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How do soil organic carbon stocks change after cropland abandonment in Mediterranean humid mountain areas?

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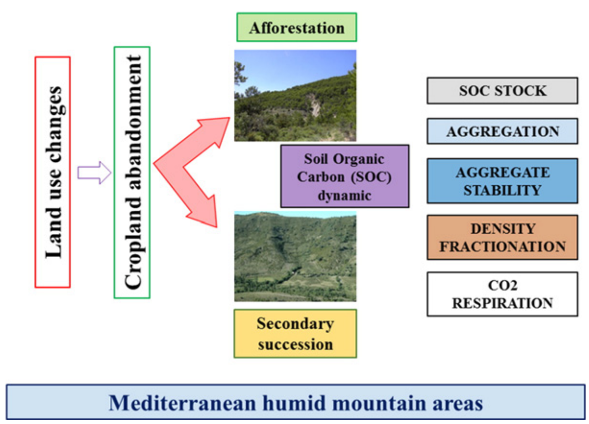
HIGHLIGHTS

- This paper examines the effects of land abandonment on soil organic carbon.
- SOC dynamics have been studied in the bulk soil and in the fractions.
- Only afforestation with *Pinus nigra* accelerates the recovery of SOC in the topsoil.
- Aggregation is an important mechanism promoting SOC stocks after afforestation.
- Pastureland should be considered in land management due to the importance in SOC stocks.

GRAPHICAL ABSTRACT



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ABSTRACT

The effects of land use changes on soil carbon stocks are a matter of concern stated in international policy agendas on the mitigation of greenhouse emissions. Afforestation is increasingly viewed as an environmental restorative land use change prescription and is considered one of the most efficient carbon sequestration strategies currently available. Given the large quantity of CO_2 that soils release annually, it is important to understand disturbances in vegetation and soil resulting from land use changes. The main objective of this study is to assess the effects of land abandonment, land use change and afforestation practices on soil organic carbon (SOC) dynamics. For this aim, five different land covers (bare soil, permanent pastureland, secondary succession, *Pinus sylvestris* (*PS*) *and Pinus nigra* (*PN*) afforestation), in the Central Spanish Pyrenees, were analysed. SOC dynamics have been studied in the bulk soil, and in the fractions separated according to two methodologies: (i) aggregate size distribution, and (ii) density fractionation, and rates of carbon mineralization have been determined by measuring CO_2 evolution using an automated respirometer. The results showed that: (i) SOC contents were higher in the *PN* sites in the topsoil (10 cm), (ii) when all the profiles were considered no significant differences were observed between pastureland and *PN*, (iii) SOC accumulation under secondary succession is a slow process, and (iv) pastureland should also be considered due to the relative importance in SOC stocks. The first step of SOC stabilization after afforestation is the formation of macro-aggregates promoted by large inputs of SOC, with a high

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contribution of labile organic matter. However, our respiration experiments did not show evidence of SOC stabilization. SOC mineralization was higher in the top layers and values decreased with depth. These results gain insights into which type of land management is most appropriate after land abandonment for SOC.

1. Introduction

Soils are the primary terrestrial pool for organic carbon and account for >75% of the Earth's terrestrial organic carbon (Lal, 2004). However, soils can render either a sink or a source of carbon and atmospheric carbon dioxide (CO_2) with direct influence on the greenhouse effect (Lugo and Brown, 1993; Lal et al., 1995). The role played by soils in the sequestration of soil organic carbon (SOC) is so crucial that the Kyoto Protocol (article 3.3) and the Paris agreement included it as an important element for managing (see also Montanarella, 2015; Keesstra et al., 2016). The effects of land use changes on SOC stocks are a matter of concern stated in international policy agendas on the mitigation of greenhouse emissions. Anthropogenic activities have led to an increase in atmospheric concentration of CO_2 (Reicosky, 2002; WMO, 2008), and forestry is recognised as major sink for carbon but as well as accumulating carbon above ground (Lal, 1997). In that sense, afforestation is increasingly viewed as an environmental restorative land use change prescription and is considered to be one of the most efficient carbon sequestration strategies currently available (Farley et al., 2005). Since the United Nations Framework Convention on Climate Change there has been increasing interest in afforestation to sequester CO_2 from the atmosphere.

Cropland abandonment has been the main land use change in the northern rim of Mediterranean humid mountain areas over the last decades (Lasanta et al., 2016) leading to the expansion of forestland and scrublands (Sluis et al., 2014). These abandoned areas can be left to undergo secondary succession (passive restoration) or be subjected to afforestation (active restoration) that mostly consists of tree and shrub planting. Large areas of Europe are increasingly abandoned (Navarro and Pereira, 2015). After farmland abandonment, old fields are spontaneously slowly colonised by annual and perennial herb communities and then are partially or completely replaced by perennial grasses, shrubs, and/or trees (secondary succession, after 10–60 years), and >100 years are necessary to observe a forest stage (Molinillo et al., 1997). Nowadays, abandoned fields present a very dense vegetation cover composed mainly of shrubs and an herbaceous layer (Lasanta-Martínez, 2005; Reiné et al., 2014). At the same time, due to the idea of land degradation after abandonment, the slow process of secondary succession, and with productive and environmental objectives, extensive afforestation programs were conducted by national forest services all over the Mediterranean region (Ortigosa et al., 1990; Yaşar Korkanç, 2014). On the other hand, some scientists suggest that secondary succession has negative impacts on environment, landscape and socio-economy (García-Ruiz and Lana-Renault, 2011; San Román Sanz et al., 2013) and suggest keeping abandoned land in the first pastureland stage (Conti and Fagarazzi, 2005; Lasanta et al., 2015). Conversely, other scientists prefer rewilding methods (Navarro and Pereira, 2015).

Different studies have reported that soil properties recover slowly after land abandonment (Ruiz-Sinoga and Martínez-Murillo, 2009; Nadal-Romero et al., 2016b), and many recent studies have shown that SOC is strongly affected by land abandonment and revegetation processes in Mediterranean mountain areas (Muñoz-Rojas et al., 2011; Novara et al., 2014; Gabarrón-Galeote et al., 2015a). Observations about the effects of afforestation on SOC have been synthesized and reviewed worldwide by Post and Kwon (2000), Guo and Gifford (2002), Paul et al. (2002) and Li et al. (2012). Moreover, given the large quantity of CO_2 that soils release annually, it is important to understand disturbances in vegetation and soils resulting from land abandonment.

SOC consists of different fractions with contrasting resistance to decomposition (Schimel et al., 1985) that are differently affected by land abandonment and revegetation processes (Trigalet et al., 2016). Soil aggregation provides physical protection of SOC against rapid decomposition (Razafimbelo et al., 2008), and aggregate formation and stability is really closely linked with SOC storage (Salome et al., 2010). However, the role of soil aggregation and SOC fractions is far from well understood in Mediterranean humid mountain areas. Density fractionation is a useful tool to study the relevance of SOC stabilization in aggregates and in association with minerals. The use of physical density fractionation methods of soil organic matter has been successfully applied in different environments (Six et al., 2000; Cerli et al., 2012; Wang et al., 2014), but it has rarely been applied to full soil profiles. The method proposed by Golchin et al. (1994) (combining dispersion and sonication) became a widely applied method: (i) the Free Light Fraction (FLF) comprises relatively undecomposed labile organic matter (unprotected), (ii) the Occluded Light Fraction (OLF) comprises organic matter stabilized by aggregation, and (iii) the Heavy Fraction (HF) is strongly associated with soil minerals (Cerli et al., 2012). Knowing the degree of protection of the SOC is crucial because it allows a better understanding of SOC dynamics, aggregation and stabilization.

