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Original article

Stable isotope insights into arthropod food chains and nitrogen cycling in a rehabilitated tailings chronosequence

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ABSTRACT

Field studies in post-mining sites on epigeic invertebrate groups are scarce despite their importance in pedogenesis and ecosystem multifunctionality. This research investigated the diversity, abundance and succession of aboveground invertebrates in a rehabilitated Pb/Zn tailings chronosequence of 5, 20 and 35 years. The study also explored the trophic relationship of selected arthropods and characterized the nitrogen (N) cycle using stable isotope measurements. The abundance and species richness in most investigated groups and the dominance index Berger–Parker (BP) of aboveground invertebrates increased with rehabilitation age from 0.17 BP in early to 0.31 BP in late stage. Elemental and stable isotope ratio analysis showed that N and C soil content increased and the C/N ratio decreased with age, yet despite this increased N availability in the system, the maturing N-cycle used N efficiently. The lack of large N losses from the system despite substantial atmospheric deposition inputs was indicated by the fact that N isotope ratios ($\delta^{15}N$) in plants and animals became significantly more negative with rehabilitation age, −6.0 $\delta^{15}N$ for plants, −5.0 $\delta^{15}N$ for herbivores and 3.0 $\delta^{15}N$ for carnivores. The length of the invertebrate food chain expanded by more than half a trophic level (2.7‰ $\delta^{15}N$) for top predator Coleoptera from early to late stage, probably reflecting more complex food webs including intra-guild predation in older communities. In conclusion, $\delta^{15}N$ measurements in plants and animals provided novel insights into the N-cycle, accumulative N flows and the trophic position in post-mining sites. It is proposed that isotope ratio measurements could be used as easy-to-measure, integrating indicators of nutrient cycling and the soil food web complexity of rehabilitated mine tailings and similar soil ecosystems.

1. Introduction

Mining supplies numerous raw minerals that are crucial for economic activities. However, the increasing demands for extraction of metals such as Pb and Zn and disposal of mine tailings cause environmental degradation and thus make ecosystem reconstruction a fundamental part for the future of industrial activities [1]. Nowadays, capping or covering the surface of the tailings with a layer of soil/subsoil materials is one of the widely accepted approaches to metalliferous mine tailings rehabilitation. These lands are usually left unmanaged and natural and weathering processes take over. Nevertheless, industries are increasingly concerned about the long-term sustainability of the rehabilitated tailings, which is currently raising the necessity for more field assessments [2,3]. Traditionally, rehabilitation assessment in post-mining sites have focused on vegetation performance [4], substrate physicochemical analysis [5] and sometimes, vertebrate communities [6]. In contrast, few studies have assessed the successional patterns [7] and the role in soil multifunctionality of invertebrate animals [8] despite their importance in processes such as decomposition and nutrient cycling [9]. A more holistic ecosystem approach is needed to evaluate the success of rehabilitation programmes and to inform rehabilitation strategies [4,10].

For such a holistic approach, invertebrate faunal groups can provide helpful information about the entire soil's capability to support its ecological functions [11]. For example, ants (Hymenoptera: Formicidae) have been associated with recovery processes of rehabilitation in bauxite [12] and in coal mines [13,14], and some species were indicative of later stages of succession in gold mining [15]. Additionally, ants have been found to directly and indirectly control resource availability for other organisms, they are important predators and influence vegetation in rehabilitated bauxite sites [8] and soil chemistry in post-mining coal sites [16].

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Different invertebrate species contribute to the ecosystem metabolism and act on different trophic levels, together controlling the cycling of C and N in the system [17]. C and N flow through the system either by way of plant consumption, i.e the herbivore food chain; or via the saprotrophic food chain, i.e decomposers. Invertebrates interact with environmental resources and exploit them to meet their niche, e.g. Coleoptera and Aranea are well known predators, whereas Isopoda and Gastropoda are litter transformers. As in other terrestrial ecosystems, invertebrates also play an important role in soil C and N dynamics in post-mining substrates. The effect of some invertebrates on C and N have been studied by Frouz et al. [18] who showed that soil macrofauna contributes to soil microstructure formation and organic matter accumulation by faecal pellets in a chronosequence of post-mining sites. Frouz et al. [19] studied changes in soil chemistry in rehabilitated sites from 4 to 45 years old and found that changes in soil N content were similar to changes in soil C content, and both increased over time. However, less is known about the links between invertebrate functions, soil processes such as C and N cycling, invertebrate trophic ecology, and changes over time in C and N dynamics in rehabilitated metalliferous mine tailings.

The geochemical technique of stable isotopes ratio analysis has proven useful for the study of invertebrate soil food webs and trophic niches [20]. In natural systems the ratio of the stable isotopes of N (^{15}N and ^{14}N, expressed as δ^{15}N) becomes slightly more enriched in the heavier ^{15}N along a food chain [21,22]. Consumers feed on their resources and thus δ^{15}N can be used independently of traditional dietary methods to infer trophic positions: organisms more enriched in ^{15}N feed higher up in the food chain [23]. δ^{15}N in plants and soils have been used as indirect indices of N cycling across habitats and associated with N losses [24]. For example, very positive δ^{15}N values have been shown to reflect sustained ecosystem N losses in Craine et al. [24], whereas very negative δ^{15}N values have been associated with atmospheric N deposition [25].

