

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

Irene Ruano (✉ [irene@pvs.uva.es](mailto:irene@pvs.uva.es))

Universidad de Valladolid <https://orcid.org/0000-0003-4059-1928>

**Celia Herrero**

instituto universitario de investigacion gestion forestal sostenible

**Felipe Bravo**

Universidad de Valladolid

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## Research

**Keywords:** Mixed stands, Mediterranean ecosystem, Dirichlet regressions, biomass equations, root system

**Posted Date:** September 28th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-903312/v1>

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6

7 **Authors**

8 Irene Ruano<sup>1,2</sup>, Celia Herrero<sup>1</sup>, Felipe Bravo<sup>1,2</sup>

9

10 **Affiliations**

11 <sup>1</sup> Instituto Universitario de Investigación Gestión Forestal Sostenible-*iuFOR*.  
12 Universidad de Valladolid-INIA. Avda. Madrid s/n. 34004 Palencia. Spain

13 <sup>2</sup> Departamento de Producción Vegetal y Recursos Forestales. ETS de Ingenierías  
14 Agrarias. Universidad de Valladolid. Palencia. Spain

15

16 **Corresponding author**

17 Irene Ruano (irene@pvs.uva.es)

18

19 **ABSTRACT**

- 20 • Background: Forest resilience should be improved to promote species adaptation  
21 and ensure the future of forests. Carbon stock is considered an indicator of  
22 resilience, so it is necessary to determine forest carbon stocks and how to improve  
23 them through forest management. The main objective of this study was to analyse  
24 biomass production and distribution among the components of four-year-old  
25 *Pinus pinaster* and *Pinus halepensis* trees. Young trees from a Nelder wheel  
26 experimental site were harvested and analysed. The effect of density could be  
27 included in the biomass analysis thanks to the Nelder wheel design. We tested  
28 densities from 1000 to 80000 seedlings/ha and analysed biomass by fitting  
29 different equations: (i) linear regressions to analyse biomass production; (ii)  
30 Dirichlet regressions to estimate the biomass proportions of each component and  
31 (iii) allometric equations to predict the biomass content of each component.
- 32 • Results: Results from this innovative approach showed that density was a  
33 significant factor for *Pinus halepensis*. We observed a general increase of total  
34 biomass at lower densities and this positive effect increased root biomass  
35 proportion at the expense of aboveground biomass. Also, a new set of equations  
36 was developed for estimating above- and below-ground biomass in young *Pinus*  
37 *pinaster* and *Pinus halepensis* trees.
- 38 • Conclusions: we note the importance of belowground biomass and its value in  
39 total biomass production (approximately 20% of total biomass for both species).  
40 The effect of density on biomass production was only significant for *Pinus*  
41 *halepensis*, but the effect of density would have been different if root biomass had  
42 not been considered in the present study. Lower densities increased root biomass

43 proportion at the expense of aboveground biomass. Currently, this positive effect  
44 is especially important in promoting management to improve tree resilience.

45

46 **Keywords**

47 Mixed stands; Mediterranean ecosystem; Dirichlet regressions; biomass equations; root  
48 system

49

50 **BACKGROUND**

51 The role of forests in mitigating climate change is quite clear and much research has been  
52 focused on quantifying it in terms of carbon sequestration (Djomo and Chimi 2017; Khan  
53 et al. 2018; Vinh et al. 2019...). Still, we lack knowledge about some aspects of the  
54 process, such as the carbon sequestered by belowground components or carbon stocks in  
55 Mediterranean ecosystems (Herrero et al., 2014; Alvarez et al. 2016). Carbon stocks are  
56 significant because of the increased atmospheric concentration of carbon dioxide, which  
57 has contributed to climate change (IPCC 2007). Climate change might also negatively  
58 affect forests by reducing forest productivity, standing biomass or carbon stocks as trees  
59 die off (Resco De Dios et al. 2007, Lindner and Calama 2013). This decreases the  
60 mitigating capacity of forests, so it is important to link mitigation and adaptation to  
61 understanding the long-term role of forests in climate change. Carbon stocks, species  
62 diversity and harvest rates are considered indicators of resilience, which can be enhanced  
63 by improving species adaptation and subsequent mitigation capacity (Hof et al. 2017).  
64 Forests face an uncertain future, but managed forests adapt more efficiently to climate  
65 change. Thus, adaptive management should consider future site conditions to ensure  
66 correct decision-making (Jandl et al. 2019).

