Physical and biochemical characterisation of soil organic carbon in topsoil and subsoil of Irish grasslands

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Physical and Biochemical Characterisation of Soil Organic Carbon in Topsoil and Subsoil of Irish Grasslands

A thesis submitted for the award of Doctor of Philosophy

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Abstract

Thesis Title: Physical and Biochemical characterisation of Carbon in topsoil and subsoil of Irish grasslands

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Soil plays a key role in the global carbon (C) cycle, since it holds 3.5 times more C than the atmosphere, and is the largest C pool after the oceans. Therefore, changes in soil organic carbon (SOC) can contribute to important emissions or sequestration of CO_2 from the atmosphere. Grassland soils have are one of the world biomes with a greater C sequestration potential. More than 46% of SOC can be found below 30 cm, and the dynamics and factors affecting subsoil SOC are different than the topsoil. The aim of this thesis is to physically and biochemically characterise SOC in topsoil and subsoil of Irish grasslands, up to 1 m depth, and to understand if there is a soil type effect on SOC sequestration potential.

A subset of soils sampled for National Soil Survey project (the Irish Soil Information System) in 2012/2013 were selected, with the aim of being representative of the main soil types occurring under grassland systems in Ireland. For each horizon, a 1 kg sample was taken, and several analysis were carried out: 1) separation of four aggregate sizes (Large and small macroaggregates, microaggregates and silt and clay), 2) isolation of fractions within small macroaggregates (particulate organic matter, microaggregates and silt and clay within macroaggregates), and 3) biochemical characterisation: non-hydrolysable C and N, as a measure of the biochemically recalcitrant fraction, and hot water extractable C and N, as a measure of the most labile fraction. Topsoil only showed differences between soil types in the case of percentage of microaggregates within macroaggregates, which was higher in Typical Sufrace-water gley than in Typical Brown Earth, while all the other analysed characteristics were equal between soil types. Subsoil horizons showed significant differences in many of the fractions. Differences in SOC characteristics were mainly influenced by clay content and stagnation properties.

Declaration

I, the undersigned, declare that the work in this project is entirely my own and to the best of my knowledge contains no material previously written, published or submitted for merit or award by this university or any other academic establishment, except where acknowledgment has been made in the text.

Signed: Gemma Torres Sallan

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A l'avi

Quan surts per fer el viatge cap a Itaca,
has de pregar que el camí sigui llarg,
ple d'aventures, ple de coneixences.
Que siguin moltes les matinades
que entraràs en un port que els teus ulls ignoraven,
i vagis a ciutats per aprendre dels que saben.
Lluís Llach

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Abbreviations

C	Carbon
CEC	Cation Exchange Capacity
HBE	Humic Brown Earth
HCl	Clorhydric Acid
HGG	Humic Groundwater Gley
HWEC	Hot Water Extractable Carbon
HWEN	Hot Water Extractable Nitrogen
mwM	microaggregates within macroaggregates
N	Nitrogen
POM	Particulate Organic Matter
RIc	Recalcitrance index for carbon
RI_N	Recalcitrance index for nitrogen
SBE	Stagnic Brown Earth
scwM	Silt and clay within macroaggregates
SLu	Stagnic Luvisol
SOC	Soil Organic Carbon
TBE	Typical Brown Earth
TLi	Typical Lithosol
TLu	Typical Luvisol
TSWG	Typical Surface-water Gley

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Chapter I

Introduction

1.1 Overview

The greenhouse effect is an anthropogenic phenomenon caused by the increase of greenhouse gases (GHGs) in the atmosphere (IPCC, 2014a). Carbon dioxide (CO_2) is one of the GHGs contributing to climate change. Soils hold nearly four times more carbon (C) than the atmosphere, and contain 82% of the C in the terrestrial pool (Lal, 2008). Carbon sequestration is defined as the process of increasing the carbon content of a pool other than the atmosphere (IPCC, 2007a).Given the large amount of C that soils hold, small changes in land use can lead to large emissions or sequestration of C from the atmosphere. Therefore, soils play a key role in C sequestration, and subsequently to the reduction to the GHG effect.

Soil organic matter (SOM) is composed mainly of C, hydrogen (H) and oxygen (O), and in smaller amounts, by sulfur (S), nitrogen (N), phosphorous (P), potassium (K), calcium (Ca) and magnesium (Mg) (Bot and Benites, 2005). The increase of soil organic matter (SOM) has several advantages for soils. Fertility is enhanced with increasing amounts of SOM, since it holds generally nutrients in a plant available form and avoiding its leaching (Bot and Benites, 2005). This soil nutrient retention occurs by increasing the cation exchange capacity (CEC) which determines a soil's ability to retain positively charged plant nutrients. It also increases soil moisture retention (Guimarães et al., 2013) and enhances soil structure through the increase of aggregation (Haynes and Beare, 1996; Martin et al., 1955; Tisdall and Oades, 1982). Improvements in terms of soil structure lead to better soil aeration, infiltration and water holding capacity, reduced bulk density which reduces compaction and more resistance to erosion and crusting (Bot and Benites, 2005). The increases in aggregation also separate physically SOM from microorganisms and their enzymes, which protects SOM from mineralization and decreases the subsequent CO_2 emissions to the atmosphere (Cambardella and Elliott, 1992; Golchin et al., 1994). Therefore, incorporation of SOC in soils is a win-win strategy since it has benefits both for the environment and for plant development, support and growth, hence productivity.

Temperate grasslands cover 22% of Europe's land area (EEA, 2005). The global SOC stock for grasslands is 343 Gt C, which is 50% more C than that stored in forests (FAO, 2010) . Under the appropriate management, grassland systems can act as a sink of C

(Conant et al., 2001). Globally, these systems have a C sequestration potential of 0.01 to 0.3 Gt C yr^{-1} (Lal, 2004a). Therefore, they play a key role on the global C cycle.

In order to understand how changes in management can influence SOC sinks it is important to understand the dynamics of SOM. Historically, SOM characterisation has been done by several different approaches: from biochemical characterisation by extracting SOM compounds with aqueous solutions, organic solvents or hydrolysing SOM with different acids, to physical fractionation, involving the isolation of various aggregate sizes or isolation of the fraction not associated with minerals through immersion in different densities liquids (see review by von Lützow et al., 2007).

Most research regarding this topic focuses on the topsoil (Denef et al., 2004; Leifeld and Kögel-Knabner, 2005; Li et al., 2007; Reicosky et al., 2002). Furthermore, the Good Practice Guidance for Land Use, Land Use Change and Forestry made by the Intergovernmental Panel on Climate Change (IPCC, 2006) recommends sampling the top 30 cm to account for SOC stocks and changes associated with land-use or land management change. Thus, the SOC located under this depth and its dynamics is still mostly unaccounted for and unknown, although in the recent years the importance of this "hidden" SOC pool has recieved more attention, and the mechanisms controlling it are starting to be elucidated (Fontaine et al., 2007; Rumpel and Kögel-Knabner, 2011; Salomé et al., 2010; Veldkamp et al., 2003).

1.2 Objectives

The main objective of this study is to characterise the factors influencing SOC vertical distribution and assess how these vary between soil types. This is crucial in order to design management systems that are adapted to optimise SOC/SOM retention and enhance productivity. Therefore, the specific objectives of this thesis are:

(i) To measure the physical protection of SOC against mineralisation in the most common soil types occurring in Irish grasslands.

(ii) Assess the biochemical characteristics of SOC, including recalcitrant and the readily-decomposable SOC of the main soil types under grassland systems in Ireland.

(iii) Evaluate the proportion of microaggregates contained in the small macroaggregate fraction, as a measure of aggregate turnover and disturbance of the system.

(iv) Assess the SOC distribution within small macroaggregates in order to have a better understanding of the potential SOC losses of Irish grasslands.

(v) Identify which are the soil characteristics influencing SOC chemical and physical properties.

(vi) Define the effect of depth in all characteristics described in objectives i, ii, iii and iv, with the aim of understanding if the commonly used sampling depth of 30 cm provides enough information to enable us to elucidate differences between soil types, or if, on the contrary, the topsoil is similar within soil types under grassland systems but different characteristics between soils arise in deeper horizons.

1.3 Thesis Layout

This thesis is divided in six chapters, three of which are experimental chapters. Chapter 1, the introduction, gives a wide overview of the importance of studying SOC characteristics and dynamics, and defines the objectives of this thesis. Chapter 2 consists of a literature review, and gives more detailed information about the topics covered in chapters 3, 4 and 5 of this thesis. The first of the experimental chapters, chapter 3, assesses the carbon distribution within aggregates of the whole profile of 31 grassland sites, divided between the six main soil types occurring in Irish grasslands. The idea behind this methodology is to assess the degree of protection of SOC against mineralisation, which increases when SOC is associated to smaller aggregate sizes (Del Galdo et al., 2003). Chapter 3 covers objectives I and VI. Chapter 4 also focuses on the level of aggregation, this time analysing the proportion of microaggregates within the small macroaggregate fraction, and assessing the SOC location within the different fractions associated with small macroaggregates (particulate organic matter, microaggregates and the silt and clay fraction). With this information, we assess whether the process of aggregate formation is similar between soil types and at depth, and if there are differences between soil types in terms of the distribution within the different factions within small macroaggregates. This provides insights into the differences between soil types in terms of SOC incorporation into soil aggregates (i.e. C sequestration). It will also inform about the potential of SOC

loses or changes in aggregation, since SOC located in POM has a higher decomposition rate than SOC in microaggregates or the silt and clay within macroaggregates (Del Galdo et al., 2003). Objectives III, V and VI are covered in chapter 4. Chapter 5 assesses the SOM characterisation from the biochemical point of view, both in the topsoil as well as the subsoil. With this chapter we aim to assess the chemical lability and recalcitrance of SOM, and clarify which are the main soil characteristics influencing these. In this chapter, objectives II, V and VI are covered.

1.4 Irish Soil Information System

The Irish Soil Information System was a project established in 2008, with the aim of creating a soil map at 1: 250,000 scale, and conduct research into the national distribution of soil types. To complete this, 228 pits were dug, all the horizons were described and the soil type of each point was identified according to the Irish Soil Classification System (Simo et al., 2014) and Wold Reference Base (WRB) (WRB, 2006). Several characteristics were analysed for each horizon: pH, total C, total N, organic C, SOM content (through loss-on-ignition), Fe/Al (through oxalate extraction), texture and bulk density. In addition, extra samples were taken to be analysed by different PhD students, who focused on diverse aspects of soil: Microbiology, Meso-fauna and C sequestration. This thesis is based on the assessment of C in the different soil types. Samples were chosen with the aim of representing the main soil types occurring under grassland systems in Ireland. For reasons of time restriction, not all analysis were conducted in all the soil types

Table 1. 1 *Number of Soil types in which each analysis was conducted, and the corresponding chapter of this thesis where the results are displayed. (numbers refer to number of profiles where analysis were carried on)*

	Chapter III	Chapter IV	Chapter V	
	Aggregate associated C	Fractions associated to small macroaggregates	Non-hydrolysable C	Hot water extractable C
Typical Brown Earth	7	7	7	7
Humic Brown Earth	6		6	6
Stagnic Brown Earth	6	5	6	6
Typical Luvisol	3	3	3	3
Stagnic Luvisol	3		3	3
Typical Surface-water Gley	6	6	6	6
Typical Lithosol			5	
Humic Groundwater Gley			5	

Chapter II

Literature Review

2.1 Climate change

2.1.1 Introduction

The term "global change" refers to planetary-scale changes in the Earth's system. This includes changes to atmospheric circulation, oceanic circulation, climate, biogeochemical cycles, water cycle, sea-ice changes, sea-level changes, food webs, biological diversity, pollution, health, fish stocks, and more (IGBP, 2015).

Global warming is a crucial and evident factor of global change. There are many long term observed trends that have made the evidence for climate change apparent. Levels of atmospheric CO_2 have increased, with current levels nowadays the highest in 650000 years, as determined by ice cores (NASA, 2016). Over the last century world's temperature has increased 0.85 °C, with glacier areas diminishing (IPCC, 2014a), and the sea level has increased 17 cm, with a rate of increase from the last decade that doubles that of the last century (Church and White, 2006). Ocean temperatures have increased on the surface (top 75 m) at a rate of 0.11 °C every decade from 1971 to 2010 (IPCC, 2014a). As a consequence, CO_2 levels in the ocean have increased, which causes a higher acidity, leading to coral bleaching and greater difficulty for some organisms to build and maintain skeletons and shells (Kurihara, 2008).

2.1.2 Greenhouse gas effect

The atmosphere contains naturally occurring GHG, which have the capacity to absorb infrared radiation. The main GHGs in the atmosphere are water vapour, carbon dioxide (CO_2), methane (CH_4), ozone (O_3), and nitrous oxide (N_2O).

The Greenhouse effect is a natural process that warms the earth surface. When energy is irradiated by the sun, a portion of it passes through the atmosphere without being absorbed, and approximately 31% of the energy directly irradiated back to the space. Part of the remaining energy is absorbed by the atmosphere (approximately 20%), and part is absorbed by the earth surface (49% of the original energy). As a consequence, the earth surface warms up and it re-irradiates energy as infrared thermal radiation. This low energy radiation is absorbed by the atmosphere. A small percentage of this re-irradiated energy will go back to space, and the major part is radiated from the atmosphere back to the earth, resulting in surface warming. Global warming occurs when the concentration of GHGs accumulate in the atmosphere. Since GHGs have the capacity to absorb large amounts of

infrared radiation, a higher percentage of the energy re-emitted by the earth is absorbed by the atmosphere, which causes additional warming of the surface and is termed radiative forcing (Khalil, 1999).

During the last century, the industrial revolution resulted in the large-scale burning of fossil fuels, land use change for agriculture and deforestation (IPCC, 2014b). This caused an increase of anthropogenic GHG emissions to the atmosphere, resulting in increased radiative forcing (RF) and consequently increased global temperatures. The IPCC define measure radiative forcing, in units of power per unit area (W/m2). In order to assess and compare different anthropogenic drivers of climate change, RF is used. The IPCC (2007) describes RF as a measure of the influence each factor (e.g. CO_2, CH_4, N_2O, halocarbons, land use change, aerosols, and other human activities) has in altering the balance of incoming and outgoing energy in the earth-atmosphere and is an index of the importance of the factor as a potential climate change mechanism. Positive RF values imply a warming of Earth's surface, while negative RF values represent cooling factors. In order to compare the capacity of a gas to contribute to radiative forcing, the Global Warming Potential (GWP) is used. This GWP is defined as the amount of heat trapped by a certain mass of gas indexed relative to CO_2 (which therefore has a GWP of 1) over a defined time horizon (usually 100 years). The need for a defined time horizon is due to the fact that individual GHG's have varying residence time in the atmosphere. Methane, for example has a 12 year residence time, whereas the residence time for CO_2 is indeterminate (can be absorbed via photosynthesis a few minutes after emission, or can last 10,000 years (Forster et al., 2007)

2.1.3 Carbon dioxide

Based on global emissions of 2004, CO_2 is 77% of the total amount of GHG, followed by CH_4 (14%), and N_2O (8%) (IPCC, 2007b). CO_2 emissions to the atmosphere have increased by 80% since 1970 to 2004 (from 21 to 38 Gt). In pre-industrial times CO_2 atmospheric concentration was approximately 280 ppm, while in 2005 the concentration was 379 ppm. This is a much higher concentration than the natural range between the last 10000 years and the year 1750 (IPCC, 2007b). The rate of increase per year was of 0.92 $GtCO_2$-eq per year from 1995 to 2004, which is a much higher rate than the one from 1970 to 1994 (0.43 $GtCO_2$-eq per year) (IPCC, 2014a).

Of total CO_2 emissions, 22% are a consequence of land use change, mainly deforestation, decay of biomass and peat burning. The biggest source of CO_2 is fossil fuel use, contributing 74% of total CO_2 emitted to the atmosphere. Nearly 63% of industrial CO_2 and CH_4 emitted between 1751 and 2010 is attributable to 90 entities (http://carbonmajors.org) , with 50% of these emissions having been emitted since 1986 (Heede, 2014).

2.1.4 International response

In order to limit GHG emissions, the United Nations (UN) and the World Meteorological Organization (WMO) created the Intergovernmental Panel on Climate Change (IPCC) in 1988, which is an expert body that assesses scientific information relating climate change.

In 1994 the UN created the intergovernmental negotiating committee for a Framework Convention on Climate Change (UNFCCC). The objective of the UNFCCC was to stabilise the concentration of GHGs in the atmosphere at a level that prevents dangerous anthropic interference with the climate system. After three Conventions of the Parties (COP) and negotiations, the Kyoto Protocol was agreed in 1997. According to this protocol, by the end of the first commitment (2008-2012) developed countries had to reduce emissions to an average of 5.2% below 1990 levels. The second commitment period is to the year 2020. In the eight-year period of the second commitment, the parties agreed to reduce GHG emissions to at least 18% below 1990 levels.

The emissions considered were CO_2, CH_4, N_2O, hydrofluorocarbons, perfluorocarbons and sulphur hexafluoride. An important part of this agreement is that commitments are calculated on a net basis, which means that sources, but also sinks are considered. As a consequence, C sinks in soils can be a key point when accounting for CO_2 emissions.

Europe has also promoted actions against climate change. In 2007, the European Council approved the European Commission Strategic Energy Review, which led to the "20-20-20" agreement. This sought to increase the proportion of renewable energy to 20%, improve energy efficiency by 20%, and reduce CO_2 emissions by 20% relative to 1990 levels. Subsequently the EU Climate and Energy Package was agreed last year, with a target of a 30% reduction in GHG's by 2030.

The Paris agreement during COP21 in December 2015 set a long-term goal of limiting the increase in global average temperature to below 2°C above pre-industrial levels. The

agreement made one step further for C sequestration in soils, since it will be allowed to use the land use, land use change and forestry (LULUCF) sector to offset emissions from other sectors, which is currently not permitted. Furthermore, if a member state has net C sequestration, the extra sequestered C in the first compliance period (2021-2025) can be either used in the next period (2026-2030) or sold to other member states. This encourages countries to increase soil C sequestration beyond their own commitment (European Comission, 2016). The 4 per 1000 initiative launched by the French ministry of agriculture, food and forest during the COP21 ("4 pour 1000," 2015) is an initiative that has the objective of increasing the existing C content in the topsoil by a 0.4% annually. The initiative aims to include all stakeholders and promote international research and scientific cooperation.

2.2 Soil Carbon

Soil contains 1550 Pg of organic C, and 780 to 950 Pg of inorganic C (Batjes, 1996; Lal, 2008). The amount of C in soils is 5 times greater than the C in the biosphere and 3.5 times the C in the atmosphere (Lal, 2008). Therefore, soils hold an important part of global C cycle. Terrestrial ecosystems are attractive for carbon sequestration because they can be managed. The focus has been on woody vegetation, essentially trees, because methods exist that can provide reasonable estimates of carbon accrual at commercially viable costs. Soil carbon is now receiving increased attention because of its potential significance but measurement difficulties have limited its value in carbon trading. Protocols allow for soil carbon in agricultural lands to be added to trading schemes but this depends on the availability of cost effective and reliable means of measurement.

2.2.1 Carbon cycle

The gross primary production (GPP) of an ecosystem represents the gross uptake of CO_2 that is used in photosynthesis minus any loss in efficiency via photorespiration (Lanigan et al., 2008, Ciais et al., 2009a). A proportion of the assimilated C is consumed via autotrophic respiration (Ra), in order to maintain tissue growth resulting in an emission of CO_2-C (Luyssaert et al., 2007). The net quantity of photoassimilates not utilsed is defined as net primary production (NPP) and relates to GPP and Ra as: GPP = NPP + Ra. Net primary production stays in the biosphere and is incorporated to soil through decomposition of root exudation, leaves, animal remains or fungus. Decomposition is a biological process that includes the physical breakdown and biochemical transformation

of complex organic molecules of dead material into simpler organic and inorganic molecules (Bot and Benites, 2005; Juma, 1999; Wetterstedt, 2010) . Once incorporated into the soil, SOC can be released back to the atmosphere through heterotrophic decomposition. Other processes that lead to a transfer of C between terrestrial and atmospheric pools are changes in land use (agriculture, deforestation and reforestation), and fossil fuel burning, which is only one-directional (biosphere to atmosphere). The biggest C pool are the oceans, with 38000 Pg of C, followed by the Geologic pool (5000 Pg C), the soil pool, with 2500 Pg of C, the atmospheric and the bionic pool, with 760 and 560 Pg of C, respectively (Lal, 2004).

2.2.2 Carbon sources and fluxes in soils

In ecosystems, the biggest gross CO_2 flux is CO_2 uptake via photosynthesis, followed by CO_2 respired from soils (Goulden et al., 1996; Longdoz et al., 2000). As reviewed by Kuzyakov (2006), there are three main broad groups of C pools that act as sources of C: (i) soil organic matter (SOM), (ii) dead plant residues, and (iii) rhizodeposits, which are organic substances released by living roots such as exudates, mucilage and sloughed-off root cells (Johnen and Sauerbeck, 1977; Johnson et al., 2006). These C pools do not have sharp boundaries in soils, and can be further divided into SOC pools with differing turnover times in soils (see below). Heterotrophic and autotrophic organisms in soils will contribute differently in decomposing these C pools, and hence to CO_2 fluxes coming from soils. The contribution of autotrophical and heterotrophical respiration to CO_2 emissions from soil varies during the year, and generally have similar levels except during the active vegetation growing season, which has a larger proportion of autotrophic respiration to the total CO_2 efflux (Saiz et al., 2007). The most important heterotrophs in terms of CO_2 emissions from soil are soil microorganisms and soil macrofauna (Kuzyakov, 2006; Reichstein et al., 2003). Soil microorganisms include bacteria, fungi, and with a minor contribution to CO_2 emissions, protozoans. Soil macrofauna includes macroscopic invertebrates and small mammals. Their contribution to CO_2 fluxes is minimal compared to those of microorganisms (Andrén and Schnürer, 1985; Ke et al., 2005; Konaté et al., 2003; Kuzyakov, 2006). Nevertheless, these groups can contribute indirectly to the microbial respiration by fragmentation of plant residues (Bot and Benites, 2005; Kuzyakov, 2006) and predation of some groups of microorganisms (Bonkowski, 2004).

Autotrophs also contribute to CO_2 emissions from soil: plants, through root respiration, are the most important ones. Algae are considered less important, as they are only active in flooded soils or soils highly influenced by water, and will only affect the top two or three centimetres. Chemolitotrophs also contribute to increase the CO_2 emissions from soil to the atmosphere, but their contribution is marginal compared to the other groups (Kuzyakov, 2006).

Kuzyakov (2006) defined five SOC pools associated with different CO_2 efflux processes (Figure 2. 1): Plant assimilates, rhizodeposits, plant residues, SOC in the rhizosphere, and SOC in root free soil. These C pools have different turnover rates (TR), which is the rate of cycling of C in a pool or a system. The smaller the turnover rate, the bigger mean residence time (MRT) in soil.

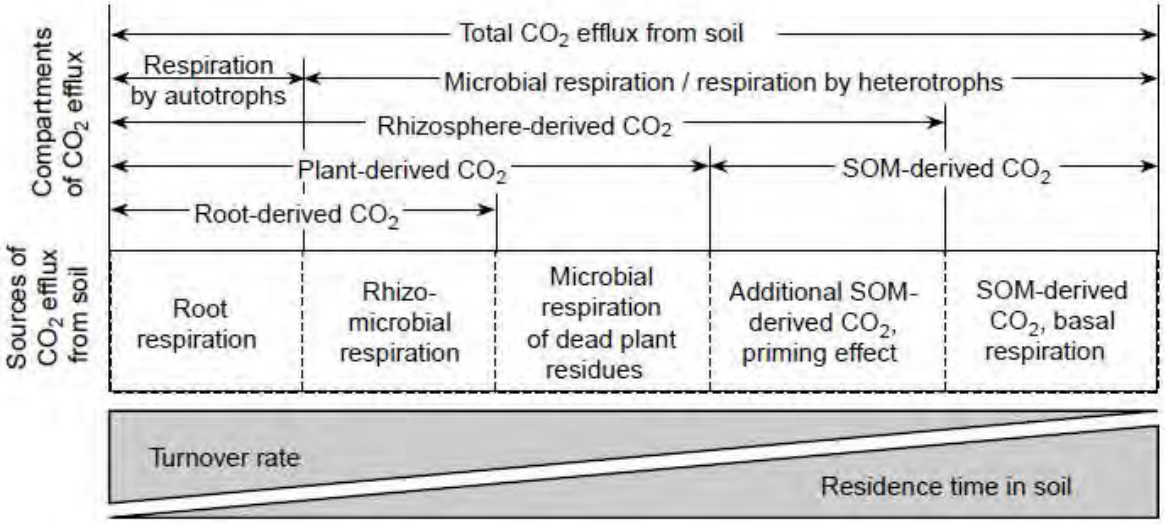


Figure 2. 1 *Five main sources of biogenic CO_2 efflux from soil, ordered according to the turnover rates and mean residence times of C in soil. The sources and compartments of the CO_2 efflux consider C pools with different TR and MRT, the localization of C pools and the agents of CO_2 production. Source: Kuzyakov (2006)*

The SOC pool with the lowest MRT in soil consists of plant assimilates, which is the only CO_2 source produced exclusively by autotrophs and contributes to CO_2 emissions through root respiration, with a MRT of minutes to a few hours, and longer periods of up to 4 days for tree roots (Ekblad and Hogberg, 2001; Kuzyakov, 2006) . The second C pool, rhizodeposition consist mainly of organic substances with low molecular weight like sugars, amino acids and organic acids (Nguyen, 2003). Rhizodeposits are decomposed

through rhizomicrobial respiration after a few hours of being excreted by roots (Kunc and Macura, 1966; Kuzyakov and Demin, 1998; Verburg et al., 1998).

The third pool, plant residues, contributes to CO_2 emissions through microbial respiration. These need weeks to months to be completely decomposed (Hadas et al., 2004; Hahn and Buchmann, 2004; Kuzyakov, 2006). The rate of decomposition will depend on the composition of the residues (Kögel-Knabner, 2002). If plant detritus has a high lignin and cellulose content, like needles of conifers, it will take from two to four years to decompose. If by contrast, there is a high N content and low lignin content, the residues will only take a few weeks to decompose (Gaudinski et al., 2000).

The SOC pool with the second highest MRT is SOC in the rhizosphere. The SOC located in this area is more subject to be affected by priming effect as compared to the SOC outside the rhizosphere. Priming effect is defined as the increase in the rate of decomposition of SOM after fresh organic matter inputs, which causes an increase in microbial activity (Fontaine et al., 2003). In the case of rhizosphere priming effect, the increase of microbial activity occurs around the rhizosphere as a result of the presence of rhizodeposits, which are a source of easily available C (Kuzyakov, 2002). The MRT of SOC in the rhizosphere is from months to decades (Kuzyakov, 2006).
Inorganic sources like soils with $CaCO_3$ can also contribute to CO_2 efflux. Globally, there are 722 Pg (Batjes, 1996) to 950 Pg (Lal, 2008) of C in form of $CaCO_3$. The dissolution of this $CaCO_3$ only contributes to increase the CO_2 emissions over a geological time scale. The abiotic CO_2 flux contributions are marginal in terms of annual or decadal time scales (3-4 g C m^{-2} y^{-1} according to Kuzyakov (2006)) if compared to biotic contributions.

2.3 Soil organic matter

Soil organic matter is defined by Bot and Benites (2005) as any material produced originally by living organisms that is returned to the soil and goes through the decomposition process. Is composed of a range of materials at a different levels of decomposition, from undecomposed plant or animal tissues to humus (Gregorich et al., 1996). When SOM decomposition occurs, nutrients contained in SOM are released into the soil matrix.

The composition of SOM consist on around 58% of C, oxygen (O), hydrogen (H), and in smaller amounts, sulphur (S), nitrogen (N), phosphorus (P), potassium (K), calcium

(Ca) and magnesium (Mg)(Bot and Benites, 2005; Stockmann et al., 2013). During the decomposition of SOM by soil organisms, its original structure its broken down, which causes a release of different nutrients that were in the SOM macromolecules, into a plant-available mineral form (Bot and Benites, 2005).

A steady state, in terms of SOC, refers to the system where inputs of C into the soil are equal to the rate of decomposition. Nevertheless, addition of C to soil can be lower than the rate of decomposition (causing a decrease of SOC), or inputs of C can be higher than the rate of decomposition, which leads to an increase of SOC (Bot and Benites, 2005).

2.3.1 Benefits of soil organic matter

There are several reasons why SOM is beneficial for soils. Raw plant residues that remain on the soil surface protect soil from the effect of the rain, wind and sun. SOM has the potential to sequester the greatest amount of SOC and for the longest time period (Del Galdo et al., 2003). The increase of SOC in soils is therefore, a win-win strategy as: it both reduces atmospheric CO_2 concentration and enhances soil fertility. As pointed by Haynes and Beare (1996), the increase of soil fertility is reached through different ways: SOM is a sink of nutrients such as N, P and K due to its mainly plant derived origin. After microbial mineralization, these nutrients are in a plant-available form and adsorbed by the humus, which is the most stable SOM fraction (Bot and Benites, 2005). The adsorption to the humus surface avoids the leaching of nutrients and ensures that they will be available for the plant when needed. These more stable organic matter compounds (humic substances) also contribute to soil nutrient retention by increasing the CEC which determines a soil's ability to retain positively charged plant nutrients (Guimarães et al., 2013).

The formation and stabilisation of soil aggregates is enhanced by SOM, which leads to an improvement of soil structure (Tisdall and Oades, 1982). As a result, soil has better aeration, water infiltration and water holding capacity, and more resistance to erosion and compaction (Bot and Benites, 2005; Lal, 2004b). The fact that SOM enhances aggregate formation and stabilization is important from the point of view of GHGs and carbon sequestration, since the SOM inside aggregates is not easily available to microorganisms (Bronick and Lal, 2005), thus avoiding its mineralization and the consequent CO_2 emissions to the atmosphere.

2.3.2 Nature and characterisation

In nature, SOM acts as a continuum and not as different fractions with sharp borders between them. Despite this, in order to be able to study its dynamics and characteristics, it can be partitioned into different fractions that relate to its degradability and therefore MRT in soils.

In soil, SOM can be physically found with three different natures: free, occluded and mineral-associated SOM. Based on five different studies made by diverse authors, von Lützow et al. (2007) concluded that the stability of SOM increases from free particulate organic matter (POM) to occluded POM, and further to mineral-associated SOM. Besnard et al. (1996) also concluded that occluded POM is less decomposable than free POM, looking at the differences in ^{13}C content of fractions from soils where C3 vegetation had been replaced by C4 vegetation. Focusing on occluded SOM, the size of the aggregate where SOM is located has an influence on SOM availability and has been widely used to characterise SOM (Bongiovanni and Lobartini, 2006; Six et al., 2000b; Six and Paustian, 2014). This is based on the assumption that SOM stored in macroaggregates has a faster turnover time than SOM stored in microaggregates and silt-sized aggregates (von Lützow et al., 2006).

The C:N ratio is also used to indicate the type of material. High C:N ratios correspond to materials that are more difficult for bacteria to decompose, like conifer needles or plants with high cellulose or lignin content. By contrast, materials with low C:N ratio, such as detritus from deciduous plant leaves or legumes with high N content, are more easily decomposable by soil microorganisms (Bot and Benites, 2005; Porta et al., 2010a). This index can also be used to assess the degree of decomposition, with lower C:N ratio indicating a higher degree of decomposition (Baisden et al., 2002)

2.3.3 Factors controlling soil organic matter in temperate European climate

2.3.3.1 Temperature

Temperature is a key factor controlling decomposition of SOM as soil respiration and temperature are exponentially correlated (Lloyd and Taylor, 1994). Soils in cold climates have more SOM due to a slower mineralization rate, while in warmer climates like the tropics, it is difficult to achieve a high SOM content as warmer temperatures induce faster decomposition rates (Bot and Benites, 2005).

As already mentioned, root respiration and microbial decomposition are the main factors responsible for CO_2 production in soils. These processes are temperature-dependent as they consist of biochemical reactions (Davidson and Janssens, 2006). For this reason, SOM decomposition increases mainly with increasing mean annual air temperature, occurring more rapidly in the tropics than in temperate areas (Bot and Benites, 2005).

2.3.3.2 Humidity

Since high soil moisture leads to greater biomass production, the total amount of SOM increases with increasing precipitation (Porta et al., 2010a). As a consequence, there is a greater input of plant residues into the soil, which becomes a source of energy for soil biota (Bot and Benites, 2005). Therefore, soil respiration also increases with higher mean annual precipitation. While an increase of precipitation leads to an increase of polysaccharide content in soils (Amelung et al., 1997), low soil moisture will negatively affect soil respiration. Davidson et al. (1998) found that respiration dropped 50% with soil matric potentials between -0.5 and -1.5 MPa. Following the same trend, Wildung et al. (1975) determined that respiration declined when soil water content reached levels below -1.3 MPa in dry grasslands. Other laboratory-based studies found that the matric potential at which respiration decreased was -0.01 MPa, although this was done in sieved and rootless soil (Orchard and Cook, 1983).

Field capacity is described by the Soil Science Society of America (2008) as "the content of water, on a mass or volume basis, remaining in a soil 2 or 3 days after having been wetted with water and after free drainage is negligible". Water contents around 40 to 60% of field capacity is optimal for microbial activity (Bot and Benites, 2005). Higher water content can saturate the soil and reduce O_2 diffusion. As most of the soil organisms need oxygen, the decrease of oxygen within the profile will result in a reduction of SOM decomposition (Doran et al., 1990; Linn and Doran, 1984; Skopp et al., 1990). If the saturation of the soil is continued over a longer period, this can lead to an accumulation of SOM (Bot and Benites, 2005).

It is widely accepted that the interaction of temperature and precipitation give the best estimation to predict soil respiration (Raich and Schlesinger, 1992), and that soils with a high moisture content have a greater content of SOM as a result of enhanced plant growth and decreased rates of SOM decomposition (Janzen et al., 2002). Hence, the vast majority of empirical models predict soil respiration using temperature and soil water content or

precipitation (Davidson et al., 2000; Hanson and Wullschleger, 2003; Raich and Potter, 1995; Reichstein et al., 2003; Schlentner and Cleve, 1985).

2.3.3.3 Texture

Clay is a colloid, and the most important characteristic of colloids is their small size, large surface area and electric charge. This allows them to interact with a large number of compounds at the same time (Porta et al., 2010a).

Clay content is directly related to SOC content. Power and Prasad (1997) found that, under similar conditions, SOC content was two to four times greater in high activity clay soils compared to sandy soils. The increasing SOC content associated to increasing clay contents is a consequence of (i) the bonds that form between the clay surface and the SOC, which retard the decomposition process, and (ii) due to the fact that clay presence increases the aggregate formation potential, and consequently, the physical protection of SOC (Rice, 2002). The adsorbing capacity of clay particles also depends on the presence of particles with different sizes in soils. Hassink (1997) reported that the same amount of clay and silt particles can hold more SOC in a sandy soil than in a fine sized textured soil. This is a result of the coagulation occurring in fine textured soil. By contrast, in sandy soils clays are in the form of individual particles, having a greater available surface to interact with SOC.

2.3.3.3.1 Clay mineralogy

Clay mineralogy also affects SOC content. Clays are formed by sheets, which are built of octahedral or tetrahedral structures with a central atom. Normally this atom is Silicon (Si^{4+}) for tetramers and Aluminium (Al^{3+}) for octahedrons. Nevertheless, isomorphic substitutions can occur. These consist of replacement of the central cation with a different cation with a similar size. As an example, if the soil is rich in Al^{3+}, when the clay is formed, aluminium can occupy the place of Si^{4+}. This leads to a less stable tetrameter due to the size difference, and it also causes a permanent negative charge on the surface, as the charge of Al^{3+} is smaller than the charge of Si^{4+} (Bleam and Hoffmann, 1988). As a consequence, clays will have a higher cation attracting force. In octahedrons isomorphic substitutions, Al^{3+} is substituted by magnesium (Mg^{2+}) or Iron (Fe^{2+}) (Porta et al., 2010a).

