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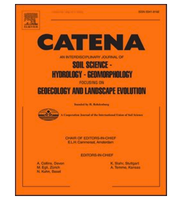

Effects of agricultural land abandonment on soil organic carbon stocks and composition of soil organic matter in the Central Spanish Pyrenees

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ABSTRACT

The Mediterranean mountainous landscapes have undergone large-scale exploitation for millennia. In the Central Pyrenees, land abandonment has occurred since the 1950s, leading to a process of woody encroachment. The main objective of this paper is to shed light on the effects of different land use and land covers (LULCs) on soil organic carbon (SOC) and nitrogen (N) stocks, and on the composition of soil organic matter (SOM) after land abandonment in the Central Pyrenees. Five LULCs (8 specific sites) were selected through detailed land use change mapping. Soil and litter samples (n = 160) were collected and analyzed, including Tetramethylammonium hydroxide pyrolysis–gas chromatography. Organic carbon (Corg)/N ratios, Carbon Preference Index and Average Chain Length indices were calculated based on the distribution of fatty acids in order to determine the molecular composition and degradation of SOM. The results showed: (i) an increase in the dominance index and forest cover, at the expense of shrublands and agricultural fields, and a decrease in Shannon's Diversity and Evenness indexes; (ii) LULC and depth had significant effects on SOC and N contents and stocks; (iii) SOC and N contents and stocks were higher in the meadows and young forests; (iv) significant differences were observed between meadows and young forests and the first stages of land abandonment considering the soil profile; (v) total decomposition of lignins and an omnipresence of unsaturated and saturated straight-chain fatty acids; and (vi) Corg/N values indicate that the origin of SOM is microbial. This study confirms that SOC accumulation after abandonment is a slow process, and the first stages of the woody encroachment decrease SOC stocks. Woody encroachment should be managed with measures based on scientific knowledge, but also considering the historic adaptation of agro-pastoral activities to the environment to ensure the proper functioning of ecosystem services and promote SOC storage in Mediterranean mountain soils.

1. Introduction

Agroecosystem management has become of great importance for humans as these systems occupy large extensions of territory and provide many ecosystem services such as water regulation and soil quality (Bernués et al., 2014). For millennia, Mediterranean mountain landscapes have undergone large-scale exploitation by people, through deforestation, agriculture, and livestock grazing, and for using natural resources for agrosilvopastoral activities (Lasanta et al., 2020a). However, since the beginning of the 20th century, a large part of Mediterranean mountain areas has been abandoned as a result of urban migration. This trend led to a decrease in livestock numbers and agricultural exploitation areas. Furthermore, the abandonment of mountain agricultural and pastoral activities during the last several decades induced the multiplication of scrublands and forests (woody encroachment), creating a new, more uniform landscape (Lasanta et al., 2016; García-Ruiz et al., 2020). In the Mediterranean basin, during the second part of the 20th century, agricultural land abandonment in mountain areas has been the most important land use and land cover change (LULCC) (e.g., Weissteiner et al., 2011). Fuchs et al. (2013) estimated that, from 1950 to 2010, about 8% of Southern Europe has been transformed from grazing land to woody vegetation. These figures are higher when considering only Mediterranean mountains, affecting more than 80% of cultivated land in the Spanish Pyrenees (Lasanta et al., 2017) and around 70% in the eastern Alps (Tasser et al., 2007).

Soil is a vital natural resource that contributes to multiple prominent

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ecosystem services, such as plant development and water control (Franzluebbers, 2002). Soil also plays a crucial role, as far as the decomposition and stabilization of organic matter (OM) is concerned (Nierop and Filley, 2007). In addition, soils represent the main compartment of organic carbon (OC) in most terrestrial ecosystems, containing globally about 1550 Pg C, which roughly is twice the amount of OC in the atmosphere (760 Pg C) and three times the amount in the biomass (550 Pg C) (Lal, 2008). Soil organic carbon (SOC) is an indicator of soil quality and is correlated with biodiversity, erosion, water infiltration and soil texture (Jobbágy and Jackson, 2000). Likewise, soil organic matter (SOM), is primarily made up of complex organic compounds in several stages of decomposition, being closely related to soil quality (Franzluebbers, 2002). The composition and quantity of SOM depends on inputs and subsequent degradation processes regulated by microorganisms. Inaccessibility of SOM for microorganisms as mediated by organo-mineral interactions and occlusion in aggregates are generally seen as key factors for the latter (Lehmann and Kleber, 2015). While the intrinsic recalcitrance of SOM due to its molecular composition is increasingly dismissed as a main determinant of SOM stabilization, molecular composition does play an important indirect role through its effects on sorption processes and aggregation (Schmidt et al., 2011; Lehmann and Kleber, 2015). In addition, the molecular composition of SOM can serve as an important measure of its degradation state (e.g. Brock et al., 2019). Pyrolysis, coupled with gas chromatography-mass spectrometry, is broadly used to study changes in the composition of OM (Pico and Barceló, 2020). Lignins and lipids have been extensively used for the determination of the source of OM (Derrien et al., 2017; Campo et al., 2019). Lignins form almost 20% of litter input in soils. Fatty acids (FAs) are the most abundant lipids in plants, microorganisms and SOM used for the assessment of different sources of OC through the analysis of the molecular ratios (Wiesenberg et al., 2012; Yang et al., 2020). FAs of higher chain lengths ($>C_{20}$) originate predominantly from terrestrial higher plants in a clear even-over-odd chain-length predominance (e.g. Dinel et al., 1990). This even-over-odd chain-length predominance, as well as the average chain-length is altered upon degradation and input of smaller chain-length FAs of microbial origin in the soil. Thus, it can be used as indicator of SOM degradation as well, specifically via the derived parameters of the Average Chain Length (ACL) and Carbon Preference Index (CPI) (widely used to characterize soils and to assign the biological sources and maturity of SOM) (e.g. Schmidt et al., 2011; Wiesenberg et al., 2012).

While the impacts of agricultural land abandonment and LULCC on landscapes and other ecosystem services (e.g. fire risk, water regulation, soil erosion, etc.) are evident (e.g., Ursino and Romano, 2014; Pisabarro et al., 2019; Khorchani et al., 2020), the effects on SOC dynamics and stocks and SOM composition are less apparent (e.g., García-Pausas et al., 2017; Campo et al., 2019; Bell et al., 2020). In addition, despite the high number of studies focused on agricultural land abandonment and LULCC and SOC, only a limited number of studies have focused on cropland abandonment in the humid Mediterranean mountains (e.g., Chiti et al., 2018; Gispert et al., 2018; Badalamenti et al., 2019). Thus, the effects of agricultural land abandonment and LULCC on SOC dynamics and stocks and SOM composition are still a major uncertainty in the global carbon balance (Smith et al., 2015). Some authors stated that differences in SOC stock changes after agricultural land abandonment and woody encroachment among sites are mainly driven by climatic conditions, especially by the mean annual precipitation (Jackson et al., 2002; Alberti et al., 2011). However, land management before and after agricultural abandonment and the revegetation processes can considerably affect SOC dynamics. The scientific literature shows a high variety of results: most of the studies agree that woody encroachment over croplands often lead to a significant increase in SOC stocks (e.g., Lasanta et al., 2020b; Djuma et al., 2020); however, there are more contrasting results related to the effects of grazing abandonment (positive or negative effects or even no changes) (Gosheva et al., 2017; Nadal-Romero et al., 2018). During recent decades, it has been highlighted that the persistence of SOM is crucial to sustaining the large SOC pool in the context of Global Change. Thus, the molecular composition of SOM and its relationship to LULCC is an emerging hot-topic in the scientific literature (Pisani et al., 2016; Campo et al., 2019; Yang et al., 2020).

In that sense, the consequences of agricultural land abandonment and natural vegetation succession (woody encroachment) on SOM composition, SOC dynamics and stocks is of great significance in Mediterranean mountains due to the large surface area abandoned. The main objective of this study is to assess the effects of agricultural land abandonment and natural revegetation processes on soil organic carbon and nitrogen stocks and on soil organic matter composition in different land uses and land covers and at different soil depths, after more than 50 years of abandonment and changes in the Central Spanish Pyrenees. The specific objectives are: (i) to quantify land use changes and analyze landscape structure, (ii) to analyze the effects of LULCC on SOC and N dynamics, and (iii) to assess the effects of LULCC on SOM composition. This leads to the following research hypotheses: (i) LULCC have a significant influence, especially in the topsoil, on SOC and N dynamics and stocks; (ii) in the long-term, SOC stocks will increase after land abandonment, when forests become older and contribute higher amounts of litter; and (iii) LULC and depth affect soil organic matter composition.

