Effect of Density on Mediterranean Pine Seedlings Using the Nelder Wheel Design: Analysis of Biomass Production

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Research

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TITLE PAGE

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ABSTRACT

• **Background:** Forest resilience should be improved to promote species adaptation and ensure the future of forests. Carbon stock is considered an indicator of resilience, so it is necessary to determine forest carbon stocks and how to improve them through forest management. The main objective of this study was to analyse biomass production and distribution among the components of four-year-old *Pinus pinaster* and *Pinus halepensis* trees. Young trees from a Nelder wheel experimental site were harvested and analysed. The effect of density could be included in the biomass analysis thanks to the Nelder wheel design. We tested densities from 1000 to 80000 seedlings/ha and analysed biomass by fitting different equations: (i) linear regressions to analyse biomass production; (ii) Dirichlet regressions to estimate the biomass proportions of each component and (iii) allometric equations to predict the biomass content of each component.

• **Results:** Results from this innovative approach showed that density was a significant factor for *Pinus halepensis*. We observed a general increase of total biomass at lower densities and this positive effect increased root biomass proportion at the expense of aboveground biomass. Also, a new set of equations was developed for estimating above- and below-ground biomass in young *Pinus pinaster* and *Pinus halepensis* trees.

• **Conclusions:** we note the importance of belowground biomass and its value in total biomass production (approximately 20% of total biomass for both species). The effect of density on biomass production was only significant for *Pinus halepensis*, but the effect of density would have been different if root biomass had not been considered in the present study. Lower densities increased root biomass
proportion at the expense of aboveground biomass. Currently, this positive effect is especially important in promoting management to improve tree resilience.

Keywords
Mixed stands; Mediterranean ecosystem; Dirichlet regressions; biomass equations; root system

BACKGROUND
The role of forests in mitigating climate change is quite clear and much research has been focused on quantifying it in terms of carbon sequestration (Djomo and Chimi 2017; Khan et al. 2018; Vinh et al. 2019…). Still, we lack knowledge about some aspects of the process, such as the carbon sequestered by belowground components or carbon stocks in Mediterranean ecosystems (Herrero et al., 2014; Alvarez et al. 2016). Carbon stocks are significant because of the increased atmospheric concentration of carbon dioxide, which has contributed to climate change (IPCC 2007). Climate change might also negatively affect forests by reducing forest productivity, standing biomass or carbon stocks as trees die off (Resco De Dios et al. 2007, Lindner and Calama 2013). This decreases the mitigating capacity of forests, so it is important to link mitigation and adaptation to understanding the long-term role of forests in climate change. Carbon stocks, species diversity and harvest rates are considered indicators of resilience, which can be enhanced by improving species adaptation and subsequent mitigation capacity (Hof et al. 2017). Forests face an uncertain future, but managed forests adapt more efficiently to climate change. Thus, adaptive management should consider future site conditions to ensure correct decision-making (Jandl et al. 2019).
High densities in mature stands generally cause competition for resources and reduce tree growth, but competitive and facilitating effects can occur simultaneously in other phases of stand development, such as seedling establishment. For example, seedlings might compete with their own or other species for resources like water or light even as wind velocity or transpiration are reduced by mutual shading or other species provide protection against herbivores (Jactel and Brockerhoff 2007; Zamora et al. 2008; Uhl et al. 2015). Moreover, positive interactions can become negative as seedlings grow (Callaway et al. 1996; Callaway and Walker 1997; Zamora et al. 2008; Uhl et al. 2015). Some models predict that facilitation and competition interactions will vary across abiotic stress gradients and that facilitation interactions will be dominant under stressful conditions, though this is debated (Maestre et al. 2005, 2006; Lortie and Callaway 2006). Therefore, the net effect of intra- and inter-specific interactions among seedlings under high densities is a key issue to analyse.

Most studies look at intra- and inter-specific interactions from a productive point of view, analysing productive species in mature stands (Piotto et al. 2004; Kuehne et al. 2013; Vanclay et al. 2013). The discussion tends to be theoretical due to the lack of studies that compare pure and mixed stands on similar sites (Pretzsch 2009), though long-term experimental plots have made it possible to study mixed mature stands more recently (Pretzsch et al. 2015, 2019a, b; Riofrío et al. 2019; Aguirre et al. 2019…). Some authors have analysed inter-specific interactions between seedlings and other weed species or shrubs (Helliwell and Harrison 1979; Scholes and Archer 1997; De las Heras et al. 2002; Gómez-Aparicio et al. 2005; Rodríguez-García et al. 2011) but little is known about less productive species and ecosystems or other forest stages, such as regeneration.

Two of the most typical pine species in Mediterranean ecosystems were studied in the present work: Pinus pinaster Aiton and Pinus halepensis Mill. Both species cover
extensive areas in the western Mediterranean basin, while *Pinus halepensis* also grows in the eastern Mediterranean. From timber, food and energy to soil protection, cultural services, hydrological control, habitat for biodiversity, or climate regulation, forests provide vital ecosystem services (Science for Environment Policy 2015). Pine plantations have been questioned in semi-arid areas in terms of soil fertility, erosion or ecological succession, but they provide more ecosystem services than grasslands, abandoned agricultural fields or other unrestored landscapes (Derak and Cortina 2014). *Pinus pinaster* and *Pinus halepensis* have high ecological importance because of their ability to grow in extremely poor soils and survive multiple droughts (Alía and Martín 2003; Fady et al. 2003). The two species share niches, but there are no studies about inter-specific interactions between them. Climate projections for Mediterranean forests predict negative effects at different levels in the future, including reduction of forest and site productivity, increment of disturbances (pests, pathogens, fires…) and shifting species distributions or even extinctions (Resco De Dios et al. 2007, Lindner and Calama 2013). The changes in species distribution and structure that are expected in Mediterranean areas (Thuiller 2003) make it especially timely to study how the selected species will behave together.

One of the main issues in this kind of research is the experiment design. The most frequent designs for analysing mixed stands consist of growing two species in varying proportions while keeping stand density constant (Vanclay 2006). Mixed mature stands have been studied in recent years thanks to long-term experimental plots (Pretzsch et al. 2015, 2019a, b; Riofrío et al. 2019; Aguirre et al. 2019…) but little is known about seedling stands or young forests. The Nelder wheel design makes it possible to test multiple densities in a single plot (Nelder 1962; Kerr 2003; Uhl et al. 2015). It consists of a circular plot containing concentric rings radiating outward with spokes that connect the centre with the furthest ring (Figure 1 – experimental design). At the intersections of spokes and
arcs, a tree is planted. This creates variable tree densities along the length of the spokes within a single plot.

The main objective of this study was to analyse biomass production and distribution among the components of four-year-old *Pinus pinaster* and *Pinus halepensis* trees. Young trees from a Nelder wheel experimental site were harvested and analysed. We expected that: (i) biomass production would be different for *Pinus halepensis* and *Pinus pinaster*; (ii) biomass production would be affected by density; (iii) the effect of density would be different for each species.

**MATERIAL AND METHODS**

**Experimental design**

The present study was carried out in a density experiment following the design proposed by Nelder (1962). The experimental site consisted of five Nelder wheels – four permanent and one temporary – in which 10 densities were tested: (Ruano et al 2021). The temporary Nelder wheel plot was installed to obtain two seedling harvests for dry biomass analysis. The results of first harvest are presented here.

The experimental site was established during the autumn of 2013 in Calabazanos (Palencia – central Spain), on approximately 1 ha of land located on an old forest nursery site belonging to the Castile-and-Leon Government. The site currently houses the Forest Health Centre (Figure 1). The experimental design was implemented according to the explanations of Parrott et al. (2012). All the Nelder wheel plots have 26 spokes, constant angles of 13.85° and 12 rings, housing a total of 312 seedlings per wheel. Trees in the outermost and innermost rings served as buffers for edge effects and were not included in the study sample. In the temporary Nelder wheel plot, two spokes were also considered as buffers for the harvests (Figure 1). *Pinus pinaster* and *Pinus halepensis* seedlings were
mixed along the spokes. Two regions of provenance – the ‘Meseta Castellana’ (Castilian High Plains) for *Pinus pinaster* and ‘Repoblaciones de la Meseta Norte’ (North Plains Reforestation) for *Pinus halepensis* – were selected to avoid the site effect (Figure 1).

One of the most important parameters to obtain in the Nelder wheel plot was the ‘rectangularity’ proportion (Parrott et al. 2012). This is defined as a proportional relationship between the arc length between spokes and the radial length between arcs, where the numerator represents the arc length and the denominator represents radial distance (Nelder 1962). Extreme ‘rectangularity values can cause bias by creating an unreasonably asymmetric arrangement of space around trees, so a rectangularity value of 1 was defined in the present experiment (Parrott et al. 2012).