Negative charges caused by isomorphic substitutions are permanent, but clays can also have positive or negative temporary charges depending on the pH of the soil. Clay

surfaces have hydroxyl groups which protonate or deprotonate depending on the concentration of protons in the soil solution. If the soil is acidic, the surfaces of the clays will protonate, and as a consequence the clay will have a positive charge. If the soil is basic, the surface of the clay will not be protonated and the clay will have a net negative charge (Porta et al., 2010a; Schoefield, 1950).

Clays are classified from a structural point of view. Kandites are clays formed by one tetrahedral and one octahedral sheet (1:1). They have a low cation exchange capacity and specific surface. Two examples of this type of clays are Kaolinite and Hallosyte. Kandites are typically present in highly weathered tropical soils. Clays can also have two tetrahedral layers and one central octahedral layer (2:1). These types of clays are present in most soils, and have a higher cation exchange capacity and greater specific surface. Muscovite, Illite, Biotite, Vermiculite and Esmectite are 2:1 clays. If the space between two sheets is filled by an octahedron, the type of clay is 2:1:1 (Porta et al., 2010a)

High specific surface and CEC of soils leads to a greater adsorption capacity (Tate and Theng, 1980). Therefore, as suggested by Six et al. (2000b), clay mineralogy will determine soil behaviour in terms of aggregation. This is a consequence of the different capacities to adsorb organic materials associated with clays with different mineralogy.

In highly weathered soils clay mineralogy can have a greater influence than SOM in aggregate formation and stabilization. These soils are characterized by a predominance of 1:1 minerals and Fe and Al oxides. The positive charge associated to oxides and the negative charges from the clay minerals generate electrostatic interactions that promote aggregation. Therefore, the primary binding agent in this soil types is through electrostatic interactions and not via SOC binding (Denef et al., 2004). Some illustrative examples of this can be found in the literature: Denef et al. (2002) described a higher aggregate formation in soils dominated by 1:1 clays and oxides when the concentration of SOC was low. In contrast, with increasing SOC concentration, soils with mixed mineralogy (2:1 and 1:1 clays) had the greatest aggregate formation. This is a consequence of both electrostatic interactions and binding through SOM occur between 2:1 and 1:1 minerals in these soil types. Equally, Denef et al. (2004) described a minor influence on aggregate formation of SOC decrease in soils with increasing amount of 1:1 clay minerals and oxides.

2.3.3.4 Organo-mineral interactions

SOM can be physico-chemically stabilised by adsorption onto the surface of clay minerals (Tisdall and Oades, 1982), which is a way of stabilizing SOM, against microbial decomposition (Kahle et al., 2004; Mikutta et al., 2007). The interaction of SOM with mineral surfaces can occur through different binding mechanisms, which will vary depending on the properties of the mineral and of the organic matter. The strongest interaction is ligand exchange, which consists on the replacement of a ligand by an adsorbate molecule, which must have a stronger chelation capacity than the ligand (Tan, 1998). This type of binding increases with decreasing soil pH, with a maximum sorption between 4.3 and 4.7, and are able to last over 100 years (von Lützow et al., 2006). According to Mikutta et al. (2007), 92% of interactions between SOM and goethite (FeOOH) occur via ligand exchange, while for pyrophyllite ($Al_2Si_4O_{10}(OH)_2$), the fraction of organic matter bound by ligand exchange is 13%, and 7% for vermiculite.

Weaker SOM-mineral interactions can also occur through bridges of polyvalent cations. Polyvalent cations have more than one positive charge. One of these positive charges can interact with a negative mineral surface (e.g. clay), and the other with an acidic functional group of SOM (e.g.COO^-, OH^-), neutralising the charge and creating a bridge between the two entities. The most common polyvalent cations in neutral or alkaline soils are Ca^{2+} and Mg^{2+}, and Al^{3+} and Fe^{3+} in acidic soils. Trivalent cations will create stronger combination complexes than bivalent ones (von Lützow et al., 2006). According to Puget et al. (1995), microbial polysaccharides are strongly adsorbed to clay surfaces through polyvalent cation bridges.

Hydrophobic interactions create groups of non-polar molecules by excluding them from water molecules. Van der Waals forces are effective at very short distance, and consist of a temporary change of orbital electrons between two nearby atoms. Hydrogen bonds involve an interaction between hydrogen atoms with a partially positive charge and a partially negatively charged O or N atom. These last three binding mechanisms are the weakest of all (Porta et al., 2010a)

2.3.4 Topography

In the landscape, some areas are more likely to have lower amounts of organic matter than others. It is more likely that the highest altitudes have a lower SOM content (Burke et al., 1995; Gregorich et al., 1998; Hook and Burke, 2000; Schimel et al., 1985). This is

a consequence of different processes: Firstly, on the lower altitudes there is usually a higher moisture and nutrient content, which enhances plant production. Due to reduced aeration and higher clay content in the lower parts as compared to the highest locations in the landscape, the decomposition of the SOM is diminished. Due to erosion, the high part of a slope will have SOM losses. In contrast, in valley bottoms, floodplains or lower part of a slope, an influx of SOM from the upper parts will happen (Cheng et al., 1998; Schimel et al., 1985).

In addition, water conditions vary with topography. In the lower parts of the landscape, there is larger soil water content. This leads to a greater plant growth (and consequently bigger OM inputs to the soil). The higher water content in lower positioned soils also causes lower soil aeration. Due to the lack of oxygen, mineralization in flat groundwater fed landscapes will be slower, leading to an accumulation of SOM.

Nevertheless, in some mountain areas peat soils can be found. In Ireland, blanket peats are found in relatively flat areas above 200 m of altitude, with high rainfall and low evaporation (Simo et al., 2014).

The effect of the topography is also evident when comparing slope orientation. In the Northern Hemisphere, slopes facing south have more sun hours. As a result, temperatures and SOM mineralization rates are higher, leading to lower SOM content than in North-facing slopes (Bot and Benites, 2005; Porta et al., 2010a).

2.3.5 Land use and management

Land use change has an effect on the amount of SOC stored in soil, leading to a release or sequestration of C. For example, conversion of cropland to grassland leads to a net increase in total values of SOC due to reductions in disturbance and a higher C input resulting from a longer growing period (Soussana et al., 2004). The conversion of grassland to forest causes a decrease SOC content, as shown by Parfitt et al. (1997), who observed a decrease of SOC after a pine plantation in grassland soils. Cultivation decreases SOC content, as shown by a 50% decrease of SOC after 30 to 50 years of cultivation (Post and Kwon, 2000). Tillage systems have been extensively reported to diminish SOC content (Chrenková et al., 2014; DeGryze et al., 2004; Six et al., 2000a). It decreases levels of SOC through reducing its physical protection (Balesdent et al., 1988; Chung et al., 2008), and increasing its mineralization speed, which impedes soils to reach the maximum C sequestration potential (Chung et al., 2008; Stewart et al., 2007). Once

tillage ceases, some signs of recovery can be noticed after four years, but the physical and chemical characteristics of these soils are still not the same as native forest even after 40 years of no-tillage (Acín-Carrera et al., 2013). Many studies indicate the value of grassland soils as an effective C sink, since the conversion of forest or cropland to grasslands lead to an increase of the SOC pool, as compared to the conversion of grasslands to forest or cropland, or cropland to forest (Parfitt et al., 1997; Ruiz-Navarro et al., 2009; Wei et al., 2012)

2.4 Soil aggregates

2.4.1 Soil aggregate formation

Soil aggregates are groups of soil particles that bind to each other more strongly than to adjacent particles (Martin et al., 1955). More stable aggregates can resist disruptive forces without disintegrating (USDA, 2011).

Two physical units are generally accepted in the model of aggregation: microaggregates (<250 μm), and macroaggregates (>250 μm) (Edwards and Bremner, 1967; Tisdall and Oades, 1982). The first hierarchical model of aggregate formation proposed by Tisdall and Oades (1982) is, with some modifications, still accepted. In this model there are three different binding agents which have an influence on different stages of the hierarchical model of aggregation: temporary, transient and persistent. The persistent pool consists of humified organic matter and polyvalent metal cation complexes. It binds together free primary particles and silt-sized aggregates to form microaggregates. Both the temporary pool (fungal hyphae and roots), and the transient pool (polysaccharides derived from microbes and plants) are associated with macroaggregate formation, and bind together the microaggregates.

While this first model describing aggregate formation proposed by (Tisdall and Oades, 1982) was unidirectional, Oades (1984) made a modification to this model, and firstly explained that microaggregates are actually formed within macroaggregates. In soils, the size of roots and hyphae decrease as they undergo the decomposition process, becoming particulate organic matter (POM). When bacteria or fungi mineralise the POM, they create a sticky mucilage that becomes encrusted with clays. Eventually, POM decreases in size and becomes encapsulated by the mineral fraction, resulting in the formation of microaggregates within macroaggregates (mwM) (Golchin et al., 1994). This POM

located inside the microaggregates is intra-POM. Several microaggregates form macroaggregates when they are bound together by inter-particulate organic matter. Given that this last fraction is more available to microorganisms, it gets decomposed faster. This eventually causes a break-up of the macroaggregates, which releases free microaggregates. These serve in turn as building blocks for the formation of new macroaggregates (Six et al., 2000a). Figure 2. 2 presents a scheme of the soil aggregate structure, with the different aggregate sizes and types of POM.

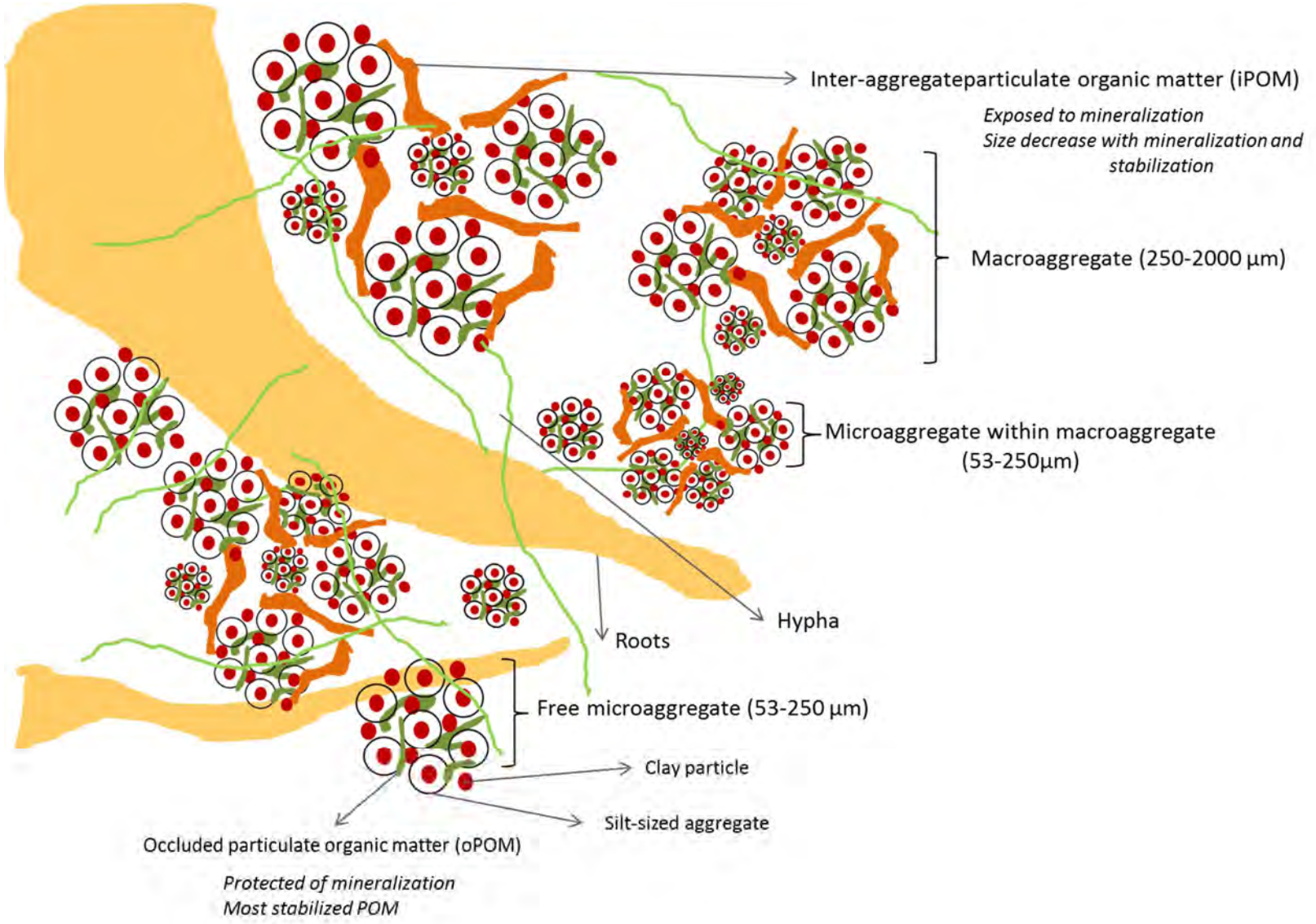


Figure 2. 2 *Soil aggregate structure*

Macroaggregates are formed by microaggregates and POM that acts as an intra-macroaggregate binding agent (Cambardella and Elliott, 1993; Jastrow et al., 1996; Puget et al., 1995). Therefore, macroaggregates contain more SOM than microaggregates, and SOM in macroaggregates is also younger and more labile than SOM associated with microaggregates (Elliott, 1986; Jastrow et al., 1996). This theory has been corroborated (Jastrow and Miller, 1998). For example, Angers et al. (1997) traced C and N in a field experiment using labelled ^{13}C and ^{15}N wheat straw. This allowed them to demonstrate that during initial decomposition, ^{13}C was accumulated in macroaggregates. After a period of time, the ^{13}C content of macroaggregates decreased, and there was a stabilization of ^{13}C in microaggregates. This demonstrates that SOM is firstly occluded within macroaggregates, and with time it relocates into microaggregates.

Based on the above aggregate model dynamics and their interaction with the organic matter, Six et al. (2000a) explained the influence of the disturbance on SOC stabilization rates and on the dynamics of the aggregates. A soil with no disturbance leads to a slower turnover of the macroaggregates, which allows a higher rate of formation of mwM. Conversely, soil disturbance such as tillage or animal pressure can destroy the macroaggregates. This causes an exposure of POM, and a faster decomposition, which increases the turnover time of macroaggregate formation. This increased turnover of macroaggregates impedes the slow mineralization necessary for microaggregate formation within macroaggregates. Therefore, a faster turnover time of macroaggregates decreases the stabilization of C in mwM (Six et al., 2000a). Subsequently, free microaggregates derived from mwM have a lower accumulation of new crop derived C in conventional tillage systems than in no tillage systems (Six et al., 1999). Following the same trend, macroaggregates of soils under conventional tillage are formed by 27% of microaggregates, while in no-tillage soils 47% of the macroaggregate weight consist of microaggregates (Six et al., 2000a).

Hence, the proportion of mwM gives a measure of the turnover of aggregates, and SOC associated with mwM is a good indicative fraction of early changes in SOC concentrations as a consequence of changes in management practices such as tillage (Denef et al., 2004; Six et al., 2000a). Nevertheless, not all soils follow the hierarchy model. Oades and Waters (1991) concluded that this model is only followed in soils where SOM directs the formation of aggregates. There are some soils, like oxisols, where electrostatic interactions between oxides and clay minerals are the dominant binding agents rather than SOM, mainly at the microaggregate level (Muggler et al., 1999), but

also in the formation of macroaggregates (Imhoff et al., 2002). This is corroborated by different studies (Blauwet and Leyssens, 2002; Six et al., 2000b), where Mollisols had an increasing SOC concentration with increasing aggregate size due to the hierarchy model, but this rule was not followed by the Alfisols and the Oxisols. Nevertheless, an increase of SOC in mwM associated with no-tillage soils in comparison with conventional tillage can be observed in soils with different mineralogies, although soils with 1:1 clays and oxides showed a lower effect of SOM decrease on formation and stabilization of mwM (Denef et al., 2004).

2.4.2 Aggregate formation and C sequestration

In terms of C sequestration, POM is important because it acts as nucleation sites for the formation of new macroaggregates and microaggregates, but microaggregate formation is crucial for C sequestration as it protects and stabilises SOC in the long term (Denef et al., 2004). The MRT of SOC located inside microaggregates in topsoils ranges from 10 to more than 100 years as the aggregate size decreases, in comparison with SOC located in macroaggregates, which has a MRT of 1 to 10 years in the topsoil (von Lützow et al., 2006). Therefore, incorporation of SOC in microaggregates is a key factor in C sequestration (Skjemstad et al., 1990) because of its higher stability and slower turnover as compared to macroaggregates (Jastrow et al., 1996). As pointed by Six et al. (2000a), no tillage leads to C sequestration as compared to conventional tillage systems, as a consequence of the slow macroaggregate turnover, which causes a greater accumulation of fine intra-POM held within stable mwM. However, more recent analyses have suggested that no-till only results in negligeable amounts of C sequestration (Corbeels et al., 2016)

Different factors determine the low mineralisation rates of SOM within microaggregates. Firstly, the size of the aggregate determines its level of stability. As demonstrated by Oades and Waters (1991), aggregate sizes smaller than 20 µm are only disrupted by ultrasonic treatment, while aggregates smaller than 2 µm have a greater stability, since they cannot be broken with ultrasounds (Chenu and Plante, 2006). Soil structure determines several soil characteristics that influence microbial activity. Aggregate size fixes the size of the pores, their length and the size of the pore necks, which is the space between the pore and the aggregate (Elliott and Coleman, 1988). Pore space restricts the movement of organisms and water. Elliott and Coleman (1986) defined four pore sizes according to their location in the soil matrix:

i) Macropores created by roots and earthworms (Lee, 1985) or by periods of shrink-swelling in soils, which create cracks in some soil types. These pores are important for the fast movement of water through the profile at field capacity, and are a habitat for microarthropods.

ii) Pores occurring between macroaggregates, which are large enough to fit nematodes, and retain water at field capacity.

iii) Pores between microaggregates. They can contain small nematodes, protozoa and are the main habitat for fungi.

iv) Pores within microaggregates, which are smaller than 1 μm and are inhabited mostly by bacteria (Kilbertus, 1980).

The fact that smaller pore sizes restrict the presence of certain organisms and regulates the amount of water contained inside affects the rate of decomposition of the SOM (Elliott and Coleman, 1988). In addition, oxygen concentration has been reported to decrease at small distances from the surface of the aggregates (Hojberg and Revsbech, 1994; Sexstone et al., 1985; Sierra, 1996), These anaerobic conditions inside macroaggregates and microaggregates party explain the stabilisation of SOC within aggregates, as the decomposition of C and N by aerobic bacteria is limited. Nevertheless, this phenomena increases denitrification rates, thus increasing gaseous N emissions (Hojberg and Revsbech, 1994; Sexstone et al., 1985). In terms of silt and clay sized aggregates, SOC associated with this fraction has been widely linked to the capacity of soils to permanently sequester C (Chantigny et al., 1997; Guggenberger et al., 1999; Puget et al., 1999).

2.5 Factors enhancing soil aggregate formation

Aggregate formation is influenced by 5 principal factors: roots, microorganisms, soil fauna, inorganic binding agents and environmental variables (Greenland, 1965; Harris et al., 1966; Martin et al., 1955).

2.5.1 Soil fauna

Soil is a large reservoir of biodiversity. It contains several communities that influence C cycling and nutrient fluxes (Swift et al., 1979). Soil fauna is divided in mesofauna and macrofauna. Mesofauna ranges from 200 μm to 10 mm and include microarthropods or enchytraeids, which plays a role in C cycling since they feed on organic materials, microflora, microfauna and other invertebrates (FAO, 2008). Soil macrofauna includes

millipedes, centipedes, ants, coleopteran, isopods, spiders, slug, snails, termites, dermaptera, lepidopters, and earthworms (FAO, 2008).

From all soil fauna groups, earthworms is the one that plays the most important role in aggregate formation. In temperate pastures their populations may reach 1000-2000 m^2 (Lee, 1985). They mediate soil aggregates in three ways: (i) burrowing, which creates pressure in the soil; (ii) mucus excretion (Edwards and Bohlen, 1996) where clays get oriented (Jeanson, 1964); and (iii) cast formation, which enhances macroaggregate and microaggregate formation when organic material and mucus gets intimately mixed in their guts (Shipitalo and Protz, 1989, 1988).

2.5.2 Roots

Roots contribute to aggregate formation by different mechanisms. Root penetration locally increases bulk density (Young, 1998), which stimulates the contact between roots and SOM necessary for aggregate formation. Roots also exude polysaccharides, which act as transient binding agents for soil aggregates (Yousefi et al., 2008). Similarly, dead root decomposition also enhances aggregate formation by stimulating the formation of microbial-derived binding agents (Six et al., 2004). Roots dry the adjacent soil, which stimulates the binding of root exudates to clay particles, and therefore enhances aggregation (Reid and Goss, 1982). Entanglement of roots also enhances aggregate formation as it physically holds together SOM and the mineral fraction (Jastrow and Miller, 1998; Miller and Jastrow, 1990; Tisdall and Oades, 1982).

Through root exudation and by drying the soil, roots stabilize surrounding aggregates (Six et al., 2004). While roots are temporary binding agents, plant-derived polysaccharides act as transient binding agents, mainly at a scale of <50 μm within macroaggregates (Six et al., 2004).

2.5.3 Microorganisms

Microbial biomass and water-extractable carbohydrates, which are derived from a mixture of plant and microbial polysaccharides (Cheshire and Hayes, 1990), have been positively correlated to aggregation (Degens, 1997). The effect of microorganisms in soil aggregation is caused by polysaccharides from plant and microbial sources, which play a key role in stabilization of soil microaggregates (Cheshire, 1979; Martin, 1971). Polysaccharides are transitory binding agents (Tisdall and Oades, 1982), as they can be

easily metabolized by microorganisms unless they become physically or chemically protected from microorganisms and enzymes (Burns and Davies, 1986). When microorganisms decompose polysaccharides, a positive feedback occurs, as these produce extracellular polysaccharides themselves. These new polysaccharides will be degraded rapidly by other microorganisms, leading to an increase in the microbial biomass (Haynes and Beare, 1996). This labile fraction acts as a binding agent between SOM and the mineral fraction, thus enhancing soil aggregation, and increasing aggregate sizes (Yousefi et al., 2008).

As well as promoting aggregate formation, microorganisms increase aggregate stability through increasing their water repellence, and thus reducing their wetting rate (Arcenegui et al., 2008; Chrenková et al., 2014; Mataix-Solera and Doerr, 2004). While microaggregate formation is mainly related to the production of mucilage by bacteria (Chenu, 1989; Oades, 1993), macroaggregate formation is more related to fungi (Bossuyt et al., 2001; Tisdall et al., 1997).

Fungal hyphae participate in aggregate formation by bringing together soil particles with organic material and binding agents. They also produce extracellular polysaccharides that participate in aggregate binding (Oades and Waters, 1991). As their specific surface area is about 100 times the specific surface of a root (Harley, 1989), with a small amount of material they develop a large surface where soil particles may become attached. Vesicular-arbuscular mycorrhizae extend 10-30 mm from the root surface (Tisdall and Oades, 1979), and their length has been found to be related to aggregate stability (Tisdall and Oades, 1980). Fungal mycelium has been found to slow down the macroaggregate turnover, because the mycelium that brings together soil particles counteracts disruptive forces that could break up macroaggregates (Six et al., 2004).

2.5.4 Environmental variables

Humidity affects aggregate formation: drying and wetting have been related to the formation of soil aggregates. According to Dorioz et al. (1993) non-aggregated soils, like compacted arable soils, will respond to dry-wetting cycles by a break up of soil clods and aggregation of smaller particles. However, in aggregated soils, drying and wetting decreases the proportion of aggregates (Denef et al., 2001a).This is due to the fact that hydration of an aggregated soil is not uniform, and when water enters rapidly in the pores, a compression of the air inside occurs. As a results there is a breakdown of the aggregates (Grant and Dexter, 1990). However, if the water enters the soil through capillary wetting,

the air in the pores is allowed to escape, and the disruption is minimal (Kemper and Rosenau, 1986).

2.5.5 Inorganic binding agents

Inorganic binding agents contribute to bind together primary particles to form aggregates (Whalen and Sampedro, 2010). Inorganic binding agents include aluminosilicates, iron and aluminium oxides and polyvalent cations such as calcium and magnesium. Aluminosilicates enhance aggregation increasing the surface reactivity of layered silicate clays. This happens when a metallic ion such as Al, Fe or Ca is adsorbed in the surface of an amorphous silicate, thus increasing the reactivity of its surface (Whalen and Sampedro, 2010). Oxides of iron and aluminium contribute to microaggregation, but also in macroaggregation in some oxide rich soils (Denef et al., 2004). There are three mechanisms by which aggregate formation is enhanced as a consequence of iron and aluminium oxides: (i) electrostatic attraction occurring between negatively charged clay surfaces and positively charged oxides, (ii) formation of organo-mineral complexes by adsorbing organic materials, (iii) promoting interactions between primary and secondary particles by oxide coating on mineral surfaces (Huang et al., 2011; Whalen and Sampedro, 2010). Finally, calcium and magnesium enhance microaggregate formation by acting as the bridge between negatively charged clay surfaces and negatively charged SOM, which forms clay-polyvalent cation-SOM complexes (Huang et al., 2011; Porta et al., 2010a)

2.6 Soil Carbon equilibrium

Several long-term experiments show increasing SOC concentrations with increasing C inputs under different treatments (Gerzabek et al., 1997; Huggins et al., 1998; Kong et al., 2005; Larson et al., 1972; Paustian et al., 1997). Nevertheless, in some field experiments with higher initial SOC stocks, there is little or no increase of SOC concentrations with inputs of C (Campbell et al., 1991; Gulde et al., 2008; Paustian et al., 1997; Reicosky et al., 2002; Solberg et al., 1997). The concept of SOC saturation assumes that there is an upper limit to the capacity of soils to store SOC, and under equilibrium conditions, there is an asymptotic response on SOC to C inputs (Carrington et al., 2012; Chung et al., 2008) (Figure 2. 3a).

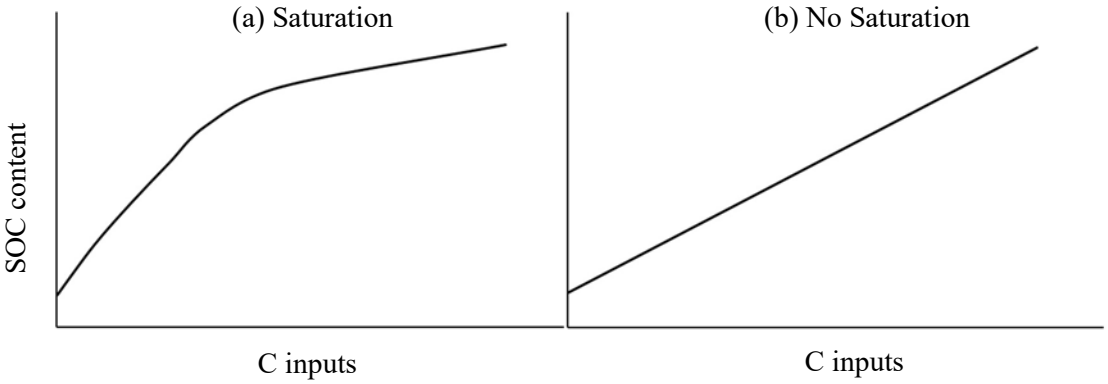


Figure 2. 3 *SOC content variations with C inputs in soils, according to the saturation model (a) and the no saturation model (b)*

Most of the biogeochemical models, such as DayCENT, DNDC or EPIC assume a direct linear effect between SOC inputs and SOC concentration (Figure 2. 3b) (Carrington et al., 2012; McGill, 1996), which means that the storage efficiency is constant, and SOC levels can increase without limit (Stewart et al., 2007). While these models are useful in predicting SOC dynamics in some soils (Parton et al., 1987; Rasmussen and Parton, 1994), they don't work for all systems. Models that take into account C saturation fit better with soils with high SOC stocks or high input levels (Stewart et al., 2007; West and Six, 2007). Stewart et al. (2007) determined, after analysing three SOC saturation models in data obtained from 11 different sites, that the SOC saturation model proposed by Six et al. (2002) was the best in predicting SOC dynamics. This model suggests that the total SOC saturation is determined by the saturation of four measurable pools that each have their own dynamics and stabilising mechanisms. These pools are: i) biochemically protected SOC pool (or non-hydrolysable), ii) silt and clay protected SOC pool, iii) microaggregate protected pool, and iv) the unprotected pool, which consists of POM (labile pool). This model implies that, since each soil has an individual upper SOC concentration limit, this maximum SOC stabilisation potential limits the effectiveness of SOC storage (Six et al., 2002; West and Six, 2007). The further we are from the saturation point, the greater the capacity for C sequestration (Stewart et al., 2007). This is in concordance with Hassink and Whitmore (1997), who determined that SOC accumulation depended both on the protective capacity of the soil, and the degree to which this protective capacity was already occupied by SOC. Hassink et al. (1997) concluded that soils can reach a maximum SOC associated with the silt and clay fraction after observing

the same amounts of SOC in this fraction in grasslands and arable analog soils. They also observed further accumulations of SOC in the POM fraction, which is in concordance with the model proposed by Six et al. (2002).

The mechanisms that lead to SOC saturation are still not clear. While some studies indicate that an important factor affecting SOC accumulation is the quality of the residue added to the soil as a result of its biochemical composition (Melillo et al., 1982; Trinsoutrot et al., 2000; Vanlauwe et al., 1996), this theory is mostly rejected now, and other studies suggest, after showing that inherent lignin recalcitrance is not affecting SOC accumulation, that it is the quantity and not the biochemical characteristics the main factor that controls SOC saturation (Carrington et al., 2012).

2.7 The impact of depth on Soil Organic Carbon distribution

The vast majority of SOC research and national SOC inventories focuses on the A horizon or the top 20 cm of the soil as a result of the difficulties of sampling and analysing deeper profiles (Harrison et al., 2011; James et al., 2014). Nevertheless, research analysing the SOC content in subsoil indicates that a substantial proportion of SOC is found at depth (Diochon and Kellman, 2009; Harrison et al., 2011; Kaiser, 2002). Jobbágy and Jackson (2000) concluded after analysing three global databases that 56% of SOC can be found below 1 m depth, while Batjes et al. (1996) described that between 46 and 63% of SOC is located from 30 cm to 1 m of global soils, with the exception of Podzoluvisols, which contained 30% of SOC below 30 cm.

Using a sampling depth of 20 cm causes an underestimation of the SOC stocks (James et al., 2014). Several studies indicate the need for sampling at deeper horizons. For example, Hamburg (2000) suggests a sampling depth of 1 m or the top of the C horizon. A sampling depth of at least 1 m has also been suggested by James et al. (2014), who concluded that this would increase the accuracy of SOC content estimates.

Subsoil SOC comes mainly from plant roots, root exudates, dissolved organic matter (DOM), and bioturbation. The contribution of each of these sources depends on climate, land use and inherent soil processes (Rumpel and Kögel-Knabner, 2011). There is increasing evidence that the subsoil has the capacity to sequester large amounts of SOC (Jobbágy and Jackson, 2000; Liski and Westman, 1995; Lorenz and Lal, 2005; Richter and Markewitz, 1995), since SOC located in deeper horizons has a longer MRT than the

topsoil, as seen by the increased presence of heavier C and N stable isotopes (Eusterhues et al., 2003; Krull et al., 2003). Subsoil C:N ratio is generally lower when compared with the topsoil, which indicates that it is highly processed (Rumpel and Kögel-Knabner, 2011). Indeed, Agnelli et al. (2004) showed that there is a higher proportion of microbial C at depth, while topsoil has more plant derived SOC.

The factors that control SOC stability at depth are still poorly understood, because most research focuses on the topsoil (Rumpel and Kögel-Knabner, 2011). Some of the mechanisms that have been found to have a large influence on SOC stability at depth are the sorption of SOC to the mineral phase (Eusterhues et al., 2005, 2003; Schöning and Kögel-Knabner, 2006), and the occlusion in silt sized aggregates (Moni et al., 2010).

Research has shown that subsoil SOC should not be considered an unchangeable pool, since changes in topsoil cause changes in subsoil SOC. For example, fresh SOC inputs could stimulate the decomposition of old SOC (Fontaine et al., 2007). Similarly, some land use changes have an effect on SOC stocks to below 100 cm depth (Guo and Gifford, 2002). Veenstra and Lee Burras (2015) demonstrated that there was translocation of SOC from topsoil to deeper horizons after 50 years of cultivation. Global warming could also have an effect on subsoil SOC, since subsoil SOC seems to be more sensitive to changes of temperature than SOC located in the topsoil (Fierer et al., 2003).

2.8 Soil Organic Carbon evaluation methods

Although SOM is a continuum of components without defined boundaries (Bot and Benites, 2005), several methodologies have been applied in order to try to classify it into components with distinct characteristics (Davidson et al., 1987; DuBois et al., 1956; González-Prieto and Carballas, 1988; Six et al., 2000a; Trumbore and Zheng, 1996). In the following sections some of these methods are described.

2.8.1 Physical fractionation

2.8.1.1 Aggregate sizes fractionation

The basis of applying physical fractionation methods remains on the description of the hierarchical model of aggregation proposed by (Tisdall and Oades, 1982), previously explained in section 2.4.1 of this chapter. To evaluate the presence of large macroaggregates, small macroaggregates, microaggregates and silt and clay, Six et al.

(1998) adapted a method from Cambardella and Elliott (1993) (Figure 2. 4). Moist soil is sieved at 8.0 mm and air dried. A 100 g subsample is submerged in deionized water on the top of a 2.0 mm sieve for five minutes. The water disrupts the aggregates because its rapid entrance into the aggregate pore creates internal air pressure. After five minutes, the sieve is moved up & down over a height of three cm with 50 repetitions during two minutes. The floating organic material (>2000 μm) is discarded since is not considered SOM. The material remaining on the top of the 2.0 mm sieve is dried at 50 °C, weighed and stored. This material consists of stones and large macroaggregates (2-8 mm).

Water plus soil that goes through the 2.0 mm sieve is poured of into a 250 μm sieve, and the same procedure is repeated. The material remaining on the 250 μm sieve are the small macroaggregates and the stones of the same size fraction (250-2000 μm). Finally, the same procedure is repeated again with the material that previously passed through the 250 μm sieve, this time with a 53 μm sieve, going through the same procedure again. With this last step, microaggregates stay on the top of the 53 μm sieve together with the same sized stones (53-250 μm), and the silt and clay fraction pass through the sieve into the final container. All the fractions are oven-dried at 50 °C, weighed and stored at room temperature. After removal of the carbonates where present, SOC is analysed for each one of the aggregate fractions with a C analyser such as LECO truspec C:N analyser.

At the end of the procedure, the following fractions are obtained: large macroaggregates (2-8 mm), small macroaggregates (250-2000 μm), free microaggregates (53-250 μm) and silt and clay (<53 μm). For the three bigger aggregate fractions, SOC concentration needs to be normalised to a sand-free basis following the Equation 2. 1 equation proposed by Six et al. (1998):

Equation 2. 1*. Calculation of sand-free C or N*

$$sandfree\ (C\ or\ N)_{\text{fraction}} = \frac{(C\ or\ N)_{\text{fraction}}}{1-(sand\ proportion)}$$

This method gives an understanding of the spatial accessibility of SOC, and therefore its potential degradability by bacteria. Different aggregate fractions are associated to SOC pools with different MRT in soils (von Lützow et al., 2007, 2006).

