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RESEARCH ARTICLE

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Carbon content of forest floor and mineral soil in Mediterranean *Pinus* spp. and Oak stands in acid soils in Northern Spain

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Abstract

Aim of study: The aim of the study was to determine the baseline carbon stock in forest floor and mineral soils in pine and oak stands in acid soils in Northern Spain.

Area of study: The study area is situated in northern Spain (42° N, 4° W) on "Paramos y Valles" region of Palencia.

Material and methods: An extensive monitoring composed of 48 plots (31 in pine and 17 in oak stands) was carried out. Litter layers and mineral soil samples, at depths of 0-30 cm and 30-60 cm, were taken in each plot. An intensive monitoring was also performed by sampling 12 of these 48 plots selected taken in account species forest composition and their stand development stage. Microbial biomass C (C_{MB}), C mineralization (C_{RB}), and soil organic C balance at stand level were determined in surface soil samples of intensive monitoring.

Main results: No differences in soil C content were detected in the two forest ecosystems up to 60 cm depth (53.0±25.8 Mg C ha^{-1} in *Pinus* spp. plantations and 60.3±43.8 Mg C ha^{-1} in oak stands). However, differences in total C (C_T), C_{MB} and C_{RB} were found in the upper 10 cm of the soils depending on the stand development stage in each species forest composition (*Pinus nigra*, *Pinus pinaster*, *Pinus sylvestris* and *Quercus pyrenaica*). Plots with high development stage exhibited significant lower metabolic quotient (qCO_2), so, meant more efficient utilization of C by the microbial community. The C content in the forest floor was higher in pine stands (13.7±0.9 Mg C ha^{-1}) than in oak stands (5.4±0.7 Mg C ha^{-1}). A greater turnover time was found in pine ecosystems *vs.* oak stands. In contrast, forest floor H layer was nonexistent in oak stands.

Research highlights: Results about litterfall, forest floor and mineral soil dynamics in this paper can be used strategically to reach environmental goals in new afforestation programs and sustainable forest management approaches.

Keywords: C stocks; pine; *Quercus pyrenaica*; litter; metabolic quotient (qCO_2).

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Introduction

Soil organic carbon (SOC) is the largest terrestrial pool of carbon and, at least, three times larger than the pool of atmospheric CO_2 (Jobbágy & Jackson, 2000). All signatory countries in the United Nations Framework Convention on Climate Change have to implement a national system for reporting carbon stock changes in the agriculture, forests and other land uses sector (Schulp *et al.*, 2008). As part of such a system, countries must quantify the size, spatial distribution and changes to their soil organic carbon (SOC) stocks. Understanding the mechanisms and factors of SOC dynamics in forest soils is important for identifying and enhancing natural sinks for C sequestration to mitigate climate change effects (Lal, 2005).

Carbon storage in forest soils is influenced by soil properties (Zou *et al.,* 2005), climate (Turrión *et al.,* 2009) and topography (Vande Walle *et al.,* 2001). In addition, forest species composition (Oostra *et al.,* 2006),

stand development stage (Turner & Lamber, 2008), land use (Oliver *et al.,* 2004), litter production and decomposition (Kavvadias *et al.,* 2001), disturbances and silviculture or forest management (Oostra *et al.,* 2006; Jandl *et al.,* 2007) play an important role in SOC balance (Lal, 2005). On the other hand, forest floor is an essential component in the relations among soil and vegetation in the wooded ecosystem and litterfall is a principal pathway for the return of nutrients to the soil (Smith *et al.,* 2015). It has been shown that the amount of litter accumulated in the forest floor is influenced by the nutrient capital of the stand as well as the decomposition rate of the litterfall. As the soil C pool is mostly determined by the balance between C input by litterfall and rhizodeposition and the release of C during decomposition; this part of the C cycling must also be taken into account.

Inventories of SOC provide suitable data at different details levels depending on the research objectives and resources. So, while assessing SOC at large scale enables us to define carbon levels considering all the spatial variability, estimating SOC stock at stand level is also necessary to obtain relevant information about the parameters affecting C storage and to adopt appropriate soil management practices. In this sense, studying microbial and C mineralization fractions allowed us to assess the microbial soil activity and to monitor soil changes in a relatively short term (Powlson *et al.,* 1987). Soil microbial biomass carbon (C_{MB}) and the specific respiratory activity of soil microbial biomass (C_{RB}) are sensitive to changes in the quantity and quality of soil organic matter and ecosystem stability (Insam, 1990). The metabolic quotient (qCO_2) indicates the energy requirements of soil microorganisms (Anderson, 2003), the level of soil microbial stress (Wardle & Ghani, 1995) and the efficiency of soil microbial populations for substrate utilization. Along with the ratio between the soil microbial biomass C and the total C (C_{MB}/C_T), the metabolic quotient reflects organic matter input and availability in the soils, efficiency of conversion to microbial C, C losses from soil, C stabilization by the mineral fractions and maintenance requirements of the soil microbial community (von Lutzow *et al.,* 2002; Turrión *et al.,* 2012).

Knowing the soil cycling and the actual capacity of C sequestration of the forest ecosystems would be necessary for calculating the maximum amount of C that is potentially able to return from the trees to the soil. In Spain, there have been three attempts to estimate SOC stocks for the whole country, conducted by Rodríguez-Murillo (2001), Chiti *et al.* (2012) and Doblas-Miranda *et al.* (2013). Although these studies presented an assessment of SOC stocks in forests, shrublands and grasslands of Peninsular Spain based on field measurements in a high amount of soil profiles, in general, no data from forest soils of "Paramos y Valles" Region in Northern Spain were considered. On the other hand, in those studies three types of forests were considered (conifer, broadleaf and evergreen broadleaf forests), however, the species composition was not taken account. Finally, litter layers were not included in the mentioned analyses.

Knowing the actual C stocks and the dynamics on different forests ecosystems in a region combined with the information on spatial distribution of these ecosystems can be used to improve insight in spatial distribution of SOC stocks under forest land use systems and to estimate the potential capacity of C sequestration of forest soils. Differences in SOC stocks between tree species could give an indication of the effects of future management changes, which can have a strong impact on SOC sequestration. In general, soils reverted to natural ecosystems and under climax vegetation have more soil organic carbon contents than those under managed ecosystems (Lal *et al.,* 1995). Our hypothesis was that the oak stands would show more soil C content because they constituted the climax vegetation in this region. Additionally, tree species are expected to differ in mitigation potential (Schulp *et al.*, 2008). This knowledge may help the manager's choice of in case of afforestation /reforestation with the aim of enhancing soil C stock (Lal, 2005).

