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

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HIGHLIGHTS

- A century-old Scots pine afforestation in a beech forest is studied.
- Changes in stable C and N isotope composition surrogated to the afforestation were analysed.
- Mineral soil horizons are ^{13}C and ^{15}N-enriched compared with organic layers
- Limited changes in soil surface SOM can be detected 100 years after afforestation.
- The soil depth to which the introduced Scots pine SOM is incorporated has been detected.

ABSTRACT

The replacement of native European beech forests (*Fagus sylvatica*) by Scots pine (*Pinus sylvestris*) afforestation may exert changes in soil properties, particularly in soil organic matter (SOM). Stable isotope composition of light elements ($\delta^{13}C$, $\delta^{15}N$) in soils are known proxies for the characterization of SOM genesis and dynamics. In this work C and N isotope composition of organic layers (fresh litter OL, fragmented litter OF and humified litter OH) and first mineral horizon (Ah) from an original beech domain and from afforested forest with pine were analysed by EA–IRMS. Additionally, C and N isotope signature was studied in complete soil profiles representative for each forest. Pine OL litter was found ^{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 from 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 ‰) as compared with 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 but for the E horizon that showed 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

INTRODUCTION

Forest soils play an important role in the context of global warming as they store large amounts of C and N, therefore 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 in 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 deciduous (broad leaved) forest such as the European beech forests (*Fagus sylvatica*) by 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, Spain) nearing 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 to protect soil and control erosion. In the short run, the establishment of the new conifer vegetation improves soil physical and chemical properties. However, the studies tackling this fact in the long-term are limited (Ruiz Navarro, 2009).

Due to its positive effects on a wide array of physical, chemical and biological properties, SOM is an important component regarding 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, mainly from 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 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 degree 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 developed under coniferous forests, presenting thicker organic layers (O–layers) than in beech forests. Leuschner *et al.,* (2013), 51–128 years after afforestation, 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 60 cm depth, a decrease in SOC and N (50 and 80 %, respectively) was detected. Berthrong *et al.,* (2009) also observed a decrease of soil C (15 %) and N (20 %) content in the mineral horizons after afforestation with pine. Although, there are many studies dealing with quantitative aspects, very few tackle the qualitative effects exerted by pine reforestations on SOM.

There is a wide range of analytical techniques for characterising SOM by 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 & Khan, 1972; Stevenson, 1982). However, most of these technique 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 & Lajtha, 2005) is applied to measure 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 C4 photosystem are ^{13}C-enriched with high $\delta^{13}C$ values around –6 and –19 ‰ (Deines, 1980). In this way, variations in SOM $\delta^{13}C$ values can be related to vegetation changes. Additionally, factors like temperature, salinity and moisture can induce variations in soil C signature (Farquhar 1984 and refs therein). Recently in temperate forests, Brunn et al., (2014) related the ^{13}C enrichment in beech leaves with environmental temperature increase, 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 & Werner, 2004; Werth & Kuzyakov, 2006). Regarding the OM components; alkanes and lipids have light C isotopic signaturesi.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 & 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.,* 2007 and refs therein) and as a consequence, SOM isotopic signature normally increases with soil depth (Brunn *et al.,* 2014; Krull *et al.,* 2002) being also a valid proxy to study soil C dynamics in soils.

Nitrogen isotopic analysis ($\delta^{15}N$) provides relevant information about the N cycle (Pardo & Nadelhoffer, 2010; Makarov, 2009). Plants are commonly depleted in ^{15}N as compared to soil and the upper soil horizons are depleted in relation to deeper ones (Högberg, 1997) being this 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, $\delta^{15}N$ values increases can be explained by the accumulation of nitrogen-containing organic materials enriched in ^{15}N that are produced by microbial activity. This ^{15}N-enrichment effect is mitigated in the soil surface by new plant biomass contributions (Billings & 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 & Nadelhoffer, 2010).

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

MATERIALS AND METHODS

Study site

The area of study is located in the Moncayo Natural Park (Iberian Range, NE–Spain 41°47’N, 1°48’W) at altitudes between 1360 and 1475 m above sea level, comprising the mature original European beech (*Fagus sylvatica*) and the 100-year-old afforested Scots pine (*Pinus sylvestris*) forests (Figure 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 & Echeverría, 2004). The studied soil profiles are developed over quartzitic sandstones (*Lower Triasic*) and present a series of common properties as high stoniness, sandy loam or loamy textures, extreme acidity, very low base content (Badía *et al.,* 2016) and classified as *Typic Haplorthod* (SSS, 2014).

