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Initial soil carbon losses may offset decades of biomass carbon accumulation in Mediterranean afforestation

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ABSTRACT

Afforestation of degraded areas was suggested as CO_2 sink, contributing to climate change mitigation. Yet, few studies have assessed this sink by combining measurements on carbon (C) in the biomass and the soil, despite it being crucial to properly estimate the mitigation potential. Here, we assessed the combined C stocks of afforestation plots of different ages on former cropland in a Cambisol landscape in Extremadura, Spain. The plots were afforested with two native tree species (*Quercus ilex* L. and *Quercus suber* L. in a density ratio of 3:1), planted at several occasions between 1998 and 2011. Stocks of afforested areas in 2022 were compared to non-afforested negative controls on arable land, to a closeby olive grove and a forest with signs of degradation. Tree biomass was estimated from allometric equations, soil organic carbon (SOC) stocks were measured to 30 cm depth, based on equivalent soil mass. The biomass C accumulation rate in afforested plots increased with tree density and elevation ($p < 0.05$; range: 25 to 75 g C m^{-2} yr^{-1}). SOC stocks, in contrast, were not significantly different in afforested and non-afforested plots at any depth and in tendency even lower in afforested plots younger than 20 years. Consequently, total (biomass plus soil) C stocks in afforested plots were not significantly higher than in non-afforested ones. Nevertheless, SOC stocks and contents between the tree rows were significantly lower compared to soil next to the trees in the olive grove (about 1200 vs. 2200 g C m^{-2} in the top 30 cm) and in tendency in the afforested plots (about 1200 vs. 1500 g C m^{-2} in the top 30 cm; $p < 0.1$). The fact that the degraded forest (about 6800 g C m^{-2}) and the olive grove (about 5300 g C m^{-2}) did have significantly higher total C stocks than the afforested and non-afforested sites (about 2300 and 1800 g C m^{-2}) could indicate that afforestation could soon become a C sink. However, our study clearly shows that afforestation is not automatically a C sink. Timing of different C pools` losses and gains affect net ecosystem carbon sequestration. While improved soil management in afforestation may reduce SOC losses, afforestation with Mediterranean *Quercus* trees under current management practices may require decades before being a C sink. This finding should temper expectations that afforestation with such tree species is a rapid solution to combat climate change.

1. Introduction

At the current speed of reducing global greenhouse gas (GHG) emissions, it will not be possible to meet the 1.5 °C global warming limit without compensating residual and unavoidable GHG emissions, e.g. by using land as a carbon (C) sink (Beuttler et al., 2019; IPCC, 2021; Pires, 2019). Terrestrial ecosystems store about four times the amount of C that is currently in the atmosphere (Lal, 2010), most of it in soils (i.e., about 2′400 Gt of C to 2 m of soil depth; Batjes, 1996; Minasny et al., 2017). Yet, Lal (2004) postulates that most arable soils store much less C than they potentially could. As a consequence, soils may have a huge potential to act as a sink of GHG. Estimates are that soils in France and in the EU could store an additional 28.5 Mt. CO_2 per year (Bamière et al., 2023) and 150–300 Mt. CO_2 per year (Bellassen et al., 2022),

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respectively. Some claim that the largest potential may be found in arid and semi-arid ecosystems with low initial C content (between 0.8 and 1.7%) (Minasny et al., 2017; Segura et al., 2016). Afforestation, i.e., planting trees in areas where no trees existed before (Nave et al., 2013), has been positioned as a promising land-based C sequestration strategy, since additional C can be stored in tree biomass and soils (Beillouin et al., 2023; Hong et al., 2020; Liu et al., 2018; Pires, 2019; Reyna-Bowen et al., 2020; Simón et al., 2013). This potential arises from the higher primary production of forest compared to arable land (Lorenz and Lal, 2018), the increased litter input above- and/or belowground due to the lack of regular biomass extraction (Bárcena et al., 2014; Rahman et al., 2017) and the less frequent disturbance of soil, e.g., via plowing (Six et al., 1999).

Spain is one of the EU countries with the largest afforested areas (Pérez-Silos et al., 2021). In the last 80 years, >6′000’000 ha of abandoned land were afforested (Gallardo, 2016). The rates in Spain reached worldwide highs within the last century, soaring further in 1992 with the reform of the Common Agricultural policy (Vadell et al., 2016). Currently, the afforested area occupies, depending on the source and due to some deforestation happening in parallel (Pérez-Silos et al., 2021), between five and six million ha, or over 12% of the national territory (Gallardo, 2016; Pérez-Silos et al., 2021). One of the most frequently used species is holm oak (*Quercus ilex* L.) (Quinto et al., 2021); cork oak (*Quercus suber* L.) is also relevant but used less frequently (Vadell et al., 2016). Both oaks are broadleaf, evergreen, slow-growing, long-living, indigenous trees of Spain (Fischbach et al., 2002; Vadell et al., 2016) and, in particular, ecologically valuable native species of the Extremadura region. Although they are pioneering species in many ecosystems, in Mediterranean ones they are considered as late successional species that follow the pioneer pine stage (Ne’eman and Osem, 2021). Both oaks have a deep (Moreno Marcos et al., 2007; Plieninger et al., 2010) and at the same time a very large lateral (Moreno et al., 2005) rooting system. *Quercus ilex* is the most widespread evergreen oak species in the Iberian Peninsula (Pulido et al., 2001) and it is considered a valuable constituent of the Spanish vegetation for economic and ecological reasons (Quinto et al., 2021). It has a high edaphic plasticity, flourishing in a wide range of temperature, moisture and soil pH values (González González et al., 2012).

Studies in the Spanish *dehesa*, a traditional agroforestry system with oaks, showed a positive effect of oak trees on SOC, with higher SOC beneath tree canopies compared to the surrounding herbaceous matrix and open areas (Gallardo, 2003; Moreno Marcos et al., 2007; Pulido-Fernández et al., 2013; Simón et al., 2013). However, because *dehesas* are typically several centuries old, it is not clear if and how fast such a positive effect on SOC can be observed in afforested plots. For example, both losses and gains in SOC stocks following afforestation have been reported (Cunningham et al., 2015). Afforested plots are often characterized by an initial SOC loss immediately after planting (Bárcena et al., 2014), followed by a gradual regain of SOC only once the trees are fully established. In light of viewing afforestation as a C sink to reach climate neutrality by 2050, both the timing of SOC dynamics and the effect of management on these dynamics (Nave et al., 2013) are important, especially considering that SOC losses could offset C sinks in tree biomass. Consequently, there is a need to monitor SOC changes within and beyond the first decades after afforestation (Bárcena et al., 2014; Poeplau et al., 2011). It is also of special importance to develop integrative approaches, linking above- and belowground C storage (Cardinael et al., 2020). Further, it is relevant to consider the spatial distribution of SOC, because SOC in afforested systems can vary with distance to trees (Howlett et al., 2011). Generally, few studies investigated SOC stocks as a function of tree age (Cardinael et al., 2017). Studies about the estimation and dynamics of C stocks and its spatial distribution following afforestation have been lacking in the Mediterranean context (Cunningham et al., 2015; Pulido-Fernández et al., 2013), where soils have distinctly low SOC contents and are mostly degraded or at least susceptible to soil degradation. Particularly, the sequestration potential of ecosystems with *Quercus ilex* and *suber* and the influence of plant biomass on SOC dynamics in these ecosystems have not yet been satisfactorily addressed (Pulido-Fernández et al., 2013; Reyna-Bowen et al., 2020; Simón et al., 2013).

