

**Changes in soil organic matter composition after Scots pine afforestation in a native European beech forest revealed by analytical pyrolysis (Py-GC/MS)**

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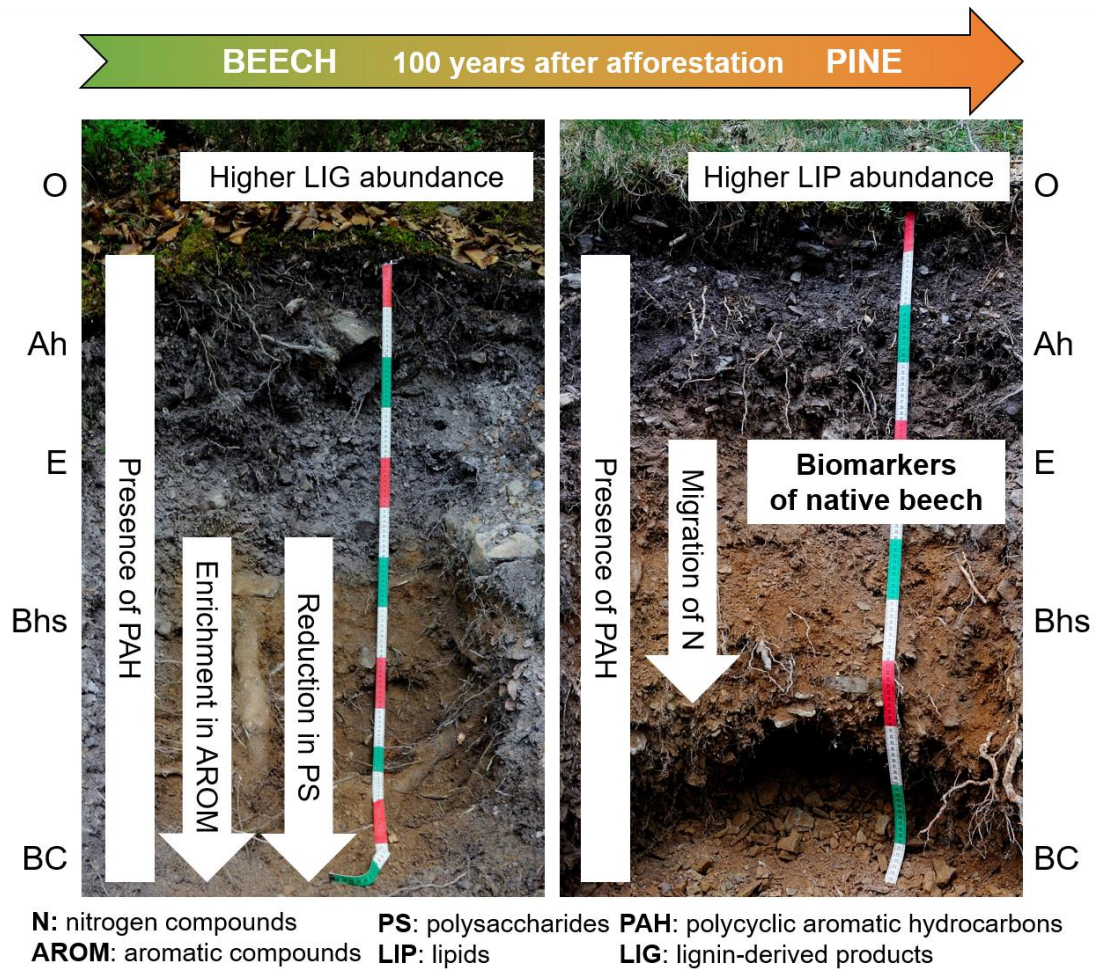
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**Full bibliographical details: Girona-García, A.; Badía-Villas, D.; Jiménez-Morillo, N.T.; González-Pérez, J.A. 2019. Changes in soil organic matter composition after Scots pine afforestation in a native European beech forest revealed by analytical pyrolysis (Py-GC/MS). Science of the Total Environment 691: 1155-1161. DOI: 10.1016/j.scitotenv.2019.07.229**

## Graphical Abstract



## Highlights

- A total of 128 pyrolysis compounds were detected in the soil organic matter (SOM)
- Polysaccharides in Bhs and BC under pine may indicate migration of complexed forms
- Natural beech forest alkane series point to higher SOM stabilization
- Biomarkers in pine show permanence of beech-derived SOM 100 yrs after afforestation

