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# Impact of tree species replacement on carbon stocks in a Mediterranean mountain area, NE Portugal

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ABSTRACT

Forest species replacement can influence significantly the amount of carbon stored in the several compartments that compose the terrestrial ecosystems (biomass, forest floor and mineral soil). This study intends to evaluate the influence of the replacement of the *Quercus pyrenaica* species (QP), which represents the climax vegetation of Serra da Nogueira, NE Portugal, by the *Pseudotsuga menziesii* (PM) and *Pinus nigra* (PN) plantations (fast-growing species). For this purpose, three plots of 314 $m^2$ were established in each stand (9 plots in total) and the height and diameter at the breast height of all trees were measured, in order to characterize the stands and estimate the tree biomass. Herbaceous vegetation and forest floor were collected in areas of 0.49 $m^2$ in 15 points under each tree species (5 per plot). At the same points, disturbed and undisturbed soil samples were collected at depths 0–5, 5–10, 10–15, 15–20 and 20–30 cm. Thirty years after the climax vegetation replacement, carbon gains are observed in forest species biomass and forest floor (1.3 Mg C $ha^{-1}$ $year^{-1}$ in PN and 4.0 Mg C $ha^{-1}$ $year^{-1}$ in PM) and significant losses were recorded on soil carbon pool (about 2.2 Mg C $ha^{-1}$ $year^{-1}$). Total carbon accumulated is significantly higher in PM (331 Mg C $ha^{-1}$) compared to PN (246 Mg $ha^{-1}$) and QP (273 Mg C $ha^{-1}$), which present statistically similar values. Tree biomass and mineral soil constitute the major carbon pools.

## 1. Introduction

The increase in atmospheric carbon content, as expected considering actual trends, draws attention to the highly valuable role of forest ecosystems in the global carbon cycle (Eswaran et al., 1993; Díaz-Pinés et al., 2011). Increasing carbon sequestration by increasing forest area, mainly through plantations of fast-growing species, has been suggested as an effective measure to mitigate atmospheric carbon concentration, which may contribute to the prevention of global warming (IPCC, 2001).

The majority of the native vegetation in Iberian Peninsula (Portugal and Spain) has been replaced by other forest species, particularly fast-growing coniferous plantations. Although this substitution may have beneficial economic consequences, it is essential to understand environmental effects such as those in carbon sequestration for mitigation of greenhouse gases. The knowledge of the differences among species in what regards carbon sequestration and effects produced in each carbon pool of the ecosystem, should be a decision support tool when introducing new forest species and can be used strategically to reach environmental goals (Oostra et al., 2006; Schulp et al., 2008; Vallet et al., 2009; Herrero et al., 2016; Chen et al., 2016). Species replacement implies changes in carbon stocks in forest biomass and soils (Peltoniemi et al., 2004; Park, 2015), because tree species and litter quantity, quality and distribution in soil horizons have high influence in carbon storage (Lal, 2005; Oostra et al., 2006; Fonseca and Figueiredo, 2018). Decomposition rate of plant residues can be slower or faster, depending on their nature. In general, it is accepted that organic residues from coniferous species decompose more slowly than broadleaf species, for example, due to the presence of non-hydrolysable polyphenolic compounds in litter (Faulds and Williamson, 1999; Díaz-Pinés et al., 2011; Chen et al., 2016). On the other hand, fast-growing species would accumulate carbon more rapidly than slow-growing species, but the literature is controversial regarding the carbon stocks and distribution in the different compartments of the system (tree biomass, understory vegetation and soils), when the former species were replaced by the latter ones. Several studies shown that substitution leads to a global carbon loss (e.g. Schroth et al., 2002; Fonseca et al., 2004; Wang and Wang, 2007; Vallet et al., 2009; Ferré et al., 2014), while others studies reported carbon gains in the systems (e.g. Díaz-Pinés et al., 2011). This diversity of results may be linked to the biotic factors,

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such as the vegetation, which influenced strongly the carbon stocks in forest biomass and soils (Vesterdal et al., 2013; Chen et al., 2016), and abiotic factors, as soil properties (Percival et al., 2000), time period considered after afforestation (Dick et al., 1998; Peichl and Arain, 2006), historical past of soil management (Post and Kwon, 2000; Ma et al., 2015) and site climate. Due to the great importance of soil in terrestrial ecosystems and the proportion of carbon stored there, slight changes resulting from disturbances, such as modifications in vegetation cover, fire and site preparation can influence the ecosystems sustainability in the long term (Percival et al., 2000; Lal, 2005; Park, 2015). Within the Kyoto Protocol and with the aim of generating carbon credits, some countries have been increasing the areas occupied by forests, improving management practices in existing areas or reorienting their production, an alternative for valuing of forests in relation to traditional logging (Cairns and Lasserre, 2004). Adequate species selection is a management option to increase carbon storage in forest systems (Vallet et al., 2009; Chen et al., 2016; Herrero et al., 2016).

The research hypothesis was that the forest species replacement affects the total carbon storage and its distribution in different compartments of the ecosystem. In this sense, the main objective of the present study was to quantify the impact of replacing a native broadleaf species (*Quercus pyrenaica*) by a fast-growing conifers plantations, *Pseudotsuga menziesii and Pinus nigra*, on carbon stocks in tree biomass, herbaceous vegetation biomass, forest floor and mineral soil, during three decades after stands establishment.

