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Storage of organic carbon and Black carbon in density fractions of calcareous soils under different land uses

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ABSTRACT

The association between soil particles and their spatial arrangement plays a key role in soil organic matter (OM) dynamics. Density fractionation combined with ultrasonic dispersion enables separation and study of soil OM fractions, considered on the basis of the mechanisms of physical protection: non-physically protected OM (FF), OM occluded into aggregates (OF), and OM stabilized in organo-mineral complexes (DF). In the present study, whole soils and density fractions of calcareous soils under three different management systems — native *Quercus ilex* forest, a *Pinus halepensis* plantation and cropped land — were analyzed for organic C (OC), total N, and Black carbon (BC) content. Black carbon is often considered as a very recalcitrant pool in the soil. However, as well as BC content of soils has seldom been quantified, long-term studies on BC stability are scarce and conclusions about BC stability are not widespread.

About 67% of the total C in the topsoil was lost as a result of converting the natural *Q. ilex* forest to cropped land, 100 years ago. After crop abandon, the stock of OC in the topsoil greatly increased upon reforestation of the studied plot with *P. halepensis*. An average recovery of 71% of the previously lost OC had been recovered, after 40 years of pine plantation. The changes in OC stocks affected mainly the free fraction (FF). Black carbon represented between 1.2 and 2.3% of the TOC of soil with the highest concentrations in OF. The maintenance of BC proportion through land uses suggests an equilibrium between inputs and outputs, and leads to the suspicion that BC could be less stable and less resistant to biodegradation than is often taken for granted.

1. Introduction

The reservoir of soil organic matter (SOM) has been proposed as both a significant source and sink of atmospheric CO_2. Because of this, the capacity of soils to accumulate and stabilize organic carbon has received great attention in recent years. In particular, SOM is recognized as an important compartment in C-driven climate change (Sanderman et al., 2003). The long-term storage of C in soil ecosystems is determined by the balance between the rate of incorporation of new organic matter (OM) in soil and the decomposition of SOM (Johnson, 1995). The properties of the soil matrix play an important role in the protection of SOM against biodegradation. It is generally accepted that Black carbon (BC) represents a long-term carbon sink in soil (Kuhlbusch, 1998; Schmidt and Noack, 2000) and that, therefore, BC plays an important role in the global carbon cycle. The amount of available data about BC content of soils is already considerable, but for Mediterranean ecosystems it is still scarce, particularly in calcareous soils.

Calcareous soils, which represent approximately 12% of the world soil resources (FAO, 1996), are of particular interest because of the high stability of their OM. Some studies of the decomposition dynamics of SOM in calcareous soils (García et al., 1997; Llorente et al., 2008; Llorente and Turrión, 2010) have shown that the decomposition rates in such soils are lower than those in other soils with similar organic carbon content.

SOM is heterogeneous and it is possible to distinguish OM fractions that vary in their stability and therefore in their intrinsic decay rates, ranging from a few days to hundreds of years (Schimel et al., 1985). The location of SOM within the soil matrix is considered a major factor determining its turnover (Tamon et al., 2006). There are two main mechanisms of SOM physical stabilization based on the location of the SOM within the soil matrix: (1) physical protection by aggregates; and 2) OM stabilization by organo-mineral complex formation. Physical fractionation methods, such as density fractionation (after ultrasonic dispersion or not), enable separation and study of SOM fractions differing in dynamics, structure and function (Golchin et al., 1994a; Six et al., 2001). Several studies have addressed the effect of land use on the size and composition of different SOM fractions (e.g., Preston et al., 1994; Golchin et al., 1995; Guggenberger et al., 1995; Gregorich et al., 1996; Helfrich et al., 2006). Subdividing SOM according to physical properties highlights the observation that

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physical location within the soil matrix is a key factor determining turnover (Balesdent, 1996).

SOM fractionation by density following ultrasonic dispersion enables separation and study of three different fractions according to the different mechanisms of physical protection (Sohi et al., 2001). Based on these stabilization mechanisms, it is possible to distinguish three SOM fractions: (1) "free" OM (free fraction, FF), isolated before ultrasonic break-down of stable aggregates; (2) OM occluded within aggregates (occluded fraction, OF), isolated after ultrasonic dispersion to break the aggregates; and 3) organo-mineral fraction (dense fraction, DF) recovered as the residual (heavy) material. Many studies (e.g. Golchin et al., 1994b; Six et al., 2002b; John et al., 2005) have shown that the unprotected FF represents a labile SOM pool with a rapid turnover rate. In contrast, OF and DF are more stable pools, with turnover times ranging from decades to centuries. Therefore, density fractions may be affected differently by changes in land use.

In Mediterranean forests, very prone to wildfires, Black carbon is expected to be found in relevant amounts. Thus it is worth to quantify its presence in the overall soil, and also its distribution among the density fractions.

In this study, carried out with soils from a calcareous moor in the region of *Castilla y León* (northwestern Spain), we studied 36 soils, taken from plots under contrasting land uses: native *Quercus ilex* stands, cereal crops, and *Pinus halepensis* plantations. Density separation was carried out in combination (or not) with ultrasonic dispersion in order to obtain SOM fractions according to different mechanisms of physical stabilization. The objectives of the present study were a) to compare the effect of land use on SOM content, b) to study the effect of land use/land cover on the distribution and characteristics of density fractions, and c) to quantify and to compare the effect of land use on BC content and distribution on whole soil and density fractions.

