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Subsoiling for planting trees in dehesa system: long-term effects on soil organic carbon

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Abstract Incorporating trees into agricultural systems, including grasslands, increases the soil organic carbon sequestration and contributes to climate change mitigation. Site preparation for tree establishment is a common practice that can involve a variety of techniques and agricultural implements such as subsoiling. This study aimed to evaluate the long-term effects of subsoiling on soil organic carbon (SOC) concentrations and stocks in a Mediterranean grassland afforested with holm oaks 22 years ago and now converted into a Dehesa agroforestry system. The study was conducted in a dehesa farm in Southwest Spain. Soil samples were taken at six depths under 10 tree canopies within and outside the original subsoiling line. Subsoiling significantly decreased SOC concentration. Mean SOC concentration in the first 20 cm was 30% lower at the subsoiling line. SOC stocks for the first 60 cm were 2660 g m^{-2} within the subsoiling line and 4320 g m^{-2} outside the line. There was a clear reduction in SOC concentration and stock with increasing depth. Root abundance and deeper rooting increased with subsoiling but did not translate into sufficient carbon accumulation in the soil, which is moderate even after 22 years. This study reveals that, in the long term, there is a trade-off in CO_2 sequestration between tree planting and soil subsoiling, highlighting the need for further research into the potential benefits and detriments of subsoiling.

Keywords Holm oak · Agroforestry system · Tilling · Bulk density · Soil condition

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Introduction

Soils can be a sink or source for atmospheric CO_2, and therefore changes in soil organic carbon (SOC) stocks have profound implications for the exacerbation or mitigation of climate change (Feng et al. 2020; Francaviglia et al. 2017). Minasmy et al. (2017), surveying 20 regions worldwide, found that, with the best management practices, soil C sequestration rates could exceed 4 per thousand. This finding makes the "4 per mille Soils for Food Security and Climate" initiative, launched at COP21 to offset global anthropogenic greenhouse gas emissions, feasible. These authors also stated that the potential to increase SOC mostly hinges on topsoil within agricultural lands, given that they may have a low initial SOC stock and

are more responsive to agronomic practices. Therefore, finding the best management practices that can increase SOC stocks on agricultural land is a challenge for both farmers and policymakers.

The conversion of agricultural systems to agroforestry systems through the incorporation of trees on croplands and grassland could play an important role in mitigating climate change (De Stefano and Jacobson 2018; Shibu 2009). Tree planting encourages carbon sequestration in above- and below-ground woody biomass (Feliciano et al. 2018). Moreover, decomposition of leaves, branches and fine roots of the tree together with root exudates constitute an important input of organic matter into the soil (Cardinael et al. 2017).

Site preparation for tree establishment is a common practice that can involve a variety of techniques and agricultural implements. One of these techniques is subsoiling. Subsoiling is a non-inversion tillage of the soil below depths of 30–45 cm. Some authors indicate that this is the most suitable technique for tree planting on agricultural land (Querejeta et al. 2008; Smal et al. 2019). Normally, deep soil tillage aims to reduce soil compaction, break up the plow pan and reduce the bulk density of the soil, conditions that can hamper successful tree establishment by slowing down root penetration (Iglesias Ranz 2004). Reduced soil strength, and improved aeration, water infiltration and nutrient availability (Raper and Bergtold 2007; Zhang et al. 2020) enhance plant survival and overall growth (Sun et al. 2017) and encourage deeper rooting of plants (Schneider et al. 2017). In Mediterranean agroforestry systems, subsoiling has been shown to improve tree establishment and growth (Iglesias Ranz 2004; Ovalle et al. 2020; Querejeta et al. 2008).

Increased root and tree growth due to deep tillage could result in an increase of SOC. Feng et al. (2020), in a meta-analysis based on global data from 43 studies, found that deep tillage of arable land enhanced SOC, especially when the soil was subsoiled. However, the response of SOC to deep tillage for planting trees is highly dependent on site-specific conditions, related agronomic practices for tree maintenance (Feng et al. 2020), time elapsed since tree planting (Pardon et al. 2017; Reyna-Bowen et al. 2020) and the tree species planted (Shaojun et al. 2016). On the other hand, several studies have shown that, in the short term, tillage can cause SOC losses due to due to a decrease in physical protection of SOC and an increase in soil aeration and microbial activity that enhances soil organic matter decomposition, especially when implements such as ploughs or disc harrows are used (Cid et al. 2013; Rutkowska et al. 2018; Sithole et al. 2019).