The objectives of this paper were:

A) to quantify the C content of forest floor and mineral soil under four Mediterranean pine species (*Pinus pinaster* Ait., *Pinus sylvestris* L., *Pinus nigra* Arn.) and under oak ecosystems (*Quercus pyrenaica* Willd.) in acid soils in Northern Spain.

B) to determine the microbiological factors most directly related to the C fixation in the studied soils.

C) to estimate the balance of the soil carbon stock, considering litterfall and organic matter decomposition.

Material and methods

Site description

The study area is situated in northern Spain (42° N, 4° W) on "Paramos y Valles" region of Palencia (Figure 1). Geomorfologically is a wide platform, commonly denominated "acidic plateau", with mean slope of 3% dismembered in a series of tentacles by the erosive action of the current fluvial net. Altitude ranges from 800 to 1000 m asl, while climate can be classified as Humid Temperate Mediterranean. Mean annual temperature is 10.3ºC and mean annual rainfall is 630 mm (mean summer precipitation equal to 107 mm). The soils can be classified mainly as Cambisols and Regosols (JCyL, 2013).

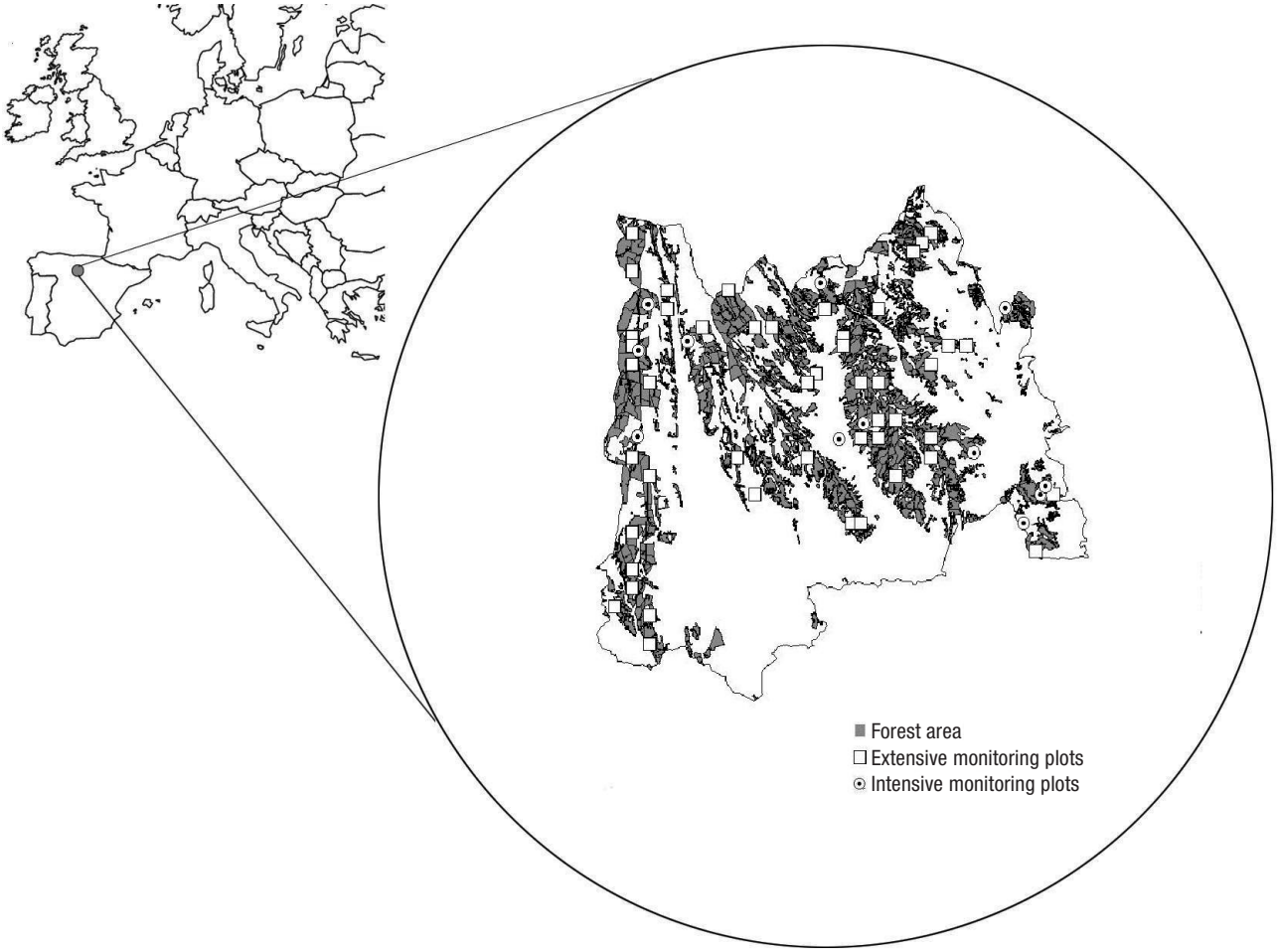

Figure 1. Location of the study area and the sampled plots of both inventories (extensive and intensive monitoring) at the "Paramos y Valles" region, north of Palencia (Spain).

Forests cover 61,570.5 ha (33.0% of total area). The main natural forest types are extensive stands of pyrenean oak (*Quercus pyrenaica* Willd.), holm oak (*Quercus ilex* L.) and portuguese oak (*Quercus faginea* Lam.). As a result of an extensive pine plantation program carried out mostly during the 1960's in non-arable lands, *Pinus* stands cover 41.5% of the total forest area. These are young and middle-aged plantations (around 30-60 years old) of *Pinus sylvestris* L. (23%), *Pinus nigra* Arn. (21%) and *Pinus pinaster* Ait. (5%). The plantations are mainly a mixture of the three species, although there are also pure stands. Isolate gaps can be found inside these plantations where mosaics of heather (*Erica* spp.) and rock roses (*Cistus* spp.) are present.

Sampling procedures

Two different soil sampling procedures, extensive and intensive monitoring (Figure 1), were carried out in order to attain the objectives.

