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Effect of land use change on contents and distribution of monosacharides within density fractions of calcareous soil

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ABSTRACT

In the last century, many calcareous soils in Castilla León (northwestern Spain) have been transformed from natural *Quercus ilex* forest to cropped land. During the past 40 years, cropping was abandoned and some of these soils has been reforested with *Pinus halepensis*. We studied how thEse land use changes affected the soil organic matter amount (C stocks) and characteristics. 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 were analyzed for organic C amount (OC) and for carbohydrate content. The study aimed to describe the carbohydrate content in whole soils and its SOM density fractions to find out whether the carbohydrates can be used to explain the origin of SOM fractions and if they can depict its response to land management. We found a loss of 67% of the topsoil OC as a result of converting the natural *Quercus ilex* forest to cropped land, ~100 years ago. After crop abandonment and soil reforestation with *Pinus halepensis*, the stock of topsoil OC increased. After ~40 years of pine plantation, an average recovery of 71% of the previously lost OC had been recovered (43% loss of reference topsoil). The changes in OC stocks affected mainly the free fraction (FF) but also the organo-mineral (DF) one. Our results point to the role of physical protection in the C stocks but also that organo-mineral associations could be disrupted under a land use change. Monosaccharide content was significantly different ($p < 0.01$) among land uses. Gas chromatography analysis revealed significant differences in monosaccharide composition between land uses and also among density fractions. Whatever the fraction and land use considered, glucose was the dominant sugar monomer, followed by mannose and xylose.

1. Introduction

Soil organic matter (SOM) is recognised as an important factor in C driven climate change (Sanderman et al., 2003). Because of this, the capacity of soils to accumulate organic C has received great attention in recent years. Land use and management influence the amount and the dynamics of SOM (Zhang et al., 1999). Numerous studies have shown that conversion of native ecosystems to agriculture disturbs the ecological balance, disrupting the C cycle and generally results in net loss of soil C (e.g., Paustian et al., 1997). However, when arable land is converted to permanent vegetation the soil organic carbon (OC) increases gradually (Haynes and Beare, 1996). That increase of soil C stock is probably due to a greater incorporation of OM and also to the lack of tillage. Adsorption to soil minerals and occlusion within soil aggregates have been universally demonstrated to shield SOC from decomposition. However, the role of minerals in SOM adsorption and stability depends on their mineralogy and soil characteristics. Studies of the effects of land use change on soil OC in calcareous soils are scarce. However, calcareous soils are of particular interest because they represent approximately 12% of the world soil resources (FAO, 1996).

Changes in management not only influence SOM quantity but may also affect its quality. Of the various components of the SOM, carbohydrates have an important role in nutrient cycling (Hu et al., 1997). Carbohydrates are one of the main components of SOM and are considered readily degradable materials that can provide

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information about SOM dynamics (Sposito, 2008). Carbohydrates comprise about 50–70% of the dry weight of most plants, which makes them the most introduced compounds to soil (Stevenson, 1994). In soil systems, carbohydrates exist in various forms, from simple sugars or oligosaccharides to humic-polysaccharide complexes (Cheshire et al., 1992). The relative composition of carbohydrates can provide information on the origin of carbohydrates (Hu et al., 1997) and the relative content of carbohydrates in organic matter fractions can depict the nature of this fractions and their cycling pattern and response to land management (Abdelrahman et al., 2016). Monosaccharides (MS) in soil are subject to rapid turnover and are involved in biochemical and organo-mineral reactions. They represent a primary source of nutrients and energy for soil micro-organisms and contribute to microbial activity and, therefore, may be a sensitive indicator of several biological properties (Larré-Larrouy et al., 2003). Carbohydrates also play a role in enhancing soil structural stability because they are of particular importance in promoting aggregate stability (Feller and Beare, 1997). However, the effects of land use changes on carbohydrates are largely unknown. Information on the nature and distribution of the soil MS in the soil density fractions is thus important for the understanding of the role of carbohydrates in organic matter stability. Based on the observation that a predominance of hexoses represents microbial derived materials while a predominance of pentose represents plant-derived materials (Guggenberger et al., 1994), the ratios C6/C5 ((mannose + galactose)/(xylose + arabinose)) and Desoxy/C5 ((rhannose + fructose)/(xylose + arabinose)) is frequently used as a proxy to determine the share of microbial OM (Oades, 1984; Six et al., 2006 and Ludwig et al., 2015).

Soil molecular structure of SOC does not necessarily predetermine the persistence of OC in soils and adsorption to soil minerals and occlusion within soil aggregates have been universally demonstrated to shield SOC from decomposition (Han et al., 2016). Soil organic matter density fractionation following ultrasonic dispersion enables the 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., 1994a; Six et al., 2002; 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.

The aims of the present study were (1) to assess and compare land use effects on soil C content and its distribution among density fractions; (2) to determine the content and origin (microbial or plant-derived) of monosaccharides in whole soils and soil density fractions.

2. Material and methods

2.1. Site description

The study was carried out in calcareous soils in the region of *Castilla y León* (northwestern Spain), UTM: 30T 384465 E 4639001 N. The mean annual rainfall in the region is around 400 mm, under a xeric moisture regime, and the mean annual temperature 12.3 °C. The altitude of the moor is between 800 and 900 m above sea level, with low slopes (<7%). The soils (Calcixerept) are quite homogeneous in spite of differences in their land use history. The native vegetation in the studied calcareous soils is Holm-oak wood (*Quercus 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 *Pinus halepensis* has been carried out on abandoned agricultural land. Agricultural land is currently cropped with cereals (usually barley), managed by conventional tillage system. The average basal area of the *Quercus ilex* forest is 13 m^2 ha^{-1} with an average density of 3200 trees ha^{-1}. The average basal area of the pine plantation is 50 m^2 ha^{-1}, with an average density of 1184 trees ha^{-1}. For the characterization of the selected plots we support the previous information using historical aerial photos, historical maps and also through discussions with local residents.