To test these hypotheses and objectives, 5 LULCs (8 specific sites) in the Central Spanish Pyrenees were selected. Soil samples (litter and mineral samples) were obtained in the field and analyzed in the laboratory. There is a broad spectrum of techniques that are used to measure SOM quality or composition, such as fractionation and analytical/instrumental chemical methods. In this research, soil samples were analyzed by means of thermally assisted hydrolysis and methylation (THM) with TMAH, followed by analysis by pyrolysis coupled with Gas Chromatography/Mass Spectrography (Py-GC/MS).

2. Materials and methods

2.1. Study area

This study was carried out in the Southern part of the Central Pyrenees (Spain), in the Valley of Puértolas and near Bestué village (Figs. 1 and 2). The altitude in the valley is between 558 and 2797 m.a.s.l. More specifically, the area chosen for soil samplings is near Bestué village and has an altitude of ~1200 m.a.s.l. (Figs. 1 and 2).

The climate is defined as a sub-Mediterranean mountain with continental influence, and climate conditions are influenced by both the Mediterranean Sea and the Atlantic Ocean, which in combination with topography and altitude, determine rainfall and temperature patterns. The mean annual temperature is 11.5 °C and mean rainfall is around 1300 mm, with dry summers and high precipitation values during spring and autumn. Soils are classified as Cambisols, characterized for having a weak horizon differentiation, moderate soil development (shallow soils not deeper than 60 cm) and low clay translocation. In the study area where samples were taken, limestones were predominant. Agricultural terraces were the most common field type in the study area. Since the middle of the 20th century, some terraces were abandoned, and others were still cultivated with meadows (Fig. 1a). Meadows are nourished with organic animal manure and synthetic (chemical) fertilizer: yearly when enough manure is available, or every two years when there is not enough manure. Organic manure is rich in nitrogen and potassium, although poor in phosphorus. In addition, to overcome potassium shortages, chemical fertilizer is sometimes used, adding phosphor-potassium fertilizer favoring the presence of leguminous plants in the meadows and improving the quality of fodder. After agricultural abandonment, a natural revegetation succession process started in the terraces: grassland (Fig. 1b), young shrublands (*Genista scorpius)* (Fig. 1c), old shrublands *(Juniperus communis and Buxus sempervirens)* (Fig. 1d) and forests (*Quercus faginea*) (Fig. 1e).

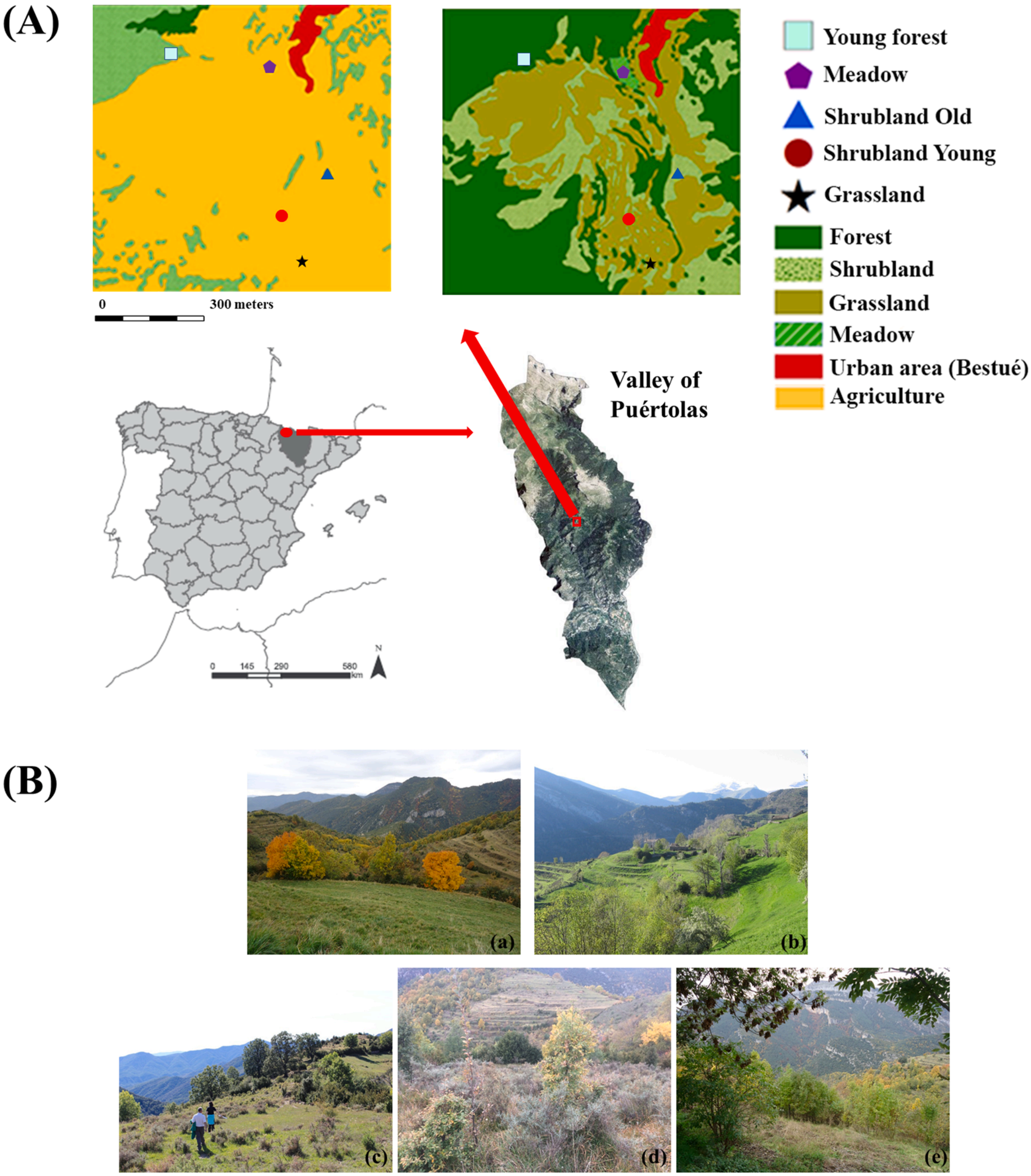

Fig. 1. (A) Information on study area location (Valley of Puértolas), and land uses and land covers in 1956 and 2016 in the sampling location (near Bestué village). (B) Overview of the present land uses and land covers. (a) meadows (M), (b) grasslands (G), (c) young shrubland (YS), (d) old shrubland (OS), and (e) young forest (YF).

2.2. Landscape analysis

Two land use and land cover (LULC) maps based on 1956 and 2016 orthophotos were created by digitizing the main LULC with ArcMap 10.4.1 (Fig. 2). Digitalization of LULC was carried out through visual comparison, by overlapping maps and then classifying in six categories: (i) agricultural land, (ii) shrublands, (iii) subalpine pasture, (iv) forest, (v) bare rock and (vi) urban areas. Additionally, four detailed maps based on orthophotos (1:50,000) from 1956, 1977, 2006 and 2016 were created through a digitalization process to show in more detail the extent of secondary succession in the sampling area, next to the village of Bestué.

The landscape analysis was carried out by ArcGis extension V-LATE considering three landscape indicators: (i) the Shannon's Diversity Index (SDI), indicating how diverse is the distribution of LULC types (values ranged between 1 and 2; values close to 1 indicate less diversity); (ii) the Shannon's Evenness Index (SE) (values ranged between 0 and 1), showing how evenly are land cover types are represented; and (iii) the Dominance (D), measuring to what extent one of a few land covers dominate the landscape (higher values indicate higher dominance in the landscape).