Ten densities were tested, ranging from 1000 to 80000 seedlings/ha (Table 1). Minimum and maximum densities were also defined, to measure the effects of low and high regeneration densities (Matney and Hodges 1991; Calvo et al 2007; Orozco et al 2007; Ruano et al 2013). Matney and Hodges (1991) identified a recruit density of 2000 seedlings/ha as the minimum requirement for successful natural regeneration. However, a density of 1000-1500 seedlings/ha can be considered satisfactory in stands with abiotic stress (Rodríguez-García et al. 2010), so the minimum density tested was 1000 seedlings/ha. At the other end, high densities have been reported in studies on post-fire natural regeneration in Mediterranean ecosystems (Calvo et al. 2007; Orozco et al. 2007; Ruano et al. 2013). Ruano et al. (2013) observed densities of 80000 seedlings/ha after a fire in *Pinus halepensis* stands, which served to establish the maximum density at 80000 seedlings/ha in the present study. The same densities were tested for all seedlings of each concentric ring and can be expressed in terms of stand density (trees/ha) or the ‘growing space’ (m²/tree) available to each tree. In the present work, this will be referred to as ‘growing space’, which is related to stand density (Table 1).
Seedlings were one growing season old at time of planting. Basal diameter and total height (length of the main stem) were measured for each plant. The same measurements (basal diameter and total height) were repeated after one year (2014) and after four years (2017). Theoretically, 120 seedlings would have been harvested each time, but only 105 seedlings were available for the first harvest (2017) due to mortality. When a seedling dies, the growing space of surrounding seedlings changes so the experimental design changes from the initial design. Options for resolving this issue include double planting, removing the seedlings surrounding the dead seedlings from the analysis, competition indexes… (Parrott et al. 2012; Kuehne et al. 2013; Uhl et al. 2015). In this case, we choose to re-calculate the growing space based on Voronoi polygons, using R Core Team 2017 software with the ‘deldir’ package (Turner 2020) for 2014 and 2017 (Supplementary Figure 1) (Ruano et al., 2021).

After four years, 37 Pinus pinaster and 68 Pinus halepensis seedlings were felled in spring of 2017 and their root systems were extracted with a backhoe. They were divided into biomass fractions following the methodology of Montero et al. (2005): root, stem, needles and thin branches (diameter smaller than 2 cm). The thinnest root fraction could not be retrieved and there were no thick branches (diameter greater than 2 cm) on the trees, so these elements were not included in the analysis. Samples of each fraction were oven dried at 80 °C until constant weight was reached. Total biomass was defined as the sum of the aboveground biomass (stem, thin branches and needles) and belowground biomass (roots). The belowground/aboveground ratio was also calculated.

**Analysis of biomass production**

The biomass production analysis was developed following the methodology of Dahlhausen et al. (2017). Four equations were fitted as linear regressions according to
Snell’s (1892) power equation, which is commonly used to predict biomass: \( y = b_1 x^{b_2} \), where \( y \) is biomass and \( x \) are the explanatory variables. This equation can be fitted as a linear regression by transforming it into \( \ln y = \ln b_1 + b_2 \ln x \). The following models were fitted for each species:

1. \( \ln W_i = b_1 + b_2 \ln (D) \)  
2. \( \ln W_i = b_1 + b_2 \ln (D) + b_3 \ln (H) \)  
3. \( \ln W_i = b_1 + b_2 \ln (D) + b_4 \ln (GS) \)  
4. \( \ln W_i = b_1 + b_2 \ln (D) + b_3 \ln (H) + b_4 \ln (GS) \)

Where \( W_i \) is the estimated biomass weight, \( D \) is the basal diameter, \( H \) is the total height and \( GS \) is the growing space for the seedling. The four models were fitted for total biomass, aboveground biomass, belowground biomass and belowground/aboveground ratio. The normality of the residuals was tested graphically with Q-Q plots of residuals and the adjusted R-squared was estimated to select better fittings.

Dirichlet regressions were then fitted to analyse the biomass and estimate proportions for each species. Biomass compartments can be estimated as proportions or percentages of total biomass. The Dirichlet distribution is a multivariate generalization of the beta distribution (Poudel and Temesgen 2016) and takes the following form:

\[
D(y|\alpha) = \frac{1}{B(\alpha)} \prod_{c=1}^{C} y_c^{(\alpha_c - 1)}
\]

Where \( \alpha_c \) are shape parameters for each variable. For this distribution, the constraints \( \alpha_c > 0 \forall \ c, \ y_c \in (0, 1) \) and \( \sum_{c=1}^{C} y_c - 1 \forall \ c \) must hold. The multinomial beta function, \( B(\alpha_c) \), serves as the normalizing constant (Maier 2014).
The following equations were fitted as single equation models for the four compartments (roots, stem, thin branches and needles: c=1, 2, 3, 4):

\[
\ln(\alpha_c) = \alpha_{1c} + \alpha_{2c} \ln(D)
\]  
(5)

\[
\ln(\alpha_c) = \alpha_{1c} + \alpha_{2c} \ln(D) + \alpha_{3c} \ln(H)
\]  
(6)

\[
\ln(\alpha_c) = \alpha_{1c} + \alpha_{2c} \ln(D) + \alpha_{3c} \ln(GS)
\]  
(7)

\[
\ln(\alpha_c) = \alpha_{1c} + \alpha_{2c} \ln(D) + \alpha_{3c} \ln(H) + \alpha_{4c} \ln(GS)
\]  
(8)

\[
\ln(\alpha_c) = \alpha_{2c} \ln(D)
\]  
(9)

\[
\ln(\alpha_c) = \alpha_{2c} \ln(D) + \alpha_{3c} \ln(H)
\]  
(10)

\[
\ln(\alpha_c) = \alpha_{2c} \ln(D) + \alpha_{3c} \ln(GS)
\]  
(11)

\[
\ln(\alpha_c) = \alpha_{2c} \ln(D) + \alpha_{3c} \ln(H) + \alpha_{4c} \ln(GS)
\]  
(12)

All compartment combinations between equations (5) and (7) were also tested.

With common parametrization, the expected values could then be defined as \(E[y_c] = \alpha_c / \alpha_0\), where \(\alpha_0\) is the sum of all \(\alpha_c\) (Maier 2014).

Moreover, AIC differences (\(\Delta_i\)) were estimated for all candidate models to ascertain empirical support for each model. Accordingly, \(\Delta_i\) is estimated as: \(\Delta_i = AIC_i - AIC_{\text{min}}\) and models with a \(\Delta_i > 10\) essentially have no support and can be omitted (Burnham and Anderson 2002).

Both analyses were performed with R Core Team 2020 software and the Dirichlet regressions were fitted using the DirichReg package (Maier 2020).

Lastly, a system of compatible allometric equations was fitted to predict the biomass content of each biomass component. The first step consisted of testing 13 biomass models.
obtained from forestry literature (Table 2). Each biomass fraction was fitted individually using the SAS 9.4 MODEL procedure of the SAS/STAT statistical program (SAS Institute Inc. 2020). The different fractions were fitted as unique fractions in the analysis. The statistical parameters (the sum of squared estimated of errors (SSR) and the determination coefficient ($R^2$)) from the 13 models fitted for each fraction were then compared to choose the best model. Weighted fitting using the inverse of the variance of the residuals ($\sigma_i^2$) was applied to correct the heteroscedasticity problem (Parresol 2001). Exponent values of $k$ were added to the fitting program. After fitting, the models were again subjected to heteroscedasticity tests to verify their correctness. The best models for each fraction were fitted simultaneously by the seemingly unrelated regressions method (SUR) to guarantee the additivity of the system (SAS Institute Inc., 2020). The SUR estimates were obtained by applying the SAS 9.4 MODEL procedure (SAS Institute Inc., 2020), using the parameters obtained in the individual fitting as initializers.

**RESULTS**

In 2017, 37 *Pinus pinaster* and 68 *Pinus halepensis* seedlings – all four years old – were harvested to analyse biomass production but 6 *Pinus pinaster* and 10 *Pinus halepensis* seedlings were not included in the biomass analysis because they served as buffers for edge effects. The *Pinus halepensis* seedlings were bigger than *Pinus pinaster* in terms of basal diameter and total height: with a mean basal diameter of almost 34 mm and a mean total height of approximately 165 cm. *Pinus pinaster* had a mean basal diameter of almost 10 mm and mean total height of 45 cm for (Table 3 and Supplementary Figure 2). *Pinus halepensis* seedling biomass was also higher, with 1504 g mean total biomass compared to approximately 39 g for *Pinus pinaster* (Table 4). In both cases, needle biomass was
higher than the other compartments, but root biomass was lower than the other compartments for *Pinus halepensis* and branch biomass was lower than the other compartments for *Pinus pinaster* (Figure 2). This was reflected in belowground/aboveground biomass ratio values of 0.23 for *Pinus halepensis* and 0.41 for *Pinus pinaster* (Table 4 and Figure 3).