The main characteristics of the soil samples are shown in Table 1. We can observe a systematic higher % of coarse material in soils under oak forest related to a stone removal management applied to the cropped lands.

2.2. Sampling procedures

A land use map of the calcareous moor of Castilla y León was elaborated with a GIS (ArcGis 10.3 for Windows) following the process shown in Fig. 1.

The map was used to select the sampling plots on the basis of the following criteria: a) *Quercus ilex* forest, cropped land, and *Pinus halepensis* plantations in adjacent areas; b) minimum area of each land use of 1 ha. For this study, plots of the above-mentioned land uses were selected in three different regions, and four representative plots were sampled (0–10 cm depth) in each region. A total of 36 topsoils (0–10 cm depth) (3 regions x 3 land uses x 4 plots) were thus sampled. A composite sample (~2 kg) by joining 3 soil subsamples, was obtained for each plot. Soils under forest uses were sampled in points under canopy trees.

Visible plant residues and roots were removed; soil was air-dried, sieved (<2 mm) and stored in plastic bags until analysis. For bulk density determinations, all of the soil extracted from the soil pit was weighed. The volume of the soil was calculated from the volume of water required to fill the hole (after impermeabilization of the soil pit with plastic sheeting) following USDA (1999).

2.3. Density fractionation of soil

A density fractionation procedure was applied. The method follows the concept of Golchin et al. (1994b), 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 were 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 residue remaining 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 and N.

Table 1
Physicochemical properties of the soil samples (0–10 cm). COURSE: mineral fragments >2 mm, BD: bulk density; HC: soil holding capacity. Means and standard error for n = 4.

Area	Land use	Texture	$CaCO_3$ (%)	Coarse (%)	BD (g ml^{-1})	HC (%)	pH
Ampudia	Quercus forest	Clay loam	13.4 ± 1.7	58.1 ± 5.4	1.35 ± 0.13	26	8.1 ± 0.1
Ampudia	Cropped land	Clay loam	10.1 ± 1.0	44.9 ± 5.5	1.25 ± 0.17	23	8.1 ± 0.1
Ampudia	Pinus plantation	Clay loam	22.2 ± 3.2	48.2 ± 13.1	1.10 ± 0.21	30	8.2 ± 0.1
Cerrato	Quercus forest	Clay loam	20.9 ± 4.6	59.0 ± 5.4	1.32 ± 0.11	28	7.9 ± 0.1
Cerrato	Cropped land	Clay loam	19.9 ± 8.8	45.3 ± 5.3	1.28 ± 0.19	21	8.1 ± 0.1
Cerrato	Pinus plantation	Clay loam	18.4 ± 1.8	48.9 ± 15.2	1.01 ± 0.17	26	7.9 ± 0.1
Monte viejo	Quercus forest	Clay loam	31.1 ± 7.3	58.4 ± 5.4	1.30 ± 0.10	25	7.9 ± 0.1
Monte viejo	Cropped land	Clay loam	59.8 ± 9.8	45.1 ± 6.2	1.22 ± 0.14	22	8.3 ± 0.1
Monte viejo	Pinus plantation	Clay loam	42.0 ± 6.9	49.3 ± 18.0	1.05 ± 0.18	32	8.1 ± 0.1

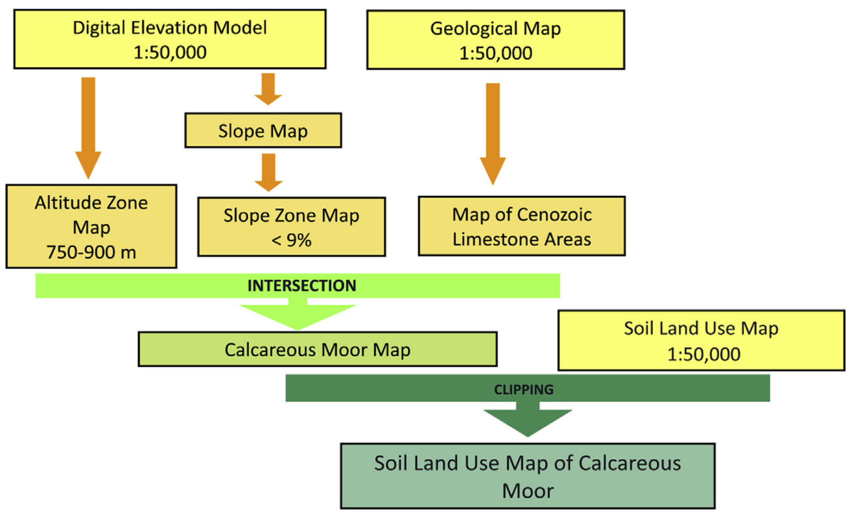

Fig. 1. Mapping of soil land uses of the calcareous moor of Castilla y León.

2.4. Analyses

Total concentrations of soil C and N were determined with an automated C/N analyzer (CHN-2000, Leco). Organic C was calculated as the difference between total and carbonate C. Soil total calcium carbonates were determined by use of 1M HCl titrated with 0.5M NaOH (FAO, 2007). For soil characterization, % of carbonates, texture, % of coarse soil materials (Ø > 2 mm), electrical conductivity (EC), Water Holding Capacity (HC) and pH were determined. Carbohydrate concentration and composition were determined for whole soils and soil density fractions by the analysis of sugar monomers released by acid hydrolysis.