## Abstract

The introduction of coniferous species in former deciduous forests may exert changes in soil organic matter, particularly in its molecular composition. In this work, pyrolysis-gas chromatography-mass spectrometry was used to study changes in SOM quality related to the centennial afforestation of Scots pine in an area formerly covered by European beech forest in the NE-flank of the Moncayo Natural Park (NE-Spain). For each soil profile three organic layers (fresh litter, fragmented litter and humified litter) and mineral soil horizons (Ah, E, Bhs and C) were studied. A total of 128 compounds were identified in the pyrograms, and composition differences were detected among the organic and mineral soil layers as well as between soils under beech and pine, for the main compound classes: nitrogen compounds, aromatics, lignin methoxyphenols, polycyclic aromatic hydrocarbons, lipids and polysaccharide-derived moieties. Such chemical differences were found to be derived from the biomass composition of the predominant vegetation type that was incorporated into the soil and from its progression into the soil profile. The analysis of the distribution of alkanes indicated higher SOM stabilization in the native beech forest soil. The signal of beech biomarkers (long chain *n*-alkanes C<sub>31</sub>-C<sub>33</sub>) found in the pine E horizon indicates the permanence of SOM derived from the natural forest ca. 100 years after the afforestation.

**Keywords:** biogeochemistry; biomarkers; alkanes; lignin; methoxyphenols; litter layers

## 1. Introduction

Forest soils play an important role in biogeochemical cycles since they store large amounts of C and N (IPCC, 2014; Marty et al., 2011). The stocks of C and N can be affected not only by climate change but also by shifts in land use such as the replacement of tree species (Leuschner et al., 2013). The processes involved in the soil-vegetation interaction are complex; vegetation influences the soil, among other factors, by the diverse composition of the produced litter, which together will influence the chemical composition of the soil organic matter (SOM) (Binkley, 1995). For this reason, it is

expected that changes in the vegetation cover will result in SOM contributions of different nature and therefore, contrasted chemical composition.

In the late 19<sup>th</sup> century, as a consequence of uncontrolled logging for charcoal production, the population of native European beech (*Fagus sylvatica*) in the Moncayo Natural Park (NE-Spain) was reduced to near disappearance. This also increased soil erosion (García Manrique, 1960) and in order to stop this phenomenon in the areas where the beech forests were decimated, afforestation of Scots pine (*Pinus sylvestris*) was conducted in the first decades of the 20<sup>th</sup> century. Consequently, shifts in vegetation such as those that were undertaken in that area ca. 100 years ago may exert remarkable changes in SOM composition. Such changes have been previously detected in the Moncayo Natural Park in a study by Girona-García et al. (2018a), which found differences in SOM C and N stable isotope composition between the natural beech and the afforested pine forests.

Organic matter is considered to be the most essential constituent of soils because it virtually influences all physical, chemical and biological properties of soil (Weil & Brady, 2017). Litter deposition is usually the main source of SOM, so changes in the amount and composition of litter will influence the complexity and stability of the SOM once incorporated into the soil (Kögel-Knabner et al., 2008). For this reason, it is important to study how forest management activities, such as afforestation, can influence the composition of SOM, especially when broad-leaved forests are replaced by coniferous species, whose litter is more difficult to decompose, resulting in litter accumulation on the topsoil (Labaz et al., 2014; Badía-Villas & Girona-García, 2018; Girona-García et al., 2018a, 2018b).

Pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS) analysis is a technique widely used for the structural characterization of SOM (González-Pérez et al., 2012; Rumpel et al., 2012; Derenne and Quénéa, 2015). It is a powerful tool that allows the separation and identification of the SOM constituents without prior chemical treatment (Moldoveanu, 1998; González-Pérez et al., 2007). Py-GC/MS generates a fingerprint that allows the quantification of the relative abundance of the different chemical

compounds that constitute the SOM (White et al., 2004; González-Pérez et al., 2007; Almendros et al., 2018). The main compounds produced by pyrolysis are of known biogenic origin; for example, by pyrolysis, polysaccharides produce anhydrosugars, lignin originates methoxyphenols, proteins result in certain nitrogen compounds, and lipids produce alkanes, alkenes, waxes, terpenes, and steranes (González-Vila et al., 2009; De la Rosa et al., 2012). The detailed study of the results obtained by Py-GC/MS allows to determine the sources and contributions of SOM in the soil, evaluate the preservation of certain biogenic materials and the selective decomposition of others that come from vegetation or that are transformed into the soil (González-Vila et al., 1999; González-Pérez et al., 2003; Almendros et al., 2018). Among the compounds originating from pyrolysis, molecular markers such as aliphatic, aromatic and heteroaromatic compounds can be found, which are related to shifts in land use and/or vegetation cover as well as perturbances such as wildfires or pollutant discharges (Bucó et al., 2004; Faria et al., 2015; Miralles et al., 2015; Santana et al., 2015; Jiménez-Morillo et al., 2016).

The aim of this study was to analyze by Py-GC/MS the qualitative changes in SOM related to the afforestation of Scots pine in areas formerly populated by native European beech forests, ca. 100 years after the afforestation was conducted. We hypothesized that the different nature of the litter produced by both species will result in different decomposition paths of SOM and will allow the depth to which the SOM of the introduced species has been incorporated into the soil to be traced.