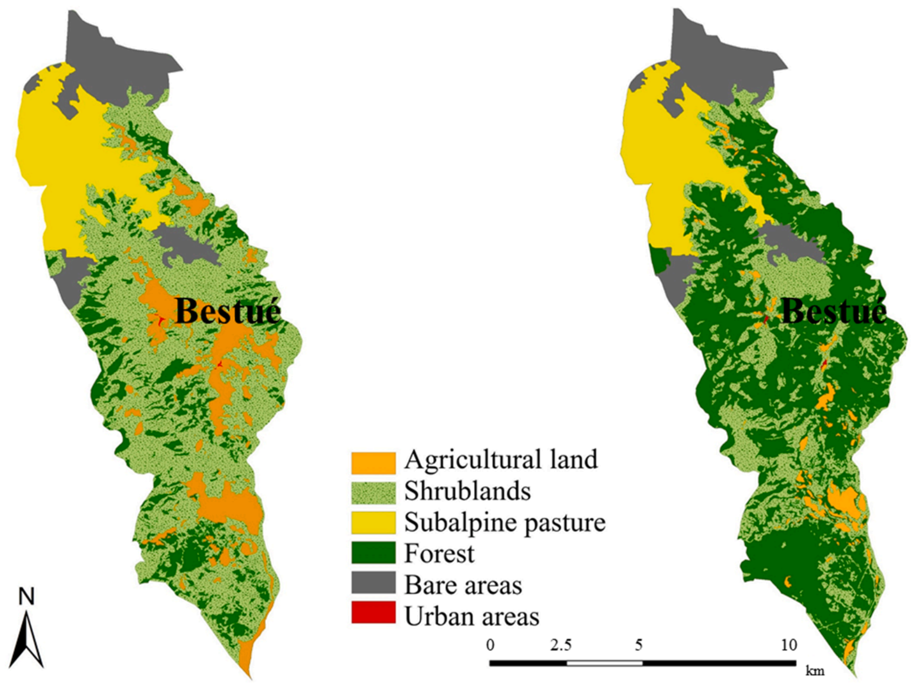

Fig. 2. Study area location, Valley of Puértolas and Bestué village (Central Pyrenees); Land use and land cover in 1956 (left) and 2016 (right).

2.3. Experimental sampling design

The sampling plots were located in areas with identical topography (slope <10°), altitude (1160 m.a.s.l.), lithology (limestones) and exposition (NW) in order to compare the results and to limit inaccuracies and errors to the lowest extent (Fig. 1). Five different areas were selected for sample collection, each one representing a different LULC and being part of a chronosequence of vegetation succession (see Fig. 1). In addition, samples were taken from the soil beneath the plant canopies (closed) and between the plants (open). In that sense, samples were selected from 8 different specific sites (corresponding to 5 different LULCs): (i) grassland that has been mowed and animal and synthetic fertilized (meadows, M); (ii) grassland that has been grazed (G); (iii and iv) young shrubland (YS), divided in open space (YSO) and underneath a closed plant canopy (YSC) and occupied mainly by *G. scorpius*; (v and vi) older shrubland (OS), divided in the same way as the young shrubland (OSO and OSC) dominated by *G. scorpius* and some *J. communis* along with few *B. sempervirens*; and, finally (vii and viii) young deciduous forest (YF), also divided in open and closed (YFO and YFC, respectively), and occupied mainly by *Q. faginea.*

Sampling depth per LULC was divided in four: litter sample, 0–10 cm, 10–20 cm and 20–30 cm. Five replicates were taken per LULC and depth interval. As a result, a total of 160 samples were taken from the field to the laboratory. Additionally, 160 undisturbed samples of mineral soil were taken to measure bulk density values using the core method with 100 cm^3 volume metal rings (Blake and Hartge, 1990). At the same locations, litter thickness and volume was determined in the field, using a square open metal frame (surface area 472.5 cm^2), and all ectorganic material on top of the mineral soil within the metal frame was collected.

2.4. Laboratory analysis: carbon and nitrogen

Bulk density of the mineral soil was determined gravimetrically through undisturbed samples, by drying a known volume of sample at 105 °C. Disturbed samples were air dried or dried at 30 °C for 7 days. Litter bulk density was also determined gravimetrically by drying and weighting the collected litter. The dry sample weight divided by the in situ measured field sample volume revealed the litter bulk density. The mineral soil material was dried (big roots and stones were removed), sieved over 2 mm mesh, and milled before further analysis. The dried ectorganic material was milled and dried at 70 °C before further analysis.

Total carbon (TC) and total nitrogen (N) in both mineral soil and ectorganic material were analyzed using an Elemental Analyzer (Elementar, Germany). In addition, carbonate concentration ($CaCO_3$) was also determined through the Wesemael method (van Wesemael, 1955) by weight loss upon dissolution by HCl, and from which the total inorganic carbon was calculated. Soil organic carbon (SOC) was calculated by subtraction of total inorganic carbon from the total carbon. SOC and N stocks were calculated multiplying each value by the respective depth and bulk density. The Corg/N ratio was also calculated.

2.5. Soil organic matter composition: Curie-point pyrolysis and thermally assisted hydrolysis and methylation (THM)

From total sample set, 72 samples were selected for thermally assisted hydrolysis and methylation (THM). The process was similar to the one proposed by Campo et al. (2019): 5 mg of soil for each sample were weighed, and 6 μl of an internal standard were added for quantification, in our case being 5α-androstane (100ng/μl in cyclohexane) and 20 μl of tetramethylammonium hydroxide (TMAH) solution (25% in water) (Sigma-Aldrich, Schnelldorf, Germany). All contents were mixed for 1 min and then a ferromagnetic wire was dipped into the solution. The wire was left to dry at 10 cm under a halogen lamp for 20 min for each side, and was later put inside a glass capillary. 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 × 0.32 mm i.d.) coated with DB-1 (film thickness 0.50 μm) 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 min^{-1} to 320 °C and maintained at that temperature for 15 min. The column was coupled to a Finnigan Trace mass spectrometer (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.

The Xcalibur software was used for the identification of lignin monomers and fatty acids (FAs), as well as for their integration in the samples. NIST Library (National Institute of Standards and Technology) and literature information from Poerschmann et al. (2008) were used for the identification.

From the FA data obtained through the process two indexes were calculated. The Carbon Preference Index (CPI) is an index that reflects the degradation of soil organic matter (Matsuda and Koyama, 1977). Average Chain Length (ACL) indicates the differentiation origin of SOM, providing an indication of the relative contribution of microbial versus higher plant derived FAs (e.g. plant-derived organic matter shows higher values than organic matter that derives from microorganisms). Shifts in the ACL may reflect changes in the input of higher plant-derived organic matter, as the relative abundance of FAs of various chain lengths between C_{20}-C_{34} may vary between different groups of plants. Thus, when the plant biomass is degraded, the long-chain FAs that are included in it are preserved, thus constituting a way of source identification (Wiesenberg et al., 2012). CPI was calculated for the entire carbon range (C_{16}-C_{30}) following the methodology proposed by March (2013) and ACL was calculated for the entire range of even-numbered FA chain-lengths (Wang et al., 2015).

2.6. Statistical analysis

First, an explanatory analysis was carried out where the mean, standard deviation and coefficient of variation were calculated for all the variables (i.e. SOC, N, Corg/N ratio, all CPI and ACL indices) for all LULCs and depths. Secondly, the data was checked for the normality of distribution using the Shapiro-Wilk normality test and Homogeneity of variance using Levene's test. Analysis of variance, a two-way ANOVA was used to compare the differences among LULCs and depths (see Table 2). The Tukey Post-Hoc test was conducted in order to determine the specific differences among different depths in the same LULC, and among different LULCs in the same depth (see Table 3). In all cases, we considered differences to be statistically significant at $p < 0.05$. The statistical analyses were conducted using SigmaPlot software (14.0) and IBM SPSS Statistics 25.

3. Results

3.1. Landscape analysis

Shrubland was in 1956 the most extensive LULC occupying about 45.1% of the area, while agriculture represented about 13%. The expansion of forest (from 13.7 to 48.7%) at the expense of shrubland (−24.8%) and agriculture (−3.2%) was the main LULCC that occurred in the area (Fig. 2, Table 1).

Table 1 summarizes the Shannon's Diversity Index, Shannon's Evenness Index and Dominance of both 1956 and 2015. Results show that in 2015 LULCs are less diversely distributed in the landscape (lower SDI) and less evenly represented (lower SE), as well as, more dominated by a lesser number of land cover patches (higher D).

Table 1
Land use and Land covers and Landscape Indexes in 1956 and 2016 in the Valley of Puértolas (Central Pyrenees, Spain).

Land cover type	Surface occupied by each LULC (%)	
	1956	2016
Shrublands	48.14	24.75
Subalpine grassland	15.21	12.99
Forests	13.68	48.69
Agriculture – Open grasslands	12.55	3.15
Bare rock	10.39	10.39
Urban	0.03	0.03
Landscape Index		
Shannon's Diversity Index	1.409	1.309
Shannon's Evenness Index	0.786	0.730
Dominance	0.383	0.483

3.2. Soil organic carbon and nitrogen dynamics

Table 2 shows the values of two-way ANOVA analysis. The results showed that the majority of the variables were significantly different depending on LULC and depth.