Samples of 500 mg of soil, spiked with 100 µl internal standard solution (containing 80 µl myo-inositol), were hydrolysed with 10 ml of 4M trifluoroacetic acid in closed 25 ml hydrolysis flasks at 105 °C for 4 h (Guggenberger et al., 1994). Following filtration through glass fibre filters (GF 6, Whatman) the hydrolysates were dried using a rotatory evaporator. Saccharides were re-dissolved using 5 × 5 ml deionised water and dissolved humic-like substances were removed passing the solution through XAD-7 adsorption resin (conditioned and purified with 1 M NaOH, deionised water, isopropanol, deionised water, 0.1 M NaOH, deionised water, 0.1 M HCl, deionised water). A second purification step was performed by dropping the solution through a column (1 × 20 cm) filled with 4 g of cation exchange resin Dower 50W X 8 (conditioned and purified with 10 ml 2 M NaOH, deionised water, 10 ml 2 M HCl, deionised water until pH approached neutrality). After freeze drying, we re-dissolved samples with 200 µl of a solution containing 100 µg ml-1 3-*O*-methylglucose (as second internal standard) in *N*-methyl-pyrrolidone, then added 200 µl *O*-methylhydroxylaminehydrochloride. The solution was heated to 75 °C for 30 min and subsequently cooled at room temperature. Final derivatisation was accomplished with 400 µl bis-(trimethylsilyl)-trifluoroacetamide at 75 °C for 5 min.

Gas chromatography was performed on a Hewlett Packard 6890 gas chromatograph equipped with a flame ionisation detector. A capillary column, HP-5 (30 m × 0.32 mm x 0.25 µm film) was used with nitrogen as the carrier gas. The operating conditions were: injection temperature 300 °C, detection temperature 300 °C column temperature programmed from 100 °C to 250 °C at a rate of 20 °C min^{-1}and from 250 °C to 300 °C at a rate of 10 °C min^{-1}. The split ratio was 50:1.

The identity of each sugar peak in the chromatograms was determined by comparing the retention times observed for standard solutions with that of the peaks observed in the chromatograms. The relative concentration of the sugar was calculated by means of response factors relative to that of a myo-inositol internal standard added to each sample before derivatization. The carbon content of each MS was calculated from its chemical formula. Litter

Table 2
Monosaccharide C (MSC), % of OC represented by monosaccharide C (MSC_OC (%)), galactose + mannose/arabinose + xylose (C6/C5) and Rhamnose + Fructose/arabinose + xylose (Desoxy/C5) in Quercus forest litter, Pinus plantation litter and crop residues. Means for n = 4.

	MSC (g C kg^{-1})	C6/C5	Desoxy/C5
Cropped land	39.46	0.28	0.02
Quercus forest	33.75	1.48	0.09
Pinus plantation	22.19	0.89	0.10

of oak forest and pine plantation and plant residues over the cropped lands were analysed for carbohydrate concentration and its corresponding monosaccharide composition. Data is shown in Table 2.

2.5. Statistical analyses

Analysis of variance (ANOVA) was used to compare variables. In case of significant F-statistics ($p > 0.05$), differences between means were tested with the Tukey procedure for multiple comparisons. Data were tested for normality and homoscedasticity with the Kolmogorov-Smirnov and Levene's statistics respectively. The statistical analyses were performed with IBM SPSS Statistics 23.0.

3. Results

3.1. Land use effect on soil OC content and monosaccharide content in whole soils

The organic C varied with the land use. The cropped land displayed the lowest content of organic C (Table 3). Percentage of OC in topsoil (from 0 to 10 cm depth), under a land use change from a natural *Quercus ilex* forest to cropped land, after approximately one century, showed an average net drop of ~67%. The subsequent reforestation of the studied calcareous soils with *Pinus halepensis* resulted in a great recovery of OC. An average OC recovery of ~71% of the OC (referring to % of OC in soils under *Quercus ilex* forest) was measured in topsoil (from 0 to 10 cm depth) after ~ 40 years of pine plantation. As regards to OC/N ratio, differences among land uses were not significant, however, soils under *Pinus halepensis* plantations appear to have the highest OC/N ratio. MS content in whole soils varied from 0.87 to 6.14 g C kg^{-1} (Table 3). Cultivated soil showed lower MS contents than forested soils. Significant differences ($p < 0.01$) were found among soils under different land use type in the following order: QF > PP > CL. The MS constituted from 6.26 to 15.00% of total organic C content of the soils, with the highest values in soils under the *Quercus ilex* forest (Table 3), however, no significant differences between land use types were found for this variable.

3.2. Monosaccharide composition of whole soils

Results of the analyses of neutral sugars in whole soils are listed in Table 4. As regard to each sugar content relative to total sugar contents, glucose was dominant ranging from 42.1 to 48.3% of the total sugar (Fig. 2). For any given sugar, except for glucuronic acid, differences in abundance among land use types were significant, always with lower contents in soils under cropping. As regards to C6/C5 ratio, significant differences ($p < 0.05$) were found among land use types with the highest values in soils under pine plantation (Table 3). C6/C5 ratio ranged from 0.82 to 1.57. Desoxy/C5 ratio was not significantly different among the topsoils under different land uses with a mean value of 0.29.

