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## ORIGINAL ARTICLE

# The effects of land abandonment and long-term afforestation practices on the organic carbon stock and lignin content of Mediterranean humid mountain soils

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Afforestation is an important strategy that can decrease atmospheric carbon by sequestering carbon in biomass and soil. In Spain, an active afforestation programme was adopted in the 1950s when the soil was severely eroded after widespread abandonment of arable land. The Araguás catchment (Central Spanish Pyrenees) is a good example of this programme because it was afforested with both *Pinus sylvestris* L. (PS) and *Pinus nigra* J.F. Arnold (PN). The soil organic carbon (SOC) stock and lignin content (based on the vanillyl, syringyl and cinnamyl contents) of these afforested soils were examined and compared to those of bare soil, secondary succession and meadow soils. Both the SOC stock and lignin content were used to evaluate the effects of land-use changes on soil. Curie-point pyrolysis with tetramethylammonium hydroxide was used to assess the lignin content. In the bare soil, there was none of the lignin compounds. The largest SOC stock and lignin content occurred under PN and secondary succession sites. A decreasing trend for the lignin content, related to the limited organic matter input and the longer degradation period, was observed at deeper horizons in all soils except meadows. These meadow soils also showed increased SOC stocks in deeper horizons. Land abandonment reduced the SOC stock although no significant differences were observed in the organic carbon incorporation assessed through lignin content (and if this was so it was restricted to the top centimetre or so). According to the results, PN was the best afforestation practice for increasing SOC stock and lignin content in soil. *Pinus sylvestris* afforestation was less successful than secondary succession at increasing SOC sequestration and lignin content.

## Highlights

- Effects of long-term afforestation and land abandonment assessed in Mediterranean humid mountain soils.
- Soil organic carbon (SOC) stock and lignin content were used as indicators.
- Bare soil had the smallest SOC stock and lignin content.
- Afforestation with *Pinus nigra* was the best practice, increasing SOC stock and lignin content.

## KEYWORDS

bare soil, cinnamyl, meadow, *Pinus*, secondary succession, soil organic carbon, syringyl, vanillyl

## 1 | INTRODUCTION

Land use and land-use change (LUC) are among the most important factors governing carbon fluxes between the terrestrial biosphere and atmosphere (Guo & Gifford, 2002). Soil dynamics are responsible for whether an ecosystem is considered a net source or sink of atmospheric CO<sub>2</sub>. In the conversion from natural vegetation to cultivated fields, soil is known to decline rapidly in soil organic carbon (SOC) because of reduced inputs of organic matter, increased decomposition of crop residues and tillage (Post & Kwon, 2000). On the other hand, creating forests on depleted cultivated soils is used to restore soils and sequester organic carbon. A widely used technique is afforestation, which is the process of planting forests in areas where none has been before, with productive (to achieve self-sufficiency in the supply of pulp and paper) and environmental objectives (to reduce flood frequency and magnitude, as well as soil erosion). In Europe, North America and Russia large areas are being remodelled into fresh new forests that can act as a carbon sink and diminish global carbon levels (Pérez-Cruzado et al., 2014). For this reason, afforestation is one of the most credited strategies according to the Kyoto Protocol (Cerli et al., 2008; Savy, Nebbioso, Córdor, & Vitullo, 2012). In Spain, an active afforestation programme was adopted in the 1950s where the soils were severely eroded (FAO, 2015) after widespread abandonment of arable land. In the Central Spanish Pyrenees, for example, these abandoned fields were then afforested with Scots Pine (*Pinus sylvestris* L.) and Black pine (*Pinus nigra* J.F. Arnold), or covered with natural or secondary succession or with prolonged fodder cultivation (meadows) (Lasanta, Vicente-Serrano, & Cuadrat, 2005).

Afforestation influences the composition of soil organic matter (SOM) (Cong, Ren, & Li, 2016; Perez-Cruzado et al., 2014; Schellekens, Buurman, & Kuyper, 2012). It represents the largest carbon pool in terrestrial environments and its composition and quantity depend on: (a) the quality (or the nature) of the inputs, which are derived from organic materials added to the soil primarily by the breakdown of plant residues, (b) degradation processes regulated by microorganisms and (c) organo–mineral interactions (Zech et al., 1996). Although some studies have assessed the quantity of SOM in afforested areas (Korkanc, 2014), its quality has been less studied. Some authors have suggested a relation between land use and lignin content and composition (Pisani, Haddix, Conant, Paul, & Simpson, 2016; Thevenot, Dignac, & Rumpel, 2010). Accordingly, lignin substitutes have been used to obtain a general indication of the SOM source, as well as the rates of degradation, of organic compounds in the soil (de Baets et al., 2012; Schellekens et al., 2012).

Lignins are a major constituent of plant-synthesized compounds of terrestrial origin (Adler, 1977; Han, Sun, Jin, & Xing, 2016). They are transferred from plants to soil,

from aboveground (i.e. shoots and leaves) or belowground (root system) litter (Thevenot et al., 2010). Lignin consists of phenyl-propanoid units and monolignols with several linkages: coniferyl, synapyl and *p*-coumaryl alcohols (Pisani et al., 2016; Poerschmann, Rauschen, Langer, Augustin, & Gorecki, 2008). The study of lignin composition is based on the quantification of specific single-ring phenol compounds known as vanillyl (V), syringyl (S) and cinnamyl (C). The sum of these units (V + S + C) is used to quantify lignin content in the soil. The relations between the S:V and C:V ratios of soils and sediments vary according to their source plants, reflecting a partial preservation of characteristic lignin patterns, from plants to soils and sediments (Thevenot et al., 2010). Several studies have dealt with these ratios to assess the state of decomposition of lignin and SOM (e.g. Castro, Fortunel, & Freitas, 2010; Cortez, Garnier, Pérez-Harguindeguy, Debussche, & Gillon, 2007).

The effects of abandonment of arable land and afforestation on lignin compounds have been tested in the literature. Most studies have been carried out in temperate environments (Cerli et al., 2008; Mueller & Kögel-Knabner, 2009) and Mediterranean semiarid areas (Castro et al., 2010; Cortez et al., 2007; de Baets et al., 2012). Soil lignin studies related to the abandonment of arable land and revegetation processes in humid Mediterranean mountains are currently lacking. Although there is consensus among researchers on the effect of different types of vegetation and land uses on the lignin content, and on the occurrence of the different lignin monomers, there is still considerable variation within these studies.

The present study was carried out in a homogeneous area with regard to soil type, parent material, topographic conditions and climate. Accordingly, the differences were attributed solely to the different land covers, even though these may affect soil humidity and temperature differently and, consequently, the composition of the soil microbial community, which has a major effect on the dynamics of soil carbon. Examining the influence of afforestation on soil properties and the hydrological response over time is part of a larger research project (López-Vicente, Nadal-Romero, & Cammeraat, 2017; Nadal-Romero, Cammeraat, Pérez-Cardiel, & Lasanta, 2016a, 2016b; Nadal-Romero, Cammeraat, Serrano-Muela, Lana-Renault, & Regüés, 2016).

The main objective of this study was to assess the effects of land abandonment (bare soil), afforestation practices (*Pinus nigra* and *Pinus sylvestris*) and different vegetation covers (secondary succession and meadows) on the stock of SOC and the content and composition of lignin in a representative area of the Central Spanish Pyrenees (the Araguás catchment). Accordingly, this study aimed to (a) determine the SOC stock and lignin content at different depths of soil profiles after more than 50 years of LUC, (b) establish relations between the physical and chemical soil properties and lignin content under the different land uses, and more generally, (c) shed some light on the current discussion about the

fate of lignin to provide soil lignin values in Mediterranean humid environments affected by land abandonment and afforestation, required to validate SOC models.

This led to the following research hypotheses: in Mediterranean humid environments (a) afforestation practices (after land abandonment) might lead to significant increases in SOC stock and lignin content, (b) lignin content may be larger in topsoil horizons and may decrease with depth, and (c) lignin degradation might be influenced by soil properties and environmental conditions.