2. Materials and methods

2.1. Site description

The study was carried out in a calcareous moor in the region of Castilla y León (northwestern Spain), UTM: 30T 384465 E 4639001N. The mean annual rainfall in the region is below 400 mm, under a xeric moisture regime, and the mean annual temperature is approximately 12.3 °C. The altitude of the moor is between 800 and 900 m, with low slopes (<7%). 'The soils (Xerepts, according to USDA, 2006) are quite homogeneous in spite of differences in their land use history. The native vegetation in the studied calcareous soils is Holm-oak wood (*Q. ilex* subsp *ballota*). In the 19th century, most of the natural forest was converted into agricultural land (cereal crops), but since the 1950s, reforestation with *P. halepensis* has been carried out on abandoned agricultural land.

2.2. Sampling procedures

A land use map of the calcareous moor of Castilla y León was elaborated with a GIS (ArcGis 9.0 for Windows) (Fig. 1). The map was used to select the sampling plots on the basis of the following criteria: a) *Q. ilex* forest, cropped land, and *P. halepensis* plantations in adjacent areas; b) minimum area of each land use, 1 ha; and c) establishment of each land use for at least 40 years. For this study, plots of the above-mentioned land uses were selected in three different regions of the calcareous moor, and four representative plots were sampled (0–10 cm depth). A total of 36 topsoils (0–10 cm depth) (3 regions × 3 land uses × 4 plots) were thus sampled. A composite sample, by joining 3 soil subsamples, was obtained for each plot.. Visible plant residues and roots were removed; soil was air-dried, sieved (<2 mm) and stored in plastic bags until analysis.

For soil characterization, % of carbonates, texture, pH, total N, total C, organic C, and C/N were determined. Total concentrations of soil C and N were determined with an automated C/N analyzer (CHN-2000, Leco). Organic carbon was calculated as the difference between total and carbonate carbon. Soil total calcium carbonates were determined by use of 1 M HCl titrated with 0.5 M NaOH (FAO, 2007).

2.3. Ultrasonic equipment

We used a Branson 450 W Sonicator, equipped with a titanium probe. The probe depth was fixed in 15 mm. The sonicator was calibrated by determining the real power output calorimetrically (North, 1976). The probe output energy was calculated from:

$$P = (m_w c_w + C_{cont})\ \Delta T\ t^{-1} + Ht,$$

where P is the calculated power (W), m_w is the mass of water (g), c_w is the specific heat of water (4.18 J g^{-1} K^{-1}), C_{cont} is the heat capacity of the container (J K^{-1}), ΔT is the temperature change (K), t is the sonication time (s) and H is the heat loss (J s^{-1}). The heat capacity of the glass beaker (C_{cont}) was determined using the method of mixtures (Morra et al., 1991), according to:

$$C_{cont} = m_1 c_w \left((T_1 - T_2)\ (T_3 - T_2)^{-1} \right) - m_2 c_w,$$

where C_{cont} and c_w are as above and m_1 is the mass (g) of an amount of water heated to T_1 (K), which is added to the beaker that already contained an amount of water m_2 (g) at room temperature (K). The final equilibrium temperature (K) of the water in the beaker is T_3.

The wanted energy output was 300 J ml^{-1} and the corresponding time was calculated from:

$$t = m_s E\ P^1,$$

where t is the sonication time (s), m_s is the mass of soil (g), E is the energy (J g^{-1}) and P is the power output (W).

2.4. Density fractionation of soil

A density fractionation procedure was applied to the topsoils (0–10 cm depth) of the 36 sampled plots. A flow diagram of the method used is provided in Fig. 2. The method follows the concept of Golchin et al. (1994a), who differentiated three degrees of physical protection of OM: FF, non-protected; OF, occluded within aggregates — extractable by sonication; and DF, retained in the dense residual material after sonication.

Briefly, 5 g of soil sample was placed in small centrifuge bottles (50 ml capacity), and 35 ml of NaI at 1.8 g ml^{-1} density were added. The bottles were shaken gently and the floating material, considered as the FF, was then recovered by centrifugation at 8000 g for 30 min at 18 °C, and filtered over a vacuum filter, using a glass fiber filter (Whatman GF/F), by washing with deionized water. The recovered NaI (not mixed with the washing water) was added to the residue remaining in the bottle and the solution was fitted to 1.8 g ml^{-1} density. The bottle was placed in an ice bath and sonicated at 300 J ml^{-1} with a probe-type ultrasonic disintegrator (Branson 450 W).

The floating material, considered as the OF, was recovered by centrifugation and washed in the same way as the FF. The remaining material, considered the DF, was washed with deionized water. All fractions were dried at 40 °C, weighed, ground in a mortar and pestle, and analyzed for C, N and Black carbon contents. Carbonates were analyzed for the whole soils and it was assumed that all carbonates were recovered in the DF. To obtain enough sample to perform analysis, it was necessary to accumulate several replicates of the fractions.2.5. Black carbon analysis.