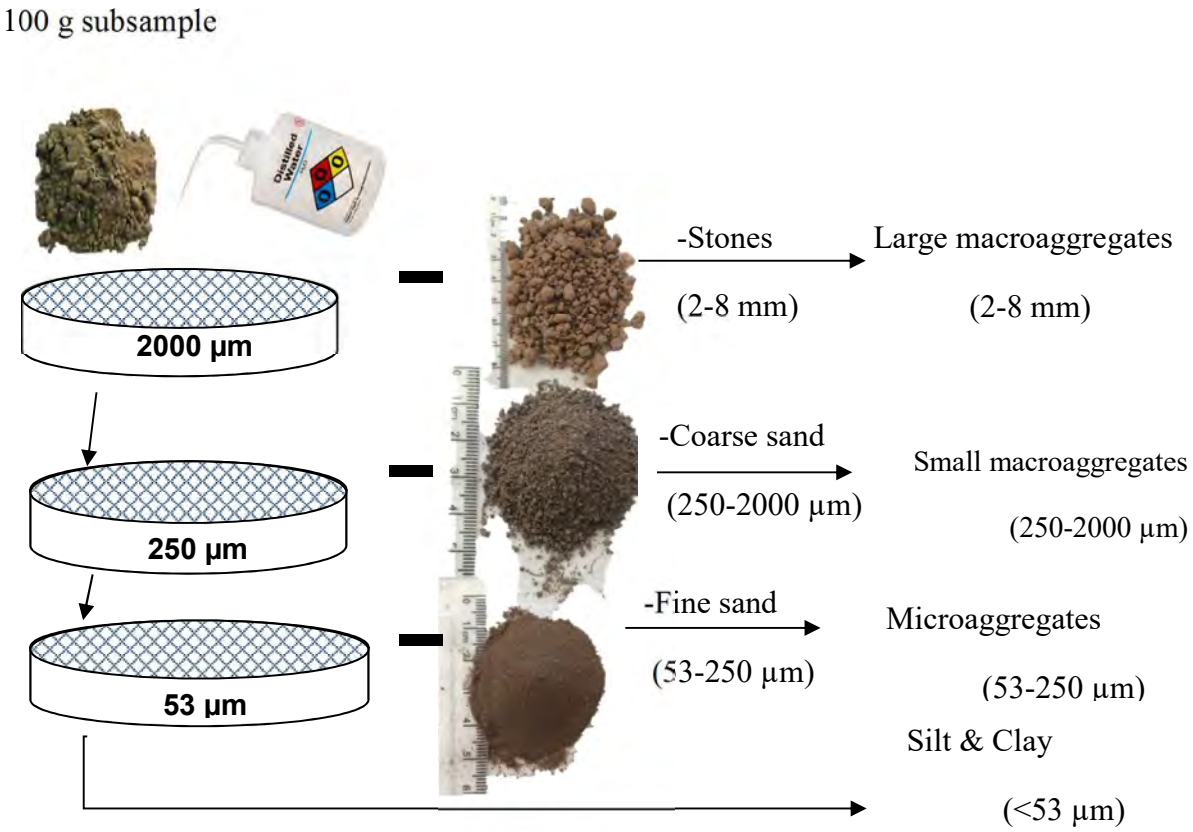


Figure 2. 4 *Aggregate fractionation method (Cambardella and Elliott, 1993)*

2.8.1.2 Microaggregates within Macroaggregates

This method separates the POM, microaggregates and the silt and clay fraction contained inside macroaggregates, based on the theory described by Six et al. (2000a), and explained in section 2.4.1 of this chapter. This method is applied to the macroaggregate fraction isolated within the method described above (section 2.8.1.1). Macroaggregates (>250 µm) are physically separated into its different components (Figure 2. 2) by placing them on the top of a 250 µm sieve located in a rotatory shaker and disrupting them by shaking with 50 glass beads of 4.0 mm (diameter). A continuous water flow washes the microaggregates and silt and clay fraction into a 53 µm sieve. This flow ensures that microaggregates are not mechanically disrupted by the glass beads. To ensure that only water stable microaggregates are isolated, the 53 µm sieve is wet sieved at a constant rate and time. At the end of the process, the material remaining on the 250 µm sieve (coarse POM + stones), the material on the top of the 53 µm sieve (mwM) and the silt and clay sized aggregates are dried at 50 °C, weighted and analysed for SOC. Aggregate weight needs to be corrected for the same-sized sand with the following

Equation 2. 2 *Calculation of percentage of mwM*

$$\frac{\text{microaggregate weight} - \text{weight of 53 to 250µm sized sand}}{\text{macroaggregate weight} - \text{weight of 250 to 2000 µm sized sand}}$$

2.8.2 Chemical fractionation

Based on the premise that SOC can be stabilised by chemical recalcitrance, physical protection or interaction with soil minerals (Helfrich et al., 2007; von Lützow et al., 2007), chemical characterisation of SOC is used to isolate SOC fractions with different degradability rates**.**

2.8.2.1 Hot water extractable carbon

Hot water extractable carbon (HWEC) is frequently used to measure the potentially bioavailable SOM in the short term (i.e. the most readily decomposable SOC pool) (Chodak et al., 2003; Davidson et al., 1987; von Lützow et al., 2007). It consists of an heterogeneous pool that contains carbohydrates, N-containing compounds such as amino-N and amides, and many of the constituents in the soil solution or weakly adsorbed to mineral surfaces of humic macromolecules (Haynes, 2005). Compared to cold water extracts, HWEC is greater in soils since extraction with water at temperatures higher than 70 °C breaks vegetative cells of microorganisms (Landgraf et al., 2006). Therefore, HWEC contains both microbial and non-microbial compounds (Haynes and Francis, 1993; Leavitt et al., 1996; Paul et al., 2000).

There are different methodologies based on the same premise, with different exposure times, temperatures and extraction steps (Chodak et al., 2003; Dodla et al., 2012; Ghani et al., 2003; Landgraf et al., 2006; Rovira and Vallejo, 2007). For this thesis, the method proposed by Rovira and Vallejo (2007) was followed: 1 g of sieved sample was boiled at 105 ºC for an hour, centrifuged and filtered through Whatman 2 filter paper. Extracts were analysed using a Shimadzu TOC analyser.

2.8.2.2 Non-hydrolysable SOC

Hydrolysis isolates the most stable SOC by removing potentially biodegradable compounds such as proteins, nucleic acids, polysaccharides, carboxyl C (Helfrich et al., 2007), leaving intact more recalcitrant compounds such as alkyl C and aryl C, highly aromatic compounds and with longer aliphatic chains, such as lignin, suberin, cutine, waxes and fatty acids (Goering and Van Soest, 1970; Leavitt et al., 1996; Martel and Paul,

1974; Minderman, 1968; Scharpenseel and Schiffmann, 1977; Schnitzer, 1978; Torn et al., 1997).

The non-hydrolysable pool is the SOC pool with the lowest young SOC content (Pelz et al., 2005), although it contains some young SOC (Balesdent, 1996). The MRT of this pool has been described to be 1500 years older than the hydrolysable fraction in surface, and older in subsurface soils (Paul et al., 1997). Although some papers determine that the non-hydrolysable fraction gives a meaningful estimate of the passive pool (Leavitt et al., 1996b; Paul et al., 1997), this pool should not be considered unalterable, since it has shown to change after changes in soil management (Paul et al., 2006).

The methodology to assess the non-hydrolysable fraction varies across papers. Different acids and exposure times are used for the hydrolysis. While some studies use H2SO4 (Oades et al., 1970; Rovira and Vallejo, 2002), other studies use HCl (Dodla et al., 2012; Rovira et al., 2008; Sollins et al., 1999). From these last studies, the most common methodology on the past years was to hydrolyse the sample by boiling it with 6M HCl during long periods of time (Leavitt et al., 1996; Martel and Paul, 1974). Nevertheless, some research shows that during hydrolysis of carbohydrates, new compounds can be formed through the Maillard reaction (Maillard, 1912) and precipitate, thus overestimating the non-hydrolysable fraction (Greenfield et al., 2013). Since hydrolysis times longer than 2 hours do not increase SOC release (Silveira et al., 2008), the methodology chosen for this thesis is to treat the samples with 1M HCl during 3h at 105 ^{o}C (González-Prieto and Carballas, 1988; Rovira et al., 2008; Yonebayashi and Hattori, 1980).

Chapter III

Carbon distribution in aggregates of topsoil and subsoil of temperate grassland systems

Note: This chapter is accepted in the Journal Nature Scientific Reports.

3.1 Abstract

Soil organic carbon (SOC) sinks play a key role in the global carbon (C) cycle. Most current assessments of SOC stocks and the guidelines given by Intergovernmental Panel on Climate Change (IPCC) focus on the top 30 cm of soil. Our research shows that, when considering only total quantities, most of the SOC stocks can indeed be found in this top layer, irrespective of soil type. However, not all forms of SOC are equally valuable as long-term, stable stores of carbon: the majority of SOC is available for mineralisation and can potentially be re-emitted to the atmosphere. SOC associated with small soil aggregates and the clay fraction is more stable and therefore represents a long-term carbon store. Our research shows that most of this stable carbon is located at depths below 30 cm, specifically in soils that are subject to clay illuviation. This has implications for land management and land-use decisions in temperate grassland regions, particularly in terms of trade-offs between primary productivity and carbon emissions following artificial drainage of clay-illuviated soils. Therefore, climate smart land management needs to consider the balance between SOC stabilisation in topsoils for productivity versus sequestration in subsoils for climate mitigation.

3.2 Introduction

The Intergovernmental Panel on Climate Change (IPCC) provides good practice guidance on the methodology to account for the impact of land use management and land use change on organic carbon stocks in soils (IPCC, 2003). Generally for Tier 1 (default) or Tier 2 (national-specific) approaches, a stock change is calculated by assessing the reference soil organic carbon (SOC) level down to 30 cm and measuring again after a period of at least 3 to 5 years (IPCC, 2003). However, this approach neither accounts for different turnover rates of different fractions of SOC, nor recognises that substantial pools of recalcitrant SOC exist below 30 cm, which may be affected by land management. For example, artificial drainage of intensive grassland systems is a common practice in many temperate countries to increase the grazing capacity of soils and extend the grazing season. The application of artificial drainage systems has been found to significantly affect the SOC recalcitrance in soils (Meersmans et al., 2011; Ruark et al., 2009). However, very few studies exist in grassland systems where empirical data is available for SOC fractions below 30 cm (Don et al., 2007; Garcia-Pausas et al., 2008).

Sophisticated biogeochemical cycling models are commonly applied to extrapolate SOC pools and recalcitrance to estimate potential source/sink reservoirs at lower depths. These include; the RothC carbon model (Jenkinson and Rayner, 1977), CENTURY model (Parton, 1996), and the DeNitrificationDeComposition (DNDC) model (Li, 2008), which all divide SOC inputs into easily decomposable and recalcitrant pools, which are subsequently input into labile, recalcitrant and inert/passive SOC pools following mineralisation. These models include the simulation of physical protection via the clay fraction, which diverts SOC flows to the humic fraction rather than CO_2 mineralisation (Falloon et al., 2006). However, these pools are usually defined kinetically and without empirical data regarding the mean residence times associated with the different chemical and physical properties (Kleber, 2010).

There have been attempts to directly relate these modelled pools to the different aggregate fractions (Del Galdo et al., 2003; Paustian et al., 1992; Zimmermann et al., 2007). Aggregation occurs when particulate organic matter (POM) starts to be decomposed. Bacteria mineralize POM, generating a mucilage that serves as an adhesive between the mineral particles of the soil and the POM (Tisdall and Oades, 1982). Through the encrustation of POM with mineral particles, SOC becomes enclosed into large

macroaggregates (2-8 mm) and small macroaggregates (250-2000 μm). As POM undergoes further decomposition, it decreases in size and gets encapsulated into microaggregates within macroaggregates (mwM) (Six et al., 2000a). This structure may weaken and break, creating free microaggregates (53-250 μm). Soil organic carbon also binds chemically to clay minerals, the smallest particles in soils (<20 μm), which due to their large surface area, have the capacity to adsorb high amounts of SOC, effectively protecting it against microbial decomposition through different binding mechanisms (Helfrich et al., 2007; Kahle et al., 2004; Mikutta et al., 2007).

The fractionation process assumes that SOC associated with microaggregates is older and more stable than SOC contained in macroaggregates, with a much slower turnover time (Angers and Giroux, 1996; Besnard et al., 1996; Elliott, 1986; Jastrow, 1996; Puget et al., 2000; Six et al., 1998; von Lützow et al., 2008, 2006). The turnover time of SOC contained in macroaggregates is reported as 1 to 10 years, while SOC associated with microaggregates is considered stable up to 100 years in soil. Silt and clay associated SOC (<53 μm) is stable in excess of 100 years (von Lützow et al., 2006). Therefore, encapsulation of SOC within microaggregates or association of SOC with silt and clay particles is considered long-term C storage (Del Galdo et al., 2003; Johan Six et al., 2002; Skjemstad et al., 1990).

Our research aimed to elucidate the patterns of SOC fractionation in typical temperate grasslands in Ireland, and particularly the following two key research questions: 1) Does depth matter in determining the long-term recalcitrance of SOC in soils? 2) How does this vary across soil types, specifically between freely draining soils and those subject to clay illuviation over depth?

3.3 Materials and Methods

3.3.1 Soil selection and sampling

Thirty-one grassland sites were sampled, representing six different soil types. These include six Humic Brown Earths (HBE), six Stagnic Brown Earths (SBE), seven Typical Brown Earths (TBE), six Typical Surface-water Gleys (TSWG), three Stagnic Luvisols (SLu) and three Typical Luvisols (TLu). These soil types represent a range of SOC and drainage characteristics typical of grassland soils occurring in Ireland.

At each location, a profile pit was dug to a depth of 1 m where possible. All horizons were described according to the FAO field handbook (FAO, 2006) and classified by means of the Irish Soil Information System (Simo et al., 2014). A 1 kg sample was taken from the centre of each horizon and immediately stored at 4 ºC, until use. Following the coning and quartering technique (Massey et al., 2014), a 300 g subsample was sieved at 8 mm and dried at 40 ºC for 7 days. Samples for soil bulk density measurement were taken in triplicate using (5 x 5 cm) cores from each horizon.

3.3.2 Aggregate fractionation

An adaptation of the wet sieving method (Denef et al., 2001a) was followed to separate each sample into four aggregate sizes: large macroaggregates, small macroaggregates, microaggregates and silt and clay (Figure 2.4). An 80 g subsample was placed on the top of a 2 mm sieve and submerged in distilled water for five minutes. This causes a slaking of the aggregates as the water enters into the pores, increasing the pressure and breaking the less stable aggregates. Subsequently, the sieve was manually moved up and down for a three minute period at a rate of 33 movements per minute, in order to make it a constant movement. Large macroaggregates and stones were retained on the 2 mm sieve. The material that passed through the sieve was further separated at 250 µm and 50 µm using a modification of the Eijkelkamp wet sieving apparatus (Figure 3. 1).

Figure 3. 1 *Original Eijkelkamp wet sieving apparatus (left) (source: Eijkelkamp online catalog) and adapted version (right), which allows to process a larger amount of sample.*

The modified wet sieving apparatus was designed to fit four sieves of 10 mm diameter, to facilitate an overall sample size of 80-100 g of <2 mm sieved soil. Soil was placed on

the top of the 250 μm sieves and moved up and down in water for a period of three minutes. The suspension collected through the sieve was then added to a 53 μm sieve and the process repeated. All material remaining on each sieve was washed into plastic containers, dried at 50 ºC, weighed and ball-milled.

For each sample, the proportion of stones (>2 mm), coarse sand (250-2000 μm) and fine sand (53-250 μm) was analysed with a modification of the ISO 11277: 1998 for particle size analysis. The percentage of each aggregate size was calculated by subtracting same-sized sand content from the total fraction weight of each fraction.

3.3.3 Soil organic carbon analysis

For each aggregate fraction, carbonates were removed (Harris et al., 2001). SOC of each fraction was analysed with a LECO Truspec CN analyser according to the ISO 10694:1195, and expressed on a sand-free basis (Six et al., 1998). To calculate the bulk soil SOC proportions throughout the profile, total SOC content of each horizon was multiplied by the bulk density of that horizon, thus obtaining g SOC cm^{-3}. Since the data was collected on a per horizon basis, the weighted average of the SOC content of the different horizons was used to calculate the proportion of SOC in the first 30 cm.

3.3.4 Statistical analysis

Analysis was performed at horizon level for each soil profile described. The physical fractionation process resulted in four relative aggregate size proportions for each horizon and four associated SOC contents. The relative distribution of SOC associated with each fraction was calculated by multiplying the proportion of each of the fractions by its SOC content, and dividing the result by the total amount of SOC in that sample. The “m” value was calculated in order to synthesise the data as follows: we summarised the distribution of SOC across the aggregate sizes by applying a normal distribution to each sample against the log-transformed aggregate size, and deriving the m-statistic, which is the mean of the distribution, and thus the natural logarithm of the aggregate size that cumulatively contains 50% of the SOC in the sample, starting from the silt and clay fraction and progressively including the SOC located in the bigger size fractions (Figure 3.2). When m is smaller than 5.5 more than 50% of the SOC at that depth is located in the silt and clay and microaggregate fractions, given that 5.5 is the natural logarithm of the upper boundary of microaggregate size class (250 μm). In the example presented in Figure 3.2, the decrease of m in deeper horizons indicates that most of the SOC is associated with

smaller aggregate size classes in deeper soil layers. It can then be observed that, in the case of Typical Surface-water Gley, m is decreasing markedly at depth, in contrast with Humic Brown Earth. Hence, m is a good tool to distinguish between soil types.

Stepwise backwards regression was performed to assess the dependence of m on depth, soil type and interaction between them.

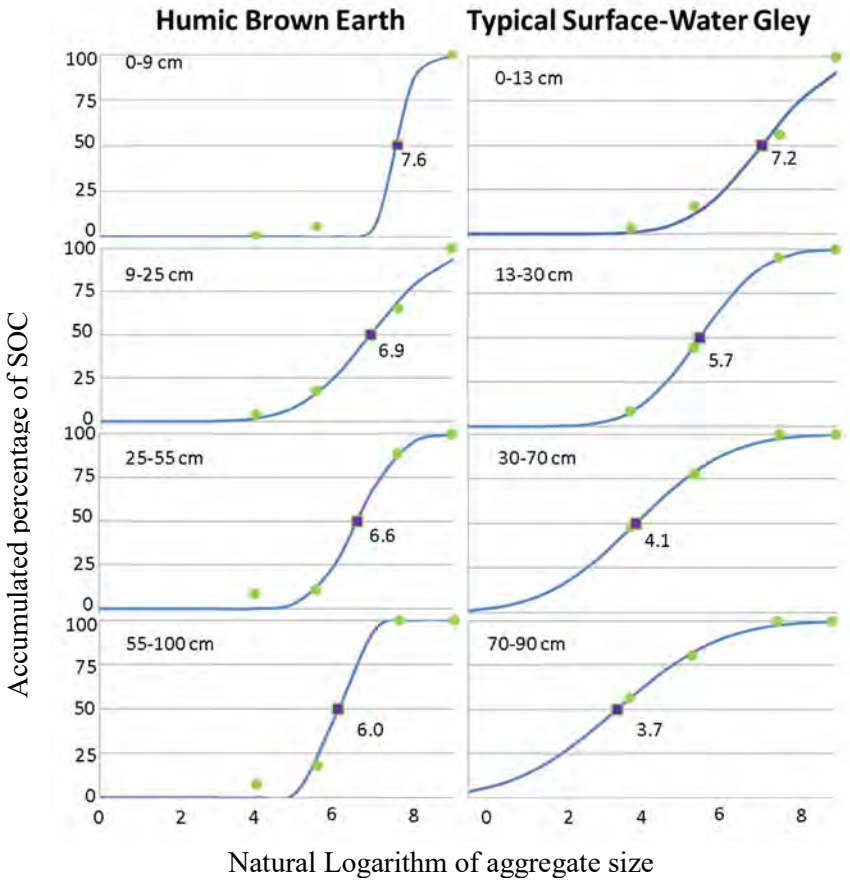


Figure 3. 2 *Example of m value for two soils at depth. The square (m) is the point of the fitted curve where 50% of the SOC is reached. Green dots represent the actual observations. The blue line is the fitted curve*

3.4 Results

3.4.1 SOC distribution within the soil profile

In this thesis, topsoil is defined as the horizons in which the mid-point is smaller than 30 cm, and subsoil for deeper horizons. This limit was decided based on the standard limit settled by the IPCC recommendations for SOC stock assessments.

The distribution of SOC across the four different aggregate size classes in all six soil types (Figure 3. 3) reveals three patterns:

1. The gross amount of SOC declines with depth in all soils;
2. The proportion of SOC associated with large and small macroaggregates declines with depth in all soils;
3. A larger proportion of the SOC is associated with microaggregates and silt and clay fractions in soils affected by clay illuviation (namely: Typical Luvisols (TLu), Stagnic Luvisols (SLu) and Typical Surface-water Gleys (TSWG)) than in Brown Earths (Humic Brown Earths (HBE), Typical Brown Earths (TBE) and Stagnic Brown Earths (SBE)).

As a result, most (68.9% ± 11.5) of the SOC was found within the top 30 cm of the soil profiles, and a significant proportion (84% ± 9.5) of this topsoil SOC was located within large and small macroaggregates, as indicated by the predominance of the 'large bubbles' in quadrant A.

Very little (16 ± 9.5%) of the SOC in the top 30 cm was associated with the microaggregates and silt plus clay, as indicated by the 'empty' quadrant B. In contrast, SOC associated with these smaller fractions equates to 45 ± 17.7% of the whole profile (down to 1 m depth) SOC stock.

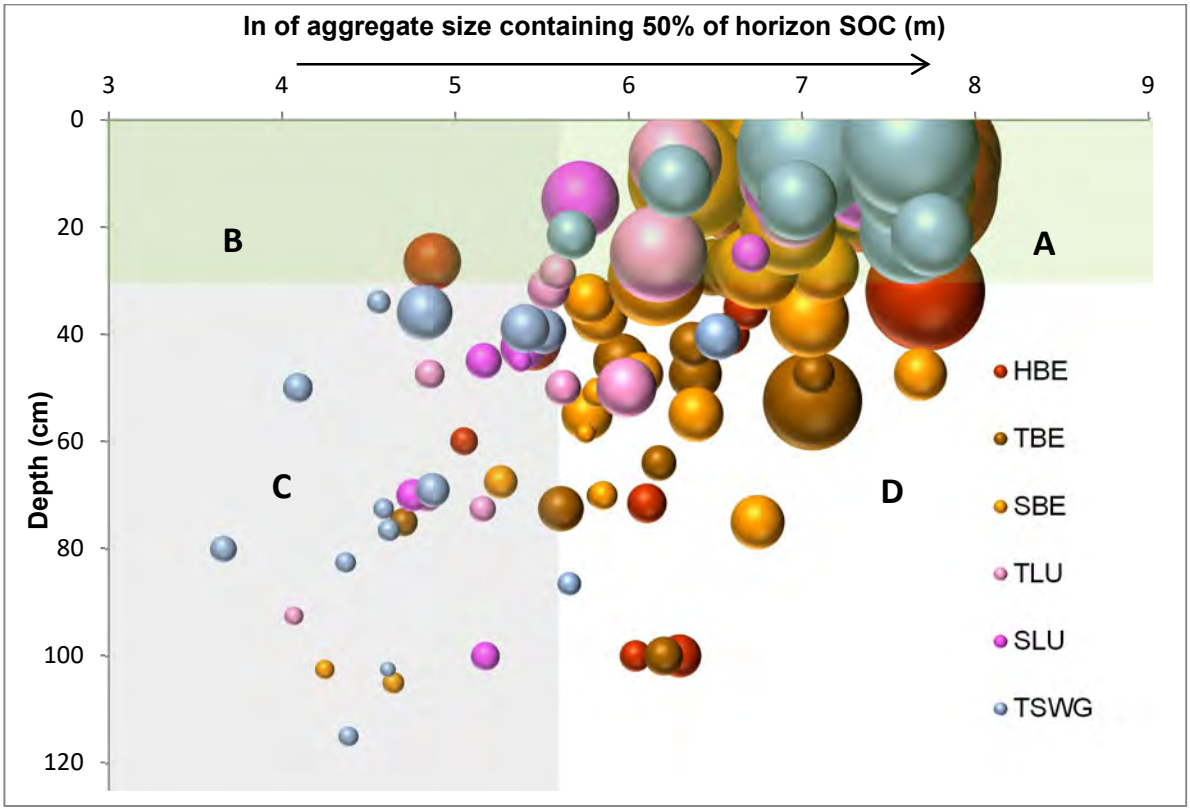


Figure 3. 3 *Relative distribution of SOC within aggregates by depth. The X-axis indicates m (natural logarithm of the aggregate sizes where 50% of the accumulated C is located, from smaller to larger sizes – indicating lability as C associated with smaller aggregates is more recalcitrant). Y-axis is the soil depth (cm). The green background shading indicates the top 30 cm of all soil profiles. Grey background shows the SOC located in silt and clay and microaggregate fractions. Bubble size represents total SOC of the bulk sample at that depth. Colours indicate individual soil subgroups: HBE = Humic Brown Earth; TBC = Typical Brown Earth; SBE = Stagnic Brown Earth; TLu = Typical Luvisol; SLu = Stagnic Luvisol; TSWG = Typical Surface-water Gley.*

3.4.2 Differences in SOC distribution between soil types

Below 30 cm, differences between soil types emerge: quadrant C shows that in the subsoils affected by clay illuviation (namely: TLu, SLu and TSWG), more than 50% of the SOC is associated with microaggregates and silt and clay fractions. At the same time, quadrant D shows that this is not the case for the Brown Earths (HBE, TBE, SBE); in the Brown Earths, the proportion of SOC in microaggregates and silt and clay fractions increases with depth but the majority of SOC remains associated with large and small macroaggregates, even at depth.

The differences in SOC distribution between Brown Earths (TBE, SBE, HBE) (Cambisols, WRB, 2006) and soils subject to clay illuviation (TSWG, SLu, TLu) (Stagnosols and Luvisols, WRB, 2006) are significantly different, but within these groups, differences between individual soil subgroups were not significantly different (Table 3.1)

Table 3. 1 *Final regression coefficients from the backward regression of m as a function of depth, soil type and their interaction. Coefficients in blank cells were not significantly different from 0 ($p<0.05$) and thus progressively removed from the model. The main effect of depth was significant for all soil types.*

Explanatory variables	Soil type used as the baseline in the backward regression																	
	TSWG			SLu			TLu			SBE			TBE			HBE		
Intercept	6.83			6.83			7.00			7.51			7.27			7.27		
Depth	-0.0244			-0.0244			-0.0271			-0.0246			-0.0195			-0.0195		
	main effect		Xdepth	main effect		xdepth	main effect		xdepth	main effect		xdepth	main effect		xdepth	main effect		xdepth
TSWG										-0.652				-0.012			-0.012	
SLu										-0.608			-0.586			-0.586		
TLu										-0.728				-0.016			-0.016	
SBE	0.600			0.600			0.543											
TBE	0.676			0.676			0.608											
HBE	0.742			0.742					0.0156									

3.5 Discussion

The soils used in this study are important soils for livestock production systems in temperate grassland regions. For example, Typical Brown Earths (TBE), Typical Luvisols (TLu) and Typical Surface-water Gleys (TSWG) account for 762k, 448k and 311k hectares of the agricultural land area in Ireland, respectively, which equates to 47.9% of the total grassland area (3176k hectares). Whilst the total quantity of SOC stocks of these soils are similar, the results from our fractionation show that the quality and stability of the SOC stocks do vary significantly between soil types, specifically soils that are subject to clay-illuviation versus free-draining Brown Earths. This has significant implications both for soil organic carbon storage over the long term and land-use management of these soils, not just in an Irish context, but worldwide, as these soils are often the dominant agricultural soils of many grassland and tillage regions of the world.

In this context, our results indicate that it is important to consider soil depth when measuring and/or modelling the SOC sequestration potential of soils in grassland systems. This finding concurs with several studies that point to the importance of identifying the relationship between aggregates and SOC in order to have a better understanding of SOC dynamics (Denef et al., 2004; Elliott, 1986; Jastrow, 1996; Six et al., 1998).

A sampling depth of 30 cm has been frequently applied to quantify the stock of SOC and the associated quality of that stock through fractionation studies. In this study, we found that sampling to a depth of 30 cm only captures 69% of the SOC located within the first meter, this is comparable to other studies in this field (Batjes, 1996; Poeplau and Don, 2013). In addition, we clearly demonstrate that the majority of this topsoil carbon is associated with macro-aggregates, on all grassland soils. We postulate that SOC found within the rhizosphere (0-30 cm) is primarily influenced by vegetation type and root exudates. Particulate organic matter (POM) stocks of the rhizosphere is quite consistent across grasslands and determines the fractions found at this depth (Rumpel and Kögel-Knabner, 2011).

At lower depths in the soil profile, SOC is increasingly associated with smaller aggregate sizes. Here, differences between soils become more pronounced: in Brown Earths, the majority of SOC is still associated with macro-aggregates, while in soils subject to clay-illuvation, the majority is associated with micro-aggregates and silt and clay fractions.In the subsoil (below 30 cm depth) mineral properties, such as clay content and chemical composition, play a stronger role in the aggregation process and exert a much stronger influence on the SOC sequestration process (Schrumpf et al., 2013).

We also found that sampling to 30 cm causes a bias in the quantification of SOC sequestration potential of these soils. If we only consider the surface 30 cm of a soil profile, up to 84% of the SOC is considered labile, as it is located in larger aggregate fractions. Hence, while the most dynamic fraction of the soil is well represented, the most stable fraction is remarkably underrated when the sampling is limited to 30 cm. This finding highlights the importance of sampling deeper soil horizons, which concurs with other recent studies (Harrison et al., 2011; Jobbágy and Jackson, 2000; Rumpel and Kögel-Knabner, 2011; Veenstra and Lee Burras, 2015).

An illustrative example of this is the higher total values of SOC of HBE as compared to SLu throughout the profile. Nevertheless, below 30 cm, SOC is located mainly in the

large aggregate fractions in HBE, while in SLu SOC is mainly associated with smaller fractions. Given that SOC occluded in macroaggregates has a turnover time of 1 to 10 years, as compared to the 10 to 100 years, and >100 years for SOC in microaggregates and silt and clay fractions, respectively (von Lützow et al., 2006), SLu has a higher degree of protection of its SOC than HBE. Therefore, HBE has a higher predisposition to SOC loss in the long-term compared to SLu.

Soils that have undergone clay illuviation are found to have a higher proportion of SOC associated with the smaller aggregate fractions and therefore longer storage potential of SOC sequestered. Many temperate grassland clay-illuviated soils are associated with poor or imperfect drainage due to the higher clay content with depth, causing stagnation of water percolating through the soil profile. The abolition of the EU milk quota in 2015 has resulted in significant investment in agricultural management practices with a projected increase in production from the dairy sector of 50% by 2020 (DAFF, 2010). Management practices include extended grazing seasons and increased grass utilisation. To achieve this, there is a renewed emphasis on the installation of arterial drainage systems in imperfectly and poorly drained soils under grassland production. O'Sullivan et al. (2015) assessed the financial trade-off between drainage for increased primary production and carbon sequestration in grassland soils, exploring a range of hypothetical carbon prices for SOC. They highlighted that at current carbon prices there is no incentive for farmers to maintain current SOC stocks. EU and national policies highlight the importance of SOC sequestration in mitigation of climate change. At the recent meeting of the "@Agreement at the Convention of the Parties" (COP) 21 in December 2015, the French Government put forward the *4 per 1000 initiative* (http://4p1000.org/understand) which proposes soil management options for the sequestration and preservation of SOC and yet no support is currently available to land managers to not drain land in order to preserve the long-term storage of SOC sequestered at depth in the clay-illuviated soils. This chapter summarises that for the long-term storage and preservation of sequestered carbon, we must also look to the potential of the sub-soil compartment and the soil management practices which influence this part of the soil profile.

3.6 Conclusions

The current IPCC guidelines account for SOC stocks in the top 30 cm of soil. While this may adequately reflect the magnitude of soil carbon sinks, this fails to capture differences in the quality of these sinks, expressed in terms of the stability and hence residence time of the SOC.

While some models, such as RothC, CENTURY, and DNDC already simulate SOC pools with different turnover times as a function of the clay fraction of the topsoil, these do not consider changes in clay content with depth below 30 cm, which may significantly affect the stability of SOC or vertical re-distribution of dissolved C, specifically in soils subject to clay illuviation

Land management practices, such as the installation of arterial drainage systems on these latter soils, have a significant influence on the long-term stability of sequestered SOC at depth. In these soils, drainage for increased productivity may reduce the capacity for longer-term storage of SOC. Incentives to maintain these stocks do not presently exist and should be considered in the formulation of agricultural policies.

Chapter IV

Effects of soil type and depth on the carbon distribution within small macroaggregates of undisturbed grassland systems

4.1 Abstract

Grassland soils have been highlighted as a global carbon (C) sink, and have the potential to sequester additional C. Sequestration of C occurs through incorporation of SOC within microaggregates and the silt and clay fraction. The distribution of SOC within macroaggregate fractions gives a good understanding about SOM dynamics and incorporation of new SOC into the soil.

IPCC recommendations account for SOC stocks in grassland systems, with a focus on the C sequestration in the topsoil (0-30 cm). The aim of this chapter is to understand the effect of soil type and depth on aggregate formation and to assess the factors affecting incorporation of SOC within macroaggregates in horizons up to 1 metre depth. To do this, we focused on the fractions associated with small macroaggregates (particulate organic matter, microaggregates, and silt and clay within small macroaggregates). Twenty-one grassland sites, covering four soil subgroups (according to the Irish Soil Classification System, Simo et al. (2015)) (Typical Surface-water Gley, Typical Luvisol, Stagnic Brown Earth and Typical Brown Earth) were selected, and fractions associated with small macroaggregates of all horizons up to 1 m depth (where possible) were isolated and analysed for SOC.

Results show that Typical Surface-water Gleys have a decreased level of mwM in the topsoil, possibly driven by a lower cation exchange capacity caused by lower pH. SOC associated with the different macroaggregate fractions in topsoil samples were similar for all soils, while in subsoil samples, differences between soil types were clear. Soils with stagnic or gleyic (reducing) conditions had a decreased content of SOC in microaggregates and silt and clay within small macroaggregates.The silt and clay fraction of the Typical Surface-water Gley samples was lower in SOC content. The small soil size fractions are the first showing SOC saturation, and the furthest a soil is from the saturation point, the greatest is the C sequestration capacity. The decreased SOC concentration in the silt and clay sized aggregates in soils with clay illuviation may indicate that these soils are further from their C saturation point, and therefore they may have a higher C sequestration potential. Increasing cation exchange capacity, clay, polyvalent metal cations increase the proportion of SOC in the fractions, and low levels of pH seemed to be limiting POM decomposition. These results contribute to elucidate the mechanisms driving aggregate formation (and thus C sequestration in microaggregates and silt and

clay) in topsoil and subsoils, and show that dynamics of SOC in subsoil horizons is soil-type dependant and therefore should be taken into account when accounting for SOC stocks, and management practices should be adapted to the individual characteristics of each soil type, in order to reduce SOC loses.

4.2 Introduction

Soil aggregation has several benefits for agriculture and the environment: It enhances aeration, structure, water holding capacity and infiltration, which improves root establishment and plant growth (Bot and Benites, 2005; Lal, 2004a; Stevenson, 1994). Aggregation physically partitions soil organic matter (SOM) from microorganisms and their enzymes, thus limiting mineralisation of SOM and CO_2 emissions to the atmosphere (Cambardella and Elliott, 1992; Six et al., 2002). Therefore, it plays an important role in carbon (C) and nitrogen (N) sequestration.