Carbon sequestration in Mediterranean grasslands can be enhanced by integrating oak trees (*Quercus ilex* L. and *Quercus suber* L.). These practices have been promoted in Spain within the context of the Common Agricultural Policy (CAP) through different afforestation programmes (EU 1992), turning grasslands into Dehesa system. In many cases, the incorporation of trees has been done by subsoiling, working the subsoiler implement along a line or at isolated points. Dehesa is one of the most representative and important agroforestry systems of the Iberian Peninsula, also known as Montado in Portugal (Pinto-Correia et al. 2011). Dehesa is a savanna-like system composed of scattered oak trees over grassland and devoted to extensive livestock farming (Moreno and Pulido 2009). Furthermore, Dehesas supports traditional livelihoods and rural economies, while maintaining a high biodiversity and key ecosystem services such as soil and water conservation or the reduction of risk of forest fires (Plieninger et al. 2021; Surová et al. 2018; Torralba et al. 2018). Despite there being many studies demonstrating how subsoiling modifies SOC in arable land of different world regions, there is a gap of knowledge on its effects on SOC of Mediterranean grasslands when used as site preparation method for conversion into agroforestry systems. Therefore, the objective of this study was to determine the long-term impact of subsoiling on SOC in a Mediterranean grassland afforested with holm oaks 22 years ago and now transformed into a Dehesa agroforestry system. We hypothesized that soil in subsoiled areas show higher concentrations and stocks of SOC, since subsoiling should have facilitated tree root growth, leading to higher carbon accumulation after 22 years from root decomposition and root exudates.

Materials and methods

Study site

The study was conducted on an experimental farm at Hinojosa del Duque, Los Pedroches, Cordoba, Spain

(38°29′46″N, 05°06′55‴W). The area has a mean annual rainfall of 437 mm and an average annual temperature of 15.1 °C (average for 2010–2017 at the meteorological experimental station in Hinojosa del Duque). The area is located at 543 m above sea level. According to the FAO soil classification system, the soil is classified as a Eutric Cambisol with a shallow depth and rocky outcrops (CSIC-IARA 1989).

The site is a dehesa with Holm oaks (*Q. ilex*) planted in 1995 with a 12 m × 12 m spacing and a density of 70 trees ha^{-1}. Planting included soil preparation by subsoiling with a three-legged curved subsoiler, 50 cm apart, on a V-shaped frame, working to a depth of 60 cm. Growth of herbaceous vegetation was controlled with a disc plough in the first five years after tree planting. Prior to 1995, the management of the area involved the cultivation of vetch and oats for hay in a three-year cycle, where the soil was fertilised with 20 t ha^{-1} of dairy manure prior to sowing, and where a chisel plough was used for soil tillage. Since 2000, the area was grazed by Merino sheep at a stocking rate of 3 sheep ha^{-1}. Grazing is rotational, with at least four grazing periods per year, adding up to an average of six months a year. In 2016, natural pastures were fertilized with 40 kg of P_2O_5 ha^{-1} using a centrifugal spreader. The amount of fertilisation was equal both within and outside the subsoiling line. At the time of soil sampling in March 2017, trees showed a mean diameter at breast height (DBH) of 17.2 cm and canopy cover of around 10%.

Soil characterisation and sampling

Soil profiles and their main characteristics were described by establishing two soil trenches. One trench was located along the subsoiling line axis, and the other was perpendicular to the subsoiling line, in different trees (see Fig. S1). The trenches started near the tree trunk and crossed beyond the tree crown projection area. Profiles were described according to the National Resource Conservation Services (NRCS) guidelines (Soil Survey Staff 2012). The main soil properties for each horizon within and outside the subsoiling line are shown in Table 1. Root density by root size (fine < 2 mm; medium 2–5 mm; coarse 5–10 mm and very coarse > 10 mm) was visually assessed in four representative units at each horizon within each trench. The unit area required for root density assessment changes according to root size: 1 cm^2 for fine roots, 1 dm^2 for medium and coarse roots, and 1 m^2 for very coarse roots. Further information on the assessment method and quantity classes can be found in the NCRS guidelines (Soil Survey Staff 2012).

Soil bulk density (BD) was assessed at four points (two below and two outside the tree canopy) in each trench. At each point, undisturbed soil samples were taken at four depths (0–5 cm, 20–40 cm, 40–60 cm and 60–100 cm) using a hand-held soil bulk density sampler. The samples were oven-dried at 105 °C for 72 h to a constant mass. Soil bulk density was calculated by dividing the dry mass of the soil by the 98.2 cm^3 volume of the soil sampler (Hao et al. 2008). At each point, bulk density at different depth layers (the same as core soil samples) was estimated by interpolation using mass-conserving splines (Malone et al. 2017).

