Soil C and N isotope composition after a centennial Scots pine afforestation in podzols of native European beech forests in NE-Spain

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ABSTRACT

The replacement of native European beech forests (*Fagus sylvatica*) with Scots pine (*Pinus sylvestris*) afforestation may exert changes in soil properties, particularly with respect to soil organic matter (SOM). Stable isotope composition of light elements ($\delta^{13}C$, $\delta^{15}N$) in soils are known proxies for the characterisation of SOM genesis and dynamics. In this research, C and N isotope composition of organic layers, classified as OL (fresh litter), OF (fragmented litter) and OH (humified litter), and the first mineral horizon (Ah) from what was, originally, a beech domain and from a domain of afforestation with pine were analysed by using EA–IRMS. Additionally, C and N isotope signatures were studied in complete soil profiles that were representative of each forest. Pine OL was found to be ^{13}C enriched ($\delta^{13}C$=–28.08 ± 0.49 ‰) compared with beech (–29.87 ± 0.27 ‰). Along the soil profile, C isotope composition mirrors that of the standing vegetation down to the first mineral Ah horizon, with significantly higher $\delta^{13}C$ in pine than in beech. Deeper in the soil, from the eluvial E horizon, no significant $\delta^{13}C$ differences were found between soils, indicating a limited pine influence in depth, years after afforestation. Pine litter tended to be ^{15}N enriched ($\delta^{15}N$=4.43 ± 2.65 ‰) compared to beech (1.43 ± 2.80 ‰). Along the soil profile, a consistent ^{15}N enrichment was observed with depth in the organic layers (O–layers) down to OH. No significant $\delta^{15}N$ differences were found in the mineral horizons between soils, except for the E horizon that showed a lower $\delta^{15}N$ in the beech than in the pine profile. This N trend could be explained by 1) a progressive biomass alteration and a concomitant ^{15}N-enrichment being, in general, more pronounced in O–layers under alien pine than under beech, and 2) migration of more humified SOM forms from eluvial to deeper Bhs horizons, causing a relative accumulation of ^{15}N-depleted SOM in the beechwood E horizon. The accumulation of fungal and root biomass in pinewood OF horizons could be reflected in its ^{15}N-depleted signature.

Keywords: Carbon stocks, Nitrogen cycle, Soil organic matter, Stable isotopes, EA–IRMS

1. Introduction

Forest soils play an important role in the context of global warming as they store large amounts of C and N, thereby, regulating biogeochemical cycles (IPCC 2014; Marty et al*.,* 2011). Stocks of C and N can be affected, not only by changes in climate and soil properties but also by forest management and the replacement of tree species (Leuschner et al*.,* 2013). The set of processes that characterise the soil–vegetation interaction is complex. Vegetation exerts an influence on soil properties, (among other factors, due to the amount and diverse composition of the litter) which have a significant bearing on the chemical composition and soil organic matter (SOM) properties (Binkley 1995). Therefore, it is expected that the replacement of a (broad-leaved) deciduous forest such as a European beech forest (*Fagus sylvatica*) with Scots pine (*Pinus sylvestris*) afforestation may exert changes on soil properties, especially in SOM quality. In the late 19th century, uncontrolled logging for charcoal production reduced beech forests in the Moncayo Natural Park (Northwest Zaragoza, northern Spain) near disappearance. This dramatically increased soil erosion rates in the area (García Manrique, 1960) and in the first decades of the 20th century, large areas were afforested with Scots pine in order to protect the soil and to control erosion. In the short run, the establishment of the new conifer vegetation improves soil physical and chemical properties. However, there are only a limited number of studies in the literature that tackle this fact of improvement with respect to the long-term (Ruiz Navarro, 2009).

Due to its positive effects on a wide array of physical, chemical and biological properties, SOM is an important component in terms of soil quality and ecosystem dynamics (Badía et al*.,* 2013; González-Pérez et al*.,* 2012). SOM is composed of a heterogeneous mixture of substances, with different degradation rates, that are mainly of vegetal origin in the form of litter, roots and exudates and, to a lesser extent, from animal and microbial sources (Schnitzer, 1999).

The amount of litter, its composition and properties are essential factors in SOM formation. Once litter is deposited on the soil surface, it undergoes important transformation processes that are mainly mediated by soil biological (heterotrophic) activity. As decomposition progresses, vegetal molecules may interact with other organic compounds or with the soil mineral fraction, resulting in organo-mineral complexes with variable degrees of complexity and stability (Kögel-Knabner et al*.,* 2008).

Previous studies (Labaz et al*.,* 2014; Leuschner et al*.,* 2013; Schulp et al*.,* 2008) indicate a trend towards litter accumulation in soils that have developed under coniferous forests, presenting thicker organic layers (O–layers) than in beech forests. Leuschner et al*.* (2013) note that, following a period of 51–128 years after afforestation, they detected a 75% increase of SOM in the soil O–layers in the afforested pinewoods as compared to the original beech forests. On the other hand, along the soil mineral horizons down to a depth of 60 cm, they detected a decrease in SOC and N (50 and 80%, respectively). Furthermore, Berthrong et al*.* (2009) observed a decrease of soil C (15%) and N (20%) content in the mineral horizons after afforestation with pine. Although, there are many studies in the literature that deal with quantitative aspects, very few studies tackle the qualitative effects on SOM that are exerted by pine reforestations.