## 2. Methods

The study area was located in Serra da Nogueira, northeast of Portugal (41°44′N and 6°52′W), in the range between 1000 and 1150 m altitude (Fig. 1). The annual average temperature is 12 °C and annual average precipitation is 1100 mm, concentrated from October to March (Agroconsultores and Coba, 1991). The native vegetation is *Quercus pyrenaica* (QP), which occupies about 6000 ha and represents the most extensive area of QP in Portugal. Over the last decades, some of the QP area has been replaced by fast-growing species, mainly *Pseudotsuga menziesii* and *Pinus nigra*, a process where wildfires had an important role. Soils are classified as Umbric Leptosols derived from maphic rocks (FAO/UNESCO, 1988; Agroconsultores and Coba, 1991), with high stoniness, normally subacid, moderate to high in organic matter content, low base exchange capacity, very low P and moderate to high K contents (Fonseca et al., 2004). Although the *Quercus pyrenaica* sampling area is about one kilometer away from the remaining sampling areas (Fig. 1), it is located in the same altitude range and on the same soil type and parent material, so it is to be expected similar soil and climatic conditions. The roads near the sampling areas (Fig. 1) are an integral part of the forest and are used mainly in stands management operations. Besides, a buffer strip was left in the border of each sampled plot (fifty meters width), where no sample was collected. Accordingly, the effects of both factors on the results were considered negligible.

To assess the impact of species replacement on carbon stocks in four compartments (tree biomass, herbaceous biomass, forest floor and mineral soil), three sampling areas were selected in adjacent locations with similar soil and climate conditions. The first area, covered by *Quercus pyrenaica* (QP), represents the original soil. The second area is in a 30 years old stand of *Pseudotsuga menziesii* (PM), and the third one, under *Pinus nigra* (PN), is also 30 years old. In each sampling area, three plots of 314 $m^2$ were randomly defined, as 10 m radius circles (following common procedures in forest inventories). In each plot, trees were counted and height and diameter at the breast height (DBH) were measured of all trees, in order to characterize the stands and estimate the amount of above and belowground biomass of forest tree species. PM and PN species show differences in density (trees $ha^{-1}$), height and diameter, aspects that translate into higher productivity for PM species (basal area and volume) (Table 1). QP stands result from natural regeneration, and trees are relatively thin and short, displaying the lowest

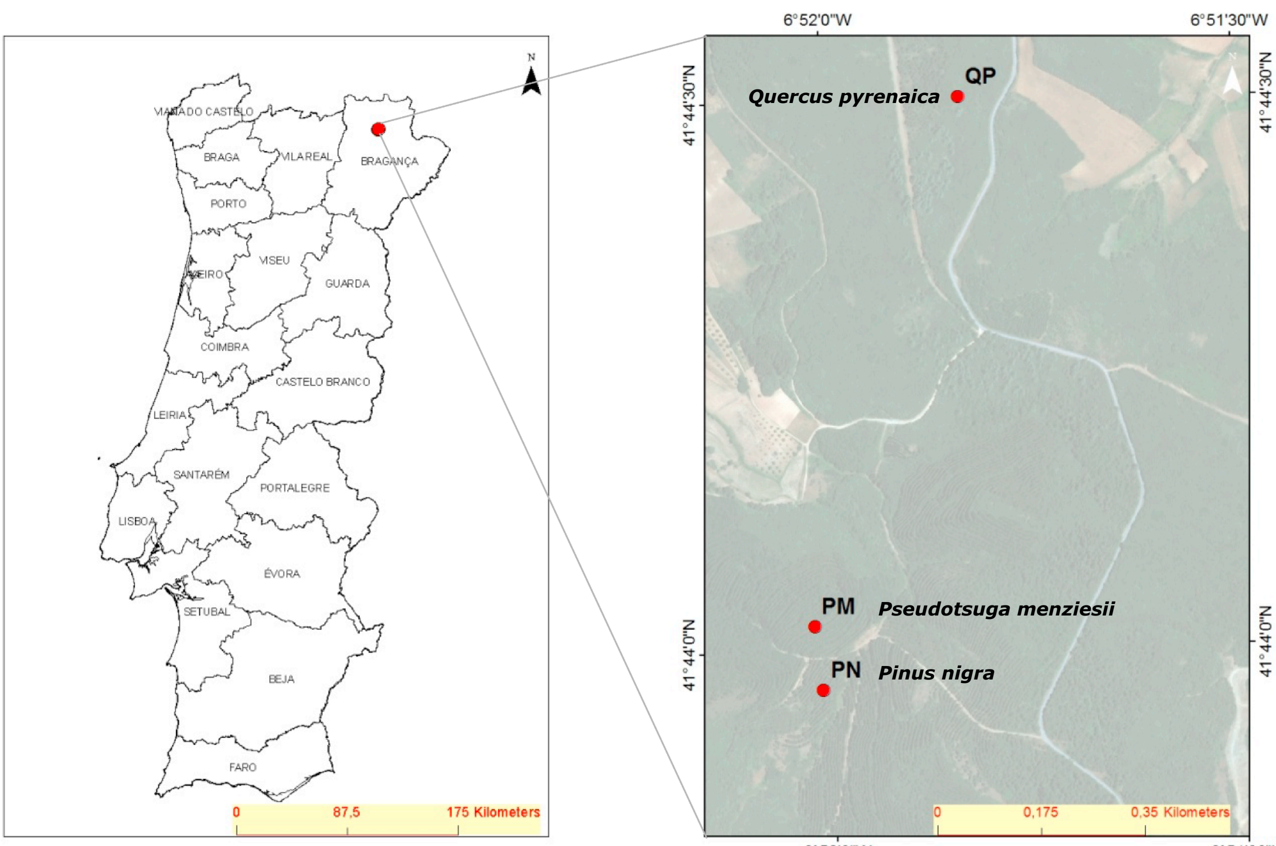


**Fig. 1.** Location of the study area in Serra da Nogueira, NE Portugal.

**Table 1**
Silvicultural characteristics of the forest species *Pseudotsuga menziesii* (PM), *Pinus nigra* (PN) and *Quercus pyrenaica* (QP).

