and legume species sown for grassland has been influenced by seed availability, species suitability, and management factors (Newton, 1993). Conventionally managed grassland, for example, relies heavily on applications of mineral fertilizers, for which varieties of *Lolium perenne* have been specially bred (Newton, 1993). Other grasses such as *Alopecurus pratensis*, *Cynosurus cristatus*, *Poa pratensis*, *Holcus lanatus*, *Dactylis glomerata*, *Agrostis stolonifera*, *Festuca ovina*, and *Poa annua*, may be recommended for their various individual qualities. Some grass species such as *Cynosurus cristatus* and *Holcus lanatus* were more productive than *Lolium* in trials under zero nitrogen fertiliser conditions (Newton, 1993). However, many grasses have been overlooked in favour of *Lolium perenne*, perhaps because of its suitability for intensive grazing, its nutritional quality because of digestibility, and its persistence under different management conditions (O'Sullivan, 1968; Newton, 1993; Whitehead 1995). Both Frame (1992) in the general grassland context, and Newton (1993) in the specific context of organic farming, suggest that the farmer should choose

the particular seed mixture in the knowledge of species suitability for his/her particular farm. However, in 1991, *Lolium perenne* constituted over 90% of all grass seed sold (Newton, 1993).

In the absence of mineral fertilizers, organic grassland depends on nitrogen returns from organic manures and on the fixation of atmospheric nitrogen by legumes in a properly managed grass sward (Newton, 1993). Therefore, legumes are an essential component of the sward in organically managed grassland. Legumes suitable for grassland are principally the red and white clovers, *Trifolium pratense* and *Trifolium repens*, lucerne and sainfoin, while other less well-known species occur (Newton, 1993). Although different legumes are useful, the 'standard' seed mixtures available locally in Ireland consist principally of *Lolium perenne* and *Trifolium repens*, as recommended over a hundred years ago by Sir George Stapledon for land improvement purposes (Newton, 1993).

The diversity and dominance of either sown and or native species are affected by farm management practices such as nutrient applications and grazing, and by soil conditions (O'Sullivan, 1968; Mullen, 1973; Tivy, 1990; McAdam, 1995). A study of the botanical composition of the grasslands in County Limerick, conducted by O'Sullivan (1968), describes the species abundance in the grassland plant communities, and the soil conditions and farm management effects manifest at that time. Where human management intervened, particularly in the fertile and most intensive pastures, stands were dominated, not surprisingly, by *Lolium perenne* and *Trifolium repens* (O'Sullivan, 1968). Average species number in the more intensively managed pastures was 25, the lowest number of species found among lowland grassland communities in that particular study. However, it was noted that grassland was then seldom managed intensively in County Limerick, and it was suggested that grassland yield could be doubled through applications of lime and fertilizer without recourse to reseeded (O'Sullivan, 1968). Since then, farm management in Ireland, and in the study region, has intensified with remarkable increases in livestock numbers and in associated fertilizer use (Lee and Diamond, 1972; Coulter and Tunney, 1996; Central Statistics Office, 1999). The impact of grassland management will be considered in the current study.

4.3 Materials and methods

The selection of study farms has been described in Chapter 2, Section 2.3.1. The following paragraphs describe how botanical analysis was carried out on each of the sixteen study farms.

4.3.1 Sampling dates

The representative field on each of the study farms was sampled for botanical analysis purposes during a two-week period in early February 1998.

4.3.2 Field sampling

A quadrat measuring 0.25 m x 0.25 m was thrown at random in a selected area of vegetation that was regarded as representative of the sampled field. Paths, gateways, and margins were avoided. The vegetation within the perimeter of the quadrat was clear-cut using a sharp knife. All clippings were placed in a polythene bag, tied, and labelled with the sampling date and farm code.

4.3.3 Sample storage

All samples were placed in cold storage at approximately 4°C on return to the laboratory, until analysis. Samples were also placed in cold storage for intervals between any analysis procedures.

4.3.4 Preparation of samples for analysis

Contents of individual sample bags were washed with cold running water to remove soil residues, taking care to retain biomass in the process. Washed vegetation was placed in its original bag and stored again at 4°C until analysis.

4.3.5 Sub-sampling for botanical composition analysis

The contents of a sample bag were emptied onto a clean laboratory bench surface, mixed well, and a sub-sample, approximately a quarter of the vegetation, was obtained for the analysis of botanical composition. Each item in the sub-sample was subsequently

separated, identified, and members of similar species were combined in bundles for analysis of their percentage content relative to the vegetation as a whole.

4.3.6 Identification of species in vegetation samples

Each grass may be identified easily by its flowering head. However, samples in this study were collected in early February, when the plants were in their vegetative stage, and flowering heads were not apparent. Identification was therefore relatively difficult. Nevertheless, the plant species found in both the random and representative vegetation samples were identified as follows.

Each item was examined carefully against a white background and using a hand lens, recording the presence, absence and appearance of identification characteristics.

Identification of grasses was based on the different vegetative structures of the plants, with particular reference to the identification key edited by Hubbard (1984).

When identifying grass species, attention was paid to the following key vegetative characteristics:

Growth form: stoloniferous or rhizomatous, loose or dense tufts;

Culms: erect, curved, or prostrate; size, rigidity, and number of nodes;

Ligules: absent, pointed, toothed or blunt;

Blades: flat or stiff, rolled or folded;

Blade tips: finely pointed, abruptly pointed, blunt;

Hairs: present or absent;

Sensory aspects: colours, flavours or smells.

Identification of other plant species was based on identification characteristics and reference keys (Webb, 1977).

4.3.7 Vegetation mass, dry weight, and botanical species abundance measurements

The mass of the vegetation sub-sample was initially measured in its moist condition, and recorded as vegetation mass. Then, grouped members of individual plant species, which had been separated from the vegetation sub-sample, identified and bundled (Sections 4.3.5 and 4.3.6) were placed in brown paper bags, labelled, and dried in a fan oven at 100°C overnight. The dry weights of each species were measured and recorded, and their sum calculated to be vegetation dry weight. As a basis for statistical analysis,

the sward content of each botanical species was then calculated as a percentage of vegetation dry weight.

4.3.8 Statistical analysis

The summary statistics describing the minimum, maximum, mean and standard deviation obtained for each plant species were determined using SPSS (Norušis, 1992) as described in Section 2.3.5.1. Spearman correlation coefficients ($r = 0.000$ or $r = -0.000$) and associated probabilities ($P < 0.05$; $P < 0.01$) were also analysed, as described in Section 2.3.5.3, to explore relationships between the botanical composition of the grassland and those farm management features and soil physical and chemical properties and site related farm parameters described in Chapters 2 and 3.

Zero values were recorded for botanical species presence on farms in which those particular plant species were absent. Regardless of this, SPSS computed the significance of relationships between botanical species and other variables such as soil organic matter, for example, based on sixteen 'plant presence' values. Unless otherwise stated, correlations mentioned throughout the thesis were attributed some significance based on there being sixteen values for computation purposes. However, based on critical values for the Spearman's rank correlation (Zar, 1996), significance could not be established at $P < 0.05$ and at $P < 0.01$ for relationships between variables and those plant species present on fewer than five and six farms respectively (See Table 4.2). Only the rank correlation coefficient is given for such instances here in Chapter 4.

4.4 Results and discussion

4.4.1 Introduction

Prior to commenting on botanical analysis results and on possible grassland management effects, and prior to a comparison of current grassland composition with what was recorded by O'Sullivan (1968) for County Limerick in the 1960s, some analytic constraints encountered in the course of this work should be mentioned.

Firstly, the botanical analysis was carried out in early February. At that time, flowering heads of species were not available as a means of identification, and temperature and light effects were evident in the sward.

Furthermore, grazing management and soil sampling data obtained for 1995 predate the botanical analysis by approximately three years, and, other than records of fertiliser applications made from 1995-1997, management of the grassland immediately prior to botanical analysis was not specifically investigated. For the purposes of discussion, it is presumed that similar grazing management practices and soil conditions prevailed in the interim between the earlier soil analyses, and later botanical analysis, dates.

4.4.2 Botanical composition of the study farms

The botanical composition of the grassland plant communities in the sixteen study farms is described in Table 4.1. Summary statistics given in Table 4.2 describe the minimum, maximum and mean values obtained for particular botanical species, and give the number of farms on which each species had been recorded. Values describing the vegetation mass and dry weight of sub-samples can be found in the data spreadsheet in Appendix D.

4.4.2.1 *Trifolium* species

Overall, clover species, which may have included both red and white clover, constituted approximately 8% of the vegetation, which according to Frame (1992) constitutes a 'good' proportion of the sward. Clover content was neither significantly correlated with soil conditions nor with farm management features. Clover content was negatively correlated with presence of *Holcus lanatus*, ($r = - 0.721$, $P < 0.01$) concurrent with the view that this grass is not a good companion for *Trifolium repens* (Newton, 1993).

4.4.2.2 *Lolium perenne*

The sward content of *Lolium perenne* ranged from 0 to 63%, with a mean content of approximately 18%. *Lolium perenne* was by far the most constant grass type, being found in all but one of the study farms, farm N, in which *Juncus* species predominated. Maximal sward *Lolium perenne* content was found on an organically managed dairy farm, farm L, in which animals graze for brief intense three-day periods followed by three weeks rest.

Table 4.1

Abundance of botanical species in the sward as percentage dry-weight of the vegetation sampled from each selected study farm field

Plant Abundance (%) Farm		A	B	E	F	G	H	J	L	M	N	O	P	Q	R	S	T	
Legume	Clover species	1.6	0	32.5	ND ^a	2.3	18.8	32.6	10.2	0.1	0.7	3.2	12.4	7.0	0	0.3	1.1	
Grass	<i>Lolium perenne</i>	19.8	0.8	41.7	9.3	51.0	6.6	1.8	63.4	15.7	0	4.8	11.7	27.6	3.6	8.4	21.7	
	<i>Agrostis stolonifera</i>	1.6	0	0	85.1	12.4	4.6	3.7	0	45.3	26.5	48.6	20.0	0	0	5.9	50.9	
	<i>Alopecurus pratensis</i>	0	32.2	2.1	0	17.5	0	21.0	10.2	0	1.4	0.8	0	0	0	0	4.2	0
	<i>Holcus lanatus</i>	0	14.1	0	1.8	3.3	0	0	0	0.2	0	0	0	0	0	5.0	80.7	0
	<i>Festuca ovina</i>	0	0	0	0	0	0	29.6	0	0	0	0	0	0	0	58.2	0	17.1
	<i>Dactylis glomerata</i>	0	0	0.8	0	1.8	0	0.9	1.29	0	0	0	0	0	0	0	0	0
	<i>Poa pratensis</i>	11.5	8.3	17.8	2.9	11.7	20.1	0	0	0	0	1.0	0	36.0	61.9	0	0	0
	<i>Poa annua</i>	0	0	0	0	0	0	13.4	0	0	0	0	0	0	3.5	0	0	0
	<i>Cynosurus cristatus</i>	0	0	0	0	0	0	0	0	0	38.7	0	0	0	0	0	0	0
Grasses	Combined percentage	32.9	55.4	62.5	ND ^a	97.7	44.7	56.9	75.2	99.9	28.8	54.2	67.7	93.0	66.9	99.2	89.7	
Forbs	<i>Plantago lanceolata</i>	40.0	0	0	0	0	9.1	0	0	0	0	2.7	0	0	28.3	0	0	
	<i>Potentilla anserina</i>	0	0	0	0	0	0	0	2.1	0	0	0	0	0	0	0	0	
	<i>Rumex acetosa</i>	0	0	0	0	0	0	0	0	0	0	0.2	0	0	4.0	0	0	
Weeds	<i>Ranunculus repens</i>	1.1	26.5	2.2	0	0	6.9	4.0	10.8	0	0.1	1.4	0.2	0	0	0.5	0.8	
	<i>Bellis perennis</i>	2.7	0	0	0	0	6.1	0	1.6	0	0	0	0	0	0	0	0	
	<i>Cirsium</i> spp.	0	18.2	0	-	0	0	0	0	0	0	0	0	0	0	0	0	
	<i>Cardamine pratensis</i>	0.7	0	0.7	0.9	0	2.4	0.9	0	0	0	0.1	0.5	0	0.4	0	0	
	<i>Taraxacum officinale</i>	0	0	2.1	0	0	1.5	0	0	0	0	1.3	0	0	0.4	0	0	
Rushes	<i>Juncus</i> spp.	0	0	0	0	0	0	0	0	0	69.0	37.0	0	0	0	0	0	
Mosses	<i>Bryophyte</i> spp.	21.2	0	0	0	0	10.5	5.6	0	0	1.5	0	19.3	0	0	0	8.3	
Diversity	Number of species	9	6	8	5	7	11	9	7	5	7	10	7	4	7	6	6	

a. ND refers to values that were not determined.

Table 4.2

The percentage dry weights of botanical species found on the study farms and the numbers of farms on which each plant type was found.

Plant type	Species	Min.	Max.	Mean	SD	Number of farms
Legume	<i>Trifolium spp.</i> ^a	0	32.6	8.1	11.3	14
Grass	<i>Lolium perenne</i>	0	63.8	18.0	19.1	15
	<i>Agrostis stolonifera</i>	0	85.1	19.0	25.5	11
	<i>Alopecurus pratensis</i>	0	32.2	5.6	9.7	8
	<i>Holcus lanatus</i>	0	80.7	6.6	20.1	6
	<i>Festuca ovina</i>	0	58.2	6.6	16.1	3
	<i>Dactylis glomerata</i>	0	1.8	0.3	0.6	4
	<i>Poa annua</i>	0	13.4	1.0	3.4	2
	<i>Poa pratensis</i>	0	61.9	10.7	17.1	8
	<i>Cynosurus cristatus</i>	0	38.7	2.4	9.7	1
Grass	% Grass content ^a	28.8	99.9	68.3	23.7	15
Forbs	<i>Plantago lanceolata</i>	0	39.9	5.0	11.8	4
	<i>Potentilla anserina</i>	0	2.1	0.1	0.5	1
	<i>Rumex acetosa</i>	0	4.0	0.3	1.0	2
Weeds	<i>Ranunculus repens</i>	0	26.5	3.4	6.9	11
	<i>Bellis perennis</i>	0	6.1	0.6	1.6	3
	<i>Cirsium spp.</i> ^a	0	18.1	1.2	4.7	1
	<i>Cardamine pratensis</i>	0	2.4	0.4	0.6	8
	<i>Taraxacum officinale</i>	0	2.05	0.3	0.6	4
Rushes	<i>Juncus spp.</i>	0	68.9	6.6	19.0	2
Moss	<i>Bryophyte spp.</i>	0	21.2	4.1	7.1	6
Species Diversity	Number of species	4.0	11.0	7.1	1.9	16

a. Loss of some bundled vegetation samples for farm F meant that only fifteen values were available for the measurements annotated.

Although five of the farms had been reseeded with the typical standard *Lolium perenne*-*Trifolium repens* mix, there was no significant correlation between the presence of *Lolium perenne* and reseeding amongst the study farms overall. *Lolium perenne* presence correlated significantly and positively with mineral P and K applications ($r = 0.630$, $P < 0.01$, in both cases).

Extensive grazing values, explicitly time spent on the land in a rotation and the ratio of days on to of the land during a rotation, correlated negatively with *Lolium* content ($r = -0.542$, $P < 0.05$; $r = -0.507$, $P < 0.05$, respectively). It could be inferred that the *Lolium perenne* content of the study farms was associated with more intensive grazing, as is commonplace (Frame, 1992).

4.4.2.3 Grasses other than *Lolium perenne*

As can be seen in Table 4.2, *Lolium perenne* was the most constant type of grass. Other grass species occurred, but to varying extents, and the total percentage sward grass content varied considerably.

Individual grasses correlated significantly with other botanical aspects, soil conditions and farm management features, as follows: *Agrostis stolonifera* was positively associated with root mass ($r = 0.592$, $P < 0.05$), and with the vegetation mass ($r = 0.508$, $P < 0.05$). *Agrostis stolonifera* content was negatively linked with reseeding ($r = -0.569$, $P < 0.05$) and was positively correlated with the number of years since farms had been reseeded ($r = 0.823$, $P < 0.05$). Each of the reseeded farms had applied the standard *Lolium perenne* plus *Trifolium repens* mix, and the suggestion here may be that reseeding interrupts *Agrostis*, which subsequently recovers. According to O'Sullivan (1968), this grass is indicative of poorly aerated soil. Soil may have been aerated if mechanically disturbed by ploughing operations prior to reseeding. Since that time, soil may have become more anaerobic creating suitable conditions for *Agrostis stolonifera* presence. Furthermore, a negative relationship was found between *Agrostis stolonifera* and soil nitrogen ($r = -0.731$, $P < 0.01$). If soil conditions are anaerobic as the presence *Agrostis stolonifera* suggests, nitrogen loss may well have occurred through denitrification which is an anaerobic process (Coyne, 1999).

Poa pratensis was positively correlated with the type of animal farmed ($r = 0.542$, $P < 0.05$), being most clearly associated with some dairy and beef farms (B, E, H, P and Q), while presence was lower or zero in other mixed grazing, sheep or goat systems generally. Although this grass is very digestible and acceptable to stock, it is disadvantaged by very slow establishment and is regarded more as an amenity grass (Frame, 1992). It was also correlated significantly with earlier additions of phosphate fertilisers prior to 1995 ($r = 0.608$, $P < 0.05$).

Alopecurus pratensis was less noticeable in reseeded farms ($r = - 0.569$, $P < 0.05$). As with *Agrostis stolonifera*, reseeding had a negative influence. *Alopecurus* was positively associated with exposure to livestock, measured as numbers of livestock multiplied by the days grazed in the year prior to soil sampling, three years earlier ($r = 0.526$, $P < 0.05$). Newton (1993) notes that this grass is persistent, and makes a good aftermath.

Holcus lanatus, present on six farms, was linked with grazing activity measured as the total number of animals multiplied by the number of days in the year ($r = 0.615$). Here, as in other instances where species were present on fewer farms, rank correlation significance has been verified using statistical tables from Zar (1996), and only statistically significant probabilities are noted. Normally, *Holcus lanatus* prevails around dung pats (O'Sullivan, 1968) and although this was not particularly noted in the present study, it may explain correlation of *Holcus* with the numbers of animals. This grass was negatively correlated with clover content ($r = - 0.721$), as mentioned, and with moss content of the sward ($r = - 0.559$).

Dactylis glomerata was present on four farms, along with *Alopecurus pratensis* ($r = 0.642$). *Dactylis glomerata* was negatively correlated with aggregate ratio in those farms ($r = - 0.531$). It was positively associated with soil magnesium content ($r = 0.498$). Frame (1992) attributes soil fertility restoration in 'tired soils' to the deep rooting nature of this grass species, and so perhaps its soil mining tendency explains the link with soil magnesium content.

Festuca ovina, occurring on farms J, R and T, was positively linked with the cation exchange capacity of those soils ($r = 0.518$) and negatively associated with the ratio of

silt to clay particles ($r = - 0.550$) and with ratio of small to large aggregates ($r = - 0.682$).

Two grasses, *Poa annua* and *Cynosurus cristatus* were found on so few farms (2 and 1 respectively) that their correlation with grassland conditions could not be established.

4.4.2.4 Less-desirable species

Plantago lanceolata was predominant in farm A, comprising 40% of the vegetation there. Farm A is the least intensively grazed of all the farms. The spreading growth habit is a disadvantage according to Frame (1992) and may have adversely affected other sward species in this farm, A, allowing *Plantago lanceolata* to prevail. It was also present in farms O and R, both of which are goat-farming enterprises. Goats generally browse, but graze if necessary (Cooper, 1995). Whether the tendency for this plant to form a flat rosette when grazed hard (Newton, 1993) allows it to escape browsing by goats is unclear. A relationship with grazing may exist, however *Plantago lanceolata* can also persist when soil conditions are adverse (Newton, 1993).

Only 2% of vegetation in farm L consisted of *Potentilla anserina*. Otherwise, it was not found on other study farms.

Rumex acetosa was found only on the two goat farms, though present in small proportions of the sward, 0.2% and 4%, respectively, on farm O and R. The general absence of *Rumex acetosa* may be attributed to the time at which sampling was carried out, or the selection of a single random sample. As *Rumex acetosa* infestation typifies intensive, highly fertilised grassland (Frame, 1992), its absence may also reflect the relatively low input farms of the current study.

Ranunculus repens was found on eleven of the study farms. O'Sullivan (1968) suggests that this plant indicates poor soil aeration, possibly linked with compaction by grazing animals. In this study, *Ranunculus repens* content in the sward was not significantly correlated with measured soil conditions. However grazing appeared to influence its presence. The numbers of animals per hectare, either in a grazing interval, or during a year's grazing, appeared to have been a negative influence, ($r = - 0.544$, $P < 0.05$, and r

= - 0.531, $P < 0.05$) suggesting that under-grazing encourages *Ranunculus repens* persistence, as reported in the literature (Lampkin, 1992).

Bellis perennis was found in just three farms, H, A, and L, at 6%; 3%, and 2% respectively, where their numbers correlated positively ($r = 0.557$) with the percentage soil organic carbon contents of 10%, 7% and 6%, respectively, in those farms.

Cirsium species were present only in farm B. Farm B also had almost negligible *Lolium perenne* presence (0.8%), and a relatively high content of *Alopecurus pratensis* (33%) while having the highest buttercup content (26.5%). *Cirsium* content is best controlled by encouraging a dense, vigorous and competitive sward (Frame, 1992), not apparent in farm B.

Taraxacum officinale was found on two of the suckler beef farms and on the two goat farms, with maximal vegetation content of 2%. *Taraxacum officinale* has high digestibility, and may increase milk production by 10% in the dairy herd (Newton, 1993), however their contribution in the present study farms may not be substantiated because of the small sample size.

Juncus species were found in quantity on two farms, N, 68%, and O, 38%, which are the only two representatives of the Gortaclareen soil series. This soil series is of a clay loam type, and is poorly drained, ideal for *Juncus* species (Frame, 1992). No *Juncus* species were detected elsewhere amongst well-drained farms. LU ha⁻¹ capacity of the Gortaclareen soil series is estimated as 1.48. Farms N and O carry 1.15 and 4.35 LU ha⁻¹, respectively. Of all the study farms, N is exposed to the heaviest treading in a grazing rotation and in a year's exposure to grazing livestock. Cattle should ideally be grazed in a *Juncus*-rich field when risk of poaching is negligible (Frame, 1992). However, in farm N, cattle are grazed year round in a month on, month off rotation, and poaching is likely to occur if farm N were grazed continuously in wet weather. Widespread poaching may even occur in summer, according to O'Sullivan (1968) if this type of plant community is grazed by mature animals. It is likely that heavier cattle would have more damaging effect on farm N than the lighter but more numerous goats on farm O.

Drainage and liming are key management options for this type of sward, however the root mass may decay in response to fertiliser applications, leaving the underlying soil even more vulnerable to poaching (O'Sullivan, 1968, Frame 1992). Fertiliser nitrogen has not been applied for a number of years on either farm, and only basic slag additions have been made in the case of farm O. Without lime additions, soil pH is much lower in N at pH 4.9, while soil in farm O was at pH 6.4, having had lime applied about six months prior to soil sampling. As noted, the percentage *Juncus* content in farm O in 1998 was about half that on farm N, so perhaps liming has had the desired effect.

Bryophyte species were found in six farms, and were associated with botanical composition, soil conditions and farm management factors. *Bryophyte* species content was negatively correlated with *Holcus lanatus* in the sward ($r = - 0.559$). *Bryophyte* species are associated with low pH (Lampkin, 1990), corroborated in the negative link with soil pH in this study ($r = - 0.705$). *Bryophyte* species were more prevalent when animals spent longer on the land in a rotation, as in more extensive grazing. The frequencies of grazing events were negatively correlated with moss content of the sward. Correlations were as follows with numbers of animals per hectare ($r = - 0.820$), livestock units per hectare ($r = - 0.571$), numbers during a rotation ($r = - 0.776$) and numbers grazing the field on an annual basis ($r = - 0.598$). *Bryophyte* species content was also negatively linked with excreta nutrients, notably excreta phosphate ($r = - 0.512$). *Bryophyte* species indicate poor soil fertility, and poor soil fertility in turn may be the basis for the negative correlation between moss content and grazing events. However, the fact that defoliation encourages more vigorous sward growth (Frame, 1992) may be a limiting factor in *Bryophyte* species development. Reseeded farms were likely to have lower *Bryophyte* species presence than other farms ($r = - 0.579$). As with grazing and defoliation, an increased sward vigour following reseeding may inhibit *Bryophyte* species, however these possible explanatory factors have not been specifically measured in the present study.

4.4.5 Grass content and botanical diversity

The percentage content of each of the various grasses in the sward was added for each farm to derive a value for the total percentage grass content of the vegetation. This varied from less than a third 28.8% in farm N, to almost the entire vegetation, 99.9%,

the mean grass content being approximately 68.3% (Table 4.1). The number of botanical species in the grassland was also measured, and ranged from a minimum of 4 species in the conventional farm, Q, to a maximum number of 11 species in farm H, which was in its second year of conversion to organic farming, although organic management was not significantly associated with plant biodiversity overall. Soil conditions and grassland management features were not significantly correlated with percentage grass content of the study farms. Grass content was negatively correlated with the numbers of plant species recorded ($r = -0.702$, $P < 0.01$). Grass content correlated negatively with some individual non-grass plant species, namely *Cardamine pratensis*, *Plantago lanceolata*, *Ranunculus repens*, and *Taraxacum officinale*, and these non-grass species were positively associated with the plant biodiversity found on the study farms. In contrast, *Lolium perenne* was positively associated with grass content and negatively correlated with plant biodiversity. This evidence suggests that other plant species are inhibited in a grassy sward.

The relatively low mean number of plant species present, 7, is in sharp contrast to numbers found in similar lowland grassland, thirty years earlier, when O'Sullivan (1968) found the lowest number of species to be 25, found in the more intensively farmed pastures. The lowest number of species encountered in the current study amongst the sixteen study farms was on the conventionally managed farm Q, in which only four plant species were found. From a management perspective, O'Sullivan (1968) reported that fertiliser nitrogen applied over a three-year period had not obliterated the natural ecological mixtures in grassland plant communities at that time. However, recent evidence suggests that fertilisers are amongst a few major factors affecting the botanical content of agricultural grassland, along with other management factors and site related characteristics such as climate, drainage, and soil base status (Yeo et al., 1998). In 1996, it was reported that the effects of phosphorous were more important than nitrogen in determining botanical change (Kirkham et al., 1996). However, while neither phosphorous nor nitrogen fertilisers were correlated with plant biodiversity in the present study, species number was positively correlated with the number of years elapsed since mineral nitrogen had been applied ($r = 0.603$, $P < 0.01$). Presumably, the plant communities have had opportunity to recover in the years during which nitrogen fertiliser, which promotes productive grass species, was not applied. Recovery of plant communities was indicated in the establishment of a low input meadow system in

Essex, where increased numbers of up to 42 plant species were reported over a ten-year period (Snow et al., 1997).

The single and most significant soil characteristic correlating with plant species number was the content of soil organic carbon ($r = 0.672$, $P < 0.01$). Species diversity may be an indicator of soil carbon storage capacity. The correlation between soil organic carbon and grass content was negative, but not significant ($r = -0.296$). Grassy swards favour defoliation for animal production, and the ensuing respired carbon dioxide is a source of carbon loss from the ecosystem.

4.4.6 Vegetation mass and dry weight

Vegetation dry weights and mass measurements were closely correlated ($r = 0.976$, $P < 0.01$), as would be expected. Significant correlations were found between vegetation dry weight and: soil depth to bedrock ($r = -0.580$); root mass ($r = -0.526$, $P < 0.05$); and sward content of *Lolium perenne* ($r = -0.506$, $P < 0.05$). Their correlations with vegetation mass were similar.

Vegetation mass correlated significantly with the absence of mineral nitrogen applications recorded for six years prior to botanical analyses ($r = -0.518$, $P < 0.05$). Correlations with four-year fertiliser applications prior to 1995 were insignificant, and it is likely that the six-year fertiliser figures are more pertinent. From 1995 to 1997, included in the six-year values, fertiliser application had continued on some farms and had changed on others. Later in Chapters 6 and 7, higher levels of vegetation mass and dry weight values were found to indicate poorer production conditions and lower nutrient input status.

4.4.7 Comparison of plant communities

It should be noted that attempts had been made during this study to conduct a cluster analyses of species abundances for botanical community analysis purposes using the multivariate statistical package, MVSP (Kovach, 1998). The application of this package is described in more detail in Chapter 7. However, associations found in the resultant dendrograms seemed unrelated to those communities identified earlier by O'Sullivan (1968). Lack of comparability may be attributable to the absence in 1998 of many

species diagnostic for the community associations as described by O'Sullivan (1968). When associations were compared directly, however, some of the plant community alliances described by O'Sullivan (1968) may have been represented in the grassland examined in the present study.

For example, the Molinio-Arrhenatheretea grassland communities associated with agricultural use is uniquely represented by farm R. The predominant species in farm R was found to be *Festuca ovina*, similar to *Festuca rubra*, common to the Molinio-Arrhenatheretea class (O'Sullivan, 1968). In addition, *Plantago lanceolata*, *Taraxacum officinale* and *Cardamine pratensis*, character-species of this class were also found, together with some *Lolium perenne*, and other species. *Festuca* communities are not particularly noted in County Limerick (O'Sullivan, 1968) and are found nowadays in relatively few areas for example in central Wales (Yeo, et al., 1998). Farm R lacks definitive clover and ryegrass content (Table 4.1), and is more closely allied to the Molinio-Arrhenatheretea than to the Lolio-Cynosuretum association of this class, described below.

One alliance of the Molinio-Arrhenatheretea, the *Cynosurion cristati*, observed by O'Sullivan (1968) in Limerick, appears to be well represented by the study farms. This type of plant community has been almost exclusively used as permanent pasture in county Limerick. Within the *Cynosurion cristati* alliance, two associations, namely Lolio-Cynosuretum and Centauro-Cynosuretum have been determined (O'Sullivan, 1968). Lolio-Cynosuretum association is characterised by abundant *Lolium perenne* and *Trifolium repens*, favoured by high fertility and by intensive grazing of the sward (O'Sullivan, 1968). Both species were found in farms E and L, together with *Holcus lanatus*, *Ranunculus repens*, and *Poa pratensis*, characteristic of the Lolium-Cynosuretum association. Other farms such as M, S, G and F also contain some species that are characteristic of this association, but have lower *Lolium* and lower clover contents than farms E and L.

Although each of farms T, J, Q, P H and A has some claim to the Lolio-Cynosuretum community structure, *Lolium perenne* and *Trifolium repens* and different combinations of diagnostic species are present in each community, though not necessarily abundant. As species numbers are fewer, particularly in farm Q, links are difficult to ascribe.

O'Sullivan (1968) noted that with minimal applications of fertilisers, many *Lolio-Cynosuretum* pastures show little signs of deterioration. However, management may have taken its toll and may account for the lower biodiversity found overall, especially in farm Q, which was the most conventionally managed.

Nine species differential for *Centaureo-Cynosuretum*, an association described as being the most widespread grassland type in Ireland in the 1960s (O'Sullivan, 1968), were not found on the study farms. According to O'Sullivan (1968), this *Centaureo-Cynosuretum* association typifies a pronounced soil mineral deficiency, low rate of mineral fertiliser applications, and the presence of continuous grazing throughout the year. Based on information from interviews with participant farmers during which mineral deficiencies were investigated, no substantial deficiency was likely to have remained at the time of botanical analysis, having been addressed by fertiliser applications or by the use of feed supplements ordinarily, or particularly prior to the conversion of farms to organic management. Grazing was intermittent on all farms, without exception. Could grassland nutrient and grazing management have altered soil conditions, eradicating grassland associations *Centaureo-Cynosuretum* in the process? Such changes in species diversity seem likely, based on recent research (Yeo et al., 1998).

Farm N and O from the Gortaclareen soil series appear to match the *Calthion palustris* alliance, with its single association, *Senecioni-Juncetum*, fairly well. Plant communities on farms N and O are characterised by an abundance of *Juncus* species, being as O'Sullivan (1968) describes, 'rush rich'. *Trifolium repens*, *Alopecurus pratensis*, and *Ranunculus repens*, as found in this association, are also present to some extent in both farms. However, average species number in this association was 33 in the 1960s, whereas only 7 and 10 species were enumerated in 1998 on farm N and O.

Farm B lacks the definitive *Lolium* and *Trifolium* species almost completely and instead has an abundance of another grass, meadow foxtail or *Alopecurus pratensis*, together with *Holcus lanatus*, some *Poa pratensis*, numerous buttercups and thistles. While Newton (1993) describes meadow foxtail as an excellent grass, it doesn't get particular mention in the grassland communities described by O'Sullivan (1968), and was not consistently present in the grassland studied at that time. In the current study, it has been found in abundance also in other farms, namely G, J and L, and to a lesser extent in

some other farms. Farm B was not clearly associated with any grassland plant community alliance described by O'Sullivan (1968).

To conclude, it can be stated that a range of grassland botanical species have been found in the study farms, yet many diagnostic species accounted in Limerick by O'Sullivan (1968) were not represented, and the botanical diversity of their grassland communities is considerably less than recorded thirty years ago (O'Sullivan, 1968). While a February sampling date may have been a disadvantage, reduced species numbers are probably attributable to more intensive grassland management practices than noted by O'Sullivan (1998).

There is ample evidence from this study, and elsewhere, that both mineral fertiliser inputs and grazing management practices impact on grassland botanical composition. Botanical diversity results of the study farms probably reflect what Yeo et al. (1998) call the 'relentless losses' of grassland diversity elsewhere in many parts of Europe. Losses may be stalled however, because although low botanical diversity was found on the conventional farm Q, botanical diversity was positively correlated with the interval since mineral nitrogen fertiliser application on other farms. These results suggest that although the grassland communities may be affected by mineral nitrogen inputs, grassland biodiversity may recover, depending on grassland management practice.

Chapter 5

Chapter 5

Soil carbon and nitrogen transformation biology in relation to influential grassland factors on the study farms

5.1 Introduction

The provision of plant-available nitrogen is a crucial determinant of soil fertility. Nitrogen fixation is carried out by soil microorganisms either as independent asymbiotic processes or in symbiotic association with leguminous plants, most commonly clover, in grassland (Newton, 1993). Low input and organic grassland systems depend primarily on nitrogen fixation in the grass-clover sward, followed by nitrogen being made available to grasses through its mineralisation and immobilisation from legumes and from other organic sources (Brady, 1990; Lampkin, 1990; Newton, 1993).

The mineralisation and immobilisation of nitrogen by soil microorganisms are pivotal activities in the provision and storage of plant available nitrogen (Barraclough, 1997). Nitrogen mineralisation occurs when nitrogenous organic compounds are used for energy, giving off ammonia in the process (Cunin et al., 1986). Carbon mineralisation occurs when organisms derive energy from organic material in respiration, giving off carbon dioxide in the process (Coyne, 1999). In order to use organic carbon for growth, heterotrophic microorganisms derive nitrogen primarily from soil ammonia (Coyne, 1999). Also, by manipulating soil organic matter, biological processes contribute to soil structure (Brady, 1990). The role of microbial nitrogen transformations has been virtually bypassed in conventional farming practice, where nitrogen is supplied in a soluble mineral fertiliser form (Whitehead, 1995). In contrast, low-input and organic farming systems recognise and depend on the microbial transformations of carbon and nitrogen for soil fertility and structural quality. There is a growing awareness that management influences soil microbial transformations with consequences both for soil fertility and structural quality, but that our knowledge about how soil processes are influenced is limited (Skinner, 1976; Parr et al., 1992; Degens et al., 2000).

The relevance of some biological contributions, in particular nitrogen fixation, has been acknowledged, with the result that the impact of conventional farming practices on

biological nitrogen fixation may be taken into account in grassland management systems. Optimal nutrient applications and grazing practices have been established that allow farmers to avail of biologically fixed nitrogen either under low input, organic or conventionally managed conditions (Lampkin, 1990; Frame, 1992; Newton, 1993; Whitehead, 1995). In contrast, by 1991, the possible effects of mineral fertilisers on carbon and nitrogen mineralisation processes had not been considered (Glendining and Powelson, 1991). According to Newton (1993), exactly when and how fixed nitrogen becomes available through mineralisation processes to grasses in the sward has not been understood, and therefore is not well controlled. Fundamentally, the underlying effects of plant growth, grazing, excreta and fertiliser applications on mineralisation processes are unknown (Bardgett and Leemans, 1996). Lack of knowledge, and its unsuccessful transfer to agricultural practice highlighted by Newton (1993), is symptomatic of single discipline studies where interdependent factors are not adequately considered. As Bardgett et al. (1998b) remarked, “most terrestrial ecosystems have explicit above-ground and below-ground compartments but these have usually been considered in isolation from each other and relatively little is known about the degree to which they interact”. In contrast to the quantity of material written on nitrogen fixation and its management (for example Ladha and Peoples, 1995), there has been no broadscale quantitative study of the functional diversity of the biota responsible for mineralisation, or of the factors that influence this diversity (Degens et al., 2000).

Understanding the biological transformation of carbon and nitrogen should have practical application. Because soil biological processes play a crucial role in soil structural quality and fertility, many authors and researchers (Nannipieri et al 1990; Lampkin, 1990; Sparling, 1997) have suggested that biology could be used as an analytical tool in determining soil quality. However difficulties in both methodology and interpretation have obscured study of influential factors, and have impeded the development of soil biology as a tool in soil analysis to date (Skinner, 1976; Lampkin, 1990).

Chapter 5 focuses on the biology of carbon and nitrogen transformations, and mineralisation in particular, in grassland, in relation to grassland management parameters, soil conditions, and botanical composition. The chapter begins with the literature outlining the biological transformation of soil organic matter, and arguments

for the biological evaluation of soils in agriculture are presented. Each of the components involved in mineralisation and immobilisation is described clearly from analytical, microbial and biochemical perspectives. The agricultural interpretation of each biological activity is also considered. Current literature on the influences of soil environmental factors, botanical composition, and nutrient and grazing practices on mineralisation biology is reviewed.

The soil microbial biomass, catabolically diverse bacterial populations, microbial activity and soil enzymes are examined in the laboratory in soils from sixteen grassland farms. Based on grassland farms under low nutrient input, organic and conventional managements, relationships between mineralisation, grassland management parameters, soil conditions, and the botanical composition of the grassland (as described in Chapters 2, 3 and 4), and amongst the biological parameters (described in Chapter 5), are examined statistically.

This study affirms the complexity of the interactions between soil management and soil biology. However, because the fundamental biological and agricultural principles are defined at the outset and are echoed in the behaviour of the study farm soils, a greater understanding of carbon and nitrogen mineralisation processes in managed grassland is achieved.

5.2. Review of literature: organic matter transformations

5.2.1. Mineralisation of carbon and nitrogen

Mineralisation of carbon and nitrogen occurs as part of global ecological processes termed the detritus food web. The detritus food web is made up of primary decomposers, some of which are microbial such as the bacteria, actinomycetes and fungi. Decomposers also include plant parasitic nematodes, predators, grazers and saprophagous fauna of varying sizes that consume the bacteria and fungi (Pankhurst, 1997), but the microbial heterotrophs are responsible for most of the decomposition that occurs in soils (Brady, 1990). In mineralisation, heterotrophic organisms and their enzymes hydrolyse organic substrates, so releasing nitrogen, potassium, phosphorus,

sulphur and other organically bound ions from soil organic matter (Richards, 1987; Brady, 1990).

In carbon mineralisation, the oxidation of organic matter to carbon dioxide and water provides energy for heterotrophs. Carbon also provides the molecular skeletons for microbial biosynthesis (Coyne, 1999). Nitrogen, required for cell protein and other components, although present in relatively smaller quantities (Brady, 1990), regulates the microbial transformation of organic carbon in soil (Chantigny et al., 1999; Henriksen and Breland, 1999). Nitrogen mineralisation occurs when heterotrophs resort to using nitrogenous compounds for energy, a process that is normally avoided by microorganisms but happens when carbonaceous sources are depleted (Zubay, 1993; Coyne, 1999). Overall, mineralisation is regulated by environmental factors, particularly by substrate composition, and by carbon and nitrogen availabilities (Cunin et al., 1986; Richards, 1987; Martens, 2000), and the mineralisation of carbon and nitrogen are inextricably linked.

Sources of nitrogen are limited in that livestock depend on plants, which in turn depend on microorganisms, for the conversion of atmospheric nitrogen into an available form. The provision of plant-available nitrogen is crucial in grassland farming (Richards, 1987; Brady, 1990; Frame, 1992; Whitehead, 1995). In the absence of synthetic fertilisers, plant-available nitrogen is derived primarily from fixation by legumes in the sward, and from the return of nitrogen in excreta (Whitehead, 1995). In nitrogen fixation, bacterial nitrogenases catalyse the reduction of atmospheric nitrogen, forming ammonia. Ammonia also arises when microorganisms mineralise nitrogenous compounds in plant material, excreta or other form of nitrogenous detritus in soil. Plants assimilate ammonia either directly from soil or, in the case of legumes, from their microbial endosymbiont. Plants may also avail of nitrate present in soil as a result either of mineral fertiliser applications, or from the microbial oxidation of ammonia to nitrate in an additional step catalysed by autotrophic, rather than heterotrophic, microorganisms (Alef, 1995b). Ammonia plays a key role in provision of nitrogen for plant and animal protein, but may be temporarily removed from the soil environment into the microbial biomass, in a process termed microbial immobilisation. Physical and chemical processes in soil also affect the retention and loss of soil ammonia (Brady, 1990).

5.2.1.1 Sources of substrates for microbial mineralisation and immobilisation

In mineralisation, organic matter is acted on by microorganisms, which use the organic matter as a substrate for their energy and growth. Substrates come in various guises, and because plants are primary producers, it is the plant canopy, litter, and root exudates that are the initial sources of soil organic matter (Richards, 1987; Brady, 1990; Coyne, 1999).

In grassland, grazing animals alter soil organic matter through dung and urine excreta which return substantial amounts of nutrients in a labile form, readily available for recycling, and are significant soil organic matter sources in grazed pasture (Lovell and Jarvis, 1996). Grazing animals may also alter available substrates indirectly through plant responses to herbivory (Bardgett et al., 1998b; Tracy and Frank, 1999).

Large soil organisms, or macrofauna, macerate and mix organic matter into soil, which accelerates its decomposition. This is especially true of earthworms, because of their tunnelling activity and through their fragmentation and distribution of organic matter through the topmost soil horizon, (Tivy, 1990; Coyne 1999). Soil mineral material that has passed through the earthworm gut creates relatively water-stable worm casts. In addition, the lining of the earthworm burrow, termed the drilosphere, is rich in ammonia and partly digested organic matter, providing a rich environment for microbial activity (Coyne, 1999). Dung beetles bring a substantial part of dung pat material from the soil surface into the soil within weeks of deposition and are largely responsible for the translocation of excreta below the soil surface (Brady, 1990; Frame, 1992; Coyne, 1999).

Substrates for microbial transformation arrive in soil in various shapes and forms. Smaller molecules, usually from root exudates or from the decomposition of plant materials and animal excreta include sugars, phenolic acids and amino acids and urea (Richards, 1987; Martens, 2000). Polysaccharides, such as starch, cellulose, hemicellulose and lignin, and other compounds such waxes and resins, usually arise from plant cells and tissues (Coyne, 1999) sloughed off or dropped into soil from root and shoot senescence and from litter decomposition. These molecules presumably also arise from herbage damaged underfoot by treading animals, and from partly digested

organic matter deposited in their excreta on grazed pasture. Polysaccharides arise in soils from microbial as well as plant sources (Richards, 1987).

Soil organic matter has been categorised as fractions of increasing stability ranging from labile to recalcitrant fractions (Brady, 1990; Janzen et al., 1998). Rapid mineralisation of carbonaceous and nitrogenous amendments in soils has been demonstrated in many studies. Half lives as short as 1-2 hours have been demonstrated for readily available materials, while the half-lives of more slowly utilised carbon pools were 30-75 hours (Lundquist et al., 1999). When soils had been separated from their rhizosphere nutrient source, and further nutrients had not been supplied, basal respiration rates declined linearly (Cheng et al., 1996), showing that the microbial populations require, and use up, available energy sources. Amino acid amendments were used up within one to twelve hours depending on soil type and environmental conditions (Jones, 1999). Such studies indicate the fragility of available carbon sources in soil. Yet dissolved organic carbon, which is the most labile fraction of soil organic matter and is the main energy source for soil organisms, has not received much attention in agriculture (Haynes, 2000), but is attracting increased interest recently (Grandy et al., 2000; Gregorich et al., 2000; Magill and Aber, 2000a & b).

In contrast to rapidly hydrolysed organic matter, lignin is more recalcitrant and is the least accessible plant carbon source for microorganisms, having a longer lifespan in soils. Lignin decomposition is not readily inducible and takes place only under conditions of carbon, nitrogen or sulphur starvation (Coyne, 1999). Because of its complexity, and also because it does not contain nitrogen, necessary for microbial growth, lignin decomposition slows microbial metabolism (Coyne, 1999) and, being relatively immune to microbial attack, has little immediate impact on soil chemistry (Richards, 1987; Coyne, 1999).

5.2.1.2 Substrate transformations by soil microbial activity

Extracellular enzymes, usually of microbial origin, hydrolyse plant polymers, releasing amino acids, phenolic acids and sugars into the soil environment. The smaller residues may be absorbed into microbial cells. Under anaerobic conditions, some energy may be derived in cells via fermentation, and organic acids and alcohols may be released to the soil (Coyne, 1999). Complete oxidation may occur. If oxygen is available, cells may carry out respiration, during which oxygen is used, carbon dioxide is released, and organic carbon is mineralised. Many soil organisms have the capacity to absorb other substrates such as methane from their environment and, given the right conditions, soil can sequester carbon, acting as a sink for greenhouse gases (Franzluebbers et al., 2000).

The use of nitrogenous organic compounds for energy gives rise to mineralisation of nitrogen to ammonia (Cunin et al., 1986; Zubay, 1993; Coyne, 1999). Urea is a source of soil nitrogen from excreta and organic matter breakdown, but urea is not a source of energy for heterotrophic decomposers (Coyne, 1999). Urea activity is the only example of extracellular ammonification processes in soil (Alef, 1995b). The fate of ammonia is multifaceted, as it may be taken up by plants, lost by volatilisation, or nitrified by other soil organisms during which nitrite, nitrate, and nitrous oxide form, with possible losses due to leaching with associated ground water risks, or gaseous losses of noxious gases to the atmosphere.

Microorganisms may synthesise a range of molecules that contribute to soil organic matter and soil structure, thereby affecting the soil environment. Microbial products include cell materials synthesised in the course of growth, extracellular enzymes released by some organisms for the hydrolysis of polymeric substrates, and other polymeric substances layered outside the cells as either capsules or slime layers. Extracellular enzymes are protein in nature and like other polymers act as energy sources that are subjected to microbial attack (Coyne, 1999). Extracellular polymers, such as polysaccharides, are known to contribute to soil aggregate stability (Skinner, 1976; Brady, 1990). Swift (1991) demonstrated that as polysaccharides were decomposed, aggregate stability diminished, confirming the roles of polysaccharides in aggregate stability, and confirming the role of microorganisms via either synthesis or degradation of polysaccharides.

Cell growth in individual bacterial terms is insignificant, but the elongation of fungal hyphae helps create a matrix in which soil particles are held in stable aggregates (Guggenberger et al., 1999). Micropores and channels are also created along fungal hyphae as they burrow, which changes the microenvironment. A few types of predators, namely nematodes and protozoan species, graze bacterial and fungal populations, and so microbial population numbers are curtailed. The decomposition of microbial heterotrophs, predator activities such as grazing and burrowing (Richards, 1987; Coyne, 1999) combined with root herbivory in the case of plant parasitic nematodes (Denton et al., 1999), brings further change in the composition and distribution of soil organic matter.

It is tempting to consider soil nutrient processes as sequential; however, the reality is very complex because the active components and the environment in which transformations take place, are interdependent. Ultimately, soil organic matter is totally transformed by soil microbial, physical and chemical processes. Soil organic matter may become stabilised by the condensation of phenolic and amino acids as humic substances, which are resistant to microbial breakdown because of their chemical structure, complexity and diversity (Coyne, 1999). The residence time for soil humus is reckoned in at least hundreds of years (Brady, 1990; Janzen et al., 1998). Humic substances contain both carbon and nitrogen, but tend to resist degradation and therefore may remain as long-term soil structural components and nutrient resources, depending on conditions.

5.2.2 Analysis of soil biology

5.2.2.1 Overcoming obstacles to the analysis of soil biology

Because of the particular relevance of soil biology in nutrient cycling for organic farming, Lampkin (1990) favoured the introduction of a biological assessment of soil in addition to chemical and physical analysis. He added that ‘the main problem with the application of these (biological) tests is their complexity and cost, combined with considerable variability which makes interpretation difficult’. Researchers have acknowledged difficulties associated with soil biology as a tool in soil assessment for some time. Without success by the mid 1970s, Skinner (1976) suggested that ‘perhaps

we were not asking the right questions of nature'. In a review of the ecological significance of the soil biology, Nannipieri et al. (1990) described many soil biochemistry methods as difficult, time consuming and cumbersome, and finds that "many of the primary obstacles impeding advances in soil biochemistry are associated with methodology". However, Nannipieri et al. (1990) also asserted that methodological problems in relation to soil biology might be resolved by avoiding methods that have been shown to be inadequate, and by defining exactly what is determined in the method itself, to facilitate its correct use.

The merits or otherwise of various soil biology methods have predominated recent soil biochemistry literature (Dilly and Munch, 1998; Lin and Brookes, 1999a, b & c), and application of any of the possible soil biology tests in an evaluation of soil has been slow. As yet, no researchers have found the ultimate microbial or biochemical measure of soil fertility or quality, and it is unrealistic according to Nannipieri et al. (1990) to assume that such a simple relationship exists. This is particularly true in relation to mineralisation, as pointed out by Burket and Dick (1998), who commented that "while chemical and biological tests have been used for many years in an attempt to predict the nitrogen status of soils, a satisfactory method of predicting nitrogen mineralisation continues to elude investigators".

The arguments for and against microbial indices of soil health were teased out recently by Sparling (1997) who noted that no absolute value for any microbial parameter could be of use because of differences in soil behaviour across types and management systems, and because of the absence of baseline values against which to compare soils. He cited one typical problem: that rapid decomposition might lead to loss of soil organic matter, whereas plants require available nutrients from mineralisation in time to meet their demands, which means that any mineralisation rate, per se, would not necessarily indicate soil health (Sparling, 1997). However, mineralisation is the main mechanism through which nitrogen becomes available for grasses in low input and organic farms. Knowledge of soil microbiology is therefore essential if we are to understand agriculture and ecosystem functioning (Coyne, 1999). Appropriate methods are urgently required.

5.2.2.2 Biological components responsible for carbon and nitrogen transformations, their analyses, and their interpretation in agriculture

The microbial biomass, the microbial populations, their cells and enzymes carry out transformations of soil organic matter in concert. However, as each component responds to its environment in a distinctive fashion, each is considered separately.

5.2.2.2.1 Microbial biomass

The microbial biomass is the total mass of diverse microorganisms, specifically organisms smaller than 5-10 μm^3 , in a given volume or mass of soil (Alef and Nannipieri, 1995d). Cells may be active, or dormant in soil, or may be nonviable. All microbial cells, active or otherwise, contribute to the overall biomass present. Previously viewed as a single entity, for example in a comparison of organic and conventional farms by Bolton et al. (1985), the microbial biomass is now widely recognised as having a relatively large inactive fraction, and a possibly smaller, metabolically active component that is more labile and responsive to soil conditions (Oades and Jenkinson, 1979; Dilly and Munch, 1998; Lovell and Jarvis, 1998).

The soil microbial biomass contains carbon, nitrogen and phosphorus immobilised in cell material (Alef and Nannipieri, 1995d; Coyne, 1999; Haynes, 2000). A relatively large microbial biomass is found in the rhizosphere of pasture soils (Haynes, 2000), where its nutrient contribution may be substantial. Estimates of the percentage of soil reserves contained in the biomass range from 1.7 to 3.7% of soil organic carbon (Jenkinson and Powlson, 1976), and 1 to 5% of the total soil nitrogen (Smith and Paul, 1990), in different ecosystems. Values for the percentage of soil phosphorus in the soil microbial biomass vary considerably from 11-83% (Smith and Paul, 1990). On cell death or lysis, microbial cell components become available as nutrients for the remaining heterotrophic population, and decomposition contributes to the soil pools of available nitrogen and phosphorus. Microbial biomass carbon provides energy and carbon for biosynthesis and so may be mineralised, available in the soil solution, or sequestered again in the microbial biomass. The ratio of carbon to nitrogen present within the microbial biomass depends on the composition of the microbial community in soil, because bacteria typically have a microbial biomass within the range of 5:1 to 8:1, whereas for fungi the range is wider, 4.5:1 to 15:1 (Coyne, 1999).

The microbial biomass is of interest in soil analysis and in the comparison of management systems because it is a source of nutrients and is also the driving force behind nutrient mineralisation processes in soil. It is also affected by management, and may serve as an indicator of soil health or quality (Reganold, 1995; Sparling 1997).

5.2.2.2.1.1 Laboratory analysis and interpretation of soil microbial biomass

The microbial biomass has been estimated in different ways. Indirect measurements are based on the content of cells, cell components or cellular activities in soils, which when multiplied by suitable coefficients, allow biomass to be extrapolated (Rowell, 1994; Alef and Nannipieri, 1995d). Comparing absolute biomass values that have been obtained directly, through microscopy for example, with microbial components or activities measured under similar circumstances, generates coefficients (Rowell, 1994).

Specific cell components such as ATP, amino acids, proteins, specific microbial phospholipids and bacterial peptidoglycan, originate naturally in soil from lysed microbial cells. In the laboratory, chloroform fumigation of soil causes microbial cells to lyse into the environment, deliberately releasing their cell contents. Because cellular metabolism is inactivated by chloroform-induced lysis, substrates released from burst cells are not attacked, and remain accessible for analysis. The concentration of any material released from cells in a fumigation-extraction (FE) technique is deemed proportional to the originating biomass (Alef and Nannipieri, 1995d). Cell components are assayed by various means (Alef and Nannipieri, 1995d) after their release during chloroform fumigation of the microbial biomass. For example, according to Amato and Ladd (1988) and Carter (1991), ninhydrin-reactive nitrogen is significantly related to the microbial biomass content of soil. Ninhydrin is a suitable reagent for the estimation of nitrogenous cell components as it reacts with all α -amino acids, primary amines and ammonia to give a purple coloured compound, determined in spectrophotometry (Zubay, 1993). However, chloroform lyses cells that may have been nonviable, dormant, or inactive for whatever reason, and so this method reveals more about the chloroform-sensitive microbial biomass rather than the active microbiota (Dilly and Munch, 1998; Lovell and Jarvis, 1998), as cell material from living and dead biomass components are assayed indiscriminately.

Gaseous exchange during respiration when multiplied by a suitable coefficient also approximates microbial biomass (Rowell, 1994). Respiration can be measured either as substrate-induced respiration in the presence of added substrate such as glucose or without amendment as in basal respiration. When respiration is measured, either at basal levels or when induced by a substrate, active or glucose-responsive microbial biomass components are measured, rather than the entire biomass (Lovell and Jarvis, 1998).

Rowell (1994) commented that there is uncertainty about the coefficient used to derive biomass from other values because of the difficulty of obtaining an accurate absolute measure of biomass to allow calibration of the method. Differences in soil conditions and handling impose other constraints. Correlation between biomass estimates and coefficients used in its estimation depends on soil sampling, conditioning, and soil type (Dilly and Munch, 1998). Anderson and Domsch (1978) found that different incubation temperatures influenced biomass coefficient values when compared with earlier research. According to a review by Smith and Paul, (1990) biomass coefficients vary with the microbial community content of bacteria and fungi, and are not straightforward. Original coefficients have either been adapted (Anderson and Domsch, 1978) or have been found unsuitable (Dilly and Munch, 1995). If 'microbial biomass' approaches reveal different active, glucose responsive or total aspects of biomass, or are based on different extrapolation coefficients, it is difficult to compare research results.

5.2.2.2.2 Living organisms and their functional capabilities

Given a particular substrate, microbial populations with the constitutive capacity to use that material will proliferate (Richards, 1987). In addition, many inducible or adaptive enzymes are synthesised by certain heterotrophs in response to new substrate provision, and the induction of enzyme activity in response to substrates enables population growth (Kay-Shoemake et al., 1998; Coyne, 1999; Kandeler et al., 1999a).

Smaller substrates may be absorbed directly or actively transported into the microbial cell, however heterotrophic microorganisms such as fungi and bacteria cannot absorb macromolecular substrates from their environment (Coyne, 1999). Instead, they employ extracellular enzymes that hydrolyse the macromolecular substrates present in the environment outside the cell, where they release smaller residues. The organism

responsible for hydrolysis, and indeed other microorganisms that lack the original hydrolysis capability, avail of smaller residues, in succession (Coyne, 1999). If a substrate provides energy, and if nitrogen is available, the microbial population will increase in number. Long after substrates are exhausted or conditions changed microbial populations may remain, quiescent, in soil (Richards, 1987; Atlas, 1995), until suitable growth conditions resume.

Evaluation of the microbial populations in soil, and more importantly of their functional capabilities, can give information about the catabolic diversity of organisms present, and about the effects of different management conditions on the soil decomposer populations (Nannipieri et al., 1990; Degens et al., 2000). Demonstration that a population or any particular functional capability exists in soil, does not however show that the particular function is taking place *in situ* (Richards, 1987; Coyne, 1999). Microbial activity in soils is, necessarily, demonstrated by other means.

5.2.2.2.1 Enumeration of functional populations in soil

Many methods have been employed to assess the numbers of microorganisms in soil, and to assess their functional capabilities. Each method has limitations. Direct microscopic observation has been used to assess the numbers of microbial propagules present, but does not reveal the functional status of the cells being observed. Cells, although evident in soil, may be quiescent, metabolically inactive or nonviable, or viable but non-culturable (Atlas, 1995). Ultimately, biochemical analysis is needed to verify whether the soil population is viable or is capable of a particular metabolic activity. Enumeration of viable microorganisms is considered here, and their activity is dealt with in the next section.

The numbers of viable culturable microorganisms are determined by serial decimal dilution of a known quantity of material in sterile diluent followed by spread plating and incubation on suitable solid media (Lorch et al., 1995). Following incubation each colony is assumed to have originated from one or possibly two or more adhering cells termed a colony forming unit, or cfu. The number of colonies visible after incubation approximates the number of culturable bacteria, more accurately termed cfu, in the original sample. Numbers of cfu in the original sample are calculated from the dilution and subsequent colony count data and are expressed per gram of soil. The catabolic

capabilities of microbial heterotrophs are of interest in studying mineralisation processes, and are studied by various means. By incorporating particular substrates in culture media for the plate count technique (Lorch et al., 1995), organisms capable of hydrolysing substrates such as cellulose, starch, protein, arginine or urea, may be enumerated.

This technology can be successfully applied for culturable cells. Being culturable implies that they are separable from their environment and that their growth requirements can be met in nutrient media. However, because of the complexity of soil, it is unlikely that all viable microbial propagules can be successfully dispersed from soil into solution, and furthermore, it is not possible to match the soil habitat with similar conditions on an agar plate (Richards, 1987; Lorch, et al., 1995). The viable non-culturable status has been recognised in many soil microorganisms, which defy laboratory culture methods, but which are functional in soils nonetheless, carrying out protein synthesis, respiration and other activities there (Atlas, 1995). In contrast to bacterial cfu which are usually fewer than found by direct means, fungal numbers may appear higher because the dispersal techniques in serial dilution and plate count methods lead to breakage of hyphal fragments, which grow to be counted as representing originally individual organisms (Lorch et al, 1995). Given these constraints, it is impossible to account for the total numbers of viable organisms, whether bacteria, actinomycetes or fungi, present in a sample of soil, using current technology. Most estimates of viable culturable microbial populations fall far short, at 0.1-10% of the numbers of propagules counted by direct means such as microscopy (Lorch et al., 1995; Riis et al., 1998).

Rather than use the plate count technique, which is problematic, other commercially available systems such as Biolog plates (Burket and Dick, 1998) are available with a range of simple carbon and nitrogenous substrates to test the range of catabolic functions available in soil samples. Biolog results tell whether that catabolic function is present or absent, but give no information about the numbers of species or organisms involved. The test can be carried out rapidly because bacterial growth manifests quickly, but the test occludes contributions by slower-growing fungi for which bacterial growth may need to be suppressed by the inclusion of antibiotics in the growth medium (Lorch et al., 1995; Grayston et al., 1998). Individual fungal isolates that have been purified

and freed from the background of bacterial contaminants may be tested in the Biolog plate system, but in any event, the test tells us whether or not a population or individual type of organism can carry out a function, and, in contrast to the plate count methods, is not enumerative.

Other methods differentiate microbial populations of bacteria and fungi in soils, and are used to quantify their proportion in soil community structure studies. Some methods are based on the fact that different microorganisms produce distinct molecules in soil, the proportions of which help to distinguish population contents. For example, a distinct nucleic acid fingerprint is associated with individual species; peptidoglycan is uniquely prokaryotic, and different phospholipid fatty acids (PLFA) typify fungal and bacterial populations. Molecules that help to differentiate species, organisms, and populations are regarded as biomarker molecules, and have been applied in the analysis of the soil microbiota (Alef and Nannipieri, 1995b) and in comparison of management effects on the structure of the microbial populations in soil (Bardgett et al., 1997).

Even though the drawbacks of the dilution plate count procedure are well known (Tiunov and Scheu, 2000), some researchers contend that it is worthwhile to isolate and count soil microorganisms, as it may provide useful information about the effects of environmental factors on the microbial community at the sites studied (Alef and Nannipieri, 1995a). Overall, the analysis of microbial populations in soils for enumerative, catabolic diversity or community structure purposes is possible, but is subject to methodological and interpretative constraints.

5.2.2.2.3 Microbial activities in the transformation of carbon and nitrogen

The transformation of carbon and nitrogen in soils is carried out by microbial cells and by cell-free extracellular enzymes that are either in the soil solution or stabilised onto soil colloids. Certain metabolic reactions are more likely to take place within intact cells either because they require an intact membrane (Alef and Kleiner, 1987) or cofactors; are membrane components; are dependent on proton gradients; or are regulated there by allosteric controls (Zubay, 1993). In this thesis, activities carried out by intact cells are termed ‘microbial’, rather than simply ‘biological’ to distinguish cellular from non-cellular soil biological activity.

The microbial population capable of carrying out a reaction may well be present in soil, but whether the population, or even the individual microbial cell is active, will depend on circumstances. In particular, cellular activities are constrained by the available energy (Kay-Shoemaker et al., 1998), available nitrogen (Coyne, 1999), and by the intracellular controls governing that activity (Zubay, 1993; Coyne, 1999). In soils, the rate of mineralisation or decomposition is governed by the C:N ratio of added residues in particular (Alef, 1995b) and by the C:N ratio of the surrounding soil (Whitehead, 1995). In cells, allosteric enzymes respond to available nitrogen and carbonaceous energy sources (Cunin et al., 1986), governing the rate at which organic material is transformed. Microbial activity measurements may therefore provide information about those factors influencing organic matter transformation processes, such as the available carbon and nitrogen in the soil environment. Extracellular enzymes are not affected by inhibitors of microbial metabolism (Coyne, 1999) and are dealt with separately. Other general environmental effects such as substrate provision, enzyme concentration, pH, temperature, redox potential and water activity govern cellular and acellular activities to varying degrees in soils as well (Coyne, 1999; Whitehead, 1995; Zubay, 1993; Richards, 1987).

5.2.2.2.3.1 Regulation of microbial transformation of carbon and nitrogen

Microbial mineralisation activity inevitably involves carbon provision for energy and nitrogen provision for cell growth, and the relative provision of both elements has a strong regulatory role, seen as the C:N ratio effect in relation to decomposition of organic matter. For example, ammonification rates depend on the C:N ratio of the organic compound (Alef, 1995b). High rates of ammonification occur at low C:N ratios (Alef, 1995b), and immobilisation of nitrogen occurs when high C:N residues are incorporated in soils (Brady, 1990). The availability of organic carbon and mineral nitrogen resources is required for decomposition to occur, and depletion of either can slow the process remarkably (Chantigny et al., 1999; Henriksen and Breland, 1999).

While it is true that that C:N ratio is relatively constant in soils (Brady, 1990), and that the C:N ratios of residues being decomposed have particular importance (Brady, 1990; Alef, 1995b; Coyne, 1999), the soil also contributes C:N ratio effects and should be considered as an integral factor (Whitehead, 1995). In some research, at least (Alef, 1995b), the relevance of the soil as contributing to the C:N ratio seems to have been

overlooked. Although Alef and Kleiner (1987) refer to limitation of ammonification when energy sources are not limiting, the possibility that mineralisation might be controlled by dynamic concentrations of labile carbon and nitrogen sources (Cunin et al., 1986) does not seem to have occurred to many soil biologists. The regulation of mineralisation by intracellular allosteric controls, as a basis for at least some of the C:N ratio effect, has not been fully considered.

In a striking example of this omission, Magill and Aber (2000a) had expected that glucose would increase nitrogen mineralisation rates in soils, and expressed surprise that mineralisation was reduced in the presence of glucose, which they attributed to lowering of apparent ammonification rates by immobilisation. The immobilisation of nitrogen is of course likely in the presence of glucose, but the researchers (Magill and Aber, 2000a) made no reference to the possibility that catabolite repression might reduce deamination of nitrogenous compounds in the presence of glucose (Cunin et al., 1986), thereby reducing mineralisation. Measurement of microbial activity using arginine ammonification as a simple gauge (Barraclough, 1997; Lin and Brookes, 1999b; O'Dowd et al., 1999) has also been reported without a thorough consideration of the biochemistry of amino acid catabolism and its intracellular regulation.

Contributory to the C:N ratio effect, allosteric regulation of carbon and nitrogen metabolism provide a context in which the effects of farm management practices on mineralisation processes might be understood in grassland soils. Because of its pivotal role, the biochemical intracellular regulation of carbon and nitrogen transformations is synthesised here.

5.2.2.2.3.2 Intracellular regulation of carbon and nitrogen metabolism

Primarily, microorganisms require energy in order to carry out their activities and for growth. Whether the microbial population will decompose that energy -supplying material also depends on nitrogen availability for the biosynthesis of cell components such as proteins, enzymes and nucleic acids (Kay-Shoemaker et al., 1998; Chantigny et al., 1999; Henriksen and Breland, 1999).

The main source of nitrogen for soil microorganisms is inorganic, as ammonia or nitrate, but ammonia is the preferred option, and relatively few use nitrates by

comparison (Coyne, 1999). Ammonia is assimilated in organic material through either of two key pathways. In the simplest mechanism, a few life forms can add ammonia directly to ketoglutarate, forming glutamate. The enzyme, glutamate dehydrogenase, is responsible, and its regulation has been studied in a range of organisms. Glutamate dehydrogenase can catalyse the assimilation of ammonia nitrogen at what are considered to be relatively high ammonia concentrations, greater than a half milligram per kilo of soil (Coyne, 1999). However, when the microbial cell senses starvation conditions through allosteric modulation mechanisms that respond to depletion of carbonaceous sources, glutamate dehydrogenase works best in the opposite direction and then tends to strip ammonia from glutamate (Zubay, 1993), releasing ammonia from a nitrogenous organic compound as in mineralisation, and releasing ketoglutarate for oxidation. So, whether organisms can use carbon for growth depends on available nitrogen, and if energy is not available from carbonaceous sources, available organic but nitrogenous options are sought and used instead.