The aim of this study was thus to understand the dynamics of SOC and biomass C stocks in afforested plots and to comprehend how stocks change as a function of time since afforestation. To further study the sink potential, afforested plots were compared to typical adjacent arable land and permanent vegetation. To investigate the spatial heterogeneity of SOC stocks related to gradients of C inputs from trees, SOC stocks at different distances to the oak trees were assessed. A final objective of this study was to understand which other factors, such as elevation, slope and texture, were shaping SOC and biomass C stocks and how. Finally, the aim was to determine whether afforested plots stored more C than the corresponding control plots.

The following hypotheses were tested:

- In the C-poor degraded arable soils of our study site, both SOC and tree biomass C stock experience an increase with time since afforestation.
- However, the total C stock of plots afforested 10–25 years ago is still lower than the total C stocks of nearby old land uses.
- Because tree root inputs are thought to positively influence SOC stocks, the SOC stock is highest close to the trees.

2. Materials and methods

2.1. Study site

This study was conducted in a privately-owned farm called “Mari-blanca” (38°40′41.9”N 5°16′02.0”W) located in the South-East of Extremadura, Spain (Fig. 1). The mean annual precipitation is around 475–500 mm yr^{-1}, most of which is occurring from October to May. The mean annual temperature is 17 °C, with July and August being the hottest months, with maximum temperatures surpassing 40 °C, while in December the temperature can drop to around 2 °C. The potential evapotranspiration has an estimated average value of 900–950 mm yr^{-1} (Lorenzo Álvarez et al., 2005). The elevation of the study area ranges from about 460 to 630 m asl., the slope from about 1.5 to 26% (Table 1).

The soil types in the study area are classified as Cambisols, according to the World Reference Base for Soil Resources soil classification system (IUSS Working Group, 2014). The study site is characterized by quartzite (in the Northern part) and slate parent material (in the Southern part). Soils contain up to 67% of sand and are mostly poor in clay (Table 1). The soils in the study area are shallow (around 0.5 m depth and less) and their pH is low (Table 1), secondary carbonates are absent.

The afforestation campaigns were conducted in the years 1998, 2002, 2005 and 2011. *Quercus ilex* L. (holm oak) and *Quercus suber* L. (cork oak) trees were planted in a 3:1 density ratio in the afforested plots. The oak density is variable between the plots, ranging from 400 (4 × 4 m) trees ha^{-1} for plots afforested in 2005 and 2011, to 500 trees ha^{-1} for plots afforested in 1998, to 625 (5 × 5 m) trees ha^{-1} for plots afforested in 2002. Oaks were planted following the topography of the terrain and along contour lines, to avoid erosion. Prior to afforestation, subsoiling to 50–70 cm was done to fragment the soil, improve water infiltration and tree root development. Until the year 2014, surface tillage (20–30 cm depth) was done two to three times a year in the afforested plots to control competition for soil water by shrub and grass encroachment. From the year 2014 on, tillage for shrub control was replaced by sheep grazing from April to June and from November to December. Occasional tillage was continued where needed to control encroachment.

2.2. Sampling design

The sampling design followed a space-for-time substitution,

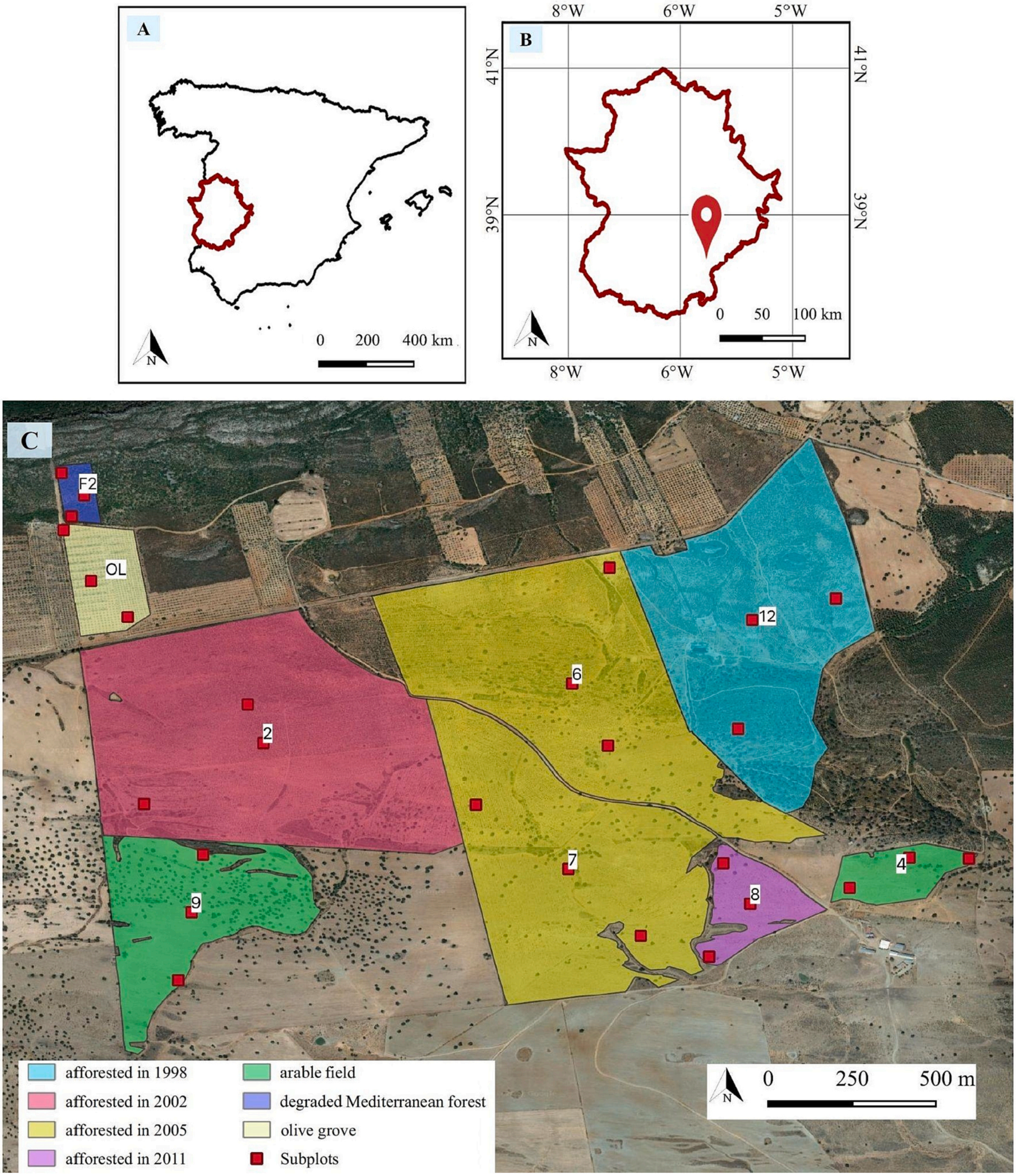

Fig. 1. Geographic illustration of the study site. A: borders of Spain in black (without the Canary Islands) showing the location of Extremadura, in red. B: borders of the Extremadura autonomous region and the location of the Mariblanca farm. C: Satellite image of the Mariblanca farm. The plots of the data collection, with their respective codes, are illustrated in different colors (representing the different land uses). The small red squares represent the subplots (three for each plot).