Considering all the mean values from the entire profile, in all LULC, SOC mean concentrations ranged from 1.68% to 22.23% (Fig. 3). Significant differences among LULC were obtained at the litter layer: the highest concentrations were recorded in the YFC, YFO and OSO. At 0–10 cm, significant differences were observed between M, G and OSO, and YSO and YSC. Meadows and grasslands showed significantly higher SOC concentrations than YSO and YSC in the 10–20 cm layer. Below 20 cm, significant differences were only observed between YSO and YSC (lower values) and OSC and meadows. Significant differences were also observed between different depths, especially between litter layer values and depth values (Fig. 3 and Table 2).

Significant differences related to N concentrations were also observed (Table 3). Mean N values ranged from 0.24% to 1.39% (Table 3). N concentrations decreased with depth and, in general, values were significantly higher in the meadow and grassland sites than in the YSO and YSC (Table 3).

Mean Corg/N ratio ranged from 7.0 to 24.8 (Fig. 3 and Table 3). As expected, higher Corg/N ratio values were found in the most superficial layers and progressively decreased with depth. Some significant differences were observed at different depths between LULC: (i) within the litter layer, no significant differences were observed; (ii) at 0–10 cm, significant differences were only observed between YFC and grasslands and meadows; and (iii) below 10 cm, YSO shows significant differences with YFC and OSC. For each LULC, significant differences were found between litter and the 10–20 cm layer (except in meadows and grasslands) (Table 3).

Mean total SOC stocks ranged between 58.9 Mg ha^{-1} (YSO) to 113.4 Mg ha^{-1} (meadows) (Fig. 4). When considering the entire profile, for each LULC, meadows showed the highest SOC stocks following the approximate order: M > YFC > OSO> OSC > G > YFO > YSC > YSO. Significant differences were observed between LULC considering the soil profile (30 cm): (i) YSO showed lower values than YFC, OSO, OSC and meadows; and (ii) YSC and YFO stocks were lower than the values recorded in the meadows sites. The proportion of SOC in the first 10 cm averaged 34.9, 35.7, 37.3, 42.7, 44.5, 46.5, 47.8 and 48.0% for YSC, YFC, OSC, YFO, M, OSO, G, and YSO, respectively.

Significant differences within the same depth between different LULC existed. At the litter layer, SOC stocks of YFO and YFC were significantly higher than the other LULC. Within the 0–10 cm, YSO and YSC stocks were lower than the values recorded in the meadow sites. Considering 10–20 cm, YSO was significantly lower than YSO, OSC, OSO and meadows. Finally, below 20 cm, only YFC was significantly higher than SAO.

Total N stocks ranged from 7.1 Mg ha^{-1} (YSO) to 12.7 Mg ha^{-1} (meadow) (Fig. 4). Taking into account the entire soil profile meadows had the highest N stocks, followed in descending order by M > YFC > OSO > OSC > G > YSC > YFO > YSO. Significant differences were observed between meadows and YFO, YSO and YSC considering the entire soil profile. The proportion of N in the first 10 cm averaged 30.6, 33.0, 36.0, 40.2, 42.4, 42.5, 42.8, and 44.7 for YSC, YFC, OSC, YFO, M, YSO, OSO, and G, respectively. Significant differences were observed between LULC at different depths. N stocks in the litter layer from YFO and YFC were significantly higher than in the other LULC. At 0–10 cm, results showed that meadows and grassland stocks were higher than the other LULCs. Concerning the 10–20 cm, the only significant difference occurred between meadows and other LULCs. Finally, below 20 cm, YSO

Table 2
F values and significance (p) of ANOVA analysis. LSD post-hoc tests are shown in Table 3 and were used to confirm where the differences occurred between groups.

		Total C	OC	N	SOC stock	N stock	Corg/N ratio	BD
Land cover	F	1.711	3.379	5.729	9.362	8.189	5.258	6.894
	p	0.112	*0.002*	*<0.000*	*<0.000*	*<0.000*	*<0.000*	*<0.000*
Soil depth	F	271.040	277.353	79.170	34.055	16.366	143.325	309.069
	p	*<0.000*	*<0.000*	*<0.000*	*<0.000*	*<0.000*	*<0.000*	*<0.000*
Land cover × Soil depth	F	1.892	1.667	2.697	1.464	1.746	4.029	2.082
	p	*0.016*	*0.045*	*<0.000*	*0.040*	*0.050*	*<0.000*	*0.007*

Note: Total Carbon (Total C), Organic carbon content (OC), nitrogen content (N), SOC stocks and N stocks, and Corg/N ratios and bulk density (BD).

Table 3
Land covers and depths values of soil physical and chemical properties (means, standard deviation and significant differences).