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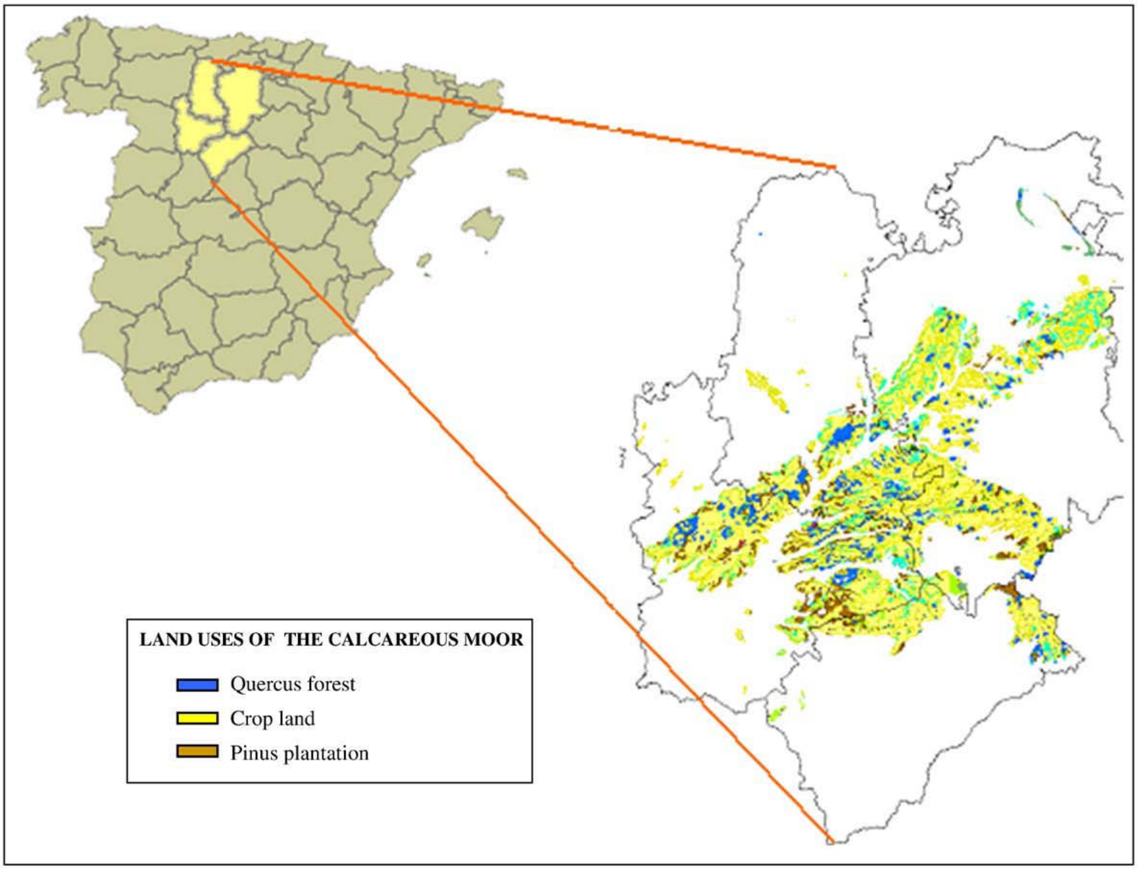

Fig. 1. Land use map of the calcareous moor of Castilla y León.

Black carbon was analyzed using benzenepolycarboxylic acids (BPCA) as a molecular marker, following the indications of Glaser et al. (1998). Briefly, 0.5 g of ground sample was digested with 10 ml of 4 mol l^{-1} trifluoroacetic acid (TFA) for 4 h at 105 °C (modification proposed by Brodowski et al., 2007).

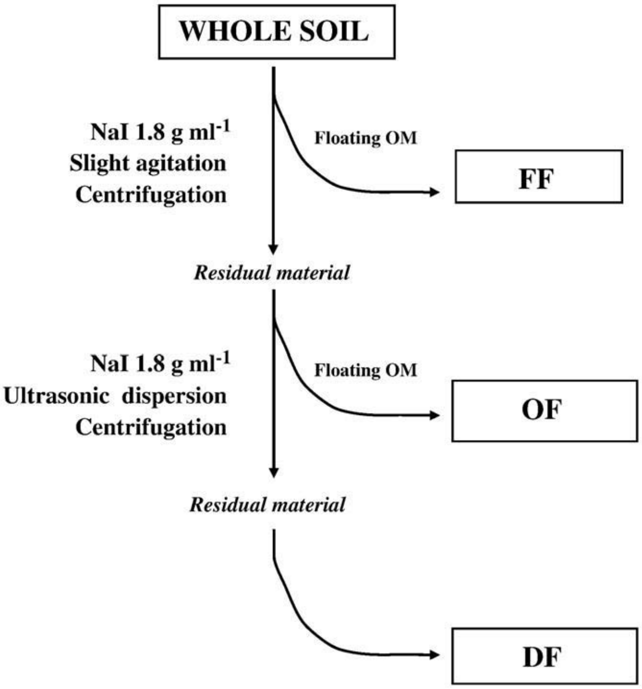

Fig. 2. Flow diagram of the density fractionation method.