67 High densities in mature stands generally cause competition for resources and reduce tree  
68 growth, but competitive and facilitating effects can occur simultaneously in other phases  
69 of stand development, such as seedling establishment. For example, seedlings might  
70 compete with their own or other species for resources like water or light even as wind  
71 velocity or transpiration are reduced by mutual shading or other species provide  
72 protection against herbivores (Jactel and Brockerhoff 2007; Zamora et al. 2008; Uhl et al.  
73 2015). Moreover, positive interactions can become negative as seedlings grow (Callaway  
74 et al. 1996; Callaway and Walker 1997; Zamora et al. 2008; Uhl et al. 2015). Some  
75 models predict that facilitation and competition interactions will vary across abiotic stress  
76 gradients and that facilitation interactions will be dominant under stressful conditions,  
77 though this is debated (Maestre et al. 2005, 2006; Lortie and Callaway 2006). Therefore,  
78 the net effect of intra- and inter-specific interactions among seedlings under high densities  
79 is a key issue to analyse.

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

90 Two of the most typical pine species in Mediterranean ecosystems were studied in the  
91 present work: *Pinus pinaster* Aiton and *Pinus halepensis* Mill. Both species cover

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

108 One of the main issues in this kind of research is the experiment design. The most frequent  
109 designs for analysing mixed stands consist of growing two species in varying proportions  
110 while keeping stand density constant (Vanclay 2006). Mixed mature stands have been  
111 studied in recent years thanks to long-term experimental plots (Pretzsch et al. 2015,  
112 2019a, b; Riofrío et al. 2019; Aguirre et al. 2019...) but little is known about seedling  
113 stands or young forests. The Nelder wheel design makes it possible to test multiple  
114 densities in a single plot (Nelder 1962; Kerr 2003; Uhl et al. 2015). It consists of a circular  
115 plot containing concentric rings radiating outward with spokes that connect the centre  
116 with the furthest ring (Figure 1 – experimental design). At the intersections of spokes and

117 arcs, a tree is planted. This creates variable tree densities along the length of the spokes  
118 within a single plot.

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

## 125 **MATERIAL AND METHODS**

### 126 **Experimental design**

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

132 The experimental site was established during the autumn of 2013 in Calabazanos  
133 (Palencia – central Spain), on approximately 1 ha of land located on an old forest nursery  
134 site belonging to the Castile-and-Leon Government. The site currently houses the Forest  
135 Health Centre (Figure 1). The experimental design was implemented according to the  
136 explanations of Parrott et al. (2012). All the Nelder wheel plots have 26 spokes, constant  
137 angles of 13.85° and 12 rings, housing a total of 312 seedlings per wheel. Trees in the  
138 outermost and innermost rings served as buffers for edge effects and were not included  
139 in the study sample. In the temporary Nelder wheel plot, two spokes were also considered  
140 as buffers for the harvests (Figure 1). *Pinus pinaster* and *Pinus halepensis* seedlings were

141 mixed along the spokes. Two regions of provenance – the ‘Meseta Castellana’ (Castilian  
142 High Plains) for *Pinus pinaster* and ‘Replantaciones de la Meseta Norte’ (North Plains  
143 Reforestation) for *Pinus halepensis* – were selected to avoid the site effect (Figure 1).

144 One of the most important parameters to obtain in the Nelder wheel plot was the  
145 ‘rectangularity’ proportion (Parrott et al. 2012). This is defined as a proportional  
146 relationship between the arc length between spokes and the radial length between arcs,  
147 where the numerator represents the arc length and the denominator represents radial  
148 distance (Nelder 1962). Extreme ‘rectangularity values can cause bias by creating an  
149 unreasonably asymmetric arrangement of space around trees, so a rectangularity value of  
150 1 was defined in the present experiment (Parrott et al. 2012).