Extensive monitoring

A total of 48 plots (circular plot of radii = 15 m) were selected on the systematic grid from the National Forest Inventory with an intensity of one plot per 2 km, taking the species composition into account (Forsee, 2005). In the region, stands established at the same time, with homogeneous site conditions and similar land use history were sampled. There were 31 plots established on *Pinus* spp. plantations and 17 on oak stands. The main characteristics of the plots are shown in Table 1.

Mineral soil samples were taken in each plot at depths of 0-30 cm and 30-60 cm, as Forsee project established in its protocol (Forsee, 2005). Each plot was divided in four parts, and from 6 to 8 individual randomized samples were obtained for each part. The forest floor accumulated on the ground above the mineral soil was sampled at each point. This was carried out by using a 900 cm^2 square sampling frame and collecting all organic material without distinguishing

Table 1. Main characteristics of the extensive monitored plots.

Forest stands	n	trees ha^{-1}	BA (m^2 ha^{-1})	QMD (cm)	Ho (m)
Pinus spp.	31	803±344	23.2±8.3	22.2±13.2	9.92±3.81
Oak stands	17	457±501	6.6±8.4	11.6± 8.1	5.12±3.87

Where BA is the stand Basal Area; QMD is quadratic mean diameter; Ho is dominant height (following Asmann definition); n: number of plots. NFI consider as minimum diameter 7.5 cm as DBH to be recorded.

among different layers. Both soil and forest floor samples were mixed, homogenized and composited to form one sample of each type per plot (forest floor, 0-30 cm mineral soil and 30-60 cm mineral soil). Samples were transported to the laboratory. Mineral soil samples were air-dried, sieved and % of coarse soil materials ([Ø > 2 mm]) was calculated.

Intensive monitoring

To obtain a better understanding of the factors most directly related to C content on forest floor and mineral soil, twelve of these 48 plots were selected for an intensive monitoring. These points were chosen taking into account two criteria: the forest species composition and stand development stage to consider the different stand situations. Species composition was described by dominant species: *P. nigra, P. pinaster, P. sylvestris* and *Q. pyrenaica* (target species basal area ratio higher or equal to 90%). The stand development stage was determined by the dominant tree height and other stand characteristics like stem vigor and quality, tree density and the intensity of the silvicultural treatments applied. In each species composition category, there were three stages of stand development: high, medium and low. High stage was characterized by the best conditions in stand quality and tree size (higher dominant height and higher vigor and quality of the trees, because of a higher intensity of silvicultural treatments). In the opposite case, low stage, was the stage where the worst stand and tree quality characteristics were found. Medium stage is the intermediate level between high and low. So, one plot by each species (n=4) and stand development status (n=3) were considered in this monitoring (n=12, Table 2). A soil profile was opened in each intensive monitoring plot. Soil horizons were described and soils were classified (WRB, 2006).

In each intensive monitoring plot, floor layers and 0-10 cm mineral topsoil were sampled. Five sampling points were established on each plot, located in the center of the plot and at a distance of 5 m to North, South, East and West directions. In these points the different organic horizons of the forest floor (undecomposed litter fraction, L; fragmented fraction, F; and humified fraction, H) were differentiated and collected on an area basis, using a 30 cm x 30 cm wooden frame. The samples were mixed, homogenized and composited to form one sample per plot and type of horizon.

Litter traps and decomposition litter bags were installed. Litter traps were installed to assess annual litterfall dynamics. Only litterfall was collected. On the other hand, within each plot, three square 30 cm x

Table 2. Main characteristics of the intensive monitored plots (n =12) in the study area.

Plot	Forest stands	Stand dev.	Lith.	Soil type	trees ha^{-1}	BA (m^2 ha^{-1})	QMD (cm)	Ho (m)	Age (years)
1	*P. nigra*	High	23	CMca	1367	40.8	19.49	14.4	36
2	*P. nigra*	Medium	27	CMdy	1833	40.0	16.67	11.1	24
3	*P. nigra*	Low	27	CMdy	689	21.0	19.68	7.5	33
4	*P. pinaster*	High	27	CMdy	478	38.6	32.09	15.2	44
5	*P. pinaster*	Medium	27	CMdy	689	36.7	26.05	13.1	28
6	*P. pinaster*	Low	33	RGdy	667	29.3	23.65	12.7	41
7	*P. sylvestris*	High	30	CMdy	733	34.0	24.29	15.9	32
8	*P. sylvestris*	Medium	27	CMdy	1100	37.4	20.81	13.2	35
9	*P. sylvestris*	Low	27	CMdy	678	31.0	24.14	6.5	41
10	*Q. pyrenaica*	High	27	CMdy	322	13.6	23.21	12.1	88
11	*Q. pyrenaica*	Medium	27	CMdy	778	24.0	19.82	9.8	51
12	*Q. pyrenaica*	Low	30	CMdy	677	2.0	6.11	4.0	14

Where Stand dev. is the stand development stage; Lith is lithology type by geologic map (JCyL, 1995) [23: detritic and carbonate sediments (*Garumnian facies*), 27: conglomerates and microconglomerates, 30: River texture edges, sands, clays and silts and 33: eolic sands]; Soil type (WRB, 2006) [CMca: Calcaric Cambisol, CMdy: Dystric Cambisol, RGdy: Dystric Regosol]; BA is the stand Basal Area; QMD is quadratic mean diameter; Ho is dominant height (following Asmann definition); Age is the stand age.

30 cm litter traps placed randomly under the canopy, to take into account spatial variability at the plot level. The traps were established in May 2008 and were collected every two months throughout the entire year (5 times). The sampling ended in May 2009. The total trap capacity was extracted each time, weighted and dried until constant weight in the laboratory. On the other hand, to estimate the decomposition litter rate, foliar litter bags were installed in the each plot. A total of 144 litter bags were prepared using polyvinyl screen mesh (0.5 mm in the bottom and 2 mm in the top) of approximately 25 cm × 25 cm. In each plot, air-dried leaf litter (7 g), obtained previously from the upper soil in each species composition plot, was put into the bags. Three 3 litter bags were extracted from each plot every 2 months after the start of the decomposition study (the same 5 times).

Contents of litter traps, decomposition litter bags and organic horizon samples were oven-dried at 70ºC, weighed in the laboratory and an aliquot was ground by a mill to plant samples.