To date, there has been no extensive study on the feeding ecology of arthropods in rehabilitated mine tailings, and no study has assessed trophic niches and N cycling using stable isotope ratio analysis of invertebrates and plants. This study aimed to investigate, invertebrate diversity, abundance, and colonization patterns along rehabilitation stages, together with trophic niches and characterization of N cycle can contribute to the development of more appropriate and long-term sustainable rehabilitation strategies in post-mining sites [19]. The specific research objectives were: a) to analyze and compare diversity and abundance of aboveground invertebrates change over time in a rehabilitated Pb/Zn tailings chronosequence of 5, 20, and 35 years? b) to determine if a shift in nitrogen isotope ratios (δ^{15}N) in plants and invertebrates occurs with rehabilitation age, and c) to assess N cycling in the rehabilitated sites. We hypothesized that the older rehabilitated site would support greater invertebrate diversity and abundance, with isotope analysis indicating a more complex and mature food web.

2. Methodology

2.1. Site description

The study was carried out on a rehabilitated Pb/Zn tailings management facility (TMF) in Ireland. Three areas in the TMF rehabilitated approximately 5, 20 and 35 years previously were selected for the study, identified herein as early-stage (5 years), mid-stage (20 years) and late-stage (35 years) rehabilitation stages. Each selected area from the three stages was approximately 1500 m^2, distributed on flat areas and also slopes of up to 30°, and covered by vegetation (Table 1). More details on species-specific vegetation composition and coverage can be found in Table S1. In the selected chronosequence, rehabilitation work involved capping with a limestone rock layer (30–40 cm depth) followed by placement of a thin subsoil material (20–30 cm depth), no seeding or planting was undertaken. Subsequent colonization by vegetation and soil fauna in the soil cover was due to a combination of the soil seed bank and fauna dispersal from adjoining lands. In this study, the area was classified as passive rehabilitation or left to natural succession.

Table 1
Stage characterisation, vegetation coverage, and soil chemical properties. Results from one-way ANOVA and multiple-mean comparisons between rehabilitation stages. Data on soil chemical properties are means, within a row value followed by different lower-case letters are significantly different at $p < 0.05$. For atmospheric deposition and Nitrogen (N) stock kg N ha^{-1} tot years = kg of Nitrogen per hectare accumulation in total years since rehabilitation (5, 20 and 35 years), calculations were done respectively for each column early, mid and late stage, data retrieved from Doyle et al. [26] for a minimum and a maximum concentration of NH_3 (μg/m^3). Data on fixation by legumes refers to values in Suter et al. [27] with estimations using the coverage of legumes in the current study. Early = early rehabilitation stage, Mid = mid rehabilitation stage, Late = late rehabilitation stage. Total vegetation coverage (%) = percentage of total vegetation coverage, pH = potential hydrogen, EC = electrical conductivity, Corg(%) = Carbon organic percentage, Norg(%) = Nitrogen organic percentage.

Stage Characterisation	Early	Mid	Late
Years rehabilitated	5	20	35
Total stage size (m^2)	1376	1892	3147
Vegetation type coverage (%):			
Forbs	3.5	24.3	46.7
Rushes/Sedges	–	10.1	15.5
Grasses	32.0	19.0	3.0
Mosses	26.6	11.4	15.0
Legumes	28.3	7.0	6.6
Trees	–	–	2.4
Total vegetation coverage (%)	90	72	89
Soil chemical properties *(means ± SD)*			
pH	8.0 ± 0.15 a	7.8 ± 0.08 b	7.6 ± 0.05 c
EC (mS/cm)	0.33 ± 0.08 b	0.36 ± 0.03 b	0.50 ± 0.05 a
C_{org} (%)	0.4 ± 0.1 b	0.9 ± 0.1 b	3.0 ± 0.8 a
N_{org} (%)	0.03 ± 0.008 b	0.07 ± 0.02 b	0.24 ± 0.07 a
C:N ratio	17.0 ± 3.0 a	13.0 ± 1.0 b	12.0 ± 1.2 b
Minimum N atmospheric deposition (Min kg N. ha^{-1} tot years)	75	300	525
Maximum N atmospheric deposition (Max kg N. ha^{-1} tot years)	100	400	700
Total N fixation by legumes (kg ha^{-1} year^{-1})	46	11	11
N stock (Min kg ha^{-1} tot years)	304	528	897

2.2. Invertebrate sampling and diversity indices

The sampling scheme was designed to target the seasonal peak of epigeic invertebrates which occurs in summer, from July to August and sampling was conducted over two consecutive years (2019 and 2020). Data from both years was analysed together to get a better representation of invertebrates groups, and to help account for fluctuations and variations that may occur in a single year. An area of approximately 700–1000 m^2 within each rehabilitation stage (early, mid and late) was divided into a grid system of 50 m^2; then, ten pitfall traps (Ø 9 cm, 14 cm depth) were installed within each rehabilitation stage using a random number table within the grid, at least 2 m apart. All traps were filled to 2 cm with ethanol (70%) as a preservative; buried and placed flush to the ground surface; covered by a stone tile held 2 cm above the opening with pebbles, and emptied after seven days, approximately n = 40 were collected per stage per year over two years. Aboveground invertebrates were sampled at each campaign using D-vac suction (Tanaka pro-force model) for approximately 20 s. Epigeic invertebrates were sorted by Class, Order, Family, Genus and Species when possible and otherwise assigned to morphospecies. The major groups of terrestrial invertebrates were identified using Field Study Council (FSC) keys for UK and Ireland. Only adult forms were considered for the diversity analysis. Collembola nomenclature followed Hopkin [28]. For species names in tables and figures, codes were assigned using the first three letters of the genus followed by the first two letters of the species when known, or morphospecies number were used. For orders of invertebrates, codes refer to the first three letters of taxonomic order.