151 Ten densities were tested, ranging from 1000 to 80000 seedlings/ha (Table 1). Minimum  
152 and maximum densities were also defined, to measure the effects of low and high  
153 regeneration densities (Matney and Hodges 1991; Calvo et al 2007; Orozco et al 2007;  
154 Ruano et al 2013). Matney and Hodges (1991) identified a recruit density of 2000  
155 seedlings/ha as the minimum requirement for successful natural regeneration. However,  
156 a density of 1000-1500 seedlings/ha can be considered satisfactory in stands with abiotic  
157 stress (Rodríguez-García et al. 2010), so the minimum density tested was 1000  
158 seedlings/ha. At the other end, high densities have been reported in studies on post-fire  
159 natural regeneration in Mediterranean ecosystems (Calvo et al. 2007; Orozco et al. 2007;  
160 Ruano et al. 2013). Ruano et al. (2013) observed densities of 80000 seedlings/ha after a  
161 fire in *Pinus halepensis* stands, which served to establish the maximum density at 80000  
162 seedlings/ha in the present study. The same densities were tested for all seedlings of each  
163 concentric ring and can be expressed in terms of stand density (trees/ha) or the ‘growing  
164 space’ (m<sup>2</sup>/tree) available to each tree. In the present work, this will be referred to as  
165 ‘growing space’, which is related to stand density (Table 1).

166 Seedlings were one growing season old at time of planting. Basal diameter and total  
167 height (length of the main stem) were measured for each plant. The same measurements  
168 (basal diameter and total height) were repeated after one year (2014) and after four years  
169 (2017). Theoretically, 120 seedlings would have been harvested each time, but only 105  
170 seedlings were available for the first harvest (2017) due to mortality. When a seedling  
171 dies, the growing space of surrounding seedlings changes so the experimental design  
172 changes from the initial design. Options for resolving this issue include double planting,  
173 removing the seedlings surrounding the dead seedlings from the analysis, competition  
174 indexes... (Parrott et al. 2012; Kuehne et al. 2013; Uhl et al. 2015). In this case, we choose  
175 to re-calculate the growing space based on Voronoi polygons, using R Core Team 2017  
176 software with the 'deldir' package (Turner 2020) for 2014 and 2017 (Supplementary  
177 Figure 1) (Ruano et al., 2021).

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

### 187 **Analysis of biomass production**

188 The biomass production analysis was developed following the methodology of  
189 Dahlhausen et al. (2017). Four equations were fitted as linear regressions according to

190 Snell's (1892) power equation, which is commonly used to predict biomass:  $y=b_1x^{b_2}$ ,  
 191 where  $y$  is biomass and  $x$  are the explanatory variables. This equation can be fitted as a  
 192 linear regression by transforming it into  $\ln y = \ln b_1 + b_2 \ln x$ . The following models were  
 193 fitted for each species:

$$194 \quad \ln W_i = b_1 + b_2 \ln(D) \quad (1)$$

$$195 \quad \ln W_i = b_1 + b_2 \ln(D) + b_3 \ln(H) \quad (2)$$

$$196 \quad \ln W_i = b_1 + b_2 \ln(D) + b_4 \ln(GS) \quad (3)$$

$$197 \quad \ln W_i = b_1 + b_2 \ln(D) + b_3 \ln(H) + b_4 \ln(GS) \quad (4)$$

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

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

$$207 \quad D(y|\alpha) = \frac{1}{B(\alpha)} \prod_{c=1}^c y_c^{(\alpha_c-1)}$$

208 Where  $\alpha_c$  are shape parameters for each variable. For this distribution, the constraints  
 209  $\alpha_c > 0 \forall c$ ,  $y_c \in (0, 1)$  and  $\sum_{c=1}^c y_c = 1 \forall c$  must hold. The multinomial beta function,  $B(\alpha_c)$ ,  
 210 serves as the normalizing constant (Maier 2014).