Analyses procedures

Physical and chemical soil properties

Some physical and chemical soil properties (bulk density, texture, pH, cation exchangeable capacity, Ca, Mg, K and Na exchangeable cation concentrations, sum of bases total C, total N and C/N) were determined in mineral soil samples of extensive monitoring. Bulk density was measured through the core method (Blake & Hartge, 1986) in the field with volumetric steel rings and soil dry weight. Texture was determined by Bouyoucos Hydrometer Method (MAPA, 1994), pH (soil:solution ratio of 1:2.5) was measured using a pH-meter. Cation exchange capacity (CEC) was determined by the Bascomb method (Bascomb, 1964). Exchangeable cations were extracted with 0.1 M $BaCl_2$, Ca and Mg were determined by atomic absorption spectrophotometry and K and Na by emission spectrophotometry (MAPA, 1994). The sum of the bases (SB) was calculated as sum of Ca, Mg, K and Na exchangeable. Soil organic matter was analyzed by the Walkey and Black method. Soil total C concentration was determined multiplying easily oxidable carbon concentration with a factor of 1.3 (MAPA, 1994). Total N was analysed by Kjeldahl method (Jones *et al.,* 1991). The C/N relationship was calculated as the ratio between soil C and total N. Accumulated SOC in each depth was calculated taking into account C concentration, bulk density, their thickness and the percentage of gravels.

Microbiological soil analyses

Microbiological soil analyses were carried out in topsoil samples from the intensive monitoring.

Microbial biomass C (C_{MB}) was determined by the fumigation-extraction procedure of Vance *et al.* (1987). Before extracting the microbial biomass, air dried soil (< 2 mm) was incubated at 60% field-capacity and room temperature for one week. Chloroform fumigation of the soils (24h) released microbial cytoplasm into the soil environment. After subsequently extracting the cell material with 0.5 M K_2SO_4 from fumigated and non-fumigated samples, total organic carbon was quantified as a reference for the microbial biomass. The extracts were analyzed for organic C by using SKALAR TOC/TN automatic analyzer. The microbial biomass was calculated by biomass C = EC / k_{EC}, where EC is the organic C extracted from fumigated soil minus that extracted from non-fumigated soil, and k_{EC} is the extractable fraction of microbial biomass C after fumigation. A k_{EC} value of 0.38, recommended by Joergensen *et al.* (1995), was used.

Potential soil respiration (C_{RB}) was determined in closed jars and under laboratory-controlled conditions following the Isermeyer method (Alef, 1995). Soil samples were wetted to 60% of water holding capacity and incubated in 1 L jars at 29ºC for 3 days. Evolved CO_2 was trapped in 0.5 M NaOH and it was determined by means of back-titration of the remaining NaOH with HCl.

The metabolic quotient (qCO_2) represents the potential soil respiration per unit microbial biomass, and was calculated as the quotient between C_{RB} and C_{MB}. Microbial quotient (C_{MB}/C_T) represented the fraction of microbial biomass C with respect to the total soil organic C (Anderson & Domsch, 1993).

All results were obtained by triplicate determinations and were expressed on the basis of oven-dry weight soil.

Organic horizons, litter pool and topsoil analysis

In all samples from intensive monitoring (organic horizons, litterfall from traps, litter bags and upper mineral soils), total C and total N were determined by dry combustion using the automated C/N analyzer (CHN-2000 LECO).

Statistical analyses

A Linear Mixed Model (LMM) with a between-subjects factor (forest ecosystem, with two levels *Pinus*

spp and oak) and a within-subjects factor (depth, with two levels, 0-30cm and 30-60cm) and their interaction, was applied to the soil parameters in the extensive inventory. When the effect studied turned out to be significant, differences among levels were evaluated using the Tukey test.

In the intensive inventory, a LMM with two between-subjects factors and their interaction was fitted to assess the differences in the C fractions of the topsoil and forest floor layers. The factors considered in the model were the species forest composition with four levels: *Pinus pinaster, Pinus sylvestris, Pinus nigra,* and *Quercus pyrenaica*, and the stand development stage with three levels: high, medium and low. So, the total effects were 12. When the effect studied turned out to be significant, differences among levels were evaluated using the Tukey test. All the statistical analyses were performed using Proc MIXED from SAS 9.1 (SAS Institute Inc., 2010).

A descriptive study was carried out to ascertain the annual fluxes of inputs and decomposition of litter in forest ecosystems. Differences in the parameters litterfall amount, C concentration and C/N relationship were evaluated among forest ecosystems and sampling time through Tukey's procedure by Proc GLM from SAS 9.1 (SAS Institute Inc., 2010).

Decomposition litter rate constants (*k,* in year^{-1}) were calculated from the different sampling time data by using Equation 1 [Eq. 1]

$$X = X_0\, e^{-kt} \qquad [1]$$

where Xo is the initial weight of needles in the litter bags, X is the weight of needles after time t in years and *k* is the decomposition rate constant (Olson, 1963).

Results

General soil parameters

In Table 3 some physical and chemicals properties of the studied soils by depths (0-30 cm) and (30-60 cm) and forest ecosystems (*Pinus* spp. and oaks) are shown. So, in oak forest ecosystems, significant higher values of clay content were found in the second depth [30-60 cm] than in the upper depth. Also, between depths, in both forest ecosystems (*Pinus* spp. and oak stands), significant higher values were found in 0-30 cm respect to 30-60 cm in exchangeable K, C_T and N concentrations. However, in oak stands, smaller values were found in CEC and C/N in the upper depth in comparison to 30-60 cm depth. On the other hand, between forest ecosystems, in the upper depth, while significant smaller values were found in pH, CEC, exchangeable K and Mg, SB and N concentration in *Pinus* spp. than in oak stands, a significant higher value of C/N was found at this depth in *Pinus* spp. In contrast, at 30-60 cm of depth, all the significant values were smaller in *Pinus* spp. than in oak stands (clay content, pH, CEC, exchangeable K and Mg, and SB). The interaction depth*forest ecosystems was significant only in Ca concentration. In this cation, in the second depth, significant higher values were found in oak than in *Pinus* spp. forests, while in the upper depth the values were similar.

Finally, the soil C content did not present significant differences by depth and by forest ecosystems, either by the interaction between depth and forest ecosystems. Soil C accumulation was 53.0 (±25.8) Mg C ha^{-1} in *Pinus* spp. plantations and 60.3 (±43.7) Mg C ha^{-1} in oak stands (Table 3).

Table 3. Physical and Chemicals properties of the soils by depths and forest ecosystems.