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 pine forest and 5 in the beech forest). For each site, O–layers (fresh litter, OL; fragmented litter, OF; humified litter, OH) and the first 10 cm of the first mineral horizon (Ah) were sampled (Figure 2). In addition, one soil profile per forest type (composed of OL–OF–OH–Ah–E–Bhs–BC horizons) was sampled and described. Mineral samples were air–dried until constant weight and sieved through a 2 mm mesh. Before analysis the samples were ground to fine powder and homogenised using an agate mortar aided with liquid nitrogen.

Elemental and isotopic analysis

Total carbon andnitrogen 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 coupled via ConFlo IV interface 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 taken correspond to total organic C (TOC).

Isotopic ratios are reported as parts per thousand deviations (expressed as δ values) referred to 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.

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 to analyse 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). Normal distribution of values was verified by a Kolmogorov–Smirnov test. All statistical analyses were carried out using StatView for Windows version 5.0.1 (SAS Institute Inc, Cary, North Carolina, USA). Data presented in the text are reported as Mean ± Standard Deviation unless otherwise stated.

RESULTS

Organic layers morphology

Under beechwood, OL (fresh litter horizons) layer with thicknesses between 1 and 4 cm was composed of recent, poorly transformed litter. Underneath, an OF horizon (1–2 cm thickness) is found that is formed mainly by fragmented plant residues, 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 structure with thinner OL horizons (1–2 cm thick), followed by potent and thicker OF layers (4–10 cm) as compared 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).

Soil organic C and total N content

TOC content of pine OL–layer tended to be higher (464 ± 19 g/kg) than for beech litter (440 ± 19 g/kg) whereas no differences with forest type was found for OF and OH horizons. On the other hand, 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). Along beech forest the N content in O–layers was similar and only the first mineral Ah horizon presented a significant lower N content. On the other hand, 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 as compared to the OL layers (10.3 ± 1.3 g/kg). The C/N ratio in OL pine layer (48.0 ± 8.1) was significantly higher than in beech OL (32.2 ± 3.5), not showing 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 as compared with the overlying horizon with a subsequent accumulation in the underlying Bhs horizons (Buurman & Jongmans 2005).

Soil C isotopic signature (δ^{13}C)

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

A consistent enrichment in ^{13}C is observed from OL to the E horizons along both soil profiles ranging from –28.68 to –27.91 ‰ and –28.44 to –26.30 ‰ in 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 $\delta^{13}C$ values tend to equal those of the original beech (Figure 3). Additionally, in both forest types, a depletion in ^{13}C was observed in Bhs and BC horizons with respect to the E horizon.

Soil N isotopic signature ($\delta^{15}N$)

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

DISCUSSION

Organic layers morphology

Coniferous litter contains compounds that make its biomass more difficult to decompose than that of broad–leave forests. This 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 induce soil acidification limiting bacterial and macroinvertebrates growth and facilitating the predominance of fungus (Ponge, 2013). This explains the abundant fungal mycelium observed in pinewood OF–layer. In this way, beechwood O–layers provide an environment more prone to SOM mineralization and humification, denoted by lower O–layer thickness as opposed 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 & Vallejo, 1996). This organic layer distribution corresponds to mull–moder humus forms in the beechwood that evolved to moder–mor forms transitions with the Scots pine afforestation (Jabiol *et al.,* 2013).

These observations match the results obtained by previous works (Marty *et al.,* 2015; Labaz *et al.,* 2014, Leuschner *et al.,* 2013) that indicate the propensity to thick O layers formation under coniferous stands as compared to natural beech forests, 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 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 to the mineral soil. This may be due in part to a selective preservation of more stable OM forms in the beechwood mineral soil layers.

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

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

Beech forest O–layers and Ah first mineral horizons showed significant differences in $\delta^{13}C$ values, decreasing gradually from OL to Ah as opposed to the pinewood forest, where no differences were found between OL–OF and OH–Ah. In this way, the $\delta^{13}C$ 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 $\delta^{13}C$ values between forest types and horizons can be observed from OL layers down to 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 profile, indicating a limited influence of the afforested species with depth 100 years after the afforestation.