	Depth (cm)	YFO	YFC	YSO	YSC	OSO	OSC	M	G
Total C (%)	Litter	20.84 ± 3.09^{A}	22.23 ± 6.70^{A}	13.95 ± 2.80 A	19.36 ± 7.39^{A}	22.45 ± 7.74^{A}	19.10 ± 5.30^{A}	17.40 ± 4.82^{A}	14.46 ± 3.21^{B}
	0–10	4.97 ± 1.25$^{B\ a}$	5.17 ± 0.23$^{B\ a}$	5.39 ± 0.42^{B} ab	5.24 ± 1.21^{B} ab	6.91 ± 1.03$^{B\ b}$	5.91 ± 1.04$^{B\ ab}$	6.14 ± 0.38$^{B\ ab}$	6.10 ± 0.97^{B} ab
	10–20	3.20 ± 0.69^{BC} a	3.55 ± 0.14$^{B\ a}$	4.08 ± 0.12^{B} a	4.08 ± 0.31$^{B\ a}$	4.51 ± 0.34$^{B\ b}$	4.62 ± 0.75$^{B\ b}$	4.16 ± 0.70$^{B\ a}$	4.39 ± 0.60^{BC} b
	20–30	2.61 ± 0.63$^{C\ a}$	2.66 ± 0.31$^{B\ a}$	3.68 ± 0.15^{B} b	3.63 ± 0.19$^{B\ b}$	3.61 ± 0.15$^{B\ b}$	3.83 ± 0.55^{Bb}	2.93 ± 0.21$^{B\ a}$	3.37 ± 0.51$^{C\ a}$
OC (%)	Litter	20.79 ± 3.10^{A} a	22.17 ± 6.71^{A} a	13.09 ± 2.96 A b	18.51 ± 7.72^{A} ab	22.23 ± 7.72^{A} a	19.06 ± 5.29^{A} a	17.36 ± 4.82^{A} ab	14.41 ± 3.22^{B} b
	0–10	4.92 ± 1.23$^{B\ a}$	5.13 ± 0.22$^{B\ a}$	3.86 ± 0.49^{B} a	3.73 ± 1.38$^{B\ a}$	6.65 ± 0.99$^{B\ b}$	5.73 ± 1.10$^{B\ a}$	6.09 ± 0.38$^{B\ ab}$	6.03 ± 0.95^{B} ab
	10–20	3.16 ± 0.69^{B} ab	3.51 ± 0.12$^{B\ a}$	2.31 ± 0.16 BC b	2.36 ± 0.41$^{B\ b}$	3.86 ± 0.51$^{B\ a}$	4.15 ± 1.10$^{B\ a}$	4.13 ± 0.69$^{B\ a}$	4.03 ± 0.48$^{B\ a}$
	20–30	2.58 ± 0.63^{B} ab	2.62 ± 0.31^{B} ab	1.68 ± 0.08^{C} a	1.80 ± 0.28$^{B\ a}$	2.72 ± 0.72$^{B\ a}$	3.15 ± 0.96$^{B\ b}$	2.90 ± 0.21$^{B\ ab}$	2.48 ± 0.31^{Ca}
N (%)	Litter	1.05 ± 0.16^{A} a	1.05 ± 0.10$^{A\ a}$	0.74 ± 0.08^{ab}	0.74 ± 0.11^{A} a	1.39 ± 0.41$^{B\ ac}$	1.21 ± 0.37^{a}	1.33 ± 0.28$^{B\ ac}$	1.18 ± 0.43^{B} ac
	0–10	0.48 ± 0.11^{B}	0.47 ± 0.02^{B}	0.41 ± 0.03	0.38 ± 0.09^{B}	0.63 ± 0.05^{B}	0.57 ± 0.11	0.66 ± 0.03^{B}	0.67 ± 0.09^{B}
	10–20	0.35 ± 0.06^{BC}	0.36 ± 0.03^{C}	0.29 ± 0.01	0.29 ± 0.03^{BC}	0.41 ± 0.06^{BC}	0.43 ± 0.11	0.46 ± 0.11^{BC}	0.49 ± 0.04^{BC}
	20–30	0.30 ± 0.06^{C}	0.29 ± 0.02^{C}	0.24 ± 0.01	0.24 ± 0.02^{C}	0.31 ± 0.07^{C}	0.41 ± 0.20	0.29 ± 0.16^{C}	0.33 ± 0.03^{C}
SOC stock (Mg ha^{-1})	Litter	2.38 ± 0.36^{A} a	3.23 ± 0.98$^{A\ a}$	0.24 ± 0.06^{A} b	1.10 ± 0.46^{A} b	0.67 ± 0.23$^{A\ b}$	0.90 ± 0.25$^{A\ b}$	0.25 ± 0.07$^{B\ b}$	0.02 ± 0.05^{A} b
	0–10	32.19 ± 8.17^{B} abc	36.46 ± 7.63^{B} abc	28.27 ± 3.07$^{B\ ac}$	23.41 ± 5.83^{B} a	46.10 ± 11$^{B\ bc}$	36.69 ± 15.14^{B} abc	50.40 ± 13.40$^{B\ bc}$	40.71 ± 2.33^{B} abc
	10–20	23.06 ± 4.85^{B} ab	31.42 ± 8.16^{B} a	15.70 ± 2.20$^{C\ b}$	22.55 ± 3.69^{B} ab	29.67 ± 9.07 BC a	33.09 ± 11.38^{B} a	35.28 ± 7.04^{C} a	27.03 ± 6.68^{C} ab
	20–30	17.65 ± 6.88^{B} ab	31.03 ± 10.64$^{B\ a}$	14.69 ± 3.13$^{C\ b}$	20.04 ± 2.40^{B} ab	22.71 ± 12.36$^{C\ ab}$	27.57 ± 7.08^{B} ab	27.44 ± 4.06^{C} ab	17.16 ± 4.07^{D} ab
N stock (Mg ha^{-1})	Litter	0.12 ± 0.02^{A} a	0.15 ± 0.01$^{A\ b}$	0.01 ± 0.00^{A} c	0.04 ± 0.01^{A} d	0.04 ± 0.01$^{A\ d}$	0.06 ± 0.02$^{A\ d}$	0.02 ± 0.00$^{B\ c}$	0.02 ± 0.01$^{A\ c}$
	0–10	3.14 ± 0.76^{B} ab	3.39 ± 0.83^{B} ab	3.01 ± 0.35^{B} ab	2.42 ± 0.47$^{B\ a}$	4.38 ± 0.87$^{B\ ab}$	3.66 ± 1.55$^{B\ a}$	5.39 ± 1.30$^{B\ b}$	4.51 ± 0.17$^{B\ b}$
	10–20	2.53 ± 0.46^{B} ab	3.29 ± 0.99^{B} ab	1.97 ± 0.30^{C} a	2.74 ± 0.33^{B} ab	3.18 ± 1.01$^{C\ ab}$	3.46 ± 1.11$^{B\ ab}$	3.92 ± 1.02$^{C\ b}$	3.28 ± 0.87^{C} ab
	20–30	2.05 ± 0.70^{B} ab	3.45 ± 0.91$^{B\ b}$	2.10 ± 0.50^{C} a	2.71 ± 0.32^{B} ab	2.64 ± 1.28$^{C\ ab}$	2.99 ± 0.71$^{B\ ab}$	3.39 ± 0.50$^{C\ ab}$	2.28 ± 0.58^{D} ab
Corg/N ratio	Litter	20.16 ± 4.46	21.21 ± 7.08	17.45 ± 2.18 A	24.78 ± 8.70^{A}	15.83 ± 1.54^{A}	15.87 ± 1.10^{A}	13.02 ± 0.00^{B}	12.86 ± 0.01^{A}
	0–10	10.23 ± 0.36 ab	10.85 ± 0.51^{a}	9.40 ± 0.41^{B} ab	9.62 ± 1.03^{B} ab	10.49 ± 0.73^{B} a	10.08 ± 0.71$^{B\ a}$	9.29 ± 0.34$^{B\ ab}$	9.01 ± 0.28^{B} ab
	10–20	9.08 ± 0.43^{a}	9.68 ± 0.54^{a}	7.98 ± 0.24 BC b	8.21 ± 0.58^{B} ab	9.36 ± 0.26^{BC} a	9.51 ± 0.37$^{B\ a}$	9.19 ± 0.92$^{B\ a}$	8.21 ± 0.27$^{B\ b}$
	20–30	8.51 ± 0.44^{ab}	8.88 ± 0.63^{a}	7.03 ± 0.22^{C} b	7.40 ± 0.55 Bab	8.52 ± 0.80$^{C\ a}$	8.72 ± 0.39$^{B\ a}$	7.96 ± 0.34$^{B\ a}$	7.49 ± 0.46$^{B\ a}$
BD (g cm^{-3})	Litter	0.04 ± 0.02^{A} a	0.03 ± 0.01^{A} ab	0.02 ± 0.01^{A} b	0.04 ± 0.01^{A} a	0.02 ± 0.01$^{A\ b}$	0.01 ± 0.00$^{A\ b}$	0.01 ± 0.00$^{A\ b}$	0.02 ± 0.01^{A} b
	0–10	0.74 ± 0.17^{B}	0.88 ± 0.17^{B}	0.84 ± 0.08^{B}	0.84 ± 0.25^{B}	0.78 ± 0.11^{B}	0.80 ± 0.24^{B}	0.98 ± 0.20^{B}	0.77 ± 0.11^{B}
	10–20	0.84 ± 0.18^{B} ab	1.03 ± 0.20^{B} ab	0.77 ± 0.10^{B} a	1.16 ± 0.16^{BC} b	0.86 ± 0.19$^{B\ ab}$	0.92 ± 0.09^{BC} ab	0.98 ± 0.11$^{B\ ab}$	0.78 ± 0.23$^{B\ a}$
	20–30	0.78 ± 0.17$^{B\ a}$	1.39 ± 0.35$^{C\ b}$	0.96 ± 0.22^{B} ab	1.36 ± 0.26$^{C\ b}$	0.90 ± 0.29$^{B\ a}$	1.01 ± 0.14$^{C\ ab}$	1.06 ± 0.07$^{B\ ab}$	0.86 ± 0.20$^{B\ a}$

Note: Total Carbon (Total C), Organic carbon content (OC), nitrogen content (N), SOC stocks and N stocks, and Corg/N ratios and bulk density (BD). Young forest open (YFO), young forest closed (YFC), young shrubland open (YSO), young shrubland closed (YSC), old shrubland open (OSO), old shrubland closed (OSC), meadow (G), grassland (H).
Note: Means with different upper case letter superscripts within a column are significantly different at 0.05 level of significance ($p < 0.05$) (depth).
Means with different lower case letter superscripts within a row are significantly different at 0.05 level of significance ($p < 0.05$) (land use).