**Biomass production – linear regressions – the Dahlhausen et al. (2017) method**

Four linear regression models were fitted for total, aboveground and belowground biomass along with the belowground to aboveground biomass ratio for each species (Table 5). For *Pinus halepensis*, Equation (4) \( \ln W_i = b_1 + b_2 \ln(D) + b_3 \ln(H) + b_4 \ln(GS) \) showed better fit based on the level of significance of the parameters and adjusted R\(^2\) for all fitted biomass except the ratio. None of the equations was considered for the belowground to aboveground biomass ratio because the predictors were not significant at all. In the case of *Pinus pinaster* however, equation (2) \( \ln W_i = b_1 + b_2 \ln(D) + b_3 \ln(H) \) showed better fit for all the biomass except the ratio. Once again, the predictors were not significant and none of the equations was considered for this variable. In summary, basal diameter, total height and seedling growing space were better predictors for estimating *Pinus halepensis* biomass, whereas basal diameter and total height were better for *Pinus pinaster*.

**Biomass proportions - Dirichlet regressions**

Dirichlet regressions were fitted to analyse the impact of basal diameter, total height and growing space on the biomass of the compartments considered for each species. Twenty-one models were fitted using equations (5) to (12) and combinations of them (Supplementary Tables 1 and 2). Based on the significance of the parameters and AIC differences (\( \Delta_i \)), models 1, 6, 9 and 10 for *Pinus halepensis* and model 2 for *Pinus pinaster*
could be considered useful. Model 9 provided the best fit for *Pinus halepensis*. In this case, basal diameter was significant in the relative biomass of tree compartments, total height was not significant in any case and growing space was only significant for root biomass. In Model 2 for *Pinus pinaster*, basal diameter and total height were significant in the biomass of relative compartments. According to Maier (2014), the expected values of the biomass proportions can be estimated as follows for *Pinus halepensis*:

\[
\alpha_0 = 0.125 \times D^{2.498} \times GS^{0.008} + 0.135 \times D^{2.490} + 0.161 \times D^{2.457} + 0.119 \times D^{2.522}
\]

\[
E_{\text{roots}} = \frac{0.125 \times D^{2.498} \times GS^{0.008}}{\alpha_0}
\]

\[
E_{\text{stem}} = \frac{0.135 \times D^{2.490}}{\alpha_0}
\]

\[
E_{\text{needles}} = \frac{0.161 \times D^{2.457}}{\alpha_0}
\]

\[
E_{\text{thin branches}} = \frac{0.119 \times D^{2.522}}{\alpha_0}
\]

For *Pinus halepensis*, the growing space was only significant for the \( \alpha \) coefficient of root biomass, but all biomass proportions can be estimated considering the growing space because of \( \alpha_0 \). Fitted biomass proportions against growing spaces are represented in Figure 4, were the minimum and maximum basal diameter of the harvested seedlings were considered for calculating fitted proportions (Table 3). Basal diameter only affects stem and root proportions, but higher diameters increased thin branches proportions and decreased needle proportions. Growing space had a small effect on biomass proportions.

According to Maier (2014), the expected values of the biomass proportions can be estimated as follows for *Pinus pinaster*:
\[ \alpha_0 = 1.07 \times 10^{-11} \times D^{7.798} \times H^{2.596} + 3.12 \times 10^{-11} \times D^{7.593} \times H^{2.463} + 6.53 \times 10^{-11} \times D^{7.502} \times H^{2.409} + 5.233 \times 10^{-12} \times D^{7.514} \times H^{2.875} \]

\[ E_{\text{roots}} = 1.07 \times 10^{-11} \times D^{7.798} \times H^{2.596} / \alpha_0 \]

\[ E_{\text{stem}} = 3.12 \times 10^{-11} \times D^{7.593} \times H^{2.463} / \alpha_0 \]

\[ E_{\text{needles}} = 6.53 \times 10^{-11} \times D^{7.502} \times H^{2.409} / \alpha_0 \]

\[ E_{\text{thin branches}} = 5.233 \times 10^{-12} \times D^{7.514} \times H^{2.875} / \alpha_0 \]

For *Pinus pinaster*, basal diameter and total height were significant but growing space was not. Fitted biomass proportions of each component are represented in Figure 5 considering minimum, average and maximum values for tree size (basal diameter and total height) of harvested *Pinus pinaster* seedlings (Table 3). In general, higher sizes increased biomass proportions for roots and thin branches but decreased needle and stem biomass.

**Biomass components – SUR method**

A system of compatible allometric equations for estimating biomass for tree components was obtained for both species. Results of the individual models showed the best models for each biomass fraction (roots, stem, needles and thin branches) of each species (Table 6).

For *Pinus halepensis*, Model 9 gave the highest value of $R^2$ in total biomass, thin branches and needles, while models 7 and 10 were the best for roots and stem, respectively.

For *Pinus pinaster*, though the highest values of $R^2$ were obtained from Model 12 for thin branches and root fractions, Model 10 for the stem, Model 13 for needles and Model 9
for total biomass; the best options for subsequent simultaneous fitting were Model 1 for needles and stem fractions, Model 2 for thin branches and Model 7 for the root fraction. They were chosen because they had the smaller number of variables linked to the small amount of data. Anyway, they have an appropriated biological behaviour.

The results of the final simultaneous fitting and the statistics for bias and precision are presented in Table 7. All parameters were significant at the 95% confidence level and all models included basal diameter and total height as independent variables.

**DISCUSSION**

Interest in biomass analysis for young trees is growing because natural regeneration stands are currently seen as significant carbon sinks (Pugh et al. 2019). Annighöfer et al. (2016) fitted aboveground biomass equations for 19 species in Europe. Cotillas et al. (2016) fitted above- and below-ground biomass equations of *Quercus ilex* and *Quercus cerrioides* in the Mediterranean basin. Alfaro-Sánchez et al. (2015) studied the effect of age or forest management on biomass accumulation and biomass allocation in post-fire *Pinus halepensis* regeneration. Dahlhausen et al. (2017) studied biomass production in young *Quercus robur* trees, also using Nelder rings. Kuznetsova et al. (2011) analyzed aboveground biomass of eight-year-old *Pinus sylvestris* and *Pinus contorta* trees in Estonia… In the present work, above- and below-ground biomass of four-year-old *P. pinaster* and *P. halepensis* trees were studied using various statistical methods. Results from this innovative approach showed that growing space, which could be related to density thanks to the Nelder wheel design, was significant for *Pinus halepensis* in the fitted linear regressions to estimate biomass production and in the Dirichlet regressions for biomass proportion estimations. These results confirmed our hypothesis that biomass production would be affected by density, but only for *Pinus halepensis*. Other researchers
have observed a relationship between the biomass of young trees and stand density. Dahlhausen et al. (2017) found that relative biomass of tree compartments was dependent on stand density, especially for belowground biomass, as we found for *Pinus halepensis*. Alfaro-Sánchez et al. (2015) also observed an effect of tree density on biomass proportions of post-fire *Pinus halepensis* regeneration, though their results differed slightly from ours. They reported higher stem and crown biomass in moderate densities (800 trees/ha) along with an increase of stem biomass and a decrease of crown biomass when tree density increased. In contrast, our findings indicate higher stem and crown densities along with lower root biomass at higher tree densities, but without the decreasing effect for the crown. However, total, aboveground and belowground biomass production were generally higher at lower densities. These minor differences between the two studies may be due to age differences in trees; the earlier study included trees ranging from five to 16 years old, while the present study looked only at four-year-old trees.

Our results showed a general increment of total biomass for *Pinus halepensis* at lower densities and this positive effect increased root biomass proportion at the expense of aboveground biomass. Our findings showed the importance of root biomass in carbon stock estimations, especially when considering the intrinsic characteristics of Mediterranean forests. In these ecosystems, disturbances such as fire or grazing intensify soil erosion, extreme climate conditions compromise natural regeneration and global changes in climate and land use directly affect fire dynamics (Scarascia-Mugnozza et al. 2000; Moriondo et al. 2006). Some of the recommendations to improve forest adaptation to climate change in Spain are based on silvicultural experience with promoting mixed stands or keeping densities relatively low (Serrada et al. 2011). Pre-commercial thinning is considered a good option for increasing the size, reproductive potential and biomass of trees in high-density post-fire regeneration stands in Mediterranean ecosystems (Madrigal
et al. 2004; Tsitsoni et al. 2004; Navarro et al. 2010; Ruano et al. 2013; AlfaroSánchez et al. 2015). Our results confirmed this recommendation, because increased root biomass promotes resilience against climate change. For instance, Mayoral et al. (2016) found that higher root biomass was related to drought adaptation in a study on the effect of water availability in the establishment phase of three coexisting Mediterranean species.

No big differences in biomass fractions were found between species, but differences in biomass production and component analysis were observed, confirming our hypothesis that biomass production would be dissimilar between species. Kuznetsova et al. (2011) also found differences between species in terms of growth and biomass allocation when analyzing aboveground biomass of eight-year-old Pinus sylvestris and Pinus contorta trees in Estonia. In the present work, the effect of density varied between species and was significant for Pinus halepensis but not significant for Pinus pinaster. Accordingly, we can also confirm the hypothesis that the effect of density would be different for each species. The non-significant effect for Pinus pinaster in our study contrasts with results from Ritson and Sochacky (2003), who observed an increase in root:shoot ratio for young Pinus pinaster trees of the same age or size in open spaces, when compared to closed spaces.