## **2. Materials and methods**

### *2.1. Area of study*

The study site was located in the NE flank of the Moncayo Massif (Iberian Range, NE-Spain) at an elevation between 1360 and 1475 m a.s.l., where natural European beech (*Fagus sylvatica*) and afforested Scots pine (*Pinus sylvestris*) forests can be found. The understory of the beech forest mainly consists of *Vaccinum myrtillus* L. and *Erica arborea* L. whereas in the pine forest, *Ilex aquifolium* L. and *Deschampsia flexuosa* L. can also

be observed. The mean annual rainfall is 1060 mm and the mean annual temperature is 9.2 °C (Ibarra & Echeverría, 2004; Martínez del Castillo et al., 2012). Soils were developed over Lower Triassic quartzitic sandstones and showed a series of common properties such as high stoniness, extreme to strong acidity, low base content, high SOM content, and sandy loam or loamy textures (Badía et al., 2016; Girona-García et al., 2018b). The general physical and chemical properties of the soil profiles are provided in Table 1. The studied soil profiles were classified as Skeletic Umbric Albic Podzol (Loamic) (IUSS WRB, 2014) and Typic Haplorthod (SSS, 2014).

Table 1. General chemical and physical properties of the studied soil profiles

Soil Profile	European beech				Scots pine			
	Ah	E	Bhs	BC	Ah	E	Bhs	BC
Horizon	Ah	E	Bhs	BC	Ah	E	Bhs	BC
Depth (cm)	0-25	25-55	55-75	75-100	0-30	30-60	60-90	90-120
pH (H <sub>2</sub> O, 1:2.5)	4.0	4.2	4.9	4.9	4.7	4.6	5.0	5.3
pH (KCl, 1:2.5)	2.9	3.2	4.2	4.3	3.7	3.3	3.9	4.3
CEC (cmol+ kg <sup>-1</sup> )	10.6	3.7	8.9	6.1	20.7	7.7	9.8	7.6
Total Organic C (g kg <sup>-1</sup> )	61.1	27	40.9	40.5	92	35	42	38
Total N (g kg <sup>-1</sup> )	5.6	4.7	5.00	4.9	7.6	4.9	5.5	4.9
C/N	11	5.7	8.2	8.3	12.1	7.0	7.6	7.8
Clay (g kg <sup>-1</sup> )	117	71	159	113	215	170	234	81.2
Silt (g kg <sup>-1</sup> )	276	309	291	328	329	406	314	447
Sand (g kg <sup>-1</sup> )	607	620	550	559	456	424	452	471
Textural class	Sandy loam				Loam			
WHC (g kg <sup>-1</sup> )	175	141	242	218	217	160	190	n.d.
Coarse fraction (g kg <sup>-1</sup> )	809	831	725	951	642	852	732	568

n.d.: not determined

## 2.2. Sampling and sample preparation

Sampling was conducted in September, 2014. Two sampling sites (Figure 1), one in the natural beech forest (41° 47' 32.15" N; 1° 48' 24.89" W) and one in the afforested pine forest (41° 47' 01.85" N; 1° 48' 16.66" W) were selected based on the similarity of the soil horizon distribution, elevation, and geomorphological characteristics. In each of the sampling sites, one soil profile in each forest type was selected and a substantial and representative amount of the organic layers and the mineral horizons was sampled. The organic layers were separated in fresh litter (OL), fragmented litter (OF) and humified litter (OH) and the mineral horizons were classified as Ah, E, Bhs and BC (Figure 2).

All samples were air-dried until constant weight and the mineral samples were sieved through a 2 mm mesh. Prior to analysis, all samples were ground to fine powder and homogenized using an agate mortar aided with liquid nitrogen.