Moreover, it has been demonstrated that although SOC concentration is generally higher in the top layers, the total soil volume of the subsurface horizons is usually much higher. Jobbagy and Jackson (2000) showed that 60% of the total SOC is stored below a depth of 20 cm. However, most studies of SOC dynamics and stability were restricted to topsoil layers, and our understanding of subsoil SOC is still limited, even when subsoils are really sensitive to land use changes (Rumpel and Kögel-Knabner, 2011).

Despite the extensive land abandonment and the consequent revegetation process, and the time occurred after the first afforestation plans, few investigations have studied the consequences of land abandonment in Mediterranean humid mountains related to soil properties. Nadal-Romero et al. 2016b showed that there is still considerable uncertainty about the effects of afforestation practices on soil property dynamics, and so far, no comparing study has been carried out for the effects of secondary succession and afforestation in Mediterranean humid mountain areas after land abandonment.

The main objective of this study is to assess the effects of land abandonment and revegetation processes (secondary succession and afforestation practices) on SOC dynamics. The specific objectives were to (i) quantify total SOC in different land use soils and depths, (ii) isolate SOC in aggregate sizes and density fractions, and (iii) determine carbon mineralization by measuring CO_2 evolution.

This leads to the following research hypothesis: an increase in vegetation cover due to secondary succession and afforestation practices in Mediterranean humid mountain areas (after the abandonment of agricultural land and grazing activities) may lead to significant increases in SOC stocks and SOC sequestration. To test these hypothesis, five different land cover types (bare soil, permanent pasturelands, secondary succession and *Pinus sylvestris* and *Pinus nigra* afforestations), in the Central Spanish Pyrenees were analysed.

2. Materials and methods

2.1. Site description

A small catchment in the Central Pyrenees (Araguás catchment, 12.4 ha, 694,800, 4,719,400, 920 to 1105 m a.s.l.) was selected to carry out the soil sampling. The area was cultivated (in terraced-slope fields) until the middle of the 20th century with cereal crops (wheat (*Triticum spp.*) and barley (*Hordeum vulgare L.*)). The area was abandoned in 1950s and most of it was afforested with *P. sylvestris* (*PS*) and *P. nigra* (*PN*), although some areas underwent to a process on natural plant colonization (secondary succession) with *Genista scorpius*, *Juniperus communis*, *Rosa gr. canina* and *Buxus sempervirens* (Nadal-Romero et al., 2016b). The present landscape is a complex mosaic (in a small area with a homogenous lithology, climatology and former land use) in which afforested patches alternate with dense and open shrubs, bare areas (that were not afforested) affected by sheet wash erosion, and permanent pasturelands (defined by Allen et al. (2011) as land on which vegetation is composed of perennial or self-seeding annual forage species which may persist indefinitely) (Fig. 1). The bedrock is Eocene Flysch with alternating sandstones with carbonate cementation and marl layers. The soils are stony and thin following centuries of cultivation practices and erosion processes. The soils are classified as Calcaric Regosols with a silt loam texture (FAO, 2014).

The climate in the area is sub-Mediterranean with oceanic and continental influences, and annual rainfall varies between 500 and 1000 mm (average annual rainfall approximately 800 mm). The average temperature is 10 °C (minimum −14 °C, maximum > 30 °C).

2.2. Sampling and design

Soil samples were collected from five different representative land cover types: bare lands, permanent pasturelands, secondary succession, afforestation with *PS* and *PN*. Three plots per five land cover types were selected, all with similar topographic conditions (slope and exposition). At each plot, organic (if present) and mineral soil samples were sampled at two randomly chosen locations. Mineral soil samples were obtained in the field at 5 cm increments for the 0–20 cm depth, and at 10 cm increments between 20 and 50 cm (20–30 cm, 30–40 cm and 40–50 cm, total samples 96). The subsamples were pooled into a composite sample, giving one sample per sampling depth and land use ($n = 32$ samples). Two separate samples per depth and land cover were collected for determination of the bulk density with the help of steel cylinders ($n = 64$).

2.3. Soil characterization

Bulk density was determined gravimetrically (unaltered samples) by drying a known volume of sample at 105 °C. A subsample from the composite samples was dried at 40 °C and then sieved through 2 mm mesh sieve in the laboratory (remaining roots and stones were carefully removed and quantified). Soil pH and electrical conductivity (EC) were measured in a deionized water suspension (1:2.5) using a pH meter and a conductivity meter. Total carbon (C_{total}) and total nitrogen (N) were determined by dry combustion (Elementar Vario El Cube). Carbonate concentration ($CaCO_3$) was determined using the Wesemael method (van Wesemael, 1955) from which also the total inorganic carbon was calculated (C_{inorg}). Soil organic carbon (SOC) was calculated by subtraction of total inorganic carbon (C_{inorg}) from the total carbon (C_{total}). SOC and N concentrations were expressed in g kg^{-1} soil, while SOC and N stocks were expressed in Mg ha^{-1} (calculated weighting each value by the respective depth and bulk density). The C_{org}/N ratio was calculated using SOC and N. Organic matter (OM) was determined using the loss on ignition method (at 375 °C). Soil texture, after removing organic matter (adding H_2O_2), was determined using a particle size analyser (Micromeritics, SediGraph 5100, Nocross, USA).

2.4. Aggregate size distribution

Samples for the different land covers were subjected to aggregate size fraction. A soil subsample of 150 g of sample was air-dried at room temperature and then fractionated using 2 mesh sieves to obtain

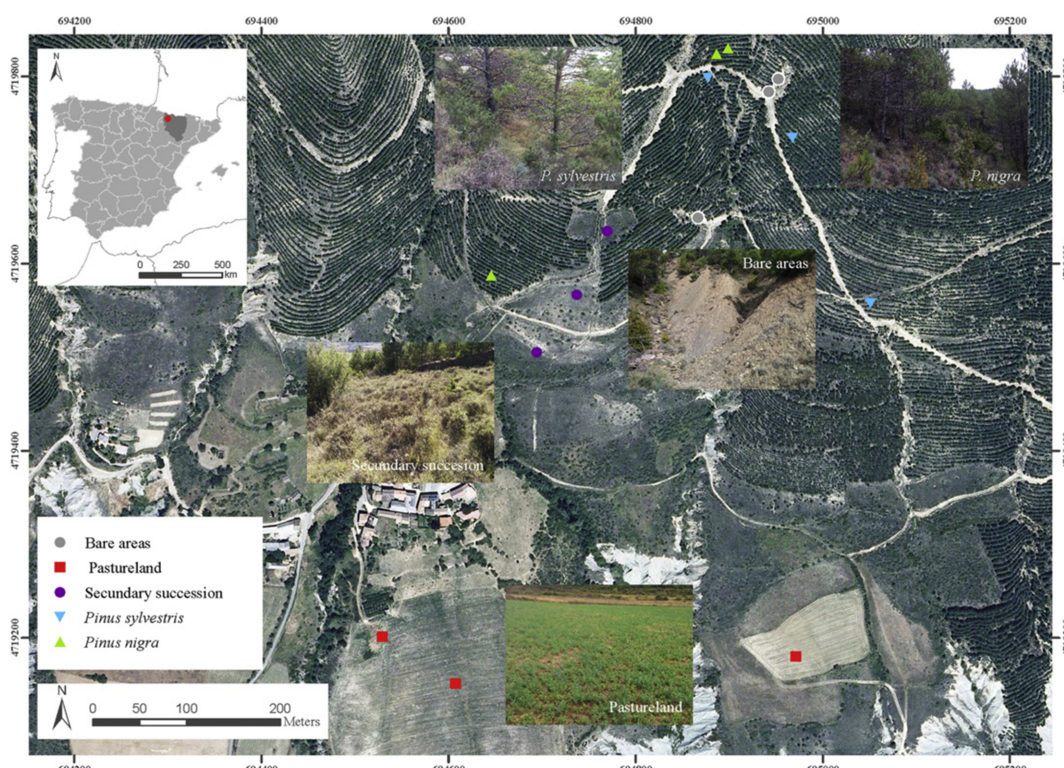

Fig. 1. Location of sampling points in the Araguás catchment.