In Bhs and BC horizons from both profiles, a depletion in ^{13}C was observed, not matching the results reported by previous studies (Billings & Richter and refs therein, 2006; Compton & Boone, 2000) 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. Possible explanations include i) the leaching of organic-mineral complexes depleted in ^{13}C i.e. including isotopically light leaf wax copmponents /or ii) the inputs from roots (depleted in ^{13}C) that, in such podzolic iluvial horizons (Bhs and BC) usually present a higher root density due to the accumulation of water and nutrients (Buurman & Jongmans, 2005; Diefendorf *et al.*, 2015; Lichtfouse et al., 1998).

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 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 matching the N content in beech OF layers. This increase could be due to fungal and root biomass inputs, this is supported by the high density of roots and fungal mycelia (rhizomorphs) observed in the field for pinewood OF layers. As with C content data, N content values obtained stand against the results obtained by Leuschner *et al.,* (2013) and Berthrong *et al.,* (2009) as no decrease in N content was observed in the mineral horizons of the afforested forests.

Although not statistically significant, it is worth to mention that the N isotope composition (δ^{15}N) along beechwood O–layers tend 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 pine OH horizons. Humification is known to cause ^{15}N- enrichment, particularly in forest soils (Szpak, 2014) and on the contrary, ^{15}N-depletion may occur during inorganic N intake by vegetation (Högberg, 1997). However, in natural systems where N availability is a limiting factor, as could be the case, such discrimination against ^{15}N is rare (Billings & Richter, 2006). Therefore, the observed trend could be best explained in terms of the alteration of biomass by heterotrophic organisms known to produce ^{15}N depleted compounds and a aprogressively ^{15}N-enriched biomass over time (Szpak, 2014; Makarov, 2009; Billings & Richter, 2006). The differences observed of 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, rich in ^{15}N-depleted chitin (Hobbie & Högberg, 2012), as observed “de visu” in the pinewood OF layer.

The meaning of δ^{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 identified in the Ah horizons is kept steady in depth (δ^{15}N ≈ -7‰), showing no differences except for the beech E horizon, where ^{15}N depletion is observed. The isotopic depletion observed in the beechwood E horizon could be explained by the leaching of more humified SOM (^{15}N-enriched) towards Bhs horizons, causing a relative accumulation of less evolved materials (depleted in ^{15}N), resulting in lower $\delta^{15}N$ values for the SOM in the eluvial horizon.

CONCLUSIONS

Soil under Scots pine presented moder–mor humus forms with remarkable accumulations of litter in the surface whereas soil O–layers in the beechwood corresponded to mull–moder forms indicating an environment 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 found 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 and afforested pinewood forests down till E horizons. Deeper in the soil, the differences between both forests isotopic signatures disappear, indicating a limited influence with depth of the afforested pinewood SOM contribution. The consistent $\delta^{15}N$ enrichment observed in 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 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 from 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. Beechwood and Pinewood soil profiles Total Organic Carbon (TOC) and total N (N) content and C/N ratio.

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. Lowercases 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