## 2 | MATERIALS AND METHODS

### 2.1 | Study area and sampling

The Araguás catchment (2° 36' 8" N, 0° 37' 28" W (4719488 and 694873 UTM); 45 ha) is in the Central Spanish Pyrenees and was selected as a representative Mediterranean mountain area (elevation ranging between 900 and 1160 m a.s.l.) for this study (Figure 1). The climate is sub-Mediterranean with Oceanic and Continental influences. Annual rainfall varies between 500 and 1000 mm (average 800 mm) and the average annual temperature is 10°C (minimum -14°C and maximum >30°C). The bedrock is Eocene Flysch and the soil is classified as a Leptic Calcaric Regosol with a silt loam texture (FAO, 2014). The soil is stony and thin, and has been modified following centuries of cultivation practices and erosion processes.

The area was intensively cultivated until the 1950s, and afterwards it was afforested with *P. sylvestris* and *P. nigra* during the 1960s and 1970s. Some areas underwent processes of secondary succession or natural plant colonization with *Genista scorpius* (L.) DC., *Juniperus communis* L., *Rosa gr. canina* L. and *Buxus sempervirens* L. Other areas were not afforested and were used for grazing meadows or were degraded to bare areas because of intense sheet wash erosion (Figure 1). Accordingly, soils were collected from five different land covers: bare lands (B), meadows (M), secondary succession (S), and afforestation with *P. sylvestris* (PS) and *P. nigra* (PN). Three plots with the same slope gradient and aspect were selected for each type of land cover (Figure 1). At each plot, litter (if present) and mineral soil subsamples were taken at two randomly chosen locations. Mineral soil from 0–20-cm depth was sampled every 5 cm, and soil from between 20- and 50-cm depths every 10 cm. These subsamples were pooled into a composite sample and one sample per depth and land use was obtained (seven depths and five land covers;  $n = 30$ ; deep soil samples were not collected in the bare areas because of the presence of parent material, and litter was collected at the afforested sites only). One separate sample at each depth and land cover was collected with steel cylinders to determine bulk density ( $n = 30$ ). Once the samples were analysed in the laboratory, these composite samples were grouped by land cover into

depth classes: 0–10-cm ( $n = 2$ ), 10–20-cm ( $n = 2$ ) and soil samples >20 cm ( $n = 3$ ). This was done to enable statistical analysis with the limited number of samples.

### 2.2 | Soil characterization

Samples were dried at 40°C and then sieved through a 2-mm mesh in the laboratory (roots and stones were carefully removed). Soil pH and electrical conductivity (EC) were measured in a deionized water suspension (1:2.5) with pH and conductivity meters. Total carbon (TC) and total nitrogen (TN) were determined by dry combustion using an elemental analyser (Vario EL Cube Elementar, Langensfeld, Germany). Carbonate content ( $\text{CaCO}_3$ ) was determined using the Wesemael method (van Wesemael, 1955), from which the total inorganic carbon was also calculated ( $C_{\text{inorg}}$ ). Organic carbon ( $C_{\text{org}}$ ) was calculated by subtracting  $C_{\text{inorg}}$  from TC. The  $C_{\text{org}}$  and TN contents were expressed in  $\text{g kg}^{-1}$  soil, whereas SOC stock was expressed in  $\text{Mg ha}^{-1}$  (using the respective depth and bulk density). The C/N ratio was calculated using  $C_{\text{org}}$  and TN (C to N mass ratio). The bulk density (BD) was determined gravimetrically by drying a known volume of sample at 105°C. Soil texture, after removing SOM, was determined with a particle-size analyser (Micromeritics, SediGraph 5100, Norcross, GA, USA).

### 2.3 | Curie-point pyrolysis and thermally assisted hydrolysis and methylation (THM)

#### 2.3.1 | Chemicals, reagents and pyrolysis products

Tetramethylammonium hydroxide (TMAH) and 5 $\alpha$ -Androstane ( $\text{C}_{19}\text{H}_{32}$  CAS# 438-22-2), used as internal standards, were purchased from Sigma-Aldrich (Schnellendorf, Germany). A total of 14 lignin compounds were analysed, including six vanillyl (V) ( $V_4$ ,  $V_6$ – $V_8$  and  $V_{14}$ – $V_{15}$ ), six syringyl (S) ( $S_4$ ,  $S_6$ – $S_8$ ,  $S_{14}$ – $S_{15}$ ) and two cinnamyl (C) ( $C_{18}$  and  $V_{18}$ ) (Table 1). These phenols indicate the lignin-derived material in the soil samples. These compounds were selected because they are definitely derived from lignin, whereas other monomers that were found might originate from other sources as well (Cerli et al., 2008).

#### 2.3.2 | Sample preparation

The TMAH was used for methylation of monomers produced simultaneously with pyrolysis that changed phenolic acid and phenols into methyl esters and methyl ethers, identified by gas chromatography–mass spectrometry (Kögel-Knabner, 2000; Mason, Filley, & Abbott, 2009). The TMAH-thermochemolysis was selected because it can trace lignin when it has been degraded severely, whereas in other methods, such as CuO oxidation, it remains undetected (Kögel-Knabner, 2000).

Soil samples (~1–2 mg) were dried at low temperatures (ca. 40°C), ground and homogenized, and then derivatized on a small glass dish for 2 min with 20  $\mu\text{L}$  of 25% (w/w)