211 The following equations were fitted as single equation models for the four compartments  
212 (roots, stem, thin branches and needles:  $c=1, 2, 3, 4$ ):

$$213 \quad \ln(\alpha_c) = \alpha_{1c} + \alpha_{2c} \ln(D) \quad (5)$$

$$214 \quad \ln(\alpha_c) = \alpha_{1c} + \alpha_{2c} \ln(D) + \alpha_{3c} \ln(H) \quad (6)$$

$$215 \quad \ln(\alpha_c) = \alpha_{1c} + \alpha_{2c} \ln(D) + \alpha_{3c} \ln(GS) \quad (7)$$

$$216 \quad \ln(\alpha_c) = \alpha_{1c} + \alpha_{2c} \ln(D) + \alpha_{3c} \ln(H) + \alpha_{4c} \ln(GS) \quad (8)$$

$$217 \quad \ln(\alpha_c) = \alpha_{2c} \ln(D) \quad (9)$$

$$218 \quad \ln(\alpha_c) = \alpha_{2c} \ln(D) + \alpha_{3c} \ln(H) \quad (10)$$

$$219 \quad \ln(\alpha_c) = \alpha_{2c} \ln(D) + \alpha_{3c} \ln(GS) \quad (11)$$

$$220 \quad \ln(\alpha_c) = \alpha_{2c} \ln(D) + \alpha_{3c} \ln(H) + \alpha_{4c} \ln(GS) \quad (12)$$

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

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

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

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

230 Lastly, a system of compatible allometric equations was fitted to predict the biomass  
231 content of each biomass component. The first step consisted of testing 13 biomass models

232 obtained from forestry literature (Table 2). Each biomass fraction was fitted individually  
233 using the SAS 9.4 MODEL procedure of the SAS/STAT statistical program (SAS  
234 Institute Inc. 2020). The different fractions were fitted as unique fractions in the analysis.  
235 The statistical parameters (the sum of squared estimated of errors (SSR) and the  
236 determination coefficient ( $R^2$ )) from the 13 models fitted for each fraction were then  
237 compared to choose the best model. Weighted fitting using the inverse of the variance of  
238 the residuals ( $\sigma_i^2$ ) was applied to correct the heteroscedasticity problem (Parresol 2001).  
239 Exponent values of k were added to the fitting program. After fitting, the models were  
240 again subjected to heteroscedasticity tests to verify their correctness. The best models for  
241 each fraction were fitted simultaneously by the seemingly unrelated regressions method  
242 (SUR) to guarantee the additivity of the system (SAS Institute Inc., 2020). The SUR  
243 estimates were obtained by applying the SAS 9.4 MODEL procedure (SAS Institute Inc.,  
244 2020), using the parameters obtained in the individual fitting as initializers.

245

## 246 **RESULTS**

247 In 2017, 37 *Pinus pinaster* and 68 *Pinus halepensis* seedlings – all four years old – were  
248 harvested to analyse biomass production but 6 *Pinus pinaster* and 10 *Pinus halepensis*  
249 seedlings were not included in the biomass analysis because they served as buffers for  
250 edge effects. The *Pinus halepensis* seedlings were bigger than *Pinus pinaster* in terms of  
251 basal diameter and total height: with a mean basal diameter of almost 34 mm and a mean  
252 total height of approximately 165 cm. *Pinus pinaster* had a mean basal diameter of almost  
253 10 mm and mean total height of 45 cm for (Table 3 and Supplementary Figure 2). *Pinus*  
254 *halepensis* seedling biomass was also higher, with 1504 g mean total biomass compared  
255 to approximately 39 g for *Pinus pinaster* (Table 4). In both cases, needle biomass was

256 higher than the other compartments, but root biomass was lower than the other  
257 compartments for *Pinus halepensis* and branch biomass was lower than the other  
258 compartments for *Pinus pinaster* (Figure 2). This was reflected in  
259 belowground/aboveground biomass ratio values of 0.23 for *Pinus halepensis* and 0.41 for  
260 *Pinus pinaster* (Table 4 and Figure 3).