according to which contemporary spatial patterns are used to understand and model temporal processes that are otherwise unobservable (Pickett, 1989). We applied the concept of a "chronosequence", in which a series of plots in plantations of different ages are studied to study plant succession (Bárcena et al., 2014; Pérez-Cruzado et al., 2012). The basic assumption is that each afforested site shares the same biotic and abiotic history. A formal statistical analysis of differences in measured site and soil properties gave no indication that this assumption was violated (see supplement, Table S1). The data collection took place in nine different plots of different land uses and afforestation ages. Five of these plots

Table 1
Table summarizing average, maximum, minimum values of different terrain and soil variables (textural classes, pH and stone content), $n = 186$. The stone content was calculated on a weight-basis. The standard deviation (SD) of each variable is also presented.

	Mean	Median	Min	Max	SD
Elevation (m a.s.l.)	512.2	505.0	461	627	37.3
Slope (%)	8.5	7.1	1.5	26.4	6.1
% Sand	25.3	12.6	5.2	66.98	21.5
% Clay	16.0	16.6	7.0	22.3	5.1
pH (H_2O)	5.5	5.5	3.7	7.5	0.9
Stones (mass %)	30.5	29.6	4	83.9	9.6

were afforested, the other four plots served as control sites and represented the neighboring ecosystems. They included a negative control, i.e., two arable fields with extensive grasslands for hay production, which have not been afforested, and positive controls, i.e., a nearby occurring Mediterranean forest with signs of human activities and degradation (including species such as *Olea europaea* var. *sylvestris* and *Juniperus oxycedrus*) and an olive grove (Fig. 2). We manually selected three representative subplots of 20 × 20 m (400 m^2) within each of the plots for the data collection, resulting in a total of 27 subplots (Fig. 1, Fig. S1). One subplot was in the middle of the plot (centroid, extracted with QGIS version 2; QGIS Development Team, 2014). The other two subplots were manually selected so that subplots covered the spatial and covariate heterogeneity (i.e., slope and elevation), aiming for a stratified sampling approach. The coordinates of every subplot represented the position of the tree in the North-West corner of the subplot (tree number one). From this tree, an area of 400 m^2 was delimited along the South-East diagonal, by using a measuring band.

The olive grove, which represents the dominant crop of Extremadura (Gallardo, 2016), included only olive trees (*Olea europaea* var. *europaea*) and some grasses. The olive trees had a mean density of 175 trees ha^{-1} and an approximate age of 150 years, according to the linear relationship between diameter at breast height (DBH) and age (Arnan et al., 2012). In the subplots within the degraded Mediterranean forest, all recorded trees belonged to two tree species, namely *Olea europaea* var. *sylvestris* (wild olive) and *Juniperus oxycedrus* (cade). Mean tree density was 375 trees ha^{-1}.

2.3. Field data collection

The following variables were recorded for every tree present in each of the subplots: DBH, height of the tree and tree species. Dead trees were excluded because they were few (3 of 274 assessed in total), very small, and they were not considered to represent a long-term C sink. The diameter was measured with a tree caliper. In case of branching or splitting of the trunk below 1.3 m, the diameter was recorded directly below the branching. Tree height for most trees was measured with a measuring band connected to a rod (for trees up to 4 m), higher trees were assessed by field expert estimation, which was validated with a hypsometer roughly every 10th tree. The tree species was identified based on tree bark and leaves. Further measurements included diversity and abundance of other non-woody plants and shrubs, the slope of the terrain measured with a clinometer, and some qualitative field observations were noted (e.g. plantation scheme, tree vitality and survival rate, or signs of erosion).

The SOC stocks (g C m^{-2}) were assessed through samples collected by a closed cylindric soil corer of 55 mm diameter and 300 mm length (Giddings Machine Company, Windsor, USA). To achieve a representative sample and at the same time assess the effect of trees on SOC stocks, soil samples were taken next to three randomly chosen trees in each subplot. For each tree, three soil cores down to 30 cm of depth were taken along the South-East diagonal at different positions: close to the tree (0.5 m distance to trunk), interrow, approximately in the middle of the row (4 m distance) and in between those two points at mid distance (2 m distance; Fig. S1). Such a design was suggested by Cardinael et al. (2017) to detect positive effects of trees on SOC in a Mediterranean agroforestry system. The soil cores were divided into three different depth intervals (0–5, 5–15, and 15–30 cm). Each set of three samples from the same depth and distance in the same subplot was combined into a composite sample. The maximum sampling depth of 30 cm was a practical choice based on the fact that 91 ± 15% of the total SOC stock change under land use change occurs in the top 30 cm (Poeplau and Don, 2013) and the recommended minimum sampling depth by the IPCC (IPCC, 2006). In the non-afforested plots, only one composite soil sample made of three insertions was taken, since there were no tree rows and hence no distances from the rows. Similarly, only one composite soil sample was taken in the degraded Mediterranean forest, because of the irregularity of the terrain and impossibility of sampling close to the trees due to the presence of many branches. For both of these land uses, the sampling positions were determined randomly inside the subplot. In the degraded Mediterranean forest, we could not extract the 15–30 cm soil layer in any subplot, due to shallow parent rock. In total we collected 186 composite soil samples. Due to the arid environment, there was no distinct layer of undecomposed litter, and thus the 0–5 cm soil layer just included any litter that was small enough to pass through a 2 mm sieve. After collection the soil samples were air-dried in open plastic bags for later processing.

2.3.1. Soil analyses

Before laboratory analysis, soil samples were sieved through a 2 mm sieve. The air-dry weight of stones and particles >2 mm and soil were recorded. To save time, stone content was assessed from a representative

Fig. 2. Picture of a part of the study site. The olive grove is visible in the foreground. In the background there are oak afforested plots (on the left) and arable plots (on the right) and some dehesa systems (in the far background).

subsample. The following laboratory analysis consisted of SOC content and pH in water for all samples, while soil texture was assessed at the subplot level. For every subplot, a soil sample of the 15–30 cm layer (5–15 cm in the degraded forest) was randomly selected for texture analyses. SOC was determined by dichromate wet oxidation (Walkley and Black, 1934). The absence of carbonates in samples with a pH ≥ 6.5 was assured by adding 1 M HCl solution to the samples; a pressure calcimeter indicated no carbonates.