3.3. Land use effect on OC content and OC/N ratio in density fractions

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

Most of the fine soil mass was located in the DF, 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 kg^{-1} fraction) among density fractions revealed the significantly lowest values in DF, as the latter was dominated by minerals, and the highest values in OF (Table 5). Cropped land presented the lowest OC concentration in all fractions with a great depletion in both FF and DF. As regards the % of OC in soil that is represented by each fraction, the largest portion of OC was associated with DF irrespective of the % of OC in a soil. The DF accounted for ~83% of TOC under cropped land, but for ~53% of TOC in topsoils (0–10 cm depth) under tree cover, with not significant differences among soils under *Pinus halepensis* or *Quercus ilex* vegetation (Table 4). 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).

3.4. Monosaccharide in density fractions

DF was significantly depleted in MS, while FF contained the highest concentrations of MS (Table 6). For FF and OF the effect of land use change in MSC concentration was significant ($p < 0.001$). However, the proportion of the OC represented by MS was not significantly different ($p < 0.05$) among fractions and neither

Table 3
OC, OC/N, Monosaccharide C (MSC), % of OC represented by monosaccharide C (MSC_OC (%)), galactose + mannose/arabinose + xylose (C6/C5) and Rhamnose + Fructose/arabinose + xylose (Desoxy/C5) in whole topsoils (0–10 cm). Means for n = 4.

	OC (%)	OC/N	MSC (g C kg^{-1})	MSC_OC (%)	C6/C5	Desoxy/C5
Quercus forest	a		a		b	
Ampudia	5.12	12.42	4.03	7.87	0.82	0.25
Cerrato	5.16	12.31	3.83	7.82	1.05	0.29
Monte viejo	6.51	12.56	6.14	9.43	1.04	0.28
Cropped land	c		b		b	
Ampudia	1.70	11.73	2.47	15.00	1.07	0.32
Cerrato	2.51	11.91	1.95	7.76	1.03	0.26
Monte viejo	1.39	11.32	0.87	6.26	0.92	0.23
Pinus plantation	b		a		a	
Ampudia	3.89	14.30	2.55	6.35	1.16	0.25
Cerrato	4.95	13.88	4.83	9.76	1.52	0.35
Monte viejo	3.70	14.46	4.09	11.05	1.57	0.37
Analysis of variance						
Land use	**	n.s.	**	n.s.	*	n.s.

Land uses indicated with the same lower case letter are not significantly different.
For ANOVA analysis, values of $p > 0.05$: n.s., $p < 0.05$:*, $p < 0.01$:** and $p < 0.001$:*** are reported.

Table 4
Monosaccharide composition of whole topsoils (0–10 cm). Means for n = 4.

	Xylosa	Arabinose	Ribose	Rhamnose	Fucose	Fructose	Mannose	Galactose	Glucose	Glucuronic acid	Galacturonic acid
	mg C kg^{-1}										
Quercus forest	a	a	a	a	a	a	a	a	a		
Ampudia	442.34	395.20	88.90	188.44	79.80	19.86	341.78	345.24	1982.26	5.93	141.81
Cerrato	336.92	372.49	85.10	184.85	79.84	19.20	362.57	385.85	1835.77	10.63	158.92
Monte viejo	601.42	565.94	128.78	297.02	131.19	28.88	606.04	608.32	2938.05	14.75	220.51
Cropped land	b	b	b	b	b	b	b	b	b		
Ampudia	268.83	210.50	28.15	132.47	39.47	19.69	212.87	300.92	1343.02	–	–
Cerrato	195.16	242.91	18.00	106.87	51.37	8.91	232.40	219.14	819.11	12.96	40.33
Monte viejo	106.13	123.07	15.44	47.23	27.07	4.87	101.60	110.34	327.00	–	10.06
Pinus plantation	ab	a	a	a	a	ab	a	a	a		
Ampudia	196.27	363.99	22.99	123.61	69.52	15.93	331.62	316.03	977.35	8.21	46.03
Cerrato	358.94	424.52	94.48	294.40	144.59	26.13	629.96	558.32	2195.92	20.37	129.57
Monte viejo	277.93	344.27	75.15	210.16	104.51	20.10	516.35	457.80	1989.52	20.69	71.75
Analysis of variance											
Land use	**	**	*	*	*	*	*	*	**	n.s.	n.s.

Land uses indicated with the same lower case letter are not significantly different.
For ANOVA analysis, values of $p > 0.05$: n.s., $p < 0.05$:*, $p < 0.01$:** and $p < 0.001$:*** are reported.

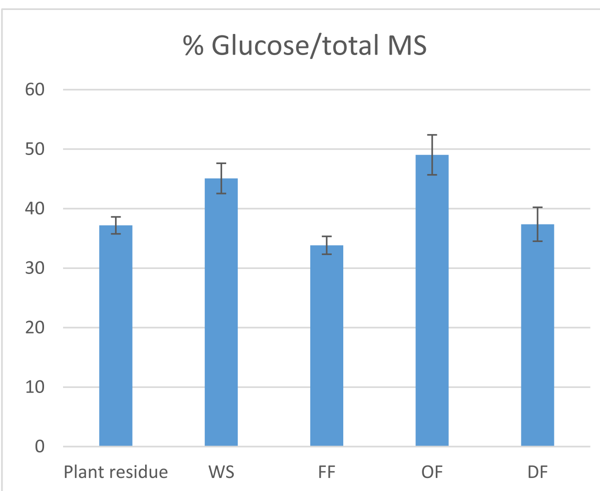

Fig. 2. Mean and standar desviation of glucose relative contribution to total monosacharide content. WS: whole soil; FF: free fraction; OF: occluded fraction; DF: organo-mineral fraction.