Soil samples were collected across the study site at ten random trees (Fig. 1) for SOC and stoniness assessment. Soil was collected at two points below the tree canopy: one within the subsoiling line, and another point outside the line. Soil samples were taken at six different depth intervals at each sample point (0–2, 2–5, 5–10, 10–20, 20–40, 40–60 cm), based on the strong, surface-skewed stratification of soil organic carbon in Mediterranean grassland systems (Francaviglia et al. 2017; Lozano-García and Parras-Álcantata 2013; Lozano-García et al. 2016). Grass and mulch surfaces were previously removed. Soil samples were taken manually (for the three topsoil layers) and using a hydraulic soil sampler with a 38.1 mm diameter soil core (Giddings®; see Fig. S1). A total of 120 soil samples were taken. Samples outside and within the subsoiling line were collected in March 2017 and June 2018. After the analysis of the soil samples, data from the first two soil layers were combined, showing the results at five depths (0–5, 5–10, 10–20, 20–40, 40–60 cm).

Soil analysis

The soil samples were separated into coarse and fine particles using a 2 mm sieve. Clods were mechanically separated. Both fractions were weighed to determine stoniness, which was defined as the percentage weight of coarse material whose diameter is greater than 2 mm. SOC concentration was estimated by Vis-NIRS spectroscopy. Once coarse

Table 1 Soil properties outside and within the subsoiling line

Position	Horizon	Depth (m)	pH1/2'5	OM%	N%	K	P	CEC (meq/100 g)	Ca	Mg	Na	Clay (%)	Sand	Silt	S.T.C
Outside subsoiling line	A	35.0	6.68	0.94	0.051	115	51.6	10.52	7.07	2.63	0.47	13.2	75.6	11.2	Sandy loam
	B	57.0	7.26	0.40	0.024	137	33.7	12.07	7.32	3.94	0.51	12.4	80.6	7.0	Sandy loam
	BC	> 57.0	8.29	0.14	0.008	98	12.4	7.73	4.12	2.88	0.52	6.4	89.4	4.2	Sand
Within subsoiling line	A	38.3	6.41	0.61	0.033	80	35.2	5.25	1.63	2.96	0.43	2.7	80.4	16.9	Loamy sand
	B	68.3	7.12	0.48	0.030	136	30.3	21.97	17.34	3.73	0.63	30.4	59.9	9.7	Sandy clay loam
	BC	> 68.3	7.66	0.05	0.007	55	8.4	9.76	6.13	2.89	0.62	5.3	87.9	6.9	Sand

Please note data for the BC horizon indicates the depth at which this horizon begins

OM: Organic matter; N: Organic Nitrogen; K: Available Potassium ppm; P: Available Phosphorus (Olsen) ppm; CEC: Cation exchange capacity; S.T.C: Soil Textural Class

particles were removed, Vis-NIRS spectra of each soil sample was recorded using a LabSpec 5,000 spectrometer (ASD Inc., Boulder, Colorado, USA) and IndicoPro 6.0 spectrum acquisition software. This equipment has a nominal spectral resolution of 3 nm at 700 nm (visible and near-infrared region) and 10 nm at 1,400 and 2,100 nm (short-wavelength infrared region). The internal data sampling rate of spectrometer varies between 1.4 nm at 350–1000 nm and 2.2 nm at 1001–2500 nm, although it interpolates to 1 nm over the entire spectral range (350–2500 nm). Soil samples were scanned using a Contact Probe device, model A122317 (ASD Inc.). Soil samples were placed in plastic bottles and scanned from overhead. Three replicates of each soil sample were scanned (each being an average of 50 internal scans). The final spectrum was obtained by averaging the three composite scans. White reference scans (Spectralon panel) were taken between every soil sample scan. SOC concentration was predicted using Vis-NIRS equations which were previously calibrated using data from 440 spectra. These spectra belonged to soil samples from the same study area analysed for SOC by Walkley–Black method (Reyna-Bowen et al. 2020; Walkley 1947). A sub-sample of soil was also analysed by Walkley–Black method. Comparisons between chemically analysed SOC and NIRS-predicted SOC revealed the Vis-NIRS equations predicted SOC concentration accurately ($R^2 = 0.94$, RMSE = 0.168).

Soil organic carbon stocks for each soil depth interval and for the whole soil profile, were calculated following the procedure described by Penman et al. (2003):

$$\mathrm{SOC}_{\mathrm{stock\ i}} = 10,000\ SOCi \cdot BDi \cdot d \cdot (1 - \delta) \quad (1)$$

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

where $\mathrm{SOC}_{\mathbf{stock\ i}}$ is the total soil organic carbon in a given layer (g m^{-2}). $\mathrm{SOC}_{\mathbf{i}}$ is the organic carbon concentration (g g^{-1}), $\mathrm{BD}_{\mathbf{i}}$ is the bulk density of the soil layer (g cm^{-3}), d is the thickness of the depth interval (cm), δ is the fraction coarse particles within the layer (0–1), and n is the number of soil layers. Thus, Eq. (2) gives the total soil organic carbon stock in the whole soil profile, discounting the effect of stoniness.