2.3.2. Allometric equations for biomass estimation

Species- and site-specific allometric equations (see Table S2) were chosen to estimate biomass, because tree diameter and height are easy-to-measure variables in the field (He et al., 2018) and because these equations are considered a reliable method to determine tree biomass and C stocks (Beckert et al., 2016; He et al., 2018; Pasalodos-Tato et al., 2015; Petersson et al., 2012). Further, they are especially robust for trees of smaller diameters, as was the case for this study (Ameztegui et al., 2022). For oaks and wild olive trees, we used the allometric equations developed for Spanish ecosystems (Gertrudix et al., 2012). These equations calculate the following biomass values: stem, thick branches, medium branches, thin branches with leaves, and roots. With DBH, the equations also include tree height, which enables more accurate biomass estimation compared to DBH-only equations (Gertrudix et al., 2012). These equations can also estimate the belowground plant biomass, a crucial component especially in Mediterranean forests where C allocation patterns are strongly biased towards the belowground compartment (Cotillas et al., 2016). The biomass estimates were transformed into biomass C stocks. The percentage of C in the tree biomass is 47.2% for *Q. suber* (Montero et al., 2005), 46.7% for *Q. ilex* and 50.8% for *Olea europaea* var. *sylvestris* (Montero et al., 2020). For cade (*Juniperus oxycedrus*), we used the allometric equation from Montero et al. (2005) which was developed in Málaga and only relies on DBH, and a C content of 51.5% (Montero et al., 2020). For olive trees, we used the power-law equation proposed by Brunori et al. (2017), developed for *Olea europaea* 'Leccino' in Italy, as a more local equation was not found. We used DBH as a substitute for the 80 cm diameter given in their equation, given the more or less constant diameter of olive trees with height at the study site. The percentage of C tree biomass for *Olea europaea* is 44.5% (Velázquez-Martí et al., 2014). For all tree species, the biomass C stock (g C m^{-2}) was calculated by dividing the weight of the respective biomass C by the area of a subplot (namely 400 m^2).

2.3.3. Soil organic carbon stocks

The total SOC stocks (g C m^{-2}) were calculated based on the equivalent soil mass (ESM) approach, which relies on comparisons to a constant (reference) mass of soil and not to a constant depth (Ellert and Bettany, 1995; Lee et al., 2009). With fixed soil masses instead of depths the method is robust even in case of compaction (Wendt and Hauser, 2013) during soil coring, presence of stones in the sample or imprecise cutting during soil sample segmenting (von Haden et al., 2020). For doing the ESM correction, we fit a quadratic spline describing the relationship between the cumulative stone-free soil mass (independent variable) and the cumulative SOC stocks (dependent variable; Wendt and Hauser, 2013). The function was then used to predict the cumulative SOC stocks for the chosen reference soil mass (g C m^{-2}). For the reference soil mass, the median of all soil masses from the same soil depth was used, i.e., 50, 160, and 270 kg m^{-2} for 0–5, 0–15 and 0–30 cm, respectively. From here on, they will be referred to as 50, 160, and 270 kg_{ESM} m^{-2} layers. Some soil samples could only be taken to 5 or 15 cm soil depth because the soil was too shallow or stony to collect cores to 30 cm depth. To not overestimate SOC stocks of such incomplete samples, we adjusted the reference soil mass to which SOC stocks were rescaled for the 160 and 270 kg_{ESM} m^{-2} layer of these:

$$270\ kgESM\_adj\ \left(kg\ m^{-2}\right) = 270 - \frac{x}{3}*(270-160) - \frac{y}{3}*(160-50) \quad (1)$$

$$160\ kgESM\_adj\ \left(kg\ m^{-2}\right) = 160 - \frac{y}{3}*(160-50) \quad (2)$$

where x is the number of cores that missed the 15–30 cm layer, and y the number of cores that missed the 5–15 cm layer. Including all soil samples at all positions, cumulative SOC stocks at 0–270 kg_{ESM} m^{-2} (or 0–160 kg_{ESM} m^{-2} in case of the degraded Mediterranean forest) were available for 63 soil cores (18 subplots $\times$ 3 cores $+$9 subplots $\times$ 1 core).

2.4. Statistical analysis

As a first step of data curation, data were screened for potential outliers but none were found. Where multicollinearity ($r > 0.7$) existed between explanatory covariates, we only included one of the two or more correlated covariates (Graham, 2003). The influence of different covariates on tree C and SOC stocks was determined by using linear mixed effects models including random intercepts per subplot. We compared biomass C, SOC, and C stocks in biomass and soil combined by land use. For the latter we joined all data from SOC based on 0–270 kg_{ESM} m^{-2} with data of measured biomass. Further, we compared SOC stocks by afforestation age, land use and distance to trees in the different ESM layers (0–50, 0–160, and 0–270 kg m^{-2} ESM). Finally, the effect of distance to trees (interrow, mid distance and next to tree) on the SOC stocks and SOC contents was only assessed in the afforested plots and the olive grove (as these were the only land uses where we could sample at different distances).

We combined different ways to account for the autocorrelation from repeated measurements in the data. To account for autocorrelation arising from repeated SOC stock measurements in the same experimental subplot we added a random intercept per subplot. Field observations showed that the plots were very heterogeneous (e.g., in slope, depth, but also in management, i.e., we observed for several of the larger plots that part of the plot was freshly tilled and another part not tilled for years). We therefore also added a gaussian spatial autocorrelation of the residual error term to allow for the autocorrelation of unexplained variation for points close to each other. The combined assessment of maximum likelihood-based model AIC, histograms and quantile-quantile plots of residuals showed that this was in general the most suitable model. For consistency, we used the same autocorrelation structure for all statistical models.

The explanatory covariates tested to explain the variation of the dependent variable were land use, age of afforestation, elevation, slope, texture, distance to the trees, where applicable, and tree density. To estimate the temporal change of C stock with plantation age (afforested plots only), we used the age of the afforested plot as the main independent continuous covariate and added interactions with the other covariates (e.g., tree density and texture) with age, as well as a random slope for age at the subplot level as residual error term, which was nested in the plot. This biomass C against age model omitted an intercept and any main effect of other covariates, based on the knowledge that prior to afforestation the tree biomass of *Quercus* trees was zero.

All statistical analyses were performed using R version 4.0.5 (R Core Team, 2021), at a significance level of $p < 0.05$, if not explicitly stated otherwise. We used the *nlme* package (Pinheiro et al., 2016) to create the statistical models. For each model we started with a set of all possible explanatory covariates (and interactions), which was followed by a backward elimination (Zuur et al., 2009), keeping only significant covariates ($p < 0.05$; Type III tests). During the backward selection, model fitting was performed using the maximum likelihood method. All final models were fit with the restricted maximum likelihood method. Post hoc comparisons of means were computed using the *emmeans* package with the "containment" method to estimate degrees of freedom and the "sidak" method to adjust confidence levels (Lenth, 2020). To illustrate the compact letter display of pairwise comparisons we used the *multicomp* package (Hothorn et al., 2008). Model diagnostic was performed visually, as suggested by Kozak and Piepho (2018). Standardized residuals were assessed against fit values and against every covariate present in the final model. The normality of the residuals was also evaluated visually with quantile-quantile plots and histograms.