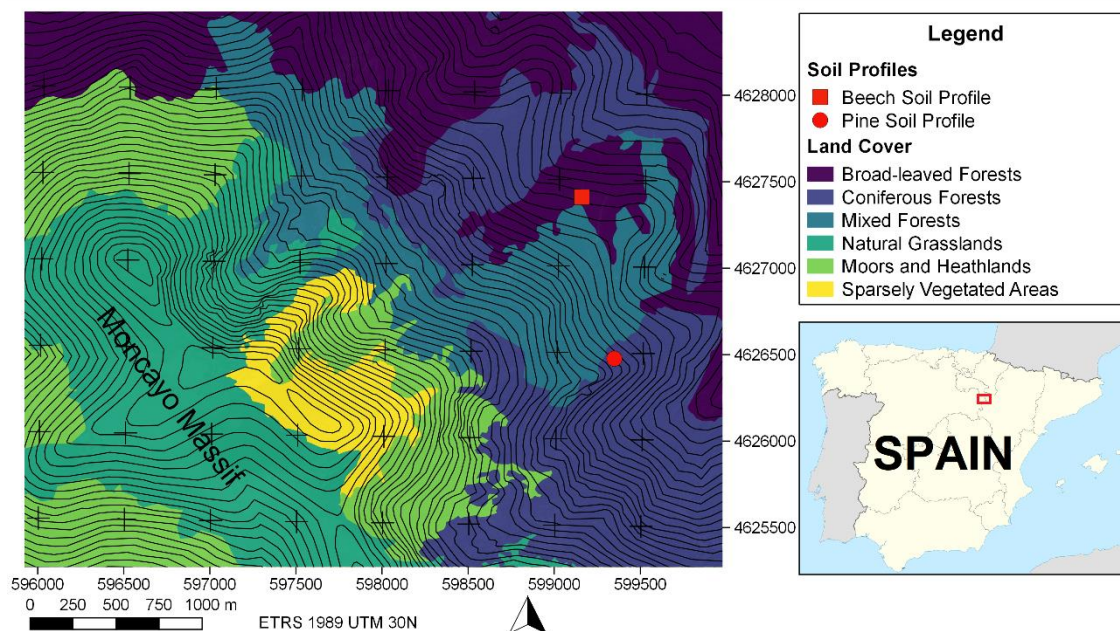


Figure 1. Location of the sampling sites in the Moncayo Natural Park

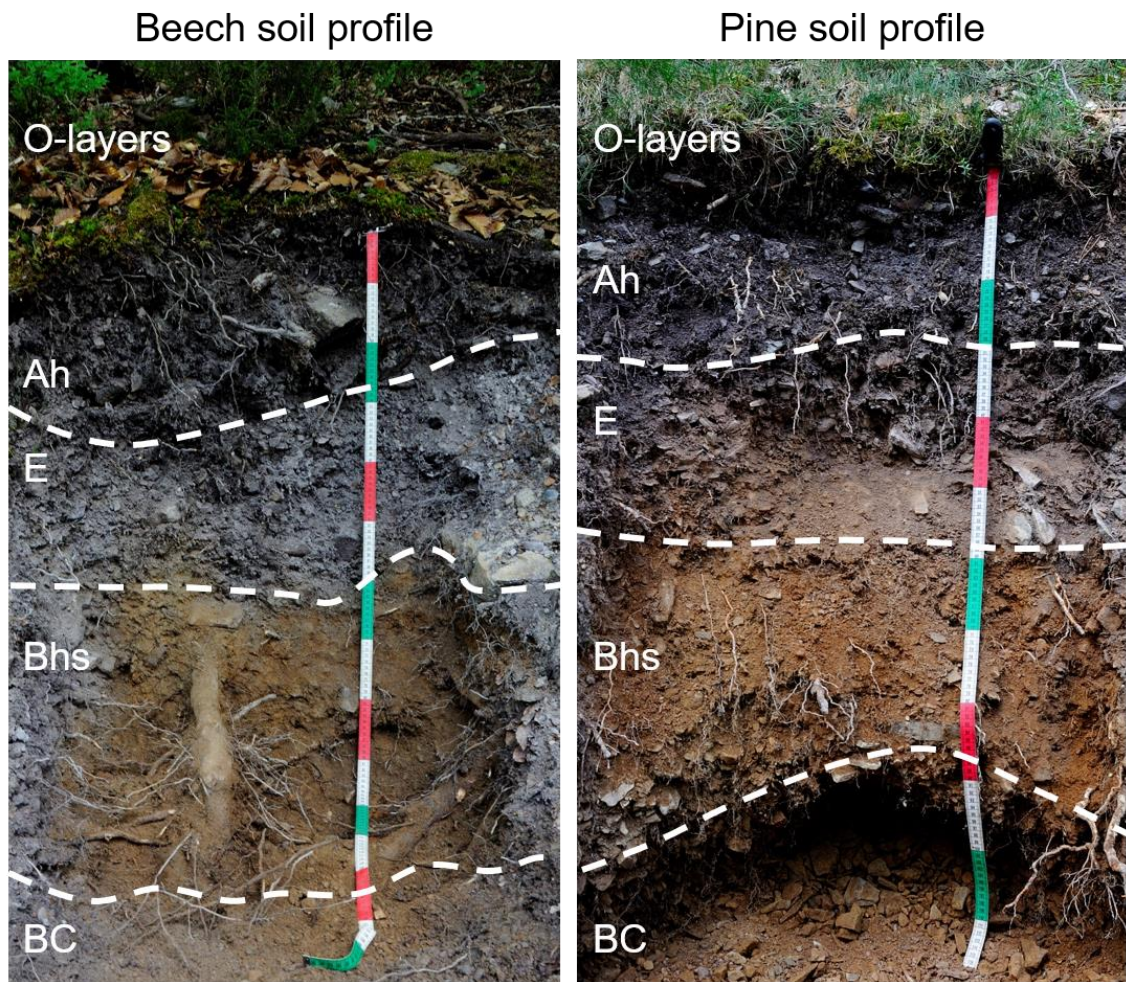


Figure 2. Sampled soil profiles in the native beech (left) and afforested pine (right) forests