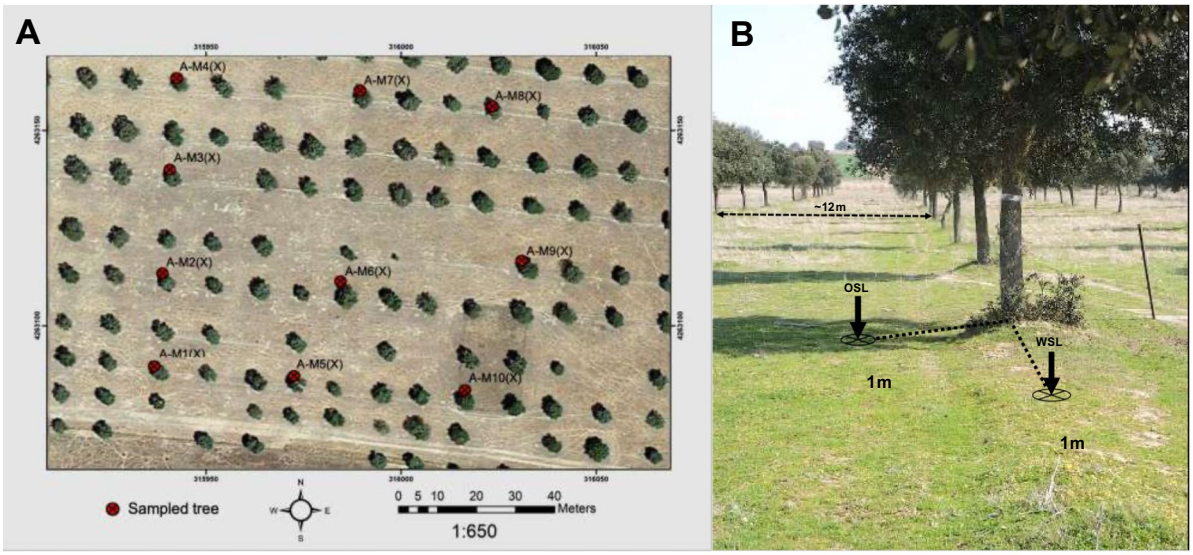


Fig. 1 Location of the soil core sampling points used to determine SOC concentration, stock and stoniness. **a** Distribution of the sampled trees across the pastured dehesa field. **b** Location of the sampling points with respect to the individual tree. Note samples were taken both within the subsoiling line (WSL) and outside the subsoiling line (OSL)

Statistical data analysis

Non-parametric Kruskal–Wallis Tests were conducted to examine differences in stoniness across soil depth and position with respect to the subsoiling line. Overall effects of depth and position on stoniness were evaluated using separate tests. Depth's effect on stoniness was then separately evaluated at each position. Bulk density and root density were analysed descriptively due to the small sample size per depth and size classes in the case of root (n = 4).

Linear mixed models (LMMs) were used to describe variation in SOC concentration and stock across the depth categories and position with respect to the subsoiling line. Three models were constructed for each response variable. The first model just included depth as a fixed explanatory variable. A second model included depth and position (within/outside the subsoiling line) as fixed effects with an additive effect. The third model incorporated both depth and position and acknowledged an interaction between them. Tree identity was always included as a random effect to account for non-independence between same-tree samples. The models were evaluated using information criteria (AIC, Akaike's Information Criterion; AICc, second order AIC; and BIC, Bayesian Information Criterion), and the best-quality model was subsequently selected. Normality of residuals was checked using the Shapiro–Wilk test, and the Bartlett test was used to verify variance was homogeneous across all depth and position categories. SOC concentration and SOC stock variables were log transformed $-\log_{10}(\mathrm{SOC}_{\mathrm{concentration}}+0.1)$ and $\log_{10}(\mathrm{SOC}_{\mathrm{stock}}+1)-$ to meet the requirements of linear models. A post-hoc comparison between the model categories was performed using the Tukey HSD test to determine which soil layers within each position showed significantly different levels of SOC stock and concentration. Statistical analyses were undertaking using R Statistical Software (version 4.2.1), and specific R packages 'eemeans' (1.8.3) and 'lme4' (1.1–31).

Results

Stoniness, bulk density and root distribution

Stoniness showed an average value of 6.8% across all profiles and positions (Table 2). Non-parametric Kruskal–Wallis Tests were conducted to examine differences in stoniness across soil depth and position with respect to the subsoiling line. No significant differences were found among the general soil depth

($\chi^2 = 2.11$, DF = 5, p = 0.7158) or position categories ($\chi^2 = 0.344$, DF = 1, p = 0.5573), or among soil depth categories at each of the separate positions (outside subsoiling line: $\chi^2 = 7.05$, DF = 4, p = 0.1332; within subsoiling line: $\chi^2 = 7.78$, DF = 5, p = 0.0998). It is important to note that stoniness outside the subsoiling line was much more variable, both within and among soil depth categories. There were smaller bulk densities for profiles within the subsoiling line (Table 2). On average, bulk density within the subsoiling line was 14% smaller than outside the line. Bulk density within the subsoiling line appears to increase with depth by about 13%, from 1.42 g cm^{-3} at 0–5 cm to 1.61 g cm^{-3} at 40–60 cm. There was no evident trend of change in bulk density across soil depth for samples outside the subsoiling line.