Ammonia is assimilated mainly via an alternative route involving glutamine synthase, which can take up ammonia at lower concentrations, probably less than one tenth of a milligram of ammonia per kilo of soil, (Coyne, 1999). This key enzyme, glutamine synthase, senses the cell's need for nitrogen-containing compounds (Zubay, 1993). The regulation of glutamine synthase activity has been studied in Gram-negative bacteria where it is controlled by a cascade of reactions, each of which is responsive to the intracellular concentration of two metabolites, glutamine and α -ketoglutarate. In effect, either a high concentration of glutamine, or a high ratio of glutamine to α -ketoglutarate, means that sufficient nitrogen-containing compounds are available in the cell (Zubay, 1993). So, if cellular organic nitrogen sources are sufficient, glutamine synthase activity is inhibited and ammonia nitrogen is not assimilated for cell material. Nitrogen starvation on the other hand is signalled by a high ketoglutarate to glutamine ratio, and glutamine synthase activity is stimulated, leading to nitrogen assimilation and immobilisation in organic cell components. In addition, glutamine synthase activity is fine-tuned by the intracellular concentrations of a further eight nitrogen-containing compounds (Zubay, 1993). So, cellular immobilisation of nitrogen from soil reserves is more likely when organic carbon is readily available, and when nitrogen is in relatively short supply, as occurs at higher C:N ratios.

If soil nitrogen is not readily available for decomposers, carbon mineralisation may be limited (Henriksen and Breland, 1999). A decrease in soil mineral nitrogen content was consistently associated, in a logarithmic fashion, with an increase in water-soluble plant-derived carbon, which accumulated when mineral nitrogen fell below 60 mg kg^{-1} (Chantigny et al., 1999). At low mineral nitrogen contents, carbon mineralisation was limited, and what are normally labile substrates in soil were not decomposed (Chantigny et al., 1999). These studies indicate that nitrogen availability regulates carbon mineralisation (Henriksen and Breland, 1999) and also influences soil organic carbon content (Chantigny et al., 1999). Conversely, if sufficient carbonaceous energy sources are available, nitrogenous sources need not be sacrificed in order to oxidise their carbon skeletons, with accompanying nitrogen mineralisation, a process that is wasteful in terms of both the immediate nitrogen loss and the energy required to recreate organic nitrogen compounds later (Zubay, 1993). Soil nitrogen and carbon reserves are therefore interdependent.

Under conditions of carbohydrate starvation, microorganisms can strip nitrogen from amino acids and alter the carbon skeleton of amino acids to form acetate, pyruvate, ketoglutarate, succinate or oxaloacetate, each of which is oxidised through the tricarboxylic acid cycle. In this way, the carbon skeleton of the amino acid is used to provide energy. For example, breakdown of five amino acids, glutamine, glutamic acid, proline, arginine and histidine happens in a progressive fashion through glutamine itself, which is then deaminated releasing ammonia and forming α -ketoglutarate. The α -ketoglutarate can enter the tricarboxylic acid cycle if energy is still required by the cell (Zubay, 1993). Effectively, in the absence of a suitable carbonaceous energy source, deamination releases ammonia nitrogen from a cellular nitrogenous compound, and mineralisation of nitrogenous soil organic matter occurs (Coyne, 1999).

5.2.2.2.3.3 Laboratory measurement of microbial activity

Finding more 'microbial activity' in organic than conventional farming (Bolton et al., 1985; Reganold, 1988) has led to the presumption that 'microbial activity' *per se* is beneficial in soil, and that it is a quantity worth measuring. However, microbial activities in soil are so rich and diverse it is impractical to measure them fully (Degens et al., 2000), and many of the techniques are time consuming and expensive (Alef and Kleiner, 1986a).

So, for simplicity, researchers have sought one parameter that was easy to measure and reliably associated with microbial activity in a range of soil types. In order to fulfil this requirement, any measure of microbial activity should be based on intact, metabolically active, microbial cells (Alef and Nannipieri, 1995c). Alef and Kleiner (1987) demonstrated that arginine ammonification was strongly disrupted by toluene, known to disrupt cell membranes; that it was rapid in arginine-amended soil samples; and was significantly correlated with respiration in arginine-amended soils. They therefore suggested that *in vitro* arginine ammonification represented soil microbial activity (Alef and Kleiner, 1987). In addition, because the arginine ammonification rate was immediate and linear for more than one hour, it indicated that organisms respond at once to the substrate without changing their physiology (Alef and Kleiner, 1987), implying that the enzymes were constitutive rather than induced, and also that it was the active population that was involved rather than a dormant fraction (Kay-Shoemaker et al., 1998; Coyne, 1999). As similar results were obtained in over thirty soils differing in pH, organic carbon and clay contents, Alef and Kleiner (1987) suggested that arginine ammonification could be applied as an indicator of microbial activity in different soils. Later it has been shown that, under certain conditions, the relationship between arginine ammonification and the microbial biomass is significant, suggesting that arginine ammonification might represent the quantity of microbial biomass present (Lin and Brookes, 1999b).

Since proposed by Alef and Kleiner (1987), numerous researchers have adopted arginine ammonification as a means of comparing the microbial activity in soils exposed to different grazing animal behaviour (Haynes and Williams, 1999), arable vegetable production intensities (Haynes and Tregurtha, 1999), management systems (Gunapala and Scow, 1998), arable and grassland systems (Dilly et al., 1997), and wet and dry conditions (Dilly and Munch, 1995), and it has also been applied in the analysis of disturbed landfill soils (Bogner et al., 1995) and sewage-sludge amended soils (Dar, 1996). However, the research to date has sought to equate arginine ammonification, representing microbial activity, with soil quality or other soil attributes such as biomass (Lin and Brookes, 1999b), rather than with nitrogen mineralisation and immobilisation potential. The application of arginine ammonification as a mineralisation assay has

particular significance in this study (Chapters 6 and 7), and is given further consideration in the following sections.

5.2.2.2.3.4 Arginine ammonification represents mineralisation, and is under intracellular control

In the soil environment, arginine would arise as one of twenty or more amino acids when proteolytic enzymes hydrolyse the protein in soil organic matter extracellularly. Following its transport across the cell membrane, arginine hydrolysis then takes place in the cell via biochemical pathways that involve arginase, arginine deiminase, or other enzymes (Abdelal, 1979; Cunin et al., 1986). Enzymes in the arginine deiminase pathway are induced by energy source depletion and catabolite repression of the arginine deiminase pathway occurs in the presence of a good energy source such as glucose (Cunin et al., 1986). When arginine is used for energy, ATP can be generated in intermediate steps during arginine breakdown via the deiminase pathway, and the carbon skeleton from arginine may be excreted as ornithine or citrulline into the microbial environment (Cunin et al., 1986). Ornithine may also be processed in the cell, entering the tricarboxylic acid cycle through ketoglutarate, providing energy via central metabolism (Zubay, 1993). Oxygen provision is required for arginine ammonification (Alef and Kleiner, 1987), confirming its contribution as a carbon source in cellular respiration. Arginine is one of the few amino acids from which nitrogen is not removed by transamination or transferred to other amino acids intracellularly (Zubay, 1993), but the ammonia is removed by oxidative deamination rather than transamination and is either liberated or used within the microbial cell. In some exceptional cases, microorganisms cannot use arginine for energy, and use it purely as a nitrogen source instead (Cunin et al., 1986).

In the laboratory, arginine ammonification is measured as the rate at which ammonia is released from arginine-amended soil samples during incubation at elevated temperatures. The highest ammonification values imply that arginine, a nitrogen source, has been used for energy, releasing ammonia as occurs in nitrogen mineralisation activity. Ammonification could also mean that labile carbonaceous compounds are not available, and that arginine is used as an energy source in the deiminase pathway, which is either induced and or freed from catabolite repression.

At lower intermediate apparent ammonification rates, ammonia nitrogen may have been withdrawn into the microbial biomass in immobilisation, resulting in apparently lower levels of ammonia free in the soil environment. When organic matter (such as arginine) is added to soil, microbial growth and respiration occurs. Arginine provides carbon initially, and on its deamination ammonia nitrogen is released for use by other organisms. If the organic amendment does not supply enough nitrogen for microbial growth, additional nitrogen may be immobilised from soil reserves, leading to apparently negative ammonification values when compared to unamended controls. Low and negative values for arginine ammonification rates have been reported by relatively few researchers such as Forster et al., (1993) and Lin and Brookes, (1999b) to date. An amendment may incur a priming effect, where the outcome differs from the response found with either the added material or the soil itself (Coyne, 1999). Immobilisation of nitrogen from soil on the addition of arginine as an amendment is an example, because the added carbon available in arginine may facilitate use of the mineral nitrogen present in soil, and so its concentration changes. Kuzyakov et al. (2000) have since remarked that negative priming effects are seldom reported in the literature.

Nitrogen mineralisation results from ammonification, which is a cellular activity (Alef, 1995b). Ammonification has been defined as the liberation of ammonia from nitrogenous compounds that are used as sources of carbon or nitrogen (Alef and Kleiner, 1986a). However, this definition may lead to the assumption that microorganisms use organic nitrogenous compounds, such as amino acids, to provide nitrogen, which is not necessarily correct. In fact organisms use mineral nitrogen in preference. Lin and Brookes (1999b) quoted Alef and Kleiner (1987) as recently as 1999, stating that arginine was 'both a C and N source'. However, amino acids such as arginine primarily provide carbon for energy when allosterically controlled enzymes sacrifice nitrogenous sources. This occurs only when other energy sources are depleted (Cunin et al., 1986; Zubay, 1993; Haynes, 1999). Ammonification should not be observed in samples with non-limiting energy source according to a subsequent work by Alef and Kleiner (1987), however, the underlying basis for this statement is not discussed in that particular paper. Later, when referring to this point from Alef and Kleiner (1987), Lin and Brookes (1999b) mention the total organic carbon content, rather than the available carbonaceous energy sources (Cunin et al., 1986) on which this

catabolite repression effect would depend. Both papers allude to the inhibitory effect of energy sources, but are not sufficiently precise as to how that effect might either manifest in practice or be measured appropriately. In effect, researchers have sought to measure microbial activity, but have neglected to see that the ‘activity’ which they are measuring depends on the dynamic availability of carbonaceous compounds for energy, and that the rate of activity therefore might reflect soil carbon and energy status because carbon reserves regulate nitrogen mineralisation potential intracellularly.

Although a few microorganisms cannot use arginine for energy but use it as a nitrogen source instead, (Cunin et al., 1986) arginine is not necessarily used for nitrogen in every case. Nitrogen that has been mineralised in the course of energy provision may be used intracellularly, and only the surplus released (Barraclough, 1997). The cell’s nitrogen requirement will determine whether nitrogen is immobilised in the microbial biomass or is released as surplus mineralised nitrogen to the surrounding soil. Overall, ammonification, or release of nitrogen from an organic source, is more likely at relatively low C:N ratios (Coyne, 1999), or when carbohydrate energy sources are depleted. The relationship with C:N ratio seems, in some reported research to be solely dependent on the amino acid or organic molecule being degraded (Alef, 1995b). However, C:N ratio is also a feature of the entire soil environment (Whitehead, 1995), which is sometimes overlooked.

Although arginine ammonification actually represents nitrogen mineralisation and immobilisation, this representation of N-mineralisation has been somewhat overlooked. Instead, more lengthy incubation procedures lasting days to weeks, followed by estimates of ammonia or nitrate or both, are used to determine soil mineralisation potential estimates (Rowell, 1994; Alef, 1995b; Rasmussen et al., 1998). None have been found satisfactory to date (Burkett and Dick, 1998; Rasmussen et al., 1998; Abril et al., 2001a). According to Nannipieri et al. (1990), attention should be paid to what is being measured so as to facilitate its correct use. If, instead of viewing arginine ammonification as a measure of microbial activity, arginine ammonification values were interpreted as mineralisation and immobilisation tendencies, relevant information about soil nitrogen transformations might be obtained. Precise analysis of soil organic matter and of available carbonaceous energy sources would help researchers consider its presumably regulatory role in nitrogen mineralisation more thoroughly. The labile

carbon and nitrogen contents, and the C:N ratios of both incorporated residues and the surrounding soil should be taken into account. Intracellular regulation of carbon and nitrogen mineralisation by available nitrogen and by carbonaceous and nitrogenous metabolites should be given careful consideration.

5.2.2.2.4 Enzyme activity in soil

Microbial enzymes are present in soil either as cellular components, or are released from bacterial and fungal cells during growth and / or on cell death. While some enzymes, such as urease, may continue mineralisation processes outside the cell, many of the cellular processes cannot take place once the cell is disrupted, and enzymes from disrupted pathways spill into the environment where they simply contribute protein to soil organic matter. In some soils, intracellular enzymes predominate, whereas in others the extracellular component, either stabilised or diffused in the soil matrix, constitutes a major percentage (Richards, 1987; Klose and Tabatabai, 1999). In an extracellular capacity cell-free enzymes are not controlled by metabolic pathway inhibitors (Coyne, 1999). Cell-free enzymes are therefore regarded in this study as capable of discrete function. Whether or not their enzymatic activity takes place will depend on substrate and coenzyme availabilities for the particular reaction, and on environmental conditions such as pH, and soil temperature.

Enzymes free in soil may associate with soil colloids, which changes the conformation of the protein, possibly blocking its breakdown by microorganisms, thereby stabilising the enzyme against attack (Coyne, 1999). Because of this stability, enzymes in soil may retain their biological activity long after the microbial population has changed. This stability has negative connotations too in that bound enzymes may react more slowly because of conformational changes (Coyne, 1999).

Urease enzyme activity is of particular interest as it is the only means through which ammonification occurs outside the cell (Alef, 1995b). Urease enzyme originates partly from plants. It also originates from soil microorganisms, about one to two thirds of which can use urea as a nitrogen source (Richards, 1987; Coyne, 1999). Urease catalyses the hydrolysis of urea to ammonium carbonate, which rapidly releases ammonia, especially at higher pH, induced by urease activity itself (Richards, 1987; Coyne, 1999). The reaction provides little energy and, as a result, urea cannot be used as

an energy source by soil microorganisms. The enzyme, which comprises a group of urea aminohydrolases, also exists in an extracellular form (Klose and Tabatabai, 1999), which may be stabilised by soil colloids (Richards, 1987; Kandeler et al., 1999), or by phosphate fertiliser derived hydroxyapatite (Marzadori et al., 1998). The percentage of intracellular and extracellular urease activity differs amongst soil types (Klose and Tabatabai, 1999). Extracellular urease is not subject to carbon and nitrogen responsive intracellular regulation (Coyne, 1999), in contrast to arginine ammonification activity of the cell. Uniquely, soil urease activity catalyses the extracellular ammonification of soil organic matter (Alef, 1995b), and because of the increasing use of urea fertilisers (Richards, 1987), urease activity is of particular interest.

5.2.2.2.4.1 Laboratory analysis of soil enzymes

Urease enzyme activity is measured as the rate at which ammonia is released from urea-amended soil during incubation in the laboratory. By including toluene, which causes cell death, any cell-associated urease activity is inhibited in the reaction mixture, and the measured activity is regarded as purely enzymatic, rather than 'microbial'. Urease activity can also be determined without toluene addition, and then includes cellular and non-cellular activities. The proportions of cellular and extracellular urease activities vary in different soils (Klose and Tabatabai, 1999).

5.2.2.5 Sampling, treatment and environmental factors influencing soil biology

Soil is a very complex and dynamic substance, which is subject to change and varies considerably because of differing genesis, parent materials, vegetation, and other factors. Differences in regard to location, depth, soil temperature all impact on soil characteristics, and as they influence soil biology, need to be taken into account.

5.2.2.5.1 Spatial variation

Soil characteristics such as depth, texture, structure, mineral and organic contents differ according to location and conditions, as described for the study farms in Chapter 3. Microorganisms require substrates that generally become more dilute with soil depth (Whitehead, 1995). Sampling depth is, therefore, a factor in the analysis. In arable soil, sampling to plough depth is recommended, and in grassland sampling to 100 mm in the most intensively rooted A horizon is the norm (Forster, 1995a).

Parkin (1993) defines regional, field or landscape, plot and microscale, as four scales of spatial variability applicable to soil biology. Difference in scale has particular relevance when examining microbial activity in relation to the landscape as a whole, as illustrated in the following example. Numerous landscape-scale observations showed that soils were not waterlogged and, approaching 20% porosity, seemed adequately oxygenated. On closer inspection, anaerobic microsites were found with associated denitrification (Parkin 1993). This type of contrast between the agricultural and microbial environments means that extrapolation from analysis in the laboratory to field levels may be inappropriate. However, knowledge of factors controlling activities at microscale level help researchers to understand how microorganisms interact with their environment, and this understanding is crucial for predictive capabilities at larger scale (Parkin, 1993).

5.2.2.5.2 Temporal variation

Because temporal differences in biological activities in soil occur, field measurements are generally analysed with reference to temperature, water content, and seasonal

variations in the soil environment, so that the effects of those particular environmental variables might be taken into account. Forster (1995a) recommends that in order to have a comprehensive view of soil biology, samples should be taken repeatedly throughout the year. However, for *in vitro* microbiological analyses, soil is normally sampled on a single occasion, and transported to the laboratory for microbiological and biochemical analysis. Frequently, a single sampling date is chosen, and is accepted practice as demonstrated in a recent volume of Soil Biology and Biochemistry (31, vol.14, 1999) which featured ten soil biology research papers, each of which was based on a single sample event. Only two authors mention sampling dates. No reference to soil sampling dates was given in three papers presented by Lin and Brookes (1999a, b & c) on arginine ammonification and various biomass determinations.

Once-off sampling is acceptable for the analysis of soil microbial components and their activities on the basis that most of the microorganisms in soil may be inactive at any one time, being dependent upon available substrates and on prevailing soil temperature, pH, and reduction-oxidation potential (Coyne, 1999). Moreover, extreme stimuli such as elevated incubation temperatures and or substrate concentrations are imposed in the laboratory to evaluate the potential microbial populations and activities (Ste-Marie and Paré, 1999). Given the fact of microbial dormancy in soil and the elevated conditions imposed *in vitro* to study their 'behaviour', the sampling dates are for the most part regarded as inconsequential. Extrapolation to field conditions is also avoided, and, where laboratory conditions differ from those in the field, are more appropriately considered as potential, rather than actual, activity values (Alef, 1995a).

However, conditions known to affect the particular microbial population at the time of sampling are sometimes taken into account. For example, reference was made to the gravimetric water content at the time of sampling bog and forest soils in a particular study, but in what was an undated sampling event (Dickens and Anderson, 1999).

In particular, it is advised that periods immediately after nutrient applications and during freeze-thaw cycles should be avoided because of the variation imposed by soil temperature changes on nutrient availability within soils (Forster, 1995a).

5.2.2.5.3 Sample treatment variation

Sampled soils are transported to the laboratory, treated in various ways, and stored for varying periods of time prior to analysis. Soil samples may be sieved and air dried as for example in the study by Bolton et al. (1985), or sieved under field moist conditions (Alef and Kleiner, 1987), crumbled, bulked (Lovell et al., 1995), mixed thoroughly, or studied in isolated soil containers *in situ* (Abril et al., 2001) depending on the circumstances. According to Gunapala et al. (1998), field characteristics of soils are likely to be drastically altered by the breaking of soil aggregates and by sieving treatments, leading to the oxidation of organic matter. This has been demonstrated in a study when sieving and drying both increased rates of initial carbon mineralisation observed for three days after treatment (Franzluebbers, 1999a), presumably because of labile carbon sources released or exposed from soil organic matter during sample treatment.

The analysis of soil organic carbon is usually carried out on sieved, air-dried soil, by hydrolysis and reaction with substances such as anthrone or potassium dichromate (Rowell, 1994; Grandy et al., 2000). For example, an analysis of sugars and uronic acids in Irish soils from permanent pastures was carried out following sieving, air drying, and acid hydrolysis (McGrath, 1973), on soils of 'average' organic carbon contents for that time (5.3% carbon). Polysaccharide content and organic carbon were significantly correlated (McGrath, 1973). However, some of the available fractions of soil organic matter may have been lost through oxidation during the drying and sieving stages in soil treatment in which a flush of microbial activity may be induced (Franzluebbers, 1999a). Some researchers in contrast reported higher values for dissolved organic carbon content following air-drying relative to field-moist samples (Haynes, 2000). It was suggested that the release of soluble organic matter may have been induced by lysis of bacterial cells on soil desiccation, or by the release of the soluble fraction from organo-mineral complexes, both of which had been found elsewhere (Haynes, 2000). Luo et al. (1996), also noted a possible increase in available carbon on air-drying of soil samples, in contrast to a decrease on storage of moist soil, as a basis for differences in microbial activity in their sampled soils.

It appears that any treatment of soil induces change in soil organic matter quality and content. Ideally, oxidation opportunities should be avoided during soil storage and handling if the more labile organic matter fractions of soil, such as polysaccharides, are

to be maintained at original levels for analysis. Whatever the treatment, possible changes in soil organic matter fractions and their contents should be acknowledged.

Fractionation of soil organic matter into its components, such as labile dissolved organic carbon (Haynes, 2000) and microbial biomass carbon (Carter, 1991; Lin and Brookes, 1995b), are strongly correlated with soil carbon content, and regarded as interdependent. However division of soil organic matter into fractions based on size, density, fragility or recalcitrance, has been somewhat arbitrary, and has proved useful only under certain soil conditions in studies of carbon mineralisation (van den Pol-van Dasselaar and Oenema, 1999).

Samples may be stored under conditions ranging from air dried (Bolton et al., 1985), which is now not recommended for microbiological samples because generally microbial activity declines (Forster, 1995a), to the more current practices of refrigeration of field moist samples at 4°C (Alef and Kleiner, 1987), deep-frozen, at -16°C to -18°C, or pre-incubated and 'conditioned' (Lin and Brookes, 1999a). Storage condition effects have been studied in relation to single parameters from different soil horizons and, where possible, are taken into account (Forster, 1995a). Rather than examining the particular effects of soil treatments and storage in each case, authors sometimes rely on comparing like with like within a particular study, for example choosing to compare only sandy soils with similar humus content (Dilly and Munch, 1998). The narrow range of soil types tested is a limitation, and makes extrapolation to other soils difficult. In any event, differences in treatment and soil types should be acknowledged when comparing results.

5.2.2.6 Interpreting *in-vitro* soil biology data for agricultural purposes

To interpret laboratory analysis for practical purposes, it must be understood that what is measured in the laboratory is potential, rather than actual, activity (Alef, 1995a). In reality, the extent to which a microbial activity actually takes place in soil is difficult to ascertain. For example, many microorganisms in soil are thought to exist in a dormant phase in the absence of either substrates or appropriate conditions (Richards, 1987; Degens et al., 2000). When suitable conditions are resumed, the dormant population is reactivated. This is what frequently happens in the laboratory, as for example in the analysis of catabolic function in Biolog plates, or in substrate-induced respiration, or in

plate count assays, where substrates are provided. Spatial variability in microbial processes is particularly problematic, and variability in mineralisation, immobilisation and denitrification along with losses due to leaching, are all possible sources of error in mineralisation estimates (Whitehead, 1995; Watkins and Barraclough, 1996).

Differences between incubation experiments and field conditions have precluded finding an ideal prediction of mineralisation potential, for example (Rowell, 1994; Rasmussen et al., 1998).

In vitro investigation creates bias in soil biological activity, because the soils are disrupted, taken from their environment, and incubated at temperatures above those normally encountered in the field (Ste-Marie and Paré, 1999). Even with the disruption entailed in laboratory-based soil analysis, Ste-Marie and Paré (1999) suggest that a useful comparison of different soils can be achieved under similar test conditions. Even though laboratory-based microbiological descriptions of soil may enable us to study and compare the behaviour of soils from different sites, the descriptions obtained may be insignificant under field conditions (Dilly and Munch, 1998), because of the extremes to which soil are exposed in the laboratory analysis which are unlikely to prevail in nature. In a review of the ecological impact of soil biological activity, Nannipieri et al. (1990) argued that the extrapolation of *in vitro* measurements to field scale is unreliable and misleading.

While analysis under similar laboratory conditions seems to be worthwhile for comparison purposes (Ste-Marie and Paré, 1999), differences in soil conditions may, at least in some instances, be resolved by the expression of the results in a way that is free from that particular constraint. For example, soils vary in moisture content and it is customary to convert results to an equivalent oven-dry weight basis (Lorch et al. 1995). However, when comparing sites with a range of values, the order in which sites were ranked depended on whether the measurements had been expressed according to either the dry weight or volume equivalent (Dilly and Munch, 1995). The authors therefore suggest that data should be expressed per horizon and hectare, and related to soil volume, to facilitate comparison of different sites. Alvarez et al. (1998) also recommend that results obtained for sites differing in density should be expressed in mass per unit of area, rather than per unit of weight, from an ecological viewpoint.

Attention should be paid to what exactly is being measured (Nannipieri et al., 1990), particularly as this may differ according to the method used. For example, if different aspects of microbial biomass are being assessed and converted using coefficients to determine total biomass, it is likely that different biomass estimates will be obtained. This was exemplified Kaiser et al. (1992) who used three different approaches to measure soil microbial biomass carbon. The absolute values for biomass carbon deviated considerably. Although the ranking of soils within sites was the same for all three methods, and although biomass values for the same sites were highly correlated with each other, correlation of data with soil organic carbon depended on the method used (Kaiser et al., 1992).

As an alternative to absolute values, Dilly and Munch (1998) recommended the use of ratios between microbial parameter estimates. When using two methods (substrate-induced respiration rate and fumigation-extraction) along with their respective coefficients to estimate soil microbial biomass, Dilly and Munch (1998) found that site biomass values were inconsistent. However the ratio between the substrate-induced and fumigation extraction values differed from site to site, according to field conditions. Because the ratio reflected the balance between the active glucose-respiring cells and total biomass and because this balance reflected site conditions, Dilly and Munch (1998) concluded that the ratio between microbial variables would be “more useful than individual microbiological features related to soil weight when evaluating microbial populations and microbially mediated processes in soils”.

5.2.2.7 Conclusions

Having considered the analysis of soil biology, the following conclusions can be drawn. A fundamental understanding of the underlying biochemistry is prerequisite to using soil biology for agricultural purposes. Components differ in response to their environment, and should therefore be interpreted as discrete but interdependent facets. Particular microbial biomass measurements represent the active or inactive fractions depending on how measurement is made. The biomass itself is part of soil organic matter, and behaves both as a biological component and nutrient source. Finding a capable population in soil does not mean that that activity is taking place there (Coyne, 1999), but perhaps indicates the potential for that activity to occur (Alef, 1995a). In the laboratory, the potential biological activity is demonstrated in response to added

substrates under elevated or accelerated measurement conditions, which are unlike those encountered in the field. Microbial activity refers to intracellular processes governed by either inducible or allosteric enzymes responsive to carbon and nitrogen compounds in the cell (Cunin et al., 1986).

Nitrogen mineralisation, specifically ammonification, is most likely to be a cellular process (Barraclough, 1997). If cellular, ammonification would presumably be governed by cellular constraints as described by Cunin et al., (1986). Soil urease is largely extracellular and having been synthesised and released from the cell, questions of either enzyme induction or repression no longer arise. For some time now, arginine ammonification has been regarded as a way to measure microbial activity simply; however, its potential in describing mineralisation and immobilisation potential in soils needs consideration because of possible catabolite repression and allosteric controls of nitrogen mineralisation by available carbonaceous energy sources.

Each of these biological components can be measured in the laboratory, and if done appropriately according to clearly defined measurement and reliable methods, should be worthwhile (Nannipieri et al., 1990). However, they represent the potential rather than actual activity, which needs field measurement verification. Baseline values are not available because of variation in soil conditions (Sparling, 1997), and so interpretation is often site-dependent. However expressing results on a per volume basis could facilitate comparison (Dilly and Munch, 1998). No single laboratory method replicates field conditions, and so satisfactory laboratory estimate of *in situ* mineralisation potential has not yet been achieved (Burket and Dick, 1998; Rowell, 1994; Abril et al., 2001).

5.2.3 Environmental and management effects on C and N mineralisation

The influences of substrates, carbon and nitrogen on mineralisation have been outlined from a biological viewpoint. In the soil environment, many factors regulate the development of microbial biomass, and determine the rate at which heterotrophic metabolism, and its associated mineralisation of carbon and nitrogen, takes place in the agroecosystem. Fundamental effects of oxygen and water availabilities, pH, temperature, soil texture and soil pores have been understood for some time (Richards, 1987; Brady, 1990; Coyne, 1999), and are not considered in depth in this thesis. Instead,

some influential factors in relation to carbon and nitrogen transformations have been researched more recently and are presented here.

5.2.3.1 Substrate availability and C:N ratio

Microbial biomass is resource limited, being influenced by soil organic matter quality in particular (Bauhus et al., 1998), and like soil organic matter, is dependent on soil conditions (Sparling, 1997). In a study of soil microbial biomass in stored soils, basal respiration by the soil microbial biomass declined, probably in response to reducing availability of recently returned plant material over thirty weeks (Lovell and Jarvis, 1998). However, when glucose was added as a substrate after that time, the induced respiration rate was restored to original values (Lovell and Jarvis, 1998). This indicates that the microbial activity of the biomass is substrate-limited, and that the active biomass is responsive to fresh substrates. Biomass itself consists of fungi and bacteria in what is termed their community structure (Alef and Nannipieri, 1995b), which is also subject to change. When a mixture of the same compounds that are associated with plant exudates was added at increased concentrations to soil samples, the community structure of soils changed consistently, and, based on molecular fingerprint analysis, fungi dominated at higher concentrations (Griffiths et al., 1999). This showed that one factor, the concentration of organic matter, influenced community composition, even though the quality or type of substrates exuded was unchanged experimentally. Similarly, copiotrophic and oligotrophic organisms have been found in association with rich and sparse environments (Hu et al., 1998). The mechanisms for interactions between increased concentrations and populations are undefined as yet, and should be examined in order to understand possible effects of increased organic matter inputs on soil community structure and function (Griffiths et al., 1999).

Whether microorganisms can be active in soils depends the types of substrate available and on the relative environmental contents of carbon and nitrogen. The carbon to nitrogen ratio of organic matter has a profound effect on whether mineralisation takes place in soil or not (Whitehead, 1995), as considered previously from a biochemical viewpoint. From an agricultural perspective, an increase in soil carbon content makes mineral nitrogen less available, as relatively more mineral nitrogen is immobilised by microorganisms availing of high carbon, low nitrogen content substrates (Newton, 1993). Soil amended with low-nitrogen composts had reduced mineralisation rates,

implying perhaps that soil nitrogen reserves were being immobilised by the microbiota (Forster et al., 1993), leading to perceptibly lower levels of the test substance, ammonia, in the amended soils. Immediate immobilisation has also been demonstrated *in vitro* in a study in which nitrogen availability influenced carbon mineralisation and wheat straw decomposition rates (Henriksen and Breland, 1999). Generally, organic materials with high C:N ratio of 30:1, or above, are slow to release mineral nitrogen and possibly lead to immobilisation. For intervening ratios of 20-30:1, immobilisation may manifest in the short term, but release of a proportion of immobilised nitrogen may occur in the longer-term (Whitehead, 1995) presumably from the microbial biomass. Substrates with a low C:N ratio of less than 20:1 tend towards more rapid mineral nitrogen release because, as the substrates are relatively low in carbohydrate content, soil organisms avail of nitrogen sources instead for energy, which are deaminated in the process.

At least part of the effect that C:N ratio has on the rate of organic matter decomposition is probably brought about by intracellular regulation of microbial activity by relative carbon and nitrogen availabilities. Given the cellular nature and control of ammonification (described in section 5.2.2.3 with reference to Cunin et al., 1986 and Zubay, 1993), it seems surprising that a relationship between C:N ratio effects on decomposition rates, catabolite repression, enzyme induction and allosteric enzymes has not been found in the soil biology, composting, or agricultural literature. Instead, most C:N ratio-mediated effects can be explained on the basis that use of either carbon or nitrogen demands the presence of the other element in a microbe-available form. It is well known, for example, that if microbial C is not limiting, soil microorganisms compete with plants for the inorganic nitrogen present in soil, and mineral nitrogen becomes immobilised in the microbial biomass temporarily (Whitehead, 1995). Joint requirements for C and N, in differing proportions, serve as a basis for the threshold C:N ratio values at which either soil N mineralisation or immobilisation are likely to occur.

The plant community, which in grassland is affected by grazing, will determine substrate provision for transformation in grassland soils. Concerns in regard to the ecological effects of intensified sheep grazing, and in regard to management-induced plant community change, have sparked interest in their combined below-ground microbial consequences (Bardgett and Leemans, 1996; Bardgett et al., 1997, 1998a &

b). To date, *in situ* mineralisation rates have not been examined extensively in relation to grassland vegetation. *In situ* rates of other microbial transformations, such as nitrification, have been associated with vegetation and soil types in forest and agricultural systems (Bauhus et al., 1998). Ste-Marie and Paré (1999) found that a relationship existed between the stand type and net nitrification potential in their comparison of forest species such as spruce, aspen, jack pine and cedar in northern Quebec. The effect differed according to the species, nitrate accumulation being favoured by aspen and absent from jack pine stands. Because substrate availability and composition are key factors influencing the decomposition of plant residues in soils (Martens, 2000), and plant materials differ in chemical composition, nitrogen content and C:N ratio, it could be presumed that plant species might also affect mineralisation rates in grassland soils, as demonstrated for nitrification in forest situations (Ste-Marie and Paré, 1999). Plants apparently affect the outcome of their decomposition. For example, the specific phenolic acid composition of different plant species had greatest effect on residual carbon content and on resultant soil aggregate size (Martens, 2000). Generally, the C:N ratio of herbage detritus in a grass-clover sward, without mineral nitrogen applications, approximates 24:1, which as described, is intermediary in terms of threshold C:N values, and may be immobilised first, and mineralised later (Whitehead, 1995).

In addition to substrates from grazed vegetation, organic amendments vary in their effect on soil fractions and microbial components. Evidence from soil fraction studies suggests that organic amendments protect urease enzyme and the microbial biomass in the clay-sized soil particles (Kandeler et al., 1999a). Differences in microbial population criteria such as C:N ratios were apparent in soil fractions following organic amendment but not in untreated soils. Further study is required to establish whether fungi or bacteria are best protected in the clay fractions (Kandeler et al., 1999a).

Particular nutrients may be required for the expression of certain cellular functions and for the biosynthesis of specific compounds by microorganisms. For example, polysaccharide production may be restricted by nitrogen availability (Elliott and Lynch, 1984, cited in Lynch and Elliott, 1997). Lundquist (1999) found that the ratio of monounsaturated to saturated microbial fatty acids increased with increasing organic inputs in a comparison of conventional and organic farming systems, possibly being

nutrient determined. In some instances, new soil additives designed to limit erosion damage alter the microbial response by induction of microbial enzyme systems (Kay-Shoemaker et al., 1998). For example, synthesis and release of the extracellular adaptive enzyme, polyacrylamide amidase, is induced at four to fifteen times the rate in soil if polyacrylamide, an anti-erosion additive is present in irrigation water. However production of the enzyme was reduced in a laboratory culture when energy sources were exhausted, and were restored on the addition of a carbohydrate source to the medium, suggesting that whether or not an organism excretes extracellular enzymes may depend not only on the inducer being present, but also on available resources (Kay-Shoemaker et al., 1998).

5.2.3.2 Complex effects of some environmental factors on soil carbon and nitrogen transformations

Some abiotic factors such as soil depth govern the rate at which mineralisation can occur. Substrates are provided from plant materials and from excreta, and so the majority of soil biological activities take place in the upper horizons, availing of nutrients and organic matter in the rhizosphere and from above ground inputs. Soil biological activities generally decrease with depth as suitable substrates become more dilute (Whitehead, 1995). An additional ecosystem effect is created in the rhizosphere, where root exudates greatly enhance microbial numbers and activities (Richards, 1987). In temperate grassland, the rhizosphere extends to a depth of only ten centimetres of the topmost soil horizon (Forster, 1995a).

Both cellular and non-cellular enzymes behave as proteins, and therefore their rates of activity will be subject to conformational change, brought about by soil pH, salt concentration and temperature. Binding to soil colloids also affects soil proteins (Coyne, 1999). In addition, the soil microenvironment differs spatially in texture, oxygen availability, available water, porespace, and soil temperature to all of which microorganisms respond, and which have immediate consequences not necessarily apparent on the larger scale (Parkin, 1993; Coyne, 1999).

The microbial response to earthworm activities is poorly understood, but organisms appear to proliferate in fresh casts with no increase in overall microbial biomass, possibly because of predation by eukaryotes, which also proliferate in the worm cast

environment (Tiunov and Scheu, 2000). Initially, numbers and activity in worm casts appear greater than in the surrounding soil, although Tiunov and Scheu (2000) suggest that the microbial population in fresh *Lumbricus terrestris* faeces is stressed, and in a transitional state, representing perhaps the early stages of microbial succession in soil. However following high-density earthworm introduction, soil organic matter may decrease, together with microbial biomass, as seen in a pine forest ecosystem (McLean and Parkinson, 1997).

In accordance with basic biological principles, whether particular microbial processes occur in soils, and, if so to what extent, are likely to be determined by substrate type, available energy and C:N ratio. In addition, the combination of microbial environment and activity together is dynamic and susceptible to change, because microbial and enzyme activities happen best under optimal conditions, as influenced by environmental factors. As microbial activity, growth and respiration, takes place, the substrate concentration, pH, available space and oxygen fluctuate, and the immediate environment is itself altered. Where nutrients are limited, the changing environment in response to growth manifests as the stationary phase observed in batch culture in the laboratory, and a stationary, quiescent, or resting phase in which the organism is in survival mode is thought to be the principle status of soil microorganisms in the nutrient-sparse environments commonly found in nature (Atlas, 1995).

Soil microbial ecology therefore is characterised by flux, and by non-linear relationships. According to Mathes and Ries (1995) microbiological field data often fail to satisfy assumptions of classical statistical analysis, and are better examined either using distribution-free statistical methods such as ranking, or transforming data to conform with assumptions, or by taking the non-compliance with assumptions into account when testing significances.

5.2.3.3 Influence of nutrient inputs, liming and grazing on carbon and nitrogen transformations in managed grassland

Adding mineral or organic nutrients as fertilisers and manures, the practice of liming, and the activity of grazing animals influence the soil environment and therefore affect mineralisation activity. It is likely that the influence of management is mediated by changes either in the substrates available for microbial growth and decomposition, in

soil pH, or in the vegetation. However, no broadscale study of mineralisation and influential factors has been conducted (Degens, 2000). Comparative studies of management systems were reported initially over fifteen years ago (Bolton et al, 1985) and continued by Reganold, (1995). More in-depth analysis of individual management parameters in relation to mineralisation processes is now being carried out as exemplified in the literature (Lovell and Jarvis, 1996; Gunapala and Scow, 1998; Rasmussen et al., 1998; Haynes and Tregurtha, 1999; Haynes and Williams, 1999; Degens et al, 2000; Franzluebbers et al., 2000; Martens, 2000).

5.2.3.3.1 Comparison of basic management systems

Conventional management had been practiced for almost a century before studies comparing the effects of organic and conventional farming on microbial heterotrophic processes were initiated. Finding sites that differed only in respect of management, and not in respect of soil genesis and topography, was the primary obstacle blocking research of this type (Reganold, 1988). In the 1980s, studies began on a pair of farms, one organically, the other conventionally managed. The farms approximated the ideal for comparison purposes, being on adjacent sites of similar genesis, and differing just in terms of their seventy-year management histories (Bolton et al., 1985; Reganold, 1988). The two farms differed in fertiliser applications, crop rotations and farm managers, however they had been exposed to the same crop residues and tillage operations (Bolton et al., 1985). Studies by Bolton et al., (1985) and Reganold (1988) focussed on those particular farms.

On comparison, urease, phosphatase and dehydrogenase enzyme activities were higher in the organically farmed soil, which was interpreted as more ‘microbial activity’ (Bolton et al., 1985). No differences in plate counts were found. Higher levels of microbial biomass carbon and nitrogen were also recorded in the organic soil. However on one sampling date, three-fold higher levels of *Nitrosomonas*, the bacterial species responsible for the initial step in the nitrification of ammonia, were recorded in the conventional farm, attributed to the higher content of mineral nitrogen measured as ammonia and nitrate in the fertilised soils (Bolton et al., 1985). In the study conducted by Bolton et al. (1985), organic carbon content was described as slightly higher in the organically managed site. Reganold (1988) later noted that the organic farm had 25-60% higher soil organic matter levels than the conventional farm. Polysaccharide

content, cation exchange capacity, extractable phosphate and potassium were higher in the organic farm, whereas calcium and magnesium levels were similar (Reganold, 1988).

Researchers have since found other differences between organic and conventionally managed soils. Arginine ammonification was higher in organic and low input systems than in conventional systems on most sampling dates (Gunapala and Scow, 1998).

Methodology and its interpretation have changed since the initial comparisons were made in the 1980s. Urease activity has been shown to be a stable activity in soil, which, because it can be largely cell-free (Klose and Tabatabai, 1999), need not necessarily correlate with what was then termed 'microbial activity' (Nannipieri et al., 1990). Microbial biomass methods have also been updated. It is likely that the fumigation methods applied by Bolton et al. (1985) measured the total microbial biomass and therefore included active and inactive biomass components. Although increased *Nitrosomonas* (which could presumably lead to undesirable loss of nitrates to ground water) was found in the conventional farm, benefits of 'more microbial activity' were unquestioned (Bolton et al., 1985).

Earlier studies, as reviewed by Reganold (1995), described management in general terms, broadly contrasting biodynamic, organic, or no-till farms, with conventionally managed counterparts. Although specific values for nutrient applications or liming were not analysed statistically in the original comparisons of the two management systems, the studies were noteworthy because they demonstrated that similar organically managed soil had more organic matter (Reganold, 1988) and more 'microbial activity' (Bolton et al., 1985) than a conventional farm. Increased biological activity became associated with an overall management approach rather than with any single parameter, although the greater organic matter content associated with organic farming has been presumed responsible in some instances (Bolton et al., 1985; Reganold, 1988). This evidence lent support to organic farming as a sustainable soil quality system.

However, differences in interpretation drawn from microbial analyses then and now highlight the requirement stated by Nannipieri et al (1990), for a definition of what is being measured, so that it can be applied correctly. Precisely what affects mineralisation

by the soil biota, and in what way, has not been considered until recently. Specific effects related to tillage, liming, mineral nutrients, dung, urine and grazing events, and botanical composition, are documented in more recent literature, and are reviewed briefly here.

5.2.3.3.2 Specific effects of management history

Each management intervention brings change in soil. Those that have particular soil organic matter influence are considered here.

5.2.3.3.2.1 Tillage, pasture and soil organic matter issues

There is no doubt that organic carbon content is affected by management history, as seen in arable systems in which organic matter is oxidised, reducing both carbon and nitrogen contents (Brady, 1990; Tivy, 1990; Whitehead, 1995, amongst others).

Aeration in tillage operations encourages microbial oxidation activity, leading to loss of soil organic matter. In contrast, long-term accumulation of organic matter is associated with increased microbial presence in undisturbed permanent pastures (Burket and Dick, 1998).

Soil organic matter has profound regulatory effects. Lin and Brookes (1999b) observed carbon contents ranging from 0.7 to 6.65%, and correlation of biomass with other variables such as substrate-induced respiration and arginine ammonification rates occurred only when the soil of high carbon content (6.65%) was omitted from the analysis. Haynes and Tregurtha (1999) found that microbial activity declined in a curvilinear manner when soil organic carbon content fell below a critical concentration of about 4.5%. This suggests that organic carbon content may limit biomass, one of its components. Carter (1991) also found significant correlation between biomass and organic carbon contents in arable and pasture soils, but the range of organic carbon contents was not reported in that study, except to say that it was higher in pasture soils.

The relationship between organic carbon content and biomass is complex. While biomass development needs carbon skeletons from soil organic carbon for its development, whether organic carbon is sequestered in the biomass, or respired and mineralised, depends on circumstances. Reganold (1993) interpreted respiration as representing a biologically active good quality soil, in response to which Wardle (1994)

argued that respiration could lead to organic carbon losses and inefficiencies in organic carbon storage, as seen for example in arable soils where respiration has led to carbon mineralisation and loss.

Significantly higher levels of enzyme activities and microbial biomass have been found in management regimes that minimised soil disturbance such as pasture, sod, wetland and forest than in more disturbed soils (Burket and Dick, 1998). In a range of soils under pasture and arable managements, although there was a significant relationship between the organic carbon content and labile fraction, considerable variation in values was obtained for the labile fraction at any given organic carbon value (Haynes, 2000). The water-soluble fraction was extremely variable, and Haynes concluded that this labile fraction was more strongly determined by the immediate cropping history of the field than by soil organic matter, even though organic matter was probably also a determinant of the labile fraction. The labile fraction represents readily mineralizable organic matter, and as it increased markedly under pasture and declined under arable conditions in the short term, it reflected change in soil organic carbon status (Haynes, 2000). Relevance of microbial responses to readily available carbon sources, and their effects on soil carbon content, is being acknowledged.

5.2.3.3.2.2 Lime application

The soil biology responds to lime applications, as observed and reported by relatively few research groups. Liming significantly increased soil microbial activity as measured by factors including arginine ammonification (Eitarabily et al., 1996), and it has been found that the bacterial population increased ten-fold and numbers remained high after liming (Neale et al., 1997).

The different analyses and responses of microbial components in relation to environmental change should be taken into account so that the effects seen here can be interpreted appropriately. For example, arginine ammonification is a measure of potential activity measured by adding arginine as a carbon source to soil samples in the laboratory. The addition of any substrate which provides energy, as arginine does, is likely to arouse the quiescent microbial population (Richards, 1987). The microbial population then appears as being active (Eitarabily et al., 1996), more activity being logically associated with the larger populations found by Neale et al. (1997) after

liming. The facts that numbers remained high after liming, and that microbial populations did not correlate particularly with pH (Neale et al., 1997), highlight the issue of quiescence, which should be acknowledged in soil analysis and its interpretation. Populations measured in response to added substrate have probably been 'woken up' in the laboratory analysis, and do not necessarily reflect prevailing soil conditions, as in the study by Neal et al. (1997). Microbial activity, on the other hand, is governed by existing soil conditions that it readily reflects, as observed by Eitarabily et al. (1996). From an agricultural perspective, the population may not be active *in situ*, but because of previous liming history, may respond more readily to organic matter applications or deposited excreta. Liming events should be taken into account when attempting to predict the mineralisation potentials of managed grasslands.

5.2.3.3.2.3 Mineral nitrogen

The effects of mineral nitrogen fertiliser applications, and available nitrogen, have been studied in relation to some biological components and activities in soils. Lower microbial biomass has been reported in conventional than in organic farming systems (Bolton et al., 1985; Lovell et al., 1995). Higher N inputs in fertilised grass and clover treatments resulted in lower soil microbial biomass contents than in lower N-input treatments (Lovell et al., 1995). Later, it was found that the active components of the microbial biomass were similar in soils under different nutrient managements, but that the inactive proportion of the biomass had been influenced by previous nitrogen inputs (Lovell and Jarvis, 1998). These authors could not offer any explanation for their findings, but concluded that the active microbial biomass appears to be unaffected by nutrient input history and responds quickly to changing conditions.

When nitrogen inputs were examined in relation to microbial biomass, Gunapala and Scow (1998) found that microbial biomass declined with fertiliser nitrogen applications in the conventional system, and increased with mineral nitrogen availability in the organic system. Again, Gunapala et al. (1998) found that potentially mineralizable nitrogen was increased in the organic system, which they attributed to the organic amendments prior to study.

The mechanisms through which nitrogen inputs might affect microbial biomass are unclear. Henriksen and Breland (1999) found that numbers of bacterial cellulase-

producing cfu declined with nitrogen availability in straw amended soil, although effects were opposite in control sites. They also found that, shortly after nitrogen resources in straw-amended soil had been depleted, carbon mineralisation rates dropped considerably too. Cellulase producers carry out an initial process in carbon mineralisation, and so the researchers suggested that nitrogen availability regulates carbon mineralisation in soils (Henriksen and Breland, 1999). Similarly, Chantigny et al. (1999) found that water-soluble organic carbon accumulated when mineral N was not available. Losses of biomass and organic carbon, associated with conventional farming systems (Gunapala and Scow, 1998) might be explained by N-fertiliser application that seems to accelerate carbon mineralisation.

5.2.3.3.2.4 Differences in effect between organic and mineral nitrogen sources

Researchers have found that mineral and manure sources of nitrogen differ in effect. Lin and Brookes (1999b) noted that the experimental soil that had received inorganic fertiliser had lower microbial biomass than the same soil that had received farmyard manure. Gunapala et al., (1998) found that amounts of microbial biomass carbon were higher in conventional plots than in organic plots; however, both soils in that experiment had been amended with vetch, a source of organic matter. Importantly, higher levels of potentially mineralizable nitrogen were consistently found in the organic than the conventional plots after vetch amendment, possibly because of continuous organic amendment applications in the organic system prior to soil extraction for study purposes (Gunapala et al., 1998). It would appear that the addition of vetch had cushioned the conventionally managed soil against biomass losses incurred without organic amendments elsewhere.

Farmyard manure apparently exhibits another important mineral-N cushioning effect (Willison et al., 1996). Because some soil organisms can use methane as a source of energy and as a carbon source, soil has the capacity to absorb and fix atmospheric methane, and has been acknowledged as a valuable methane sink. However, applications of mineral nitrogen have been associated with reduced rates of methane oxidation, because of inhibition by ammonia (Willison et al., 1996). Paradoxically, it was found that methane was as strongly oxidised in sites receiving farmyard manure in the long term as in sites receiving zero nitrogen. In other words, farmyard manure was not inhibitory, even though the concentration of ammonia nitrogen applied in manure

was similar to that in mineral fertilisers. However, when mineral nitrogen was applied to sites that had already received farmyard manure, methane oxidation rates dropped. It was thought that elevated levels of microbial biomass in plots receiving farmyard manure had acted as a buffer against the inhibition of methane oxidation by the ammonia present in the manure (Willison et al., 1996), allowing methane oxidation to occur in soil at control site levels. In contrast, mineral nitrogen additions would not have had the integral biomass buffer content of farmyard manure, and would be inhibitory.

Furthermore, microbial biomass is affected by mineral nitrogen, but effects may take time to be observed. Lovell et al. (1995) observed that effects of either withdrawal of fertiliser treatments or of new fertiliser applications were not immediately evident in microbial biomass parameters during a one-year trial, but that biomass and microbial plate counts were both significantly higher in soils that had had no past fertiliser history. On average, biomass C and N were at least 60% higher in the unfertilised treatments than in fertilised swards (Lovell et al., 1995). Other research has established that nitrogen availability determines the extent of carbon mineralisation (Henriksen and Breland, 1999). If this is true, the provision of nitrogen as mineral fertiliser is likely to accelerate organic matter oxidation leading ultimately to biomass loss, as cautioned by Wardle (1994).

In addition to biomass effects, other microbial variables such as nitrogen mineralisation are influenced by nitrogen applications. Biomass nitrogen and nitrogen mineralisation were increased with long-term additions both of farmyard manure and mineral nitrogen, and of phosphorus and potassium (Kandeler et al., 1999a). Rasmussen et al. (1998) found that nitrogen mineralisation *in situ* increased linearly as a function of past fertiliser N applications. This was interpreted as recovery of nitrogen that had been applied previously, presumably from soil organic matter and microbial biomass, over time. Similar nitrogen mineralisation trends happened *in vitro*, but the rate was different, possibly because the soil had been disrupted for laboratory analysis. They concluded that previous nitrogen applications all affect nitrogen mineralisation potential (Rasmussen et al., 1998).

In a comparison of fertiliser applications on mineralisation and enzyme activities, nitrogen mineralisation was three to four times higher in plots that had been treated long-term with both farmyard manure and mineral fertilisers, than in control plots (Kandeler et al., 1999a). Organic amendment alone increased urease activity three-fold, which was attributed to greater stability of urease in the presence of organic matter in soils, whereas urease was unaffected by mineral fertiliser applications. For other enzymes, activities were increased with fertiliser applications, regardless of fertiliser type. At low levels of mineral nitrogen amendment, nitrogen mineralisation rates depended on the type of crop (Kandeler et al., 1999). The same authors established that the type of fertilisation, organic versus inorganic, had far more significant influence on microbial biomass and on carbon and nitrogen cycling processes than had crop type, and that the additions of farmyard manure had an overriding effect. In general, farmyard manure increased microbial biomass, nitrogen mineralisation, urease activity and arginine ammonification, whereas the effects of additional mineral fertilisers differed according to biological function (Kandeler et al., 1999).

Overall, nitrogen fertilisers seem to alter the soil organic matter content, according to the type of fertiliser, organic or mineral, added. Microbial biomass seems to be reduced by mineral fertiliser addition, and microbial activity may be accelerated by added nitrogen. The negative effects of mineral nitrogen applications may take time to manifest in soil, and may be cushioned by organic amendments.

5.2.3.3.2.5 Effects of phosphate fertilisers

Effects of phosphate fertilisers have been recorded recently in relation to soil enzyme activity. Marzadori et al. (1998), found that hydroxyapatite, which is a calcium phosphate derivative formed from phosphate fertilisers and soil calcium and which attracts organic molecules such as amino acids, plays an enzyme-stabilising role in soil and sediments. While the urease enzyme that was free in the soil solution had lost its activity, the absorbed enzyme had retained 60% of its activity value after four days (Marzadori et al., 1998). The absorbed enzyme was more stable even in the presence of additional proteolytic enzymes. It was concluded that hydroxyapatite could be an effective carrier of extracellular urease in alkaline soils (Marzadori et al., 1998).

5.2.3.3.2.6 Effects of grazing

Grazing introduces change in the botanical composition of the grassland, but also affects soil structure through treading, adds organic matter and urine, and induces plant response to foliar herbivory. Although soil microorganisms are affected by grazing, little is known about how grazing influences microbial processes (Tracy and Frank, 1999). Intensification of hill grazing systems has become a cause for concern (Bardgett et al., 1997) and, combined with the lack of knowledge of nutrient cycling effects of grazing, has spurred research efforts.

As with organic and conventional farming systems, comparisons between grazed and ungrazed pastures have been undertaken. In a comparison of grazed and ungrazed hill grasslands, Bardgett et al. (1997) found that microbial biomass and activity declined significantly following the removal of sheep from different grassland sites. The authors attributed this to the removal of readily utilizable substrates in the form of sheep excreta and urine, and to changes in the quantity and quality of root exudates induced in the plant by the effects of grazing. Bardgett et al. (1997) also found that nematode numbers were greatly reduced in ungrazed grassland, which they attributed to changes in the supply of plant litter, pathways of organic matter decomposition from faeces and urine, and soil physicochemical conditions. They concluded, in agreement with global literature, 'that herbivory by larger animals is one of the most important determinants of soil microbial processes in grassland ecosystems'.

The patterns of grazing also distribute nutrients differentially throughout a field, as excreta are deposited most frequently at night-lying sites, feed and water troughs, paths and gateways (Frame, 1992). Haynes and Williams (1999) found that microbial activity (measured as arginine ammonification) was higher in camp than non-camp soils. Camp soil is used as a term to describe soil in night-lying areas, where animals congregate, and where consequently dung and urine deposition is relatively increased. The cell-free enzymes protease, histidase, urease, acid phosphatase and arylsulphatase, were all higher in camp soils but the rate of another parameter related to cellular activity, dehydrogenase, remained unaffected (Haynes and Williams, 1999). Soil pH, organic matter and nutrient content were also increased in camp versus non-camp soils. Prolonged application of diluted dung and urine from cattle had a similar effect, in increasing soil organic matter content, nutrient accumulation, and soil biological activity. It was concluded that the camping activities of grazing animals result in

increased fertility in camp soils at the expense of those properties on the main grazing areas (Haynes and Williams, 1999).

Specific attention is being given to the effects of urine, dung and manure on the functional microbiota in soil. The effects of dung and urine have been studied by applying excreta to the soil surface (Lovell and Jarvis, 1996; Bardgett et al., 1998), and by incorporation of dung in laboratory soil samples (Lovell and Jarvis, 1996). Bardgett et al. (1998) found that when dung was applied to the soil surface in a microcosm experiment, microbial biomass increased following dung additions, but the effect was not significant, and accounted for only ten percent of microbial biomass variation. Dung addition did not affect microbial activity, as measured by basal respiration rates. Field experiments where dung pats were left in place on the soil surface yielded similar results even though over a ten-week period the dung pats had lost approximately sixty percent of their total carbon and nitrogen (Lovell and Jarvis, 1996). The extent to which carbon and nitrogen had entered the soil was not ascertained; however, it was found that soil mineral nitrogen content increased initially under the dung pats, possibly because grass growth had been curtailed. The capping effect and increased soil temperature under the dung pats may have increased mineralisation rates, as the underlying levels of nitrogen returned to background levels when the encrusted dung pat became broken up after about six weeks (Lovell and Jarvis, 1996).

In contrast, when the organic material from excreta is in contact with soil itself, rather than just left on the soil surface, labile components are readily hydrolysed by the soil biota, depending on conditions (Lovell and Jarvis, 1996). Increased microbial activity has been found in response to more prolonged additions of diluted dung and urine (Haynes and Williams, 1999) or following the direct incorporation of dung mixed with sieved soil which was accompanied by a sixty percent increase in microbial biomass, which with increased respiration indicated that dung was being used as a substrate for microbial metabolism and growth (Lovell and Jarvis, 1996). This was perceived as a short-term localised effect of substrate incorporation, unlike field situations where breakdown is relatively slow. The direct incorporation of labile substrates appears to have a very different effect than when they are left on the surface of soil, but it is likely that field conditions may promote a more stable organic carbon pool (Lovell and Jarvis, 1996).

As well as affecting both the botanical and chemical composition of the sward, plant defoliation and treading by grazing animals probably affect the soil organic matter content by the addition of damaged herbage onto or into soil. For example, the organic matter derived from herbage that has been damaged by the grazing animal tends to have a higher concentration of nitrogen and a lower C:N ratio than senescent material (Whitehead, 1995). According to Tracy and Frank (1999), induced plant responses to herbivory and grazing events are likely to change the composition of shoots, roots and plant exudates, presenting a different substrate profile for microbial attack. Tracy and Frank (1999) hypothesised that microbial populations in grazed grassland are sustained mainly by inputs of labile carbon from dung and by the increased root turnover or root exudation beneath grazed plants. Although grazed pasture may present higher levels of organic matter to soil heterotrophs, little is known really about how dung and plant responses to herbivory affect either the soil biota or their nutrient turnover processes (Lovell and Jarvis, 1996; Bardgett et al., 1998; Tracy and Frank, 1999). Studies of grazing event effects on denitrification in the field have just begun (Luo et al., 1999 a & b).

As an overall effect of grazing, recent work by Haynes (2000) has shown that the water-soluble labile carbon content ranged from lower values in arable soils to higher values in grazed pastures. Haynes (2000) attributed this to the build-up of organic matter under grazing, and to losses incurred under arable conditions, and to the fact that the organic matter returned to soil under arable conditions is much less than that returned to pasture soils.

It is likely that the effects of farm management, soil physical and chemical environment, and botanical composition each influence mineralisation through the varying provisions of carbon and nitrogen. However, the quality of inputs and the flux of nitrogen in the system must be taken into account if the effects of carbon and nitrogen on mineralisation processes are to be understood from a managed grassland viewpoint.

Materials and methods

The following methods were chosen having considered soil biological analysis.

5.3.1 Introduction

As described in preceding sections, components involved in the biology of nitrogen and carbon transformations have been examined in depth. In the present study, management of each sampled field was documented in detail so that the nutrient and grazing influences could be taken into account, as recommended (Bardgett and Leemans, 1996). Temporal variation between the sixteen study farms was minimised by sampling over a relatively short time span in summertime. Samples stored for biological analysis were deep-frozen to minimise biological activities and accompanying changes in the soil samples prior to laboratory analyses.

Soils were analysed in a field-moist condition without sieving so as to reduce disturbance and reduce the possibility of aeration induced oxidation losses in either soil organic matter or activity. Roots were retained so as to include rhizosphere soil.

Simple methods were applied as much as possible to reduce cost and to maintain clarity for interpretation purposes.

5.3.2 Farm selection

Sixteen study farms were selected, as described in Section 2.3.1.

5.3.3 Soil sampling for biological analysis

Farms were visited in the summer of 1995 and ten soil samples were removed from the selected field on each farm in a random manner avoiding gateways and paths. Intact soil core samples were taken from 0-100 mm depth using a hand trowel. The trowel was inserted into the soil three to four times to loosen a clod of at least 10 cm diameter, and 6-10 cm in depth, and each clod was extracted and placed in an individual plastic bag. Clods were numbered 1-10 in the order of sampling from each field. Additional samples were taken on the same day, within a half-metre radius of samples taken for biological analysis, for soil physical and chemical analyses (Chapter 3).

5.3.4 Storage of soil samples for biological analysis

On return to the laboratory, the clod samples were sealed in plastic Ziplok[®] bags and stored at -16 to -18°C. Later, five of the deep-frozen clods, numbered 2, 4, 6, 8, and 10 in their order of field sampling, were selected for biological analysis. The remaining five clods were maintained at -16 to -18°C as reserve material.

5.3.5 Preparation of soil samples for biological analysis

Prior to analysis, five clods were allowed to defrost for 24 hours in their sealed Ziplok[®] bags at room temperature. Once defrosted, soil from a depth of 30-50 mm from each clod was cut away from the clod, bulked and homogenised in a Waring blender for five minutes to achieve homogeneity prior to sub-sampling. This bulked homogenised field-moist soil was used for the analysis of all biological parameters. When necessary, the homogenised soil was sealed in a further Ziplok[®] bag and stored at -16 to -18°C between analytical procedures.

5.3.6. Estimation of soil dry weight equivalent

Results of biological analyses were expressed per gram equivalent oven dried soil (g^{-1} ds), and per gram equivalent field moist soil, referred to as g^{-1} s or simply g^{-1} for study purposes. Dry weight equivalent measures were implemented so as to conform to the soil biology literature. However, as it was found that relationships between other study parameters and certain biological values expressed per field moist and dry weight equivalent differed in this particular study, moist weight values were of interest.

Measured 50 g quantities of field-moist homogenised soil were dried for 24 hours at 104°C, and their content of soil moisture recorded. Soil moisture content was expressed as a percentage of the field-moist sample. Biological analyses were invariably carried out on sub-sampled field-moist soil and results were expressed both per gram of field-moist soil g^{-1} s and per gram equivalent oven-dried soil, g^{-1} ds, for ease of comparison with the soil biology literature.

5.3.7 Parameters measured

Each of the measured parameters has been introduced and the basis for their measurement has been considered in previous sections.

5.3.7.1 Microbial biomass

Microbial biomass was indirectly evaluated using two methods. The nitrogen present in microbial cell components was measured as the ninhydrin reactive nitrogen released from lysed cells during chloroform fumigation (Section 5.3.6.1.1). Ninhydrin reactive nitrogen then represents the chloroform-sensitive nitrogenous component, and probably includes the majority of microbial cell material, whether inactive or active (Dilly and Munch, 1998; Lovell and Jarvis, 1998), whether heterotrophic or other in terms of their metabolism. The substrate-induced respiration rate was measured as the carbon dioxide released during incubation of soil amended with aqueous glucose. Substrate-induced respiration values represent the active, glucose-responsive portion of the microbial biomass (Lovell and Jarvis, 1998).

5.3.7.1.1 Ninhydrin reactive nitrogen

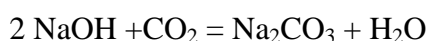
The methods of Amato and Ladd (1988) and Rowell (1994) were adapted significantly and the simpler protocol is described here. Bulk homogenised soil was defrosted for 24 hours at room temperature. Duplicate 10 g samples were placed in 25 ml screw-capped glass bottles. In a one-step fumigation procedure, 2 ml of chloroform was added to one flask and both were closed and incubated at room temperature for 10 days. Samples were then transferred to 250 ml flasks for extraction with 50 ml of 2 mol KCl, and extracts were filtered through Whatman No. 1 filter paper. A 2 ml aliquot of the filtrate was mixed with 1 ml of ninhydrin reagent, heated in a boiling water bath for 25 minutes, cooled and mixed with 2 ml of a solution containing ethanol and water in equal proportions. Absorbance was read at 570 nm using 1 cm light path cells. The concentration of ninhydrin-reactive nitrogen was estimated as $\mu\text{g N ml}^{-1}$ with reference to L-leucine standards (Rowell, 1994). The total extracted ninhydrin-reactive nitrogen released during fumigation was calculated as follows to give the ninhydrin-reactive nitrogen released on fumigation:

$(50 \text{ ml} + \text{moisture in } 10 \text{ g sample}) \times (\text{fumigated-non fumigated } \mu\text{g N ml}^{-1})$ was divided by the equivalent weight of oven-dried soil in the 10 g sample, and expressed as the number of micrograms of nitrogen per gram dry soil, $\mu\text{g N g}^{-1} \text{ ds}$.

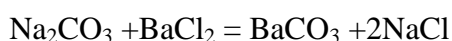
5.3.7.1.2 Substrate-induced respiration

The measurement of the respiration rate of soil following amendment with aqueous glucose was adapted from Rowell (1994). Bulk soil that had been homogenised as described in Section 5.3.5 and stored deep-frozen in the interim between analyses was defrosted at room temperature for 4 hours. Duplicate 50 g samples were placed in 250 ml conical flasks. Samples of sand each weighing 10 g were distributed to two control flasks. The soil samples were amended with 2 mg ml⁻¹ aqueous glucose at a rate of 2 ml glucose solution per gram equivalent dry weight of soil. A vial containing 5 ml of 0.3 mol NaOH was suspended with thread in the flask headspace and flasks remained open to the air for 30 minutes after which they were sealed with Parafilm[®]. Flasks were incubated at ambient temperature in the laboratory in the dark for two weeks with daily opening to the air for 2 minutes to prevent progression to anaerobic conditions. After 14 days incubation, the NaOH from each vial was emptied into a 250 ml conical flask and the vial rinsed into the conical flask with 10 ml distilled water. The solution was mixed with 10 ml of 1 mol BaCl₂ solution and 6 drops of phenolphthalein indicator and was titrated with 0.1 mol HCl. A colour change from red to colourless was regarded as the end point.

The analysis of respiration rate is based on the following principles. Two sodium hydroxide molecules react with each carbon dioxide to give sodium carbonate and water as in the equation:



Barium chloride precipitates the carbonates from the solution as follows:



In a subsequent titration with 0.1 mol HCl, any sodium hydroxide remaining in the flasks reacts with molar acid equivalents. Respired CO₂ decreases the amount of sodium hydroxide available for titration in the test sample compared to controls. The respiration rate was calculated as follows: The titre obtained for a test sample was subtracted from the average of two control values and the number of mol of sodium hydroxide was calculated. As 2 mol NaOH is equivalent to 1 mol CO₂, the concentration of remaining NaOH is halved and then multiplied by the molar mass of carbon dioxide, 44 g, to quantify the carbon dioxide released from the 50 g of soil in grams. This value is

divided by the duration of incubation in hours, and the equivalent oven dry weight of the soil (ds), and the respiration rate is expressed as $\mu\text{g CO}_2 \text{ g}^{-1} \text{ ds h}^{-1}$.

5.3.7.2 Laboratory estimation of viable bacterial populations using the plate count method

Bulked homogenised moist soil was prepared as described (Section 5.3.4) and 10 g were measured into 100 ml sterile water in a 200 ml screw capped bottle. Shaking the mixture up and down by hand through a 30 cm arc vigorously for five minutes dispersed the soil particles (Case and Johnson, 1984). Subsequent serial dilution was carried out to 10^{-6} using 1 ml sterile pipettes and transferring 1ml aliquots into 9 ml sterile tap water. Each dilution was shaken for a few moments on a Rotamixer De Luxe rotary tube shaker and plated immediately as 0.1 ml surface aliquots spread with a sterile Drigalsky glass stick. The Drigalsky stick was methanol-sterilised and flamed between each application. This spread plate method was used throughout.

Media was prepared and sterilised as directed by the manufacturer, with amendments as described. The media, temperatures and duration of incubation are described for the total and functional bacterial cfu counts below.