Aggregates are formed through the association of SOM with the mineral fraction of the soil. When bacteria decompose particulate organic matter (POM) a sticky mucilage is secreted. This mediates POM encrustation by the mineral fraction, creating a macroaggregate (>250 μm) (Oades, 1984; Tisdall and Oades, 1982). When POM gets further decomposed, it decreases in size (Guggenberger et al., 1994) and it becomes encapsulated by mineral compounds in smaller structures: microaggregates (53-250 μm) and silt and clay sized aggregates and particles (<53 μm), which serve as building blocks for new macroaggregates when the POM is too small to keep the microaggregates together (Six et al., 1998). Oxygen diffusion is often reduced at the centre of microaggregates and further at the centre of silt and clay sized aggregates (Sexstone et al., 1985), hence the SOM contained inside is not easily accessible for microorganisms, and the mean residence time of SOM associated with microaggregates and the silt and clay fraction is higher than that in macroaggregates (Six et al., 2002). SOM can be associated with mineral particles through electrostatic cation bridges, between SOM functional groups and a polyvalent cation such as Fe, Al or Ca, or through stronger bonds with ligand exchange (von Lützow et al., 2006). Therefore, the incorporation of SOC into microaggregates and the silt and clay fraction is a mechanism for C sequestration (Skjemstad et al., 1990). C associated with mwM has been highlighted as an early indicator of SOC changes associated to management practices (Six and Paustian, 2014).

Some management practices or soil characteristics can have an influence on macroaggregate turnover. Slower turnover of macroaggregates enhances microaggregate formation within macroaggregates, which increases C sequestration (Six et al., 2000a). In contrast, if the soil is disturbed, for example during cultivation, macroaggregates are broken, releasing microaggregates and POM, which becomes a source of SOC for

bacteria and causes a depletion of SOC (Six et al., 2000a). Several studies have elucidated the dynamics of this fraction and its variability across soil types, climates and management (Álvaro-Fuentes et al., 2009; Chung et al., 2008; Denef et al., 2004).

Clay content is positively correlated with aggregate formation (Porta et al., 2010b). Different clay mineralogies also have different effects on aggregation: while soils with 2:1 clays or mixed clay mineralogy show increased aggregation with increased SOC inputs, 1:1 minerals have a greater potential for aggregate formation with low levels of SOC, since SOC is not the primary binding agent in these soil types (Denef et al., 2004, 2002). Microaggregate formation is also influenced by land management (Cambardella and Elliott, 1993; Six et al., 2000a; Zibilske and Bradford, 2007): conversion of grassland to cropland diminishes SOC associated with small aggregates. In cropping systems, conventional tillage increases macroaggregate turnover, thus reducing aggregation and C sequestration. In contrast, no-tillage practices enhance SOC stabilisation into microaggregates and silt and clay within macroaggregates (scwM) (Six et al., 2000a). Dry-wet cycles also affect microaggregate formation, by enhancing or decreasing aggregation depending on other soil conditions, such as SOC and moisture (Denef et al., 2001a; Haynes and Swift, 1990).

While some studies assess the C distribution within the different aggregates sizes in grassland soils (Bach et al., 2010; Wang et al., 2015), most of the research regarding microaggregate formation within macroaggregates focuses on the effects of different management practices, such as tillage (Denef et al., 2004; Mikha and Rice, 2004; Zibilske and Bradford, 2007). Very few studies have adressed microaggregate formation within macroaggregates comparing temperate grassland soils (O'Brien and Jastrow, 2013).

The findings of several studies indicate the importance of studying the dynamics of SOM in subsoil: Salomé et al. (2010) revealed that the regulation of SOC cycling was different in subsoil to topsoil. The mechanism activating SOC mineralisation in topsoil was the input of fresh SOM, while in the subsoil, the contact between degraders (or their exoenzymes) and the substrate was the limiting factor for SOM mineralisation. Other studies indicate that the chemical composition of SOM in subsoil is affected by pedological processes and is therefore soil-type specific (Eusterhues et al., 2005; Rumpel et al., 2002). Some management practices such as draining or plowing have an impact in deeper horizons (Baker et al., 2007; Tan et al., 2002; Veenstra and Lee Burras, 2015).

The dynamics and mechanisms affecting this pool of SOC are starting to be elucidated, but to date, most of the research concerning SOC dynamics and aggregation has focused on the topsoil. Rumpel and Kögel-Knabner, (2011) suggested that there were knowledge gaps regarding the mechanisms affecting aggregation and SOC stabilisation in subsoils.

This chapter addresses this knowledge deficit by focusing on (i) the percentage of microaggregates within macroaggregates (mwM) and distribution of SOC within macroaggregate fractions (i.e. Particulate organic matter (POM), mwM and silt and clay within macroaggregates (scwM)) both in the topsoil and whole profile and (ii) the soil characteristics driving these differences, if they exist. The aim of this chapter is to understand the effect of soil type and depth on aggregate formation and to assess the factors affecting the incorporation of SOC within macroaggregates in horizons up to 1 metre depth. This will provide a better understanding of differences in SOC dynamics within soil types, and inform the discussion on how management practices can be adapted to increase SOC storage in soils. The hypothesis is that different soil types will show different distributions of C within macroaggregate fractions, since different soils have different characteristics that will influence differently the process of aggregate formation.

4.3 Materials and Methods

4.3.1 Soil selection and sampling

Twenty one grassland sites were selected with the aim of being a representative set of the main four soil types occurring in Irish grasslands. These are six Typical Surface-water Gleys (TSWG) three Typical Luvisols (TLu), five Stagnic Brown Earths (SBE), and seven Typical Brown Earths (TBE). For each site, a pit was dug up to 1 m depth where possible, and each horizon was described following the FAO field handbook (FAO, 2006) and sampled. Soils were classified according to the Irish Soil Information System (Simo et al., 2014). For each horizon, one kg sample was taken and stored at 4 ºC until use.

4.3.2 Aggregate separation

A 300 g subsample was gently passed through an 8 mm sieve, and dried at 40 °C for one week. Following an adaptation of the method described by Cambardella and Elliott (1993), small macroaggregates (250-2000 μm) were isolated, dried, and stored at room temperature until use.

The isolation of POM, mwM and scwM was done following the methodology proposed by Six et al. (2000). Briefly, 10 g of small macroaggregates were placed on the top of a 250 µm mesh with 50 glass beads (4 mm diameter), and gently shaken under a continuous water flow that washes the smaller material into a 53 µm sieve in order to avoid breaking up of the mwM. To ensure that only water-stable mwM were isolated, the material remaining on the 53 µm sieve was wet sieved. During this process, mwM were separated from the scwM fraction. All fractions were dried, weighed, ball milled and stored for SOC analysis.

The proportion of mwM was calculated accoding to Six et al. (2000), with the equation shown in the literature review section (equation 2.2):

The proportion of SOC in each fraction within macroaggregate were calculated as follows:

$$\frac{g\ SOC\ in\ fraction}{g\ fraction\ (including\ same\ sized\ sand)} \times \frac{g\ fraction\ (including\ same\ sized\ sand)}{g\ macroaggregate}$$

Equation 4. 1. *Calculation of the SOC content in a fraction by g of aggregate*

4.3.3 Soil organic carbon analysis

Carbonates within each sample were removed (Harris et al., 2001), and SOC associated with each fraction was analysed with a LECO truspec CN analyser following the ISO 10694:1195.

4.3.4 Statistical analysis

The effect of depth and soil type on the percentage of aggregation and the SOC distribution within fractions was analysed using PROC MIXED in SAS (version 9.3). After checking the residuals, log transformations were needed to ensure that the data fitted the assumptions required for the analysis. The Duncan test was used to check the differences within groups.

The explanatory variables used were obtained from the dataset associated to each one of the profiles analysed for the Irish Soil Information System (explained in chapter 1).

4.4 Results

4.4.1 Microaggregates within small macroaggregates

Microaggregate content within small macroaggregates (mwM) only differed between soil types in the topsoil samples (midpoint < 30 cm) (p=0.018), Table 4. 1, this being higher in Typical Brown Earths (TBE) compared to Typical Surface-water Gleys (TSWG). No distinction between soil types was evident for the subsoil or the whole profile.

Table 4. 1 *mwM (%) by depth for each soil type, divided by areas in the soil profile. (TBE=Typical Brown Earth, SBE=Stagnic Brown Earth, TLu=Typical Luvisol, TSWG=Typical Surface-water gley). Lowercase letters indicate significant differences between soil types (p<0.05).*

	TBE	SBE	TLu	TSWG
Topsoil	53.15±2.15 **a**	49.3±2.29 **ab**	46.58±3.35 **ab**	43.7±0.99 **b**
Subsoil	44.16±3.58 **a**	42.09±5.53 **a**	41.03±7.05 **a**	35.55±5.63 **a**
Whole profile	48.65±3.2 **a**	44.71±4.71 **a**	43.6 ± 5.55 **a**	38.5 ± 4.63 **a**

All soil types have a similar SOC content in the topsoil (Table 4. 2). SOC was not significantly correlated to mwM (p=0.18), and no differences in SOC concentrations were found between soil types (Table 4. 2). Typical Surface-water Gley soils have a significantly lower CEC than the other soils (Table 4. 2). The linear correlation between CEC and mwM in the topsoil of Typical Surface-water Gley is significant (R^2=0.49) (p=0.0035). pH is significantly higher in Typical Brown Earths compared to Typical Surface-water Gleys (Table 4. 2), but it is not significantly correlated with mwM on the topsoil (p=0.27).

Table 4. 2. *Mean and standard errors of TOC (%), Cation exchange capacity (C.E.C) and pH on the topsoil, divided by soil type. Different letters indicate significant differences between soil types (p<0.05)*

	SOC (%)	CEC (cmol kg^{-1})	pH
TBE	2.64±0.32 **a**	11.95±1.39 **a**	6.16±0.2 **a**
TLu	2.14±0.47 **a**	11.05±1.61 **a**	6.28±0.25 **a**
SBE	3.3±0.58 **a**	10.79±1.16 **a**	5.75±0.14 **ab**
TSWG	2.96±0.57 **a**	6.9±0.95 **b**	5.41±0.08 **b**

4.4.2 Carbon associated to macroaggregate fractions

This section presents the SOC associated to each isolated macroaggregate fraction expressed on a gram of macroaggregate basis, calculated using equation 4. 1 shown in the Materials and Methods Section.

Neither the topsoil nor all horizons analysed together showed differences in macroaggregate-associated SOC between soil types. Only the subsoil (midpoint of horizon deeper than 30 cm) showed distinction between soil types for mwM and scwM (figure 4.1). In the subsoil, macroaggregates of Typical Surface-water Gleys had a significantly lower content of SOC located in mwM than Stagnic Brown Earth, and significantly less SOC in the scwM than both Brown Earth subgroups.

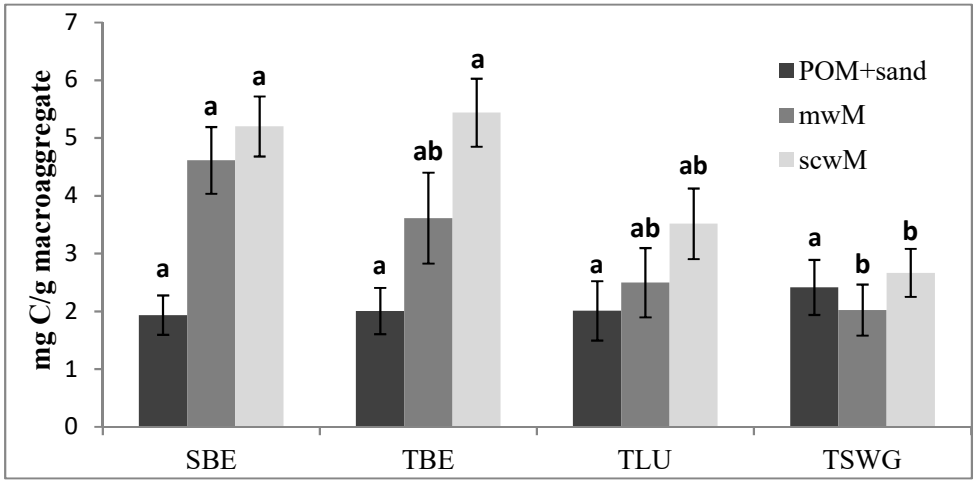


Figure 4.1*.Carbon content in each fraction expressed as mg per g of macroaggregate. Displayed results correspond to back transformed data of the subsurface horizons. Letters represent significant differences between soil types. Error bars represent standard error of the mean.*

4.4.3 Soil characteristics influencing aggregation and SOC distribution within fractions

Soil characteristics influencing SOC distribution within soil fractions were analysed across the entire soil profile. Significant results for Spearman correlations between SOC content in each fraction and the different soil parameters are shown in Table 4. 3. Content of SOC in the different macroaggregate fractions was correlated with most of the soil chemical characteristics. CEC had a positive influence on all fractions, particularly scwM and mwM. pH was negatively correlated to SOC content in POM and mwM. From all

cations affecting CEC, SOC in scwM and mwM was mostly influenced by Ca, and POM was more influenced by K content. Increasing amounts of Al were influencing positively SOC content in the mwM and scwM, and Fe had a positive influence on SOC in all fractions. Increasing amounts of SOC were positively correlated to the SOC content in all fractions, but with a higher correlation with SOC in mwM and scwM.

Table 4. 3. *Coefficients of determination (R^2) of regressions between SOC located in (i) POM, (ii) microaggregates, (iii) silt and clay within small macroaggregates from all soils, and different soil properties that could potentially have an influence on aggregation. Only significant interactions are displayed.*

Parameter	mgC-POM/g M	mgC-mwM/g M	mgC-sc/g M
Na (cmol/kg)		0.26	0.28
K (cmol/kg)	0.5	0.52	0.52
Mg (cmol/kg)	0.3	0.41	0.45
Ca (cmol/kg)	0.4	0.67	0.66
C.E.C (cmol/kg)	0.44	0.66	0.68
pH	-0.44	-0.25	
Clay (%)	0.42	0.36	0.37
Al (cmol/kg)		0.4	0.47
Fe (cmol/kg)	0.33	0.57	0.56
SOC (%)	0.69	0.95	0.92

4.4.4 Soil organic carbon per gram of fraction

While data presented up to this point is plotted per gram of aggregate, this section focuses on the concentration of SOC per gram of each individual fraction. This allows direct comparison of the SOC enrichment in each fraction by soil type, without taking into account the quantity of that fraction present in the soil. For example, it allows comparison of % of SOC in microaggregates, per gram of microaggregates. If there is more SOC in one gram of microaggregates of soil type A in comparison soil type B, it means that this fraction is enriched in SOC in soil type A.

None of the fractions in the topsoil showed SOC enrichment when compared between soil types. For the whole soil, there was no difference in POM and mwM soil types. Nevertheless, the scwM in Typical Surface-water Gleys and Typical Luvisols had

significantly lower concentration of SOC than Typical Brown Earths, this being 2.17 and 2.38% in the Typical Luvisols and Typical Surface-water Gleys, respectively, as compared to 3.56% in Typical Brown Earth (Figure 4.2).

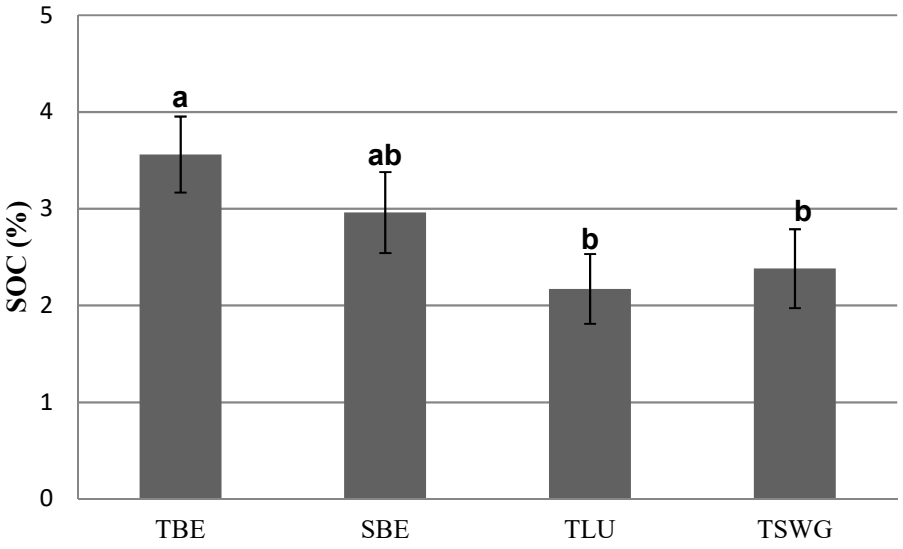


Figure 4. 2. *SOC concentration in the scwM fraction, for the different soil types. Letters represent significant differences between soil types. Error bars represent standard error of the mean.*

4.5 Discussion

4.5.1 Microaggregates within small macroaggregates

There were no significant differences in the percentage of mwM across the entire soil profile between soil types. For the topsoil, the overall level of aggregation (48.57%) is similar to that described by Six et al. (2000) for soils under no-tillage systems (47.1%). However, the data presented by Six et al. (2000) corresponds to the top 5 cm of soil of croplands, while our data for the topsoil corresponds to horizons with a mid-point shallower than 30 cm and grassland sites. Since the level of mwM decreases significantly with depth, it is likely that the top 5 cm of the grassland soils shown in this study have a higher level of aggregation compared to the no tillage systems studied by Six et al. (2000).

The fact that the differences of mwM among soil types are only found in the top 30 cm and not in the subsoil suggests that it could be a function of the SOC content or grassland

management. When looking at the soil characteristics that differ between soil types, it can be seen that SOC levels are similar in the top 30 cm of all soils. While it has been reported that aggregate formation is promoted in soils with high SOC inputs as compared to low SOC inputs (Denef et al., 2002), our results indicate that under similar management conditions with similar amounts of SOC inputs, other soil characteristics will play an important role in aggregate formation. The two factors that are significantly lower in Typical Surface-water Gleys compared to Typical Brown Earths and therefore could have an influence on the different formation of mwM are pH and CEC. CEC exhibits a strong positive correlation with mwM (R^2=0.49, p=0.0035). We suggest that this could be the factor that is driving aggregate formation in undisturbed soils with similar amounts of SOC. This is possibly due to the fact that although pH is not directly related to the percentage of mwM-associated SOC, it does affect the CEC (Jones and Jacobsen, 2005). Increasing pH increases linearly the CEC of clay and SOM (Helling et al., 1964). At lower pH, the protons are neutralising the negative charges of the SOM, and therefore the CEC is lower. In contrast, at higher pH the negative charges of SOM are neutralised by polyvalent cations such as Ca^{2+}, Mg^{+2}, which increases the CEC of SOM. The bivalent cations adsorbed to the surface of a molecule of SOM will also interact with other SOM molecules and clay surfaces, and therefore enhance aggregation of SOM and aggregate formation (Porta et al., 2010b).

We suggest that under similar SOC content and disturbance, increases in CEC of the soil, affected by increases in the pH, have a positive influence on aggregate formation. Liming is a common practice on acidic grassland soils in Ireland to reduce acidity, decrease toxicity by Fe and Al, and increase the mobility of some nutrients such as P. According to our results, this could have a positive influence on aggregation, which is in concordance with Haynes and Naidu (1998). Nevertheless, the increase of pH also causes an increase of microbial activity (USDA, 1999). Therefore, it should be further investigated if the C sequestered by a supposed enhanced aggregate formation after liming applications is higher or lower than the losses of SOC caused by SOM decomposition after increased microbial activity.

4.5.2 Distribution of carbon within macroaggregate fractions

Topsoil horizons have similar SOC contents in all the fractions, and the same results are found when all horizons are analysed together. When looking at subsoil samples, there

are two contrasting soil types that differ in all fractions: Stagnic Brown Earths and Typical Surface-water Gleys.

Horizons deeper than 30 cm of the soil type Typical Surface-water Gleys had lower content of SOC in mwM than Typical Brown Earths, and less SOC in the scwM than both Typical and Humic Brown Earth soils. Nevertheless, the POM fraction is similar between all soil types. This has different possible explanations. The first possibility is that there are similar inputs of fresh residue into the subsoil in all soil types (because of the similar amounts of POM), but the decomposition of POM and the subsequent incorporation of SOC into the smaller fractions is happening at a slower rate in Typical Surface-water Gleys. Since the amounts of total SOC in the studied soil types are similar, and so is the anthropological disturbance, the low incorporation of SOC into smaller fractions may be due to reduced mineralisation. The main characteristics of Typical Surface-water Gleys are: (i) increasing clay content in subsoil and (ii) reducing conditions in a sub-surface horizon caused by surface-water stagnation (Simo et al., 2014). The anoxic conditions caused by the waterlogging in Typical Surface-water Gleys could be limiting POM mineralisation by reducing the microbial activity (Reddy and Patrick Jr., 1975). This reduced mineralisation of the POM could be limiting its size reduction and the subsequent incorporation into mwM and the association with the silt and clay fraction (Six et al., 2000a). Nevertheless, if this was the case, it would lead to an increase of SOC in POM within macroaggregates.

Another possible explanation for the similar proportion of C in POM, and the decreased SOC content in mwM and scwM in TSWG could be that POM inputs, as well as POM decomposition, are lower in TSWG. Given that root length is limited in soils with low oxygen diffusion (Bengough et al., 2011), the anoxic conditions that characterise the soil type TSWG could be limiting the rooting depth, which would lead to a lower incorporation of POM in the subsoil. In this scenario, there would also be a lower degree of POM decomposition caused by the anoxic conditions. This explains (i) the similar proportion of C in POM in the different soil types, since the lower POM inputs are offset by lower decomposition, and (ii) the reduced proportion of C in mwM and scwM, as a result of lower POM inputs into the system, and consequently lower amounts of C being incorporated into the smaller aggregate sizes.

Drainage (involving the installation of sub-surface pipes) is common practice in grassland systems in Ireland, particularly on Typical Surface-water Gley soils. This practice decreases the water content of the soil, which increases soil redox potential. The consequences of this in aggregate formation in subsoil should be further investigated, since it could lead to a decrease or an increase of incorporation of C into the smaller aggregate sizes. It is possible that the increase of oxygen in subsurface horizons would also allow roots to grow deeper (Bengough et al., 2011), which could increase the POM inputs into the subsoil, and consequently the incorporation of C into the smaller aggregate sizes. Nevertheless, given that decomposition of SOM is faster under aerobic conditions (Acharya, 1935; Alexander, 1961), and that POM is less physically protected than mwM and scwM, its decomposition rate could be faster (Chan et al., 2002). Therefore, the increased oxygen availability caused by the drainage could cause a fast mineralisation of the POM and a reduced in the incorporation of SOC within mwM and scwM, which would lead to a reduced C sequestration, or it could also cause an increase of POM inputs, and if the mineralisation rate is slow enough, incorporation of this POM into the mwM and scwM. The consequences of the two possible scenarios are opposed, and it should be further investigated which would be the consequences of increasing the oxygen availability (e.g. by installation of drainage) in macroaggregate turnover in subsoils.

Since the top 30 cm of all soils sampled, contained similar amounts of SOC within macroaggregate fractions, we suggest that the depth proposed by the IPCC guidelines to monitor SOC stocks (IPCC, 2006) is not useful to elucidate the different aggregate dynamics occurring in the different soil types. Our results show that there are differences between soil types in the distribution of carbon within fractions in subsoil horizons, therefore the dynamics stabilising SOC in subsoils differ between soil types. Since human management can have an effect on subsoil horizons (Veenstra and Lee Burras, 2015), we suggest that subsoil should be considered when accounting for SOC stocks and planning for adequate management practices for each soil type.

4.5.3 Soil characteristics influencing aggregation and SOC distribution within fractions

When looking at the soil characteristics that are correlated to each one of the small macroaggregate fractions, it can be seen that the CEC of soils play an important role, especially in the scwM. Increasing amounts of CEC lead to increasing SOC content in all

fractions, but especially in the scwM and the mwM. As explained above, this is due to the enhanced interactions between the mineral and the organic fractions of the soils through polyvalent cations caused by increased CEC, which enhance microaggregate formation (Helling et al., 1964; Porta et al., 2010b). From all cations used to calculate the CEC of soils (Na, K, Mg, Ca), Ca seems to be the one that is further contributing to incorporation of SOC within the smaller fractions. The positive effect of Fe and Al on aggregation has been previously described: Clay bonds with the organic fraction of soil through polyvalent metals to form large organomineral complexes (Edwards and Bremner, 1967; Tisdall and Oades, 1982). Unsurprisingly, clay content is positively correlated with C content in all Macroaggregate fractions. It has been widely reported that increasing amounts of clay enhance aggregate formation (Franzluebbers and Arshad, 1996; Wagner et al., 2007) and SOC stabilisation (Hassink, 1997).

pH is negatively correlated with the presence of carbon in POM and in mwM (with a lower R^2). Decomposition of SOC is reduced in acidic soils (Amato and Ladd, 1992; Motavalli et al., 1995), since lower pH has a negative effect on microbial biomass, soil microbial community and enzymatic activity (DeLaune et al., 1981; Nodar et al., 1992). In our soils, as pH increases there is more decomposition of POM, and thus there is less SOC associated with this fraction in soils with higher pH. We hypothesise that SOC in POM is more affected by changes in pH because it's more labile and readily available for microorganisms, while SOC in mwM is protected from mineralisation through other mechanisms (Six et al., 1998), which limit the decomposition also at higher pH.

4.5.4 Soil organic carbon per gram of fraction

This section focuses on the concentration of SOC per gram of each individual fraction in order to compare SOC concentrations without taking into account the weight of each fraction within macroaggregates. Thus, this is useful to check if a fraction is "enriched" in SOC in a soil type when comparing to another, which means that it has an increased SOC content per gram of fraction.

All soil types have the same C concentration in POM and mwM. The scwM differs between soil types, this being higher in Typical Brown Earths than in Typical Luvisols and Typical Surface-water Gleys. The SOC saturation model implies that there is an upper limit of SOC content in the whole soil. Once this limit is reached, stabilisation ceases (Chung et al., 2008). There are four SOC pools that reach the saturation point at different

SOC concentrations: (i) Non-protected pool (measured as the light fraction or POM), (ii) biochemically protected, (iii) microaggregate protected and (iv) silt/clay occluded carbon. The saturation level of the whole soil is reached once all the individual fractions are SOC saturated, and the capacity of each soil to sequester SOC depends on how far the soil is from its saturation point (Six et al., 2002; Stewart et al., 2007). The smallest soil size fractions are the ones that show saturation at lower SOC inputs given that they have a smaller C sequestration potential and a slower SOC turnover (Chung et al., 2008; Hassink, 1997). Chung et al. (2008) showed that, after 30 years of SOC inputs, the silt + clay fraction within small macroaggregates reached a saturation point, while SOC concentration was still increasing with increasing SOC inputs in the mwM fraction.

Our study does not include SOC inputs, and therefore we cannot directly compare the response of the fractions within small macroaggregates to a constant SOC input. Nevertheless, the fact that the silt and clay fraction within small macroaggregates has a greater SOC concentration in Typical Brown Earths than in Typical Luvisols and Typical Surface-water Gleys may indicate that Typical Surface-water Gleys and Typical Luvisols are further from their saturation point than Typical Brown Earths. Therefore, Typical Surface-water Gleys and Typical Luvisols have a greater capacity for SOC sequestration if clay mineralogy doesn't differ within soil types, which would implicate a different SOC sequestration capacity (Denef et al., 2004).

The common characteristic of Typical Luvisols and Typical Surface-water Gleys is a clay increase at depth. Therefore, the lower SOC concentration in these soils can be due to a "dilution effect", since there is a bigger ratio of reactive clay surface for similar amounts of carbon.

4.6 Conclusions

The IPCC guidelines for SOC measurements recommend a sampling depth of 30 cm. At this depth, the soils in our study show no differences between them, except a decrease in the percentage of mwM in Typical Surface-water Gleys when compared to Typical Brown Earths.

Only when looking at deeper horizons, can patterns between soil types be observed: (i) Soils with reducing conditions caused by waterlogging contain a decreased percentage of protected carbon in mwM and scwM. Draining of these soils will become an increasing

practice in order to increase production since the EU milk quota finished in 2015 (Schulte et al., 2016), and to meet the aspirations for increased food production and exportations set by the Food Wise 2025 (DAFM, 2015). Our results suggest that these soils are more prone to have a SOC depletion at depth if this happens, since they have a smaller percentage of their SOC in the most protected fraction of their small macroaggregates. We hypothesize that the increased oxygen availability would lead to a fast mineralisation of the POM, which would cause a reduction of the SOC stabilisation in microaggregates and the silt and clay fraction. This should be further investigated. (ii) the scwM of Typical Surface-water Gley contain a lower percentage of SOC than in the other soil types, which suggest that is further from the saturation point, and therefore has a higher C sequestration potential.

The factors influencing the proportion of SOC into the different small macroaggregates fractions are increased CEC, increased clay content, the presence of polyvalent metal cations that act as binding agents between SOC and the mineral fraction, and the pH, which limits POM decomposition at its lower levels.

Chapter V

Biochemical characterisation of soil organic carbon in temperate grassland soils

5.1 Abstract

Grasslands have the greatest topsoil carbon (C) sequestration potential amongst all land uses. In soils, C can be sequestered through aggregate formation, association with clay particles or it can be biochemically stabilized through the formation of recalcitrant SOM compounds, which are more resistant to microbial attack than labile fractions of SOM. In this chapter, the biochemical recalcitrance of C and nitrogen (N) was analysed with a hot water extraction procedure (for the labile fractions), and analysing the non-hydrolysable C and N (as a measure of the recalcitrant fractions), for each horizon of 40 grassland sites up to 1 m depth. Results show that topsoil does not show significant differences between soil types. Subsoil contained more recalcitrant N, labile C per gram of C, and less labile N per gram of N than the topsoil. Soils with stagnation properties showed an increased amount of recalcitrant C and N, which could be a consequence of a slower lignin degradation and lower aerobic respiration rates. Increasing clay content and exchangeable Ca contribute to diminish the amount of labile C and N, while the recalcitrant nitrogen is being stabilised by Fe and Al. Results indicate that there is C and N leaching and re-deposition occurring down through the soil profile, combined with a later stabilisation of those in the subsoil, which causes an increased amount of recalcitrant SOM. This indicates the substantial value of Irish grasslands as sinks of recalcitrant C and N. Since individual characteristics within each soil type can only be observed when looking across the whole profile, we suggest that this C and N pool should be taken into account for future land management planning. For example, a way of preserving or increasing amounts of recalcitrant C and N in subsoils would be the avoidance of drainage installation, since stagnation properties have been shown to be increasing this SOM pool.

5.2 Introduction

Temperate grasslands contain between 247 and 264 billion tonnes of soil organic carbon (SOC) and have the greatest soil carbon (C) sequestration potential among all land uses (Conant et al., 2001; Lal, 2004a). Although a large proportion of SOC is found in subsurface horizons (Diochon and Kellman, 2009; Harrison et al., 2011; James et al., 2014), the SOC inventories generally focus on those stocks to a depth of 30 cm, ignoring both the quantity and quality of SOC at deeper horizons (Fontaine et al., 2007; Ward et al., 2016).

Conceptual models of carbon pools partition SOC based on fast and slow rates of turnover, with active, slow and passive pools (Parton et al., 1987; Smith et al., 1997). However, it is difficult to relate these conceptual pools back to empirical measurements based on physical or biochemical characteristics. Physical protection and SOC biochemical characteristics have been used to characterise SOC in order to determine the rate of SOC mineralisation and mean residence time. Particle size and density fractionations are widely used, but have been shown to have marginal correlation with turnover rates *in vivo* (Christensen, 2001; Roscoe et al., 2001; Tiessen and Stewart, 1983). Partitioning between water soluble and acid non-hydrolysable may be the simplest method for distinguishing between labile and recalcitrant carbon pools.

Hot water extractable carbon (HWEC) and nitrogen (HWEN) have been used as a measure of the most readily mineralisable SOC and N fraction (i.e. the most labile fractions) (Chodak et al., 2003; Davidson et al., 1987; von Lützow et al., 2007). This is an heterogeneous pool that consists of both microbial (Haynes and Francis, 1993) and non-microbial compounds, since the treatment with >70 °C water kills the cells of microorganisms (Landgraf et al., 2006). Its composition consists mainly of carbohydrates and N-containing compounds such as amino sugars and amides (Haynes, 2005).

In order to assess the most biochemically recalcitrant SOC and N pool, hydrolysis with acid has been proposed (Stout et al., 1981) and widely used (Leavitt et al., 1996; Paul et al., 2011, 1997; Rovira and Vallejo, 2007; Cheng et al., 2007). Hydrolysing soils with 6M HCl chemically removes the more labile fraction of SOC: proteins, nucleic acids, polysaccharides, carboxyl C; while the most chemically stable fraction such as lignin, fats, waxes, resins, suberins, and predominately molecules with alkyl C, highly aromatic and longer aliphatic chains remain as the non-hydrolisable fraction (Paul et al., 2000;

Rovira and Vallejo, 2002; Silveira et al., 2008). There is a significant correlation between ^{14}C radiocarbon dating with residue fractions post-hydrolysis, with the residence time of acid-hydrolysis residue fractions ranging from 1300 to 1800 years (Leavitt et al., 1996; Paul et al., 2000), and at deeper horizons, residence times can be even longer (Anderson and Paul, 1984; Stout et al., 1981). Soil N consists mainly of amino acids, peptides and proteins (Morier et al., 2008; Sjöberg et al., 2004), and in a lower quantity, of nitrils and heterocyclic N, formed mainly during advanced humification through abiotic reactions (Knicker et al., 2002), or by changes in SOM after fire episodes (Almendros et al., 2003; Knicker, 2007). The N compounds removed by acid hydrolysis consist mainly of amino acids and amino sugars (Kelley and Stevenson, 1996), but the non-hydrolysable fraction is not yet clearly defined (Knicker et al., 2001) and labile compounds such as peptide-like components have been found in the non-hydrolysable fraction (Knicker et al., 2001). Initially, acid hydrolysis was conducted with highly concentrated acids and long hydrolysis time (Sowden, 1966). However, some studies have shown that prolonged hydrolysis with 6M HCl creates artefacts that overestimate the recalcitrant pool (James, 1972), and hydrolysis with different steps and concentrations were procedures were proposed (González-Prieto and Carballas, 1988; Janel et al., 1979). Silveira et al. (2008) concluded that hydrolysis times longer than 2 hours did not significantly increase SOC release. For this reason, less destructive analytical methods need to be applied in order to give a better estimation of the recalcitrant pool (Greenfield et al., 2013). In this study, samples were treated with 1M HCl during 3 h (González-Prieto and Carballas, 1988; Rovira et al., 2008; Yonebayashi and Hattori, 1980). Nevertheless, there may still be a portion of more recent SOC in the non-hydrolysable fraction (Balesdent, 1996).

Although several studies use this approach to characterise the SOC in the topsoil (Chodak et al., 2003; Landgraf et al., 2006; Spohn and Giani, 2010), there is less information about biochemical characteristics of SOC located in the subsoil.

The aim of this chapter is (i) to assess the variation of biochemically recalcitrant and labile SOC and N down the soil profile to 100 cm depth and between common soil types of temperate grasslands in Ireland, and (ii) to determine which soil characteristics have a greater influence on SOC stability.

5.3 Materials and methods

5.3.1 Site selection and sampling

Forty-one grassland sites were sampled representing six soil types: seven Typical Brown Earths (TBE), six Stagnic Brown Earths (SBE), six Humic Brown Earths (HBE), three Typical Luvisols (TLu), three Stagnic Luvisols (SLu), six Typical Surface-water Gleys (TSWG)), five Typical Lithosols (TLi), and five Humic Groundwater Gleys (HGG) with the objective of covering the main soil types on which Irish grasslands are found.

At each site, a profile pit was dug to a depth of 1 m, where possible. All horizons were described according to FAO (2006), and the profile was classified according to the Irish Soil Information System (Simo et al., 2014). For each one of the horizons, one kg sample was taken and dried at 40 ^{o}C for 7 days. Once dried, each sample was manually sieved to 2 mm and a subsample was ball milled for further analysis.