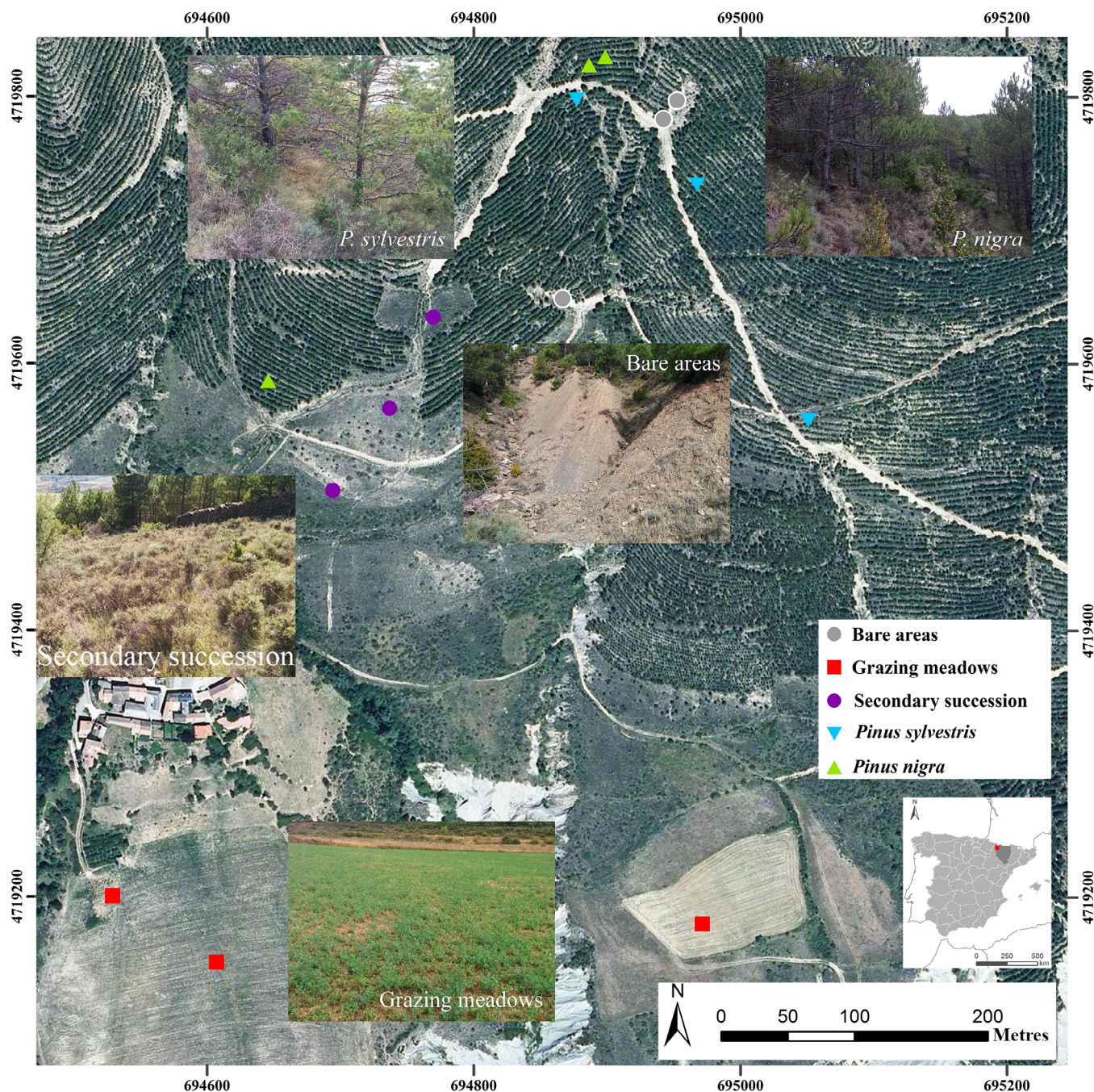


FIGURE 1 Location and overview of sampling points in the Araguás catchment

freshly prepared TMAH solution in water. About 6  $\mu\text{L}$  of the internal standard, 5 $\alpha$ -Androstane solution (100  $\mu\text{g l}^{-1}$  in cyclohexane), was also added to the samples. The mixture was spread on a ferromagnetic wire (Curie-point pyrolysis wire 600°C) with a spatula. The amount loaded on to the wire depended on the amount of carbon in the sample, which determined the quality of the chromatogram. The wire was dried under a halogen lamp and then placed in a glass capillary tube (the distance between the end of the wire with the sample and the end of the glass capillary tube was 1 cm). The glass capillary tube was placed in the probe of the pyrolysis unit and inserted into the GC system.

### 2.3.3 | Thermally assisted hydrolysis and methylation (THM)

For the THM, the sample on the Curie-point wire was heated at 600°C for 5 s. The Curie-point unit (Horizon Instruments, Heathfield, UK) was connected to a Thermo-Quest Trace GC 2000 gas chromatograph (Thermo Fischer, Milan, Italy), the products were separated by a fused silica column (Agilent J&W Scientific, Santa Clara, CA, 30 m  $\times$  0.32 mm i.d.) coated with DB-1 (film thickness 0.50  $\mu\text{m}$ ) and helium was used as the carrier gas. The oven was initially kept at 40°C for 1 min, then it was heated at a rate of 7°C  $\text{min}^{-1}$  to 320°C and maintained at that temperature for 15 min. The column was coupled to a Finnigan Trace mass spectrometer

**TABLE 1** Lignin compounds used for calculating the amount of lignin in the soils studied

	ID code	$t_R^a$ (min)	Fragment ion(s) -, molecular ion <sup>b</sup>
<b>Vanillyl</b>			
3,4-dimethoxybenzaldehyde	V4	20.5	151-165- <u>166</u>
3,4-dimethoxybenzoic acid methyl ester	V6	22.4	165-181- <u>196</u>
cis-1-(3,4-dimethoxyphenyl)-2-methoxyethylene	V7	23.2	151- <u>179</u> -194
trans-1-(3,4-dimethoxyphenyl)-2-methoxyethylene	V8	23.4	151- <u>179</u> -194
threo/erythro-1-(3,4-dimethoxyphenyl)-1,2,3-trimethoxypropane	V14	26.0	166- <u>181</u> -270
threo/erythro-1-(3,4-dimethoxyphenyl)-1,2,3-trimethoxypropane	V15	26.1	166- <u>181</u> -270
<b>Syringyl</b>			
3,4,5-trimethoxybenzaldehyde	S4	22.6	125- <u>181</u> -196
3,4,5-trimethoxybenzoic acid methyl ester	S6	24.5	195-211- <u>226</u>
cis-1-(3,4,5-trimethoxyphenyl)-2-methoxyethylene	S7	25.4	181- <u>209</u> -224
trans-1-(3,4,5-trimethoxyphenyl)-2-methoxyethylene	S8	25.7	181- <u>209</u> -224
threo/erythro-1-(3,4,5-trimethoxyphenyl)-1,2,3-trimethoxybenzene	S14	27.3	<u>211</u> -300
threo/erythro-1-(3,4,5-trimethoxyphenyl)-1,2,3-trimethoxybenzene	S15	27.5	<u>211</u> -300
<b>Cinnamyl</b>			
trans-3-(4-methoxyphenyl)-3-propenoic acid methyl ester	C18	24.1	133- <u>161</u> -192
trans-3-(3,4-dimethoxyphenyl)-3-propenoic acid methyl ester	V18	25.8	191- <u>207</u> -222

<sup>a</sup> $t_R$ , retention time.<sup>b</sup>Quantification ions underlined.

(MS) with the following operating conditions: 70 eV ionization potential of the electron impact source, 250°C ion source temperature, cycle time 1 s and data acquisition in full scan mode covering a mass range  $m/z$  47–600.

Lignin breakdown products were identified using Xcalibur Software to interpret the mass spectra with the help of the National Institute of Standards and Technology, (NIST) 2014 library, and also by considering their retention times or by comparison with data in the literature, or both (Poerschmann et al., 2008). Quantification of individual breakdown products was based on the ratios of the peak areas of selected diagnostic ions (underlined in Table 1) to those of the diagnostic ion  $m/z = 188$  amu of the internal standard.

The syringyl:vanillyl (S:V) and the cinnamyl:vanillyl (C:V) ratios are used to distinguish the vascular plant source material according to plant (gymnosperm and angiosperm) and tissue (woody and non-woody) types. Thus, gymnosperms (conifers like *P. sylvestris* and *P. nigra*) contain mainly V phenols and essentially no S phenols (S:V ~ 0), whereas angiosperms (such as grasses, deciduous trees and flowering plants) produce both V and S phenols (S:V > 0). In addition, C phenols are derived from non-woody tissues (grasses, leaves and needles, C:V > 0), but not from woods in any appreciable amount (C:V = 0) (Ertel & Hedges, 1984). We also calculated the acid:aldehyde (Ad/Al) ratio, indicated by components of the vanillyl monomers (V6/V4 = 3,4-dimethoxybenzoic acid methyl ester/3,4-dimethoxybenzaldehyde) and syringyl monomers (S6/S4 = 3,4,5-trimethoxybenzoic acid methyl ester/3,4,5-trimethoxybenzaldehyde) (see Table 1). These ratios indicate the oxidative state of lignin, which correlates with the rate of

lignin degradation in the soil. The larger is the Ad/Al ratio, the larger is the rate of degradation (i.e. Ertel & Hedges, 1984; Poerschmann et al., 2008).

## 2.4 | Statistical analysis

Normality of the data was tested using the Shapiro–Wilk test and homogeneity of variances was tested by Levene's test. Pearson correlations were carried out to investigate the relations among variables. Differences in SOC stocks and lignin content (in total and in V, S and C contents) between the various land-cover types and between the sampled soil-depth classes were determined by the analysis of variance (a one-way ANOVA). When the *F* test was significant, means were compared and confirmed *a posteriori* with Fisher's least significant difference (LSD). In all the cases, differences were considered to be statistically significant at  $P < 0.05$ . We highlight the limitations of the dataset used (because of the limited true replication and the grouping of the samples at different depths) when general comments on the effect of land use on the soil properties and lignin content are made.