Roots were concentrated within the A horizon (see Table 1, Fig. 2). Very fine roots in the A horizon were common throughout all soil profiles. Within tree canopy, medium, coarse and very coarse roots were also common, although very coarse roots were less common within the subsoiling line. Beyond-canopy, very coarse roots were not present, and medium and coarse roots were only found within the subsoiling line. Very fine roots were common within the B horizon although they were found in low abundance under the canopy outside the subsoiling line. Medium-sized and coarse roots were scarce in this horizon. No roots were found in the BC horizon (more than 60 cm approximately) at any of the profiles (Fig. 2).

SOC concentration

Several LMMs describing the change in SOC concentration were comparatively evaluated (Table 3). The best model incorporated both the depth and position variables (subsoiling line, not subsoiling line) but did not acknowledge an interaction between them. All the coefficients in the model were statistically significant (see Table S1). SOC concentrations were significantly lower at the subsoiling line (Fig. 3). Depth-weighted mean value of SOC concentration in the first 20 cm was 30% lower at the subsoiling line (0.54% in subsoiled line and 0.78% for profiles not subsoiled). SOC concentration followed a negatively exponential distribution with depth, with an approximate fivefold decrease from 0–5 cm to 40–60 cm depths (Fig. 3). This decrease is concentrated at the first 10 cm. SOC concentration remains below 0.5% beyond 10 cm depths.

SOC stock

Overall, SOC stocks for the first 60 cm were 2660 g m^{-2} within the subsoiling line and 4320 g m^{-2} outside the line (Fig. 4). The best LMM that describes cumulative variation in SOC stocks across soil depth included both depth and position with an additive effect (Table 3). This model revealed that log-transformed SOC stocks within the subsoiling line were 0.258 smaller than across profiles outside the line (Table S2). SOC stocks were also heavily stratified and showed evident cumulative increases with increasing depth (Fig. 4). Outside the subsoiling line, SOC stock within the first 20 cm was about three times as large as the stock at the last 20 cm (2415 g m^{-2} at 0–20 cm compared to 874 g m^{-2} at 40–60 cm depth; Fig. 4, Table S2). This difference was reduced to twice as large for profiles within the

Table 2 Mean values and standard error for soil bulk density and stoniness across soil depth, both within and outside the subsoiling line

Depth (cm)	Bulk density (g cm^{-3})		Stoniness (%)	
	Outside subsoiling line	Within subsoiling line	Outside subsoiling line	Within subsoiling line
0–5	1.73 ± 0.03	1.42 ± 0.10	7.30 ± 0.67	7.01 ± 0.26
5–10	1.69 ± 0.03	1.41 ± 0.07	4.65 ± 2.06	7.45 ± 0.46
10–20	1.64 ± 0.04	1.39 ± 0.10	5.31 ± 1.52	7.53 ± 0.62
20–40	1.61 ± 0.07	1.42 ± 0.17	7.41 ± 1.36	5.99 ± 0.56
40–60	1.70 ± 0.06	1.61 ± 0.22	8.31 ± 1.28	6.02 ± 0.74

Bulk density includes below and outside tree canopy data. Bulk density values were not taken at all of the soil layers, and some were instead derived via interpolation (see Methods for more information)