Diversity in the three stages was calculated using the Berger–Parker dominance (BP) index [29] which has been used for monitoring the biodiversity of disturbed soils [30,31]. It expresses the relative significance of the most widely spread species and is determined by the formula: BP = N_{max}/N, where N_{max} is the number of invertebrates of the most widely spread species, and N is the total number of species of invertebrates. Herein, abundance refers to the total number of individuals collected per stage from both years analysed together. The above diversity and abundance were calculated using the whole dataset of invertebrates. Lepidoptera and Orthoptera did not have a representative sample size since only one individual per year was captured and D-vac and pitfalls are not systematic methods to sample those groups [32]. Therefore, they were excluded from the diversity measurements calculations.

2.3. Site analysis: soil chemical properties, vegetation, N inputs, isotope ratio analysis

To characterise the sites from which the invertebrates were sampled, selected chemical properties of the substrate and botanical composition were determined. Six soil samples of approximately 20 cm × 20 cm from the top 20 cm were extracted from each stage, air-dried, sieved (Ø 0.15 mm) and characterised for soil organic C and N using a Thermo FlashEA 1112 Elemental Analyser®. The soil C:N ratio was calculated from the mass of organic C to the mass of organic N. Soil pH and electrical conductivity (EC) in water were determined using 1:2 suspensions after shaking for 30 min at 200 rpm; the pH was measured with a WTW-pH 115 330i/set meter and the EC with a WTW 3110/set meter.

Plant species growing on the study sites were identified and grouped to genus level and species when possible. Nomenclature for plants followed Stace [33] and Fitter [34] for sedges and rushes. Plant community and vegetation cover percentage within the different rehabilitation stages was assessed using ten quadrats (1 m × 1 m) per stage evenly distributed in 2019. Herein, the abundances of plant species were measured by vegetation cover (n = 10 per stage).

Published annual N deposition data were acquired from the Environmental Protection Agency (EPA) database, reported in Doyle et al. [26], with calcareous seminatural grasslands selected as the most similar habitat. Total inputs of N and accumulated N stocks were estimated approximately as the sum of atmospheric deposition and N fixation by legumes derived from vegetation cover percentage, separately for each rehabilitation stage. The total N deposition since time from rehabilitation was calculated for both, the lowest 15 kg ha^{-1} and the highest 20 kg ha^{-1} (Table 1). Total N fixation by legumes per stage, was estimated using data from Suter et al. [27] and adjusted to the percentage of legumes coverage in each stage and time since rehabilitation, by estimating total N (kg $ha^{-1}year^{-1}$) fixated by legumes at maximal realized legume proportion 100% seeded and calculated to legumes coverage per stage and years in the sampling areas. N stocks (kg N ha^{-1}) were calculated as the sum of all inputs, over the years since rehabilitation, assuming no losses occurred.

For the stable isotope ratio analysis, 17 adult invertebrate species and morphospecies common to the three rehabilitation stages were selected. Arthropods selection was based on specimens available across the three stages and on having species representing the main trophic levels; predators, herbivores and omnivores. Additionally, *Ocypus olens* (Coleoptera, Staphylinidae) juveniles were included for their potential role as control predators across the stages at the sampling time. Fresh plant material (including leaves, shoots and flowers) of three plant species (*Centaurium erythraea*, *Leontodon saxatilis* and *Holcus lanatus*) common to the three stages were collected. When possible, at least three replicates from different specimens of flora and fauna were selected for all the species and morphospecies. For each stage, the site-specific isotopic baseline (i.e., measured $\delta^{15}N$ of herbivores) was used to calculate $\Delta^{15}N$ spacings of consumers (predators and omnivores) that can be used to infer food chain lengths [35]. The boundaries for all consumer levels were assumed to span 3.4‰, as defined by Post [36]. $\Delta^{15}N$ was calculated as $\Delta^{15}N$= ($\delta^{15}N$ predators/omnivores - $\delta^{15}N$ mean of herbivores per stage).

In the laboratory, invertebrates were dried in a desiccator at 30 °C for 72 h; plant samples were oven-dried at 65 °C for 40 h. A sample mass of 0.5–1.0 mg dry weight for arthropods (n = 190) and up to 3.0 mg for plants (n = 38) was encapsulated in ultra-clean tin capsules (6 mm × 4 mm). To ensure representative samples, both plants and large arthropods were homogenized into fine powder in a ball mill. For small arthropods, the whole body was included; for some Cicadellidae, half a body was included on a vertical section. Samples were analysed by continuous flow isotope ratio mass spectrometry (CF-IRMS) using a Europa Scientific Stable Isotope Analyser at Iso-Analytical Ltd. (Cheshire, UK). The analysis was carried out in the dual isotope mode, allowing $\delta^{15}N$ and $\delta^{13}C$ to be measured simultaneously on the same sample. Stable isotope abundance is expressed using the δ notation with a unit of ‰. δX was calculated as δX= (Rx/Rstd − 1), where X is the notation of the heavy isotope of a pair. The reference material used during $\delta^{13}C$ and $\delta^{15}N$ analysis of the samples was powdered tuna protein (IA-R069) ($\delta^{13}C_{V\text{-}PDB}$ = −18.8‰, $\delta^{15}N_{Air}$ = 11.69‰). The reference standards included with the samples were soy protein (IA-R068) ($\delta^{13}C_{V\text{-}PDB}$ = 25.21‰, $\delta^{15}N_{Air}$ = 1‰) and a mixture of ammonium sulphate (IA-R046) and oxalic acid (IAEA-C7) ($\delta^{13}C_{V\text{-}PDB}$ = −14.51‰, $\delta^{15}N_{Air}$ = 21.79‰). The analytical precision (S.D.) for these standards was 0.03‰ for C, and 0.08‰ for N in tuna protein (n = 10); S.D., was 0.02‰ for C, and 0.03‰ for N in soya protein (n = 30), and 0.07‰ for C and 0.08‰ for N in the mixture of ammonium sulphate and oxalic acid (n = 10).