The data (physical and chemical soil properties, lignin content and rates of degradation) were also analysed by a principal component analysis (PCA). The PCA was carried out on the correlation matrix. The largest proportion of the variation in these variables (61.1%) was concentrated in the first two principal components (PCs). They described most of the variation in the data associated with the different land-cover types. On this basis, the PCA was used to identify which variables accounted for most of the variation on PC1 and PC2, and what combinations of variables were associated with each other, and to determine the degree to which

LUC affects the soil characteristics under study. All statistical analyses were carried out using SPSS Statistics 20.

### 3 | RESULTS

#### 3.1 | Physicochemical soil properties

The main characteristics of the soil are reported in Table 2 (and the mean values used for statistical analysis are shown in Table S1, Supporting Information). The largest BD values occurred in the top 10 cm of the bare soil. Bulk density increased with depth in the soils from meadow and PN sites. In general, pH values increased with soil depth, but in the meadows there were no major changes in the profile. The largest values of EC were obtained in the soil from the PN sites, and the smallest values were in the bare areas. A

general decrease in EC values occurred with increasing soil depth for all land uses. There were large contents of  $\text{CaCO}_3$  in all soil profiles, reflecting the influence of the parent material. In general,  $\text{CaCO}_3$  tended to increase with soil depth, although no clear pattern was observed. The texture of the soils can be classified as silt loam and there was little variation in the soil particle-size distribution either along the profiles or among land covers, except for the PN soil, which had larger sand contents.

The smallest  $\text{C}_{\text{org}}$  contents were in the bare soils and the largest ones in those under PN. The  $\text{C}_{\text{org}}$  content decreased with soil depth and there were significant differences between land uses (Table 3). The largest TN values were in the meadow soils and in the top layer of the PN sites. The C/N ratios were large in the afforested sites, particularly in PN samples (Table 2). The largest SOC stock (considering the whole profile, Figure 2) was under PN (more than

**TABLE 2** Main characteristics of soil samples in the different land uses and at the different depths

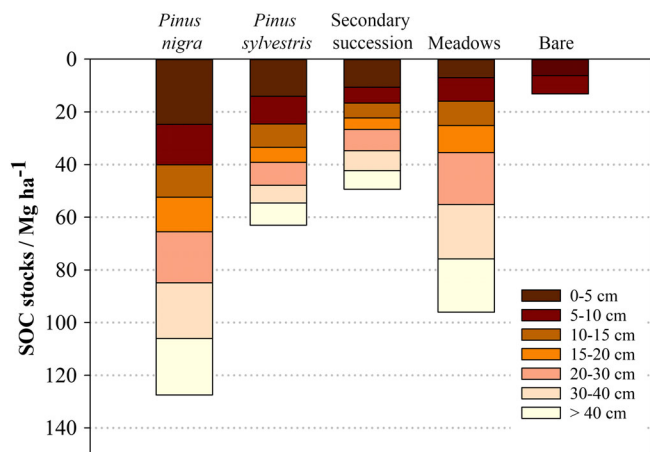
Land use	Soil depth (cm)	BD ( $\text{g cm}^{-3}$ )	pH	EC ( $\mu\text{S m}^{-1}$ )	$\text{CaCO}_3$ (%)	Sand (%)	Silt (%)	Clay (%)	$\text{C}_{\text{org}}$ ( $\text{g kg}^{-1}$ )	TN ( $\text{g kg}^{-1}$ )	C/N ratio	SOC ( $\text{mg ha}^{-1}$ )
Bare lands	0–5	1.73	8.0	146	34.4	10.1	70.1	19.8	7.23	0.94	7.79	6.24
	5–10	2.00	7.9	152	37.3	10.3	66.2	23.5	6.95	0.92	7.52	6.96
Meadows	0–5	0.63	7.6	297	41.7	12.4	68.4	19.2	22.24	2.50	8.90	7.04
	5–10	0.98	7.6	294	40.8	11.8	71.4	16.8	18.20	2.25	8.08	8.94
	10–15	1.07	7.7	272	41.5	12.3	70.8	16.9	18.17	2.27	8.02	9.2
	15–20	1.00	7.6	317	41.3	12.6	68.6	18.8	20.41	2.53	8.07	10.26
	20–30	1.23	7.6	308	42.2	12.7	69.9	17.4	19.61	2.50	7.83	19.74
	30–40	1.46	7.5	299	42.5	11.0	71.3	17.7	19.21	2.44	7.89	20.6
	>40	1.56	7.6	303	40.8	11.8	71.0	17.2	16.51	2.28	7.26	20.3
Secondary succession	0–5	1.09	7.7	277	37.1	6.4	74.5	19.1	19.67	2.22	8.85	10.71
	5–10	0.83	7.8	222	38.1	6.6	74.1	19.3	14.31	1.73	8.27	5.94
	10–15	0.90	7.9	229	39.2	6.6	74.1	19.3	13.84	1.69	8.20	5.66
	15–20	1.11	8.0	194	39.5	5.0	77.5	17.5	9.15	1.28	7.17	4.35
	20–30	0.82	8.2	181	40.4	4.2	76.2	19.6	7.31	1.01	7.21	8.09
	30–40	1.18	8.4	168	40.6	3.8	76.8	19.4	6.38	0.92	6.95	7.52
	>40	1.18	8.4	164	40.3	4.2	84.3	11.5	6.02	0.86	7.00	7.09
Afforestation: <i>Pinus sylvestris</i>	Litter	–	7.8	273	44.3	–	–	–	22.60	1.79	12.63	14.14
	0–5	1.32	7.7	281	38.0	8.6	74.0	17.4	21.44	1.81	11.82	10.45
	5–10	1.14	7.7	297	37.2	10.3	71.6	18.1	18.39	1.47	12.47	8.84
	10–15	1.23	7.7	277	40.9	8.4	73.7	17.9	14.42	1.27	11.39	5.7
	15–20	1.27	7.8	229	40.6	6.1	72.6	21.3	8.99	0.96	9.37	8.78
	20–30	1.21	7.9	235	37.4	7.9	72.5	19.6	7.26	0.91	7.94	6.7
	30–40	1.23	8.2	179	45.2	6.0	73.4	20.6	5.46	0.72	7.61	8.42
	>40	1.16	8.2	182	43.8	6.0	75.6	18.4	7.26	0.71	10.21	24.7
Afforestation: <i>Pinus nigra</i>	Litter	–	7.2	594	20.0	–	–	–	78.68	2.68	29.36	15.4
	0–5	0.93	7.5	541	13.4	19.1	76.6	4.4	53.36	1.52	35.20	12.31
	5–10	1.00	7.6	433	32.6	25.6	59.8	14.6	30.94	1.91	16.24	13.08
	10–15	1.02	7.6	348	31.5	24.5	60.6	14.9	24.18	1.62	14.93	19.37
	15–20	1.21	7.7	303	28.4	22.7	61.0	16.3	21.60	1.49	14.47	21.23
	20–30	1.23	7.9	256	30.6	26.8	56.8	16.4	13.84	1.29	10.70	21.38
	30–40	1.29	7.8	260	25.9	23.1	58.1	18.8	16.49	1.36	12.11	6.24
	>40	1.40	7.7	275	17.8	17.1	59.2	23.7	15.32	1.26	12.18	6.96

BD: bulk density;  $\text{CaCO}_3$ : calcium carbonate content;  $\text{C}_{\text{org}}$ : organic carbon content; C/N ratio: C to N mass ratio; SOC: soil organic carbon stocks; TN: total nitrogen content.