| Parameters (units) | PM | PN | QP |
|---|---|---|---|
| Density (trees $ha^{-1}$) | 1189 | 967 | 2433 |
| Age (years) | 30 | 30 | – |
| Dominant height (m) | 23.3 | 16.1 | 10.4 |
| Average height (m) | 22.6 | 15.4 | 8.7 |
| Quadratic mean diameter (cm) | 25.7 | 23.8 | 12.3 |
| Basal area ($m^2$ $ha^{-1}$) | 62.1 | 43.4 | 28.7 |
| Volume ($m^3$ $ha^{-1}$) | 532.5 | 278.5 | 156.9 |
| Canopy cover (%) | 98 | 85 | 80 |

**Table 2**
Regression parameters (a and b) and standard error (SEE) used to estimate the above (ABG) and belowground (BGB) biomass (kg of dry matter) for *Pseudotsuga menziesii* (PM), *Pinus nigra* (PN) and *Quercus pyrenaica* (QP) forest species.

| Species | Biomass | Parameters | | SEE |
|---|---|---|---|---|
| | | a | b | |
| PM | AGB | −2.21637 | 2.35162 | 0.073801 |
| | BGB | −2.46359 | 2.13727 | 0.229940 |
| PN | AGB | −2.7773 | 2.51564 | 0.134416 |
| | BGB | −3.76193 | 2.38784 | 0.179241 |
| QP | AGB | −2.59695 | 2.53453 | 0.247318 |
| | BGB | −2.4543 | 2.13346 | 0.242145 |

productivity. Canopy cover was evaluated by direct observation in the field for PM (98%), PN (85%) and QP (80%) stands (Table 1). Allometric relations with DBH as the independent variable (Table 2, Eq. (1)), developed by Montero et al. (2005), were used to estimate above and belowground biomass of tree species (PM, PN and QP):

$$B= CF \times A\times DHB^{b} \quad (1)$$

where B is above or belowground biomass of forest species; CF is a correction factor; $CF = e^{\left(\frac{SEE^2}{2}\right)}$, SEE is the standard error of estimation (see Table 2); $A = e^{a}$; DHB is quadratic mean diameter.

Herbaceous aboveground biomass and forest floor were entirely collected in 5 areas of 70 × 70 cm randomly established over each plot (following common procedures used to assess herbaceous vegetation and organic horizons in forest areas). Herbaceous root biomass was quantified based on a root-to-shoot ratio of 0.23 ± 0.03 obtained by Gonçalves et al. (2013) in a stand located approximately 15 km from the place where the current study took place. Forest floor material was separated according to morphological criteria into L-layer (Litter layer, composed by organic material recently fallen that is readily identifiable as to origin), F-layer (Fragmentation layer, comprised by organic material partly decomposed, but yet recognizable as to origin) and H-layer (Humus layer, comprised by well-decomposed organic material in which plant structures are generally not recognizable, containing considerable amount of mineral matter) (Wesemael, 1993; van Delft et al., 2006; Fonseca and Figueiredo, 2018). Herbaceous biomass and forest floor samples were dried at 65 °C for 72 h to determine dry matter. In the same areas (70 × 70 cm), disturbed and undisturbed soil cores were collected in the 0–5, 5–10, 10–15 15–20 and 20–30 cm of soil layers. Bulk density was determined in undisturbed samples, weighting oven-dried soil (at 105 °C), collected in 100 $cm^3$ cylinders. Soil samples were air dried and sieved with a 2 mm sieve, to determine the coarse elements content. All forest floor and mineral soil samples were analyzed for total C by dry combustion (ISO, 1995).

The carbon stored in tree biomass (Mg C $ha^{-1}$) and herbaceous biomass (Mg C $ha^{-1}$) was determined by multiplying the biomass values by 0.5, average assumed as carbon concentration in dry matter, as accepted by several authors (e.g. Laclau, 2003; Petrokofsky et al., 2012; Gonçalves et al., 2013). Forest floor mass values were converted to carbon (Mg C $ha^{-1}$) by multiplying these values by the carbon concentration in dry matter. Soil organic carbon contents ($C_{SOC}$; Mg C $ha^{-1}$) were calculated by multiplying carbon concentration (Cc; g $kg^{-1}$) by bulk density (BD; g $cm^{-3}$) and thickness (z; cm) of the mineral soil layer with a correction for coarse elements content (CE; v $v^{-1}$), using the following equation (Percival et al., 2000; Sil et al., 2017):

$$C_{SOC} = z\, Cc(BD - 2.65\ CE/100) \quad (2)$$

The total carbon storage (TC) per unit area (Mg C $ha^{-1}$) was estimated by summing the mean amount of carbon in different pools:

$$TC = C_{FS} + C_{HV} + C_{FF} + C_{SOC} \quad (3)$$

where $C_{FS}$ is carbon content in above and belowground forest species biomass; $C_{HV}$ is carbon content in above and belowground herbaceous vegetation biomass; $C_{FF}$ is carbon content in forest floor, $C_{SOC}$ is carbon content in mineral soil.

Statistical analysis comprised one-way ANOVA and multiple comparisons of averages (Tukey, 5%) for assessing the effects of species replacement on carbon pools.

## 3. Results and discussion

### 3.1. Carbon stocks in biomass of forest species

The carbon stocks in the tree stratum biomass show clear differences after 30 years replacement of the native species (QP) by the PM and PN species (Fig. 2). Values for PM (with a mean of 196 Mg C $ha^{-1}$) were significantly higher than those found for PN (114 Mg C $ha^{-1}$) and QP (80 Mg C $ha^{-1}$). Despite the statistically similar results obtained for PN and QP, PN records an increase in aboveground tree biomass carbon pool as compared to QP. It should be noted that the PN is also considered a fast-growing species; nevertheless, the characteristics of this stand differ from those of the PM stand, the latter showing larger tree density (number of trees per hectare) and higher canopy cover (Table 1), both factors affecting carbon storage (Fonseca et al., 2004; Park, 2015). Fast-growing species, such as PM and PN, are more effective in carbon accumulation in biomass (Vallet et al., 2009; Gonçalves et al., 2013; Fonseca et al., 2014). According to Park (2015), modifications in vegetation cover, even when replacement involves species closely connected to each other, may produce important differences in total carbon stocks, as well as in the distribution of carbon across ecosystem compartments. The same author observed significant differences on tree carbon pool between *Pinus resinosa* and *Pinus banksiana* species. Identical results are reported by Chen et al. (2016) for the compared species (*Pinus davidiana* vs. *Pinus sylvestris*).

