Carbon accumulation over 70 years of old-field succession driven by live biomass in a Mediterranean continental environment

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ABSTRACT

Forest expansion in abandoned agricultural lands entails the fixation of atmospheric CO_2 in the live biomass, the soil as well as in other ecosystem compartments, and in turn, has strong implications for C budgets and the design of actions to mitigate climate change all over the world. However, changes in C stocks in these compartments are still poorly understood. We assessed the main patterns and drivers of C accumulation over spontaneous colonization of abandoned agricultural lands, considering the above- and below-ground biomass (AGB and BGB, respectively), the woody debris (WD), the litter and the surface soil (SS; 0–6 cm depth), in an area of Central-North Spain. To attain this objective, we established a chronosequence of 30 plots (0.0625 ha) located in agricultural lands abandoned in 1956–1977, 1977–2005 and 2005–2017, in four different forest types. We found that the AGB and the SS accounted for the major proportion of the total C stock, but the BGB was also relatively important. Carbon stocks in all these major compartments increased linearly with the age of tree colonization. But C in the SS did not significantly differ among plots abandoned in the three periods considered and showed decreasing rates of change from youngest (i.e. those in plots abandoned in 2005–2017) to oldest (i.e. those in plots abandoned in 1956–1977) stands. Carbon accumulation was mainly driven by adult stem density and age of tree colonization in the AGB and the BGB, and by the total nitrogen content in the SS. Our findings indicate that, in these new forests, C accumulation in the live biomass is still ongoing and proceeds relatively fast and at an increasing rate, which points toward a clear potential for atmospheric CO_2 fixation over the next few decades. This process, however, proceeds more slowly and shows signals of an apparent deceleration in the SS. The C stock in the surface soil is, in fact, largely disconnected from that of live biomass and varies depending on pre-abandonment land-use differences among the study sites.

1. Introduction

Over the last few decades, many areas of the Northern Hemisphere have experienced an unprecedented increase in forest cover. This increase is mainly due to the deliberate planting of trees in areas that were once covered (i.e. reforestation) or not covered (i.e. afforestation) by forests, but also through the spontaneous colonization of lands that were formerly cultivated or pastured (Fuchs et al., 2016). This forest expansion in abandoned agricultural lands can be explained by the rural-–urban migration during the XXth century that led to the abandonment of less productive or *marginal* lands (i.e. those characterized by shallow soil depths, high stone contents, poor drainage and or high steepness of

the slopes; Rey-Benayas et al., 2007). Once abandoned, these former agricultural lands (also termed *old-fields*) underwent secondary succession, are spontaneously colonized by shrubs and trees and increase in ecosystem C stocks (Schimel et al., 2001). In Europe, forest expansion in abandoned agricultural lands had resulted in smaller changes in carbon (C) fluxes than afforestation and reforestation, but it has occurred over larger areas (Fuchs et al., 2016), having strong implications for regional C budgets (Velázquez et al., 2022) and in turn contributing to mitigate climate change (Uri et al., 2012).

Most studies of abandoned agricultural lands have reported a net increase on the C accumulated (i.e. C stock) in the above-ground biomass (C_{AGB}) over the first 50–75 years after land abandonment

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(Alberti et al., 2011; Uri et al., 2012). Such an increase, however, is slower during the first few decades, but much faster afterwards, once trees become dominant (e.g. Wang et al., 2016). Carbon accumulation in the AGB is positively driven by factors that stimulate tree growth such as mean annual rainfall (MAR) and temperature (MAT; Anderson et al., 2006). It is also positively affected by contents in soil nutrients such as nitrogen (N; Bose et al., 2014) and phosphorous (P; Aggenbach et al., 2017), as well as by and fine-textured soils, which are related to a larger water holding capacity (Johnson et al., 2000). Stand density has a positive effect on C accumulation until a certain density is reached, which leads to competition for light and nutrients and in turn negatively affects biomass production (Uri et al., 2012; Bose et al., 2014).

During secondary succession a large proportion of the C accumulates in the AGB, but also in other compartments such as below-ground biomass (BGB), woody debris (WD) and litter. Below-ground biomass plays an important role on forest C storage, particularly in regions characterized by a pronounced seasonality in rainfall (Vargas et al., 2008) such as those of Mediterranean climate, and mainly because of the development of coarse roots (Hooker and Compton 2003). In abandoned agricultural lands, the C stock in the BGB (C_{BGB}) increases more slowly than in the AGB (C_{AGB}). However, the main determinants of this increase are not widely known (Clemmensen et al., 2013). Carbon stocks in woody debris and litter (C_{WD} and C_{lit}) also increase over secondary succession (Novara et al., 2013).

Soil is another important pool for C accumulation in the forests, woodlands and other wooded areas developed after agricultural land abandonment (hereafter; recovering lands). In particular, pools in the most superficial mineral soil (i.e. up to 5–10 cm depth) are usually more active at sequestering C than those in deepest ones (Foote and Grogan 2010; Uri et al., 2012; La Mantia et al., 2013; Nave et al., 2013; Wang et al., 2016). Surface soil C stocks (C_{SS}) usually increase slowly over the first few years (or decades) following abandonment, and much faster afterwards (Richter et al., 1999), mainly as a consequence of the increasing C inputs from the live biomass (Foote and Grogan 2010; Nóvak et al., 2014; Novara et al., 2013; Badalamenti et al., 2019). In temperate-cold environments, C_{SS} use to experience major increases in favorable environments with medium values of MAR and MAT that enhance litter decomposition (Thuille and Schulze 2006). Another important driver of soil C accumulation is primary productivity: the larger the C_{AGB}, the more woody debris and litter to decompose (Castro et al., 2010), and in turn, the larger the C_{SS}. Nonetheless, the role of AGB as a determinant factor of soil C accumulation in abandoned agricultural lands is usually mediated by the type of vegetation. Indeed, in Mediterranean environments, lands colonized by deciduous trees and shrubs, have usually better-quality litter whose decomposition leads to larger C_{SS} (La Mantia et al., 2013). Larger contents in soil nutrients such as nitrogen (N) and phosphorous (P) also contribute to increase C_{SS} through favoring woody plants growth, and therefore, the production of plant residues (Alberti et al., 2011; Novara et al., 2013). Surface soil C also accumulates more in fine-textured soils (i.e. those with a high proportion of clay), because they are better protected than coarse-textured ones against C losses (Foote and Grogan 2010; Nóvak et al., 2014), and in soils with high pH, since it increases organic matter decomposition rates (Aggenbach et al., 2017).

Over the last few years, our understanding of the main patterns and drivers of C accumulation over spontaneous colonization in abandoned agricultural lands has increased, particularly in temperate cold climates (e.g. Foote and Grogan 2010; Uri et al., 2012; Bose et al., 2014; Nóvak et al., 2014). In Mediterranean environments, were forest expansion in agricultural land abandonment has been pervasive (Rey-Benayas et al., 2007), many studies have assessed changes in species and functional types richness, species composition and plant-plant interactions as a consequence of this process (e.g. Bonet and Pausas 2007) and those analyzing changes in C stocks have mostly focused on the soil, without considering relationships with other compartments (but see Badalamenti et al., 2019).

In this study we assess the main patterns and drivers of the process of C accumulation as a consequence of spontaneous revegetation of abandoned agricultural lands, in a region of Mediterranean-continental environment; *Castilla y León* (Central-North Spain). We consider the C stocks in above and below-ground woody biomass, woody debris, litter and surface soils, over three periods of abandonment (i.e. 1956–1977, 1977–2005 and 2005–2017), in recovering lands currently dominated by four different tree species (i.e.; *Juniperus thurifera* L., *Quercus ilex* L., *Q. faginea* Lam. and *Q. pyrenaica* Willd.). Specifically, we asked the following questions; (i) What is the relative importance of the different compartments as C stocks?, (ii) What are the main patterns of change in the C stocks of the entire stand and in each compartment?, (iii) What are the main driving factors of C accumulation over the study period in the entire stand and in each compartment? We also examined if the relative importance of each compartment as C stock, and the main patterns and drivers of change in the C stocks varied depending on the above-mentioned four forest types.

2. Material and methods

2.1. Study area and selection of sampling sites

We performed our study in *Castilla y León* (Central-North Spain; Fig. 1a) a 9.4 million ha region formed by a high plateau (600–700 m a.s. l) surrounded by mountain ranges with altitudes usually over 2000 m a. s.l. The climate of this region is Mediterranean continental, with hot summers and cold winters, and extreme day-night temperature oscillations. Mean annual rainfall is relatively high (i.e. >1000 mm•yr^{-1}) near the mountain ranges, particularly in the Northern and Westernmost areas (Ninyerola et al., 2005). Over the last half of the XXth century, this region experienced a strong process of agricultural land abandonment that has in turn provoked a steady increase in the forest cover (Velázquez et al., 2022).