**TABLE 3** The  $F$ - and  $P$ -values from a one-way analysis of variance ANOVA between different soil depths for each land use (grouping factor land use). Samples were grouped by depth: 0–10, 10–20 and 20–50 cm

ANOVA		$C_{org}$ ( $g\ kg^{-1}$ )	SOC ( $mg\ ha^{-1}$ )	Lignin ( $\mu g\ mg^{-1}$ )	V ( $\mu g\ mg^{-1}$ )	S ( $\mu g\ mg^{-1}$ )	C ( $\mu g\ mg^{-1}$ )	S/V	C/V	Ad/Al <sub>V</sub>	Ad/Al <sub>S</sub>
Meadows	$F_{2,4}$ -values	0.468	107.6	61.37	12.84	3.061	1.429	0.172	0.424	1.947	4.635
	$P$ -values	0.657	<0.001	0.001	0.018	0.156	0.340	0.848	0.681	0.257	0.091
Secondary succession	$F_{2,4}$ -values	9.99	2.254	5.567	4.87	5.712	1.493	2.930	2.237	4.079	2.337
	$P$ -values	0.028	0.221	0.068	0.084	0.067	0.340	0.165	0.223	0.108	0.213
<i>Pinus sylvestris</i>	$F_{2,4}$ -values	19.75	4.044	2.602	3.388	1.029	1.429	1.652	1.124	0.143	0.731
	$P$ -values	0.009	0.110	0.189	0.138	0.436	0.340	0.300	0.410	0.871	0.536
<i>Pinus nigra</i>	$F_{2,4}$ -values	6.849	4.392	52.47	40.52	21.17	104.4	0.172	7.980	0.779	1.056
	$P$ -values	0.050	0.098	0.001	0.002	0.007	<0.001	0.848	0.040	0.518	0.728

Note. An *a posteriori* LSD test was carried out (data not shown in the Table). Ad/Al<sub>S</sub>: acid to aldehyde ratio for the syringyl units; Ad/Al<sub>V</sub>: acid to aldehyde ratio for the vanillyl units; C: cinnamyl;  $C_{org}$ : organic carbon content; S: syringyl; SOC: soil organic carbon stock; V: vanillyl.



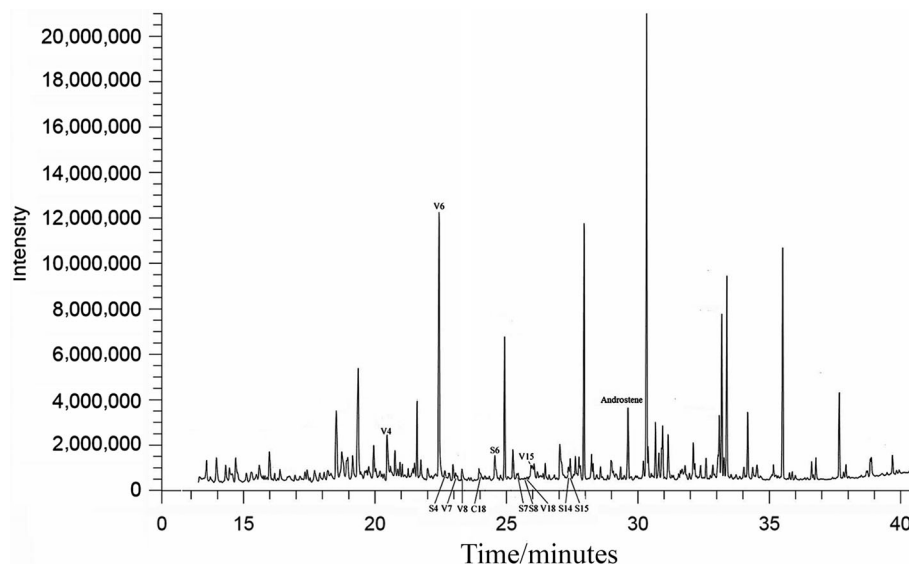
**FIGURE 2** Soil organic carbon (SOC) stocks in the different soil profiles

120  $Mg\ ha^{-1}$ ), followed by meadows ( $\sim 96\ Mg\ ha^{-1}$ ) and PS ( $\sim 65\ Mg\ ha^{-1}$ ). Bare soil had the smallest SOC stock ( $\sim 13\ Mg\ ha^{-1}$ ). Comparing the sites, afforestation with PN produced a larger increase in the SOC stock than afforestation with PS. There were significant increases in SOC stock with depth for the different land uses (Table 3), particularly

in meadow and PN sites, which also had the larger BD values. Consequently, the largest differences between land uses were observed in the deepest horizons (Table S2, Supporting Information).

### 3.2 | Lignin

A total of 14 lignin compounds were analysed in this study (Figure 3). Most of them occurred in soil samples from the different land uses, but there was none in the bare soil samples. In the uppermost 5 cm of all the soils, V6 was a dominant phenol, except for the meadows sites where it matched with V18 and C18, which indicated graminaceous input. Vanillyl 14 and V15 and S14 and S15 were present in the surface horizons only. The values of C3 and V3 were larger in the soils of the meadows and secondary succession sites, indicating non-woody graminaceous input. In the meadow soil, the vanillyl and syringyl compounds increased with depth, whereas the cinnamyl compounds barely changed (detailed information about the concentrations of these compounds under each land use at each soil depth is available in Table S3, Supporting Information). Although there were no



**FIGURE 3** Chromatogram of the 14 compounds identified in the 0–5-cm depth *Pinus nigra* sample (see Table 1 for the name of the compounds)



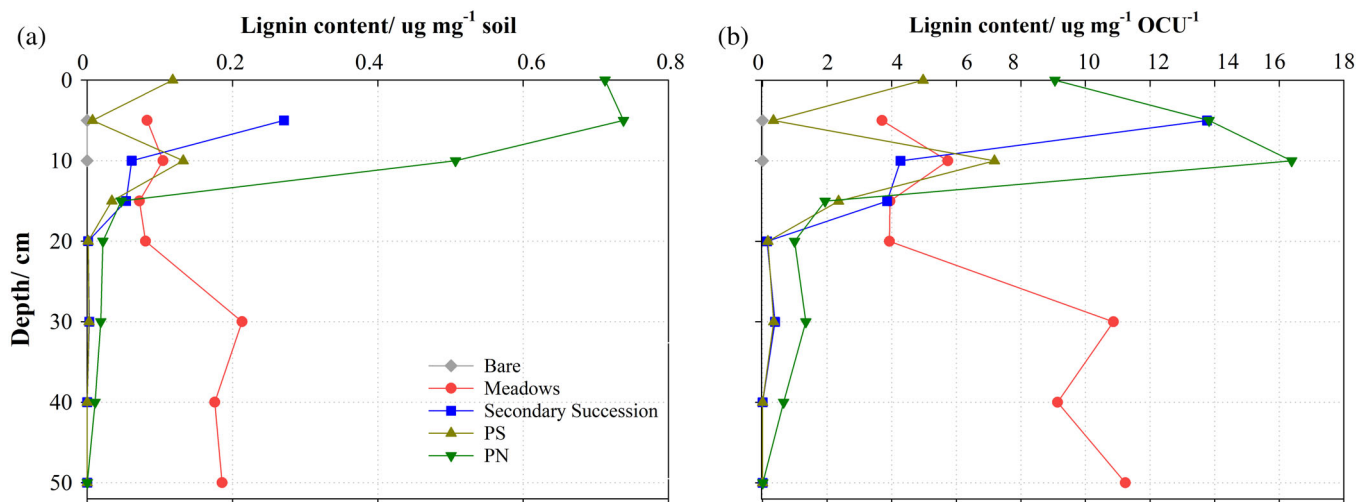


FIGURE 4 (a) Lignin content ( $\mu\text{g mg}^{-1}$  soil) and (b) lignin content per organic carbon unit ( $\mu\text{g mg}^{-1} \text{OCU}^{-1}$ ) in the different soil profiles. PS, *Pinus sylvestris*; PN, *Pinus nigra*

clear patterns in the lignin content under the different land uses, larger values were recorded at the PN sites (Figure 4a). In the secondary succession and PS sites, there was no lignin in the samples below 30 cm, whereas at the PN sites it was present below 40 cm.