More than 70% of the carbon is stored in the aboveground biomass: 71% (57 Mg C $ha^{-1}$), 72% (141 Mg C $ha^{-1}$) and 80% (91 Mg C $ha^{-1}$) to

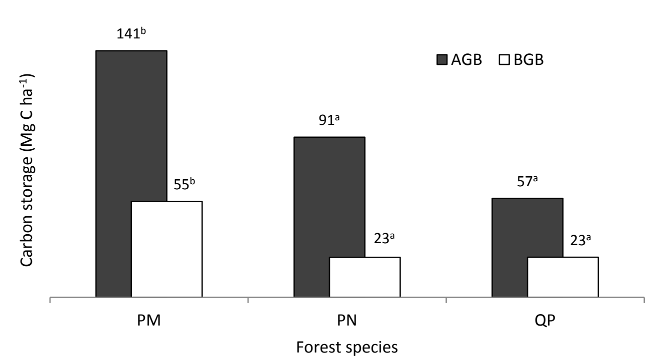


**Fig. 2.** Carbon storage in aboveground (AGB) and belowground (BGB) biomass of forest species *Pseudotsuga menziesii* (PM), *Pinus nigra* (PN) and *Quercus pyrenaica* (QP). Average values of each biomass component with the same letter are not significantly different ($p > 0.05$).

the QP, PM and PN species, respectively. Similar percentages were reported by Gonçalves et al. (2013) in a mixed stand of *Pseudotsuga menziesii* and *Castanea sativa* located in Northern Portugal. Carbon stocks in PM belowground biomass (55 Mg C ha$^{-1}$, equivalent to 1.8 Mg C ha$^{-1}$ year$^{-1}$) exceeded twice that found in the PN and QP stands (23 Mg C ha$^{-1}$ for both species). It should be noted that, in temperate forest ecosystems, a greater accumulation of carbon in the aboveground biomass, as compared to the belowground, is commonly referred (e.g. Peichl and Arain, 2006; Vallet et al., 2009; Fonseca et al., 2014; Chen et al., 2016).

The carbon stored in tree biomass increased for both introduced species (PM and PN), reaching values that exceed those recorded for QP in 35 Mg C ha$^{-1}$ or an average of 1.2 Mg C ha$^{-1}$ year$^{-1}$ in PN (all increment in aboveground biomass) and 116 Mg C ha$^{-1}$ or an average of 3.9 Mg C ha$^{-1}$ year$^{-1}$ in PM (84 Mg C ha$^{-1}$ in aboveground biomass and 32 Mg C ha$^{-1}$ in belowground biomass). In contrast, Vallet et al. (2009) recorded carbon losses in tree biomass when the *Quercus petraea* (117 Mg C ha$^{-1}$) was replaced by *Pinus nigra* (67 Mg C ha$^{-1}$). It seems that, the carbon storage in tree biomass is controlled by species-specific development patterns.

### 3.2. Carbon stocks in biomass of herbaceous vegetation

Carbon storage in above and belowground biomass of herbaceous vegetation is not relevant (Fig. 3). In PN and QP species, herbaceous vegetation is scarce and dispersed owing to the presence of forest floor layers, which hindered their growth. Identical observations are reported by Gonçalves et al. (2013). In PM species, the presence of herbaceous vegetation was not observed since the high canopy cover of this species (Table 1), prevents sunlight reaching stand's ground surface. Park (2015) emphasize that the higher canopy cover in *Pinus resinosa* stands contributed to a low carbon stored in herbs and short shrubs understorey, accounting for less than 2% of the total carbon stored. The same author also noted that herb carbon decreased linearly with age while litter carbon increased linearly with age, which corroborates the observations recorded in the present study. Vallet et al. (2009) ignored the contributions of the herbaceous stratum in carbon stocks since this compartment represents roughly 4% in French forests.

As mentioned, the contribution of carbon stocks in herbaceous vegetation to the total carbon stored is almost negligible (0, 0.2 and 0.3% for the PM, PN and QP species, respectively), adding in the system 0.48 Mg C ha$^{-1}$ in PN and 0.84 Mg C ha$^{-1}$ in QP. According to Gonçalves et al. (2013), the growth of herbaceous vegetation can be conditioned by the tree species that, over time, give rise to an organic horizon formed by litterfall and other parts of the trees, which, in turn, prevents understory vegetation development. Despite the reduced contribution of herbaceous vegetation to global carbon storage, the development of annual and perennial herbaceous plant communities played an important role in the system productivity, and their rapid growth and death provided a significant source of organic carbon and nutrients to the soil (Mun and Whitford, 1998; Nicolini and Topp, 2005).

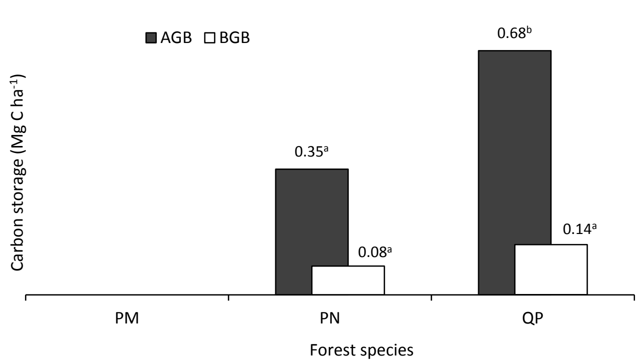


**Fig. 3.** Carbon storage in aboveground (AGB) and belowground (BGB) biomass in herbaceous vegetation. Average values of each biomass component with the same letter are not significantly different ($p > 0.05$).