2.4. Statistical analysis

Differences in dominance index Berger Parker, abundance, richness, soil chemical properties and isotope ratio data across stages were first tested by Shapiro–Wilk test for normality and examined using one-way ANOVA, followed by Tukey's test; correlation analysis was also explored for $\delta^{15}N$ in plants, N_{org} and C/N ratio in soil across stages. Statistical analyses were performed using GraphPad Prism 9.1.0. To investigate possible clustering and patterns of epigeic invertebrate group abundance with soil chemical properties and vegetation type Canonical Correspondence analysis (CCA) were used using CANOCO 5.0. Prior to CCA, it is necessary to investigate how data on abundance are arranged along the gradient of age using indirect gradient analyses or CA and proceed with CCA if results suggest a long gradient (>3 groups with gradient responds) [37]. For CA and CCA only the Formicidae family from Hymenoptera were included, due to their role in ecosystem development [38,39].

3. Results

3.1. Dominant vegetation type coverage and soil chemical properties

Total vegetation cover was above 72% in all stages (Table 1). Dominant species for each stage were; Early - *Trifolium repens*, *Bryum capillare* and *Festuca rubra,* Mid - *B. capillare*, *F. rubra* and *Leucanthemum vulgare*, and in late *B. capillare*, *L. vulgare* and *Ranunculus acris*, (Table S1). After 35 years (late stage), grass coverage decreased from 32% to 3%, as woody vegetation, mainly *Crataegus monogyna,* forbs and rushes/sedges started colonising. Overall, these results indicate that vascular plant abundance increased as coverage of grasses and mosses decreased with years since rehabilitation.

Selected soil chemical properties are shown in Table 1. Soil pH was close to neutral, and significant differences were found between early and late and early and mid. Salinity was low, ca. 0.3–0.5 mS/cm, in the three stages, and slightly increasing with age, with significant differences between early and late and mid and late. C_{org} (%) and N_{org} (%) increased with years since rehabilitation; for C_{org}, significant differences were found between early and late and mid and late; for N_{org} significant differences were found between early and late and mid and late stages. The C/N ratio decreased with years, and significant differences were found between early and late and early compared with mid. N atmospheric deposition and accumulated N stocks increased with years, whereas N fixation by legumes decreased (Table 1).

3.2. Diversity and abundance of invertebrates

In total, 1408 individuals and 91 species and morphospecies belonging to 10 orders of invertebrates were collected. Table 2 shows the total abundance and number of species/morphospecies per group. A detailed list of all groups and morphospecies is shown in Table S2. The most numerous groups were Hymenoptera, followed by Collembola, Isoptera, Araneae, Hemiptera and Coleoptera. The highest richness was found in Araneae, Diptera, Coleoptera and Hymenoptera (Table 2).

Berger Parker index (BP) indicated that dominance increased with rehabilitation age, while the BP diversity measure decreased, and significant differences were found between early and late stages. Thus, less diverse communities were found in the late stage. Richness was highest in early and late stages and decreased in the mid stage. Total abundance also increased with years since rehabilitation. No significant difference ($p < 0.05$) was found between stages for richness and abundance (total) (Table 2).

3.3. Invertebrate groups and relation with soil chemical properties and vegetation type

The analysis of abundance group response to gradient (rehabilitation age) or CA indicated that four groups presented a long gradient: Opiliones, Diptera, Chilopoda and Hymenoptera (Formicidae) (Fig. 1). Opiliones, Diptera and Chilopoda were associated with early rehabilitation stages, and Hymenoptera with mid and late stages. N_{org} and C_{org} was associated with increasing coverage of forbs, trees and rushes/sedges in the late rehabilitation stage. Canonical correspondence analysis (CCA) for the soil chemical properties, vegetation type coverage and association with the abundance of invertebrate groups, showed that Opiliones and Diptera abundance was positively associated with grasses and legumes in the early stage. Chilopoda was positively associated with mosses coverage increase in the early stage. Hymenoptera was positively associated with C_{org} and N_{org}, and with coverage of rushes, sedges and forbs in the late stage. No other groups presented an association with soil properties and vegetation type. CCA1 explained 94% of accumulated variance. The dispersion of the species along the ordination axis 1 and axis 2 was not significant, Eigenvalue was below 0.3. Legumes (94%) and N_{org} (6%) explained most of the variability but simple effects were not significant ($p > 0.05$).