The residue was collected by filtration through a glass fiber filter, rinsed several times with deionized water and dried at 40 °C for 2 h. The residue was quantified and transferred to a glass digestion tube and oxidized with 2 ml of 65% HNO_3 for 8 h at 170 °C at high pressure. The digestion solution was poured through an ash-free cellulose filter into a 10 ml volumetric flask, and the volume made up with deionized water. An aliquot of 2 ml was diluted with 4 ml of deionized water and 100 µl of the first internal standard containing 100 µg citric acid were added. The solution was cleaned using a cation exchange resin (Dowex 50WX8, 200–400 mesh). The BPCAs were freeze-dried in conical flasks, then re-dissolved in methanol and transferred to reactivials. Then 100 µl of a second internal standard solution containing 100 µg of biphenyl-2,2-dicarboxylic acid were added prior to drying the solvent by evaporation. The BPCAs were derivatized to trimethylsilyl derivatives by adding 125 µl of pyridine and 125 µl of N,O-bis(trimethylsilyl)-trifluoroacetamide and heating to 80 °C for 2 h. Capillary gas chromatography was performed in an HP 6890 instrument equipped with an HP-5 capillary column (30 m × 250 µm × 0.25 µm film thickness) and a flame ionization detector (FID). For correct data acquisition, standard solutions of 20, 50, 100, 250 and 500 µg of BPCAs in methanol were used. The sum of the yields of BPCA was multiplied by a correction factor of 2.27 for BC quantification (Glaser et al., 1998).

2.5. Statistical analyses

Analysis of variance (ANOVA) was used: a) to compare the yields, C and N contents, and C/N ratio, of the fractions, overall or for a given land use type, and b) to compare different land use for a given

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Table 1
Physicochemical properties of the soil samples.

Area	Land use	Texture	$CaCO_3$ (%)	OC (%)	N (%)	OC/N	pH
Cerrato	Cropped land (CL)	Clay loam	19.9 ± 8.8	2.78 ± 0.62	0.25 ± 0.12	11.0 ± 2.1	8.1 ± 0.1
Cerrato	*Quercus* forest (QF)	Clay loam	20.9 ± 4.6	5.42 ± 0.74	0.41 ± 0.19	13.1 ± 2.3	7.9 ± 0.1
Cerrato	*Pinus* plantation (PP)	Clay loam	18.4 ± 1.8	5.45 ± 0.71	0.38 ± 0.11	14.1 ± 2.6	7.9 ± 0.1
Ampudia	Cropped land (CL)	Clay loam	10.1 ± 1.0	1.59 ± 0.38	0.18 ± 0.09	10.6 ± 2.1	8.1 ± 0.1
Ampudia	*Quercus* forest (QF)	Clay loam	13.4 ± 1.7	5.87 ± 0.82	0.46 ± 0.18	12.7 ± 2.4	8.1 ± 0.1
Ampudia	*Pinus* plantation (PP)	Clay loam	22.2 ± 3.2	3.47 ± 0.51	0.21 ± 0.08	21.0 ± 3.1	8.2 ± 0.1
Monte Viejo	Cropped land (CL)	Clay loam	59.8 ± 9.8	1.56 ± 0.29	0.26 ± 0.07	10.6 ± 1.7	8.3 ± 0.1
Monte Viejo	*Quercus* forest (QF)	Clay loam	31.1 ± 7.3	6.51 ± 0.80	0.50 ± 0.20	11.9 ± 1.9	7.9 ± 0.1
Monte Viejo	*Pinus* plantation (PP)	Clay loam	42.0 ± 6.9	3.70 ± 0.67	0.36 ± 0.08	13.1 ± 1.9	8.1 ± 0.1

Means ± standard error for $n = 4$.

fraction. In case of significant *F*-statistics ($p > 0.05$), differences between means were tested with the Tukey multiple comparisons procedure. Data were tested for normality and homoscedasticity with the Kolmogorov–Smirnov and Levene statistics, respectively.

The statistical analyses were performed with the Systat 14.0 Statistical Software Package (SPSS for Windows).

3. Results

3.1. Effect of land use on C storage in whole soils and density fractions

The main characteristics of the samples are shown in Table 1.

Percentage of OC in topsoil (from 0 to10 cm depth), under a land use change from a natural *Q. ilex* forest to cropped land (100 years ago) showed an average net drop of 67%. The subsequent reforestation of the studied calcareous soils with *P. halepensis* resulted in a great recovery of OC. An average OC recovery of 71% of the OC (referring to % of OC in soils under *Q. ilex* forest) was measured in topsoil (from 0 to10 cm depth) after 40 years of pine plantation.

The free and intra-aggregate fractions obtained by density fractionation were visually distinct. The FF comprised recognizable plant material, whereas the intra-aggregate was amorphous dark material. The recovery of fractions with respect to the initial soil weight varied between 98 and 103% (Table 2). Recoveries higher than 100% may be due to incomplete removal of NaI.

As expected, most of the fine soil mass was located in the DF (Table 3), and OF represented a minor part of the soil mass. The yields of all the density fractions were significantly different among land uses, with a significantly greater amount of FF in soils under tree cover than in the soils under agricultural land use, due to greater incorporation of organic matter.

Comparison of OC concentration ($g\ C\ g^{-1}$ fraction) among density fractions revealed the lowest values in DF, as the latter was dominated by minerals, and the highest values in OF (Table 4). Cropped land presented the lowest OC concentration overall fractions. As regards the % of OC in soil that is represented by each fraction, the major portion of OC was associated with DF (Table 4). The DF accounted for about 83% of TOC under cropped land, but for about 53% of TOC in topsoils (0–10 cm depth) under tree cover, with no-significant differences among soils under *Pinus* or *Quercus* vegetation.

