# The influence of tree and soil management on soil organic carbon stock and pools in dehesa systems

Lizardo Reyna-Bowen[1,2], Pilar Fernandez-Rebollo[2], Jesús Fernández-Habas[2], José A. Gómez [1,*]

[1] Institute for Sustainable Agriculture, IAS.CSIC, Avenida Menéndez Pidal S/N. 14004. Córdoba, Spain.

[2] Department of Forestry Engineering, University of Córdoba, University Campus of Rabanales, Madrid-Cádiz Road Km. 396, 14014 Córdoba, Spain.

[*]Corresponding author.joseagomez@ias.csic.es

## Abstract

This study evaluated the effect on SOC concentration, stock and fractions in a dehesa divided into two areas of similar soil type but different soil management. The first area was a pastured dehesa (P) with young Holm oaks, planted in 1995 (70 trees ha$^{-1}$, 12 m x 12 m) and, since 2000, grazed by sheep (3 sheep ha$^{-1}$) with an average period of grazing of six months a year. Prior to this it was managed in the same way as the second adjacent area. The second area was a cropped dehesa (C) with widely spaced mature Holm oak (14 trees in a 12-ha dehesa), on which a mixture of vetch and oats was cultivated every three years and tilled with a chisel plough. After 22 years both dehesas showed similar SOC stock distribution amongst areas with different soil management, with approximately 40 t ha$^{-1}$ in the top 100 cm of the soil. The P dehesa only showed higher SOC stock than the C dehesa on the surface 0-2 cm (5.86 ± 0.56 t ha$^{-1}$ *vs* 3.24 ± 0.37 t ha$^{-1}$). The influence of the trees, increasing SOC concentration and content when compared to the area outside the canopy projection, was only detected under the mature trees in the C dehesa. In the area outside the tree canopy, both systems showed a similar distribution of soil organic carbon among their different fractions, with the unprotected fraction being the dominant one, followed by the physically and chemically protected fractions. In the C dehesa, the mature trees' presence significantly modified the distribution of soil organic carbon in their surroundings, increasing the relevance of the unprotected fraction. The distribution of soil organic carbon in the unprotected and physically and chemically protected fractions were strongly correlated to the overall organic carbon concentration in the soil, indicating the rapid response of these three fractions to management, with the biochemically protected fraction showing no correlation, suggesting a high resilience to the changes in carbon budget.

**Keywords:** organic carbon fractions, agroforestry, shift from cultivation to grazing, crop rotation, tree plantation.

## 1. Introduction

Agro-silvo-pastoral systems are a form of land use where trees, crops, pasture and livestock share the same plot of land (Cubbage *et al.,* 2012). The interaction of these four elements of agro-silvo-pastoral systems provides a variety of benefits and ecosystem services, including $CO_2$ fixation in the woody tissues of trees and in the soil as organic carbon (Nair *et al.*, 2008; Nair and Nair, 2014). However, it is well known that soil organic carbon storage and dynamics depend on region, parent material, time, cover vegetation, and soil management (Jenny 1980; Munoz-Rojas et al. 2012). Therefore, local studies are necessary to appraise the potential of soil to store carbon properly. Dehesas are important agro-silvo-pastoral systems in Mediterranean areas, particularly south-western Spain, as well as Portugal and Sardinia in Italy (Eichhorn *et al.*, 2006; Caballero *et al.*, 2009; Cappai *et al.*, 2017).

In Spain, dehesas cover a total of around 4 million ha of land in the south-western provinces (Junta de Andalucia, 2017). Dehesa is a system developed on poor land and aimed mainly at extensive livestock raising and in which forestry is not concentrated on timber production, but rather on increasing the crown cover per tree, and producing acorns and fuelwood. It has been used for many centuries (Olea and San Miguel-Ayanz, 2016), with the first written reference from year 924 AD (Olea et al., 2005). Beef cattle, sheep and Iberian pigs roam freely in the dehesa and feed on pasture and acorns, all of which contribute to soil fertility (Cappai *et al.,* 2017). The Holm oak (*Quercus ilex*) is the most typical tree in dehesas (Costa *et al.,* 2006); trees may also provide firewood and cork (Carbonero and Fernández-Rebollo, 2014; Cappai *et al.,* 2017). In some flat areas, dehesas are cultivated in rotation with natural pastures. Cereals or a mixture of legumes and cereals are common crops for hay, silage or for grain and straw.

Several papers have addressed the effect of soil management on carbon storage in the dehesa; however, it remains a topic which requires further research in this land use system. For instance, Parras-Alcántara *et al.* (2015) when evaluating dehesas in southern Spain found no significant difference in soil organic carbon stock between the conventional and organic management systems, with 74.9 t ha$^{-1}$ and 76.4 t ha$^{-1}$ respectively. Corral-Fernández *et al.,* (2013), also in Spain, found that soil organic carbon concentration was only slightly different after 20 years of two types of tillage management in dehesas, which was lower in less-intensively farmed land, but also showed a strong influence of soil depth on soil organic carbon storage and concentration. In our review, no studies were found evaluating the impact of grazing on soil organic carbon content in dehesas, but compared to similar systems suggests that this might be dependent on climate type and grazing intensity. So, under arid or semi-arid climates (cool or warm), only low- and medium-grazing intensities were associated with increased soil organic carbon stocks when compared to ungrazed land, while in a warm, moist climate, all grazing intensity increased soil organic carbon stocks (Abdalla *et al.,* 2018). Differences in land use and its understory may have a greater impact on soil organic carbon in dehesas than grazing intensity within the range of stocking density usually described. Pulido-Fernández *et al*. (2013), in Southern Spain, found the highest soil organic carbon stock in soils from dehesas (42.3 tha$^{-1}$), being almost twice that of tree-less grasslands (23.9 tha$^{-1}$) and degraded units (23.7 tha$^{-1}$). Another case study by Upson *et al.* (2016) showed how the conversion of a grassland site in lowland England to either woodland or a silvopastoral-system modified the concentration of carbon in the top 150 cm of the soil. Fourteen years after tree planting, the organic carbon content in the 10 cm surface soil layer remained higher in grassland (6.0%), lower in the woodland (4.6%), and intermediate in the silvopastoral system (5.3%), with no differences among treatments below the 20 cm soil depth. Other examples of land use conversion show significant increases in soil organic carbon after the conversion of pasture to forest (8%), cultivation to pasture (19%), cultivation to plantation (18%) and cultivation to secondary forest (53%) (Guo and Gifford, 2002; Paul *et al.,* 2000; Post and Kwon, 2000).

Lozano-García *et al.* (2016) indicated that in studies on soil organic carbon in the Mediterranean area it is necessary to address the spatial variability induced by slope, aspect, and the position of the trees. The influence of the tree below the canopy area is an important factor in the spatial determination of soil organic carbon. The horizontal and vertical extent of its roots affects the soil organic carbon content below and beyond the tree crown differently. Howlett *et al*. (2011) found that in a silvopastoral oak dehesa system (mix of *Quercus ilex* and *Q. suber*), with native pasture and livestock production, there was a higher soil carbon below mature cork oak trees when compared to the area outside the tree canopy projection. Simón *et al.,* (2013) highlighted the positive correlation between tree presence and soil organic carbon stocks up to distances of 8 m away from the tree trunk. The presence of the Holm oak had been noted by previous studies as an important factor for organic carbon sequestration throughout the soil profile (Gallardo, 2003; López-Carrasco *et al.*, 2015). In other forest systems, it has been shown that the presence of trees increased the soil microbial biomass carbon pool in the first layer (Kara *et al.*, 2008), and improved the soil water holding capacity (Joffre and Rambal, 1993). However, it is unclear how long it takes to develop these differences after tree establishment and the interaction with specific land uses in the dehesa.

Soil organic carbon is a key attribute to soil quality. The impact of soil organic carbon on soil quality can be evaluated more precisely by its distribution amongst different organic carbon fractions with different degrees of protection against physical, chemical, and biochemical degradation. Six *et al.*, (2002) proposed a fractionation method into four classes to evaluate soil quality in arable, afforested, and forest ecosystems. Among these fractions, the physically protected carbon associated with the 53-250 micro aggregates and the chemically protected carbon associated with aggregates <53 microns, are mostly responsible for the changes in long-term accumulation and stability of the carbon pool associated with changes in land use and management. In the same study, Six et al. (2002) showed how the unprotected carbon in macro-aggregates >250 microns are more sensitive to changes in agricultural soil management, while physically or chemically protected carbon greatly contributed to increased SOC stock in afforestation of cultivated land. Vicente-Vicente *et al*. (2017) analyzed the impact of management factors on soil organic carbon fractions in olive groves managed with a temporary cover crop, which can be considered a similar forest system. Their results suggested that an increase in soil cover by the temporary cover crop increases the capacity to store carbon in three different compartments (unprotected, physically and chemically protected), while the biochemically protected carbon is the most stable throughout the entire soil profile, regardless of management or vegetation cover.

We planned the study presented in this manuscript with the aim of developing new knowledge that could contribute to a better quantification of the potential of dehesas to store organic carbon in the soil using different management options. Therefore, the study described in this manuscript encompassed three specific objectives:

1) To quantify the soil organic carbon (SOC) stock in a dehesa with a similar soil type and history, but two different soil managements for 22 years.