The lignin contents ranged between 0 and  $0.74 \mu\text{g mg}^{-1}$  soil,  $0\text{--}16 \mu\text{g mg}^{-1}$  lignin per unit of organic carbon (hereafter OCU) (Figure 4a,b). Large values were recorded for the lignin content per OCU for all the surface soil samples (0–5 cm) except for bare soils (Figure 4b). Afforestation with PN and PS resulted in a litter layer with more than 9 and  $5 \mu\text{g mg}^{-1}$  lignin  $\text{OCU}^{-1}$ , and an increase of 4.6 and 1.5% at the 0–5-cm depth, respectively, relative to this layer in the bare soil. On the other hand, the lignin content in soil of the secondary succession at 0 cm was around  $14 \mu\text{g mg}^{-1}$  lignin  $\text{OCU}^{-1}$ , and at 0–5 cm the increase was about 1.3% compared to bare soil. These contents decreased with depth, as shown in Figure 4b.

Table 3 indicates the significant differences in lignin content between soil depths for the meadow and PN sites. In relation to land uses, there were significant differences at 0–10 cm (PN lignin content was significantly larger than at the other sites) and at 20–50 cm (in meadows it was significantly larger than for the other land uses) (Table S2, Figure 4a). By testing the different compounds individually, significant differences between depths were observed in the vanillyl content in meadow and PN soils, whereas for syringyl and cinnamyl, there were significant differences only between depths of PN soils (Table 3). There were also significant differences between land uses at 0–10 and 20–50 cm for vanillyl and cinnamyl, but only at 20–50 cm for syringyl (Table S2).

Figure 5 presents the results of the cinnamyl/vanillyl (C/V) and of the syringyl/vanillyl (S/V) ratios for the different land uses. The boxes identify the categories that can be differentiated according to the methodology used by

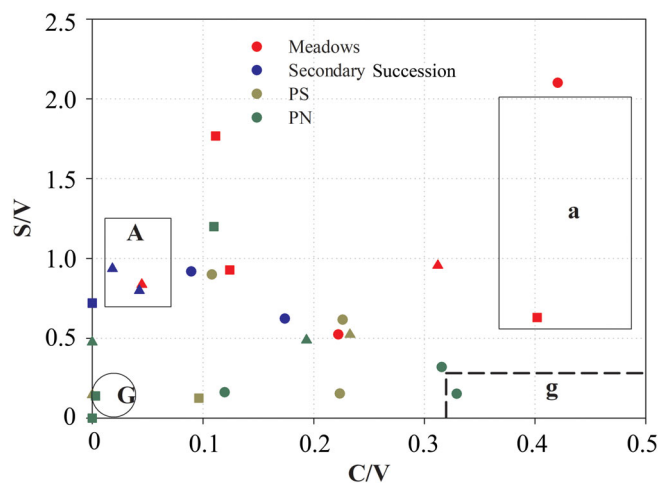
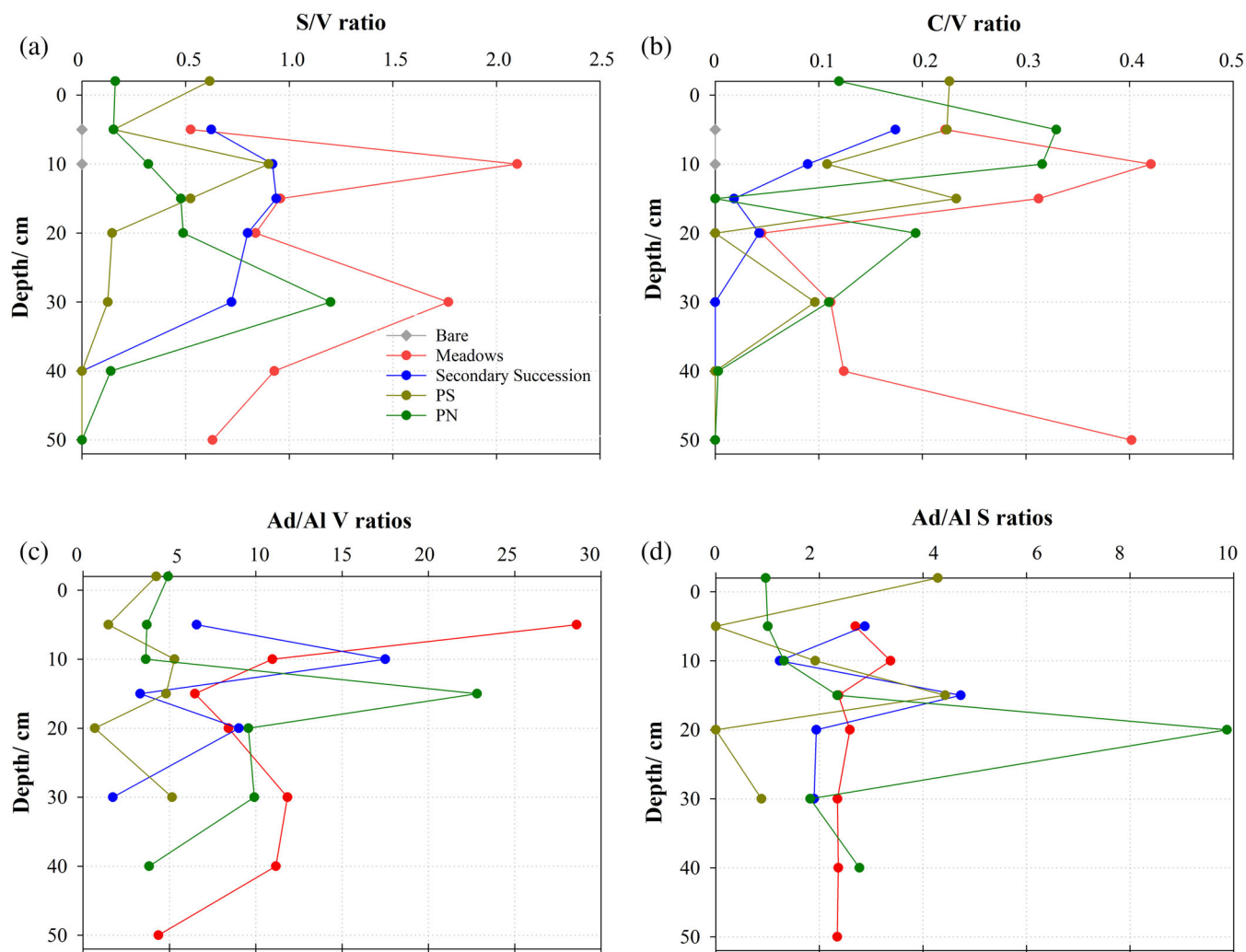


FIGURE 5 Cinnamyl/vanillyl (C/V) and syringyl/vanillyl (S/V) ratios of soil samples from different land uses and depths. A, woody angiosperm material; a, non-woody angiosperm material; G, woody gymnosperm material; g, non-woody gymnosperm material. Note that in all land uses, circles represent the 0–10-cm depth, triangles the 10–20-cm depth and squares the >20-cm depth soil samples. PS, *Pinus sylvestris*; PN, *Pinus nigra*

Ertel and Hedges (1984) and Cerli et al. (2008). The different categories are: gymnosperm wood (G), non-woody gymnosperm tissue (g), angiosperm wood (A) and non-woody angiosperm tissue (a). Accordingly, the S/V ratios were larger for the meadow and secondary succession sites, and the C/V ratios were in general larger at the afforested sites (Figures 5, 6a,b). The C/V and S/V ratios ranged from 0 to 0.42 and 0 to 2.1, respectively, and there were no consistent trends with depth. The C/V and the S/V ratios did not show significant differences with soil depth (Table 3). There were significant differences between land-cover types at 0–10 cm for C/V and 10–20 cm for S/V (Table S2).