There is a wide range of analytical techniques for characterising SOM by way of physical, chemical and biological methods that allow the determination of its chemical structure and composition (Almendros, 2008; Almendros et al*.,* 2010; de la Rosa et al., 2011; Schnitzer and Khan, 1972; Stevenson, 1982). However, most of these techniques imply previous physical or chemical extraction of distinct fractions of SOM. In recent years, progress has been made regarding techniques that allow SOM characterisation without previous fractionation of its components. Among these techniques, Isotope Ratio Mass Spectrometry (IRMS) (Michener and Lajtha, 2005) has been applied to the measurement of soil stable isotope composition ($\delta^{13}C$ and $\delta^{15}N$), representing a widespread technique that can be used as a proxy to identify and understand SOM biogeochemical and environmental processes.

Natural ^{13}C abundance has been widely used as an organic tracer for SOM dynamics research. The majority of terrestrial plant species have a C3 photosystem with $\delta^{13}C$ values ranging between –24 and –34‰, whereas, plants from tropical, arid and saline environments with a C4 photosystem are ^{13}C-enriched with high $\delta^{13}C$ values of around –6 and –19‰ (Deines, 1980). In this way, variations in SOM $\delta^{13}C$ values can be related to vegetation changes. Additionally, factors such as temperature, salinity and moisture can induce variations in soil C signature (Farquhar, 1984 and references therein). Recently, in temperate forests, Brunn et al. (2014) related the ^{13}C enrichment in beech leaves with increases in environmental temperature, which should affect soil moisture and stomatal opening. The shape of the leaves also affects $\delta^{13}C$ and there are slight differences in isotopic composition between different plant parts and organs (Hobbie and Werner, 2004; Werth and Kuzyakov, 2006). Regarding the OM components; alkanes and lipids have light stable element (C) isotopic signatures, i.e. they are depleted in ^{13}C (Collister et al*.,* 1994; Diefendorf et al., 2015), whereas, cellulose and lignin present similar values to those from the original vegetation (Hobbie and Werner, 2004). Therefore, the degradation of certain labile SOM compounds, i.e. polysaccharides, may induce additional isotope fractionation in the soil (Balesdent et al., 1988). On the other hand, it is known that during decomposition in soil and evolution/humification processes, SOM is progressively ^{13}C enriched (Zech et al*.,* 1997 and references therein) and as a consequence, SOM isotopic signature normally increases with soil depth (Brunn et al*.,* 2014; Krull et al*.,* 2002), which is also a valid proxy with which to to study soil C dynamics in soils.

Nitrogen isotopic analysis ($\delta^{15}N$) provides relevant information about the N cycle (Pardo and Nadelhoffer, 2010; Makarov, 2009). Plants are commonly depleted in ^{15}N in comparison to soil and the upper soil horizons are depleted in relation to deeper horizons (Högberg, 1997), with this being particularly pronounced in forest soils (Szpak, 2014). This variation with depth can be explained by the strong isotopic fractionation that occurs during ammonification, nitrification and denitrification processes, resulting in ^{15}N-depleted ions (NH_4^+, NO_3^- and N_2O) and a residual N enriched in ^{15}N (Makarov, 2009). In general, increases in $\delta^{15}N$ values can be explained by the accumulation of nitrogen-containing organic materials that are enriched in ^{15}N and which are produced by microbial activity. This ^{15}N-enrichment effect is mitigated in the soil surface by new plant biomass contributions (Billings and Richter, 2006). Additionally, soil $\delta^{15}N$ values can also vary depending on previous land uses (i.e. forest, pastures, agricultural crops and practices), plant species, as well as rain regimes (Pardo and Nadelhoffer, 2010).

This study aims to detect the changes which occur in soil C and N that has been surrogated to the centennial afforestation of Scots pine in the European beech forest domain of Moncayo Natural Park (Northwest Zaragoza, northern Spain) using the stable isotopic composition of light elements ($\delta^{13}C$ and $\delta^{15}N$) as proxies for SOM quality and dynamics.

2. **Materials and methods**

2.1. Study site

The area of study is located in the Moncayo Natural Park (Iberian Range, northeast Spain) with coordinates of 41°47’N, 1°48’W, at altitudes between 1360 and 1475 m above sea level, comprising the original and mature European beech (*Fagus sylvatica*) and the 100-year old afforested Scots pine (*Pinus sylvestris*) forests (Fig. 1). Beech forest understory is composed mainly of *Vaccinum myrtillus L.* and *Erica arborea L.* while *Ilex aquifolium L.* and *Deschampsia flexuosa L.* can also be found in the pinewood. Mean annual precipitation is around 1060 mm and mean annual temperature is 9.2 °C. Soil moisture regime in the area is udic and the temperature regime is mesic (Martínez del Castillo et al*.,* 2012; Ibarra and Echeverría, 2004). The studied soil profiles are developed over quartzitic sandstones (*Lower Triasic*) and present a series of common properties, such as high stoniness, sandy loam or loamy textures, extreme acidity, very low base content (Badía et al*.,* 2016) and the soils are classified as *Typic Haplorthod* (SSS, 2014).