2) To evaluate the effect on SOC stock of a strategy for tree regeneration in the dehesa based on plantations with a higher tree density, which were introduced in southern Spain as part of the reforestation programme promoted by the European Commission in 1992.

3) To explore the implications of two different types of soil management and the influence of the trees on the distribution of SOC in different fractions that differ in stability.

## 2. Material and methods

### 2.1. Area description

This study was conducted on an experimental farm located at Hinojosa del Duque, Cordoba, Spain (38º 29' 46" N and 05º 06' 55" W, see Fig. 1), at 543 meters above sea level. The area has a mean annual rainfall of 437 mm and an average annual temperature of 15.1 ºC (average for 2010 to 2017; meteorological experimental station, Hinojosa del Duque). The soil type in the study area is classified as Eutric Cambisol with a shallow depth and rocky outcrops (CSIC-IARA, 1989). This farm was selected because it covers two adjacent areas (Fig. 1) with different features, and maintains detailed records about soil management for the last few decades. The first area is a pastured dehesa with young Holm oaks (hereinafter, P). Holm oaks were planted in 1995 at a density of 70 trees ha$^{-1}$ (in a regular frame of 12 m x 12 m). This planting included soil preparation by subsoiling and, during the first five years, the control of herbaceous vegetation with a disc plough. At the time of our sampling, March 2017, the mean diameter at breast height (DBH) was 17.2±2.6 cm and canopy cover was around 10%. The area had been grazed by Merino sheep since 2000, at a stocking rate of 3 sheep per hectare. Grazing is conducted rotationally with at least four grazing periods per year (with the average period of grazing being six months a year). In 2016, natural pastures were fertilized with 40 kg of $P_2O_5$ ha$^{-1}$. Prior to 1995 the management of this area was similar to the second area described below.

The second area is a cropped dehesa with widely spaced (1.2 trees ha$^{-1}$) mature Holm oaks (hereinafter, C). A historical aerial photograph shows that in 1956 these trees, already matured, were already present with the same spatial structure, so it is at least 90-100 years old. At the time of sampling, the mean DBH of Holm oaks was 78.1 ± 13.6 cm and the tree canopy cover was less than 1%. Every three years, a mixture of vetch and oats is cultivated for hay. Prior to sowing, the area is fertilized with 20 t ha$^{-1}$ of dairy manure. A chisel plough is used for soil tillage, with tillage covering all the plots except the immediate vicinity (0.3-0.4 m approximately) of the tree trunk. After harvest, and for the following two years of the three-year period between consecutive sowings, the area is grazed, keeping to a similar scheme to that of the pastured area. The main soil properties for both areas, taken from four soil pits made at the time of sampling (two for each area, see section below), are shown in Table 1.

## 2.2 Soil characterization and sampling

Four soil profiles were described by digging four pits, two in each area and with all the pits starting near the tree trunk and crossing below and outside the tree crown projection area. The description of the profile, including visual assessment of root density by root size, was made according the NRCS guidelines, Soil Survey Staff (2012), distinguishing between the areas below and beyond the tree crown projection in all the pits.

Undisturbed soil samples were taken with a hand soil sampler to determine bulk density (BD). The samples were taken at the 4 pits, distinguishing between pit zone (below and outside the tree canopy) and at four depths (0-5 cm, 20-40 cm, 40-60 cm and 60-100 cm), with two replications totalling 64 samples. The samples were oven-dried at 105 ºC for 72 hours to a constant mass. The bulk density of the soil was calculated by dividing the dry mass of soil by the volume of the bulk density sampler (98.2 $cm^3$), according to Hao *et al*. (2008).

In each area (P and C), 10 random sampling points were selected outside the tree canopy projection. Additionally, the nearest tree to each point was identified and a new sampling point under the canopy was selected, but at a distance from the trunk that located this point within the ploughed area in the C dehesa. Therefore, a total of 20 sampling points per area were sampled. Samples were taken at 8 different depth intervals (0-2 cm, 2-5 cm, 5-10 cm, 10-20 cm, 20-40 cm, 40-60 cm, 60-80 cm and 80-100 cm, where possible) at each sampling point, having previously removed the grass and mulch surface. Soil samples were taken combining a manual soil sampler (for the three top-soil samples) and a hydraulic soil sampler (Giddings®) with a 38.1 mm diameter soil core. Overall a total of 266 soil samples were taken (area x sampling points x depth) because the soil depth at the hard C horizon was less than 100 cm at some sampling points.

## 2.3 Soil analysis

The soil samples were ground, passed through a 2 mm sieve, and homogenised. Stoniness, defined as coarse material whose diameter is >2 mm, was determined as a % of mass. Soil organic carbon (SOC), a concentration of fine earth (<2 mm diameter) was determined according to Walkley and Black (1947).

Soil organic carbon stocks for each soil depth interval ($\mathrm{SOC}_{\mathrm{stock\ i}}$), and for the whole soil profile, were calculated according to Penman *et al*. (2003) and IPCC (2003):

$$\mathrm{SOC}_{\mathrm{stock\ i}} = 10000\ SOC_i\ x\ BD_i\ x\ d\ x\ (1-\delta) \qquad (1)$$

$$\mathrm{SOC}_{\mathrm{stock}} = \sum_{i=1}^{i=n} SOC_{stock\ i} \qquad (2)$$

where $\mathrm{SOC}_{\mathrm{stock\ i}}$ is the total soil organic carbon in a given layer (t ha$^{-1}$). $\mathrm{SOC}_{\mathrm{i}}$ is the organic carbon concentration (g g$^{-1}$), $\mathrm{BD}_{\mathrm{i}}$ is the bulk density of the soil (t m$^{-3}$) as defined above, d is the thickness of the depth interval (m), δ is the fraction (0 - 1) of gravel larger than 2 mm in the soil sample, and n is the number of soil layers. Therefore, equation 2 gives the total soil organic carbon, $\mathrm{SOC}_{\mathrm{stock}}$ (t ha$^{-1}$) in the whole soil profile discounting the effect of stoniness. BD values for soil depths not sampled were interpolated using mass-conserving splines (Malone *et al*. 2017). Mean values of BD in each area, below and outside the tree canopy, were used in equation 1.

## 2.4. Soil organic carbon fractionation

Soil organic carbon fractionation was used on a subset of selected samples. For our exploratory analysis, we chose samples from the 0-2 cm, 2-5 cm, 20-40 cm and 40-60 cm depth intervals, and from amongst them, the samples with the maximum and minimum organic carbon concentration values were chosen in both study areas, in the zone below the tree crown and beyond it. Fractionation was carried out following the method proposed by Six *et al*. (2002). This method, which combines physical, chemical and biochemical fractionation, allows the determination of four different pools of soil organic carbon: (i) the unprotected fraction, which is the particulate organic carbon in aggregates measuring 2000-250 µm, separated by sieving, plus the light fraction (LF) of the 250-53 µm aggregates, separated by flotation and centrifugation of the >53 µm aggregates; (ii) the physically protected fraction, which is the organic carbon in the 250-53 µm aggregates that remains stable after centrifugation, once we have discarded the light fraction by flotation; (iii) the chemically protected fraction, which is the hydrolysable portion, after acid hydrolysis, of organic carbon in aggregates measuring < 53 µm, the slime-sized fractions and clay isolated during the initial sieving and dispersion; and (iv) the biochemically protected fraction, which is the non-hydrolysable organic carbon remaining in the slime and clay fractions after the acid hydrolysis. The concentration of organic carbon in each pool was determined by wet oxidation using sulphuric acid on samples between 0.3–0.5 g using potassium dichromate with an absorbance spectroscope in the range of 600 µ (Vicente-Vicente *et al.*, 2017, Jindo *et al.*, 2016).

## 2.5 Statistical data analysis

In each soil depth sampled, two-factors ANOVAs were performed to evaluate the effects of (i) the soil management, (ii) the tree presence (below and outside the canopy projection) and (iii) their interactions on BD, stoniness, SOC concentration, SOC stock and soil organic carbon fractions. SOC and SOC stock data were transformed to fulfil the ANOVA requirements (inverse and logarithmic functions for SOC and SOC stock respectively). Data of soil organic carbon fractions were grouped in two depths: top (0-2cm, 2-5cm) and deep (20-40 cm, 40-60 cm) layers. The relationship between soil organic carbon fractions was explored by Pearson coefficient correlation. The overall effect of depth on BD, stoniness and SOC was evaluated using a Kruskal-Wallis test and Mann Whitney U test, in the case of soil organic carbon fractions.