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

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

#### 274 **Biomass proportions - Dirichlet regressions**

275 Dirichlet regressions were fitted to analyse the impact of basal diameter, total height and  
276 growing space on the biomass of the compartments considered for each species. Twenty-  
277 one models were fitted using equations (5) to (12) and combinations of them  
278 (Supplementary Tables 1 and 2). Based on the significance of the parameters and AIC  
279 differences ( $\Delta_i$ ), models 1, 6, 9 and 10 for *Pinus halepensis* and model 2 for *Pinus pinaster*

280 could be considered useful. Model 9 provided the best fit for *Pinus halepensis*. In this  
 281 case, basal diameter was significant in the relative biomass of tree compartments, total  
 282 height was not significant in any case and growing space was only significant for root  
 283 biomass. In Model 2 for *Pinus pinaster*, basal diameter and total height were significant  
 284 in the biomass of relative compartments. According to Maier (2014), the expected values  
 285 of the biomass proportions can be estimated as follows for *Pinus halepensis*:

$$286 \quad \alpha_0 = 0.125 * D^{2.498} * GS^{0.008} + 0.135 * D^{2.490} + 0.161 * D^{2.457} + 0.119 * D^{2.522}$$

$$287 \quad E_{roots} = 0.125 * D^{2.498} * GS^{0.008} / \alpha_0$$

$$288 \quad E_{stem} = 0.135 * D^{2.490} / \alpha_0$$

$$289 \quad E_{needles} = 0.161 * D^{2.457} / \alpha_0$$

$$290 \quad E_{thin\ branches} = 0.119 * D^{2.522} / \alpha_0$$

291 For *Pinus halepensis*, the growing space was only significant for the  $\alpha$  coefficient of root  
 292 biomass, but all biomass proportions can be estimated considering the growing space  
 293 because of  $\alpha_0$ . Fitted biomass proportions against growing spaces are represented in  
 294 Figure 4, were the minimum and maximum basal diameter of the harvested seedlings  
 295 were considered for calculating fitted proportions (Table 3). Basal diameter only affects  
 296 stem and root proportions, but higher diameters increased thin branches proportions and  
 297 decreased needle proportions. Growing space had a small effect on biomass proportions.  
 298 According to Maier (2014), the expected values of the biomass proportions can be  
 299 estimated as follows for *Pinus pinaster*:

$$\alpha_0 = 1.07 * 10^{-11} * D^{7.798} * H^{2.596} + 3.12 * 10^{-11} * D^{7.593} * H^{2.463} + 6.53 * 10^{-11} * D^{7.502} * H^{2.409} + 5.233 * 10^{-12} * D^{7.514} * H^{2.875}$$

$$E_{roots} = 1.07 * 10^{-11} * D^{7.798} * H^{2.596} / \alpha_0$$

$$E_{stem} = 3.12 * 10^{-11} * D^{7.593} * H^{2.463} / \alpha_0$$

$$E_{needles} = 6.53 * 10^{-11} * D^{7.502} * H^{2.409} / \alpha_0$$

$$E_{thin\ branches} = 5.233 * 10^{-12} * D^{7.514} * H^{2.875} / \alpha_0$$

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.

### 312 **Biomass components – SUR method**

313 A system of compatible allometric equations for estimating biomass for tree components  
314 was obtained for both species. Results of the individual models showed the best models  
315 for each biomass fraction (roots, stem, needles and thin branches) of each species (Table  
316 6).

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

319 For *Pinus pinaster*, though the highest values of R<sup>2</sup> were obtained from Model 12 for thin  
320 branches and root fractions, Model 10 for the stem, Model 13 for needles and Model 9

321 for total biomass; the best options for subsequent simultaneous fitting were Model 1 for  
322 needles and stem fractions, Model 2 for thin branches and Model 7 for the root fraction.  
323 They were chosen because they had the smaller number of variables linked to the small  
324 amount of data. Anyway, they have an appropriated biological behaviour.

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

## 328 **DISCUSSION**

329 Interest in biomass analysis for young trees is growing because natural regeneration  
330 stands are currently seen as significant carbon sinks (Pugh et al. 2019). Annighöfer et al.  
331 (2016) fitted aboveground biomass equations for 19 species in Europe. Cotillas et al.  
332 (2016) fitted above- and below-ground biomass equations of *Quercus ilex* and *Quercus*  
333 *cerrroides* in the Mediterranean basin. Alfaro-Sánchez et al. (2015) studied the effect of  
334 age or forest management on biomass accumulation and biomass allocation in post-fire  
335 *Pinus halepensis* regeneration. Dahlhausen et al. (2017) studied biomass production in  
336 young *Quercus robur* trees, also using Nelder rings. Kuznetsova et al. (2011) analyzed  
337 aboveground biomass of eight-year-old *Pinus sylvestris* and *Pinus contorta* trees in  
338 Estonia... In the present work, above- and below-ground biomass of four-year-old *P.*  
339 *pinaster* and *P. halepensis* trees were studied using various statistical methods. Results  
340 from this innovative approach showed that growing space, which could be related to  
341 density thanks to the Nelder wheel design, was significant for *Pinus halepensis* in the  
342 fitted linear regressions to estimate biomass production and in the Dirichlet regressions  
343 for biomass proportion estimations. These results confirmed our hypothesis that biomass  
344 production would be affected by density, but only for *Pinus halepensis*. Other researchers