2.2. Sampling and sample preparation

Sampling was conducted in September 2014, following North–East oriented rectilinear slopes with similar inclination (20%). Ten sampling sites were selected (5 in the pine forest and 5 in the beech forest). For each site, O–layers classified as OL (fresh litter), OF (fragmented litter) and OH (humified litter) and the first 10 cm of the first mineral horizon (Ah) were sampled (Fig. 2). In addition, one soil profile per forest type near the aforementioned sampling points (composed of OL–OF–OH–Ah–E–Bhs–BC horizons) was sampled and described. Mineral samples were air dried until constant weight and then sieved through a 2 mm mesh. Before analysis, the samples were ground to a fine powder and homogenised using an agate mortar aided with liquid nitrogen.

2.3. Elemental and isotopic analysis

Total carbon and nitrogen, as well as the bulk isotopic composition of light elements (C and N), were analysed by dry combustion in a Flash 2000 elemental micro–analyser (Thermo Scientific) coupled via ConFlo IV Universal Continuous Flow Interface (Thermo Scientific) to a Delta V Advantage isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany). Given the absence of carbonates in the parent material composition, the total C measurements that were taken correspond to total organic C (TOC).

Isotopic ratios are reported as parts per thousand deviations (expressed as δ values) with respect to the appropriate IAEA standards (VPBD and V–Air for C and N, respectively):

$$\delta = \left[\frac{\mathbf{R}\,sample\ - \mathbf{R}\,standard}{\mathbf{R}\,standard}\right] \mathbf{x\ 1000} \qquad (1)$$

where R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio. The standard deviations of $\delta^{13}C$ and $\delta^{15}N$ were typically less than ± 0.05‰, ± 0.2‰, respectively.

2.4. Statistical analysis

In order to identify the differences in the studied soil properties surrogated to forest and horizon type, one–way ANOVA tests were used. The forest types (European beech *vs.* Scots pine) were considered as fixed factors when analysing the effect of vegetation change, splitting data by soil horizons (OL, OF, OH, Ah). Additionally, changes in soil properties with horizon type were checked using the horizon type (OL, OF, OH, Ah) as fixed factor, splitting data by forest type (European beech and Scots pine). The Nnormal distribution of values was verified by using a Kolmogorov–Smirnov test. All statistical analyses were carried out by using StatView for Windows version 5.0.1 (SAS Institute Inc., Cary, North Carolina, USA). The statistical analysis was performed for the data of the field replicates (n=5 for each forest type) and values presented in the text are reported as Mean ± Standard Deviation, unless otherwise stated. However, the values obtained for the soil profiles did not allow for statistical comparisons, hence, results are expressed as Mean ± Standard Deviation of the analytical replicates; in this way, they are considered as observations that support the data which were subjected to statistical analysis.

3. Results

3.1. Morphology of organic layers

Under beechwood, the OL (fresh litter horizons) layer with thicknesses between 1 and 4 cm was composed of recent, poorly transformed litter. Underneath the litter, an OF horizon (1–2 cm thickness) was found that is mainly formed of fragmented plant residues that were, generally, of foliar origin. Below these horizons, thicker OH layers (2–5 cm), consisting of well–decomposed litter, were observed (Table 1). The pinewood O–layers presented different structures with thinner OL horizons (1–2 cm thickness), followed by potent and thicker OF layers (4–10 cm) in comparison to the beechwood O–layers, and with a remarkable density of roots and fungal mycelia.

Additionally, no differences were detected in pH values of the uppermost mineral Ah horizon (0–10 cm) between beech (4.6 ± 0.5) and pine (4.1 ± 0.4) forests (Table 1).

3.2. Soil organic C and total N content

TOC content of the pine OL–layer tended to be higher (464 ± 19 g/kg) than for the beech litter (440 ± 19 g/kg), whereas, no differences with respect to forest type were found for OF and OH horizons. However, for each vegetation type, all horizons showed significant differences among them in TOC content, reflecting a decrease from the upper layers (OL) down to the first mineral horizons (Ah 0–10 cm) (Table 2).

Regarding N content, only OL–layers showed significant differences between forest types, being higher in beech litter (13.9 ± 2.0 g/kg) than in pine needles (10.3 ± 1.3 g/kg). Throughout the beech forest, the N content in O–layers was similar and only the first mineral Ah horizon presented a significantly lower N content. But, in the pinewood a significant N enrichment was observed in the OF (15.4 ± 2.6 g/kg) and OH (13.8 ± 1.5 g/kg) layers in comparison to the OL layers (10.3 ± 1.3 g/kg). The C/N ratio in the OL pine layer (48.0 ± 8.1) was significantly higher than in the beech OL (32.2 ± 3.5), but there were no evident differences among the other horizons or between forest types.

Along the soil profiles, TOC and N content distribution (Table 3) matched that of a podzol, as it showed a TOC and N content decrease in the E horizons compared to the overlying horizon, with a subsequent accumulation in the underlying Bhs horizons (Buurman and Jongmans, 2005).