Depth (cm)	Forest ecosystem	Bulk density (g cm^{-3})	Clay (%)	pH	CEC (cmol$_c$ kg^{-1})	Exchangeable cations (cmol$_c$ kg^{-1})				SB (cmol$_c$ kg^{-1})	C (g kg^{-1})	C (Mg ha^{-1})	N (g kg^{-1})	C/N
						K	Ca	Mg	Na					
0-30	*Pinus* spp.	1.10 aA	9.1 aA	5.4 aA	12.00 aA	0.35 aA	2.62 aA	0.52 aA	0.04 aA	3.52 aA	21.0 aA	30.0 aA	0.9 aA	23.6 aA
	Oak stands	1.09 aA	12.8 aA	5.8 aB	13.92 aB	0.61 aB	4.02 aA	0.73 aB	0.04 aA	6.68 aB	21.1 aA	38.1 aA	1.2 aB	17.7 bA
30-60	*Pinus* spp.	1.25 aA	11.1 aA	5.4 aA	10.63 aA	0.25 bA	1.89 aA	0.33 bA	0.04 aA	2.50 aA	14.0 bA	22.9 aA	0.6 bA	25.0 aA
	Oak stands	1.22 aA	18.8 bB	5.9 aB	14.14 bB	0.43 bB	5.99 aB	0.75 aB	0.04 aA	6.15 aB	13.1 bA	22.2 aA	0.7 bA	18.3 aA
FACTOR:														
Depth		ns	**	ns	ns	**	ns	ns	ns	ns	***	ns	***	ns
Forest ecosystems		ns	***	***	***	***	***	***	ns	***	ns	ns	**	*
Depth* Forest ecosystems		ns	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns

Note: SB: Sum of bases; CEC: cation exchangeable capacity,

Different lower letters showed significant differences in each forest ecosystems between depths at 95% Tukey's test. Upper letters showed significant differences in each depth between forest ecosystems forest at 95% Tukey's test. Significant levels: ***: (p<0.01); **: (p<0.05); *: (p<0.1) ; ns: no significant.

Soil microbial activity and C mineralization

Microbial biomass C represented between 0.56-1.37 % of total C (mean 0.94 ± 0.30 %). Significant differences were found (Table 4) in the interaction between species composition and stand development stage in the C_T, the ratio C_{MB}/C_T , C_{RB}/C_T and the metabolic quotient (qCO_2). So, these variables, in the studied soils, were different in the different species composition depending on the stand development stage considered. In *Pinus nigra* stands, the significant lowest C_T value was found in the high stand development stage plot. In contrast, significant higher C_T values were found in the high development stage stands than in the others for *Pinus sylvestris* and *Quercus pyrenaica* forests. For *Pinus pinaster* significant higher C_T values were found in low development stages in comparison to the other two stages. For the ratio C_{MB}/C_T, different patterns were found. So, no significant differences were found in *Pinus nigra* stands, and significant different values were found in each stand development stage in *Pinus pinaster* stands. In *Pinus sylvestris*, a significant higher value was found in lower stand development stage than in the others, however in *Quercus pyrenaica,* the significant higherst values were found in the highest stand development stage. For the C_{RB}/C_T ratio significantly higher values were found in the high than in the medium and low stand development in *Pinus nigra* plots. For the other forest species the significant lowest C_{RB}/C_T values were found in the plots with high stand development stage. For the qCO_2, no significant differences were found in *Pinus nigra* stands. However, in the other forests, low stand development plots showed significant higher qCO_2 values than high stand development stages.

Forest floor Carbon

The average amount of forest floor was 16.5 Mg ha^{-1}, showing average values of 26.9 (±7.4) Mg ha^{-1} in pine ecosystems and 11.8 (±6.5) Mg ha^{-1} in oak stands. The carbon content in this pool was significantly higher in pine [13.7 (±3.8) Mg C ha^{-1}] than in oak stands [5.4 (±3.0) Mg C ha^{-1}].

The amount and the carbon content in forest floor varied among species composition, stand development stage and the interaction between the two factors (Table 5). Analyzing the different forest floor layers

Table 4. Values of C_T content, C_{MB}/C_T ratio, C_{RB} and metabolic quotient (qCO_2) in the upper 10 cm soil by species forest composition and stand development stage

Species Forest Composition	Stand Development	C_T (gC $100g^{-1}$)		C_{MB}/C_T (gC_{MB} kgC_T^{-1})		C_{RB}/C_T (gC_{RB} kgC_T^{-1})		qCO_2 (gC_{RB} gC_{MB}^{-1})	
Pinus nigra	High	1.32	b	8.42	a	9.15	a	1.11	a
	Medium	2.27	a	6.86	a	5.06	c	1.05	a
	Low	2.55	a	7.33	a	7.21	b	1.05	a
Pinus pinaster	High	2.15	b	11.21	b	4.72	c	0.43	c
	Medium	2.56	b	13.52	a	7.48	a	0.55	b
	Low	3.20	a	8.62	c	6.29	b	0.73	a
Pinus sylvestris	High	4.08	a	5.57	b	1.90	c	0.37	c
	Medium	3.42	b	5.21	b	4.70	b	1.17	a
	Low	3.39	b	12.93	a	9.19	a	0.71	b
Quercus pyrenaica	High	4.37	a	13.66	a	4.29	c	0.32	b
	Medium	2.92	b	11.27	b	10.44	a	0.93	a
	Low	2.72	b	8.74	b	9.44	b	1.22	a
FACTOR									
Species Forest Composition		***		***		***		***	
Stand Development		ns		***		***		***	
Species Forest Composition * Stand Development		***		***		***		***	

Where C_T is the total organic C content; C_{MB} is the soil microbial biomass C and C_{RB} is the biomass respiration C; q_{CO2} is the metabolic quotient (C_{RB}/C_{MB}). Different letters means significant differences among stand development stages within each species forest composition. Significance levels: ***: ($p<0.001$). ns: not significant.

Table 5. Values of C concentration, Carbon content and C/N ratio of the different litter layers (L, F and H) by species forest composition and stand development.