We assessed the process of C accumulation in abandoned agricultural lands through a chronosequence or *space-for-time substitution* approach (Johnson and Miyanishi 2008; Walker et al., 2010). According to this approach, first, we considered the four forest types occurring over a largest area within the study region, according to the *Spanish Forest Map 1*:50, 000 (*Área Banco de Datos de la Naturaleza*, 2006); those dominated by *Juniperus thurifera*, *Quercus ilex*, *Quercus pyrenaica* and *Q. faginea*. Second, within the area occupied by these forest types, we selected five study sites, one for each *Q. ilex*, *Q. faginea* and *Juniperus thurifera*, and two for *Q. pyrenaica*, as this species covered a much larger area than the other three. To comply with the assumptions of chronosequence studies (Walker et al. 2010), environmental factors (i.e. lithology, soils, elevation, steepness of the slope, climate, and land-use history) were different between sites but similar within sites (Table A.1). Second, for the areas in which these study sites were located, we obtained aerial photographs for the periods 1956–1957, 1977–1980, 2005–2007 and 2015–2017 from the *Centro Nacional de Información Geográfica*. The aerial photographs of each period (e.g. 1956–1957) were superposed with those of the immediately subsequent one (e.g. 1977–1980), comparing among both within the areas occupied by the four main forest types selected (Fig. 1c–f). When a land showing signs of cultivation in the aerial image of one period became vegetated in the aerial image of the subsequent one, we assumed that secondary succession had occurred on that land (Aggenbach et al., 2017) and it was digitized. Third, once digitized these lands, in each study site, we randomly established two 0.0625-ha plots located on those lands abandoned in each, 1956–1977, 1977–2005 and 2005–2017 periods, obtaining two chronosequences of three plots (six plots per site, 30 plots in total; Fig. 1f). Although most plots were squared (i.e. 25 × 25 m^2), some of them were rectangular (i.e. 20 × 30 m^2) in order to adapt to the shape of the fields in which they were installed. All these plots had also adjacent mature forests and/or trees (i. e. a similar distance to seed sources; Johnson and Miyanishi 2008). In order to minimize the impact of spatial structure on statistical analyses
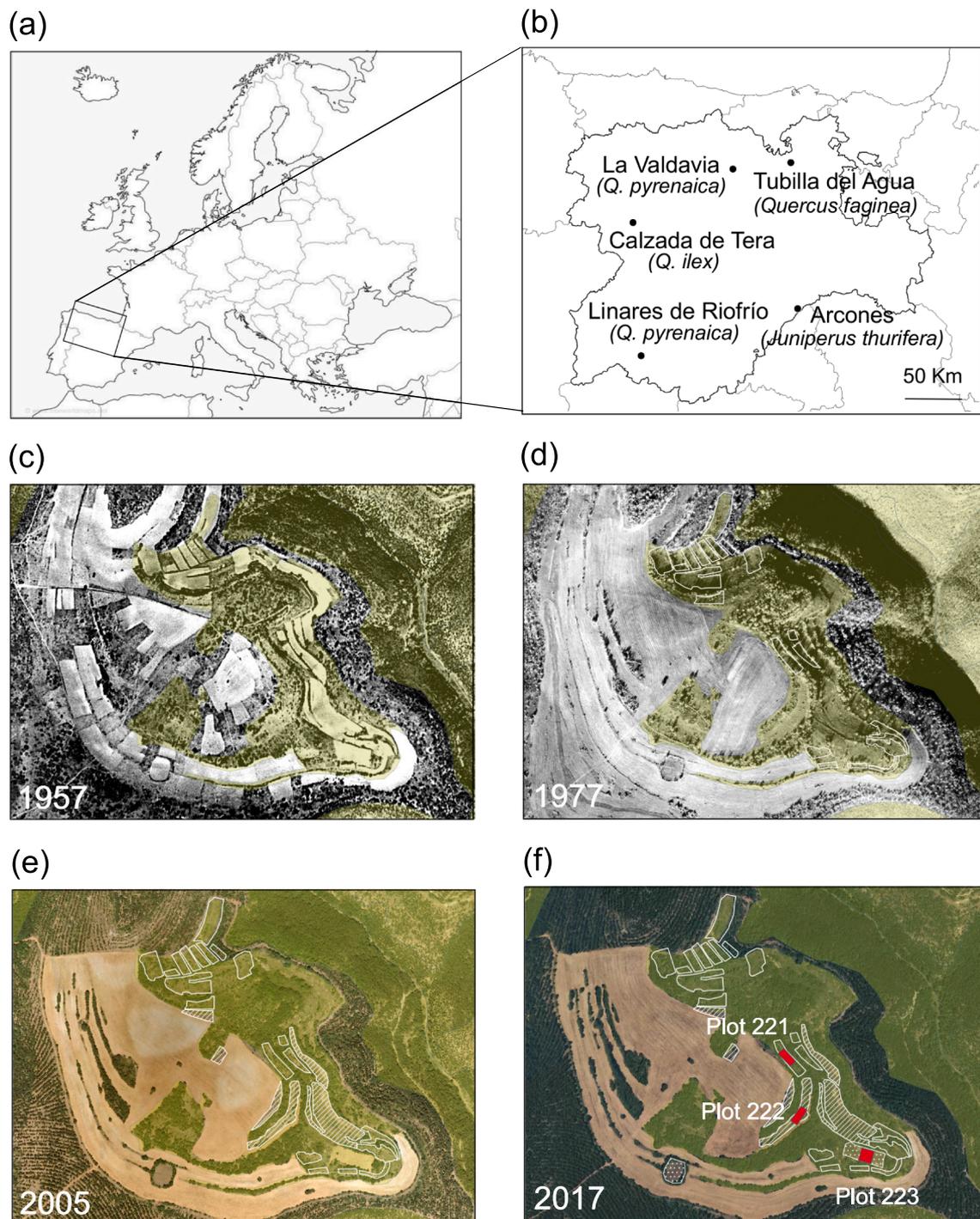
Fig. 1. (a) Location of the study area. (b) Location of the study sites within the study area, indicating the forest type as defined by the dominant tree species. (c-f) Selection of sampling sites for the *Cueto Sombrío* chronosequence (*Tubilla del Agua*), showing the ortho-corrected images of (c) 1957, (d) 1977, (e) 2005 and (f) 2017, and the fields abandoned in 2005–2017, 1977–2005 and 1956–1977, respectively, within the area occupied by *Q. faginea*-dominated forests according to the Spanish Forest Map 2006, in light green. In f, location of sampling plots 221, 222 and 223 is also indicated.

(Legendre and Legendre 2012) we devise plots location so that plots on lands abandoned in different periods were closer one to another than plots on lands abandoned in the same period.

2.2. Field sampling

In each plot, all tree and shrub individuals with ≥0.25 and 0.50 m height were identified to species, labelled and mapped. In all trees ≥1.3 m height, we recorded diameter at breast height (DBH; cm) and height

(H; m), by using a large caliper and a Vertex (Häglof, Sweden), respectively. In shorter trees and shrubs, we measured diameter at root collar (DRC; cm) through a caliper, as well as height (H; m) and the two main canopy diameters (CD; cm) by means of a measuring tape. In the four corners and in the centre of each plot we located five quadrats (1 × 1 m^2) in which all woody debris (i.e. deadwood on the forest floor with diameter >2 cm; Alberti et al., 2011) was collected. Within these quadrats, we also randomly place a smaller one (0.25 × 0.25 m^2) to collect all the litter (i.e. leaves, dead moss, partially decomposed organic

material and deadwood with diameter <2 cm; Herrero et al., 2016). Once the litter was removed, we took one soil sample (five soil samples per plot), from the top 0–6 cm of the soil profiles using a stainless steel cylinder (d = 5 cm and volume 118 cm³). To determine the age of woody colonization, in all plots abandoned in 1956–1977 and 1977–2005, we took two wood cores in the five trees with the largest DBH, at 1.3 m above the ground, in North-South and East-West directions, using a 5.15 mm increment bore (Häglof, Sweden; Hooker and Compton 2003).

2.3. Laboratory analyses

To estimate C_{AGB} and C_{BGB} in each plot, we first calculated above- and below-ground biomass (AGB and BGB, respectively; Mg•ha⁻¹) per woody individual through allometric equations relating DBH and H with dry biomass weight (Table B.1). Second, we summed the single tree or shrub biomass values obtained, to find plot-level estimates of AGB and BGB (Mg•ha⁻¹), and transformed these estimates into C_{AGB} and C_{BGB} (Mg C•ha⁻¹) by means of species-specific conversion factors (e.g. Montero et al., 2013). For those species for which these factors were not found, we multiplied the woody biomass values by 0.5 (Penman et al., 2003). To estimate C_{WD} and C_{lit} we first oven-dried WD and litter samples at 70 °C for 72 h and weighed them (Bueis et al., 2018). These dried samples were subsequently grounded in a Retsch MM 301 ball mill, and their C contents were estimated by using a LECO CHN-2000 analyzer. We finally expressed these contents as stocks, calculating the mean of the five samples per plot, and referring to a unit of area; i.e. Mg C•ha⁻¹. We also calculated the adult stem density (ASD; number of adult stems•ha⁻¹) per plot, considering as *adults* all tree and treelet stems ≥1.3 m height.