### 3.3. Carbon stocks in the forest floor

The forest floor dry matter accumulated on the soil surface under the three species is significantly higher for PM (31.8 Mg ha$^{-1}$) and PN (27.1 Mg ha$^{-1}$), compared to QP (18.4 Mg ha$^{-1}$), when considering the L, F and H organic layers together (Table 3). These differences can be related to the decomposition rate of organic residues input at soil surface (Vesterdal et al., 2008; Vallet et al., 2009; Kooch et al., 2017; Fonseca and Figueiredo, 2018). Actually, large quantities of weakly decomposed organic material were observed under PM and PN species (conifer species), whilst under QP (broadleaf species) the organic residues show a much more advanced decomposition stage and incorporation in mineral soil. Identical results were obtained by other authors (Trum et al., 2011; Bargali et al., 2015; Chen et al., 2016; Fonseca and Figueiredo, 2018). Although the PN stand presents lower density (trees ha$^{-1}$) than the PM stand (see Table 1), the high pine cones production by the PN may explain the absence of statistically significant differences between the two species, which is in agreement with Martins et al. (2009).

For all the tree species studied, carbon concentration decrease from L to H layers, showing similar values in L layer and significantly higher in F and H layers in PN and QP species (Table 3). The decrease of carbon concentration can be associated with the humification degree and with the addition of mineral particles at these layers, mainly owing to the soil biological activity (Wardle, 1993; Martins et al., 2009; Fonseca and Figueiredo, 2018).

Carbon stocks in whole forest floor (L, F and H layers) were significantly higher under PM (11.1 Mg C ha$^{-1}$) and PN (13.1 Mg C ha$^{-1}$) than QP (8.2 Mg C ha$^{-1}$) (Fig. 4), which corresponds to an additional accumulation of 97 kg ha$^{-1}$ year$^{-1}$ and 163 kg ha$^{-1}$ year$^{-1}$ for PM and PN species, respectively, as compared with QP. These amounts of carbon were higher when compared to 5.4 Mg C ha$^{-1}$ stored in the forest floor of a 30-year-old *Pinus strobus* plantation (Peichl and Arain, 2006). It is also observed that, as compared to PN, PM species presents lower C stocks in all organic layers. PM forest floor is richer in calcium and magnesium, which favours the decomposition rate (Fonseca et al., 2004; Martins et al., 2009). In a research work on the effects of several forest species on carbon storage in temperate region, Vesterdal et al. (2013) reported that conifers species (*Picea, Tsuga* and *Larix*) accumulated more carbon in the forest floor than *Quercus* spp. The L layer recorded a slight increase statistically not significant in the carbon storage, 3.7, 3.8 and 3.2 Mg C ha$^{-1}$ for the species PM, PN and QP,

**Table 3**
Forest floor layers (L, F and H) carbon concentration and dry matter under *Pseudotsuga menziesii* (PM), *Pinus nigra* (PN) and *Quercus pyrenaica* (QP) forest species, expressed as mean and standard deviation. For each forest floor layer means with the same letter across rows are not significantly different ($P > 0.05$).

| Forest floor | Species | | |
|---|---|---|---|
| Layers | PM | PN | QP |
| *Carbon concentration (g kg$^{-1}$)* | | | |
| L | 520.3 ± 24.7$^{a}$ | 552.6 ± 11.3$^{a}$ | 539.4 ± 5.8$^{a}$ |
| F | 426.7 ± 38.7$^{a}$ | 524.0 ± 41.5$^{b}$ | 500.2 ± 38.0$^{b}$ |
| H | 216.7 ± 63.9$^{a}$ | 390.3 ± 59.2$^{b}$ | 385.2 ± 70.1$^{b}$ |
| *Dry matter (Mg ha$^{-1}$)* | | | |
| L | 7.2 ± 3.3$^{a}$ | 6.9 ± 3.6$^{a}$ | 5.9 ± 1.1$^{a}$ |
| F | 9.5 ± 2.9$^{b}$ | 8.5 ± 6.0$^{b}$ | 3.8 ± 1.3$^{a}$ |
| H | 15.1 ± 5.5$^{b}$ | 11.7 ± 5.6$^{ab}$ | 8.7 ± 3.6$^{a}$ |
| Total | 31.8$^{b}$ | 27.1$^{b}$ | 18.4$^{a}$ |

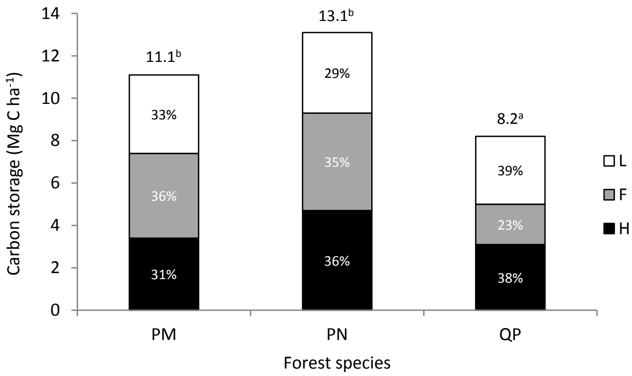


**Fig. 4.** Carbon storage in L, F and H organic layers (forest floor). Average values with the same letter are not significantly different ($p > 0.05$).

respectively (Fig. 4). The F layer is the one with the highest carbon storage and the values are similar among PM (4.0 Mg C ha$^{-1}$) and PN (4.6 Mg C ha$^{-1}$), and significantly higher than those found for QP species (1.9 Mg C ha$^{-1}$). Finally, the H layer shows some discrepancies among the three species but without significant differences. A similar pattern of carbon distribution in L, F and H layers was obtained by Fonseca and Figueiredo (2018) when comparing conifers species (*Pinus pinaster*, *Pinus nigra* and *Pseudotsuga menziesii*) vs. a broadleaf species (*Castanea sativa*).