Species Forest Composition	Stand Development	L	F	H	C_L		C_F		C_H		$(C/N)_L$	$(C/N)_F$	$(C/N)_H$
		(Mg ha^{-1})			(g kg^{-1})	(Mg ha^{-1})	(g kg^{-1})	(Mg ha^{-1})	(g kg^{-1})	(Mg ha^{-1})			
P. nigra	High	8.7 a	5.4 a	7.0 a	497 a	4.3 a	464 a	2.4 ac	325 a	2.4 a	139.1 a	84.6 a	53.6 a
	Medium	3.9 bc	4.1 a	9.8 a	497 a	1.9 b	425 a	1.7 c	327 a	3.4 a	113.9 b	54.9 b	45.5 a
	Low	4.9 b	4.9 a	11.0 a	546 b	2.6 a	544 b	2.5 a	425 b	6.5 a	98.4 b	80.0 a	53.1 a
P. pinaster	High	4.2 a	4.5 a	13.8 a	494 a	2.1 a	438 a	1.9 a	223 a	3.6 a	131.3 a	63.8 a	46.5 a
	Medium	7.9 c	4.4 a	9.6 a	497 bc	3.9 b	463 ab	2.0 a	270 ab	2.5 a	100.7 bc	65.7 a	47.0 a
	Low	5.9 b	3.9 a	7.2 a	455 ac	2.7 a	473 b	1.8 a	316 b	2.4 a	129.1 ac	71.4 a	46.5 a
P. sylvestris	High	8.3 a	5.4 a	16.7 a	503 a	4.2 a	466 a	2.5 a	311 a	5.5 ac	98.3 a	60.5 a	47.7 a
	Medium	4.4 b	2.8 b	15.8 a	496 b	2.2 b	470 a	1.3 b	397 c	6.3 c	102.9 a	50 b	38.0 b
	Low	8.2 a	4.2 a	13.4 a	497 b	4.1 a	475 a	2.0 a	273 b	3.6 a	128.6 b	74.2 c	51.2 a
Q. pyrenaica	High	2.6 a	4.5 abc	0.0	473 a	1.2 a	393 a	1.9 a	-	0.0	63.2 a	46 a	-
	Medium	4.1 b	2.3 ac	0.0	436 b	1.7 bc	339 a	0.7 c	-	0.0	48.8 b	46.4 a	-
	Low	4.3 b	4.4 b	0.0	439 b	1.9 b	319 a	1.5 a	-	0.0	51.3 b	46.3 a	-
FACTOR													
Species Forest Composition		***	ns	*	***	***	***	**	***	**	***	***	***
Stand Development		ns	***	ns	**	**	ns	***	**	ns	***	***	***
Species Forest Composition * Stand Development		***	**	ns	***	***	**	**	***	ns	***	***	***

Where L is the amount of undecomposed litter fraction; F is the amount of fragmented fraction and H is the amount of humified fraction; C_L, C_F and C_H are carbon concentration (g kg^{-1}) and amount (Mg ha^{-1}) in L, F and H fractions, respectively; $(C/N)_L$ is the C/N relationship on the L layer; $(C/N)_F$ is the C/N relationship on the F layer; $(C/N)_H$ is the C/N relationship on the H layer. Significance levels: *: ($p<0.05$). **: ($p<0.01$). ***: ($p<0.001$). ns: not significant.

(L, F and H), the mass was substantially greater in the L, H layers than in F layer in most of the plots (Table 5). The C content in H layer showed significantly smaller values in oak stands. In most of the plots of the four species compositions, the carbon concentration ranged in the following order: L>F>H. Within each forest composition, the stand development stage showed different C amounts and contents of L and F layers without a clear tendency, except for H layer. While a higher C was found in L layer in the highest stand development stage for *P. nigra* and *P. sylvestris*, the opposite behavior was found for *P. pinaster* and *Q. pyrenaica*. However, in the different stand development stages of *Pinus* spp. species compositions, similar values were found in H layer. The values of the relation C/N in pine ecosystems (L>98; F>50 and H>38) contrasted to those obtained in oak stands (>46 in L and F layers).

Litterfall and litter decomposition

Figure 2 shows the temporal evolution of C in litterfall at the four forest ecosystems. The average annual litterfall carbon was 8.9 (±1.8) and 2.9 (±0.7) Mg C ha^{-1} year^{-1} in *Pinus* spp. and oak stands, respectively. Foliage peaked in autumn (Figure 2), where more than 50% of the total litter was fallen in that season in the four species compositions. Litterfall showed different values of C/N along the sampling period in the different forest stands (Figure 3). Smaller values were found in oak stands along the year.

The litter bag experiment showed that degradation of dry organic matter decreased along the year in the different forest stands (Figure 4). The models ($p<0.0001$) allowed us to determine the value of the decomposition rate constant k (year^{-1}). The k value was different between *Pinus* spp. and oak stands. They were 0.18 and 0.46 years^{-1} for the dry organic matter in *Pinus* spp. and oak, respectively, and 0.16 and 0.32 years^{-1} for the C content in *Pinus* spp. and oak stands, respectively.

Discussion

In this paper, C stock in forest floor and mineral soils in pine and oak stands in acid soils in Northern Spain has been determined. The study area showed significant

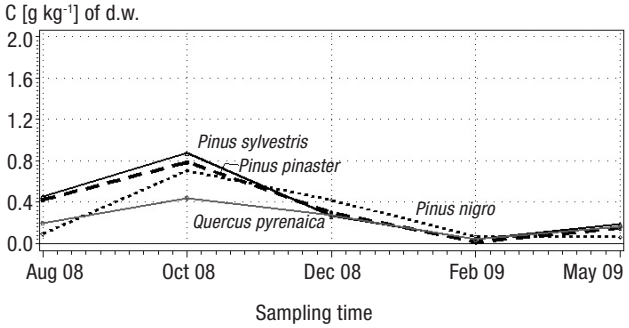

Figure 2. C content (g kg^{-1}) in litterfall picked up by sampling time.

Note: d.w. is dry weight. Aug is August; Oct is October; Dec is December; Feb is February.

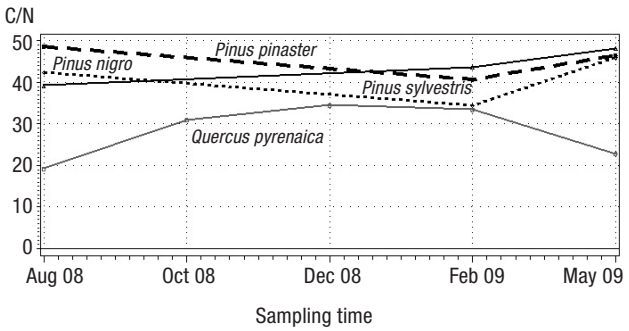

Figure 3. Relation C/N of litterfall picked up in the traps by sampling time in the different species compositions.

Note: Aug is August; Oct is October; Dec is December; Feb is February.