BD and stoniness are involved in the calculation of SOC stock and small differences in both variables could affect the final results of carbon stock and mask the differences induced by soil management. Additionally, it is known that BD and stoniness can experience large spatial fluctuation, even at close distances. We used the non-parametric bootstrap approach to assess the uncertainty in SOC stock estimation in C and P related to stoniness and BD (Efron and Tibshirani, 1986). We pooled all stoniness data and, per depth, a new random sample of a size similar to the number of sampling points we had at that depth (10 or lesser) was extracted by resampling with replacement. This bootstrap sample of stoniness was then used to calculate SOC stock at each sampling points (10) following equation 1 and 2, where SOC concentration and BD were the original values resulted from samplings. Finally, we averaged the SOC stock from the 10 sampling points and the resulted mean was denoted as SOC stock*. We repeated this process 500 times and compiled the bootstrap distribution of SOC stock* in each area P and C. The uncertainty was quantified by the confidence interval at the 95% level, using the 2.5 and 97.5 percentiles of the bootstrap distributions of SOC stock*. If the confidence interval included the original mean of SOC stock resulting from the sampling, then the effect of stoniness in SOC stock calculation was said to be negligible. A similar procedure was followed with BD, except for the resampling stage. In this case, only BD data from the same area were pooled, due to the significant influence of soil management on BD. The bootstrap sample of BD was then used to calculate SOC stock, keeping in this case the original values of SOC concentration and stoniness from samplings.

Calculations and analysis were performed using the R language for statistical computing (R Core Team, 2013) and Statistica SE 14.

## 3. Results

### 3.1 SOC concentration

The SOC concentration was highly stratified, with a clear trend to an exponential decrease in line with depth, particularly in the top 20 cm of the soil (Fig. 2). Overall, mean values decreased from 1.81% near the surface to 0.14% between 80 and 100 cm. Soil management affected SOC concentration only in the uppermost 2 cm, reaching average values of 1.30 ± 0.41% in C dehesa and 1.99 ± 0.57% in P dehesa. Holm oak increased SOC concentration in C dehesa by up to 20 cm depth. This increase ranged between 53% for the top 2 cm to 142% for the 10 cm-20 cm soil layer. However, in P dehesa the presence of the Holm oaks had an insignificant effect on SOC concentration. Roots were concentrated in the A horizon, very fine and fine roots were common under the Holm oak canopy of both dehesas C and P, being more abundant beyond the tree canopy zone in P dehesa (Supplementary material 1, S1). Also, in the A horizon, medium and coarse-sized roots were more abundant. In the B horizon, the presence of very fine and fine roots was common under the Holm oak canopy in both areas, but scarce beyond the tree canopy projection area. Very few coarse roots were found in this horizon. In the BC horizon (more than 60 cm depth) no roots were found in either the C or P dehesa.

### 3.2 SOC stock

SOC stock presented a similar depth-distribution amongst areas with different soil managements (P and C) and between zones (below and beyond tree canopy) as those already discussed for SOC concentration (Supplementary material 2, S2). Therefore, P dehesa only showed a higher SOC stock than C dehesa at the surface 0-2 cm (5.86 ± 0.56 t $ha^{-1}$ *vs* 3.24 ± 0.37 t $ha^{-1}$). Mature trees in the C dehesa contributed to a significant increase in the carbon stock in the topsoil layer (0-20 cm) by an average of 87%; however, young trees had no significant effects on P dehesa.

Figure 3 depicts the SOC stock accumulated up to a depth of 20 cm, 40 cm and the total sampled profile. The mean soil depth ranged between 89 cm in P dehesa outside the tree influence, to 81 cm in C dehesa, under the tree canopy. Soil depth reached 100 cm only at 36% of the sampled points. P dehesa showed significantly higher SOC stock than C dehesa in the 0-20 cm profile. However, the differences were not significant when, for carbon stock calculation, 40 cm depth or the total sampling depth were considered. Up to a depth of 20 cm, P dehesa stocked, on average, 6.5 t $ha^{-1}$ more than C dehesa, which represents an increase of 42%. Mature trees significantly increased carbon stocked in C dehesa (up to 20 cm, 40 cm and at total soil depth). Total carbon stocks were 39.9 t $ha^{-1}$ and 38.8 t $ha^{-1}$ in P and C dehesa respectively, and 45.6 and 52.5 t $ha^{-1}$ under the tree canopy (Fig 3). The top 40 cm held, on average, more than 70% of the carbon stocked in the soil profile.

Stoniness increased with depth and was significantly higher in C dehesa (8% and 12% on average in P and C dehesas) (Table 2). This slight difference in stoniness slightly reduces the total carbon stock in C dehesa compared to P dehesa. However, resampling stoniness led to a similar mean of total carbon stock in C and P dehesa (39.7 and 40 t $ha^{-1}$ respectively), given that mean values were within the confidence interval of the bootstrap distribution of SOC stock* (Fig 4, A and B), and there was therefore a lack of statistically significant differences.

Like stoniness, soil bulk density increased significantly with depth (Table 2). On average, BD ranged from 1.49 t $m^{-3}$ in the top layer (0-2 cm) to 1.64 t $m^{-3}$ at the deepest depth interval measured (80-100 cm). The effect of different types of soil management on bulk density was restricted to the topsoil layer (0-5 cm), with higher values in the P dehesa. In both areas (P and C), trees significantly decreased the soil bulk density of the top layer under their canopy. Figure 4 (C and D) showed bootstrap distribution of SOC stock* after BD resampling. According to the position of the mean value in the range of confidence interval, the use of raw data of BD from each area instead of mean value, resulted in a slightly higher value of stock in C dehesa but similar in P dehesa (39.7 t ha$^{-1}$ in P dehesa and 39.4 t ha$^{-1}$ in C dehesa).

### 3.3. Soil organic carbon pools

Figure 5 depicts the SOC concentration in the different pools by soil management (C and P) and location to the tree canopy at top and lower soil layers. The absence of the tree effect on SOC concentration in the different pools, as has been already shown for bulk SOC concentration, is apparent from the analysis in P dehesa. In the topsoil of C dehesa, there is a higher concentration of SOC in the unprotected and the chemically protected pools in samples located below the tree canopy. No differences were detected between types of dehesa in the SOC concentration of the different pools with the exception of the biochemically protected. Additionally, Figure 5 compares the SOC concentration of the different pools between the under-tree canopy of both types of dehesa. It is apparent how the differences in SOC concentration are concentrated in the unprotected pool, which tends to be higher in C dehesa. Overall, there was a clear effect of depth on SOC concentration of all the fractions except the biochemically protected ones (Table 3).

There was a clear correlation between SOC concentration of the unprotected, and physically and chemically protected, fractions with SOC concentration in the bulk soil (Table 4). SOC concentration for the physically and chemically protected pools was higher than that of the bulk soil, particularly the chemically protected pool, while the unprotected fraction presented a lower concentration than that of the bulk soil. There was no correlation between the SOC concentration of biochemically protected pool and that of the bulk soil.

The distribution of the total SOC among the different pools is depicted in Figure 6. In P dehesa the largest pool of organic carbon was in the unprotected fraction, at around 30%-45%, although the other three pools had a slightly lower contribution, ranging from 15% to approximately 30%, with the exception of the biochemically protected pool in the topsoil itself beyond the tree canopy, which represented a small fraction of around 3%. There was a tree effect on the relative contribution of the unprotected pool, which tended to decrease under the tree canopy, and in the biochemically protected pool, which tended to increase in relative contribution under the tree. In C dehesa, the unprotected carbon pool was also the fraction with the largest contribution to the total SOC, although this time with a higher magnitude than in P dehesa, ranging from 30% to 75%. The other two organic carbon fractions with a larger contribution were the physically and chemically protected fractions, both ranging from 10% to approximately 25%. The biochemically protected pool contribution presented a wider variation, ranging from 2% in the top layer of the under-canopy area up to 20% in the lower soil layer. In C dehesa there was a significant tree-effect on the relative contribution of the unprotected carbon pool, which tended to have a higher contribution under the tree canopy. Table 3 showed that depth does not affect the relative contribution of each fraction to the overall SOC stock, which only varies in the biochemically protected fraction due to the increase of their relative contribution as the soil depth increases.

## 4. Discussion

### 4.1. SOC concentration in dehesa

Our results clearly show that SOC concentration decreases with depth in the dehesa system. These results are consistent with the literature (González González *et al.*, 2012; Pulido-Fernández *et al*., 2013; Francaviglia *et al.*, 2017). In fact, the highest SOC concentration was found in the top 0-5 cm, although with a high variability, ranging from 1.2% to 2.0 %. These results are similar to those reported by Pulido-Fernández *et al*. (2013) for dehesas in Leptosols, 2.3 % and Luvisol 1.1 %. The authors attributed this variation in the first 5 cm to the type of land use. Higher values may be found in other dehesas, an example being a study consisting of 36 different dehesas in Mainland Spain featuring Holm oaks with scattered tree cover (González González *et al.*, 2012). The authors reported the same mean SOC concentration as in our study (1.6%). However, this was in the first 20 cm of soil, suggesting even higher values in more superficial layers. Such a discrepancy may be explained by differences in the climatic conditions, as our study site was more arid, and also had lower thickness and clay content in the surface layer.

The decreases in SOC concentration were clearly stratified at depths of up to the 20 cm, after which the trend continued without significant differences. This 20 cm depth threshold, in which the depth-dependent relationship of SOC concentrations was strongest, coincides with the A horizon in our soils. It has previously been shown that both SOC and depth of the A horizon are highly correlated with the soil type (Premrov *et al.*, 2017), as well as the rhizosphere depth, the historical use of the land, and the orography (Marinho *et al.*, 2017). The type of vegetation has a further influence on the rhizosphere width due to significant differences in the root systems, such as woody plants having deeper root systems than herbaceous plants (Pulido-Fernández *et al*. 2013; Zhou, Boutton & Wu 2017). In our experiment, most of the roots of the trees and herbaceous vegetation were found in the A horizon.