Ruiz-Peinado et al. (2011) fitted biomass equations for estimating the carbon sinks of some Spanish species, among which were Pinus pinaster and Pinus halepensis. The same biomass equations and the same fitting method (SUR method) were applied, but our selected biomass equations were different than biomass equations defined by Ruiz-Peinado et al. (2011). Moreover, in that study, thin branches (maximum diameter > 2 cm) and needles were fitted together for adult trees, while our findings were obtained using one model for each fraction. Tree age might explain the differences. Vanninen (2004) observed tree age and tree size were significant for allocation in the biomass components.
of *Pinus sylvestris*. A graphical comparison between their models and ours, however, showed a similar trend in exponential increase for estimated biomass in relation to diameter increase (Supplementary Figure 3).

Our results regarding biomass proportions were similar to those of Alfaro-Sánchez et al. (2015) for post-fire regeneration of *Pinus halepensis*. They also observed the highest biomass for the crown fraction (branches, cones and needles), though the second-highest biomass fraction was the roots in their case and the stem in our case. However, our results for *Pinus pinaster* biomass proportions differed from those of Ritson and Sochacky (2003). They reported a decrease in root:shoot ratio as tree size increased while our results indicated an increase for larger trees. The biomass proportions also varied between adult and young trees, though no significant differences were found between species. Both had proportions of around 20% allocated to stem biomass, less than 60% to crown biomass with thin branches and needles, and around 20% to root biomass. Ruiz-Peinado et al. (2011) found differences between species, mainly for stem and crown biomass. Specifically, more than 60% was allocated to stem biomass and only 15% to crown biomass for *Pinus pinaster* but almost 40% to stem biomass and almost 40% to crown biomass for *Pinus halepensis*. The main difference we found between species was in the crown (branches and needles), with approximately 23% in thin branches biomass and almost 35% in needles biomass for *Pinus halepensis* compared to around 8% thin branches biomass and almost 50% needles biomass for *Pinus pinaster*. This could be due to the size differences between the two species (Vanninen 2004; Ruano et al. 2021).

Fitted equations included basal diameter and total height as independent variables, based on their potential to produce models for large-scale applications and because they explain most of the variability in observed tree biomass. Diameter at breast height is used more often for biomass analysis but none of the *Pinus pinaster* seedlings in our study was
higher than 1.3 m. As an alternative, diameter at root collar seems to be more appropriate for young trees (Annighöfer et al. 2016). It was indicated as the best variable for estimating biomass for young *Picea Abies* L. Karst trees in Slovakia (Pajtík et al. 2008) or *Pinus pinaster* young trees with a diameter at breast height (DBH) below 10 cm in Australia (Ritson and Sochacki 2003). Other tree or stand level variables have also been used to fit biomass equations to accommodate the specific characteristics of each forest or species (Herrero et al. 2014; Risio et al. 2014).

The Nelder design has its strengths and weaknesses. Most traditional designs require much larger areas to analyse different stand densities compared to the Nelder design (Vanclay et al. 2013). It provides an advantage over factorial designs because the experimental unit is the tree rather than the plot. Its main weakness for this study was mortality but double planting was unfeasible because one of the aims of the experimental site was to simulate natural conditions. In the present work, the growing space was recalculated using Voronoi polygons to avoid removing the seedlings surrounding dead seedlings. This made it possible to analyse biomass production in seedlings and young trees by simulating natural conditions. In other words, the Nelder design allows for optimal analysis of tree dynamics in a single plot, but conceptual and statistical changes were necessary for some of the analyses (Uhl et al. 2015).

**CONCLUSIONS**

A new set of biomass equations for young *Pinus pinaster* and *Pinus halepensis* trees was developed and presented in the present work. These equations allowed us to estimate above- and below-ground biomass for young trees with higher accuracy, using four-year-old trees and competition statuses ranging from low-density values favoring successful natural regeneration (1000 trees/ha) to the high-density values found in postfire natural
generation (80000 trees/ha). The Nelder wheel design made this possible, marking a new research milestone.

Further research is needed on biomass production and proportions for seedlings and saplings because biomass equations for adult trees cannot be extrapolated to younger trees.

Once again, we note the importance of belowground biomass and its value in total biomass production (approximately 20% of total biomass for both species). The effect of density on biomass production was only significant for *Pinus halepensis*, but the effect of density would have been different if root biomass had not been considered in the present study. Lower densities increased root biomass proportion at the expense of aboveground biomass. Currently, this positive effect is especially important in promoting management to improve tree resilience.

**DECLARATIONS**

Consent to participate: yes

Ethics approval: not applicable

Consent for publication: not applicable

Availability of data and material: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Authors' contributions

Irene RUANO: designed the experiment, conducted field measurements, ran the first version of the data analysis, drafted the manuscript and improved it by incorporating the comments and suggestions of the other authors.

Celia HERRERO: ran the data analysis and improved the manuscript with helpful comments.

Felipe BRAVO: defined the hypothesis, designed the experiment, supervised the work, improved the manuscript with helpful comments and coordinated the research project.

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REFERENCES

Aguirre A, del Río M, Condés S (2019) Productivity Estimations for Monospecific and Mixed Pine Forests along the Iberian Peninsula Aridity Gradient. Forests 10:430. https://doi.org/10.3390/f10050430

Alfaro-Sánchez R, López-Serrano FR, Rubio E, et al (2015) Response of biomass allocation patterns to thinning in Pinus halepensis differs under dry and semiarid Mediterranean climates. Ann For Sci 72:595–607. https://doi.org/10.1007/s13595-015-0480-y
Alvarez S, Ortiz C, Díaz-Pinés E, Rubio A (2016) Influence of tree species composition, thinning intensity and climate change on carbon sequestration in Mediterranean mountain forests: a case study using the CO2Fix model. Mitig Adapt Strateg Glob Chang 21:1045–1058. https://doi.org/10.1007/s11027-014-9565-4

Annighöfer P, Ameztegui A, Ammer C, et al (2016) Species-specific and generic biomass equations for seedlings and saplings of European tree species. Eur J For Res 135:313–329. https://doi.org/10.1007/s10342-016-0937-z

Callaway RM, DeLucia EH, Moore D, et al (1996) Competition and facilitation: Contrasting effects of Artemisia tridentata on desert vs montane pines. Ecology 77:2130–2141. https://doi.org/10.2307/2265707

Callaway RM, Walker LR (1997) COMPETITION AND FACILITATION: A SYNTHETIC APPROACH TO INTERACTIONS IN PLANT COMMUNITIES. Ecology 78:1958–1965. https://doi.org/10.1890/0012-9658(1997)078[1958:CAFASA]2.0.CO;2

Calvo L, Santalla S, Valbuena L, et al (2007) Post-fire natural regeneration of a Pinus pinaster forest in NW Spain. Plant Ecol 197:81–90. https://doi.org/10.1007/s11258-007-9362-1

Chirino E, Bonet a., Bellot J, Sánchez JR (2006) Effects of 30-year-old Aleppo pine plantations on runoff, soil erosion, and plant diversity in a semi-arid landscape in south eastern Spain. Catena 65:19–29. https://doi.org/10.1016/j.catena.2005.09.003

Cotillas M, Espelta JM, Sánchez-Costa E, Sabaté S (2016) Aboveground and belowground biomass allocation patterns in two Mediterranean oaks with
contrasting leaf habit: an insight into carbon stock in young oak coppices. Eur J For Res 135:243–252. https://doi.org/10.1007/s10342-015-0932-9

Dahlhausen J, Uhl E, Heym M, et al (2017) Stand density sensitive biomass functions for young oak trees at four different European sites. Trees 31:1811–1826. https://doi.org/10.1007/s10342-017-1586-7

De las Heras J, Martínez-Sánchez JJ, González-Ochoa AI, et al (2002) Establishment of Pinus halepensis Mill. saplings following fire: effects of competition with shrub species. Acta Oecologica 23:91–97. https://doi.org/10.1016/S1146-609X(02)01138-4

Derak M, Cortina J (2014) Multi-criteria participative evaluation of Pinus halepensis plantations in a semiarid area of southeast Spain. Ecol Indic 43:56–68. https://doi.org/10.1016/j.ecolind.2014.02.017

Djomo AN, Chimi CD (2017) Tree allometric equations for estimation of above, below and total biomass in a tropical moist forest: Case study with application to remote sensing. For Ecol Manage 391:184–193. https://doi.org/10.1016/j.foreco.2017.02.022

Gómez-Aparicio L, Valladares F, Zamora R, Luis Quero J (2005) Response of tree seedlings to the abiotic heterogeneity generated by nurse shrubs: an experimental approach at different scales. Ecology (Cop) 28:757–768. https://doi.org/10.1111/j.2005.0906-7590.04337.x

Helliwell DR, Harrison AF (1979) Effects of light and weed competition on the growth of seedlings of four tree species on a range of soils. Forestry 31:37–46

Herrero C, Juez L, Tejedor C, et al (2014) Importance of root system in total biomass
for Eucalyptus globulus in northern Spain. Biomass and Bioenergy 67:212–222.