three size fractions (>5 mm, 5–2 mm and <2 mm) (macro-aggregates were defined as aggregates being >2 mm). Sieves were placed on a sieve shaker and shaken at 30 Hz for 5 min using a horizontal shaking machine (Retsh, AS 200 basic, Haan, Germany). Aggregates retained at each sieving level were fully weighted after the removal of small stones and plant residues (these were weighted for final corrections). Aggregation analysis was done twice per sample. For each fraction, the C_{total}, N, SOC, C_{org}/N ratios and the $CaCO_3$ content were determined. The relative distribution of SOC in aggregates was calculated from the SOC content per aggregate fraction multiplied by the mass of aggregates per fraction.

Aggregate stability of top (0–5 cm) macro-aggregates (4–4.8 mm diameter) for air-dried aggregates and pre-wetted aggregates (at pF 1 for 24 h before commencing the analysis) was determined using the drop test (Counting the Number of Drops) (Imeson and Vis, 1984). 20 aggregates per land cover and moisture condition were randomly selected, and we counted the number of drop impacts required to disrupt the aggregate sufficiently to pass through the 2 mm sieve (up to a maximum of 200 drops).

2.5. Density fractionation

Density fractionation was applied on non-sieved soil samples after the manual removal of the large roots and stones following the methods of Golchin et al. (1994) and Cerli et al. (2012). Ten grams of soil were weighted in a centrifuge tube and 50 ml of sodium polytungstate (NaPT) of a density of 1.6 g cm^{-3} was added. The suspension stood for 1 h and after this time it was centrifuged at 6800 *g* for 20 min at room temperature. The floating material (free light fraction, FLF) was separated and collected on a 0.7 μm pore glass-fibre filters (Whatman GF/F filter), by using a rubber spatula, and washed with deionized water till the conductivity of the washing water was <200 μS cm^{-1}. The remaining soil was re-suspended into 50 ml of NaPT and then dispersed by ultrasound at 150 J mL^{-1} (Sonopuls HD 3200 with VS70 probe), calibrated according to Schmidt et al. (1999) in an ice-bath to keep the temperature <40 °C). The amount of energy was pretested according to Cerli et al. (2012) to ensure a proper separation between the organic matter enclosed into aggregates and associated with minerals. After the dispersion process, the samples were again centrifuged; the floating material (constituted the occluded light fraction, OLF) was then separated, filtered and washed with deionized water (as above described for the FLF). The remaining sample was washed by repeated addition of deionized water, shaked and centrifuged (10,000 *g* to ensure complete sedimentation of the smallest clay-size particles), until the conductivity of the wash water was <500 μS cm^{-1}. The soil material (heavy fraction, HF) was then transferred into dark containers.

Density fractionation was done twice per sample. All fractions were freeze-dried, homogenized (the HF was milled) and used for the determination of SOC, N, C_{org}/N ratio. In the HF the $CaCO_3$ content was also determined using the Wesemael method. The average recovery of soil mass and SOC after density fractionation was 98.7 ± 0.7% and 92.0 ± 7.8%.

2.6. Soil CO_2 fluxes

CO_2 efflux of each sample was continuously assessed using an automated respirometer (Respicond V) in the laboratory (at 20 °C temperature and darkness). We used about 15 g of soil that were rewetted (before the experiment) to their field capacity moisture. The main principle of the Respicond has been described previously by Nordgren (1988). It consists of water baths with inserted hermetically closed vessels. Each vessel contains a smaller open vessel with KOH solution and platinum electrodes inserted into the solution. These electrodes are connected to a digital volt-amperemeter. The CO_2 released from the soil samples is absorbed by the KOH solution and the amount of absorbed CO_2 is determined by measuring changes in conductivity of the KOH solution. Data were collected every 30 min (during 30 days). The experiment was done twice per sample. Carbon (C) mineralization rates were calculated for each sample as the ratio between accumulated CO_2-C released during 30 days and total SOC of soil (mg CO_2-C g C^{-1} d^{-1}).

2.7. Statistical analysis

All data were tested for normal distribution for all measured properties using the Chi-square test. Pearson correlations were used to investigate the relationships among the variables, and analysis of variance, a one-way ANOVA, was used to compare the differences among depths and land covers.

To determine the differences for the aggregate stability results, due to the non-normality of the data, the non-parametric Kruskal–Wallis test was used. In all cases, we considered differences to be statistically significant at $p<0.05$.

Principal component analysis (PCA) was also performed to determine first correlations among the measured variables and to elucidate major variation patterns in terms of land covers. The position of different soil samples in the factorial plane will be shown. All statistical analyses were carried out using SPSS Statistics 20.

3. Results

3.1. Physico-chemical soil properties and soil profiles

The main characteristics of soil samples are reported in Table 1. Soil profiles have been modified due to the old agricultural activities and subsequent afforestation soil preparation techniques. After 50 years of afforestation practices, soils still showed truncation signs in the surface horizons, and only a thin organic horizon was observed in the *PS* and *PN* sites.

The pH increased with depth, but in the pastureland sites no major changes in pH throughout the profile were found. EC decreased with depth, apart from pasturelands, and the highest values were found in the *PN* topsoils.

High contents of $CaCO_3$ were found in all soil profiles, reflecting the influence of the parent material. In general, $CaCO_3$ tend to increase with depth, although a clear pattern was not observed ($p = 0.656$).

OM content decreased with depth. The highest values were recorded in the *PN* topsoil sites. Soils were dominated by the silt fraction and there was little variation in the particle size either along the profile or among land covers, except for the *PN* that showed higher sand contents.

3.2. SOC and N content. SOC stocks

The largest SOC contents were found in the *PN* sites in the first 20 cm and the lowest values were recorded in the bare areas and secondary succession sites (Fig. 2A). Significant differences were observed: (i) at 0–10 cm between *PN* and the different land covers ($p<0.001$), (ii) at 10–20 cm between secondary succession and *PN* ($p<0.001$), and *PS* and *PN* ($p<0.001$), and between pasturelands, and secondary succession and *PS* ($p = 0.002$ and $p = 0.003$ respectively), and (iii) below 20 cm, between pasturelands and *PN* and secondary succession and *PS* ($p<0.001$). Below 20 cm similar contents were observed in *PN* and pasturelands. Significant differences related to depth, between 0 and 10 cm and samples below 20 cm appeared for the different land covers (secondary succession, $p<0.001$; *PS*, $p<0.001$; *PN*, $p = 0.001$), except in the pastureland sites.

N contents decreased with depth (Fig. 2B). Values were higher in the pasturelands, although the highest values were recorded in the organic horizon of the *PN* sites. Significant differences were observed: (i) at 0–10 cm between bare lands and pasturelands with the different land covers ($p<0.001$), (ii) at 10–20 cm and below 20 cm between pasturelands and the different land covers ($p<0.001$).

Table 1
Physico-chemical soil properties in the different land covers.