5.3.2 Hot water extractable carbon and nitrogen

Hot water extracts were done to all soil types except Typical Lithosols and Humic Groundwater Gleys, with the method described by Rovira and Vallejo, 2007. Briefly, 20 mL of distilled water was added into 1 g of 2 mm sieved sample and boiled for one hour at 105 ^{o}C. Three analytical replicates were used for each sample, and one blank was added every 19 samples. To obtain the extract, the samples were centrifuged and filtered with filter paper Whatman 2. Total organic carbon (TOC) was measured using a Shimadzu TOC analyser.

5.3.3 Acid hydrolysis

The recalcitrant SOC and N pool was analysed in all soil types, by hydrolysing 0.5 g of ball milled soil with 20 mL of 1M HCl at 105°C for three hours. After cooling, the remaining sample was washed three times with distilled water by transferring the sample and the hydrolysate into centrifuge tubes, centrifuging during 10 minutes, discarding the hydrolysate and adding 20 mL more of distilled water.

Samples were subsequently transferred into previously weighed beakers and dried at 105 °C overnight. The remaining residue was analysed with a LECO Truspec CN analyser.

The recalcitrance index was calculated according to Rovira and Vallejo (2002) with the following equations:

Equation 5. 1 *Calculation of recalcitrant C and N*

$$RI_C = (\text{unhydrolysed C} / \text{SOC}) * 100$$
$$RI_N = (\text{unhydrolysed N} / \text{total N}) * 100$$

5.3.4 Statistical analysis

Since RI_C and RI_N are percentages, values were transformed with the following formula prior to analysis: $y' = \text{asin}(\sqrt{y/100}$, being y the RI_C or RI_N, and y' the transformed value.

The effect of depth and soil type on RI_C, RI_N, HWEC and HWEN was analysed using PROC MIXED in SAS (version 9.3) after checking the residuals. Duncan's Multiple Range test was used as a multiplicity adjustment for comparing group means. Correlations were analysed using Spearman test.

5.4 Results

5.4.1 Depth distribution of RIc, RI_N, HWEC and HWEN, and its relation to SOC and total N

Contents of labile C and N, as represented by the hot water extractable fraction and recalcitrant N were affected by their position in the profile, as shown in Table 5. 1.

Table 5. 1. *Average and standard errors of RIC, RIN, HWEC and HWEN, divided according to the position within the profile of each sample. Data for all soils grouped. Letters indicate significant differences ($p<0.05$)*

		RI_C	RI_N	mg HWEC gOC^{-1}	µg HWEN gN^{-1}
Position in profile	Topsoil	71.28±1.19	53.81±1.93 b	41.81±1.76 b	33.65±2.26 a
	Subsoil	70.89±1.53	61.52±2.67 a	61.56±3.36 a	19.40±1.49 b

Labile C and N showed contrasting patterns at depth (Figure 5. 1): While absolute amounts of HWEC per g of soil decreased at depth (data not shown), there was a significant increase of levels of HWEC when plotted on a gram of SOC basis (mgHWEC

gOC^{-1}) in subsoil horizons ($p<0.0001$). There was a negative correlation between mgHWEC gOC^{-1} and SOC: Horizons with lower amounts of SOC had increasing amounts of mgHWEC gOC^{-1} (R^2= -0.625, $p<0.0001$). Conversely, the amounts of HWEN per gram of N in the horizon (µgHWEN gN^{-1}) decreased at deeper horizons and it was positively correlated to the amount of N in the soil profile (R^2= 0.4358, $p<0.0001$).

Recalcitrant C was not significantly correlated with the amount of SOC in the profile, while RI_N decreased significantly with increasing amounts of total N (R^2=-0.345, $p<0.001$). There was no correlation between the amounts of recalcitrant and labile C, or between recalcitrant and labile N. The C:N ratio of the extracts increased from 11.35±0.3 in the topsoil to 18.02 ±1.67 in horizons deeper than 30 cm, suggesting les processed organic matter.

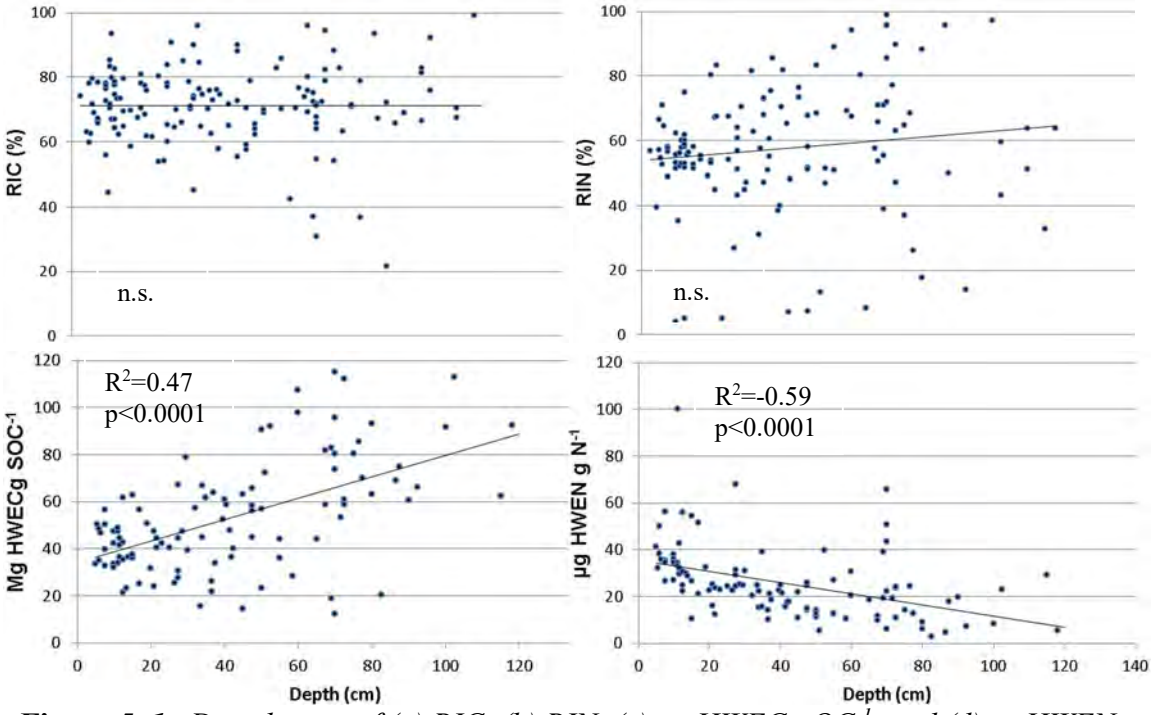


Figure 5. 1. *Distribution of (a) RIC, (b) RIN, (c) mgHWEC gOC^{-1}, and (d) µgHWEN gN^{-1} by depth. Data of all soils plotted together.*

5.4.2 Effect of soil type on recalcitrant and labile Carbon and Nitrogen

The labile fraction (i.e. mgHWEC gOC^{-1} and µgHWEN mgN^{-1}) did not differ between soil types, nor in the topsoil, neither in subsoil or the whole profile (data not shown). In the recalcitrant fraction, all soil types had similar amounts of RI_C and RI_N in the topsoil (Table 5. 2). The subsoil only differed between soil types in the case of the RI_C values, while RI_N was similar across all soils.

Table 5. 2. *RIC and RIN values by soil type, dividing by the position within the profile of each sample. Data are averages ± standard errors. Analysis were performed on the previously transformed values by arc-sine of squared root. In a given column, values followed by different letters (a, b, c) indicate significant differences($p<0.05$). Group splitting was performed according to Duncan's test.*

	Topsoil		Subsoil	
Soil type	RIC (%)	RIN (%)	RIC (%)	RIN (%)
HBE	66.28±3.80	51.16±5.21	73.39±2.47 ab	65.28±5.57
HGG	77.49±1.48	62.51±2.97	71.64±3.87 ab	73.77±6.47
SBE	71.99±2.57	54.10±1.59	73.92±2.93 a	57.63±5.40
SLU	75.75±9.00	59.04±0.57	70.31±1.56 ab	76.50±8.44
TBE	72.70±2.77	43.35±7.86	71.22±2.62 ab	51.64±7.79
TLU	67.41±5.34	58.17±3.08	58.64±7.26 bc	65.74±10.4
TSWG	70.68±2.80	59.45±3.73	79.55±3.06 a	59.00±6.50
TIi	70.28±1.15	51.66±1.37	49.63±8.28 c	55.92±8.97
All	71.28±1.19	53.81±1.93	70.89±1.53	61.52±2.67
p > F	0.3868	0.1457	0.0018	0.248

When the whole profile was taken into account differences were observed for both RI_C and RI_N, but not for the labile fraction. The differences between soil types for the whole profile are displayed in Figure 5. 2. The soil types Typical Surface-water Gleys, Humic Groundwater Gleys and Stagnic Brown Earths have significantly higher RI_C contents than Typical Lithosol and Typical Luvisols. Stagnic Luvisols, Typical Brown Earths and Humic Brown Earths have RI_C values between the two extremes.

Values of RI_N also showed differences between soil types when all horizons are analysed. Nevertheless, there are no clear patterns in terms of soil characteristics and RI_N values.

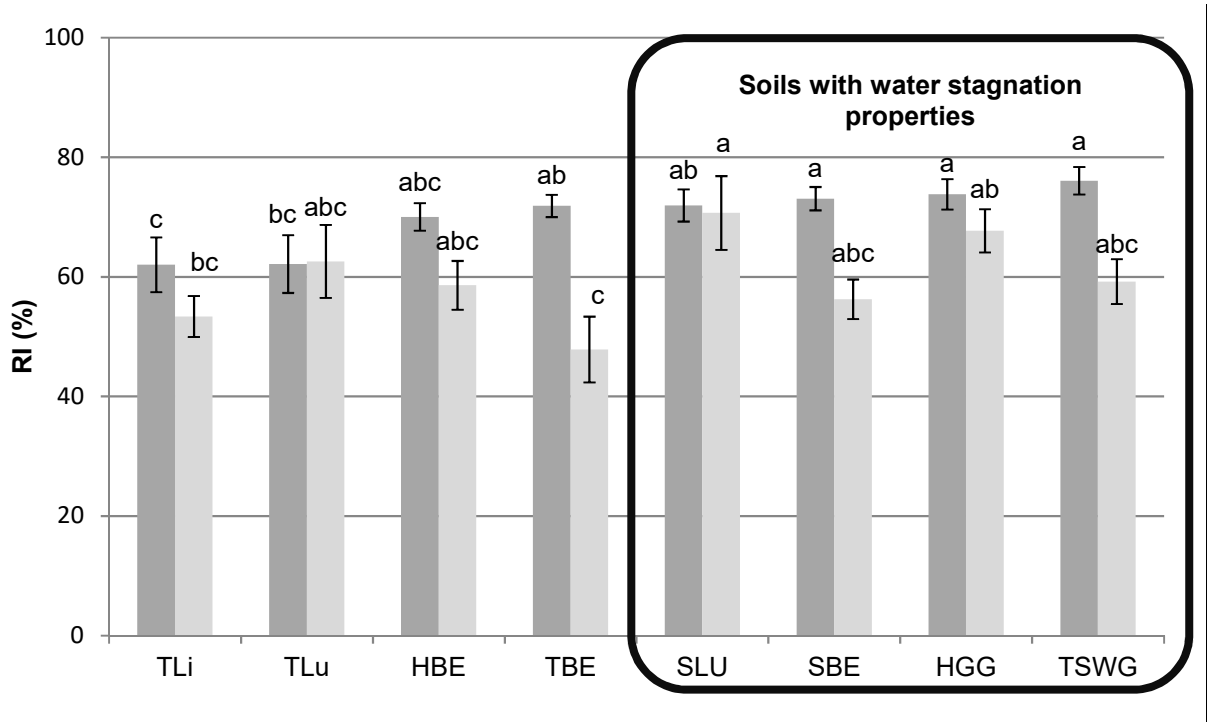


***Figure 5. 2**. RI_C (dark bars) and RI_N (light bars) values of the overall profile, by soil type. Bars indicate standard error. RI_C p= 0.009, RI_N p= 0.0219. Analyses were performed on the previously transformed values by arc sine of squared root. Values followed by different letters (a, b, c) differ, at P = 0.05. Group splitting according to Duncan's test.*

The influence of stagnation properties within the recalcitrant and labile fraction is further analysed in Table 5. 3. Soils with stagnation conditions had a significantly higher amount of recalcitrant C and N, while the contents of mgHWEC gOC^{-1} and µgHWEN gN^{-1} were not affected by waterlogging.

***Table 5. 3**. Average and standard errors of RIC, RIN, HWEC and HWEN divided in soils with or without stagnation properties. Letters indicate significant differences (p<0.05). Group splitting was performed according to Duncan's test.*

		RIC	RIN	HWEC	HWEN
Water influence	Non-stagnic	67.82±1.60 b	54.55±2.71 b	51.06±2.84	26.39±2.04
	Stagnic	74.03±1.17 a	61.49±2.06 a	55.08±3.54	24.7±2.1

5.4.3 Soil chemical characteristics influencing SOM recalcitrance and lability

The correlation indices between RIC, RIN and different soil parameters that could potentially have an effect on SOC lability are shown in table 5.4. Results are given for all horizons grouped (whole soil), topsoil and subsoil. It can be observed that amounts of recalcitrant C were not affected by any of the studied characteristics. This was not the case for recalcitrant N, which was negatively correlated with aluminium oxalate throughout the whole profile and particularly at soil surface, where Al was the only parameter affecting levels of RIN. Only in subsoil horizons, did increasing amounts of clay contribute to an increase in the recalcitrant nitrogen. For all horizons grouped together, iron oxalate significantly reduced the recalcitrant nitrogen content, although with a weaker correlation than aluminum oxalate.

The labile fraction of C and N were negatively affected by increasing amounts of Clay on the whole soil and subsoil. Labile C was also decreasing with increasing amounts of oxalate Fe when the whole soil was analysed. Exchangable Ca was causing a decrease of the extractable C in all the positions of the profile, and an increase of HWEN when the whole soil was considered.

Table 5. 4. *Correlation indices (r) between RIC, RIN, HWEC (mgHWEC gOC-1), HWEN (µgHWEN gN-1) and several parameters related to the mineral matrix of the soil ç samples: clay content, free iron, free aluminum, and exchangeable calcium. Significant correlations in bold*

	Whole soil				Topsoil				Subsoil			
Parameter	RI_C	RI_N	HWEC	HWEN	RI_C	RI_N	HWEC	HWEN	RI_C	RI_N	HWEC	HWEN
Clay	0.01	0.13	**-0.36**	**-0.22**	-0.21	0.09	0.12	0.11	0.15	**0.2**	**-0.31**	**-0.67**
Free Fe	-0.06	**-0.25**	**-0.28**	0.12	-0.06	-0.10	0.07	0.15	-0.06	-0.10	-0.24	-0.12
Free Al	-0.01	**-0.34**	-0.07	-0.07	-0.05	**-0.41**	0	-0.25	0.07	**-0.31**	-0.24	-0.04
Exch Ca	-0.09	-0.09	**-0.25**	**0.22**	-0.05	-0.08	**-0.45**	0.01	-0.07	0.03	**-0.52**	-0.19

5.5 Discussion

5.5.1 Depth distribution of RIC, RIN, HWEC and HWEN, and its relation to SOC and total N

5.5.1.1 Carbon fractions

The amounts labile fraction (both C and N) and recalcitrant nitrogen are affected by the position within the soil profile, while total amounts of recalcitrant C are maintained at depth. Decreases in HWEC at depth when expressed on a per unit soil basis has been widely reported in a range of soil types and land uses (Ćirić et al., 2016; Yang et al., 2012). This is most probably due to an increase of bulk density in deeper horizons, and a decrease TOC content as a function of depth. Hamkalo and Bedernichek (2014) reported a decrease in the percentage of HWEC as a percentage of TOC in arable lands, while in forest systems, HWEC was maintained throughout the profile. Our results show a significant increase of labile C through the profile in undisturbed grassland soils, with an increase of 20 mgHWEC gOC^{-1} in the subsoil as compared to the topsoil. A similar phenomenon has also been observed in *Sorghum* stands under a Free Air CO_2 Enrichment (FACE) experiment, where increases in labile SOC were observed between 80-100 cm. This was hypothesised to be due to organic matter contributions from deeper roots (Cheng et al., 2007).

Although long extractions with hot water can extract considerable amounts of old SOC, during the first hour these extracts are enriched in young SOC (Balesdent, 1996), which is observed in this study. HWEC is highly correlated with microbial biomass (Ghani et al., 2003; Sparling et al., 1998), and is formed mainly of readily available molecules like carbohydrates, phenols, glucans and lignin monomers originating from biomass, rhizodeposits (Landgraf et al., 2006), and extracellular microbial polysaccharides (Haynes and Francis, 1993). Given that HWEC kills vegetative cells of microorganisms, extracting those as well as non-microbial compounds (Landgraf et al., 2006), is possible that the increase of mgHWEC gOC^{-1} is partially related with an increase of microbial by-products at depth. This is in line with Agnelli et al. (2004), who described an increasing proportion of microbial biomass per g of SOC at depth. However, the elemental analysis of the extracts show that there is an increasing C:N ratio with depth, which indicates that the easily extractable fraction is less microbially processed in deeper horizons than in the topsoil. Considering that part of the HWEC was already dissolved organic carbon (DOC),

the fact that there is an increase of mgHWEC gOC^{-1} at depth, and that the hot water extracts have a higher C:N ratio in deeper horizons might indicate that there is leaching of non-processed SOC throughout the profile. It has been previously shown that DOC can be adsorbed to mineral surfaces, metal oxides and hydroxides. This reduces its rate of mineralisation and is considered a process of SOC stabilisation in subsoil (Kaiser and Guggenberger, 2000; Kalbitz et al., 2005; Lorenz and Lal, 2005). This adsorbance and leaching of SOM with mineral surfaces may be the mechanism taking place in the studied sites, and it is in line with chapter III, where a higher presence of SOC was found to be associated with small aggregate sizes (micro- and silt/clay-associated C) in soils with clay illuviation at depth.

Acid hydrolysis removes the most labile compounds of SOC such as carbohydrates, polysaccharides and simple aromatic C, and leaves a more chemically recalcitrant residue with alkyl-C and aliphatic-C (Silveira et al., 2008). Most research has observed a decrease of SOC recalcitrance with depth (Joergensen and Meyer, 1990; Rovira and Vallejo, 2007, 2002; Tan et al., 2004). However, our results indicate a maintenance in recalcitrant C in the subsoil, which is similar to the results reported by (Montané et al., 2007), where the proportion of recalcitrant carbon was maintained through the profile of mountain grasslands. Although there is a small content of young SOC in the non-hydrolisable pool, in general this is much older than the hydrolysable pool (Leavitt et al., 1996; Stout et al., 1981). A possible explanation for the high presence of non-hydrolysable SOC in subsoil could be the previously mentioned sorption of labile SOC in mineral surfaces and subsequent leaching and stabilisation of SOC down the profile. The leached SOC down to the subsoil is not mineralised, which concurs with the study of Garcia-Pausas et al. (2008), who observed a high presence of SOC available for microbes that was not being used, and concluded that increases in available SOC inputs does not always cause an increment in mineralisation rates.

5.5.1.2 Nitrogen fractions

Nitrogen extractable by hot water represents a relatively labile part of the total N (Curtin et al., 2006). Our results indicate that there is a lower proportion of labile nitrogen in deeper horizons when compared to the topsoil. This could be due to a mineralisation and denitrification of the labile N compounds, but given the high amount or recalcitrant N found at depth, we hypothesise that probably an important stabilisation of labile N compounds is happening in subsoil. Recalcitrant N slightly increases at depth, this being

significantly higher in subsoil than topsoil. However, the increase is not as evident as the trends happening in the labile fraction. Previous research is contradictory, with studies describing a maintenance (Rovira and Vallejo, 2007), a slight increase (Dou et al., 2016) or a decrease (Rovira and Vallejo, 2002) of RI_N at depth. The slight increase of RI_N at deeper horizons may have different causes: one is the fixation of ammonium by clay compounds (Yariv and Cross, 2002), which occurs by replacing the original cations associated with clay minerals for ammonium cations. Indeed, our results show a positive correlation between RI_N and clay in the subsoil. Other mechanisms by which N-containing compounds such as ammonium or amino acids get stabilised in soils is through the association with the C=O groups of lignins, which form stable complexes (Knicker, 2004; Verma et al., 1975; Waksman and Iyer, 1932), or the condensation with phenols or quinones. Both of these types of compounds are derived from lignin degradation (Theis, 1945), or by secondary metabolites of microorganisms in the case of phenols (Haider and Martin, 1967; Waksman and Iyer, 1932).

Knicker et al. (2001) showed that after acid hydrolysis, some labile compounds such as peptides or proteins were not fully hydrolysed. The internal part of big globular proteins such as enzymes may need longer hydrolysis times to allow the acid to access the most internal, hydrophobic part. For this paper we used a short hydrolysis with a low concentration of acid to avoid the formation of artefacts occurring when proteins and polysaccharides react and condense through the Maillard reaction (Allard et al., 1997; Maillard, 1912). Therefore, we hypothesise that a portion of the recalcitrant N is actually formed by amino acids, peptides and proteins that would be destroyed by the acid hydrolysis if they were part of smaller proteins, but have resisted the hydrolysis because they are forming bigger globular proteins that have not yet been broken down by soil microorganisms.

The increase of RI_N and maintenance of RI_C could also be caused by a movement of fresh SOM through the profile. Given that all the studied soils are under undisturbed grassland systems, the high presence of earthworms could be contributing to the movement of SOM through the profile. Earthworms can build burrows meters deep in soil (Benckiser, 1997; Lorenz and Lal, 2005). While building the burrows, earthworms transport fresh SOM from the topsoil into the subsoil (Andriuzzi et al., 2016; Hale et al., 2005). The walls of the burrows made by anecic earthworms get covered with raw and decomposed plant

residues and casts (Nielsen and Hole, 1964), which could be an explanation of the increased presence of the recalcitrant C and N at depth as compared to other studies.

Another possible explanation for the increased proportion of RI_N and RI_C as compared to other studies is the previous land use of Irish grasslands: forestry. The forest cover for Ireland has dropped from 80% 6000 years ago to 11% at present. A 60% of this clearance occurred 1 to 2 thousand years ago, and the 40% occurred within the last 4 centuries. This was caused mainly by the development of farming and the growth of blanket bogs (DAFF, 2008; Teagasc, n.d.). Since rooting depth in temperate forests are much deeper than in temperate grasslands (Jackson et al., 1996), it is possible that part of this recalcitrant C and N at depth is derived from the decomposition of ancient forests roots.

Both the biochemical and physical characterisation (Torres-Sallan et al., under review) indicate that there is a high proportion of recalcitrant SOM in temperate grasslands subsoils, both in terms of physically and chemically recalcitrant C and N. The accumulation of stable SOC in Irish grassland subsoils is possibly caused by the relatively low temperatures (Lloyd and Taylor, 1994; Raich and Potter, 1995) and more importantly a considerable lack of disturbance (Bot and Benites, 2005; Dick and Gregorich, 2004), which cause lower mineralisation rates of the SOC being leached. Nevertheless, this sink of SOC at deeper horizons could become a source of SOC if any change (physical, chemical or biological) occurs in the soil, as it can affect the decomposition, retention or leaching rates of SOC (Kalbitz and Kaiser, 2008). Indeed, changes in soil temperature have a higher effect in the respiration rates of subsurface horizons (Fierer et al., 2003).

5.5.2 Effect of soil type on recalcitrant and labile carbon and nitrogen

Firstly, it is important to highlight that there were not any differences in labile or recalcitrant fractions when only the topsoil horizons were analysed. Again, this indicates that more attention should be paid at subsoil horizons, which agrees with several recent studies indicating the importance of subsoil sampling (James et al., 2014; Rumpel and Kögel-Knabner, 2011; Salomé et al., 2010; Veenstra and Lee Burras, 2015) The most labile fraction of C and N is similar between all soil types. However, the recalcitrant fraction of both C and N does differ between soil types. Recalcitrant nitrogen is only different between soil types when all horizons are included in the analysis. Still, the resulting groups do not follow any clear pattern that can be related to pedogenesis. This is not the case of RI_C, where soils influenced by waterlogging show a higher presence of

RI_C when all horizons are analysed together. However, this is only a trend, and significant differences are only seen between Stagnic Brown Earths, Humic Groundwater Gleys and Typical Surface-water Gleys, which all have significantly higher amounts of RIC than Typical Luvisols and Typical Lithosols. This trend is corroborated when the means of soils with and without stagnation properties are compared, since soils influenced by waterlogging show a significantly higher amount of RI_N and RI_C.

The anoxic to anaerobic properties seem to have an effect on the selective degradation of labile compounds, leaving a more recalcitrant residue. Accumulation of recalcitrant compounds were also found under rice fields, which showed increasing amounts of phenolic compounds in young SOM as anoxic conditions increased (Olk et al., 2002, 1996). Lignins and the phenol compounds contained in lignins are highly resistant to degradation under anoxic conditions (Zeikus, 1980), therefore there is a slower decomposition and a subsequent accumulation of lignin and its phenolic compounds happening under conditions which lack oxygen (Sahrawat, 2003). Anoxic conditions also help to increase the recalcitrant SOM by promoting the formation of stable complexes of SOM and Fe^{2+} (Jansen et al., 2003; Sahrawat, 2003; Schnitzer and Skinner, 1966), and the association of amino acids or other compounds containing organic N with lignin and phenols (Bondietti et al., 1972; J. P. Martin et al., 1980; Sahrawat, 2003), which are more resistant to microbial mineralisation (Sahrawat, 2003).

5.5.3 Soil chemical characteristics influencing SOM recalcitrance and lability

Soil characteristics influencing recalcitrance or lability of C and N in the topsoil were different than those in the subsoil. This may indicate that the dynamics controlling SOM in topsoil are different to those in the subsoil (Salomé et al., 2010). None of the studied parameters seemed to be affecting the amounts of RI_C. Clay content, oxalate extracted Fe and Al were affecting the RI_N contents in different ways and positions within the soil profile. Possibly, the increasing amounts of RI_N with higher clay contents found in the subsoil are due to (i) the formation of clay-ammonium complexes (Yariv and Cross, 2002), which increases its resistance to acid hydrolysis (ii) enhancement of microaggregate formation (Huang et al., 2011; Porta et al., 2010b), which causes an encapsulation of SOM within stable microaggregates and reduces SOM physical availability to soil microorganisms and their enzymes, thus causing a preservation of

SOM from mineralisation (von Lützow et al., 2006). Nevertheless, if this mechanism was the main cause of the increment in RI_N in soils, it would probably have also an influence on RI_C.

The presence of Al and Fe is usually linked to increasing amounts of SOC (Baldock and Skjemstad, 2000; Sombroek et al., 1993). Our results show no correlation between Fe and Al with amounts of RIc. Unexpectedly, increasing amounts of recalcitrant N are affected negatively by Al mainly in topsoil, but also in subsoil and for the whole profile analysed together, where Fe also has a negative influence on RI_N, although with a lower influence. Oxalate extractable Fe is also diminishing the amount of labile C when the whole profile is taken into account. A well-known mechanism by which SOM can reduce its mineralization is its capacity for precipitating by forming organo-mineral complexes (Martin et al., 1966; Muneer et al., 1989). Indeed, many studies have shown a positive correlation between metal content and SOC in different soil types (Hughes, 1982; Johnson and Todd, 1983; Kaiser and Guggenberger, 2000). Many organic salts with Ca, Fe or Al have low solubility (Cronan and Aiken, 1985). Hence, precipitation in a salt form is a way of preservation of the soil labile compounds. Therefore, it is no surprise that increasing amounts of Fe are contributing to decrease the amount of HWEC.

Increasing amounts of Ca have a big influence on decreasing HWEC. As with Fe, Ca is a well-known compound stabilizing SOM (Baldock and Skjemstad, 2000). The association of SOM with Ca is a commonly-known way of protecting SOM against mineralization (Baldock and Skjemstad, 2000), since it decreases the solubility of SOM (Muneer et al., 1989). Humic acid forming organo-mineral complexes with Ca are older, have a slower turnover time (Legorreta-Padilla, 2005) and are more humified (Olk et al., 1996), and less responsive to land use changes than humic acids not associated with Ca (Olk, 2006). The dynamics of HWEN are different to HWEC, since HWEN levels are not affected by Fe and surprisingly, increasing amounts of exchangeable Ca are related to increased contents of HWEN.

While clay content does not affect the amounts of recalcitrant C and N, it diminishes the contents of labile C and N content in both the whole soil and in the subsoil. This is caused by the adsorption of the labile compounds to the clay surfaces, which protects them from microorganisms or their enzymes (Chenu et al., 2002; Christensen, 1996; Mayer, 1994; Six et al., 2002)

5.6 Conclusions

Our results show that the proportion of labile N and recalcitrant C and N are different in the topsoil and subsoil. In fact, there was no distinction for topsoil within soil types in terms of recalcitrant or labile SOM, while RI_C and RI_N were distinct between soil types when analysing the whole profile.

Stagnation properties contributed to generate a greater pool of recalcitrant C and N, probably because of a slowing down of the degradation of lignin, resulting in an accumulation. Results suggest that leaching of SOC and N is occurring down the soil profile with a subsequent stabilisation of these in deeper horizons in temperate grasslands. Nevertheless, part of the fraction accounted as recalcitrant N probably consists of peptides or proteins with high molecular weight. Increasing clay content and exchangeable Ca contribute to diminish the amount of labile C and N, while the recalcitrant nitrogen is being diminished by Fe and Al.

The high proportion of biochemically protected SOC and N is in accordance with our previous study, where we showed that there is an increase of SOC physical protection below 30 cm in the same sites, especially in soils with clay illuviation. Therefore, we conclude that i) The sink of SOC located in deeper horizons should not be ignored when accounting for SOC stocks, as it is an important factor in terms of SOC stabilisation and therefore GHG emissions reduction; ii) the vertical movement of SOC and N through the profile is a real and important phenomenon affecting SOM dynamics and equilibrium, and the factors affecting it should be further investigated.

Chapter VI

Overall discussion, conclusions and future work

6.1 Main findings

This PhD has highlighted a number of important findings, some of which are summarised in figure 6. **1**. The main findings discussed in this chapter are:

- **Topsoil** SOC does not allow distinction between soil types in terms of SOC dynamics. Neither aggregate associated SOC, nor SOC biochemical characteristics show significant differences at this depth (see section 6.1.1, and chapters III,IV,V).
- **Clay illuviation** has an influence on SOC aggregation in deeper horizons, with clay illuviated soils containing a greater proportion of their SOC in smaller aggregate sizes, and a lower concentration of SOC per gram of silt and clay sized aggregates, thus indicating that they are further from the saturation point than soils without clay increase at depth (see section 6.1.2 and chapters III and IV).
- **Stagnic properties** contribute to C and N stabilisation in soils, as shown by the increased proportion of recalcitrant C and N in soils with stagnic properties, and cause a lower transference of SOC from the POM fraction to microaggregates or the silt and clay aggregate fraction. (see section 6.1.3 and chapter IV and V).
- Some results indicate that there is a **vertical movement of SOC** down through the profile: there is a higher presence of SOC associated to microaggregates and silt and clay in the soil type Typical Surface-water Gley when compared with Brown Earths. However, there is also a contrasting lower incorporation of SOC into microaggregates and silt and clay within macroaggregates in Typical Surface-water Gleys. This may indicate that the microaggregates found in deep horizons are actually formed on the topsoil, and leached through the profile (see section 6.1.4 and chapters III, IV, V).

6.1.1 Sampling of topsoil: is it enough?

Chapter III shows that by focusing on the topsoil, we are mainly accounting for the most labile fraction of SOC, since 84% of SOC is located in small and large macroaggregates (>250 μm). The SOC associated to macroaggregates has a higher turnover time, and a lower mean residence time (MRT) than SOC associated to microaggregates and the silt and clay fraction, which are more protected from microbial attack (Denef et al., 2002; Six et al., 2002; von Lützow et al., 2006). Therefore, limiting sampling to the topsoil horizons means analysing the most labile fraction of the profile, and underestimating the more

recalcitrant SOC (i.e. that associated to microaggregates and the silt and clay fraction), since only 16% is associated to these fractions on the topsoil.

All three experimental chapters indicate that by looking only at topsoil (0-30 cm), all soil types have a similar degree of protection against mineralisation, both physical or biochemical. There is no difference in the distribution of SOC within aggregates, nor in the amount of recalcitrant and labile C and N, as shown in chapter III. When focusing on the fractions within macroaggregates, chapter IV shows that the only difference in topsoil is that Typical Brown Earths have an increased percentage of microaggregates within small macroaggregates (mwM) compared to Typical Surface-water Gleys. This seems to be driven by an increased cation exchange capacity due to higher levels of pH. Although there is a higher percentage of mwM, none of the fractions (Particulate organic matter (POM), mwM and silt and clay within macroaggregates (scwM)) show differences in terms of SOC content in fractions per gram of macroaggregate, or SOC per gram of fraction. Once the focus is set to subsoil horizons, or to all horizons from 0 to 100 cm together (where possible), different trends can be seen, and in some cases differences between soil types emerge. Hence, sampling in lower horizons is necessary to elucidate the particularities of each soil types in terms of SOM stocks and dynamics.

This is in line with several studies that point at the importance of studying the characteristics and dynamics of SOM located in subsoil (Diochon and Kellman, 2009; Harrison et al., 2011; Kaiser, 2002). Batjes et al. (1996) concluded that between 46 and 63% of global SOC is located between 30 cm and 1 m. Jobbágy and Jackson (2000) described that 56% of global SOC was located below 1 m depth. In the case of the soils studied in this thesis, 31% of the SOC was located below 30 cm. The dynamics and the factors controlling subsoil SOC are different in subsoil than in topsoil (Salomé et al., 2010), and although SOC located in subsoil has a greater mean residence time (MRT) than that on the topsoil (Rumpel and Kögel-Knabner, 2011), management practices or land use change also have an effect on the subsoil SOC (Don et al., 2007; Guo and Gifford, 2002; Mueller and Koegel-Knabner, 2008; Veenstra and Lee Burras, 2015). Therefore, it is crucial to take this into account in order to understand how different management practices will affect each soil type in the subsoil as well as in the topsoil.

6.1.2 Clay illuviation

Chapter III shows that as depth increases, SOC is located within smaller aggregate sizes, but the distribution of SOC within aggregates is different within soil types: soils with clay illuviation hold an increased proportion of their carbon in microaggregates and silt and clay sized aggregates, in comparison with Brown Earths, which contain more SOC in larger aggregate sizes even at depth. Encapsulation of SOM within aggregates protects it against mineralisation (Elliott, 1986; Moni et al., 2010; Six et al., 1998). The level of protection increases with decreasing aggregate size. This is as a result of (i) lower oxygen diffusion (Sexstone et al., 1985) since anaerobic conditions limit SOM decomposition due to the presence of fewer and slower degradative enzymatic pathways (Davidson and Janssens, 2006), and (ii) limited access from soil organisms to the centre of the aggregates, as a result of the smaller pore size happening in smaller aggregate sizes (Elliott and Coleman, 1988; Kilbertus, 1980). In addition, adsorption to the smaller aggregates or clay and silt sized particles makes SOC unavailable to microorganisms and their enzymes (Chenu et al., 2002). Therefore, encapsulation of SOC within microaggregates and the silt and clay fraction is a mechanism of C sequestration (Denef et al., 2004; Six et al., 2000a). The mean residence time of C in microaggregates has been described as 100 years by von Lützow et al. (2006), and of 209 years on average by Six et al. (2002). Our results show that in soils where clay content increases at depth, a higher proportion of SOC is located in these smaller aggregate sizes with higher MRT associated. Therefore, SOC in these soils is further protected from mineralisation. Taking into account that MRT of both macroaggregates (small and large) and microaggregates of temperate pastures have been shown to have a much higher MRT than other land uses such as tillage systems or pastures from tropical climate (Six et al., 2002), we can conclude that possibly clay-illuviated soils under temperate grassland systems are one of the mineral soils that are further contributing to the sequestration of C over longer periods of time. Clay content is also affecting the biochemical recalcitrance of SOM, as seen in chapter V, where increasing amounts of clay are related to lower contents of labile C and N.