3.3. Soil C isotopic signature ($\delta^{13}C$)

The obtained results showed a consistent and significantly heavier C isotopic composition under the pinewood O–layers than in the beechwood O–layers (Table 4). These values are in accordance with previously published data, indicating that the beech C isotopic signature (Nahm et al*.,* 2007) is less-extreme than the pine C isotopic signature (Llorente et al*.,* 2010). For the Ah mineral horizon, the difference between beechwood and pinewood forest types was significant at $P = 0.05$.

A consistent enrichment in ^{13}C is observed from the OL to the E horizons along both soil profiles, ranging from –29.68 to –27.91‰ and –28.44 to –26.30‰ in the beech and pine O–layers, respectively. Regarding the mineral horizons, the values ranged from –26.99 to –25.70‰ and –26.70 to –25.67‰ for beech and pine, respectively. At deeper horizons (E, 30 cm), pine δ^{13}C values tends to equal those of the original beech (Figure 3). Additionally, in both forest types, a depletion in ^{13}C was observed in the Bhs and BC horizons with respect to the E horizon.

3.4. Soil N isotopic signature (δ^{15}N)

No significant differences were observed in terms of forest types or O–layer type in δ^{15}N values, although some trends were found (Table 5). In the OF layers, a slight depletion of ^{15}N was observed in comparison with the overlying (OL) and underlying (OH) layers. δ^{15}N values presented significant variations between the OH layers and Ah horizons in both forests, highlighting the shift from the organic layers to the mineral horizon. Along the beech soil profile (Fig. 4), a progressive enrichment in ^{15}N can be observed from the OL to the OH layers, significantly increasing towards the Ah mineral horizon. No differences were observed in the depth, except for the E horizon, where δ^{15}N values significantly decreased. Regarding the pinewood soil profile, a depletion in

^{15}N is observed in the OL horizon in comparison to the OF horizon, followed by a significant enrichment towards the OH horizon, whereas, no differences were observed in depth along the mineral soil horizons.

4. Discussion

4.1. Morphology of organic layers

Coniferous litter contains compounds that make its biomass more difficult to decompose than that of broad–leaved forests. This fact normally results in the accumulation of plant residues and production of acidic compounds under pine forests (Schulp et al*.,* 2008). The combined action of the acid compound production with the low base content in the parent material induces soil acidification which limits bacterial and macroinvertebrate growth and facilitates the predominance of fungus (Ponge, 2013). This explains the abundant fungal mycelium that is observed in the pinewood OF–layer. In this way, beechwood O–layers provide an environment hat is more prone to SOM mineralisation and humification, denoted by lower O–layer thickness in comparison to pinewood O–layers, characterised by fragmented plant biomass accumulations packed with roots and fungal mycelia (Leuschner et al*.,* 2013; Schulp et al*.,* 2008; Carceller and Vallejo, 1996). This organic layer distribution corresponds to mull/moder humus forms in the beechwood that evolved to moder/mor form transitions with the Scots pine afforestation (Jabiol et al*.,* 2013).

These observations match the results that were obtained in previous research (Marty et al*.,* 2015; Labaz et al*.,* 2014, Leuschner et al*.,* 2013) that indicate the propensity to thick O layer formation under coniferous stands, in comparison to natural beech forests, thus, increasing SOM pools in the surface. Nonetheless, Girona-García et al*.* (2015) (when studying SOM composition and structure by analytical pyrolysis (Py–GC/MS) of both beech forests and pine forests in the Moncayo Natural Park down to 100 cm depth), found a more stable and well-preserved SOM under the beech forest once the OM is incorporated into the mineral soil. This may be due, in part, to a selective preservation of more stable OM forms in the beechwood mineral soil layers.

4.2. Soil organic C content and C isotopic signature ($\delta^{13}C$)

The TOC content in pinewood OL layers was found to be significantly higher than in beechwood, and this fact has already been noted by Carceller (1995) with respect to Moncayo Natural Park. Although TOC content presented no differences in the OF, OH and Ah horizons between forest types, the values tend to be higher in pine horizons. In a comparative study between beechwood and Scots pinewood, Schulp et al*.* (2008) found no significant differences in C content in the first 10 cm of mineral soil. However, Leuschner et al*.* (2013) and Berthrong et al*.* (2009) note significant decreases in soil C content after Scots pine afforestation.

Beech forest O–layers and Ah first mineral horizons showed significant differences in δ^{13}C values, decreasing gradually from OL to Ah as opposed to the same horizons in the pinewood forest, where no differences were found between OL–OF and OH–Ah. In this way, the δ^{13}C values for the beechwood might indicate an environment in which SOM degradation is gradual, and not as limited as in the pinewood (higher C/N ratios, lower pH and nutrient content), where a more heterogeneous mixture of undecomposed and decomposed SOM is found.

Along the soil profiles, a clear and progressive differentiation in δ^{13}C values between forest types and horizons can be observed from the OL layers down to the Ah horizons, increasing with depth as SOM is decomposed, which usually produces an enrichment in ^{13}C (Brunn et al*.,* 2014; Krull et al*.,* 2002). In the mineral E horizons of the pinewood profile, δ^{13}C values tend to equal those of the beech forest profile, indicating a limited influence of the afforested species with depth, 100 years after the afforestation.