Table 5
OC, % OC of total OC of the soil, and OC/N ratio in density fractions. Means for n = 4.

	OC (g C Kg^{-1}fraction)			%OC (of total OC of soil)			OC/N		
	FF	OF	DF	FF	OF	DF	FF	OF	DF
Quercus forest									
Ampudia	266	364	28	47.1	5.2	54.9	16.1	20.7	15.1
Cerrato	276	386	25	50.6	4.3	44.6	18.9	20.6	12.1
Monte viejo	259	376	33	43.2	4.2	50.9	17.0	18.6	14.6
Cropped land									
Ampudia	229	331	14	14.3	2.1	82.4	15.0	9.4	15.7
Cerrato	227	360	21	10.9	3.8	84.7	15.1	13.4	10.6
Monte viejo	227	343	12	17.9	2.1	82.9	15.9	14.9	10.0
Pinus plantation									
Ampudia	319	391	26	37.6	2.1	62.5	26.9	12.6	16.1
Cerrato	296	383	27	44.1	3.5	53.2	22.2	20.4	14.1
Monte viejo	295	389	20	48.7	2.3	50.2	23.9	16.9	13.0
Analysis of variance									
Use		n.s.			n.s.			n.s.	
Fraction		***			**			n.s.	
*Use*fraction*		***			***			*	

FF: free fraction; OF: occluded fraction; DF: organo-mineral fraction.
For ANOVA analysis, values of $p > 0.05$: n.s., $p < 0.05$:*, $p < 0.01$:** and $p < 0.001$:*** are reported.

among land uses. As regards to C6/C5 sugar ratio and Desoxy/C5 ratio no significant differences ($p < 0.05$) were found among density fractions. However, significant differences were detected among land uses for a given fraction. (Table 6).

Sugar composition differed significantly between density fractions, however for all density fractions glucose was the most abundant MS (Table 7). Comparing the effect of land use on MS composition of each density fraction, greater differences were found in FF. In soils under cultivation FF was depleted in arabinose, fructose and galactose. In soils under pinus plantation FF was enriched in fructose and glucose. OF appears to be the less affected fraction for land use changes as regards to abundance or relative contribution of sugars. As regards to DF, in cultivated soils xylose appeared to have higher relative contribution to total sugar than in other land uses types. FF contained the highest relative variation in relative abundance of glucose.

Glucose varied from 29.9 to 48.1% of the total sugar content of FF with the highest values in soils under pine plantations. In comparison to the other density fractions, in FF underlines the high relative contribution of mannose, which represents from 17.7 to 27.1% of the total sugars content of the fraction (Fig. 3). The free fraction presents significant less relative abundance of rhamnose than the other fractions. In OF, glucose represented from 48.2 to 54.1% of the total sugar content (Fig. 2), also with the higher values in soils under *Pinus halepensis* plantation.

DF showed values of glucose from 36.2 to 42.2% of the total sugar content. Compared to the other density fractions, DF was significantly ($p < 0.05$) enriched in arabinose and galactose, the OF was significantly depleted in xylose and FF was significantly and greater enriched in mannose (Fig. 3).

4. Discussion

4.1. Land use effect on soil OC content, monosaccharide content in whole soils

Land use change from a natural *Quercus ilex* forest to cropped land (~100 years ago) resulted in an average net drop of 67% in the % of OC in the topsoil (from 0 to 10 cm depth). These results are consistent with results reported by Llorente et al. (2010) for those calcareous soils. 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

Table 6
Monosaccharide C (MSC), % of OC represented by monosaccharide C (MSC_OC), galactose + mannose/arabinose + xylose (C6/C5) and rhamnose + fructose/arabinose + xylose (Desoxy/C5) in density fractions. Means for n = 4.

	MSC (g C kg^{-1})			MSC_OC (%)			C6/C5			Desoxy/C5		
	FF	OF	DF	FF	OF	DF	FF	OF	DF	FF	OF	DF
Quercus forest												
Ampudia	12.66	4.55	0.37	4.75	1.25	1.32	2.36	1.11	1.14	3.59	4.78	2.97
Cerrato	7.46	3.74	0.75	2.70	0.97	3.00	1.04	1.08	1.14	4.27	3.81	2.91
Monte viejo	13.56	2.66	0.89	5.23	0.70	2.69	1.51	0.91	1.22	2.22	2.93	2.99
Cropped land												
Ampudia	46.22	5.80	1.94	20.18	1.75	13.85	0.84	0.58	1.25	5.66	2.53	2.70
Cerrato	30.28	–	0.26	13.34	–	1.24	0.89	–	0.79	3.56	–	1.57
Monte viejo	5.27	5.02	1.82	2.32	1.37	15.17	2.01	–	1.23	2.28	–	2.75
Pinus plantation												
Ampudia	18.17	1.07	–	5.69	0.27	–	1.82	1.46	1.62	5.04	4.55	3.90
Cerrato	14.31	–	1.31	4.83	–	4.85	3.29	2.28	1.33	4.69	7.78	2.64
Monte viejo	8.51	2.56	0.82	2.88	–	–	2.03	1.84	1.93	7.16	6.62	4.83
Analysis of variance												
Use		n.s.			n.s.			n.s			n.s.	
Fraction		***			n.s.			n.s.			n.s.	
*Use*fraction*		***			n.s.			**			**	

FF: free fraction; OF: occluded fraction; DF: organo-mineral fraction.
For ANOVA analysis, values of $p > 0.05$: n.s., $p < 0.05$:*, $p < 0.01$:** and $p < 0.001$:*** are reported.