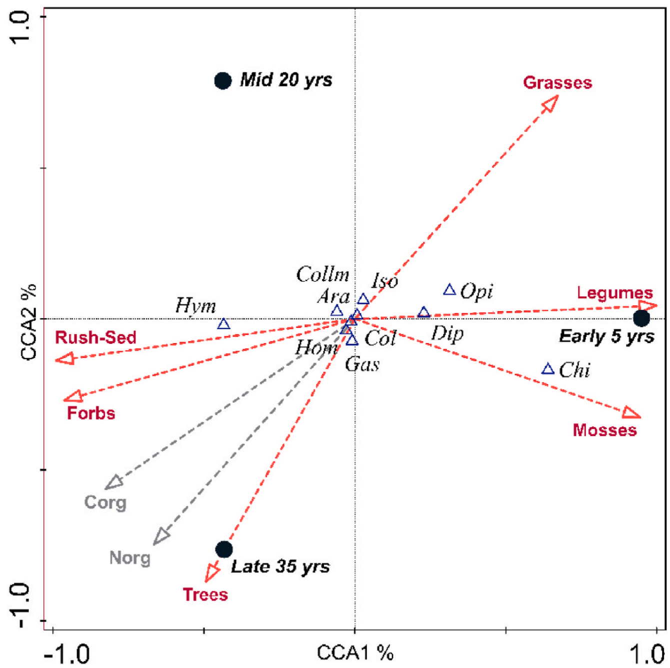

Fig. 1. Biplot from canonical correspondence analysis (CCA) based on invertebrate groups community similarity (Chi-square distance), soil chemical properties and vegetation coverage type in the chronosequence of rehabilitated stages. Ara = Aranea, Chi = Chilopoda, Col = Coleoptera, Collm = Collembola, Dip = Diptera, Gas = Gastropoda, Hom = Homoptera, Hym = Hymenoptera (Formicidae), Iso = Isoptera, Opi = Opilion; Corg (%) = Carbon organic percentage; Norg (%) = Nitrogen organic percentage; Grasses = % coverage of grasses, Mosses = % coverage of mosses, Legumes = % coverage of legumes; Rush-Sedge = % coverage of rushes and sedges; Forbs = % coverage of forbs.

3.4. Stables isotope ratio analysis ($\delta^{13}C$ and $\delta^{15}N$) of soil invertebrates and fresh plants

A selection of invertebrate species and plants $\delta^{15}N$ and $\delta^{13}C$ (mean ±

Table 2
Orders of invertebrates, data on the total abundance, number of species, and morphospecies found in the study and abundance at each rehabilitation stage. Data on diversity measures are means ± SD. Results from one-way ANOVA and multiple-mean comparisons between stages of succession are shown for diversity measurements, within a row followed by different lower-case letters are significantly different at $p < 0.05$.

Invertebrates Groups	Order	Abundance	Species/Morphospecies	Abundance Rehabilitation Stages			Richness (S) Rehabilitation Stages		
				Early	Mid	Late	Early	Mid	Late
Hymenoptera	Hymenoptera	443	13	8	168	267	7	2	6
Collembola	Entomobryomorpha	241	7	31	117	93	3	4	5
Isoptera	Isoptera	170	3	54	81	35	3	2	2
Aranea	Araneae	132	15	37	48	47	6	10	11
Homoptera	Hemiptera	124	12	29	41	54	7	8	5
Coleoptera	Coleoptera	95	13	19	26	50	8	7	7
Gastropoda	Stylommatophora	94	8	21	18	55	4	5	7
Diptera	Diptera	65	14	50	8	7	11	3	3
Opiliones	Opiliones	36	3	29	5	2	3	1	1
Chilopoda	Lithobiomorpha	11	3	10	–	1	3	–	1

Diversity measures *(means ± SD* for total invertebrates	Early	Mid	Late
Berger-Parker dominance (BP)	0.17 ± 0.05 a	0.22 ± 0.03 ab	0.31 ± 0.06 b
Abundance (ind./rehabilitation stage)	193 ± 88 a	344 ± 153 a	409 ± 181 a
Richness (S)	41 ± 13 a	36 ± 8 a	41 ± 12 a

SD) values, number of replicates and C:N ratio are shown in Table S3. The full data of all species included in the analysis are shown in Table S4. Significant differences for $\delta^{15}N$, but not for $\delta^{13}C$ values, were found across the chronosequence (Table S3). All species selected presented more negative values of $\delta^{15}N$ with time since rehabilitation, with a single exception (Phaop $\delta^{15}N$ was lower in early compared to mid stage but not significantly so). ANOVA results for differences between arthropod species across the chronosequence are shown in Table S3.

For plants, *Centaurium erythraea* was depleted in $\delta^{15}N$ from early to mid (5.0‰), from mid to late (1.9‰) and from early to late (6.9‰). *Holcus lanatus* from early to mid (3.8‰), from mid to late (0‰) and from early to late (3.8‰). *Leontodon saxatilis* from early to mid (4.9‰), from mid to late (2.1‰) and from early to late (6.7‰). For all plants, it was observed that $\delta^{15}N$ was lower with age and significant differences were found between early and mid, and early and late rehabilitation stage. The mean depletion for the three species was 5.0‰ from early to mid, 1.0‰ from mid to late and 6.0‰ from early to late (Table S3).

The three trophic guilds identified in the study (Herbivores, Omnivores and Carnivores) and their mean $\delta^{15}N$, $\delta^{13}C$, $\Delta^{15}N$, along with C:N ratio of plants and invertebrates, are shown for each rehabiliatation stage (Table S3). ANOVA results revealed that for all the trophic guilds, significant differences were found for $\delta^{15}N$ and $\Delta^{15}N$, but not for $\delta^{13}C$ or C/N ratio. For the selection of common species across stages, $\delta^{15}N$ was depleted with rehabilitation age and significant differences were found between early and mid, early and late but not between mid and late (Table S3). Significant differences were found for carnivore $\Delta^{15}N$ content in mid compared with late stage. Differences between $\delta^{15}N$ values in carnivores and their potential prey was 6.9 ‰ in early, 6.3‰ in mid and 8.0‰ in the late rehabilitation stage. Assuming an average enrichment of 3.4‰ of $\delta^{15}N$ in animals versus their food, two trophic steps were found in the carnivore community and their food for the early and mid rehabilitation stages, and two and half for late stage (Table S3) (calculations not shown in table). Fig. 2 shows strong patterns for $\delta^{15}N$ values for invertebrates grouped into invertebrate predators (IP), including both omnivores and carnivores, invertebrate consumers (IC), including plant-feeder herbivores and omnivores, across the rehabilitation chronosequence. Mean $\delta^{15}N$ values in predators, consumers and plants (P) became more negative with time since rehabilitation. Significant differences were found for mean $\delta^{15}N$, within each group per stage, in predators for early, mid and late stages, for consumers between early and late and in plants between early and mid and early compared with