In the Bhs and BC horizons from both profiles, a depletion in ^{13}C was detected. However, this observation is not in line with the results that are reported by previous studies in the literature (Billings and Richter and references therein, 2006; Compton and Boone, 2000), and that showed a general ^{13}C enrichment trend with depth due to the presence of older SOM in deeper horizons among different soil types, including podzols. The possible explanations include i) the leaching of organic-mineral complexes that are depleted in ^{13}C, i.e. including isotopically light leaf wax components, or ii) the inputs from roots (depleted in ^{13}C) that, in such podzolic illuvial horizons (Bhs and BC) that usually present a higher root density, due to the accumulation of water and nutrients (Buurman and Jongmans, 2005; Diefendorf et al., 2015; Lichtfouse et al., 1998).

4.3 Soil N content and N isotopic signature (δ^{15}N)

Soil N content was similar among the O–layers in both forest types and significantly different compared to the Ah (0–10 cm) mineral horizons. On the other hand, in the pinewood OF and OH layers there showed a similar N content and significant differences were found between OL and Ah horizons. Paying attention to the differences found between forest types, although beech leaves showed a higher N content than pine needles, a N enrichment in pine OF layers was observed which matches the N content in beech OF layers. This increase could be due to fungal and root biomass inputs, since a high density of roots and fungal mycelia (rhizomorphs) was observed in the field for pinewood OF layers. These N inputs are also reflected in the mineral soil results, while not observing any N decrease after pine afforestation, as reported by previous studies in the literature (Leuschner et al*.,* 2013; Berthrong et al*.,* 2009).

Although having no statistical significance, it is noteworthy that the N isotope composition (δ^{15}N) along the beechwood O–layers tends to increase with depth, whereas, in pinewood a different trend is observed with lower δ^{15}N values in the OF layer. Enrichment in ^{15}N may be caused by N losses (ammonification, nitrification and denitrification processes) and a selective biomass^{15}N enrichment, especially in the pine OH horizons. Humification is known to cause ^{15}N enrichment, particularly in forest soils (Szpak, 2014), however, ^{15}N-depletion may occur during inorganic N intake by vegetation (Högberg, 1997). But, in natural systems where N availability is a limiting factor, as could be the case with respect to the observations in the above, such discrimination against ^{15}N is rare (Billings and Richter, 2006). Therefore, the observed trend could be best explained in terms of the alteration of biomass by heterotrophic organisms that are known to produce ^{15}N-depleted compounds and a progressively ^{15}N-enriched biomass over time (Szpak, 2014; Makarov, 2009; Billings and Richter, 2006). The differences observed in the pinewood ^{15}N-depleted OF layer, may well also reflect a limited SOM humification in this conspicuously potent layer (4-10 cm thick) of litter accumulation, in addition to the presence of fresh root and fungal biomass, that is rich in ^{15}N-depleted chitin (Hobbie and Högberg, 2012), as observed de visu in the pinewood OF layer.

The meaning of $\delta^{15}N$ values can be adequately observed along the soil profile horizons as they show the same trend as topsoil samples (Fig. 4). The enrichment in the heavy isotope that was identified in the Ah horizons is kept steady at depth ($\delta^{15}N \approx -7‰$), and no apparent differences were observed, except for the beech E horizon, where ^{15}N depletion is detected. The isotopic depletion that was observed in the beechwood E horizon could be explained by the leaching of more humified SOM (^{15}N-enriched) towards the Bhs horizons, causing a relative accumulation of materials that are less evolved (depleted in ^{15}N), resulting in lower $\delta^{15}N$ values for the SOM in the elluvial horizon.

5. Conclusions

Soil under Scots pine presented moder/mor humus forms with remarkable accumulations of litter at the surface, whereas, soil O–layers in the beechwood corresponded to mull/moder forms, indicating an environment that is more suitable for biological activity. Due to the limited biological activity, SOM is accumulated on the pinewood surface, providing higher C stocks and thicker O–layers, but no quantitative differences were observed in depth between both forest types. The soil pH was not significantly affected by the change of vegetation although a slight acidification was observed under the pinewood. The C isotope ratio ($\delta^{13}C$) allowed us to trace SOM evolution along the soil profile and revealed differences between natural beech forest and afforested pinewood forest downward until the E horizons. Deeper in the soil, the differences between both forest isotopic signatures disappear, indicating a limited influence with depth of the afforested pinewood SOM contribution. The consistent $\delta^{15}N$ enrichment observed at depth along soil profiles is probably related to N mineralisation, tending to be higher in the pinewood than in the beechwood OH layers and, apparently, not presenting differences in depth. The accumulation of fungal and root biomass in the pinewood OF horizons is reflected in its ^{15}N-depleted signature.

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Table 1. Description of the organic horizons and humus types in the beech and pine forests of Moncayo Natural Park.