https://doi.org/10.1016/j.biombioe.2014.04.023

Hof AR, Dymond CC, Mladenoff DJ (2017) Climate change mitigation through adaptation: the effectiveness of forest diversification by novel tree planting regimes. Ecosphere 8:e01981. https://doi.org/10.1002/ecs2.1981

Jactel H, Brockerhoff EG (2007) Tree diversity reduces herbivory by forest insects. Ecol Lett 10:835–848. https://doi.org/10.1111/j.1461-0248.2007.01073.x

Jandl R, Spathelf P, Bolte A, Prescott CE (2019) Forest adaptation to climate change— is non-management an option? Ann For Sci 76:1–13.

https://doi.org/10.1007/s13595-019-0827-x

Kerr G (2003) Effects of spacing on the early growth of planted Fraxinus excelsior L. Can J For Res 33:1196–1207. https://doi.org/10.1139/x03-041

Khan MNI, Shil MC, Azad MS, et al (2018) Allometric relationships of stem volume and stand level carbon stocks at varying stand density in Swietenia macrophylla King plantations, Bangladesh. For Ecol Manage 430:639–648.

https://doi.org/10.1016/j.foreco.2018.09.002

Kuehne C, Kublin E, Pyttel P, Bauhus J (2013) Growth and form of Quercus robur and Fraxinus excelsior respond distinctly different to initial growing space: results from 24-year-old Nelder experiments. J For Res 24:1–14.

https://doi.org/10.1007/s11676-013-0320-6

Kuznetsova T, Tilk M, Pärn H, et al (2011) Growth, aboveground biomass, and nutrient concentration of young Scots pine and lodgepole pine in oil shale post-mining landscapes in Estonia. Environ Monit Assess 183:341–350.
Lortie CJ, Callaway RM (2006) Re-analysis of meta-analysis: support for the stress-gradient hypothesis. J Ecol 94:7–16. https://doi.org/10.1111/j.1365-2745.2005.01066.x

Madrigal J, E MH, Hernando C, et al (2004) Respuesta a Corto Plazo del Regenerado Post-Incendio de Pinus pinaster Ait . a Clareos Mecanizados Intensos. Silva Lusit 12:1–14

Maestre FT, Cortina J (2004) Are Pinus halepensis plantations useful as a restoration tool in semiarid Mediterranean areas? For Ecol Manage 198:303–317. https://doi.org/10.1016/j.foreco.2004.05.040

Maestre FT, Valladares F, Reynolds JF (2005) Is the change of plant-plant interactions with abiotic stress predictable? A meta-analysis of field results in arid environments. J Ecol 93:748–757. https://doi.org/10.1111/j.1365-2745.2005.01017.x

Maestre FT, Valladares F, Reynolds JF (2006) The stress-gradient hypothesis does not fit all relationships between plant-plant interactions and abiotic stress: further insights from arid environments. J Ecol 94:17–22. https://doi.org/10.1111/j.1365-2745.2005.01089.x

Maier MJ (2014) DirichletReg: Dirichlet Regression for Compositional Data in R

Mayoral C, Pardos M, Sánchez-González M, et al (2016) Ecological implications of different water use strategies in three coexisting mediterranean tree species. For Ecol Manage 382:76–87. https://doi.org/10.1016/j.foreco.2016.10.002
Moriondo M, Good P, Durao R, et al (2006) Potential impact of climate change on fire risk in the Mediterranean area. Clim Res 31:85–95

Navarro FB, Jiménez MN, Cañadas EM, Gallego E (2010) Effects of different intensities of overstory thinning on tree growth and understory plant-species productivity in a semi-arid Pinus halepensis Mill. afforestation. 19:410–417

Pajtik J, Konopka B, Lukac M (2008) Biomass functions and expansion factors in young Norway spruce (Picea abies [L.] Karst) trees. For Ecol Manage 256:1096–1103. https://doi.org/10.1016/j.foreco.2008.06.013

Parresol BR (2001) Additivity of nonlinear biomass equations. Can J For Res 31:865–878. https://doi.org/10.1139/x00-202

Parrott DL, Brinks JS, Lhotka JM (2012) Designing Nelder wheel plots for tree density experiments. New For 43:245–254. https://doi.org/10.1007/s11056-011-9278-4

Piotto D, Viquez E, Montagnini F, Kanninen M (2004) Pure and mixed forest plantations with native species of the dry tropics of Costa Rica: a comparison of growth and productivity. For Ecol Manage 190:359–372. https://doi.org/10.1016/j.foreco.2003.11.005

Poudel KP, Temesgen H (2016) Methods for estimating aboveground biomass and its components for Douglas-fir and lodgepole pine trees. Can J For Res 46:77–87. https://doi.org/10.1139/cjfr-2015-0256

Pretzsch H, del Rio M, Ammer C, et al (2015) Growth and yield of mixed versus pure stands of Scots pine (Pinus sylvestris L.) and European beech (Fagus sylvatica L.) analysed along a productivity gradient through Europe. Eur J For Res 134:927–947. https://doi.org/10.1007/s10342-015-0900-4
Pretzsch H, del Río M, Biber P, et al (2019a) Maintenance of long-term experiments for unique insights into forest growth dynamics and trends: review and perspectives. Eur. J. For. Res. 138:165–185

Pretzsch H, Steckel M, Heym M, et al (2019b) Stand growth and structure of mixed-species and monospecific stands of Scots pine (Pinus sylvestris L.) and oak (Q. robur L., Quercus petrea (Matt.) Liebl.) analysed along a productivity gradient through Europe. Eur J For Res 1–19. https://doi.org/10.1007/s10342-019-01233-y

Pugh TAM, Lindeskog M, Smith B, et al (2019) Role of forest regrowth in global carbon sink dynamics. Proc Natl Acad Sci U S A 116:4382–4387. https://doi.org/10.1073/pnas.1810512116

Resco De Dios V, Fischer C, Colinas C (2007) Climate change effects on mediterranean forests and preventive measures. New For. 33:29–40

Riofrío J, del Río M, Maguire D, Bravo F (2019) Species Mixing Effects on Height–Diameter and Basal Area Increment Models for Scots Pine and Maritime Pine. Forests 10:249. https://doi.org/10.3390/f10030249

Risio L, Herrero C, Bogino SM, Bravo F (2014) Aboveground and belowground biomass allocation in native Prosopis caldenia Burkart secondaries woodlands in the semi-arid Argentinean pampas. Biomass and Bioenergy 66:249–260. https://doi.org/10.1016/j.biombioe.2014.03.038

Ritson P, Sochacki S (2003) Measurement and prediction of biomass and carbon content of Pinus pinaster trees in farm forestry plantations, south-western Australia. For Ecol Manage 175:103–117. https://doi.org/10.1016/S0378-1127(02)00121-4
Rodríguez-García E, Bravo F, Spies TA (2011) Effects of overstorey canopy, plant–
plant interactions and soil properties on Mediterranean maritime pine seedling
dynamics. For Ecol Manage 262:244–251. https://doi.org/10.1016/j.foreco.2011.03.029

Rodríguez-García E, Juez L, Bravo F (2010) Environmental influences on post-harvest
natural regeneration of Pinus pinaster Ait. in Mediterranean forest stands submitted
to the seed-tree selection method. Eur J For Res 129:1119–1128. https://doi.org/10.1007/s10342-010-0399-7

Ruano I, Rodríguez-García E, Bravo F (2013) Effects of pre-commercial thinning on
growth and reproduction in post-fire regeneration of Pinus halepensis Mill. Ann
For Sci 70:357–366. https://doi.org/10.1007/s13595-013-0271-2

Ruiz-Peinado R, del Río M, Montero G (2011) New models for estimating the carbon
sink capacity of Spanish softwood species | Ruiz-Peinado | Forest Systems. For
Syst 11:176–188

Scarascia-Mugnozza G, Oswald H, Piussi P, Radoglou K (2000) Forests of the
Mediterranean region: gaps in knowledge and research needs. For Ecol Manage
132:97–109. https://doi.org/10.1016/S0378-1127(00)00383-2

Scholes RJ, Archer SR (1997) Tree-Grass Interactions in Savannas. Annu Rev Ecol
Syst 28:517–544

Thuiller W (2003) BIOMOD - optimizing predictions of species distributions and
projecting potential future shifts under global change. Glob Chang Biol 9:1353–
1362. https://doi.org/10.1046/j.1365-2486.2003.00666.x

Tsitsoni T, Ganatsas P, Zagas T, Tsakaldimi M (2004) Dynamics of postfire
regeneration of Pinus brutia Ten. in an artificial forest ecosystem of northern Greece. Plant Ecol (formerly Veg 171:165–174.

https://doi.org/10.1023/B:VEGE.0000029385.60590.fc

Uhl E, Biber P, Ulbricht M, et al (2015) Analysing the effect of stand density and site conditions on structure and growth of oak species using Nelder trials along an environmental gradient: experimental design, evaluation methods, and results. For Ecosyst 2:17. https://doi.org/10.1186/s40663-015-0041-8