There is an upper limit of C that soils can hold. Once this limit is reached, the capacity of soil to stabilise new C inputs decreases (Chung et al., 2008). Six et al. (2002) proposed a C saturation model in which four SOC pools contribute to C saturation in a hierarchical manner: the first SOC pool that shows saturation is the silt and clay occluded pool,

followed by microaggregate protected, then biochemically protected and finally the non-protected pool (i.e. light fraction or POM). The further a soil is from the saturation point, the greater capacity of sequestering C it has (Stewart et al., 2007). In chapter IV, two of the three soils analysed in chapter III that present clay illuviation at depth were further investigated, this time looking at the compounds forming small macroaggregates (POM, mwM and scwM), and an important finding was met: Typical Surface-water Gleys and Typical Luvisols show a depletion of SOC in their scwM. From our data it cannot be clarified if Typical and Stagnic Brown Earths are or not at their saturation point, but the fact that there is less SOC in the silt and clay fraction in soils with clay illuviation (Typical Surface-water Gley and Typical Luvisol) as compared to none clay illuviated soils, indicates that these are further from their saturation point than the two Brown Earths, which means that they have a higher potential of sequestering additional SOC (Six et al., 2002). This should be further investigated, and the first option that should be looked at is the possible difference in clay mineralogy between the different analysed soils. If this was the case, this could be an explanation of the different capacities of holding SOC in different soils, since clays with different mineralogies have different capacities to adsorb SOC (Bronick and Lal, 2005; Martin and Haider, 1986). For example, Kandites are clay minerals more common in tropical soils, and are formed by one tethraedral and one octahedral sheet (1:1). Their surface area and low cation exchange capacity (CEC) is lower than 2:1 clays such as illites, vermiculites or smectites (Bronick and Lal, 2005; Porta et al., 2010b). Within the 2:1 clays there are also differences in CEC and surface area, which controls the SOC adsorption capacity (Mayer, 1994). Indeed, Chenu and Plante (2006) showed that SOM was predominantly linked to 2:1 clays. Another possible cause of the different SOC concentration in clays of soils with a higher clay content that should be further investigated are differences in Fe oxides within soils. Such a difference would lead to differences in the level of sorbed SOM, and hence, different degrees of protection against microbial degradation (Eusterhues et al., 2005).

In summary, our results indicate that Irish grassland soils with clay illuviation are (i) currently sequestering a larger proportion of their SOC in deep soils, since they are holding a higher part of their SOC in smaller aggregate sizes as compared to Brown Earths. The association of SOC to these smaller aggregate sizes leads to a higher mean residence time of SOC (Six et al., 2002; von Lützow et al., 2006). This is as a result of an increased degree of protection against microbial mineralisation and the subsequent C

loses to the atmosphere (Denef et al., 2001b; Six et al., 2000a) . The results also indicate that Irish grassland soils with clay illuviation have a greater capacity to store more SOC in their smaller sizes, as indicated by the depletion of SOC found in scwM in clay-iluviated soils. If other aspects such as clay mineralogy does not differ between soil types, the depletion of SOC in scwM in clay-iluviated soils may indicate that these soils are further from their saturation point, and therefore that they have a higher C sequestration capacity (Six et al., 2002; Stewart et al., 2008).

6.1.3 Stagnic properties

Stagnation properties have also appeared to be an important factor for SOC distribution within macroaggregates, as well as to the most recalcitrant fraction of SOC and N.

Since macroaggregates are formed by microaggregates held together by POM (Elliott, 1986; Six et al., 2000a), in chapter IV this fraction was further investigated in order to have an idea of the degree of incorporation of new SOC into the smaller fractions. This chapter revealed that when looking at the whole profile, the soil type Typical Surface-water Gley had a lower content of SOC in microaggregates per g of macroaggregate than Stagnic Brown Earths, and less SOC in silt and clay per g of macroaggregate than Stagnic Brown Earths and Typical Brown Earths. The content of SOC in POM per g of macroaggregate was slightly higher, but not enough to show a significant difference. The initial source of SOC in mwM and, in later stages, scwM is POM, which decreases in size and becomes coated by the mineral fraction as a result of the decomposition process (Oades, 1984; Six et al., 1998). This process is enhanced by the sticky mucilage segregated by the decomposers (Guggenberger et al., 1994). Its well-known that anoxic conditions reduce microbial activity (Reddy and Patrick Jr., 1975). Therefore, the reduced proportion of SOC located in mwM and scwM can be due to (i) similar POM inputs, but reduced mineralisation rate of the POM caused by the stagnic properties happening in Typical Surface-water Gleys, or (ii) reduced POM inputs, as a consequence of the limitation of rooting depth caused by the stagnic properties, accompanied by a reduced mineralisation of this POM, which explains both the lower percentage of C in mwM and scwM, and the similar proportion of C in POM found in the TSWG.

This thesis has also demonstrated in chapter V that the proportion of recalcitrant SOC and N is significantly higher in soils that show some kind of stagnic properties. We hypothesise that this is because (i) lignin degradation, as well as the degradation of lignin-

derived compounds such as phenols and quinones is highly resistant to mineralisation under reduction conditions (Bot and Benites, 2005; Zeikus, 1980), which leads to an accumulation of those acid-hydrolysis resistant compounds in the soil. (ii) the association of SOM with Fe^{2+} is enhanced under anoxic conditions, and (iii) the association of N containing compounds such as amino acids with lignin and phenols is also promoted by reduced conditions (Bondietti et al., 1972; Martin et al., 1980; Sahrawat, 2003). All these mechanisms protect SOC against microbial attack (Sahrawat, 2003), which leads to an accumulation of recalcitrant compounds in soils.

The findings of chapters IV and V suggest that stagnation properties are influencing (i) the amount of recalcitrant SOC and N in subsoils, by increasing it in comparison with free-draining soils, (ii) incorporation of C into mwM and scwM, by regulating the inputs and slowing down the mineralisation of POM. These results indicate that a change on the water regime of these soils, for example by draining them, could lead to a modification on the degree of protection and the dynamics of SOC. The impact that this would have on macroaggregate turnover, microaggregate formation and mineralisation of the highly recalcitrant SOC found in temperate grassland soils are unknown, and deserve to be further investigated. While it is true that a slow enough decomposition of the POM leads to the incorporation of POM into microaggregates (Six et al., 2000a), we suggest that in the case that POM inputs were similar, the sudden increase of oxygen caused by draining would cause a peak of mineralisation of SOC, since SOC breakdown is faster through aerobic decomposition (Alexander, 1961). Nevertheless, it is also possible that POM inputs increase as a result of an increase of root length. In this case, it should be further investigated is this increases in POM would be enough to offset the C loses caused by the increased mineralisation rate that would probably happen in drained soils, and the consequences that this would have in terms of C inputs into the smaller aggregate fractions.

As mentioned above, one of the possible reasons for the higher recalcitrant SOC and N content found in waterlogged soils is the slower decomposition of lignin occurring under anaerobic conditions (Bot and Benites, 2005). An increase of the oxygen availability as a consequence of the installation of drainage in the fields would enable microorganisms to decompose this fraction, thus reducing the percentage of both total and recalcitrant SOC. Both phenomena could in turn contribute to C emissions to the atmosphere, and to a reduction of the total and protected SOC stocks.

6.1.4 Vertical movement of Carbon

Results of chapter III indicate that the soil type Typical Surface-water Gley, which has clay illuviaton and stagnation properties, has a high percentage of its carbon located in the free microaggregates as well as in the silt and clay free fraction. Free microaggregates are firstly formed within macroaggregates, and then released when the POM is too small to keep the macroaggregates together (Elliott, 1986; Six et al., 2000a). Therefore, it would be logical to think that an increased presence of SOC within microaggregates and the silt and clay fraction is a consequence of an increased incorporation of C into microaggregates when these are formed in the core of macroaggregates. Despite this, when looking at the results of chapter IV, it can be seen that soil type Typical Surface-water Gley has a lower content of SOC in mwM and scwM than the Brown Earth per gram of macroaggregate. This indicates that the incorporation of SOC in mwM and scwM is lower in Typical Surface-water Gley, which arises a question: Where does the high percentage of free microaggregate and silt and clay associated SOC come from in these soils? Our hypothesis is that there is a vertical movement of SOC down the profile: the microaggregates are formed in the topsoil and leached down the profile through macropores. The corroboration of this hypothesis, and the rate at which this is happening should be further investigated. Other studies have shown a vertical movement of SOC associated through the profile (Kaiser and Kalbitz, 2012; Rumpel and Kögel-Knabner, 2011), and transport of SOC as part of organo-mineral complexes suggesting as a possible SOC source in Alisols, Luvisols, Acrisols and Lixisols (Rumpel and Kögel-Knabner, 2011).

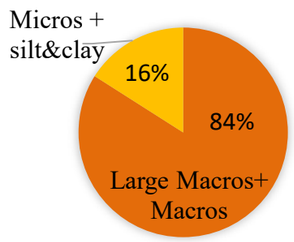
Another factor that might be indicating that there is a vertical movement of SOM through the profile is the increased presence of both recalcitrant SOC and N in deeper horizons. Results of chapter V indicate that: (i) Recalcitrant SOC is not affected by the position of the profile, but is maintained in deeper horizons, which is in contrast with most of the research indicating that at depth, recalcitrant SOC diminishes (Joergensen and Meyer, 1990; Rovira and Vallejo, 2007, 2002; Tan et al., 2004). Montané et al. (2007) also showed a maintenance of RI_C and an increase of RI_N through the profile of mountain grasslands, which they hypothesised to be a consequence of increased root exudates or leaching of labile nitrogenous compounds from litter. (ii) There is an increase in hot water extractable SOC at depth, which is the most labile SOC fraction and is highly correlated with microbial biomass and rhizodeposits (Landgraf et al., 2006). (iii) Labile N is

decreasing at depth, which results in an increased C:N ratio of the hot water extracts in deeper horizons. All these three points might be indicating that there is a leaching or movement of SOM through the profile: Firstly, the high amounts of recalcitrant SOC and N in the subsoil could be partly caused by the effect of burrowing by earthworms. This causes a movement of fresh SOM from the topsoil, and transports it into the subsoil (Hale et al., 2005; Lorenz and Lal, 2005). Second, while a decrease of the labile fraction of N at depth might be partly related to a mineralisation of the SOM that is being leached down, the fact that the increase of labile N is accompanied by an increase in recalcitrant N in deeper horizons might indicate that is also due to an adsorption of N compounds to mineral surfaces (Yariv and Cross, 2002), or the association with lignins, phenols or quinones (Knicker, 2004; Waksman and Iyer, 1932).

These results have several implications, and create many new questions, which are analysed in the sections; Conclusions and Future Work.

68,9% of SOC in profile

% SOC in fractions

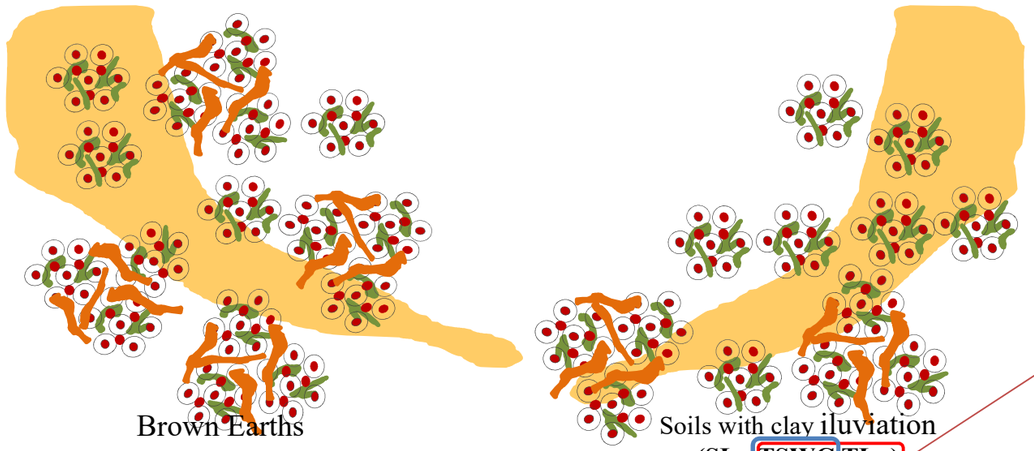


No ≠ between soil types in:

- Aggregate Distribution
- Fractions associated to macroaggregates
- Biochemically recalcitrant or labile SOC and N

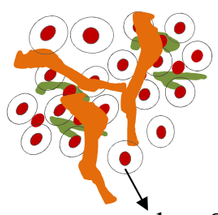
TBE:

+ mwM than TSWG
= C in fractions



Brown Earths

Soils with clay iluviation
(SLu TSWG TLu)



less SOC by g of macroaggregate in mwM than SBE

less SOC by g of macroaggregate in scwM than SBE and TBE

n findings of this thesis.

\+

Labile N (HWEN)

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Recalcitrant N (RIN)

Labile C (HWEC)

TBE > TSWG TLu

C enrichment scwM

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6.2 Conclusions

This thesis has demonstrated that SOC fractions in topsoil (0-30 cm) samples does not indicate any differences between soil types in temperate grasslands (except a higher percentage of microaggregates within macroaggregates in Typical Surface-water Gleys compared to Typical Brown Earths). Analyses from subsoil samples shows different trends in both the physical, biochemically protected and labile fractions. This research suggests that the current sampling recommendations given in the Good Practice Guidance for the Land Use, Land Use Change and Forestry document from the IPCC (IPCC, 2003) of sampling the surface 30 cm only, in order to account for SOC stocks and changes should be revised. Factors driving SOM dynamics on the topsoil are different than the subsoil (Salomé et al., 2010). This thesis has demonstrated that when looking at the whole profile up to 1 m depth (where possible) there are differences between soil types in terms of SOM dynamics and protection against mineralisation. Therefore, sampling in deeper horizons is necessary in order to understand individual SOM dynamics associated to each soil type, and assess how changes on the management that would primarily affect topsoil, could affect subsoil on a longer term.

The research carried on in this thesis indicates that there is a high presence of recalcitrant SOC and N in subsoils of temperate grasslands, and that there seems to be potential for further long term storage, especially in soils that undergo clay illuviation. Management practices to keep and increase this pool should be applied. Our results indicate that part of the high recalcitrant content in deeper horizons may be due to the reducing conditions happening in some of soils under temperate grassland systems, which directly points at the installation of drainage systems in grasslands.

These findings have become of special importance now that the Milk quota has been abolished. The milk quota was a tax established by the European Comission in 1984, in order to control milk production, as a response to the over-production in Europe. It consisted on the payment of a levy when the producers exceeded a certain volume of milk (or fat content in their dairy products) (DAFM). On April 2015, this quota expired and now farmers can produce more milk without having to pay any fees. The optimum stocking rate for a dairy farm depends on the level of grass production, therefore, in order to increase the number of dairy cows, an increase of the productivity is needed. In temperate climates with high precipitation like Ireland, this is mainly achieved through

increasing soil fertility and draining soils, amongst others (Teagasc, 2015). This suggest that in the upcoming years draining of soils in which fertility or traffickability is limited by stagnic conditions will increase, we propose that while this may result in an increase in local productivity, it will have a consequence for decreasing SOC stocks. Until now, the increase of C sequestration in the LULUCF sector could not be used to offset the emissions of CO_2 when accounting for the GHG reduction targets set by the European Union Climate and Energy Package 2020 (European Comission, 2009). This is now going to change for the period 2020-2030, after the European Comission proposal made in concordance with the EU commitments in the Paris Agreement on climate change (European Comission, 2016). The new proposal requires that each Member State of the EU compensates the accounted CO_2 emissions from one land use, with an equivalent removal of CO_2 from the atmosphere in the same sector, which is known as "no debit rule". If a Member State removes more CO_2 from the atmosphere than their own commitment, this can be sold to other Member States. Therefore, specially in this new frame, sequestering C from the atmosphere is of economical importance as well as environmental.

We suggest some incentives should be given to the farmers to avoid the instalation of draining systems in more vulnerable soils, or the soils more capable of sequestering C, in order to preserve and increase this "hidden" SOC pool (O'Sullivan et al., 2015; Schulte et al., 2016).

6.3 Future work

Although this thesis has elucidated serveral useful characteristics of temperate grasslands in relation mainly to SOC, but also to N, it has also raised many new questions that are worth highlighting for future research:

- Effect of drainage on the microaggregate fractions: There is less incorporation of POM into microaggregates and the silt and clay fraction within macroaggregates in Typical Surface-water Gleys. Our hyphothesis is that this is a consequence of the reducing conditions caused by waterlogging in this soils. It would interesting to investigate if the increased concentration of oxygen would lead to a sudden mineralisation and loss of the POM fraction, or if by contrary it would lead to a

slow mineralisation, which would result into a translocation of most of the SOC located in POM into the smaller fractions.

- Effect of drainage on the recalcitrant SOC and N fractions: Our results show that there is a higher presence of recalcitrant SOC and N in soils with stagnation properties. The effect that a sudden increase of oxygen would have in these fractions should be further investigated under field conditions. Our hyphothesis is that this would enable the mineralisation of lignin, and decrease the association of N compounds with lignins or Fe, which would enable its mineralisation and consequent losses to the atmoshpere. Since installation of drainage systems is a common practice in Irelandand is very likely going to increase in the near future, we suggest that these first two points should be investigated.
- Isotope analysis on the fractions: While this thesis has been useful to have a picture of the current situation of the studied soils, it is worth investigating at which rate SOC is being incorporated into the different aggregate fractions. In other words, at which rate is C sequestration happening. To do this, it is necessary to use isotope dating analysis.
- Assess the effect of different management practices under grassland systems on the aggregate associated SOC. This is actually ongoing, as a result of a collaboration with the Soil Quality Asessment Research Project (SQUARE project), which is a national Irish project investigating the management effects of management practices on grasslands and tillage soils, focusing on different aspects: soil nutrients, biology, water infiltration, structure and C sequestration. With the results of this colaboration , the effect of different management practices such as grazing intensity or stocking rate affect the SOC distribution within macroaggregates .

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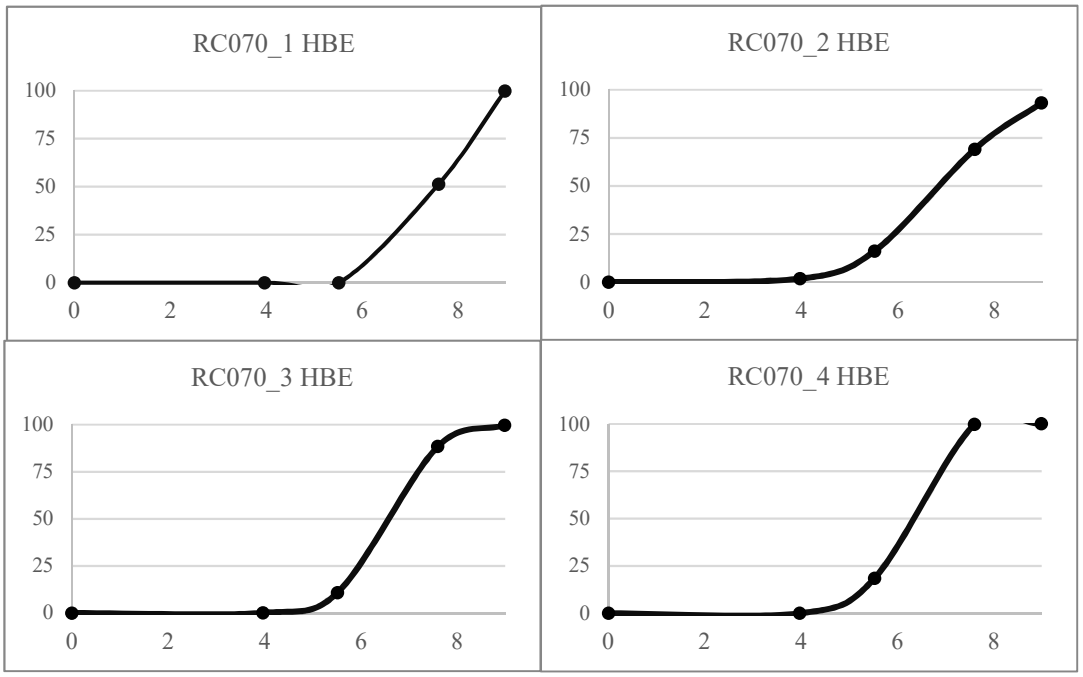
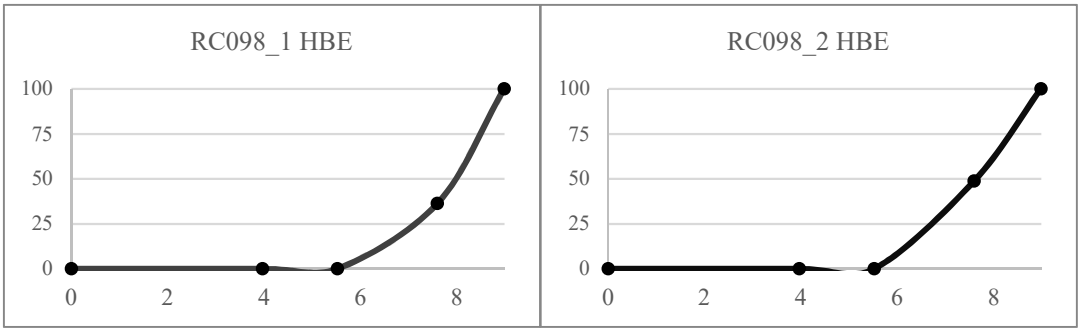
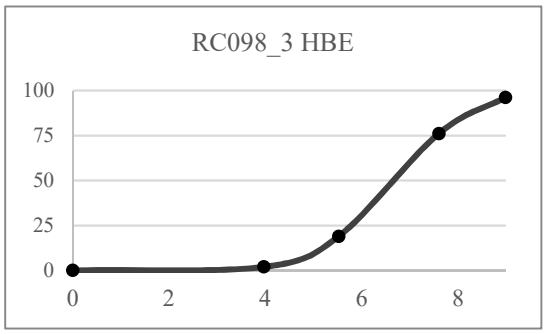
Apendix

The following figures plot some of the data of chapter III. In the figures, the accumulative SOC distribution within aggregate sizes is plotted. For each graph, the title indicates the profile number (E.g: RC070), followed by an underscore and the horizon number (e.g:_1), and the abbrevation for the soil type (HBE=Humic Brown Earth, TBE=Typical Brown Earth, SBE=Stagnic Brown Earth, TLu= Typical Luvisol, SLu= Stagnic Luvisol, TSWG=Typical Surface-water Gley). For all the presented graphs, the x axis is the natural logarithm of the aggregate sizes, and the Y axis is the accumulated percentage of SOC. For each graph, the "m" value can be extrapolated by checking the x value in the fitted curve where y value is 50.