Forest type	Beechwood	Pinewood
Elevation (masl)	1360	1470
O–Horizon thickness (cm)		
OL	1–4	1–2
OF	1–2	4–10
OH	2–5	1–6
Humus type	Mull/moder	Moder
pH of Ah horizon (0-10 cm)	4.6 ± 0.5	4.1 ± 0.4

Table 2. Total Organic Carbon (TOC), Total N (N) and C/N ratio (C/N) of O–layers and Ah (0–10 cm) mineral horizons (Mean ± standard deviation). P indicates significant differences ($P < 0.05$) between forest types for each horizon. Lowercase letters refer to significant differences between horizons for each forest system.

	TOC (g/kg)			N (g/kg)			C/N		
Soil horizons	Beechwood	Pinewood	*P*	Beechwood	Pinewood	*p*	Beechwood	Pinewood	*P*
OL	440 ± 19 a	464 ± 19 a	0.007	13.9 ± 2.0 a	10.3 ± 1.3 a	0.013	32.2 ± 3.5 a	48.0 ± 8.1 a	0.004
OF	385 ± 32 b	414 ± 19 b	0.147	14.6 ± 1.4 a	15.4 ± 2.6 b	0.555	26.5 ± 2.4 ab	27.3 ± 3.8 b	0.695
OH	316 ± 48 c	366 ± 46 c	0.160	13.7 ± 2.7 a	13.8 ± 1.5 b	0.947	23.3 ± 2.6 b	26.7 ± 4.1 b	0.170
Ah (0–10 cm)	86.9 ± 15 d	94.1 ± 5.6 d	0.471	3.48 ± 1.8 b	4.61 ± 3.1 c	0.528	27.8 ± 7.8 ab	25.6 ± 11.5 b	0.740

Table 3. Total Organic Carbon (TOC) and total N (N) content and C/N ratio for Beechwood and Pinewood soil profiles.

Soil profile	Horizon	Depth (cm)	TOC (g/kg)	N (g/kg)	C/N
Beechwood	OL	-9	470 ± 3	17.0 ± 1.0	27.6 ± 1.7
	OF	-7	407 ± 4	17.1 ± 0.1	23.8 ± 1.7
	OH	-2	371 ± 27	17.0 ± 0.6	21.8 ± 1.7
	Ah	0–25	61.1 ± 2	5.6 ± 0.2	11.0 ± 0.6
	E	25–55	27.0 ± 14	4.7 ± 0.0	5.7 ± 0.5
	Bhs	55–75	40.9 ± 5	5.0 ± 0.2	8.2 ± 0.5
	BC	75–100	40.1 ± 1	4.9 ± 0.1	8.3 ± 0.5
Pinewood	OL	–7	474 ± 7	12.4 ± 0.0	38.1 ± 1.2
	OF	–6	413 ± 38	18.6 ± 0.5	22.2 ± 1.9
	OH	–1	311 ± 23	15.1 ± 0.3	20.7 ± 1.5
	Ah	0–30	92.2 ± 7	7.65 ± 0.1	12.1 ± 0.8
	E	30–60	34.5 ± 3	4.95 ± 0.0	7.0 ± 0.5
	Bhs	60–90	41.6 ± 5	5.49 ± 0.2	7.6 ± 0.6
	BC	90–120	38.1 ± 6	4.91 ± 0.1	7.8 ± 0.5

Table 4. $\delta^{13}C$ (‰) values of O–layers and Ah (0–10 cm) mineral horizon (mean ± standard deviation, n=5). P indicates significant differences ($P < 0.05$) between forest types for each horizon. Lowercase letters refer to significant differences between horizons for each forest system.

Soil horizons	$\delta^{13}C$ (‰)		*P*
	Beechwood	Pinewood	
OL	−29.87 ± 0.27 a	−28.08 ± 0.49 a	0.0001
OF	−28.93 ± 0.19 b	−27.64 ± 0.62 ab	<0.0001
OH	−28.08 ± 0.37 c	−26.99 ± 0.53 bc	0.0081
Ah (0–10 cm)	−27.51 ± 0.65 d	−26.30 ± 0.52 c	0.0556

Table 5. $\delta^{15}N$ (‰) values of O-layers and Ah (0–10 cm) mineral horizons (mean ± standard deviation, n=5). p indicates significant differences ($P< 0.05$) between forest types for each horizon. Lowercase letters refer to significant differences between horizons for each forest system.

Soil horizons	$\delta^{15}N$ (‰)		*P*
	Beechwood	Pinewood	
OL	1.43 ± 2.80 a	4.43 ± 2.65 a	0.1204
OF	1.15 ± 2.23 a	3.44 ± 2.70 a	0.2362
OH	3.84 ± 3.33 a	6.47 ± 3.12 a	0.2409
Ah (0–10 cm)	17.0 ± 7.60 b	17.6 ± 7.50 b	0.8611