Table 7
Monosaccharide composition of fractions. Means for n = 4.

		Xylose	Arabinose	Ribose	Rhamnose	Fucose	Fructose	Mannose	Galactose	Glucose	Glucuronic acid	Galacturonic acid
		mg C kg^{-1}										
Quercus forest												
Ampudia	FF	699.89	1770.01	375.84	283.61	191.76	121.20	4593.78	1222.96	3051.06	158.84	195.42
	OF	7.01	205.70	55.02	30.14	408.15	392.64	2648.45	12.71	72.92	7.01	205.70
	DF	22.66	59.20	12.74	17.07	15.35	5.88	53.25	40.10	149.51	0.00	0.00
Cerrato	FF	762.33	975.40	68.02	122.28	835.17	61.95	422.40	687.00	3064.31	162.94	305.59
	OF	8.05	178.49	57.07	26.36	311.34	442.24	1913.45	13.02	97.74	8.05	178.49
	DF	46.43	120.52	11.45	33.31	26.98	9.41	90.18	99.39	296.26	0.00	19.03
Monte viejo	FF	2167.97	1421.35	99.93	132.64	1411.26	87.42	4543.08	871.57	2544.29	166.06	118.43
	OF	317.28	280.02	0.00	152.08	38.42	21.89	271.63	271.67	1209.06	12.15	88.81
	DF	61.16	127.77	16.24	63.66	21.88	12.73	128.59	102.78	333.02	0.00	25.92
Cropped land												
Ampudia	FF	4260.29	2228.20	123.02	1166.19	327.07	278.96	2403.64	3067.77	31280.65	208.22	879.65
	OF	16.44	230.25	85.59	32.62	446.20	401.18	2883.19	38.88	184.60	21.58	215.30
	DF	145.40	319.18	42.34	99.43	45.73	31.92	287.25	294.50	671.03	0.00	0.00
Cerrato	FF	3233.98	1383.51	56.51	728.35	336.62	174.32	2017.14	2093.80	12312.71	7704.82	248.12
	OF	–	–	–	–	–	–	–	–	–	–	–
	DF	22.76	65.35	8.68	10.00	10.96	4.98	29.91	39.91	68.15	0.00	0.00
Monte viejo	FF	690.64	775.19	32.86	229.16	200.00	44.90	2000.50	747.92	388.69	53.22	108.89
	OF	11.29	235.49	65.55	43.62	347.22	530.11	2860.19	26.81	196.77	15.29	231.49
	DF	107.23	321.52	33.02	105.83	58.15	23.08	257.49	269.04	651.65	0.00	0.00
Pinus plantation												
Ampudia	FF	720.45	1993.93	169.91	374.57	220.14	191.02	3218.78	1719.93	8752.99	324.39	493.27
	OF	83.74	92.46	3.24	51.49	13.19	13.70	146.93	110.01	543.99	0.00	11.33
	DF	261.50	784.53	51.27	337.95	167.56	77.64	889.18	808.75	2384.54	0.00	35.24
Cerrato	FF	256.11	907.53	353.10	234.86	4397.69	2606.52	2963.04	182.19	284.16	256.11	907.53
	OF	950.50	816.81	11.99	895.45	231.13	152.68	2496.25	1535.77	9720.60	34.96	395.80
	DF	71.55	240.25	30.23	61.54	59.98	30.62	204.27	209.42	408.96	0.00	0.00
Monte viejo	FF	129.80	158.20	98.24	46.79	1138.97	748.26	4761.01	196.58	309.11	129.80	158.20
	OF	184.58	128.39	3.34	121.75	36.31	18.90	305.21	272.02	1494.04	0.00	0.00
	DF	31.77	89.03	10.13	45.17	25.57	12.15	116.50	116.51	349.70	6.45	21.05
Analysis of variance												
Use		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Fraction		***	***	***	***	n.s.	***	**	***	***	*	**
*Fraction*land use*		*	**	***	n.s.	n.s.	***	*	**	**	n.s.	**

FF: free fraction; OF: occluded fraction; DF: organo-mineral fraction.
For ANOVA analysis, values of $p > 0.05$: n.s., $p < 0.05$:*, $p < 0.01$:** and $p < 0.001$:*** are reported.

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). The acceleration of organic matter oxidation is, at least in part, due to the disruption of the soil aggregates, which exposes once-protected organic matter to decomposition (De Gryze et al., 2006; Grandy and Robertson, 2007). Larré-Larrouy et al. (2003) found, on a tropical Vertisol, that intensive cultivation caused a 56% reduction in soil concentration from the level originally found in the soil. The subsequent reforestation of the studied calcareous soils with *Pinus halepensis* resulted in a great recovery of OC. An average OC recovery of 71% of the OC (referring to % of OC in soils