With regard to the soil samples, we weighed them before and after air-drying (3 days at 21 °C), and subsequently, sieved to 2 mm, separating the fine earth from the remaining roots and stones. Dry soil bulk density was calculated as mass of dry soil per steel cylinder volume (1.18•10⁻⁴ m³). In the fine earth, we measured the proportions (g/100 g) of sand, silt, and clay fractions (diameters 0.2–2, 0.002–0.2 and <0.002 mm, respectively) by the Bouyoucos hydrometer method, and the pH (soil:solution ratio of 1:2.5) using a pH-meter. We obtained the proportion of soil organic matter (SOM; g/100 g) by the Walkley and Black (1934) method, and the C content by dividing by the Van Ben-melen factor (1.724). With these data, the surface soil organic carbon stocks (C_{SS}; Mg C•ha⁻¹) were determined through the formula (López-Marcos et al., 2018);

$$C_{SS}\left(MgC \cdot ha^{-1}\right) = C_{conc} * BD * D * CF_{coarse} * 10^2 \tag{1}$$

where C_{conc} is the concentration of C in the fine earth of the soil (g C•g fine earth soil⁻¹), BD is the bulk density (kg soil•m⁻³), D is the depth of the horizon (0.06 m), and CF_{coarse} is the correction factor of the coarse fraction (g fine earth•g soil⁻¹). Surface soil N (N; g•kg soil⁻¹) was obtained by the Kjeldahl method (Jones 1991), and available P in the surface soil (P; g•kg soil⁻¹) by the Olsen method (Olsen et al., 1954). We extracted exchangeable cations (Ca^{+2}, Mg^{+2}, K^+, Na^+) with 1 M ammonium acetate at pH = 7 (Schollenberger and Simon 1945) and subsequently, determined their concentrations by atomic absorption spectrophotometry (Ca_{ex}, Mg_{ex}; cmol$_{(+)}$•kg soil⁻¹) and emission spectrophotometry (K_{ex} and Na_{ex}; cmol$_{(+)}$•kg soil⁻¹). We also calculated an overall measure of surface Soil Fertility (SF; cmol$_{(+)}$•kg soil⁻¹) by means of the sum of bases; i.e. summing up Ca_{ex}, Mg_{ex}, K_{ex}, and Na_{ex}, as well as the C:N relationships in the WD, litter and surface soil (CN_{WD}, CN_{litter} and CN_{SS}). Finally, the total C stock of each stand (C_{all}; Mg C•ha⁻¹) was calculated by summing up C_{AGB}, C_{BGB}, C_{WD}, C_{lit} and C_{SS} per plot. We also calculated the rates of change for the different C stocks (Mg C•ha⁻¹•yr⁻¹), per plot, dividing the C stocks by the number of years over which spontaneous colonization had occurred; i.e. from the first year of the period of abandonment (1956, 1957, 1977, 1980, 2005 and 2007), or if absent, the first year of such period in which there were

available climatic records, to the last year before sampling (i.e. 2017).

To estimate the age of tree colonization (ATC) per plot, we prepared the 89 valid cores obtained in the field following standard dendro-chronological techniques (Stokes and Smiley 1996); (i) drying, mounting and gluing them firmly on grooved wooden sticks, (ii) sanded them with successively finer grades of sandpaper until optimal surface resolution allowed annual rings to be distinguished under magnification (x10) and (iii) scanning the cores with a high precision scanner (Epson Expression 1640XL) and (iv) counting annual rings per core. The ATC was then estimated through subtracting the number of tree rings to the sampling year of each plot (i.e. 2018–2019), and then subtracting 5 years more (the mean estimated time for tree individuals to reach 1.3 m height). For those plots abandoned over the period 2005–2017, without ≥1.3 height trees, and in which wood cores were subsequently not taken, we estimated the ATC by analyzing all ortho-photos for such period and determined the year in which woody vegetation first appeared. Then we subtracted this year to the sampling year of each plot.

Using records taken on the nearest meteorological stations (Table A.1), we calculated MAR (mm) and MAT (°C), to consider the average climatic conditions. We also estimated Mean Monthly Rainfall of the Driest Month (MMRD; mm) and Mean Minimum Temperature of the Coldest Month (MMTC; °C) to take into account the negative effects on plant's emergence and growth as a consequence of summer drought and winter freeze. All these variables were calculated per plot, considering the time period number of years over which spontaneous colonization had occurred.

2.4. Statistical analyses

To test for the relative importance of the C stocks in the different compartments, and to check whether it varied over the different periods of abandonment and forest types or not, we just calculated the proportions of C accumulated in the different compartments with regard to the total C stock, in the different periods of abandonment. These estimations were made for all plots located on each forest type, separately. To describe the main patterns of change in the total C stock and in those of the different compartments, we checked if the relationships between C_{all}, C_{AGB}, C_{BGB}, C_{WD}, C_{lit} and C_{SS}, and the ATC, followed a linear model (and when not, logarithmic or S-curve ones; Kashian et al., 2013) as well as the significance and sign of such relationships. Additionally, we tested if the abovementioned C stocks significantly differed between the different abandonment periods and forest types by using an ANOVA test considering a threshold *P*-value of 0.05. We also followed a similar approach for rates of change in the C stocks (hereafter; rC_{AGB}, rC_{BGB}, rC_{WD}, rC_{lit}, rC_{SS} and rC_{all}. In those tests in which general significant differences between different periods or forest types were detected, we carried out a *post-hoc* Tukey test in order to determine the pairwise abandonment periods or forest types among which the most significant differences were.

To determine the main driving factors of C accumulation, we first selected the most independent and meaningful environmental factors by means of a Pearson's product-moment correlation considering all factors, and a principal component analysis (PCA) for each group of climatic, C:N relationships, soil-fertility and soil-texture factors separately (Ihaddaden et al. 2013). In each group, amongst pairs of highly correlated factors (Pearson's $r \geq 0.6$), those with the highest factor loadings (>0.5) on the first two PCA axis were selected. Second, we tested for the effects of these finally selected factors, together with ATC, ASD and pH, on C accumulation in the entire stand and in each compartment, through Multiple Regressions (Appendix C). We used the backward procedure based on Akaike Information Criterion (AIC), in order to define the overall best (and most parsimonious) explanatory model (Burnham and Anderson 1998). We also used analysis of covariance (ANCOVA; Huitema 1980) to test if the relationships between changes in the different C stocks and their main driving factors (i.e. those in finally selected

Multiple Regressions), varied between the different forest types. As the response function between any of C stocks and their driving factors was linear, a linear generalization of the analysis of variance was used and linear models were fitted (Kutner et al., 2004). As all dependent variables were continuous, we selected a normal distribution function and an identity link function (Agresti 2002).

When necessary, variables were log, square-root, arc-sin or box-cox transformed to achieve normality, equality of variances and linearity assumptions. In Pearson's product-moment correlations, and in order to avoid type I errors, Rice's sequential correction was applied. We used R packages *measuRing* (Lara et al., 2015) to identify and count tree rings on scanned cores, *nlstools* (Baty et al., 2015) and *leaps* (Miller 2020) to perform linear and multiple linear regressions, and *rstatix* (Kassambara, 2021) to perform ANCOVAs. We compared among different models with AIC by using the R package *MASS* (Venables and Ripley 2002).

3. Results

3.1. Carbon accumulation in the different compartments

Among the four compartments considered, the AGB comprised the largest C stock (i.e. C_{AGB}; 13.07 ± 15.41 Mg C•ha^{-1}), followed by the surface soil (i.e. C_{SS}; 8.18 ± 2.34 Mg C•ha^{-1}) and the BGB (i.e. C_{BGB} 4.13 ± 5.38 Mg C•ha^{-1}). In contrast, those of the FWD and the litter were extraordinarily low (i.e. < 0.5 Mg C•ha^{-1}). The larger relative importance of biomass and surface soil as C pools with regard to WD and litter was also confirmed when considering plots abandoned over different periods, separately (Table 1). In all forest types considered, the relative proportion of C_{AGB} and C_{BGB} over the total C stock increased from plots abandoned in 2005–2017 to plots abandoned in 1977–2005 and 1956–1977, whereas the opposite occurred for C_{SS} (Fig. D.1).

3.2. Patterns of change

The relationships between the C stocks in the AGB, the BGB and the SS, and ATC could be fitted through linear models, and were all positive

Table 1
Mean and standard deviation of the mean carbon stock (Mg C•ha^{-1}) and the mean rates of change (Mg C•ha^{-1}•yr^{-1}) on forests, woodlands and other wooded lands developed in abandoned agricultural lands of the *Castilla y León* region (Central-North Spain) abandoned in 2005–2017, 1977–2005 and 1956–1977. We measured C pools in above- and below-ground biomass (C_{AGB} and C_{BGB}), surface soil (C_{SS}; 0–6 cm depth), as well as the entire stand (C_{all}). The mean values and rates of change of the main drivers of C accumulation in the above mentioned pools, as identified by multiple regressions, namely; adult stem density (ASD; number of adult stems•ha^{-1}), Mean Monthly Rainfall of the Driest Month (MMRD; mm), concentration of N (N; g•kg soil^{-1}), and proportion of sand (*sand*; %) in the surface soil.