### 3.4. Carbon stocks in the mineral soil

The introduction of PM and PN species replacing QP (climax vegetation) reduced significantly the carbon concentration in all soil layers (Table 4). Although, PM species presents higher carbon concentration than PN species, both do not differ significantly from PN. The significant differences found between the introduced conifer (PM and PN) and the native broadleaf (QP) species are seemingly due, at first, to soil disturbances when conifers stand was installed (PM and PN), which favoured soil organic matter mineralization (Schulp et al., 2008; Gonçalves et al., 2013; Ma et al., 2015). Conversely, broadleaf species (QP) store carbon in more stable form (Vesterdal et al., 2013).

**Table 4**
Soil carbon concentration (g C kg$^{-1}$), soil bulk density (g cm$^{-3}$) and coarse fragments under forest species *Pseudotsuga menziesii* (PM), *Pinus nigra* (PN) and *Quercus pyrenaica* (QP), expressed as mean and standard deviation. For each soil layer means with the same letter across rows are not significantly different ($P > 0.05$).

| Depth (cm) | Species | | |
|---|---|---|---|
| | PM | PN | QP |
| *SOC concentration (g kg$^{-1}$)* | | | |
| 0–5 | 58.7 ± 13.9$^a$ | 52.4 ± 10.2$^a$ | 100.0 ± 23.1$^b$ |
| 5–10 | 55.4 ± 10.6$^a$ | 50.9 ± 9.6$^a$ | 80.4 ± 7.6$^b$ |
| 10–15 | 54.5 ± 17.6$^a$ | 45.4 ± 5.3$^a$ | 74.7 ± 8.0$^b$ |
| 15–20 | 48.4 ± 12.0$^a$ | 44.3 ± 5.3$^a$ | 66.9 ± 7.4$^b$ |
| 20–30 | 44.5 ± 12.0$^a$ | 40.4 ± 8.5$^a$ | 60.9 ± 10.3$^b$ |
| *Bulk density (g cm$^{-3}$)* | | | |
| 0–5 | 1.02 ± 0.12$^a$ | 1.01 ± 0.13$^a$ | 0.84 ± 0.13$^b$ |
| 5–10 | 1.11 ± 0.16$^a$ | 1.18 ± 0.15$^a$ | 0.90 ± 0.07$^b$ |
| 10–20 | 1.18 ± 0.21$^a$ | 1.19 ± 0.15$^a$ | 0.94 ± 0.09$^b$ |
| 15–20 | 1.24 ± 0.19$^a$ | 1.23 ± 0.19$^a$ | 1.03 ± 0.08$^b$ |
| 20–30 | 1.37 ± 0.13$^a$ | 1.25 ± 0.16$^a$ | 1.08 ± 0.07$^b$ |
| *Coarse fragments (%)* | | | |
| 0–5 | 40.1 ± 11.4$^a$ | 48.1 ± 8.5$^b$ | 37.2 ± 10.0$^a$ |
| 5–10 | 44.3 ± 14.3$^b$ | 44.5 ± 5.6$^b$ | 32.0 ± 12.4$^a$ |
| 10–20 | 48.7 ± 16.5$^b$ | 42.5 ± 7.6$^b$ | 25.5 ± 11.7$^a$ |
| 15–20 | 51.4 ± 12.3$^b$ | 43.3 ± 7.2$^b$ | 26.7 ± 15.1$^a$ |
| 20–30 | 53.7 ± 15.2$^b$ | 46.3 ± 7.3$^b$ | 25.3 ± 5.5$^a$ |

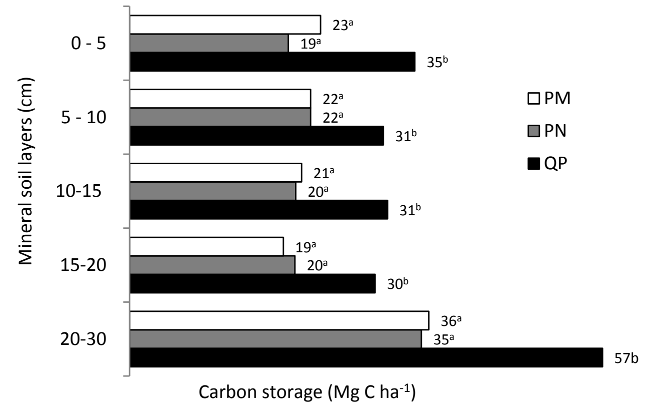


**Fig. 5.** Carbon storage in mineral soil layers of forest species *Pseudotsuga menziesii* (PM), *Pinus nigra* (PN) and *Quercus pyrenaica* (QP). Average values of each soil layer with the same letter are not significantly different ($p > 0.05$).

Also, tree species composition controls the inputs and biochemical characteristics of soil organic carbon (Díaz-Pinés et al., 2011). The carbon concentration decreased with soil depth while bulk density increased (Table 4), following a common variation pattern. Soil coarse elements showed a variation pattern with depth different according to forest species, but always with values higher than 25% of soil total dry mass (Table 4). These high coarse elements contents are commonly found in soils of the Mediterranean basin, in general, and specifically for the study area regional context (Díaz-Pinés et al., 2011; Fonseca et al., 2012; de Figueiredo, 2012).