## 4.2. Effect of soil management on SOC concentration

With respect to soil management, in our study cereal-legume cultivation in rotational 3-year cycles, plus sheep grazing, resulted in a similar SOC concentration to no-tillage farming and exclusively sheep grazing, albeit with the exception of the topsoil layer (0-2 cm). It is also generally accepted that a change of land use from agriculture into permanent grassland can increase soil organic carbon concentration, at least in the first years after the transformation (Mohanty *et al.,* 2017; Abdalla *et al.,* 2018). The relevance of our study is to show that, for the conditions studied, this transformation from three-year rotation into permanent grassland has not increased the SOC concentration after 20 years. This might be explained by the relatively low tillage intensity of the three-year rotation employed together with the organic fertilization applied to the crop, by the moderate productivity of the permanent grassland under rain-fed Mediterranean conditions in the study area which did not receive additional fertilization, and by the fact that the plantation of trees in the P dehesa required periodical tillage during the first five years to control competition for soil water by the weeds growing naturally on the farm. In the latter explanation, such intense work on the soil could result in a decrease in SOC concentration during the first few years, which the soil may still be recovering from. Nevertheless, there are other examples in the literature that did not find the expected increase to changing soil management under Mediterranean conditions, particularly in more arid areas. For instance,  Romanyà *et al*. (2010) already stated ~~that state~~ that after abandoning agricultural lands, the capacity of C sequestration would be greater in wet areas and lowest in semiarid. Low levels of organic C in semiarid and Mediterranean soils suggest that the recovery of C after the abandonment of arable may not take place mainly as a result of ecological and soil constraints existing in dry and semiarid areas it can take a very long time.  Romanyà *et al.* (2000) estimated that the original SOM content can be reached after 60-100 years after reforestation with P. radiata of cereal fields.

### 4.3. Effect of the trees on SOC concentration

SOC concentration not only depends on depth, but also on other factors relating to the presence of trees. However, in this study, higher SOC concentrations were only found in the presence of mature trees, up to a depth of 40cm, but not in the presence of younger trees. This finding echoes several research works carried out in dehesas (Howlett *et al*. 2011; Pulido-Fernández *et al.*, 2013; Simón *et al.*, 2013; González González *et al*. 2012; Gallardo 2003) and in other agroforestry systems (Fernández-Ondoño *et al*. 2010; Monroe *et al*. 2016; Upson *et al*. 2016). Since the trees' influence on SOC is strongly related to their roots, their presence was evaluated in the A horizon of both dehesas in our study. Thus, lower root density was found in the open area compared to the area below the tree canopy of both young and mature trees (samples from P and C dehesa, respectively), but the highest root density was found under the mature trees. We also documented a sharp decrease in root density from the A to B horizon. Although several studies have recorded that oak roots can extend to deep soil horizons (Moreno *et al.*, 2005), many studies have found that most of the root biomass is concentrated in the top 30cm of the soil horizons (Canadell, *et al.* 1996;Moreno y Obrador 2007; Ojeda*et al.*2018; Meier *et al*. 2018). This might explain why Pulido-Fernández *et al*. (2013) found differences between SOC concentration from samples taken beneath tree canopies when compared to those solely from the 0cm-5cm layer in open areas. Moreover, another factor affecting the topsoil underneath the canopies is the accumulation of fallen leaves, as was studied in a sessile oak site (Kara *et al*. 2008). Nevertheless, this effect is more evident in soils under deciduous trees rather than evergreen trees.

Our results reinforced the hypothesis that trees can significantly increase SOC concentration after a relatively long period of time (more than 22 years, but less than 90 years in our case), with it being necessary to incorporate a temporal dimension when appraising the impact of trees on SOC concentration in the dehesa system. This is in line with results obtained in other agroforestry systems, such as those studied by Upson *et al.* (2016) in England, where the introduction of ash trees (*Fraxinus excelsior*) into pasture grazed by beef cattle had not increased SOC 14 years after planting. Accordingly, introducing trees onto grasslands does not necessarily increase SOC content in the one or two decades after planting. In fact, after tree plantation, SOC could even decrease because of the priming effect of new root exudates and dead roots (Cardinael *et al*., 2018). In this connection, Haile *et al.* (2010), indicate that agroforestry systems can help soil carbon sequestration in the long-term, while, in the short-term, carbon conservation may be unstable, mainly due to certain soil management practices. This seems to be the case with the P dehesa in our study, where the dominant source of organic carbon in the soil remains herbaceous vegetation and animal manure. Several studies indicate that the fine roots of herbaceous crops and pastures are more abundant than those of trees, while also having a higher renovation rate (Persson 1983; Canadell *et al.* 1996; Moreno et al. 2005). It is easier for these fine roots to integrate into the soil in a more protected form of carbon (Rasse *et al.*, 2005).

### 4.4. SOC fractions

Our study is one of the few studies evaluating the effect of management and tree influence on SOC fractions at several depths in dehesa. In P dehesa, after more than 22 years' afforestation, the oak trees did not have a significant influence on the SOC concentration of the different carbon pools, or on the relative contribution of each pool to the total SOC, when compared to the area outside the tree canopy.

Poeplau and Don (2013) indicate how most of the changes in soil organic carbon tend to happen a few decades after afforestation. This lack of differentiation might be due to the homogenising effect of permanent grazing across the whole plot, which is a dominant factor overcoming the moderately higher presence of tree roots in the below canopy areas, particularly of sparse coarse roots (S1). Moreover, the overall moderate SOC concentrations mean that none of the depths and areas in the P dehesa are carbon saturated, with carbon saturation deficits from 0.20 to 0.81, as defined by Steward *et al.* (2009). A different situation was observed in the C dehesa which holds much older trees. In this dehesa, the under-canopy area presented a higher SOC concentration in the unprotected and chemically protected pools, resulting in a higher fraction of the carbon being contained in the unprotected pool, when compared to the area beyond the canopy projection. The unprotected pool is the most sensitive to changes in SOC (Poeplau and Don, 2013), which suggests that these differences might be due to a higher addition of fresh plant and root material in the vicinity of the trees, as is apparent due to the higher tree root density in this dehesa (S1). Given the correlation between the bulk SOC content and that of all the pools except the biochemically active pool (shown in Table 4), the absence of a difference in organic carbon concentration translates into the lack of differences in the different carbon pools. The biochemically protected carbon pool has a very slow response to changes in SOC content (Steward et al., 2009) and this seems to be the case here.

The dominant organic carbon pool in both dehesas tended to be the unprotected pool, represented from 29% to 77% of the total carbon pool depending on the dehesa, depth and sampling point. There were only in two situations, both in the pastured dehesa, where another pool displayed a similar share of the total carbon pool. This was in the lower soil layer outside the canopy projection, where the biochemically protected form represented more than 25% of the total organic carbon, and in the topsoil layer under the canopy in which the chemically protected pool was 30% of the total carbon pool (slightly higher than the 29% share of the unprotected fraction). This proportion of unprotected carbon (which might average around 35-40% for the two dehesas and depths sampled) in the higher ranges of the share of the unprotected fraction in the total soil organic carbon pool is one reflected by Poeplau and Don (2013) for some grassland and forest areas in Northern Europe. Although there were some slight differences in the method to determine the different carbon pools, our results suggest that in the edaphic-climactic conditions of our study, and despite a relatively high carbon saturation deficit, the two strategies for dehesa management store a significant fraction of soil organic carbon in the unprotected pool. The fraction of SOC stored in the unprotected pool is higher than that reported for other agricultural systems (e.g. Poeplau and Don, 2013) even for another typical Mediterranean cropping system, that of olives, for which Vicente-Vicente *et al.* (2017) reported an average share of the unprotected fraction ranging from 35%, in the top 5 cm of the soil, to 22%, in the 5 cm-15 cm soil depth. The other two dominant pools are the physically and chemically protected fractions which ranged from a 10% to a 35% share, similar to that shown by Vicente-Vicente et al. (2017) for olives. The dehesas studied displayed biochemically protected fractions which were slightly lower (particularly in the topsoil) than those reported for other agricultural systems (Poeplau and Don, 2013; Vicente-Vicente *et al.* 2017). As a result, these dehesa systems are more sensitive to changes in conditions (e.g. more intensive management, warmer climate, etc) which can cause rapid depletion of the other less protected pools (Six et al., 2002b).

### 4.5. SOC stock

The total SOC stock in our work reached values from 38.8 t ha$^{-1}$ to 52.5 t ha$^{-1}$. These values are within the observed range in other studies carried out on dehesa systems. For instance, Howlett *et al.* (2011), in a dehesa with a similar soil depth, up to 100cm, found a total SOC stock of 28 t ha$^{-1}$. In a dehesa in a relatively more humid area (582 mm of annual rainfall) used as permanent grassland, Roman *et al.* (2018) found a mean value of 43.8 t ha$^{-1}$, sampling a soil profile at a depth of 30 cm which held, on average, 70% of the total SOC stock. Nonetheless, as these authors highlighted, SOC stock varied considerably across the landscape, in which Cambisol, Regosol and Leptosol soil types were alternated (17.0 to 94.1 t ha$^{-1}$). Corral-Fernández *et al.* (2013), found a mean total SOC stock of 77 t ha$^{-1}$ in different dehesa farms with Cambisol, with this value decreasing to 58 t ha$^{-1}$ in cases where Leptosols were present.