3. Results

3.1. Tree density and carbon stocks across different land uses

The average number of oaks trees per ha ranged from 225 to 650 (mean of 453; Table 2) and was quite variable due to initial differences in planting densities and due to tree mortality in some plots. For the olive grove and the degraded forest, the average number of trees per plot was 175 and 375, respectively (Table 2), and the degraded forest was mainly composed of *Olea europea* var. *sylvestris* (60% of relative abundance).

Biomass C stocks of afforested plots were significantly lower than in the olive grove and the degraded Mediterranean forest, and significantly higher than in arable fields (Fig. 3). Elevation was the only other covariate having a significant (positive) effect on biomass C stocks. For a median elevation (505 m) model the estimated mean biomass C stocks were 93 ± 294 g C m^{-2} (mean ± standard error) for arable land, and around 1216 ± 197, 5453 ± 1079 and 4400 ± 617 g C m^{-2} for afforested plots, degraded forest and the olive grove, respectively. In contrast to biomass C stocks, SOC stocks in 0–270 kg_{ESM} m^{-2} were not significantly different between any of the land uses. Yet, mean SOC stocks at median elevation, while not significantly different from each other, tended to be lower in afforested plots (1140 ± 107 g C m^{-2}) compared to arable plots (1511 ± 187 g C m^{-2}). For the combined biomass C and SOC stocks, no significant difference between afforested (2333 ± 171 g C m^{-2}) and arable plots (1789 ± 331 g C m^{-2}) could be detected. On the other hand, the combined biomass C and SOC stocks in afforested and arable plots were significantly lower than in the degraded forest (6784 ± 913 g C m^{-2}) and the olive grove (5316 ± 386 g C m^{-2}).

Similar to the biomass C stocks, the mean growth rates of biomass C stocks within the afforested plots were significantly ($p < 0.005$) increasing with higher elevation (Fig. 4). Also, the observed tree density did play a significant role in the tree growth rates (estimate of an additional 8.8 g C m^{-2} yr^{-1} per increase of 100 trees per ha in tree density; $p < 0.05$). At an elevation of 500 m asl, they were around 43.3 ± 3.6 g C m^{-2} yr^{-1}, while at an elevation of 550 m asl, they were at 71.2 ± 7.4 g C m^{-2} yr^{-1} for an assumed median tree density of 450 trees per ha.

3.2. Soil carbon stocks by land use, depth and distance from trees

By comparing only data from the managed plots containing trees at regular distances (afforested and olive grove) we found a significant interaction between land use type and the distance to the tree. This manifested in significantly higher SOC stocks next to the tree in the olive grove in all three ESM depths considered (Fig. 5). The same was true for SOC contents.

In contrast, there were no significant differences between distances to trees in afforested plots in 0–50 and 0–160 kg_{ESM} m^{-2} depths and SOC stocks only tended to be ($p < 0.1$) higher close to the trees in 0–270 kg_{ESM} m^{-2}. The SOC contents were significantly higher next to the tree compared to the other two distances in the 5–15 cm depth. However, there was also a tendency for higher SOC contents next to the trees compared to the inter-row in the other two depths of the afforested plots ($p < 0.1$).

A more detailed analysis of SOC stocks by land use including afforestation age, distance from trees and ESM depth interval, revealed that differences in SOC stocks were present in both the 0–50 and the 0–160 kg_{ESM} m^{-2} layer (Fig. 6) but not in the 0–270 kg_{ESM} m^{-2} layer (not shown in detail due to absence of significant differences; see Fig. 3). The SOC stocks also depended strongly on the distance to the tree. For example, the inter-row distance SOC stocks of the olive grove were significantly lower than those of the degraded forest in 0–50 kg_{ESM} m^{-2}. However, while no significant difference between the SOC stocks of the olive grove and any afforested sites could be detected in the inter-row distance, the olive grove did have significantly higher SOC stocks than the 17 year old afforested plots in the 0–50 kg_{ESM} m^{-2} and higher SOC stocks than the 17 and 20 year old afforested plots in the 0–160 kg_{ESM} m^{-2}.

Table 2
Summary table of descriptive statistics (mean, median, minimum, maximum values, and standard deviation) regarding total (i.e. above- and belowground) tree biomass.

Biomass-related variable	mean	median	min	max	SD
Afforested					
Tree height (m)	2.6	2.6	0.5	6.0	0.9
DBH (cm)	9.1	9.1	1.0	24.3	3.6
Trees per ha	453	450	225	650	113
C stock (g C m^{-2})	904	834	41	1900	456
Olive					
Tree height (m)	3.9	4.0	2.6	5.0	0.6
DBH (cm)	29.3	32.6	12.3	40.0	7.9
Trees per ha	175	150	150	225	43
C stock (g C m^{-2})	3219	2848	1727	5080	1707
Forest (degraded)					
Tree height (m)	3.4	3.2	1.7	5.5	1.0
DBH (cm)	12.6	12.0	6.0	28.0	4.1
Trees per ha	375	350	200	575	188
C stock (g C m^{-2})	2443	2164	1304	3860	1301

4. Discussion

4.1. SOC losses potentially offset biomass C stocks gains in early years after afforestation

Despite higher biomass C stocks in afforested compared to arable plots, the lack of a significant difference of combined biomass C and SOC stocks in the top 30 cm is striking. It seems thus likely that topsoil SOC losses have been offsetting the biomass C gains, which have not (yet) translated into sufficient inputs of C to reverse the trend. A reason for the loss could be a lower root C input from the afforested trees compared to the extensive grasslands in the arable land especially in the first years after afforestation, when the trees do not explore the full potential rooting area. Additionally, the loss might result from soil disturbance during afforestation and in the following years to eliminate competition from the understory vegetation. In fact, initial SOC losses in afforestated soils are common due to soil disturbances at planting and initially lower C inputs in the first years (Jandl et al., 2007; Menichetti et al., 2017; Poeplau and Don, 2013; Vesterdal et al., 2002). For instance, a Spanish study found C losses during the first 10 years after afforestation in the 0–15 cm layer (Pérez-Cruzado et al., 2012). In the 11–15 age period, the SOC contents of afforested plots were similar to those of former pastures. Similarly, Segura et al. (2016) did not find a significant difference in SOC stocks between 20 year-old plots afforested with *P. halepensis* and arable plots in South-Eastern Spain. Yet, in the same study, SOC stock started to increase in the 0–5 cm soil depth in plantations older than 20 years. In the worst case, it may take up to two decades just to return to the initial pre-afforestation SOC stocks (Laganière et al., 2010). The lower SOC stocks in the 17 and 20 year old plantations compared to the other fields (Fig. 6) seem to indicate that this also happened in our study. It could be because the root system of *Quercus ilex* trees is typically more profound than grasses, being almost uniformly distributed in the top 150 cm of soil, whereas grass roots are heavily concentrated in the top 30 cm (Moreno et al., 2005). Therefore, afforestation likely shifted the root C inputs towards the subsoil. With the soils of Mariblanca being shallow (we reached the parent material stones in 6 out of 21 afforested plots with at least one core), it is unlikely that the majority of C input was very deep, but inputs below 30 cm are a possible explanation for the