Land cover	Depth (cm)	BD (g cm^{-3})	pH	EC (μS cm^{-1})	CaCO3 (%)	OM (%)	Sand (%)	Silt (%)	Clay (%)
Bare lands	0–5	1.73	8.0	146	34.4	1.45	10.1	70.1	19.8
	5–10	2.00	7.9	153	37.3	1.36	10.3	66.2	23.5
	0–5	0.63	7.6	297	41.7	4.45	12.4	68.4	19.2
	5–10	0.98	7.6	294	40.8	3.64	11.8	71.4	16.8
	10–15	1.07	7.7	272	41.5	3.63	12.3	70.8	16.9
Permanent pastureland	15–20	1.00	7.6	317	41.3	4.08	12.6	68.6	18.8
	20–30	1.23	7.6	308	42.2	3.92	12.7	69.9	17.4
	30–40	1.46	7.5	299	42.5	3.84	11.0	71.3	17.7
	>40	1.56	7.6	303	40.8	3.30	11.8	71.0	17.2
	0–5	1.09	7.7	277	37.1	3.93	6.4	74.5	19.1
	5–10	0.83	7.8	222	38.1	2.86	6.6	74.1	19.3
	10–15	0.90	7.9	229	39.2	2.77	6.6	74.1	19.3
Secondary succession	15–20	1.11	8.0	194	39.5	1.83	5.0	77.5	17.5
	20–30	0.82	8.2	181	40.4	1.46	4.2	76.2	19.6
	30–40	1.18	8.4	168	40.6	1.28	3.8	76.8	19.4
	>40	1.18	8.4	164	40.3	1.20	4.2	84.3	11.5
	0-layer	–	7.8	273	44.3	4.73	–	–	–
	0–5	1.32	7.7	281	38.0	4.29	8.6	74.0	17.4
	5–10	1.14	7.7	297	37.2	3.68	10.3	71.6	18.1
Afforestation: *Pinus sylvestris*	10–15	1.23	7.7	277	40.9	2.88	8.4	73.7	17.9
	15–20	1.27	7.8	229	40.6	1.80	6.1	72.6	21.3
	20–30	1.21	7.9	235	37.4	1.45	7.9	72.5	19.6
	30–40	1.23	8.2	179	45.2	1.09	6.0	73.4	20.6
	>40	1.16	8.2	182	43.8	1.45	6.0	75.6	18.4
	0-layer	–	7.2	594	20.0	15.74	–	–	–
	0–5	0.93	7.5	541	13.4	10.67	19.1	76.5	4.4
	5–10	1.00	7.6	433	32.6	6.19	25.6	59.8	14.6
Afforestation: *Pinus nigra*	10–15	1.02	7.6	348	31.5	4.84	24.5	60.6	14.9
	15–20	1.21	7.7	303	28.4	4.32	22.7	61.0	16.3
	20–30	1.23	7.9	256	30.6	2.77	26.8	56.8	16.4
	30–40	1.29	7.8	260	25.9	3.30	23.1	58.1	18.8
	>40	1.40	7.7	275	17.8	3.06	17.1	59.2	23.7

BD: bulk density; EC: electrical conductivity; $CaCO_3$: carbonate content; OM: organic matter.

The C_{org}/N ratios (Fig. 2C) in the *PN* sites were significantly higher than those of the other sites ($p<0.001$). No significant differences were found due to soil depth, except for the *PN* sites ($p<0.001$).

Total SOC stocks ranged between 13.2 Mg C ha^{-1} and 136.5 Mg C ha^{-1} of which 78–93% were stored below the first 5 cm (Fig. 3). The highest SOC stocks were recorded in the *PN* sites. The percentage of SOC in the top 10 cm averaged 17%, 23%, 42% and 36% for pasturelands, secondary succession, *PS* and *PN* respectively.

Significant differences related to land cover at 0–10 cm were found between afforestation sites and the different land covers ($p<0.05$). At 10–20 cm and below 20 cm significant differences were found between pastureland and *PN* and *PS* and secondary succession ($p<0.05$) for SOC stocks. Below 10 cm no significant differences were observed between pastureland and *PN* sites. If we consider the complete soil profile, SOC stocks were only moderately affected by the afforestation, and no significant differences were observed between pasturelands and *PN* sites.

3.3. Aggregation and stability of macro-aggregates

Afforestation with *PN* significantly increased macro-aggregate proportions ($p<0.001$). The average percentage of macro-aggregates in the soil profiles were 51%, 37%, 55% and 67% for pasturelands, secondary succession, *PS* and *PN* respectively (data not shown).

The contribution of macro-aggregates to total SOC ranged between 8% in the bare soils to >60% in the *PN* (Fig. 4). These values were significantly higher in the pasturelands than in the secondary succession sites ($p<0.001$), and significantly higher in the afforested sites than in the secondary succession sites (*PS* $p = 0.001$; *PN* $p<0.001$), and tend to increase with depth. The micro-aggregates at 0–5 cm contained the highest SOC concentrations especially in the *PN* topsoil (higher than 50 g kg^{-1}).

The results of the aggregate stability showed that bare soils had the least stable aggregates both in dry and wet conditions. On average, soils beneath *PS* and *PN* were the most stable in both situations (Fig. 5). Significant differences were observed in dry conditions between

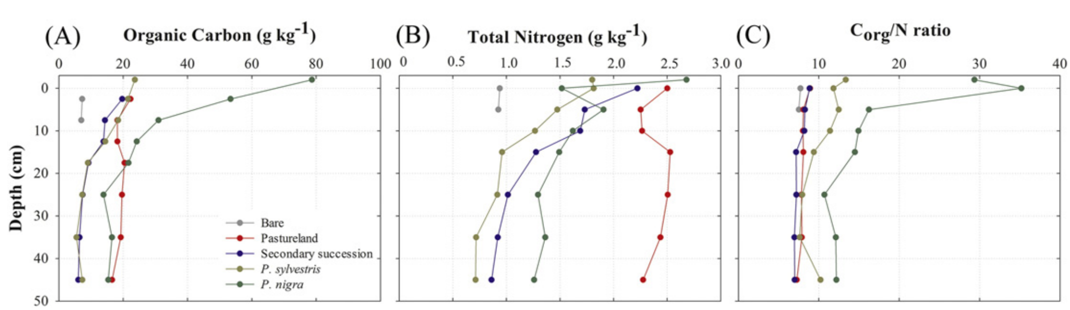

Fig. 2. (A) Organic carbon, (B) total nitrogen concentrations and (C) C_{org}/N ratios in the different land covers and depths.

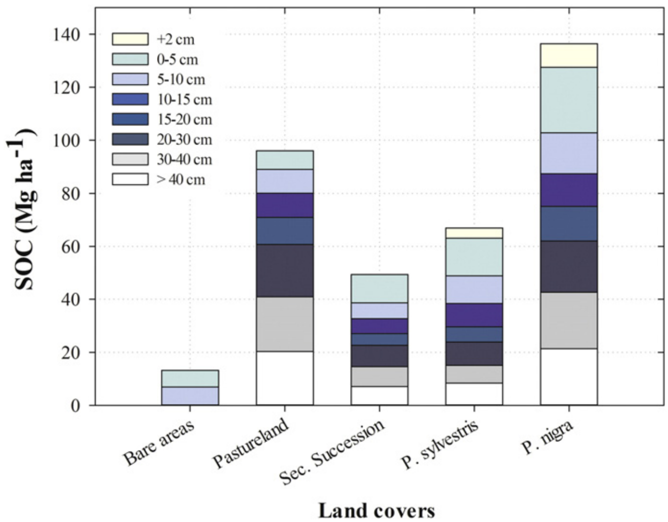

Fig. 3. Soil organic carbon stocks (Mg ha^{-1}) in the different land covers in the soil profile.

aggregates from bare areas and all land covers ($p<0.05$). In wet conditions, significant differences were observed between afforested sites and the other land cover types.