5.3.7.2.1 Numbers of bacterial cfu

Nutrient agar (Oxoid[®] CM3) plates were prepared and were spread plated as described above (Section 5.3.7.2) and incubated in the dark at 25°C for two days. All visible colonies were counted and expressed as bacterial cfu per gram equivalent oven-dried soil.

5.3.7.2.2 Numbers of bacterial cfu with protease activity

Nutrient Agar (Oxoid[®] CM3) was prepared containing 2 percent skim milk powder, which was autoclaved separately at 121°C for 5min and gently mixed into the somewhat cooled nutrient agar at 45-50°C prior to pouring. Plates were spread plated as described (Section 5.3.7.2). Following incubation at 25°C for two days, colonies surrounded by clear zones where proteolysis had occurred were counted and calculated and expressed as the number of cfu bacteria with protease activity per gram equivalent oven-dried soil.

5.3.7.2.3 Number of bacterial cfu releasing ammonia from arginine

Media containing in g l⁻¹: Agar Technical, 15; K₂HPO₄, 7.0; KH₂PO₄, 2.0; trisodium citrate, 0.5; glucose, 1.0; MgSO₄, 0.1, and L-Arginine 1.0 was prepared, sterilised, spread plated (Section 5.3.7.2) and incubated at 30°C for 5-7 days. A loop-full of each resultant colony was transferred to a porcelain tile and tested with Nessler's reagent for ammonia. Numbers of ammonia-releasing colonies were calculated and expressed as cfu releasing ammonia from arginine per gram equivalent oven-dried soil.

5.3.7.2.4 Numbers of bacterial cfu with urease activity

Urea agar base (Oxoid[®] CM651) was prepared with urea added to 2 percent. Plates were spread plated as described (Section 5.3.7.2) and incubated in the dark at 25°C for 5-7 days. Deep pink coloured colonies were counted and expressed as numbers of cfu bacteria with urease activity per gram equivalent oven-dried soil.

5.3.7.2.5 Numbers of bacterial cfu with exopolysaccharide activity

Sugar-rich Czapek Dox agar base plates were spread plated as described (Section 5.3.7.2) and incubated at 25°C for 5 days. Muroid (gummy) colonies assumed to have synthesised and accumulated extracellular polysaccharides were counted as cfu bacteria with exopolysaccharide production capability per gram equivalent oven-dried soil.

5.3.7.2.6 Numbers of bacterial cfu with cellulase activity

Nutrient agar (Oxoid[®] CM3) was prepared with 1 percent carboxymethylcellulose dissolved by boiling prior to the addition of nutrient agar powder. Plates were spread plated as described (Section 5.3.7.2) and incubated at 25°C for two days and then flooded with iodine solution (1 g of Iodine crystals; 2g of Potassium Iodide dissolved in 300ml distilled water and left for 24 hours before use). Colonies surrounded by paler brown halos where cellulose digestion had occurred were counted as numbers of cfu bacteria with cellulolytic capability per gram equivalent oven-dried soil.

5.3.7.2.7 Numbers of bacterial cfu with starch hydrolysis activity

Nutrient agar (Oxoid[®] CM3) was prepared containing 2 percent starch, which had been dissolved by boiling prior to the addition of nutrient agar powder. Plates were spread plated as described in Section 5.3.7.1, and incubated at 25°C for two days and then

flooded with iodine solution (1 g of Iodine crystals; 2g of Potassium Iodide dissolved in 300ml distilled water and left for 24 hours before use). Colonies surrounded by clear halos indicating that the hydrolysis of starch had occurred were counted as numbers of cfu bacteria with amylolytic capability per gram equivalent oven-dried soil.

5.3.7.3 Microbial activity as arginine ammonification

Homogenised bulk soil was defrosted at room temperature for 24 hours. The method of Alef (1995b) was followed, with modifications. Six 2 g sub-samples of each soil were placed in individual 12 ml volume centrifugation tubes, capped, and incubated in a water bath at 30°C for 15 minutes to equilibrate. Then, 0.5 ml of a 0.125% arginine solution was added drop-wise to 3 samples that were incubated for 3 hours at 30°C. Meanwhile, 0.5 ml of distilled water was added to the remaining three tubes, which were immediately immersed in liquid nitrogen, therefore forestalling ammonification in the controls. After three hours, 8mls of 2 mol KCl were added to each of the tubes, which were shaken for 30 minutes to extract ammonia adsorbed on the soil particles. Soil particles were precipitated out by centrifugation at 2500 rpm for 7 minutes, leaving a clear supernatant, 0.5 ml of which was mixed with 1.5 ml 2 mol KCl, 1ml phenolate solution (2% sodium phenoxide tetra-hydrate), 0.5 ml nitroprusside solution (0.005% nitroprusside) and 0.5 ml NaOH/NaOCl solution (25 ml NaOCl, containing at least 15% active Cl₂, in 0.125 mol NaOH). Samples were incubated at 30°C for 30 minutes to allow the reaction to proceed and colour to develop. The absorption was measured at 630 nm against a 2 mol KCl blank. Ammonium standard solution (0.00072 mol NH₄Cl) equivalent to 10µg NH₄-N ml⁻¹ was placed in volumetric flasks in 0, 2.5, 5.0, 12.5, 20, 30, 40 and 50 ml quantities and were brought to 50 ml volume with 2 mol KCl, corresponding to 0, 0.5, 1.0, 2.5, 4.0, 6.0, 8.0 and 10.0 µg NH₄-N ml⁻¹. Quantities (0.5 ml) of ammonia solutions were mixed with 1.5 ml KCl, 1ml phenolate solution, 0.5ml nitroprusside solution, and 0.5ml NaOH/NaOCl solutions, incubated at 30°C in a water bath for 30 minutes, and the absorbance of the solutions were read at 630 nm against a 2 mol KCl blank. A calibration curve was plotted.

Absorbance values obtained for the controls were subtracted from those of the test samples to correct for existing ammonia in soils prior to incubation. The ammonia released per gram of soil per hour is calculated via the following equation:

$$\text{NH}_4\text{-N } (\mu\text{g g}^{-1} \text{ h}^{-1}) = \frac{\text{NH}_4\text{-N } (\mu\text{g ml}^{-1}) \times 4.25}{T \times 2}$$

where T is the incubation time in hours, 4.25 the total volume of solution added, and 2 represents the moist weight of the soil (s) used. Results were expressed as micrograms of ammonia nitrogen released per gram equivalent moist soil (not annotated) per hour as well as per gram equivalent oven-dried soil (annotated ds) per hour.

5.3.7.4 Urease enzyme activity

Urease enzymes catalyse the hydrolysis of urea to CO₂ and NH₃. In this study, urease activity was assayed by titration of the ammonia released from urea-amended soil. The methods of Klein and Koths (1980) and UCG (1970) were adapted and the modified method is described below. Urease enzyme activity in soils is strongly associated with soil organic matter but is not necessarily cell-associated. Toluene was included in the incubation to inhibit cellular activity so that non-cellular urease activity could be estimated.

Bulked, homogenised soil that had been stored at -16°C was defrosted at room temperature for 24 hours prior to sub-sampling and analysis. Triplicate samples of 10 g field- moist soil were placed in 100 ml flasks. 1.5 ml of 0.2 mol urea, 9ml distilled water and 0.9 ml toluene, were added to each flask and the contents mixed by swirling momentarily. The flasks were plugged with cotton wool and incubated for 4 hours at 30°C. 0.1 ml of 1% mercuric chloride was added to inhibit enzymatic activity and ammonia was extracted with 2 mol KCl (8 ml). The extract was filtered through Whatman No. 1 filter paper. Then, 5-6 drops of universal pH indicator was added to the filtrate and ammonia was determined by titration with 0.05 mol HCl (UCG 1970). A colour change from green to pink was noted as the end point. The initial titration value was discounted and an average result obtained from two replicates. Results were calculated on the basis of molar equivalents and expressed as micrograms of ammonia nitrogen released per gram moist soil and per equivalent oven-dried soil per hour.

5.3.8 Statistical analysis

Descriptive statistics were analysed as described in Section 2.3.5.1, and Spearman's rank correlation coefficients and their significance determined as in Section 2.3.5.3.

5.4 Results and discussion

Particular aspects of the soil biology, namely microbial biomass, populations, activities, and soil enzymes were examined and found to differ considerably amongst the sixteen study farms. Results obtained for biological analyses of soils from the sixteen study farms are summarised here in Chapter 5, and details for each farm are given in Appendix D, in the data spreadsheet. Significant relationships with farm management, soil conditions, botanical and biological variables are considered in the light of similar research.

5.4.1 Microbial biomass

The active aspects of the microbial biomass capable of metabolising glucose were measured as substrate-induced respiration (SIR). Ninhydrin reactive nitrogen was extracted and measured following chloroform fumigation (NRN) to represent nitrogenous material stored in the chloroform-sensitive microbial biomass. Although controversy surrounds the coefficients used for extrapolation of biomass values (Rowell, 1994), the likely microbial biomass carbon SMBC and biomass nitrogen SMBN contents were derived, for comparison purposes. Values for microbial biomass estimated as SIR, NRN, as well as the derived soil microbial biomass carbon, nitrogen and microbial biomass C:N ratio values, are detailed for each farm in Appendix D.

Table 5.1 Microbial biomass related values

Microbial biomass estimated indirectly as the ninhydrin-reactive nitrogen released following chloroform fumigation NRN and as the rate of carbon dioxide released during substrate-induced respiration SIR.

	Min.	Max.	Mean	SD
^a NRN, $\mu\text{g N (g}^{-1}\text{ds)}$	1.3	54.0	37.0	14.4
^b SIR, $\mu\text{g CO}_2 \text{(g}^{-1} \text{ds h}^{-1})$	32.8	177.0	122.0	37.7
^c Biomass C:N (ratio), n=14	4.15	7.4	5.7	1.1
Microbial biomass C as percent of soil organic C (%), n = 15	0.41	2.90	1.71	0.67

^aNRN may be multiplied by 4.6 to estimate SMBN (Rowell, 1994).

^bSIR may be multiplied by 8.15 to estimate SMBC (Lin and Brookes, 1999).

^c Biomass C:N values were summarised here without farms E and F, values for which were beyond the norm.

Ninhydrin reactive nitrogen, and substrate induced respiration rates have not been reported for similar Irish soils. Although differences in procedures and soil conditions preclude direct comparison with soils elsewhere, key research papers are used as a basis for discussion. For example, similar biomass-related variables have been studied on sandy soils but using different analytical procedures in arable rather than pasture soils (Lin and Brookes, 1999 a, b & c). Cropping history affects soil microbial biomass content and pasture soils probably have higher SMBC levels (Smith and Paul, 1990). However as the research of Lin and Brookes (1999 a, b & c) includes one of the most recent appraisals of microbial biomass and activity measurements, it is used as a basis for comparison. In addition, the study of Lovell et al. (1995) is commented on, in which biomass was evaluated in long-term fifty-year old pasture. However as soils in their study: contained at least 36% clay; were very poorly drained; and were regularly waterlogged throughout the winter, they differ considerably from the well-drained sandy loam soils in this study. In a further contrast, pasture plots were cut (Lovell et al. 1995) rather than exposed to grazing animals, and presumably differed in organic matter returns.

Substrate-induced respiration rate in the study farm soils was substantially higher than basal respiration rates reported elsewhere. Basal respiration rates of 22-118 $\mu\text{g CO}_2 \text{g}^{-1} \text{h}^{-1}$ were reported by Lin and Brookes (1999a), and 0.9-2.6 $\mu\text{g CO}_2 \text{-C g}^{-1} \text{h}^{-1}$ reported

by Lovell et al. (1995). A coefficient of 8.15 (Lin and Brookes, 1999 c) was applied to SIR values whereby the mean microbial biomass carbon content of $994 \mu\text{g g}^{-1}\text{ds h}^{-1}$ was arrived at for the study farm soils. On that basis, microbial biomass C content is relatively high compared to levels observed elsewhere. Sandy to clay soils studied by Lin and Brookes, (1999a, b and c) ranged from approximately 20 to $1150 \text{ SMBC } \mu\text{g g}^{-1}$ soil based fumigation extraction, however the mean value was not reported in their analysis. Elsewhere, biomass C ranged from 98 to $754 \mu\text{g g}^{-1}$ in arable and pasture soils based on a different fumigation incubation method (Carter, 1991). Lovell et al. (1995) report a range of 1009 to $1716 \mu\text{g g}^{-1}$ soil microbial biomass C values for long-term pasture, but in clay soils. Overall, SMBC contents of study farm soils appear to be slightly higher than in similar soil types, but lower than values elsewhere for soils of higher clay contents. This difference is not surprising, as clay content is known to have protective organic matter effects (Brady 1990; Franzluebbers et al., 1996), and organic matter, including microbial biomass, might be expected to attain higher values under higher clay content conditions.

Unlike much of the literature (Alef and Kleiner, 1987; Carter, 1991; Lin and Brookes, 1999; Haynes and Tregurtha, 1999) no correlation was found between either SIR or NRN biomass-related values and soil organic carbon content. However the organic carbon contents of soils here (mean 6%, maximum 9.61%, Section 3.4.4.1) were substantially higher than in some studies mentioned (Alef and Kleiner, 1987; Haynes and Tregurtha, 1999; Lin and Brookes, 1999a). Organic carbon content of soils studied by Carter (1991) was not reported. The fact that significant reductions in biomass-related values are reported elsewhere, at concentrations of organic carbon below certain values, above which values relationships with organic carbon content were either confused or insignificant (Alef and Kleiner, 1987; Haynes and Tregurtha, 1999; Lin and Brookes, 1999), suggests that biomass size and activity is limited by lower organic carbon contents in the soils examined in those particular studies. In contrast, the lack of correlation observed between microbial biomass values, which were measured in soils of relatively higher soil organic carbon content in the sixteen farms used in the present study, may mean that at higher levels of soil organic carbon content, soil organic carbon content is not a biomass-limiting factor.

Carbon storage inefficiencies as warned by Wardle (1994), and biomass reduction, have been observed, for example, in response to mineral fertilisers (Bolton et al., 1985; Lovell et al., 1995; Gunapala et al., 1998; Lin and Brookes, 1999b). In this study, organic carbon contents ($r = 0.581$, $P < 0.05$) were higher and total microbial biomass values increased according to the intervals since mineral nitrogen had been applied on the study farm soils ($r = 0.585$, $P < 0.05$). Generally, the higher microbial biomass values and organic carbon contents found here seems to reflect relatively low mineral nitrogen input levels applied to the study farms as reported in Chapter 2. However, microbial biomass may have recovered in the interim without mineral N applications, although no specific correlation was established between NRN and mineral nitrogen applied in the four years prior to sampling. In addition to the mineral nitrogen applied on each of the three farms, organic manure had been applied at different rates, and so any singular effects of mineral nitrogen cannot be ascertained. There may have been cushioning effects of manures as found by Gunapala et al., (1998), however this cannot be ascertained from current data.

Research has also shown that mineral and organic amendments differ in effect, and that organic material may buffer against the mineral nitrogen effect which otherwise appears to incur organic matter loss, and to inhibit methane oxidation and storage in soils (Willison et al., 1996). Whether organic inputs buffer against biomass loss is not clear in the present study, as all three farms adding mineral nitrogen had added organic matter as well. Henriksen and Breland (1999) suggested that mineral nitrogen encourages carbon mineralisation in soils, and that labile carbon accumulates in the absence of mineral nitrogen. Reduced decomposition rates may support the accumulation of biomass and soil polysaccharides as observed in the present study in the interval since mineral nitrogen had been applied to the study farm soils, and discussed in Chapter 3. In addition, total biomass or NRN values increased relative to soil polysaccharides ($r = 0.550$, $P < 0.05$), suggesting that available carbon substrates might facilitate biomass development and create the particular biomass-cushioning effect of organic manures as observed by Willison et al. (1996).

The correlation in the present study with fertiliser history spanning eight years, rather than with additions in the four most recent years prior to sampling is perhaps because it may take time for the effects of fertilisers to be observed in the biomass (Lovell et al.,

1995). Effects of either of fertiliser treatment withdrawal or new applications were not evident during a one-year trial although biomass was significantly higher in soils that had had no past fertiliser history (Lovell et al., 1995). On average, biomass C and N were at least 60% higher in the unfertilised treatments than in fertilised swards (Lovell et al., 1995). In an elaboration of earlier work, Lovell and Jarvis (1998) showed that mineral nitrogen inputs reduced the inactive biomass fraction, however the active fraction did not reflect nitrogen input history. Similar results were observed in the present study, as NRN values that include the active and inactive biomass fractions were correlated with mineral nitrogen history, and increased with the interval since mineral nitrogen applications, whereas the active biomass fraction values for SIR were not significantly correlated with mineral fertilisers. The inactive microbial biomass may consist largely of non-viable non-responsive cells, in contrast to the glucose-responsive cells measured in the SIR analysis. Differences in the active and inactive biomass fractions may account for the delay described above.

Additional relationships have emerged. The active biomass fraction was significantly correlated with root mass ($r = 0.592$, $P < 0.05$). Again, interactions with fertiliser inputs are likely because root mass values were significantly lower in N and P fertilised swards in this study (see Section 3.3.4.1). It has been proposed by other researchers that reduced biomass occurs because of lower root mass presence and because of lower C content found in N-fertilised swards (Lovell et al., 1995). As O'Sullivan (1968) noted in the study of County Limerick grasslands in 1968, "when lime or fertilisers or both are spread on the poorer grasslands, the decay of the old sward and root mat is accelerated". Whilst O'Sullivan (1968) may have been referring particularly to a decline in botanical diversity of the sward, the softening effect of fertiliser on the grass root mass to which he refers is probably induced by ready nitrogen availability, leading to organic matter turnover. Available carbon is likely to be the link between root mass and biomass according to Lovell et al. (1995), biomass being limited if carbon is insufficient for its development.

In the present study, the root mass, polysaccharides and biomass may be interrelated through carbon availability in the rhizosphere, as influenced by mineral nitrogen inputs, such that loss of organic carbon, microbial biomass and root mass may have been accelerated in nitrogen-fertilised soils. Levels of NRN also increased with the C:N ratio

of the soil ($r = 0.657$, $P < 0.05$). Additional correlations with soil organic matter, examined later, show that the relationship between biomass and soil organic carbon is quite complex.

Neither NRN nor SIR values were significantly linked with soil pH in the sixteen study farm soils investigated in this present study. Lack of correlation between microbial biomass measurements and soil pH have been observed elsewhere (Lin and Brookes, 1999b). It is interesting that the dormant or quiescent microbial biomass components, which are hidden in both NRN and SIR measurements, did not reflect current soil conditions in the sixteen farms. In the study of grazed and ungrazed hill grasslands conducted by Bardgett et al. (1997), a negative correlation between basal respiration rate and soil pH was noted. However soil pH values were lower, at 4.4 and 5.4 (Bardgett et al., 1997), than encountered generally amongst the sixteen farms in the present study (Section 2.4.1). Researchers sometimes interpret the lack of correlation with organic carbon as being pH related, because the high organic carbon content was noted in an acidic soil (Lin and Brookes 1999b). However soil pH was unrelated to microbial biomass in the present study. Data from the present study suggests, instead, that because biomass is a fraction of soil organic matter, it is inherently reduced at lower organic carbon contents. At higher, presumably non-limiting, organic carbon contents, possibly above 5-6%, biomass content may depend instead on factors other than soil carbon content. In addition, the inactive biomass may not reflect current soil conditions such as pH, but because of quiescence, may be related instead to past management events.

Contrasting current and temporal responses are noteworthy in agriculture because they may help to differentiate between management conditions, and may act as signposts for short and long term soil responses to management change. As considered, soil microbial biomass values in the sixteen study farms seems unrelated to current soil pH but may have been influenced by fertiliser input history. Further work is needed to determine whether significant increases in biomass (NRN) values with intervals since fertiliser N applications is a temporal effect, similar to a time-dependent response of biomass to lime applications observed by Neale et al. (1997) and temporal differences in biomass in relation to fertiliser applications as observed by Lovell et al (1995).

Grazing management and other factors were associated with the active glucose-responsive microbial biomass. SIR rates increased with the duration of rest intervals in the grazing rotation ($r = 0.502$, $P < 0.05$), and relative to root mass ($r = 0.592$, $P < 0.05$), and decreased with the presence of *Cardamine pratensis* ($r = -0.529$, $P < 0.05$).

The increase in SIR with the length of rest intervals in the grazing rotation seems to contrast with a decline in microbial biomass and activity found in ungrazed hill grasslands (Bardgett et al., 1997), and with increased biomass in grazed pastures found by Tracy and Frank (1999). Bardgett et al. (1997) suggested that the decline in biomass and activity could be due to the removal of readily utilizable substrates in the form of sheep excreta and urine, and to changes in the quantity and quality of root exudates induced in the plant by the effects of grazing. However, Bardgett et al. (1997) excluded sheep from the *Agrostis-Festuca* and *Nardis* dominated upland pastures for a year prior to analysis, and rest intervals in the present study pastures ranged from zero in extensively grazed sites that were set stocked, to 72 days at most in summer-grazed lowland farms (See Appendix D for detailed grazing information). Although it is difficult to compare the studies directly, interactions between the grazing events and biomass observed in the study farm soils are probably related to substrates from damaged herbage, excreta and plant responses to herbivory in grazed pastures. Evidence from the present study that grazing, amongst other factors, influences microbial substrate provision is elaborated in Chapter 7.

Ratios of parameters may correspond more with soil conditions than absolute values (Dilly and Munch, 1998). Because of this possibility, ratios between variables, and their relative values expressed as a percentage, were explored from time to time, out of curiosity. When the microbial biomass carbon content was calculated as a percentage of soil organic carbon (Table 5.1) and used as a variable in rank correlation analysis, far more significant relationships were found than with either absolute value. It would appear that the larger the percentage of soil organic carbon contained in the microbial biomass, the lower the percentage water stable aggregates ($r = -0.876$, $P < 0.05$), for example. Water stable aggregate content already correlates with respiration rate ($r = -0.578$, $P < 0.05$) and with organic carbon content ($r = 0.806$, $P < 0.05$). Because microbial processes are known to increase soil stability through polysaccharide

secretion and hyphal extension (Richards, 1987; Brady, 1990; Coyne, 1999), and microbial activity reduces aggregate stability when polysaccharides are decomposed in soil (Swift, 1991), a negative correlation between the relative microbial biomass carbon content in soil with aggregate stability should be considered in more detail in future. The value for soil microbial biomass was extrapolated from the SIR figures, which represent glucose responsive biomass or the active microbial biomass fraction. A relatively more active microbial biomass may oxidise organic matter, detracting from the organic carbon required for aggregate stability, for example, as suggested by Swift (1991).

In addition, relative microbial biomass content calculated as the ratio between microbial biomass C and soil organic carbon content was significantly related to the numbers of livestock grazing in the year prior to sampling ($r = 0.653$, $P < 0.05$). In Chapter 3, a negative association between grazing intensity in a rotation and aggregate stability was noted. Grazing intensity is seen, later in this chapter, to correlate with the cellulase-producing microbial population, so perhaps grazing animals alter substrates available and indirectly affect aggregates through the substrate-activated biomass. The percentage content of soil organic carbon contained in the biomass was highest at low organic carbon contents, as indicated by the strongly negative correlation between these two variables ($r = -0.679$, $P > 0.01$). Such a relationship between one of the smaller organic matter pools, namely the microbial biomass carbon content, and soil organic carbon which presumably is the larger pool, is indicative of ceiling levels within soil organic matter, reminiscent of Russian dolls, where the size of successive containers is limited by the one above. Threshold levels of soil organic matter are strongly indicated by this observation, and by the absence of correlation between contained pools as found in this study compared with multiple correlations at lower soil organic carbon contents, discussed above.

In future, use of ratios may help to uncover how grazing animals could influence soil microbial biomass, organic matter, and soil structure in grassland.

SIR rates and NRN values corresponded significantly ($r = 0.580$, $P < 0.05$). Biomass values often related to each other as in earlier studies (Carter 1991; Lin and Brookes, 1999b), and a relationship is reasonable because the population capable of glucose use is a proportion of the entire biomass. However, the balance between active and dormant fractions probably depends on soil conditions at any time, as determined by factors such

as grazing. It seems that the microbial biomass is a reflection of nutrient inputs and grazing events, or management history, rather than soil activity potentials, because biomass was not correlated with either microbial populations or with cellular activities. However, as microbial biomass includes viable and non-viable cells, discrepancies between biomass, populations and activities are possible.

Relationships with the C:N ratio of the microbial biomass were calculated on the basis of all sixteen farms, and also by excluding farms E and F for which values were beyond the norm as their NRN contents were unusually low. On the basis of all farms, with the exception of farm Q for which soil C:N ratio values were not available, soil C:N ratio was negatively correlated with the microbial biomass C:N ratio ($r = -0.623$, $P < 0.05$). Correlation was also negative but not significant when farms E and F were excluded. The inverse relationship observed here, that is between the C:N ratios of soil and the soil microbial biomass that soil contains, suggests that microbial biomass carbon is least when organic carbon content is relatively high. This result is in agreement with the finding that the relative proportion of active microbial biomass (SIR) on which its microbial biomass carbon estimates are based, increases as soil organic carbon declines. It is difficult to interpret microbial biomass estimates, generally, because its absolute value cannot be confirmed using current methods (Richards, 1987) and coefficients for indirect estimation are in dispute (Rowell, 1994). However, observations in the present study suggest that active pools of organic matter change in proportion to soil organic matter content, and that the balance of soil resources influence both soil structure and soil biological function. The positive association between biomass, measured as NRN, and soil C:N ratio is further indication that a balance exists between soil organic matter pools, and that their proportions of C and N are interrelated.

Overall, carbon content in the study farm soils appears adequate to support the microbial biomass, which does not appear to be carbon-limited. Aspects of previous management history such as nutrient inputs probably influence biomass content. Furthermore, the active biomass component relative to soil organic matter is probably affected by grazing events. Management history is more closely reflected in the biomass than soil pH. Microbial biomass may also influence soil structure in a dynamic fashion, because aggregate stability was least in soils that had a greater proportion of active biomass relative to soil organic carbon content. Ratios between

absolute values should be explored as an additional means to describe organic matter transformation in soils. Factors such as fertiliser application and grazing histories influence biomass in specific ways and should always be taken into account in soil microbial biomass studies. Studies should be specifically constructed so as to provide an understanding of the effects of mineral nitrogen additions on the overall microbial biomass because of its contribution to soil nutrient pools. The effects of fertiliser nitrogen on microbial biomass deserve further scrutiny, because if biomass loss is incurred, as results imply, then biomass carbon, nitrogen, and phosphate will diminish with negative consequences in mineral-fertilised soils.

5.4.2 Bacterial populations, and their catabolic and biosynthetic capabilities

Bacterial populations for each of the study farms are described the data spreadsheet in Appendix D, and are summarised in Table 5.2 for the sixteen study farms. The results for general bacterial populations and those capable of particular substrate hydrolysis are summarised in Table 5.2. Catabolic function populations are listed after general bacterial populations in the order of their mean population size because relative population size may influence soil biological function (This is elaborated on in Chapter 7).

Table 5.2 Bacterial populations, their catabolic and biosynthetic capabilities

Total culturable bacteria and colony forming units displaying cellulase, amylase, protease production, urease and arginine ammonification and extracellular polysaccharide production activities *in vitro*.

Bacteria and respective functions (x 10 ⁵ cfu g ⁻¹ ds)	Min.	Max.	Mean	SD
Total bacteria on nutrient agar	8.4	784.0	261.7	288.5
Bacteria with cellulase	4.1	106.9	46.0	34.0
Bacteria with amylase	3.5	158.0	41.0	44.3
Urea ammonifiers	1.1	112.4	20.0	30.9
Arginine ammonifiers	0.3	16.1	7.1	4.8
Bacteria with protease	0.4	10.3	5.5	3.3
Extracellular polysaccharide producers	0.8	17.3	7.6	5.9

The bacterial populations (Table 5.2) are similar in range to those described for other soils in the literature. For example, researchers have reported bacterial cfu of 37×10^5 (Dodds et al., 1996) and $38 - 52 \times 10^5$ (Asmar et al., 1992), and total aerobic counts of $212-613 \times 10^5$ (Bolton et al., 1985).

Total bacterial populations were significantly higher at higher particle density values ($r = 0.568$, $P < 0.05$) and lower in relation to organic carbon content ($r = -0.585$, $P < 0.05$). The latter finding is at variance with the results of Lovell et al. (1995), who found higher bacterial counts at higher levels of organic matter. Greater numbers of bacteria were associated with higher *Juncus* spp. presence ($r = 0.537$). This correlation was originally deemed significant ($P < 0.05$) on the basis of sixteen farm values, but because *Juncus* spp. were found on only two farms, the statistical significance of their relationship with other variables cannot be established, as discussed in Chapter 4.

In this particular study, the larger bacterial population was generally found on nutrient agar than was enumerated for any particular functional subgroup. This makes sense, because the total count of bacterial cfu would be expected to include, and therefore be larger than, hydrolytic subgroups.

Microbial populations in the study farm soils differed considerably in regard to their catabolic potentials. Mean numbers of organisms with the potential to initiate carbon mineralisation were greater than those with potential nitrogen mineralisation, decreasing according to substrates used, as follows: total bacterial population as measured on nutrient agar, > cellulose, > amylose, > urea, > arginine, > protein. Higher numbers of bacteria capable of using cellulose than arginine makes sense too, because energy provision is a determinant, and it is likely that carbonaceous sources are used for energy in preference to nitrogenous organic molecules (Cunin et al., 1986). The predominance of carbohydrate using organisms also reflects the 24:1 substrate C:N ratio characteristic of herbage detritus in grazed unfertilised grassland (Whitehead, 1995).

Generally, total bacterial numbers on each farm were larger than other groups. The ranking of catabolic populations was similar within and across farms, with total > cellulolytic > amylytic populations. Some exceptions occurred. Cellulolytic bacteria outnumbered the total bacterial population in farm B, amylytic bacteria and arginine

ammonifiers outnumbered the total population in farm S, and urea ammonifiers comprised the largest bacterial population in farm E. It seems illogical that smaller groups outnumbered total bacterial populations. However, as only four discrepancies were encountered from over one hundred triplicate plate count estimates, errors may be attributable to inaccuracies inherent in soil plate counts (Lorch et al., 1995; Tiunov and Scheu, 2000). Of course the plate count figures may represent the situation, because the particular substrates in the catabolic function media could have induced enzyme systems in bacteria extracted from soil, thereby encouraging the appearance numbers over and above those found on more general purpose nutrient agar media.

Numbers of cellulolytic bacteria found by Henriksen and Breland, (1999) were between 0.8 and $2.8 \times 10^6 \text{ g}^{-1} \text{ ds}$ in soils of differing nitrogen availabilities. Numbers in this study were far higher- from 4.1 to $108 \times 10^6 \text{ g}^{-1} \text{ ds}$. Henriksen and Breland (1999) had found that the numbers of cellulolytic bacteria increased with increasing nitrogen availability, measured as ammonium and nitrate concentrations in the sampled soils. Carbon mineralisation rates were also related to nitrogen availability and cellulolytic organisms in their study (Henriksen and Breland, 1999). Levels of available nitrogen were not established on the sixteen farms in the present study, and no apparent correlation existed here between total nitrogen content and cellulase producers. However, the numbers of cellulolytic bacteria correlated significantly with larger grazing animals ($r = 0.550$, $P < 0.05$). Bulkier animals may lead to greater amounts of maceration underfoot by treading, and the accompanying introduction of damaged herbage into soil would presumably encourage cellulose utilising organisms. Tracy and Frank (1999) have suggested that higher microbial biomass is associated with grazing because of increased organic matter availability. It may be that cellulose that is trodden into soil from damaged plant material, together with nitrogen inputs from excreta influence the soil microbial population dynamically in the present study. Contrasting relationships found between grazing and biomass in the literature (Bardgett et al., 1997; Tracy and Frank, 1999) and the present study suggest that grazing affects the microbial transformations of carbon and nitrogen in a complex fashion, and whether grazing by heavier animals promotes carbon mineralisation in grassland requires further investigation. Cellulase producers were associated also with particle density ($r = 0.660$, $P < 0.05$). Inverse correlation has already been noted between particle density and organic matter (Section 3.4.4.1). Whether the activity of cellulolytic organisms incurs organic carbon

losses and contributes to higher relative particle density should be investigated, because of the importance of soil organic matter in relation to soil structure and texture. Cellulase producers were significantly correlated with abundance of the botanical species *Poa pratensis* ($r = 0.722$, $P < 0.05$). Cellulase producers were correlated negatively with presence of *Plantago lanceolata* ($r = - 0.498$), however as this plant species was found on only four study farms, statistical significance of association with its presence cannot be established.

Amylase producers were reduced relative to clay content ($r = - 0.587$, $P < 0.05$). Because numbers of catabolic populations have generally not been examined in soils, comparisons are not available. It is possible, that like soil enzymes (Marzadori et al., 1998), amylose and other carbohydrate substrates are shielded by clay, and perhaps they are, like urease enzyme, less accessible to degradation there (Marzadori et al., 1998). Without corroborative evidence, it may be that amylase producers are denied access to their substrate in soils with higher clay contents.

Amylase producers were higher in shallower soils, as their numbers correlated negatively with the depth of the A-horizon ($r = - 0.796$, $P < 0.05$) and with depth to the underlying parent material ($r = - 0.587$, $P < 0.05$). Researchers have observed that biological activities decrease with soil depth because of changing soil conditions, notably substrate dilution (Whitehead, 1995; Abassi and Adams, 1998). However, microbial numbers and activities were measured for the sixteen farms in a single bulked sample spanning from 30-50 mm depth of soil, and measurements were not made for samples at different soil depths. Depth measurements in the present study therefore relate to the amount of soil on site, either in the topmost horizon, or to the underlying parent material. In this study, any relationship with soil depth is probably substrate related, as substrates are diluted with soil depth (Whitehead, 1995), and would be expected to be more concentrated in relatively shallower and or drainage-impeded soils. Dodds et al. (1996) found that plate counts did not relate to soil depth, in that research, sampling depth was the focus and the depth of soil to parent material at each site was not considered. No references were available to substantiate site-depth related effects on microbial populations; however, amylase producers were also more numerous at higher altitudes ($r = 0.726$, $P < 0.05$) and at higher soil polysaccharide content ($r = 0.591$, $P < 0.05$). Substrates, as in polysaccharides, are clearly influential. Whether the influence of

altitude is climatic, with decreased decomposition at higher elevations is open to question. However, altitude and depth are related, soils in this study being shallower at higher elevations (Section 3.2.4.2.1). The hypothesis that site depth influences substrate concentration could be checked either by substrate analysis across differing site depths of soil, or in the laboratory under controlled conditions. Other researchers have suggested that soil characteristics should be described on a per volume basis (Dilly and Munch, 1995), for ecological purposes, and to compare sites of differing bulk densities. As clarification, depth should be given as both sampling depth and site depth, so that the relationship to sampling depth and available soil volume could be distinguished as factors in the substrate-dependent processes.

Amylase producers are associated with the abundances of clover, ($r = 0.674, P < 0.01$), *Taraxacum officinale* ($r = 0.578, P < 0.05$); and *Poa annua* ($r = 0.537, P < 0.05$). Amylase producers were more numerous in botanically diverse grassland farms ($r = 0.546, P < 0.05$). Grayston et al. (1998) studied the selective influence of plant species on microbial diversity in the rhizosphere, and found that wheat had consistently higher populations of bacteria and fungi than all samples except for the clover rhizosphere in which bacterial counts exceeded all others. Soil type was not influential (Grayston et al., 1998). In addition, catabolism of six groups of carbon sources was greater in the rhizosphere communities from wheat, clover and ryegrass than from either bentgrass or sucrose-amended and unamended soils. This was attributed to the differential availability of carbon sources exuded by the plants in the crop rhizosphere. The authors, however, stated that possible effects of plant exudates needed further study (Grayston et al., 1998).

The large range of urea hydrolysing bacteria observed in the sixteen study farms correlated significantly with the number of animals present together on the discrete grazed area ($r = 0.552, P < 0.05$), which was calculated without reference to the grazed acreage. It is tempting to speculate that higher animal numbers disperse urea in a way that would provide substrate and stimulate the ureolytic population. However, the urea content of soil was not evaluated in the present study, and may have varied considerably according to grazing events, urine patch location, and so on, as suggested by studies of camp and non-camp soils elsewhere (Haynes and Williams, 1999). Urea had been added as a fertiliser to only one farm, Q, which had been conventionally managed. However

the number of animals contributing to urine output on the farm may be a contributing factor. The numbers of organisms capable of urea hydrolysis were greater on Farm E than were any other microbial populations in that particular soil, including total enumerated bacteria. Urea ammonifiers were more numerous with increased clover content in the sward ($r = 0.518$, $P < 0.05$). The response to substrate, either as urea from animals or higher nitrogen availability from clover, is a possible basis for both relationships.

The numbers of arginine ammonifiers correlated significantly with the ratio of grazed-to-rested days in a rotation ($r = -0.526$, $P < 0.05$), and with the number of days grazed in the year prior to soil sampling ($r = -0.574$, $P < 0.05$). Continuity of grazing seems to have negative influence on this particular type of bacterial population. Grazing animals recycle relatively labile forms of both carbon and nitrogen in their excreta, which usually enhance microbial activities there (Abassi and Adams, 1998). Since it appears that the absence, rather than presence, of grazing animals stimulates arginine ammonifying populations, it may be that arginine use occurs when substrates diminish. This would concur with the population densities, cellulolytic organisms being higher in number than arginine-using populations in what are presumably more trodden and substrate-rich soils. Total grass species content was also associated with arginine ammonifiers ($r = 0.557$, $P < 0.05$). Reasons for this association are not understood, however responses to particular root exudates may be involved.

Proteolytic organisms were linked with liming, ($r = 0.548$, $P < 0.05$), which has been shown elsewhere to increase net nitrogen mineralisation initially (Neale, et al., 1997). In each soil studied by Neale et al. (1997), liming induced net nitrogen mineralisation over the first few days of incubation. The authors noted a ten-fold increase in bacterial numbers that remained evident after liming which, they suggested, had induced a proliferation of bacteria that had been either dormant or suppressed in the original acid soils. Proteolytic organisms were not enumerated specifically by Neale et al. (1997), but would initiate nitrogen mineralisation, and were increased in limed soils in the present study. Neale et al. (1997) reported that although the bacterial population increased ten-fold and numbers remained high after liming, nitrogen mineralisation activity decreased over time, returning to rates observed in the unlimed condition depending on soil factors. No correlation was found between protease producers and soil pH in the sixteen

study farms and, as before (Neale, et al., 1997), management history appeared to have more significant effect on the soil biomass and bacteria than do current soil conditions. However, changes in soil pH were not taken into account on that occasion (Neale et al., 1997). Mineralisation decreased after liming in the study conducted by Neale et al., (1997). However, unlike the quiescent microbiota, microbial activity is probably a response to prevailing soil conditions. It is likely that the microbial population can become quiescent when conditions change, when correlation between management-heightened bacterial populations and current soil pH is not expected.

Proteolytic organisms were positively associated with soil organic carbon ($r = 0.626$, $P < 0.05$) and with soil C:N ratio ($r = 0.546$, $P < 0.05$). Proteolytic organisms also appeared to be associated with soil magnesium content ($r = 0.544$, $P < 0.05$).

Magnesium limestone applied by Neale et al. (1997) increased net nitrogen mineralisation rates initially, with probable population changes. No reference was made to the magnesium content of their study soils, and changes in microbial populations and activities were attributed to the initial pH effects of added lime rather than to magnesium content. In the present study, an association with plant-available soil magnesium content is relevant.

Protease producers are more prevalent with stands containing *Ranunculus repens* ($r = 0.506$, $P < 0.05$), *Bellis perennis* ($r = 0.557$), and *Taraxacum officinale* ($r = 0.676$), although, statistical significance can be established only in the case of *Ranunculus* because of other species presence on relatively few farms. Botanical species diversity was associated with both protease producers ($r = 0.630$, $P < 0.05$) as were amylolytic organisms, mentioned above.

In seven farms, A, B, E, L, R, S, and T, extracellular polysaccharide producer numbers were lower than all other bacterial populations, whilst they outnumbered just the proteolytic populations and arginine and urea ammonifying organisms on most of the remaining farms. Lower extracellular polysaccharide producing populations than those with carbonaceous substrate hydrolysis is probably also related to substrate availability, as in general, extracellular polysaccharides are, like extracellular enzymes (Kay-Shoemaker et al., 1998), probably not synthesised unless spare energy supplies are available for storage. Extracellular polysaccharide producers may indicate carbonaceous

energy source abundance, however more information is needed to substantiate this claim.

There was a 200-fold difference in numbers of exopolysaccharide producing organisms across the study farms, numbers of which correlated with total bacterial counts ($r = 0.606$, $P < 0.05$), cellulase producing organisms ($r = 0.518$, $P < 0.05$), and amylase producing organisms ($r = 0.541$, $P < 0.05$). Relationships with cellulolytic populations are probably rooted in substrate provision, cellulose breakdown by another function of the microbiota providing a ready supply of substrate for exopolysaccharide producers. A similar explanation is likely for the relationship with amylolytic microorganisms, although it is likely that amylolytic microorganisms include exopolysaccharide producers, and / or avail of the substrate provided by the bacterial mucous in soils as well.

As mentioned earlier, biomass C:N values for farms E and F were in doubt because of being beyond the norm. Correlations with biomass C:N values were established with and without those farms. On the basis of fourteen values, correlation ($P < 0.05$) with extracellular polysaccharide producing populations of bacteria was slightly greater ($r = -0.551$) than with all sixteen values ($r = -0.507$). Extracellular polysaccharide producing bacteria may indicate available-carbon sufficiency, and the inverse correlation is in line with that observed earlier in the present study between soil organic carbon content and microbial biomass C:N ratio (Section 5.4.1).

Significant relationships between exopolysaccharide producers and altitude ($r = 0.626$, $P < 0.05$), and soil phosphate content ($r = 0.520$, $P < 0.05$) were found. The link between altitude and exopolysaccharide producers is possibly also through substrate provision in the immediate soil environment, because altitude is inversely associated with soil polysaccharide content ($r = 0.576$, $P < 0.05$, Chapter 3). Indeed, the higher amylolytic population at higher altitudes and in the environs of exopolysaccharide producers may explain why polysaccharide content itself doesn't correspond with the exopolysaccharide producer population, but does with the amylolytic population that probably avails of polysaccharide as a substrate.

In this study, a few interrelationships were found amongst soil biology measurements. For example, total numbers of cfu correlated positively with cellulolytic bacteria ($r =$

0.668, $P < 0.05$) and negatively with proteolytic bacteria ($r = - 0.538$, $P < 0.05$). In contrast, Asmar et al. (1992) found significant positive correlation between the total bacterial count and proteolytic activity; however, protease-producing organisms were not enumerated in that study. An inverse relationship between bacterial and proteolytic populations could exist in soil if proteolytic organisms reduce the viable population in soil by using the microbial biomass, active or otherwise, as a substrate for growth.

Few, if any, catabolic studies have analysed the soil microbial populations by plate count methods, probably because of inherent inaccuracies. Donnison et al. (2000) recorded catabolic diversity on the basis of the presence or absence of particular functions amongst microbial isolates from their sampled soils. They concluded that changes in microbial communities, in respect of their bacterial and fungal proportions, were related to changes in plant productivity and composition, or the form and quantity of fertiliser applied. However because microbial capabilities and plant species were noted only on a presence or absence basis, and fertiliser regimes were described only in broad terms, the study lacked quantitative information, and results cannot be satisfactorily compared with an enumerative analysis such as the present study.

Based on statistical analysis of the catabolic and biosynthetic populations in the study farm soils, many factors such as substrate availability, liming, grazing events and botanical species were found to be influential. Relationships between grazing events and microbial populations could be explained on the basis of energy source provision, from cellulose or polysaccharides. Similarly, Burket and Dick (1998) found that metabolic diversity corresponded with organic matter inputs in differing management systems, and concluded that management is readily reflected in the microbial biomass. Interestingly, and unlike the case with microbial biomass values (Section 5.3.7.2), no association was found between mineral fertiliser applications and microbial populations. Based on observations, it is proposed that organic substrate availability, in particular energy provision, is a major determinant of catabolic and biosynthetic populations of heterotrophs remaining in soil.

5.4.3 Microbial activity as arginine ammonification

As in the literature to date (Alef and Kleiner, 1987; Lin and Brookes, 1999b), microbial activity was measured *in vitro* as the rate of ammonia release from arginine-amended

soil samples. The arginine ammonification rates measured *in vitro* for the sixteen study-farm soils are described in Table 5.3. In an alternative interpretation opted for in the present study, positive values were regarded as representing nitrogen mineralisation rates, and negative values were regarded as nitrogen immobilisation. Both are given individual attention. The full results of arginine ammonification, N-mineralisation and N-immobilisation rates for each farm are available in the data spreadsheet in Appendix D.

Table 5.3 In vitro arginine ammonification interpreted as microbial activity and as the mineralisation and immobilisation of nitrogen

The rate of ammonia-nitrogen released per hour following arginine amendment, per gram moist soil per hour. Positive values have been interpreted as arginine mineralisation, and negative values as nitrogen immobilisation from soil reserves.

Arginine ammonification (NH ₄ -N, $\mu\text{g}^{-1}\text{s}^{-1}\text{h}^{-1}$)	Min.	Max.	Mean	SD
Arginine ammonification, n = 15	-2.15	17.07	4.07	5.77
^a Mineralisation, n = 10	0.89	17.07	6.56	5.55
^a Immobilisation, n = 5	0.00	2.15	0.31	0.597

^a Positive and negative values have been treated as individual activities.

A wide range of values for ammonia released per hour per gram of soil was encountered amongst the sixteen study farms. Overall arginine ammonification rates were not significantly correlated with farm management features, soil characteristics, biological or botanical variables.

Relationships have been reported between soil organic matter content and arginine ammonification rates, rates generally falling where soils contain less than 4.5 percent organic carbon according to Haynes and Tregurtha (1999). Linear decreases in arginine ammonification rates in soils ranging from 4.9 to 0.9 percent organic carbon content have been reported elsewhere (Alef and Kleiner, 1987). However, the sixteen soils examined in the present study had relatively higher organic carbon contents than described by Alef and Kleiner (1987), ranging from 3.2 to 9.6 with a mean value of 6.0 percent (Section 3.4.4, Table 3.15), and organic carbon content in this study bore no

significant relationship to overall ammonification values obtained *in vitro* for the sampled soils. It may be, as seen in Alef and Kleiner's (1987) report, that the linear relationship does not exist in soils above 5% organic carbon such as those which predominated in the current study. Lack of correlation between arginine ammonification rates and organic carbon content of the soils examined in the present study may also mean that organic carbon of the study farm soils is not limiting, as discussed in relation to microbial biomass estimates.

Researchers have also reported relationships between arginine ammonification rates and oxygen uptake following glucose addition, which failed to correlate when soils of 8.0% organic carbon contents were included (Alef and Kleiner, 1987). Others have found that correlation between arginine ammonification and biomass carbon, soil ATP content and substrate-induced respiration rate, failed to occur when soils of 6.65% organic carbon were included (Lin and Brookes, 1999b). No correlation was found between arginine ammonification rates and microbial biomass in a study of sewage-sludge amended soils although sewage amendment enhanced arginine ammonification by 8-12% (Dar, 1996). Both Alef and Kleiner (1987) and Lin and Brookes (1999b) report correlation between organic carbon content and arginine ammonification at organic carbon content values below 4.5-6.6 percent. Gunapala and Scow (1998) found higher levels of arginine ammonification under organic and low input management than under conventional management on most sampling dates, and arginine ammonification was substantially increased in the organic plots following the input of organic residues. The overwhelming conclusion drawn by Gunapala and Scow (1998) was that the amount of carbon entering the system was the most important factor differentiating the microbial communities. While not all researchers describe the relationship between arginine ammonification and soil organic carbon as a curvilinear one, most report the failure of arginine ammonification to correlate with both organic carbon and other variables at higher carbon backgrounds. In the present study, failure to find significant correlations ($P < 0.05$) with arginine ammonification may be due to the high content of soil organic carbon, around and above which relationships have failed in the literature. It is likely that at higher organic carbon contents, biomass is not limited as suggested in this thesis (Section 5.4.1). If biomass is not limited, arginine ammonification, which is carried out by the active biomass fraction, is likely to correspond with factors other than with either biomass or organic carbon instead.

Even though the arginine ammonification assay of microbial activity has been tried and tested since first advocated by Alef and Kleiner (1986b), negative values have been reported only recently by a few authors such as Lin and Brookes (1999b). A closer look at what might underlie ammonification rates is warranted because both negative and positive ammonification values were found in the present study, and because ammonification measurements arise from a combination of two key functions, mineralisation and immobilisation. So, unlike approaches found in the literature to date, a novel approach was adopted and the ammonification values were separated into component parts. Positive arginine ammonification values were interpreted as mineralisation, and negative values were regarded as immobilisation. When this distinction was applied, significant relationships between nitrogen mineralisation and immobilisation, farm management, soil conditions and other variables became apparent.

Arginine ammonification values were positive in farms, A, B, E, F, O, P, Q, R, S and T, indicating that mineralisation predominated in those farms. Mineralisation was significantly related to a number of key management features. Mineral nitrogen supplements in the four years prior to sampling were significantly associated with reduced *in vitro* mineralisation rates ($r = -0.683$, $P < 0.05$). This is in general agreement with the research of Gunapala and Scow (1998) who have reported significantly lower arginine ammonification values in farms under conventional rather than organic management.

Burket and Dick (1998) suggest that several mechanisms may be involved and that, for example, the addition of mineral nitrogen in the absence of organic inputs may cause more rapid oxidation of organic matter, which reduces the carbon pool available for the microbial biomass. They add that without sufficient carbon inputs, the microbial biomass will be depressed regardless of the nitrogen available. Lin and Brookes (1999b) found that soil that had received inorganic fertilisers had lower levels of arginine ammonification than soils with farmyard manure, which they thought reflected the smaller biomass in the former case. Even though no direct correlation between biomass and arginine ammonification was found to exist in the present study, it may still be that biomass size, lowered by carbon loss when mineral fertilisers are applied, might limit arginine ammonification in some way. While it is unlikely that soil organic carbon

content could have limited the overall size of the biomass fraction in the study farm soils, (as inferred from higher organic carbon content and lack of its correlation either with biomass or arginine ammonification values in contrast to other studies (see Sections 5.4.1 and this section) an increase in organic carbon content (Section 3.4.4.1), and in the microbial biomass measured as NRN (Section 5.4.1) has been associated with the interval elapsed since mineral nitrogen was applied to study farm soils, and biomass size, as part of the overall soil organic matter pool, may be a contributory factor.

Factors in addition to biomass size may intervene between mineral nitrogen provision and arginine ammonification. If, for example, mineral nitrogen activates carbon mineralisation (Henriksen and Breland, 1999), and if root matter is more rapidly turned over in fertilised soils (Lovell et al., 1995), labile carbon may be more readily available from root decomposition in fertilised soils. Catabolite repression and allosteric regulation of ammonification are likely to occur where carbonaceous energy sources are adequate (Cunin et al., 1986), and the presence of labile carbon sources released from accelerated root breakdown could account for reduced arginine ammonification rates in fertilised soils. This would explain the higher levels of arginine ammonification found in organically than in conventionally managed soils (Gunapala and Scow, 1998). Further evidence to the effect that available carbon regulates soil N-mineralisation rates is found in the present study and provided later in Chapter 7. However the presumption is speculative, and the existence and content of labile carbonaceous energy sources needs to be explored in the study farm soils.

Mineralisation rates were significantly greater in farms with longer intervals since aeration as would have accompanied mechanical disturbance ($r = 1.000$, $P < 0.05$). Carter (1991) reported that ploughed soils had lower levels of organic carbon and nitrogen in the plough layer than direct drilled or pasture soils, and that microbial biomass was reduced in the ploughed soils. Root mass significantly correlated with mineralisation, and was also increased relative to years since mechanical disturbance. Mechanical disturbance breaks up root material, which is rapidly decomposed reducing soil organic matter content temporarily. The reduction in microbial biomass, and its recovery as organic matter accumulates in the pasture phase, may simply mean that the microbial biomass size and its associated activity are limiting in disturbed soils, but recover along with polysaccharide content, also connected with pasture age.

Alternatively, as pastures age, soil organic carbon content becomes resistant to degradation (Franzluebbers et al., 2000), and readily available carbon sources dwindle, allowing greater opportunity for ammonification of nitrogenous carbon added as arginine *in vitro* to occur. In the study conducted by Franzluebbers et al. (2000), the ratio between microbial biomass carbon and organic carbon content fell as pasture aged, and the authors attributed this to substrate recalcitrance. In other words, as pasture aged, less carbon was being accumulated or immobilised in the microbial biomass (Franzluebbers et al., 2000).

Whether arginine ammonification could have been biomass-limited in fertilised or mechanically disturbed soils is not clear, as arginine ammonification and biomass values were not correlated in the current study. Further analysis is needed to see whether catabolite repression of arginine ammonification is responsible for reduced mineralisation rates observed in fertilised and more recently disrupted soils. Labile organic carbon content of the soils should be examined further to determine its origin and role in relation to arginine ammonification rates. If intracellular allosteric controls prevail, the provision of labile carbon is likely to regulate nitrogen mineralisation activity in managed grasslands.

Neither allosteric regulation nor catabolite repression of arginine ammonification has been fully considered in the soil biology literature. Lin and Brookes (1999b) found that the highest rates of arginine ammonification were associated with lower arginine concentrations, and that while arginine ammonification rates declined rapidly at higher values, no ammonia was detected at larger levels of amendment in arginine ammonification experiments. When arginine is deaminated, it releases metabolites that act as a carbon source (Cunin et al., 1986; Zubay, 1993). Although Lin and Brookes (1999b) referred to the suggestion made by Alef and Kleiner (1987) that arginine ammonification is inhibited in soils that contained much readily available substrate, they did not offer an explanation. Furthermore, no reference was made to the possibility that at higher arginine concentrations, metabolites released through arginine use could lead to either allosteric modulation or catabolite repression, and thereby reduce arginine ammonification rates. It is likely that the induction, repression and allosteric control of enzymes was overlooked in this research because organic carbon was assayed in air-

dried ground soil by the dichromate method, from which labile fractions may have been lost, and no reference was made to more available carbon fractions in their reports.

In the present study, negative values, interpreted as immobilisation occurred in arginine-amended samples from five of the study farms, namely H, J, L, M and N. There was insufficient sampled material available from farm G to determine its arginine ammonification rate. Immobilisation figures were expressed as positive values so that statistically significant relationships and trends could be interpreted more easily.

Microbial activity measured as the amounts of ammonia released from soil following arginine amendment *in vitro* were inversely but insignificantly correlated with pH ($r = -0.243$), when viewed as a single parameter, and the relationship with pH was unclear. When examined as separate functions, it was found that pH related strongly to both immobilisation and mineralisation tendencies. There was a strong curvilinear response shown with immobilisation, increasing in the pH range of 4.91 to 6.12. Correlations between pH and immobilisation were highly significant ($r = 1.000$, $P < 0.05$). Soils in which mineralisation occurred ranged from pH 5.2 to 7.2. Where mineralisation occurred, its rate declined with soil pH ($r = -0.796$, $P < 0.05$) in a complex manner, unlike the curvilinear response of immobilisation within the similar pH range. Separation of ammonification values into two distinct functions brought an independent and highly significant relationship with pH to light. Immobilisation was also significantly correlated with soil calcium content ($r = 1.000$, $P < 0.05$).

Farms with frequent treading in the longer term, measured as the numbers of livestock units per hectare multiplied by the number of days grazed in the year prior to sampling, had significantly lower rates of N-immobilisation ($r = -0.900$, $P < 0.05$).

Because only five of the study farms exhibited immobilisation, it was not possible to establish the significance of any correlation at Spearman's correlation coefficient values less than 0.900 (Zar, 1993). However, while other associations with immobilisation were not demonstrably significant ($P < 0.05$), some relationships with immobilisation seem noteworthy. For example, mineral nitrogen had not been applied to the 'immobilising farms' for four years prior to sampling, and, in line with this observation, immobilisation may have increased in the interval since mineral nitrogen applications (r

= 0.564). Indeed organic farming may encourage immobilisation ($r = 0.564$). Organic manures ($r = 0.335$) are not inhibitory, and may have a slight positive effect. Because immobilisation signifies biomass development, each correlation observed between immobilisation and fertiliser application history concurs with literature in which biomass and soil organic matter are reportedly cushioned by organic manures and decline in mineral fertilised soils (Willison et al., 1996; Lovell and Jarvis, 1998; Gunapala et al., 1998; Henriksen and Breland, 1999).

The additions of lime seem to influence immobilisation ($r = 0.667$), while immobilisation may fall away in the years after liming ($r = -0.866$). Immobilisation may also be influenced by the proportions of silt and clay in soil ($r = 0.718$), and by bulk density ($r = -0.821$), and by the abundance of grass species in the sward ($r = 0.700$).

Although available energy sources and nitrogenous compounds regulate ammonification intracellularly (Cunin et al., 1986), correlations of immobilisation and mineralisation with soil carbon and nitrogen content were not statistically significant. Organic carbon and nitrogen measurements in the present study might not take account of the labile organic matter fractions because air-dried and sieved soils were used in their analyses. It is likely that recalcitrant non-labile fractions of carbon and nitrogen were included in, but that intracellular availabilities were probably not represented by, the carbon and nitrogen fractions examined in this study to date. Any expected correlation of either nitrogen immobilisation or mineralisation with C:N ratio could have been masked by the methods used. Moreover, research elsewhere suggests that relationships with C:N ratio are complex. Gunapala and Scow (1998) found that microbial activity and biomass variables correlated negatively with mineral nitrogen in conventional systems, whereas positive associations were observed in organic farms. A closer look at the dynamic soil organic matter fractions and at their mineral nitrogen and organic carbon contents and inputs is warranted, in order to understand their underlying relationship to microbial carbon and nitrogen transformations in managed grassland.

5.4.4 Urease enzyme activity in soils

Summarised data for soil urease activity, measured *in vitro* on the sixteen study farms, is presented in Table 5.4. Detailed urease values for each farm can be found in the data spreadsheet, in Appendix D.

Table 5.4 In vitro urease enzyme activity

Soil urease enzyme activity measured as ammonia released from urea-amended soil *in vitro*.

Urease activity in urea amended soil	Min.	Max.	Mean	SD
NH ₄ -N ($\mu\text{ g}^{-1}\text{ ds h}^{-1}$)	0.6	88.1	38.1	27.1

Soil urease activity corresponded significantly with numbers of protease producing organisms found in soil ($r = 0.544$, $P < 0.05$). Urease enzyme, which is a protein, might be hydrolysed by proteolytic activity, carried out by protease producing microorganisms in soil. However, it is known that urease is stabilised against proteolysis by absorption to soil colloids (Marzadori et al., 1998).

Urease activity was also related to cumulative phosphate added from mineral preparations, basic slag and manure sources to the sixteen study farm soils ($r = 0.550$, $P < 0.05$). Marzadori et al. (1998) found that, in the presence of hydroxyapatite, which is a calcium derivative of phosphate fertilisers in soils, urease exhibited far greater stability than when free in the soil solution. The activity and stability was also proven in the presence and absence of added proteolytic enzymes (Marzadori et al., 1998). Whether from plants or microbial sources, any urease released into the soil solution is quickly degraded, and so it is the adsorbed enzyme that probably accounts for a significant proportion of urealytic activity in soil (Marzadori et al., 1998). As phosphate additions were associated with higher levels of urease activity in the present study farm soils, it may also have stabilised urease enzyme there. Urease stability may explain its continuity in the sampled soils, despite the increased background populations of proteolytic organisms. Why soil urease enzyme might be associated with proteolytic organisms is not clear. Urea may be derived from amino acid breakdown by microorganisms through the arginase pathway (Zubay, 1993), more urea therefore being

released by more proteolytic activity perhaps. Urease activity releases ammonia nitrogen from urea, and the most significant link between urease activity and any other factor was its correlation with the cumulative presence of grazing animals in the year prior to sampling ($r = 0.712$, $P < 0.05$). The urease enzyme activity was probably extracellular, being measured in the presence of toluene to disrupt microbial cells, and would arise from urease producers, which appeared to have been more prevalent in farms with larger numbers of grazing animals. There may be a connection between continuity of substrate for urease hydrolysing organisms and their enzymes, but this is unsubstantiated.

Urease also corresponded to the abundance of the plant species *Bellis perennis* ($r = 0.531$), presence of which was recorded, however, on only three of the sampled fields.

5.5 A summary of the soil biology of the sixteen study farms: analyses, influential factors, and recommendations

Earlier comparisons of the biological characteristics of soils from organic and conventional farms had found that the microbial biomass, numbers of culturable bacteria, microbial and enzyme activities were greater in soils under non-conventional management systems (Bolton, 1985; Reganold et al., 1993), suggesting that an analysis of soil microbiology might help to describe soil quality. From a more applied agricultural setting, the farming press recommended that, in particular, mineralisation processes and influential factors needed to be understood (Newton, 1993) and that soil biology could be used as a tool in soil analysis (Lampkin, 1990). However, attempts to understand mineralisation in the broader scale (Degens, 2000) and to apply biological analysis as a gauge of soil quality or fertility have been largely unsuccessful to date (Sparling, 1997). In a comprehensive review (Nannipieri et al. 1990), the main obstacles to the analysis of soil biological activities were cited as methodological, but it was asserted that soil biological analysis would be worthwhile if methods that had been shown to be faulty were avoided, and if what was being measured was defined to facilitate its correct use.

5.5.1 Underlying principles

As a preliminary to this study of biological parameters and influential factors, underlying biochemical and microbiological principles were set out, as summarised here. Decomposition activity is carried out largely by microbial heterotrophs (Brady, 1990)

and is regulated by the provision of carbonaceous energy sources (Cunin et al., 1986), and by available mineral nitrogen to support bacterial growth (Chantigny et al., 1999). Heterotrophs may respond to substrates through enzyme induction (Kay-Shoemaker et al., 1998), leading to population increase. Microorganisms use soil carbon in respiration (Wardle, 1994), and in a nutritionally sparse environment, populations are subject to quiescence (Atlas, 1995). Viable organisms can resume activities once conditions and substrates allow (Lovell and Jarvis, 1998). The microbial biomass consists of active and inert fractions, which respond differently to management (Lovell and Jarvis, 1998), and when degraded, biomass releases carbon, nitrogen, and phosphate from sequestration into the soil nutrient pool (Smith and Paul, 1990). Microbial activity is regarded as cellular (Alef and Kleiner, 1987; Nannipieri et al., 1990) and as such is governed by intracellular regulation of metabolism by carbonaceous and nitrogenous compounds (Cunin et al., 1986). Extracellular enzymes are not regulated by metabolic inhibitors (Coyne, 1999) but may be stabilised on soil colloids (Marzadori et al., 1998).

All too often, these underlying principles have not been acknowledged in the soil biology literature. For instance, researchers have suggested that amino acids could be a source of nitrogen for microorganisms in mineralisation (Alef and Kleiner, 1986; Lin and Brookes, 1999b). However this is not strictly the case as in most instances microorganisms use carbonaceous sources for energy and sacrifice nitrogenous sources only under starvation conditions (Cunin et al., 1986), releasing ammonia in the process. Instead, microorganisms use ammonia as a nitrogen source in preference to organic compounds (Coyne, 1999). Possible regulation of amino acid catabolism by available energy sources has been alluded to (Alef and Kleiner, 1987) but not elaborated on. Other authors refer to the possibility that readily available substrate might limit ammonification (Lin and Brookes, 1995b), but do so with imprecise reference to total carbon content which is likely to have included senesced material that would be less available (Franzluebbers et al., 2000; Haynes, 2000), and therefore unlikely to incur either allosteric or catabolite repression effects. In the most striking oversight of all, Magill and Aber (2000) expected to find higher N-mineralisation rates in the presence of added glucose, but found reduced N-mineralisation rates instead, which they attributed to immobilisation. Although nitrogen immobilisation in the presence of glucose is plausible, no reference was made to the possibility that catabolite repression

of nitrogen mineralisation by an available energy source (Cunin et al., 1986) might have occurred, and controls to that effect were not included in the analysis.

While the intracellular control of nitrogen mineralisation has not been fully accounted for, researchers have also overlooked or neglected to mention the facts that (1) the microbial population is subject to quiescence; (2) the biomass contains active as well as passive fractions; (3) the biomass is part of soil organic matter and might limit biomass size. As fundamental principles, these facets govern microbial activity too. The literature has been remiss in some respects, which may account for the lack of knowledge transfer from mineralisation biology to agricultural purposes, noted by Newton in 1993. Underlying biochemical principles of carbon and nitrogen transformation were considered in this study and, as a result of observations, mechanisms are suggested through which influential factors might interact with and affect the mineralisation of carbon and nitrogen, and plant growth, in managed grassland.

5.5.2 Defined biological parameters assessed

The advice of Nannipieri et al. (1990) that what is measured should be defined to facilitate its correct use, was taken. Distinct biological components such as biomass fractions, catabolically diverse populations, cellular activity and cell free enzymes were analysed in a relatively simple way in soils. Far from simply finding 'more biological activity' under any general type of management system, distinct and varied relationships emerged between the biological components of mineralisation and specific factors such as liming, fertiliser inputs and grazing events, as well as with soil conditions and vegetation species. Findings and recommendations in regard to microbial biomass, populations, cellular and enzyme activities and their analyses in grassland soils are summarised here.

Microbial biomass was regarded as a single entity when the initial comparisons of organic and conventionally managed soils (Bolton et al, 1985) were carried out, but differentiation of microbial biomass into active, passive or total fractions has helped to differentiate between soils and management systems. In the sixteen study farms in the present study, larger total biomass fractions were recorded on farms with greater intervals since mineral nitrogen applications. The active biomass appeared to respond to

grazing events and to plant root mass. Such observations concur with the findings that biomass is management influenced, and that higher levels are generally associated with non-conventional farming systems or with absence of mineral fertilisers (Bolton et al., 1985; Gunapala and Scow, 1998). However, as the effects of mineral fertilisers and manures vary (Gunapala and Scow, 1998), and both were applied on the study farms in this instance, more critical analysis is necessary.

The mechanisms underlying biomass increase are uncertain, but it may be that mineral nitrogen facilitates carbon mineralisation (Henriksen and Breland, 1999) leading to root mass and organic matter turnover (Lovell et al., 1995), leaving little available carbon for the microbial biomass pool (Franzluebbers et al., 2000). This explanation is plausible in the present study because root mass and organic carbon content were negatively associated with mineral nitrogen applications (Chapter 3). Lower organic carbon content was associated with lower microbial biomass values reported elsewhere (Lin and Brookes, 1999b). However, decline in biomass size with organic carbon content reported elsewhere (Alef and Kleiner, 1987; Carter 1991; Lin and Brookes 1999 c) suggests carbon resources limit biomass size, in agreement with Bauhus et al. (1998). No correlation between microbial biomass and activity and organic carbon was found at the higher, presumably non-limiting, organic carbon concentrations in the sixteen farms. Organic carbon on the sixteen study farms does not appear to constrict microbial biomass.

Lower soil aggregate stability values were observed with higher active biomass proportions of soil organic carbon content in the present study. The proportion of active biomass was also related to grazing, which in turn had correlated somewhat with aggregate stability. The role of the microorganisms in contributing to and in detracting from soil aggregate stability has been demonstrated (Swift, 1991). Results of the present study suggest that microbial interaction with soil structure is a function of the active biomass component relative to soil organic matter, and needs further investigation. This example supports the view expressed by Dilly and Munch (1998) that ratios, rather than absolute values, might help to describe how the microbial biomass interacts with environmental variables.