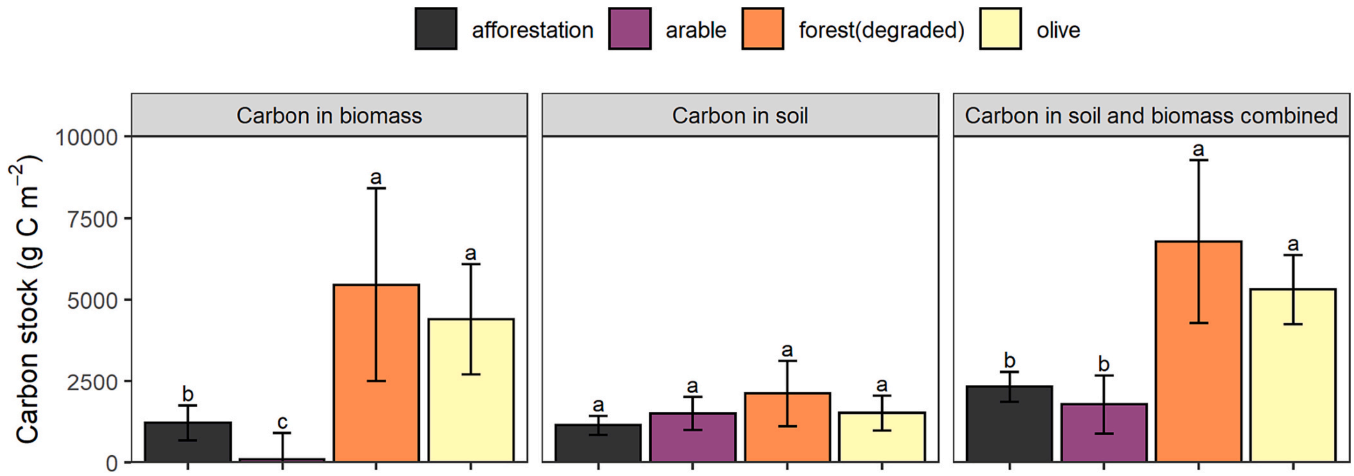

Fig. 3. Least square means of carbon stocks in biomass, soil (0–270 kg_{ESM} m^{-2}) and soil and biomass combined (g C m^{-2}) by land use. Due to a significant effect of elevation, results are given for a median elevation of 505 m. Means sharing the same capital letter are not significantly different ($p < 0.05$). Vertical error bars represent the 95% confidence intervals.

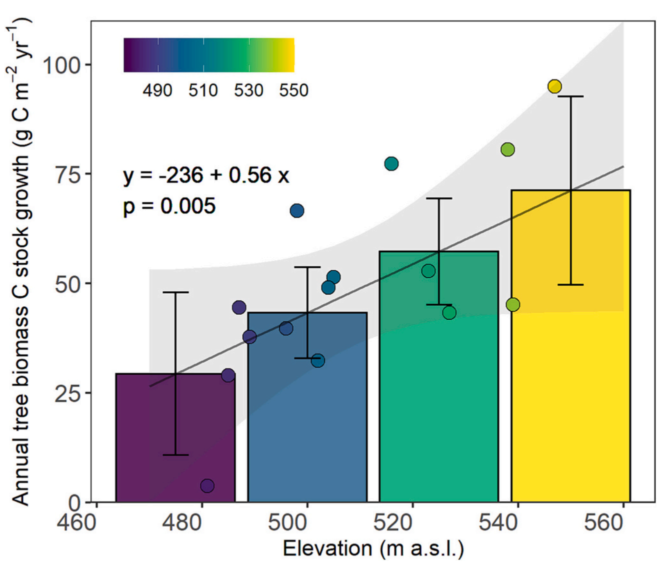

Fig. 4. Least square means of the annual biomass stock growth (g C m^{-2} yr^{-1}) as a function of elevation. Vertical error bars represent the 95% confidence intervals. The means are given for a median tree density of 250 trees per ha.

low SOC stocks in afforested plots. As such, the fact, that the differences in SOC stocks between different ages did not prove significant, could be due to confounding factors typical in space-for-time approaches, such as different soil depths that roots could explore, leading to higher concentrations in topsoils of shallower soils. Nevertheless, the literature reports that offsetting initial losses, and even more so, reaching significantly higher SOC stocks than the former arable land, may take several decades (Cunningham et al., 2015; Poeplau and Don, 2013; Smith, 2004), and usually at least 30 years (Bárcena et al., 2014; Hoogmoed et al., 2012; Paul et al., 2002; Segura et al., 2016; Ťupek et al., 2021). It might take at least 40 to 45 years (Laganière et al., 2010; Poeplau and Don, 2013) and even >100 years (Poeplau et al., 2011) to reach a new SOC equilibrium, especially for slow growing trees like *Quercus ilex* and *suber*. Consequently, a long time horizon is needed if afforestation projects with the latter species should serve as net C sinks.

4.2. A potential not yet reached in afforested plots?

Our results clearly show that the combined soil and biomass C stocks of afforested plots (2333 ± 170 g C m^{-2}) were significantly lower and far from the level of older land uses, such as the adjacent degraded forest (6784 ± 913 g C m^{-2}) and the olive grove (5316 ± 386 g C m^{-2}). At the same time, there was a clear accumulation of biomass C in afforested plots (around 30 to 70 g C m^{-2} yr^{-1}), which is likely to continue because *Quercus ilex* and *suber* are slow growing tree species, the area is rather arid, and typical European tree species continue to accumulate biomass until at least 150 years of age (Guillemot et al., 2015). Hence, the afforested sites are probably far from their potential C storage. The absence of a significant difference in total C stocks of afforested sites compared to arable land (2333 ± 170 vs 1790 ± 330 g C m^{-2}) suggests that the former are only beginning to act as SOC sinks. Their sink potential is not only in the form of biomass, but also in the form of soil, as potentially shown by significantly higher SOC stocks in the degraded forest in the first two soil layers. However, an alternative explanation for the higher SOC stocks in the degraded forest in the first two layers could be that the same amount of SOC had to accumulate in less soil material due to a shallower soil.

Yet, a future SOC sink potential of the afforested sites seems likely when considering how low their SOC stocks were compared to other sites in Extremadura. For example, Pulido-Fernández et al. (2013) observed higher SOC stocks in *dehesas* (4600 g C m^{-2}) compared to treeless grasslands (2430 g C m^{-2}) and adjacent crops (970 g C m^{-2}) in the top 10 cm of soil. Simón et al. (2013) measured SOC stocks of 1263 ± 396 g C m^{-2} close to trees and 521 ± 133 g C m^{-2} away from trees in the uppermost 5 cm of the soil of a *dehesa* system. Higher SOC values were, however, found in other studies on *dehesas*: 3600 g C m^{-2} down to 20 cm of soil depth (González González et al., 2012), 4450 g C m^{-2} down to 10 cm of soil depth (Pulido-Fernández et al., 2013). Pérez-Cruzado et al. (2012) also identified rather high SOC contents (7480 g C m^{-2} to a depth of 30 cm) under plantations. Calvo de Anta et al. (2020) even estimated SOC in degraded forest soils in the first 30 cm of the mineral layer to be between 7000 and 10,000 g C m^{-2} in semi-arid and dry sub humid regions. Yet, due to the high stone content and the semi-arid climate, such high SOC stocks are probably out of reach at our study site.