The different management carried out on P and C dehesas had resulted in a different SOC stock, when a soil depth of 20 cm was taken into account for calculation, with the area devoted to permanent grassland stocking more carbon - 6.5 more t ha$^{-1}$. As we discussed above, there were no significant differences in SOC concentration between different types of management in most of the soil layers considered in this study, with the exception of the upper topsoil layer (0-2 cm). Therefore, this difference in carbon stock could be due to the contribution of other variables involved in SOC stock calculation, such as stoniness or soil bulk density, the latter depending on soil management. In fact, as a result of grazing, P dehesa showed higher soil bulk density at the surface than C dehesa, in which the occasional tilling reduced BD in the layer worked by farming implements. The simulation of the calculation of SOC stock through the permutation of the raw data of stoniness and BD, albeit referring to the total soil profile, led to slight variations on SOC stock, somewhat more accentuated in C dehesa where the measured stoniness was higher and the BD lower. Although these differences, within the range of 0.5-1 t ha$^{-1}$, were small in the context of total stock, they may have importance when comparing different land uses and soil management. Along these lines, Mohanty *et al.* (2017), found that SOC stock increases when arable land was devoted to permanent grassland (from 28 t ha$^{-1}$ to 32 t ha$^{-1}$). Moreover, these variables can also be important to the dynamics of the organic matter, by regulating processes such as the flow of water, circulation of air in the soil, or even the formation of micro-aggregates (Uribe *et al*. 2015; Ferreiro-Domínguez *et al.* 2016; Seddaiu *et al*. 2018). However, the differences disappeared when increasing the depth for stock calculation. The carbon stocked in the first 20 cm of the soil represented around 55% of the total SOC stock in P dehesa, and 40% in C dehesa, which coincides with the results of Corral-Fernández *et al.* (2013), who found more than 41% of the total carbon stored in a Cambisol soil type (100cm deep), or 48% in a leptosol (65cm deep), in this layer. Authors such as Parras-Alcántara *et al*. (2015) and Roman *et al.* (2018), reported an average of 70-80% of the total carbon accumulated as being in the first 25-30 cm of the soil.

Several studies demonstrate that the presence of trees has a big impact on SOC stock in dehesa (Howlett *et al*. 2011; Simón *et al.* 2013). In our study, we found contrasting results depending on the maturity of the trees. In P dehesa, with relatively young trees (22-year-old) there was no impact on SOC stock, while in C dehesa with mature trees, SOC stock increased by 35% under the tree canopy (from 38.8 to 52.5 t ha$^{-1}$). These results are in line with the findings of Upson *et al.* (2016) in a temperate agroforestry system where trees were 14 years old. Howlett *et al.* (2011) found an increase of 22 t ha$^{-1}$ on SOC stock under the canopy of mature cork oaks in comparison with areas beyond the tree canopy (from 28 to 50 t ha$^{-1}$). Furthermore, Uribe *et al.* (2015) highlighted that trees adjacent to grazing had a positive influence on the ability of soils to store soil C and N. Young trees with low growth, as occurs with the Holm oak, have still a constricted root system and canopy and this fact may limit the accumulation of SOC in the area underneath tree. However, this represents a clear future opportunity for P dehesa to fix organic carbon in soil and woody tissues. To do so, some kind of tree density control would be necessary in the future to overcome possible competition amongst the trees for soil nutrients and water that would collapse growth.

The carbon soil stock in C dehesa, with current tree canopy cover, reached a value of 38.9 t ha-1, while in P dehesa was 40.5 t ha-1. Although the difference was not significant, the change in land use from a crop-pasture rotation to a permanent grassland with holm oaks, have resulted in an increment of SOC stock in the order of 2 per mil per year, which mean a 50% of the target proposed by "4 per 1000" initiative.

## 5. Conclusions

Our results provide insight into the impact of specific types of management in dehesa on the modification of SOC and SOC stock, as well as its effect on its distribution among the different organic carbon fractions. It is apparent that 22 years after transformation of crop-pasture rotation and low tree density into permanent grassland used exclusively for low intensity grazing, and with a high tree density (70 trees ha$^{-1}$), both dehesas presented a similar SOC stock of approximately 40 t ha$^{-1}$ in the top 100 cm of the soil. The dehesa with permanent grassland only showed higher SOC stock than the crop-pasture rotation dehesa on the surface (5.86 ± 0.56 t ha$^{-1}$ *vs* 3.24 ± 0.37 t ha$^{-1}$). The influence of the trees, increasing SOC and SOC stock when compared to the area outside the canopy, was only detected in mature trees in the cropped dehesa. 22 year-old Holm oaks were still not able to induce an increase in SOC and SOC stock. The lack of differences in SOC stock between the two dehesas can be explained by ~~1~~ the lack of differences in SOC stock between the two managements, crop rotation vs grazing at low density, especially away from the trees, and ~~2~~ that the significant increase of SOC stock, found only beneath old tree canopies, did not translated in significant differences at plot scale because the low tree density.

Permanent grassland and crop-pasture rotation presented a similar distribution of soil organic carbon amongst different functional fractions, with the unprotected fraction being the dominant one (30%-45%), followed by the physically (15%-25%) and chemically (15-25%) protected fractions. The presence of mature trees significantly modified the distribution of soil organic carbon in their surroundings, increasing the importance of the unprotected fraction (40%-70%), and decreasing the relative importance of the physically (10%-25%) and chemically protected (9%-29%) fractions, probably for the higher contribution of fresh organic matter from roots and leaves to the soil. The distribution of soil organic carbon in the unprotected, and physically and chemically protected fractions was strongly correlated to the overall organic carbon concentration in the soil, with the biochemically protected fraction showing no correlation.

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***Highlights (for review)**

The main highlights of this paper are as follows:

1. Changing tree density, from 1.2 to 72 t ha$^{-1}$, did not increase soil OC stock after 22 years.

2. Mature oak trees have influence the distribution of soil organic carbon.

3. The unprotected organic carbon fraction is the dominant one.

4- The mature trees’ presence modifies the distribution of organic carbon fractions.

**Table 1.** Soil properties of the two areas - pastured dehesa with young trees (P) and cropped dehesa with mature trees (C). For BC horizon, data shown in this column indicate the depth at which this horizon begins; CEC: Cation exchange capacity; K: Available Potassium ppm; N: Organic Nitrogen; P: Available Phosphorus (Olsen) ppm, S.T.C: Soil Textural Class.

| **Dehesa** | **Hz** | **Depth (m)** | **pH 1/2'5** | **OM %** | **N %** | **K** | **P** | **CEC** | **Ca** | **Mg** | **Na** | **Clay** | **Sand** | **Silt** | **S.T.C.** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | **(meq/100g)** | | | | **(%)** | | | |
| P | A | 0.37 | 6.55 | 0.78 | 0.04 | 117.50 | 43.40 | 7.89 | 4.35 | 2.80 | 0.45 | 8 | 78 | 14 | Loamy sand |
| | B | 0.63 | 7.19 | 0.44 | 0.03 | 136.50 | 32.00 | 17.02 | 12.33 | 3.84 | 0.57 | 21 | 70 | 8 | Sandy clay loam |
| | BC | > 0.63 | 7.98 | 0.10 | 0.01 | 76.50 | 10.40 | 8.75 | 5.13 | 2.89 | 0.57 | 6 | 89 | 6 | Sand |
| C | A | 0.36 | 6.54 | 1.40 | 0.07 | 205.00 | 28.10 | 6.27 | 2.86 | 2.45 | 0.47 | 7 | 83 | 10 | Sand |
| | B | 0.77 | 7.75 | 0.49 | 0.02 | 155.50 | 4.95 | 19.55 | 13.07 | 5.36 | 0.80 | 23 | 68 | 9 | Sandy clay loam |
| | BC | > 0.77 | 8.65 | 0.16 | 0.01 | 103.00 | 3.40 | 14.62 | 10.36 | 3.11 | 0.96 | 13 | 81 | 6 | Loamy sand |

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**Table 2.** Mean values and standard errors of soil bulk density and stoniness according to depth in P and C dehesa, below and outside the tree canopy projection. At each depth, different letters indicate a significant difference according to two-factors ANOVA ($p<0.05$).