### 2.3. Pyrolysis-gas chromatography-mass spectrometry analysis

Pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS) analysis was performed for SOM characterization on both organic and mineral horizons using a double-shot pyrolyzer (Frontier Laboratories, 2020i) attached to a GC/MS system (Agilent 6890N). The samples were capsuled and introduced in the micro furnace previously heated to 500 °C for 1 min. Then the pyrolysis products were automatically injected into the GC/MS for analysis. The gas chromatograph was equipped with a low polar capillary column (Agilent J&W HP-5ms UI; 30 m × 250 μm × 0.25 μm). The oven temperature was held at 50 °C for 1 min and then increased to 100 °C at 30 °C min<sup>-1</sup>, from 100 °C to 300 °C at 10 °C min<sup>-1</sup>, and stabilized at 300 °C for 10 min. Helium was used as the carrier gas, at a constant rate of 1 mL min<sup>-1</sup> and the total analysis time was 32 min. The detector



consisted of a mass selective detector (Agilent 5973 Technologies 5973N) and mass spectra were acquired at ionization energy of 70 eV.

#### *2.4. Data analysis*

The collection and processing of data was performed using the Agilent ChemStation Data Analysis software. Compound assignment was achieved by single-ion monitoring (SIM) for the major homologous series and by comparison with published data reported in the literature or stored in the digital NIST 14 (Maryland, USA) and Wiley 7 (Weinheim, Germany) libraries. This procedure allowed the identification of the main organic compounds in the samples as well as a semi-quantitative assessment in terms of relative abundances of compounds in the pyrolysates. The relative abundance of the pyrolysis products was calculated by normalizing the peak areas of each individual compound to the total area for all the peaks of the detected products. The identified compounds were grouped according to their probable origin in the following families: nitrogen compounds, aromatic compounds, polysaccharides, lignin-derived products, polycyclic aromatic hydrocarbons and lipids. In this way, the summation of the normalized peak areas of the individual compounds for each family allowed their relative abundances to be calculated.

### **3. Results and discussion**

#### *3.1. Soil organic matter composition revealed by Py-GC/MS*

Well-resolved chromatograms were obtained for both organic and mineral soil layers with a grand total of 128 different compounds detected; including 96 under beech and 89 under pine (Supplementary Material).

In the soils developed under both forest types, the N-derived compounds showed a similar distribution pattern with depth (Figure 3), increasing from the OL to Ah horizons (6.47 to 13.3 % and 5.68 to 19.0 % in beech and pine, respectively) and decreasing in the E horizons down to 8.12 and 11.6 % for beech and pine, respectively. The increase in N-derived compounds in the Ah horizon could be explained by a higher SOM degradation once incorporated into the mineral soil (Vancampenhout et al., 2009). On

the other hand, the decrease detected in the E mineral horizon could be a consequence of the migration of N compounds towards deeper soil layers. This is a probable explanation for the pine profile, since a higher relative abundance of N-compounds was observed in the Bhs and BC horizons compared to the E. However, this effect was not detected in the beech profile, as no N compounds were identified in the Bhs and BC horizons.