Figure captions

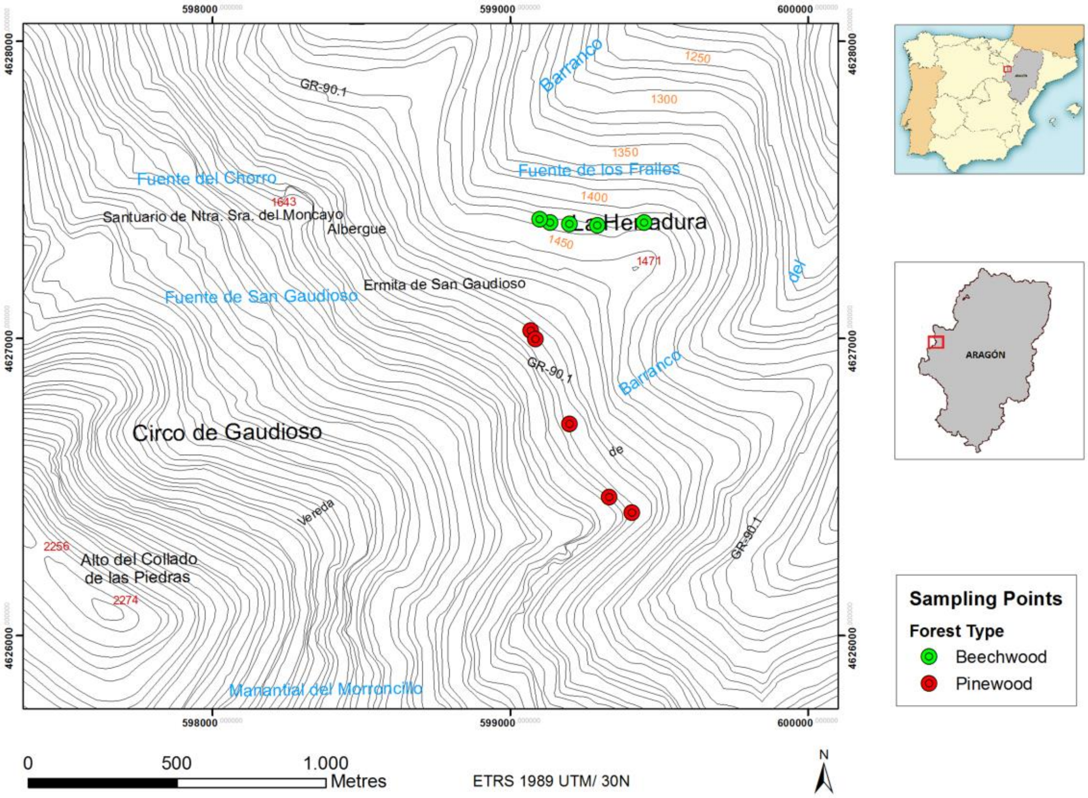

Fig. 1. Location of the study area in the Moncayo Natural Park (Zaragoza, NE Spain). Sampling points are indicated in green for the beech forest and in red for the Scots pine forest.

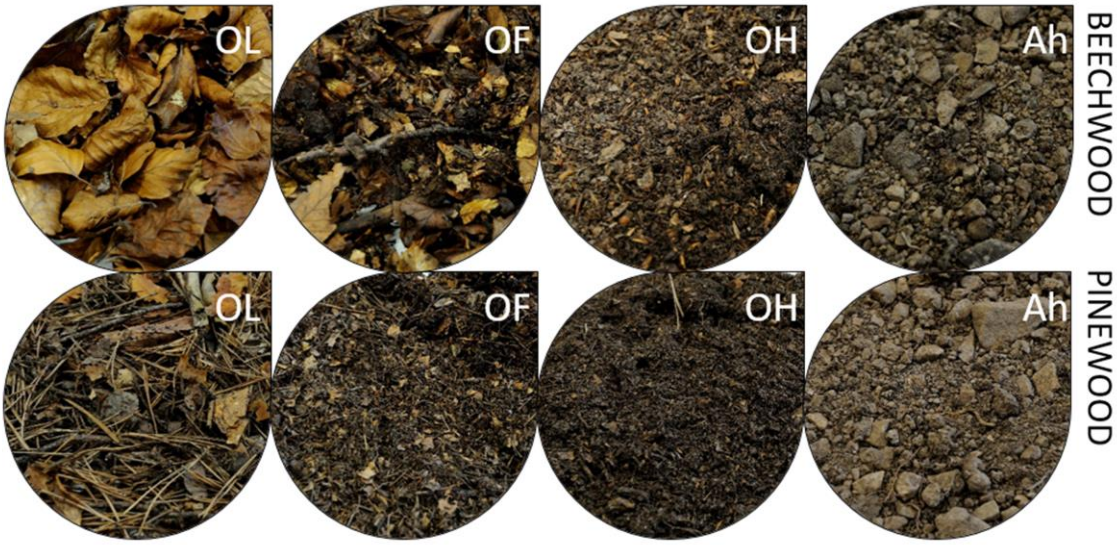

Fig. 2. Plain view of the O–layers and Ah horizons that were morphology sampled in the European beech (up) forest and Scots pine (down) forest.