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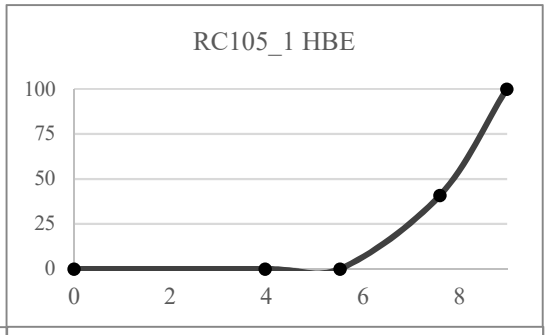
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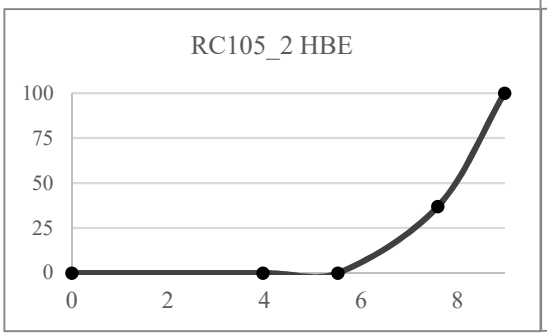
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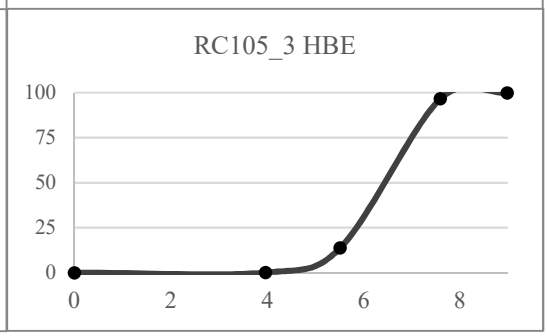
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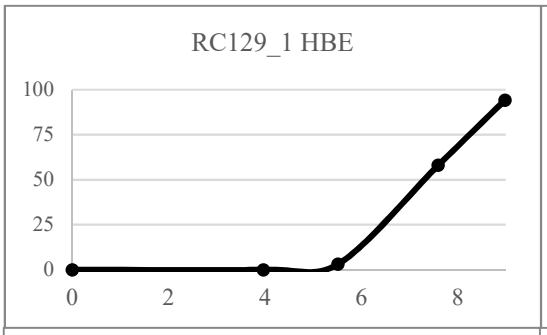
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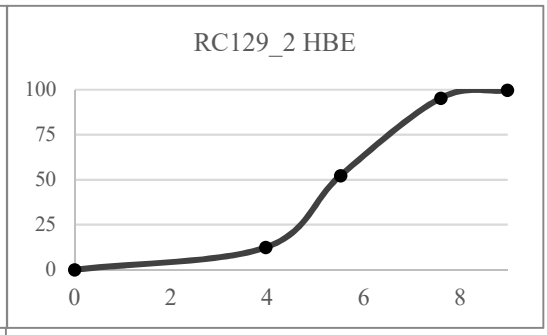
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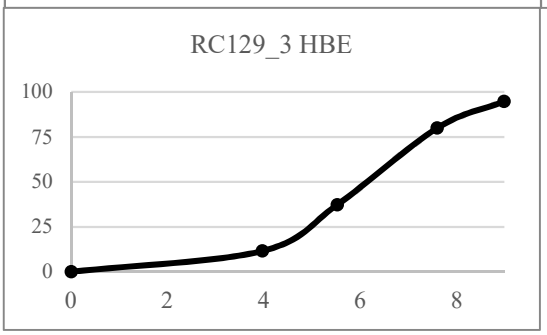
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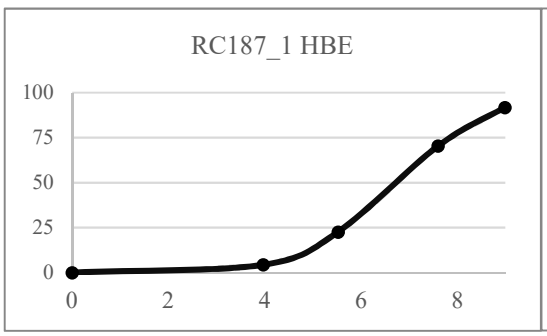
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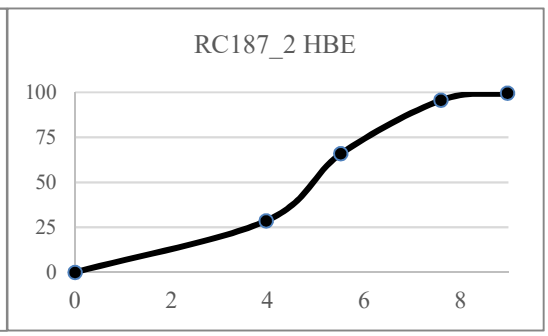
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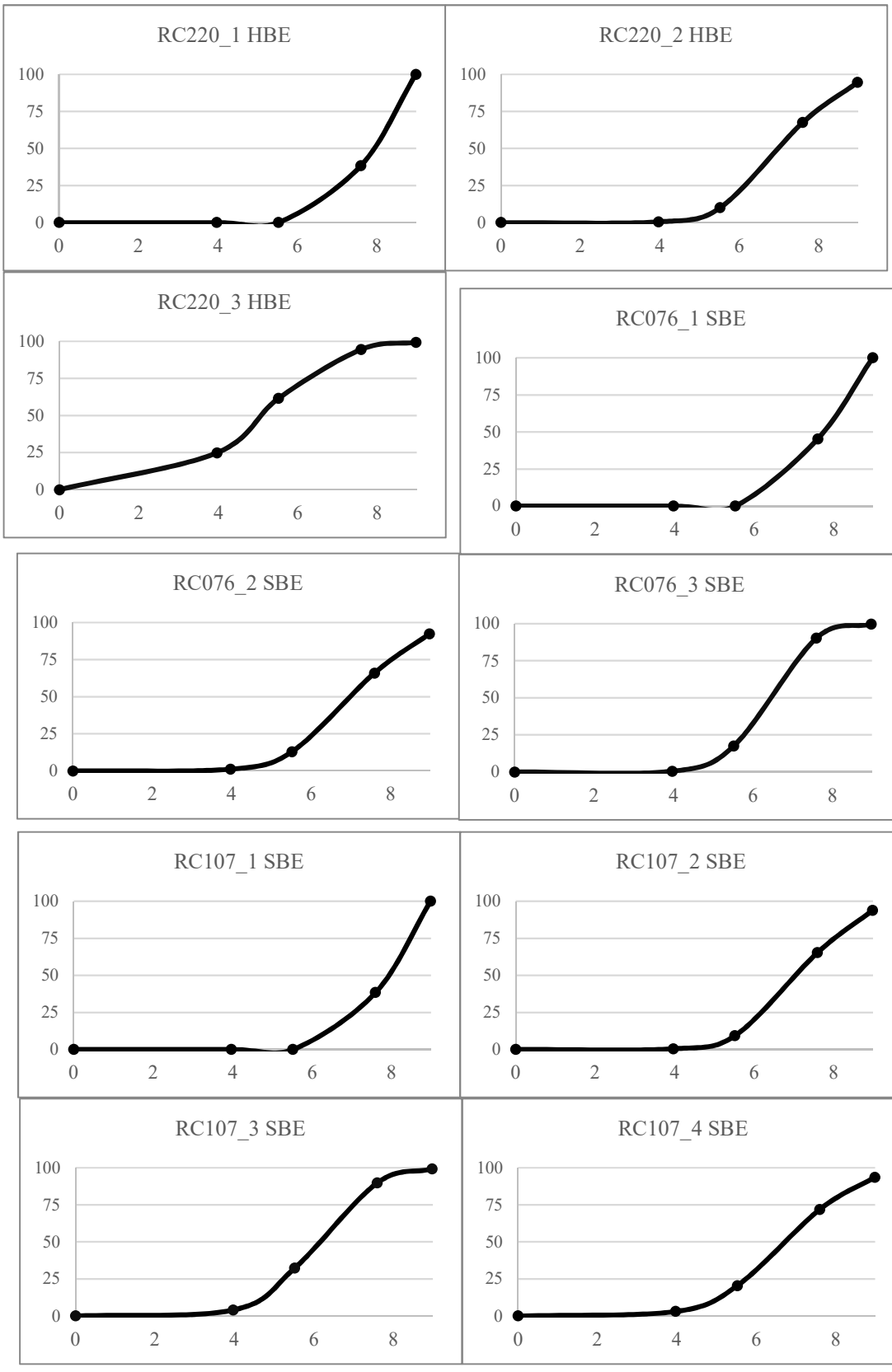
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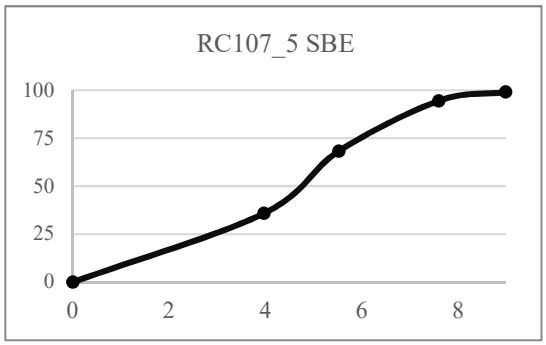
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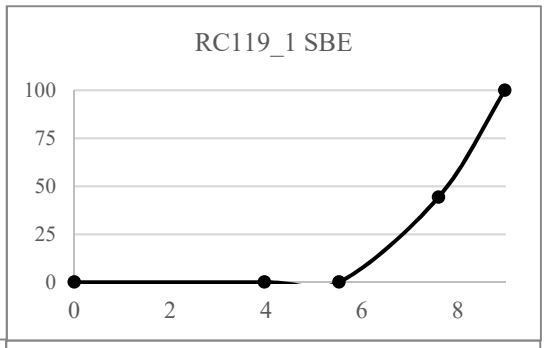
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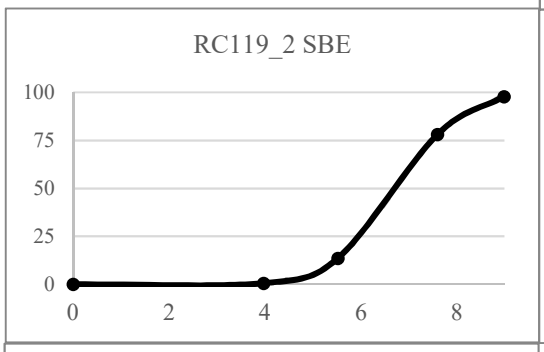
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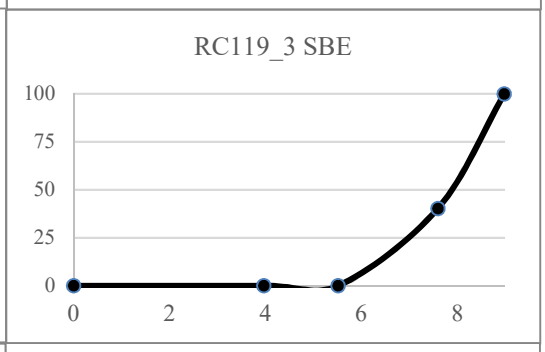
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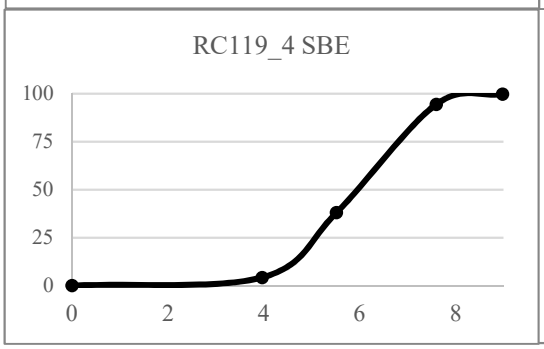
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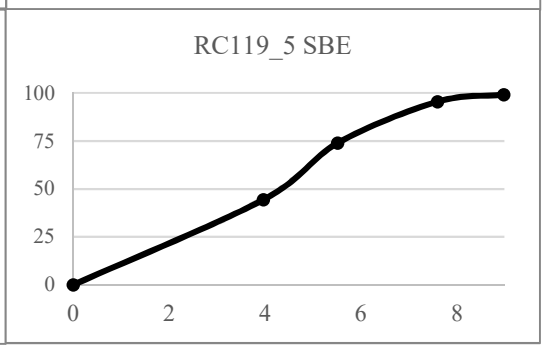
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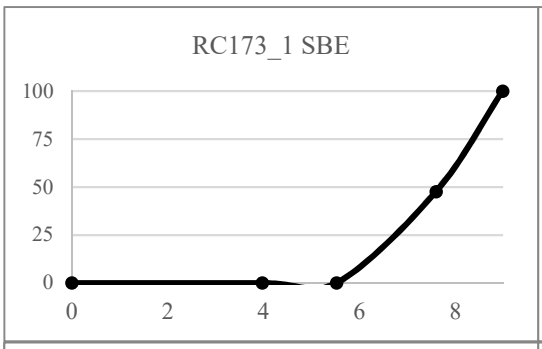
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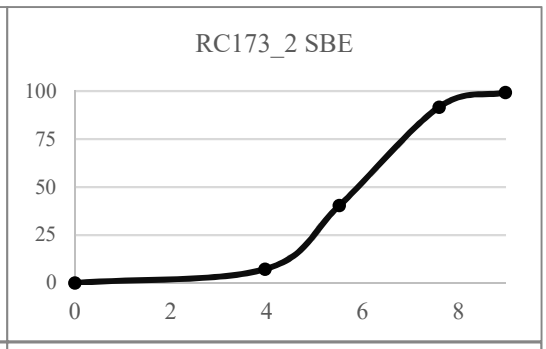
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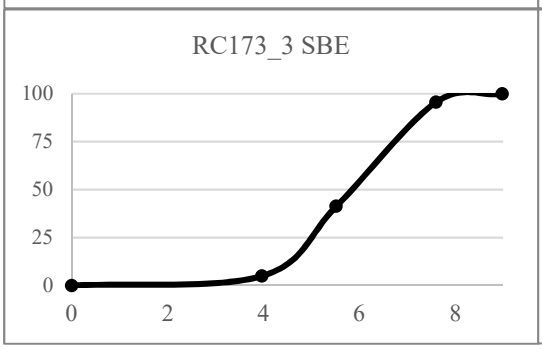
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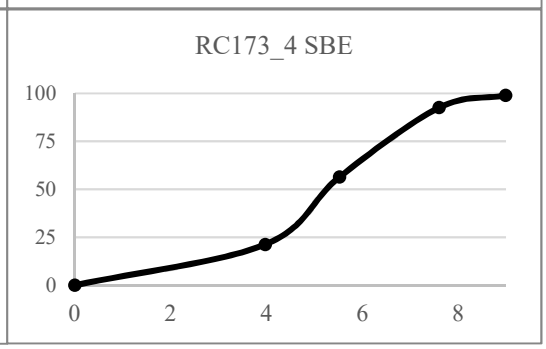
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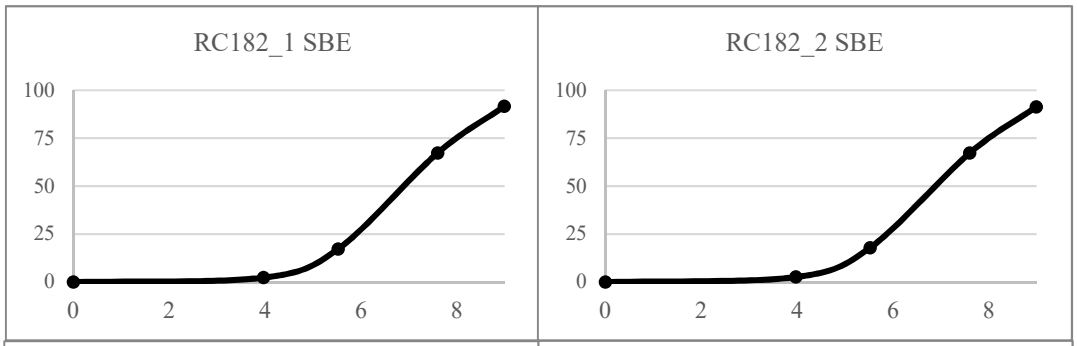
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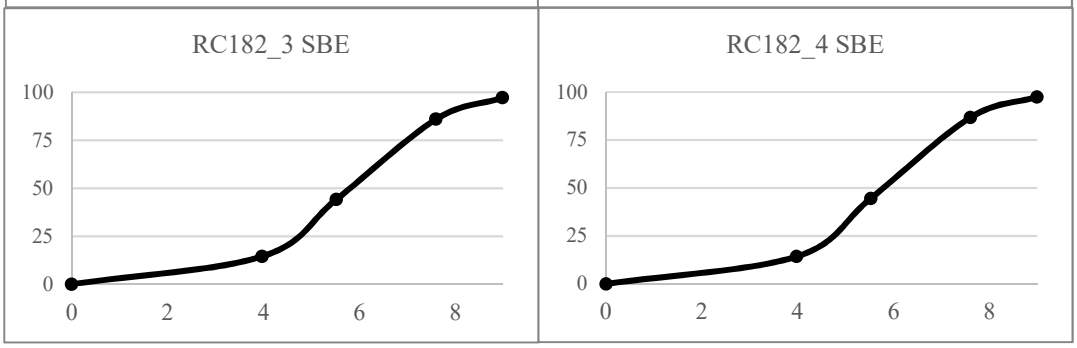
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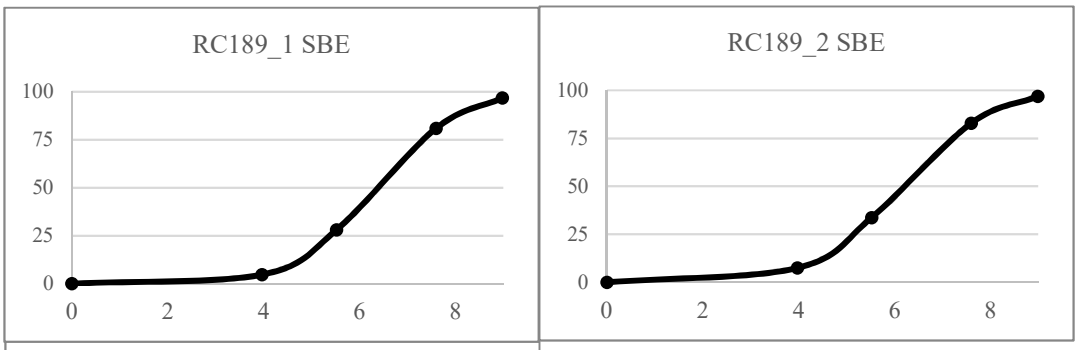
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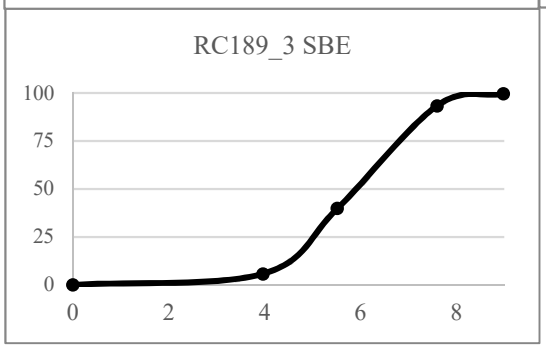
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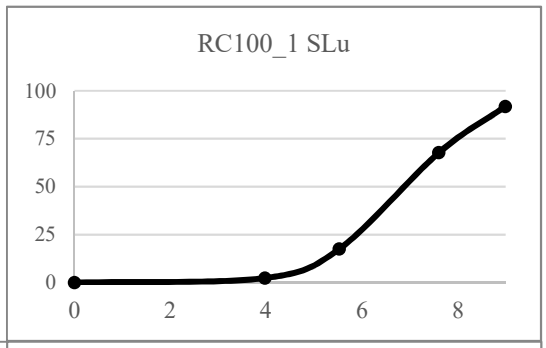
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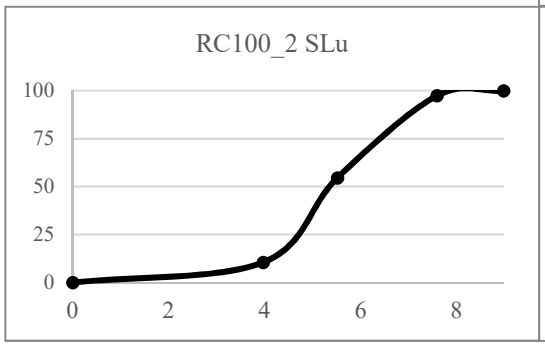
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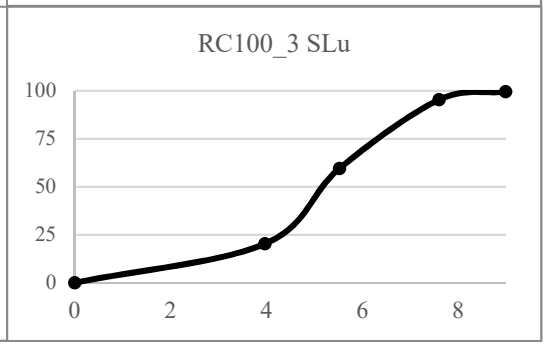
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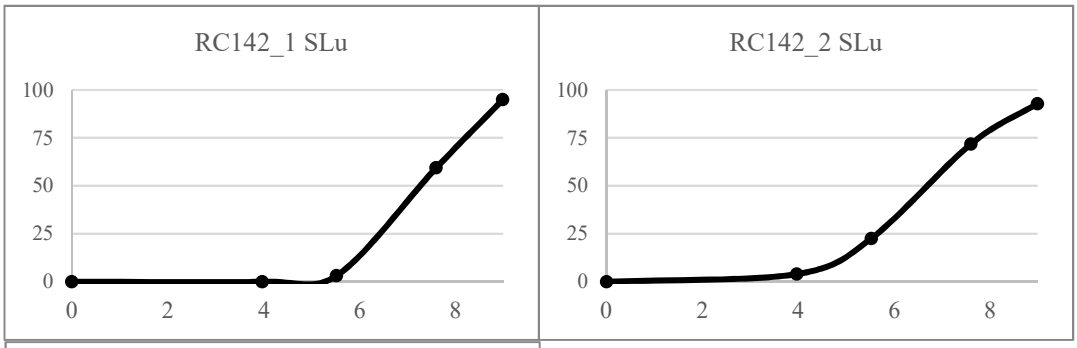
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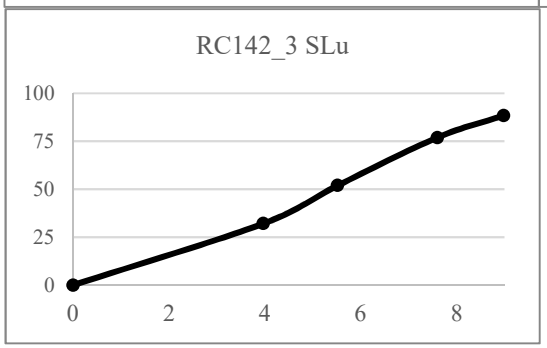
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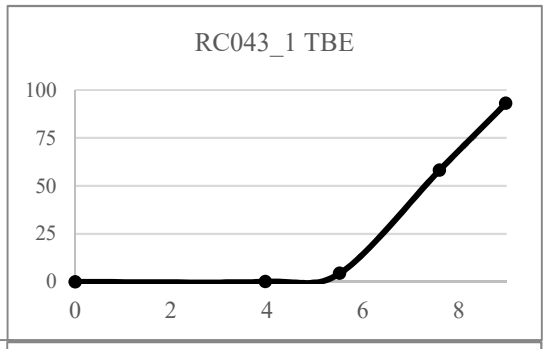
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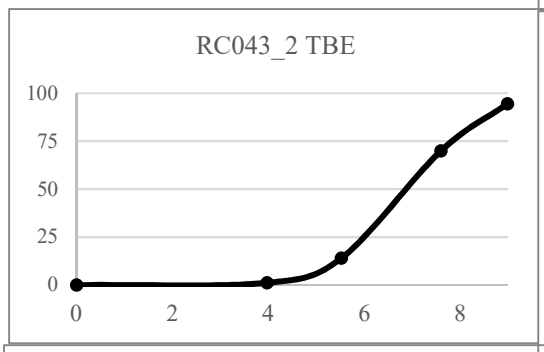
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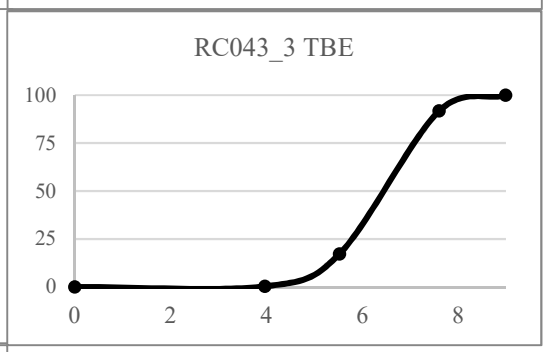
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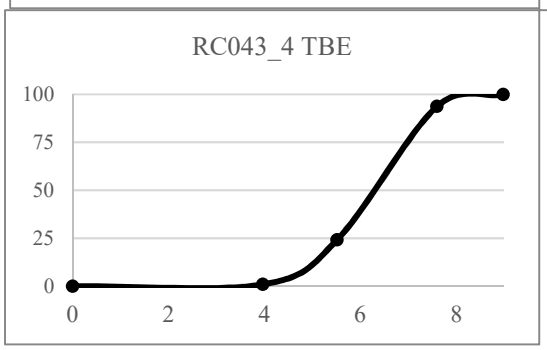
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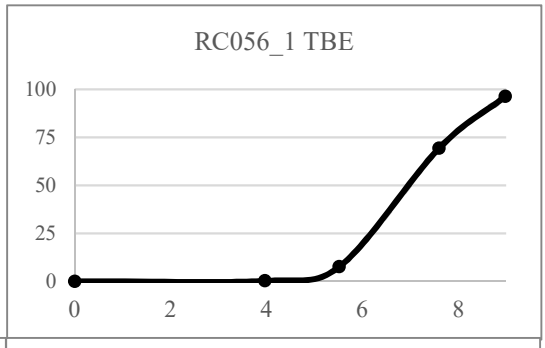
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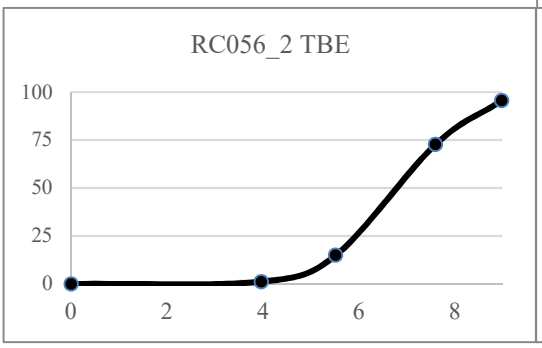
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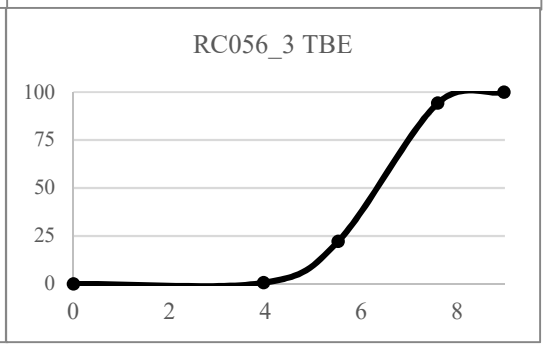
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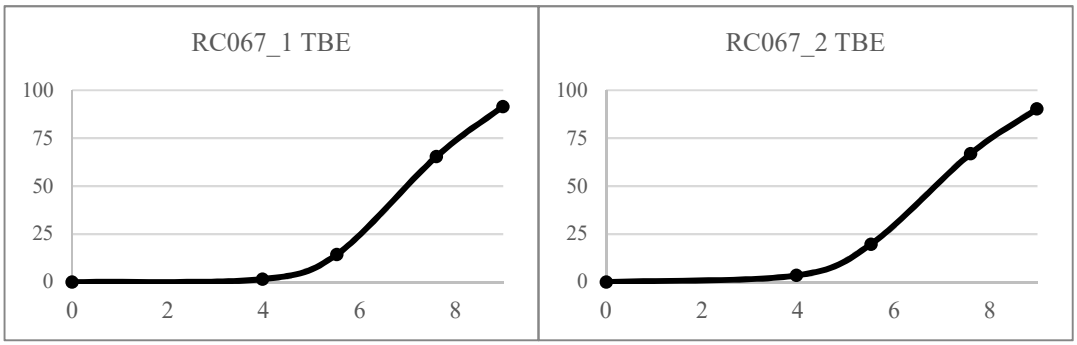
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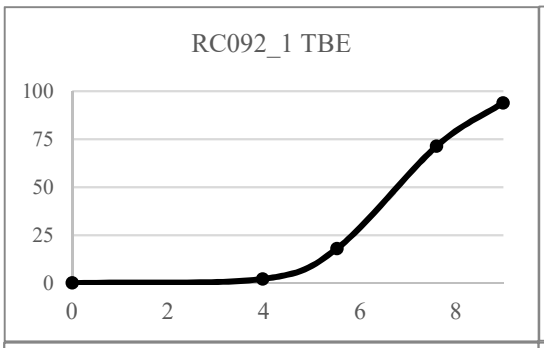
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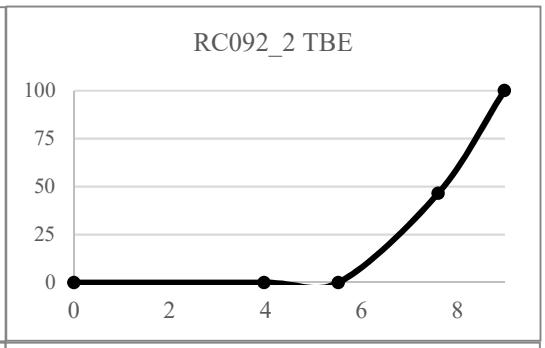
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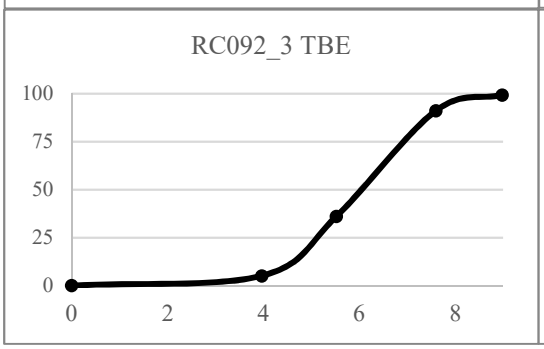
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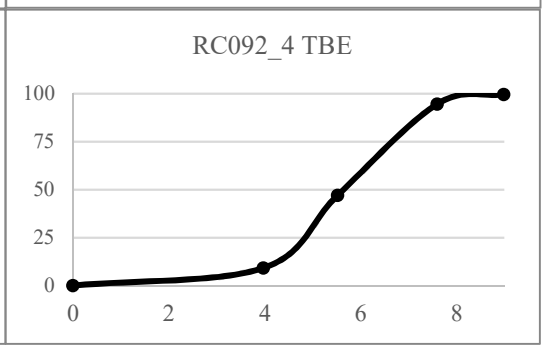
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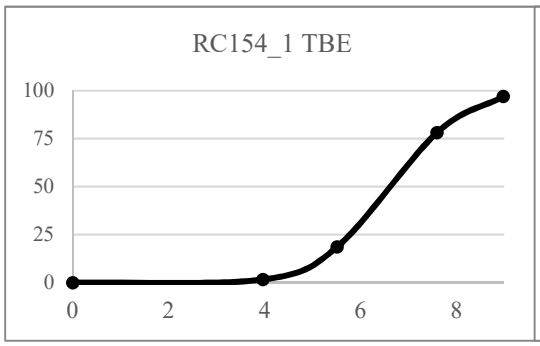
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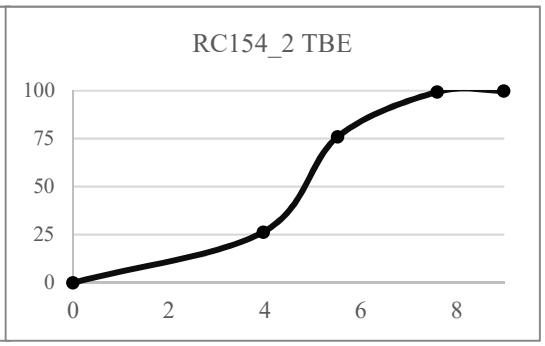
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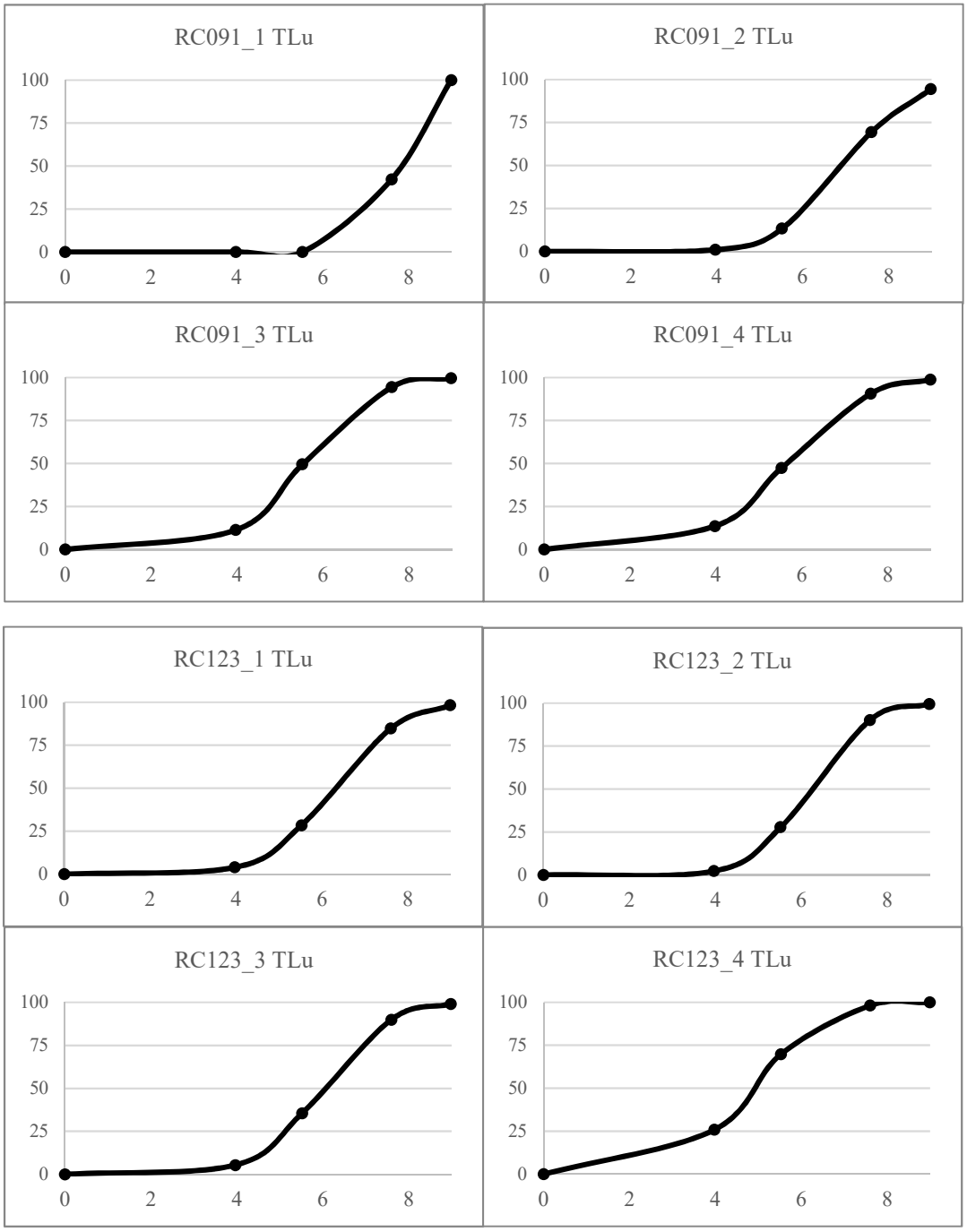
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RC091_2 TLu
RC091_3 TLu
RC091_4 TLu
RC123_1 TLu
RC123_2 TLu
RC123_3 TLu
RC123_4 TLu
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2
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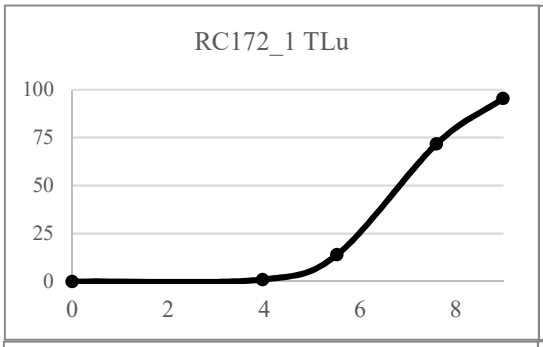
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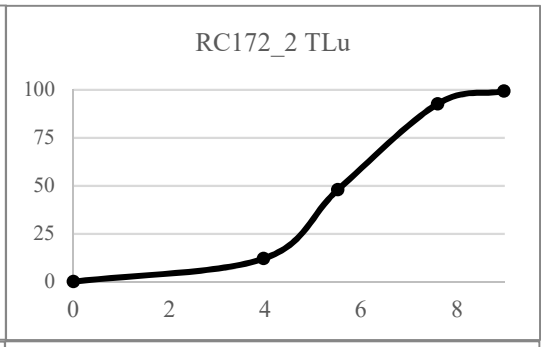
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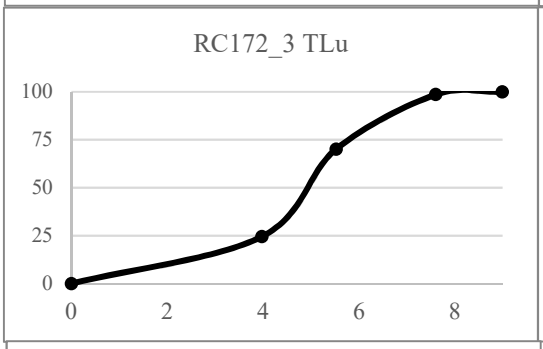
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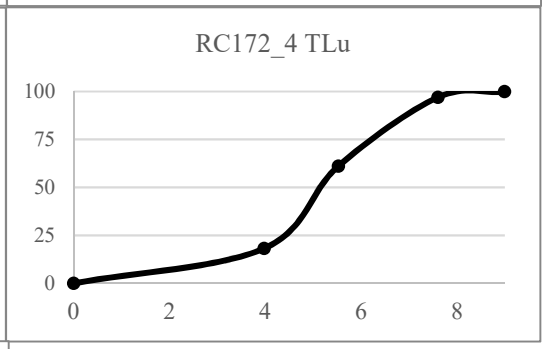
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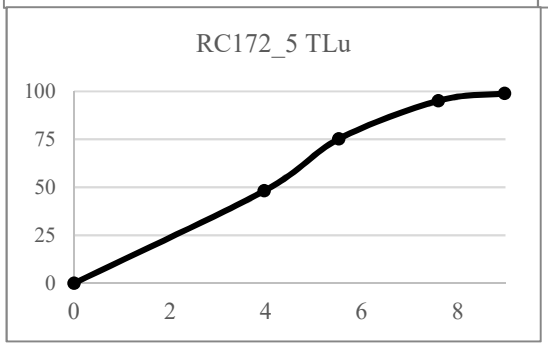
RC172_5 TLu
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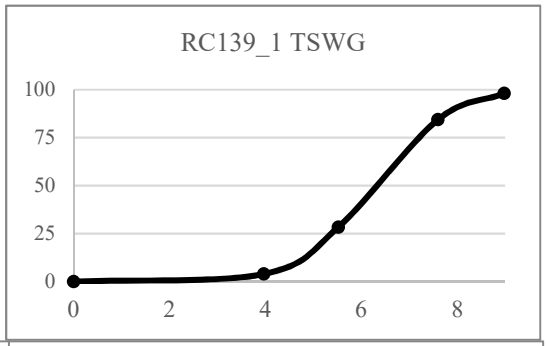
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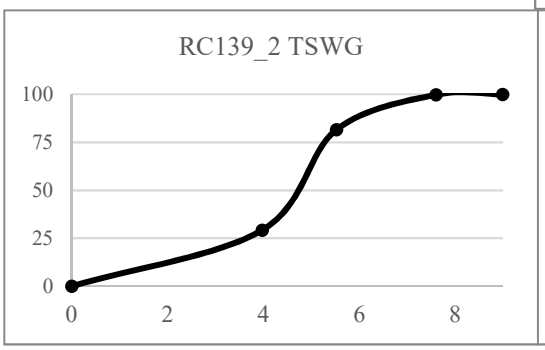
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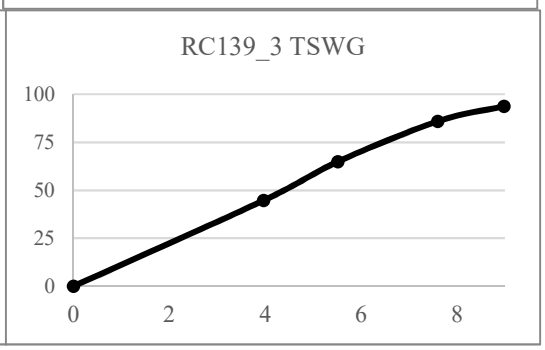
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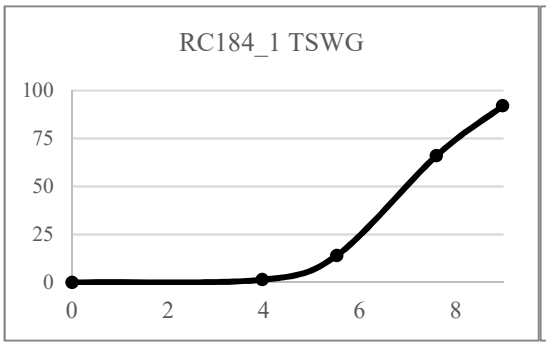
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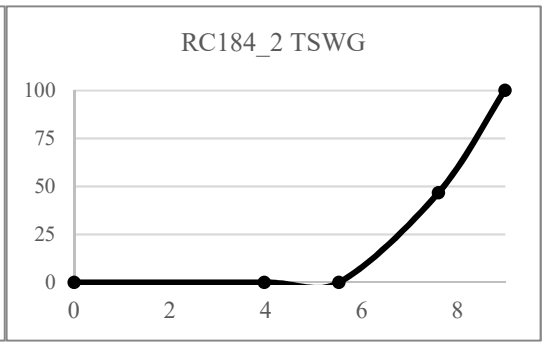
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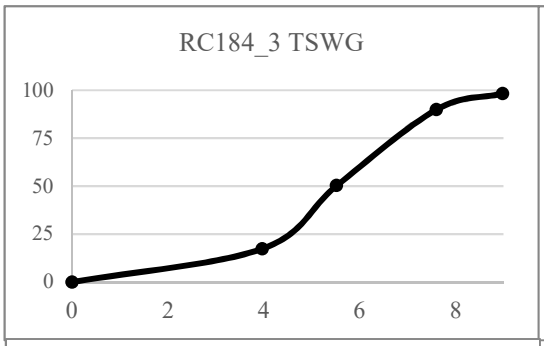
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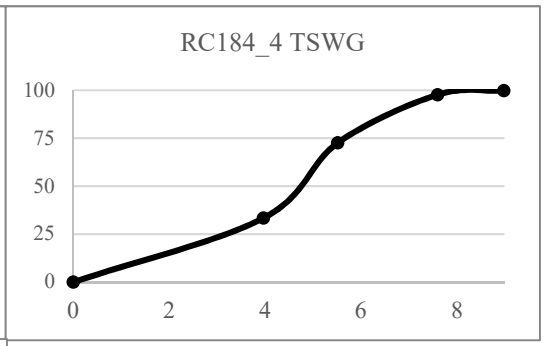
RC184_4 TSWG
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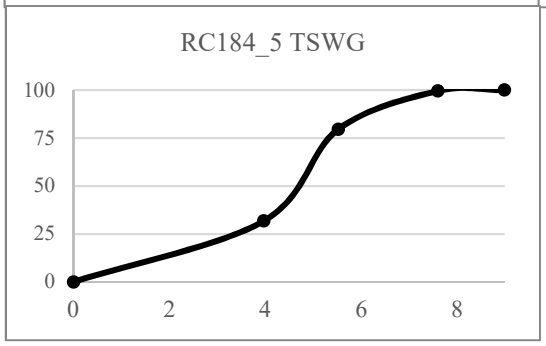
RC184_5 TSWG
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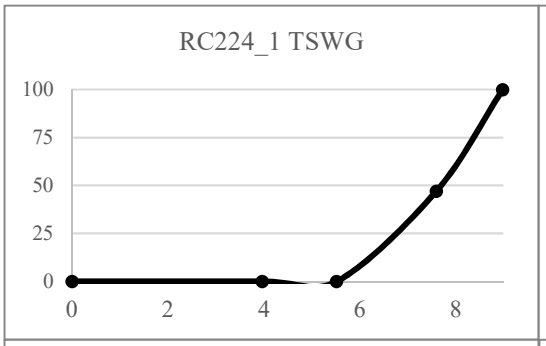
RC224_1 TSWG
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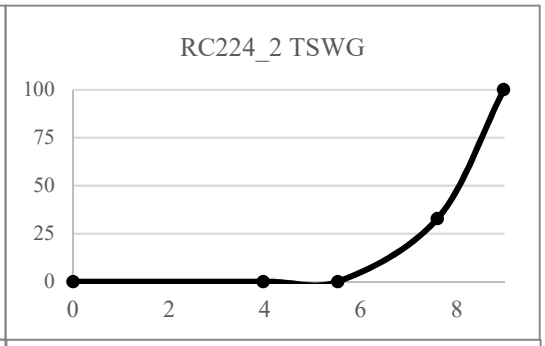
RC224_2 TSWG
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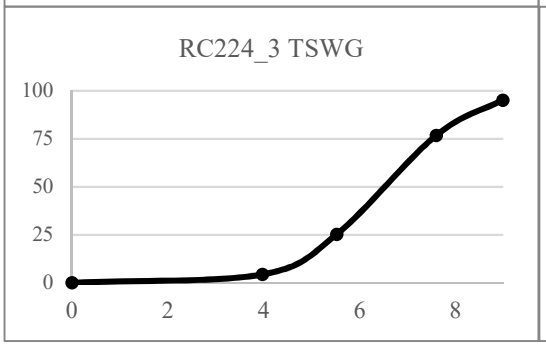
RC224_3 TSWG
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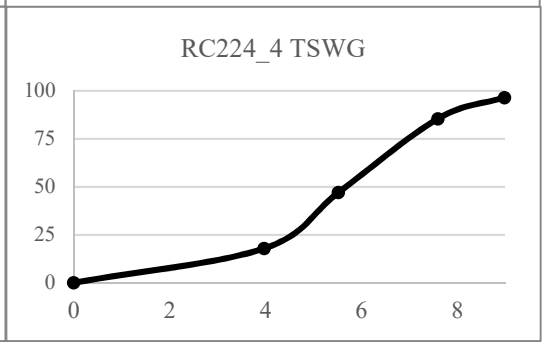
RC224_4 TSWG
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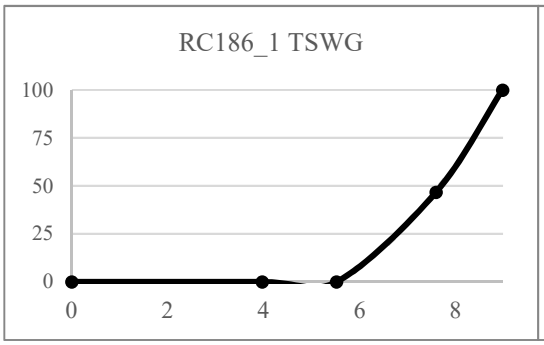
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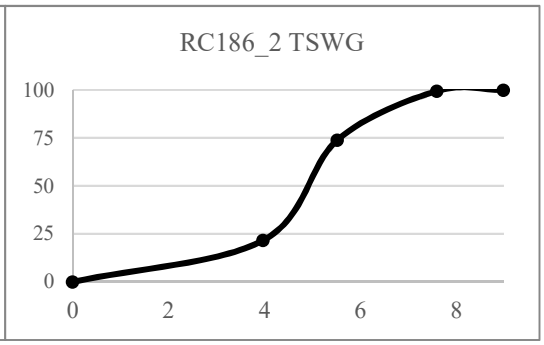
RC186_2 TSWG
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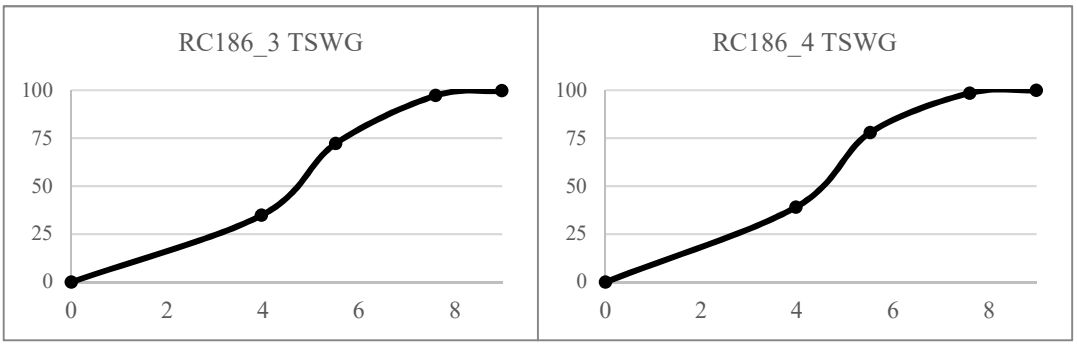

The following data corresponds to the regressions presented in chapter IV, but not shown in there:

Apendix table 1. Coeficients of determination between microaggregates within macroaggrestes (mwM) and TOC, CEC and pH in the topsoil of Typical Surface-water Gley

	R^2	p value
TOC	0,34247914	0,18
CEC	0,48636	0,0035
pH	0,05131164	0,27

Apendix table 2. Differences between soil types on the C distribution within small macroaggregate fraction, of the topsoil samples. Different letters indicate siginificant differences. Group Spliting was performed according to Duncan's test, in log transformed data (original values are displayed on the table)

	mg C in POM/ g small macroaggregate	mg C in microaggregate/ g small macroaggregate	mg C in silt+clay/ g small macroaggregate
SBE	3,18 a	20,33 a	14,50 a
TBE	2,75 a	17,57 a	12,36 a
TLU	2,88 a	13,65 a	10,23 a
TSWG	7,00 a	20,34 a	13,42 a
p value	0.0649	0,287	0,103

Apendix table 3. Differences between soil types on the C distribution within small macroaggregate fraction, of all horizons grouped together. Different letters indicate siginificant differences . Group Spliting was performed according to Duncan's test, in log transformed data (original values are displayed on the table).

	mg C in POM/ g small macroaggregate	mg C in microaggregate/ g small macroaggregate	mg C in silt+clay/ g small macroaggregate
SBE	1,79 a	10,46 a	8,61 a
TBE	1,92 a	11,37 a	9,11 a
TLU	1,91 a	7,23 a	6,27 a
TSWG	4,01 a	8,47 a	6,27 a
p value	0.258	0,166	0,127

Apendix table 4. Differences between soil types on the percentage of SOC in small macroaggregate fraction, of topsoil samples. Different letters indicate siginificant differences . Group Spliting was performed according to Duncan's test, in log transformed data (original values are displayed on the table).