| **Depth (cm)** | **Bulk density (t m$^{-3}$)** | | | | **Stoniness (%)** | | | |
|---|---|---|---|---|---|---|---|---|
| | **P-dehesa** | **P-dehesa below canopy** | **C-dehesa** | **C-dehesa below canopy** | **P-dehesa** | **P-dehesa below canopy** | **C-dehesa** | **C-dehesa below canopy** |
| **0-2** | 1.62 (0.05) a | 1.53 (0.13) b | 1.53 (0.02) b | 1.28 (0.05) c | 6.8 (1.22) a | 7.5 (0.77) a | 10.7 (1.45) b | 9.2 (1.29) b |
| **2-5** | 1.61 (0.05) a | 1.53 (0.12) b | 1.53 (0.01) b | 1.23 (0.04) c | 8.4 (0.97) a | 7.1 (1.15) a | 11.3 (1.85) b | 10.6 (1.79) b |
| **5-10** | 1.57 (0.06) a | 1.54 (0.01) a | 1.53 (0.01) a | 1.35 (0.05) b | 2.1 (0.94) a | 4.6 (2.06) a | 9.8 (2.96) b | 7.7 (1.55) b |
| **10-20** | 1.49 (0.09) a | 1.54 (0.07) a | 1.52 (0.03) a | 1.44 (0.04) a | 5.0 (0.97) a | 5.3 (1.52) a | 12.4 (2.75) b | 10.7 (2.97) b |
| **20-40** | 1.46 (0.09) a | 1.56 (0.06) a | 1.55 (0.05) a | 1.59 (0.05) a | 8.0 (0.78) a | 7.4 (1.36) a | 8.5 (1.70) a | 11.4 (2.23) a |
| **40-60** | 1.70 (0.03) a | 1.61 (0.11) a | 1.65 (0.02) a | 1.67 (0.04) a | 9.9 (1.52) a | 8.3 (1.28) a | 12.4 (1.82) a | 11.3 (2.28) a |
| **60-80** | 1.69 (0.08) a | 1.55 (0.12) a | 1.69 (0.05) a | 1.69 (0.02) a | 14.2 (2.97) a | 9.6 (2.33) a | 10.0 (2.08) a | 11.4 (1.51) a |
| **80-100** | 1.63 (0.09) a | 1.50 (0.11) a | 1.71 (0.08) a | 1.71 (0.05) a | 9.5 (1.95) a | 7.0 (2.50) a | 13.9 (2.85) b | 17.0 (2.00) b |

**Table 3**. Mann-Whitney U test of the soil organic carbon (SOC) concentration and stock at each fraction with soil depth as independent factor. Significance is noted as: ns: not significant; *: p< 0.05; **: p<0.01; ***: p<0.001.

| Effect | Variable | Z | p-value |
|---|---|---|---|
| *Depth* | *SOC unprotected* | 2.669 | ** |
| | *SOC physically protected* | 2.947 | ** |
| | *SOC Chemically protected* | 3.829 | ** |
| | *SOC Biochemically protected* | -1.137 | ns |
| | | | |
| | *Stock unprotected* | 1.880 | ns |
| | *Stock physically protected* | 0.859 | ns |
| | *Stock Chemically protected* | -0.580 | ns |
| | *Stock Biochemically protected* | -3.133 | ** |

**Table 4.** Pearson correlation coefficient between the soil organic carbon concentration in the bulk soil and in the different soil fractions. Significance is noted as: ns: not significant; *: p< 0.05; **: p<0.01; ***: p<0.001.

| | SOC fractions | | | |
|---|---|---|---|---|
| | Unprotected | Physically protected | Chemically protected | Biochemically protected |
| Bulk soil | 0.98*** | 0.72*** | 0.87*** | -0.29 ns |
| Unprotected | | 0.61** | 0.82*** | -0.30 ns |
| Physically protected | | | 0.85*** | -0.25 ns |
| Chemically protected | | | | -0.45* |

# Figure

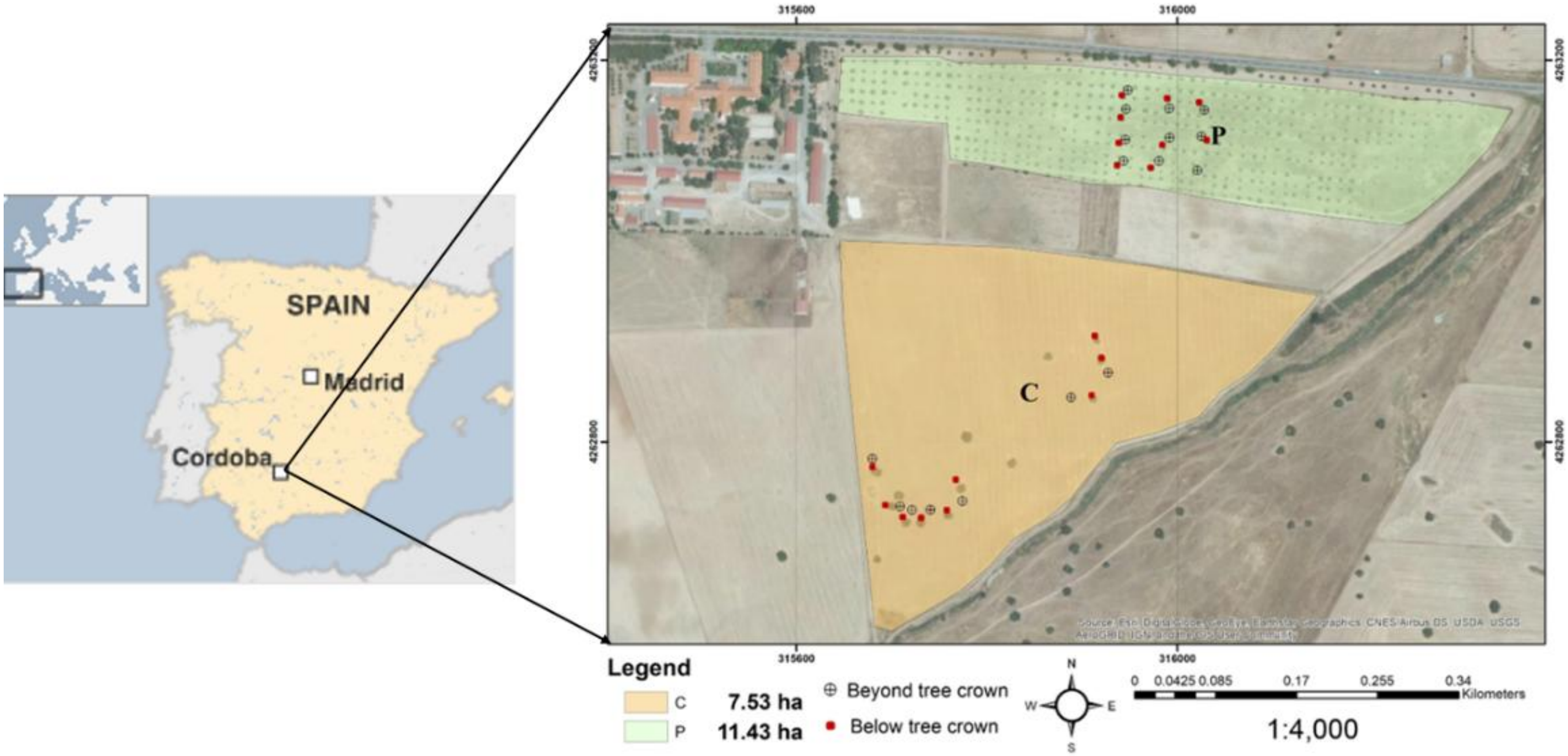

**Figure 1:** Location of the experimental farm at Hinojosa del Duque, Córdoba, Spain. Study areas are highlighted with different colours: Pastured dehesa with young trees (P) in green, and cropped dehesa with mature trees (C) in brown. Red circles represent soil sampling points below the tree crowns, and dark circles with a cross inside mark sampling points beyond the tree crown projection.