Compartment	2005–2017		1977–2005		1956–1977	
	Mean ± SD	Mean ± SD	Mean ± SD	mean r. ± SD	Mean ± SD	Mean ± SD
C_{AGB}	0.3 ± 0.3	0.04 ± 0.03	8.6 ± 5.6	0.4 ± 0.2	30.1 ± 12.7	0.5 ± 0.14
C_{BGB}	7.8 ± 1.6	0.01 ± 0.01	3.1 ± 2.1	0.1 ± 0.05	9.3 ± 2.2	0.1 ± 0.04
C_{SS}	7.5 ± 1.1	0.6 ± 0.1	8.3 ± 1.1	0.2 ± 0.03	9.0 ± 2.3	0.1 ± 0.04
C_{all}	7.8 ± 1.4	0.6 ± 0.1	20.0 ± 7.5	0.5 ± 0.2	48.4 ± 10.6	0.8 ± 0.2
ASD	144 ± 67.8	6.6 ± 0.6	662 ± 248	1.8 ± 0.2	1139 ± 593	18.9 ± 7.5
MMRD	4.44 ± 0.0	0.4 ± 0.0	4.5 ± 0.0	0.1 ± 0.0	4.8 ± 2.8	0.1 ± 0.0
N	1.6 ± 0.3	0.1 ± 0.02	1.8 ± 0.3	0.1 ± 0.01	2.2 ± 0.6	0.04 ± 0.01
sand	67.5 ± 7.6	4.8 ± 0.5	68.3 ± 9.9	2.5 ± 0.5	69.8 ± 7.1	1.16 ± 0.1

and significant, but not strong ($R^2 < 0.5$; Fig. 2; Table E.1). With regard to the relationships between the rates of change in such C stocks, those for C_{AGB}, C_{BGB} and C_{SS} were also significant and not strong (Table E.1), however, while those for C_{AGB} and C_{BGB} were positive (Fig. E.1a–c), that for C_{SS} was negative (Fig. 4a). We did not consider such relationships for C_{FWD} and C_{lit} (and their rates) because of the negligible contribution of these two compartments to the total C stock. With regard to the ANOVAs, plots located in lands abandoned over the three periods considered differed significantly in their C_{AGB} ($F_{2,27} = 71.1$, $P < 0.001$), C_{BGB} ($F_{2,27} = 55.2$, $P < 0.001$) and C_{all} ($F_{2,27} = 68.0$, $P < 0.001$), but not on their C_{SS} (Fig. 3a–c). They also differed significantly in their rates of change; rC_{AGB} ($F_{2,27} = 33.2$, $P < 0.001$), rC_{BGB} ($F_{2,27} = 31,5$, $P < 0.001$), rC_{all} ($F_{2,27} = 4.5$, $P < 0.05$: Fig. E.1d–f) and rC_{SS} ($F_{2,27} = 71.8$, $P < 0.001$: Fig. 4b). Pair-wise Tukey comparisons also revealed that plots in agricultural lands abandoned in 1956–1977 had significantly larger C_{AGB}, C_{BGB} and C_{all} than those abandoned in 1977–2005, and that the latter had a significantly larger C_{AGB}, C_{BGB} and C_{all} than those abandoned in 2005–2017, respectively (Fig. 3a–c). We obtained very similar results for rates of change in C_{AGB} and C_{BGB} (Fig. E.1c-d), whereas rates of change in C_{SS} were, in plots abandoned in 2005–2017, significantly larger than in plots abandoned in 1956–1977 and 1977–2005 (Fig. 4b). With regard to forest types, significant differences were just detected for C_{SS} ($F_{3,26} = 5.93$, $P < 0.001$), which was larger in areas dominated by *Q. faginea* than in areas dominated by *Q. ilex* and *Q. pyrenaica* (Fig. 3d).

3.3. Driving factors

According to Pearson's product moment correlations and PCA analyses, and in order to perform multiple regressions, we selected MMRD, CN_{SS}, N and *sand*, as factors associated with climate, C:N relationships, soil-fertility and soil-texture, respectively (Appendix F). We included ATC, ASD and soil pH as factors in all models. According to best fitting (i. e. those with the lowest AIC; Table C.1) multiple regression models (Table 2), C accumulation, in the AGB, was mainly (and positively) driven by ASD and ATC, but negatively affected by MMRD, whereas in the BGB it was just positively driven by ATC and ASD (in this order). Carbon accumulation in the SS, however, was positively determined by N and *sand*, whereas in the entire stand, it was driven by all above-mentioned variables with the exception of *sand*. Any of the selected variables, at any of the models, had VIF > 5 values, which indicates that there were no multicollinearity problems in our models. The results of the ANCOVAs (Table 3) revealed that, for C_{AGB}, C_{BGB} and C_{all} in the analyses considering ASD and ATC as covariates, and for C_{SS} and C_{all} in the analyses considering N as covariate, just these covariates (and not the interaction terms) had a significant effect on the dependent variable. Contrastingly, for C_{AGB} and C_{all}, in the analysis considering MMRD as covariate, and for C_{SS} in the analyses considering *sand* as covariate, the interaction terms (i.e. ftype:MMRD and ftype:*sand*) were also significant. Tukey *post-hoc* comparisons showed that the relationships $C_{AGB} \sim$ ATC significantly ($P \leq 0.001$) differed between plots located in areas dominated by *Q. ilex* and those located in areas dominated by *Q. faginea*, while the relationships $C_{SS} \sim$ *sand* significantly ($P \leq 0.05$) differed between plots located in areas dominated by *Q. ilex* and those located in areas dominated by either, *Q. faginea* and *Q. pyrenaica*.

4. Discussion

4.1. Importance of the different compartments

Our results clearly pointed that AGB and the SS accounted for the major C stocks, which corroborates findings of former studies performed in recovering lands. In fact, our values of C_{AGB} (i.e. 13.7–56.9 Mg C•ha^{-1}), were very similar to those found in 75-year old forests developed after agricultural land abandonment in the Italian pre-Alps (48.0–61.5 Mg C•ha^{-1}; Alberti et al., 2011) and to that estimated for Spanish forests (45 Mg C•ha^{-1}; Vayreda et al., 2011). However, they
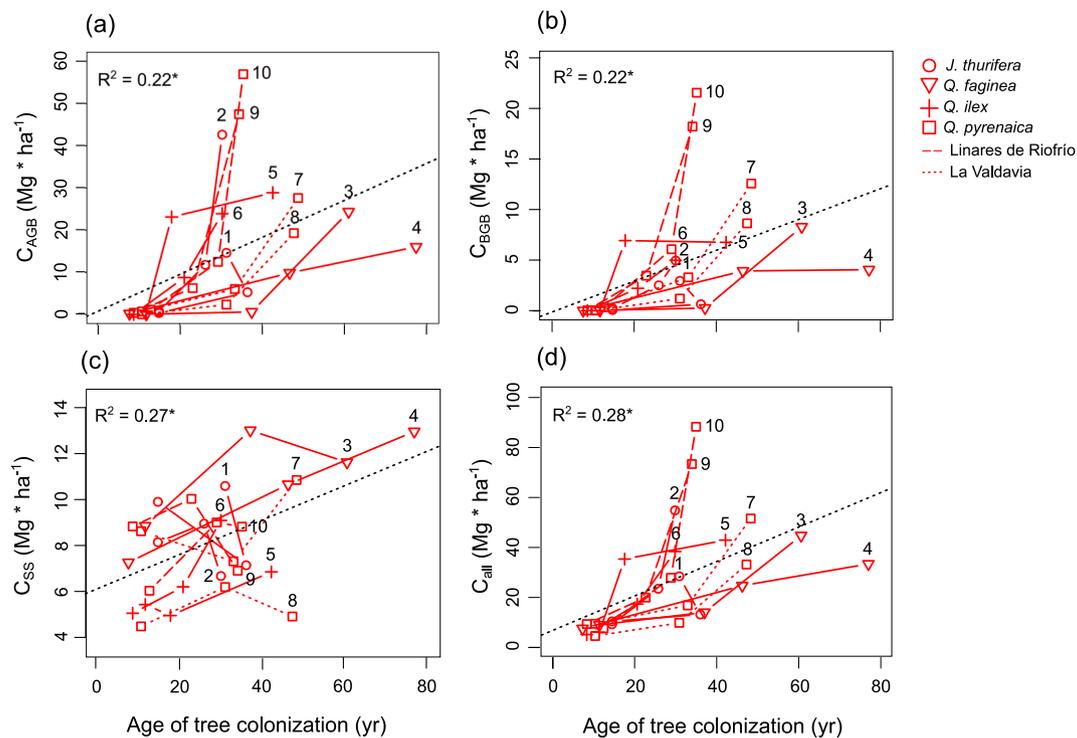
Fig. 2. Linear regressions between C stocks (Mg C•ha^{-1}) in (a) above-ground biomass (C$_{AGB}$), (b) below-ground biomass (C$_{BGB}$), (c) surface soil (C$_{SS}$) and (d) the entire stand (C$_{all}$), and age of tree colonization (ATC; yr), in the 0.0625-ha plots, showing the adjusted coefficient of determination (R^2). The forest types considered (i.e. dominated by *Juniperus thurifera*, *Quercus faginea*, *Q. ilex* and *Q. pyrenaica*) as well as the trajectories of change between plots of the same chronosequence are also indicated with different symbols and lines, respectively. Chronosequences are represented by numbers: (1) Cueva de San Frutos and (2) Las Andolejas, (Arcones), (3) La Tenada and (4) Cueto Sombrío (Tubilla del Agua), (5) La Dehesa 1 and (6) La Dehesa 2 (Calzada de Tera), (7) Trasotero y Rabanillo and (8) Matorro y Vargas (La Valdavia), and (9) La Cuesta and (10) Nava de la Dehesa (Linares de Riofrío)

were much lower than that found in conifer (275 Mg C•ha^{-1}; Thuille and Schulze, 2006) and deciduous forests (82 Mg C•ha^{-1}; Bose et al., 2014) developed over similar periods, in temperate cold environments. Surface soil C concentration values (i.e. 8–56 g•kg^{-1}) in plots abandoned in 1956–1977, were similar to those detected by Badalamenti et al., (2019; 17.1 g•kg^{-1}) and La Mantia et al., (2013; 45 g•kg^{-1}), in Mediterranean forests of Southern Italy, developed for <70 and >50 years, at 0–15 and 0–10 cm depth, respectively. However, C$_{SS}$ values (i. e. 4.5–13.0 Mg C•ha^{-1}; Table 1) were much lesser than those registered at 0–10 cm in silver birch dominated <60 yr-old forests in temperate cold environments (i.e. Estonia; Uri et al., 2012). It is also important to note that studies performed in forests of similar age (i.e. 14–80 years) and dominated by *Q. pyrenaica* nearby our study sites in Linares de Riofrío and La Valdavia, but in which soils were sampled up to larger depths (0–100 cm), detected much larger C stock values; i.e. 60 and 74 Mg C•ha^{-1} (Turrión et al., 2009; Herrero et al., 2016), respectively. This evidence suggests that, the C stock accumulated in the full soil profile could be larger than that in the AGB. Interestingly, C$_{BGB}$ values (i.e. 0.3–21.5 Mg C•ha^{-1}) were at least one half of those of C$_{AGB}$, which points to a major importance of the BGB as C pool in Mediterranean-continental environments such as those of our study sites. With regard to the negligible contribution of C$_{lit}$ and C$_{WD}$ to the total C stock, it may be explained by the observed lack of tree mortality in early to mid-successional forests such as ours (Hooker and Compton 2003). In fact, nearby and relatively mature forests dominated by *Q. pyrenaica* yielded slightly larger C$_{lit}$ values (i.e. 0.5–1 Mg C•ha^{-1}; Turrión et al., 2009; Herrero et al., 2016) than ours.