Table 2
Recoveries of mass, total organic C (TOC) and total N (TN) after density fractionation.

	% of soil weight	% of TOC	% of TN
Cerrato			
CL	100.33 ± 1.33	99.42 ± 3.81	95.20 ± 5.12
QF	102.72 ± 3.43	99.51 ± 2.12	98.90 ± 5.93
PP	102.14 ± 2.12	100.78 ± 1.21	84.54 ± 3.16
Ampudia			
CL	98.46 ± 5.10	98.77 ± 4.27	99.51 ± 3.12
QF	102.33 ± 3.52	107.19 ± 9.11	80.95 ± 7.74
PP	100.92 ± 4.11	102.28 ± 2.69	97.94 ± 5.60
Monte Viejo			
CL	99.88 ± 2.14	102.81 ± 3.15	98.88 ± 2.98
QF	102.63 ± 5.04	99.20 ± 4.29	90.30 ± 9.02
PP	101.63 ± 1.44	101.23 ± 3.37	89.89 ± 7.84

CL: Cropped land; QF: *Quercus* forest; PP: *Pinus* plantation; FF: free fraction; OF: occluded fraction; DF: dense fraction. Means ± standard error for $n = 4$.

The recovery of organic C after fractionation ranged from 97% to 107%, with an average of 101% (Table 2). These recoveries are too much high, taking into account that the repeated soil washing and decanting is expected to result in losses of soluble organic matter, especially if an ultrasonic treatment is applied. Such high recoveries may be due to organic impurities contained in the NaI solution, and/or to the possible presence in OF and/or FF of small amounts of carbonates, whose carbon could have been taken as organic C. Owing to all these constraints, the data for C cannot be taken as precise estimations.

3.2. Effect of land use on N storage and C/N ratio in whole soils and density fractions

As regards N content, FF was the most sensitive fraction to land use change.

The highest concentration of N was found in the OF. However this fraction represented the lowest contribution to total N (always $<3\%$ of total N). In contrast, the DF shows the lowest N concentration but contributed the most to total N (Table 5).

The OC/N ratio was significantly higher ($p < 0.05$) in FF than in the DF. Comparison of the OC/N ratio in FF among the different types of land use, revealed significantly higher values ($p < 0.01$) in soils under pine forest (Table 5).

The recovery of N after fractionation ranged from 81% to 99% (Table 2), expectable recoveries because of the extraction of soluble N from repeated washing during the fractionation process.

3.3. Black carbon content in whole soils and density fractions

Black carbon represents a small proportion of the whole soil (from 0.4 to 3.1 $mg\ g^{-1}$ of soil) and from 1.2 to 2.3% of the soil TOC (Table 6).

A large part of BC was not associated with the mineral phase. As shown in Table 7, there were differences among fractions, both in BC concentration and in % of BC with respect to TOC, in the following order: DF < FF < OF. Between 0.9 and 2.1% of the OC of FF corresponded to BC, and between 1.2 and 3.4% of the OC in OF. Only BC content in OF was sensitive to changes in land uses, showing higher concentration in cropped land than in soils under tree cover.

4. Discussion and conclusions

4.1. Effect of land use on C storage in whole soils and density fractions

Land use change from a natural *Q. ilex* forest to cropped land (100 years ago) has resulted in an average net drop of 67% in the % of

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Table 3
Density fraction yields.

	FF	OF	DF
	(% of soil weight)		
Cerrato			
	b	*c*	*a*
CL	1.20 ± 0.02 C	0.27 ± 0.11 B	98.86 ± 0.38 A
QF	9.44 ± 0.37 A	0.58 ± 0.28 A	92.70 ± 1.91 B
PP	7.38 ± 0.33 B	0.45 ± 0.09 AB	94.31 ± 3.43 AB
Ampudia			
	b	*c*	*a*
CL	1.06 ± 0.50 C	0.11 ± 0.02 B	97.30 ± 2.00 A
QF	9.06 ± 0.20 A	0.73 ± 0.32 A	92.54 ± 3.53 B
PP	4.32 ± 1.38 B	0.21 ± 0.11 AB	96.39 ± 3.52 AB
Monte Viejo			
	b	*c*	*a*
CL	1.09 ± 0.12 C	0.08 ± 0.58 B	98.71 ± 0.34 A
QF	10.85 ± 1.94 A	0.72 ± 0.16 A	91.06 ± 1.16 B
PP	7.20 ± 0.14 B	0.16 ± 0.10 AB	94.27 ± 3.65 AB

CL: Cropped land; QF: *Quercus* forest; PP: *Pinus* plantation; FF: free fraction; OF: occluded fraction; DF: dense fraction. Fractions indicated with the same italic letter are not significantly different ($p<0.05$). Land uses indicated with the same capital letter are not significantly different ($p<0.05$) for a given fraction. Means ± standard error for $n=4$.

OC in the topsoil (from 0 to 10 cm depth). These results are consistent with results reported by Llorente and Turrión (2010) for the same region. Such loss is also consistent with the findings of Burke et al. (1989), who reported 50% of SOC loss for land use transformation from grassland to crop land, and the findings of other studies (Prior et al., 2000) showing that cultivation generally decreases the amount of organic matter. Soil tillage induces soil C loss by acceleration of organic C oxidation, which results in the release of large amounts of CO_2 to the atmosphere (La Scala et al., 2008). Another tillage-related factor that contributes to soil C losses is disruption of the soil aggregates, which exposes once-protected organic matter to decomposition (De Gryze et al., 2006; Grandy and Robertson, 2007).