345 have observed a relationship between the biomass of young trees and stand density.  
346 Dahlhausen et al. (2017) found that relative biomass of tree compartments was dependent  
347 on stand density, especially for belowground biomass, as we found for *Pinus halepensis*.  
348 Alfaro-Sánchez et al. (2015) also observed an effect of tree density on biomass  
349 proportions of post-fire *Pinus halepensis* regeneration, though their results differed  
350 slightly from ours. They reported higher stem and crown biomass in moderate densities  
351 (800 trees/ha) along with an increase of stem biomass and a decrease of crown biomass  
352 when tree density increased. In contrast, our findings indicate higher stem and crown  
353 densities along with lower root biomass at higher tree densities, but without the  
354 decreasing effect for the crown. However, total, aboveground and belowground biomass  
355 production were generally higher at lower densities. These minor differences between the  
356 two studies may be due to age differences in trees; the earlier study included trees ranging  
357 from five to 16 years old, while the present study looked only at four-year-old trees.

358 Our results showed a general increment of total biomass for *Pinus halepensis* at lower  
359 densities and this positive effect increased root biomass proportion at the expense of  
360 aboveground biomass. Our findings showed the importance of root biomass in carbon  
361 stock estimations, especially when considering the intrinsic characteristics of  
362 Mediterranean forests. In these ecosystems, disturbances such as fire or grazing intensify  
363 soil erosion, extreme climate conditions compromise natural regeneration and global  
364 changes in climate and land use directly affect fire dynamics (Scarascia-Mugnozza et al.  
365 2000; Moriondo et al. 2006). Some of the recommendations to improve forest adaptation  
366 to climate change in Spain are based on silvicultural experience with promoting mixed  
367 stands or keeping densities relatively low (Serrada et al. 2011). Pre-commercial thinning  
368 is considered a good option for increasing the size, reproductive potential and biomass of  
369 trees in high-density post-fire regeneration stands in Mediterranean ecosystems (Madrigal

370 et al. 2004; Tsitsoni et al. 2004; Navarro et al. 2010; Ruano et al. 2013; Alfaro-Sánchez  
371 et al. 2015). Our results confirmed this recommendation, because increased root biomass  
372 promotes resilience against climate change. For instance, Mayoral et al. (2016) found that  
373 higher root biomass was related to drought adaptation in a study on the effect of water  
374 availability in the establishment phase of three coexisting Mediterranean species.

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

387 Ruiz-Peinado et al. (2011) fitted biomass equations for estimating the carbon sinks of  
388 some Spanish species, among which were *Pinus pinaster* and *Pinus halepensis*. The same  
389 biomass equations and the same fitting method (SUR method) were applied, but our  
390 selected biomass equations were different than biomass equations defined by Ruiz-  
391 Peinado et al. (2011). Moreover, in that study, thin branches (maximum diameter > 2 cm)  
392 and needles were fitted together for adult trees, while our findings were obtained using  
393 one model for each fraction. Tree age might explain the differences. Vanninen (2004)  
394 observed tree age and tree size were significant for allocation in the biomass components

395 of *Pinus sylvestris*. A graphical comparison between their models and ours, however,  
396 showed a similar trend in exponential increase for estimated biomass in relation to  
397 diameter increase (Supplementary Figure 3).