Figure 6c,d shows the acid to aldehyde ratio for the vanillyl units ( $\text{Ad}/\text{Al}_V$ ) and syringyl units ( $\text{Ad}/\text{Al}_S$ ). The

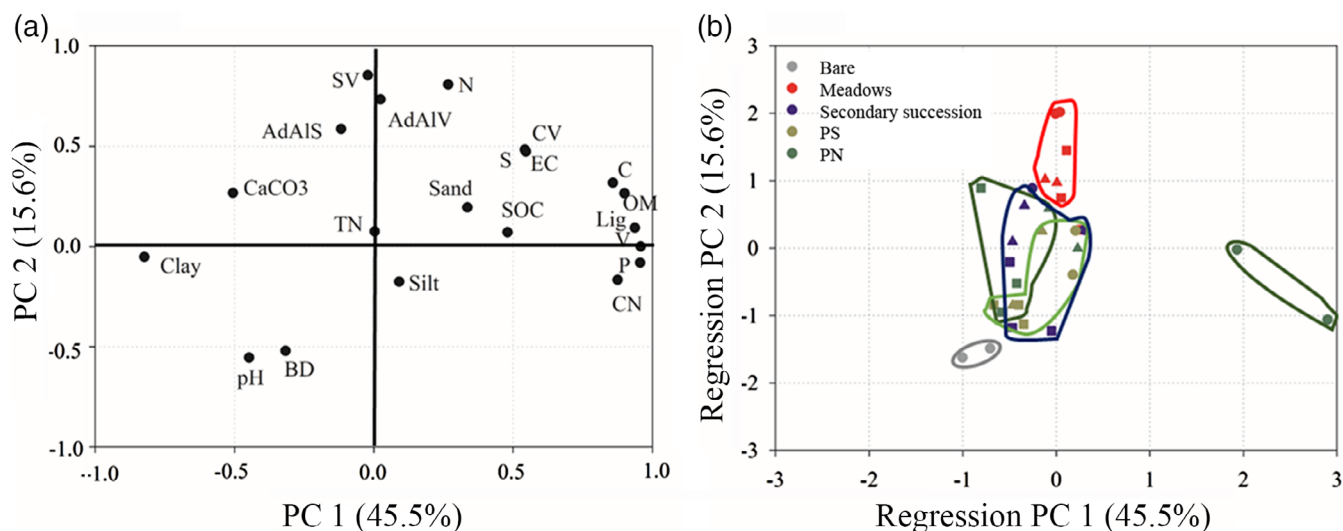


**FIGURE 6** (a) Syringyl/vanillyl (S/V) and (b) cinnamyl/vanillyl (C/V) ratios in the different soil profiles, and acid to aldehyde ratio for (c) the vanillyl units (Ad/Al<sub>V</sub>) and (d) syringyl units (Ad/Al<sub>S</sub>). PS, *Pinus sylvestris*; PN, *Pinus nigra*

Ad/Al<sub>V</sub> values are generally larger than those for Ad/Al<sub>S</sub>, which could indicate greater alteration of V units than of S units. The results of the ANOVA (Table 3) show that through the entire profile there were no significant differences between depths for each land use. However, the LSD values indicated that there were significant differences between PS and PN sites at 20–50 cm for the Ad/Al<sub>V</sub> ratio (Table S2).

The results of the correlation analyses are given in Table S4 (Supporting Information). The lignin content was positively correlated with EC, C<sub>org</sub>, TN, C/N ratio and SOC stock, and negatively correlated with pH, CaCO<sub>3</sub> and clay content. Similar patterns were observed for the different individual components, although the correlation coefficients with the soil characteristics were larger for the vanillyl compounds. The S/V ratio was correlated with TN content only, and the C/V ratio was positively correlated with EC, C<sub>org</sub> and TN, and negatively so with clay content. Finally, the rate of degradation Ad/Al<sub>V</sub> was correlated with TN only, and Ad/Al<sub>S</sub> with the sand and silt contents.

Figure 7a shows a plot of the eigenvectors in the plane of the first two components. The eigenvector values are large and positive for lignin compounds and vanillyl, C<sub>org</sub>, SOM, C/N ratio and P, and large and negative for clay on the first component, which explained 45.5% of the variance. The second component explained 15.6% of the variance; it has large positive eigenvector values for SV and rates of lignin degradation. Figure 7b shows the PC scores in the plane of PC1 and PC2. Although no strong discrimination is evident, there are some differences. The surface soil horizons from PN are on the positive side of PC1, clearly separated from the other sites and with larger values of C<sub>org</sub>, SOM, lignin compounds, P and C/N ratio. Samples from the meadow sites are also clearly distinguishable; they are on the positive side of PC2, in particular the surface soil horizons, and related to larger rates of degradation. The bare soil samples are on the negative side of both components and are also clearly separate (see Table S5, Supporting Information). The sites for secondary succession, *P. sylvestris* and depth layers of *P. nigra* are clustered together close to the centre of both components.



**FIGURE 7** (a) Eigenvectors from the principal component analysis (PCA) and (b) PC scores plotted in the plane of the first two components. Note that for all land uses, circles represent the 0–10-cm depth, triangles the 10–20-cm depth and squares the >20-cm depth of soil samples. BD, bulk density; EC, electrical conductivity; CaCO<sub>3</sub>, calcium carbonate content; C<sub>org</sub>, organic carbon content; TN, total nitrogen content; SOC, soil organic carbon stocks; V, vanillyl; S, syringyl; C, cinnamyl; Ad/Alv, acid to aldehyde ratio for the vanillyl units; Ad/Als, acid to aldehyde ratio for the syringyl units

## 4 | DISCUSSION

### 4.1 | Soil properties and lignin content

Changes in soil properties after land abandonment in Mediterranean environments were limited, even if afforestation practices were carried out (Ruiz-Sinoga & Martinez-Murillo, 2009). In this study, SOC stock was larger in the topsoil (0–10 cm) of the afforestation sites, which was also observed by other studies (Korkanc, 2014), and its accumulation under secondary succession was a slow process (i.e. Gabarrón-Galeote, Trigalet, & van Wesemael, 2015).

The lignin contents reported here (0–0.74  $\mu\text{g mg}^{-1}$  soil, 0–16  $\mu\text{g mg}^{-1}$  lignin OCU<sup>-1</sup>) (Figure 4 a,b) were within the range reported in the review by Thevenot et al. (2010), although smaller than those found in other studies: de Baets et al. (2012), 14–37  $\mu\text{g mg}^{-1}$  OCU<sup>-1</sup>, and Grünewald, Kaiser, Jahn, and Guggenberger (2006), 22–49  $\mu\text{g mg}^{-1}$  OCU<sup>-1</sup>. The decline of lignin concentration with soil depth reported here for all land-use types, except the meadows, has also been observed elsewhere (Cerli et al., 2008; Heim & Schmidt, 2007; Otto & Simpson, 2006; Wiesmeier et al., 2009). The cause of this phenomenon is considered to be the limited input of organic matter at depth and the longer periods of degradation (de Baets et al., 2012). The increase in lignin concentration with depth under the meadows suggests input of these compounds at depth from roots and the reduced rate of degradation at depth. The Pearson correlations (Table S4) show that there were strong positive relations between lignin and SOC and SOC stock values. However, the soils of the meadow sites followed a different trend: the lignin content increased below 20 cm, whereas the C<sub>org</sub> content was constant. At these sites, roots provide fresh input continuously at depth, and therefore the lignin content is relatively large. Accordingly, there were significant

differences in lignin content in  $\mu\text{g mg}^{-1}$  per OCU<sup>-1</sup> between soil profiles of the different land covers as a function of depth (Figure 4b). With increasing depth, organic matter inherited from former agricultural practices was evident, providing signature residues at depth and from forest undergrowth (showing different sources of vegetation at greater depth). The presence of undergrowth under PS and PN showed accelerated decomposition following afforestation (e.g. the Ad/Als ratio under PN, Figure 6d, where strongly degraded grass lignin is present at 20-cm depth).

Soil characteristics and environmental properties also correlate with lignin content and lignin degradation in soils. Thevenot et al. (2010) and de Baets et al. (2012) suggested that the lignin content in soil was associated with organic carbon and nitrogen contents.