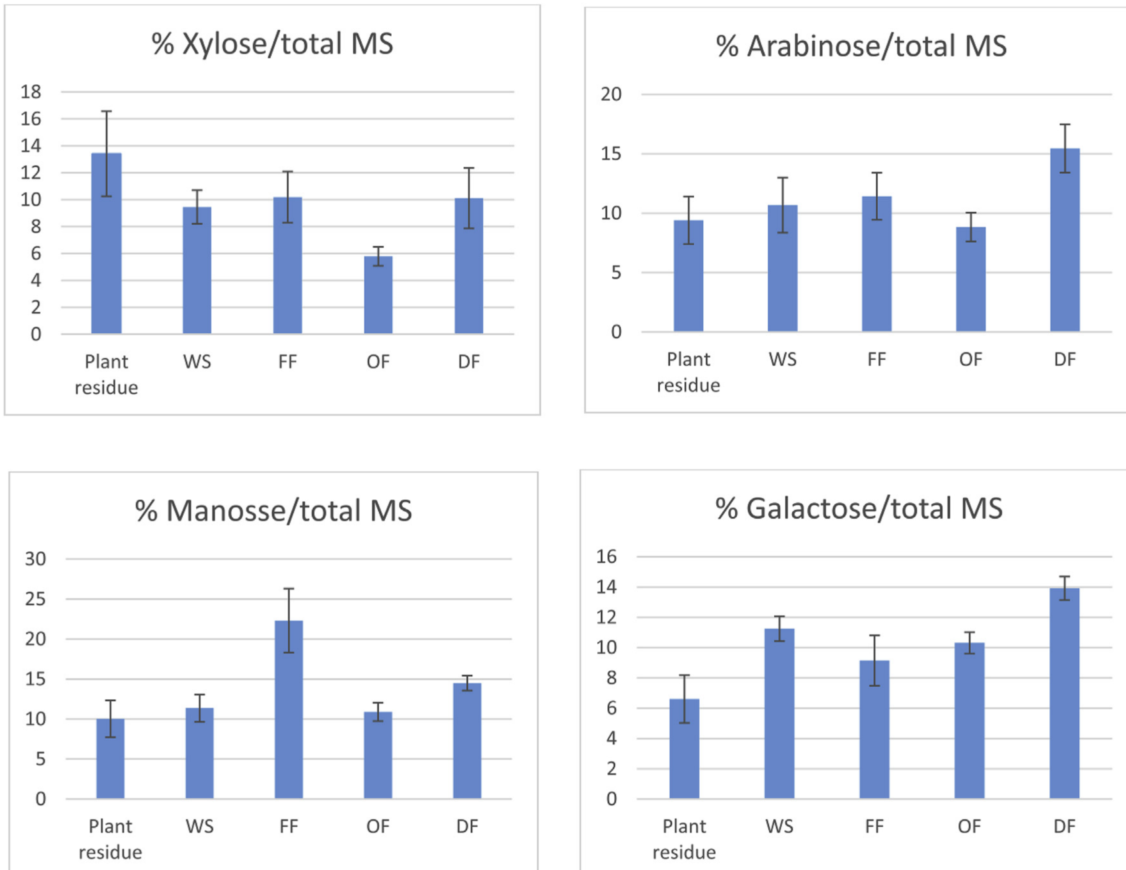

Fig. 3. Mean and standar desviation of Arabinose, Xylose, Manose and Galactose relative contribution to total monosacharide content. WS: whole soil; FF: free fraction; OF: occluded fraction; DF: organo-mineral fraction.

under *Quercus ilex* forest) was measured in topsoil (from 0 to 10 cm depth) after 40 years of pine plantation. That increase of soil C stock is probably due to a greater incorporation of OM and also to the lack of tillage. In the Vertisols studied by Larré-Larrouy et al., a pasture installation resulted 19 years later in an accretion of its C concentration amounting to near 90% that the original soil. Differences of our results with those found by Larré-Larrouy et al. could be atributed to the different vegetation but also to the different soil conditions. Soil conditions would influence the stock of OC associated with the minerals and therefor into its stability (Han et al., 2016). Soils under tree cover (oak or pine) showed significantly higher MS concentration than soils under cultivation. Several studies documented the impact of land-use changes on soil carbohydrates (e.g., Sanger et al., 1997; Trouvé et al., 1996; Turrión et al., 2002). Decreasing amounts of labile substances are usually observed after cultivation of native soils with little return of plant debris to soil (Christensen, 1992). Forest clearing and cultivation induced a rapid decrease in total soil organic C and carbohydrate content (Hu et al., 1997). The % of OC represented by MS presented the highest values in soils under *Quercus ilex* forest, however no significantly differences were found among land uses. The comparison of the % of OC represented by MS between plant residues (ranging from 22.19 to 39.46 g/kg) and whole soil samples (ranging from 0.87 to 4.09 g/kg) support the fact that carbohydrates are a labile component of organic matter and are usually rapidly metabolized by soil microorganism (Martens et al., 2004). Carbohydrates are the major C contribution to terrestrial soils and provide the major source of energy for microbial processes in soil.

Among the monosaccharides that were identified in the whole soils, glucose was always dominant, ranging from 40 to 50% of the total carbohydrates concentration, which reflected the dominant contribution of glucose in the cellulose and hemicellulose plant fractions added to soils. For any given sugar, except for glucuronic acid, differences in abundance among land use types were significant, with higher contents in soils under *Quercus ilex* forest and lower in cultivated soils. Most of the existing differences in relative amounts of the monosaccharides appeared to be associated with xylose, arabinose, glucose, and fructose. Polysaccharides of microbial origin normally have higher C6/C5 ratios due to a major abundance of galactose and mannose, while soils with plant derived polysaccharides, typically have a low C6/C5 because of the xylose and arabinose abundance (Oades, 1984; Six et al., 2006 and Ludwig et al., 2015). The studied soils showed C6/C5 ratio from 0.82 to 1.57 suggesting a major contribution of plant residues to soil carbohydrates with respect to the microbial one. Desoxy/C5 ratio was from 0.23 to 0.37. Land use had a significant influence in both ratios and therefor in the carbohydrates origin. The studied soils showed the lowest values of C6/C5 ratio under *Quercus ilex* forest and the highest ones in soils under the pine forest. These results indicate a major contribution of plant residues to soil carbohydrates in cropped soils and soils under oak forest compared with soils under pine forest where microbially derived monosaccharides are grater. These results are in contrast with those found by Conti et al. (2016) who reported that these ratios seem to be not significantly affected by ecosystem types. Besides if we compare the C6/C5 ratio in soils and in the plant residues, the changes in the composition of the carbohydrates is even more interesting inasmuch as *Quercus ilex* litter show the higher C6/C5 ratio. These results may be related, on the one hand with a more readily available C substrate for microbes in pine litter than in oak one and, on the other hand, with a larger surface area of pine needles compared to the *Quercus ilex* leaves.