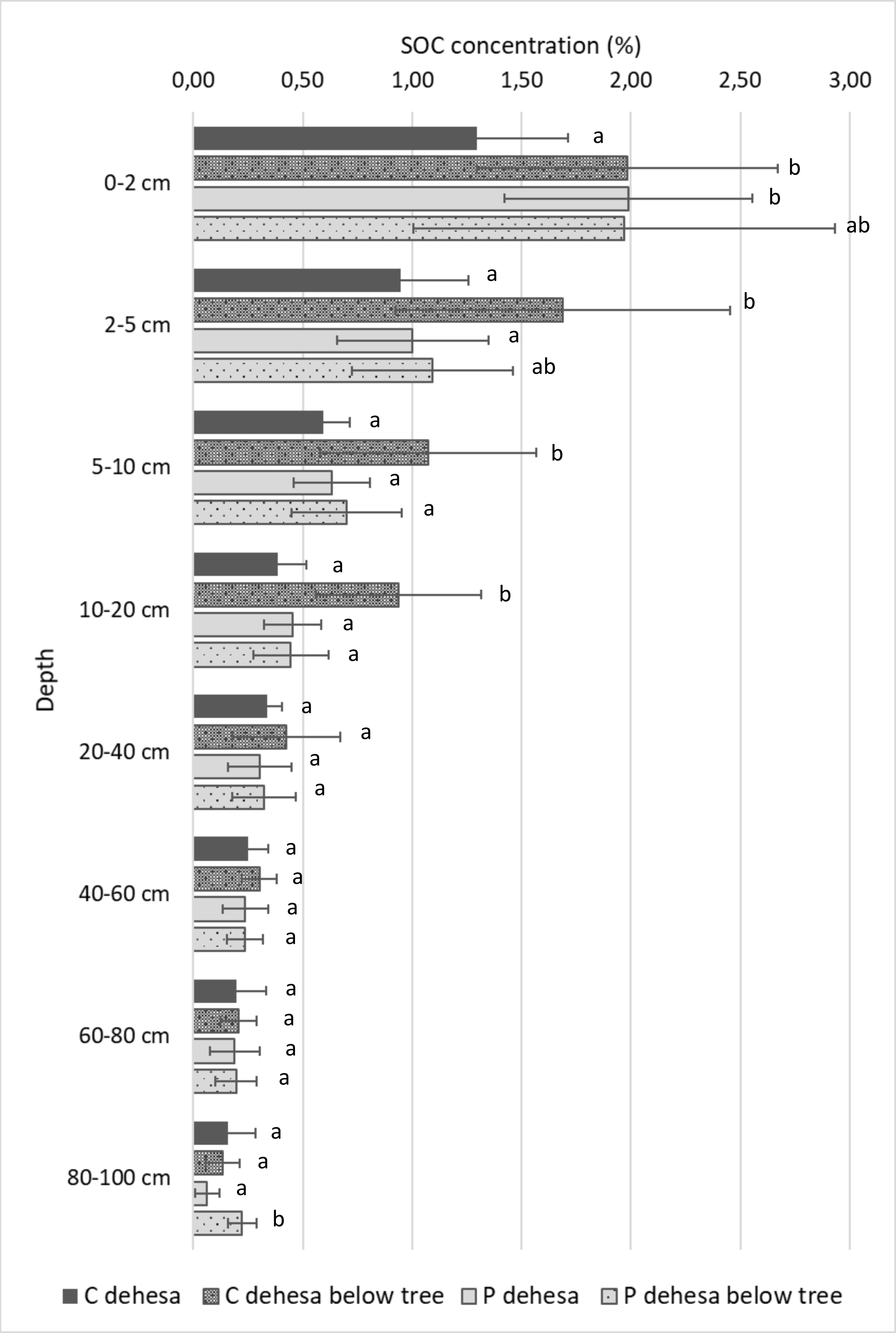

**Figure 2:** Distribution of SOC concentration with depth at C and P dehesas outside and under the tree canopy (mean and standard deviation). At each depth, different letters indicate a significant difference between soil management and tree influence (beyond-below tree canopy).

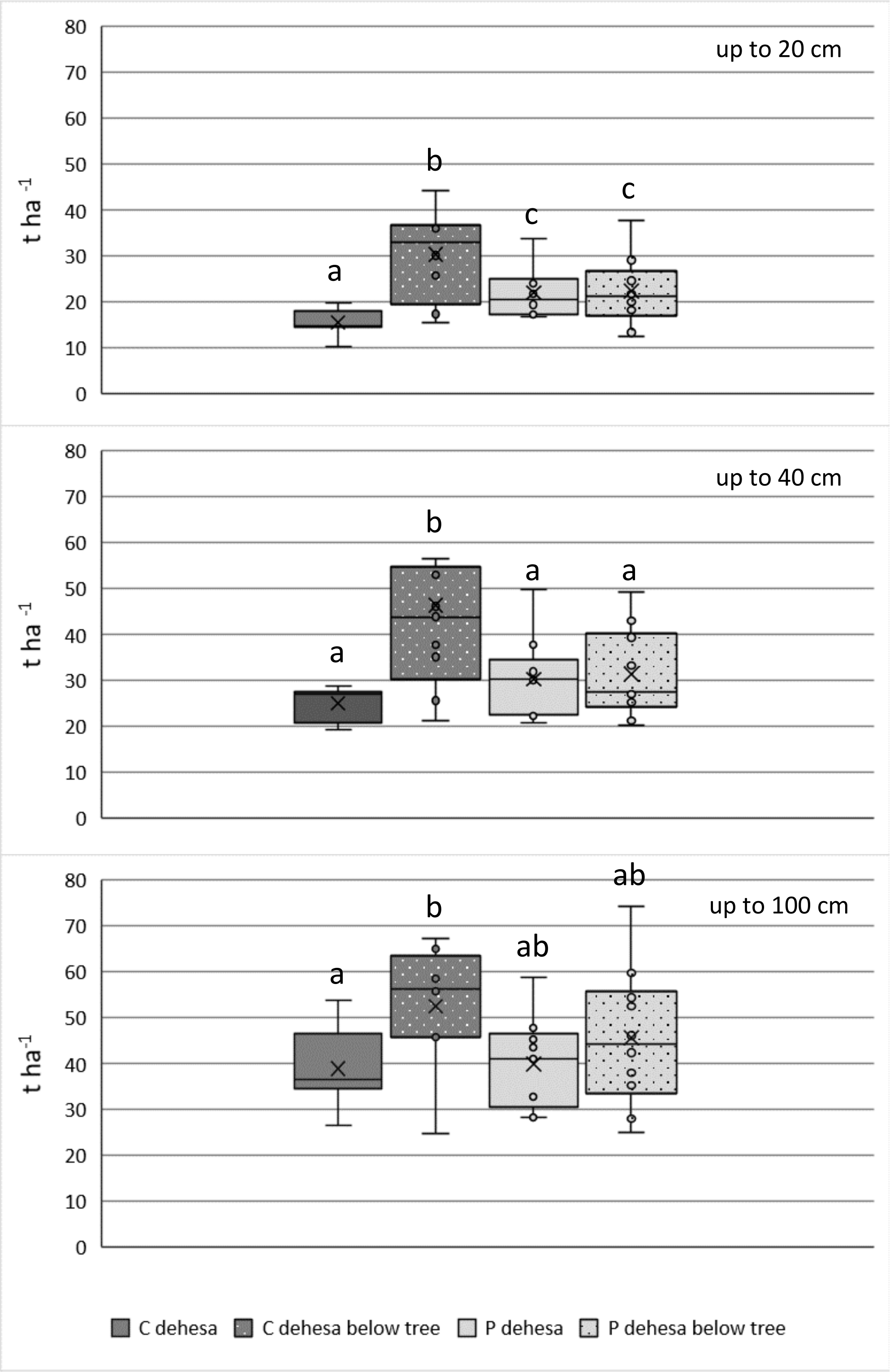

**Figure 3:** SOC stock at different depths at C and P dehesas outside and under the tree canopy. Different letters indicate a significant difference between soil management and tree influence (beyond-below tree canopy at P and C dehesa).

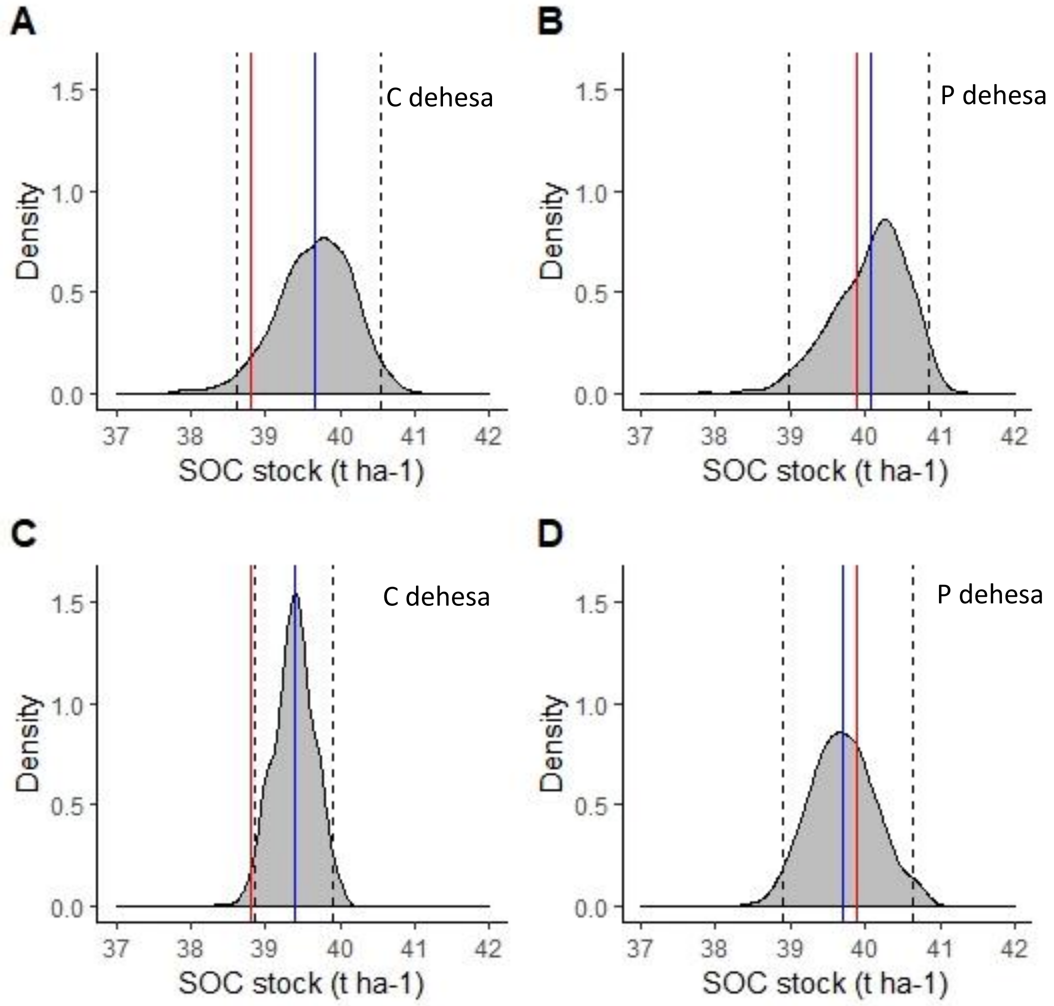

**Figure 4:** Bootstrap distribution of SOC stock after stoniness (A and B) and bulk density resampling (C and D) showing the mean (blue lines) and the confidence interval (2.5 and 97.5 percentiles; dotted lines). The red line shows the mean SOC stock resulting from sampling points.