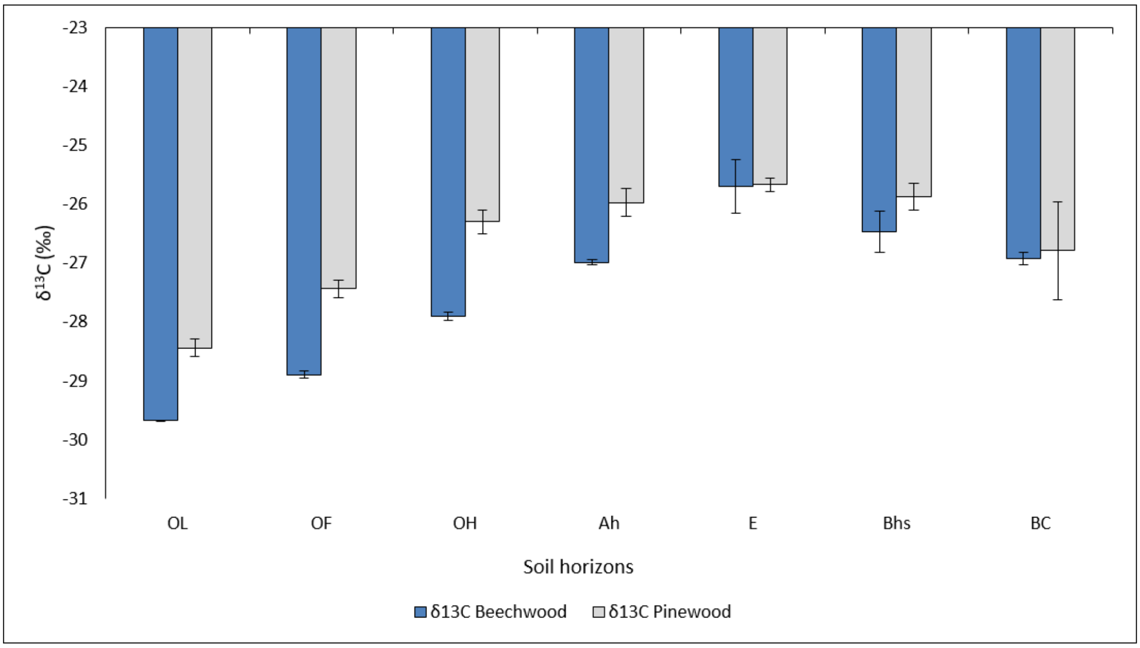

Fig. 3. Beechwood (blue) and pinewood (grey) C isotopic composition ($\delta^{13}C$) for each of the O–layers (OL, OF, OH) and the mineral horizons (Ah, E, Bhs and BC) of the sampled soil profiles. Error bars indicate the standard deviation of analytical replicates.

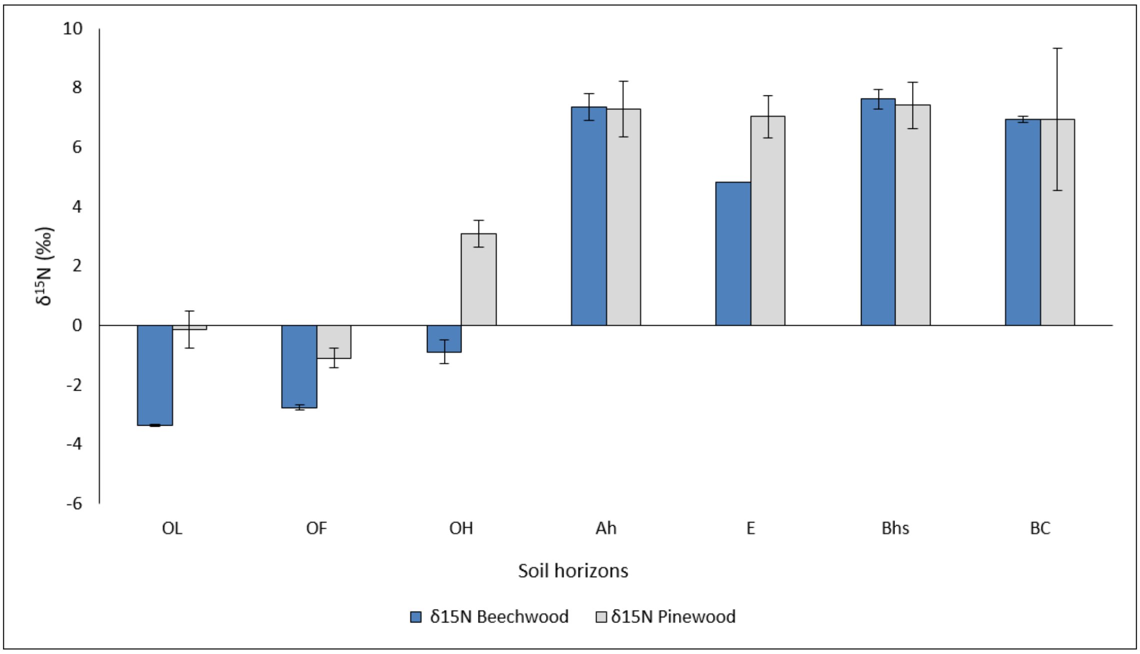

Fig. 4. Beechwood (blue) and pinewood (grey) N isotopic signature ($\delta^{15}N$) for each of the O–layers (OL, OF, OH) and the mineral horizons (Ah, E, Bhs and BC) of the sampled soil profiles. Error bars indicate the standard deviation of analytical replicates.