3.4. Density fractionation

FLF and OLF represented a small proportion of the soil mass (Table 2). Both fractions are composed of almost pure organic material. Most of the soil mass, with little variation was recovered in the HF. In all the samples, both FLF and OLF had a SOC content higher than 20 g kg^{-1}, and the HF contained only a small percentage of SOC, lower than 2 g kg^{-1}, except in the topsoil of *PN* (Table 2).

In all the land cover types, the HF represented the most important part of the total SOC at all depths (Table 2 and Fig. 6). The contribution of the HF to SOC was slightly lower in the afforested sites, indicating a large contribution of FLF and OLF in these sites. Significant differences were only found in the first 10 cm between afforestation sites and the different land covers ($p<0.05$). The contribution of HF increased with depth, although no significant differences were observed, except in the *PN* sites between the topsoil and the deepest soils ($p<0.05$).

C_{org}/N ratios of the HF tended to decrease with depth, whereas an opposite pattern was observed for the FLF and OLF (Fig. 6). Higher C_{org}/N ratios were found in the afforested sites (FLF and OLF) suggesting lower quality of the OM (litter).

3.5. Soil CO_2 fluxes and C mineralization

C mineralization ranged from 4.9 and 24.5 mg CO_2–C g C^{-1} (0.15 to 0.81 mg CO_2–C g C^{-1} d^{-1}) (Fig. 7). Topsoil samples from the pasturelands and the afforested sites showed higher mineralization values than subsurface horizons, although no significant differences were observed between different depths or land covers (Fig. 7). It is also interesting to note that C mineralization rates were only correlated with the FLF ($p<0.01$).

Finally, a PCA was performed for the complete soil samples dataset, taking into account all the studied variables. The two PC explained around 64% of the variance. Fig. 8 showed the position of soil samples in the factorial plane. The different land covers were successfully discriminated along PC1 and PC2. Bare, pasturelands, secondary succession and *PS* samples were distributed in a similar position in the factorial plane, and soil samples from the *PN* sites were separately distributed in the positive side of PC2.

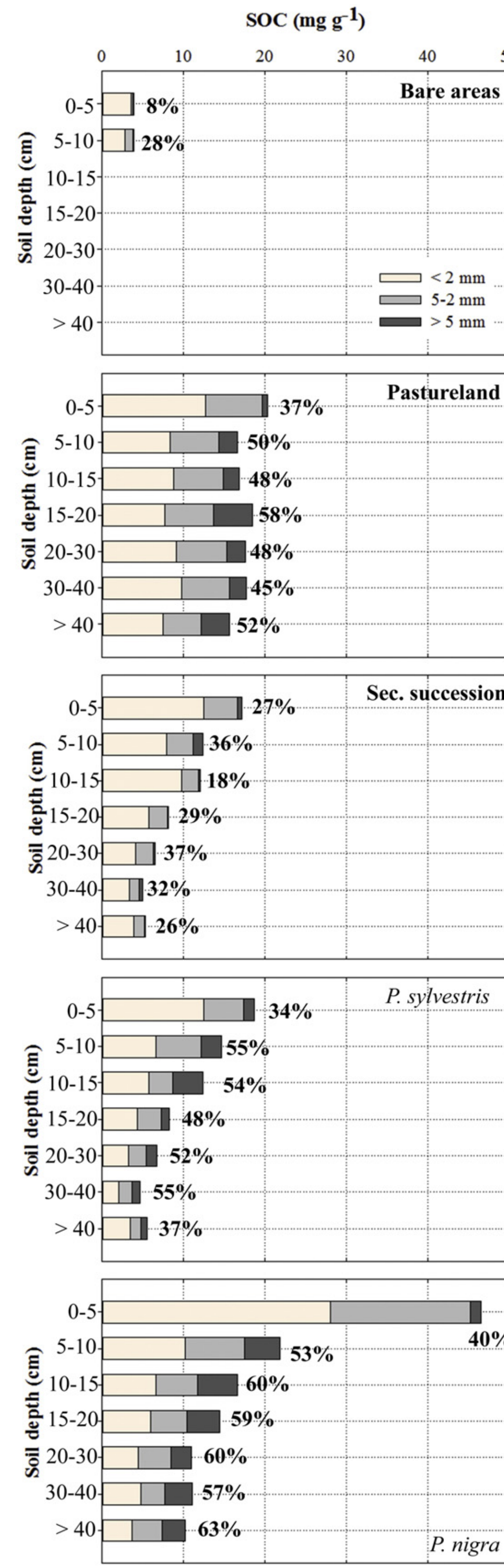

Fig. 4. Soil organic carbon (SOC) in the three different aggregate size fractions (<2 mm; 5–2 mm; >5 mm). Numbers in the figure refer to the contribution of the macro-aggregate to SOC (total SOC = 100%) in the respective depth.

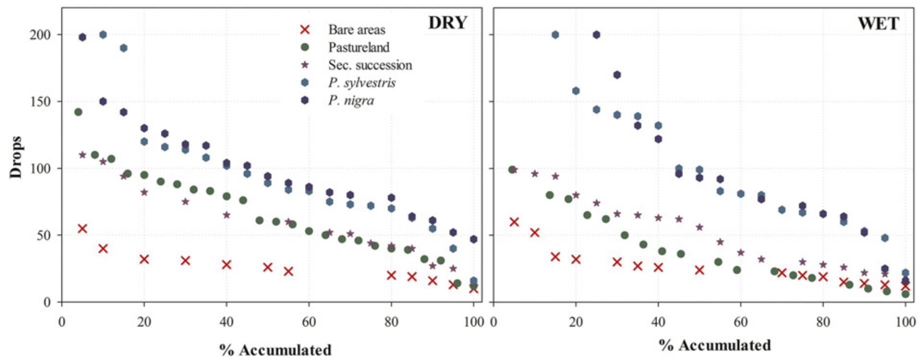

Fig. 5. Drop test results in dry and wet conditions in the different land covers. Accumulative curves that connect the percentage of resistant aggregates with the number of drops.

4. Discussion

4.1. Land abandonment and revegetation processes

This study has addressed the question of how SOC stocks change after land abandonment in Mediterranean humid mountain areas.

Several studies have shown that changes in land cover involved changes in SOC (Guo and Gifford, 2002). However, there is no agreement in the literature in relation to the effects of revegetation and afforestation in SOC (Cui et al., 2012). It has often been reported that afforestation of former agricultural fields can increase significantly SOC and N stocks/concentrations after few decades (Paul et al., 2002). Our study showed that after afforestation, litter accumulation (dense litter layer) and the presence of an organic horizon were the most remarkable difference between land covers. It seems evident that significant changes in SOC occur when degraded areas were afforested. The bare areas presented the soils with the lowest SOC contents due to the scarce and discontinuous litterfall supplies. Maestre et al. (2003) observed similar results in semi-arid Mediterranean afforested areas.

SOC and N contents tended to be more abundant in the topsoil (organic layer and 0–10 cm) than in deeper soils, showing significant differences in most cases, except in pasturelands. Our results also showed that below 20 cm similar SOC contents were observed in *PN* and pasturelands. In that sense, Bayramin et al. (2009) demonstrated that the differences of the SOC contents and stocks between pasturelands and *PN* were insignificant, indicating the ineffectiveness of pine plantations on changing the SOC content.