Fig. 5 presents carbon stocks in the mineral soil layers analysed. At all depths, soil carbon accumulation under PM and PN species is significantly lower than that under QP species, which can be justified by several effects as site preparation (Fonseca et al., 2014; Ferré et al., 2014), management practices (Zhang et al., 2016) and the nature of the litterfall produced by each tree species (Vesterdal et al., 2013; Cools et al., 2014; Zhang et al., 2016; Fonseca and Figueiredo, 2018). These factors may promote the breakdown of soil aggregates, affect soil biological activity and, consequently, the mineralization rate of organic residues in the forest floor and of soil organic matter (Gonçalves et al., 2013; Herrero et al., 2016; Fonseca and Figueiredo, 2018). In general, carbon storage displays a vertical gradient, tending to decrease with increasing mineral soil depth. As stressed before, total carbon stored in the soil compartment (0–30 cm) is significantly higher under QP (184 Mg C ha$^{-1}$) than under PM (121 Mg C ha$^{-1}$) or PN (116 Mg C ha$^{-1}$) (Fig. 5), meaning values 34% (PM) and 36% (PN) lower in the introduced than in the native species (QP). Similar results are presented by Ferré et al. (2014), showing an overall decrease on soil organic carbon stocks, roughly 40%, after 37 years of *Populus euramericana* plantation in areas formerly covered by natural forest (*Quercus* spp.), in northern Italy. Also, according to Lal (2005) soils under natural forest, in general, have more soil organic carbon contents than those under managed forest plantations. Herrero et al. (2016), in a study carried out in northern Spain, observed similar carbon content in soils when comparing plantations of *Pinus* spp. with natural *Quercus pyrenaica* areas (53 Mg C ha$^{-1}$ vs. 60 Mg C ha$^{-1}$), these values being, however, clearly lower than those found in the present study. In contrast, a work developed in Central Spain, shows that *Pinus sylvestris* soils stored almost the double of carbon than *Quercus pyrenaica* soils at their ecotone (Díaz-Pinés et al., 2011). Disturbances that may occur in the soil such as changing vegetation cover, fires and management practices, influence in a long term the ecosystems sustainability and their ability to provide ecosystem services, such as carbon storage (e.g. Lal, 2005; Fonseca et al., 2014; Ferré et al., 2014; Chen et al., 2016; Sil et al., 2017; Fonseca and Figueiredo, 2018). The highest loss was recorded in the surface layer (0–5 cm; 11–15 Mg C ha$^{-1}$), which may be related to a faster process of organic matter mineralization and gas exchanges with the atmosphere (Fernández et al., 1993; Gonçalves et al., 2013). The losses observed in each layer in the PM and PN species are identical, ranging from 0.0 Mg C ha$^{-1}$ (5–10 cm) to 4.0 Mg C ha$^{-1}$ (0–5 cm). Comparing the values obtained for the whole mineral soil (0–30 cm) under the introduced with those under native species, carbon losses observed were of 62.7 Mg C ha$^{-1}$, equivalent to 2.1 Mg C ha$^{-1}$ year$^{-1}$, for PM and of 66.8 Mg C ha$^{-1}$, equivalent to 2.2 Mg C ha$^{-1}$ year$^{-1}$ for PN. The difference between PM and PN being only 6%. Lower results, yet with a similar trend (1.5 Mg C ha$^{-1}$ year$^{-1}$), were obtained by Ferré et al. (2014), when studying a natural forest that was replaced by *Populus euramericana* cultivation. Taking into account the results obtained and since the organic matter content is essential in the maintenance of soil properties and, hence, in the conservation of the entire ecosystem, species substitution may be contributing to temporary and long-term ecosystem degradation.

### 3.5. Total carbon stocks in the system

The total amount of carbon accumulated in the system ranged from 245.6 Mg C ha$^{-1}$ (PN) to 272.6 Mg C ha$^{-1}$ (QP) and to 333.1 Mg C ha$^{-1}$ (PM) (Fig. 6). Overall, total carbon stocks increased in PM (55.4 Mg C ha$^{-1}$, equivalent to 1.8 Mg C ha$^{-1}$ year$^{-1}$) and decreased in PN (27.4C Mg ha$^{-1}$ equivalent to 0.9 Mg C ha$^{-1}$ year$^{-1}$) (Fig. 7), yet not significantly differing from the native QP species. Total carbon stock is relatively higher than that found by Vallet et al. (2009) when *Quercus petraea* (221 Mg C ha$^{-1}$) was replaced by *Pinus nigra* (175 Mg C ha$^{-1}$) over a 60 years period in Central France. In the present study, mineral soil and tree biomass were the largest carbon pools for all species, but with different pattern of distribution for the native (QP) and for introduced species (PN and PM). In QP, mineral soil was the most important carbon stock compartment and in PM and PN carbon stored in tree biomass assumed a more relevant importance, mainly in PM species. Carbon stocks in introduced tree biomass showed a large increase after 30 years replacement of the native forest, since in QP it represents 29% (80 Mg C ha$^{-1}$) of the total, passing to 60% (196 Mg C ha$^{-1}$) in PM and to 47% (114 Mg C ha$^{-1}$) in PN. In the introduced species, carbon increases due tree biomass and forest floor contributions were sufficient to cover the losses in the soil down to 30 cm depth, reaching similar values (PN) and significantly higher (PM), when compared to the original situation (QP).

The contribution of carbon stocks in herbaceous vegetation is very low, less than 1 Mg C ha$^{-1}$, representing 0, 0.18 and 0.28% of the total carbon stored for PM, PN and QP species, respectively. Although not expressive, there were losses in the contribution of herbaceous

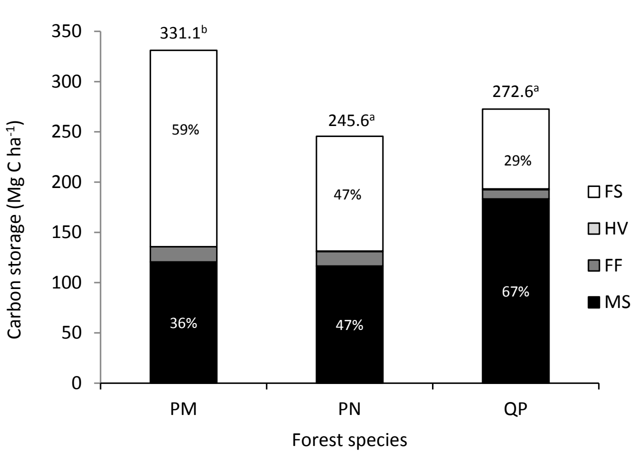


**Fig. 6.** Total carbon storage in the whole compartments of forest species *Pseudotsuga menziesii* (PM), *Pinus nigra* (PN) and *Quercus pyrenaica* (QP). Forest species (FS), herbaceous vegetation (HV), forest floor (FF) and mineral soil (MS). Average values of each tree species with the same letter are not significantly different ($p > 0.05$).

vegetation to total carbon storage after 30 years replacement of QP species by PM and PN species. Overall, the herbaceous vegetation stratum is often less developed in forest plantations than in natural forests (Peichl and Arain, 2006).