Properties that correlated negatively with the lignin content were CaCO<sub>3</sub>, pH and clay content. Other authors have observed similar patterns. Thevenot et al. (2010) conducted a meta-analysis of 29 studies suggesting that the lignin content decreased with decreasing particle size, which is supported by the results of the present study (i.e. smaller lignin content with increasing clay content). According to Thevenot et al. (2010), the lignins present in the finest fractions seemed to be more stable, degraded slowly and were less altered than those in the coarse fraction. Grünewald et al. (2006) also concluded that accumulation of SOC in calcareous soils was mainly a result of the attachment of partly oxidized SOM to positively charged clays, resulting in stabilization against further decomposition. Relations in the present study between lignin content and CaCO<sub>3</sub> can be related to the results of Grünewald et al. (2006), who assumed that partly degraded lignin components, not adsorbed to layered double hydroxides, undergo further microbial oxidation until they form complexes with calcite.

The negative correlation between increasing pH and lignin content suggests such a degradation, which was observed in deeper horizons. In the meta-analysis, the authors found significant relations between lignin degradation states ( $Ad/Al_V$  and  $Ad/Al_S$  ratios) and pH and mean annual temperature. They suggested these correlations could reflect the impact of pH on the activity of fungi in soils and consequently on lignin degradation.

#### 4.2 | Lignin and land cover

The effect of land-use changes on the lignin compounds has been tested (Cong et al., 2016; de Baets et al., 2012; Schellekens et al., 2012), but studies of lignin related to the abandonment of arable land and revegetation processes in humid Mediterranean mountains are currently lacking. In addition, although there is a consensus among researchers on the effect of different vegetation types and land use on the lignin content and occurrence of the various lignin monomers, there is still considerable variation within these studies. Furthermore, the effect of LUC on SOM composition is still not well understood (Han et al., 2016). The analyses carried out in the present study have some limitations because of the size of the dataset (lack of true replication). Therefore, comments on the effect of land use on the soil properties and lignin content are limited to a small region and should not be extrapolated to similar land covers in other areas.

In this research, the bare soil can be considered the degraded soil, and there were no lignin compounds in these soil samples. Accordingly, the effect of afforestation on this former abandoned agricultural land is notable, and the topsoil of afforested sites had the largest lignin contents, associated with larger organic matter inputs and reduced rates of lignin decomposition (Figure 4a,b). This could be related to the resilience and unpalatability of conifer needles, which are avoided by grazing animals and are decomposed slowly by fungi (Virzo De Santo, De Marco, Fierro, Berg, & Rutigliano, 2009). Similarly, de Alcantara et al. (2004) observed a significant increase in lignin content in the topsoils of afforested land when evaluating soil sustainability in areas surrounding hydroelectric reservoirs planted with riparian forest. Cerli et al. (2008) analysed the changes in SOM in an aged sequence of former agricultural soils in Norway. They observed increased C in the mineral layers of the oldest stands related to the increasing input of coniferous litter and changes in soil properties (with stand age). Thevenot et al. (2010), after the evaluation of 29 different studies, concluded that soils of arable land had the largest lignin content, followed by that of grassland and forest soils. de Baets et al. (2012) also indicated that abandoned sites in SE Spain were characterized by the largest soil lignin contents. In the present study, the meadows had the largest lignin content in the soil profile, followed by PN, secondary succession and PS. Panettieri, Rumpel, Dignac, and Chabbi (2017) also found that soils under permanent grasslands stored large

amounts of SOM. Grassland roots account for the main litter input and the accumulation of SOM, which is mainly related to belowground litter.

The largest SOC stock was at the PN site, followed by that of meadow, PS and secondary succession. Both the SOC stocks and the lignin contents showed that afforestation with PN would provide the best potential for increasing the amounts of organic carbon in the soil. Differences between the effects of both pine species on soil are clear, although we cannot explain this. Some hypotheses about the origin of such differences could be: (a) different planting systems were used 50 years ago, (b) tree density or (c) greater hydric stress of PS. In Mediterranean mountain areas *P. nigra* and *P. sylvestris* frequently form mixed forests that are close to the southern distribution limit of PS forests, but at the ecological optimum for PN (Barberó, Losiel, Queézel, Richardson, & Romane, 1998). Various studies have analysed differences between these two pines (for example differences in tree rings, Martin-Benito, Beeckman, & Cañellas, 2013), but there has been no research to analyse differences in their effects on soil properties and SOC and lignin dynamics. In our research, the co-occurrence of these two species in the study area has enabled a comparison of such soil characteristics. Further research should be carried out to continue investigating the effects of afforestation with these species on the soil system.

The  $Ad/Al$  ratios generally increase during lignin degradation. At both pine sites, the acid/aldehyde ratios were rather small in the first few centimetres, indicating fresh litter input, whereas below 15 cm the ratio tended to be larger. This result suggests that the lignin input from PN and PS is large but the rate of degradation is slow in the top layers and large in the deeper layers, resulting in smaller amounts of lignin with depth. The large ratios found in the deep layers of both pine sites were significantly different, suggesting a possible greater rate of degradation of vanillyl compounds in PN than in PS.

The relatively large lignin content in the soil below 20 cm at the meadow sites, compared with that at the same depth for other sites, probably resulted from the large SOC stocks of the meadow sites and the root systems present at this depth (Jobbagy & Jackson, 2000). Crow et al. (2009) also suggested that roots of a particular species could be important sources of soil lignin and so exert some control on its content. Thevenot et al. (2010) attributed increased lignin content with depth in the subsoil to vertical transport and to its protection there.

The ratios  $S/V$  and  $C/V$ , which can indicate the origin of the plant, have been used to assess the source of lignin in soils (Otto & Simpson, 2006). The  $S/V$  ratio, used as a substitute for selective degradation of syringyl lignin as opposed to vanillyl lignin (van der Heijden & Boon, 1994), showed a decrease with the progressive transformation of SOM. The smallest  $S/V$  ratios at the afforested sites accorded with the

findings of de Baets et al. (2012). The similarity of S/V and C/V ratios with depth in meadow soils did not suggest any variation in the vegetation responsible for providing residues within the profile. The large lignin content in the subsoil suggested a slow rate of degradation of these residues but this was not supported by the consistent acid/aldehyde ratios with depth (Table 3). This might be a result of the decomposed remains of older more deeply rooted vegetation and contributions of acids from non-lignin phenolic compounds in non-woody tissues such as grass.

Multivariate analysis has been shown to be an important tool for pattern recognition in soil properties and pyrolysis mass spectrometry. In that sense, PCA illustrated both the similarities and differences between soil samples. Bare soils, surface soils from the PN and meadow sites were clearly identifiable in the plot of PC scores (Figure 7b). In this plot, meadow soil samples were distributed evenly, suggesting similar chemical composition (tight grouping). The PN samples, however, were scattered, showing differences between topsoil and subsurface samples.

## 5 | CONCLUSIONS

Afforestation with *Pinus nigra* was the best afforestation practice for increasing SOC stocks and lignin contents in the soil (particularly in the top 10 cm of the soil), whereas *P. sylvestris* was less successful than natural secondary succession in a humid mountain area in increasing SOC stocks and lignin (Central Pyrenees).

Most land-cover types followed the trend of decreasing lignin content with depth, except for the meadow sites. Our results showed, contrary to our hypothesis, that after more than 50 years of abandonment of arable land and afforestation, soil organic matter showed a similar composition under the different land-cover types, and only small significant differences were recorded under *P. nigra*, both in the topsoil and with depth. The latter could be related to the larger lignin input and reduced rates of lignin decomposition.

In general, there was no consistent variation in the indicators of lignin degradation across the different land-cover types (apart from in the top few centimetres of the soil), suggesting that long-term chemical stabilization of lignin was not greatly affected by land abandonment and consequent land-use changes.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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