398 Our results regarding biomass proportions were similar to those of Alfaro-Sánchez et al.  
399 (2015) for post-fire regeneration of *Pinus halepensis*. They also observed the highest  
400 biomass for the crown fraction (branches, cones and needles), though the second-highest  
401 biomass fraction was the roots in their case and the stem in our case. However, our results  
402 for *Pinus pinaster* biomass proportions differed from those of Ritson and Sochacky  
403 (2003). They reported a decrease in root:shoot ratio as tree size increased while our results  
404 indicated an increase for larger trees. The biomass proportions also varied between adult  
405 and young trees, though no significant differences were found between species. Both had  
406 proportions of around 20% allocated to stem biomass, less than 60% to crown biomass  
407 with thin branches and needles, and around 20% to root biomass. Ruiz-Peinado et al.  
408 (2011) found differences between species, mainly for stem and crown biomass.  
409 Specifically, more than 60% was allocated to stem biomass and only 15% to crown  
410 biomass for *Pinus pinaster* but almost 40% to stem biomass and almost 40% to crown  
411 biomass for *Pinus halepensis*. The main difference we found between species was in the  
412 crown (branches and needles), with approximately 23% in thin branches biomass and  
413 almost 35% in needles biomass for *Pinus halepensis* compared to around 8% thin  
414 branches biomass and almost 50% needles biomass for *Pinus pinaster*. This could be due  
415 to the size differences between the two species (Vanninen 2004; Ruano et al. 2021).

416 Fitted equations included basal diameter and total height as independent variables, based  
417 on their potential to produce models for large-scale applications and because they explain  
418 most of the variability in observed tree biomass. Diameter at breast height is used more  
419 often for biomass analysis but none of the *Pinus pinaster* seedlings in our study was

420 higher than 1.3 m. As an alternative, diameter at root collar seems to be more appropriate  
421 for young trees (Annighöfer et al. 2016). It was indicated as the best variable for  
422 estimating biomass for young *Picea Abies* L. Karst trees in Slovakia (Pajtík et al. 2008)  
423 or *Pinus pinaster* young trees with a diameter at breast height (DBH) below 10 cm in  
424 Australia (Ritson and Sochacki 2003). Other tree or stand level variables have also been  
425 used to fit biomass equations to accommodate the specific characteristics of each forest  
426 or species (Herrero et al. 2014; Risio et al. 2014).

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

## 438 **CONCLUSIONS**

439 A new set of biomass equations for young *Pinus pinaster* and *Pinus halepensis* trees was  
440 developed and presented in the present work. These equations allowed us to estimate  
441 above- and below-ground biomass for young trees with higher accuracy, using four-year-  
442 old trees and competition statuses ranging from low-density values favoring successful  
443 natural regeneration (1000 trees/ha) to the high-density values found in postfire natural

444 generation (80000 trees/ha). The Nelder wheel design made this possible, marking a new  
445 research milestone.

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

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

#### 456 **DECLARATIONS**

457 Consent to participate: yes

458 Ethics approval: not applicable

459 Consent for publication: not applicable

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

462 Competing interests: The authors declare that they have no competing interests

#### 463 **Funding:**

464 Spanish Economy and Competitiveness Ministry, ‘Mixed Forest Complexity and  
465 Sustainability: Dynamic, Silviculture and Adaptative Management Tools’ Project

466 (AGL2014-51964-C2-1-R) and European Union, ‘Mixed Species forest management.  
467 Lowering risk, increasing resilience- REFORM’ Project (PCIN-2017-027)

468 **Authors' contributions**

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

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

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

476 **Acknowledgments:**

477 The authors thank the Castile-and-Leon Forest Services for the use of their facilities, their  
478 help installing the Calabazanos Nelder wheel experimental site, and their ongoing  
479 support.

480

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668

669 **TABLES**

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

Ring	Radial length (m)	Growing space for a seedling (m <sup>2</sup> )	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	-	-

672

673 Table 2. Biomass models tested for different tree components using seemingly unrelated  
 674 regressions (SUR). W<sub>i</sub>: biomass component (g); D: basal diameter (mm); Ht: total height  
 675 (cm); b<sub>i</sub>: 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	$W_i = b_1 * (D * H)^{b_2}$

676

677 Table 3. Characteristics of the harvested seedlings: sampling size (n), mean, maximum  
678 and minimum values of basal diameter (mm) and mean, maximum and minimum values  
679 of total height (cm).