4.2. Patterns of change

The positive and significant relationships between the C stocks in each compartment and ATC (Fig. 2, Table E.1), as well as the significant

differences found for C$_{AGB}$ and C$_{BGB}$ between periods of abandonment (Fig. 3a-b), point toward a general, net increase in C accumulation over the first 70 years after abandonment. These results corroborate those found in both, Mediterranean (Alberti et al., 2011; Badalamenti et al., 2019) and temperate cold (Uri et al., 2012) environments. The weakness (i.e. R^2 ≤ 0.5) of the abovementioned relationships may be related to the fact that, for C$_{AGB}$ and C$_{BGB}$, the lines joining plots of the same chronosequence that were colonized by trees 40–70 yr ago with plots that were colonized 20–40 years ago, generally showed steeper slopes than the lines joining plots colonized by trees 20–40 and 0–20 years ago (Fig. 2a, b). These results, together with the larger rate of change in plots abandoned in 1956–1977 and 1977–2005 than in plots abandoned in 2005–2017 for C$_{AGB}$ and C$_{BGB}$ (Table 1) indicate that, in the biomass, C stocks increased slowly during the first few decades after abandonment, but much faster afterwards. In contrast, the negative and significant relationship between the rate of change in C$_{SS}$ and ATC (Fig. 3a) together with the fact that rates of change in C$_{SS}$ had significantly larger values for plots abandoned in 2005–2017 than for plots abandoned in 1977–2005 and 1956–1977 (Fig. 3b), suggest a deceleration in C$_{SS}$ accumulation through time. Such patterns of change in AGB and BGB can be explained as a consequence of tree growth (Wang et al., 2016). Patterns of change detected in the SS, however, contrast with those detected in meta-nalyses considering soil C accumulation as a consequence of forest recovery in Europe (Bárcena et al., 2014) and North-America (Nave et al., 2013), consisting of major gains ≥30 years after abandonment, and are probably derived from the low C inputs from litter and woody debris (Yang et al., 2014). Surprisingly, C$_{SS}$ values were significantly larger in areas dominated by *Q. faginea* than in areas dominated by *Q. pyrenaica* (Fig. 3d), when both are marcescent species with easy-to-decompose litter (Thuille and Schulze 2006) and occurring in relatively cold and wet places (La Mantia et al., 2013). These results can be explained because in Linares de Riofrío, a *Q. pyrenaica*-dominated
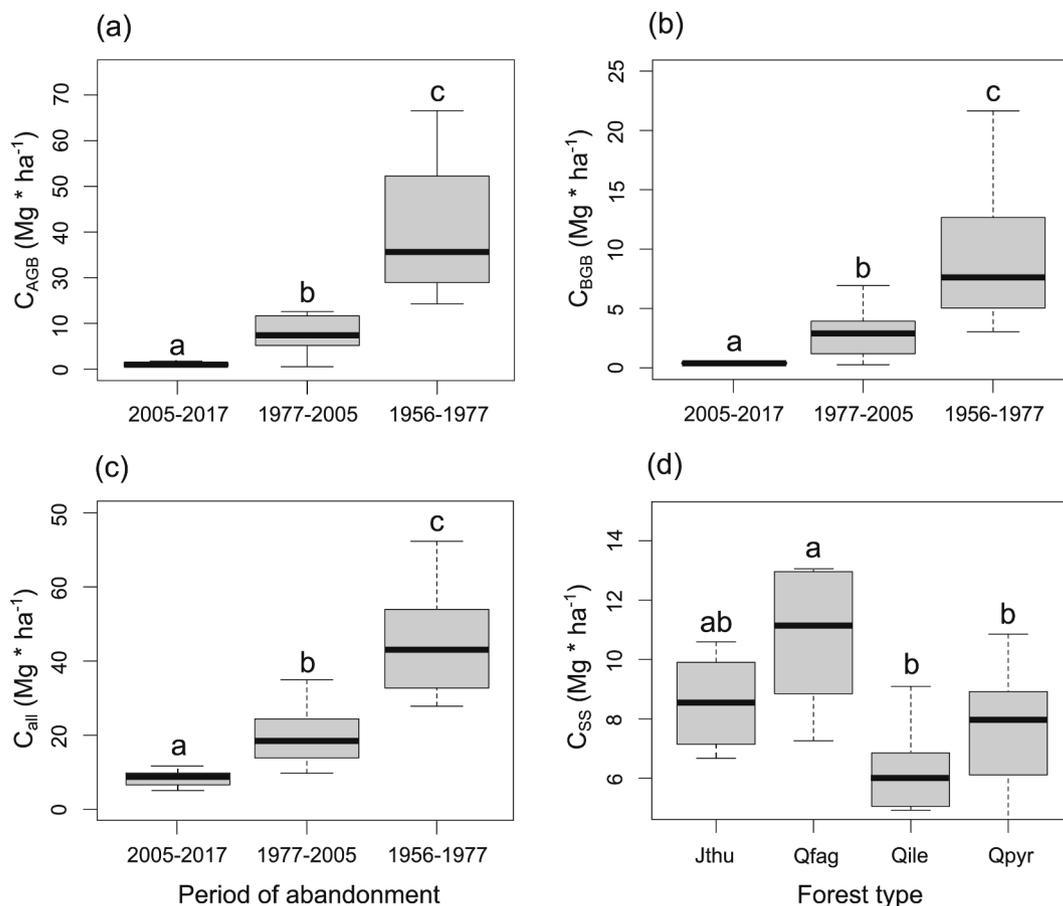
Fig. 3. Differences in carbon accumulated in the (a) above- and (b) below-ground biomass, (c) the entire stand, among those plots located in recovering lands abandoned in 2005–2017, 1977–2005 and 1956–1977. Differences in (d) carbon accumulated in the soil among those plots located in recovering lands dominated by *Juniperus thurifera* (Qthu) *Quercus faginea* (Qfag) *Q. ilex* (Qile) and *Q. pyrenaica* (Qpyr). Different letters indicate significant differences according to Pair-wise Tukey HSD post hoc comparisons).
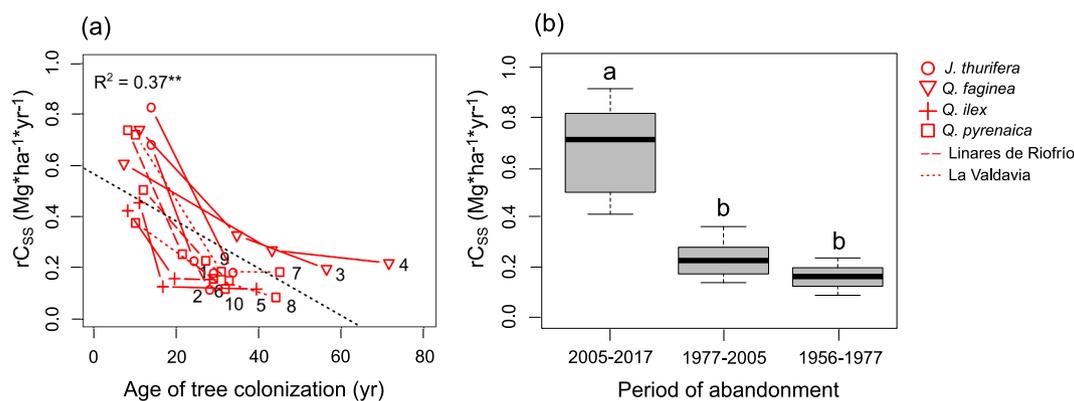
Fig. 4. (a) Linear regressions between rates of change in C stocks (Mg C·ha^{-1}·yr^{-1}) in the surface soil (rC$_{SS}$) and age of tree colonization (ATC; yr), in the 0.0625-ha plots, showing the adjusted coefficient of determination (R^2). (b) Differences in rC$_{SS}$ among those plots located in recovering lands abandoned in 2005–2017, 1977–2005 and 1956–1977. The rest of specifications are as in Figs. 2 and 3.

forest, lands were alternatively used as pastures before abandonment (T. Zarza, Pers. communication). Therefore, in the latter site, as well as it has occurred in other abandoned pastures (Alberti et al., 2011; Nadal-Romero et al., 2018), during the early stages of succession C losses derived from the ending of C inputs from herbaceous root litter, organic amendments and cattle droppings, could have been bigger than the new (and low) C inputs from litter and woody debris supplied through woody plants colonization, whereas in the first one, C$_{SS}$ had just increased as a

consequence as the aforementioned inputs.