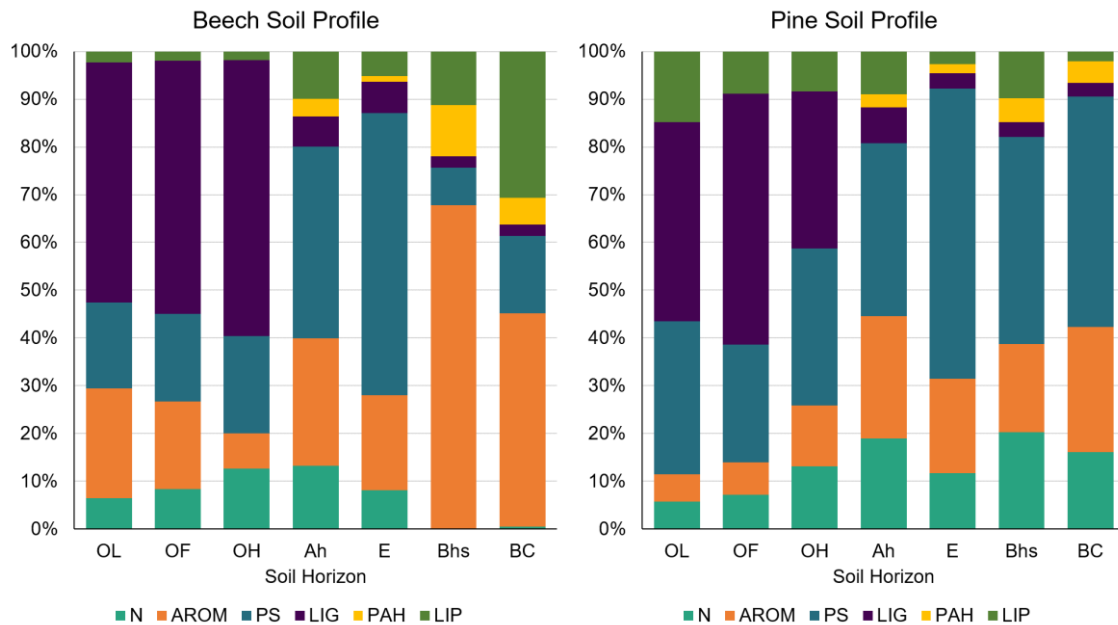


Figure 3. Relative abundance of the main families of compounds released by Py-GC/MS for each horizon and soil profile: nitrogen compounds (N), aromatic compounds (AROM), polysaccharides (PS), lignin-derived products (LIG), polycyclic aromatic hydrocarbons (PAH), and lipids (LIP).

The relative abundance of lipids (Figure 3) in the organic horizons released by pyrolysis was higher in the pine profile (8.40-14.9 %) than in the beech profile (1.80-2.31 %). Lipid preservation in the organic layers of the pine profile may indicate differences in its biomass composition compared to the organic layers of the beech profile. It has been previously observed that this type of compound is present and abundant in the litter and in the suberin of the barks of coniferous trees (Derenne & Quénéa, 2015). Another probable explanation for this process could be the delay in microbial degradation related to low-quality (high C/N ratio) SOM contributions (Vancampenhout et al., 2009). On the other hand, no differences were detected between forest types for the mineral horizons

except for the BC horizons, where the relative abundance of lipids was notably higher in the beech profile (30.7 %) than in the pine profile (2.02 %). This can be associated with root contributions at that depth.

The relative abundance of aromatic compounds in the organic layers showed an opposing trend in both soil profiles (Figure 3). In the beech profile, the OL layer was enriched in aromatic compounds (22.9 %), which decreased to 7.47 % in the OH layer. These results contrast the values obtained for the pine profile, where the OH layer was enriched (12.8 %) in aromatic compounds compared to the OL layer (5.83 %). The concentration of aromatic compounds in the OH layer of the pine profile may be related to a decrease in the lignin content, since some compounds that come from the degradation of lignin could be categorized as aromatic compounds (Ferro, 2010). Mineral Ah and E horizons showed similar relative abundances in both, the beech (26.6 and 19.9 %) and pine (25.6 and 19.8 %) profiles. The enrichment in aromatic compounds detected in the Bhs and BC horizons compared to the overlying horizons in both profiles could be a consequence of alkylphenol illuviation rather than *in situ* lignin degradation (Ferro, 2010), which is remarkably higher in the beech profile than in the pine profile.

Polycyclic aromatic hydrocarbons (PAH) were only detected in the mineral horizons, with a relative abundance ranging from 1.21 to 10.7 % in the beech profile and from 1.90 to 4.93 % in the pine profile (Figure 3). The appearance of PAH has traditionally been identified as a product of the analytical pyrolysis of charred materials (González-Pérez et al., 2007; De la Rosa et al., 2008), which may be related to the occurrence of wildfires and/or charcoal production in the area. The higher PAH content detected in the Bhs horizons is probably related to the translocation from the uppermost horizons and later accumulation in the spodic horizon.

The differences in the relative abundance of the lignin-derived pyrolysis products (methoxyphenols) were mainly detected in the organic layers, whereas their content remained similar in the mineral horizons (Figure 3). The relative abundance of lignin compounds was higher in the OH layer of the beech profile (57.9 %) compared to the pine profile (32.8 %), whereas their values for the OL and OF layers were similar in both

cases (ca. 50 % of the total pyrolysis products). This higher lignin content in the beech organic layers compared to pine was previously observed in temperate forests (Vancampenhout et al. 2009). In the organic layers of the pine profile, a dominance of lignin compounds related to guaiacol was observed, which is a characteristic trait of gymnosperm softwood that was also detected in the Ah mineral horizon. On the other hand, in the beech profile, syringol-derived compounds were also detected, which is characteristic of angiosperm vegetation (Amelung et al., 2008).