It has also been possible in the present study, by describing management and botanical contexts, and using enumerative techniques, to show that grazing management and botanical composition influence catabolic populations in soil, in specific ways. Amongst the heterotrophs responsible for decomposition (Brady, 1990), bacteria were easier to quantify. Bacterial populations with particular catabolic function in the breakdown of cellulose starch urea amino acids and protein were enumerated. Such bacterial heterotrophs are involved in soil carbon and nitrogen mineralisation processes, and their enumeration facilitated the comparisons of study farm soils and of influential conditions. All of the tested catabolic functions were represented to some extent on each farm, suggesting that soil on each farm is capable of carbon and nitrogen mineralisation processes. Carbon decomposing organisms predominated in contrast to those that could use organic nitrogenous compounds, a finding in line with the expected 24:1 C:N ratio of herbage detritus in unfertilised grassland (Whitehead, 1995). The fact that bacterial populations increase in response to substrates, and may remain quiescent, may explain why populations in study farm soils were influenced more by management events such as liming and grazing than by soil pH. Bacterial populations and activities should be regarded as different entities, because quiescence implies that the population need not reflect immediate conditions. This was seen in lack of correlation between bacterial populations and soil pH. In contrast, cellular activity is usually influenced by prevailing environmental constraints, as was seen here in the cases of mineralisation and immobilisation which were strongly correlated with soil pH values.

Substrate availability is a key factor regulating microbial populations. For example, soil polysaccharides correlated with amylolytic organisms in the present study. Grazing values such as the presence of larger animals, grazing intensity, continuity of grazing animals, and durations of grazing intervals were associated with particular catabolic populations, and it is likely that grazing events influenced microbial presence in the study farm soils through provision of substrates from damaged herbage, excreta and possible plant herbivory responses. In future work, microbial substrates should be identified and measured in the study farm soils to see whether the availability of particular substrates could account for variations in population size and catabolic function, and to see whether substrate availability has been influenced by particular grazing events.

Similarly, the many relationships found between microbial populations, botanical species, and botanical diversity, are probably substrate based, and an analysis of the plant exudates and rhizosphere chemistry associated with particular species would help to clarify how these associations might come about in practice. While population studies show that catabolically capable organisms are present, carbon and nitrogen transformation activity of the heterotrophic populations as affected by prevailing environmental conditions *in situ* remains to be seen.

Arginine ammonification was proposed as a simple measure of microbial activity (Alef and Kleiner, 1987) and has been used as such for many years. When arginine ammonification data were regarded as just ‘microbial activity’ in the present study, no significant relationship was found with soil or management parameters. In contrast, when separated into positive and negative values representing N-mineralisation and N-immobilisation, significant correlations were found. Longer intervals since mineral nitrogen applications are accompanied by reduced mineralisation rates. Nitrogen mineralisation rates increased with increasing root mass, and as pastures aged. Immobilisation occurred only in farms to which no mineral nitrogen had been applied in the four years prior to sampling. Looked at in conjunction with mineralisation, it is likely that organic carbon availability facilitates nitrogen immobilisation in the biomass, as suggested by Magill and Aber (2000). Immobilisation also tended to decline with exposure to grazing livestock. Mineralisation and immobilisation correlated with soil pH in a complex fashion within the same pH range.

In contrast to cellular activities, soil enzyme activity was related not to nitrogen or carbon related inputs but to use of phosphate fertilisers, which on closer inspection may be attributable to the stabilising effect of hydroxyapatite (Marzadori et al, 1999). Urease is likely to relate to factors that determine the originating urease producer population, but overall soil urease activity seemed to reflect its free extracellular quality.

5.5.3 Recommendations for biological analyses of grassland soils

The following recommendations should facilitate the interpretation and analysis of soil biology in managed grasslands.

1. Specific rather than general system effects of influential management practices should be taken into account. Individual and combined effects of mineral and organic fertiliser applications should be examined.
2. Biomass should be regarded as part of soil organic matter, having active and possibly inert fractions. The role of biomass as a nutrient reserve should be considered in relation to management effects. The role of organic carbon as a sink for immobilised nitrogen needs to be addressed.
3. Underlying principles, such as intracellular regulation of nitrogen mineralisation, should be acknowledged.
4. The practice of analysing soil organic carbon content on sieved air-dried soil should be examined. Specific labile and recalcitrant soil organic matter fractions should be accounted for.
5. Whether labile carbon regulates nitrogen mineralisation should be examined in laboratory and in field experiments.
6. Ratios between soil biological parameters should be considered as a means to describe dynamic relationships between for example soil biological and structural properties.

5.5.4 Overall conclusions regarding the biological analysis of grassland soils

According to Degens et al. (2000), no broadscale study has been carried out on the functional diversity of the soil microbiota, and according to Newton (1993) factors regulating mineralisation of legume nitrogen had not been understood. A broad analysis of soil, site-related, botanical and management parameters in sixteen grassland farms under a range of management systems forms the basis for this unique study. Results and observations have established that nutrient inputs, liming, grazing events and botanical species influence factors (microbial biomass, heterotrophic populations and their activities) responsible for mineralisation processes in defined ways that are as yet not fully understood. Underlying mechanisms are probably based on biological phenomena, such as the provision of mineral nitrogen and carbonaceous energy sources as suitable substrates for the heterotrophic organisms that are themselves subject to enzyme induction, intracellular regulation, population quiescence, and biomass degradation.

Further precise and controlled investigation is required to identify underlying influential mechanisms, and to extrapolate this information to grassland soil management for practical purposes.

Chapter 6

Chapter 6

Grassland production on the study farms in relation to influential factors

6.1 Introduction

This study comprised sixteen farms, most of which had had either no or low levels of fertiliser inputs or were managed organically. Their farm fertility had generally not been augmented by the mineral nutrient additions characteristic of conventional farming practice, in contrast to one study farm managed conventionally. Grassland production on the majority of study farms would have depended, therefore, on biological processes that released plant-available nitrogen from soil organic matter available as legumes, detritus and excreta. However, when legume-fixed nitrogen becomes available to grasses in the sward is not well understood and therefore not controlled (Newton, 1993), and there are as yet no satisfactory methods of predicting *in situ* nitrogen mineralisation potential (Burket and Dick, 1998). In other words, although the link between soil biology and productivity is presumed, the role of soil biological properties has not been elucidated satisfactorily for grassland production purposes.

Because of its particular role in low input and organic farms, soil biological analysis has been suggested as an adjunct to existing physical, chemical, textural and structural soil analysis regimes (Lampkin, 1990). An ambition to determine the suitability of land for organic farming motivated the present study. In the past, land capability classes have been determined based on limitations posed by climate, gradient, soil structure and texture, wetness, erosion and vegetation factors (Gardiner and Radford, 1980; Frame, 1992), and land capability classes have been linked with potential production. Soil biological factors have not been taken into account for reasons of experimental difficulty (Lampkin, 1990; Nannipieri et al., 1990). Furthermore, as baselines for soil biological criteria had not been established (Sparling, 1997), the ambition to determine suitability of land for organic farming was put on hold. The present study therefore set out to clarify the role of soil biology in grassland production under low input, organic and conventional nutrient conditions. In addition, by using grassland production as an objective measure, relationships with management, a range of soil and land properties,

botanical composition and soil biological components and processes were considered. Possible soil property indicators of grassland production were also sought.

6.2 Review of literature

In the search for grassland production indicators, it is useful to consider the criteria that soil properties, in particular the soil biology, have been thought to indicate until now. Soil biological properties have been examined particularly in relation to three criteria, namely: plant growth or yield (Moore and Russell, 1972; Skinner, 1976); soil quality (Bolton et al., 1985; Reganold, 1988; Parkinson, 1991; Reganold et al., 1993; Haynes and Tregurtha, 1999), and, more recently, soil health, regarded in part as the soil biological response to environmental challenges such as management change (Pankhurst et al., 1997a & b; Doran and Safley, 1997; Lovell et al., 1995; Griffiths et al., 2001, amongst others). Each of these measures presents methodological and conceptual difficulties, however their advancement through crop yield soil quality and soil health reflects changes in the understanding and exploitation of soil as a natural resource.

Attempts to indicate crop yield using soil biology have been unsuccessful. For example, Moore and Russell (1972) found no relationship between soil fertility, as measured by dry matter yield, and dehydrogenase activity. The term ‘dehydrogenase’ describes enzymes that work intracellularly in the transfer of electrons from metabolites towards their terminal electron transport (Zubay, 1993), and has been thought to represent microbial activity in soil (Casida et al., 1964; Nannipieri et al., 1990). However, Moore and Russell (1972) found that correlation coefficients between dehydrogenase activity and eleven measured soil properties known to influence plant yield, other than pH (0.45) and particle size (0.48), were low, which the authors interpreted as lack of useful correlation. Direct comparison of this research with the present grassland study is unlikely because their statistical procedures were not described and their experiments were conducted as pot trials, using *Phaseolus lathyroides* as the experimental crop. Although Moore and Russell (1972) came to the conclusion: “that there were no grounds for optimism that there is a relationship between dehydrogenase activity and soil fertility as measured by plant growth”, they qualify this assertion on the grounds that “plant yield reflects integrated processes operating over the whole of the plant

growth period". Skinner (1976) attributed the lack of success in earlier attempts to correlate soil biology with soil fertility, measured on the basis of crop yield, to the possibility that researchers 'had not asked the right questions of nature". In their comprehensive review of the ecological significance of biological activity in soil, Nannipieri et al. (1990) concluded that it was unlikely that a single measure of microbiological activity would relate simply to production measurements. Additional biological, anthropogenic and natural criteria were needed (Nannipieri et al., 1990). Apparently, any correlation of soil biology with crop yield will require a comprehensive information base that takes integrative processes of management, soil biology and crop growth into account (Moore and Russell, 1972; Skinner, 1976; Nannipieri et al., 1990).

If crop yield is to be considered as a production measure, nonetheless, in the present study, its measurement needs consideration. Grassland yield is a complex entity, and is measured in different ways. For example, the farmer may judge the amount of herbage available to stock on a day-to-day basis by simply walking the land, judging the sward by eye and using past experience (Frame, 1992). However, sward density is difficult to quantify, usually because of sward height and composition influences. Clover, for example, is often underestimated in the grass-clover sward because of its broad-leafed format (Frame, 1992). Weighted disc and graduated stick measurements have been devised to measure sward height, but uptake of disk or other sward-measuring devices by farmers has been slow in the UK (Frame, 1992). Sward height may also be measured by walking in a 'W' across a field, avoiding gateways, troughs and paths, and taking at least 30 to 50 measurements, using a sward height measuring stick designed for that purpose (Frame, 1992). Sward height and density help to determine the grazing rotation, and a minimum height is usually observed. For fodder record, representative samples of hay can be measured and amounts made per field estimated. Similarly, silage weights can be estimated using large-scale trailer weighing devices. However, farmers seldom weigh grass, and are unlikely to be aware of grassland output as tonnes per hectare (Brockman, 1995b). Neither forage measure, however, takes account of losses during curing in the case of hay, or in feeding out of either silage or hay (Frame, 1992). The measurements of sward and fodder outlined here are applicable to working farms. However, because grassland is subjected to different uses such as grazing and cutting, and grass is converted to products such as milk, meat or wool in processes that depend

on grass utilisation efficiency, discrete harvest estimates of grass or fodder are inadequate (Brockman, 1995b; Frame, 1992).

Stocking density methods based on livestock units per hectare LU ha^{-1} , that were devised in the 1960s, are also used to evaluate grassland production (Brockman 1995b; Frame, 1992). Grassland production and its potential have been evaluated in Ireland using stocking density as a gauge (Lee and Diamond, 1972). It (LU ha^{-1}) is a convenient measure, because although grassland production may include different grazing animals such as goats, sheep, cattle, dairy cows and horses, the production of each type of grazing animal may be compared on the basis of their dairy cow equivalent value, established for each animal type (IOFGA, 1996; Frame, 1992).

Use of stocking density as a measure of grassland production or its potential, however, may have spurred production increases, with consequences for soil quality. Lee and Diamond (1972) suggested that increased livestock production was possible in Irish grasslands on the basis of what was then being achieved on similar soil associations in Ireland and elsewhere. Increased mineral nitrogen inputs and application of current technologies such as more digestible higher yielding grass varieties were then advised, in order to achieve what was seen as potential capacity, (Lee and Diamond, 1972). Since that time, however, intensified grazing and silage cutting have been recognised as problematic (Frame, 1992). Specifically, vegetation change in response to grazing intensification is known (O'Sullivan, 1968; Bardgett et al., 1997, amongst others). Intensification effects in Ireland were forewarned by O'Sullivan (1968) who noted that the grassland "community poorest in species is that where human and animal influence seem to be of major importance". Differences also in the soil biota have been found in grazed and ungrazed hillsides (Bardgett et al., 1997). Although soil and botanical changes occur in response to intensification, the functional mechanisms that underlie change in vegetation and in organic matter transformation are not well understood, and the ecological implications of these changes are not yet known (Bardgett et al., 1997). Quantifiable measures are needed to evaluate effects of management change and increased production on soil quality itself.

Improved soil and environmental quality is one of the major reasons for adoption of organic farming strategies (Reganold et al., 1993). Earlier comparisons of the soil

biology present in conventional, biodynamic and organic farms have tended to equate soil microbial activity and organic matter contents with 'soil quality' (Bolton et al., 1985; Reganold, 1988; Reganold et al., 1993). Soil microbial activity and organic matter have been connected with soil quality on the presumption that more microbiological activity would be required to deal with the higher levels of mineralisation that are expected in organically managed soil (Gunapala et al., 1998). However such presumptions that 'more is better' have been applied in the past (Bolton et al., 1985) without apparent concern for the likely negative effects of higher *Nitrosomonas* populations, found in a conventional farm in that study, and likely to incur soil nitrogen loss. Whilst each microbial activity should be defined and interpreted correctly (Nannipieri et al., 1990), each analysis should be based on quantifiable and objective parameters rather than presumed benefits.

The most frequently used concept of soil quality has been 'suitability for chosen uses' (Doran and Safley, 1997). However, if suitability is based on potential rather than actual production, the consequences of intensification to meet that potential may be catastrophic unless effects of intensification on soil quality are taken into consideration. Spurred by intensification-related problems, and by a growing awareness of the function of soil in the biosphere, definitions of soil quality and health have evolved over time (Doran and Safley, 1997). Soil health, for example, has been defined as the "continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, promote the quality of air and water environments, and maintain plant, animal, and human health" (Pankhurst et al., 1997a). While there is some disagreement as to what constitutes either soil health or quality (Pankhurst et al., 1997a & b; Sparling, 1997), it is agreed that soil quality comprises biological criteria as well as physical and chemical attributes (Lampkin, 1990) and descriptive variables (Smith et al., 1993). Generally, the term 'soil quality' is acceptable when the intended use of a soil is defined, as soil of suitable quality for the production of one crop may not match other plant requirements (Pankhurst et al., 1997b). According to Smith et al. (1993), definition of soil quality criteria should be followed by "the identification of specific soil parameters that can be measured to adequately quantify the terms of the soil quality definition". The authors suggest that soil quality can be defined in terms of productivity amongst other factors (Smith et al., 1993).

However, to define soil quality in terms of productivity now seems inappropriate, in view of known management intensification effects.

As the intention to evaluate soil suitability for organic farming motivated this study but criteria were lacking, it was decided to choose grassland production itself as a measure against which suitable criteria might be identified. However, it was evident from the literature that soil biological properties had not correlated with crop yield (Moore and Russell, 1972), and that comprehensive information was needed to take integrative plant production processes into account (Moore and Russell, 1972; Skinner, 1976; Nannipieri et al., 1990). With this in mind, and in view of the inadequacies of single grassland harvest measures (Brockman 1995 b; Frame, 1992) a more comprehensive grassland production figure was sought against which production criteria might be evaluated.

Modern grassland production values incorporate grass and livestock production in a single measure when interpreted as metabolisable and utilised energy (Frame, 1992). The amount of energy required by an animal of certain type and age and weight to attain a particular live weight gain, offspring and milk production can be found in published animal nutrition tables or reference texts such as ADAS, 1984; Evans et al., 1990; Frame, 1992; and Cooper, 1995, which were availed of in the present study. The metabolisable energy (ME) available from each type of fodder is also known (Brockman, 1995b; Frame, 1992). The net energy output from grassland over a year usually starting in spring, is calculated based on animal requirements for maintenance, growth, offspring, and milk production. Energy from grassland- produced fodder sold off the farm or left over at the end of the year is added in the calculation, and energy from bought in feeds and from on-farm grassland fodder fed to animals during that year is deducted. Net values for energy necessary to produce livestock, their offspring, milk and energy in unused fodder are calculated on a per hectare basis for the production year. Energy produced is then expressed as Gigajoules per hectare, GJ ha^{-1} (Frame, 1992). Production figures for lowland and hill grassland have been published for the UK (Frame, 1992), and such figures allow a comparison of farms and of their soil and management effects.

Financial performance has also been applied as a production indicator (Frame, 1992). Financial data for the comparison of conventional and organic farms has been lacking

(Lampkin, 1990), and compared to cash crop incentives in cereal production, the difficulties inherent in conversion of grass to livestock profits may have been a disincentive for farmers (Frame, 1992). Financial performance has been used as a gauge of conventional versus biodynamic farms, however (Reganold et al., 1993; Reganold, 1995). In a study of paired conventional and biodynamic farms in New Zealand, biodynamic farms were deemed “as financially viable as their conventional counterparts” (Reganold, 1993). In the later review of soil quality and profitability of biodynamic and conventional farms, Reganold (1995) summarised data from published and unpublished theses and concluded that biodynamic farms have better soil quality, lower crop yields, and equal or higher net returns per hectare than their conventional counterparts. Where found, comparability of financial performance in the studies reviewed by Reganold (1995) was attributed to the frequently lower cost of biodynamic farming, and higher prices for biodynamic produce. Soil quality was generally better on the biodynamic farms, which for example were reported to have higher organic matter content and microbial activity, lower bulk density and thicker topsoil than their conventional neighbours (Reganold, 1993). Since financial performance comparability is largely determined by premium prices (Reganold, 1995) it remains to be seen whether financial performance will differentiate between farms and between soil properties as soil quality or production indicators in the present study.

In the present study, grassland production was assessed initially in terms of stocking density, energy produced per hectare and gross margin. Their values served mainly as a vehicle for the examination of the relationships between grassland production and soil properties. Grassland production was also employed as a tool for assessing the merit of biological and other soil properties as indicators of soil production potential.

6.3 Materials and methods

6.3.1 Introduction

Annual production achieved on each study farm was estimated from the questionnaire and interview data (See Chapter 2 and Appendix A). Animal and fodder production was interpreted as the total energy needed for the growth, maintenance and production of livestock and milk, and from forage sold off the farm, while taking bought-in feeds and

other non-grassland fodder crops into account. Gross margins for each of the farms were also estimated, based on income reported during interviews, including grants, premia and headage payments, taking farm costs into account. Stocking density was calculated as livestock units per hectare of grassland.

All production estimates were based on data spanning 1996 / 1997, and was preceded by soil sampling that had taken place in 1995. This facilitated evaluation of soil properties as predictors of grassland outputs.

6.3.2 Parameters measured

6.3.2.1 Energy from grassland

Calculation of grassland production in terms of energy was based on a formula given by Frame (1992). The following items are included:

A: Ruminant livestock numbers, including their dairy and offspring production

B: Quantities of home-grown farm fodder crops sold

C: Quantities of purchased ruminant feedstuffs

D: Yields of home-grown fodder crops produced, in the formula:

$$\text{Grassland production in Gigajoules per hectare (GJ ha}^{-1}\text{)} = \frac{\text{A+B} - \text{(C+D)}}{\text{hectares of grassland}}$$

In the original formula, Frame (1992) included quantities of fodder crops in hand at end of year and quantities of fodder crops in hand at beginning of year, data that were not recorded in the present study and were presumed for the purposes of this study to cancel each other out.

Data for items A, B, C, and D were obtained from detailed analysis of information recorded in the questionnaires and interviews. Raw data was converted into metabolisable energy (ME) equivalents using annual factors for the different classes of stock, calculating the ME value of their dairy production, calf, lamb and kid outputs and for livestock growth, and using standard ME values for the feedstuffs such as silage and hay produced or bought in and other non- grassland feeds produced on the farm such as arable silage, beet and turnips. Data for the calculation of energy equivalents of livestock and fodder were obtained from the following references: ADAS, 1984; Evans

et al., 1990; Frame, 1992; and Cooper, 1995. The energy output figure for each farm incorporated the energy needed for livestock, dairy and fodder production from the grassland for either the year 1996 /1997 following soil sampling. The choice of year, either 1996 or 1997, was based on the livestock production and management history information that seemed most representative of grassland performance for that farm. For example, if weather had been a problem in any one year, or if animals were lost due to illness, that more difficult year was avoided and the more 'normal' production year examined instead. Data and their energy equivalent values were transferred to an Excel worksheet and total energy output figures were calculated in Gigajoules per hectare of grassland (GJ ha^{-1}).

It should be noted that for calculation of energy produced on the farm as a whole, the fodder outputs and animal production figures for the entire farm in the year prior to sampling were taken into account. Grazing events described earlier in this thesis were based on figures for the sampled field itself, so that the above ground activities and their effects on the underlying soil in that particular field might be explored.

6.3.2.2 Gross margin per hectare

Section 10 of the questionnaire comprised a series of questions in regard to financial matters. Farmers were asked for estimates of their total farm income including sales of produce, grants, subsidies, headage payments and premia. Fixed and variable costs including fodder, fuel, seed, paid or contracted labour, and interest on farm loans, were also reported. Organic premia were noted, as were the types of extensification grants current at the time of sampling and which may have been availed of, such as REPS scheme payments or other sources. Any grants afforded to the farm for purposes such as forestry or other non- grassland based enterprises were ignored. Data were entered into an Excel database. Income, minus variable costs for fuel feed veterinary and other requirements in that year, was calculated and expressed as gross margin on a per hectare basis ($\text{IR } \pounds \text{ ha}^{-1}$).

6.3.2.3 Livestock units per hectare

Total livestock numbers per hectare LU ha^{-1} of grassland on the farm in the year prior to sampling were estimated from figures given in relation to grazing and production for the year prior to sampling for the entire grassland area. Animal production figures were confirmed with reference to animal purchase, sale and housing data. Conversion rates, based on dairy cow equivalents for LU ha^{-1} , were obtained from IOFGA (1996) and are presented in Appendix B.

6.3.3 Statistical analysis

Descriptive statistics were determined as described in Section 2.3.5.1. As in preceding chapters, non-parametric statistical methods were used because data were not normally distributed. Spearman's Rank Correlation analysis of relationships between production, profit, and stocking density, LU ha^{-1} and each of the grassland management, soil, botanical, and biological variables was carried out using SPSS (Norusis, 1992; SPSS, 1995 & 1999). All statistically significant Spearman's Rho values in relation to various measures of grassland production components are reported as positive correlation $r = 0.000$, or negative correlation $r = -0.000$, together with an associated probability $P < 0.05$ or $P < 0.01$, as appropriate.

In addition to descriptive and bivariate statistical analysis, stepwise linear multiple regression equations were developed here, in Chapter 6, using the SPSS package (Norusis, 1992; SPSS 1999). Energy output in GJ ha^{-1} was used as the dependent variable against which each putatively independent measure describing management, soil conditions, botanical grassland composition and soil biological components and activities were tested in equation building exercises. Stepwise inclusion or removal criteria were set as probability-of-F-to-enter $\leq .050$, and probability-of-F-to-remove $\geq .100$. User-defined missing values were treated as missing. Equations were built by either excluding cases with missing values list-wise or pair-wise, or alternatively variables were replaced with the variable mean, as options in SPSS (SPSS, 1995). Treatment of missing variables is noted. Three such successfully built equations are reported.

6.4 Results and discussion

6.4.1 Energy outputs per hectare

Results of energy from grassland are summarised in Table 6.1. The value for each farm is shown in the datasheet in Appendix D.

Table 6.1

Grassland energy outputs: annual energy required for livestock and their production and for fodder sold from the land, taking bought-in and non-grassland farm produced feeds into account

	Min.	Max.	Mean	SD
Energy (GJ ha ⁻¹)	12.0	65.9	39.3	14.5

Table 6.1 shows that there is large variation in energy outputs from the grassland farms in this study. According to Frame (1992), values for dairy farms are expected to average between 60 and 80 GJ ha⁻¹, comfortably achieved by the top rating farm, L, which is an organically managed dairy farm. Farm J, which had the lowest energy output, was dedicated to summer grazing and had not been intensively managed for many years. The conventionally managed farm, Q, was also relatively productive (53 GJ ha⁻¹). Energy production levels as cited by Frame (1992) for hill farms range from 4 to 41 GJ ha⁻¹, depending on sward type and nutrients, whereas lowland grassland farms could achieve 120 GJ ha⁻¹ (Frame, 1992). As each farm in this study was below three hundred metres elevation, none could be classified as a hill farm. The highest mineral N application rates, recorded on the conventionally managed farm Q, were approximately 254 kg ha⁻¹ and were relatively moderate (MAFF, 1994). Generally, the study farms represent the lower to median range of potential energy output values for grassland, and the mean energy output (39.3 GJ ha⁻¹) probably reflects their lower nutrient input status overall. Energy outputs were significantly correlated with soil pH, ($r = 0.664$, $P < 0.01$). The mean pH value found on the study farm soils was 6.1, which falls just above the ideal range of pH 5.5 to 6 suggested for grassland (Frame, 1992). According to Lampkin (1990), soil pH should be maintained at around pH 6 or higher for optimal grassland conditions.

Fertiliser additions correlated significantly with energy output, but to varying degrees depending on the element applied and on the fertiliser format used. Fertiliser data for six years immediately prior to production assessment was more highly correlated with production than were earlier data obtained for four years prior to soil sampling for analysis. In the most extreme example of this, lack of mineral nitrogen applications over the six-year period prior to 1997 were negatively correlated ($r = -0.665$, $P < 0.01$), as was the four-year lack recorded prior to 1995 ($r = -0.650$, $P < 0.05$), however as can be seen from their probabilities, the six-year figure is of greater relevance. The greater correlation with data pertaining immediately to the production year probably reflects declining fertility in the increased interval since mineral nitrogen applications on some of the farms. It may also reflect the fact that farm G had applied mineral nitrogen in the interim after the soil had been sampled. Furthermore, certain farms (B, F, G, H, L, P and Q) continued to input fertilisers as before soil sampling, whereas inputs on farms A, E, J, M, N, O, R, S and T remained static at their previous four-year value. The effect of continued or increased application would presumably have increased soil fertility in those farms, relative to others in which fertilisers were not applied.

Mineral nitrogen applications were not themselves significantly correlated with energy outputs; however only four farms had applied mineral nitrogen in the six-year timeframe, and each of the four farms had applied varying quantities of organic manures as well. However, nitrogen applied as organic manures on six farms was significantly correlated with production ($r = 0.556$, $P < 0.05$). Combining this importance of organic nitrogen with the decline in production in the absence of mineral nitrogen applications, the importance of nitrogen fertilisers in whatever form is highlighted. Mineral phosphate and potassium applications were significantly related to energy outputs ($r = 0.509$, $P < 0.05$ in both cases). Organic forms of potassium fertiliser were beneficial ($r = 0.556$, $P < 0.05$), whereas organic phosphorous that included its basic slag application were not correlated. Farms managed organically for longer periods were more productive ($r = 0.541$, $P < 0.05$). This probably reflects their use of organic manures in contrast to the lack of nutrient applications on farms which had not been managed organically, and which were of either no, or low, input type. Along with other applications, fertiliser nitrogen, either in mineral or organic forms, is key to production outputs.

The most significant correlation of energy outputs with any management factor was found in relation to mechanical disturbance history. This feature of the study farms had been recorded as the interval, in number of years, since the soil had been ploughed, tilled for arable crop purposes, or exposed to chain harrow use (see Section 2.4.4). In those farms in which soil had had recent mechanical disturbance of any kind, energy output was significantly higher ($r = 0.903$, $P < 0.01$) than when soil had been relatively undisturbed for some time. Energy outputs were also higher in farms that had been reseeded ($r = 0.672$, $P < 0.01$) compared to other farms. Mechanical disturbance may have accounted for the effects of reseeded, because soil had been disturbed in five out of the six reseeded farms. Seed had been spread onto the soil surface from the back of a tractor on the remaining farm B probably following preparatory chain harrow use.

It would appear that mechanical disturbance boosts grassland production by a significant amount. However, on-farm analysis of production before and after reseeded was simply noted as the farmer's opinion as to its success in terms of species improvement. As the farmer's view is probably subjective, this estimate was not considered suitable for statistical analysis. There is also some suggestion that ploughing and resowing land improves yield, not specifically because of the type of seed mixture which in the six reseeded farms consisted of a standard commercially available clover and ryegrass combination, but because of the likely release of fertility accumulated in an old pasture (Newton, 1993). Inference based on higher polysaccharide content observed since mechanical disturbance (Section 3.4.2.9) suggests that mechanical disturbances may have helped to renew soil because of aeration, and that this effect dwindles over time. Similarly, longer intervals since mechanical disturbance related strongly to both lower soil pH ($r = - 0.903$, $P < 0.01$) and increased root mass content ($r = - 0.805$, $P < 0.05$), both of which are associated with reduced energy outputs. Whatever the mechanism involved, it is likely that mechanical disturbance helps to renew the sward. Furthermore, the observed correlation with production is in line with expected increases in yield following reseeded (Whitehead, 1995). Whether or not 'lean years' were experienced immediately after reseeded, or whether nitrogen applications were made specifically to offset this (Whitehead, 1995), is not known.

Aeration had presumably been accompanied by nutrient returns to soil from decomposed root material (O'Sullivan, 1968; Lovell et al., 1995), leading to sward

renewal and to the increased fertility spoken of by Newton (1993). It would be interesting to compare the production of arable ley farming systems, where land is ploughed and aerated at regular intervals, with that of permanent undisturbed pastures. However, inversion of fertile topsoil should be avoided to maintain soil fertility (Frame, 1992), and, in the long term, organic matter losses associated with ploughing (Tivy, 1990) are not advisable. It would be a pity to reduce the relatively high organic carbon content of the study farm soils without first understanding the basis for any meritorious influence that any soil disturbing and aerating operation might have. The effect of chain harrow use should be examined, because farm B, which had had regular chain harrow treatments, was particularly productive.

Greater depth of soil to the parent material was related to energy output ($r = 0.580$, $P < 0.05$). As mentioned in Chapter 3, the risk of poaching is probably reduced with greater soil depths (Tivy, 1990), which apparently also supported more intense grazing as practiced in some of the sixteen study farms (Section 3.2.4.2.1).

Only one of the variables describing the physical condition of the soil, namely root mass, correlated significantly with energy output. The relationship was strongly negative, at $r = -0.779$, $P < 0.01$. Lower productivity might simply reflect sward age (Newton, 1993), because root mass appears to be lower in reseeded fields, and was lower in fields that had been mechanically disturbed in recent years. Root mass may have accumulated in the absence of mineral nitrogen. This may be due to the tendency of roots to extend in search of nutrients (Whitehead, 1995; Sparling, 1997). Mineral nitrogen may also hasten the mineralisation of organic matter contained in roots, with carbon accumulating in the absence of mineral nitrogen (Lovell et al., 1995; Henriksen and Breland, 1999). The relationship between root mass and energy output levels may be a complex one, based on interdependent factors such as the status of the sward, nitrogen inputs, available carbon, and other parameters such as soil microbial activity.

Soil magnesium content was highly significantly ($r = 0.659$, $P < 0.01$) related to energy output. Magnesium is known to contribute significantly to both plant and animal nutrition (Brockman, 1995a), and its association with energy outputs is not surprising. Magnesium content was also inversely related with root mass values ($r = -0.711$, $P < 0.01$, see Section 3.4.2.7). This suggests that root foraging activity with resultant root

development may have occurred in response to inadequate soil nutrient conditions. Soil magnesium content is noteworthy because it could be augmented legitimately in organically managed farms by the judicious use of magnesium based liming materials (IOFGA, 1995), recognising also the apparent significance of soil pH (above).

A negative correlation was found between energy output and the botanical specie, *Festuca ovina* ($r = -0.630$), present only on three of the sampled fields from farms J, R and T where it comprised 30, 58 and 17 percent respectively of the vegetation dry weight. Moss presence, recorded on six of the study farms, was also negatively correlated with production ($r = -0.546$), as in the literature (Lampkin, 1990). Vegetation dry weight was negatively correlated with production, more strongly so ($r = -0.632$ $P < 0.01$) than moist vegetation weight ($r = -0.615$, $P < 0.05$). Correlation of energy outputs with rye grass abundance was positive ($r = 0.447$) but insignificant. The negative relationships of vegetation dry weight with both energy outputs and *Lolium* presence are evidence that a grassy sward is a production asset.

A highly negative correlation was observed between energy output and *in vitro* nitrogen mineralisation activity recorded for field-moist soil ($r = -0.855$, $P < 0.01$), derived as the positive values for arginine ammonification (Chapter 5). This result is surprising, because nitrogen mineralisation should provide nitrogen for the growing sward, and would be expected to be, as Sparling (1997) suggests, a good microbial indicator of soil health. Instead, the negative relationship observed in the present study probably points to the importance of timing, and to the need for rapid mineralisation, ideally when plants demand it (Sparling, 1997). As noted in Chapter 5, the grassland botanical composition of the study farms was not significantly correlated with nitrogen mineralisation rates, however ammonification concurs, albeit insignificantly, with vegetation dry weight. Further study is required to clarify the effects of various mineralisation rates on sward development and grassland energy production.

Rapid rates of mineralisation, without accompanying plant uptake and growth as the results of this study imply, may lead to nutrient losses from soil amended with organic nitrogen sources. If rapid ammonification leads to nitrogen loss or reduces outputs, researchers should note that more microbial activity which arginine ammonification has been presumed to imply, is not necessarily better. The precise agronomic effect of any

microbial activity, and how that activity is influenced by management history, needs to be understood.

Examining variables in relation to field conditions, rather than their abstraction to soil dry weight equivalents, may prove useful, and is suggested in the marginally more significant relationship between energy outputs and arginine ammonification or mineralisation in field moist (given above) rather than dry soil equivalent value ($r = -0.794$ $P < 0.01$). Energy outputs were positively correlated ($r = 0.600$) with immobilisation. Observed on only five study farms, the statistical significance of relationships with immobilisation could not be established at Spearman's $r < 0.900$ (see Section 4.3.8).

6.4.2 Gross margin per hectare

Table 6.2 summarises gross margin information based on figures for income and loss recorded during interview with participant farmers. Gross margin for each farm is given in the data spreadsheet in Appendix D.

Table 6.2

Gross margin per hectare estimated from income, including sales, premia, grants and headage payments, exclusive of farm costs, per hectare of grassland

	Min.	Max.	Mean	SD
Gross margin (£ ha ⁻¹)	-83.63	761.13	259.98	232.98

Gross margin differs considerably amongst the sixteen farms. One farm shared an enterprise for which the farm supplied raw materials for a business partner, thereby incurring financial losses, on paper. The financial status of the business partner was not disclosed. The identity of particularly high earning farms is not given, for reasons of confidentiality. Although confidentiality was assured, financial descriptions may not give a reliable production estimate, because of either fear of disclosure for taxation purposes, or a desire to impress the researcher as to the farm's profitability. The applicability of gross margin as a research tool and as an objective measure of grassland

production is in doubt, for many reasons. A negative balance recorded in one farm suggests that inputs exceeded outputs financially, however the year in which a financial loss characterised by reasonable good energy outputs, 37 GJ ha⁻¹, in that year. Gross margins also take financial aids, such as grants and premia into account. Therefore, as in the studies reviewed by Reganold (1995), gross margin may not necessarily reflect farm yield. Lack of association was seen in the differing directions of, and insignificant correlations between, gross margin and energy outputs ($r = 0.397$) and LU ha⁻¹ ($r = -0.564$) observed in the present study. Furthermore, as no significant correlations appeared to exist between gross margin and any of the measured management features such as pH, or grazing, or with soil physical, chemical, site related, and biological conditions, it can be concluded that gross margin is not a very sensitive measure of grassland production.

Gross margin was, however, significantly correlated with certain fertiliser applications made to the sampled field in the six years prior to farm production assessment. Grassland production correlated significantly with mineral phosphate and potassium ($r = 0.666$, $P < 0.01$); with organic nitrogen sources ($r = 0.556$, $P < 0.05$); total added nitrogen from mineral and organic sources ($r = 0.620$, $P < 0.05$); and total added potassium ($r = 0.629$, $P < 0.05$). Gross margin was inversely correlated with the immediate interval without mineral nitrogen applications to the sampled field ($r = -0.649$, $P < 0.05$). Fertilisers, calculated on a per hectare basis for an entire farm, were correlated to a lesser extent than applications made to the sampled field. Two correlations were observed, namely with mineral P and K applications on a per farm hectare basis ($r = 0.582$, $P < 0.05$) with grassland production. Why applications to the sampled field should match profits better than those made to the entire farm is not understood. However, as was observed with energy outputs, it could be inferred from correlations that gross margin reflects fertiliser applications, and may decline in the absence of mineral nitrogen application particularly.

On comparison, facilities with larger animals such as dairy cows (farm L) or cattle for suckler beef production (farm E) were more profitable than those with smaller animals such as goats (farm R).

Gross margin revealed little about soil factors that might influence production itself. The fact that profitability itself did not correlate significantly with any biological

variable supports the view that it does not serve as useful gauge of soil fertility for the purposes of comparison. The apparent disconnection between gross margin and soil biology, for example, may arise because of anthropogenic influences such as farmer ingenuity, or other conditions such as market forces, which directly affect income and costs, whilst probably having little connection with soil itself. Gross margin, therefore, is deemed unsatisfactory as a measure of either soil fertility or grassland production for the purposes of this study.

6.4.3 Livestock units per hectare

Livestock units per hectare are summarised in Table 6.3. Results for each farm are presented in the datasheet in Appendix D.

Table 6.3

Livestock units per hectare of grassland summarised for the sixteen study farms

	Min.	Max.	Mean	SD
Livestock units per hectare	0.72	4.35	1.66	0.87

Numbers range from very low, to what seems to be an unusually high figure, 4.4, LU ha⁻¹ on farm O, which together with farm R, also a goat farm with 2.8 LU ha⁻¹, appear extreme. Goats are, however, light animals, and their tendency to browse rather than graze is regarded as capable of improving hill grazing (Cooper, 1995). All other farms range from the minimum value (0.7 LU ha⁻¹) on farm P, which is grazed throughout the year, to the highest value otherwise recorded at 1.98 LU ha⁻¹ on farm E, specialising in suckler beef production.

Significant correlations between stocking density and farm management occurred only in relation to soil pH ($r = 0.599$, $P < 0.05$), and with the length of time for which farms were organically managed ($r = 0.602$, $P < 0.05$). Soil pH is a known fertility indicator, and it is likely that nutrient applications during organic management have been beneficial. The fact that both goat farms have extreme livestock numbers, and are organically managed, is likely to have strengthened the relationship between livestock

units per hectare and organic farming too. Southern aspect ($r = -0.589$, $P < 0.05$), percentage clay ($r = 0.567$, $P < 0.05$), and cation exchange capacity ($r = 0.503$, $P < 0.05$), correlated significantly. The correlation was inverse with southern aspect, meaning that the more northerly farms had lower stocking density, as might be expected, because south-facing land is usually more productive (Brady, 1990). Similarly, cation exchange capacity is a beneficial soil quality (Brady, 1990).

Some botanical species, found on too few farms to establish statistical significance, seemed important when computed in relation to stocking density on the basis of sixteen farm values. Correlations were as follows: *Rumex acetosa* ($r = 0.570$), on the two goat farms; *Taraxacum officinale* ($r = 0.566$) on two suckler beef and two goat farms; and *Bryophyte* spp ($r = -0.571$), on six more extensively grazed farms with lower pH. Mosses were found on six of the study farms, (A, H, J, N, P, and T), only one (farm H) of which had had manure applications in the years preceding botanical analysis. Values for moss-containing farms were below the mean for either soil plant available phosphorous (all except farm H) or soil plant available potassium (all except farm A). Lack of nitrogen applications together with below-mean values for the important plant-available nutrients P and K where mosses were found concurs with the general association of moss species with unfertile soil conditions (Frame, 1992), and helps explain the inverse relationship between mosses and stocking density. This was further substantiated by the correlation of moss presence with extensive grazing (Section 4.4.4). Effects of grazing animals on botanical species composition are inextricable from production measured as stocking density, because soil fertility plays an important role in both, and because botanical species are affected by grazing events.

Stocking density measurements did not correlate significantly with any of the microbial components recorded. In contrast, significant relationships emerged between grazing events and soil microbial components, as discussed in Chapter 5. It can be concluded that production output values expressed as stocking density for the entire farm are unlikely to be predicted reliably by soil characteristics that are affected by grazing duration, frequency, and animal number, as imposed by the grazing practices on any particular field.

6.4.4 The search for interactive production determinants

Although measures of grassland production have correlated significantly with soil properties, it is likely that influential factors are part of integrative processes involved for example in plant growth (Moore and Russell, 1972). Factors that influenced production may have impacted on each other in ways that were not immediately perceived using bivariate rank correlation methods in which single variables were examined in relation to production, one at a time. Other statistical tools are needed to analyse the relationships of several independent variables with production, and to establish their relative importance. Multiple linear regression analysis would serve this purpose (Gonick and Smith, 1993).

6.4.4.1 Selection of a suitable dependent variable

How production relates to soil properties is the focus of this study. Production values, therefore, will be posed as the dependent variable. For a number of reasons, observations suggested that grassland production was best represented by energy output (GJ ha^{-1}). Firstly, energy outputs encompassed the entire animal, milk and fodder production of each farm. Secondly, values were within the expected range. Thirdly, values were regarded as objective, having been calculated from farm data in contrast to financial data. Farm data, unlike financial details, had been given without apparent fear of disclosure amongst participant farmers. Energy outputs should therefore provide an unbiased gauge against which possible contributory factors could be appraised. Stocking density (LU ha^{-1}), a possible alternative production measure, fails to take account either of non-grassland fodder inputs from the farm or grassland fodder sold off the farm. This is supported by lack of correlation between stocking density and energy outputs in the present study. Similarly, profit margin correlated well with fertiliser inputs but bore no relationship to soil properties observed in the study farms. Of all three values, energy outputs seemed to represent grassland production best. The variable, energy outputs per hectare, was adopted as the dependent in multiple regression analysis so that the combined roles of several soil properties could be examined in relation to it.

6.4.4.2 Multiple regression models

Combinations of management factors, soil chemical, physical and site related parameters, botanical grassland composition features, and biological variables, were selected systematically as possible predictors for inclusion in a series of linear multiple regression equations. Linear multiple regression equations were built in a stepwise manner, as described in Section 6.3.3. All putatively independent variables describing: farm nutrient applications; grazing practices; soil physical, chemical and site related parameters; botanical composition of the grassland; microbial biomass, populations and microbial and enzyme activities of the study farms, were assembled for inclusion. Equation 1 emerged and is presented in Table 6.4.

Table 6.4

Equation 1. Built using energy outputs GJ ha^{-1} as the dependent variable with the majority of putative independent management, soil property and botanical composition variables as possible predictors

Dependent variable: energy outputs	Unstandardized Coefficients
Constant	19.404
Independent variables, in order:	
Root mass	-20.227
Biomass C:N ratio (n=14)	6.883
Arginine ammonification (n=15)	-.800
Vegetation mass	-.206
Nitrogen fertilisers applied to farm as a whole over six year period	8.276E-03
Lime applied	.558
Microbial biomass as % of soil OC	3.311
Pore space	0.272
Soil plant-available phosphorus	-.250

$R^2 = .998$

The model given as Equation 1 explains approximately 99% of the variance in the dependent variable, energy outputs.

Certain predictors, namely root mass and vegetation mass, included in Equation 1 have already been indicated as significant factors in inverse relation to production. Root mass appears to be the most important predictor, and is supported by its highly negative correlation with energy outputs. Vegetation mass influence is also negative. Variables

such as root mass and vegetation mass, that were significantly correlated with production in rank correlation analysis and discussed already, are not given detailed consideration here. Newly important variables are considered, however.

Biomass C:N ratio appears to play a positive role. This measurement involved extrapolation from substrate-induced respiration values to determine microbial biomass carbon content. Values for farms E and F had been regarded as erroneous (Section 5.4.1) and were replaced with the mean for multiple regression analysis purposes, meaning that 14 cases were available for computation. Higher biomass C:N ratio, indicated by its positive role in the equation, may imply that relatively more carbon is sequestered and available for microbial respiration in soil. It also represents a higher glucose-responsive biomass fraction compared to the total biomass content. A more active microbial biomass component may be beneficial to grassland production, however the bases for relationships mentioned are not clear, and further investigation is required.

Arginine ammonification rates, inclusive of mineralisation and immobilisation, influenced production inversely. Originally, correlations had not been established with arginine ammonification rates overall, but had been significant in the relation to mineralisation rates. Significance of arginine ammonification in Equation 1 is probably attributable to the fact that there were 15 values to compare, instead of either 10, or 5, as would have been the case with either mineralisation or immobilisation values alone. It is likely that a better match with energy outputs could be achieved from the larger database.

The total amount of nitrogen applied is a predictor in Equation 1 (Table 6.4). This quantity of nitrogen refers to amounts applied per farm hectare in the six years prior to the analysis of production output. The importance of fertiliser inputs in relation to energy and gross margin has already been discussed. Nitrogen inputs contribute positively, and although only six farms have applied nitrogen of any form, fertiliser nitrogen appears, as expected (O'Sullivan, 1968), to have been an important production factor. Lime applications were also beneficial. Correlation with lime application had not appeared in bivariate analysis.

The microbial biomass proportion relative to soil organic carbon is a positive production component. This serves as a focus for the distinction between soil quality and production assessments. Earlier, negative correlations were found between this value and soil aggregate stability as well as with organic carbon content itself (Section 5.4.1). It would seem that higher proportions of microbial biomass in soil organic matter indicate poorer soil quality conditions. Observations suggest that 'soil quality' may not be synonymous with grassland production, contrary to the definition of Smith et al. (1993). Relationships observed in the present study between production, nitrogen applications, organic carbon and relative biomass content imply that, although N-fertilised soil had higher grassland outputs, mineral nitrogen applications may lead to deterioration in soil organic matter quality. How microbial biomass functions as part of soil organic carbon and in relation to nitrogen inputs and grassland production remain to be elucidated.

Positive inclusion of pore space is unexpected because this value is highly correlated with root mass, which has negative effect. Negative influence of plant available soil phosphorus is also inexplicable. Whether the model arrived at, which was ninth in the stepwise process of entering and removing variables, ultimately included too many variables and therefore tended to 'overfit' the dependent (Legendre and Legendre, 1998; SPSS, 1995) is not known in regard to the equation presented. Furthermore, as the pore space and soil available phosphate variables were added in equation eight and nine, respectively, only increased the regression coefficient (R squared) by a minute amount, they should be eliminated (Legendre and Legendre, 1998).

6.4.4.3 Constraints of multiple regression analysis

Although Equation 1 has revealed some information additional to that available from bivariate analysis, multiple regression analysis is subject to certain constraints. Firstly, data should be complete for all variables (SPSS, 1995). Attempts were made to build a comprehensive and realistic equation from the dataset. However, values for some variables were missing in certain cases. For example instead of sixteen values, fifteen were available for arginine ammonification, ten for mineralisation, and five for immobilisation. Steps can be taken to circumvent this problem. Variables without valid values for all cases can be excluded list-wise. For pair-wise exclusion, only those cases

with complete data for the pair of variables being correlated are used to compute regression coefficients.

However, when cases with missing variables were excluded list-wise, computation failed because no valid cases remained. This may seem surprising. However, one hundred and thirty variables were selected for inclusion from the dataset, and values were missing for certain variables. Exclusion of cases pair-wise was also unworkable because variables were either constant or because correlations were missing, as annotated in the SPSS results output (SPSS, 1999). Equation 1, therefore, had been possible only when user-defined missing values were replaced with the mean. Two variables in the resultant equation, namely arginine ammonification and biomass C:N, lacked one and two values, respectively. The relative importance of variables may have been influenced by this step.

Key production-related variables such as soil pH, and plant available magnesium content have not emerged in the equation. Each of these variables, soil pH, root mass, plant available magnesium content and soil depth can be seen in Chapter 3 to be significantly related to at least one if not all of the others mentioned, or to have been correlated with variables already in the equation. Highly correlated variables are likely to cause computational difficulty because they inflate the variance of the regression coefficients (Legendre and Legendre, 1998) that indicate the proportion of variance explained by the regression model (SPSS, 1999). Similarly, when Burket and Dick (1998) used stepwise multiple regression analysis to model nitrogen mineralisation, it was found that highly correlated variables including biomass nitrogen and enzyme activities were excluded from the resultant equation because of multicollinearity, and only two variables emerged as reliable predictors in their analysis.

Furthermore, according to Legendre and Legendre (1998), if further stepwise multiple regression analysis is carried out, a different subset of variables that would explain even more of the variance might be found, and there is no guarantee that a better formula does not exist. This suggestion was put to the test in the present study, and further equations were built using different data subsets. As the highest R squared value was found in Equation 1, in other words this particular model explained the largest proportion of variance amongst equations found, it was deemed satisfactory. In

addition, multiple linear regression analysis is based on linear relationships (Gonick and Smith, 1993), with which biological responses may not be compliant (Zar, 1996).

6.4.4.4 Further linear multiple regression analysis, bearing constraints in mind

Although multiple regression analysis can be problematic, it could be applied in a more measured fashion to identify important variables. The constraints, listed above, were borne in mind. Only those soil properties for which at least 14 values were available were included in the analysis. Data pertaining to nutrient inputs, grazing and botanical aspects were excluded. Equation 2 emerged, and is presented in Table 6.5.

Table 6.5

Equation 2. Built using energy outputs GJ ha^{-1} as the dependent variable together with each soil property for which data were complete in 14 cases

Dependent variable: energy outputs	Unstandardized Coefficients
Constant	-77.945
Independent variables, in order:	
Root mass	-25.590
Biomass C:N ratio (n=14)	9.646
Pore space	1.815
Arginine ammonifying organisms	-1.192
Soil plant available magnesium	7.157E-02
Altitude	-5.750E-03

$R^2 = .993$

In order to develop this equation, cases E and F would have been excluded list-wise because of their doubtful biomass C:N value. All remaining cases would have had the full complement of data.

The importance of root mass, biomass C:N ratio and pore space has already been demonstrated in Equation 1, Table 6.4. Reappearance of pore space in a positive way here suggests that root mass and porespace, although related, have different functions. Root mass probably belies the age and condition of the sward based on earlier

observations of root mass in relation to historical reseeded and aeration or disturbance operations that had taken place previously, whereas pore space may indicate soil structural quality and aeration and or penetrability at the time of sampling. Soil content of plant available magnesium has already correlated significantly with energy outputs.

New in this equation, arginine ammonifying organisms play a negative role. Whether this is linked with actual arginine ammonification activity *in vitro* or in the field has not been established, but it is likely that microbial nitrogen mineralisation would depend on the bacterial responsible for that activity. As mentioned, presence of a bacterial population should not be equated with its activity (Coyne, 1999). Although the population would be required, arginine ammonification would be influenced by intracellular control and carbon availability (Cunin et al., 1986), amongst other things such as soil pH.

Altitude also has negative influence. The majority of the farms were of the low land variety, and none could be classified as being a hill farm. Altitude was, however, negatively correlated with soil depth, a factor that may have been excluded from the equations given because of its multicollinearity with other variables.

A final equation was built in which all soil property information for which data were complete in each case were included. Equation 3 is given in Table 6.6.

Table 6.6

Equation 2. Built using energy outputs GJ ha^{-1} as the dependent variable each soil property variable for which data were complete for each of the 16 study farms

Dependent variable: energy outputs	Unstandardized Coefficients
Constant	-9.939
Independent variables, in order:	
Root mass	-35.252
Microbial biomass C as % of soil OC	11.897
Pore space	1.480
$R^2 = .797$	

Variables that emerged as important in Equation 3 have been seen in similar roles already. However, the third model as presented here accounts for 80% of the variance in

the dependent, energy outputs. It would appear that additional variables would be required from amongst soil property data to better explain the role of soil properties as grassland production determinants.

In conclusion, it can be said that linear multiple regression analysis helps important features to emerge, but is subject to constraints such that it is unlikely that any one formula will suffice, if data are either not available or are highly correlated, as in a study such as this.

6.4.4.5 Identification of production indicators from amongst soil properties

One of the aims of this study had been to identify grassland production indicators. The high correlation of grassland production, measured as energy outputs, with soil pH, depth, soil plant available magnesium and calcium contents, suggests that the rank correlation search method has been satisfactory, as each of these elements is a known production influence. In addition to recognisable factors, two other soil properties, namely root mass and N-mineralisation rates *in vitro*, emerged, albeit negatively, as energy output predictors. Followed by linear multiple regression analyses, it was found that additional factors such as the proportion of biomass carbon present relative to soil organic carbon was influential, but this may be an artefact of carbon change in N-fertilised soils. Linear multiple regression analyses in the present study repeatedly demonstrated the importance of root mass in grassland production. Those analyses also suggested involvement of N-mineralisation processes indirectly as arginine ammonification rates and as numbers of arginine ammonifying organisms present in the sampled soils.

It appears that two parameters, namely root mass and N-mineralisation *in vitro* were consistent in their negative relationship with grassland production. Whether either of these could serve as a grassland production indicator for soil assessment purposes is of interest.

Visual assessment of grassland productivity is usually based on the above-ground crop response to changes in management for example, monitored in the field either as leaf

area index or sward height, or measured as herbage dry matter per hectare (Frame, 1992). However, root mass was strongly related to energy outputs, reseeding and soil aeration, and may be a useful indicator of sward condition and of production potential. Whitehead (1995) had contended that it was difficult to measure the weight or length of roots grown in the field, and difficult to separate living from dead roots. In the present study, general root mass was measured with relative ease, using extracted soil cores that were washed, dried overnight and weighed, with minimal disruption to the farmed soil, described in Chapter 3. Compared to the grazed sward, root mass may be less impacted by grazing events, and may be simpler to measure than any enzymatic or other microbial methods suggested as production indicators to date. This study suggests that the general extent of root mass is relatively easy to determine and may be a useful, albeit negative, indicator of grassland production potential.

In addition to root mass, it is likely that rapid nitrogen mineralisation rates predict relatively poorer grassland production outcomes. Sparling (1997) has suggested that the mineralisation of organic nitrogen “has potential as an indicator of soil health because it reflects previous biological activity in soil, general decomposer activity as in ammonification, and specialist groups required for the oxidation of ammonium and nitrite”. In this study, the strongly negative association between nitrogen mineralisation and energy outputs from grassland suggests that rapid rates of nitrogen release from soil organic matter may not be opportune for grassland production, and so has negative effect. The possibility that nitrogen mineralisation measured in arginine amended soil might indicate production should be examined, and the validity of this measure established.

For the purposes of this study, because ammonium nitrifier populations are more difficult to quantify (Nannipieri et al., 1990) and nitrate production rates require lengthy incubations (Magill and Aber, 2000), net nitrogen mineralisation was not measured but was interpreted instead from arginine ammonification assays *in vitro*. The literature to date has not relied on arginine ammonification as a measure of nitrogen mineralisation potential, but instead has applied arginine ammonification as a simple descriptor of microbial activity in soils. Nevertheless, when arginine ammonification was interpreted as mineralisation for the purposes of this study, its value, and its significant relationship

with energy outputs suggests, in agreement with Sparling (1997), that nitrogen mineralisation is a potential indicator of grassland production.

Application of arginine ammonification values in this way as nitrogen mineralisation would have distinct advantages. It would shorten assay time considerably to three hours incubation in the laboratory, whereas current mineralisation potential tests require two weeks (Abril et al., 2001). Furthermore, current nitrogen mineralisation assays are not yet proven reliable production indicators (Burket and Dick, 1998; Rasmussen et al., 1998). Whether arginine ammonification can serve this purpose should be examined.

6.5 Conclusions

Energy output estimation was a useful research tool. Unlike either gross margin or stocking density, energy output values facilitated identification of important farm and soil variables in relation to grassland production via rank correlation and multiple regression analyses. It is therefore proposed that energy outputs should be regarded as an objective measure of grassland production, and as a gauge against which productivity indicators might be assessed from amongst farm and soil properties in future.

Correlations suggest that effective grassland production requires: a good depth of soil; an appropriate soil pH; and adequate plant available soil magnesium and calcium contents. Production is less effective at relatively lower root mass contents and at higher rates of nitrogen mineralisation. It is hypothesised that rapid nitrogen mineralisation is not opportune for the growing plant, and that nutrient losses possibly occur, culminating in lower levels of grassland productivity. Poorer conditions may also be indicated by denser root mass, greater amounts of vegetation and in the relatively higher abundances of some botanical species and mosses in the sward. Many of these influences can be alleviated by established management procedures, however factors influencing nitrogen mineralisation rates in managed grassland need further exploration before specific practical recommendations can be made.

Along with the previously established influences such as soil depth, magnesium and calcium content and soil pH, new variables namely root mass and arginine ammonification rates, representing N-mineralisation *in vitro*, emerged as possible

production indicators. Both have merits. Root mass may be easier to quantify reliably than sward development, as it would be less exposed to grazing effects. The mineralisation assay adopted on this occasion is simpler to perform and less time consuming than mineralisation assays used to date (Alef, 1995b). Furthermore, no existing mineralisation assay has proved a useful predictor of production potential (Burkett and Dick, 1998; Rasmussen et al., 1998). More information is needed about the relationship between soil microbial biomass, organic carbon content and nutrient management. Although found important in multiple regression equations, and important relative to soil organic carbon too, the proportion of biomass present is more difficult to quantify and more subject to controversy (Rowell, 1994) than either the arginine ammonification assay or derivation of soil root mass. Soil microbial biomass may prove a better indicator of soil quality change and of limiting thresholds within soil organic matter pools, as neither soil organic carbon nor biomass contents correlated with production, but were interrelated.

It can be concluded that it is possible to assess grassland production and soil properties in terms of energy outputs, and that grassland production in the study farms is influenced by soil biology. However, the impact of soil biology is probably affected by nutrient and grazing practices to which the soil has been exposed, and integrative processes should be accounted for in the search for production indicators. Two emerging factors, root mass and N-mineralisation *in vitro*, warrant investigation as grassland production indicators and may have application in soil assessment programmes in future. In addition, the roles of the microbial pools in relation to soil organic matter content and function should be elucidated.

Chapter 7

Chapter 7

Explanatory mechanisms, general discussion, recommendations and conclusions

7.1 Introduction

The purpose of this research was to identify key factors and explanatory mechanisms for grassland production at low fertiliser levels or in the absence of mineral nitrogen. Key production determinants have been identified, satisfying the first objective. They are: nutrient inputs, soil pH, soil depth, magnesium, calcium, root mass, N-mineralisation *in vitro*, and soil disturbance history. It seems reasonable that in the absence of mineral nitrogen fertiliser applications, production would depend on soil biological function, but soil biological function and associated nutrient returns from legumes in the sward are not well understood (Parr et al., 1992; Newton, 1993; Degens et al., 2000). Explanatory mechanisms were therefore sought for non-fertiliser production determinants so that their roles in soil could be understood from biological and production perspectives. This research has been conducted by looking at all measured variables, particularly the soil biology, in relation to others that described grassland management, soil physical and chemical environment, grassland botanical composition, and production.

Many interactions occurred between biological and other soil components, explanations for which were inferred from observed statistical significance, known biological principles, and likely agricultural consequence. One argument, that available carbon is used before organic nitrogen, ('AC is used before ON') is proposed as the main explanatory mechanism governing nitrogen availability in grassland soils, in theory. The argument is based on the allosteric regulation and catabolite repression of arginine degradation for energy in the presence of readily accessible carbonaceous energy sources as reviewed by Cunin et al. (1986), and is supported by the significant correlations that occurred between ammonification and soil attributes in this study. High rates of ammonification are associated with lower levels of production, and suggest that ammonia may be released in a way inopportune for grass growth under certain conditions. However practical questions are as yet unanswered. For example, why

should ammonification rates and grassland production of two organically managed farms, L and R, differ so remarkably at the same soil pH?

The reason why questions are unanswered probably lies at the heart of the data set, because it encompasses over one hundred variables impossible to reconcile visually with each other in tabular form; yet examining relationships two by two gives a fragmented view. Ideally, multivariate methods should be employed where more than two variables are being considered simultaneously (Fowler et al., 1998), but many multivariate statistical methods would be unsuitable (Manly, 1986) because the majority of the data does not conform to the normal distribution, as considered in Chapter 2. Ordination methods could resolve these numeric difficulties for three reasons: (1) they do not rely on tests of statistical significance; (2) they expose patterns that reasonably explain variation and (3) they reduce the data set to manageable proportions (Fowler et al., 1998; Legendre and Legendre 1998). The present study is exploratory rather than deterministic and so ordination analyses are entirely appropriate (Legendre and Legendre, 1998; Palmer, 2000 a).

Section 7.2 describes the ordination methods implemented in this study. Results of the ordination analyses are presented with supporting evidence for production influences and explanatory mechanisms in Section 7.3. A general discussion follows in which outcomes, uniqueness and limitations of the study are considered, with recommendations. Conclusions are also presented.

7.2. Ordination analyses

Ordination procedures in multivariate analysis are means by which complex data can be calculated and displayed, relationships between variables discerned numerically, and results portrayed graphically in relation to the ordination axes (Legendre and Legendre, 1998; Kovach 1998; Palmer, 2000b). The resultant map in ordination space enables communication about patterns found. Two methods, namely principle components analysis (PCA) and canonical correspondence analysis (CCA) were employed. Terminology in ordination reflects its adoption for community analysis in ecology. Here, the terms ‘case’, ‘sample’ and ‘site’ mean ‘farm’; ‘species’ applies similarly for

botanical composition as in other ecological studies. The term ‘variable’ refers to attributes of soil; farm management; vegetation and or production.

7.2.1 Principal components analysis

PCA rotates a complex cloud of data points so that the maximum variance becomes visible in ordination space (Kovach, 1998; Palmer, 2000b). Each principal component or axis is a new variable that consists of linear combinations of contributory variables. The importance of each contributory variable is given as its component loading or eigenvalue for that axis. Together, those variables comprising that axis account for a percentage of the variance in the data set. Ideally, the largest proportion of the data should be described by the first principle component, while the proportion declines with subsequent axes. Principle components explain as much of the information in the data set as possible (Fowler et al., 1998), and help to identify the most important environmental gradients (Mathes and Ries, 1995; Kovach, 1998).

The relative positions of sample points or farms in ordination space are also informative. Farms that rate highly for a particular variable will be in extreme positions relative to the extracted axes (Kovach, 1998), while farms with low values for that variable, but high values for another, will be positioned at a distance on the opposite side and therefore easily discerned from the diagram, or from their positive and negative eigenvalue signs for that axis, reported in the results output.

In PCA, the axes are linear combinations of data, and assume that the data are in continuous measurement format (Kovach, 1998). PCA is useful for either linear or monotonic relationships that either increase or decrease but not both, however species do not usually relate with each other in this way (Palmer, 2000b & c). When PCA is calculated for variables that peak at optimal environmental conditions (Palmer, 2000 b, c, & g), an artefact termed the ‘horseshoe effect’ results. This artefact is serious because the horseshoe effect distorts the second PCA axis which then does not represent a true secondary gradient (Palmer, 2000b). In nature, the peak or unimodal response is typical of species that come and go across an environmental gradient. It is also seen in the way that enzymes respond to increased temperature environments. Similarly, soil biological components probably have optimal conditions for their activity (Coyne, 1999). The

unimodal response should be considered in the present study, but using methods other than PCA.

According to Palmer (2000 b), most environmental variables are likely to be monotonically related to underlying factors and to each other, in which case PCA is quite appropriate for analysis of samples, or farms, in environmental space. In the present study, several significant rank correlations have been found between variables. This suggests that linear relationships exist, and that PCA would be suitable for display purposes.

7.2.2 Canonical correspondence analysis

Unlike PCA, canonical correspondence analysis, CCA assumes a unimodal response and is best suited to the examination of species abundance in relation to environmental change (Palmer, 2000 c). CCA analysis is mathematically complex as it involves weighted averaging with multiple regressions (Kovach, 1998), and the axes in CCA are constrained to represent linear combinations of the environmental factors together with each of the other variables such that the two sets of data are directly related, and ‘correspond’ (Legendre and Legendre, 1998).

Arrows (Euclidean vectors) represent environmental data in CCA diagrams and show the trend for that variable; the length of the arrow indicates the degree of that environmental change encountered amongst cases in the study; and the direction of the arrow is associated with an ordination axis. Drawing perpendicular lines from any variable to an arrow indicates the environmental preference for that component (Kovach, 1998), even if arrows are extended to facilitate this (Qiu et al., 2001). Closeness in any CCA diagram will indicate similarity (Kovach, 1998; Chamberlain et al., 1999) of farms with respect to those environmental variables implemented in that ordination. An arch effect may occur in CCA diagrams across a long environmental gradient, and results from data reduction processes whereby the two axes, supposedly independent, become mathematically related. This may be overcome by detrending whereby axes are divided into a chosen number of segments and rescaled so as not to curl results back towards each other (Kovach, 1998).

Because each site in the CCA map is derived from species' as well as site' scores for each variable measured so that they correspond, it is 'synthetic' and doesn't give an absolute measure of distances in relation to mapped variables (Micheloud, 1997). Yet because each site point incorporates all variables, and because CCA deals appropriately with unimodal responses, it may reveal aspects not seen by other means.

7.2.3 Applicability of ordination procedures

Although ordination methods are best suited to relatively long environmental gradients, the apparent predominance of linear relationships in this study suggests that only portions of environmental gradients may have been sampled. Short gradients are understandable in working grassland farms that are necessarily non-extreme, as exemplified by pH range from 4.9 to 7.2 in the study farm soils (Section 2.4.1) Nevertheless, farms varied in many respects, and useful patterns can be anticipated.

Patterns can be recognised in ordination space if there is a scatter of known entities along a gradient. Different species serve this purpose in community ecology, and act as reference points (Palmer, 2000 d) especially for indirect gradient analysis where the environmental gradients are not specified by vectors. Here, farm points were identified as either circles, triangles or squares for high, average and low production levels respectively, and were used as signposts for production-related gradients in ordination space.

A systematic 'data diving' (Palmer, 2000 a) approach was adopted in order to contain as much information as possible yet at the same time reduce the data set sensibly. Data diving involves making inroads into the data set to try out combinations of variables in relation to each other. As much of the data can be included as the researcher deems fit, or a selection of data can be processed based on sheer curiosity, hunches, or existing significant relationships (Palmer, 2000a & e). Data diving is acceptable for exploratory research, as it is a means by which the researcher can learn more about the data set, find important factors contributing to variation, identify important environmental gradients, and begin to formulate hypotheses (Palmer, 2000 a). Hypotheses generated in this way may be subjected to objective and rigorous experimental and statistical scrutiny, and may serve as the basis for further work (Palmer, 2000a).

7.2.4 Implementation of ordination procedures

7.2.4.1 Ordination methods

PCA and CCA were implemented with the Multivariate Statistical Package, MVSP-Plus version 3.1 (Kovach, 1998).

7.2.4.2 Treatment of data prior to ordination

Data were included according to the requirements of each procedure. Categorical variables were excluded from CCA as advised (Kovach, 1998), and included occasionally in PCA where their contribution was thought appropriate. Data were not manipulated or transformed because ordination analysis does not assume data to be normally distributed (Mathes and Ries, 1995), and because CCA is based on a randomisation test (Palmer, 2000 f).

Data had originally been measured on different scales and varied in magnitude, and calculations in PCA were based on an eigenanalysis of the correlation, rather than covariance, matrix (Kovach, 1998). Data were standardised and centralised prior to PCA analysis as an MVSP option (Kovach, 1998), so that with a standard deviation of one, and mean equal to zero (Legendre and Legendre, 1998), all variables were implemented as having equal importance, to avoid errors of scale (Fowler et al., 1998; Kovach, 1998). CCA analysis was carried out on data without manipulation, and results were examined both with and without detrending (Kovach, 1998).