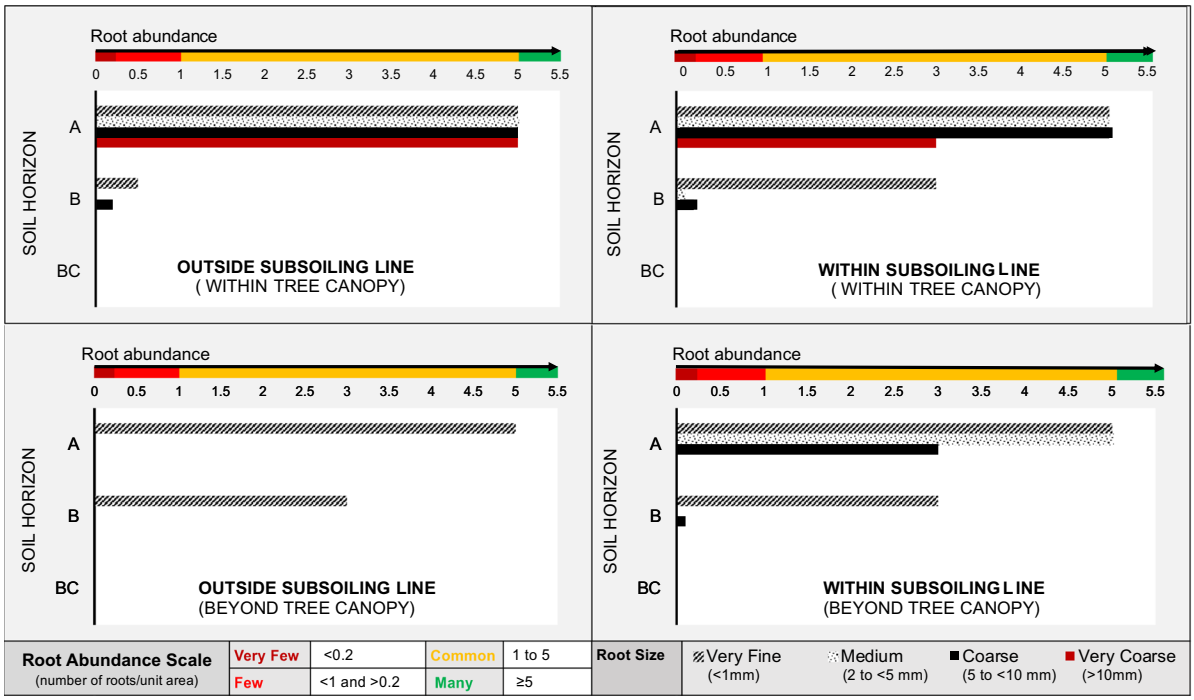


Fig. 2 Mean root abundance by size across the soil horizons. Abundance was assessed visually following the National Resource Conservation Services (NRCS) guidelines (Soil Survey Staff 2012)

Table 3 Information criteria values for the linear mixed models (LMMs) used to describe the distribution of SOC concentration and SOC stock across soil depth (and at different positions with respect to the subsoiling line)

Model variables	AIC	AICc	BIC
concentration ~ depth + (1|tree)	10.78	11.80	30.18
concentration ~ depth + position + (1|tree)	**0.69**	**2.02**	**22.86**
concentration ~ depth * position + (1|tree)	16.01	18.98	49.26
stock ~ depth + (1|tree)	4.25	5.41	14.56
stock ~ depth + position + (1|tree)	**-23.74**	**-22.09**	**-11.38**
stock ~ depth * position + (1|tree)	-13.97	-11.03	2.51

AIC Akaike's Information Criterion, *AICc* second order AIC, *BIC* Bayesian Criterion Models with the highest quality (best fit and least complexity) are presented in bold. '(1|tree)' indicates tree is treated as a random factor to account for non-independence

subsoiling line (1363 g m^{-2} at 0–20 cm compared to 756 g m^{-2} at 40–60 cm depth; Fig. 4, Table S2).

Discussion

SOC concentration

Contrary to expectation, SOC concentration was lower in the subsoiling line. Subsoiling appears to have encouraged a higher presence of roots in the A and B horizon when compared to the non-subsoiled area, which is in line with the expected effect of deep tillage on root distribution. Indeed, Madeira et al. (1989) observed that shallow tillage caused the concentration of tree roots in the 0–20 cm layer and deep tillage caused increased root distribution to shift to the 20–75 cm layer of the soil. However, observed changes in root growth and distribution have not been matched by increases in soil carbon concentration, as reported by some authors (Shaojun et al. 2016; Upson and Burgess 2013). This result suggests that

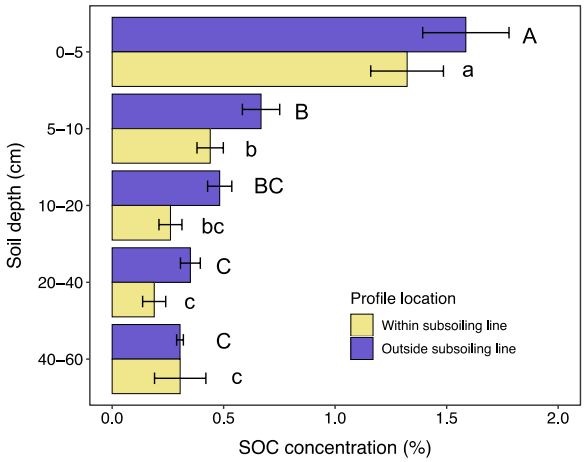


Fig. 3 Distribution of SOC concentration across soil depth within and outside the subsoiling line (mean ± standard error). Different letters indicate a significant difference in SOC concentration between the depth categories in the same position according to mixed effect model. Upper case letters: outside subsoiling line; lower case letters: inside subsoiling line