It is also important to point out that mean rates of change in C$_{all}$ (i.e. 0.50——0.81 Mg C•ha^{-1}•yr^{-1}) were lower than those reported for recovering lands in Central Italy (1.18 Mg C•ha^{-1}•yr^{-1}; Alberti et al., 2011) and the North-Eastern USA (2.10 Mg C•ha^{-1}•yr^{-1}; Hooker and Compton 2003), but similar to those reported in a *Nothofagus* chronosequence of the Southern hemisphere (0.55 Mg C•ha^{-1}•yr^{-1}; Davis et al., 2003). These results indicate that the process of C accumulation in

Table 2

Best-fitting multiple regression models considering the C accumulated in the above- (C_{AGB}) and the below-ground biomass (C_{BGB}), the surface soil (C_{SS}) and the entire stand (C_{all}) as dependent variables, and the age of tree colonization (ATC; years), the adult stem density (ASD; inds. ha^{-1}), the Mean Monthly Rainfall of the Driest Month (MMRD; mm), the C:N relationship (CN$_{soil}$; mg •kg soil^{-1}), the nitrogen content (N; g•kg soil^{-1}) and the proportion of sand (sand; %) in the soil as factors. The adjusted r^2 for each model, as well as the standardized coefficients (β) and significance (* and ** indicate $P \leq 0.05$ and 0.001, respectively) of each factor are also given.

Models	Adj-r^2
$C_{AGB} \sim 0.56$ (ASD**) + 0.41 (ATC*) − 0.23 (MMRD*)	0.79**
$C_{BGB} \sim 0.57$ (ATC**) − 0.38(ASD*)	0.76**
$C_{SS} \sim 0.83$ (N**) + 0.30 (sand*)	0.68**
$C_{all} \sim 0.56$ (ASD**) + 0.32 (ATC*) + 0.22 (N) − 0.17 (MMRD*)	0.77**

Table 3

Results for one-way analyses of the covariance (ANCOVA) to test whether relationships between C_{AGB}, C_{BGB}, C_{SS} or C_{all}, and their main drivers of change varied significantly among the different forest types considered. Bold characters indicate significant effects (* and ** indicate $P \leq 0.05$ and 0.001, respectively) on the dependent variable. The main drivers are age of tree colonization (ATC; years), adult stem density (ASD; inds. ha-1), Mean Monthly Rainfall of the Driest Month (MMRD; mm) nitrogen content (N; g•Kg soil^{-1}) and sand proportion (sand; %) in the soil. Forest types are those dominated by *Juniperus thurifera*, *Quercus ilex*, *Q. faginea* and *Q. pyrenaica*, abbreviated as *jthu*, *qile*, *qfag* and *qpyr*, respectively. Significant pairwise differences among forest types according to *post-hoc* Tukey tests are also indicated.

Dep. var.	Effect	F$_{(2,33)}$
C_{AGB}	ftype	1.51n.s.
	ASD	**59.43****
	ftype:ASD	0.55n.s.
	ftype	2.79n.s.
	ATC	**50.23****
	ftype:ATC	1.03n.s.
	ftype	0.18n.s.
	MMRD	0.04n.s.
	ftype:MMRD	**7.31****
C_{BGB}	ftype	1.46n.s.
	ATC	**49.09****
	ftype:ATC	0.99n.s.
	ftype	3.11n.s.
	ASD	**68.24****
	ftype:ASD	1.09n.s.
C_{SS}	ftype	2.49n.s.
	N	**21.84****
	ftype: N	1.90n.s.
	ftype	**8.64****
	sand	0.005n.s.
	ftype:sand	**5.38***
C_{all}	ftype	0.17n.s.
	ASD	**47.92***
	ftype:ASD	0.95n.s.
	ftype	0.75n.s.
	ATC	**41.83***
	ftype:ATC	0.94n.s.
	ftype	0.52n.s.
	N	**4.84***
	ftype:N	0.37n.s.
	ftype	0.08n.s.
	MMRD	0.16n.s.
	ftype:MMRD	**6.3***

our study area is relatively slow, probably as a consequence of the slow (and progressively smaller) accumulation in the soil (Fig. 3). Moreover, the difference between the age of abandonment, defined from aerial ortho-photos and the ATC, obtained from tree cores, indicates the

existence of a 10–20 yr time lag for tree colonization in our study plots. These time lags are commonly mentioned in studies of secondary succession, where they are usually related to the inhibition of tree germination and establishment, as a consequence of the development of thicket-forming shrubs (Walker et al., 2010). In our case, however, this explanation seems unlikely because, although some of the shrub species detected (e.g. *Cytisus scoparius*) may form dense thickets, all over the study sites, trees were already present in plots abandoned in 2005–2017 (i.e. the early stages of ecological succession), and represented >75% of the C_{AGB} in plots abandoned in 1956–1977. Such time-lags could be better explained by the negative effects of the hydric and temperature stress characteristic of Mediterranean-continental environments (Moreno et al., 2011) together with herbivory (García-Cervigón et al., 2017) on tree germination, growth and survival.

4.3. Driving factors

We identified adult stem density as a major driver of changes in C_{AGB} and C_{BGB} because, in early- and mid-stages of succession, this factor usually increases in parallel to the live biomass as a result of considerable total tree growth and low mortality of tree stems (Kashian et al., 2013). These results provide support for those of Vayreda et al. (2011), who identified stand structure as one of the main determinants of C accumulation in Spanish forests, and suggest that the new forests, woodlands and other wooded lands developed in our study sites after agricultural land abandonment, have not yet reached their mature phase. With regard to the MMRD, its negative and significant effect on C_{AGB} may be explained on the basis of the significant, positive and very strong correlation between C_{AGB} and C_{BGB} ($R^2 > 0.9$; Table F.1). In Mediterranean-continental environments, marcescent trees, usually invest in deep root systems that allow them to maintain their high evapotranspiration rates during summer months, when water availability is limited (Moreno et al., 2011). In fact, these types of trees occur commonly in areas in which, although MMRD can be low, MAR is relatively high (i.e. > 900 mm•yr^{-1}), such as those in Tubilla del Agua, La Valdavia and Linares de Riofrío (Table A.1). Amongst those sites, the lesser the MMRD is, the more stressful the summer droughts are, and in turn, the more the development of the C_{BGB}. Moreover, these sites have also the least fertile soils (i.e. Leptosol, Dystric and Humic Cambisols; Table A.1), which in turn also enhances root development on trees in order to obtain a larger amount of nutrients (Schneider et al., 2001).

Changes in C_{SS} were not explained by either C_{AGB} or ATC, which implies that C accumulation in this compartment is relatively independent from the process of spontaneous revegetation (Uri et al., 2012) or require long periods to become apparent (Alberti et al., 2011; Nadal-Romero et al., 2018). Surface soil C accumulation responded primary to N, which is also strongly correlated to K$_{ex}$ and Ca$_{ex}$, and overall soil fertility (Appendix F). These results confirm that the sustained increase in C_{SS} along successional gradients is supported by a parallel increase, or at least, an accrual of N (Alberti et al., 2011; Novara et al., 2013; Nóvak et al., 2014; Yang et al., 2014; Nadal-Romero et al., 2018). This thigh relationship between C and N accumulation is particularly important over the earlier stages of succession (Dickie et al., 2013), which suggests that soils on our recovering lands are still developing. Interestingly, C_{SS} was also significantly and positively driven by *sand*, which might be considered counterintuitive since soil organic matter seems to be more protected from decomposition in fine- than in coarse-textured soils (Foote and Grogan 2010; Novak et al., 2014). However, it had also relatively large values in forests dominated by *J. thurifera* and *Q. pyrenaica*, where soils were much sandier (Fig. 3; Table S6). These results may be explained by the fact that, in Arcones and Linares de Riofrío, where recovering lands are dominated by the aforementioned species, agricultural lands were regularly used as pastures, and subsequently, just after abandonment, their C stocks might have been larger than in the other sites (Alberti et al., 2011; Nadal-Romero et al., 2018). Thus, the importance of *sand* as C_{SS} driver highlights the importance of

prior land-use on soil C accumulation over old-field succession (Hooker and Compton 2003; Nave et al., 2013; Bárcena et al., 2014). Finally, and noteworthy, C_{all} was mainly driven by ASD and ATC (Table 2), which suggests that it basically depends on biomass C accumulation.

4.4. Limitations

In this study, we have used a chronosequence approach to assess changes in C stocks through time recovering lands. According to this approach, study sites have been carefully selected, the process of agricultural abandonment has been well documented through comparing aerial photographs from different periods or consulting owner's descendants, and several forest types have been tested, which is mandatory in a heterogeneous study region such as ours. However, due to budget and time constraints, replication within certain forest types was low (i.e. two chronosequences for each, *J. thurifera*, *Q. faginea* and *Q. ilex* –dominated forests), which could impede us to properly know the true variance within each forest type. However, relationships between C accumulation in any of the compartments studied and their main drivers of change varied significantly among the different forest types just for C_{AGB} and MMRD (Table 3). These findings indicate that within-forest type variations are not so important, or, in other words, that the main patterns and driving factors detected for C accumulation equally apply for all forest types considered. Thus, our results demonstrate that, though imperfect, chronosequences remain an adequate way to study forest recovery, particularly to assess changes on major structural attributes of the vegetation, such as C stocks (Johnson and Miyanishi 2008), and in slow-growth forests (Kashian et al., 2013) such as those of our study region.