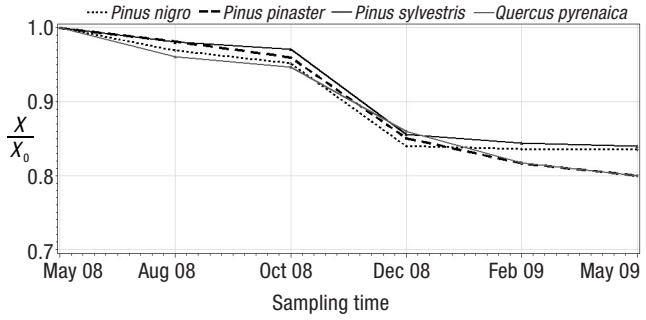

Figure 4. Evolution of the ratio $\frac{X}{X_0}$ by sampling time by species compositions in litter bag experiment.

Note: Aug is August; Oct is October; Dec is December; Feb is February. X is the weight of needles after time; X_0 is the initial weight of needles in the litter bags.

higher clay contents at 30-60 cm depth in oak stands due to the presence of a cambic horizon at this depth. In contrast, in *Pinus* spp., clay contents didn´t show significant differences between depths probably due to mixing of soil horizons when the afforestation was performed. The higher clay contents found in the second depth in oak stands could explain the higher values of CEC found. This fact, join the significant smaller values of C/N in oak stands reflected the higher organic matter quality in this type of forest ecosystems in comparison to *Pinus* spp. stands.

On the other hand, our results showed that similar soil C stock exists underground, while different forest ecosystems grow aboveground. Our hypothesis was that the oak stands would show more soil C content because they constituted the climax vegetation. However, the oak stand degradation causes that this ecosystem accumulates C in a manner similar to middle-aged plantations that are growing and are being managed for wood production. Selvicultural prescriptions to improve the vigor of oak stands would be necessary to increase the C fixation in this type of forest. In addition, the C fixed allowed us to assess the repercussions of pine plantations on soil C in this area. Plantations were established in non-productive lands. In the study area, Mulas *et al.* (2015) assessed C budgets under different land use systems. Their results showed that C concentration in surface horizon (0-10 cm) was 16.9 g kg^{-1} under shrubs and 19.0 g kg^{-1} under crops. The comparison of C in the different land uses reflects the importance of forest ecosystems in the region. Afforestation programs have to consider this fact. The lack of litter inputs from the previous ecosystem is progressively compensated by litter inputs from the newly-established forest plantation. After several years, a new soil C equilibrium is eventually reached, when carbon outflows from decomposition are balanced by carbon inflows from litter production (Jandl *et al.,* 2007).

Amounts of SOC accumulation found in this study agree with previous works. Rodriguez-Murillo (2001) estimated the soil C under different types of land use and soil in peninsular Spain obtaining under conifers mean values of 75 Mg C ha^{-1} and under broadleaves forests mean values of 93.6 Mg C ha^{-1}. Turrión *et al.* (2009) found that soils under *Q. pyrenaica* forests in Western Spain (Sierra de Gata Mountains) had a high capacity to accumulate SOC (between 33 and 185 Mg C ha^{-1}), while higher values were found in chestnut coppice forest (195 Mg C ha^{-1}) by Gallardo & González (2004).

In this paper, two different inventories have been carried out. The first one, the extensive inventory allowed us to consider soil spatial variability and to know the importance of the soil C in the ecosystem. Bernoux *et al.* (2002) emphasized the importance of large-scale studies to refine global estimations obtained by the aggregation of local estimates. Previous studies carried out on the tree biomass in this region (Herrero & Bravo, 2012) showed that *Pinus* spp. tree biomass fixed an average static value of 42.6 Mg C ha^{-1}, while oak stands fixed 5.2 Mg C ha^{-1}. Total C fixed in the different pools

(tree biomass, dead wood, scrub, forest floor and soil) of the two types of forest ecosystems (pine and oak stands) was 110.7 Mg C ha^{-1} and 73.9 Mg C ha^{-1}, respectively (unpublished data). This meant that soil C was the main pool of C in natural oak stands in the region. It represented 84% of the total C because of the scarcity of vegetation in this forest ecosystem. In *Pinus* spp. plantations, soil C content represented almost 48% of the total C fixed in the ecosystems. These values showed the importance of these young/middle-aged forests in mature soils characterized by absence of carbonates. In comparison to the value reported by Dixon *et al.* (1994) for temperate forest soil carbon (60%), these types of plantations reflected that their accumulation could be higher in the next succession stage.

On the other hand, the second inventory, characterized by studying microsite conditions, revealed additional information about soil C content and characteristics. Measurement of the microbiological properties of soil, such as C_{MB} and C_{RB}, provided a more sensitive appraisal and indication of the management practices on SOC contents (Eleftheriadis & Turrión, 2014). Mahía *et al.* (2006) obtained for soil under *P. sylvestris* forest average values for microbial C of 0.74% of C_T, and under *P. pinaster* forests 0.56% of total C, in the North-western Spain, both into the range of our values. Eleftheriadis & Turrión (2014) found that C_{MB} represented 3.7% of C_T in soils under *Q. pubescens* in Greece calcareous soils. The data showed that the studied forest soils had a relatively low C_T concentration in topsoil 0-10 cm (between 1.3 and 4.4%), and only around 1% of this C_T is microbial biomass C.

Since organic matter content is often one of the most influencing factors in microbial biomass and soil respiration, and organic matter showed a wide range of variation on soil samples analyzed (Table 4), the C_{MB} and C_{RB} values were expressed as relative values of total soil C to increase the information. According to diverse authors (Mahía *et al.,* 2006) the ratio C_{MB}/C_T is related to substrate quality. Then, this parameter would be expected to increase with increasing the stand development stage and it should also be higher in soils under *Q. pyrenaica* assuming that substrate quality was higher in soils under climax vegetation. Oak stand with higher development stage showed significant higher C_{MB}/C_T values than stands with medium and lower ones, and *P. pinaster* stand with lower development stage showed significant lower values of this ratio than higher and medium development stage stands. The type of soil under the *P. pinaster* is a Regosol, soil with lower development than Cambisol, which characterized the other forest plots, and it could explain the different pattern observed for the ratio in this soil. Bueis *et al.* (2016) found that soil physical, chemical and biochemical parameters, as well as physiographic parameters, were driving factors in determining site index for *Pinus sylvestris* in acidic plateau plantations of northern Spain.