7.2.4.3 Interpretation of ordination diagrams

Farms are labelled according to their capital letter. As patterns need identifiable reference points, grassland production was arbitrarily assigned as being at high ($>50 \text{ GJ ha}^{-1}$), medium ($30\text{-}50 \text{ GJ ha}^{-1}$) or low ($< 30 \text{ GJ ha}^{-1}$) levels and marked with circular, triangular and square markers respectively, throughout Chapter 7. Markers are identified in the first of these ordination diagrams, Fig. 7.1. The explanation for each variable can be found in the key given in Appendix C, and data for each farm can be found in the datasheet in Appendix D.

The percentage variance explained by the axes is noted in the text describing each PCA diagram. However, PCA and CCA results are presented without reference to eigenvalues because relationships between farms and variables in PCA are self evident in the diagrams presented, and because the most important environmental gradients in CCA, identifiable as long vectors close to the axes, are abundantly clear.

7.3 Results of ordination analysis

7.3.1 What drives production, and how?

Individual variables that may ‘drive’ production have been identified in Chapter 6. In this section, patterns that help to explain how important factors influence production and interact with each other are sought.

PCA and CCA analyses were implemented according to an ordered data diving approach, beginning with larger numbers of variables. It was hoped that by including as many variables as possible, factors would not be unwittingly omitted, and relevant patterns could manifest (Palmer, 2000e & f). However CCA ordinations were extremely noisy, and uninterpretable for that reason at least (Legendre and Legendre, 1998; McCune, 1997). In PCA ordinations, the magnitude and direction of relationships between variables found throughout Chapters 2 to 6 were generally upheld in ordination diagrams. For example, greater intervals elapsed since mineral nitrogen had been applied were close to unproductive farm positions in ordination space; arginine ammonification was associated with farms where cellulolytic populations were lower. Data reduction was negligible because numerous axes were necessary to reveal the successive layers in the clouds of data points. Eigenvalues were very low, meaning that little of the variation was explained by the numerous variables included. The resultant diagrams were far too cluttered. The first two axes of 15 from a PCA implemented with most of the data set are included as Fig. 7.1, to illustrate. These two axes accounted for almost one third of the variation.

Fig. 7.1 shows that productive farms to the right of the diagram can be separated from farms diagonally opposite in which grazing is extensive, and root mass is higher, in agreement with nutrient inputs, soil pH, and grazing practices. Farm Q, conventionally managed, is in an extreme outlying position. Although implemented, arginine

mass, soil plant-available magnesium and calcium, and N-mineralisation *in vitro*, were implemented one by one, each with its company of variables significantly related to it. Table 7.1 gives the implemented variables, the number of axes extracted, and the percentage variation explained.

PCA is free from the problems of multicollinearity as found in multiple regression analyses in Chapter 6. Recall that variables such as soil pH, which should feature as production predictors did not appear in linear regression equations, probably because of being highly correlated with other variables already in the equation. In contrast to linear multiple regression analysis, PCA benefits from correlations between key variables because their relationships bring repeated and recognisable patterns into view. As such, PCA displays related variables unreservedly in relation to each other in ordination space. A production-related gradient was observed in each of diagrams listed in Table 7.1. Overlap, in which farms of contrasting production levels were sited close together, occurred to varying degrees along what were otherwise reasonable production-related inclines. Ordinations around soil pH and arginine ammonification are illustrated to shed light on how key factors interact and how soil might work in practice. Additional ordinations from Table 7.1 were similar and can be found in Appendix F. The best production-related gradients are presented here.

Table 7.1

PCA ordinations of key factors with variables significantly related to them

Key factor	Study variables significantly related to the key factor	Number of Axes extracted	Variance explained
Soil pH	Mineral N and P inputs; grazing intensity; interval since aerated; duration of organic management; root mass; plant available magnesium and calcium; N-mineralisation <i>in vitro</i> .	3	74%
Interval since mechanical disturbance	Soil pH; interval since mineral N applied; soil type; root mass, bulk density; porosity; polysaccharide content; N-mineralisation <i>in vitro</i> ; urease activity <i>in vitro</i> for moist and dry soil.	4	77 %
Soil depth	Interval since mineral N applied; days off land per rotation; days grazed in the year; grazing frequency in year; ratio of days grazed to a rotation; slope; depth of A horizon; <i>Cardamine pratense</i> ; dry weight of vegetation; amylytic population; N-mineralisation <i>in vitro</i> .	4	78%
Root mass	^a Soil pH; Organic and mineral N and K inputs to sampled field; P additions to farm; days on land in a rotation; number of animals per hectare; organic farming history; pore space.	4	80%
Magnesium	Soil pH; organic P added to farm; interval under organic management; root mass; % water stable aggregates; <i>Dactylis glomerata</i> ; proteolytic population, plant available calcium	3	78%
N-mineralisation <i>in vitro</i> (n=10) ^b	Soil pH; Mineral N applied to sampled field and to farm; interval since mineral N applied; interval since aerated; whether reseeded or not; soil depth, root mass; N-mineralisation <i>in vitro</i> .	3	81%

a. Originally, PCA implemented on 20 variables that correlated significantly with root mass resulted in 7 axes that together explained 87% of the data. The number of variables was reduced, for simplicity.

b. All of the ordinations except that for N-mineralisation *in vitro* were applied to sixteen farms. N-mineralisation *in vitro* was implemented first with 10, then 15 and 16 farms, as explained in context in this chapter.

7.3.2.1 Soil pH

Soil pH is one of the most important production parameters. As expected, however, it is highly correlated with other production influences, and is part of the integrated plant growth processes mentioned by Moore and Russell (1972). How soil pH relates with production, environmental factors and with soil biology is examined here.

7.3.2.1.1 How does soil pH ‘work’ in relation to the production environment?

Fig. 7.2 represents a principal component ordination of soil pH in relation to variables that correlated significantly with it. Three axes were extracted, accounting for a total of 74% of the variation. Axes 1 and 2, presented, explain 63%. A clearly defined production gradient has emerged across ordination space, and variables are characteristically placed relative to others, and to production. Correlated variables that would have been redundant in multiple regressions contribute meaningfully here, and can be “considered a blessing” (Palmer, 2000g) because they help patterns emerge.

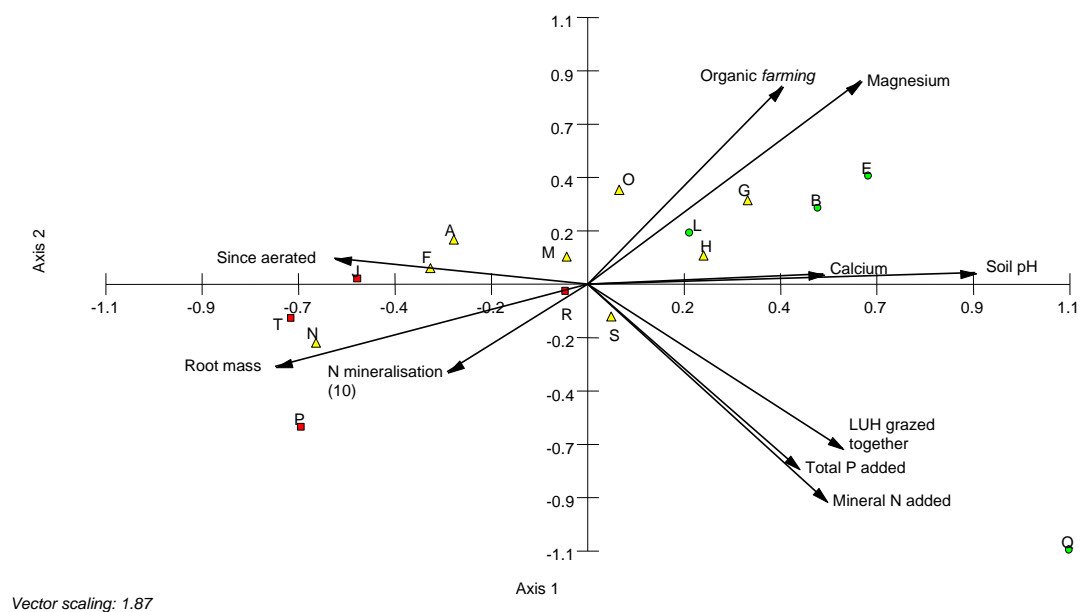


Fig. 7.2 Principal components analysis of soil pH together with variables significantly related to it for the sixteen study farms. Farms labels are capital letters. Square, triangular and circular markers refer to farms of lower, average and higher relative levels of production, respectively. Vectors are plotted as Euclidean arrows, the direction and relative length of which reflects the degree of correlation; variables closest to outermost arrow tips are most strongly correlated. A key to variable names is given in Appendix C.

Farm Q, the only conventionally managed site, is positioned in ordination space next to both high fertiliser inputs and soil pH, in an extreme outlying position. Other productive

farms, such as farm E and B in which mineral fertilisers have not been applied to the same extent, however, are closer to variables describing soil plant available magnesium and organic farming instead. It is likely that fertilisers give Q a ‘bypass option’ because of externally supplied nutrients, whereas other productive farms are positioned in a way that they appear to depend more on organic management and on intrinsic soil nutrient content for their production status.

Farms N, R, H and G overlap with farms of dissimilar production; however, most farms are distributed along a gradient that could be described by decreasing root mass, and simultaneously increasing soil pH, magnesium and calcium contents. The gradient is not absolute because farms at different production levels are close and their relative production levels overlap.

Fig. 7.2 shows that soil pH is not an isolated factor but contributes jointly with others such as magnesium content and mineral fertiliser additions that each have a role to play. Production had correlated significantly with soil pH, and might have been interpreted as a direct pH response. However, the correlation with soil pH and its relationships with other key factors deserve closer scrutiny.

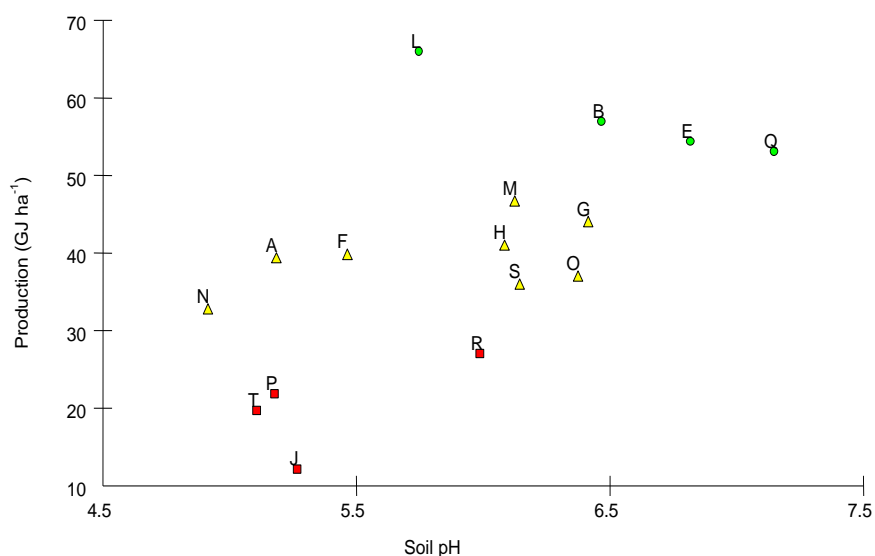


Fig. 7.3 Scatter graph of soil pH (x axis) and farm production (y axis).

The effect of soil pH is usually examined by posing it as an independent variable, x , as in fig. 7.3. It can be seen that while production increases relative to soil pH, farms at

similar pH values, for example farms R and L, have contrasting production outcomes. This “messy scatter graph” is typical of response curves in which linear regression is carried out to fit the best line (Gonick and Smith, 1993). In reality, variation is accounted for statistically, and the so-called ‘linear’ response is just a good approximation. The strength of the relationship is affirmed by the positive correlation between them (Section 6. 4.1)

Ordination (Fig. 7.2) and scatter graph (Fig.7.3) results suggest that soil behaviour fluctuates in a way that singular variables cannot account for. However, because PCA ordination diagrams are multivariate and portray interactions between combined factors, they may represent the grassland production environment better than the relationship between just two variables displayed in either a response curve or scatter graph, or calculated as a statistically significant correlation.

7.3.2.1.2 Is soil pH influential or influenced?

Soil pH is expected to influence grassland production, and that it does has been observed here. For the most part in soil research, pH is regarded as ‘cause’, and the ‘response’ to pH as ‘effect’. This is understandable as soil pH affects plant nutrient uptake (Brady, 1990; Parkinson, 1995). Soil pH was also found to be a significant regulatory factor of organic matter decomposition in such varied environments as for example the Park Grass Experiment at Rothamsted, UK (van Bergen et al, 1998) and in humid tropical soils sampled in Costa Rica, Columbia, Peru and Brazil (Motavalli et al., 1995).

However, soil pH is also a consequence of environmental forces. Contributors to soil acidity and alkalinity such as precipitation, leaching, base saturation, and adsorbed cations are well documented (Brady, 1990). Yan et al. (1996), the first to explain soil pH changes during legume growth and application of plant material, found that soil pH declines initially due to the release of protons from plant roots, matched by reciprocally increased alkalinity within the plant shoot. Later, return of this plant material to soil increased pH again (Yan et al., 1996). Microbial processes also change soil pH. Without plant material being present, microbial activities such as ammonification and the reduction of organic anions produced alkaline conditions, whereas nitrification created

acidic conditions in acidic mineral soils (Conyers et al., 1995). Soil pH may be due in part to soil bacterial populations, because they alter the pH of their environment during substrate metabolism (Castro et al., 2000). Bacteria may influence soil pH indeed, as seen in the scatter graph based on observations in the present study (Fig. 7.4).

Under controlled experimental conditions, Fig. 7.4 might represent a unimodal ‘response’ of pH to bacterial population size. However, the graph arose from incidental observation and not from purposeful experiments, conditions were not controlled, and the population was not normally distributed, and so the prerequisite conditions for establishing a response curve (Zar, 1996) were not satisfied. Nonetheless, this was the clearest evidence that any unimodal response might occur amongst the myriad combinations of variables examined out of curiosity and not reported in the present study.

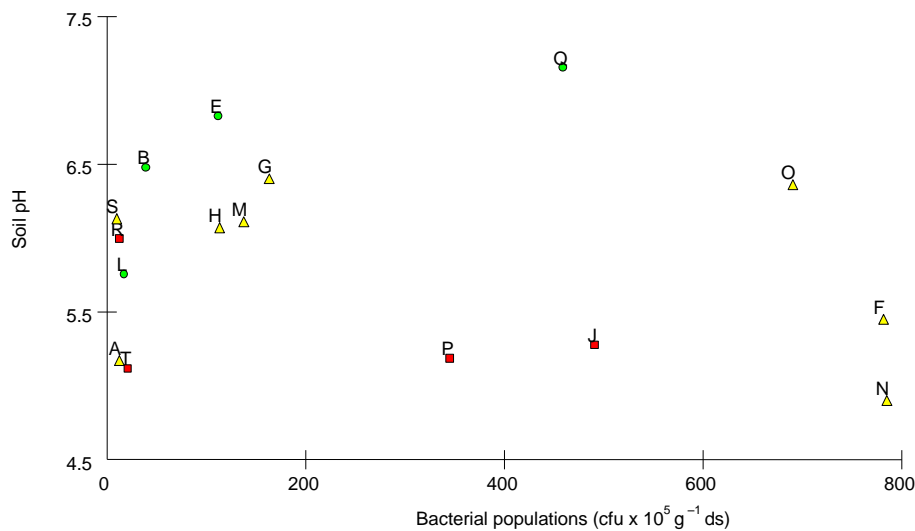


Fig. 7.4 Scatter graph of bacterial populations (x axis) and soil pH (y axis)

Even though their soil pH values vary, productive farms are clustered at non-extreme bacterial populations, except for farm Q. Higher bacterial population densities are characteristic of higher inputs (Bardgett and Cook, 1998), which may account for this. Two notable exceptions to the possible unimodal response, farm J and P, have the highest root mass values and are set stocked, unlike remaining farms have less root mass and were grazed and rested alternately. Root mass accrual suggests that turnover of soil organic matter is not taking place, and that available carbon is in short supply. Excretal deposits, as well as treading and herbage damage underfoot are probably

irregular. If substrates are not forthcoming from soil organic matter, excreta or detritus, as may be the case in farms P and J, bacterial metabolism and its environmental effects may be reduced. Whether environmental elements are causal or not is not established, but some interaction is likely, and it seems that soil pH is an interdependent aspect of the whole environment.

7.3.2.1.3 Does soil pH influence soil biology, and if so, in what way?

On the other hand, possible effects of soil pH on soil biological function should be elucidated in order to understand, and therefore manage, soil nutrient cycling processes. Correlations itemised in Chapter 5 were those significant ($P < 0.05$) correlations of other parameters with biological components. Lack of correlation of any parameters with soil pH may have gone unnoticed. Examined afresh but from a soil pH perspective, biological responses were found to have been individualistic and unpredictable, as summarised here. Microbial biomass, populations, and urease enzyme activity showed no correlation with soil pH. Some bacterial populations may survive best under optimal soil pH conditions but this was unclear. Arginine ammonification declined significantly with soil pH; however, the following scatter graph shows that mineralisation happens in farm R, and immobilisation in farms H and M, at roughly similar pH values (Fig.7.5)

Usually, pH is posited as the independent variable, x . Here in Fig. 7.5, the graph is inverted. Drawn in this way, it resembles a third order polynomial, and some sort of reciprocity may be going on between the two variables.

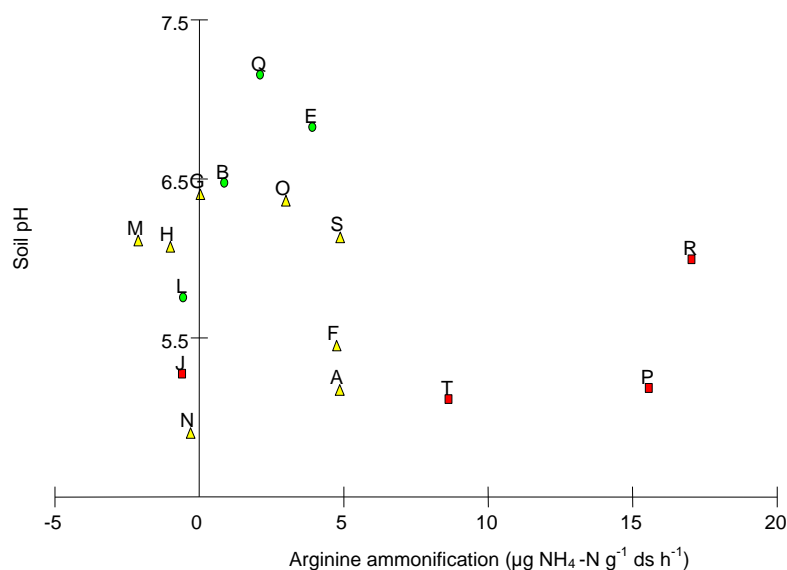


Fig. 7.5 Scatter graph of arginine ammonification (x axis) against soil pH (y axis)

7.3.2.1.4 Making sense of soil pH

The scatter graphs in Figs. 7.3 to 7.5 inclusive and incomplete correlations indicate non-absolute relationships between soil pH and other variables. Zar (1996) states that, for many kinds of paired biological variables, the magnitude of both variables changes simultaneously. So, to regard one or other variable as ‘independent’ would not be reasonable. Therefore, response curves may not be the best way to interpret soil biological function. Questions may also apply if soil pH and its environment are truly interdependent such that their ‘causes and results mutually give rise to one another’ (Kalu Rinpoche, 1997). Indeed the precise mode of soil pH influence has yet to be fully established (Motavalli et al., 1995). Mutual responses of soil pH and biology ought to be examined under controlled experimental conditions that satisfy statistical requirements.

In conclusion, it can be stated that all of the correlation, graph and ordination results suggest that soil pH acts in conjunction with other soil factors to influence production positively, but in an interdependent manner. Although key factors are highly correlated with each other (Chapter 6), ideal analytical methods are to hand however, because PCA displays interactive factors such as soil pH, soil plant available magnesium content and so on, unreservedly. Furthermore, key factors appeared in reasonable relation to each other along what emerged as a production-related gradient in ordination space.

7.3.2.2 Exploring N- mineralisation *in vitro* as a production indicator

The importance of N-mineralisation cannot be overemphasised, as it is the means through which plant nitrogen is made available biologically from legumes, herbage and detritus. Its significance has been substantiated in this study by high correlation of an N-mineralisation assay, carried out using arginine as substrate *in vitro*, with grassland production. It, like soil pH, has correlated significantly with other production influences (Table 7.1). Arginine mineralisation and organisms responsible for this activity have appeared as production predictors in multiple regression equations (Section 6.4.4.2, Tables 6.5 & 6.6). N-mineralisation *in vitro* may therefore have a role as a production indicator. This possibility is examined using ordination analysis here. Given their significant inverse relationship, N-mineralisation *in vitro* was examined as a potential bioindicator for grassland production in a series of ordination analyses.

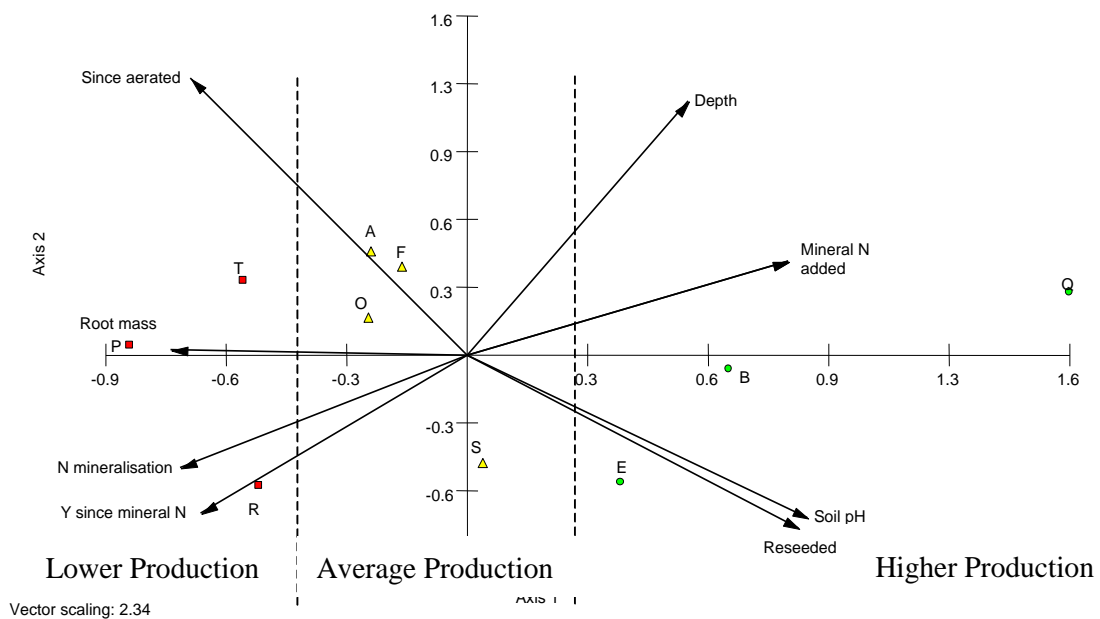


Fig. 7.6 Principal components ordination of factors related to N mineralisation *in vitro* for those ten farms in which positive values were recorded in the laboratory. Square, triangular and circular markers refer to farms of lower, average and higher relative levels of production, respectively. Vectors are plotted as Euclidean arrows, the direction and relative length of which reflects the degree of correlation; variables closest to outermost arrow tips are most strongly correlated. A key to variable names is given in Appendix C. Sectors are indicated in which farms have had apparently lower, average or higher relative levels of production, as estimated for the year following biological analysis.

Figure 7.6 is a PCA ordination of N-mineralisation *in vitro* together with factors significantly related to it. Three axes were extracted, the first two of which are shown. They account for 66% of the variation in the data; the third axis explains a further 15%, three axes accounting for 81% in total. The ordination involves just those ten farms with positive arginine ammonification values. Statistical relationships are upheld in that the greatest N-mineralisation rates occur in unproductive situations that have not been aerated or have been without fertiliser applications, and have higher root-mass. N-mineralisation rates are lower in productive, deep or reseeded sites, and where mineral nitrogen has been added, in soils at higher pH. Farms of average production are located midway in ordination space between productive and unproductive situations. Production declines without overlap along Axis 1, from right to left. The ordination is evidence that a production gradient exists in sampled soils. As was the case for the pH -related gradient, the N-mineralisation gradient is not absolute with respect to any single variable. Instead, production related gradients derived either from soil pH or N-mineralisation relate to a suite of variables mapped in ordination space.

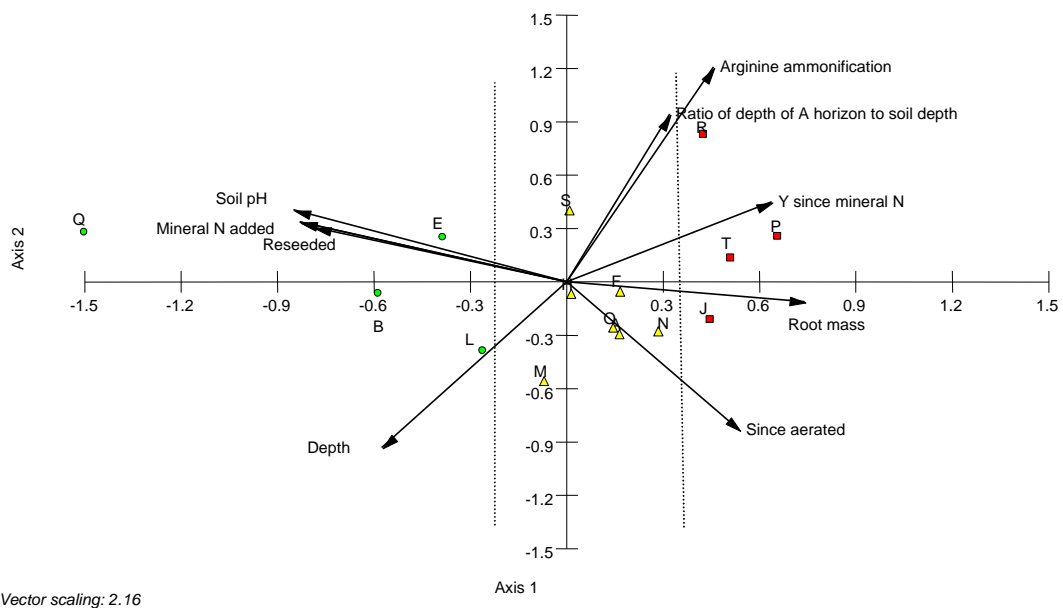


Fig. 7.7 PCA ordination of arginine ammonification for fifteen farms for which values were available, with factors significantly related to N-mineralisation *in vitro*. Legend is as for Fig. 7.7, except that all arginine ammonification values are included.

The first of the ordinations using N-mineralisation as the basis (Fig. 7.6) has been implemented with only those 10 farms for which positive arginine ammonification values had been obtained. Ordination was repeated, but with all values for arginine

ammonification to see whether a similar gradient might apply to those farms where N-immobilisation had occurred *in vitro* (Fig.7.7). Fifteen farms were implemented, because arginine ammonification values were not available for farm G.

In Fig. 7.7, production was successfully differentiated along Axis 1 and, although farms were in the opposite order to that found in Fig.7.6, the relative positions of farms and variables were unchanged. Again, three axes were extracted, in total accounting for 73% of the variation. The first two, shown, explain 59%.

One further ordination (Fig. 7.8) was implemented without arginine ammonification values but with farm G, so that its position relative to other farms could be established, and the reliability of the production related gradient explored.

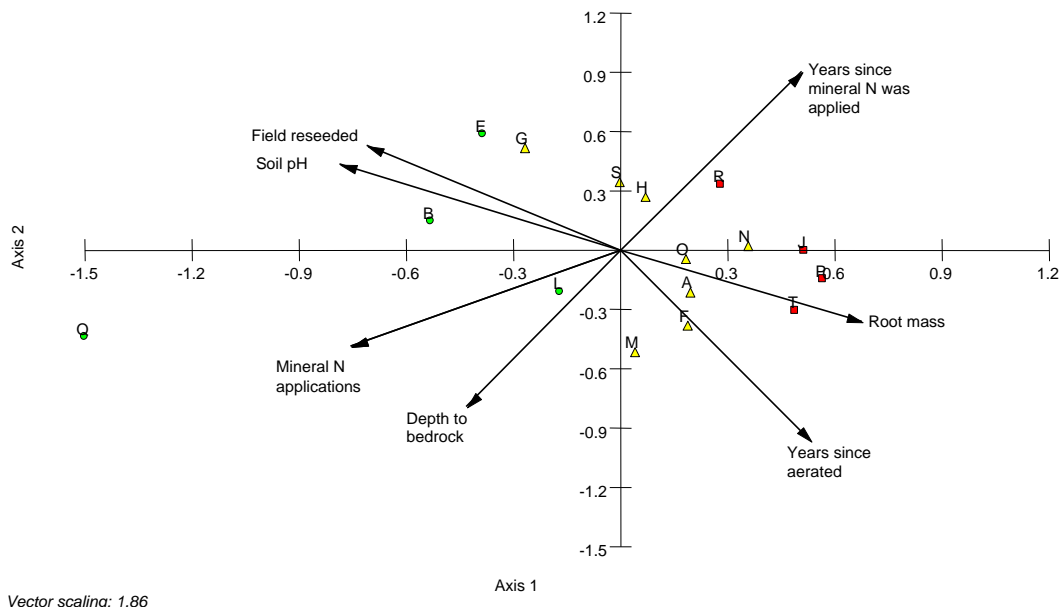


Fig. 7.8 PCA ordination of all sixteen farms with variables significantly related to N-mineralisation *in vitro*. Legend is the same as that for Fig. 7.7, except that arginine ammonification values have not been included.

The PCA ordination portrayed in Fig. 7.8 was implemented without direct use of arginine ammonification values and with all farms. The ordination extracted three axes as before, accounting for 75% of the variation. The first two, shown, explain 62%. This fresh ordination (Fig. 7.8) shows that the production gradient seen in Figs. 7.6 and 7.7 still holds, albeit at an angle to the first Axis. In fact farms G and M, closest to productive farms, rated best amongst the intermediate group at 44 GJ ha⁻¹ and 46 GJ ha⁻¹ respectively, while others range from 30 GJ ha⁻¹ upwards. Productive farms were rated from 50 GJ ha⁻¹ upwards. Results suggest that N-mineralisation and variables significantly related to it describe the production environment fairly well, whether arginine ammonification is present as a value or not.

In conclusion, it can be stated that N-mineralisation and its significantly related factors should be examined as a grassland production indicator suite in PCA.

7.3.3 How does the soil biology ‘work’?

In the present study, arguments that ‘AC is used before ON’ and substrates might regulate bacterial populations, have been proposed, supported by bivariate analyses. An environmental view might help to unravel how soil biology functions.

7.3.3.1 Environmental factors and soil biology

All variables that had correlated significantly with biological components were listed in Table 7.2 in order to facilitate data diving for their ordination analysis.

Individualistic responses of different biological components were exposed in this tabulation process even prior to ordination. Distinctions are noteworthy, and are summarised in this paragraph. Biomass behaved as part of soil organic matter and played a dynamic role in soil structure. While the overall bacterial population may have depleted organic carbon through its respiration, proteolytic organisms may have required soil organic matter as substrate. Biomass associated more with the total organic matter pool than with events or situations likely to change transient pool components. Biomass contrasted with bacterial populations that reflected previous events such as lime inputs. The response of activity to environment (e.g. soil pH) was the most immediate of all cellular (biomass, populations and arginine ammonification) aspects. Urease, a soil enzyme, was not specifically substrate related, but may depend on stabilising factors.

Because biomass reflects organic matter, populations seem to recollect events, and cellular response is more immediate, it can be concluded that their temporal relationships differ. Such distinct temporal responses may have practical application in the biological assessment of soil change in the long and short term.

Table 7.2

Environmental variables related to soil biological components

Significant environmental variables	Associated with biological component	
	Directly	Inversely
Related primarily to biomass		
Soil C: N ratio; polysaccharide content; Ratio of biomass N to soil N	Total biomass (NRN)	
Root mass; days rested in a rotation; ^a Ratios of biomass C&N to soil C&N	Responsive biomass (SIR)	
% Water stable aggregates		^a Responsive biomass
Related primarily to population		
Organic carbon; Lime inputs		Bacterial population
Larger animals & treading; Particle density	^a Cellulolytic population	
Soil P	^a Gummy population	
Altitude, slope; polysaccharide content; Clover; Botanical diversity ^a All depth values	^a Amylytic population	^a Amylytic population
Time farmed organically; ^a Lime & organic phosphate inputs; Since ploughed (n=5); Organic Carbon; Soil C:N; ^a Magnesium; Botanical diversity	^a Proteolytic population	
^a Ratio of rotation rested to grazed days; ^a % Grasses in vegetation	^a Arginine ammonifiers	
^a Grazing continuity in rotation & year		^a Arginine ammonifiers
Related primarily to cellular activity		
Root mass; Intervals since mineral N added and since soil disturbance	^b N-mineralisation	
Soil pH; Mineral N fertiliser inputs Soil depth		^b N-mineralisation
Ratio between A horizon and depth	Arginine ammonification	Bacterial population
Soil pH	^b N-immobilisation	
Related primarily to enzyme activity		
Urease	^a Phosphate inputs; ^a Proteolytic population	

a. Terminology and references to sets of similar environmental factors have been condensed for clarity.

b. Nitrogen mineralisation refers to N-mineralisation *in vitro*.

7.3.3.2 Gradients suggest an ecological succession in grazed soil

Ordinations were implemented with soil biological components (Fig. 7.9) and with substrate gain and loss factors (Fig. 7.10). Crucial C and N arguments of this thesis were supported.

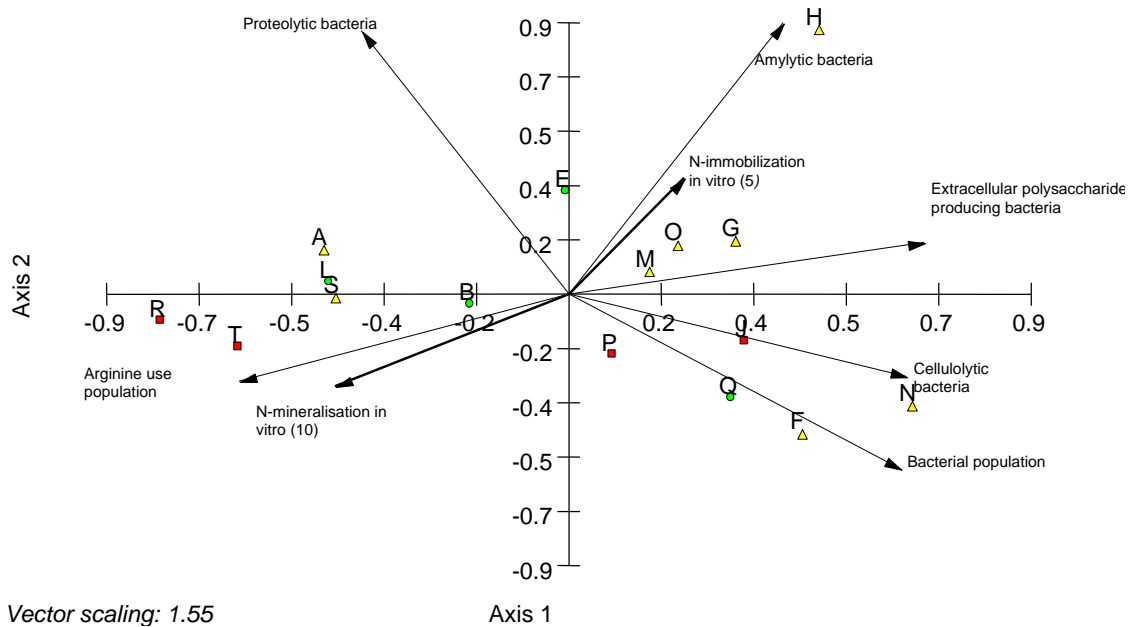


Fig. 7.9 PCA ordination of bacterial populations, N mineralisation and N immobilisation *in vitro* for sixteen study farms. A key to variable names is given in Appendix C. Five farm values were available for immobilisation, ten for mineralisation.

Figure 7.9 shows that grassland production was not strongly associated with particular bacterial populations and that bacterial populations were likely to be non-extreme in productive farms. Instead, a biological gradient spanned from what could be described as carbon source use and abundance on the right to nitrogenous energy source use and carbon scarcity on the left. Cellulose-using organisms predominate in some farms, but are smaller in farms L, A, R, S, and T where arginine use and ammonification predominates. Populations capable of using other substrates such as starch and protein predominate between those capable of cellulose and arginine use. Immobilisation of nitrogen is more likely when C is abundant, as indicated by amylytic populations. This is the first known graphic indication that a succession of bacterial populations and activities might exist in grazed grasslands, as could be inferred from this diagram. The authors van Bruggen and Semenov (2000) stated that: “Any kind of soil disturbance is bound to lead to a succession in bacterial and fungi and the associated food web”.

Indeed succession in grazed grasslands is a plausible explanation for observations made in the present study. Whether or not a temporal succession is represented here could be established experimentally in further work.

Evidence that resource availability may regulate soil bacterial populations and soil microbial biomass exists elsewhere. Bacterial populations have been compared at high and low levels of available carbon and have been found to be copiotrophic (preferring abundance) and oligotrophic (preferring scarcity) respectively (Hu et al., 1999). In addition, Bardgett et al. (1997) described a decline in microbial biomass after removal of sheep from different grassland sites. Those two studies compared environmental extremes. In contrast, working grassland farms presented a range of conditions across which environmental gradients could manifest in the present study. Bacterial populations capable of specific carbonaceous or nitrogenous energy source uses were quantified here too, and therefore a C-N function related gradient can be inferred from the bacterial and substrate-related diagrams presented. Neither Hu et al., (1999), nor Bardgett et al (1997), examined heterotrophic C and N functionality of soil bacterial populations.

What evidence is there to suggest that carbon source use is depleted in some soils leading to presumed scarcity here? Other studies have demonstrated that change in available carbon can happen rapidly, and that the rate of change varies with environmental factors. A linear decline of basal respiration occurred over two hours when soils were separated from their rhizosphere nutrient source (Cheng et al., 1996), and soils used up amino acid amendments within one to twelve hours depending on soil type and environmental conditions (Jones, 1999). Depletion may well occur in grazed soils when animals have been moved elsewhere in the rotation and the land is rested.

7.3.3.3 Substrate gain and loss factors influence heterotrophic bacteria

The following ordination is based on the inclusion of ‘substrate gain and loss factors’ together with the heterotrophic bacterial populations and their activities examined in the present study. Substrate gain and loss factors have been identified here because they have been significantly associated with either bacterial populations or their activities in

rank correlation analysis in Chapter 5, and are thought to interact with bacteria on the basis that each affects substrate provision or withdrawal from the bacterial environment, hence the term ‘substrate gain and loss factors’. For example, depth of soil might affect the dilution of substrate through the soil profile, such that amylolytic organisms that use starch substrates can be more abundant in shallow soils. Similarly, as clover is starch rich (Whitehead, 1995) and is associated with botanically diverse swards, carbon abundance is likely to provide niches suited to proliferation of either amylolytic and or extracellular polysaccharide producing organisms. Substrate gain and loss were not fully appreciable in the itemised approach to bacterial populations and activities in Chapter 5, whereas such relationships are more apparent now because of the environmental approach adopted in Chapter 7.

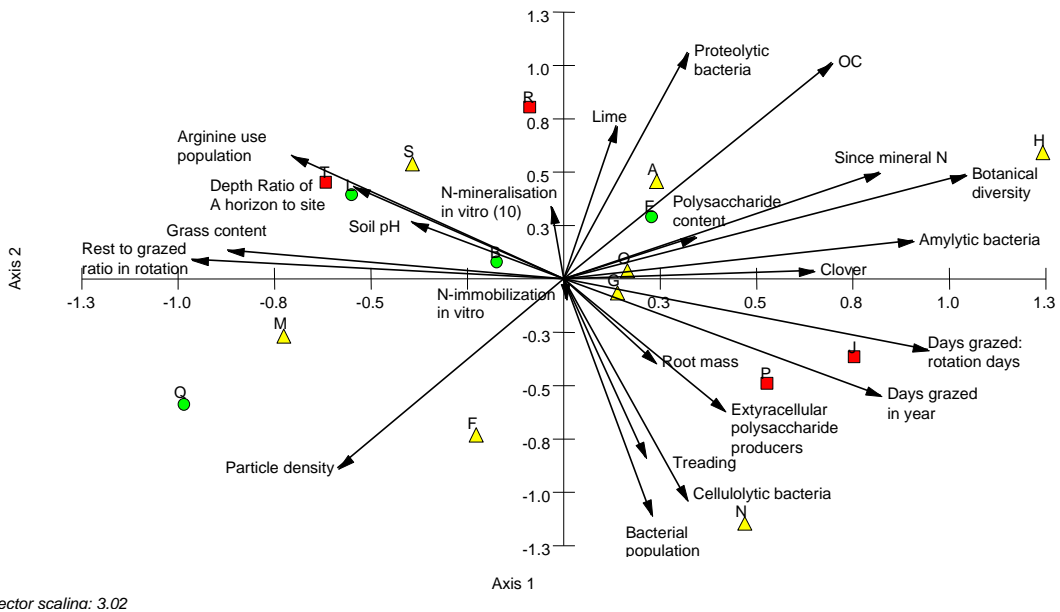


Fig. 7.10. PCA ordination of all sixteen farms. Variables include bacterial populations, N-mineralisation (10 farm values) and N-immobilisation vitro (5 farm values), together with significantly- related substrate gain and loss factors. A key to variable names is given in Appendix C. Grazing descriptors have been simplified.

That substrates link the abiotic and biotic environment is demonstrated in the following ordination. Carbon availability is implicated in the diagrams because C -rich and- sparse requirements are diametrically opposite, and biological functions relate reasonably to the ‘AC is used before ON’ argument throughout. Substrate gain and loss implications are discussed in context.

Figure 7.10 illustrates the links between bacterial populations and activities and those factors influencing substrate gain and loss from either soil organic matter or herbage. A total of eight axes were extracted and explained 90% of the variation; the first two, presented, account for 44%. Each relationship with bacterial populations and / or activities in this ordination can be explained on the basis that the corresponding environmental factor has influenced, or has itself been influenced by, relative labile substrate presence. The labile substrates may have been either gained or lost to soil from grazed herbage or to /or from soil organic matter.

Many of the presumed substrate-altering factors, namely mineral N-fertilisers, easily available organic substrates, plant rhizodeposition, and easily decomposable organic substances, encountered here were amongst those listed in a review of C and N priming effects written by Kuzyakov et al. (2000). Priming affects increase or reduce mineralisation of carbon and nitrogen beyond that expected in soils exposed to fresh substrates (Coyne, 1999; Kuzyakov et al., 2000). Observed links are considered by diagram sector.

The upper right sector of the diagram is characterised by botanically diverse swards and relatively higher organic carbon and polysaccharide content. Biological activity becomes intense when amended with leguminous residues that are rich in carbon and nitrogen compounds and that supply vitamins and more complex substrates (Altieri, 1999). Clover, for example, is rich in starch (Whitehead, 1995), explaining its association with amylolytic organisms. Organic carbon may have been augmented by diverse plant species, and by previous liming (Whitehead, 1995), and may have increased in the absence of mineral nitrogen applications. Populations capable of using protein and starch are facilitated here too. Influence of vegetation type is evident in the diagram and has been reported variously for other species elsewhere (Martens, 2000; Ste. Marie and Paré 1999; Bauhus et al., 1998).

The lower right sector is characterised by grazing intensity. Cellulose is probably trodden, and possibly macerated, from herbage into soil where it stimulates populations of cellulolytic and other bacteria there. The outlying farm Q on the lower left is conventional and has a lower organic carbon content along with higher particle density

that contrast with the organic-rich sector diametrically opposite, where farms have been lacking in mineral nitrogen for some time.

To the upper left of the diagram, farms are rested more than grazed, have a higher ratio of A horizon to soil depths, and have higher arginine-using populations and N-mineralising activities. Farms in this sector also have larger total percentage of grass species in their vegetation. Farms in this sector are characterised by organisms and activities associated with scarce available-carbon (AC) resources.

Biomass and enzymes were not clearly related to particular substrate-altering factors, and are omitted from the ordination. Tracy and Frank (1998) had found that microbial biomass could be accurately predicted by plant biomass which was unaffected by chronic grazing. Bacterial or other microbial populations were not measured in their study, and as grazing had no effect on microbial biomass, they presumed that the microbial populations would derive sufficient labile C from dung and plant roots, and that plants were the deciding factor (Tracy and Frank, 1998). However, they found that mineral N fluxes responded more to grazing than topography, and that net N mineralisation was highest in grazed grassland. Their finding of highest mineralisation rates in grazed situations may concur with higher N-mineralisation *in vitro* in grassier swards in the present study, although whether arginine ammonification values measured here could substitute for field mineralisation values is not known.

Other researchers have found that active substrate use or its mineralisation by microorganisms was stimulated by substrate additions but related poorly to antecedent conditions examined against high and low values for plant species diversity, extensive or intensive agricultural practices (Griffiths et al., 2001). Differences in response between biomass (measured as either SIR or NRN) and microbial activity (measured as arginine ammonification) in relation to environmental influences are further evidence that soil biological components relate individualistically to their environment, and that temporal change may be reflected in one component, and not in another.

It seems easier to identify substrate sources than to implicate their absence. However, substrate scarcity can be inferred from the relative positions of farms and variables in opposite sectors. To the upper right of Fig. 7.10, substrates such as starches are likely to

be in plentiful supply from clover presence. Amylytic and extracellular polysaccharide producer populations indicate that this is a more abundant sector. Across from this abundant sector, N-mineralising bacterial populations and activities to the left of Fig. 7.10, are indicative of scarcity. Other evidence supports scarcity too. If farms are rested more than grazed, concentrations of substrates may be depleted by microbial activity during the relatively longer rest interval. This suggestion is supported by evidence of rapid soil resource depletion under experimental conditions in the laboratory (Cheng et al., 1996; Jones 1999). Furthermore, a comparison of soil under grazed tussocks (Northup et al., 1999) demonstrated that organic matter inputs to the soil contents were reduced by intensive grazing, leading to lower levels of microbial biomass. In this study, and in soils under grassy conditions, cellulose is likely to predominate rather than other substrates. The grazing animal probably removes relatively more from grassy swards, thereby reducing the relative flow of nutrients to soil, leading to more oligotrophic conditions.

This implicit succession of organisms would be expected to occur through metabiosis, a non-associative process whereby one type of organism prepares the environment for another (Waid, 1999), because bacterial populations differ, and are not necessarily strongly correlated with each other in the present study. However, as noted in Chapter 5, bacterial populations decline in size, from general bacterial numbers through arginine using with proteolytic organisms having the smallest populations overall. Copiotrophic organisms might reasonably avail of nutrients from trodden herbage during the rest period in a grazing rotation. Then, when resources are depleted, growth and activity of nitrogenous energy source users would be facilitated instead. Population data from the farms in the present study are in agreement with a copiotrophic-oligotrophic gradient, because populations capable of cellulose use were smaller in farms A, L, R, S and T where arginine-using populations predominated. Similarly, oligotrophic situations were characterised by ammonification, whereas more abundant environments were likely to facilitate immobilisation.

How might depth ratios affect substrates? Three soil depth values, namely: depth to bedrock; depth to A horizon; and the ratio between these two values, correlated differently with bacterial populations. Relationships between depth values differed in direction between C-use and N-use. Depth ratio correlated significantly with arginine

ammonification. Amyltic organisms had correlated significantly with shallower soils, suggesting that dilution might play a role. However, the differences observed in the direction of correlations with populations on the basis of their C-use and N-use suggested that something other than just dilution was at play. It appears that depth ratio may represent soil layers more dynamically. To illustrate, the highest recorded *in vitro* N- mineralisation rates occurred in farm R, the shallowest soil. Farm R, a Kilfergus soil is described as well drained (Section 3.2.4.2.5), but was reportedly the wettest of all farms during interviews with farmers. Impeded drainage could have thwarted substrate flow from herbage to the soil beneath, starving soil of AC, allowing ON use. Substrates dispersal through soil is influenced partly by hydraulic conductivity, (Netto et al., 1999), and is likely to be affected by layers in the soil profile and by depth to bedrock (Falleiros et al., 1998; Keppeler and Brown, 1998; Brady, 1990). AC may not get through.

Volumetric water content may also be involved because, although the effects of soil texture are known to influence soil organic matter decomposition, a recent study found more highly significant correlation with the volumetric water content that better explained differences for both native and residual soil organic matter contents (Thomsen et al., 1999). Although slight differences between relationships with field moist and dry weight equivalent values were observed for soil biological parameters in the present study, soil moisture characteristics were not particularly examined here. Field situations are briefly considered as ‘what if’ scenarios: If carbon doesn’t reach the soil beneath, there is a risk that ammonification of organic nitrogenous energy sources may occur; on the other hand if nitrogenous energy sources do not penetrate into impeded soil, the issue of ammonia loss to the environment may arise if drainage-impeded soil is disturbed and amended in some way. Ordinations implemented with values that were significantly related to depth in rank correlation analysis, two ordinations of which are presented in Appendix F, show that depth relates to grazing practices as well.

To conclude, it can be stated that factors such as grazing, vegetation, fertiliser inputs, and soil depth may determine the dispersal of substrates through soil and ought to be considered in soil organic matter turnover studies, because they are likely to impact on

the microbial environment, on soil organic matter turnover rates, and on grassland production.

7.3.4 Patterns emerging in CCA

CCA might give a more integrated view of grassland soil and farms because of the weighted averaging of samples and species together via the CCA algorithm (Palmer, 2000 c). However, initial application of CCA was unsatisfactory.

A singular matrix, equivalent to dividing by zero and that therefore cannot be inverted, (Kovach, 1998; Legendre and Legendre, 1998; Palmer, 2000 f) was often reported in the MVSP results output for CCA. A singular matrix may have resulted when variables were linear combinations of each other, or constant. The numerous zero values for botanical species and nutrient input variables may have been regarded as constant or as linear combinations of each other in MVSP, however reasons for any singular matrix error were not identified in the MVSP results output obtained in the present study. Conventions are sometimes applied to solving related problems, for example, when computing logarithmic values, zero would be replaced with ' $x+1$ ' (Kovach, 1998). However, data for CCA purposes need not be transformed (Palmer, 2000 f). To complicate matters, CCA gives a synthetic portrayal of each farm based on all of the environmental and species variables describing it (Micheloud, 1997). Because of its complexity, CCA is regarded by McCune (1997) as "one of the most potentially misleading multivariate methods". In order to circumvent the 'zero' problem mineral fertiliser applications could have been described as ' $x + 1$ ' quantities. Because manipulation of the data for CCA might mislead, variables were best left as they were.

'Irrelevant' factors made ordinations uninterpretable due to 'noise' (Legendre and Legendre, 1998; McCune, 1997). Multicollinear variables were dropped automatically from CCA analysis in MVSP (Kovach, 1998) as from multiple regression analyses. Frequently, CCA maps failed to differentiate many variables that remained clustered at the origin. Uninterpretable CCA were otherwise attributed to the observed predominance of both linear relationships and relatively short gradients, and to the general lack of a unimodal response observed amongst variables in the data set.

However because CCA might detect the unexpected, it was implemented afresh. Two assumptions were put aside. Although a suite of multicollinear variables described the production environment together in PCA, they were now not expected to perform together in CCA. Putative independent attributes of the soil environment were implemented occasionally in a more dependent role, in other words roles of ‘species’ and ‘environment’ were swapped. New patterns emerged. Two scenarios are presented, one demonstrates production-related clusters, the other reveals farms as an environmental gradient along which optima for N-immobilisation, production, and N-mineralisation could be discerned.

By way of introduction, this paragraph describes how the two contrasting yet informative scenarios came about. Both evolved from systematic ordinations of selected variables, especially pH, root mass, soil organic carbon (OC) content, soil C:N ratio, and depth ratio, implemented in threes as was necessary to achieve dispersal of sites in ordination space. Three selected from those listed were implemented either all as ‘dependent’ or as ‘independent’ variables. When pH, root mass, depth ratio, and organic carbon were implemented together as dependent variables, the best production-related farm clusters appeared. Clusters overlapped most when C: N ratio was included. Farm production clusters remained the same, regardless of environmental variables mapped with them. No production relationship emerged when any three of the selected variables, pH, root mass, OC, C:N ratio, and depth ratio, were implemented as though they were more independent ‘environmental’ variables.

In contrast, when any three from the selection (pH, root mass, OC content, C:N ratio; depth ratio) were implemented as independent environmental variables but with immobilisation and mineralisation values as dependents, a line of farms, that was not production related emerged repeatedly. The line related to arginine use, that is to N-mineralisation and N-immobilisation, instead. The most definitive gradient of farms in relation to arginine use evolved from use of root mass, pH, and depth ratio values as environmental attributes. When implemented with OC content, its vector was shorter than that for depth ratios, and OC content didn’t give the same delineation. Linearity was best when arginine-using populations were included as the third dependent ‘species’-type variable. The order of farms in the arginine ammonification lines also

remained the same, regardless of environmental variables implemented in the ordinations.

7.3.4.1 Production-related clusters

Ordination inputs consisted of three variables, soil organic carbon content, soil pH and root mass, as dependent descriptors for all sixteen farms. Ordinations were repeated using nutrient inputs (Fig. 7.11a), grazing and botanical values (Fig.7.11.b), and bacterial populations as environmental variables (Fig. 7.11.c). Results were not detrended. In three diagrams, the three key variables remained clustered at the origin, whereas arrows extended for environmental vectors. Diagrams include just cases and vectors for clarity.

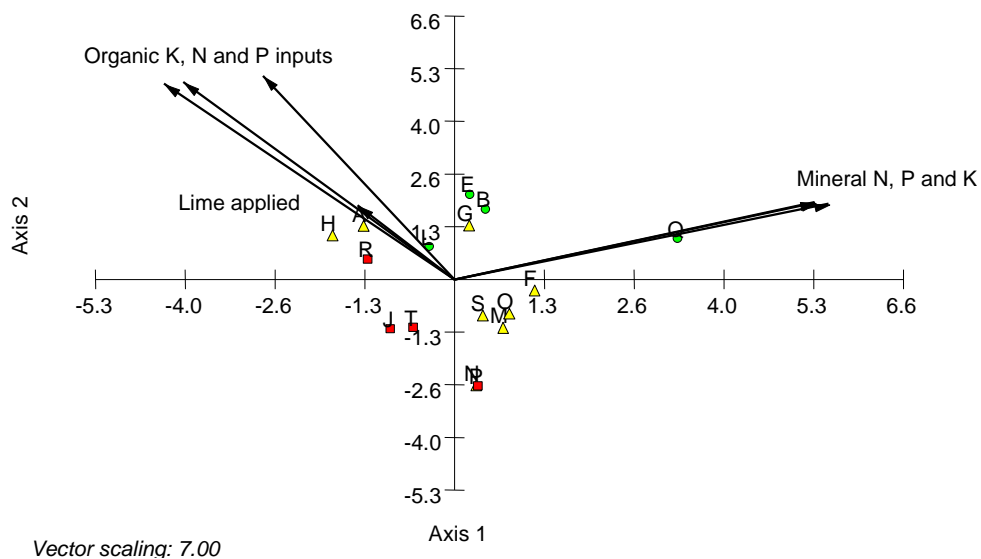


Fig. 7.11 a. CCA ordination diagram of sixteen study farms: Environmental vectors describe the nutrient inputs to the study farms in the six years preceding production- related data collation. A key to variable names is given in Appendix C. Mineral P additions were implemented but were removed from the ordination because of multicollinearity. Variables entered as ‘species’ data included soil pH, organic carbon and root mass, but these are not shown as they were clustered at the origin.

As can be seen in Figs. 7.11 a-c, the exact position of each farm differs slightly from one map to another, but its relative position is the same. The same pattern occurred even when randomly selected ‘environmental’ variables were implemented.

The bases for repeated patterns in CCA can be explained by the algorithm used, and by the ecological concept of species association. Species become ‘associated’ because they respond similarly to the environment in geographic, spatial, temporal or experimental

manner (Legendre and Legendre, 1998). In ecology, each association corresponds to its environment and so will reliably co-occur as a group of species along sampling axes. Species associations can be identified by their internal stability (Legendre and Legendre, 1998), and convergence is guaranteed according to Palmer (2000 c) because of the way in which species scores and sample scores are treated to maximally correspond in CCA. The term species is used in ecology, but in reality what has appeared as clusters in this ordination diagram are associations between farms, on the basis of their production level. If production is regarded as a 'specie' responsive to its environment, the clusters can reasonably be interpreted, because the results show that farm production is representative of its environment. Similar production outcomes occur together, with more productive farms in the upper right quadrant, less productive farms below, and average farms scattered in between.

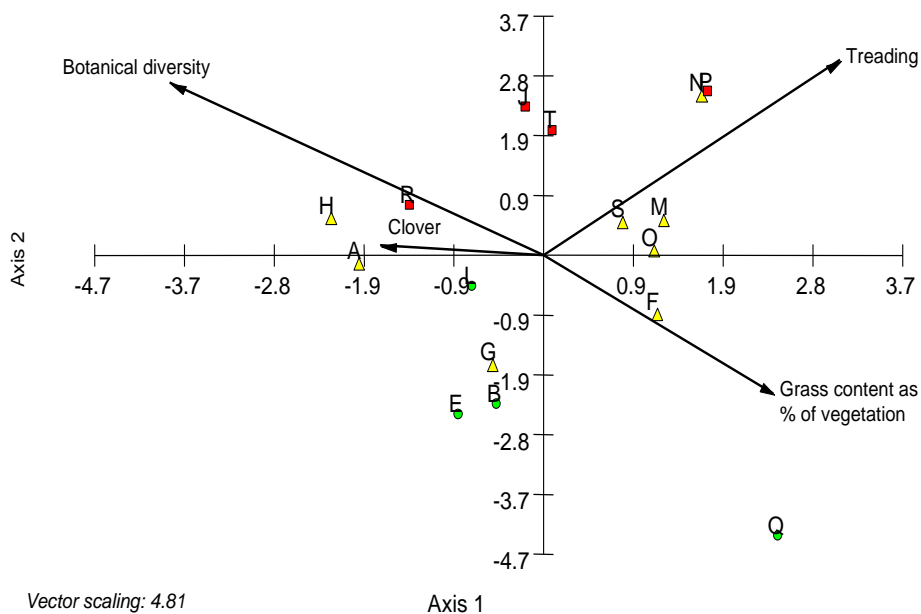


Fig. 7.11 b. CCA ordination diagram of sixteen study farms: Environmental vectors describe selected grazing and botanical data. A key to variable names is given in Appendix C. Variables entered as dependent, non-environmental data were soil pH, organic carbon and root mass, but these are not shown as they were clustered at the origin.

The cluster pattern was reliable and occurred regardless of environmental variables added in or removed. Association between farms and individual variables is not absolute because the ordination constrained farms and variables together. Nevertheless, each farm position is reasonable relative to any 'environmental' variable. Farm Q is positioned as an outlier at the tip of mineral nutrient vectors, (mineral P addition

exhibited collinearity so had been dropped from calculations but is included in the diagram) whilst L, organically managed, is closer to organic input vectors in Fig.7.11 a; farms N and P have the two highest trading values, although N is much more trodden than P; botanical species are well represented in A, R and H, and so on (Fig.7.11 b). Similarly, farm E has the highest urealytic population, while T has one of the highest arginine using populations (Fig.7.11 c).

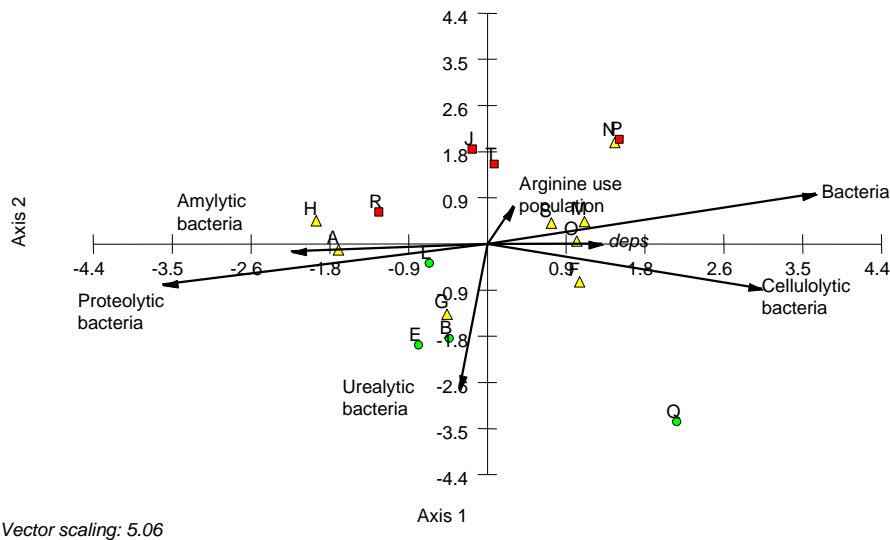


Fig. 7.11 c. CCA ordination diagram of sixteen study farms: Environmental vectors describe bacterial populations. A key to variable names is given in Appendix C. Variables entered as dependent, non-environmental data were soil pH, organic carbon and root mass, but these are not shown as they were clustered at the origin.

In Figs.7.11 a-c, nutrient inputs are associated with more productive farms; however, other variables such as grazing, vegetation, and bacterial aspects other than the urealytic population of productive farms are non-extreme.

When used as environmental variables, relationship of pH and root mass and soil OC content with production was confused, and without obvious reference either to productive environments or to particular soil conditions. Furthermore, when the CCA ordinations depicted in Figs. 7.11 a-c were repeated and detrended, the variables pH, soil OC content and root mass were not at the origin this time but appeared as vectors in ordination space. However, because all of the implemented farms were centred on the origin in the detrended examples, any production- or environmental-related clusters were not discernible.

To conclude, it may be stated that soil pH, root mass, and soil organic carbon content separated farms according to production levels in CCA, and represented the production environment fairly well when regarded as dependent variables. Furthermore, the interdependent but reproducible nature of that environment was demonstrated because the same reasonable production pattern emerged, regardless of what additional management, vegetation or biological descriptors were used to describe it.

7.3.4.2 N-function-related linear diagrams

Detrended CCA ordinations were implemented. Values for N-mineralisation and N-immobilisation *in vitro* and bacterial populations capable of arginine ammonification were used as dependent variables. Environmental variables comprised soil pH, root mass and ratio between depth to A horizon and depth to bedrock. Values were included for all sixteen farms and the resultant CCA ordination is given in Fig. 7.12.

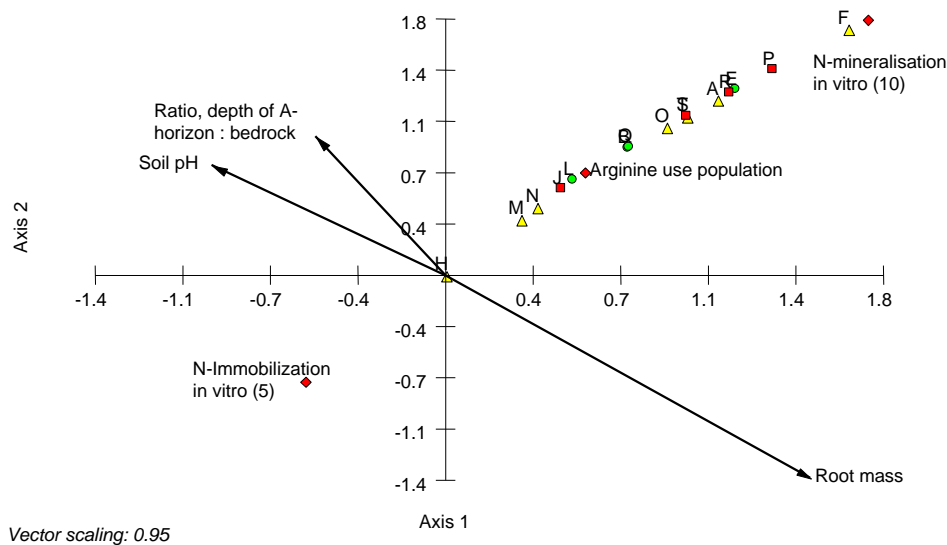


Fig. 7.12 CCA ordination of variables describing arginine use in relation to selected environmental variables, implemented for 15 farms. A key to variable names is given in Appendix C.

Instead of clusters, a consistent line of farms was observed when soil pH, root mass and ratio of A-horizon to site depths, were input as three putatively ‘independent’ or environmental variables (Fig. 7.12). From the right of the diagram, the ten farms F to B and Q are those in which mineralisation occurred. The line is then intercepted by the arginine using population variable, and continues with the remaining five farms, from L to H, in which immobilisation occurred. In the absence of an arginine ammonification value for farm G, this farm was omitted.

The rank order of farms from F to H does not match their rank order according to any of the variables that were implemented. Instead, arginine-using populations are lowest at opposite ends, and highest for farms closer in the middle. Although productive farms are positioned centrally, they are not characterised by high values for any arginine-related function. Highest rates exist at farm M for immobilisation on the one hand, and at R for mineralisation, on the other, mid way amongst five and ten farms respectively. Apparently each N-function exhibits a different unimodal response to the combined environmental conditions being mapped. Production may be unimodal too, occurring best at relatively low values for all three arginine use or N-functions. The ‘unimodal’ position for production appears to be non-extreme.

Important environmental gradients are easily identified in CCA because of their relatively long arrow length, and their high correlation with and therefore close proximity to an ordination axis. Depth ratio values satisfy these criteria. However, because CCA reflects the environment in an inclusive manner, the position of each farm and the optima for each function probably depend on combined factors, optimal values for which are difficult to discern from the diagram alone. It is likely that nitrogen cycling is a dynamic response, requiring little biological activity, as production is non-extreme in respect to all three values. For example, in the organically and very productive farm L, N-immobilisation may help conserve nitrogen from legumes in the sward, and could facilitate grass growth if released to the sward and if grazing is managed in an opportune manner.

The ordination provides evidence that nitrogen turnover matters, and that its surrounding environment includes factors such as soil pH and root mass both of which may be managed appropriately. However, the conditions for opportune temporary sequestration and release of nitrogen in grazed grasslands have not been elucidated, and both immobilisation and mineralisation occur at the same pH values, as discussed. Management in the short term is unlikely to alter depth ratios significantly, but as depth ratios may depict penetrability (Section 7.3.3.3), this could also be improved.

Because bacteria might shed light on labile carbon issues concerning immobilisation and mineralisation, additional ordinations were implemented by adding in a single

bacterial population as an environmental variable each time. The linear order of farms remained the same as before; however, the farms were positioned either as a straight line (when implemented either without bacterial population or with extracellular polysaccharide producers as the environmental descriptor) or in angular forms (when implemented with bacterial, proteolytic, cellulolytic and amylytic populations as the environmental descriptor) that progressed through more acute angles in the order of bacterial populations listed. The most acute angle was obtained when urease-producing populations were used to describe the environment. One of the resultant diagrams, implemented with amylytic bacterial populations as the third variable, is shown.

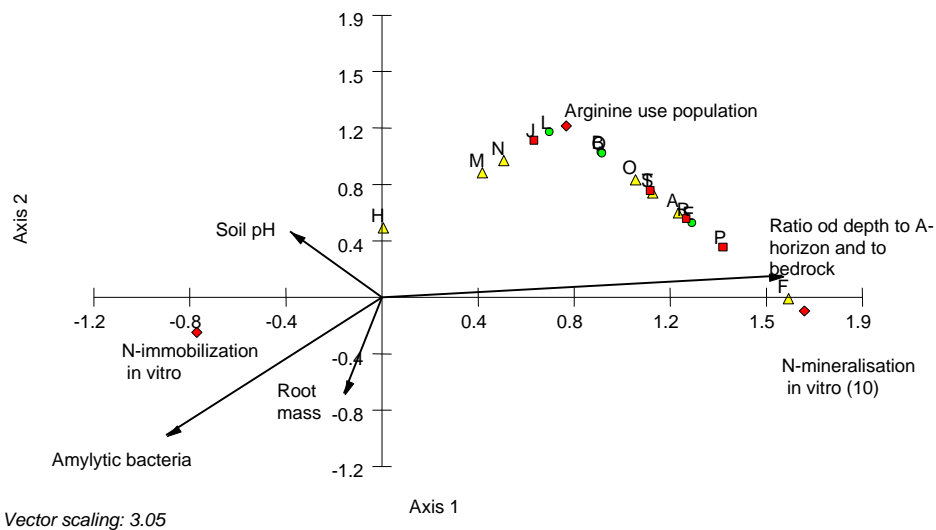


Fig. 7.13 CCA of variables describing arginine use, with amylytic bacteria included as the third dependent variable. Variable names are explained in Appendix C.