4.2. Land use effect on OC content and OC/N ratio in density fractions

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

As regards the % OC in soil that is represented by each fraction, the major portion of OC was associated with DF (Table 5). The DF accounted for about 83% of OC under cropped land, but for ~53% of OC in topsoil under tree cover, with no significant differences among soils under pine or oak vegetation. These values are consistent with the findings of John et al. (2005), who reported that 86–91% of soil OC was in 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.

OC/N ratio was significantly higher ($p < 0.05$) in FF and OF than in DF (Table 5), 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 Golchin et al. (1994a) who observed somewhat higher OC/N ratios for FF. N tends to be more protected than OC and this trend is reflected by the decrease in the C/N ratio with increasing density of the fraction. The higher values of OC/N ratio for the soils under pine forest are also consistent, with the findings of other studies on litter composition, such as that by Traversa et al. (2008), who compared the OC/N ratio of the litter under *Pinus halepensis* and *Quercus ilex*.

Cropped land presented the lowest OC concentration in all fractions with a great depletion in both FF and DF. The declination of OC in FF is according with the fact that no physically shielded fraction are more available to microbial attack. The much lower mineralization of mineral-associated OC related to free OC has been frequently reported in the literature, highlighting the potential importance of mineral on the stability of OC in soils (Han et al., 2016). The depletion of OC in the DF with cultivation agree with recent literature indicating that protection of organic materials by sorptive interactions is limited to the organic molecules directly bound to the mineral surface and organo-mineral associations could be disrupted under variable environmental conditions (Baldock and Skjemstad, 2000).

4.3. Content of monosaccharide in density fractions

C was present as MS in all the density fractions. The monosaccharide C concentration was significantly different among fractions. The highest concentration was found in FF and the lowest was detected in the DF. This is in agreement with the generally held opinion that carbohydrates are labile compounds which are degraded rapidly (e.g., Hatcher et al., 1981; Benner et al., 1984). The contribution of MS to the total soil C pool was constant in any fraction for any land use, according to Preston et al. (1994) who found that the proportion of soil organic present as carbohydrates remains markedly constant irrespective of the land use, and in spite of absolute changes in both C and carbohydrates. Our results are in agreement with the premise of Preston et al. (1994) that equate the proportion of organic materials in a given soil with soil type and not by cropping practices. In contrast, Hu et al. (1997) or Larré-Larrouy et al. (2003) stated that the contributions of carbohydrates to the total soil C pool differ significantly among ecosystems.

The degradation of sugars may be related by the presence of the mineral matrix and several studies reported a stabilisation of sugars by interaction with soil minerals (Miltner and Zech, 1998) as well as their incorporation into stable aggregates (Puget et al., 1999).

4.4. Monosaccharide composition and relative contribution of density fractions

Carbohydrate content was a suitable characteristic for distinguishing different SOM fractions. It accounted for a large proportion of the FF while it represented a relatively small proportion of the OF and DF in agreement with Abdelrahman et al. (2016). Glucose was the predominant sugar in all the fractions according with the fact that non-cellulosic saccharides are easily-available C and energy sources for microorganisms (Martin and Haider, 1986). According with the observations of Puget et al. (1999), we found a decrease of glucose associated with the mineral fraction (DF) In contrast we found a significantly greater relative contribution of glucose in OF pointing a probably contribution of cellulose and hemicellulosic carbohydrates in the organo-mineral aggregation. Plant material contains large proportions of pentose sugars (mainly xylose and arabinose). The soil microbial population, in contrast, synthesizes dominantly galactose, mannose and little, if any, arabinose and xylose (Oades, 1984). In accordance therewith, we found a enrichment of the FF in mannose because this is the OM fraction more accessible to the attack of the microorganisms. The C6/C5 and the Desoxy/C5 ratio are widely used to estimate the decomposition of plant residues and the stock of compounds synthesized. In the studied soils, C6/C5 and Desoxy/C5 ratios showed significant differences among land uses with higher values in all the fractions corresponding with soils under *Pinus halepensis* plantation.

5. Conclusions

Historical transformation of *Quercus ilex* forest to cropped lands in calcareous soils in this area has resulted in a major loss of OC (~67% of the topsoil OC). However, subsequent reforestation with *Pinus halepensis* throughout the past 40 years has resulted in good recovery of the SOC: an average recovery of ~71% of the previously lost OC had been recovered (43% loss of reference topsoil). The changes in OC stocks affected mainly the free fraction (FF) but also the organo-mineral (DF) one, indicating the role of physical protection in the C stock changes but also that organo-mineral associations could be disrupted under a land use change.

Soil carbohydrates accounted for 6–15% of the total organic C of soils. Cultivation also affected the proportion of soil C present as carbohydrates. MS analysis revealed significant differences in carbohydrate composition between land uses and also among density fractions. Therefore, carbohydrates can depict the nature and composition of SOM density fractions.

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