The increase in forest floor carbon storage from QP to introduced species ranged from 2.9 Mg C ha$^{-1}$ (PM) to 4.9 Mg C ha$^{-1}$ (PN). Their contribution to the total carbon stored in the system corresponded to 3.0, 4.8 and 2.7% for the species PM, PN and QP, respectively. However, forest floor is an important component in the relationships soil-vegetation in forest systems, a crucial resource for nutrients cycling in forest soils (Smyth et al., 2015) and a fundamental part of the carbon biogeochemical cycle (Ordóñez et al., 2008; Zhang et al., 2016).

The soil is one of the main carbon pools in forest systems. Carbon accumulation in mineral soil under introduced conifers species (PM and PN) ranged between 116 and 121 Mg C ha$^{-1}$, which represents roughly two-thirds of that stored in QP (184 Mg C ha$^{-1}$). Comparing the percent contribution of this compartment to total carbon storage in each tree species, considerable changes occurred after 30 years species replacement, since they fell down from 68% in QP to 37% in PM and 48% in PN. Changes in land use directly affect gas exchange between terrestrial ecosystems and the atmosphere, with consequences for carbon storage (Watson, 2000; Park, 2015). Carbon stocks in forest ecosystems depends fundamentally on the complex interactions between climate, soils, tree species, management practices, quantity and quality of litter produced by the dominant species in the region concerned (Post and Kwon, 2000; Paul et al., 2002; Pregitzer and Euskirchen, 2004; Lal, 2005; Peichl and Arain, 2006; Vallet et al., 2009; Ma et al., 2015).

## 4. Conclusions

The results show that the mineral soil and tree biomass are the largest carbon pools; forest floor and herbaceous vegetation are not significant contributions to total carbon storage, with values lower than 5% and 1%, respectively. Comparing the introduced species (PM and PN) with the original situation (QP), after 30 years species replacement, forest floor (2.9 Mg C ha$^{-1}$ for PM and 4.9 Mg C ha$^{-1}$ for PN) and tree biomass (116 Mg C ha$^{-1}$ for PM and 35 Mg C ha$^{-1}$ for PN) were carbon sinks, whilst mineral soil was a carbon source (62.7 Mg C ha$^{-1}$ for PM and 66.7 Mg C ha$^{-1}$ for PN). These findings highlight the effects of specific development patterns and of mechanized operations associated to PM and PN stands' installation. In this way, the research hypothesis was confirmed, since the total carbon stored and its distribution in the different system compartments was affected by the species replacement.

Vegetation cover changes, in relation to the total carbon stored in the system, was beneficial in case of PM species, presenting a gain of 16.9% as compared with the native species (QP), while PN species presented a loss of 10.1%, even though, both (QP and PN) presented statistically similar results. The significant losses in mineral soil carbon compartment were rebalanced through the carbon gain in tree biomass and forest floor. In the current context whereby greenhouse gases effect, climate change and global warming have been emphasized; the replacement of native vegetation plays an important role in the dynamics of carbon in the atmosphere and in terrestrial ecosystems. Despite the increased productivity obtained with the introduced species (PM and PN, fast-growing species), it is necessary to focus attention on the impacts of species replacement in the mineral soil. Organic matter content is often one of the most influencing factors in physical, chemical and biological soil properties, and its reduction may have consequences on soil fertility, biodiversity and ecosystem resilience, and affecting sustainability. Consequently, the protection of soil organic matter assumes primary importance and it is essential to adopt strategies that allow soil organic matter conservation. Accordingly, the selection of tree species to be used in afforestation/reforestation programmes should take into account ecosystem services provided (e.g. carbon stocks) and ecosystem sustainability.

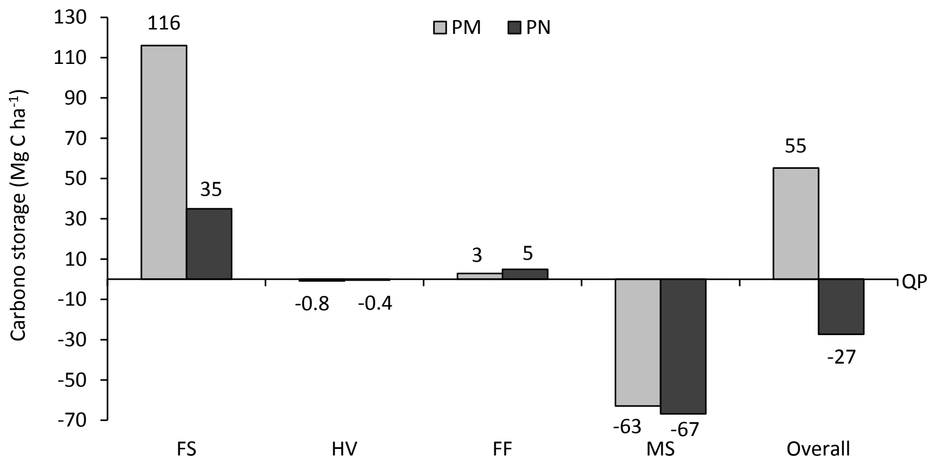


**Fig. 7.** Variation in carbon content in all compartments of the *Pseudotsuga menziesii* (PM) and *Pinus nigra* (PN) compared to original soil (QP). Forest species (FS), herbaceous vegetation (HV), forest floor (FF) and mineral soil layers.

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