The angular relationship has not been seen in CCA diagrams elsewhere, and was thought unusual, particularly as arginine ammonification rates had not correlated significantly with bacterial populations in rank correlation analysis. Relationships were examined. When scatter graphs were drawn of bacteria (x) and arginine ammonification (y), ammonification related to cellulolytic and amylytic populations and extracellular polysaccharide producers in a more unimodal way, but the direction and relationship and optimal mode differed. Relationship with arginine -using, proteolytic and urease producers seems more linear. Such observations support the central theme of this thesis, that substrate type matters. They also suggest that metabiosis occurs whereby one organism (bacterial population in this case) changes the environment for another (Waid,

1999). Metabiosis is indicated also by the non-associative nature of relationships (Waid, 1999; Andr n and Balandreau, 1999), as indicated by lack of significant correlation between bacterial populations and arginine- use values (this lack is apparent in Table 7.2).

7.3.4.3 Conclusions drawn from CCA ordinations: production

The CCA diagrams presented in Chapter 7 imply that separate optimal modes exist for each N-function and for production; however, production is non-extreme. One organic farm and the one conventional farm fared best, and both had similar paddock grazing routines. This suggests that even when mineral nitrogen is not applied, certain grazing rotations may both regulate biological activity and maximise nitrogen use by grassy swards.

CCA evidence also suggests that while abiotic factors such as depth ratios and soil pH, and factors with both abiotic and biotic effect such as root mass, influence the rate and type of cellular activity, amylytic bacteria influence immobilisation and ammonification substantially. Amylytic organisms may indicate higher levels of available C that enable immobilisation and disable ammonification. Other bacterial populations relate in a non-associative way to N-function too, as already mentioned. However, bacterial population densities probably result from environmental influence of both vegetation and grazing rotations. Grazing practices are likely to be instrumental in the supply of AC, and in the regulation of mineralisation and of immobilisation as well.

It has been thought that microbial communities would need to mineralise larger amounts of organic matter in organically managed soils (Gunapala and Scow, 1998). Low optima for N-functions suggest that more mineralisation is not necessarily the case. Mineralisation from nitrogen in the microbial biomass may simply be more opportune, stored in small quantities and released as needed, and the optimal biotic conditions for organic nitrogen turnover should be established experimentally for grazed swards.

7.3.4.4 Conclusions drawn from CCA ordinations: relative independence

The straight lines and recognisable clusters obtained with CCA are a welcome contrast to the messy scatter graph and third order polynomial response when production and or

ammonification values are paired for example with soil pH (Fig. 7.3 & 7.5). Cluster and linear responses observed in CCA also add to PCA evidence of interdependence amongst factors.

The very different straight line and cluster diagrams warrant consideration. Lines of farms never related to production directly, whereas clusters gave the first indications that CCA diagrams could display farms in relation to production. Why should production exhibit a clustered rather than linear effect? Production is a measure of many outcomes from grassland, encompassing fodder, animal offspring and growth, milk, and bought-in feeds over an entire year. It is not a singular event. Neither is it solely attributable to one influential factor such as pH or arginine ammonification, but is more likely to result from a suite of variables interacting together as in PCA, as exemplified in Figs.7.2 & 7.6. Production seems to be the product of combined variables, rather than a singular response any one environmental influence or gradient, which would presumably have emerged as a linear response instead.

In contrast to production, each arginine ammonification value is singular, describing the extent of ammonia-N release or retention. Moreover, of all biological components measured, arginine ammonification was the most responsive to factors in its immediate environment, including soil pH, root mass, and depth ratio (see Table 7.2, and accompanying text). A singular response with a continuum of values for arginine ammonification rate might be expected to reflect its environment more precisely than a composite value. A line of farms in ordination space may imply that arginine ammonification is a dependent variable that reflects similar conditions in neighbouring farms along that line, which represents an environmental gradient in ordination space, as might be expected (Kovach, 1998). The linear arrangement probably indicates that a longer gradient exists. The finer scale at which microbes respond to their environment (Parkin, 1993) may also be indicated by a relatively longer gradient, and should be considered.

Relatively few linear CCA outputs have been found in the environmental sciences literature. In one example, soil profile moisture response was linear in relation to environmental factors (Qiu et al., 2001). Note the singular attribute. In contrast, clusters appeared when composite sample sites were mapped in a comparison of bird

populations on organic and conventional farms in southern Britain (Chamberlain et al., 1999). Many of the CCA ordinations investigated throughout the present study are not reported here, but it was noted that linear responses occurred when biological variables were implemented, more so than any other type. The implication from research here and elsewhere is that CCA can differentiate singular more independent variables from more composite dependent facets because they may result in either a linear or cluster type response, respectively.

Using CCA via CANOCO (version 3.1, ter Braak, 1991, cited in Oliver et al., 2000), Oliver et al., (2000) established the relative independence of indicators of forest management effects by an iterative process, by including habitat variables one by one, and seeing their relative contributions in the resultant ordinations. They availed of the forward selection procedure available in CANOCO (version 3.1, ter Braak, 1991, cited in Oliver et al., 2000) not available in MVSP (Kovach, 1998). Data from the present study could be examined in this way.

The fact that microbial measurements have exhibited elongated relationships with the environmental variables here may imply that gradients are 'long' with respect to microbial function. Earlier in this report, comment has been made on the difficulties in spatial analyses and comparisons of fine- and broad- scale attributes particularly when extrapolating the results of soil biological analyses in the laboratory to field scale (Moore and Russell, 1972; Nannipieri, 1990; Parkin, 1993, amongst others). What CCA results suggest, however, is that while productive farms are not extreme from a microbial viewpoint, it may be worthwhile to examine biological optima in relation to production at this more refined scale. Soil seems to be managed well biologically for grassland production purposes in the organic farm L for example, which is non-extreme biologically, and therefore fine-scale understanding of productive conditions is desirable.

Meanwhile, it can be inferred from the exploratory analyses of study farm production and biology in CCA that key environmental attributes interdepend, and that biological activity is a function of its environment.

7.4 General discussion

7.4.1 Introduction

This study has sought the basis for how soil biological and other properties might drive grassland production in the absence of mineral fertiliser applications. Soil was examined from a wide variety of viewpoints, focussing on grassland management, the soil biology involved in soil organic matter turnover, and on grassland production. Substantial differences were found between farms in terms of organic matter content, soil biology and vegetation. Most of the differences could be attributed to nutrient inputs and to grazing practices.

Key production factors have been identified. How each influential factor might influence organic matter turnover in grassland soils has been considered, with particular reference to selected heterotrophic components of the soil biology and grassland production. Almost every significant relationship of grassland parameters with the bacterial populations and cellular activity responsible for soil organic matter turnover can be explained in terms of substrate availability. The relationship of arginine ammonification with soil pH may be an exception. Observations also indicate that organic matter turnover is regulated intracellularly, such that available carbon or AC is used before organic nitrogen, ON.

7.4.2 Soil biology, soil organic matter quality, and sustainability

Although soil organic carbon content was not particularly production related, it may have been sufficient for soil biological purposes in the farms studied because none of the soil biological components seem to have been limited by it. However, the percentage of organic matter residing in the microbial biomass may indicate decline in organic matter content, as that proportion increased with declining organic carbon content. Microbial biomass may be reaching a ceiling in smaller organic matter pools.

Questions arise, for example with regard to the continued sustainability of the conventional farm Q, which had the lowest OC content of the sixteen farms studied. Organic matter is very important and comes, with other soil properties, second to crop yield as an indicator of agricultural sustainability (Vance, 2000). However, in the present study, soil organic matter content, botanical diversity and certain biological

behaviours were significantly greater over time since soil had been mechanically disturbed and / or since mineral nitrogen had been applied, suggesting that certain soil quality parameters may have recovered. Management type may also influence recovery because, when long-term experiments were reviewed, Vance (2000) found that the return of crop residues was a key management factor that significantly influenced soil organic matter levels and productivity across a range of sites. Although not correlated significantly with production in the present study, soil organic carbon content helped, when implemented with soil pH and root mass, to arrive at production-related farm clusters in CCA. Soil organic carbon may play an important role, as suggested by Vance (2000). In below-threshold situations, organic carbon may limit biological function, and might then be expected to indicate production potential.

Biodiversity is sometimes regarded as an indicator for soil and ecosystem quality (Andrén and Balandreau, 1999). Biodiversity of the plant population in the present study correlated significantly with nutrient inputs and with grazing practices. However with reference to an earlier study of grassland in Co. Limerick, (O'Sullivan, 1968), plant biodiversity may have been at least halved over thirty years of more intense management, and the fact that the least number of botanical species was recorded in farm Q highlights this. According to Andrén and Balandreau (1999), biodiversity is controversial because, while it is deemed valuable, within a multiplicity of species carrying out similar activities, some may be functionally redundant. Whether reduced botanical biodiversity poses a risk of reduced botanical functionality to any of the study farms is not clear. The roles of vegetation have not been established precisely in this study, but biodiversity was associated in PCA maps with greater organic matter, hydrolytic populations and immobilisation, in contrast to N-fertilised situations. In order to evaluate the role of plant biodiversity in grassland, the precise contribution of each species should be established to determine whether their functions overlap and determine whether loss of one or more species would pose a threat to continued ecosystem function.

Below ground, hydrolytic bacterial populations were measured rather than microbial species diversity. Populations capable of the hydrolysis of polysaccharide, protein, cellulose, urea and arginine were found in each farm, where at least their C and N turnover functions are theoretically possible. Because grassland management

influenced the soil biological components, their recovery following management change or perturbation may depend on initial resources. A study of threshold levels is needed for each organic matter pool, as there may be points of no return beyond which soil can no longer serve its intended purpose.

7.4.3 'AC is used before ON', in theory

'AC is used before ON' means that ammonia is unlikely to be released from soil organic matter if available-carbon sources suffice. If soil is disturbed and organic matter released, labile carbon becomes available. Organisms use available carbon for energy and cell growth, and available carbon restricts N mineralisation intracellularly (Cunin et al., 1986; Zubay, 1993). Meanwhile, bacterial cells may use mineral nitrogen for growth, immobilising it into their biomass and temporarily removing it. These activities may predominate until labile carbon or mineral nitrogen or both are entirely sequestered, and no longer freely available. Change in labile C concentration may happen in a matter of hours, and soil organic matter turnover is likely to be dynamic.

Temporary sequestration of mineral N in the microbial biomass may explain the 'lean years' experienced post- reseeded, during which nitrogen fertilisation is needed in order to maintain plant productivity (Whitehead, 1995), and may also account for a drop in production that is expected when nitrogen fertiliser applications are reduced (Keane and Allen, 1999). For example, during conversion from conventional to organic farming, microbial communities are expected to adapt to mineralising larger amounts of organic matter (Gunapala and Scow, 1998). However, the turnover of N from soil microbial biomass is reportedly ten times faster than that from vegetation (Smith and Paul, 1990), and temporary immobilisation effects may not be so severe, but may depend on conditions. A comparison of farm M (an average farm as regards production) with the highest immobilisation rate, and farm L (the most productive farm) with low immobilisation rates illustrates the differences that may occur. Generally, biological activity was not extreme in any productive farm, and an opportune balance between N storage, release and plant uptake is likely to be required for good levels of production to occur in grassland that is reliant on soil biological processes.

The C:N ratios of residues (Alef, 1995 b) and of soil (Whitehead, 1995) are known to regulate decomposition rates of organic matter whether introduced as amendments, detritus or excreta, or already incorporated as part of a more integral soil organic matter component. It has been inferred from the present study and other research (Chantigny et al., 1999; Henriksen and Breland, 1999) that the relative labile C and mineral N pools are regulatory quantities. For example, in the present study, mineral N- fertilised soils had lower ammonification rates, possibly because of C released from soil organic matter, as indicated by the reduced root mass content too. Conversely, if mineral N were not available for AC release, higher ammonification rates would be incurred, as was found in unfertilised soils. In other words, labile AC is likely to reflect soil mineral-N fertility status.

However, if the balance of C and N is pivotal, and C:N ratio influences the balance between immobilisation and mineralisation (Whitehead, 1995), why was soil C: N ratio not related either to production or to arginine ammonification rates? Although C:N ratio influences mineralisation and immobilisation, the influence requires qualification. Whitehead (1995) suggests that readily available C and N may be more relevant than the ratio of total C to total N. According to Richards (1987), the chemical nature of amendments is important.

Observations from the present study concur with the need for refinement of C:N as a criterion, because it is likely that total carbon analysis does not reflect available material. If so, the C:N ratio cannot accurately represent the microbial activity immediately responsible for N-mineralisation. Reasons are as follows. Organic matter C and N were measured in soil samples that had been withdrawn, bagged, transported, sieved free of roots, and air-dried (Chapter 3). Given the rapid depletion of energy resources from soil (Cheng et al., 1996; Jones, 1999) transient aspects of the soil organic pool may have been lost. In addition, mineral N content as available in soils is difficult to measure, because, amongst other things, of its rapid use by soil microorganisms. If labile resources are used up or lost from sampled soil prior to analysis, methods may account for the more recalcitrant molecules that remain and that are therefore unlikely either to be easily absorbed, or to have immediate catabolite repression or allosteric function. The C:N ratio as currently measured in soils may represent a quantity that is inaccessible and therefore microbe-remote. As mineralisation

seems to be responsive to the immediate substrate (inferred) and soil pH environment, then remoteness of the C:N ratio might account for its non-correlation either with microbial activity or with associated grassland production effects.

7.4.4 Bioindicator searches: findings and prospects

N-mineralisation *in vitro*, recorded in the present study as the positive values for arginine ammonification, is a likely bioindicator candidate as it was significantly correlated, albeit negatively, with production, and was observed as a reliable production gradient with other key factors in PCA, implemented either with or without it. Soil biological assessment seems desirable for organic farming (Lampkin, 1990), but bioindicators have not been found to date elsewhere (Skinner, 1976; Nannipieri et al., 1990) for various reasons, such as lack of information about suitable comparative values or thresholds, differences between soils, and differences between laboratory and field conditions (Skinner 1976; Lampkin 1990; Sparling 1997).

7.4.4.1 A suitable gauge may have helped to identify indicators in this study

Indicators may have emerged more easily in this study because just one composite variable, energy, was applied so that all animal and fodder production elements were accounted for. It is likely that fluctuations in singular production measures such as herbage, fodder, particular botanical species, livestock and milk would be ‘flattened’ by incorporation within one representative figure, for example. A significant fluctuation in livestock and feed requirements occurred in one farm - after visits to heifers there by a neighbour’s bull, across a broken fence. Additional feed was purchased but this temporary ‘bulge’ would have been accounted for as energy in the composite production value.

Gross margin correlated with nutrient inputs, indicating that fertiliser use increased production, but gross margin did not correlate significantly with intrinsic soil factors, and so may reflect more anthropogenic concerns. Stocking density was not particularly well correlated with soil conditions either, and grazing on site was what best related to the soil condition in the sampled field. Given the between-farm differences in livestock type, intensity, and vegetation, it is unlikely that a singular measure of production

would be applicable as a means of farm comparison. However, because the level of energy produced acted as a signpost for related gradients and correlated with farming practice in an expected way, it is likely that a composite measure such as energy is a reliable and objective gauge of contributory factors.

7.4.4.2 Acknowledging definitions and constraints may have been of value

Bioindicators have been sought elsewhere to reflect organic matter quality, soil quality or crop production, or that indicate soil perturbation and recovery thereafter.

This study urges caution in the use of soil biological components as gauges of soil quality because if soil OC content limits biological activity, the limited soil organic matter pools are more likely to reflect each other, leading to significant correlations amongst them, as for example between biomass (Lin and Brookes, 1999 b) or organic carbon (Alef and Kleiner, 1987) and arginine ammonification rates reported in the literature. Below a threshold or limiting level of soil OC content, soil quality and production may be reflected more by OC itself than any of its contained or restricted component pools. However, biological activity does not appear to have been restricted by the relatively higher soil OC content in study farm soils because arginine ammonification rates were not correlated with soil OC content. Instead, rates correlated with nutrient input and disturbance history, root mass, and soil pH, rather than biomass, and restrictions other than more recalcitrant or total measures of soil OC content may come into play beyond its threshold level. If not restricted by soil organic matter content, arginine ammonification may serve a production-bioindicator role. It did not behave as an organic matter indicator in the study farm soils.

Another consideration is that bioindication by one factor is unlikely (Nannipieri et al., 1990), as demonstrated in the present study by lack of absolute correlation with production, and by interaction between variables. Nevertheless, arginine ammonification values have shown some promise as an indicator here, as described, and elsewhere because it provided valuable information about the N-status of composts (Forster et al., 1993); and its trend has matched both N-mineralisation rates in soils (Leirós et al., 2000) as well as potentially mineralizable nitrogen (Gunapala et al., 1998). It has the added advantage over existing N-mineralisation assays that it can be

performed over a matter of hours instead of weeks. Further work is needed to determine its general applicability.

7.4.5. Uniqueness of this study

This study differed from similar research in many respects. Firstly, the location of the study in Ireland, and in Counties Limerick and Clare, probably afforded an opportunity to examine soil biology in soils with higher levels of soil organic carbon than have been examined in similar studies elsewhere.

The research was exploratory, in a range of working farms rather than in contrasted management regimes (Bolton et al., 1985; Reganold, 1995), or grazing intensities (Bardgett et al., 1997) and so bias in favour of any particular farming system was unlikely. In addition, it is the only bioindicator search known of to have applied energy as a comprehensive production gauge. Numerous attributes of grassland were taken into account in order to describe nutrient inputs, grazing, soil, vegetation, biology and production comprehensively. Results could be interpreted in relation to grazing frequency, continuity and treading, for example, and results suggest that both nutrient inputs, and grazing in particular, influence substrate flow to soil biological components that affect soil organic matter turnover as well as N- mineralisation rates *in vitro*.

A range of numerical and statistical analyses were applied, and while PCA has been seen in relation to soil ecology, this is the first known use of CCA in relation to either bacterial activity or grassland production in studies of this kind.

Although sampling depth was uniform, soil depth values to the A horizon and bedrock, not usually mentioned, were taken into account. Soil sampling and analyses differed in some respects from those used in other research: for example, un-sieved soil inclusive of rhizosphere material was used as the basis for biological analysis. Although viable counts of plated bacterial numbers rarely match soil reality (Lorch et al., 1995), catabolic populations were enumerated, allowing comparison of farms and exposure of related environmental gradients. Microbial species, molecular fingerprint, or other community structure analysis was not considered. Functionality of populations was examined with reference to bacterial products such as polysaccharides as well as

ammonia released from arginine and urea, although the latter function is largely extracellular. Whether proteolytic, cellulolytic and amylolytic function take place was not determined. Arginine ammonification was interpreted as N-mineralisation and N-immobilisation *in vitro*. Because arginine was viewed as an energy source, it was possible to consider influences of substrate gain and loss factors related to carbonaceous resources, with possible allosteric and catabolite repression effects.

7.4.6 Limitations of this study

7.4.6.1. Use of working grassland farms

Using working grassland farms may have presented a blessing in disguise. The farmer, not the researcher, made management decisions that introduced variation with less risk of bias. For example, certain grassland farms were selected on the understanding that they were organically managed, but it was then found that some farmers had changed their management practices for various reasons and that one farm was conventionally managed.

Access to and use of fields for repeated measurements, and availability of farmers for interview, were constrained in working farms over which the researcher had little or no control. To illustrate, twenty farms were visited at the outset of the study in 1995 and their soil samples collected and analysed. Subsequently, it emerged that management data could not be collected for four farms C, D, I, & K, and their data were therefore excluded.

Because of time constraints and practicality, one field representative of the farm as a whole was sampled, from which bulked samples were used as the basis for biological analysis. However seasonal differences in microbial attributes and other variables were avoided by sampling once, as is the norm for biological purposes, in summer.

7.4.6.2. Analytical shortfalls

The small numbers of farms representing particular types of organic and mineral fertiliser applications, grazing practices and fodder production made specific statistical analysis difficult. Some map-derived values used, such as liability to drought, may not have reflected management change and current soil conditions. Soil moisture was not

fully characterised, and values for soil temperature, precipitation, shelter, and wind exposure were not obtained. Volatilisation of soil ammonia was not considered. More transient C and N compounds were not measured although their presence is likely to regulate organic matter turnover.

Botanical analysis was carried out at a time of year when it was difficult to identify the species present and so data presented may not be completely reliable. Soil biological analysis included microbial biomass, one enzyme and one cellular activity and seven bacterial populations, but other types of heterotrophs such as fungi were ignored. Bacterial populations were determined by plating aerobically and potential anaerobes were overlooked. Autotrophs were not examined. One value for arginine ammonification was missing because an insufficient soil sample was obtained initially.

7.4.6.3 Unanswered questions

Although key objectives have been met, some anticipated relationships were not observed.

Clover species were not particularly associated with production. However, because clover is starch rich and contains fixed nitrogen, it may have dual C and N provision purpose. Evidence from significant correlations as well as ordination results suggests that clover species, together with botanic diversity, support a wide variety of soil OM related functions in soil such as: the immobilisation of nitrogen; higher levels of biomass N; OM use and storage by proteolytic and amylolytic bacteria and by extracellular polysaccharide producers; and soil organic carbon content. If clover supplies C as well as N, it is likely to have a regulatory soil organic matter turnover function beyond its nitrogen fixation role. Its association with botanic diversity and with soil organic matter supports this notion; however, return of nitrogen from legumes in the sward is not fully understood (Newton, 1993). The role(s) of legumes should be elucidated, especially as mineral fertiliser additions have contrasting OC and botanic diversity effects.

Originally, biological components had been expressed per gram of field-moist soils while awaiting soil moisture content data. Values were later converted to the dry weight

equivalent, as seen in the literature. Moist and dry weight measurements for bacterial populations and cellular activity were implemented in statistical analyses and in ordination, out of curiosity. In some cases, correlation existed with one value, only, not both. As mentioned already, depth- and soil moisture-related factors may influence substrate dispersal, and this awaits elucidation.

There was some evidence from ordination analyses of depth-related factors (given in Appendix F) that grazing practice, although related to depth, seems to override depth as a production determinant, because farms of the same soil series (Elton) and depth values seemed to be more productive if grazed more intensively. Soil biological components and activity differed according to silage and hay abstraction history, but too few fields were examined in either case to establish statistical significance.

Interviews provided additional information about veterinary issues, animal health, social issues, farmer education, and about perceived support for organic farming from family members, the media, institutions and organisations. Opinions were given in confidence, and as they had nothing factual to offer in this study and were not collated by a trained sociologist or anthropologist, they were not considered further.

7.4.7 Further work

The hypotheses that: N-mineralisation *in vitro* indicates grassland performance; AC is used before ON; substrate gain and loss factors regulate rates of AC and ON turnover; thresholds exist below which other soil organic matter components are limited need to be tested experimentally. Ultimately, information from this research should be practically applied.

As the research in the present study was conducted in a relatively small number of farms and data were not normally distributed, its approach was exploratory. Ideally, regulation of C and N turnover in grassland soils and biological components thereof should be scrutinised under rigorously-controlled experimental conditions, in the laboratory or in field trials, as appropriate, sufficient to test hypotheses.

7.4.7.1 Work prior to hypothesis testing

All variables have been examined in some way but there may be unseen gradients and important variables that could help explain the variation in the data but that have not been identified through ‘manual’ data diving for ordination purposes. Stepwise analysis is a semi-automated option in CANOCO (version 3.1, ter Braak, 1991, cited in Oliver et al., 2000) which can be used prior to hypothesis testing. In this process, uninformative variables can be removed one at a time through iteration and inspection of the results (Palmer, 2000 e). CANOCO could also be used to test the independence of variables (Oliver et al., 2000), and to help establish their contribution relative to each other.

7.4.7.2 Does arginine ammonification *in vitro* reflect N-function in the field?

Whether arginine ammonification *in vitro* reflects actual field release of ammonia N from soil organic matter needs to be established experimentally. Although the *in vitro* measurement of arginine ammonification accounts for mineral N as ammonia and does not include nitrite and nitrate, it represents initial steps in the mineralisation of soil organic matter. Whether or not this step is rate-limiting needs to be established. Concurrent *in situ* N-mineralisation assays and arginine ammonification tests could be carried out and compared. There may be experimental difficulties encountered in their comparison because the *in vitro* test takes place in a matter of hours, while the other assays take weeks to complete.

Implementing values for the same variables for other sites, in an exploratory way, could test the N-mineralisation/ production gradient. If the gradient is applicable, farms could be expected to locate along it according to their production potential. However the significance of the ordinations should be tested for reliability.

7.4.7.3 Is it true that ‘AC is used before ON’?

According to Smith and Paul (1990), amino acid use for C by soil microorganisms depends on a complex series of controls, and carbohydrate C will be utilised rather than amino acid skeletons that can be conserved for protein synthesis (Coyne, 1999). There

is strong evidence from statistically significant correlations and apparent gradients in this study that available C (AC), is used before organic nitrogen, (ON). This is exemplified by predominance of cellulolytic bacteria where the arginine use population is smallest. Apparently too, nitrogen mineralisation occurs where C is not readily available. Such observations implicate intracellular regulation of nitrogenous energy source use, such as catabolite repression and allostery documented for arginine use by bacteria (Cunin et al., 1986), and alluded in reference to the soil environment by Smith and Paul (1990), and by Coyne (1999).

To test that this hypothesis is applicable to soil OM, labile carbonaceous energy sources such as intracellular metabolites or molecules that can be absorbed from soil for use by bacterial cells will need careful measurement because they presumably change rapidly. Reliable storage and rapid analytical methods could be applied to this problem, such as immediate liquid nitrogen temperature reduction on field sampling, and chromatographic and other techniques appropriate for molecular profile studies. Such methods are available. Innovative bioassays are offered as a solution later.

Whether or not arginine ammonification *in vitro* is repressed by catabolite presence in soil needs to be verified experimentally. This could be approached in different ways. Arginine ammonification *in vitro* could be compared for soils with different levels of available C, either occurring naturally or introduced experimentally. Soils could be amended with glucose to mimic abundance; sparse environments could be induced by pre-incubation of soil having removed it from herbage and or rhizosphere substrate sources. (Magill and Aber, 2000, have already reported the inhibitory effect of glucose.) Following amendment, arginine ammonification rates would be expected to decline initially in glucose-amended soil, and expected to increase when labile C content dwindled. However, the rate of ammonification is likely to depend on soil conditions, such as pH, that may change, and this must also be accounted for. Disappearance of AC, release or immobilisation of ammonia nitrogen, changes in the microbial populations, biomass and associated environmental conditions such as soil pH should be monitored concurrently, over time, so that soil biological and other environmental responses to change could be examined, and the 'AC is used before ON' argument scrutinised.

In other approaches to this problem, soil concentrations of two key intracellular metabolites, ketoglutarate and glutamine could be examined in relation to arginine ammonification rates *in vitro* to establish whether their concentrations are regulatory, as would be expected (Cunin et al., 1986; Zubay, 1993). Alternatively, organisms capable of hydrolysing arginine could be either enriched for or isolated from soil and their arginine ammonification kinetics explored in media with/without possible regulatory metabolites. Presumably induction and or repression effects could be inferred.

The purpose of these experiments and kinetic studies would be to clarify soil biological function and regulation in C and N turnover processes, and to examine the hub of soil C:N ratio effects, rather than to question intracellular regulations of arginine metabolism (Cunin et al., 1986) that have already been elucidated.

7.4.7.4 Substrate gain and loss factors should be explored

Factors probably determining substrate gain and loss from herbage have been itemised here, but need to be explored and verified experimentally either in field or pot trials in which experimental conditions can be controlled. For example, the influence of soil depths and ratios thereof, and presumably varying hydraulic conductivities and water contents, might be examined using artificially created soil profile effects in the laboratory. Influence of vegetation, soil depth, grazing animal treading and herbivory could also be examined in field studies, but with a sufficiently large sample base for statistical rigour.

7.4.7.5 Thresholds for soil organic matter and biology should be identified

The need for threshold data as soil quality measures has been identified (Sparling, 1997). A review of the literature would help to disclose likely threshold levels of soil organic matter pools in soils in an exploratory way. For example, perusal of research reports in this study suggests that soil OC content thresholds in temperate grasslands may hover around 4-6%, because at levels above that, relationships between pools differ or seem unconstrained. However, criteria and methodology probably need definition so that different soil conditions can be compared, and levels established for thresholds suited to soil quality and performance assessments. Thresholds may not be generally

applicable, but might be of value in measuring response and recovery of a particular soil following specific management change.

7.4.7.6 Application of analyses to field conditions

Grassland C and N turnover, N mineralisation and immobilisation and their regulation should be examined in response to grassland management change in specifically designed time-series analyses. Influences for investigation would include organic and mineral nutrient inputs, conversion to organic farming, soil perturbation, reseeded practices, vegetation type and grazing rotations. Variables such as N-mineralisation and immobilisation *in vitro*, soil pH, root mass, bacterial populations, C:N ratio, as deemed suitable should be monitored, as well as vegetation, soil organic matter and structural parameters. Ratios between variables could be implemented to examine the changing balance of components and activities over time. Specific studies could be designed to monitor soil biology during the decline and subsequent recovery of productivity observed in the wake of reseeded or during the conversion period from conventional to organic farming regimens, so that nutrient cycling processes and requirements can be elucidated.

Each field study of this kind could provide information towards: identifying threshold levels of regulatory conditions such as soil OC content and soil pH; C and N decomposition and turnover processes, their optima and regulation; environmental gradients; biological succession; and metabiotic effects. Furthermore, if concurrent sward measurements were made, optimal conditions might be determined for both biological activity and vegetation growth in grazed pasture production. If extended for environmental purposes, the conditions for minimal losses of mineral nitrogen either to undesirable plant species or as environmental pollutants could also be determined.

7.4.7.7 Developing an understanding of soil pH

Earlier in this chapter, relationships between soil pH, ammonification and bacterial populations were exposed but could not be understood because of their complexity. Soil pH appears to be a function of its environment, and may be determined in part by the soil biology with which it interacts. How soil pH interacts with and influences its environment, in particular N-mineralisation and immobilisation, both of which were directly correlated to it here, needs to be elucidated experimentally. Appropriate experiments could be devised whereby change in pH and environment could be monitored concurrently.

Influences may be inextricable, but deliberate pH change practices such as liming should be examined in relation to soil biological function so that management induced changes in soil can be understood and anticipated.

7.4.8 Bringing this information to bear in agriculture

Having identified influential factors and explanatory mechanisms, the practical application of this research is considered here.

7.4.8.1 Bioassay of ammonification, labile C and mineral N

Labile organic matter is especially subject to biological change, because of which it is difficult to measure using current analytical techniques. Heterotrophs are mainly responsible for the transience in labile organic molecules; however, their activity could be used to advantage, in a bioassay. Microbial bioassays are in common usage elsewhere. For example, microbial detection of phenylalanine is routine in the heel prick test for phenylketonuria, and there is no reason to suppose that similar relatively doable tests could not be applied in a soil analysis context. Two applications come to mind, one a direct measure of arginine ammonification rates, the other an evaluation of labile C content in sampled soils.

It is likely that arginine ammonification assay or variants thereof could be adapted for use as a simple bioassay, because the colour change in the reactants in the presence of ammonia is measured using a spectrophotometer but is discernible to the naked eye.

Furthermore, the reaction would be expected to take place with relative speed, in hours or less, and might require little more expertise than do current soil nutrient and pH test kits. Existing applications in bacterial identification kits may already include suitable arginine ammonification assays, and should be researched. If *in vitro* and *in situ* N-mineralisation rates are found to tally, bioassays of this kind could be calibrated in future against field values and conditions.

Rather than using arginine ammonification rates as a gauge, labile C could be assayed according to heterotrophic growth in response to substrate. A range of indicator microorganisms that respond to labile C across abundance to scarcity levels expected in grassland soils could be embedded in a test medium. Incubation with a soil suspension would presumably lead to a microbial growth response to AC that could be discerned as colour reactions in test chromogens. Tests could be calibrated in relation to labile substrates standards.

Similarly, bioassays could be used to test for soil mineral nitrogen. Glutamine synthase, for example, can respond to ammonia concentrations as low as one tenth of a milligram per kilogram of soil (Coyne, 1999), and could conceivably be incorporated in a bioassay based on microbial growth response to ammonia presence in soil samples.

It should be borne in mind however that the results of bioassays developed for use in soil may vary from one soil to another, and tests should be standardised and examined for their general suitability. If bioassays are likely to have practical application in soil analysis, they should ideally be simple and doable.

7.4.8.2 How might N-mineralisation be monitored and controlled in practice?

Because of the inverse relationship between production and N-mineralisation *in vitro*, producers might wonder what increases N-mineralisation rate, in order to regulate it. Meanwhile, N-mineralisation is not a visible entity, and some indication of its occurrence and rate is needed as a basis.

Root mass is probably the best, yet indirect, ammonification indicator, as it correlated significantly in the present study with ammonification and seems to accumulate in soil that has not been either disturbed and or fertilised recently. Root mass is also indicative of poor soil nutrient levels such as magnesium, another production determinant. Root mass could be a simple indicator in its own right. As it may take time to respond to management interventions, during which released carbon may have had effect, management history and current interventions such as disturbance or fertiliser applications will need to be taken into consideration. Soil pH influences ammonification too, but as contrasting production and ammonification rates occur at similar soil pH, it is not an absolute indicator of either.

The question should probably be: “what decreases ammonification? ”, in which case the answers include added mineral nitrogen, and soil disturbances that release available carbon. Because factors are interdependent, an assessment of soil should probably include key factors as implemented in the PCA diagram. By knowing how values for key soil properties relate to a production gradient ordination diagram, it may be possible to predict production, or to predict what effect change in one attribute might have overall. More work is needed to validate this type of analysis and prediction, however.

Research is needed to establish what are the ideal biological conditions for grassland production. As part of this process, values for the key production-related soil properties that have been identified here such as ammonification rates *in vitro*, root mass content, and soil pH could be included in grazing simulation and optimisation models. For example, GRASIM is available as a field-tested web resource to help farmers predict pasture management and environmental effects (Mohtar et al., 2000). GRASIM accounts for changes in water and nitrogen provision from rainfall, fertilisers and leaching, and takes account of known nutrient cycling processes and nitrogen stresses to the plant. Although GRASIM predictions were in strong agreement with field situations (Mohtar et al., 1997 cited in Mohtar et al., 2000) if key influential data were included for particular situations, the predictive quality of the model might reasonably be improved upon.

7.4.8.3 Conclusions in regard to agricultural application

The hypothesis that ‘AC is used before ON’ in grassland soils needs to be tested experimentally, and the basis for soil biological response to any management change, either in terms of C, N or soil pH, needs to be elucidated. In the likelihood that a suite of production indicators exists, suitable field methods are needed for their analysis in real situations. However, information, analytic procedures, kits or modelling resources will have to be reliable and readily doable so as to ensure their usefulness and adoption in practice. The practical aim of this work would be to understand conditions under which nitrogen is made available from legumes to grasses in the sward and facilitate information transfer so that grassland production could be optimised in a sustainable way.

7.5 Recommendations

The findings of this study agree with those of other workers in the same research area who have expressed the need for interdisciplinary studies that give a more comprehensive view in order to understand soil organic matter turnover and for knowledge transfer (Bardgett et al., 1998 b; Skinner, 1976; Newton, 1993). Specific recommendations are summarised from the present study without a great degree of elaboration here.

Ideally, studies on the relationship of soil biology with soil organic matter turnover in grassland soils should be carried out under properly designed experimental conditions that satisfy criteria for rigorous statistical analysis procedures. As many variables as practicable should be described in order to account for and make reasonable comment on, the complexity of soil. A range of specified current and historical management conditions are best examined rather than extremes compared. This would counter bias in favour of one particular system over another. Kirchmann and Thorvaldsson (2000), stated: “many European and other countries focus on organic farming as a solution, but this approach is dangerous because it does not necessarily lead to a better environment or better food products”. What is needed is a direct and scientific understanding of the particular management influences, their environmental effects, and their products, so that benefits or otherwise of any system become obvious. A reliable gauge or reference

point applicable to other studies should be used when looking for indicators of either production and or soil organic matter quality, to enable comparison.

For soil assessment purposes, each attribute should be evaluated according to well-defined criteria taking biological and field conditions into account. Only those methods known to be reliable (Nannipieri et al., 1990) or for which constraints have been taken into account in study design, should be used. Values for soil characteristics should be reported on a per-volume or per -hectare basis. Ratios may be a useful where correlation is otherwise lacking. Non -associative effects may occur as in metabiosis. The interdependence of soil factors means that their relationships may be difficult to extricate; however, CCA analysis may help in this regard. Analyses should be interpreted with multivariate tools such as PCA that offer an inclusive perspective of the dynamically interdependent and therefore changing soil resource.

Soil depth on site as well as sampling depth should be reported, as should moisture characteristics. Soil biology should be considered from first principles. Whether substrates are used for either C or N or both should be clarified, and possible allosteric and catabolite regulation should be taken into account. The connection between C and N should be examined (Kuzyakov, 2000), because it is likely that organic AC and mineral N availabilities determine each other. Available C should be measured if necessary by bioassay, because regulation at cellular level is likely.

Because each soil biological component behaves individualistically, each should be defined and not regarded as interchangeable with others. Caution is urged because soil organic matter pools may behave like regulatory containers with threshold levels beyond which their contained pools cannot expand. Soil organic carbon content may indicate production better than constituent constrained pools, but soil biology may eventually reflect soil organic matter quality, if limited by it. Because cellular activity reflected immediacy and related well to production, whereas soil organic matter content reflected management history, their different temporal response may be a useful indicator of environmental change.

Originally, mineralisation activity in organically managed soil was presumed greater than that in conventionally managed situations. Relatively higher amounts of

mineralisation were presumed necessary to cope with the higher organic matter content found, and turnover requirements expected, in organic compared to conventional situations (Gunapala and Scow, 1998). In the present study, the most productive farms appeared to be non-extreme with respect to immobilisation, ammonification, bacterial populations, microbial biomass, and respiration rates, whereas non-productive farms had highest rates of N-mineralisation *in vitro* and higher root mass contents, suggesting that more is not necessarily better in terms of production. Grassland growth might exhibit a linear 'response' to pH, lime, or fertilisers, resulting in enhanced production, but in reality environmental factors interact, and are unpredictable. Enhancing one may have negative effect elsewhere.

The best indication of production may come from key selected variables such as pH root mass, soil plant available magnesium content, arginine ammonification and disturbance history, implemented together in a PCA ordination. Because of the interdependent nature of soil factors, soil management will need informed consideration as to what expense is incurred by any intervention. The optimal conditions for maintenance and release of soil N are unlikely to be extreme, but are probably inherent in the ideal grazing rotation and amenable to fine-tuning.

Much will depend on how soil is regarded. For the short-term, mineral fertilisers may temporarily enhance production, but organic matter losses may ensue, pushing soil beyond thresholds needed to sustain biological activity. Alternatively, with appropriate management for the longer term, soil biology could continue to transform soil organic matter in a sustainable way.

7.6 Conclusions

Annual grassland production (GJ ha^{-1}) encompassing livestock and fodder was significantly related to: fertiliser inputs; soil pH; soil depth to bedrock; soil contents of plant available magnesium and calcium; and mechanical disturbance of soil, influences of which were direct. Production was inversely related to two biological variables, namely: root mass; and the positive values of ammonification in arginine-amended soil representing N-mineralisation *in vitro*.

Although production approximated a linear response with each influential factor, each relationship was non-absolute. Influential factors correlated significantly with each other and were interdependent. When influential factors were implemented together in PCA, reasonable production-related gradients emerged in ordination space. Variables that, in rank correlation analysis, are significantly related to N-mineralisation *in vitro* could serve as a useful production indicator suite in PCA.

It was inferred from statistical and ordination evidence that: the rate and type of nitrogen metabolism that took place in soil were influenced by available carbonaceous (AC) energy sources; available carbon facilitated nitrogen immobilisation; and that organic nitrogenous energy sources (ON) were used, and ammonia released in N-mineralisation, when labile carbon was depleted. This led to the hypothesis that 'AC is used before ON' in soil organic matter turnover. Observations concur with the known intracellular allosteric regulation and catabolite repression of arginine ammonification. Presence of AC was influenced by mineral nitrogen fertiliser applications. However, soil C, N and C:N ratio values were unrelated to N-mineralisation *in vitro*. The discrepancy may arise because labile resources are rapidly expended in sampled soils and because current C and N analysis methods then may capture only the more recalcitrant soil resources that then remain in the sampled soil. Available carbon should be considered as a regulatory factor in soil carbon and nitrogen turnover processes.

Soil organic carbon content was higher than in similar studies where correlations with biomass and microbial activity have been found. Uncorrelated in study farm soils, organic carbon contents seem to be above what may be biologically limiting thresholds. The proportion of carbon in the microbial biomass was inversely related to soil aggregate stability. The proportion of biomass carbon increased inversely with organic carbon content and this is further indication of a limiting organic carbon threshold.

Soil organic matter and biological components behaved individualistically. Organic carbon probably reflected fertiliser history and may have restricted biomass carbon. Proteolytic bacterial populations may have remained at elevated levels, since lime applications, because of quiescence, and was not pH related; in contrast, arginine ammonification, a cellular activity, was responsive to the immediate soil pH environment, and unrelated to liming history. The cell free enzyme, urease, was not

correlated with cellular controls and may have been stabilised in soils by extrinsic factors such as phosphate fertilisation. Biological variables may not be interchangeable for soil quality assessment purposes, but may have individual value because of their likely differing temporal responses to change.

Fertiliser nutrient inputs, grazing practices, soil disturbance histories, vegetation and soil depth influenced bacterial populations and activities, probably through substrate gain and loss from either herbage or soil organic matter. Metabiosis is implicated, whereby bacterial populations deplete cellulose and other carbonaceous resources during the interval between grazing sessions, allowing N-mineralisation and populations responsible for that activity to predominate in more extensive or rested situations. A succession of copiotrophic to oligotrophic soil bacterial populations may occur along a gradient of carbon abundance and scarcity in grazed grasslands.

In productive farms, the microbial biomass and its respiration rate, the catabolic bacterial populations and their activities namely N-immobilisation and N-mineralisation, were not extreme. As grazing practices probably regulate substrate flow to bacterial populations, the balance between N-mineralisation, N-immobilisation, and plant available N may be optimised, in future, by the type and timing of the grazing rotation. Manipulation of just one influential factor, such as soil pH, is unlikely to suffice because soil pH interacted with other soil variables in unpredictable ways.

Further work is needed to test the hypotheses that: a suite of N-mineralisation -related variables could indicate production; and that allosteric modulation and catabolite repression govern N-mineralisation in grassland soils. Whether N-mineralisation *in vitro* is relevant at field scale needs to be established. The *in vitro* method would require only three hours incubation in the laboratory, giving it a distinct advantage over the two-week incubation required for current field methods. Having correlated with production where *in situ* methods have not, N-mineralisation *in vitro* offers promise.

Appendix A

Section 1. History of the farm:

1. Is the farm in REPS (Rural Environment Protection Scheme)? Yes No

If yes, when did the farm enter the scheme? Month _____ Year _____

2. Is the farm IOFGA Symbol Standard? Yes No

If yes, when was the IOFGA symbol granted? Month _____ Year _____

If no, has the farm been in conversion to organic? Yes No

If yes, please describe the conversion state(s) which applied at the time of sampling:

[Note: Farms were sampled either during spring1995 or winter1996/7

Year 1 of 2 year:

Area in conversion: All of farm or Acres _____ or hectares _____

Was the sampled field at this stage? Yes No

Year 2 of 2 year:

Area in conversion: All of farm or Acres _____ or hectares _____

Was the sampled field at this stage? Yes No

Year 1 of 1 year:

Area in conversion: All of farm or Acres _____ or hectares _____

Was the sampled field at this conversion stage? Yes No

If a different form of conversion has applied to the farm please describe:

3. What is the total area of the farm? Acres _____ or hectares _____

What is the adjusted area of the farm? Acres _____ or hectares _____

What is the area of grassland on the farm? Acres _____ or hectares _____

What is the adjusted area of grassland? Acres _____ or hectares _____

What is the area of the sampled field? Acres _____ or hectares _____

4(a). What is the main type of enterprise on the farm?

- Dairying
- Suckler cows
- Beef production
- Goats
- Sheep
- Mixed farming
- Other, please specify: _____

(b). Is there any forestry on the farm? Yes No

If yes, what area is under forestry? Acres _____ or hectares _____

5. What farm machinery do you have ? Please list main items:

6. How has land use changed on the farm since 1987? Please describe:

Section 2: Fodder production on the farm:

Do you produce fodder on the farm? Yes No

If yes, please describe in the following tables:

7(a). Silage:

Year	Area harvested for silage Unit: acres hectares	Was red clover sown for that crop? In the sampled field?	What <i>tonnage</i> of silage was made?	Number of 4x4 bales	Number of 5x5 bales	Was silage double chopped?	Amount of silage from the sampled field	Amount of silage sold off the farm
1992	a <input type="checkbox"/> h <input type="checkbox"/>	Sown <input type="checkbox"/> Field <input type="checkbox"/>				Yes <input type="checkbox"/> No <input type="checkbox"/>	tonnes 4x4 5x5	tonnes 4x4 5x5
1993	a <input type="checkbox"/> h <input type="checkbox"/>	Sown <input type="checkbox"/> Field <input type="checkbox"/>				Yes <input type="checkbox"/> No <input type="checkbox"/>	tonnes 4x4 5x5	tonnes 4x4 5x5
1994	a <input type="checkbox"/> h <input type="checkbox"/>	Sown <input type="checkbox"/> Field <input type="checkbox"/>				Yes <input type="checkbox"/> No <input type="checkbox"/>	tonnes 4x4 5x5	tonnes 4x4 5x5
1995	a <input type="checkbox"/> h <input type="checkbox"/>	Sown <input type="checkbox"/> Field <input type="checkbox"/>				Yes <input type="checkbox"/> No <input type="checkbox"/>	tonnes 4x4 5x5	tonnes 4x4 5x5
1996	a <input type="checkbox"/> h <input type="checkbox"/>	Sown <input type="checkbox"/> Field <input type="checkbox"/>				Yes <input type="checkbox"/> No <input type="checkbox"/>	tonnes 4x4 5x5	tonnes 4x4 5x5
1997	a <input type="checkbox"/> h <input type="checkbox"/>	Sown <input type="checkbox"/> Field <input type="checkbox"/>	Is the sampled field being used for silage production currently? Yes <input type="checkbox"/> No <input type="checkbox"/>					

7(b). Hay:

Year	Area harvested for hay in acres or hectares	How many cocks were made?	Number of small square bales	Number of large round bales	Amount of hay from the sampled field	Amount of hay sold off the farm
1992	a <input type="checkbox"/> h <input type="checkbox"/>					
1993	a <input type="checkbox"/> h <input type="checkbox"/>					
1994	a <input type="checkbox"/> h <input type="checkbox"/>					
1995	a <input type="checkbox"/> h <input type="checkbox"/>					
1996	a <input type="checkbox"/> h <input type="checkbox"/>					
1997	a <input type="checkbox"/> h <input type="checkbox"/>	Is the sampled field being used for hay currently? Yes <input type="checkbox"/> No <input type="checkbox"/>				

7(c). Other fodder produced on the farm:

year	Fodder type(s)	Amount produced	Amount sold
1992			
1993			
1994			
1995			
1996			
1997			

8. How has fodder production changed on the farm since 1987? Please describe:

Section 3. Grazing:

9 (a) Do animals graze year-round on any of the farm? Yes No

If yes, what area of the farm is grazed during the winter months?

All of the grassland is used for grazing in winter

If only part of the farm is grazed please give area: Acres _____ or hectares _____

(b) Do you have an area for summer grazing only? Yes No

If yes, what area is for summer grazing only? Acres _____ or hectares _____

This area is grazed from (date: month to month) _____ to _____

(c) Do you avail of conacre? Yes No

If yes, what area of conacre is grazed? Acres _____ or hectares _____

In what months is conacre grazed? (Date: month to month) _____ to _____

10. Do you practice rotational grazing? Yes No

If yes, (a) please indicate each of the ways in which the animals are rotated:

Field by field

Through a number of paddocks

Through temporarily fenced strips

How long is a section left before animals return to graze again? _____

(b) What height is the grass when you move animals from a section or field ?

½ inch

1 inch

2 inches

4 inches

Other Please specify _____

11. Do you practice mixed grazing? Yes No

(a) If yes, are cattle and sheep always grazed together? Yes No

(b) If they are, are all cattle moved on while some sheep remain? Yes No

(c) If another mixed grazing situation applies, please describe:

12. (a) Is tight grazing deliberately practised at specific times of the year? Yes No

If yes, during what months would the sward be tight grazed ?

January

February

March

April

May

June

July

August

September

October

November

December

(b) Do you practice topping? Yes No

If yes, during which months does topping take place?

January

February

March

April

- May
- June
- July
- August
- September
- October
- November
- December

13. Is the sampled field grazed ? Yes No

If yes, (a) is it used for winter grazing? Yes No

If no, is the field used for summer grazing only? Yes No

(b) Roughly what height of sward would be in the sampled field at the start of the season before first grazing? inches _____ cm _____

(c) Roughly on what date did grazing begin in this field

in 1995 _____

in 1996 _____

and in 1997 _____

(d) How many animals graze the field together and for how long are they there before being moved from the field at different times during the year?

Types of animal/ Number	mid May	mid June	mid July	mid August	mid September	mid October
Dry Cattle						
Cows						
Suckler cows						
Sheep						
Goats						
Horses						
Moved after						

(e) Is the sampled field regularly under-grazed ? Yes No

(f) Is the sampled field regularly overgrazed? Yes No

14. Please comment on how grazing has changed on the farm since 1987:

Section 4. Animal housing and waste storage:

15. Do you have winter housing on the farm? Yes No

If yes, (a) in which years could you avail of winter housing?

1992 Yes No

1993 Yes No

1994 Yes No

1995 Yes No

1996 Yes No

(b) Please describe the housing of animals during winter of 1996-1997:

Types of Animal	Number housed	from date	to date	Is this pattern of housing usual? Comment on numbers, length of time .
Cattle				
Dairy Cows				
Suckler Cows				
Calves				
Sheep				
Goats				

16. Do you have slatted housing on the farm? Yes No

If yes, (a) what percentage of the housing is slatted? _____ %

(b) Do you store slurry on the farm? Yes No

If yes, what cubic volume of slurry can be stored? Volume _____

Do you agitate the slurry? Yes No

If you agitate the slurry, do you add additives to help agitation? Yes No

If yes, please describe additives used :

17. Do you store manure on the farm? Yes No

If yes, (a) Is the manure heap covered? Yes No

(b) Is run-off collected from the manure heap? Yes No

(c) Is straw incorporated in any way in the manure heap? Yes No

If yes please specify what type of straw is used and how it is incorporated:

18. What form of animal bedding is used ?

Symbol Standard straw

Straw from other sources

Peat and soil cubicle bases

Sawdust and wood shavings

Paper

Other: please specify

19. Please describe housing and waste storage conditions have changed on the farm since 1987:

Section 5: A description of the soil, land, and drainage:

20. Please give most recently available soil test results:

Source:

Teagasc

IOFGA

Other

please specify: _____

Test Date: Month _____ Year _____

Mineral	Amount in soil (ppm)	Index number	Recommended treatments:
N	_____	_____	_____
P	_____	_____	_____
K	_____	_____	_____
Mg	_____	_____	_____
Lime	_____	_____	_____

(a) Soil Association Number _____ Soil Series: _____

21. Has any mineral deficiency ever been recorded on the farm? Yes No

If yes, please describe _____

Was the deficiency corrected? Yes No

22. In relation to the sampled field,

(a) Please give the approximate depth of soil feet _____ inches _____

(b) Is the soil in this field ploughable? Yes No

Is the soil in the field stony? Yes No

23. Has the sampled field ever been reclaimed? Yes No

If yes, (a) when did reclamation take place: Year _____ month _____

and (b) indicate the most appropriate condition(s) which describe(s) this field prior to reclamation:

- Bog
 - Poorly drained
 - Rocky
 - Stony
 - Scrub
 - Furze
 - Other please specify:
-
-

24. Has the sampled field ever been tilled? Yes No

If yes, has a green cover crop been sown? Yes No

25. Has straw ever been burned on the sampled field? Yes No

If yes, give most recent year _____

26. Have any of the fields ever been terraced? Yes No

If yes, was the sampled field terraced? Yes No

27 (a) What area of the land on your farm is free draining? Acres _____ or hectares _____

Is the sampled field free draining? Yes No

(b) What area of the land on your farm is wet? Acres _____ or hectares _____

Is the sampled field wet? Yes No

(c) Are there turloughs on the farm? Yes No and in the sampled field? Yes No

28. Does any of the soil on the farm tend towards erosion? Yes No

If yes, (a) please describe:

(b) Does erosion occur in the sampled field? Yes No

29. Is the sampled field sheltered from the prevailing winds? Yes No

30. Have cattle ever been kept on the sampled field throughout the winter? Yes No

31. Has the sampled field ever been poached? Yes No

If yes, (a) what percentage of the sampled field has been poached? _____ %

(b) In what years was the sampled field poached?

1993

1994

1995

1996

1997

32. Were conventional tractor jobs carried out in the sampled field? Yes No

If yes, how many conventional tractor jobs were carried out on the field during

1993 _____

1994 _____

1995 _____

1996 _____

1997 _____

33. Would a conventional tractor damage the land in the sampled field? Yes No

Section 6: The Sward:

34. (a) Has all of the grassland on the farm been in permanent unimproved pasture for over ten years?

Yes No Don't know

If yes, and grassland has not been re-seeded, please go to question number 35.

(b) If no, has any of the grassland been re-seeded? Yes No

If yes, was the sampled field re-seeded? Yes No

Please describe re-seeding as applied in

the sampled field or other fields as appropriate:

(c) What was the approximate date of the most recent reseeded : month _____ year _____

(d) How was re-seeding carried out? Please tick method(s) used:

- Over-sown
- Rolled
- Drilled
- Strip seeded
- Other please specify:

(e) Did the re-seeding involve use of

- Herbicide Yes No
- Slug pellets Yes No
- Other please specify:

(f) Did the seed mix contain:

- Red Clover Yes No
- White Clover Yes No
- Perennial rye-grass Yes No
- Italian rye-grass Yes No
- Other please specify:

(g) Where was the seed supplied from?

Co-op Yes No

Organic specialist supplier Yes No

Other please specify:

(h) Was the seed treated? Yes No

(i) What percentage of success do you feel the most recent re-seeding achieved? _____ %

35. Do you intend to re-seed in the near future? Yes No

Please comment further:

36. Which in your view best describes the sward in the sampled field?

- Grassy
 - Clumped
 - Open
 - Mossy
 - Other please specify
-
-

(a) Is there clover in the sampled field ? Yes No

If yes, what type of clover predominates in the sampled field?

- Red
- White
- Mixed

(b) Roughly what % of the sward is clover?

- 0- 10
- 11- 20
- 21-30
- 31-40
- 41-50
- Other Please specify: _____

37. Please indicate the weed content as a percentage of the sward in the sampled field:

Weed type	0%	1-10	11-20	21-30	31-40	>40
Docks	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Bracken	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Buttercups	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Nettles	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Rushes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Ragwort	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Thistles	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other _____	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other _____	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other _____	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

38. (a) From 1992 onwards, what was the sampled field mainly used for?

	1992	1993	1994	1995	1996	1997
Silage						
Hay						
Grazing in spring until winter						
Summer grazing only						
Year-round grazing						

(b) Have any other activities taken place on the sampled field in the last few years? Please describe:

39. How has the activity in the sampled field changed since 1987?

40. (a) What best describes the overall productivity of the sampled field?

Highly unproductive Unproductive Average Productive Highly productive

(b) How does the sampled field compare with other fields on the farm?

Poorest field Poor Average Good The best field

(c) How has productivity of the sampled field changed in the last 10 years? Please describe:

(d) How would you rate the soil on your farm overall ?

Very poor Poor Average Good Very good

(e) How would you rate the land of your farm overall?

Very poor	Poor	Average	Good	Very good
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

(f) In your opinion, how does your farm compare with other farms?

	Very poorly	Poorly	Same as	Better	Far better
Organic farms	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Conventional farms	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

(g) Since 1992, has production changed in any of these areas?

	Decreased	Stopped	No change	Started	Increased
Beef production	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dairying	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Suckler cows	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Sheep	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Goats	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Mixed farming	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

If production has changed, please comment on what the contributory factors may have been.

Section 7: Lime and Fertiliser Application on the Farm:

41. Please complete the following tables as appropriate:

(a) Lime applications on the farm:

Description:				
	Amount applied on farm	Cost	On sampled field	Amount on sampled field
1992			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1993			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1994			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1995			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1996			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1997			Yes <input type="checkbox"/> No <input type="checkbox"/>	

(b) Manure applications on the farm:

Description:			
	Amount applied on farm	On the sampled field	Amount on field?
1992		Yes <input type="checkbox"/> No <input type="checkbox"/>	
1993		Yes <input type="checkbox"/> No <input type="checkbox"/>	
1994		Yes <input type="checkbox"/> No <input type="checkbox"/>	
1995		Yes <input type="checkbox"/> No <input type="checkbox"/>	
1996		Yes <input type="checkbox"/> No <input type="checkbox"/>	
1997		Yes <input type="checkbox"/> No <input type="checkbox"/>	

(c) Slurry applied on the farm:

Description:				
	Amount applied on the farm	Cost	on sampled field	Amount on field
1992			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1993			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1994			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1995			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1996			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1997			Yes <input type="checkbox"/> No <input type="checkbox"/>	

(d) Other fertiliser applied on the farm:

Description:

	Amount applied on the farm	Cost	on sampled field	Amount on field
1992			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1993			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1994			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1995			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1996			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1997			Yes <input type="checkbox"/> No <input type="checkbox"/>	

(e) Other fertiliser applied on the farm:

Description:				
	Amount applied on the farm	Cost	on sampled field?	Amount on field?
1992			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1993			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1994			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1995			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1996			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1997			Yes <input type="checkbox"/> No <input type="checkbox"/>	

(f) Other fertiliser applied on the farm:

Description:				
	Amount applied on the farm	Cost	on sampled field	Amount on field?
1992			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1993			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1994			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1995			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1996			Yes <input type="checkbox"/> No <input type="checkbox"/>	
1997			Yes <input type="checkbox"/> No <input type="checkbox"/>	

42. Please give quantities of straw, manure, and slurry purchased for use on the farm:

Year	Straw amount	Approx. cost	Manure amount	Approx. cost	Slurry amount	Approx. cost
1992						
1993						
1994						
1995						
1996						
1997						

43. Have you used silage additives from 1993 onwards? Yes No

If Yes, (a) please tick the type of additive used:

bacterial

molasses

other please specify

(b) What is the approximate total cost of these additives since 1993? £_____

Section 8. Inputs and outputs:

44. Please describe the types and numbers of animals born and bought onto the farm and sold from the farm including details of age, weights, prices, dates of purchase and sale from 1992 onwards:

(a) 1992

Describe the numbers and types of animals already on the farm in this year:

Animals born, bought and sold in 1992:

(b) 1993

(c) 1994

(d) 1995

(e) 1996

(f) 1997

45. Liquid Milk Production:

(a) Main milk producing animal: Please indicate whether: Dairy Cow Goat Sheep

Year	Number of animals	Yield per animal Gallons/year	Income from Liquid milk in that year	Quantity of Cheese made	Income from sale of cheese
1993					
1994					
1995					
1996					
1997					

(b) Other milk producing animal: Please indicate whether: Dairy Cow Goat Sheep

Year	Number of animals	Yield per animal Gallons/year	Income from Liquid milk in that year	Quantity of Cheese made	Income from sale of cheese
1993					
1994					
1995					
1996					
1997					

46. Please describe the types and quantities of feed purchased for use on the farm:

Year	Type of Fodder	Feed Constituents	Quantity Purchased	Cost of feed

1993	Hay			
	Silage			
	Feed:			
	Feed:			
1994	Hay			
	Silage			
	Feed:			
	Feed:			
1995	Hay			
	Silage			
	Feed:			
	Feed:			
	Feed:			
1996	Hay			
	Silage			
	Feed:			
	Feed:			
	Feed:			
1997	Hay			
	Silage			
	Feed:			
	Feed:			
	Feed:			

47. Has the pattern of fodder purchase changed since 1987? Yes No

If yes, please describe change_____

Section 9. Animal health:

48. Please describe any preventive treatments used on the farm:

(a) Please describe the numbers of *sheep dipped* and give the diseases for which dip was used:

In 1992, _____ were dipped against _____

In 1993, _____ were dipped against _____

In 1994, _____ were dipped against _____

In 1995, _____ were dipped against _____

In 1996, _____ were dipped against _____

In 1997, _____ were dipped against _____

(b). Have *probiotic treatments* been used on the farm ? Yes No

Year	Probiotic treatment description	Type and number of animals treated
1992		
1993		
1994		
1995		
1996		
1997		

(c) Please describe any *anthelmintic* (*worms, parasites*) treatments given to animals:

Year	Treatment used	against disease	Types of animals	Number of animals
1992				
1993				
1994				
1995				
1996				
1997				

(d) Please describe any *vaccinations* given to animals:

Year	Vaccination	Disease	Type of animal	Number of animals
1992				
1993				
1994				
1995				
1996				
1997				

49. Please describe any diseases on the farm in recent years in the following table:

Year	Disease	Type of animal	Number of sick animals	Number of losses	Veterinary visits	Antibiotics given		Homeopathy given	
						Yes	No	Yes	No
1992									
1993									
1994									
1995									
1996									
1997									

50. Has the health of animals on the farm changed since 1987? Yes No

If yes, in what way has animal health changed?

Animals are much more unhealthy

Animals are more unhealthy

Animals on the farm are healthier

Animals on the farm are much more healthy

51. Does the possibility of disease on the farm

(a) Limit your choice of animal ? Yes No

(b) Limit the number of animals ? Yes No

(c) Necessitate use of treatments restricted in organic farming? Yes No

(d) Affect the farm in other ways? Yes No Please specify

52. (a) Prior to 1992, have there been any recurrent disease(s) on the farm? Yes No
If yes, please specify the disease(s)

(b) Were major losses of animals incurred due to any disease prior to 1992? Yes No
If yes, please describe the diseases involved, dates, types and age of animals and losses:

Section 10. Financial matters:

Please remember that any financial information gathered in this survey will be encoded and categorised to ensure confidentiality.

53. Are you involved in any additional on-farm enterprise? Yes No

If yes, please tick any relevant enterprise

- Cheese making
- Bed and Breakfast
- Horticultural produce
- Farm machinery for hire
- Other

Please specify: _____

54. Estimate of total farm income including income from all sales of produce, farm based enterprises, variable grants, subsidies, headage payments and premia:

Year	£ 0-10,000	£11-20,000	£21-30,000	£31-40,000	£41-50,000	> £ 51,000
1992						
1993						
1994						
1995						
1996						
possible in 1997 ?						

55. Is supplementary income availed of

	By you ?	By your partner?
Full- time employment	Yes <input type="checkbox"/> No <input type="checkbox"/>	Yes <input type="checkbox"/> No <input type="checkbox"/>
Part-time employment	Yes <input type="checkbox"/> No <input type="checkbox"/>	Yes <input type="checkbox"/> No <input type="checkbox"/>
Distance to work	Miles _____	Miles _____
Retirement benefits	Yes <input type="checkbox"/> No <input type="checkbox"/>	Yes <input type="checkbox"/> No <input type="checkbox"/>
Other, please specify: _____ / _____		

56. Estimate of total farm variable costs including fodder, fuel, seed, paid or contracted labour, interest payable on loans for buildings or machinery, and any other variable costs:

Year	£ 0-5,000	£5,100-10,000	£10,100-15,000	£15,100-20,000	£20,100-30,000	> £ 30,000
1992						
1993						
1994						
1995						
1996						
possible in 1997 ?						

57.

(a) Dairy farms: What is the milk quota for your farm? Quantity: _____

Have you availed of the full milk quota on your farm? Yes No

If no, have you been able to sell any quota to other farms? Yes No

(b) Beef: What is the suckler cow quota for your farm? Number of animals: _____

Have you availed of the full quota on your farm? Yes No

If no, have you been able to sell any quota to other farms? Yes No

(c) Sheep farming: What is the sheep quota for your farm? Number of animals: _____

Have you availed of the full quota on your farm? Yes No

If no, have you been able to sell any quota to other farms? Yes No

58. Do you receive organic premium prices for organic produce ?

If yes, please describe organic premia payments received in the table below:

Received in	type of animal or produce	Percentage premium paid	quantity of produce	Amounts received
1993				
1994				
1995				
1996				
1997				

59. (a) Is the farm in receipt of any extensification grant(s) ? Yes No Please tick:

Grant type:	Shelter belt	Animal housing	Waste storage	Silage pits	Set-aside	Forestry	Other
1993							
1994							
1995							
1996							
1997							

(b) . Has the farm received headage payments: Yes No Please describe any received:

Year	types of animal	number of animals	Amounts received
1993			
1994			
1995			
1996			
1997			

(c) Has the farm received any other Premia? Yes No

If yes, please describe payments received in the table below:

Year	Type of premia	Awarded for	Amounts received
1993			
1994			
1995			
1996			
1997			

60. Is the farm : Inherited Yes No

Purchased Yes No

Shared Yes No

Other- please describe : _____

Section 11. Support :

61. (a) Which type of farm produce do you label as organic?

Live animals Yes No

Meat Yes No

Other produce Yes No

Please specify: _____

(b) What distance are your nearest organic buyers from your farm? _____miles

(c) In your experience, how does the organic label influence sales of produce?

62. (a) How do present market conditions influence your farm?

(b) Have you been affected in any way by the BSE crisis ?

63. If the farm has incurred financial losses, would you like to comment on the loss contributing factors?

64. In your opinion, how helpful are the following grant schemes?

	Very unimportant	Unimportant	Neutral	Helpful	Very helpful
REPS	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Organic top-up	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Extensification	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Shelterbelt	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Housing:	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Animal waste storage	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Set-aside	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Forestry	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Biodiversity	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other: _____	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

65. Are you a member of any organisations or associations ?

IOFGA Yes No

SHROM Yes No

Other farming organisations? Yes No

If yes, please specify _____

Environmental associations Yes No

If yes, please specify _____

Other associations Yes No

If yes, please specify _____

66. In your view, how relevant are the following as support for organic farming?

	Highly Irrelevant	Irrelevant	Don't know	Relevant	Highly Relevant
The media-newspapers	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Journals	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Television	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Education	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Family involvement	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Marketing	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Teagasc	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
IOFGA	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
SROM	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other farming organisations:	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Please specify _____					
Environmental organisations:	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Please specify _____					
Political organisation:	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Please specify _____					
Other organisation:	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Please specify _____					
Other organisation:	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Please specify _____					

Do you have other sources of support not mentioned in this survey ?

Please specify _____

67. (a) Which of the following do you: Read every issue of? Every second issue ? Now and again?