The polysaccharide-derived fraction in the soil under beech increased progressively with depth from 18.0 % in the OL layer to 59.0 % in the E horizon, followed by a remarkable decrease in the Bhs (7.86 %) and BC (16.2 %) horizons (Figure 3). In the pine soil profile, the relative abundance of polysaccharides showed a similar trend as observed in the beech profile, increasing from the OL (31.9 %) to the E (60.8 %) horizon, although no decrease was detected in the Bhs and BC horizons, where the abundance was still relatively high (43.4 and 48.3 %, respectively). The higher relative abundance of polysaccharide-derived fractions in the pine organic layer compared to those of the beech could be explained by the higher presence of microbial biomass observed in the pine profile. Fungal walls are composed of glycoproteins and polysaccharides, mainly glucan and chitin, which may explain the concomitant increase in the relative abundance of N and polysaccharide-derived compounds that were observed in deeper soil layers (Bhs and BC) of the pine profile and are known products derived from the pyrolysis of chitin (Moldoveanu, 1998). Furthermore, the more abundant fraction of the polysaccharide-derived pyrolytic compounds for all horizons of both profiles corresponded to low molecular mass compounds such as furfural. This is related to the presence of small decomposed polysaccharides such as those of chitin biopolymers (Bowman & Free, 2006).

### *3.2. Differences in the n-alkane series between forest types*

The study of the *n*-alkane ( $C_nH_{2n+2}$ ) series allowed the detection of different vegetation markers in the soil related to each forest type (Figure 4). Plants are known to preferentially produce odd-numbered carbon long chain alkanes (Eglinton et al., 1962),

with the more prominent peaks detected in the pyrograms of samples from the organic layers. Since the distribution of long chain alkanes is a characteristic trait of the vegetation type, it can be used as a biomarker or molecular tracer for each of the forest species. However, as SOM undergoes transformations in the soil, this trend shifts, and usually, the proportion of short chain even-numbered carbon alkanes increases concomitantly with depth, as has been observed in previous works (González-Pérez et al., 2007; Amelung et al., 2008; Santana et al., 2015). Throughout the beech soil profile, the influence of vegetation was mainly reflected in the organic layers. As was previously observed using conventional biomarker approaches based on the study of soil lipid extracts, the *n*-alkane distribution in the organic layers under beech vegetation was dominated by the C<sub>27</sub> hydrocarbon, accompanied by variable amounts of C<sub>25</sub> that were substituted by shorter chain homologues (C<sub>20</sub>-C<sub>25</sub>) in the mineral horizons (Marseille et al., 1999; Griepentrog et al., 2016). Additionally, a high proportion of long chain odd-numbered *n*-alkanes (C<sub>31</sub>-C<sub>33</sub>) was found in the mineral soil samples. On the other hand, in the pine soil profile short-chained molecules (<C<sub>20</sub>) prevailed with no carbon number preference, which has been traditionally associated with products of microbial biosynthesis (Dinel et al., 1990; González-Pérez et al., 2012) and may indicate a higher degree of SOM degradation with contributions of organic compounds from the microbial biomass (Assis et al., 2011).