4.5. Conclusions and further research

Summarizing, our results indicate, first, that over the process of spontaneous colonization of abandoned agricultural lands, in Mediterranean-continental environments, C accumulates mostly in the live biomass and the surface soil, but these two pools are largely disconnected, and second, that the BGB plays an important role as C pool. Third, they confirm that, in our study sites, and 70 years after abandonment, C accumulation is still occurring. As, in recovering lands, live biomass recovery use to reach a steady state after 80–100 yr (Hooker and Compton 2003; Bose et al., 2014), C stocks might still increase over the next 1–3 decades in plots abandoned in 1956–1977, and over a longer period in the rest. Such C accumulation has accelerated in the AGB, whereas, it has apparently decelerated in the SS. These results point toward a reorganization of the ecosystem along secondary succession with regard to CO_2 sequestration (Odum, 1969). They also suggest that, in order to increase the future C storage capability of these new forests, we should intervene in the AGB (as it is, apparently, the most-dynamic compartment), through management actions such as tree thinning and pruning, which improve individuals' growth (Aldea et al., 2017) and drought resistance (Sohn et al., 2016). In the near term, it would be recommendable to test if the abovementioned predictions on C accumulation are consistent with long-term changes in the vegetation through re-sampling the study plots (Walker et al., 2010). It would be also interesting to assess the relationships between biomass C accumulation and changes in plant functional types and species richness (e.g. Wardle et al., 2016). Our 30 plots provide also the basis for further studies on the underlying mechanisms of woody plant colonization and dynamics (e.g. Velázquez and Wiegand, 2020). All these possibilities will contribute to advance our knowledge on the process of forest expansion in abandoned agricultural lands in Mediterranean-continental environments, as well as their ecosystem- and regional-level implications.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2023.121422.

References

Aggenbach, C.J.S., Kooijman, A.M., Fujita, Y., van der Hagen, H., van Til, M., Cooper, D., Jones, L., 2017. Does atmospheric deposition lead to greater nitrogen and carbon accumulation in coastal sand dunes? Biol. Conserv. 212, 416–422. https://doi.org/10.1016/j.biocon.2016.12.007.

Agresti, A., 2002. Categorical Data Analysis. Wiley & Sons, New York, New York.

Alberti, G., Leronni, V., Piazzi, M., Petrella, F., Mairota, P., Peressotti, A., Piussi, P., Valentini, R., Gristina, L., La Mantia, T., Novara, A., Rühl, J., 2011. Impact of woody encroachment on soil organic carbon and nitrogen in abandoned agricultural lands along a rainfall gradient in Italy. Reg. Environ. Chang. 11 (4), 917–924.

Aldea, J., Bravo, F., Bravo-Oviedo, A., Ruiz-Peinado, R., Rodríguez, F., Del Río, M., 2017. Thinning enhances the species-specific radial increment response to drought in Mediterranean pine-oak stands. Agr. For. Meteorol. 237, 371–383. https://doi.org/10.1016/j.agrformet.2017.02.009.

Anderson, K.J., Allen, A.P., Gillooly, J.F., Brown, J.H., 2006. Temperature-dependence of biomass accumulation rates during secondary succession. Ecol. Lett. 9 (6), 673–682. https://doi.org/10.1111/j.1461-0248.2006.00914.x.

Badalamenti, E., Battipaglia, G., Gristina, L., Novara, A., Rühl, J., Sala, G., Sapienza, L., Valentini, R., La Mantia, T., 2019. Carbon stock increases up to old growth forest along a secondary succession in Mediterranean island ecosystems. PLoS One 14 (7), 1–13. https://doi.org/10.1371/journal.pone.0220194.

Bárcena, T.G., Kiaer, L.P., Vesterdal, L., Stefánsdottir, H.M., Gundersen, P., Sigurdsson, B. D., 2014. Soil carbon stock change following afforestation in Northern Europe: a meta-analysis. Glob. Chang. Biol. 20, 2393–2405.

Baty, F., Ritz, C., Charles, S., Brutsche, M., Flandrois, J., Delignette-Muller, M., 2015. A toolbox for nonlinear Regression in R: the package nlstools. J. Stat. Softw. 66 (5), 1–21. https://doi.org/10.18637/jss.v066.i05.

Bonet, A., Pausas, J.G., 2007. Old field dynamics on the dry side of the Mediterranean basin: patterns and processes in semiarid southeast Spain. In: Cramer, V.A.,

Hobbs, R.J. (Eds.), Old Fields: Dynamics and Restoration of Abandoned Farmland. Island Press, Washington DC, pp. 247–264.

Bose, A.K., Schelhaas, M.-J., Mazerolle, M.J., Bongers, F., 2014. Temperate forest development during secondary succession: effects of soil, dominant species and management. Eur. J. For. Res. 133 (3), 511–523.

Bueis, T., Bravo, F., Pando, V., Turrión, M.B., 2018. Local basal area affects needle litterfall, nutrient concentration, and nutrient release during decomposition in Pinus halepensis Mill. plantations in Spain. Ann. For. Sci. 75 (1).

Burnham, K.P., Anderson, D.R., 1998. Model Selection and Inference: A Practical Information-theoretic Approach. Springer-Verlag, New York.

Castro, H., Fortunel, C., Freitas, H., 2010. Effects of land abandonment on plant litter decomposition in a Montado system: relation to litter chemistry and community functional parameters. Plant Soil 333 (1-2), 181–190.

Clemmensen, K.E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., Stenlid, J., Finlay, R.D., Wardle, D.A., Lindahl, B.D., 2013. Roots and associated fungi drive long-term carbon sequestration in boreal forest. Science 339, 1615–1618. https://doi.org/10.1126/science.1231923.

Davis, M.R., Allen, R.B., Clinton, P.W., 2003. Carbon storage along a stand development sequence in a New Zealand Nothofagus forest. For. Ecol. Manag. 177, 313–321. https://doi.org/10.1016/S0378-1127(02)00333-X.

Dickie, I.A., Martínez-García, L.B., Koele, N., Grele, G.-A., Tylianakis, J.M., Peltzer, D.A., Richardson, S.J., 2013. Mycorrhizas and mycorrhizal fungal communities throughout ecosystem development. Plant Soil 367, 11–39. https://doi.org/10.1007/s11104-013-1609-0.

Foote, R.L., Grogan, P., 2010. Soil carbon accumulation during temperate forest succession on abandoned low productivity agricultural lands. Ecosystems 13 (6), 795–812. https://doi.org/10.1007/s10021-010-9355-0.

Fuchs, R., Schulp, C.J.E., Hengeveld, G.M., Verburg, P.H., Clevers, J.G.P.W., Schelhaas, M.-J., Herold, M., 2016. Assessing the influence of historic net and gross land changes on the carbon fluxes of Europe. Glob. Chang. Biol. 22, 2526–2539. https://doi.org/10.1111/gcb.13191.

García-Cervigón, A.I., Velázquez, E., Wiegand, T., Escudero, A., Olano, J.M., Giesecke, T., 2017. Colonization in Mediterranean old-fields: the role of dispersal and plant-plant interactions. J. Veg. Sci. 28 (3), 627–638.

Herrero, C., Turrión, M.-B., Pando, V., Bravo, F., 2016. Carbon content of forest floor and mineral soil in Mediterranean Pinus spp. and Oak stands in acid soils in Northern Spain. For. Systs. 25 (2), e065.

Hooker, T.D., Compton, J.E., 2003. Forest ecosystem carbon and nitrogen accumulation during the first century after agricultural abandonment. Ecol. Appl. 13 (2), 299–313. https://doi.org/10.1890/1051-0761(2003)013[0299:FECANA]2.0.CO;2.

Huitema, B.E., 1980. The Analysis of Covariance and Alternatives. Wiley & Sons, New York.

Ihaddaden, A., Velázquez, E., Rey-Benayas, J.M., Kadi-Hanifi, H., 2013. Climate and vegetation structure determine plant diversity in Quercus ilex woodlands along an aridity and human-use gradient in Northern Algeria. Flora 208 (4), 268–284.

Johnson, E.A., Miyanishi, K., 2008. Testing the assumptions of chronosequences in succession. Ecol. Lett. 11 (5), 419–431.

Johnson, C., Zarin, D., Johnson, A., 2000. Post-disturbance aboveground biomass accumulation in global secondary forests. Ecology 81, 1395–1401. https://doi.org/10.2307/177216.

Jones, J.B., 1991. Kjeldahl Method for Nitrogen Determination. Micro-Macro Publishing Inc., Athens (Georgia).

Kashian, D.M., Romme, W.H., Tinker, D.B., Turner, M.G., Ryan, M.G., 2013. Postfire changes in forest carbon storage over a 300-year chronosequence of Pinus contorta-dominated forests. Ecol. Monogr. 83, 49–66.

Kassambara, A. 2021. rstatix: Pipe-Friendly Framework for Basic Statistical Tests. [R programming language].

Kutner, M.H., Neter, J., Lewis, R., Shier, D.N., Butler, J.L., 2004. Applied Linear Statistical Models. McGraw-Hill, New York.