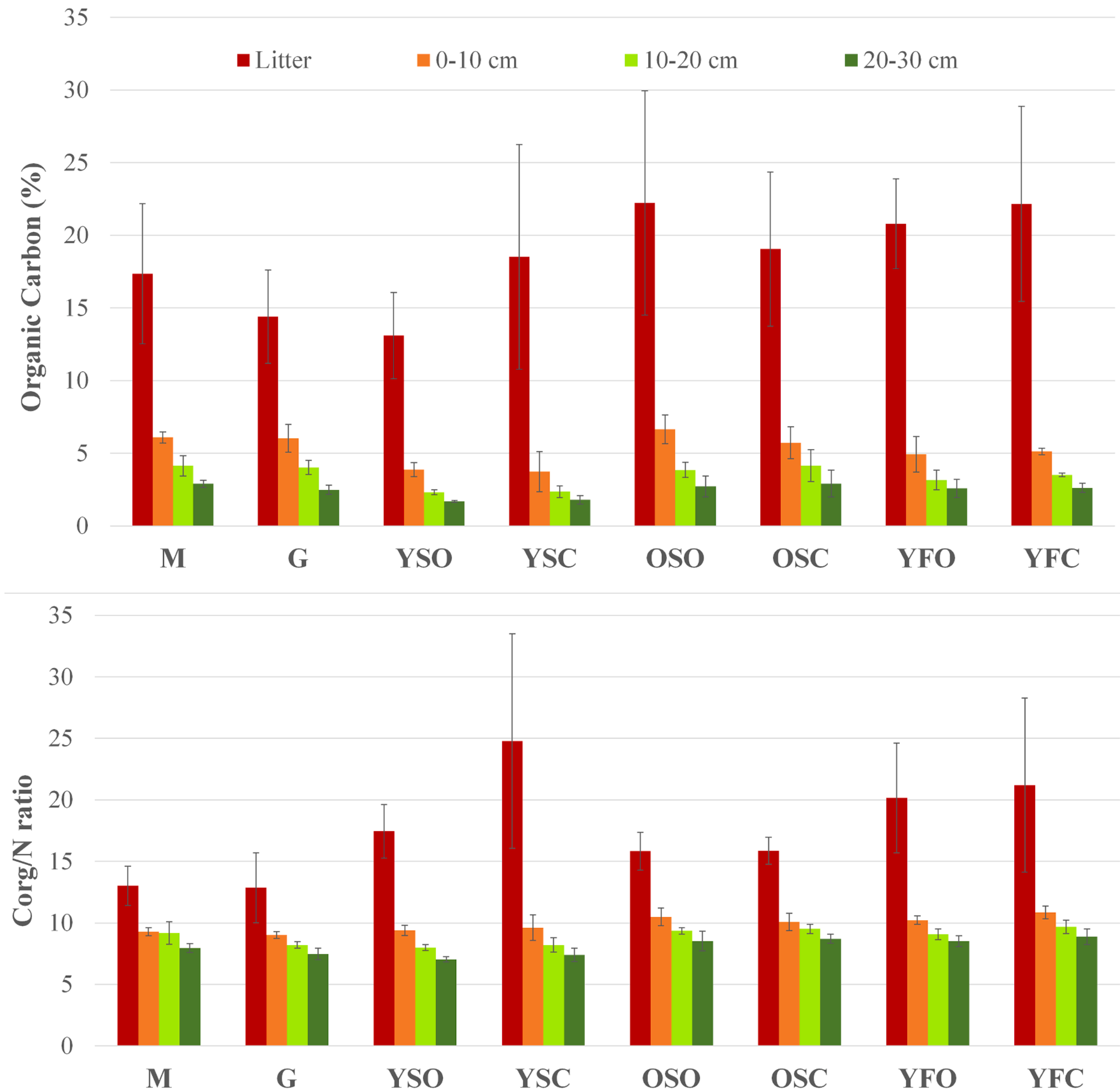

Fig. 3. Organic Carbon (%) and Corg/N ratio per land cover, depth. Values represent the averages of the 5 replicates. Error bars show standard deviation. Note: Meadows (M), grasslands (G), young open shrubland (YSO), young closed shrubland (YSC), old open shrubland (OSO), old closed shrubland (OSC), young open forest (YFO) and young closed forest (YFC).

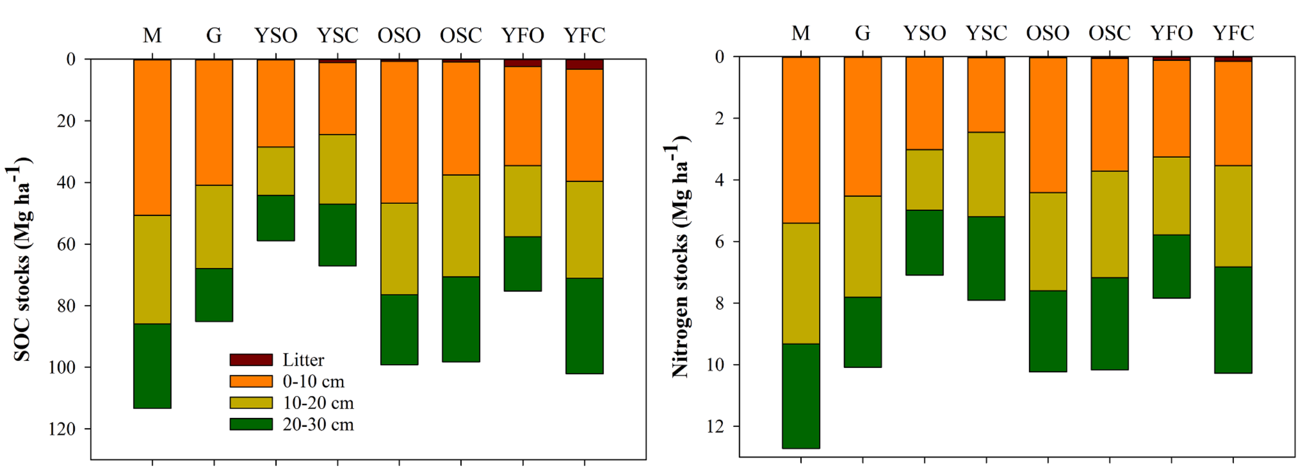

Fig. 4. SOC and N stocks throughout the profile for the different land use and land cover sites. Note: Meadows (M), grasslands (G), young open shrubland (YSO), young closed shrubland (YSC), old open shrubland (OSO), old closed shrubland (OSC), young open forest (YFO) and young closed forest (YFC).

values were lower than the ones observed in YFC.

3.3. Soil organic matter composition

Results from the thermally assisted hydrolysis and methylation (THM) analysis indicated that lignin was only present in the litter layers.

FAs, ranging between C16:0 and C30:0, were identified in the samples with the exception of C29:0 which was completely absent (Table 3 and Supplementary material). In addition, two unsaturated FAs were identified (C16:1 and C18:1). Together with the even numbered saturated FAs ranging from C16:0 to C:26:0, these were the most abundant ones. They were found in all depths and LULCs. The least abundant FAs were the longer-chain C25:0, C28:0 and C30:0, which were completely absent from the deepest layer (except in grassland sites) (Table 3 and Supplementary material) (see Table 4).

The CPI values were calculated for saturated FAs for the entire range (C16:0-C30:0). Differences between LULCs and depths were observed (Fig. 5). At the top soil layers, CPI values ranged from ~5 until ~40. Grasslands together with YSO and YSC showed the lowest values (~5), followed by M (~10) and OSO (~20). YFC showed a CPI around 20, and YFO and OSO presented the highest values. CPI values increase in the 10–20 cm layers in grasslands, meadows, YSO and YFO, and decrease again in the 20–30 cm layer. In the other sites, CPI values decreased with increasing soil depth. The ACL index was calculated for saturated FAs. ACL values ranged from 17.0 to 18.7. Similar values were observed in the different LULC and depths. No significant differences were observed

Table 4
Saturated and unsaturated fatty acids that were identified in the samples after methylation with TMAH (0–10, 10–20, and 20–30 cm). (F = Found, NF = Not Found).