4.3. Annual accumulation of biomass carbon stocks

The mean annual accumulation rates of tree biomass in the afforested plots of this study (52 g C m^{-2} yr^{-1} at the mean 515 m asl.) fit nicely to the tree biomass C stocks reported by Cotillas et al. (2016), who found biomass stocks of about 3890 g C m^{-2} for *Q. ilex* plantations of 65 years of age in Spain, corresponding to a mean accumulation rate of 59.85 g C m^{-2} yr^{-1}. The strong effect of altitude on tree growth in our

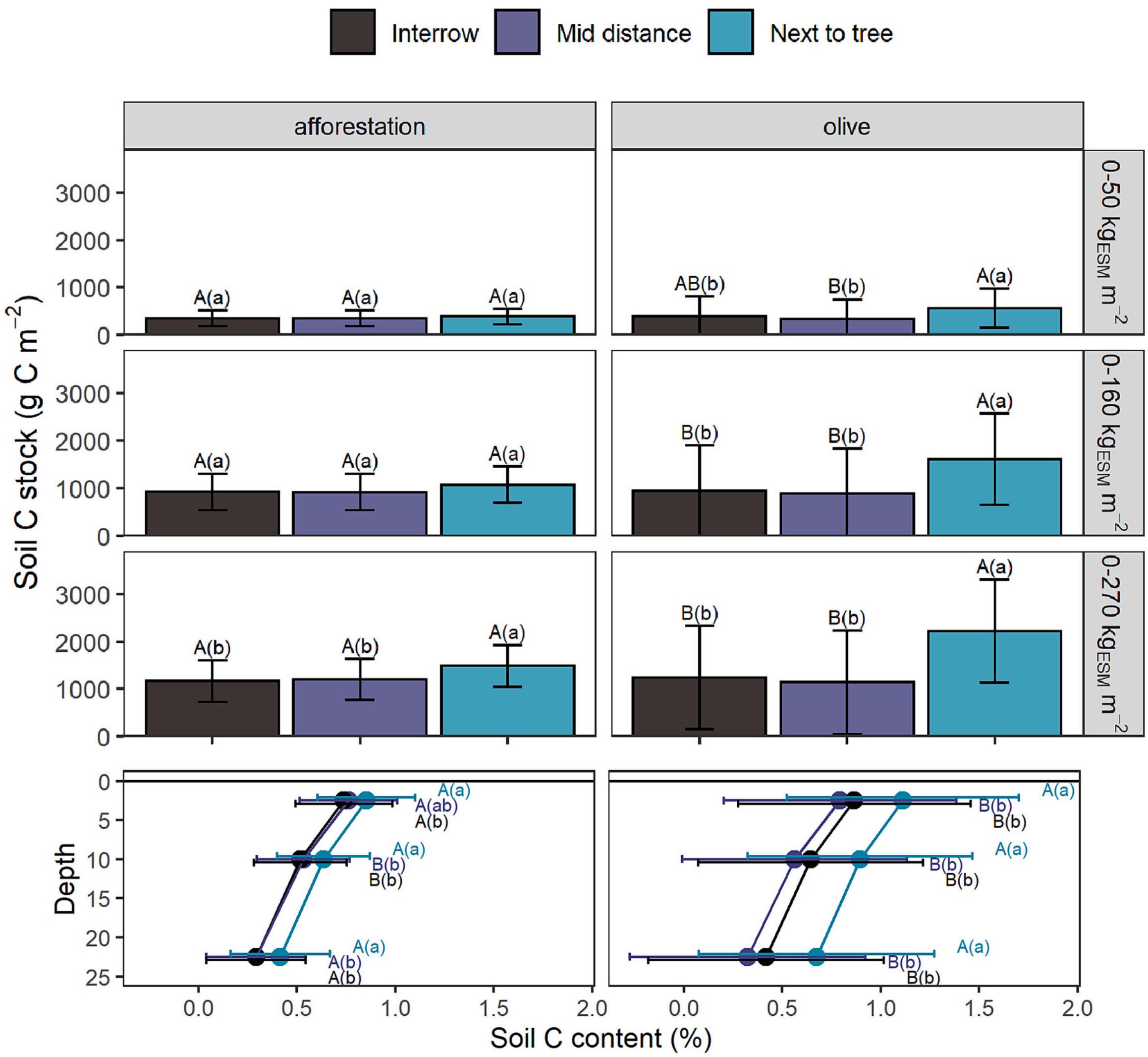

Fig. 5. Least square means of cumulative SOC stocks (g C m^{-2}) at the different equivalent soil mass depths (top) and of SOC content per depth (bottom). Comparisons are made within the same equivalent soil depth layer or depths. Means sharing the same capital letter are not significantly different ($p < 0.05$). Means sharing the same lower-case letter (in brackets) are also not significantly different at the lower threshold ($p < 0.1$). Error bars represent the 95% confidence intervals.

study is likely due to a better soil water status of higher elevated plots; lower altitude can be linked to higher temperatures and decreased moisture (Lajtha and Getz, 1993; López-Senespleda et al., 2021), both conditions known to negatively influence *Q. ilex* (Gea-Izquierdo et al., 2009). In this study, the altitude difference was only about 100 m, so the temperature effect is minimal. Yet, condensation of moisture is likely to happen when winds go from the lower altitude southern part of the study area to higher altitude north (i.e., the mountains in the north have elevations up to 800 m asl.), suggesting that the effect of soil moisture should be studied in more detail, especially because the site with 500 mm of annual rainfall is about 25% below the mean precipitation of areas where *Quercus ilex* forests appear naturally (Quinto et al., 2021).

Regarding the distribution of C pools, our study is in alignment with other studies. For instance, Johnson (1992) underlined that the majority (75%) of the C sequestered after afforestation was in the biomass. These findings were confirmed by the meta-analysis of Kim et al. (2016) (i.e., 70% of C sequestration in biomass, 30% in soils) and were also found by others (Cardinael et al., 2017; Vesterdal et al., 2007).