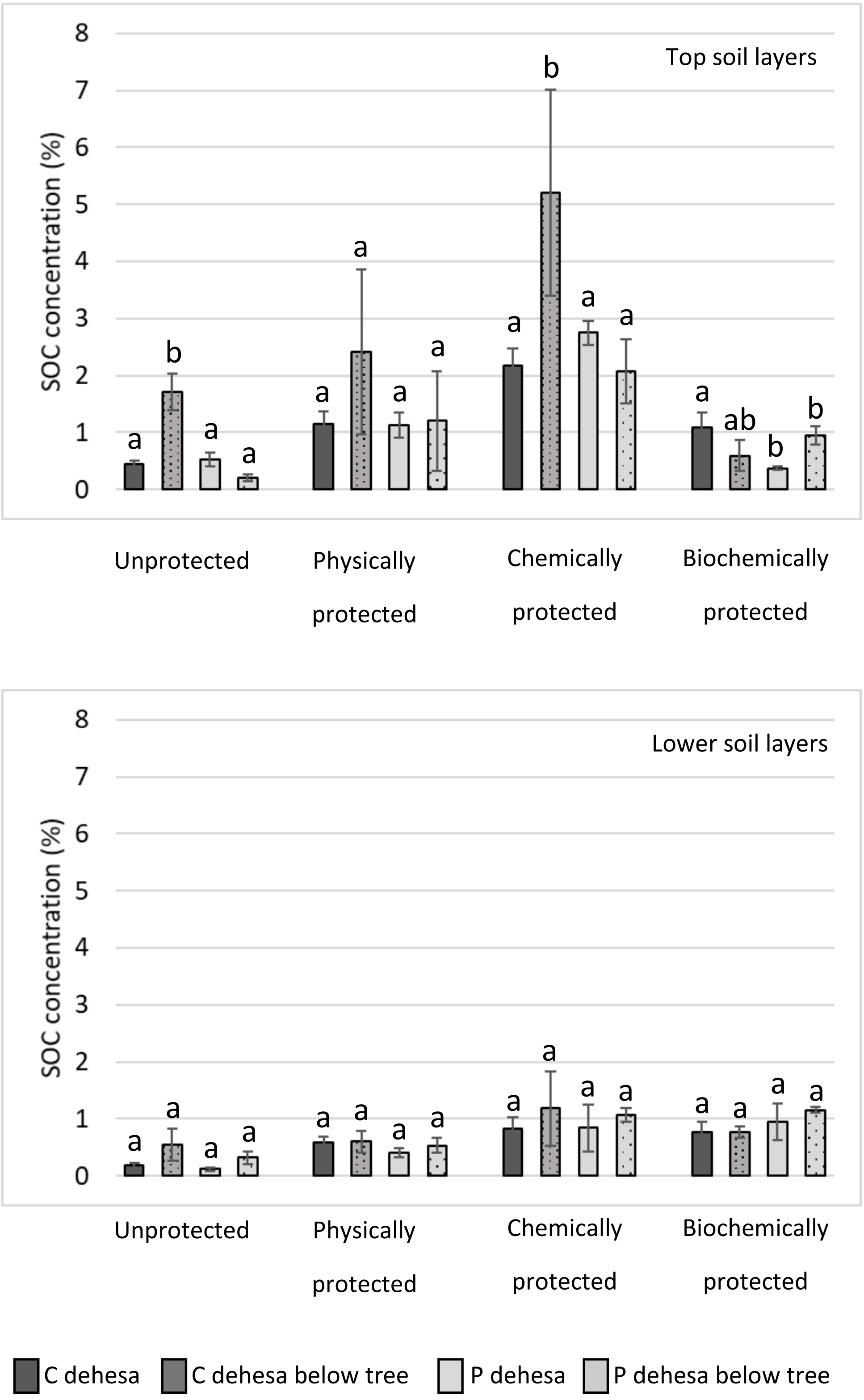

**Figure 5:** Soil organic carbon (SOC) concentration by fractions at C and P dehesa outside and under the tree canopy by depth. Top layers (0-2 cm, 2-5 cm), lower layers (20-40 cm, 40-60 cm). In each fraction, different letters indicate a significant difference according to soil management and tree influence.

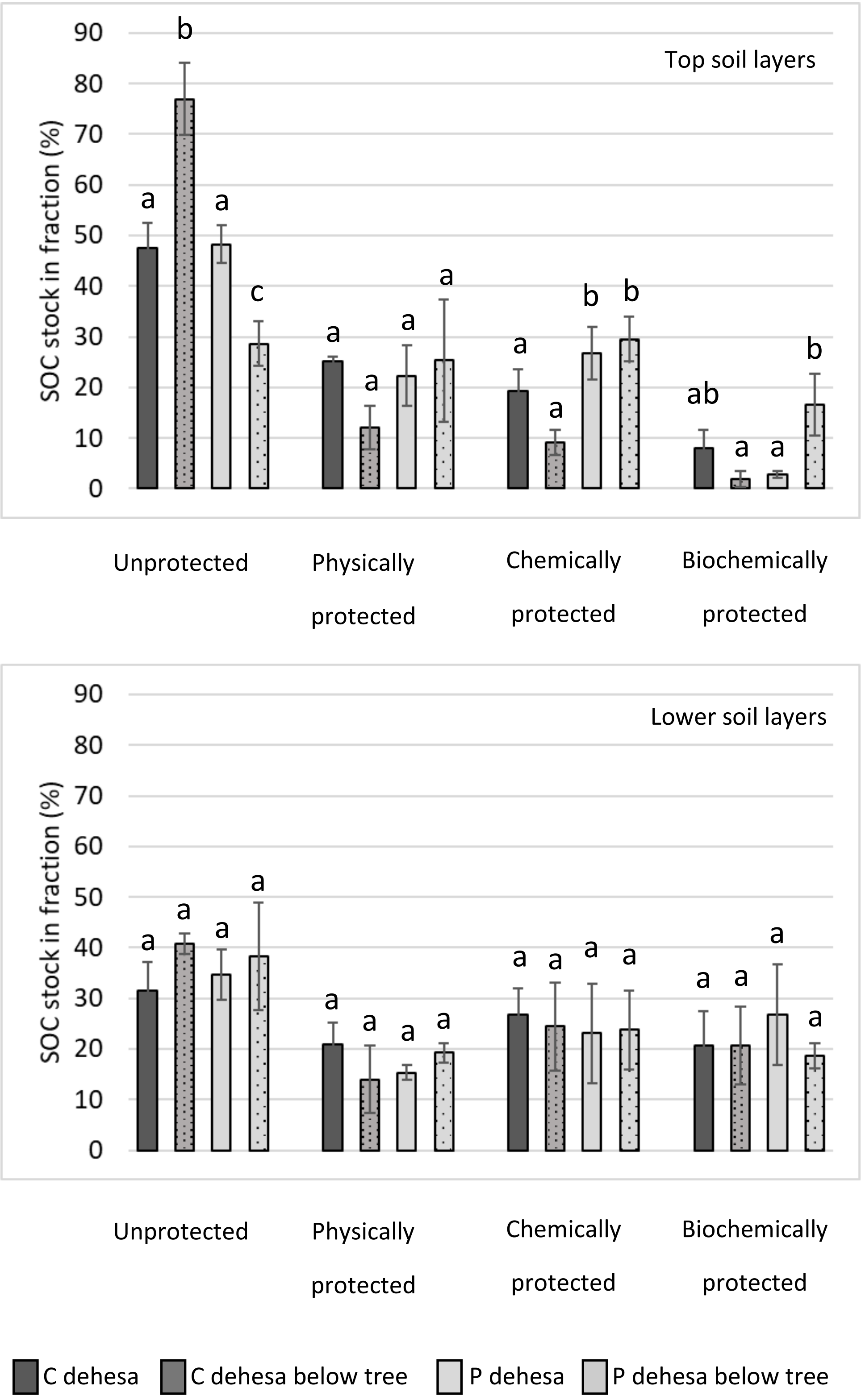

**Figure 6:** Distribution of soil organic carbon (SOC) stock by fractions according to soil management (C and P) and tree influence (beyond-below tree canopy) in tow depths, top layer (0-2 cm, 2-5 cm) and lower layer (20-40 cm, 40-60 cm). In each fraction, different letters indicate a significant difference according to soil management and tree influence