Reforestation of the studied calcareous soils with *P. halepensis* resulted in a great recovery of OC. An average recovery of 71% of the OC (referring to % of OC in soils under native *Q. ilex* forest) was measured in topsoil (from 0 to10 cm depth) after 40 years of pine plantation.

Comparison of OC concentration (g C g^{-1} fraction) among density fractions revealed the lowest values in DF, as the latter was dominated by minerals, as suggested by Golchin et al. (1994b, 1995), and the highest values of OC concentration were found in OF. It has been suggested that such high concentration is due to the physical protection of OM by aggregates attributed to compartmentalization of substrate and microbial mass (Killham et al., 1993; Six et al., 2002a).

As regards the % OC in soil that is represented by each fraction, the major portion of OC was associated with DF (Table 3). The DF accounted for about 83% of TOC under cropped land, but for about 53% of TOC in topsoil under tree cover, with no significant differences among soils under *Pinus* or *Quercus* vegetation. These values are consistent with the findings of John et al. (2005), who reported that 86–91% of total SOC was associated with the mineral-associated SOM fraction at grassland, maize and wheat sites in silty soils; in contrast, the free and occluded fraction accounted for 52% of total SOM in a spruce stand on similar soil.

4.2. Effect of land use on N storage and C/N ratio in whole soils and density fractions

The highest concentration of N was found in OF. However, this fraction represented the lowest contribution to total N (always <3% of the total N). In contrast, DF showed the lowest N concentration but contributed the most to total N (Table 4).

OC/N ratio was significantly higher ($p<0.05$) in FF than in DF. The OC/N ratio value was significantly lower in DF than in OF (Table 4), in contrast to the findings of Rovira and Vallejo (2003) who reported significantly higher OC/N in DF in soils over calcareous material and under *Quercus rotundifolia*, but in agreement with the data of Roscoe and Buurman (2003) and Golchin et al. (1994b) who observed somewhat higher C/N ratios for FF and also according to those of Kölbl and Kögel-Knabner (2004), who found no differences between FF and OF.

Differences among land uses were only significant in FF. The higher values of OC/N ratio for the soils under pine forest were quite expectable, in aggrement with references such as Duchaufour, 2001. Results are also consistent, with the findings of different studies on litter composition, such as that by Traversa et al. (2008), who compared the C/N ratio of the litter under *P. halepensis* and *Q. ilex*.

4.3. Effect of soil management on Black carbon content in whole soils and

In studied calcareous soils, Black carbon concentration ranged from 0.38 to 3.07 mg BC g^{-1} of whole soil and the proportion of the TOC represented by BC, ranged from 12.18 to 23.09 mg BC g^{-1} of TOC. These concentrations are lower than those found in the soils analyzed by Dai et al. (2005) or in the soils studied by Glaser and Amelung (2003), using the same method. However, our values are only slightly

Table 4
Organic carbon (OC), %OC of total OC of the soil and OC/N in density fractions.

	OC (mg C g^{-1}fraction)			%OC (of total OC of soil)		
	FF	OF	DF	FF	OF	DF
Cerrato						
	b	*a*	*c*	*b*	*c*	*a*
CL	227 ± 29 B	360 ± 11 B	21 ± 1 B	10.9 ± 3.9 B	3.8 ± 0.9 A	84.7 ± 3.4 A
QF	276 ± 22 AB	386 ± 7 A	25 ± 2 AB	50.6 ± 3.2 A	4.3 ± 1.4 A	44.6 ± 2.6 B
PP	296 ± 11 A	383 ± 10 A	27 ± 4 A	44.1 ± 4.3 A	3.5 ± 1.7 A	53.2 ± 3.8 B
Ampudia						
	b	*a*	*c*	*b*	*c*	*a*
CL	229 ± 27 B	331 ± 18 B	14 ± 4 B	14.3 ± 2.4 B	2.1 ± 2.5 A	82.4 ± 5.3 A
QF	266 ± 24 AB	364 ± 16 AB	28 ± 1 A	47.1 ± 3.2 A	5.2 ± 1.3 A	54.9 ± 3.7 B
PP	319 ± 31 A	391 ± 13 A	26 ± 3 A	37.6 ± 5.1 A	2.1 ± 1.7 A	62.5 ± 4.2 B
Monte Viejo						
	b	*a*	*c*	*b*	*c*	*a*
CL	227 ± 21 B	343 ± 11 B	12 ± 2 B	17.9 ± 4.8 B	2.1 ± 2.4 A	82.9 ± 6.3 A
QF	259 ± 16 AB	376 ± 9 A	33 ± 6 A	43.2 ± 3.7 A	4.2 ± 1.8 A	50.9 ± 3.2 B
PP	295 ± 22 A	389 ± 14 A	20 ± 7 AB	48.7 ± 1.2 A	2.3 ± 0.7 A	50.2 ± 1.7 B

CL: Cropped land; QF: *Quercus* forest; PP: *Pinus* plantation; FF: free fraction; OF: occluded fraction; DF: dense fraction. Fractions indicated with the same italic letter are not significantly different ($p<0.05$). Land uses indicated with the same capital letter are not significantly different ($p<0.05$) for a given fraction. Means ± standard error for $n=4$.