4.4. Positive effect of trees on soil carbon stocks

Despite the difficulty to detect differences in SOC stocks between land uses, our results corroborate the positive effect of trees on the SOC stocks through root inputs (Cardinael et al., 2017) and even more clearly on SOC contents (Fig. 5). While we are not aware of studies that measured the effect of afforestation with *Q. ilex* and *Q. suber* on SOC, our findings reflect the ones of studies in Spanish *dehesas*. For instance, the positive effect of *Q. ilex* trees on topsoil SOC has been demonstrated by several studies (Gallardo, 2003; Howlett et al., 2011; Moreno Marcos et al., 2007; Reyna-Bowen et al., 2020; Simón et al., 2013). In our study, the positive effect of trees on SOC was best visible in the olive grove, where SOC stocks next to the trees were significantly higher than SOC stocks further away from the trees in all soil layers. While the results, probably due to the younger tree age, were less clear for the afforested plots, their SOC stocks in the 0–270 kg_{ESM} m^{-2} as well as their SOC contents in all depths tended to be ($p < 0.1$) higher close to the trees compared to more distant positions. Given the evergreen nature of both trees, the high root density below the trees compared to further distances (Reyna-Bowen et al., 2020) are likely the main driver behind this gradient, also considering the high root biomass and root-shoot ratio of the afforested trees (Bárcena et al., 2014; Laganière et al., 2010). Additionally, this pattern could be the consequence of the tillage activities carried out between the tree rows. Tillage indeed promotes SOC losses due to enhanced mineralization or erosion (Abdalla et al., 2020; Francaviglia et al., 2017; Haddaway et al., 2017; Six et al., 2002; Ye et al., 2020). It likely also destroys the topsoil roots, which for *Quercus*

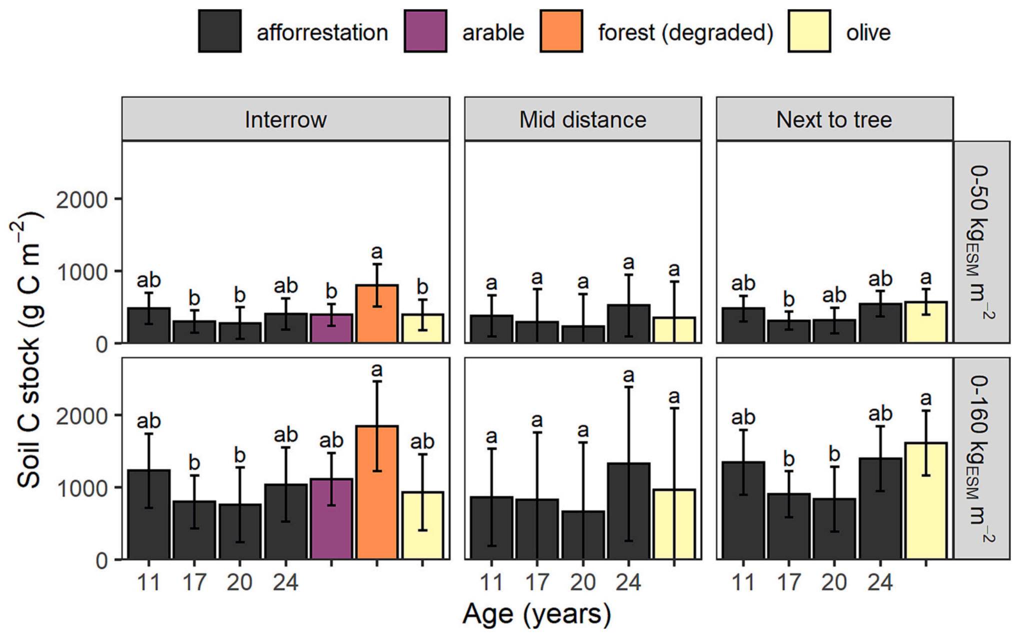

Fig. 6. Least square means of the cumulative SOC stocks (g C m^{-2}) by equivalent soil mass depth interval, land use and age, as well as distance to trees. Comparisons are made within the same equivalent soil depth layer. Means sharing the same capital letter are not significantly different ($p < 0.05$). Vertical error bars represent the 95% confidence intervals.

ilex can reach further than the canopy and explore >7 times the area (Moreno et al., 2005). This suggests that methods of less soil disturbance are needed to control competition in sustainable afforestation.

4.5. Temporal dynamics have to be considered if afforestation is to be used as a carbon sink

Our results, showing that after two decades, the combined biomass C and 0–30 cm SOC stocks were not higher in afforested than in non-afforested plots, should temper claims on the efficacy of afforestation as a method for C sequestration (Gvein et al., 2023; Zomer et al., 2008), at least for Mediterranean afforestation with *Quercus ilex* and *Q. suber* and when sticking to the IPCC recommended 0–30 cm depths for SOC stocks. The timescale considered strongly affects the potential for such afforestation projects to act as C sinks, a critical issue that is often neglected by studies that estimate the sequestration based on the difference between a fully developed forest and arable sites (Bastin et al., 2019; Pan et al., 2011). Because afforested plots of 24 years of age did not have significantly higher C stocks than the arable land, we suggest that, contrary to studies that only investigated aboveground biomass C (Chiquier et al., 2022; Gvein et al., 2023), afforestation cannot by default be considered as an immediate climate solution. Even in the most degraded sites, two to three decades are needed to reach higher than initial SOC (Menichetti et al., 2017; Nave et al., 2013), and even more so in less optimal conditions. When only focusing on C storage and neglecting other ecosystem services, afforestation can even have negative effects on landscape biodiversity, especially with tree species monocultures (Bond et al., 2019; Messier et al., 2022). At least under the conditions of this study, the first 20 and more years could not be regarded as a net sink of CO_2, which has also been reported by a recent modeling study (Fradette et al., 2021). Consequently, our results call for further research on how to implement afforestation in a way that is less disruptive to the soils, e.g., surface mulching instead tillage to suppress weeds, or even syntropic agriculture, mimicking natural succession (Andrade et al., 2020), if afforestation should be a solution to achieve rapid CO_2 sequestration. An alternative to tillage would be a transformation to agroforestry systems with livestock grazing, which also reduces wildfire risk (Damianidis et al., 2021).

5. Conclusions

Our study provided insights into an afforestation project with *Quercus ilex* and *Quercus suber* on degraded arable land in Extremadura, Spain. The afforestation led to a biomass C accumulation of 25 to 70 g C m^{-2} yr^{-1}. However, when the 0–30 cm soil and biomass C stocks together were compared between land-uses, no significant difference was found between afforested and non-afforested arable plots, even after two decades. It is thus likely that initial topsoil losses of SOC counteracted gains in biomass C. Such a temporal trend of SOC dynamic has also been reported in other studies, which suggest that the joint evaluation of SOC and biomass C stocks is the only valid measure to determine if afforestation acts as a net C sink. According to our results, it can take more than two decades for Mediterranean afforestation with *Q.ilex* and *Q. suber* to become a net C sink, according to IPCC standards, despite successful establishment and growth of the trees. If the goal of new afforestations is to mitigate climate change in the coming decades, it will be necessary to implement them in a way that is less disturbing to topsoil SOC. Future studies could focus on finding afforestation techniques that minimize soil disturbance to reduce the time needed for afforested plots to be a net C sink, and study SOC for even lower depths. Further, studying total system C stocks of a larger age range of oak forests or *dehesa* systems should help to gain a better understanding of the total C sink potential over time.

CRediT authorship contribution statement

Valeria Renna: Conceptualization, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing, Investigation, Methodology. **Pilar Martín-Gallego:** Conceptualization, Investigation, Project administration, Supervision, Writing – review & editing. **Federico Julián:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – review & editing. **Johan Six:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. **Rémi Cardinael:** Conceptualization, Supervision, Writing – review & editing. **Moritz Laub:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The raw data will is published on Zenodo: https://doi.org/10.5281/zenodo.10034403. The embargo will be released at publication.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geodrs.2024.e00768.

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