a significant reduction in SOC concentration may have occurred following mechanical soil preparation by subsoiling 22 years ago, and that the input of organic carbon from holm oak litter and fine root decomposition during this time did not counteract the effects of soil disturbance. Tilling modifies the soil structure and aerates the soil profile, leading to the release of organic soil carbon due to the respiration of heterotroph microorganisms (Balesdent et al. 2000; Rutkowska et al. 2018; Sithole et al. 2019). Indeed, some studies show one immediate effect of tillage is to induce an abrupt loss of CO_2 (Álvaro-Fuentes et al. 2007; Cid et al. 2013), although subsoiling may have a less pronounced effect than tillage with other types of implements, such as ploughs or disc harrows (Feng et al. 2020). On the other hand, tree influence on soil variables in agroforestry systems is dependent on tree age and size (Howlett et al. 2011). Pardon et al. (2017) found no significant variation in SOC concentration with distance to trees in young alley cropping fields, but observed higher SOC concentration in the vicinity of mature trees at field boundaries. Similarly, Reyna-Bowen et al. (2020) in Dehesa system found a higher SOC concentration under old oak canopies and no effect when soil was assessed under young oaks. Some authors argue that tree influence on SOC concentration is expected to occur at least 5 to 10 years after establishment, requiring more time to achieve a more homogeneous tree influence in the intercrop zone (Oelbermann et al. 2006; Pardon et al. 2017). In our case, 22-year-old holm oaks have not yet been able to significantly increase SOC concentration under their canopy and counteract the previous effects of soil tilling.

We found a clear reduction in SOC concentration as soil depth increases. These patterns are consistent with trends observed in the literature since exponential decreases in SOC concentration across depth

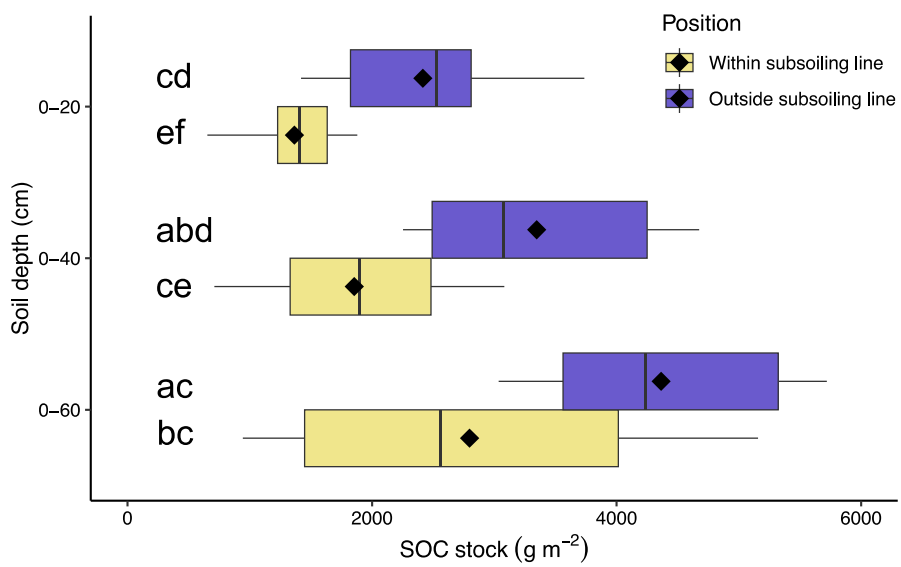


Fig. 4 SOC stock across different depths for profiles within and outside the subsoiling line. The depth categories represent cumulative layers of increasing thickness. Boxplots represent the data distribution (median, interquartile range and typical range). Rhombuses represent the categorical means. Different letters indicate a significant difference in SOC stock between the categories, which take into account both depth and position

have been observed in agricultural-ecological systems within the Mediterranean region (Francaviglia et al. 2017; González-González et al. 2012; Pulido-Fernández et al. 2013). Depth distribution of SOC was in accordance with the observed distribution of root density since higher root growth in the A horizon corresponded to higher values of carbon within the soil (Shaojun et al. 2016; Upson and Burgess 2013). Absolute values in SOC concentration are also partially consistent with values reported by other studies in dehesas and Mediterranean agroecosystems. Our study revealed that SOC concentrations in the topmost layers (on average 1.5% at the first 5 cm) are roughly similar to those reported by Pulido-Fernández et al. (2013), who respectively measured SOC concentrations of 2.3% and 1.1% within the first 5 cm of Leptosols and Luvisols dehesas. However, our mean SOC concentrations at the first 20 cm (0.78% outside the subsoiling line and 0.54% within the line) are generally smaller than those observed at related systems (Lozano-García and Parras-Alcantara 2013; Lozano-García et al. 2016). For example, González-González et al. (2012) evaluated 36 different dehesas in Mainland Spain, reporting a mean SOC concentration of 1.6% in the first 20 cm of soil. Lower SOC concentration values within the first 20 cm could be explained by processes relating to soil texture, vegetation type and previous land use management. Thus, our site held particularly sandy soils, which could explain lower carbon content since carbon is positively correlated with the proportion of soil composed by clay (Cai et al. 2016; Kong et al. 2009; O'Brien et al. 2015). Additionally, the study site had been previously cultivated in a three-year rotation cycle, meaning regular soil disturbance could have reduced the overall organic matter due to aeration and release of stored carbon (Álvaro-Fuentes et al. 2007; Balesdent et al. 2000; Cid et al. 2013).