Journal , Paper, Periodical	Read each issue of	Every other one	Now and again
Farmer's Journal			
Irish Farmers Weekly			
Organic Matters			
New Farmer and Grower			
Daily Newspaper :			
Daily Newspaper:			
Clare Champion			
Limerick Leader			
Other:			

(b) Which farming supplements do you prefer? _____

68. Please describe your education, training and qualifications

Intermediate (Junior) Cert: Yes No

Leaving Cert Yes No

With Agricultural Science? Yes No

With Biology as a subject? Yes No

Agricultural College Yes No

Green Cert Yes No

ACOT courses Yes No

Farming Qualifications Yes No

Please specify : _____

Training undertaken Yes No

Please specify: _____

Other Certificates Yes No

Please specify: _____

Diplomas Yes No

Please specify: _____

Degrees- third level Yes No

Please specify : _____

Postgraduate studies Yes No

Please specify : _____

Research: Yes No

Please specify : _____

Please describe other education or training you have had in the context of either farming or organic farming:

69. (a) How many members of your family are actively involved in intensive farming?

None

1

2

other Please specify: _____

(b)How many members of your family are actively involved in organic farming?

- None
- 1
- 2
- other Please specify: _____

70. Are you the sole person responsible for management of the farm Yes No

If no, is this responsibility shared with

- A partner
- Other family member
- Other please specify: _____

How much of the responsibility for management is yours?

- A little
- About a quarter
- About half
- Three quarters
- Other please specify: _____

71. Is the work of the farm shared Yes No

If yes, is it shared with

- Family members
- Employed labour
- Other please specify: _____

72. (a) What, for you, are the advantages of organic farming ?

(b) What, for you, are the disadvantages of organic farming ?

73. (a) What are your dreams for agriculture generally?

(b) What are your dreams for your farm ?

74. Do you have any questions about either the sampled field , the soil or the land which we might be able to address in the survey?

75. Thank you for your participation in the survey!

Appendix B

Table B.1

Conversion factors to evaluate stocking density
as cow day equivalents

Class of stock	Conversion factor to cow days, LU
Horses	1.2
Dairy cows	1.0
Suckler cows	0.75
Cattle 0-1 y	0.30
Cattle 1-2 y	0.54
Cattle > 2 y	0.65
Sheep and lambs	0.10
Hogget	0.07
Goats	0.10

Source: IOFGA, 1996.

Table B.2

Estimated daily output of N P and K from dung, urine and excreta, in grams per day
according to type and age of animal

Excreta	Dung content			Urine content			Total excreta content		
	N	P	K	N	P	K	N	P	K
Nutrient g d⁻¹									
Horse	53.1	26.6	26.6	118.0	3.5	118.0	177.0	29.5	147.5
Dairy cow	90.0	45.0	45.0	200.0	6.0	200.0	300.0	50.0	250.0
Suckler	70.0	35.0	35.0	152.0	4.6	152.0	228.0	38.0	190.0
Cattle 0-1y	25.0	13.0	13.0	56.0	1.7	56.0	84.0	14.0	70.0
Cattle 1-2 y	60.0	30.0	30.0	112.0	4.0	134.0	201.0	33.5	46.9
Cattle > 2y	70.0	35.0	35.0	152.0	4.6	152.0	228.0	38.0	190.0
Ewe	9.0	4.5	4.5	15.0	0.5	4.5	24.0	5.0	20.0
Hogget	1.5	1.5	5.0	0.2	1.5	8.0	1.7	6.7	3.0
Goat	9.0	4.5	4.5	15.0	0.5	4.5	24.0	5.0	20.0

Sources: Frame, 1992; REPS, 1993.

Table B.3

Scores given to describe the drainage characteristics of the soil series in the present study

Soil series	Drainage	Score given
Elton	Well drained	3.0
Ballylanders	Well drained	3.0
Patrickswell	Well drained	3.0
Baggottstown	Excessively drained	3.5
Gortaclareen	Poorly drained	0.0
Cooga	Excessively drained	4.0
Kilfergus	Well drained	3.0

Sources: Finch and Ryan, 1966; Finch, 1971.

Appendix C

Appendix C

Key to variables measured and mentioned in Chapters 2-7.

Related variables are close together and follow the progression of topics in the thesis with reference to the table in which they are first described. Their precise order follows that of SPSS files used for the data spreadsheet (Appendix D) and correlation table (Appendix E). The table in which the variable is first reported is given in column one; its SPSS abbreviation used in correlation table and in data spreadsheet in column two; and labels in scatter graphs and ordination diagrams in Chapter 7 and Appendix F are given in the third column.

Individual values for variable and farms can be found in Appendix D.

- a. Most of the variables have been summarised for descriptive statistics purposes, and appear in tables listed. Some variables were not used as a descriptive tool initially, but became noteworthy as the thesis developed.
 - b. Annotation has been used to shorten variable names. In regard to nutrient applications: four or 4, and six or 6, refer to four year and six year applications prior to 1995 and 1997 respectively.
 - c. Inclusion of the phrase 'farm' refers to applications to the entire farm, whereas 'sf' refers to the sampled field. Applications referred to in the text of the thesis are on the basis of the sampled field unless otherwise stated. Farm applications are mentioned in the text of Chapter 6, where they related better than field data to grassland production. Farm applications are referred to occasionally in Chapter 7 and in Appendix F.
 - d. In regard to bacterial populations and biological variables: values pertaining to dry weight equivalent of soil are prefixed d or D; moist weight values are not prefixed. Similarly, bacterial activities are either prefixed with, or contain the letter D, if pertaining to soil dry-weight equivalent values, given per gram dry weight of soil, g^{-1} ds.
-

Table	Descriptive statistics	Spreadsheet and correlation table	Scatter graphs and ordination diagrams
2.1	Soil pH		
Lime and fertiliser applications			
2.3	Years since lime application	Since lime	
2.2		Lime sf	Lime applied; lime inputs
6.5		Lime farm	
a	Years since manure or slurry applied	Since OM	Since organic;
2.3	Years since application of mineral N	4SinceMinN; 6SinceMinN	Interval since mineral N applied; since mineral N; Y since mineral N;
a, b		4Sinceferts; 6Sinceferts	Since fertilisers;
2.2, b	Nutrient and lime applications: Mineral fertilisers	4Bag sf ; 6Bag sf	Mineral inputs; Mineral N applied to sampled field; mineral N added; Mineral N, P and K; mineral N added recently;
2.2, b	Organic fertilisers	4Org sf; 6Org sf	Organic P; Organic K, P and N inputs; organic phosphate inputs
2.2	Total fertilisers		Total P added; phosphate inputs;
6.5, c		Min farm; Org farm	P additions to farm; organic P added to farm; Mineral N applied to farm
Grazing			
7.2	Type of animal (encoded 1 goat; 2 sheep; 3 mixed sheep and cattle; 4 young cattle; 5 dairy cows).	MAIN	Larger animals
2.4	Continuity Days grazed in a rotation	ONLAND	Days on land in a rotation; continuity of grazing in rotation
2.4	Days rested in a rotation	OFFLAND	Days off land per rotation
2.4	Days grazed in year	DAYGRAZE	Continuity of grazing in year
2.4	Intensity Ratio days grazed /rotation	ONONOFF	Ratio days grazed to days in a rotation
2.5	Frequency of grazing,	NUMAPERH	Number of animals per hectare
2.5	Frequency in rotation	NUMPHINT	Grazing frequency;
2.5	Frequency in year	ANSHYBDS	Grazing frequency; Grazing frequency in year
2.6	Treading, bulk of animals on field	LUH	
2.6	Treading in rotation	LUHFRDS	Treading
2.6	Treading in year	EXPOSURE	

2.6	Total estimated nutrients excreted; Excreta N, P and K	NEXCRGH, PEXCRGH KEXCRGH	Excreta N (represented all excreta values)
2.4	Ratio days grazed to rotation	ONONOFF GRAZREST	Grazing intensity
2.4	Ratio days rested/grazed	RESTGRAZE	
a	Frequency in year	ADSPERH	
a	Added days grazed in year for each type	ADDDAYS	
a	Stocking density on sampled field	INTENSE	LUH grazed together
2.5	Total number present together	NUMBERAN	Overall frequency

Mechanical disturbance and reseeded history

2.8	Mechanical disturbance	sinceair	Interval since aerated; since aerated; interval since soil disturbance;
a	Since arable crop sown Reseeded (whether reseeded or not)	w_arable RESEDED	Interval since ploughed Whether reseeded or not; reseeded
2.8		SINCEED	

Farm management system

2.9	Farm management system	ORGANICF	Duration of organic management; time farmed organically; Organic farming;
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Site-related properties

3.1	Elevation above sea level	ALTITUDE	Altitude
3.2	Slope	SLOPE	
3.3	Variation from due south	SOUTHERN	South facing;
3.4	Depth of soil, A horizon	DEPTH	A horizon; depth value
3.4	Depth to parent material	DEPTH	Depth; depth value
7.2	Ratio between A horizon and depth	ADEPTH	Ratio, depth of A horizon: bedrock; depth value
3.5	Percent clay	CLAY%	
3.6	Ratio of silt to clay	SILTCLAY	
3.7	Soil series	SOILASS	Soil type
3.8	Natural drainage	DRAINED	Well drained
a	Field regarded as wet /dry	WETDRYSF	(Farmer opinion)

Soil physical and chemical properties

3.9	Root mass	ROOTMASS	
3.10	Bulk density	BULKDENS	
3.11	Particle density	PARTICLE	
3.12	Pore space as percentage	PORESPACE	Porosity
3.13	Aggregate ratio	AGGRATIO	
3.14	Water stable aggregates	WSACENT	% WSA
3.15	Organic carbon content	OC	OC; organic carbon
3.16	Soil nitrogen	TOTALNIT	Soil N
3.17	C:N ratio	CARBNIT	Soil C:N ratio

3.18	Plant-available soil phosphorus	PHOSPHAT	Soil P
3.19	Plant-available soil potassium	POTASSIU	Soil K
3.21	Plant-available soil magnesium	MAGNESIUM	Magnesium; magnesium content
3.20	Plant-available soil calcium	CALCIUM	Plant available calcium; calcium; calcium content
3.22	Cation exchange capacity	CATION	CEC
3.23	Soil polysaccharide content	POLYSACH, DPOLYSACH	Polysaccharide content

Botanical composition of the grassland

4.1	<i>Lolium perenne</i>	RYEGRASS	Rye grass
4.4	<i>Agrostis stolonifera</i>	AGR_STO	
4.1	<i>Alopecurus pratensis</i>	ALO_PRA	
4.1	<i>Holcus lanatus</i>	HOL_LAN	
4.1	<i>Festuca ovina</i>	FES_OVI	
4.1	<i>Dactylis glomerata,</i>	DAC_GLO	
4.1	<i>Poa pratensis</i>	POA_PRA	
4.1	<i>Poa annua</i>	POA_ANN	
4.1	<i>Cynosurus cristatus</i>	CYN_CRI	
4.1	<i>Ranunculus repens</i>	RAN_REP	
4.1	<i>Bellis perennis</i>	BEL_PER	
4.1	<i>Trifolium spp.</i>	CLOVER	Clover
4.1	<i>Cirsium spp.</i>	CIR_SPP	
4.1	<i>Rumex acetosa</i>	RUM_ACE	
4.1	<i>Bryophyte spp.</i>	BRY_SPP	Mosses
4.1	<i>Cardamine pratensis</i>	CAR_PRA	
4.1	<i>Taraxacum officinale</i>	TAR_OFF	
4.1	<i>Plantago lanceolata</i>	PLA_LAN	
4.1	<i>Potentilla anserina</i>	POT_ANS	
4.1	<i>Juncus spp.</i>	JUN_SPP	Rush species
		TOT_WT	
a	Vegetation dry weight	DWT_SPB	
a	Vegetation moist weight	BOTMASS	Botanical mass
4.2	% Grass content	ALLGRASS	Grassy
4.2	Number of species	NSPECIES	Plant diversity; botanical diversity;

Soil biological properties

5.2, d	Total bacteria on nutrient agar	BACTERIA, DBACTERIA	Bacteria; Bacterial population(s)
5.2, d	Bacteria with cellulase	CELLULA, DCELLULA	Cellulolytic bacteria; cellulolytic organisms
5.2, d	Bacteria with amylase	AMYLASE, DAMYLAS	Amylytic population
5.2, d	Extracellular polysaccharide producers	EPS, DEPS	deps; extracellular polysaccharide producing; gummy population; bacteria
5.2, d	Bacteria with protease	PROTEASE DPROTEAS	Proteolytic bacteria
5.2, d	Arginine ammonifiers	ARGUSER,	Arginine using; arginine use

5.3, d	Arginine ammonification	DARGUSER CMAMONIA, CDAMONIA	population; Arginine ammonification
5.3, d	Immobilisation	CIMOBILI, CIMOBILZD	N immobilisation <i>in vitro</i> ;
5.3, d	Mineralisation	CMAMON, CDAMON,	N mineralisation; N- mineralisation <i>in vitro</i> ;
5.4, d	Urease activity in urea amended soil	ENZUREA, DENZUREA	Urease activity <i>in vitro</i> for moist and dry soil; urease;
5.4, d	Urea ammonifiers	UREASERS, DUREASRS	Urealytic population;
5.1	SIR	RESPRATE	Responsive biomass
5.1	NRN	NINHYDN	Total biomass
a	% NRN to soil N	TOTNSMBN	SMBN/Soil N; ratio of biomass N to soil N;
a	% OC as SMBC (percent of soil OC in soil microbial biomass	OCASSMBC	SMBC/OC; ratio of biomass C to soil C
5.1	Biomass C:N ratio	BIOMASCN	
<hr/>			
Grassland production			
6.3	Livestock units per hectare	LUHGRASS	This value refers to all of the grassland on the farm
6.2	Gross margin per hectare	PROFITHA	
6.1	Grassland energy outputs	ENERGYGJ	Production; grassland production

Appendix D

	farmcode	ph	moistten	sinclime	limeha	limefarm	sinceom	fnominn	snominn	fnoferts	snoferts	fbagnsf	fbagpsf
1	A	5.18	1.71	999.00	.00	.83	999.00	8.00	10.00	8.00	10.00	.00	.00
2	B	6.47	.68	.50	4.94	4.94	.50	3.00	6.00	.50	.00	29.38	.00
3	E	6.82	1.48	1.50	7.41	7.41	999.00	8.00	10.00	1.00	4.00	.00	.00
4	F	5.46	1.80	999.00	.00	.00	999.00	999.00	999.00	.50	.50	.00	.00
5	G	6.41	.80	2.50	3.09	1.32	3.00	8.00	2.00	2.00	2.00	.00	.00
6	H	6.08	1.96	.50	7.41	7.41	.50	8.00	10.00	.50	1.00	.00	.00
7	J	5.27	.78	999.00	.00	.00	999.00	8.00	10.00	.50	1.00	.00	.00
8	L	5.75	1.94	2.50	4.20	4.20	1.00	3.00	6.00	1.00	2.00	.00	37.50
9	M	6.12	.97	.50	2.47	2.47	999.00	999.00	999.00	1.00	2.00	.00	.00
10	N	4.91	1.58	999.00	.00	.00	999.00	8.00	10.00	8.00	10.00	.00	.00
11	O	6.37	1.29	.50	2.47	2.47	999.00	8.00	10.00	.50	3.00	.00	.00
12	P	5.18	.78	3.50	4.94	4.94	999.00	8.00	10.00	.50	.00	.00	.00
13	Q	7.15	1.23	999.00	.00	.00	.50	.00	.00	.00	.00	920.00	150.00
14	R	5.99	.88	1.50	4.94	4.04	999.00	8.00	10.00	1.00	4.00	.00	.00
15	S	6.14	1.08	1.50	13.59	13.59	999.00	8.00	10.00	1.00	4.00	.00	.00
16	T	5.11	1.58	999.00	.00	.00	999.00	8.00	10.00	8.00	10.00	.00	.00

	fbagsf	forgnsf	forgpsf	forgksf	ftotnsf	ftotpsf	ftotksf	sbagnsf	sbagpsf	sbagksf	sorgnsf	sorgpsf	sorgksf
1	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
2	.00	111.35	69.27	263.93	140.73	69.27	263.93	29.38	.00	.00	169.08	106.64	401.39
3	.00	.00	45.00	.00	.00	45.00	.00	.00	.00	.00	.00	45.00	.00
4	.00	.00	7.03	.00	.00	7.03	.00	.00	.00	.00	.00	49.50	.00
5	.00	.23	28.43	.62	.23	28.43	.62	56.25	18.75	37.50	.23	28.43	.62
6	.00	66.00	68.95	154.00	66.00	68.95	154.00	.00	.00	.00	99.00	86.55	231.00
7	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
8	75.00	22.28	11.88	51.98	22.28	49.38	126.98	.00	37.50	75.00	44.55	23.76	103.95
9	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
10	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
11	.00	.00	28.13	.00	.00	28.13	.00	.00	.00	.00	.00	28.13	.00
12	.00	.00	135.00	.00	.00	135.00	.00	.00	.00	.00	.00	202.50	.00
13	300.00	4.95	2.64	11.55	924.95	152.64	311.55	1380.00	225.00	450.00	7.43	3.96	17.33
14	.00	4.45	34.05	11.86	4.45	34.05	11.86	.00	.00	.00	4.45	34.05	11.86
15	.00	.00	54.00	.00	.00	54.00	.00	.00	.00	.00	.00	54.00	.00
16	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00

	stotnsf	stotpsf	stotksf	minnfarm	minpfarm	minkfarm	orgnfarm	orgpfarm	orgkfarm	totnfarm	totpfarm	totkfarm	inputs4a
1	.00	.00	.00	.00	.00	.00	.10	9.46	.28	.10	9.46	.28	2.00
2	198.46	106.64	401.39	29.38	.00	.00	150.60	82.83	352.45	179.98	82.83	352.45	4.00
3	.00	45.00	.00	.00	.00	.00	32.16	172.12	75.05	32.16	172.12	75.05	2.00
4	.00	49.50	.00	.00	.00	.00	.00	122.27	.00	.00	122.27	.00	2.00
5	56.48	47.18	38.12	56.25	18.75	37.50	73.03	108.68	170.51	129.28	127.43	208.01	3.00
6	99.00	86.55	231.00	.00	.00	.00	337.90	263.58	788.44	337.90	263.58	788.44	3.00
7	.00	.00	.00	.00	12.50	25.00	.00	.00	.00	.00	12.50	25.00	1.00
8	44.55	61.26	178.95	.00	37.50	75.00	119.51	63.74	278.85	119.51	101.24	353.85	3.00
9	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	2.00
10	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	1.00
11	.00	28.13	.00	.00	.00	.00	.00	70.31	.00	.00	70.31	.00	2.00
12	.00	202.50	.00	.00	.00	.00	.00	74.27	.00	.00	74.27	.00	1.00
13	1387.43	228.96	467.33	1044.42	264.95	567.51	6.90	3.68	16.09	1051.31	268.63	583.60	4.00
14	4.45	34.05	11.86	.00	.00	.00	179.14	113.49	255.69	179.14	113.49	255.69	2.00
15	.00	54.00	.00	.00	.00	.00	.00	47.70	.00	.00	47.70	.00	2.00
16	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	1.00

	minnfour	minpfour	minkfour	orgnfour	orgpfour	orgkfour	allnfour	allpfour	allkfour	main	onland	offland	daygraze
1	.00	.00	.00	.10	9.47	.28	.10	9.46	.28	4.00	14.00	14.00	120.50
2	29.38	.00	.00	100.40	55.22	234.97	129.78	55.22	234.97	5.00	17.00	14.00	136.55
3	.00	.00	.00	.00	154.97	.00	.00	154.97	.00	5.00	7.00	14.00	81.00
4	.00	.00	.00	.00	81.51	.00	.00	81.51	.00	2.00	14.00	10.00	192.00
5	.00	.00	.00	48.80	95.76	113.97	48.80	95.76	113.97	3.00	14.00	14.00	157.50
6	.00	.00	.00	225.27	203.51	525.63	225.27	203.51	535.63	5.00	60.00	30.00	160.00
7	.00	12.50	25.00	.00	.00	.00	.00	12.50	25.00	4.00	198.00	.00	198.00
8	.00	37.50	75.00	79.67	42.49	185.90	79.67	79.99	260.90	5.00	3.00	18.00	38.43
9	.00	.00	.00	.00	.00	.00	.00	.00	.00	3.00	18.00	72.00	40.40
10	.00	.00	.00	.00	.00	.00	.00	.00	.00	5.00	33.00	33.00	182.50
11	.00	.00	.00	.00	70.31	.00	.00	70.31	.00	1.00	7.00	28.00	35.00
12	.00	.00	.00	.00	49.51	.00	.00	49.51	.00	5.00	365.00	.00	365.00
13	909.74	176.64	378.34	4.60	2.45	10.73	914.33	179.09	389.07	5.00	3.00	21.00	11.00
14	.00	.00	.00	122.80	156.63	179.45	122.80	156.63	179.45	1.00	10.00	10.00	94.50
15	.00	.00	.00	.00	47.70	.00	.00	47.70	.00	4.00	10.00	30.00	56.25
16	.00	.00	.00	.00	.00	.00	.00	.00	.00	4.00	7.00	28.00	36.80

	ononoff	numaper	numhint	anshbyds	luhgrass	luhfords	luhfdsoc	exposure	nexcrkgh	pexcrkgh	kexcrkgh	grazrest	restgraz
1	.50	1.51	21.18	182.30	1.16	13.29	1.82	114.40	38.96	7.19	34.25	1.00	1.00
2	.55	8.10	137.63	1105.52	1.54	117.50	19.86	943.82	194.37	35.07	168.56	1.21	.82
3	.33	9.88	69.16	800.28	1.98	44.09	6.70	510.18	149.45	26.71	126.04	.50	2.00
4	.58	22.80	319.20	4377.60	1.31	30.32	7.30	415.87	90.97	18.76	34.12	1.40	.71
5	.50	21.61	302.58	3403.97	1.52	67.00	10.74	753.74	75.30	14.77	41.43	1.00	1.00
6	.67	3.46	48.41	553.28	1.50	21.79	2.27	248.98	70.82	12.73	59.94	2.00	.50
7	1.00	1.55	306.57	306.57	.85	207.30	30.53	207.30	69.42	12.37	58.49	999.00	.00
8	.14	49.89	149.68	1917.43	1.45	133.90	21.00	1715.24	482.53	85.84	410.94	.17	6.00
9	.20	34.58	622.44	1397.03	1.18	62.24	1.98	139.70	33.53	6.92	12.57	.25	4.00
10	.50	14.82	2712.06	2704.65	1.15	1017.02	222.30	1014.24	409.75	73.43	346.20	1.00	1.00
11	.20	59.28	414.96	2074.80	4.35	70.89	13.38	354.45	88.80	17.04	55.52	.25	4.00
12	1.00	1.41	512.97	512.97	.72	287.26	60.29	287.26	89.39	16.41	77.84	999.00	.00
13	.13	74.10	222.30	815.10	1.58	222.30	69.69	815.10	236.38	41.57	199.70	.14	7.00
14	.50	62.24	622.44	5882.06	2.82	62.24	7.50	588.21	141.17	29.12	52.94	1.00	1.00
15	.25	40.51	405.08	2278.58	1.88	263.30	46.40	1481.07	126.46	22.56	106.52	.33	3.00
16	.20	4.12	28.82	151.49	1.63	18.73	3.10	98.47	33.63	6.00	28.33	.25	4.00

	grasumsi	adsperh	addays	intense	numera	sinceair	w_arable	wploughd	disturbd	reseeded	sincseed	reps	organicf
1	1.00	100.45	361.00	.95	49.00	999.00	999.00	999.00	999.00	.00	999.00	.00	12.00
2	3.00	517.73	805.73	6.91	59.00	3.00	999.00	999.00	2.00	1.00	2.00	.00	36.00
3	1.00	600.21	62.00	6.30	60.00	15.00	999.00	13.00	13.00	1.00	13.00	.00	36.00
4	3.00	3546.67	120.00	2.17	120.00	999.00	999.00	999.00	999.00	.00	999.00	1.00	13.00
5	3.00	1968.28	100.00	4.79	140.00	20.00	999.00	20.00	18.00	1.00	20.00	2.00	24.00
6	1.00	368.85	95.00	1.56	21.00	150.00	150.00	999.00	150.00	.00	999.00	3.00	18.00
7	2.00	291.98	60.00	1.05	42.00	999.00	999.00	999.00	999.00	.00	999.00	.00	.00
8	3.00	1139.02	1578.02	44.63	101.00	999.00	45.00	45.00	45.00	1.00	45.00	.00	42.00
9	3.00	1397.03	14.00	3.46	14.00	999.00	999.00	999.00	999.00	.00	999.00	.00	6.00
10	1.00	1352.33	182.50	5.56	24.00	150.00	999.00	150.00	150.00	.00	999.00	.00	.00
11	3.00	1729.00	200.00	10.13	120.00	999.00	999.00	999.00	999.00	.00	999.00	.00	48.00
12	1.00	128.24	365.00	.79	52.00	999.00	999.00	999.00	999.00	.00	999.00	.00	.00
13	3.00	815.10	815.10	74.10	30.00	5.00	5.00	5.00	5.00	1.00	5.00	.00	.00
14	3.00	5882.06	70.00	6.22	63.00	150.00	150.00	150.00	150.00	.00	999.00	4.00	24.00
15	1.00	569.64	80.00	26.33	41.00	22.00	20.00	20.00	20.00	1.00	20.00	.00	24.00
16	1.00	151.49	121.00	2.68	10.00	999.00	999.00	999.00	999.00	.00	999.00	.00	.00

	altitude	slope	southern	deptha	depth	adepth	sovera	clayperc	siltclay	soilass	drained	wetdrysf	rootmass
1	61.00	13.00	145.00	38.00	101.60	.37	2.67	21.00	1.57	1.00	3.00	2.00	.92
2	25.00	88.00	105.00	38.00	101.60	.37	2.67	21.00	1.57	1.00	3.00	5.00	.76
3	105.00	694.00	25.00	19.00	63.50	.30	3.34	25.00	1.60	2.00	3.00	5.00	.67
4	47.50	233.00	102.00	38.00	63.50	.60	1.67	20.00	1.80	3.00	3.00	3.00	1.23
5	140.00	152.00	12.00	19.00	63.50	.30	3.34	25.00	1.60	3.00	3.00	5.00	.92
6	130.00	192.00	124.00	19.00	63.50	.30	3.34	25.00	1.60	3.00	3.00	5.00	1.28
7	59.00	79.00	125.00	12.70	43.18	.29	3.40	12.00	1.25	4.00	3.50	4.00	2.01
8	65.00	52.00	57.00	38.00	101.60	.37	2.67	21.00	1.57	1.00	3.00	5.00	1.12
9	75.00	70.00	50.00	38.00	101.60	.37	2.67	21.00	1.57	1.00	3.00	5.00	1.84
10	107.50	209.00	45.00	19.00	78.74	.24	4.14	22.00	1.32	5.00	.00	4.00	2.09
11	177.50	138.00	7.50	19.00	78.74	.24	4.14	22.00	1.32	5.00	.00	3.00	1.74
12	110.00	588.00	80.00	28.00	58.42	.48	2.09	14.00	1.79	6.00	4.00	5.00	2.22
13	999.00	999.00	999.00	38.00	101.60	.37	2.67	21.00	1.57	1.00	3.00	5.00	.87
14	90.00	420.00	27.00	20.00	30.48	.66	1.52	34.00	1.35	6.00	3.00	1.00	1.48
15	65.00	285.00	75.00	38.00	63.50	.60	1.67	20.00	1.80	3.00	3.00	5.00	1.78
16	35.00	42.00	5.00	64.00	86.36	.74	1.35	22.00	1.55	1.00	3.00	5.00	1.84

	bulkdens	particle	porespac	aggratio	wsacent	organicc	totalnit	carbnit	phosphat	potassiu	cation	magnesi	calcium
1	1.10	2.29	52.00	3.35	86.50	7.30	.56	13.00	2.93	222.50	7.92	220.00	1485.00
2	1.25	2.33	47.00	2.78	82.65	5.92	.49	12.00	6.20	82.50	15.77	300.00	4750.00
3	1.10	2.46	55.00	2.67	86.55	6.59	.57	11.60	2.55	122.50	13.46	350.00	7450.00
4	1.16	2.39	51.00	10.95	81.30	4.16	.44	9.44	4.50	95.00	9.20	165.00	1165.00
5	1.19	2.36	50.00	2.39	85.74	6.24	.52	12.01	7.20	62.50	13.93	310.00	3850.00
6	.99	2.13	54.00	4.26	85.96	9.61	.72	13.28	12.90	80.00	15.69	250.00	8500.00
7	1.12	2.30	51.00	1.94	88.89	6.79	.62	11.04	2.55	65.00	15.32	195.00	1925.00
8	1.05	2.35	55.00	2.44	80.88	6.38	.57	11.12	4.50	250.00	11.70	285.00	2010.00
9	.98	2.42	60.00	3.79	82.55	5.20	.42	12.23	2.65	27.50	9.89	215.00	13500.00
10	1.05	2.45	57.00	4.94	73.67	4.58	.36	12.70	1.50	72.50	5.95	60.00	310.00
11	1.02	2.35	57.00	3.73	80.54	5.30	.31	17.21	11.00	10.00	11.94	295.00	1975.00
12	1.05	2.41	57.00	7.70	78.38	4.77	.51	9.38	3.40	122.50	5.92	95.00	540.00
13	1.27	2.46	48.00	4.40	63.17	3.19	999.00	999.00	11.98	20.00	10.21	180.00	7900.00
14	1.03	2.28	55.00	1.29	88.94	8.30	.65	12.77	3.85	187.50	14.90	200.00	5800.00
15	1.17	2.31	49.00	4.82	77.82	5.65	.51	10.97	6.60	52.50	12.56	175.00	2070.00
16	1.06	2.32	54.00	2.16	79.06	6.05	.48	12.67	1.40	25.00	18.72	135.00	595.00

	polysach	dpolysac	ryegrass	agr_sto	alo_pra	hol_lan	fes_ovl	dac_glo	poa_pra	poa_ann	cyn_cri	ran_rep	bel_per
1	9.00	10.86	19.79	1.59	.00	.00	.00	.00	11.48	.00	.00	1.06	2.65
2	7.50	8.05	.83	.00	32.23	14.05	.00	.00	8.26	.00	.00	26.45	.00
3	23.00	27.00	41.74	.00	2.05	.00	.00	.82	17.83	.00	.00	2.22	.00
4	19.00	23.17	9.33	85.05	.00	1.82	.00	.00	2.88	.00	.00	.00	.00
5	30.00	32.61	51.02	12.44	17.51	3.30	.00	1.78	11.68	.00	.00	.00	.00
6	45.00	55.97	6.60	4.57	.00	.00	.00	.00	20.14	13.37	.00	6.94	6.09
7	22.20	24.10	1.80	3.71	20.95	.00	29.60	.87	.00	.00	.00	4.00	.00
8	28.00	34.74	63.75	.00	10.19	.00	.00	1.29	.00	.00	.00	10.84	1.62
9	18.50	20.49	15.67	45.28	.00	.23	.00	.00	.00	.00	38.71	.00	.00
10	44.80	53.21	.00	26.48	1.38	.00	.00	.00	.95	.00	.00	.08	.00
11	32.50	37.32	4.80	48.58	.76	.00	.00	.00	.00	.00	.00	1.42	.00
12	18.70	20.28	11.74	20.03	.00	.00	.00	.00	35.99	.00	.00	.16	.00
13	24.90	28.41	27.61	.00	.00	.00	.00	.00	61.93	3.49	.00	.00	.00
14	42.50	46.60	3.61	.00	.00	5.02	58.23	.00	.00	.00	.00	.00	.00
15	43.90	49.22	8.40	5.89	4.22	80.70	.00	.00	.00	.00	.00	.49	.00
16	22.50	26.72	21.70	50.89	.00	.00	17.12	.00	.00	.00	.00	.84	.00

	clover	cir_spp	rum_ace	bry_spp	car_pra	tar_off	pla_lan	pot_ans	jun_spp	tot_wt	dwt_spb	botmass	allgrass
1	1.59	.00	.00	21.20	.71	.00	39.93	.00	.00	5.66	9.73	15.39	32.90
2	.00	18.18	.00	.00	.00	.00	.00	.00	.00	2.42	35.40	37.82	55.40
3	32.54	.00	.00	.00	.74	2.05	.00	.00	.00	12.17	23.28	35.45	62.50
4	999.00	999.00	.00	.00	.91	.00	.00	.00	.00	14.25	16.76	31.01	999.00
5	2.28	.00	.00	.00	.00	.00	.00	.00	.00	3.94	22.82	26.76	97.70
6	18.78	.00	.00	10.49	2.37	1.52	9.14	.00	.00	5.91	27.06	32.97	44.70
7	32.62	.00	.00	5.57	.87	.00	.00	.00	.00	17.23	61.34	78.57	56.90
8	10.19	.00	.00	.00	.00	.00	.00	2.10	.00	6.18	14.04	20.22	75.20
9	.12	.00	.00	.00	.00	.00	.00	.00	.00	8.68	16.43	25.11	99.90
10	.71	.00	.00	1.46	.00	.00	.00	.00	68.94	25.34	53.33	78.67	28.80
11	3.17	.00	.22	.00	.11	1.31	2.73	.00	36.90	9.16	14.19	23.35	54.20
12	12.36	.00	.00	19.25	.47	.00	.00	.00	.00	6.39	35.88	42.27	67.70
13	6.97	.00	.00	.00	.00	.00	.00	.00	.00	3.73	14.21	17.94	93.00
14	.00	.00	4.02	.00	.40	.40	28.31	.00	.00	4.98	40.79	45.77	66.90
15	.30	.00	.00	.00	.00	.00	.00	.00	.00	26.32	82.42	108.74	99.20
16	1.12	.00	.00	8.33	.00	.00	.00	.00	.00	10.69	60.57	71.26	89.70

	nspecies	bacteria	dbacteri	logdbact	cellulas	dcellula	logdcell	amylase	damylas	logdamyl	eps	deps	logdeps
1	9.00	9.06	10.93	6.04	3.40	4.10	5.61	3.96	4.78	5.68	1.28	1.54	5.19
2	6.00	37.30	40.02	6.60	46.30	49.67	6.70	3.26	3.50	5.54	1.12	1.20	5.08
3	8.00	96.00	112.68	7.05	51.00	59.86	6.78	93.00	109.15	7.04	1.17	1.37	5.14
4	5.00	640.00	780.49	7.89	40.30	49.15	6.69	9.46	11.54	6.06	8.87	10.82	6.03
5	7.00	149.00	161.96	7.21	54.00	58.70	6.77	76.60	83.26	6.92	13.60	14.78	6.17
6	11.00	90.00	111.94	7.05	43.00	53.48	6.73	127.00	157.96	7.20	13.90	17.29	6.24
7	9.00	453.00	491.86	7.69	43.00	46.69	6.67	49.00	53.20	6.73	9.30	10.10	6.00
8	7.00	14.60	18.11	6.26	7.53	9.34	5.97	8.06	10.00	6.00	.65	.81	4.91
9	5.00	123.00	136.21	7.13	33.00	36.54	6.56	8.16	9.04	5.96	9.26	10.25	6.01
10	7.00	660.00	783.85	7.89	83.00	98.57	6.99	31.00	36.82	6.57	7.50	8.91	5.95
11	10.00	600.00	689.05	7.84	35.30	40.54	6.61	61.60	70.74	6.85	11.80	13.55	6.13
12	7.00	319.00	345.99	7.54	98.60	106.94	7.03	43.00	46.64	6.67	9.60	10.41	6.02
13	4.00	403.00	459.73	7.66	87.00	99.25	7.00	15.80	18.02	6.26	12.90	14.72	6.17
14	7.00	12.20	13.38	6.13	6.00	6.58	5.82	12.80	14.04	6.15	3.76	4.12	5.62
15	6.00	7.46	8.36	5.92	6.76	7.58	5.88	14.80	16.59	6.22	.91	1.02	5.01
16	6.00	18.50	21.97	6.34	7.30	8.67	5.94	9.73	11.56	6.06	.81	.96	4.98

	protease	dproteas	logdprot	argusers	darguser	logdargs	margheld	dargheld	celuprot	soilamon	cmamoni	cdamonia	cimobili
1	8.00	9.65	5.98	4.50	5.43	5.73	105.80	127.61	.94	999.00	4.82	5.81	.00
2	5.86	6.29	5.80	4.50	4.83	5.68	83.65	89.74	1.15	3.32	.89	.95	.00
3	6.67	7.83	5.89	2.90	3.40	5.53	78.56	92.21	1.15	5.46	3.94	4.62	.00
4	.33	.40	4.60	.28	.34	4.53	81.19	99.01	1.45	5.13	4.72	5.76	.00
5	4.16	4.52	5.66	4.43	4.82	5.68	56.03	60.90	1.20	999.00	999.00	999.00	999.00
6	8.30	10.32	6.01	.85	1.06	5.02	79.94	99.43	1.12	.64	-1.04	-1.29	-1.04
7	.82	.89	4.95	5.96	6.47	5.81	70.00	76.00	1.35	.30	-.57	-.62	-.57
8	5.20	6.45	5.81	10.40	12.90	6.11	79.25	98.32	1.03	.32	-.53	-.66	-.53
9	2.50	2.77	5.44	6.60	7.31	5.86	86.56	95.86	1.21	1.44	-2.15	-2.38	-2.15
10	.65	.77	4.89	1.41	1.67	5.22	82.04	97.44	1.43	.65	-.34	-.40	-.34
11	8.60	9.88	5.99	6.30	7.23	5.86	96.61	110.94	1.10	.71	2.96	3.40	.00
12	5.60	6.07	5.78	7.16	7.77	5.89	68.52	74.32	1.22	2.11	15.59	16.91	.00
13	2.71	3.09	5.49	9.80	11.18	6.05	108.68	123.97	1.27	.00	2.13	2.43	.00
14	7.70	8.44	5.93	14.70	16.12	6.21	53.10	58.22	.98	.38	17.07	18.72	.00
15	5.40	6.05	5.78	7.60	8.52	5.93	91.75	102.86	1.02	999.00	4.84	5.43	.00
16	4.36	5.18	5.71	12.80	15.20	6.18	50.90	60.45	1.04	999.00	8.66	10.29	.00

	cimoblzd	imobfew	dimobfew	cdamon	cmamon	enzurea	denzurea	ureasers	dureasrs	logdursr	pcascore	resprate	ninhydn
1	.00	999.00	999.00	5.81	4.82	73.06	88.12	3.20	3.86	5.59	-.53	99.22	42.34
2	.00	999.00	999.00	.95	.89	43.50	46.67	2.50	2.68	5.43	-.24	126.62	37.95
3	.00	999.00	999.00	4.62	3.94	44.81	9.30	10.92	112.42	7.05	.00	32.93	3.41
4	.00	999.00	999.00	5.76	4.72	20.90	.63	.77	81.00	6.91	.51	65.06	1.25
5	999.00	999.00	999.00	999.00	999.00	30.32	32.96	8.10	8.80	5.94	.21	138.02	51.07
6	1.04	1.04	1.29	999.00	999.00	43.31	53.87	8.36	10.40	6.02	.22	138.02	53.64
7	.57	.57	.62	999.00	999.00	9.04	9.82	11.60	12.60	6.10	.29	81.00	30.57
8	.53	.53	.66	999.00	999.00	47.26	58.64	13.60	16.87	6.23	-.54	141.98	35.12
9	2.15	2.15	2.38	999.00	999.00	9.98	11.05	1.91	2.12	5.33	-.06	138.02	38.40
10	.34	.34	.40	999.00	999.00	7.16	8.50	.88	1.05	5.02	.93	163.13	42.60
11	.00	999.00	999.00	3.40	2.96	39.54	45.41	17.70	20.33	6.31	.16	138.02	43.00
12	.00	999.00	999.00	16.91	15.59	73.43	79.64	6.00	6.51	5.81	.32	146.00	40.48
13	.00	999.00	999.00	2.43	2.13	58.37	66.59	9.30	10.61	6.03	.36	96.00	39.23
14	.00	999.00	999.00	18.72	17.07	27.30	29.93	10.60	11.62	6.07	-.64	134.15	41.84
15	.00	999.00	999.00	5.43	4.84	43.50	48.77	9.90	11.10	6.05	-.40	177.10	42.37
16	.00	999.00	999.00	10.29	8.66	16.76	19.90	7.03	8.35	5.92	-.60	141.98	42.57

	totnsmbn	ocassmb	rsirrn	smbcvsn	biomascn	microbcn	rsmbccoc	rfencsoc	rtonpops	profitha	farmsil	fieldsil	farmhay
1	3.48	1.11	2.34	4.15	4.15	4.15	11.07	14.09	.11	171.28	1.96	999.00	4.21
2	3.56	1.74	3.34	5.91	5.91	5.91	17.44	15.59	.40	260.92	24.70	24.70	4.63
3	.28	.41	.18	17.08	17.08	999.00	4.07	1.26	.64	18.53	999.00	999.00	999.00
4	.13	1.28	.29	92.53	92.53	999.00	12.75	.73	1.15	6.48	37.05	999.00	6.60
5	4.52	1.80	2.70	4.79	4.79	4.79	18.02	19.89	.85	330.80	16.35	13.89	999.00
6	3.43	1.17	2.57	4.56	4.56	4.56	11.70	13.56	.86	376.53	13.32	999.00	999.00
7	2.27	.97	2.65	4.69	4.69	4.69	9.72	10.94	.93	184.51	999.00	999.00	999.00
8	2.83	1.81	4.04	7.16	7.16	7.16	18.14	13.39	.10	759.08	24.70	24.70	999.00
9	4.21	2.16	3.59	6.36	6.36	6.36	21.64	17.96	.58	329.33	999.00	999.00	6.18
10	5.44	2.90	3.83	6.78	6.78	6.78	29.04	22.63	1.57	263.80	999.00	999.00	4.12
11	6.38	2.12	3.21	5.68	5.68	5.68	21.21	19.72	.80	999.00	5.88	5.94	999.00
12	3.65	2.50	3.61	6.39	6.39	6.39	24.96	20.64	.96	280.23	18.53	999.00	12.35
13	999.00	2.45	2.45	4.33	4.33	4.33	24.51	29.88	1.00	761.13	49.40	49.40	6.18
14	2.96	1.32	3.21	5.68	5.68	5.68	13.17	12.25	.00	145.48	3.95	999.00	12.35
15	3.82	2.56	4.18	7.40	7.40	7.40	25.55	18.24	.24	245.59	999.00	999.00	13.72
16	4.08	1.91	3.34	5.91	5.91	5.91	19.13	17.11	.04	109.65	10.19	999.00	4.94

	fieldhay	citsilag	cuthay	fieldcut	energygj	timefarm	managed	typemgt	harvest3	harvest4
1	999.00	.00	.00	.00	39.55	5.00	3.00	3.00	2.00	3.00
2	7.07	1.00	1.00	1.00	56.86	4.00	5.00	4.00	3.00	4.00
3	999.00	.00	.00	.00	54.28	5.00	5.00	3.00	3.00	4.00
4	999.00	1.00	1.00	1.00	40.00	4.00	3.00	2.00	2.00	3.00
5	999.00	1.00	.00	1.00	44.21	6.00	4.00	3.00	2.00	3.00
6	999.00	.00	.00	.00	41.18	3.00	4.00	4.00	2.00	3.00
7	999.00	.00	.00	.00	12.03	1.00	2.00	2.00	1.00	1.00
8	999.00	1.00	.00	1.00	65.89	6.00	4.00	5.00	3.00	4.00
9	6.18	.00	1.00	1.00	46.87	3.00	3.00	3.00	2.00	3.00
10	999.00	.00	.00	.00	32.97	5.00	2.00	2.00	2.00	2.00
11	999.00	1.00	.00	1.00	37.20	3.00	5.00	5.00	2.00	2.00
12	999.00	.00	.00	.00	21.76	4.00	2.00	2.00	1.00	1.00
13	999.00	1.00	.00	1.00	53.00	6.00	1.00	1.00	3.00	4.00
14	12.35	.00	1.00	1.00	26.90	6.00	4.00	4.00	1.00	2.00
15	4.94	.00	1.00	1.00	36.15	2.00	2.00	4.00	2.00	2.00
16	999.00	.00	.00	.00	19.58	1.00	2.00	2.00	1.00	1.00

Appendix E

	PH	MOISTTEN	LIMEHA	FNOMINN	FBAGNSF	FBAGPSF	FBAGKSF	FORGNSF	FORGPSF	FORGKSF	ALLNFOUR	ALLPFOUR	ALLKFOUR	ONLAND	OFFLAND	DAYGRAZE	ONONOFF	NUMAPERH	NUMPHINT	ANSHBYDS	LUHFORDS	LUHFDSOC	NEXCRKGH	GRAZREST
PH	1.00	-.27	.43	-.44	.54*	.27	.27	.43	.43	.40	.40	.60*	.32	-.40	.09	-.43	-.34	.49	-.12	.23	.04	.02	.26	-.20
MOISTTEN	-.27	1.00	-.17	.06	-.31	.23	.23	-.01	-.27	-.02	.05	.13	.03	-.32	.39	-.20	-.26	.06	-.37	-.04	-.45	-.35	.05	.08
LIMEHA	.43	-.17	1.00	.10	-.09	-.16	-.16	.33	.36	.18	.41	.13	.02	.03	.02	.12	.04	.00	.15	.09	-.02	.22	.19	
FNOMINN	-.44	.06	.10	1.00					-.04	-.62*	-.64*	-.31	-.61*	.44	-.01	.40	.40	-.40	.18	-.06	-.25	-.35	-.62*	.36
FBAGNSF	.54*	-.31	-.09	-.82**	1.00	.50*	.50*	.56*	.14	.51*	.59*	.26	.49	-.21	-.01	-.27	-.19	.23	-.20	-.08	.25	.34	.41	-.14
FBAGPSF	.27	.23	-.16		.50*	1.00		.47	-.13	.42	.51*	.34	.53*	-.58*	.09	-.50*	-.58*	.50*	-.16	-.01	.29	.38	.53*	-.62*
FBAGKSF	.27	.23	-.16		.50*	1.00**	1.00	.47	-.13	.42	.51*	.34	.53*	-.58*	.09	-.50*	-.58*	.50*	-.16	-.01	.29	.38	.53*	-.62*
FORGNSF	.43	-.01	.33		.56*	.47	.47	1.00	.42			.62*	.90**	-.15	-.01	-.12	-.04	.24	-.29	.14	.01	.07	.41	.17
FORGPSF	.43	-.27		-.04	.14	-.13	-.13	.42	1.00	.44	.28	.54*	.19	.10	-.23	.20	.32	-.05	-.01	.16	.17	.16	.31	.40
FORGKSF	.40	-.02	.36	-.62*	.51*	.42	.42		.44	1.00		.62*	.89**	-.13	-.03	-.09	-.01	.23	-.26	.17	-.01	.04	.40	.21
ALLNFOUR	.40	.05	.18	-.64*	.59*	.51*	.51*		.28		1.00	.63**	.94**	-.19	-.03	-.17	-.07	.22	-.37	.05	-.10	-.02	.31	.14
ALLPFOUR	.60*	.13	.41	-.31	.26	.34	.34	.62*	.54*	.62*		1.00	.60*	-.30	-.25	-.06	.03	.36	-.19	.28	-.13	-.01	.37	.20
ALLKFOUR	.32	.03	.13	-.61*	.49	.53*	.53*		.19			.60*	1.00	-.09	-.12	-.06	.03	.13	-.38	-.04	-.04	.04	.25	.11
ONLAND	-.40	-.32	.02	.44	-.21	-.58*	-.58*	-.15	.10	-.13	-.19	-.30	-.09	1.00	-.17				.28	-.16	.15	.02	-.36	
OFFLAND	.09	.39	.03	-.01	-.01	.09	.09	-.01	-.23	-.03	-.03	-.25	-.12	-.17	1.00	-.51*	-.56*	.30	.08	.07	.02	-.09	-.05	-.34
DAYGRAZE	-.43	-.20	.02	.40	-.27	-.50*	-.50*	-.12	.20	-.09	-.17	-.06	-.06		-.51*	1.00			.18	.01	.13	.12	-.09	
ONONOFF	-.34	-.26	.12	.40	-.19	-.58*	-.58*	-.04	.32	-.01	-.07	.03	.03		-.56*		1.00		.06	-.10	.00	.00	-.19	
NUMAPERH	.49	.06	.04	-.40	.23	.50*	.50*	.24	-.05	.23	.22	.36	.13		.30			1.00	.39		.20	.22	.45	-.55*
NUMPHINT	-.12	-.37	.00	.18	-.20	-.16	-.16	-.29	-.01	-.26	-.37	-.19	-.38	.28	.08	.18	.06	.39	1.00	.62*	.58*	.45	.17	-.10
ANSHBYDS	.23	-.04	.15	-.06	-.08	-.01	-.01	.14	.16	.17	.05	.28	-.04	-.16	.07	.01	-.10	.69**	.62*	1.00	.23	.23	.44	.19
LUHFORDS	.04	-.45	.09	-.25	.25	.29	.29	.01	.17	-.01	-.10	-.13	-.04	.15	.02	.13	.00	.20	.58*	.23	1.00		.55*	-.34
LUHFDSOC	.02	-.35	-.02	-.35	.34	.38	.38	.07	.16	.04	-.02	-.01	.04	.02	-.09	.12	.00	.22	.45	.23		1.00		-.27
NEXCRKGH	.26	.05	.22	-.62*	.41	.53*	.53*	.41	.31	.40	.31	.37	.25	-.36	-.05	-.09	-.19	.45	.17	.44	.55*		1.00	-.13
GRAZREST	-.20	.08	.19	.36	-.14	-.62*	-.62*	.17	.40	.21	.14	.20	.11		-.34		-.55*		-.10	.19	-.34	-.27	-.13	1.00
RESTGRAZ	.34	.26	-.12	-.40	.19	.58*	.58*	.04	-.32	.01	.07	-.03	-.03		.56*				-.06	.10	.00	.00	.19	
ADSPERH	.32	.01	-.01	-.11	-.07	.08	.08	.12	-.04	.15	.05	.35	-.03	-.29	.10	-.17	-.26		.56*		.05	.04	.30	.00
INTENSE		.00	.23	-.64*	.46	.58*	.58*	.38	.15	.36	.29	.30	.21		.36				.09	.44	.35	.39		-.61*
NUMBERAN	.29	-.12	.15	-.07	-.09	.02	.02	.13	.34	.16	.07	.44	.06	-.24	-.56*	.12	.11	.27	.09	.53*	.00	.07	.31	.15
DISTURBD	.36	.54	.05	.58	-.72*	-.26	-.26	-.17	-.22	-.07	-.17	.03	-.02	.37	.32	.44	.28	.00	.36	.32	-.07	-.12	-.15	.28

	PH	MOISTTEN	LIMEHA	FNOMINN	FBAGNSF	FBAGPSF	FBAGKSF	FORGNSF	FORGPSF	FORGKSF	ALLNFOUR	ALLPFOUR	ALLKFOUR	ONLAND	OFFLAND	DAYGRAZE	ONONOFF	NUMAPERH	NUMPHINT	ANSHBYDS	LUHFORDS	LUHFDSOC	NEXCRKGH	GRAZREST
RESEEDED	20	-.17	.40	-.60*	.49	.49	.49	.45	.37	.42	.37	.37	.33	-.49	.03	-.34	-.38	.34	-.28	.17	.28	.31	.56*	-.27
ORGANICF	54*	.09	.57*	-.16	-.05	.01	.01	.35	.49	.38	.21	.42	.16	-.43	.02	-.31	-.22	.39	-.13	.41	-.16	-.18	.31	.03
ALTITUDE	23	.03	.31	.43	-.43	-.09	-.09	.02	.29	.02	.02	.38	-.04	.09	.20	.02	-.02	.18	.35	.28	.19	.12	.03	-.03
SLOPE	26	-.19	.57*	.34	-.12	-.31	-.31	-.07	.62*	-.07	-.15	.51	-.25	.12	-.25	.36	.34	.14	.43	.45	.30	.35	.46	.34
SOUTHERN	-.20	.03	-.01	-.17	.25	.00	.00	.14	.08	.14	.27	-.04	.37	.58*	-.29	.58*	.63*	-.51*	-.23	-.31	.01	-.01	-.08	.54
DEPTH	15	.28	-.32	-.66*	.46	.46	.46	.21	-.36	.16	.25	-.28	.14	-.37	.51*	-.57*	-.62*	.17	-.34	-.20	-.11	-.14	.08	-.47
ADEPTH	-.18	-.02	.10	-.13	.04	.04	.04	.01	.12	.03	.04	-.04	-.09	-.22	-.19	-.14	-.09	.10	-.06	.02	-.23	-.17	-.07	-.09
CLAYPERC	24	.23	.24	.26	-.13	-.13	-.13	.32	.12	.35	.33	.41	.21	-.29	.27	-.27	-.22	.24	-.12	.25	-.41	-.37	.06	.18
SILTCLAY	25	.12	.49	.00	-.04	-.04	-.04	.03	.55*	.02	.01	.32	-.09	.05	-.09	.21	.17	-.10	-.16	.12	-.10	-.12	.05	.32
DRAINED	-.12	-.42	.14	.00	.00	.00	.00	.00	.22	.00	.00	.03	.15	.38	██	.44	.48	-.49	-.15	-.46	.06	.03	-.26	.08
ROOTMASS	██	-.15	-.18	.50	-.49	-.34	-.34	-.52*	-.23	-.50*	-.59*	-.59*	-.48	.51*	.14	.30	.23	-.22	.59*	-.07	.36	.26	-.33	-.13
BULKDENS	35	-.30	-.14	-.44	.58*	.23	.23	.14	.06	.10	.20	.13	.18	-.20	-.34	.05	.03	.01	-.32	.01	.20	.35	.29	.03
PARTICLE	26	-.07	-.19	-.31	.25	.30	.30	-.23	-.11	-.28	-.27	-.02	-.36	-.17	.12	-.13	-.33	.24	.29	.16	.37	.34	.37	-.37
PORESPAC	-.37	.18	.02	.43	-.57*	-.19	-.19	-.39	-.15	-.36	-.44	-.27	-.45	.14	.24	-.03	-.11	.00	.47	.03	.01	-.14	-.13	-.23
AGGRATIO	-.07	.23	-.01	-.05	.09	.02	.02	-.23	.15	-.27	-.21	-.05	-.33	.26	.26	.22	.12	-.01	.30	.14	.31	.28	.18	.16
WSACENT	.08	-.11	.20	.32	-.27	-.38	-.38	.14	.07	.19	.17	.25	.28	.20	-.46	.29	.42	-.26	-.26	-.07	-.55*	-.58*	-.31	.52
ORGANICC	-.05	.12	.35	.32	-.35	-.23	-.23	.28	.14	.32	.30	.22	.42	.05	-.20	.10	.24	-.30	-.47	-.26	-.49	-.50*	-.26	.32
TOTALNIT	10	.06	.43	.03	-.12	.22	.22	.47	.29	.47	.57*	.56*	██	.05	-.39	.22	.32	-.21	-.40	-.19	-.20	-.16	.04	.25
CARBNT	01	.30	-.15	.23	-.06	-.19	-.19	.19	-.23	.19	.35	.04	.22	-.15	.46	-.39	-.26	.14	-.12	-.03	-.43	-.47	-.26	.07
PHOSPHAT	61*	.04	.35	-.35	.38	.32	.32	.60*	.51*	.57*	.61*	██	.54*	-.19	.10	-.19	-.08	.41	-.08	.31	.08	.12	.19	.08
POTASSIU	-.28	.19	.26	-.05	-.23	.01	.01	.19	.26	.24	.20	.18	.21	.12	-.52*	.42	.38	-.29	-.17	.02	-.16	-.15	.30	.41
CATION	28	-.17	.28	.05	.14	-.17	-.17	.42	.21	.44	.29	.23	.38	-.13	-.02	-.16	.04	-.05	-.44	-.16	-.32	-.24	-.17	.14
MAGNESIU	██	-.05	.40	-.16	.10	.03	.03	.42	.30	.43	.37	.44	.38	-.27	-.05	-.24	-.14	.11	-.40	.05	-.33	-.39	.02	.06
CALCIUM	██	-.10	.46	-.35	.33	.22	.22	.53*	.26	.52*	.53*	.54*	.49	-.19	.26	-.38	-.28	.39	-.12	.11	-.21	-.31	.00	-.06
DPOLYSAC	06	.37	.29	.22	-.27	.12	.12	.19	.12	.20	.14	.35	.16	-.22	.47	-.18	-.22	.45	.24	.44	.20	.24	.31	.03
RYEGRASS	23	.28	.00	-.21	-.05	.48	.48	.04	-.08	.01	.11	.21	.08	-.54*	-.01	-.41	-.51*	.12	-.44	-.18	-.28	-.26	-.05	-.48
AGR_STO	-.43	.16	-.37	.59*	-.46	-.46	-.46	-.61*	-.30	-.61*	██	██	██	.26	.27	.14	.08	-.12	.32	.10	-.11	-.14	-.50*	.08
ALO_PRA	29	-.38	.22	-.21	.12	.00	.00	.19	.18	.19	-.01	-.06	.14	.01	-.12	.12	.08	-.02	-.05	.15	.42	.39	.34	.01

	PH	MOISTTEN	LIMEHA	FNOMINN	FBAGNSF	FBAGPSF	FBAGKSF	FORGNSF	FORGPSF	FORGKSF	ALLFOUR	ALLPFOUR	ALLKFOUR	ONLAND	OFFLAND	DAYGRAZE	ONONOFF	NUMAPERH	NUMPHINT	ANSHBYDS	LUHFORDS	LUHFDSOC	NEXCRKGH	GRAZREST
POA_PRA	28	.02	.12	-.19	.40	.16	.16	.27	.38	.23	.40	.48	.29	.17	-.21	.28	.28	-.31	-.32	-.24	.00	.07	.17	.34
RAN_REP	02	.14	.30	-.21	.05	.01	.01	.27	.25	.27	.13	-.01	.27	.02	-.03	.01	.14	-.40	-.54*	-.48	-.06	-.05	.10	.09
CLOVER	02	.22	.00	.07	-.18	.22	.22	-.08	.06	-.12	-.08	.33	.13	.06	-.30	.20	.18	-.34	-.30	-.47	.04	.11	-.01	-.22
CAR_PRA	-.17	.24	.06	.48	-.35	-.35	-.35	-.17	.15	-.15	-.10	.34	.01	.36	-.49	.53*		-.43	-.19	-.22	-.41	-.35	-.27	.56*
DWT_SPB	-.28	-.38	.22	.37	-.14	-.44	-.44	-.18	.16	-.16	-.31	-.26	-.23	.38	-.06	.37	.39	-.26	.24	-.03	.32	.33	-.03	.28
ALLGRASS	32	-.31	.15	-.22	.07	.28	.28	-.02	.02	-.04	-.07	.00	-.09	-.30	.12	-.41	-.47	.39	.16	.19	.10	.04	-.13	-.53
NSPECIES	-.16	.19	.20	.53*	-.47	-.31	-.31	-.04	.14	-.01	-.01	.14	.13	.23	-.14	.26	.35	-.40	-.21	-.26	-.18	-.19	-.19	.29
DBACTERI	-.06	-.07	-.48	.03	.06	-.02	-.02	-.27	-.24	-.30	-.32	-.01	-.25	.28	-.06	.31	.21	.02	.43	.17	.33	.37	.08	.05
DCELLULA	23	-.18	.03	-.19	.34	.15	.15	.12	.29	.08	.06	.29	.04	.28	-.09	.34	.25	-.16	.14	-.03	.44	.47	.34	.07
DAMYLAS	20	.01	.27	.46	-.27	-.15	-.15	-.07	.29	-.08	-.10	.43	.00	.13	-.01	.20	.22	-.09	.06	-.02	.14	.17	-.03	.07
DEPS	23	-.04	-.13	.18	.07	-.05	-.05	.09	.08	.07	.18	.44	.18	.33	-.02	.26	.28	.05	.22	.16	.00	.00	-.24	.29
DPROTEAS	21	.20	.55*	.14	-.09	-.06	-.06	.33	.49	.36	.38	.38	.31	-.22	.01	-.26	-.05	-.06	-.39	-.20	-.36	-.36	-.03	.11
DARGUSER	-.04	-.23	.03	-.25	.10	.41	.41	.07	-.07	.08	.10	-.12	.09	-.46	.00	-.57*	-.53*	.38	.11	-.10	.18	.17	.03	
CMAMONIA	-.19	-.16	.11	.32	-.13	-.22	-.22	-.29	.25	-.26	-.16	.03	-.35	-.22	-.42	.00	.06	-.01	.01	.04	-.09	.04	-.01	.05
IMOBFEW	90*	-.10	.41	.26		-.35	-.35	.11	.11	.11	.11	.10	.10	.10	.30	-.20	.10	-.10	-.30	-.60	-.80	-.90*	-.80	.00
CMAMON	-.73*	-.02	.14	.71*	-.68*	-.41	-.41	-.40	.03	-.28	-.35	-.31	-.35	.24	-.29	.27	.27	-.25	.36	-.02	-.07	-.20	-.48	.05
DENZUREA	12	.00	.24	-.38	.30	.41	.41	.39	.34	.37	.53*	.18	.46	-.11	-.03	-.21	-.12	-.09	-.34	-.35	.10	.09	.04	-.23
DUREASRS	31	.23	.17	.02	-.19	.19	.19	-.04	.17	-.04	-.09	.55*	.01	-.49	-.35	-.17	-.11	.36	-.10	.21	-.13	-.01	.22	-.12
RESPRATE	-.36	.03	.23	.20	-.29	-.04	-.04	-.07	.12	-.06	-.19	-.40	-.22	.11	.50*	-.07	-.17	.02	.34	.11	.43	.33	.04	-.24
NINHYDN	-.10	.13	.09	.44	-.20	-.24	-.24	.08	.08	.09	.15	-.02	.06	.09	.49	-.10	-.06	-.03	.02	.05	-.04	-.04	-.26	.13
TOTNSMBN	-.01	-.21	-.13	.23	.00	-.25	-.25	-.17	-.11	-.17	-.19	-.46	-.31	.05	.58*	-.30	-.35	.15	.34	.09	.27	.17	-.21	-.29
OCASSMBC	-.09	-.15	-.04	-.13	.14	.21	.21	-.13	-.01	-.16	-.18	-.35	-.30	-.05	.51*	-.25	-.39	.34	.56*	.25		.58*	.24	-.55*
SMBCVSN	-.08	.12	.28	.05	-.28	-.11	-.11	-.32	.20	-.31	-.55*	-.16	-.58*	-.10	.05	.08	-.06	.14	.32	.37	.21	.17	.38	-.02
BIOMASCN	-.08	.12	.28	.05	-.28	-.11	-.11	-.32	.20	-.31	-.55*	-.16	-.58*	-.10	.05	.08	-.06	.14	.32	.37	.21	.17	.38	-.02
PROFITHA	28	.00	.09	-.56*	.34	.59*	.59*	.56*	.13	.51	.54*	.22	.58*	.06	.39	-.17	-.23	.23	.08	.04	.43	.34	.22	-.30
ENERGYGJ		.19	.26	-.65*	.44	.48	.48	.56*	.22	.53*	.49	.42	.41	-.37	.20	-.34	-.38	.33	-.32	.17	-.11	-.15	.41	-.12
FIELDHAY	-.40	-.80	-.32	.00	.26			.74	.00	.74	.74	.80	.74	-.11	-.80	.60	.60	.20	.21	.20	-.63	-.40	.60	.60
FIELDSIL	56	-.10	-.05	-.07	.80	.80	.80	.67	-.46	.67	.97*	.41	.97*	-.39	-.13	-.36	-.46	.21	-.67	-.87	.87	.87	.72	-.46

	RESTGRAZ	ADSPERH	INTENSE	NUMBERAN	DISTURBD	RESEDED	ORGANICF	ALTITUDE	SLOPE	SOUTHERN	DEPTH	DEPTH	ADEPTH	CLAYPERC	SILTCLAY	DRAINED	ROOTMASS	PARTICLE	PORESPAC	AGGRATIO	WSACENT	ORGANICC	TOTALNIT	CARBNIT
PH	.34	.32	.32	.29	.36	.40	.54*	.23	.26	-.20	-.08	.15	-.18	.24	.25	-.12	.38	.26	-.37	-.07	.08	-.05	.10	.01
MOISTTEN	.26	.01	.00	-.12	.54	-.17	.09	.03	-.19	.03	.17	.28	-.02	.23	.12	-.42	-.15	-.07	.18	.23	-.11	.12	.06	.30
LIMEHA	-.12	-.01	.23	.15	.05	.40	.57*	.31	.57*	-.01	-.16	-.32	.10	.24	.49	.14	-.18	-.19	.02	-.01	.20	.35	.43	-.15
FNOMINN	-.40	-.11	-.64*	-.07	.58	-.60*	-.16	.43	.34	-.17	-.47	-.66*	-.13	.26	.00	.00	.50	-.31	.43	-.05	.32	.32	.03	.23
FBAGNSF	.19	-.07	.46	-.09	-.72*	.49	-.05	-.43	-.12	.25	.30	.46	.04	-.13	-.04	.00	-.49	.25	-.57*	.09	-.27	-.35	-.12	-.06
FBAGPSF	.58*	.08	.58*	.02	-.26	.49	.01	-.09	-.31	.00	.30	.46	.04	-.13	-.04	.00	-.34	.30	-.19	.02	-.38	-.23	.22	-.19
FBAGKSF	.58*	.08	.58*	.02	-.26	.49	.01	-.09	-.31	.00	.30	.46	.04	-.13	-.04	.00	-.34	.30	-.19	.02	-.38	-.23	.22	-.19
FORGNSF	.04	.12	.38	.13	-.17	.45	.35	.02	-.07	.14	.06	.21	.01	.32	.03	.00	-.52*	-.23	-.39	-.23	.14	.28	.47	.19
FORGPSF	-.32	-.04	.15	.34	-.22	.37	.49	.29	.62*	.08	-.15	-.36	.12	.12	.55*	.22	-.23	-.11	-.15	.15	.07	.14	.29	-.23
FORGKSF	.01	.15	.36	.16	-.07	.42	.38	.02	-.07	.14	.04	.16	.03	.35	.02	.00	-.50*	-.28	-.36	-.27	.19	.32	.47	.19
ALLNFOUR	.07	.05	.29	.07	-.17	.37	.21	.02	-.15	.27	.10	.25	.04	.33	.01	.00	-.59*	-.27	-.44	-.21	.17	.30	.57*	.35
ALLPFOUR	-.03	.35	.30	.44	.03	.37	.42	.38	.51	-.04	-.27	-.28	-.04	.41	.32	.03	-.59*	-.02	-.27	-.05	.25	.22	.56*	.04
ALLKFOUR	-.03	-.03	.21	.06	-.02	.33	.16	-.04	-.25	.37	-.06	.14	-.09	.21	-.09	.15	-.48	-.36	-.45	-.33	.28	.42		.22
ONLAND		-.29		-.24	.37	-.49	-.43	.09	.12	.58*	-.38	-.37	-.22	-.29	.05	.38	.51*	-.17	.14	.26	.20	.05	.05	-.15
OFFLAND	.56*	.10	.36	-.56*	.32	.03	.02	.20	-.25	-.29	.18	.51*	-.19	.27	-.09		.14	.12	.24	.26	-.46	-.20	-.39	.46
DAYGRAZE		-.17		.12	.44	-.34	-.31	.02	.36	.58*	-.44	-.57*	-.14	-.27	.21	.44	.30	-.13	-.03	.22	.29	.10	.22	-.39
ONONOFF		-.26		.11	.28	-.38	-.22	-.02	.34	.63*	-.44	-.62*	-.09	-.22	.17	.48	.23	-.33	-.11	.12	.42	.24	.32	-.26
NUMAPERH				.27	.00	.34	.39	.18	.14	-.51*	.15	.17	.10	.24	-.10	-.49	-.22	.24	.00	-.01	-.26	-.30	-.21	.14
NUMPHINT	-.06	.56*	.09	.09	.36	-.28	-.13	.35	.43	-.23	-.26	-.34	-.06	-.12	-.16	-.15	.59*	.29	.47	.30	-.26	-.47	-.40	-.12
ANSHBYDS	.10		.44	.53*	.32	.17	.41	.28	.45	-.31	-.13	-.20	.02	.25	.12	-.46	-.07	.16	.03	.14	-.07	-.26	-.19	-.03
LUHFORDS	.00	.05	.35	.00	-.07	.28	-.16	.19	.30	.01	-.21	-.11	-.23	-.41	-.10	.06	.36	.37	.01	.31	-.55*	-.49	-.20	-.43
LUHFDSOC	.00	.04	.39	.07	-.12	.31	-.18	.12	.35	-.01	-.18	-.14	-.17	-.37	-.12	.03	.26	.34	-.14	.28	-.58*	-.50*	-.16	-.47
NEXCRKGH	.19	.30		.31	-.15	.56*	.31	.03	.46	-.08	-.02	.08	-.07	.06	.05	-.26	-.33	.37	-.13	.18	-.31	-.26	.04	-.26
GRAZREST		.00	-.61*	.15	.28	-.27	.03	-.03	.34	.54	-.33	-.47	-.09	.18	.32	.08	-.13	-.37	-.23	.16	.52	.32	.25	.07
RESTGRAZ	1.00	.26		-.11	-.28	.38	.22	.02	-.34	-.63*	.44	.62*	.09	.22	-.17	-.48	-.23	.33	.11	-.12	-.42	-.24	-.32	.26
ADSPERH	.26	1.00	.44	.50*	.31	.08	.39	.30	.31	-.47	-.16	-.12	-.07	.38	-.05	-.49	-.13	.27	.16	-.01	.03	-.24	-.22	.10
INTENSE		.44	1.00	.20	-.53		.53*	.06	.10	-.46	.17	.36	-.05	.21	-.08	-.49	-.42	.28	-.19	-.07	-.35	-.24	-.16	.05
NUMBERAN	-.11	.50*	.20	1.00	-.20	.28		.24	.26	-.17	-.25	-.25	-.10	.10	.17	-.06	-.41	.06	-.10	-.16	.28	.06	.05	-.16
DISTURBD	-.28	.31	-.53	-.20	1.00		-.30	.36	.15	.15	-.42	-.49	-.11	.42	-.26	-.42		-.50	.72*	.00	.22	.42	.36	.59