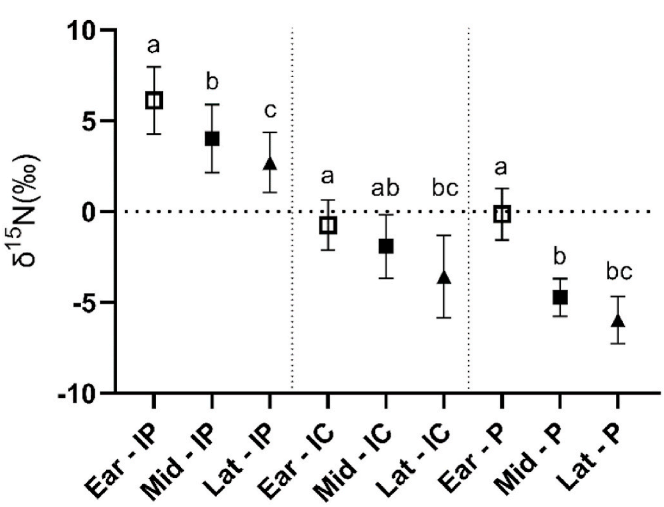

Fig. 2. Distribution of mean $\delta^{15}N$ values in invertebrate predators (IP), invertebrate consumers (IC) and plants (P) across the rehabilitation stages. Different letters (within each group IP, IC and P) indicate significant differences at $p < 0.05$. Ear = Early, 5 years. Mid = Mid, 20 years. Lat = Late, 35 years. Error bars represent standard deviation.

late (Fig. 2).

For the plant species *C. erythraea*, *H. lanatus*, *L. saxatilis*, a strong negative correlation was found between N_{org} in soils and $\delta^{15}N$ values at each stage (early, $r = -0.456$; $p > 0.05$; mid, $r = -0.746$; $p < 0.05$; late, $r = -0.887$; $p < 0.05$) (Fig. S2). However, the soil C:N ratio was strongly positively correlated with $\delta^{15}N$ values for the three plant at each stage (data not shown).

The ranking of $\Delta^{15}N$ for the carnivore and omnivore species across the rehabilitation stages are shown in Fig. 3. The variations in baseline-corrected trophic levels from early to mid stage was less than a trophic level from early to mid (1.3‰), from mid to late resulted close to a one trophic level (3.3‰) and from early to late length of the food web expand more than a trophic level (4.6‰). The length of the invertebrate food chain expanded more than half a trophic level (2.73‰) for the common Coleoptera top predator *Pterostichus niger* (Pteni) from early to late. It was also observed that *P. niger* and *Pterostichus madidus* (Ptema) expanded up to the 5th trophic levels, which did not occur in the other two rehabilitation stages. In the early stage they presented a lower $\Delta^{15}N$ compared to other predators ($Thom_1$, Ocyol juv, $Lycm_1$, $Lithm_1$, $Lithm_2$) (Fig. 3). The same trend was also visible in $\delta^{15}N$ values of *P. niger* and *P. madidus* in early versus mid and late stages (Fig. S1).

4. Discussion

4.1. Diversity, abundance and succession of invertebrate groups

Considering that the early rehabilitation stage was just 5 years after implementation, invertebrate richness was high and can be explained by habitat characteristics such as open patches, that attracts early colonizer species such as Diptera, Chilopoda and some Collembola [40]. Specifically, in mining sites, disturbances such as poor nutrient content and habitat fragmentation [41], could inhibit highly competitive species and increase diversity. This is the intermediate disturbance hypothesis and promotes diversity in early stage substrates [42], compared to mid and late stage which agrees with the present findings.

Suitability of diversity indices to assess rehabilitation in post-mining sites has been questioned. For example, Cross et al. [7] argued that diversity surveys are difficult to implement due to sampling effort, financial viability, and availability of taxonomists. Further, diversity indices alone cannot provide information about the quality or habitat conditions to indicate persistence of the faunal communities in the long term [43]. Conversely, the current study found the Berger Parker index increased with rehabilitation age, and is in agreement with Caruso et al. [30] and Zaitsev et al. [44]. Assessing functional diversity with group assemblages and their relations with soil properties, ecosystem development and nutrient cycling offers a more promising approach to evaluate rehabilitation success and succession in post-mining substrates [19]. This would account better for the fact that species assemblages and feeding relations can alter the prey-predator balance and influence ecosystem processes in post-mining sites [45,46].

The current findings showed that abundance and richness of most groups of invertebrates increased with rehabilitation age, as also reported in post-mining coal sites [46]. Exceptions included collembola abundance, whose abundance in post-mining coal sites was independent from age and vegetation coverage [45,47]. Similarly, the results for Chilopoda are in agreement with Dunger and Voigtläender [45], who observed them as both early colonizers and also dominating after 46 years in rehabilitated coal substrates. In the current study, three species of *Lithobius* were found in the early stage, but only *L. microps* occurred after 35 years. A species-specific colonization behaviour of Chilopoda toward open habitats was detected, with *Lithobius* species acting as early colonizers. Habitat preferences of Chilopoda include small, sunny open habitats with stones [48] and flat levelled areas [49], typical of the early stage. The present study also found an association between abundance of Chilopoda and mosses in the early stage, which suggest that mosses may be used for hiding or as potential refuge for preference prey such as