On the other hand, several studies demonstrated that secondary succession following land abandonment also determined the SOC content and stocks, and in general, in Mediterranean mountain areas an increase in SOC is reported due to an increase in the input or organic matter (Cammeraat et al., 2005; Lesschen et al., 2008; van Hall et al., 2016). Secondary succession is often accompanied by changes in soil structure that affect SOC storage, and the increase in litter and roots can favour

Table 2
Density fractionation results: soil recovery per fraction, SOC (Soil Organic Carbon) per fraction and contribution (%) of the different fractions to total SOC.

		Soil recovery (%)				SOC (mg g^{-1})			Recovery % SOC			
	Depth (cm)	FLF	OLF	HF	Total	FLF	OLF	HF	FLF	OLF	HF	Total
Bare	0–5	0.20	0.14	99.40	*99.70*	24.0	37.3	0.3	13.8	14.0	70.9	*98.7*
	5–10	0.12	0.10	97.70	*97.96*	20.3	31.9	0.3	7.7	10.1	78.8	*96.7*
Permanent pastureland	0–5	0.58	0.73	97.6	*98.91*	27.7	36.2	1.6	7.7	11.4	74.4	*93.6*
	5–10	0.13	0.16	99.23	*99.52*	30.8	36.0	1.5	2.2	3.5	91.5	*97.2*
	10–15	0.16	0.25	99.30	*99.71*	23.0	35.4	1.5	2.5	5.1	89.3	*96.9*
	15–20	0.37	0.20	98.50	*99.07*	28.6	36.7	1.7	5.7	4.0	86.4	*96.3*
	20–30	0.34	0.10	98.94	*99.38*	26.8	31.2	1.7	4.8	1.7	86.1	*92.6*
	30–40	0.39	0.21	97.02	*97.62*	28.6	27.0	1.8	6.0	3.0	86.2	*95.1*
	40–50	0.18	0.19	98.57	*98.93*	29.7	34.1	1.6	2.7	3.8	85.1	*91.5*
Secondary succession	0–5	0.54	0.45	97.71	*98.70*	29.1	34.7	1.5	9.0	9.1	79.3	*97.4*
	5–10	0.44	0.32	98.69	*99.45*	30.7	32.1	1.0	11.0	8.4	77.5	*96.9*
	10–15	0.30	0.38	98.47	*99.15*	26.2	28.4	1.0	7.2	8.9	82.3	*98.4*
	15–20	0.19	0.20	96.98	*97.36*	27.2	35.8	0.6	8.5	10.2	79.8	*98.5*
	20–30	0.10	0.21	98.15	*98.46*	23.0	36.1	0.5	4.1	12.5	81.5	*98.1*
	30–40	0.19	0.10	98.54	*98.83*	19.7	25.6	0.5	7.5	4.6	76.4	*88.5*
	40–50	0.20	0.57	97.35	*98.12*	27.2	25.6	0.3	9.4	30.1	59.9	*99.4*
Afforestation: *Pinus sylvestris*	0–5	1.49	1.10	97.08	*99.67*	27.5	37.7	1.2	21.2	21.6	52.7	*95.5*
	5–10	1.19	0.93	96.84	*98.96*	28.1	36.1	0.9	22.7	23.6	48.2	*94.5*
	10–15	0.48	0.87	96.08	*97.42*	28.6	36.2	0.9	10.1	27.4	59.3	*96.7*
	15–20	0.44	0.35	98.85	*99.64*	28.1	34.3	0.6	15.0	14.3	69.4	*98.7*
	20–30	0.29	0.32	97.85	*98.46*	27.9	34.7	0.4	13.0	18.5	67.1	*98.5*
	30–40	0.19	0.16	98.77	*99.12*	28.5	36.6	0.2	15.5	17.6	64.4	*97.5*
	40–50	0.15	0.24	97.59	*97.98*	24.8	29.0	0.3	9.7	18.2	80.5	*100.0*
Afforestation: *Pinus nigra*	0–5	3.40	1.83	92.87	*98.10*	32.8	37.7	3.5	26.9	15.1	61.1	*100.0*
	5–10	1.38	1.04	97.28	*99.70*	32.6	34.1	1.4	22.4	17.0	61.3	*100.0*
	10–15	0.27	1.88	96.55	*98.70*	23.7	33.3	1.1	4.6	36.8	58.5	*99.9*
	15–20	0.31	0.42	98.78	*99.51*	29.0	36.0	1.2	4.6	11.9	81.8	*98.3*
	20–30	0.15	0.36	98.98	*99.49*	23.2	35.7	0.9	3.2	13.3	83.1	*99.5*
	30–40	0.23	0.47	97.16	*97.86*	27.2	35.0	0.9	5.5	17.0	76.6	*99.1*
	40–50	0.18	0.21	97.81	*98.20*	29.4	36.5	0.8	4.0	10.1	83.7	*97.8*

FLF: free light fraction; OLF: occluded light fraction; HF: heavy fraction.