Metabolic quotient (qCO_2) showed significant lower values in the stands with high development stage for *P. pinaster*, *P sylvestris* and *Q. pyrenaica* forests. This lower values are related to more efficient utilization of C by the microbial community (Anderson, 2003; Llorente & Turrión, 2010). In principle, the qCO_2 values were expected to decrease with increasing substrate quality being higher under unfavourable than under favourable conditions. Our data clearly support this hypothesis since stands with high development stage exhibited significant lower qCO_2 values than those stands with lower development stage.

Forest management carried out in stands with higher development consisted of thinning that reduced tree density and increased the radial growth of the remaining trees. At the moment of the intervention, thinning changes the microclimate and soils become warmer and possibly wetter due to reduced evapotranspiration. It increases aeration and microbial biomass and stimulates forest floor C decomposition. An increased proportion of microbial carbon and nitrogen in the total soil organic pool indicates higher nutrient availability to the plants. Microbial biomass and respiration parameters could be therefore sensitive indices of the effect of silvicultural practices on the soil microbiological environment. In contrast, the higher value of qCO_2 and the smaller value of C_{MB}/C_T, which defined the worst conditions of soil efficiency use of microbial populations were found in the lower stand development stages (medium or low).

As well as soil properties, the extensive inventory allowed as to evaluate the role of the forest floor within the forest ecosystems at large scale. The high amount of C fixed in this pool, above all in *Pinus* spp. plantations (13.7 Mg C ha^{-1}), showed the relative importance of this pool in the total C fixed. Forest floor carbon represented 12.4% of the total C fixed in the pine ecosystem analyzed, while it represented the 7.3% of the total carbon fixed (5.4 Mg C ha^{-1}) in the oak stands. Although higher proportions were found in mixed forest of oak and beech in Belgium areas by Vande Walle *et al.* (2001), smaller values were found by previous researchers such as Gallardo & González (2004) or Ordóñez *et al.* (2008).

The smaller C/N ratio reflects that the forest floor degradation in oak is faster than the pine because micro-organisms prefer digestion of litter with a low C/N ratio (< 20) to satisfy their nitrogen needs (Vande Walle *et al.,* 2001). Previous authors agreed as to the critical

C/N ratio at which net mineralization began when C/N=20-30 (Waring & Schlesinger, 1985). In the present study, under coniferous sites, the C/N values would indicate that the net mineralization appeared to be restricted to the lower part of the forest floor. This agrees with other articles such as Kavvadias *et al.* (2001) and Vesterdal *et al.* (2013).

The C annual returns found in the studied forest were similar to those reported in other coniferous and deciduous forest, such as 2.6 Mg C ha^{-1} $year^{-1}$ by Gallardo & González (2004) in *Castanea sativa* forests, Central-Western Spain or between 3.2 to 5.2 Mg C ha^{-1} $year^{-1}$ by Cañellas & San Miguel (1998) in *Quercus coccifera,* Eastern Spain. Smaller values of litterfall C/N were found in oak stands along the year. Again the low litterfall C/N ratio was an indicator of fast decomposition rate and less forest floor C content. If the results of soil component are linked here, the higher values found in the upper depth of the mineral soils of quality elements like K, C_T and N, could probably due to due to the incorporation of organic matter from litterfall providing organic and inorganic elements for the nutrient cycling processes (Mudrick *et al.,* 1994).

Our results of litter decomposition agree with previous studies, where *k* values ranged from 0.15 $years^{-1}$ in *P. sylvestris* mountain areas, 0.39 $years^{-1}$ in chestnut coppice by Gallardo & González (2004), to 0.62 $years^{-1}$ in *Q. pyrenaica* forest by Gallardo & Merino (1993). The *k* value allows to estimate the half-life time of the litter in the studied plots ($t_{1/2} = \ln 2/k$), obtaining in our study values of 3.9 and 1.5 years for pinus and oak forests, respectively. These results corroborate the faster decomposition of oak litter, with lower C/N ratio than pinus litter (Table 5). This situation occurs when the microbial activity has the potential to respire more C that contained in above- and belowground litter production.

Climate conditions, species composition, slope position, litter supply and quality, abundance of understory vegetation, acidity, soil fertility and biological activity are contributing factors to the litter degradation process. In our case, the chemical composition (particularly the higher lignin and wax content in *Pinus* spp.), together the stand variables (tree density, soil light interception, aeration, etc.), could be affecting the organic matter decomposition process (Mudrick *et al.,* 1994; Lal, 2005).

Soil microbial biomass was an important factor in decomposing the organic matter (although not the only one), but soil properties and forest management had a great influence in stand aeration and litter decomposition in our areas (Jandl *et al.*, 2007; Eleftheriadis & Turrión, 2014). Forest management can stimulate the decomposition of the forest floor and can modify its quality in the area by 1) the tree species selection (quantity and chemical quality of litter, rooting depth) and 2) the thinning regime (microclimate). The choice of tree species for ecosystems depends on management goals and priorities. If sequestration of C into SOC was the main environmental goal for the region, then *Pinus* spp. plantations would be preferred. *Pinus* spp. showed higher values of C, higher sequestration capacity and great stability for the organic matter, which imply higher resistance to microbial attack. However, if other services were sought, oak stands would generate a high nutrient status in terms of base cations, pH and C/N ratio, and this could be favorable for climate change mitigation with a strong soil C sink. In the study area, thinning intensity has been low, removing a small proportion of stand basal area (Herrero & Bravo, 2012). The high stand density has thus maximized the forest floor C content. Therefore, new cycles of thinning operations would be needed in the *Pinus* spp. plantations to improve forest litter decomposition, which is a major pathway for providing organic and inorganic elements for the nutrient cycling processes (Mudrick *et al.,* 1994). In Mediterranean forest ecosystems, the role of litter decomposition in nutrient cycling becomes still more important when considering the degradation of forest vegetation and soils by wild fires and overgrazing (Kavvadias *et al.*, 2001).

In our study area, the SOC content in the *Pinus* spp. plantations (53.0 Mg of C ha^{-1}) seems to be an important parameter to emphasize the importance of this type of forests. Afforestation with these species enhances CO_2 stock. The SOC estimates presented here provide a baseline to estimate future changes in soil C stocks in peninsular Spain and to assess their vulnerability to key global change drivers. Likewise, they could be used to improve our ability to respond to environmental changes by informing land use management schemes aimed at promoting and conserving C stocks.

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