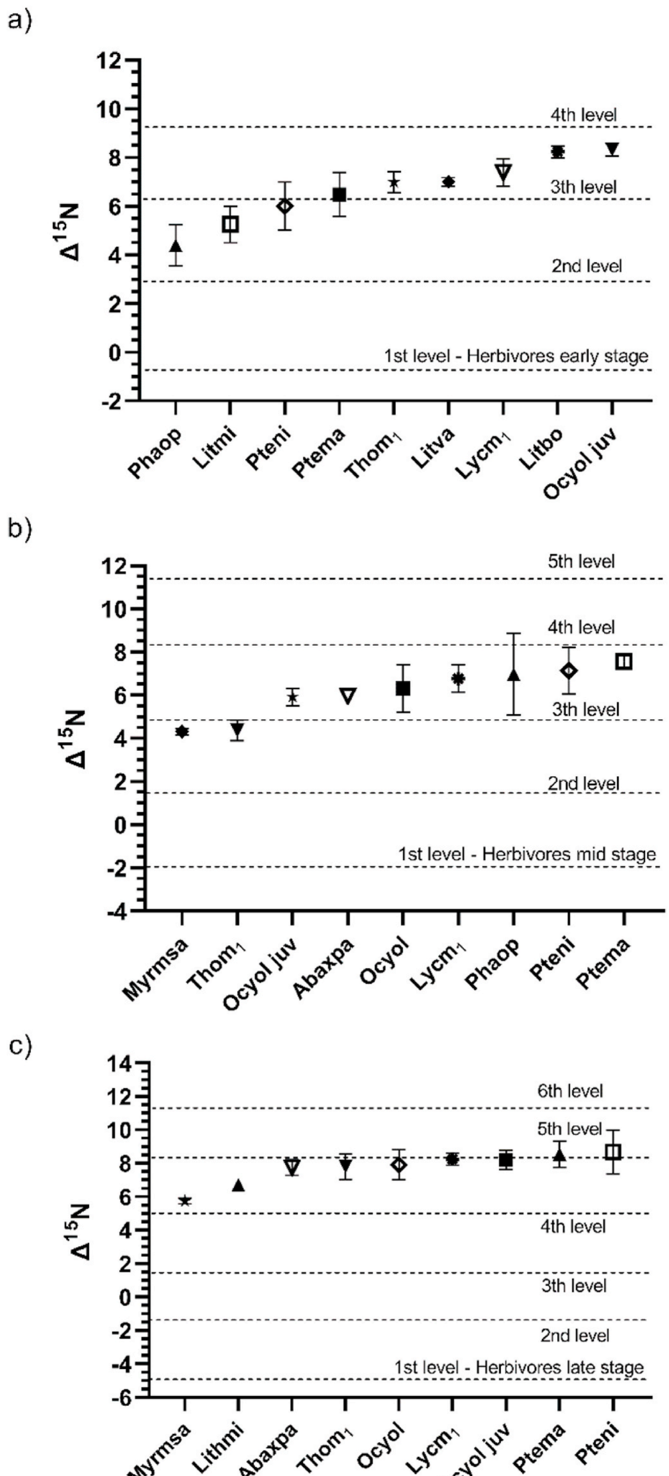

Fig. 3. Ranking of calculated $\Delta^{15}N$ values (means ± SD) for invertebrate consumer species and estimated trophic levels above herbivores across rehabilitation stages; a) early stage, b) mid stage and c) late stage. For full name of species see Table S3 and Table S2. Error bars represent standard deviation.

Isopods and Collembola [21,50]. Coleoptera had no association with vegetation in the current study. This may be due to Carabidae accounting for a large proportion of Coleoptera sampled, and they are known for having a lack of reliance on environmental factors such as particular food or habitat [51] and independent of the vegetation succession [52].

Formicidae richness and abundance increased with rehabilitation age, as previously reported for other sites [8,14]. This may be due to the increase in appropriate ground-cover, and development of woody plant communities in the older sites [53]. Holec and Frouz [13] reported that ant colonization was species-specific, requiring a combination of woody vegetation and empty patches. Absence of *M. rubra* was attributed to the environmental constraints of bare soil and absence of vegetation cover. In the current study, *Myrmica sabuleti* and *M.ruginodis* only appeared 20 years after rehabilitation and is attributed to the development of sufficient habitat conditions and change in vegetation composition and structure.

4.2. Soil chemical properties, isotopes and N-cycle

Accummulation of C and N in post-mining sites is essential for soil formation, supporting biological activity, and overall ecosystem recovery [54]. Nitrogen is a key limiting resource in rehabilitated mine sites, limiting primary productivity, which in turn limits herbivores [55] and influences detritus-based food webs [19]. Carbon is a key limiting factor for soil biological activity and soil formation [56]. The observed improvement in soil properties with rehabilitation age is similar to findings from other mine site studies. Specifically, increased C and N content with rehabilitation age was reported in rehabilitated lignite [57] and coal sites [58,59]. Accumulation of soil organic matter with rehabilitation age is typical for substrates that originally were almost free of organic matter, such as subsoil materials [60]. Lower C/N ratios are usually associated with a higher N availability, which is consistent with current findings. However, higher soil N availability is usually associated with faster decomposition rates and increased N losses from the soil. N losses as nitrate and N_2 result in ^{15}N enrichment of the residual soil N [24]. Open cycles in agricultural land with enriched N result in increased soil $\delta^{15}N$, due to isotope fractionation caused by loss of ^{15}N-depleted NO_3^- [61]. This isotope effect is so evident that $\delta^{15}N$ has been proposed as a useful, integrating indicator of N saturation and 'openness' of ecosystems [62].