	RESTGRAZ	ADSPERH	INTENSE	NUMBERAN	DISTURBD	RESEDED	ORGANICF	ALTITUDE	SLOPE	SOUTHERN	DEPTH	DEPTH	ADEPTH	CLAYPERC	SILTCLAY	DRAINED	ROOTMASS	PARTICLE	PORESPAC	AGGRATIO	WSACENT	ORGANICC	TOTALNIT	CARBNT
RESEDED	.38	.08	.72*	.28	-.34*	1.00	.46	-.07	.13	-.13	.15	.26	-.01	.04	.36	.00	-.33*	.29	-.54*	-.11	-.14	-.06	.18	-.36
ORGANICF	.22	.39	.53*	.28	-.30	.46	1.00	.20	.12	-.23	-.10	.07	-.14	.36	.15	-.36	-.54*	-.15	-.03	-.25	.29	.31	.11	.15
ALTITUDE	.02	.30	.06	.24	.36	-.07	.20	1.00	.42	-.37	-.64*	-.30	-.52*	.47	.03	-.28	.12	.22	.50	.18	-.07	.02	.02	.35
SLOPE	-.34	.31	.10	.26	.15	.13	.12	.42	1.00	-.14	-.38	.05	.05	.17	.44	.08	.05	.30	.10	.33	-.03	-.17	.13	-.32
SOUTHERN	-.63*	-.47	-.46	-.17	.15	-.13	-.23	-.37	-.14	1.00	.03	-.01	-.01	-.57*	.20	.47	-.06	-.39	-.38	.28	.26	.20	.36	-.24
DEPTH	.44	-.16	.17	-.25	-.42	.15	-.10	-.64*	-.38	.03	1.00	.60*	.05	-.29	.26	.04	-.14	.02	-.23	.15	-.39	-.31	-.29	-.19
DEPTH	.62*	-.12	.36	-.25	-.49	.26	.07	-.30	.05	-.01	.60*	1.00	-.04	-.06	-.13	-.39	-.35	.25	-.07	.10	-.36	-.27	-.44	.28
ADEPTH	.09	-.07	-.05	-.10	-.11	-.01	-.14	-.52*	.05	-.01	.05	1.00	-.19	.37	.35	.04	-.21	-.19	.01	-.11	-.07	.06	-.32	
CLAYPERC	.22	.38	.21	.10	.42	.04	.36	.47	.17	-.57*	-.29	-.06	-.19	1.00	-.19	-.52*	-.32	-.08	.22	-.40	.31	.42	.21	
SILTCLAY	-.17	-.05	-.08	.17	-.26	.36	.15	.03	.44	.20	.26	-.13	.37	-.19	1.00	.30	-.28	.16	-.27	.51*	-.12	-.16	.09	-.52*
DRAINED	-.48	-.49	-.49	-.06	-.42	.00	-.36	-.28	.08	.47	.04	-.39	.35	-.52*	.30	1.00	.14	-.16	-.22	-.14	.28	.20	.51	-.63*
ROOTMASS	-.23	-.13	-.42	-.41			-.54*	.12	.05	-.06	-.14	-.35	.04	-.32	-.28	.14	1.00	-.09	.52*	.20	-.30	-.21	-.31	-.11
BULKDENS	-.03	-.11	.24	.21			-.06	-.48	.04	.20	.21	.09	.13	-.32	.27	.21	-.49	.17		.01	-.14	-.24	.02	-.51*
PARTICLE	.33	.27	.28	.06	-.50	.29	-.15	.22	.30	-.39	.02	.25	-.21	-.08	.16	-.16	-.09	1.00	.23	.42	-.48		-.56*	-.35
PORESPAC	.11	.16	-.19	-.10	.72*	-.54*	-.03	.50	.10	-.38	-.23	-.07	-.19	.22	-.27	-.22	.52*	.23	1.00	.08	-.03	-.04	-.27	.28
AGGRATIO	-.12	-.01	-.07	-.16	.00	-.11	-.25	.18	.33	.28	.15	.10	.01	-.40	.51*	-.14	.20	.42	.08	1.00			-.48	-.22
WSACENT	-.42	.03	-.35	.28	.22	-.14	.29	-.07	-.03	.26	-.39	-.36	-.11	.31	-.12	.28	-.30	-.48	-.03		1.00			.21
ORGANICC	-.24	-.24	-.24	.06	.42	-.06	.31	.02	-.17	.20	-.31	-.27	-.07	.42	-.16	.20	-.21		-.04			1.00		.39
TOTALNIT	-.32	-.22	-.16	.05	.36	.18	.11	.02	.13	.36	-.29	-.44	.06	.21	.09	.51	-.31	-.56*	-.27	-.48	.70**		1.00	-.03
CARBNT	.26	.10	.05	-.16	.59	-.36	.15	.35	-.32	-.24	-.19	.28	-.32	.58*	-.52*	-.63*	-.11	-.35	.28	-.22	.21	.39	-.03	1.00
PHOSPHAT	.08	.25	.39	.32	-.20	.36	.44	.38	.14	.12	-.05	.05	-.09	.10	.35	-.14	-.41	-.15	-.40	.24	-.13	-.05	.12	.13
POTASSIU	-.38	-.11	-.24	.35	.27	.01	.22	-.14	.17	.43	.00	-.16	.19	.01	.22	.30	-.24	-.20	.11	-.11	.46	.44	.55*	-.20
CATION	-.04	-.09	.13	-.08	-.12	.20	.27	-.26	-.17	-.14	-.08	-.16	.06	.37	-.20	.09	-.22	-.52*	-.40		.42	.56*	.38	.21
MAGNESIU	.14	.17	.29	.48	-.46	.48		.22	-.10	-.11	-.27	.18	-.39	.44	.04	-.15		-.08	-.12	-.46	.56*	.49	.29	.25
CALCIUM	.28	.25	.38	-.08	-.20	.39	.35	.17	.10	-.03	-.04	.14	-.07	.37	.15	-.01	-.50*	-.01	-.14	-.20	.33	.26	.40	.22
DPOLYSAC	.22	.38	.39	-.05		.06	.21	.53*	.32	-.35	-.40	-.28	-.25	.51*	-.10	-.49	.15	-.16	.15	.01	-.16	.16	.18	.35
RYEGRASS	.51*	-.03	.14	.16	-.25	.45	.10	.05	-.20	-.28	.33	.30	.20	.10	.40	.17	-.42	.33	-.04	-.14	-.07	.02	.16	-.19
AGR_STO	-.08	.13	-.39	-.13	.41	-.57*	-.30	.11	-.07	-.30	.08	-.08	.05	-.15	.06	-.21	.59*	.13	.33	.40	-.37	-.46		.01
ALO_PRA	-.08	.03	.36	.35	-.50	.58*	.43	-.11	-.06	.03	-.33	-.02	-.44	-.11	-.13	-.01	-.20	.00	-.35	-.34	.14	.15	.09	-.30

	RESTGRAZ	ADSPERH	INTENSE	NUMBERAN	DISTURBD	RESEDED	ORGANICF	ALTITUDE	SLOPE	SOUTHERN	DEPTHA	DEPTH	ADEPTH	CLAYPERC	SILTCLAY	DRAINED	ROOTMASS	PARTICLE	PORESPAC	AGGRATIO	WSACENT	ORGANICC	TOTALNIT	CARBNIT
POA_PRA	-.28	-.26	-.18	.03	-.40	.23	-.19	.32	.38	.29	-.13	.00	-.15	.08	.48	.22	-.42	.34	-.25	.39	-.07	-.12	.23	-.09
RAN_REP	-.14	-.53*	.05	-.01	-.16	.17	.39	-.21	-.30	.34	-.13	.16	-.31	-.07	-.16	.07	-.19	-.37	-.09	-.27	.24	.48	.33	.06
CLOVER	-.18	-.34	-.21	.11	.01	.02	-.10	.30	.11	.17	-.50	-.28	-.44	-.13	.12	.38	-.03	.12	.05	.01	.14	.21	.41	-.20
CAR_PRA	-.18	-.18	-.59*	.17	.36	-.46	-.03	.08	.29	.49	-.43	-.55*	-.12	-.05	.17	.33	-.01	-.31	.08	.08	.56*	.40	.46	-.02
DWT_SPB	-.39	-.21	-.16	-.37	.34	-.11	-.34	-.19	.35	-.04	-.18	-.58*	.19	-.09	-.07	.26	.53*	-.24	-.13	-.10	-.04	.07	.10	-.27
ALLGRASS	.47	.25	.26	-.07	-.32	.41	-.05	-.11	-.01	-.34	.44	.06	.52*	-.19	.40	.31	.03	.27	-.12	-.06	-.26	-.30	-.07	-.52
NSPECIES	-.35	-.25	-.31	.18	.55	-.29	.27	.52*	.00	.15	-.34	-.58*	.31	-.27	-.05	.05	-.44	.29	-.28	.50	-.30	.47	.48	
DBACTERI	-.21	.31	-.13	.12	-.14	-.28	-.32	.32	.21	-.12	-.46	-.14	-.53*	-.15	-.19	-.17	.27	.57*	.21	.39	-.27	-.59*	-.47	-.10
DCELLULA	-.25	-.01	-.02	.02	-.31	.20	-.26	.43	.49	-.04	-.39	-.12	-.42	-.01	.21	.10	-.04	-.01	.43	-.31	-.45	-.12	-.26	
DAMYLAS	-.22	.04	-.07	.09	.24	.00	.02	-.51*	-.27	-.59*	-.54*	.35	.06	.00	.10	.10	.14	.01	.08	.17	.29	.10		
DEPS	-.28	.28	-.25	.11	.20	-.25	-.19	.63*	.24	.07	-.47	-.24	-.39	.12	.13	-.02	.03	.15	.02	.34	.00	-.20	-.07	.21
DPROTEAS	.05	-.21	.09	.18	.29	.03	.58*	.34	.00	.03	-.12	.01	-.06	.46	.01	-.12	-.32	-.48	.15	-.26	.35	.43	.55*	
DARGUSER	.53*	-.06	.33	-.15	-.12	.08	-.06	-.15	-.20	-.31	.44	.10	.54*	-.07	-.27	.20	.21	-.16	.09	-.38	-.21	.03	.08	-.04
CMAMONIA	-.06	-.10	-.10	.27	-.20	-.07	-.02	-.10	.37	-.26	.27	-.36	.04	.22	.14	.04	-.14	-.08	.00	-.08	-.01	.03	-.16	
IMOBFEW	-.10	.10	-.50	-.70	.00	-.35	.21	.10	-.30	.30	.21	.05	.56	-.05	.46	.45	-.30	-.40	.10	-.20	.60	.40	.40	.10
CMAMON	-.27	-.10	-.56	-.13	-.57	-.40	.15	.25	-.17	.01	-.69*	.72*	.00	.04	.47	.70*	-.49	.49	-.14	.10	.38	.45	-.18	
DENZUREA	.12	-.47	.06	-.03	-.24	.22	.08	.14	-.23	.33	.28	.29	.16	-.16	.21	.24	-.19	-.27	-.19	.10	-.19	.14	.31	.11
DUREASRS	.11	.31	.29	.55*	.00	.20	.47	.08	.32	-.15	-.23	-.39	-.01	.03	.13	.04	-.29	-.02	-.09	-.18	.24	.17	.31	-.22
RESPRATE	.17	-.09	.09	-.29	.58	-.06	-.08	.28	.01	-.28	.14	.05	.12	-.01	.03	-.18	.59*	-.08	.33	.23	-.58*	-.18	-.30	-.01
NINHYDN	.06	-.03	-.06	-.20	.53	-.20	-.02	.57*	-.10	-.30	-.21	-.03	-.21	.49	-.12	-.42	.24	-.32	.11	.03	-.21	.15	-.10	-.63*
TOTNSMBN	.35	.07	.16	-.20	.12	-.10	-.09	.43	-.19	-.48	-.01	.31	-.24	.18	-.25	-.49	.38	.19	.28	.16	-.56*	-.40	-.63*	.42
OCASSMBC	.39	.12	.34	-.29	.07	.06	-.25	.23	.05	-.37	.27	.23	.12	-.19	.00	-.27	.48	.39	.22	.49	-.30	-.30	-.06	
SMBCVSN	.06	.27	.20	.18	-.02	.18	.23	-.16	.46	-.24	.18	-.12	.24	-.18	.40	-.08	.13	.43	.25	.33	-.27	-.37	-.36	-.62*
BIOMASCN	.06	.27	.20	.18	-.02	.18	.23	-.16	.46	-.24	.18	-.12	.24	-.18	.40	-.08	.13	.43	.25	.33	-.27	-.37	-.36	-.62*
PROFITHA	.23	.04	.28	-.16	-.02	.31	-.03	.51	-.22	.08	-.07	.36	-.35	.01	.01	-.02	-.02	.16	-.01	.15	-.38	-.15	.11	.12
ENERGYGJ	.38	.24	.52*	.26	-.66	-.54*	.03	-.10	.04	.20	.58*	-.16	.18	.32	-.21	-.36	-.19	.06	.03	-.05	.04	.01		
FIELDHAY	-.60	.40	-.40	.80	.50	-.45	.32	.40	.40	-.40	-.77	-.32	.32	.95	-.95	-.60	-.40	.00	.80	.80	.80	.40	.80	
FIELDSIL	.46	-.72	.62	-.87	-.32	.73	-.67	-.95	-.74	.95	.89*	.80	.92*	-.80	.23	.73	-.67	.29	-.67	.36	-.36	-.21	.63	-.95

	PHOSPHAT	POTASSIU	CATION	MAGNESIU	CALCIUM	DPOLYSAC	RYEGRASS	AGR_STO	ALO_PRA	POA_PRA	RAN_REP	CLOVER	CAR_PRA	DWT_SPB	ALLGRASS	NSPECIES	DBACTERI	DCELLULA	DAMYLAS	DEPS	DPROTEAS	DARGUSER	CMAMONIA	IMOBFEW
PH	.61*	-.28	.28	.67*	.06	.23	-.43	.29	.28	.02	.02	-.17	-.28	.32	-.16	-.06	.23	.20	.23	.21	-.04	-.19	.90*	
MOISTTEN	.04	.19	-.17	-.05	-.10	.37	.28	.16	-.38	.02	.14	.22	.24	-.38	-.31	.19	-.07	-.18	.01	-.04	.20	-.23	-.16	-.10
LIMEHA	.35	.26	.28	.40	.46	.29	.00	-.37	.22	.12	.30	.00	.06	.22	.15	.20	-.48	.03	.27	-.13	.55*	.03	.11	.41
FNOMINN	-.35	-.05	.05	-.16	-.35	.22	-.21	.59*	-.21	-.19	-.21	.07	.48	.37	-.22	.53*	.03	-.19	.46	.18	.14	-.25	.32	.26
FBAGNSF	.38	-.23	.14	.10	.33	-.27	-.05	-.46	.12	.40	.05	-.18	-.35	-.14	.07	-.47	.06	.34	-.27	.07	-.09	.10	-.13	
FBAGPSF	.32	.01	-.17	.03	.22	.12	.48	-.46	.00	.16	.01	.22	-.35	-.44	.28	-.31	-.02	.15	-.15	-.05	-.06	.41	-.22	-.35
FBAGKSF	.32	.01	-.17	.03	.22	.12	.48	-.46	.00	.16	.01	.22	-.35	-.44	.28	-.31	-.02	.15	-.15	-.05	-.06	.41	-.22	-.35
FORGNSF	.60*	.19	.42	.42	.53*	.19	.04	-.61*	.19	.27	.27	-.08	-.17	-.18	-.02	-.04	-.27	.12	-.07	.09	.33	.07	-.29	.11
FORGPSF	.51*	.26	.21	.30	.26	.12	-.08	-.30	.18	.38	.25	.06	.15	.16	.02	.14	-.24	.29	.29	.08	.49	-.07	.25	.11
FORGKSF	.57*	.24	.44	.43	.52*	.20	.01	-.61*	.19	.23	.27	-.12	-.15	-.16	-.04	-.01	-.30	.08	-.08	.07	.36	.08	-.26	.11
ALLNFOUR	.61*	.20	.29	.37	.53*	.14	.11		-.01	.40	.13	-.08	-.10	-.31	-.07	-.01	-.32	.06	-.10	.18	.38	.10	-.16	.11
ALLPFOUR	.68**	.18	.23	.44	.54*	.35	.21	-.47	-.06	.48	-.01	.33	.34	-.26	.00	.14	-.01	.29	.43	.44	.38	-.12	.03	.10
ALLKFOUR	.54*	.21	.38	.38	.49	.16	.08		.14	.29	.27	.13	.01	-.23	-.09	.13	-.25	.04	.00	.18	.31	.09	-.35	.10
ONLAND	-.19	.12	-.13	-.27	-.19	-.22	-.54*	.26	.01	.17	.02	.06	.36	.38	-.30	.23	.28	.28	.13	.33	-.22	-.46	-.22	.10
OFFLAND	.10	-.52*	-.02	-.05	.26	.47	-.01	.27	-.12	-.21	-.03	-.30	-.49	-.06	.12	-.14	-.06	-.09	-.01	-.02	.01	.00	-.42	.30
DAYGRAZE	-.19	.42	-.16	-.24	-.38	-.18	-.41	.14	.12	.28	.01	.20	.53*	.37	-.41	.26	.31	.34	.20	.26	-.26	-.57*	.00	-.20
ONONOFF	-.08	.38	.04	-.14	-.28	-.22	-.51*	.08	.08	.28	.14	.18		.39	-.47	.35	.21	.25	.22	.28	-.05	-.53*	.06	.10
NUMAPERH	.41	-.29	-.05	.11	.39	.45	.12	-.12	-.02	-.31	-.40	-.34	-.43	-.26	.39	-.40	.02	-.16	-.09	.05	-.06	.38	-.01	-.10
NUMPHINT	-.08	-.17	-.44	-.40	-.12	.24	-.44	.32	-.05	-.32	-.54*	-.30	-.19	.24	.16	-.21	.43	.14	.06	.22	-.39	.11	.01	-.30
ANSHBYDS	.31	.02	-.16	.05	.11	.44	-.18	.10	.15	-.24	-.48	-.47	-.22	-.03	.19	-.26	.17	-.03	-.02	.16	-.20	-.10	.04	-.60
LUHFORDS	.08	-.16	-.32	-.33	-.21	.20	-.28	-.11	.42	.00	-.06	.04	-.41	.32	.10	-.18	.33	.44	.14	.00	-.36	.18	-.09	-.80
LUHFDSOC	.12	-.15	-.24	-.39	-.31	.24	-.26	-.14	.39	.07	-.05	.11	-.35	.33	.04	-.19	.37	.47	.17	.00	-.36	.17	.04	-.90*
NEXCRKGH	.19	.30	-.17	.02	.00	.31	-.05	-.50*	.34	.17	.10	-.01	-.27	-.03	-.13	-.19	.08	.34	-.03	-.24	-.03	.03	-.01	-.80
GRAZREST	.08	.41	.14	.06	-.06	.03	-.48	.08	.01	.34	.09	-.22	.56*	.28	-.53	.29	.05	.07	.07	.29	.11		.05	.00
RESTGRAZ	.08	-.38	-.04	.14	.28	.22	.51*	-.08	-.08	-.28	-.14	-.18	-.64**	-.39	.47	-.35	-.21	-.25	-.22	-.28	.05	.53*	-.06	-.10
ADSPERH	.25	-.11	-.09	.17	.25	.38	-.03	.13	.03	-.26	-.53*	-.34	-.18	-.21	.25	-.25	.31	-.01	.04	.28	-.21	-.06	-.10	.10
INTENSE	.39	-.24	.13	.29	.38	.39	.14	-.39	.36	-.18	.05	-.21	-.59*	-.16	.26	-.31	-.13	-.02	-.07	-.25	.09	.33	-.10	-.50
NUMBERAN	.32	.35	-.08	.48	-.08	-.05	.16	-.13	.35	.03	-.01	.11	.17	-.37	-.07	.18	.12	.02	.09	.11	.18	-.15	.27	-.70
DISTURBD	-.20	.27	-.12	-.46	-.20	.90**	-.25	.41	-.50	-.40	-.16	.01	.36	.34	-.32	.55	-.14	-.31	.24	.20	.29	-.12	-.20	.00

	PHOSPHAT	POTASSIU	CATION	MAGNESIU	CALCIUM	DPOLYSAC	RYEGRASS	AGR_STO	ALO_PRA	POA_PRA	RAN_REP	CLOVER	CAR_PRA	DWT_SPB	ALLGRASS	NSPECIES	DBACTERI	DCELLULA	DAMYLAS	DEPS	DPROTEAS	DARGUSER	CMAMONIA	IMOBFEW
RESEEDED	.36	.01	.20	.48	.39	.06	.45	-.57*	.58*	.23	.17	.02	-.46	-.11	.41	-.29	-.28	.20	.00	-.25	.03	.08	-.07	-.35
ORGANICF	.44	.22	.27		.35	.21	.10	-.30	.43	-.19	.39	-.10	-.03	-.34	-.05	.27	-.32	-.26	.02	-.19	.58*	-.06	-.02	.21
ALTITUDE	.38	-.14	-.26	.22	.17	.53*	.05	.11	-.11	.32	-.21	.30	.08	-.19	-.11	.52*	.32	.43		.63*	.34	-.15	-.10	.10
SLOPE	.14	.17	-.17	-.10	.10	.32	-.20	-.07	-.06	.38	-.30	.11	.29	.35	-.01	.00	.21	.49	.51*	.24	.00	-.20	.37	-.30
SOUTHERN	.12	.43	-.14	-.11	-.03	-.35	-.28	-.30	.03	.29	.34	.17	.49	-.04	-.34	.15	-.12	-.04	-.27	.07	.03	-.31	-.26	.30
DEPTH	-.05	.00	-.08	-.27	-.04	-.40	.33	.08	-.33	-.13	-.13	-.50	-.43	-.18	.44	-.70**	-.46	-.39	-.80**	-.47	-.12	.44	.27	.21
DEPTH	.05	-.16	-.16	.18	.14	-.28	.30	-.08	-.02	.00	.16	-.28	-.55*	-.58*	.06	-.34	-.14	-.12	-.59*	-.24	.01	.10	-.36	.05
ADEPTH	-.09	.19	.06	-.39	-.07	-.25	.20	.05	-.44	-.15	-.31	-.44	-.12	.19	.52*	-.58*	-.53*	-.42	-.54*	-.39	-.06	.54*		.56
CLAYPERC	.10	.01	.37	.44	.37	.51*	.10	-.15	-.11	.08	-.07	-.13	-.05	-.09	-.19	.31	-.15	-.01	.35	.12	.46	-.07	.04	-.05
SILTCLAY	.35	.22	-.20	.04	.15	-.10	.40	.06	-.13	.48	-.16	.12	.17	-.07	.40	-.27	-.19	.21	.06	.13	.01	-.27	.22	.46
DRAINED	-.14	.30	.09	-.15	-.01	-.49	.17	-.21	-.01	.22	.07	.38	.33	.26	.31	-.05	-.17	.10	.00	-.02	-.12	.20	.14	.45
ROOTMASS	-.41	-.24	-.22		-.50*	.15	-.42	.59*	-.20	-.42	-.19	-.03	-.01	.53*	.03	.05	.27	-.04	.10	.03	-.32	.21	.04	-.30
BULKDENS	.18	-.09	.13	.04	-.06	-.30	.16	-.28	.42	.32	-.05	.02	-.20	.10	.18	-.41	.00	.20	-.13	-.09	-.37	-.10	.21	-.67
PARTICLE	-.15	-.20	-.52*	-.08	-.01	-.16	.33	.13	.00	.34	-.37	.12	-.31	-.24	.27	-.44	.57*		.10	.15	-.48	-.16	-.14	-.40
PORESPAC	-.40	.11	-.40	-.12	-.14	.15	-.04	.33	-.35	-.25	-.09	.05	.08	-.13	-.12	.29	.21	.01	.14	.02	.15	.09	-.08	.10
AGGRATIO	.24	-.11		-.46	-.20	.01	-.14	.40	-.34	.39	-.27	.01	.08	-.10	-.06	-.28	.39	.43	.01	.34	-.26	-.38	.00	-.20
WSACENT	-.13	.46	.42	.56*	.33	-.16	-.07	-.37	.14	-.07	.24	.14	.56*	-.04	-.26	.50	-.27	-.31	.08	.00	.35	-.21	-.08	.60
ORGANICC	-.05	.44	.56*	.49	.26	.16	.02	-.46	.15	-.12	.48	.21	.40	.07	-.30		-.59*	-.45	.17	-.20		.03	-.01	.40
TOTALNIT	.12	.55*	.38	.29	.40	.18	.16		.09	.23	.33	.41	.46	.10	-.07	.47	-.47	-.12	.29	-.07	.43	.08	.03	.40
CARBONIT	.13	-.20	.21	.25	.22	.35	-.19	.01	-.30	-.09	.06	-.20	-.02	-.27	-.52	.48	-.10	-.26	.10	.21	.55*	-.04	-.16	.10
PHOSPHAT	1.00	-.20	.11	.36	.46	.32	.05	-.18	.03	.32	.02	.06	.00	-.34	.10	.02	-.03	.14	.21	.52*	.35	-.08	-.11	.50
POTASSIU	-.20	1.00	-.20	.14	-.13	-.18	.09	-.47	.01	.16	.26	.09	.39	-.15	-.35	.26	-.36	-.13	-.24	-.34	.31	-.11	.22	-.50
CATION	.11	-.20	1.00	.39	.36	.15	-.11	-.23	.34	-.22	.42	-.04	.00	.40	.01	.15	-.35	-.23	.16	-.17	.26	.12	-.07	.40
MAGNESIU	.36	.14	.39	1.00	.59*	-.04	.29	-.46	.44	.10	.42	.15	.05	-.47	-.08	.38	-.24	-.06	.15	.04	.54*	-.21	-.33	.30
CALCIUM	.46	-.13	.36	.59*	1.00	.15	.19	-.48	-.01	.15	-.01	-.07	-.04	-.24	.35	-.09	-.27	.00	.10	.23	.29	.05	-.40	.90*
DPOLYSAC	.32	-.18	.15	-.04	.15	1.00	-.14	-.01	.04	-.17	-.05	.03	-.07	.22	-.09	.28	.00	.00	.54*	.16	.19	.03	-.10	-.40
RYEGRASS	.05	.09	-.11	.29	.19	-.14	1.00	-.13	-.12	.25	-.13	.31	-.18	-.51*	.54*	-.18	-.25	.01	.00	-.07	.06	.22	.11	.40
AGR_STO	-.18	-.47	-.23	-.46	-.48	-.01	-.13	1.00	-.32	-.25	-.36	-.12	.01	.12	.10	-.14	.45	.00	.06	.27	-.39	-.21	.06	.40
ALO_PRA	.03	.01	.34	.44	-.01	.04	-.12	-.32	1.00	-.20	.49	.07	-.27	.22	-.03	.16	.00	.08	.10	-.30	-.10	-.16	-.32	-.56

	PHOSPHAT	POTASSIU	CATION	MAGNESIU	CALCIUM	DPOLYSAC	RYEGRASS	AGR_STO	ALO_PRA	POA_PRA	RAN_REP	CLOVER	CAR_PRA	DWT_SPB	ALLGRASS	NSPECIES	DBACTERI	DCELLULA	DAMYLAS	DEPS	DPROTEAS	DARGUSER	CMAMONIA	IMOBFEW
POA_PRA	.32	.16	-.22	.10	.15	-.17	.25	-.25	-.20	1.00	-.07	.39	.26	-.23	-.16	.04	.20	.72	.33	.49	.10	-.42	.04	-.11
RAN_REP	.02	.26	.42	.42	-.01	-.05	-.13	-.36	.49	-.07	1.00	.37	.19	.01	-.48	.54*	-.27	-.10	.04	-.39	.51*	-.12	-.34	-.30
CLOVER	.06	.09	-.04	.15	-.07	.03	.31	-.12	.07	.39	.37	1.00	.61*	-.15	-.18	.56*	.37	.45	.31	.11	-.27	-.22	-.10	
CAR_PRA	.00	.39	.00	.05	-.04	-.07	-.18	.01	-.27	.26	.19	.61*	1.00	-.03	-.53*	.55*	.16	.05	.37	.34	.26	-.46	.03	.34
DWT_SPB	-.34	-.15	.40	-.47	-.24	.22	-.51*	.12	.22	-.23	.01	-.15	-.03	1.00	.07	-.06	-.05	.04	.21	-.24	-.28	.10	.23	-.20
ALLGRASS	.10	-.35	.01	-.08	.35	-.09	.54*	.10	-.03	-.16	-.48	-.18	-.53*	.07	1.00		-.21	-.08	-.16	-.08	-.39	.56*	.13	.60
NSPECIES	.02	.26	.15	.38	-.09	.28	-.18	-.14	.16	.04	.54*	.56*	.55*	-.06		1.00	-.02	-.06	.55*	.15		-.28	-.11	-.05
DBACTERI	-.03	-.36	-.35	-.24	-.27	.00	-.25	.45	.00	.20	-.27	.37	.16	-.05	-.21	-.02	1.00		.38	.61*	-.54*	-.47	-.35	-.30
DCELLULA	.14	-.13	-.23	-.06	.00	.00	.01	.00	.08		-.10	.45	.05	.04	-.08	-.06	.67**	1.00	.52*	.52*	-.28	-.44	-.26	-.30
DAMYLAS	.21	-.24	.16	.15	.10	.54*	.00	.06	.10	.33	.04		.37	.21	-.16	.55*	.38	.52*	1.00	.54*	.16	-.31	-.08	-.10
DEPS	.52*	-.34	-.17	.04	.23	.16	-.07	.27	-.30	.49	-.39	.31	.34	-.24	-.08	.15	.61*	.52*	.54*	1.00	-.08	-.42	-.25	.80
DPROTEAS	.35	.31	.26	.54*	.29	.19	.06	-.39	-.10	.10	.51*	.11	.26	-.28	-.39		-.54*	-.28	.16	-.08	1.00	.13	.14	.50
DARGUSER	-.08	-.11	.12	-.21	.05	.03	.22	-.21	-.16	-.42	-.12	-.27	-.46	.10	.56*	-.28	-.47	-.44	-.31	-.42	.13	1.00	.39	.00
CMAMONIA	-.11	.22	-.07	-.33	-.40	-.10	.11	.06	-.32	.04	-.34	-.22	.03	.23	.13	-.11	-.35	-.26	-.08	-.25	.14	.39	1.00	
IMOBFEW	.50	-.50	.40	.30	.90*	-.40	.40	.40	-.56	-.11	-.30	-.10	.34	-.20	.60	-.05	-.30	-.30	-.10	.80	.50	.00		1.00
CMAMON	-.52	.47	-.05	-.60	-.47	.24	-.02	.26	-.50	-.33	-.46	-.12	.18	.56	.28	.16	-.44	-.38	.03	-.26	.02	.53		
DENZUREA	.49	.16	-.14	.11	.11	-.09	.27	-.39	-.15	.36	.23	.10	-.11	-.34	.03	.14	-.47	-.07	-.13	.02	.54*	.37	.17	.30
DUREASRS	.22	.11	.17	.28	.11	.24	.22	-.15	.13	-.14	.14	.52*	.44	-.13	.07	.21	.07	-.09	.35	.01	.18	.01	.16	.00
RESPRATE	-.01	-.17	-.15	-.39	-.31	.41	-.07	.34	.02	-.28	-.03	-.29	-.53*	.32	.20	-.04	-.18	-.08	.00	-.20	.02	.32	.10	-.67
NINHYDN	.31	-.38	.14	-.03	-.05	.55*	-.13	.29	-.15	.05	-.09	-.19	-.20	.11	-.17	.34	-.09	-.04	.37	.32	.34	.00	.11	.10
TOTNSMBN	.09		-.13	-.10	-.15	.18	-.12	.47	.00	-.15	-.27	-.41		.05	.09	-.06	.17	.07	.06	.19	-.06	.16	-.02	-.10
OCASSMBC	.08	-.45	-.41	-.53*	-.22	.24	-.08	.35	-.11	-.11	-.38	-.35		.16	.34	-.46	.16	.19	-.10	.00	-.31	.36	.10	-.30
SMBCVSN	-.30	.21	-.24	-.19	-.23	.03	.05	.25	.16	-.23	-.04	-.11	-.10	.20	.25	-.34	.08	.08	-.12	-.42	-.30	-.11	.13	-.60
BIOMASCN	-.30	.21	-.24	-.19	-.23	.03	.05	.25	.16	-.23	-.04	-.11	-.10	.20	.25	-.34	.08	.08	-.12	-.42	-.30	-.11	.13	-.60
PROFITHA	.54*	-.23	-.12	.15	.34	.28	.21	-.20	.10	.30	.02	.14	-.40	-.32	.25	-.06	.11	.38	.14	.35	.04	.12	-.52	.10
ENERGYGJ	.41	.17	-.01		.60*	-.10	.45	-.45	.23	.31	.20	-.02	-.23		.13	-.21	-.13	.19		-.01	.17	-.21	-.44	.20
FIELDHAY	-.40	.80	.60	.40	.40	-.40	-.60	-.74	-.21	.26	-.21	-.95	.77	-.20	-.60	.63	.20	-.20	-.40	.40	.80	.20	.40	
FIELDSIL	.05	.36	-.46	-.62	.82	-.67	.10	-.92*	-.21	.55	.00	.31	-.73	.10	.36	-.92*	-.36	.41	-.56	-.15	-.67	.46	-.32	

	CMAMON	DENZUREA	DUREASRS	RESPRATE	NINHYDN	TOTNSMBN	OCASSMBC	SMBCVSN	BIOMASCN	PROFITHA	ENERGYGJ	FIELDHAY	FIELDSIL
PH	.73*	.12	.31	-.36	-.10	-.01	-.09	-.08	-.08	.28	.66**	-.40	.56
MOISTTEN	-.02	.00	.23	.03	.13	-.21	-.15	.12	.12	.00	.19	-.80	-.10
LIMEHA	.14	.24	.17	.23	.09	-.13	-.04	.28	.28	.09	.26	-.32	-.05
FNOMINN	.71*	-.38	.02	.20	.44	.23	-.13	.05	.05	-.56*	-.65*	.00	
FBAGNSF	-.68*	.30	-.19	-.29	-.20	.00	.14	-.28	-.28	.34	.44	.26	.80
FBAGPSF	-.41	.41	.19	-.04	-.24	-.25	.21	-.11	-.11	.59*	.48	.	.80
FBAGKSF	-.41	.41	.19	-.04	-.24	-.25	.21	-.11	-.11	.59*	.48	.	.80
FORGNSF	-.40	.39	-.04	-.07	.08	-.17	-.13	-.32	-.32	.56*	.56*	.74	.67
FORGPSF	.03	.34	.17	.12	.08	-.11	-.01	.20	.20	.13	.22	.00	-.46
FORGKSF	-.28	.37	-.04	-.06	.09	-.17	-.16	-.31	-.31	.51	.53*	.74	.67
ALLNFOUR	-.35	.53*	-.09	-.19	.15	-.19	-.18	-.55*	-.55*	.54*	.49	.74	
ALLPFOUR	-.31	.18	.55*	-.40	-.02	-.46	-.35	-.16	-.16	.22	.42	.80	.41
ALLKFOUR	-.35	.46	.01	-.22	.06	-.31	-.30	-.58*	-.58*	.58*	.41	.74	
ONLAND	.24	-.11	-.49	.11	.09	.05	-.05	-.10	-.10	.06	-.37	-.11	-.39
OFFLAND	-.29	-.03	-.35	.50*	.49	.58*	.51*	.05	.05	.39	.20	-.80	-.13
DAYGRAZE	.27	-.21	-.17	-.07	-.10	-.30	-.25	.08	.08	-.17	-.34	.60	-.36
ONONOFF	.27	-.12	-.11	-.17	-.06	-.35	-.39	-.06	-.06	-.23	-.38	.60	-.46
NUMAPERH	-.25	-.09	.36	.02	-.03	.15	.34	.14	.14	.23	.33	.20	.21
NUMPHINT	.36	-.34	-.10	.34	.02	.34	.56*	.32	.32	.08	-.32	.21	-.67
ANSHBYDS	-.02	-.35	.21	.11	.05	.09	.25	.37	.37	.04	.17	.20	-.87
LUHFORDS	-.07	.10	-.13	.43	-.04	.27		.21	.21	.43	-.11	-.63	.87
LUHFDSOC	-.20	.09	-.01	.33	-.04	.17	.58*	.17	.17	.34	-.15	-.40	.87
NEXCRKGH	-.48	.04	.22	.04	-.26	-.21	.24	.38	.38	.22	.41	.60	.72
GRAZREST	.05	-.23	-.12	-.24	.13	-.29	-.55*	-.02	-.02	-.30	-.12	.60	-.46
RESTGRAZ	-.27	.12	.11	.17	.06	.35	.39	.06	.06	.23	.38	-.60	.46
ADSPERH	-.10	-.47	.31	-.09	-.03	.07	.12	.27	.27	.04	.24	.40	-.72
INTENSE	-.56	.06	.29	.09	-.06	.16	.34	.20	.20	.28	.52*	-.40	.62
NUMBERAN	-.13	-.03	.55*	-.29	-.20	-.20	-.29	.18	.18	-.16	.26	.80	-.87
DISTURBD		-.24	.00	.58	.53	.12	.07	-.02	-.02	-.02	-.66	.50	-.32

	CMAMON	DENZUREA	DUREASRS	RESPRATE	NINHYDN	TOTNSMBN	OCASSMBC	SMBCVSN	BIOMASCN	PROFITHA	ENERGYGJ	FIELDHAY	FIELDSIL
RESEEDED	-.57	.22	.20	-.06	-.20	-.10	.06	.18	.18	.31		-.45	.73
ORGANICF	-.40	.08	.47	-.08	-.02	-.09	-.25	.23	.23	-.03	.54*	.32	-.67
ALTITUDE	.15	.14	.08	.28	.57*	.43	.23	-.16	-.16	.51	.03	.40	-.95
SLOPE	.25	-.23	.32	.01	-.10	-.19	.05	.46	.46	-.22	-.10	.40	-.74
SOUTHERN	-.17	.33	-.15	-.28	-.30	-.48	-.37	-.24	-.24	.08	.04	-.40	.95
DEPTH	.01	.28	-.23	.14	-.21	-.01	.27	.18	.18	-.07	.20	-.77	.89*
DEPTH	-.69*	.29	-.39	.05	-.03	.31	.23	-.12	-.12	.36	.58*	-.32	.80
ADEPTH	.72*	.16	-.01	.12	-.21	-.24	.12	.24	.24	-.35	-.16	.32	.92*
CLAYPERC	.00	-.16	.03	-.01	.49	.18	-.19	-.18	-.18	.01	.18	.95	-.80
SILTCLAY	.04	.21	.13	.03	-.12	-.25	.00	.40	.40	.01	.32	-.95	.23
DRAINED	.47	.24	.04	-.18	-.42	-.49	-.27	-.08	-.08	-.02	-.21		.73
ROOTMASS	.70*	-.19	-.29	.59*	.24	.38	.48	.13	.13	-.02		-.60	-.67
BULKDENS	-.55	.06	.09	-.34	-.28	-.21	-.07	-.03	-.03	-.06	.19	.00	.82
PARTICLE	-.49	-.27	-.02	-.08	-.32	.19	.39	.43	.43	.16	.36	-.40	.29
PORESPAC	.49	-.19	-.09	.33	.11	.28	.22	.25	.25	-.01	-.19	.00	-.67
AGGRATIO	-.14	.10	-.18	.23	.03	.16	.49	.33	.33	.15	.06		.36
WSACENT	.10	-.19	.24	-.58*	-.21	-.56*		-.27	-.27	-.38	.03		-.36
ORGANICC	.38	.14	.17	-.18	.15	-.40		-.37	-.37	-.15	-.05	.80	-.21
TOTALNIT	.45	.31	.31	-.30	-.10	-.63*		-.36	-.36	.11	.04	.40	.63
CARBNIT	-.18	.11	-.22	-.01	.56*	.42	-.06	-.62*	-.62*	.12	.01	.80	-.95
PHOSPHAT	-.52	.49	.22	-.01	.31	.09	.08	-.30	-.30	.54*	.41	-.40	.05
POTASSIU	.47	.16	.11	-.17	-.38		-.45	.21	.21	-.23	.17	.80	.36
CATION	-.05	-.14	.17	-.15	.14	-.13	-.41	-.24	-.24	-.12	-.01	.60	-.46
MAGNESIU	-.60	.11	.28	-.39	-.03	-.10	-.53*	-.19	-.19	.15		.40	-.62
CALCIUM	-.47	.11	.11	-.31	-.05	-.15	-.22	-.23	-.23	.34	.60*	.40	.82
DPOLYSAC	.24	-.09	.24	.41	.55*	.18	.24	.03	.03	.28	-.10	-.40	-.67
RYEGRASS	-.02	.27	.22	-.07	-.13	-.12	-.08	.05	.05	.21	.45	-.60	.10
AGR_STO	.26	-.39	-.15	.34	.29	.47	.35	.25	.25	-.20	-.45	-.74	-.92*
ALO_PRA	-.50	-.15	.13	.02	-.15	.00	-.11	.16	.16	.10	.23	-.21	-.21

	CMAMON	DENZUREA	DUREASRS	RESPRATE	NINHYDN	TOTNSMBN	OCASSMBC	SMBCVSN	BIOMASCN	PROFITHA	ENERGYGJ	FIELDHAY	FIELDSIL
POA_PRA	-.33	.36	-.14	-.28	.05	-.15	-.11	-.23	-.23	.30	.31	.26	.55
RAN_REP	-.46	.23	.14	-.03	-.09	-.27	-.38	-.04	-.04	.02	.20	-.21	.00
CLOVER	-.12	.10	.52*	-.29	-.19	-.41	-.35	-.11	-.11	.14	-.02	-.95	.31
CAR_PRA	.18	-.11	.44	-.53*	-.20	-.23	-.23	-.10	-.10	-.40	-.23	.77	-.73
DWT_SPB	.56	-.34	-.13	.32	.11	.05	.16	.20	.20	-.32	-.13	-.20	.10
ALLGRASS	.28	.03	.07	.20	-.17	.09	.34	.25	.25	.25	.13	-.60	.36
NSPECIES	.16	.14	.21	-.04	.34	-.06	-.46	-.34	-.34	-.06	-.21	.63	-.92*
DBACTERI	-.44	-.47	.07	-.18	-.09	.17	.16	.08	.08	.11	-.13	.20	-.36
DCELLULA	-.38	-.07	-.09	-.08	-.04	.07	.19	.08	.08	.38	.19	-.20	.41
DAMYLAS	.03	-.13	.35	.00	.37	.06	-.10	-.12	-.12	.14	-.19	-.40	-.56
DEPS	-.26	.02	.01	-.20	.32	.19	.00	-.42	-.42	.35	-.01	.40	-.15
DPROTEAS	.02	.54*	.18	.02	.34	-.06	-.31	-.30	-.30	.04	.17	.80	-.67
DARGUSER	.53	.37	.01	.32	.00	.16	.36	-.11	-.11	.12	-.21	.20	.46
CMAMONIA		.17	.16	.10	.11	-.02	.10	.13	.13	-.52	-.44	.40	-.32
IMOBFEW		.30	.00	-.67	.10	-.10	-.30	-.60	-.60	.10	.20		
CMAMON	1.00	.02	.01	.52	.32	.02	.12	.09	.09	-.13	-.13	.50	-.50
DENZUREA	.02	1.00	-.20	.24	.27	.11	.17	-.46	-.46	.53*	.17	-.40	.87
DUREASRS	.01	-.20	1.00	-.43	-.38	-.57*	-.43	.26	.26	-.29	.09	.40	-.36
RESPRATE	.52	.24	-.43	1.00	.58*	.64*	-.21	.21	.21	.37	-.28	-.80	-.53
NINHYDN	.32	.27	-.38	.58*	1.00	.23*	.35	-.43	-.43	.32	-.28	-.40	-.56
TOTNSMBN	.02	.11	-.57*	.64*		1.00	-.19	-.19	-.19	.42	-.15	-.80	-.95
OCASSMBC	.12	.17	-.43		.35		1.00	.22	.22	.44	-.14	-.10	.21
SMBCVSN	.09	-.46	.26	.21	-.43	-.19	.22	1.00	-.34	-.34	.12	-.10	-.15
BIOMASCN	.09	-.46	.26	.21	-.43	-.19	.22		1.00	-.34	.12	-.10	-.15
PROFITHA	-.13	.53*	-.29	.37	.32	.42	.44	-.34	-.34	1.00	.41	-.40	.63
ENERGYGJ		.17	.09	-.28	-.28	-.15	-.14	.12	.12	.41	1.00	-.20	.67
FIELDHAY	.50	-.40	.40	-.80	-.40	-.80	.00*	.00*	.00*	-.40	-.20	1.00	
FIELDSIL	-.50	.87	-.36	-.53	-.56	-.95	.21	-.15	-.15	.63	.67		1.00

*. Correlation is significant at the .05 level (2-tailed).

** . Correlation is significant at the .01 level (2-tailed).

	SBAGNSF	SBAGPSF	SBAGKSF	SORGNSF	SORGPSF	SORGKSF	TOTNFARM	TOTPFARM	TOTKFARM	RYEGRASS	AGR_STO	ALO_PRA	POA_PRA	RAN_REP	CLOVER	CAR_PRA	DWT_SPB	ALLGRASS	NSPECIES	PROFITHA	ENERGYGJ	
S p e a r	SBAGNSF	1.00	.64**	.64**	.56*	.12	.56*	.62*	.46	.50	.20	-.33	.28	.48	-.18	-.13	-.44	-.18	.27	-.39	.46	.46
	SBAGPSF		1.00		.51*	-.14	.51*	.52*	.48	.54*		-.36	.18	.25	-.16	.20	-.44	-.44	.42	-.26		.51*
	SBAGKSF			1.00	.51*	-.14	.51*	.52*	.48	.54*		-.36	.18	.25	-.16	.20	-.44	-.44	.42	-.26		.51*
	SORGNSF				1.00	.35		.60*			.04	-.61*	.19	.27	.27	-.08	-.17	-.18	-.02	-.04	.56*	.56*
	SORGPSF					1.00	.35	.28	.56*	.20	-.12	-.15	.08	.41	.17	.06	.26	.16	.02	.01	.03	.20
	SORGKSF						1.00		.60*		.04	-.61*	.19	.27	.27	-.08	-.17	-.18	-.02	-.04	.56*	.56*
	TOTNFARM							1.00			.20		.05	.50*	.15	.02	-.03	-.29	-.06	.04	.44	.59*
	TOTPFARM								1.00		.31	-.46	.00	.61*	-.02	.37	.30	-.27	.06	.03	.24	.51*
	TOTKFARM									1.00	.19		.19	.38	.33	.24	.07	-.24	-.09	.17	.49	.54*
	RYEGRASS										1.00	-.13	-.12	.25	-.13	.31	-.18	-.51*	.54*	-.18	.21	.45
	AGR_STO											1.00	-.32	-.25	-.36	-.12	.01	.12	.10	-.14	-.20	-.45
	ALO_PRA												1.00	-.20	.49	.07	-.27	.22	-.03	.16	.10	.23
	POA_PRA													1.00	-.07	.39	.26	-.23	-.16	.04	.30	.31
	RAN_REP														1.00	.37	.19	.01	-.48	.54*	.02	.20
	CLOVER															1.00	.61*	-.15	-.18	.56*	.14	-.02
	CAR_PRA																1.00	-.03	-.53*	.55*	-.40	-.23
	DWT_SPB																	1.00	.07	-.06	-.32	
	ALLGRASS																		1.00		.25	.13
	NSPECIES																			1.00	-.06	-.21
	PROFITHA																				1.00	.41
	ENERGYGJ																					1.00

** Correlation is significant at the .01 level (2-tailed).

* Correlation is significant at the .05 level (2-tailed).

Appendix F

Appendix F.

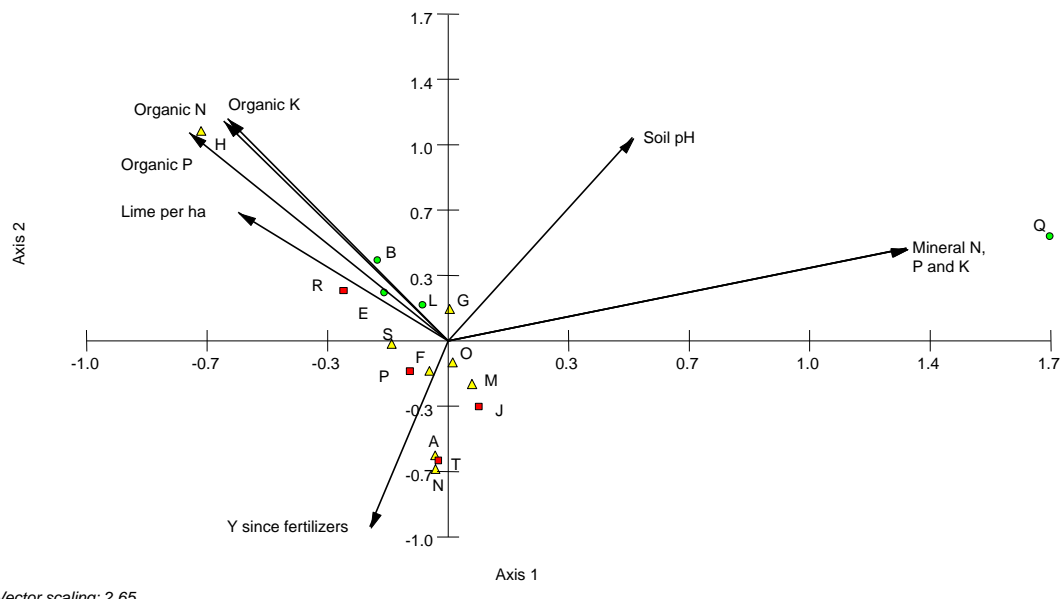


Fig. F.1 Principal component ordination of fertilizer and lime inputs and soil pH for six year period prior to production analysis of the sixteen study farms. Square, triangular and circular markers refer to farms of lower, average and higher relative levels of production, respectively. Vectors are plotted as Euclidean arrows, the direction and relative length of which reflects the degree of correlation; variables closest to outermost arrow tips are most strongly correlated. Fertilizer inputs for the six year interval prior to production estimates are termed organic or mineral N, P and K respectively. Other descriptors are self-explanatory, except Y since fertilizers refers to the number of years since the application of fertilizers of any kind.

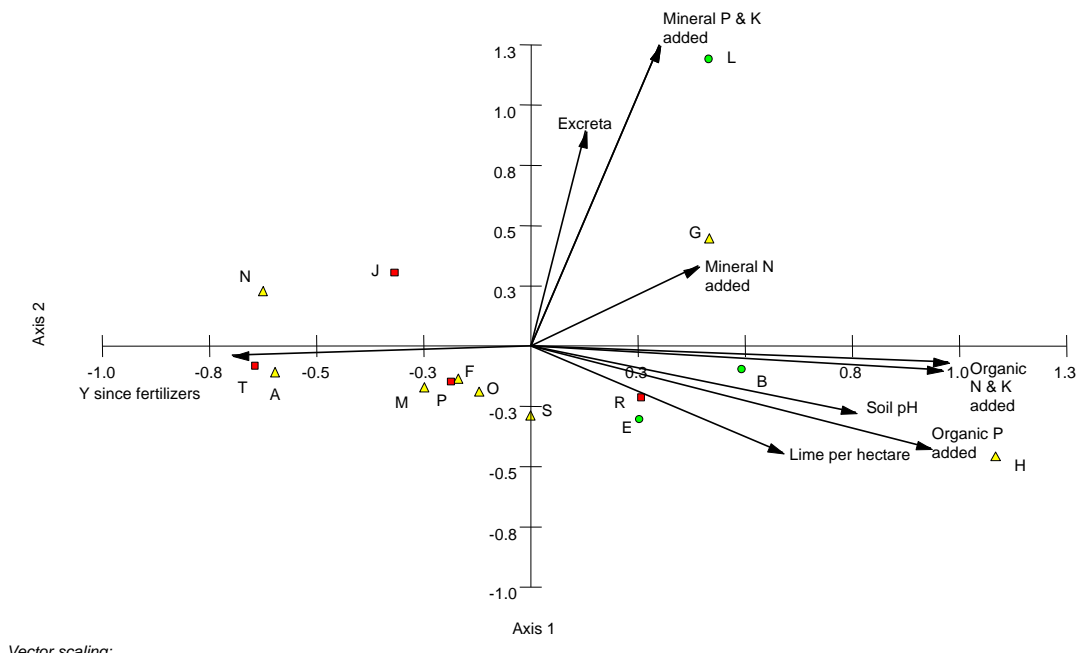


Fig F.2 Principal component ordination of fertilizer and lime inputs for six year period prior to production analysis together with annual approximate excretal nitrogen returns and soil pH, implemented with fifteen study farms. Square, triangular and circular markers refer to farms of lower, average, and higher relative levels of production, respectively. Vectors are plotted as Euclidean arrows, the direction and relative length of which reflects the degree of correlation; variables closest to outermost arrow tips are most strongly correlated. Fertilizer inputs are termed organic or mineral N, P and K respectively. Other descriptors are self-explanatory, except Y since fertilizers refers to the number of years elapsed since application of any fertilizers on the farm. Excreta inputs were represented as nitrogen from both dung and urine.

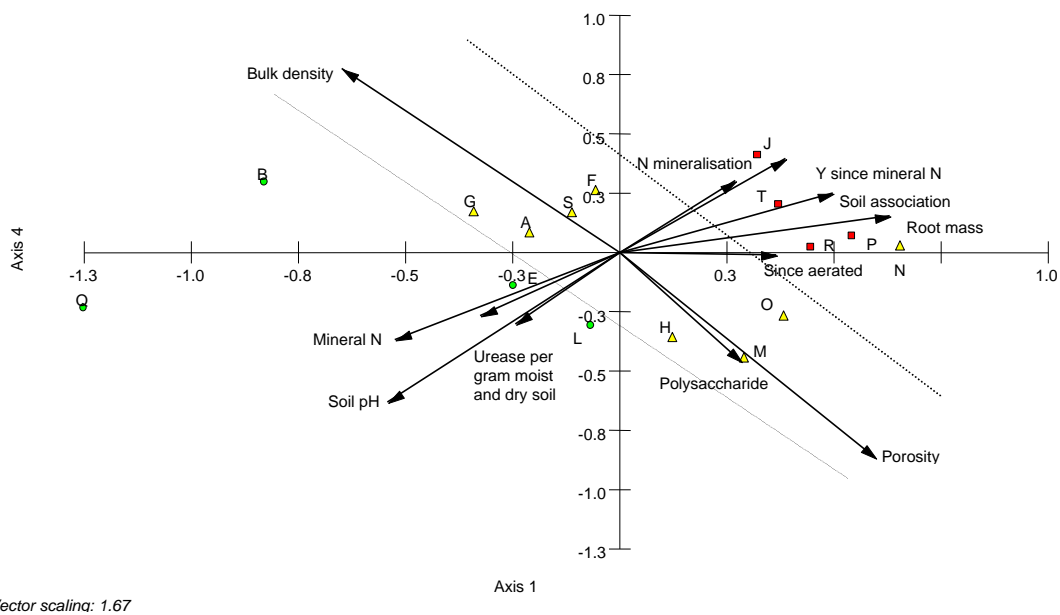


Fig. F.3 Principal components ordination of the interval since soil was aerated with associated variables for the sixteen farms. Square, triangular and circular markers refer to farms of lower, average and higher relative levels of production, respectively. Vectors are plotted as Euclidean arrows, the direction and relative length of which reflects the degree of correlation; variables closest to outermost arrow tips are most strongly correlated. *in vitro* N mineralisation measured as positive values for arginine ammonification; Except where indicated, values are for sixteen farms.

Fig. F.4 Variables related to soil depth

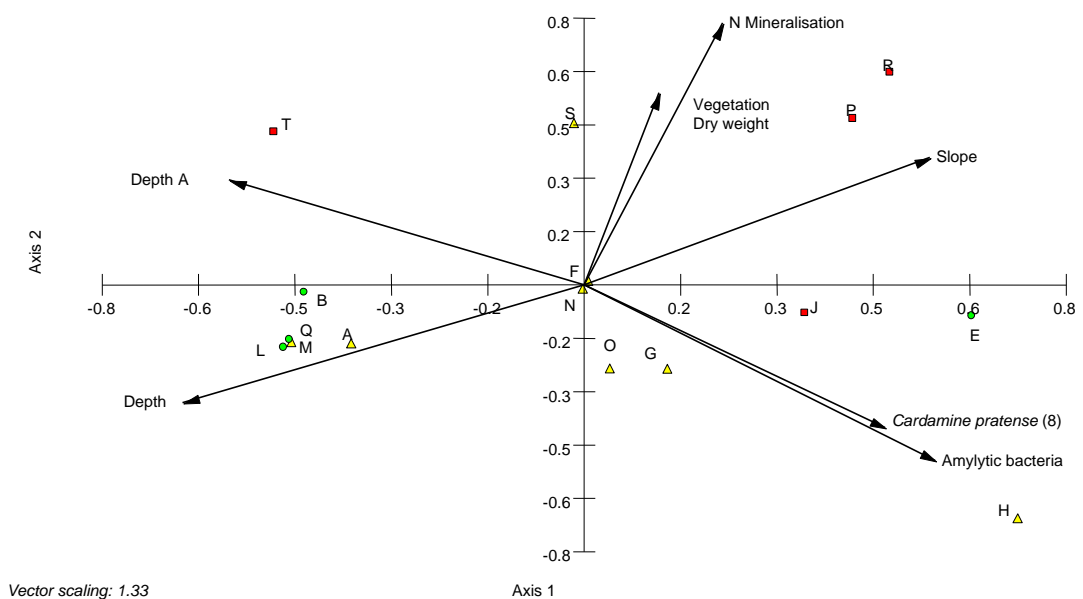
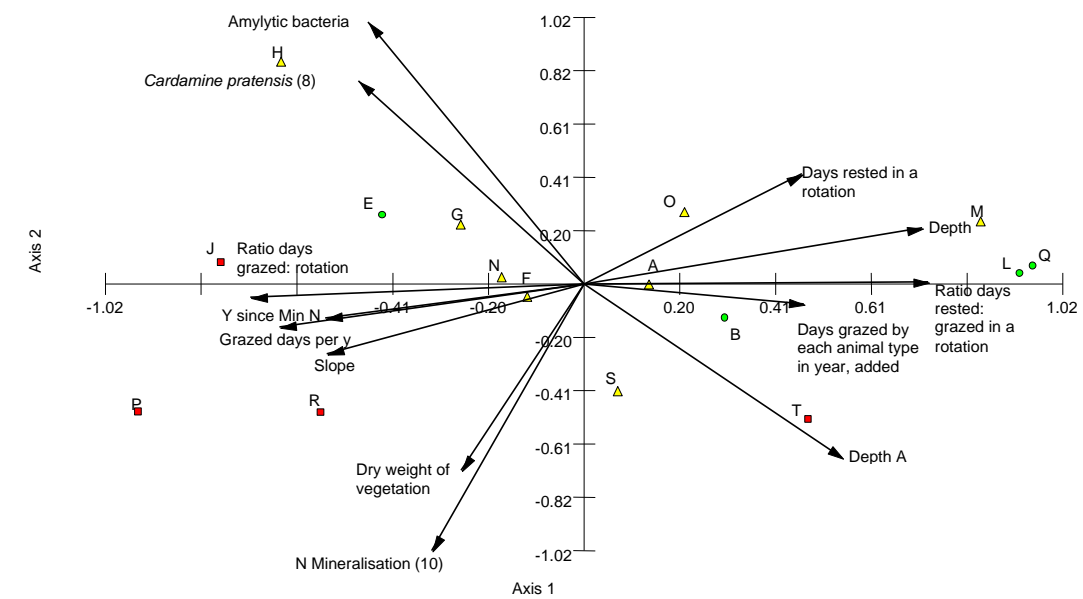


Fig. F.4-a-b Principal components ordinations implemented with depth and related variables for the sixteen study farms, (a) implemented including nutrient and grazing variables (b) implemented without nutrient and grazing variables. Square, triangular and circular markers refer to farms of lower, average and higher relative levels of production, respectively. Vectors are plotted as Euclidean arrows, the direction and relative length of which reflects the degree of correlation; variables closest to outermost arrow tips are most strongly correlated. Descriptors are self-explanatory. Values are for sixteen farms, except where stated.

Fig. F.6 Variables related to root mass

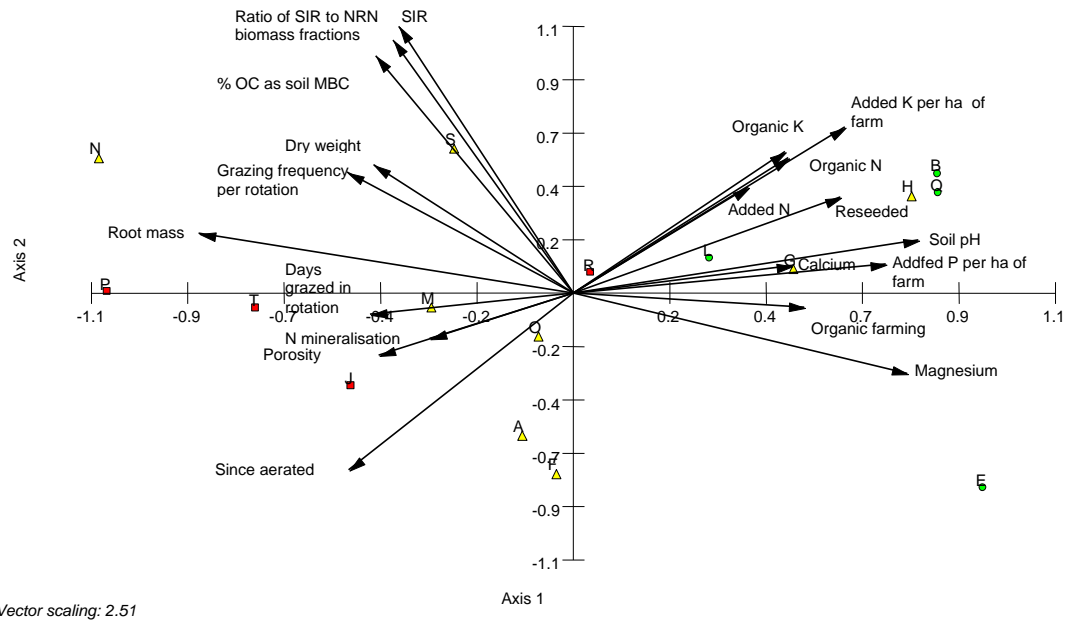


Fig. F.6 Principle component analysis of root mass and related variables for the sixteen study farms. Square, triangular and circular markers refer to farms of lower, average and higher relative levels of production, respectively. Vectors are plotted as Euclidean arrows, the direction and relative length of which reflects the degree of correlation; variables closest to outermost arrow tips are most strongly correlated. Descriptors are self explanatory, except where annotated: microbial biomass nitrogen (NRN); substrate induced respiration (SIR); percent soil organic matter as microbial biomass (% OC as soil MBC); in vitro N mineralisation derived as positive values for arginine ammonification activity; fertiliser inputs per hectare of grassland overall are noted in addition to others per hectare of the sampled field.

Fig. F.5 Variables related to soil plant–available magnesium content

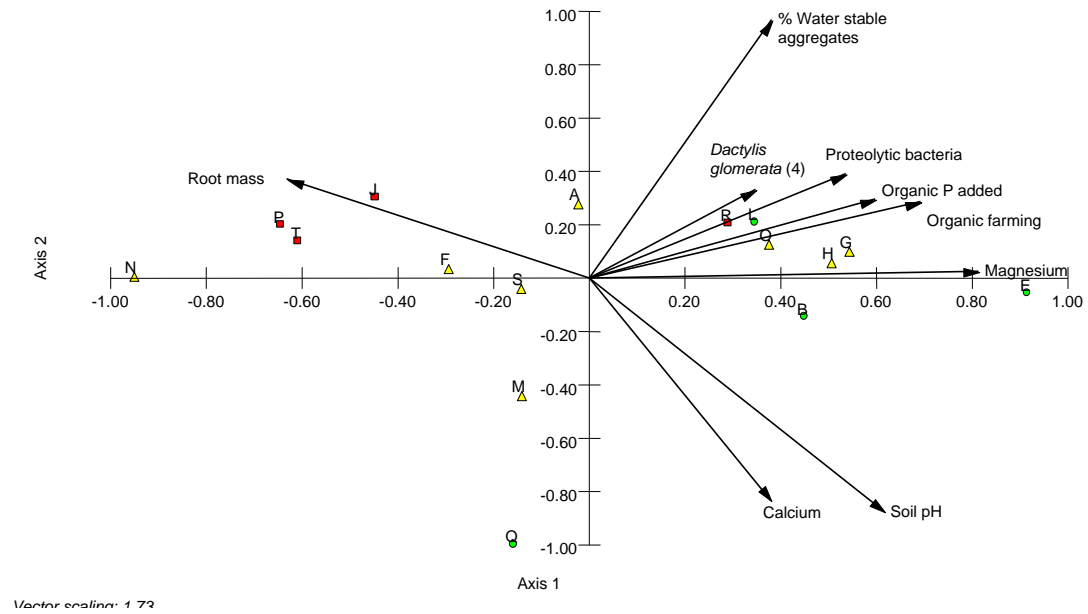


Fig. F.5. Principal components analysis of soil plant-available magnesium content together with variables significantly related to it for the sixteen study farms. Square, triangular and circular markers refer to farms of lower, average and higher relative levels of production, respectively. Vectors are plotted as Euclidean arrows, the direction and relative length of which reflects the degree of correlation; variables closest to outermost arrow tips are most strongly correlated. Descriptors are self-explanatory, except organic P added refers to ‘organic’ phosphate applied from manures and from basic slag, acceptable as an alternative to more soluble forms per hectare to the farm in the four years prior to soil magnesium analysis. Organic farming refers to the length of time for which the farm has been organically managed. Values are for sixteen farms, except where stated.