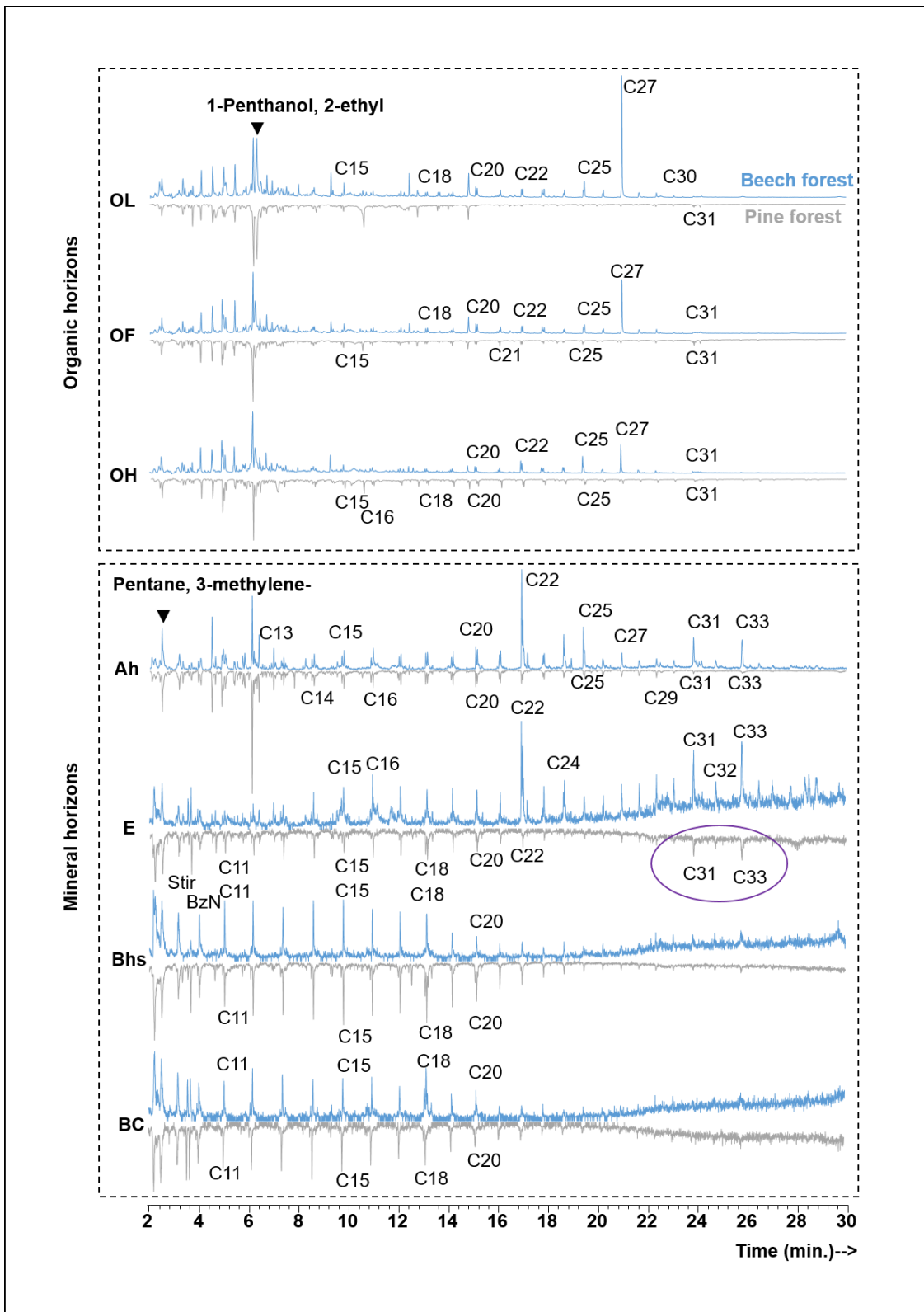


Figure 4. Normalized pyrograms obtained by Py-GC/MS for the *n*-alkane homologous series (*m/z* 85) for each horizon of the beech and pine soil profiles. The purple circle indicates beech biomarkers found in the pine profile.

As stated above, the pyrograms of both the pine and beech profiles were clearly differentiated from the OL to Ah horizons. However, in the pine E horizon an increase in the C<sub>31</sub> and C<sub>33</sub> peaks was detected, which was not observed in the overlying horizons. These C<sub>31</sub> and C<sub>33</sub> peaks were, although, detected in the Ah and E horizons of the beech profile, which suggested that these were biomarkers from the previous natural beech vegetation, which remained in the SOM for more than 100 years after the pine afforestation. Regarding the Bhs and BC mineral horizons of both forest types, a clear predominance of short chain *n*-alkanes and the disappearance of the C<sub>31</sub> and C<sub>33</sub> peaks was observed, suggesting the presence of a more evolved SOM at that depth.

These results match the findings of a previous study conducted in the same soil profiles that analyzed the SOM stable isotope composition, which indicated that SOM derived from the natural vegetation was still detectable in the E horizon of the afforested pine (Girona-García et al., 2018b).

#### **4. Conclusions**

Well-resolved chromatograms using Py-GC/MS were obtained for organic and mineral soil layers, and a total of 128 pyrolysis compounds were detected. The pyrograms showed the chemical variability of the SOM derived from the predominant vegetation type as well as its distribution down in the soil for each forest type. The SOM in organic soil layers (OL, OF and OH) consisted mainly of lignocellulosic biomass with abundant lignin and polysaccharide derived compounds. The mineral horizons Ah and E were found to be particularly enriched in polysaccharides. The Bhs and BC horizons showed an increase in aromatic compounds, PAH and lipids, the latter being steeper in the beech soil, possibly due to microbial activity. An unexpectedly higher polysaccharide content was observed in the Bhs and BC horizons under pine, most likely related to the migration of complexed forms. The analytical pyrolysis results of selected ion traces for the *n*-alkane series (*m/z* 85) showed a higher abundance of odd-numbered carbon long chain alkanes in the beech compared to the pine profile indicating a greater stabilization of

SOM. The appearance of probable beech biomarkers (C<sub>31</sub> and C<sub>33</sub>) in the pine E horizon indicated the permanence of beech-derived SOM ca. 100 years after the afforestation.

## **Acknowledgments**

This study is part of the results of the FUEGONEO (CGL2016-76620-R) and INTERCARBON (CGL2016-78937-R) projects funded by the Spanish “Ministerio de Economía y Competitividad”. Girona-García, A. was funded by an FPI research grant (BES-2014-068072) of the “Ministerio de Economía y Competitividad” (Spanish government). Thanks are due for the financial support to CESAM (UID/AMB/50017 - POCI-01-0145-FEDER-007638), to FCT/MCTES through national funds (PIDDAC), and the co-funding by the FEDER, within the PT2020 Partnership Agreement and Compete 2020. We also thank the Aragonese Agents for Nature Protection (APN) of the Moncayo Natural Park for their collaboration and for issuing the sampling permits.

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