Vanclay JK (2006) Experiment designs to evaluate inter- and intra-specific interactions in mixed plantings of forest trees. For Ecol Manage 233:366–374. https://doi.org/10.1016/j.foreco.2006.05.034

Vanclay JK, Lamb D, Erskine PD, Cameron DM (2013) Spatially explicit competition in a mixed planting of Araucaria cunninghamii and Flindersia brayleyana. Ann For Sci 70:611–619. https://doi.org/10.1007/s13595-013-0304-x

Vanninen P (2004) Allocation of above-ground growth in Pinus sylvestris - Impacts of tree size and competition. Silva Fenn 38:155–166. https://doi.org/10.14214/sf.425

Vinh T Van, Marchand C, Linh TVK, et al (2019) Allometric models to estimate above-ground biomass and carbon stocks in Rhizophora apiculata tropical managed mangrove forests (Southern Viet Nam). For Ecol Manage 434:131–141. https://doi.org/10.1016/j.foreco.2018.12.017

TABLES
Table 1. Radial length, potential growing space and associated tree density along each spoke of the Nelder wheel plots.

| Ring | Radial length (m) | Growing space for a seedling (m²) | Tested densities (seedlings/ha) |
|------|-------------------|-----------------------------------|--------------------------------|
| 0    | 1.14              | -                                 | -                              |
| 1    | 1.45              | 0.13                              | 80000                          |
| 2    | 1.85              | 0.20                              | 49162.68                       |
| 3    | 2.36              | 0.33                              | 30212.11                       |
| 4    | 3.01              | 0.54                              | 18566.36                       |
| 5    | 3.84              | 0.88                              | 11409.65                       |
| 6    | 4.90              | 1.43                              | 7011.61                        |
| 7    | 6.25              | 2.32                              | 4308.87                        |
| 8    | 7.97              | 3.78                              | 2647.94                        |
| 9    | 10.17             | 6.15                              | 1627.25                        |
| 10   | 12.97             | 10.00                             | 1000                           |
| 11   | 16.55             | -                                 | -                              |

Table 2. Biomass models tested for different tree components using seemingly unrelated regressions (SUR). \( W_i \): biomass component (g); D: basal diameter (mm); Ht: total height (cm); \( b_i \): model parameters.

| Model |
|-------|
| 1 \( W_i=b_1*D*Ht \) |
| 2 \( W_i=b_1*D^2*Ht \) |
| 3 \( W_i=b_1*D+b_2*D^2 \) |
| 4 \( W_i=b_1*D+b_2*D^2+b_3*D^2*H \) |
| 5 \( W_i=b_1*D+ b_2*H \) |
| 6 \( W_i=b_1*D^2+ b_2*D^2*H \) |
| 7 \( W_i=b_1* D^2+ b_2*H \) |
| 8 \( W_i=b_1*D^2+b_2*H+ b_3*D^2*H \) |
| 9 \( W_i=b_1*D^2+ b_2*D*H \) |
| 10 \( W_i=b_1*D^2*H+ b_2*D*H \) |
| 11 \( W_i=b_1*D^{b_2}*H^{b_3} \) |
| 12 \( W_i=b_1* D^{b_2} \) |
| 13 \( W_i=b_1*(D*H)^{b_2} \) |
Table 3. Characteristics of the harvested seedlings: sampling size (n), mean, maximum and minimum values of basal diameter (mm) and mean, maximum and minimum values of total height (cm).

| Species             | n  | mean | max | min | Mean  | max | min |
|---------------------|----|------|-----|-----|-------|-----|-----|
| *Pinus halepensis*  | 68 | 33.93| 62.00 | 11.60 | 164.78 | 250 | 67  |
| *Pinus pinaster*    | 37 | 9.36 | 24.47 | 3.85 | 45.23  | 116 | 13  |
Table 4. Biomass production of the harvested seedlings: mean, maximum and minimum values (g) of compartment biomass, total biomass, aboveground biomass (sum of stem, needles and thin branches biomass) and belowground/aboveground biomass ratio. Maximum and minimum values are given in parenthesis.

| Species       | Roots (belowground) | Stem     | Needles  | Thin branches | Total    | Aboveground | Ratio    |
|---------------|---------------------|----------|----------|---------------|----------|-------------|----------|
| *P. halepensis* | 275.22              | 505.89   | 364.78   | 1503.96       | 1228.74  | 0.23        |
|               | (20.85-802.21)      | (30.12-1312.68) | (9.53-1027.86) | (92.15-3774.04) | (63.48-3139.96) | (0.09-0.45) |
| *P. pinaster* | 10.12               | 17.03    | 3.94     | 38.53         | 28.41    | 0.41        |
|               | (0.67-107.77)       | (0.43-103.76) | (0.00-31.09) | (1.41-284.16) | (0.74-176.39) | (0.16-1.10) |
Table 5. Linear regression models (Equations 1, 2, 3 and 4) for total, aboveground and belowground biomass and belowground/aboveground biomass ratio of *Pinus halepensis* and *Pinus pinaster* seedlings: estimated parameters, significance and adjusted R-squared of each model.

| Species         | Model       | b₁         | b₂ (D)       | b₃ (Ht)       | b₄ (GS)       | R²adj |
|-----------------|-------------|------------|--------------|---------------|---------------|-------|
| *P. halepensis* | Total       | 0.1587     | 1.9954       | -4.4080       | 0.2987        | 0.7508 |
|                 |             | ns         | ***          | ***           | ***           |       |
|                 | Aboveground | -0.1196    | 2.0159       | -4.8494       | -0.0238       | 0.7387 |
|                 |             | ns         | ***          | ***           | ***           |       |
|                 | Belowground | -1.4453    | 1.9646       | -5.3117       | -1.1323       | 0.7232 |
|                 |             | *         | ***          | ***           | ***           |       |
|                 | Ratio       | -1.3256    | -0.0514      | -0.4620       | -1.1084       | -      |
|                 |             | **         | ns           | ns            | ns            | 0.0143 |
|                 |             | ns         | 0.0435       | -0.2347       | -0.1162       | 0.0159 |
| *P. pinaster*   | Total       | -2.7527    | 2.7178       | -0.9498       | -2.7629       | 0.9209 |
|                 |             | ***        | ***          | ***           | ***           |       |
|                 |             | -2.7629    | 2.7224       | 0.0212        | -0.0179       | 0.9191 |
|                 |             | ns         | ns           | ns            | ns            |       |
|                 |             | -4.0022    | 1.9327       | 0.7938        | 0.0353        | 0.9552 |
|                 |             | ***        | ***          | ***           | ***           |       |
| Model | W_needles | W_thin branches | W_stem | W_root | W_total | W_needles | W_thin branches | W_stem | W_root | W_total | W_needles | W_thin branches | W_stem | W_root | W_total |
|-------|------------|-----------------|--------|--------|---------|------------|-----------------|--------|--------|---------|------------|-----------------|--------|--------|---------|
| 1     | 0.672      | 0.6228          | 0.704  | 0.623  | 0.695   | **0.952**   | 0.875           | 0.935  | 0.814  | 0.945   | 0.841      | 0.942           | 0.924  | 0.945  |
| 2     | 0.595      | 0.5723          | 0.604  | 0.433  | 0.595   | 0.807       | **0.857**       | 0.729  | 0.979  | 0.924   | 0.814      | 0.942           | 0.966  | 0.814  |
| 3     | 0.714      | 0.6765          | 0.692  | 0.626  | 0.728   | -           | -               | -      | -      | -       | -          | -               | -      | -      |
| 4     | -          | -               | -      | -      | -       | -           | -               | -      | -      | -       | -          | -               | -      | -      |
| 5     | 0.665      | 0.5949          | -      | -      | 0.646   | -           | -               | -      | -      | -       | -          | -               | -      | -      |

Aboveground

|          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
|          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
|          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
|          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
|          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
|          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
|          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
|          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |

Belowground

|          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
|          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
|          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
|          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
|          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
|          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
|          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
|          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |

**Table 6.** Model comparison of each biomass fraction: coefficient of determination ($R^2$) of each model (Table 2). Bold type indicates the best model chosen for each biomass fraction. Bold type underlined indicates the selected model. Nc: no convergence; - denotes non-significant parameters.