	M 0–10	G 0–10	YSO 0–10	YSC 0–10	OSO 0–10	OSC 0–10	YFO 0–10	YFC 0–10	Total
C16:1	F	F	F	F	F	F	F	F	***8***
C18:1	F	F	F	F	F	F	F	F	***8***
C16:0	F	F	F	F	F	F	F	F	***8***
C17:0	F	F	F	F	F	F	F	F	***8***
C18:0	F	F	F	F	F	F	F	F	***8***
C19:0	F	F	F	F	NF	F	F	NF	***6***
C20:0	F	F	F	F	F	F	F	F	***8***
C21:0	F	F	F	NF	NF	F	NF	NF	***4***
C22:0	F	F	F	F	F	F	F	F	***8***
C23:0	F	F	F	NF	NF	NF	F	F	***5***
C24:0	F	F	F	F	F	F	F	F	***8***
C25:0	F	F	F	NF	NF	NF	NF	F	***4***
C26:0	F	F	F	F	F	F	F	F	***8***
C27:0	NF	NF	NF	F	NF	NF	NF	F	***2***
C28:0	F	F	F	NF	NF	NF	NF	NF	***3***
C30:0	F	F	NF	NF	NF	NF	NF	NF	***2***
	M 10–20	**G 10–20**	**YSO 10–20**	**YSC 10–20**	**OSO 10–20**	**OSC 10–20**	**YFO 10–20**	**YFC 10–20**	**Total**
C16:1	F	F	F	F	F	F	NF	F	***7***
C18:1	F	F	F	F	F	F	F	F	***8***
C16:0	F	F	F	F	F	F	F	F	***8***
C17:0	F	F	F	F	NF	F	F	F	***7***
C18:0	F	F	F	F	F	F	F	F	***8***
C19:0	F	F	F	F	NF	F	F	F	***7***
C20:0	F	F	F	F	F	F	F	F	***8***
C21:0	NF	F	NF	F	NF	NF	NF	F	***3***
C22:0	F	F	F	F	F	F	F	F	***8***
C23:0	F	F	F	F	NF	F	F	F	***7***
C24:0	F	F	F	F	F	F	F	F	***8***
C25:0	NF	NF	NF	NF	F	F	NF	F	***3***
C26:0	F	F	F	F	F	F	NF	F	***7***
C27:0	F	NF	F	F	F	F	NF	F	***6***
C28:0	NF	NF	F	NF	NF	NF	NF	NF	***1***
C30:0	F	NF	NF	NF	NF	NF	NF	NF	***1***
	M 20–30	**G 20–30**	**YSO 20–30**	**YSC 20–30**	**OSO 20–30**	**OSC 20–30**	**YFO 20–30**	**YFC 20–30**	**Total**
C16:1	F	F	F	F	F	F	F	F	***8***
C18:1	F	F	F	F	F	F	F	F	***8***
C16:0	F	F	F	F	F	F	F	F	***8***
C17:0	F	F	F	F	F	F	F	F	***8***
C18:0	F	F	F	F	F	F	F	F	***8***
C19:0	NF	F	NF	F	NF	F	NF	F	***4***
C20:0	F	F	F	F	F	F	NF	F	***7***
C21:0	NF	NF	NF	F	NF	NF	NF	NF	***1***
C22:0	F	F	F	F	F	F	F	F	***8***
C23:0	F	NF	NF	F	NF	F	NF	F	***4***
C24:0	F	F	F	F	F	F	F	F	***8***
C25:0	NF	NF	NF	NF	NF	NF	NF	NF	***0***
C26:0	F	F	F	F	F	F	NF	F	***7***
C27:0	F	F	F	F	F	F	NF	NF	***6***
C28:0	NF	NF	NF	NF	NF	NF	NF	NF	***0***
C30:0	NF	NF	NF	NF	NF	NF	NF	NF	***0***

Note: Meadows (M), grasslands (G), young shrubland open (YSO), young shrubland closed (YSC), old shrubland open (OSO), old shrubland closed (OSC), young forest open (YFO) and young forest closed (YFC).

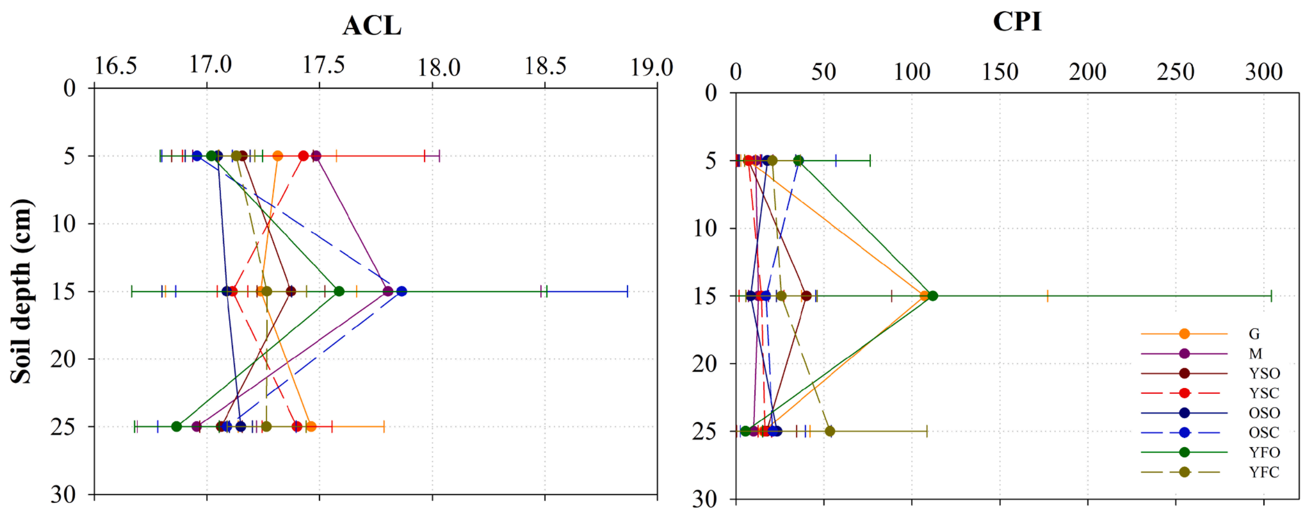

Fig. 5. ACL and CPI values (0–10 cm, 10–20 cm, and 20–30 cm) for the 8 land covers Mean values are presented with a dot and error bars (standard deviation) with lines through the dots, parallel to the X axis. Note: Meadows (M), grasslands (G), young open shrubland (YSO), young closed shrubland (YSC), old open shrubland (OSO), old closed shrubland (OSC), young open forest (YFO) and young closed forest (YFC).

between different LULC and depth for CPI and ACL values (Fig. 5).

4. Discussion

4.1. Landscape analysis

Shrub expansion after agricultural land abandonment is a worldwide phenomenon, particularly noticeable in Mediterranean mountain areas (e.g., MacDonald et al., 2000; Bracchetti et al., 2012; Peña-Angulo et al., 2019; García-Ruiz et al., 2020). In our study area in the Central Pyrenees, the landscape become less fragmented since agricultural land abandonment, with fewer patches dominating the landscape as a result of plant colonization and maturation of tree communities. The comparison between LULC of 1956 and 2015 showed an increase of forest (3.6x) at the expenses of shrublands and agricultural areas. The landscape structure analysis indicated a decrease in the Shannon's Diversity Index and Shannon's Evenness indexes (from 1.409 to 1.309 and 0.786 to 0.700, respectively) and an increase in the Dominance from 0.383 to 0.483. These landscape changes affect natural hazards and different ecosystem services: (i) soil carbon sequestration (e.g., De Baets et al., 2013; Lasanta et al., 2020b); (ii) quantity and quality of water resources (e.g., García-Ruiz et al., 2011); (iii) pastoral resources (e.g., Gartzia et al., 2016; Lasanta et al., 2019), (iv) soil conservation (e.g., Boix-Fayos et al., 2020), (v) biodiversity (e.g., Otero et al., 2015), (vi) frequency of fires, due to the increase in vegetal biomass, and consequently, increase fire risk and the extension of fires (e.g., Ursino and Romano, 2014), and (vi) touristic activity and cultural services (e.g., Bernués et al., 2014).

Related to soil organic carbon sequestration, landscape structure is well known as one of the main factors controlling the spatial distribution of SOC. However, few studies have quantified the relative importance of the spatial variability of SOC related to the landscape characteristics (Stevens et al., 2015). Landscapes can determine the lateral movements of SOC, through the spatial distribution of LULC (landscape configuration), topography, runoff and soil erosion, creating a complex structure of LULCs that accumulate or export SOC (e.g., Nadeu et al., 2015). In that sense, some studies conclude that in small and homogeneous catchments, runoff controls the temporal variability of dissolved SOC, while in the large and heterogeneous catchments, the combination of hydrological mechanisms and the main landscape elements (as LULC, ravines, wet areas…) condition SOC movement (Laudon et al., 2011). Therefore, the landscape homogenization in the Bestué Valley, since the middle of the 20th century, resulted in the loss of LULCs, lowering the relevance of LULC in regard to the spatial distribution of SOC. However, Doetterl et al. (2016) suggested that preliminary studies should be carried out to understand the role of landscape structure in the dynamics and spatial redistribution of SOC. This is necessary in future research to advance our knowledge of small-scale processes (particle, aggregates…), enabling the integration of lateral fluxes of SOC in global models. Thus, in this study, the role of LULC (landscape composition) in SOC has been studied, but further research would be needed to investigate the effects of landscape configuration in SOC and lateral fluxes.

4.2. SOC and N dynamics

It has been demonstrated that LULCC, agricultural land abandonment and woody encroachment influence soil properties and SOC and N dynamics in Mediterranean mountain areas, especially in the topsoil layers (e.g., Lizaga et al., 2019; Lasanta et al., 2020a). Nevertheless, different results have also been found in previous studies (Li et al., 2016; Hunziker et al., 2017). For example, Montané et al. (2007) and Gosheva et al. (2017) showed that SOC stocks do not increase after secondary succession; while other studies showed an increase in SOC linked to revegetation (e.g., van Hall et al., 2017; Bell et al., 2021). Most of the studies indicated higher inputs of organic matter, but a slow process after agricultural land abandonment: during the early stages, a first decline of SOC in the mineral soil normally occurs followed by an increase of SOC with time (long-term changes) (Casals et al., 2004; Lasanta et al., 2020b). In Bestué, the decreasing order of SOC stocks related to LULC is: M > YFC > OSO> OSC > G > YFO > YSC > YSO. Thus, young shrublands (YSO and YSC), as an early stage after agricultural land abandonment, showed the lowest SOC stocks, due to the short time after abandonment, the less mature plants, and a minimal litter contribution.