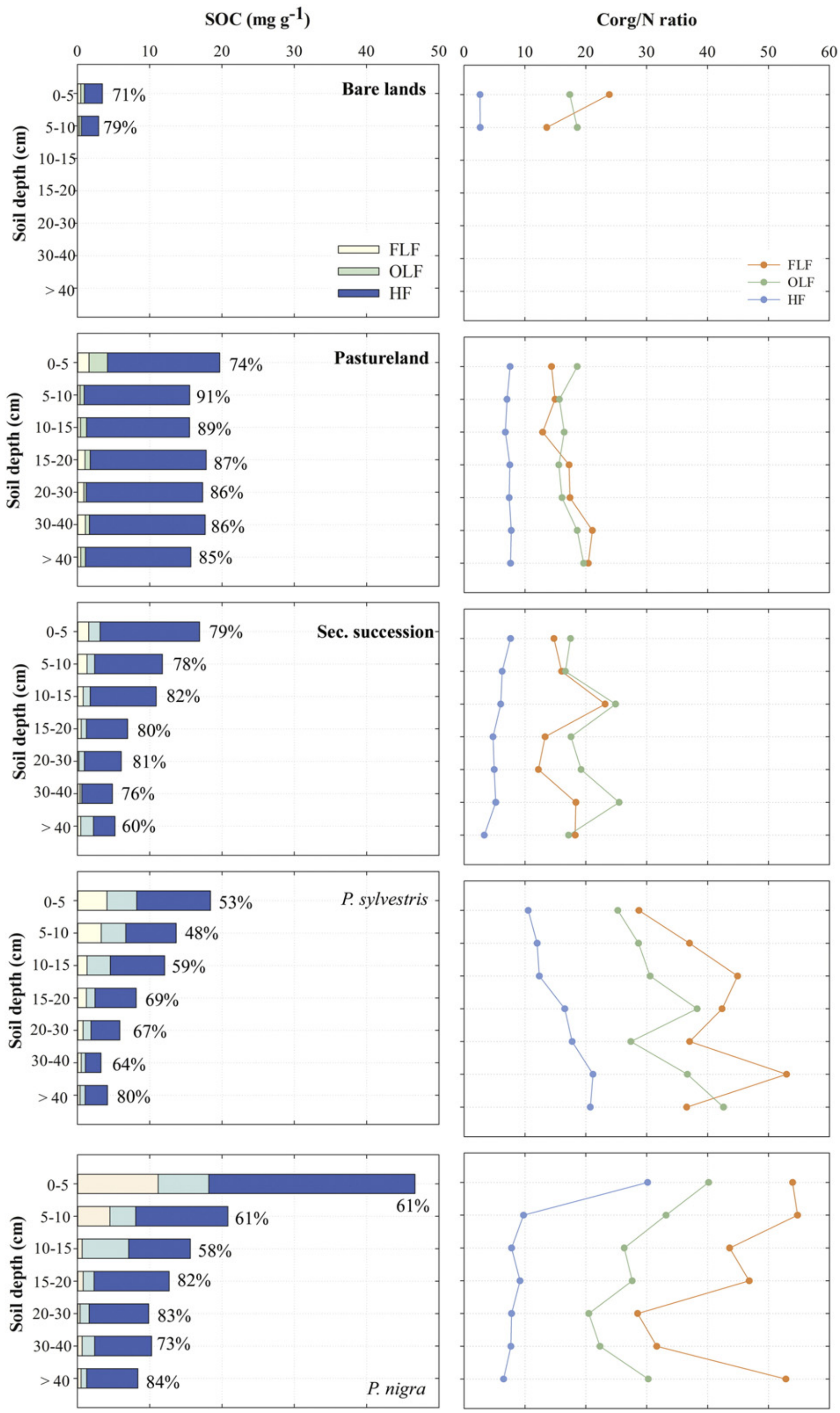

Fig. 6. Soil organic carbon and C_{org}/N ratios in the three different density fractions (FLF: free light fraction; OLF: occluded light fraction; HF: heavy fraction). Numbers in the figure refer to the contribution of the heavy fraction to SOC (total SOC = 100%) in the respective depth.

the formation of macro-aggregates and therefore an increase in the total SOC stock (Tisdall and Oades, 1982). However, our study suggested that SOC accumulation in soils under secondary succession after cropland abandonment is really slow, as it has been observed in other Mediterranean mountain areas (Novara et al., 2013; Gabarrón-Galeote et al., 2015a,b), whereas afforestation can accelerate the incorporation of SOC into the soil. In that sense, our research indicated that changes in SOC are limited to the first 10 cm after 50 years of land abandonment, and only significant changes were observed when afforestation practices were carried out. Albaladejo et al. (1998) noted the difficulty of Mediterranean ecosystems to recover their original SOC pools and cycling patterns after disturbance. Moreover, Nadal-Romero et al. 2016b showed that afforestation has not yet arrived at maximum potential as SOC sink (at least in the first 20 cm), compared with mature native

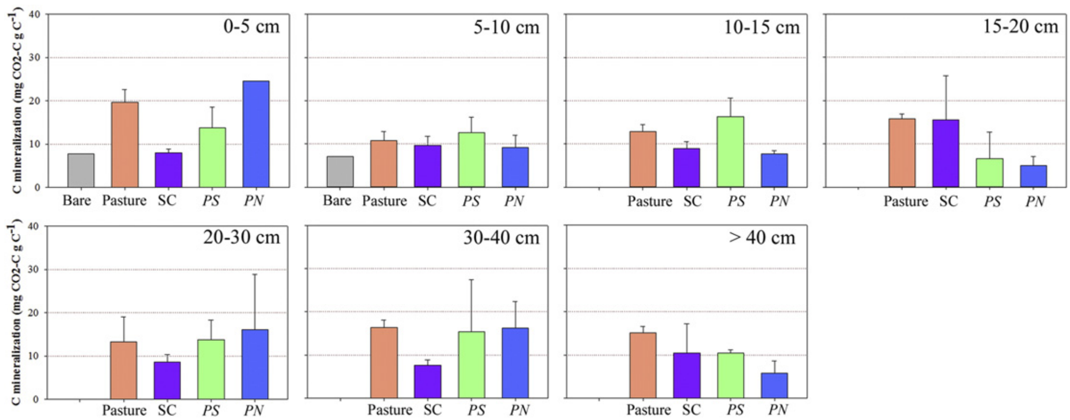

Fig. 7. C mineralization in the different soil depths and land covers. Note: SC: secondary succession; *PS*: *Pinus sylvestris*; *PN*: *Pinus nigra*.

forest (as reference system). This is consistent with earlier findings in Mediterranean regions (Fernández-Ondoño et al., 2010; Cuesta et al., 2012). Nevertheless, like most previous investigations on the land use effects on SOC, we could not know the background SOC stocks before land abandonment, which to some extent added uncertainties to our results.

Total SOC stocks ranged between 13.2 Mg C ha^{-1} and 136.5 Mg C ha^{-1}, following approximated order: *PN*>pasturelands>*PS*-secondary succession > bare areas (Fig. 3). If we had included only the organic layer and the upper mineral soils (0–10 cm) the afforestation would have significantly improved SOC stocks (13, 16, 17, 25, 40 Mg C ha^{-1} in bare, pastureland, secondary succession, *PS* and *PN* respectively). However, if the whole soil profile was taken into account the afforestation effects decreased, and only small differences were observed, showing pasturelands even larger values than *PS* sites. In that sense, Hiltbrunner et al. (2013) indicated that >80% of the studies only include topsoils, and consequently the effects of subsoils were mostly neglected, and Salome et al. (2010) estimated that in excess of 50% of the SOC stock is found in the subsoil (below 20 cm). Moreover, in pasturelands the proportion of SOC stored in depth is usually higher than in other land uses, due to their root-to shoot ratio is normally higher (Jobbagy and Jackson, 2000), highlighting the importance to include subsoil samples. Likewise, studies looking at SOC stocks confirmed the importance of pasturelands as a store of SOC in soils (Post and Kwon, 2000; Rodríguez-Murillo, 2001), and Boix-Fayos et al. (2009) indicated that SOC concentrations in pasturelands does not often differ significantly from those found in Mediterranean forest.

How SOC is stabilized after cropland abandonment? We have applied soil aggregate size and SOC fractionation techniques to isolate SOC pools. In general, cropland abandonment is often associated to aggregate formation (MacDonald et al., 2000) being a key process of soil development which promotes carbon stabilization by hindering decomposition of particulate/labile organic matter. Our results indicated that the revegetation processes due to secondary succession or afforestation practices increased the presence of macro-aggregates in the soil profile, stimulated by the high SOC concentration and the large contribution of fresh plant residues (Six et al., 2000). In the topsoils, afforestation significantly increased soil aggregation: coarser and stronger aggregates were observed coinciding with higher SOC.

Additionally, related to fractionation and SOC pools, our results demonstrated a significant higher contribution of FLF to total SOC in the afforested soils in the first 10 cm, as a consequence of: (i) higher litter input in combination with a high C_{org}/N ratio of litter in forest coniferous soils, (ii) a low microbial decomposability of coniferous forest litter (due to the enrichment of phenolic compounds), and (iii) protection in macro-aggregates (Helfrich et al., 2006; Yamashita et al., 2006). In that sense, some studies have demonstrated that FLF is more sensitive to management practices and land use changes that total SOC (Echeverría et al., 2014). The contribution of FLF decreased with depth, and significant differences were only observed in the *PN* sites between the topsoil and the subsoil. The few studies of density fractionation over the entire soil profile showed also declining contributions of FLF to total SOC with soil depth (John et al., 2005; Kögel-Knabner et al., 2008).