La Mantia, T., Gristina, L., Rivaldo, E., Pasta, S., Novara, A., Rühl, J., 2013. The effects of post-pasture woody plant colonization on soil and aboveground litter carbon and nitrogen along a bioclimatic transect. iForest 6 (4), 238–246. https://doi.org/10.3832/ifor0811-006.

Lara, W., Bravo, F., Sierra, C., 2015. measuRing: An R Package to measure tree-ring widths from scanned images. Dendrochronologia 34, 43–50. https://doi.org/10.1016/j.dendro.2015.04.002.

Legendre, P., Legendre, L., 2012. Numerical Ecology. Elsevier, New York, New York.

López-Marcos, D., Martínez-Ruiz, C., Turrión, M.B., Jonard, M., Titeux, H., Ponette, Q., Bravo, F., 2018. Soil carbon stocks and exchangeable cations in monospecific and mixed pine forests. Eur. J. Forest. Res. 137, 831–847. https://doi.org/10.1007/s10342-018-1143-y.

Miller, A. 2020. leaps: Regression Subset Selection. [R programming language].

Montero, G., Pasalodos-Tato, M., Montoto, R., López-Senespleda, E., Onrubia, R., Bravo-Oviedo, A., Ruiz-Peinado, R. 2013. Contenido de carbono en la biomasa de las principales especies de matorral y arbustedos de España. 6°Congreso Forestal Español; Vitoria-Gasteiz 10-14 Junio 2013.

Moreno, G., Gallardo, J.F., Vicente, M.A. 2011. How Mediterranean deciduous trees cope with long summer drought? The case of Quercus pyrenaica forests in Western Spain. in: Bredemeier, M., Cohen, S., Godbold, D.L., Lode, E., Pichler, V., Schleppi, P., (Eds.), Forest Management and the Water Cycle: An Ecosystem-Based Approach, Ecological Studies 212. Berlin: Springer-Verlag, Berlin, pp. 189-201.

Nadal-Romero, E., Otal-Laín, I., Lasanta, T., Sánchez-Navarrete, P., Errea, P., Cammeraat, E., 2018. Woody encroachment and soil carbon stocks in subalpine areas in the Central Spanish Pyrenees. Sci. Total Environ. 636, 727–736. https://doi.org/10.1016/j.scitotenv.2018.04.324.

Nave, L.E., Swanston, C.W., Mishra, U., Nadelhofer, K.J., 2013. Afforestation effects on soil carbon storage in the United States: a synthesis. Soil Sci. Soc. Am. J. 77, 1035–1047.

Ninyerola, M., Pons, X., Roure, J.M., 2005. Atlas Climático Digital de la Península Ibérica. Metodología y aplicaciones en bioclimatología y geobotánica. Universidad Autónoma de Barcelona, Bellaterra.

Nóvak, T.J., Incze, J., Spohn, M., Glina, B., Giani, L., 2014. Soil and vegetation transformation in abandoned vineyards of the Tokaj Nagy-Hill, Hungary. Catena 123, 88–98.

Novara, A., Gristina, L., La Mantia, T., Rühl, J., 2013. Carbon dynamics of soil organic matter in bulk soil and aggregate fraction during secondary succession in a Mediterranean environment. Geoderma 193–194, 213–221.

Odum, E.P., 1969. The strategy of ecosystem development. Science 164, 262–270. https://doi.org/10.1126/science.164.3877.262.

Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A. 1954. Estimation of available phosphorus in soils by extraction with NaHCO₃. USDA Circular 939. USDA, Washington D.C.

Penman, J., Gytarsky, M., Hiraishi, T., Krug, T., Kruge, D., Pipatti, R., Buendia, L., Miwa, K., Ngara, T., Tanabe, K., Wagner, F., 2003. Good Practice Guidance for Land Use, Land-use Change and Forestry. Intergovernmental Panel on Climate Change, Geneva.

Rey-Benayas, J.M., Martins, A., Nicolau, J.M., Schulz, J., 2007. Abandonment of agricultural land: an overview of drivers and consequences. CAB reviews. Perspect. Agr. Veter. Sci. Nutrit. Nat. Resour. 2, 1–14.

Richter, D.D., Markewitz, D., Trumbore, S.E., Wells, C.G., 1999. Rapid accumulation and turnover of soil carbon in a re-establishing forest. Nature 400, 56–58.

Schimel, D.S., House, J.I., Hibbard, K.A., Bousquet, P., Ciais, P., Peylin, P., Braswell, B.H., Apps, M.J., Baker, D., Bondeau, A., Canadell, J., Churkina, G., Cramer, W., Denning, A.S., Field, C.B., Friedlingstein, P., Goodale, C., Heimann, M., Houghton, R. A., Melillo, J.M., Moore III, B., Murdiyarso, D., Noble, I., Pacala, S.W., Prentice, I.C., Raupach, M.R., Rayner, P.J., Scholes, R.J., Steffen, W.L., Wirth, C., 2001. Recent patterns and mechanisms of carbon exchange by terrestrial ecosystems. Nature 414, 169–172. https://doi.org/10.1038/35102500.

Schneider, K., Turrión, M.-B., Grierson, P.F., Gallardo, J.F., 2001. Phosphatase activity, microbial phosphorus and fine root growth in forest soils in the Sierra de Gata, western central Spain. Biol. Fertil. Soils 34, 151–155. https://doi.org/10.1007/s003740100387.

Schollenberger, C.J., Simon, R.H., 1945. Determination of exchange capacity and exchangeable bases in soil ammonium acetate method. Soil Sci. 9, 13–24.

Sohn, J.A., Saha, S., Bauhus, J., 2016. Potential of forest thinning to mitigate drought stress: A meta-analysis. Forest Ecol. Manag. 380, 261–273. https://doi.org/10.1016/j.foreco.2016.07.046.

Stokes, M.A., Smiley, T.L., 1996. An introduction to tree-ring dating: Tucson: The University of Arizona Press. Tucson, Arizona.

Thuille, A., Schulze, E.-D., 2006. Carbon dynamics in successional and afforested spruce stands in Thuringia and the Alps. Glob. Chang. Biol. 12, 325–342. https://doi.org/10.1111/j.1365-2486.2005.01078.x.

Turrión, M.B., Schneider, K., Gallardo, J.F., 2009. Carbon accumulation in umbrisols under Quercus pyrenaica forests. Effects of bedrock and annual precipitation. Catena 79, 1–8. https://doi.org/10.1016/j.catena.2009.04.004.

Uri, V., Varik, M., Aosaar, J., Kanal, A., Kukumägi, M., Lõhmus, K., 2012. Biomass production and carbon sequestration in a fertile silver birch (Betula pendula Roth) forest chronosequence. For. Ecol. Manag. 267, 117–126. https://doi.org/10.1016/j.foreco.2011.11.033.

Vargas, R., Allen, M.F., Allen, E.B., 2008. Biomass and carbon accumulation in a fire chronosequence of a seasonally dry tropical forest. Glob. Change Biol. 14, 109–124. https://doi.org/10.1111/j.1365-2486.2007.01462.x.

Vayreda, J., Gracia, M., Canadell, J.G., Retana, J., 2011. Spatial patterns and predictors of forest carbon stocks in Western Mediterranean. Ecosystems 15, 1258–1270. https://doi.org/10.1007/s10021-012-9582-7.

Velázquez, E., Wiegand, T., 2020. Competition for light and persistence of rare light-demanding species within tree-fall gaps in a moist tropical forest. Ecology 101 (7), e03034.

Velázquez, E., Martínez-Jaraíz, C., Wheeler, C., Mitchard, E.T.A., Bravo, F., 2022. Forest expansion in abandoned agricultural lands has limited effect to offset carbon emissions from Central-North Spain. Reg. Environ. Chang. 22, 132. https://doi.org/10.1007/s10113-022-01978-0.

Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S, fourth ed. Springer, New York, New York.

Walker, L.R., Wardle, D.A., Bardgett, R.D., Clarkson, B.D., 2010. The use of chronosequences in studies of ecological succession and soil development. J. Ecol. 98, 725–736. https://doi.org/10.1111/j.1365-2745.2010.01664.x.

Walkley, A., Black, I.A., 1934. An examination of the Degtjareff Method for determining doil organic matter and a proposed modification of the chromic Acid Titration Method. Soil Sci. 37, 29–38. https://doi.org/10.1097/00010694-193401000-00003.

Wang, K., Deng, L., Ren, Z., Shi, W., Chen, Y., Shang-Guan, Z., 2016. Dynamics of ecosystem carbon stocks during vegetation restoration on the Loess Plateau of China. J. Arid. Land 8 (2), 207–220. https://doi.org/10.1007/s40333-015-0091-3.

Wardle, D.A., Jonsson, M., Bansal, S., Bardgett, R.D., Gundale, M.J., Metcalfe, D., 2016. Linking vegetation change, carbon sequestration and biodiversity: insights from island ecosystems in a long-term natural experiment. J. Ecol. 100, 16–30. https://doi.org/10.1111/j.1365-2745.2011.01907.x.

Yang, Y., Wang, G., Shen, H., Yang, Y., Cui, H., Liu, Q., 2014. Dynamics of carbon and nitrogen accumulation and C: N stoichiometry in a deciduous broadleaf forest of deglaciated terrain in the eastern Tibetan Plateau. For. Ecol. Manag. 312, 10–18. https://doi.org/10.1016/j.foreco.2013.10.028.