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Table 5
Nitrogen (N), %N of total N of the soil and OC/N in density fractions.

	N (mg N g^{-1}fraction)			%N (of total N of soil)			OC/N		
	FF	OF	DF	FF	OF	DF	FF	OF	DF
Cerrato									
	b	*a*	*c*	*b*	*c*	*a*	*a*	*ab*	*b*
CL	15.1 ± 0.1 A	26.7 ± 4.1 A	2.0 ± 0.2 A	7.6 ± 3.2 B	3.0 ± 0.3 A	84.5 ± 6.1 A	15.1 ± 3.3 B	13.4 ± 3.2 A	10.6 ± 0.7 A
QF	14.6 ± 0.3 B	18.7 ± 3.2 A	2.0 ± 0.1 A	40.3 ± 5.9 A	3.2 ± 0.5 A	55.4 ± 2.1 B	18.9 ± 0.5 B	20.6 ± 6.1 A	12.1 ± 2.3 A
PP	13.4 ± 0.8 B	18.8 ± 3.8 A	1.9 ± 0.3 A	28.3 ± 7.1 A	2.4 ± 0.2 A	53.8 ± 4.8 B	22.2 ± 0.9 A	20.4 ± 4.7 A	14.1 ± 3.1 A
Ampudia									
	b	*a*	*c*	*b*	*c*	*a*	*a*	*ab*	*b*
CL	15.3 ± 2.3 AB	35.3 ± 10.2 A	0.9 ± 0.5 A	14.7 ± 3.5 B	3.4 ± 0.3 A	81.4 ± 6.4 A	15.0 ± 2.9 B	9.4 ± 7.1 A	15.7 ± 0.4 A
QF	16.5 ± 0.6 A	17.6 ± 7.3 A	1.9 ± 0.7 A	34.7 ± 5.2 A	3.0 ± 0.2 A	43.3 ± 6.6 B	16.1 ± 0.8 B	20.7 ± 5.2 A	15.1 ± 1.2 A
PP	11.9 ± 1.3 B	31.1 ± 8.1 A	1.6 ± 0.3 A	21.8 ± 7.7 A	2.7 ± 0.5 A	60.2 ± 10.2 B	26.9 ± 1.4 A	12.6 ± 3.3 A	16.1 ± 2.1 A
Monte Viejo									
	b	*a*	*c*	*b*	*c*	*a*	*a*	*ab*	*b*
CL	14.3 ± 0.9 AB	23.0 ± 2.1 A	1.2 ± 0.7 A	12.0 ± 2.1 B	1.5 ± 1.1 A	85.4 ± 4.0 A	15.9 ± 1.1 B	14.9 ± 3.5 A	10.0 ± 3.7 A
QF	14.9 ± 0.7 A	20.3 ± 5.3 A	2.3 ± 0.5 A	27.9 ± 4.2 A	2.5 ± 0.4 A	49.9 ± 6.3 B	17.0 ± 0.4 B	18.6 ± 5.2 A	14.6 ± 2.0 A
PP	12.4 ± 1.1 B	23.0 ± 3.2 A	1.5 ± 0.6 A	26.9 ± 3.9 A	1.8 ± 0.3 A	61.1 ± 4.9 B	23.9 ± 2.7 A	16.9 ± 3.8 A	13.0 ± 0.9 A

CL: Cropped land; QF: *Quercus* forest; PP: *Pinus* plantation; FF: free fraction; OF: occluded fraction; DF: dense fraction. Fractions indicated with the same italic letter are not significantly different ($p<0.05$). Land uses indicated with the same capital letter are not significantly different ($p<0.05$) for a given fraction. Means ± standard error for $n=4$.

lower than those reported by Rovira and Vallejo (2007) for surface horizons in Mediterranean soils. In any case, our results concur with the fact that BC may be a relevant pool, but it is not a dominant one.

In whole soils, no differences in BC content among land uses were found for the calcareous soils studied. This supports the findings of Rodionov et al. (2006) who did not observe any changes in BC content related to long-term arable cropping of steppe soils of Russia. Likewise, Brodowski et al. (2007) found that land use changes from grassland to cropland decreased C content, but did not affect BC proportion. The maintenance of BC proportion through land uses is relevant because it suggests an equilibrium between inputs and outputs, and leads to the suspicion that BC could be less stable and less resistant to biodegradation than is often taken for granted (e.g., Kuhlbusch, 1998; Schmidt and Noack, 2000). In addition, BC soil content has seldom been quantified, and long-term studies on BC stability are scarce and conclusions about BC stability are not widespread (Dai et al., 2005; Rovira et al., 2009). Contradictory experimental results reported both rapid (Bird et al., 1999) and slow (Shindo, 1991) decomposition of biomass-derived BC in soils. Hockaday et al. (2006) reported an association between soil charcoal fragments and filamentous microbiota that resemble saprophytic fungi; this provides grounds for suspecting that these organisms play a role in fate of soil BC. An additional question about BC stability was introduced by Rovira et al. (2009), who reported an overall decrease in BC content with wildfires; this maybe because the wildfire itself destroys part of the pre-existing BC by combustion.

These facts suggest that, leaving aside specific cases such as “Terra Preta” soils (Glaser et al., 2001), Black carbon could be not so stable and relevant pool for C accumulation in soils, as suggested by some studies (Bird et al., 1999; Hockaday et al., 2006).

