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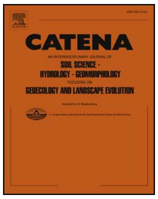

Soil C and N isotope composition after a centennial Scots pine afforestation in podzols of native European beech forests in NE-Spain

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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 (δ^{13}C, δ^{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 ($\delta^{13}C = -28.08 \pm 0.49$‰) compared with beech ($-29.87 \pm 0.27$‰). Along the soil profile, C isotope composition mirrors that of the standing vegetation down to the first mineral Ah horizon, with significantly higher δ^{13}C in pine than in beech. Deeper in the soil, from the eluvial E horizon, no significant δ^{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 ($\delta^{15}N = 4.43 \pm 2.65$‰) compared to beech ($1.43 \pm 2.80$‰). Along the soil profile, a consistent ^{15}N enrichment was observed with depth in the organic layers (O–layers) down to OH. No significant δ^{15}N differences were found in the mineral horizons between soils, except for the E horizon that showed a lower δ^{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.

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 et al., 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

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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, 2007) 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 et al., 1982 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 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