	SOC in POM+SAND (%)	SOC in mwM (%)	SOC in scwM (%)
SBE	1,73 a	4,08 a	4,82 a
TBE	1,07 a	4,10 a	4,55 a
TLU	1,41 a	3,11 a	3,22 a
TSWG	2,74 a	4,87 a	4,35 a
p value	0,572	0,32	0,127

Apendix Table 5. Data analysed for each horizon on each profile. Source: Irish Soil Information System

Reference number	Profile nº	Horizon	Na	K	Mg	Ca	Base Saturation (%)	Cation Exchange Capacity (cmol kg^{-1})	N (%)	C (%)	Org. C(%)	pH	Fe (g kg^{-1}) Oxalate	Al (g kg^{-1}) Oxalate	Sand % w/w	Silt % w/w	Clay % w/w	Bulk Density g/cm3
RPT01BR01	RC100	1(Ap)	0,08	0,22	3,05	8,35	Sat.	10,14	0,215	2,65	2,10	6,4	5,75	1,53	50	31	19	1,23
RPT01BR01	RC100	2(Btg)	0,09	0,54	3,70	8,97	Sat.	6,38	0,105	0,87	0,57	6,6	6,12	1,78	43	36	21	1,47
RPT01BR01	RC100	3(BCtg)	0,08	0,16	2,19	4,78	55	13,12	0,056	0,31	0,23	6,6	4,21	1,78	6	51	43	1,47
RPT17BR02	RC042	1(Ap)	0,09	0,44	3,08	15,49	94	20,33	0,501	5,07	3,80	6,8	13,21	4,07	32	36	32	0,83
RPT17BR02	RC042	2B	0,08	0,13	1,18	4,86	96	6,53	0,159	1,52	0,79	7,0	7,83	4,96	57	32	11	
RPT28BR01	RC043	1(Ap)	0,11	0,80	2,41	8,85	85	14,29	0,498	5,08	3,94	5,9	10,83	4,24	44	28	28	0,79
RPT28BR01	RC043	2(AB)	0,12	0,25	1,11	7,53	84	10,79	0,293	3,14	2,01	6,1	12,33	6,14	40	38	22	
RPT28BR01	RC043	3(Bt)	0,08	0,04	0,35	3,19	85	4,30	0,101	1,10	0,58	6,3	6,91	4,52	53	32	15	
RPT28BR01	RC043	4(BC)	0,08	0,03	0,21	2,39	76	3,54	0,062	0,72	0,34	6,4	4,07	3,12	62	29	9	
RPT28BR01	RC043	5(2C)	0,08	0,03	0,07	1,93	77	2,75	0,007	0,25	0,12	6,4	1,35	1,32	70	23	7	
RPV37BR01	RC089	1(Ap1)	0,35	0,11	1,59	12,38	96	15,10	0,512	6,40	5,79	5,6			44	42	14	0,82
RPV37BR01	RC089	2(Ap2)	0,17	0,04	0,72	9,37	Sat.	10,02	0,241	3,23	2,74	6,1			64	27	9	
RPV37BR01	RC089	3(Bt)	0,13	0,01	0,59	6,78	93	8,08	0,123	2,11	1,28	6,0			66	26	8	
RPV37BR01	RC089	4(CB)	0,08	0,01	0,20	2,72	96	3,14	0,041	0,74	0,38	6,2			57	33	10	
RPV37BR01	RC089	5(C)	0,08	0,01	0,10	1,37	71	2,20	0,015	0,32	0,15	6,2			56	37	7	
RPW48BR03	RC105	1(Ap)	0,08	0,13	2,96	15,16	Sat.	17,97	0,443	7,26	6,64	6,0	2,32	1,60	44	37	19	0,86
RPW48BR03	RC105	2(B/A)	0,08	0,05	1,76	11,62	Sat.	13,23	0,178	4,45	4,01	6,3	0,42	1,43	47	38	15	
RPW48BR03	RC105	3(C)	0,08	0,03	0,66	3,34	92	4,46	0,064	0,78	0,52	6,1	0,12	1,10	60	32	8	
RPW55BR01	RC123	1 (Ap1)	0,10	0,19	0,80	12,33	89	15,12	0,309	2,85	2,44	7,0	8,32	2,25	32	43	25	1,11
RPW55BR01	RC123	2 (Ap2)	0,08	0,26	0,86	11,68	88	14,59	0,308	2,85	2,65	6,9	8,16	2,17	29	40	31	1,03
RPW55BR01	RC123	3 (Bt)	0,08	0,15	0,49	6,39	76	9,30	0,140	1,32	1,01	7,0	8,57	2,31	28	46	26	1,12
RPW55BR01	RC123	4 (C)	0,08	0,06	0,14	2,18	78	3,15	0,049	0,43	0,30	7,3	1,74	1,53	39	47	14	
RPW55BR01	RC123	5 (RC)	0,08	0,07	0,17	2,44	74	3,75	0,053	0,28	0,17	7,4	1,41	1,32	38	33	29	
RPW58BR02	RC107	1(Ap1)	0,08	0,11	2,34	8,78	Sat.	11,03	0,308	2,95	2,33	6,1	8,33	2,02	41	35	24	1,12
RPW58BR02	RC107	3(Bw)	0,08	0,03	0,58	4,78	94	5,83	0,082	1,11	0,54	6,0	14,27	4,06	48	38	14	0,83
RPW58BR02	RC107	5(2C)	0,08	0,04	0,20	1,23	Sat.	1,11	0,027	0,21	0,13	6,1	1,23	1,44	50	32	18	

Reference number	Profile nº	Horizon	Na	K	Mg	Ca	Base Saturation (%)	Cation Exchange Capacity (cmol kg^{-1})	N (%)	C (%)	Org. C(%)	pH	Fe (g kg^{-1}) Oxalate	Al (g kg^{-1}) Oxalate	Sand % w/w	Silt % w/w	Clay % w/w	Bulk Density g/cm3
RPW58BR02	RC107	4(2Bt)	0,08	0,03	0,19	1,93	60	3,69	0,106	1,69	0,82	5,9	9,27	4,31	49	41	10	0,99
RPW58BR02	RC107	2(Ap2)	0,08	0,10	1,15	5,68	86	8,12	0,188	1,97	1,40	5,6	9,21	2,33	37	39	24	1,11
RPW58BR03	RC119	1 (Ap1)	0,22	1,07	3,67	9,29	82	17,38	0,517	4,88	4,33	6,1	4,56	1,91	46	33	21	0,99
RPW58BR03	RC119	2 (Ap2)	0,08	0,47	1,83	4,16	71	9,26	0,212	2,04	1,98	6,2	5,80	1,99	47	36	17	1,11
RPW58BR03	RC119	3 (Bt)	0,08	0,21	1,36	2,94	58	7,87	0,144	1,46	0,79	6,3	8,23	4,61	43	39	18	
RPW58BR03	RC119	4 (Bg)	0,08	0,10	0,51	0,83	Sat.	1,20	0,043	0,40	0,23	6,5	1,43	1,47	54	33	13	1,42
RPW58BR03	RC119	5 (BC)	0,08	0,17	0,60	0,74	59	2,69	0,034	0,16	0,10	6,7	0,75	0,59	59	28	13	1,53
RPW69BR02	RC122	1 (Ap)	0,08	0,21	0,59	3,60	43	10,41	0,415	5,89	5,89	5,1			47	33	20	
RPW69BR02	RC122	2 (Cg)	0,08	0,01	0,11	0,80	68	1,47	0,040	0,88	0,39	5,7			61	32	7	
RPC03BR01	RC138	1(Ah)	0,43	0,17	3,26	11,45	Sat.	15,31	0,46	5,26	3,94	5,7			73	17	10	0,78
RPC10BR01	RC144	1(Ap)	0,08	0,07	0,37	13,62	Sat.	14,17	0,38	6,22	4,96	6,3			59	29	12	
RPC10BR01	RC144	2(Bw)	0,08	0,02	0,06	2,73	89	3,24	0,05	1,09	0,63	6,7			64	27	9	
RPC10BR01	RC144	3(Bg)	0,08	0,03	0,05	1,90	72	2,86	0,04	0,66	0,35	6,7			71	21	8	
RPC10BR01	RC144	4(C1)	0,08	0,04	0,13	2,59	93	3,04	0,01	0,66	0,27	6,5			75	16	9	
RPC10BR01	RC144	5(C2)	0,08	0,02	0,04	1,17	73	1,80	0,01	0,48	0,16	6,4			86	10	4	
RPC11BR01	RC142	1(Ap)	0,08	0,23	0,62	9,78	Sat.	10,41	0,24	3,30	2,49	6,2	6,44	2,28	69	24	7	0,97
RPC11BR01	RC142	2(Bt)	0,08	0,15	0,19	2,92	79	4,21	0,07	0,93	0,42	6,1	12,67	4,45	55	34	11	
RPC11BR01	RC142	3(2Cg)	0,08	0,09	0,18	1,38	Sat.	1,30	0,02	0,31	0,14	6,1	4,12	2,02	62	32	6	
RPC11BR02	RC143	1(Ap1)	0,09	0,14	0,26	4,78	81	6,51	0,26	2,81	2,30	5,7	9,92	2,56	56	33	11	1,02
RPC11BR02	RC143	2(Ap2)	0,08	0,04	0,17	3,84	74	5,59	0,14	1,55	0,90	6,2	9,06	2,51	54	33	13	1,15
RPC11BR02	RC143	3(Bw)	0,08	0,02	0,08	1,86	55	3,72	0,11	1,16	0,49	5,9	8,58	4,84	58	34	8	
RPC11BR02	RC143	4(CB)	0,08	0,02	0,07	0,66	53	1,56	0,02	0,31	0,15	6,1	2,84	1,71	58	36	6	
RPC11BR02	RC143	5(Cr)	0,08	0,04	0,12	0,68	99	0,94	0,01	0,20	0,11	6,1	1,23	1,31	49	43	8	
RPC22BR01	RC139	1(Ap)	0,08	0,06	0,20	2,20	99	2,55	0,18	1,98	1,50	5,2			62	29	9	1,27
RPC22BR01	RC139	2(Bg1)	0,08	0,04	0,19	1,14	Sat.	0,79	0,02	0,21	0,16	6,6			56	36	8	1,76
RPC22BR01	RC139	3(Bg2)	0,08	0,06	0,14	1,99	Sat.	2,00	0,02	0,16	0,12	7,1			47	43	10	
RPC42BR03	RC134	1 (Ap1)	0,10	0,16	0,62	7,71	81	10,64	0,45	7,22	5,83	5,4			52	36	12	0,66
RPC42BR03	RC134	2 (Ap2)	0,08	0,04	0,25	5,38	89	6,47	0,22	4,08	3,69	5,7			50	35	15	1,09

Reference number	Profile nº	Horizon	Na	K	Mg	Ca	Base Saturation (%)	Cation Exchange Capacity (cmol kg^{-1})	N (%)	C (%)	Org. C(%)	pH	Fe (g kg^{-1}) Oxalate	Al (g kg^{-1}) Oxalate	Sand % w/w	Silt % w/w	Clay % w/w	Bulk Density g/cm3
RPC42BR03	RC134	3(Cg)	0,08	0,05	0,16	1,82	89	2,38	0,01	0,22	0,18	5,7			46	44	10	1,86
RPG62BR01	RC154	1(Ap)	0,08	0,05	0,29	4,53	75	6,64	0,20	1,86	1,24	6,0	9,83	2,52	72	17	11	1,20
RPG62BR01	RC154	2(Bt)	0,08	0,04	0,20	2,23	88	2,91	0,04	0,36	0,22	6,6	5,40	1,85	74	16	10	1,35
RPG62BR01	RC154	3(Cr)	0,08	0,05	0,37	7,10	Sat.	7,09	0,04	0,59	0,39	7,1	5,49	1,30	77	17	6	1,16
RPH61GJ01	RC184	1(Ap)	0,15	0,53	1,48	8,14	95	10,89	0,619	6,74	5,48	5,5	7,73	3,59	41	26	33	0,86
RPH61GJ01	RC184	2(Ap2)	0,08	0,71	0,73	4,87	83	7,74	0,404	4,06	3,01	5,7	8,23	3,97	39	31	30	1,01
RPH61GJ01	RC184	3(Bwg)	0,08	0,51	0,41	2,44	97	3,56	0,104	0,87	0,57	5,9	4,53	2,30	52	26	22	1,17
RPH61GJ01	RC184	4(Btg)	0,08	0,58	1,04	3,91	57	9,81	0,053	0,21	0,14	5,6	5,31	1,21	46	24	30	1,66
RPH61GJ01	RC184	5(BCg)	0,13	0,27	2,42	9,11	82	14,50	0,043	0,19	0,14	6,0	2,96	0,89	48	21	31	1,44
RPH61GJ03	RC186	1(Ap)	0,08	0,53	0,66	6,99	96	8,64	0,461	5,28	4,10	5,6			39	28	33	0,96
RPH61GJ03	RC186	2(Eg)	0,09	0,13	0,32	4,25	92	5,22	0,085	0,95	0,86	6,0			35	38	27	1,49
RPH61GJ03	RC186	3(Btg)	0,08	0,07	0,74	3,45	Sat.	3,40	0,050	0,26	0,12	5,7			46	31	23	1,42
RPH61GJ03	RC186	4(Cg)	0,11	0,12	2,40	8,07	87	12,24	0,068	0,15	0,11	6,4			44	28	28	1,57
RPH70GJ01	RC182	1(Ap)	0,08	0,25	0,58	11,50	91	13,66	0,535	6,16	5,23	5,6	9,08	4,30	44	26	30	0,74
RPH70GJ01	RC182	2(Bw)	0,08	0,17	0,27	7,58	Sat.	7,60	0,305	3,57	2,57	5,9	8,92	4,59	47	28	25	0,89
RPH70GJ01	RC182	3(Bs)	0,08	0,04	0,07	3,66	Sat.	2,23	0,148	1,67	0,72	6,0	9,52	5,42	55	30	15	0,94
RPH70GJ01	RC182	4(Btg)	0,08	0,02	0,02	1,21	96	1,40	0,030	0,25	0,10	6,2	2,92	2,63	63	26	11	
RPH90BR01	RC070	(1Ap)	0,22	0,61	1,90	31,74	97	35,50	0,885	9,63	8,98	6,2	10,34	4,32	28	38	34	
RPH90BR01	RC070	2(Ap2)	0,08	0,23	0,79	23,11	99	24,35	0,454	4,63	3,63	6,6	11,70	4,87	38	33	29	
RPH90BR01	RC070	3(Bt)	0,08	0,07	0,26	10,52	Sat.	8,73	0,108	1,06	0,52	6,4	6,21	3,88	51	34	15	
RPH90BR01	RC070	4(C)	0,08	0,05	0,27	6,86	Sat.	4,67	0,205	0,53	0,28	6,3	3,42	1,95	63	28	9	
RPJ00BR01	RC072	1(Ap)	0,11	1,90	2,48	21,89	Sat.	24,96	0,483	4,97	4,38	6,2			58	20	22	
RPJ00BR01	RC072	2(C)	0,14	1,29	0,77	6,02	Sat.	6,92	0,040	0,39	0,22	6,5			71	19	10	
RPN38GJ02	RC189	1(Ap)	0,08	0,14	0,76	9,85	73	14,80	0,492	5,92	4,23	5,3	5,38	3,42	56	23	21	0,78
RPN38GJ02	RC189	2(Bw)	0,08	0,06	0,48	7,77	85	9,92	0,265	3,20	2,62	5,4	3,53	2,67	58	24	18	
RPN38GJ02	RC189	3(Bw(g)x)	0,08	0,04	0,30	6,11	Sat.	5,80	0,055	0,43	0,26	5,9	1,39	1,17	75	14	11	
RPN58GJ01	RC187	1(Ap)	0,08	0,42	0,68	18,24	Sat.	19,05	0,637	6,67	6,12	6,4	5,59	2,89	58	26	16	0,63
RPN58GJ01	RC187	2(Bw)	0,08	0,17	0,38	5,67	Sat.	6,07	0,148	1,53	0,92	6,4	3,21	3,22	27	64	9	

Reference number	Profile nº	Horizon	Na	K	Mg	Ca	Base Saturation (%)	Cation Exchange Capacity (cmol kg^{-1})	N (%)	C (%)	Org. C(%)	pH	Fe (g kg^{-1}) Oxalate	Al (g kg^{-1}) Oxalate	Sand % w/w	Silt % w/w	Clay % w/w	Bulk Density g/cm3
RPO09BR02	RC067	2(Ap2)	0,08	0,23	0,55	14,88	Sat.	12,51	0,201	2,69	2,37	7,2	2,05	1,46	56	22	22	
RPO09BR02	RC067	1(Ap)	0,08	0,47	1,42	16,76	Sat.	17,65	0,335	3,81	3,35	7,1	2,40	1,22	44	35	21	
RPO09BR02	RC067	3(R)	0,08	0,17	0,53	17,30	Sat.	15,08	0,183	3,07	2,64	7,2	0,86	3,05	66	19	15	
RPO12BR02	RC056	1(Ap)	0,08	0,19	0,69	10,98	87	13,70	0,345	3,75	2,74	6,2	6,60	2,62	61	22	17	0,86
RPO12BR02	RC056	2(AB)	0,08	0,04	0,24	5,59	83	7,19	0,252	2,86	2,04	6,2	6,52	2,56	61	22	17	
RPO12BR02	RC056	3(C)	0,08	0,02	0,07	1,85	Sat.	0,63	0,080	0,84	0,42	6,2	3,17	3,35	73	18	9	
RPO12BR02	RC056	4B	0,08	0,04	0,07	2,63	84	3,35	0,086	0,89	0,46	6,1	5,65	4,50	60	26	14	
RPO17BR01	RC060	1(Ap)	0,08	0,13	1,23	10,91	90	13,78	0,221	2,13	1,71	6,3	3,15	1,19	40	35	25	1,30
RPO17BR01	RC060	2(Bg)	0,09	0,16	1,39	13,09	92	16,01	0,065	0,49	0,36	7,6	1,25	0,86	28	36	36	1,52
RPO17BR01	RC060	3(BC)	0,12	0,23	1,41	20,40	Sat.	21,65	0,059	0,47	0,29	8,0	1,43	0,86	19	42	39	1,59
RPO17BR01	RC060	4(Ckr)	0,10	0,25	1,30	21,64	Sat.	19,03	0,044	1,38	0,28	8,2	1,10	0,59	25	41	34	
RPQ50GJ01	RC213	1(Ap)	0,27	0,50	0,65	5,78	71	10,07	0,345	4,22	3,58	5,6			57	22	21	0,96
RPQ50GJ01	RC213	2(Bstr)	0,26	0,42	0,26	3,76	52	9,02	0,183	2,52	1,31	5,6			71	18	11	
RPQ50GJ01	RC213	3(Cr)	0,11	0,22	0,04	0,85	23	5,15	0,132	1,59	0,61	5,5			82	12	6	
RPQ61GJ01	RC215	1(Ap)	0,08	0,12	0,15	3,44	77	4,91	0,192	2,21	1,78	5,3			68	22	10	1,41
RPQ61GJ01	RC215	2(E)	0,08	0,09	0,09	3,43	Sat.	3,54	0,116	0,87	0,66	6,0			59	26	15	1,52
RPQ61GJ01	RC215	3(Bw)	0,08	0,09	0,03	1,92	Sat.	2,01	0,057	0,67	0,32	6,0			56	28	16	1,49
RPQ61GJ01	RC215	4(BC)	0,08	0,07	0,02	0,97	Sat.	0,89	0,017	0,16	0,07	6,0			75	14	11	1,73
RPQ80GJ03	RC224	1(Ah)	0,23	0,38	0,53	3,67	74	6,51	0,602	6,20	5,31	5,2	11,28	2,51	20	40	40	1,48
RPQ80GJ03	RC224	2(Bw)	0,15	0,15	0,41	2,67	78	4,35	0,279	2,40	1,93	5,4	8,83	2,64	18	45	37	0,86
RPQ80GJ03	RC224	3(Bwg)	0,10	0,09	0,31	2,12	44	5,98	0,155	0,98	0,61	5,5	8,93	2,95	29	42	29	0,99
RPQ80GJ03	RC224	4(BC)	0,08	0,11	0,25	1,54	47	4,19	0,080	0,27	0,16	5,6	2,81	1,64	46	36	18	1,24
RPQ92BR01	RC076	1(Ap)	0,26	0,99	2,58	16,72	Sat.	19,54	0,564	4,85	4,35	6,4	14,31	3,03	14	50	36	
RPQ92BR01	RC076	2(Bt1)	0,18	0,82	1,18	9,86	94	12,86	0,243	2,38	1,75	6,6	14,44	3,72	12	52	36	
RPQ92BR01	RC076	3(Bt2)	0,61	0,51	0,73	8,25	Sat.	9,89	0,155	1,55	0,84	6,5	12,55	3,87	20	55	25	
RPR11GJ01	RC220	1(Ap)	0,18	0,28	0,73	12,96	92	15,36	0,629	7,63	6,40	5,5	15,67	2,91	35	30	35	0,75
RPR11GJ01	RC220	2(Bs)	0,13	0,10	0,31	5,68	93	6,72	0,213	2,44	1,15	5,7	17,45	5,18	35	43	22	0,83
RPR11GJ01	RC220	3(C)	0,10	0,10	0,13	1,98	Sat.	1,01	0,074	0,42	0,21	6,0	1,80	2,36	47	34	19	1,19

Reference number	Profile nº	Horizon	Na	K	Mg	Ca	Base Saturation (%)	Cation Exchange Capacity (cmol kg-1)	N (%)	C (%)	Org. C(%)	pH	Fe (g kg^{-1}) Oxalate	Al (g kg^{-1}) Oxalate	Sand % w/w	Silt % w/w	Clay % w/w	Bulk Density g/cm3
RPR21BR01	RC098	1(Ap)	0,16	0,29	1,23	7,00	79	11,04	0,580	7,43	6,53	5,3	11,70	2,54	32	37	31	0,78
RPR21BR01	RC098	2(A/B)	0,08	0,07	0,76	4,29	76	6,84	0,255	3,29	2,62	5,2	8,46	2,73	35	40	25	
RPR21BR01	RC098	3(Bt)	0,08	0,03	0,37	1,67	55	3,95	0,114	1,03	0,55	5,4	5,07	2,64	36	42	22	
RPR21BR01	RC098	4(C)	0,08	0,06	0,33	1,57	66	3,08	0,098	0,53	0,30	5,4	2,70	2,49	42	36	22	
RPR30BR01	RC096	1(Ap)	0,26	0,20	1,21	9,08	Sat.	10,70	0,342	3,45	2,91	5,7			27	41	32	0,97
RPR30BR01	RC096	2(Bt)	0,08	0,05	0,34	1,37	31	5,85	0,128	1,05	0,67	5,1			32	42	26	1,19
RPR30BR01	RC096	3(Btg)	0,08	0,09	1,09	0,85	37	5,69	0,086	0,35	0,25	5,1			19	46	35	
RPR30BR01	RC096	4(BC)	0,10	0,07	0,84	0,54	42	3,67	0,073	0,28	0,20	5,1			30	41	29	
RPR30BR01	RC096	5(Cg)	0,08	0,10	1,06	0,75	44	4,51	0,081	0,27	0,19	5,1			14	64	22	
RPR30BR02	RC172	1(Ap)	0,08	0,16	0,65	8,39	Sat.	8,90	0,09	2,68	2,02	6,3	9,32	1,58	34	41	25	1,10
RPR30BR02	RC172	2(Bts)	0,08	0,03	0,26	3,45	84	4,56	0,08	0,83	0,38	6,2	7,50	2,25	32	46	22	1,14
RPR30BR02	RC172	3(Bt)	0,08	0,05	0,26	3,13	94	3,74	0,08	0,48	0,24	6,3	3,90	1,72	27	46	27	1,33
RPR30BR02	RC172	4(Bc)	0,08	0,07	0,29	3,18	99	3,65	0,08	0,37	0,19	6,4	2,70	1,55	66	21	13	1,34
RPR30BR02	RC172	5(2Cg)	0,08	0,14	0,30	3,53	Sat.	3,25	0,37	0,18	0,10	6,3	1,41	1,10	35	42	23	
RPR30BR03	RC173	1(Ap)	0,08	0,12	0,61	6,12	78	8,87	0,16	3,38	2,70	5,4	11,11	2,46	29	37	34	1,11
RPR30BR03	RC173	2(Btg)	0,08	0,04	0,31	6,29	96	6,97	0,16	1,10	0,89	6,0	7,56	2,15	31	41	28	
RPR30BR03	RC173	3(Bt)	0,08	0,05	0,25	3,96	96	4,51	0,15	1,23	0,73	6,0	9,20	3,63	53	23	24	
RPR30BR03	RC173	4(C)	0,08	0,04	0,45	4,44	94	5,34	0,13	0,61	0,31	5,9	4,70	2,46	25	46	29	
RPR31BR01	RC097	1(Ap)	0,12	0,20	1,30	14,00	Sat.	14,79	0,475	6,17	5,86	5,5			8	48	44	0,83
RPR31BR01	RC097	2(Bt)	0,08	0,07	0,62	4,77	96	5,75	0,179	1,66	1,21	5,5			7	57	36	1,18
RPR31BR01	RC097	3(Btg1)	0,08	0,08	0,55	1,51	45	4,91	0,102	0,48	0,28	5,2			9	57	34	1,49
RPR31BR01	RC097	4(Btg2)	0,08	0,10	0,48	0,57	25	5,00	0,104	0,32	0,19	5,1			12	50	38	
RPR41BR01	RC092	1(Ap1)	0,13	0,17	1,71	8,99	90	12,18	0,521	4,81	4,08	5,1	14,26	3,17	26	36	38	0,90
RPR41BR01	RC092	2(Ap2)	0,08	0,08	0,99	9,43	94	11,27	0,331	2,90	2,27	5,5	13,07	3,32	26	39	35	
RPR41BR01	RC092	3(Bt)	0,08	0,05	0,35	5,98	86	7,53	0,181	1,61	0,85	5,9	11,17	4,60	37	35	28	
RPR41BR01	RC092	4(BC)	0,08	0,06	0,32	5,85	91	6,95	0,132	0,90	0,58	6,0	7,21	3,06	49	26	25	
RPR51BR01	RC091	1(Ap)	0,16	0,24	1,99	8,40	86	12,50	0,445	4,28	3,82	5,3	6,56	1,41	47	26	27	1,04
RPR51BR01	RC091	2(Ap2)	0,08	0,14	1,56	9,47	Sat.	10,61	0,211	1,79	1,55	6,0	5,78	1,33	55	23	22	1,19

Reference number	Profile nº	Horizon	Na	K	Mg	Ca	Base Saturation (%)	Cation Exchange Capacity (cmol kg^{-1})	N (%)	C (%)	Org. C(%)	pH	Fe (g kg^{-1}) Oxalate	Al (g kg^{-1}) Oxalate	Sand % w/w	Silt % w/w	Clay % w/w	Bulk Density g/cm3
RPR51BR01	RC091	3(Bt1)	0,08	0,14	1,02	6,59	Sat.	7,44	0,083	0,62	0,49	6,5	3,59	1,38	48	25	27	1,50
RPR51BR01	RC091	4(Bt2)	0,10	0,11	0,97	7,80	98	9,13	0,063	0,44	0,35	6,8	1,74	1,07	43	28	29	1,49
RPR51BR01	RC091	5(Btk)	0,08	0,10	0,33	19,10	Sat.	9,47	0,021	3,81	0,23	8,4	0,48	0,39	57	25	18	1,75
RPR51BR01	RC091	6(2C)	0,08	0,05	0,26	19,05	Sat.	6,87	0,009	2,93	0,16	8,5	0,34	0,20	72	25	3	1,51
RPT01BR01	RC100	1(Ap)	0,08	0,22	3,05	8,35	Sat.	10,14	0,215	2,65	2,10	6,4	5,75	1,53	50	31	19	1,23
RPT01BR01	RC100	3(BCtg)	0,08	0,16	2,19	4,78	55	13,12	0,056	0,31	0,23	6,6	4,21	1,78	6	51	43	1,47
RPT01BR01	RC100	2(Btg)	0,09	0,54	3,70	8,97	Sat.	6,38	0,105	0,87	0,57	6,6	6,12	1,78	43	36	21	1,47
RPT17BR02	RC042	1(Ap)	0,09	0,44	3,08	15,49	94	20,33	0,501	5,07	3,80	6,8	13,21	4,07	32	36	32	0,83
RPT17BR02	RC042	2B	0,08	0,13	1,18	4,86	96	6,53	0,159	1,52	0,79	7,0	7,83	4,96	57	32	11	
RPV37BR01	RC089	1(Ap1)	0,35	0,11	1,59	12,38	96	15,10	0,512	6,40	5,79	5,6			44	42	14	0,82
RPV37BR01	RC089	2(Ap2)	0,17	0,04	0,72	9,37	Sat.	10,02	0,241	3,23	2,74	6,1			64	27	9	
RPV37BR01	RC089	3(Bt)	0,13	0,01	0,59	6,78	93	8,08	0,123	2,11	1,28	6,0			66	26	8	
RPV37BR01	RC089	4(CB)	0,08	0,01	0,20	2,72	96	3,14	0,041	0,74	0,38	6,2			57	33	10	
RPV37BR01	RC089	5(C)	0,08	0,01	0,10	1,37	71	2,20	0,015	0,32	0,15	6,2			56	37	7	
RPW48BR03	RC105	1(Ap)	0,08	0,13	2,96	15,16	Sat.	17,97	0,443	7,26	6,64	6,0	2,32	1,60	44	37	19	0,86
RPW48BR03	RC105	2(B/A)	0,08	0,05	1,76	11,62	Sat.	13,23	0,178	4,45	4,01	6,3	0,42	1,43	47	38	15	
RPW48BR03	RC105	3(C)	0,08	0,03	0,66	3,34	92	4,46	0,064	0,78	0,52	6,1	0,12	1,10	60	32	8	
RPW55BR01	RC123	1 (Ap1)	0,10	0,19	0,80	12,33	89	15,12	0,31	2,85	2,44	7,0	8,32	2,25	32	43	25	1,11
RPW55BR01	RC123	2 (Ap2)	0,08	0,26	0,86	11,68	88	14,59	0,31	2,85	2,65	6,9	8,16	2,17	29	40	31	1,03
RPW55BR01	RC123	3 (Bt)	0,08	0,15	0,49	6,39	76	9,30	0,14	1,32	1,01	7,0	8,57	2,31	28	46	26	1,12
RPW55BR01	RC123	4 (C)	0,08	0,06	0,14	2,18	78	3,15	0,05	0,43	0,30	7,3	1,74	1,53	39	47	14	
RPW58BR02	RC107	1(Ap1)	0,08	0,11	2,34	8,78	Sat.	11,03	0,308	2,95	2,33	6,1	8,33	2,02	41	35	24	1,12
RPW58BR02	RC107	3(Bw)	0,08	0,03	0,58	4,78	94	5,83	0,082	1,11	0,54	6,0	14,27	4,06	48	38	14	0,83
RPW58BR02	RC107	5(2C)	0,08	0,04	0,20	1,23	Sat.	1,11	0,027	0,21	0,13	6,1	1,23	1,44	50	32	18	
RPW58BR02	RC107	4(2Bt)	0,08	0,03	0,19	1,93	60	3,69	0,106	1,69	0,82	5,9	9,27	4,31	49	41	10	0,99
RPW58BR02	RC107	2(Ap2)	0,08	0,10	1,15	5,68	86	8,12	0,188	1,97	1,40	5,6	9,21	2,33	37	39	24	1,11
RPW58BR03	RC119	1 (Ap1)	0,22	1,07	3,67	9,29	82	17,38	0,52	4,88	4,33	6,1	4,56	1,91	46	33	21	0,99
RPW58BR03	RC119	2 (Ap2)	0,08	0,47	1,83	4,16	71	9,26	0,21	2,04	1,98	6,2	5,80	1,99	47	36	17	1,11

Reference number	Profile nº	Horizon	Na	K	Mg	Ca	Base Saturation (%)	Cation Exchange Capacity (cmol kg^{-1})	N (%)	C (%)	Org. C(%)	pH	Fe (g kg^{-1}) Oxalate	Al (g kg^{-1}) Oxalate	Sand % w/w	Silt % w/w	Clay % w/w	Bulk Density g/cm3
RPW58BR03	RC119	3 (Bt)	0,08	0,21	1,36	2,94	58	7,87	0,14	1,46	0,79	6,3	8,23	4,61	43	39	18	
RPW58BR03	RC119	4 (Bg)	0,08	0,10	0,51	0,83	Sat.	1,20	0,04	0,40	0,23	6,5	1,43	1,47	54	33	13	1,42
RPW58BR03	RC119	5 (BC)	0,08	0,17	0,60	0,74	59	2,69	0,03	0,16	0,10	6,7	0,75	0,59	59	28	13	1,53
RPW69BR02	RC122	2 (Cg)	0,08	0,01	0,11	0,80	68	1,47	0,04	0,88	0,39	5,7			61	32	7	
RPW69BR02	RC122	1 (Ap)	0,08	0,21	0,59	3,60	43	10,41	0,42	5,89	5,89	5,1			47	33	20	