D: basal diameter; Ht: total height; GS: growing space for the seedling. Significance values: ***: p-value <0.001; **: p-value <0.01; *: p-value < 0.05; ·: p-value < 0.1; ns: non-significant effect.
Table 7. Final simultaneous biomass equations for *Pinus halepensis* and *Pinus pinaster*. SSE: sum of squared error; MSE: mean squared error; RMSE: root mean squared error; $R^2$: coefficient of determination; $R^2_{adj}$: adjusted coefficient of determination; D: basal diameter; Ht: total height.

| Species       | Model                        | SSE    | MSE   | RMSE  | $R^2$ | $R^2_{adj}$ |
|---------------|------------------------------|--------|-------|-------|-------|-------------|
| *P. halepensis* | $W_{\text{thin branches}}=0.160399*D^2+0.025503*D*Ht$ | 14529  | 23818 | 154.3 | 0.671 | 0.671       |
|               | $W_{\text{stem}}=0.000598*D^2*Ht+0.037706*D*Ht$ | 10669  | 17490 | 132.3 | 0.727 | 0.727       |
|               | $W_{\text{roots}}=0.13254*D^2+0.529169*Ht$ | 76804  | 12590 | 112.2 | 0.613 | 0.613       |
|               | $W_{\text{needles}}=0.207597*D^2+0.039945*D*Ht$ | 20222  | 33152 | 182.1 | 0.731 | 0.731       |
| *P. pinaster*  | $W_{\text{thin branches}}=0.000679*D^2*Ht$ | 43.829 | 1.593 | 1.262 | 0.812 | 0.816       |
|               | $W_{\text{stem}}=0.014081*D*Ht$ | 61.825 | 2.248 | 1.499 | 0.873 | 0.876       |
|               | $W_{\text{roots}}=0.059415*D^2+0.040243*Ht$ | 60.620 | 2.245 | 1.498 | 0.862 | 0.862       |
FIGURES

Figure 1. Location of the experimental site and Nelder (1962) wheel design with a climate diagram (data from Sistema de Clasificación Bioclimática Mundial (1996–2009)). Species distribution along the spokes of the temporary wheel plot. Mixture levels shown by percentage examples. The first harvest occurred in 2017; the second harvest is forthcoming.

Figure 2. Relative compartment biomass composition per species.
Figure 3. Belowground/aboveground biomass ratio of each species

Figure 4. Fitted values of the Dirichlet regression model for each component of *Pinus halepensis*. Fitted values for the minimum basal diameter of harvested *Pinus halepensis* seedlings are represented by dashed lines and fitted values for the maximum basal diameter of harvested *Pinus halepensis* seedlings are represented by solid lines.
Figure 5. Fitted values of the Dirichlet regression model for each component of *Pinus pinaster*. Minimum (min), maximum (max) and average (average) values of harvested *Pinus pinaster* seedlings were considered as explanatory variables (basal diameter and total height) to calculate fitted values.
SUPPLEMENTARY MATERIAL

Supplementary Table 1. Alpha coefficients for different variables from Dirichlet regression of the models referring to Eqs. (5) to (12) for estimating relative biomass weight per compartment of *Pinus halepensis*.

| Model | Compartiment | \( \alpha_{1c} \) | \( \alpha_{2c} \) (D) | \( \alpha_{3c} \) (Ht) | \( \alpha_{4c} \) (GS) | AIC | \( \Delta_i \) |
|-------|--------------|--------------------|-----------------------|----------------------|----------------------|-----|-----------|
| 1     | Roots        | -2.421*            | 2.589***              |                      |                      | -1205 | 2         |
|       | Stem         | -2.283*            | 2.563***              |                      |                      |       |           |
|       | Needles      | -2.102*            | 2.530***              |                      |                      |       |           |
|       | Thin branches| -2.402*            | 2.595***              |                      |                      |       |           |
| 2     | Roots        | -4.086 ns          | 2.460***              | 0.423 ns             |                      | -1203 | 4         |
|       | Stem         | -4.300 ns          | 2.408***              | 0.510 ns             |                      |       |           |
|       | Needles      | -3.690 ns          | 2.406***              | 0.405 ns             |                      |       |           |
|       | Thin branches| -4.186 ns          | 2.456***              | 0.453 ns             |                      |       |           |
| 3     | Roots        | -0.256·            | 2.552***              | -0.011 ns            | -1203               | 4    |           |
|       | Stem         | -2.196·            | 2.550***              | -0.022 ns            |                      |       |           |
|       | Needles      | -1.972·            | 2.504***              | -0.016 ns            |                      |       |           |
|       | Thin branches| -2.288*            | 2.574***              | -0.018 ns            |                      |       |           |
| 4     | Roots        | -5.628 ns          | 2.115***              | 0.964 ns             | 0.076 ns             | -1200 | 7         |
|       | Stem         | -5.660 ns          | 2.105***              | 0.987 ns             | 0.065 ns             |       |           |
|       | Needles      | -5.030 ns          | 2.107***              | 0.875 ns             | 0.065 ns             |       |           |
|       | Thin branches| -5.559 ns          | 2.149***              | 0.936 ns             | 0.066 ns             |       |           |
| 5     | Roots        | 1.860***           |                      |                      |                      | -1189 | 18        |
|       | Stem         | 1.872***           |                      |                      |                      |       |           |
|       | Needles      | 1.888***           |                      |                      |                      |       |           |
|       | Thin branches| 1.872***           |                      |                      |                      |       |           |
| 6     | Roots        | 2.595***           | -0.486*              |                      | -1199               | 8    |           |
|       | Stem         | 2.553***           | -0.447·              |                      |                      |       |           |
|       | Needles      | 2.535***           | -0.423·              |                      |                      |       |           |
|       | Thin branches| 2.599***           | -0.480·              |                      |                      |       |           |
| 7     | Roots        | 1.872***           | 0.066 ns             |                      | -1191               | 16   |           |
|       | Stem         | 1.886***           | 0.053 ns             |                      |                      |       |           |
|       | Needles      | 1.902***           | 0.054 ns             |                      |                      |       |           |
|       |       |       |       |       |       |
|-------|-------|-------|-------|-------|-------|
|       |       |       |       |       |       |
| Thin branches | 1.885*** | 0.058 ns |
| 8 Roots | 2.677*** | -0.535· | -0.052 ns | -1196 | 11 |
| Stem   | 2.666*** | -0.518· | -0.052 ns |
| Needles | 2.624*** | -0.477 ns | -0.045 ns |
| Thin branches | 2.706*** | -0.547· | -0.050 ns |
| 9 Roots | -2.083* | 2.498*** | 0.008* | -1207 | 0 |
| Stem   | -2.005· | 2.490*** |
| Needles | -1.824· | 2.457*** |
| Thin branches | -2.124* | 2.522*** |
| 10 Roots | -2.398* | 2.588*** | -1206 | 1 |
| Stem   | -2.309* | 2.577*** | -0.007· |
| Needles | -2.079* | 2.529*** |
| Thin branches | -2.379* | 2.594*** |
| 11 Roots | -2.459* | 2.600*** | -1203 | 4 |
| Stem   | -2.321* | 2.574*** |
| Needles | -2.131* | 2.538*** | 0.001 ns |
| Thin branches | -2.439* | 2.606*** |
| 12 Roots | -2.411* | 2.586*** | -1203 | 4 |
| Stem   | -2.273* | 2.561*** |
| Needles | -2.092· | 2.528*** |
| Thin branches | -2.405* | 2.597*** | -0.002 ns |
| 13 Roots | -2.136* | 2.516*** | 0.006 ns | -1207 | 0 |
| Stem   | -2.080* | 2.515*** | -0.005 ns |
| Needles | -1.864· | 2.471*** |
| Thin branches | -2.164* | 2.537*** |
| 14 Roots | -2.156* | 2.522*** | 0.007 ns | -1205 | 2 |
| Stem   | -2.099* | 2.521*** | -0.004 ns |
| Needles | -1.876· | 2.475*** | 0.002 ns |
| Thin branches | -2.191* | 2.545*** |
| 15 Roots | -2.142* | 2.517*** | 0.009* | -1206 | 1 |
| Stem   | -2.073* | 2.512*** |
| Needles | -1.862· | 2.470*** | 0.004 ns |
| Thin branches | -2.191* | 2.544*** |
|    |        |        |        |        |        |
|----|--------|--------|--------|--------|--------|
| 16 | Roots  | -2.077*| 2.497***| 0.008*| -1205  |
|    | Stem   | -2.005·| 2.489***|       |        |
|    | Needles| -1.820·| 2.456***|       |        |
|    | Thin branches | -2.115*| 2.520***| 0.001 ns|        |
| 17 | Roots  | -2.357*| 2.576***| -1205  |
|    | Stem   | -2.271*| 2.566***| -0.007·|        |
|    | Needles| -2.047·| 2.520***| -0.001 ns|        |
|    | Thin branches | -2.338*| 2.583***|        |        |
| 18 | Roots  | -2.368*| 2.582***| -1206  |
|    | Stem   | -2.291*| 2.574***| -0.009*|        |
|    | Needles| -2.050·| 2.523***|        |        |
|    | Thin branches | -2.383*| 2.598***| -0.005 ns|        |
| 19 | Roots  | -2.433*| 2.592***| -1.201 |
|    | Stem   | -2.295*| 2.567***|        |        |
|    | Needles| -2.109*| 2.532***| 0.001 ns|        |
|    | Thin branches | -2.425*| 2.602***| -0.002 ns|        |
| 20 | Roots  | -2.169*| 2.526***| 0.005 ns| -1205  |
|    | Stem   | -2.112*| 2.524***| -0.006 ns|        |
|    | Needles| -1.888·| 2.479***|        |        |
|    | Thin branches | -2.204*| 2.549***| -0.002 ns|        |
| 21 | Roots  | -2.135*| 2.516***| 0.011*| -1205  |
|    | Stem   | -2.079*| 2.515***|        |        |
|    | Needles| -1.856·| 2.469***| 0.006 ns|        |
|    | Thin branches | -2.172*| 2.539***| 0.004 ns|        |

D: basal diameter; Ht: total height; GS: growing space for the seedling; AIC: Akaike’s information criterion; Δi: AIC difference; Significance values: ***: p-value <0.001; **: p-value <0.01; *: p-value < 0.05; ·: p-value < 0.1; ns: non-significant effect.