Figures

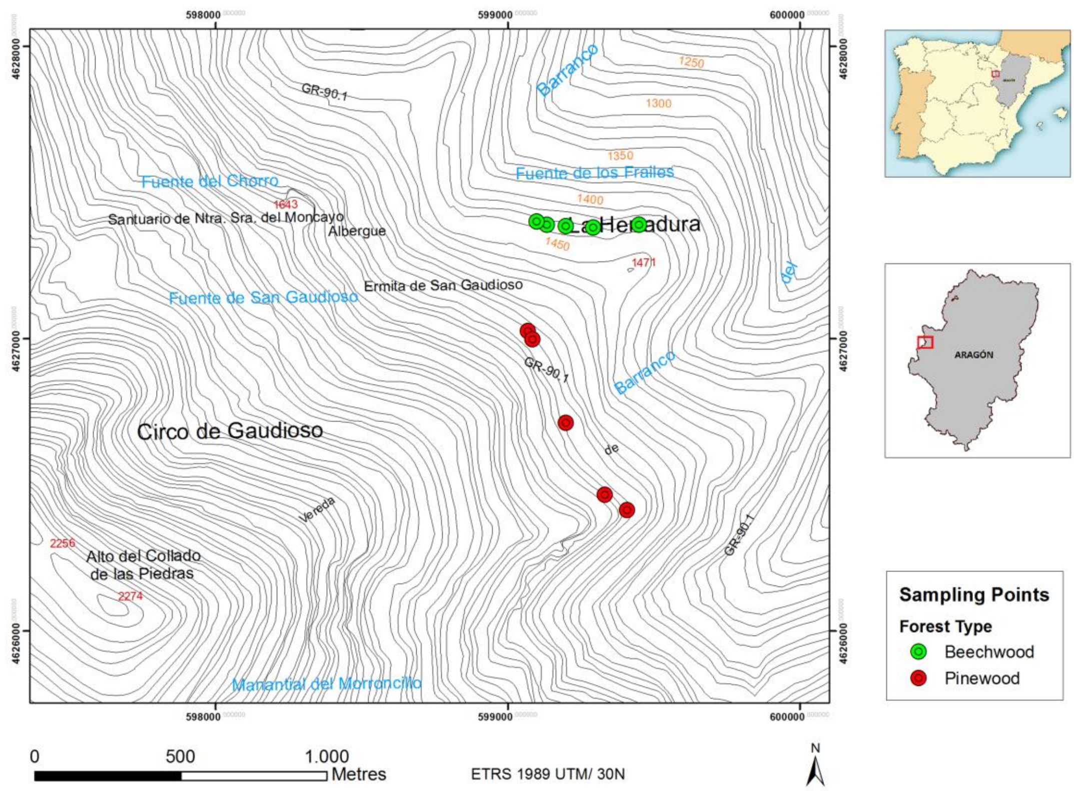

Fig. 1. Location of the study area in the Moncayo Natural Park (Zaragoza, NE–Spain). Sampling points are indicated in green for the beech forest and in red for the Scots pine forest.

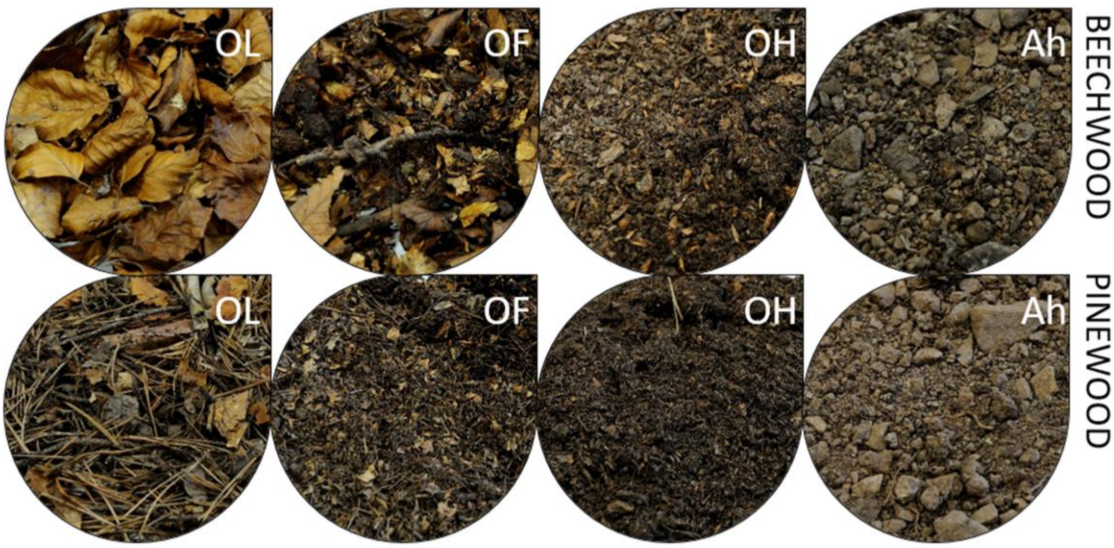

Fig. 2. Plain view of the O–layers and Ah horizons morphology sampled in the European beech (up) and Scots pine (down) forests.