The high SOC values as recorded in meadows and grasslands in the Central Pyrenees (Figs. 3 and 4) should also be noted. The relevance of these LULCs regarding SOC storage is not new in Mediterranean mountain areas (e.g. Casals et al., 2004; Nadal-Romero et al., 2018). The high values found can be related to belowground biomass throughout the roots. Roots biomass, structure and distribution could be the reason why the upper layer of meadows and grasslands showed higher concentrations compared to the young forest sites (e.g. Wang et al., 2021). Likewise, some differences related to land management, and mowing and grazing intensity, influence inputs and SOC dynamics in grassland areas. Mowing hinders senescence processes in meadows and affects not only the quality of leaf litter, but also the quantity, which is reduced after the harvest. On the other hand, differences in fertilization types - animal faeces or synthetic fertilizers - can influence SOC and N dynamics in these sites. Livestock dung, which was broadly present in grassland and meadows sites, constitutes a very important source of OM (Jarvis et al., 1996) and provides a source of labile carbon that tends to increase microbial biomass (Lovell and Jarvis, 1996).

Related to nitrogen concentrations and stocks, significant differences were also observed. Taking into account the soil profile, meadows had the highest N stocks followed in descending order by YFC > OSO > OSC > G > YFC > YFO > YSO. The significantly higher contents of N in the meadows, grasslands and forests in the different soil depths, compared to the ones obtained in the young shrublands suggest a greater capacity to fix nitrogen in these sites due to the higher presence of leguminous vegetation. This fact is linked to the growth of herbaceous vegetation with high N fixing capacity. On the contrary, when shrub vegetation increases at the first stages, the closed canopy inhibits light from reaching the surface and therefore limits grass growth, and this process could be responsible for reducing SOC and N stocks in shrubland sites.

4.3. Soil organic matter composition

The Corg/N ratio points out the degradation rate of soil organic matter (Swangjang, 2015). Our results showed that low Corg/N ratios were found on grasslands, meadows and the young shrublands, meaning higher decomposition rates. Also, higher Corg/N ratios in the upper soil layer of shrublands than in grasslands was explained by slower decomposition of the respective litter input, as has been also stated in García-Pausas et al. (2017). Forests and old shrublands had Corg/N ratios >10 in the top layer, thus implying that the SOM is probably of plant-derived origin.

Surprisingly, there were no lignins identified in any of the soil samples (except in litter samples). This could be related to important decomposition mechanisms, suggesting that all lignins are decomposed rapidly once they are incorporated into the soil. While lignins have long been regarded as recalcitrant components, our observation is in line with several other studies who found preferential degradation of lignin as reviewed by Thevenot et al. (2010). It is also in line with the conclusions of Schmidt et al. (2011) that SOM degradation is an ecosystem property and it is the specific local conditions that determine degradation rates of various compound classes rather than intrinsic molecular recalcitrance.

Our study showed a large proportion of FAs, but as other studies have already indicated, the selective preservation of lipids is unlikely to be attributed to differences in SOM input related to the vegetation (Nierop et al., 2007; Yang et al., 2020). Our study showed that FAs with a carbon chain length of 16 (C16:0) were the most abundant, followed by compounds with a carbon chain length of 18 (C18:0). Most of the FAs were saturated except for C16:1 and C18:1. The omnipresence of the two unsaturated FAs, which are predominantly microbial-derived, justify the conclusion that there is significant microbial input in the soils under study (Angst et al., 2016). Similar results were observed in Wiesenberg et al. (2012) and Yang et al. (2020).

The least abundant FAs are the longer-chain C25:0, C28:0 and C30:0, which were completely absent from the deepest layers (below 20 cm). Meadows samples are the most fatty acid-enriched samples, containing all of them. Tsutsuki and Kondo (1997) indicated that FAs with longer chain lengths are more stable than those with a shorter chain length. Thus, when plant biomass is degraded, long-chain fatty acids are better preserved than short-chain fatty acids. This leads to the conclusion that the limited presence of long-chain fatty acids implies the presence of a difference in decomposition mechanisms between the meadows and grasslands, where the organic matter could be also plant-derived non-degraded, and the other LULCs.

No significant differences were obtained between LULC and depths related to CPI and ACL indexes. However, two statements can be confirmed. The first one is that the CPI values obtained obtained in grasslands, meadows and young shrublands support a plant-derived origin of OM. This result was also confirmed by Rumpel et al. (2009), stating that SOM of grassland sites are primarily made up of materials that come from vegetation and have not been decomposed yet. The second statement is related to the range of the ACL values, that suggest that there is a strong microbial presence (also observed in Wiesenberg et al., 2012) and reworking of organic matter by microorganisms.

4.4. Implications for the management of abandoned agricultural lands in Mediterranean areas

Abandoned agricultural lands occupy a large extension in the Mediterranean mountain area, and it is foreseen that agricultural land abandoned will continue over the next decades. These abandoned agricultural areas experience a natural revegetation process that affects the provision of ecosystem services to society. In this study, we have found that natural revegetation processes produce, during the first decades, a decrease in SOC and N stock in the soil compared to the meadow areas, and changes in soil organic matter composition. Only when the forest stage is reached are the contents and stocks similar to the meadows. These results suggest that post-abandoned agricultural land management practices should be focused on the conservation of meadows, grasslands and forests, trying to limit the period in which shrublands predominate in the fields. Nonetheless, in some Mediterranean mountain areas, the shrubland stage is very long (between 60 and 100 years) due to natural limiting factors (i.e. climate, soil) or due to the low soil fertility conditions after many years of agricultural activity. In these cases, some managers remove shrublands in selected areas to favor the regeneration of pastures (García-Ruiz et al., 2020), and in that way, favor environmental (i.e. decrease forest fires) and socioeconomic (i.e. increase extensive livestock) services. The final idea is to create a mosaic landscape, with grasslands but also with forest patches in the areas with the optimum conditions.

5. Conclusions

This study has provided novel information on the effects of agricultural land abandonment and subsequent woody encroachment on soil organic carbon and nitrogen contents and stocks, and soil organic matter composition in a mountain Mediterranean area (Central Pyrenees, Spain). Our results support our initial hypothesis that agricultural land abandonment and land use and land cover changes have a significant influence, especially in the topsoil layer, on SOC and N dynamics and stocks, and that SOC storage is a slow-process after abandonment.

Landscape analysis showed a positive evolution and maturation of woody vegetation in the study area, as a result of the abandonment of agriculture activities during the last decades. The main land cover changes are the multiplication (3.5x) of forest cover at the expense of shrublands, and to a lesser extent of agricultural fields (with a decrease of 50% of shrublands cover, and the disappearance of 75% of the agricultural land). Thus, the landscape becomes less dominated by land cover patches, decreasing Shannon's Diversity and Shannon's evenness indexes, and increasing the Dominance index.

Our results also confirm that SOC accumulation after agricultural land abandonment is a slow process and that the first stages of secondary succession or woody encroachment seem to be less productive in terms of SOC. Significant differences with regard to soil profiles were observed between meadows and YFC and the first stages of land abandonment. In that sense, time is demonstrated to be a key element to consider when studying and predicting SOC storage after land abandonment. Our results also confirmed the relevance of grasslands and meadows related to SOC storage, as it has already been shown in other Mediterranean mountain areas.

The THAM-Pyrolysis-GC–Ms analysis for the soils under study revealed large relative abundances of FAs, with a major contribution of saturated FAs. Lignins were completely absent from all soil layers, and were only found in litter samples, thus implying the presence of a strong decomposition mechanism for this compound class. Unsaturated (C16:1, C18:1) and shorter-chain saturated fatty acids were omnipresent (confirming high microbial activity), and differences between meadows and grasslands and the other LULCs were observed. These results are also validated by the CPI and ACL indexes.

This study suggests that agricultural land abandonment processes and woody encroachment should be managed through adequate post-land abandonment management practices, ensuring the proper functioning of ecosystem services (such as water resources and soil), and promoting organic carbon storage and soil quality in Mediterranean mountain soils.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.catena.2021.105441.

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