Even though N availability increased in the present study, a very strong pattern with years since rehabilitation was observed for $\delta^{15}N$ depleted values of invertebrates and plants in the mid and late stage. This trend is similar to the depleted ^{15}N reported in unfertilised forest soils, since N cycling is more conservative and less open in terms of N loss, unless they are disturbed [63]. In N limiting ecosystems with increased nitrification over time, Högberg [62] proposed that plants were enriched in $[NH_4]^+$ and ^{15}N-depleted NO_3^-. The maturity of the rehabilitation stages in the present study exhibited ^{15}N depletion and this is probably from ^{15}N-depleted NO_3^- from denitrification. Negative $\delta^{15}N$ values in plants of around −2 to −3‰ are usually typical for new ecosystems with limited N content [64]. With time the stages become more mature and have low losses involving ^{15}N fractionation [65].

In the current study, depleted $\delta^{15}N$ values in both plants and invertebrates was observed as rehabilitation age progressed, alongside accumulation in atmospheric N deposition. It is proposed that measured $\delta^{15}N$ also reflects deposition inputs, following simple mass balance rules, since there were no other external inputs into the system (apart from legumes) and the area of the current study is surrounded by agricultural lands. Atmospheric ammonia has depleted $\delta^{15}N$ values, an average of -3.7 ± 3.5‰ was measured for daily-based precipitation [25]. In general, authors also found a lack of seasonality in ammonia $\delta^{15}N$. In terms of total amounts deposited, Kelleghan et al. [66] reported that average N deposition comprised of 50% dry NH_3 and 32% wet NH_4, with approximate annual average N deposition of between 15 and 20 kg N ha^{-1} in Irish grasslands [26] which could explain current findings of system N accumulations.

In summary, the current study indicates that $\delta^{15}N$ in plants (and invertebrates) reflect the N cycling in this developing ecosystem. Negative $\delta^{15}N$ in plants indicate atmospheric inputs and low N losses from the system. The N cycle in the late stage was representative of a more open N-cycle, characteristic of more mature conditions, which was supported by $\delta^{15}N$ in plants and invertebrates, and C/N ratio in soil. $\delta^{15}N$ became more negative with time and most likely reflected deposition inputs, and suggested that N losses from these systems are low. This offers a new tool for assessing the trajectory of N availability of post-mining ecosystems. However, the approach should be tested over different post-mining substrates, climatic conditions, seasonality and longer chronosequences [67,68].

4.3. Isotopes and trophic niches of invertebrates

The isotopic signatures of the basal resources of a simple food web are propagated relatively unchanged through the consumers. In general, lower values of $\delta^{15}N$ indicate feeding on plant materials (e.g. algae and vascular plant) versus higher values feeding on microbes and animals (e.g. fungi and microfauna) [20,36]. This is evident in the current findings; predators and omnivores species showed higher $\delta^{15}N$ than potential prey, such as froghoppers (*Cicadellidae* sp) and Isopods (*Armadillum vulgare*) across the rehabilitation stages. Overall results of the $\delta^{15}N$ and $\delta^{13}C$ analyses confirmed the different feeding guilds (herbivore plant-feeders, saprophagous, omnivores, and carnivore) reported in the literature. For example, the plant-feeder Cicadellidae m_1 and Cicadellidae m_2 and the saprophagous *Armadillum vulgare* were more depleted in $\delta^{15}N$ when compared to their potential predators, which is consistent with Schmidt et al. [69]. For predators, the observed higher value of $\delta^{15}N$ between Lycosidae and their diet was also previously reported [23,70]. Difference in $\delta^{15}N$ between detritivores and predators was equivalent to one trophic transfer (3.4‰) [21,71]. These trends were also observed in the present study. *Myrmica sabuleti* had a relatively high $\delta^{15}N$ in the mid stage compared to late, which could indicate a more predatory diet. High $\delta^{15}N$ previously suggested a more predatory diet in Formicidae [72]. Some ants are specialized on seed predations [19,72] and findings for *M. sabuleti* in the late stage could reflect the higher plant richness and seed diversity that could support a more seed-based diet.

Calculated $\Delta^{15}N$ spacings for consumers indicate that in the arthropod food chains elongate with time since rehabilitation, with more than one whole trophic level addition between the mid and late stages. This patterns closely resembles the pattern reported for developing invertebrate food webs on newly exposed glacier forelands [35]. This may be due to generalist predators such as *Pterostichus* ground beetles, *Ocypus* rove beetles and also wolf spiders (Lycosidae) predating more predators as food webs develop and become more mature. The $\Delta^{15}N$ isotope spacings thus suggest longer and more complex food chains (with intra-guild predation) in later rehabilitation stage, making isotopes powerful and relatively easy to measure indicators. The stable isotope analysis of the arthropod assemblages in the rehabilitated mine tailings revealed that food chains elongated and became more complex with time since rehabilitation.

5. Conclusions

The dominance index Berger Parker reflected differences across the rehabilitation stages. Future rehabilitation strategies should support habitat heterogeneity since it benefits the diversity of invertebrate groups.

Chilopoda, Diptera, Isopoda and some Collembola were characterized as early colonizers, whilst ants appeared only after 20 years from rehabilitation, being associated with age and vegetation type. Species-specific habitat requirements in post-mining sites should be further investigated, particularly for ants.

Stable isotope analysis revealed elongated and more complex food chains in late stage (35 yrs) rehabilitation. Results also indicate that the nitrogen cycle in late stage rehabilitation was representative of a more open N-cycle, typical of mature soil conditions. This was supported $\delta^{15}N$ content in plants and invertebrates and C/N ratio in soil.

Further assessment of stable isotope analysis over different post-mining substrates, climate conditions, and longer chronosequences is recommended.

CRediT authorship contribution statement

Sara Pelaez-Sanchez: Writing – original draft, Methodology, Formal analysis. **Olaf Schmidt:** Writing – review & editing, Validation, Supervision, Resources, Conceptualization. **Ronan Courtney:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejsobi.2024.103616.

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