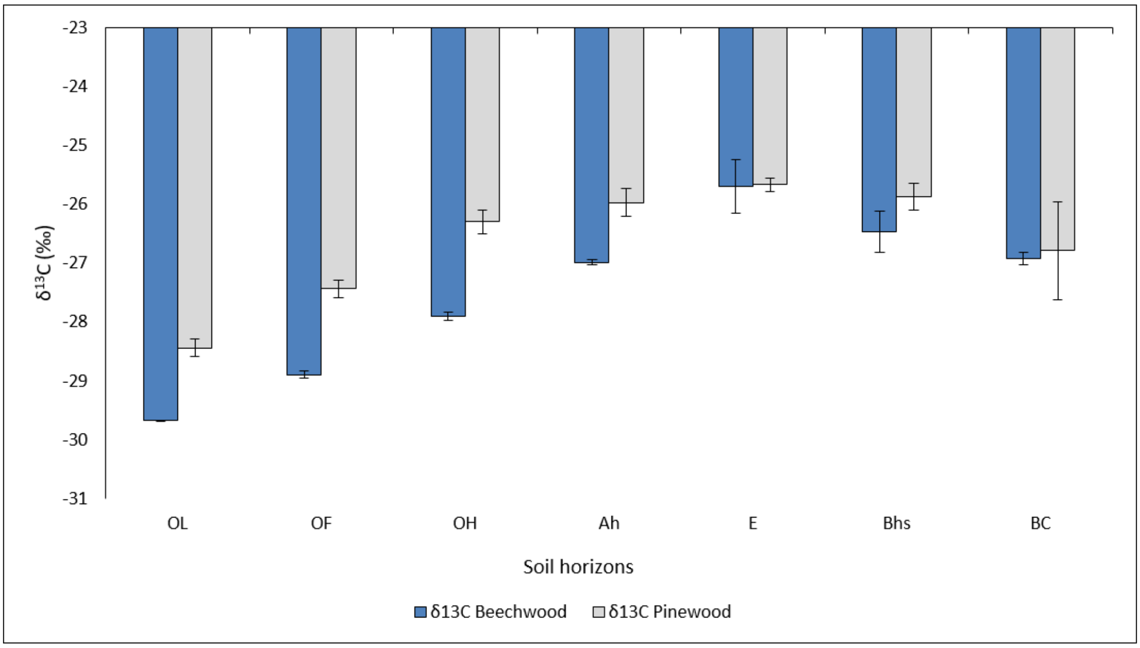

Fig. 3. Beechwood (blue) and pinewood (grey) C isotopic composition (δ^{13}C) for each of the O–layers (OL, OF, OH) and the mineral horizons (Ah, E, Bhs and BC) of the sampled soil profiles. Error bars indicate the standard deviation of analytical replicates.

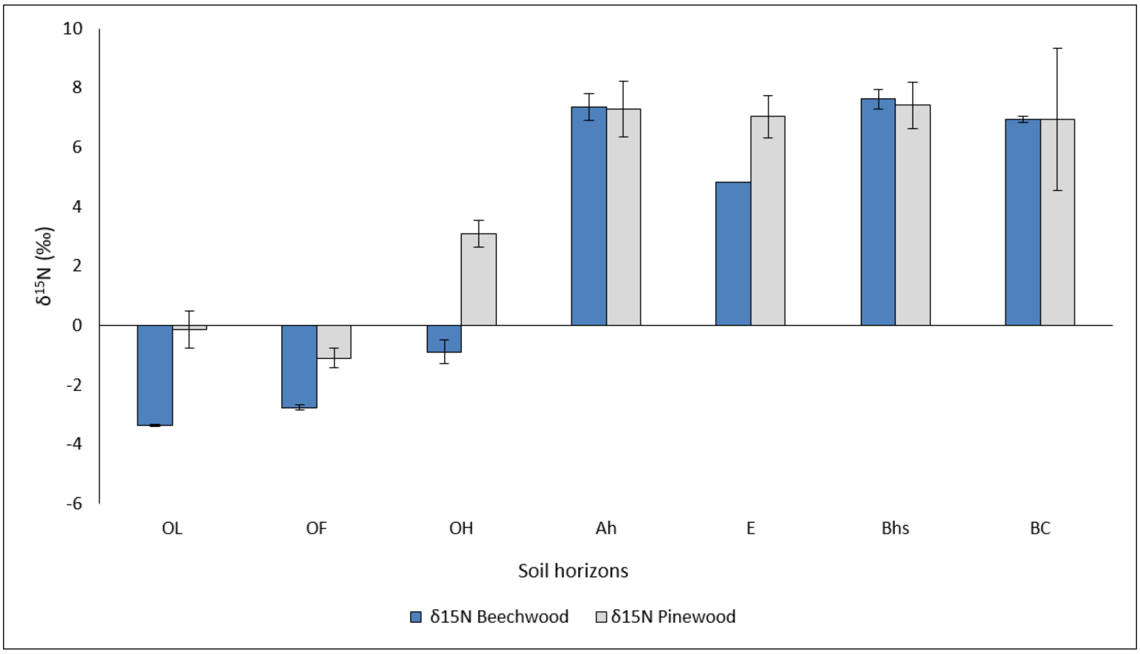

Fig. 4. Beechwood (blue) and pinewood (grey) N isotopic signature (δ^{15}N) for each of the O–layers (OL, OF, OH) and the mineral horizons (Ah, E, Bhs and BC) of the sampled soil profiles. Error bars indicate the standard deviation of analytical replicates.