Carbon mineralization ranged from 0.15 to 0.81 mg CO_2–C g C^{-1} d^{-1} and decreased with depth. *PN* showed really high values in the topsoils due to the presence of labile material (litter accumulation). Topsoil horizons showed higher CO_2 respiration values than subsurface horizons,

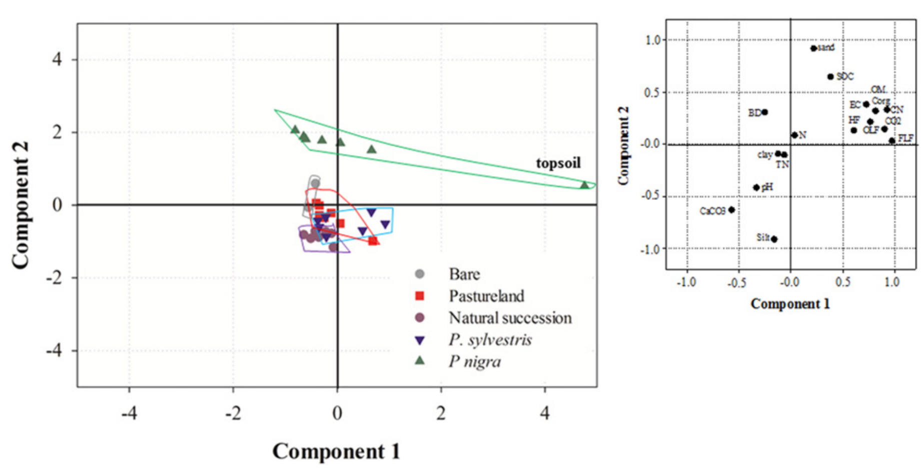

Fig. 8. Factorial analysis and distribution of soil samples upon the 1–2 factorial plane.

although no significant differences were observed between different depths or land covers. The reduction of CO_2 production with soil depth was previously reported by Ross et al. (1999). Low C mineralization rates in depth could be related to the low fresh organic matter inputs (FLF). These results were also reported by several authors (Muñoz et al., 2008; Álvarez and Álvarez, 2000). Kadono et al. (2008) indicated that the FLF of soil organic matter was one of the main soil variables explaining mineralized carbon. Such relationships could be expected, as the FLF comprises mainly freshly organic material. These results suggest that FLF is an important carbon fraction and the most sensitive to land cover changes. Additionally, C_{org}/N ratio of the HF in pasturelands and secondary succession was lower than in afforested sites (Fig. 6), which is related to an increase of the quality of the organic matter (Carrera et al., 2009; Condron et al., 2010), although further research about quality of organic matter is required. SOC in the secondary succession sites and pasturelands was rapidly stabilized due to the presence of litter with low C_{org}/N ratio. In that sense, Conant et al. (2011) indicated that the finer but denser root mat under pasturelands favours the formation of smaller but very stable aggregates in which SOC is stabilized.

4.2. Management practices and climate change

In terrestrial ecosystems, the amount of SOC is usually greater than the amount in living vegetation. It is therefore important to understand the dynamics of SOC, as well as, its role in terrestrial ecosystem, carbon balance and the global carbon cycle. Over the last few decades, afforestation has been a major strategy in Mediterranean countries, and an increase of about 18% in SOC stores is predicted due to the changes from crops to afforestation (Guo and Gifford, 2002). However, natural systems are complex and trees do not guarantee improved carbon sequestration, and although there was an increase in above and below ground biomass these gains were offset by losses in soil carbon. In that sense, to compensate the increasing atmospheric CO_2 levels and to mitigate current global warming, it is essential to increase not just SOC sequestration, but also favour its incorporation into compartments for which its stability could be assured for decades or centuries (Boix-Fayos et al., 2009; Nadeu et al., 2012).

The results of this study suggested that cropland abandonment and the subsequent revegetation processes (due to secondary succession and afforestation practices) will be of great importance in the impact of global change on SOC content and stocks in Mediterranean humid mountain areas. The findings suggested that for the time being (around 50 years), the secondary succession and the introduction of *PS* in Mediterranean humid mountain areas after land abandonment has not substantially improved SOC dynamics. However, higher SOC stocks and significant differences in aggregation processes and carbon pools were observed in *PN* sites especially in the first 10 cm. Moreover, further research should be carried out to investigate the differences observed between *PN* and *PS*. We hypothesized that the big differences in SOC stocks between the two studied conifers could be due to: (i) different planting systems, (ii) tree density, or (iii) higher hydric stress of *PS*. Finally, it is important to remark the important role played by pasturelands. The high root production provides the potential to increase SOC in pasturelands and vegetated fallow fields. Permanent pasturelands, with careful grazing management, can therefore play a vital role in sequestering carbon (also environmental, landscape and socio-economic positive effects (Lasanta et al., 2016)), and should be taken into account in land management and international agendas.

Afforestation is increasingly viewed as an environmental restorative land cover change prescription. Total areas of forest plantations can be expected to increase rapidly in the near future with carbon markets expanding demands for bioenergy increasing. However, there are still many uncertainties in the SOC dynamics, C mineralization and soil respiration after cropland abandonment in mediterranean humid mountain areas that should be addressed, and there is still a relevant question that emerges: How should we proceed? Our research has shown that after 50 years of cropland abandonment and management: (i) there is no control of extreme hydrological events due to afforestation practices (Nadal-Romero et al., 2016a), (ii) soil properties and consequently soil quality is similar in secondary succession in comparison to afforested areas (Nadal-Romero et al., 2016b), (iii) there are significant differences in SOC dynamics after afforestation with *PN* but ony limited to the first 10 cm, and (iv) permanent pasturelands should be considered in land management due to the relative importance in SOC stocks. In that sense, we suggested that the effects of cropland abandonment and afforestation practices on soil properties should be considered in the design of future forest restorations in Mediterranean humid areas, and strategies for SOC sequestration should be focused considering the complete soil profiles.

5. Conclusions

This study has provided novel information on the effects of cropland abandonment, land use changes and secondary succession and afforestation practices on SOC dynamics in a Mediterranean humid mountain area (Central Pyrenees). Our findings demonstrated that contrary to our hypothesis, only afforestation practices with *P. nigra* can accelerate the recovery of SOC in the first 10 cm, and no changes were observed under secondary succession.

The following conclusions can be made:

(i) The afforestation of former arable soils resulted in a constant SOC accumulation in the organic and mineral horizons due to litter deposition, stabilization processes, and lack of disturbance.
(ii) SOC concentrations only slightly increased in the topsoil (0–10 cm) and decreased in subsoil. SOC stocks were higher in the *PN* sites followed by pasturelands.
(iii) Most of the SOC changes occurred in the topsoil layer (10 cm), however it is necessary to include the deepest layers (subsoil), because SOC stocks below 20 cm did not change significantly between pasturelands and *PN*.
(iv) SOC accumulation under secondary succession in Mediterranean humid mountain areas is a slow process.
(v) Even if the total SOC did not change significantly over time following land abandonment and revegetation processes, aggregation processes increased, and SOC pool fractions dynamics significantly changed after afforestation practices in the first 10 cm.
(vi) The relative contribution of macro-aggregates to total SOC was significantly higher at *PN* sites and tended to increase with depth. In terms of carbon content, micro-aggregates at 0–5 cm had the highest SOC contents. In the topsoil, afforestation significantly increased soil aggregation: coarser and stronger aggregates coinciding with higher soil organic carbon.
(vii) SOC enrichment in the FLF fractions of the afforestation sites in the topsoil was the main driver of soil respiration. FLF is unprotected SOC that can easily be mineralized into CO_2.
(viii) Our results from the aggregate size distribution and density fractionation would suggest aggregation as an important mechanism promoting SOC stocks after afforestation. However, data from our respiration experiments suggested that aggregation at the afforested sites did not result in direct SOC stabilization.
(ix) Pasturelands should also be considered in land management due to the relative importance in SOC stocks.

Acknowledgments

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