Supplementary Table 2. Alpha coefficients for different variables from Dirichlet regression of the models referring to Eqs. (5) to (12) for estimating relative biomass weight per compartment of Pinus pinaster.
| Model | Compartiment | $\alpha_{1c}$ | $\alpha_{2c}$ (D) | $\alpha_{3c}$ (Ht) | $\alpha_{4c}$ (GS) | AIC | $\Delta_i$ |
|-------|--------------|---------------|-------------------|-------------------|-------------------|-----|-----------|
| 1     | Roots        | -17.497***    | 8.720***          |                   |                   | -195.5 | 10.4     |
|       | Stem         | -16.725***    | 8.408***          |                   |                   |       |           |
|       | Needles      | -16.075***    | 8.273***          |                   |                   |       |           |
|       | Thin branches| -18.027***    | 8.790***          |                   |                   |       |           |
| 2     | Roots        | -25.253***    | 7.798***          | 2.596**           |                   | -205.9 | 0         |
|       | Stem         | -24.188***    | 7.593***          | 2.463**           |                   |       |           |
|       | Needles      | -23.451***    | 7.502***          | 2.409**           |                   |       |           |
|       | Thin branches| -25.976***    | 7.514***          | 2.875***          |                   |       |           |
| 3     | Roots        | -24.567***    | 11.704***         | -0.338 ns         | -191.2 14.7       |
|       | Stem         | -23.851***    | 11.415***         | -0.321 ns         |                   |       |           |
|       | Needles      | -23.218***    | 11.288***         | -0.315 ns         |                   |       |           |
|       | Thin branches| -24.546***    | 11.588***         | -0.376-           |                   |       |           |
| 4     | Roots        | Model did not converge |  |                   |                   |       |           |
|       | Stem         |               |                   |                   |                   |       |           |
|       | Needles      |               |                   |                   |                   |       |           |
|       | Thin branches|               |                   |                   |                   |       |           |
| 5     | Roots        | 1.057***      |                   |                   |                   | -154.8 | 51.1     |
|       | Stem         | 1.032***      |                   |                   |                   |       |           |
|       | Needles      | 1.155***      |                   |                   |                   |       |           |
|       | Thin branches| 0.811***      |                   |                   |                   |       |           |
| 6     | Roots        | 0.900 ns      | 0.127 ns          | -153.0            | 52.9             |
|       | Stem         | 0.662 ns      | 0.258 ns          |                   |                   |       |           |
|       | Needles      | 0.439 ns      | 0.472 ns          |                   |                   |       |           |
|       | Thin branches| 1.375 ns      | -0.314 ns         |                   |                   |       |           |
| 7     | Roots        | 1.088***      | 0.233 ns          | -149.3            | 56               |
|       | Stem         | 1.064***      | 0.263 ns          |                   |                   |       |           |
|       | Needles      | 1.187***      | 0.263 ns          |                   |                   |       |           |
|       | Thin branches| 0.837***      | 0.277 ns          |                   |                   |       |           |
| 8     | Roots        | 0.672 ns      | 0.287 ns          | 0.254 ns          | -148.1 57.8      |
|       | Stem         | 0.420 ns      | 0.427 ns          | 0.284 ns          |                   |       |           |
|       | Needles      | 0.256 ns      | 0.605 ns          | 0.281 ns          |                   |       |           |
|       | Thin branches| 1.253 ns      | -0.224 ns         | 0.300 ns          |                   |       |           |
| 9     | Roots        | -17.400***    | 8.686***          | -0.013 ns         | -193.8 12.1      |
|       | Stem         | -16.713***    | 8.404***          |                   |                   |       |           |
|       | Needles      | -16.062***    | 8.269***          |                   |                   |       |           |
|       | Thin branches| -18.016***    | 8.786***          |                   |                   |       |           |
| 10    | Roots        | -17.497***    | 8.722***          |                   | -193.6 12.3      |
|       | Stem         | -16.775***    | 8.428***          | 0.008 ns          |                   |       |           |
|       | Needles      | -16.075***    | 8.275***          |                   |                   |       |           |
|       | Thin branches| -18.027***    | 8.791***          |                   |                   |       |           |
| 11    | Roots        | -18.041***    | 8.956***          |                   | -194.2 11.7      |
|       | Stem         | -17.264***    | 8.642***          |                   |                   |       |           |
|       | Needles      | -16.730***    | 8.549***          | 0.018 ns          |                   |       |           |
|      | Thin branches  | Roots      | Stem        | Needles    | Thin branches |
|------|----------------|------------|-------------|------------|---------------|
| 12   | -18.545***     | -18.718*** | -17.933***  | -17.280*** | -19.022***    |
|      | 9.017***       | 9.235***   | 8.919***    | 8.783***   | 9.225***      |
|      | -194.3         | 11.6       | 0.026 ns    | 1.116 ns   |               |
| 13   | -18.417***     | -17.410*** | -16.741***  | -16.064*** | -18.018***    |
|      | 9.120***       | 8.690***   | 8.415***    | 8.271***   | 8.788***      |
|      | 0.007 ns       | -0.012 ns  | 0.004 ns    | 0.007 ns   | 0.028 ns      |
| 14   | -18.417***     | -17.933*** | -17.129***  | -16.647*** | -18.862***    |
|      | 9.120***       | 8.919***   | 8.722***    | 8.515***   | 9.160***      |
|      | 0.007 ns       | 0.022 ns   | 0.016 ns    | 0.007 ns   | 0.032 ns      |
| 15   | -18.417***     | -17.387*** | -18.777***  | -16.647*** | -18.480***    |
|      | 9.120***       | 8.841***   | 9.066***    | 8.930***   | 8.989***      |
|      | 0.007 ns       | 0.022 ns   | 0.016 ns    | 0.007 ns   | 0.032 ns      |
| 16   | -18.417***     | -18.931*** | -18.277***  | -17.622*** | -19.307***    |
|      | 9.120***       | 9.335***   | 9.066***    | 8.930***   | 9.352***      |
|      | 0.007 ns       | -0.020 ns  | 0.016 ns    | 0.007 ns   | 0.032 ns      |
| 17   | -18.258***     | -18.258*** | -17.599***  | -16.989*** | -18.752***    |
|      | 9.054***       | 9.054***   | 8.783***    | 8.663***   | 9.111***      |
|      | -192.9         | 13         | 0.018 ns    | 0.025 ns   |               |
| 18   | -18.685***     | -18.685*** | -17.914***  | -17.247*** | -18.995***    |
|      | 9.222***       | 9.222***   | 9.010***    | 8.770***   | 9.214***      |
|      | 0.002 ns       | 0.002 ns   | 0.018 ns    | 0.025 ns   | 0.032 ns      |
| 19   | -18.849***     | -18.849*** | -18.063***  | -17.497*** | -19.183***    |
|      | 9.295***       | 9.295***   | 8.978***    | 8.874***   | 9.297***      |
|      | 0.013 ns       | 0.013 ns   | 0.018 ns    | 0.013 ns   | 0.020 ns      |
| 20   | -19.089***     | -19.089*** | -18.403***  | -17.800*** | -19.453***    |
|      | 9.402***       | 9.402***   | 9.121***    | 9.040***   | 9.415***      |
|      | 0.023 ns       | 0.023 ns   | 0.008 ns    | 0.023 ns   | 0.036 ns      |
| 21   | -18.949***     | -18.949*** | -18.276***  | -17.653*** | -19.325***    |
|      | 9.343***       | 9.343***   | 9.067***    | 8.942***   | 9.360***      |
|      | 0.017 ns       | 0.017 ns   | 0.008 ns    | 0.005 ns   | 0.029 ns      |

D: basal diameter; Ht: total height; GS: growing space for the seedling; AIC: Akaike’s information criterion; Δi AIC difference; Significance values: ***: p-value <0.001; **: p-value <0.01; *: p-value < 0.05; ·: p-value < 0.1; ns: non-significant effect.
Supplementary Figure 1. Effect of mortality in the temporary Nelder wheel plot: initial design, evolution and re-calculation of growing spaces using Voronoi polygons.

Supplementary Figure 2. Basal diameter (mm) in relation to total height (cm) classified by species

Supplementary Figure 3. Comparison of roots and stem biomass estimations between Ruiz-Peinado et al. (2011) models (‘adult’) and our models (‘young’). Biomass: kg for
adult trees and g for young trees. Diameter: DBH – cm for adult trees and basal diameter – mm for young trees.