SOC stocks

The most evident pattern emerging from the results is the difference in SOC stocks within and outside the subsoiling line, where soils within the subsoiling line only hold about 60% of the organic carbon outside the line. This result is a consequence of the differences in SOC concentration discussed above, as well as other factors, including bulk density, which also play a role in stock accounting. For example, Penman et al. (2003) observed that SOC stock, bulk density and stoniness vary proportionally. In our study, soil bulk density was lower in the subsoiling line (14% smaller than outside the line), which is consistent with the expected effects of subsoiling on soil structure (Raper and Bergtold 2007), contributing to a lower SOC stock computation. Stoniness also influences SOC stock, but it should not have affected the observed stock values since it did not vary significantly from one position to another. Our stoniness and bulk density results also reveal how subsoiling creates long-lasting effects within the soil profile, even 22 years after soil preparation (Evans 2009). Subsoiling appears to reduce bulk density within the first 60 cm and to modify the distribution of coarse particles, since stoniness within the subsoiling line samples is much more homogeneous, probably because subsoiling mixed and overturned the soil particles (Fonseca and Figueiredo 2016). However, the difference in bulk density could also be related to mechanical weed removal strategies, since recurrent tilling along the tree rows could have further compacted the soil outside the subsoiling line (Raper and Bergtold 2007; Upson and Burgess 2013).

Previous evaluations of SOC stocks in Dehesa system show similar values, especially those outside the subsoiling line, although many of them have been estimated far from the tree canopy. For example, Lozano-García and Parras-Alcántara (2013) found stocks for the first 100 cm under conventional tilling and organic farming were 5840 and 4870 g m^{-2}. Corral-Fernández et al. (2013) found a mean total SOC stock (up to about 1 m depth) from 7670 to 5800 g m^{-2} depending on soil type (Cambisol/Leptosol). Under the influence of mature oaks, Howlett et al. (2011) recorded mean SOC stock of 5020 g m^{-2} up to 1 m depth. SOC stocks have shown a clear stratification that is consistent with other research in this agroforestry system. For example, Corral-Fernández et al. (2013) found in Cambisol that the surface horizons held 50% more SOC than lower horizons. Reyna-Bowen et al. (2020), calculating SOC stock for the first 100 cm, found that only 4.6% of the stock was found beyond 60 cm. Despite the apparent low contribution of deeper layers to the total organic carbon in Dehesa, in agroforestry systems it is still necessary to assess

deeper soil layers (Upson and Burgess 2013), due to the impact of planting operations and trees on soil carbon at depth.

Implications towards management

The study's results have important management implications, given that subsoiling has the potential to significantly reduce the SOC concentration and stock of Dehesa soils within the subsoiling line. However, it is also important to acknowledge that the extent of subsoiling is constrained in space and time, since subsoiling only took place prior to planting and only affected the soil within the planting rows. In our case, subsoiling lines were about 1 m wide and tree rows spacing was 12 m, so that subsoiling only affected 8% of the total afforested area. Therefore, afforested land that was subsoiled hold only 30 g m^{-2} less carbon than non-subsoiled areas that evolved to grassland (see Fig. S2 in supplementary material). It is worth noting that, at the time of sampling, the decrease in SOC stock due to the initial tillage for planting was of a smaller magnitude than the carbon stored aboveground. Indeed, carbon stock in the woody structures of trees of our study site (accounting carbon in the biomass of branches and roots), is estimated to be around 698 g m^{-2}, an amount that far exceeds the loss of organic carbon due to tillage. On the other hand, SOC stock is related to the growth of the tree component and is therefore expected a continuous evolution in SOC status as trees mature.

Given that subsoiling has proved to enhance oak seedling survival and growth in Mediterranean environments (Iglesias Ranz 2004; Ovalle et al. 2020; Querejeta et al. 2008), it can be considered a suitable soil preparation method to shift grassland and arable land toward agroforestry system as part of a policy of increasing tree cover to foster CO_2 sequestration. However, this management practice requires careful consideration when implemented in order to reduce undesirable impacts on soil, understanding both optimal field condition and basic mechanics involved.

In conclusion, our results showed that planting preparation via subsoiling decreased SOC concentration and stock within the subsoiling lines. Root abundance and deeper rooting appears to be enhanced by subsoiling, but increased root density did not translate into a sufficient carbon accumulation in soil, which is moderate even after 22 years. The current state of knowledge derived from the findings of this study reveals that subsoiling represents a trade-off between CO_2 sequestration and increased seedling establishment and growth, highlighting the need for further research into its potential benefits and detriments.

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