Table 6
Black carbon (BC) content in whole soils.

	BC (mg C g^{-1} soil)	BC (mg C g^{-1} TOC)
Cerrato		
CL	1.19 ± 0.91	20.83 ± 5.11
QF	2.93 ± 1.08	19.34 ± 3.95
PP	1.94 ± 0.86	17.21 ± 2.74
Ampudia		
CL	0.89 ± 1.17	23.09 ± 4.19
QF	1.76 ± 0.79	15.17 ± 2.06
PP	0.59 ± 1.35	10.64 ± 4.83
Monte Viejo		
CL	0.38 ± 1.42	12.18 ± 3.17
QF	3.07 ± 1.30	20.79 ± 2.26
PP	1.48 ± 0.93	17.59 ± 1.99

CL: Cropped land; QF: *Quercus* forest; PP: *Pinus* plantation. Means ± standard error for $n=4$.

4.4. Black carbon content in density fractions and effect of soil management on Black carbon contents

The highest BC concentration and the highest contribution to the overall BC amount in soil were found in OF, followed by FF. The BC contents in density fractions in this study are consistent with the findings of Rumpel et al. (2006), who reported that a major part of the BC was not associated with the mineral phase, as well as, with the findings of Skjemstad et al. (1990), who reported that BC may be a major component of both FF and OF. The high proportion of BC indicates a great chemical recalcitrance on the part of the OC in FF and OF, which may explain the low decomposition rate found in these soils (Llorente and Turrión, 2010).

A significant portion of BC was localized in FF, the pool commonly assigned to labile humus. Hence, the question arises as to whether BC content also depends on different management practices. The only fraction sensitive to land use change was OF, with higher concentration both respect to the total mass and to the organic C in the fraction for cropped soils than in soils under tree cover.

5. Conclusions

Historical transformation of *Quercus* forest to cropped lands in calcareous soils in this area has resulted in a major loss of OC, as was expected. However, subsequent reforestation with *Pinus* throughout the past 40 years has resulted in good recovery of the SOC.

SOM fractionation by density and ultrasonic dispersion enables separation of fractions corresponding to different mechanisms of physical protection and determination of the effect of land use on the amount and spatial arrangement of OM in soils. Therefore, study of the density fractions and their BC content enables better understanding of the high stability of OM in calcareous soils.

Despite the different OC content of soils under different land use — higher under tree cover and lower under cultivation — the OM stabilization mechanisms were not significantly different. OM was mainly located in the organo-mineral complex, resulting in physico-chemical stabilization against further decomposition.

In our study, Black carbon always represents <3% of the TOC, far lower than the maximums reported in the literature.Furthermore, a significant proportion (between 0.9 and 3.5%) of the free and occluded OM corresponds to Black carbon.

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Table 7
Black carbon content of the different density fractions.

	BC (mg C g^{-1} fraction)			BC (mg C g^{-1} OC fraction)		
	FF	OF	DF	FF	OF	DF
Cerrato						
	b	*a*	*c*	*b*	*a*	*c*
CL	10.6 ± 1.0 A	25.6 ± 1.3 A	0.4 ± 0.1 A	20.6 ± 3.9 A	34.0 ± 4.1 A	7.4 ± 2.1 A
QF	8.7 ± 0.8 A	17.2 ± 0.9 B	0.2 ± 0.2 A	13.9 ± 2.7 A	19.7 ± 1.8 B	2.6 ± 2.7 A
PP	8.8 ± 0.9 A	18.8 ± 1.1 B	ND	13.1 ± 2.1 A	21.7 ± 2.1 B	ND
Ampudia						
	b	*a*	*c*	*b*	*a*	*c*
CL	7.4 ± 0.8 A	25.2 ± 0.9 A	0.1 ± 0.1 A	14.2 ± 1.7 A	33.4 ± 2.8 A	2.1 ± 3.1 A
QF	10.1 ± 1.0 A	14.5 ± 1.4 B	0.4 ± 0.2 A	16.7 ± 1.9 A	17.5 ± 3.1 B	5.1 ± 2.0 A
PP	8.7 ± 0.9 A	21.2 ± 1.1 B	0.4 ± 0.2 A	12.0 ± 3.1 A	23.9 ± 4.2 B	6.5 ± 2.3 A
Monte Viejo						
	b	*a*	*c*	*b*	*a*	*c*
CL	4.6 ± 1.2 A	23.4 ± 1.2 A	0.4 ± 0.1 A	8.8 ± 4.1 A	32.4 ± 3.7 A	11.8 ± 1.8 A
QF	7.7 ± 0.8 A	13.5 ± 1.5 B	0.9 ± 0.3 A	14.1 ± 3.3 A	15.8 ± 2.2 B	9.1 ± 2.3 A
PP	11.6 ± 1.1 A	10.3 ± 0.8 B	0.4 ± 0.2 A	17.3 ± 2.8 A	11.6 ± 3.4 B	9.3 ± 1.1 A

CL: Cropped land; QF: *Quercus* forest; PP: *Pinus* plantation; FF: free fraction; OF: occluded fraction; DF: dense fraction. ND: No determination. Fractions indicated with the same italic letter are not significantly different ($p < 0.05$). Land uses indicated with the same capital letter are not significantly different ($p < 0.05$) for a given fraction. Means ± standard error for $n = 4$.

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