Species	n	Basal diameter			Total height		
		mean	max	min	Mean	max	min
<i>Pinus halepensis</i>	68	33.93	62.00	11.60	164.78	250	67
<i>Pinus pinaster</i>	37	9.36	24.47	3.85	45.23	116	13

680

681

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
<i>P. halepensis</i>	275.22 (20.85-802.21)	358.07 (22.34-933.75)	505.89 (30.12-1312.68)	364.78 (9.53-1027.86)	1503.96 (92.15-3774.04)	1228.74 (63.48-3139.96)	0.23 (0.09-0.45)
<i>P. pinaster</i>	10.12 (0.67-107.77)	7.43 (0.31-41.54)	17.03 (0.43-103.76)	3.94 (0.00-31.09)	38.53 (1.41-284.16)	28.41 (0.74-176.39)	0.41 (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	b <sub>1</sub>	b <sub>2</sub> (D)	b <sub>3</sub> (Ht)	b <sub>4</sub> (GS)	R <sup>2</sup> <sub>adj</sub>
<i>P. halepensis</i>	Total	0.1587 ns	1.9954 ***			0.7508
		-4.4080 ***	1.4934 ***	1.2409 ***		0.8151
		0.2987 ns	1.9536 ***		0.0335 ns	0.7497
		-6.1915 ***	1.0329 ***	1.9015 ***	0.1552 ***	0.8648
	Aboveground	-0.1196 ns	2.0159 ***			0.7387
		-4.8494 ***	1.4960 ***	1.2853 ***		0.8051
		-0.0238 ns	1.9873 ***		0.0230 ns	0.7354
		-6.5108 ***	1.0670 ***	1.9005 ***	0.1445 ***	0.8459
	Belowground	-1.4453 *	1.9646 ***			0.7232
		-5.3117 ***	1.5395 ***	1.051 **		0.7673
		-1.1323 ·	1.8711 ***		0.0750 ·	0.7348
		-7.5581 ***	0.9594 ***	1.8826 ***	0.1954 ***	0.8467
	Ratio	-1.3256 **	-0.0514 ns			- 0.0143
		-0.4620 ns	0.0435 ns	-0.2347 ns		- 0.0159
		-1.1084 *	-0.1162 ns	0.0521 ·		0.0227
		-1.0473 ns	-0.1076 ns	-0.0179 ns	0.0509 ns	0.0046
<i>P. pinaster</i>	Total	-2.7527 ***	2.7178 ***			0.9209
		-0.9498 ***	1.9479 ***	0.7710 ***		0.9541
		-2.7629 ***	2.7224 ***		0.0212 ns	0.9191
		-4.0022 ***	1.9327 ***	0.7938 ***	0.0353 ns	0.9552

Aboveground	-3.5617	2.9356			0.9065
	***	***			
	-4.9006	2.0744	0.8624		0.9412
	***	***	***		
Belowground	-3.5697	2.9391		0.0166	0.9037
	***	***		ns	
	-4.9486	2.0606	0.8833	0.0324	0.9037
	***	***	***	ns	
Ratio	-3.0143	2.2280			0.8788
	***	***			
	-3.8067	1.7183 **	0.5104 *		0.8969
	***				
Ratio	-3.0267	2.2335		0.0258	0.8766
	***	***		ns	
	-3.8591	1.7031	0.5332 *	0.0353	0.8971
	***	***		ns	
Ratio	-1.9153 *	0.2828 ns			0.0546
	-1.2021	0.6772 ns	-0.4182		0.1522
	ns		ns		
	-1.9652	0.3022 ns		-0.0468	0.1066
	ns			ns	
	-1.2320	0.7104 ns	-0.4316	-0.0496	0.2103
ns		ns	ns		

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 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.

	<i>Pinus halepensis</i> n=68					<i>Pinus pinaster</i> n=37				
Model	$W_{needles}$	$W_{thin\ branches}$	$W_{stem}$	$W_{root}$	$W_{total}$	$W_{needles}$	$W_{thin\ branches}$	$W_{stem}$	$W_{root}$	$W_{total}$
1	0.6729	0.6228	0.7047	0.6238	0.6958	<b>0.9526</b>	0.8758	<b>0.9358</b>	0.8140	0.9450
2	0.5950	0.5723	0.6043	0.4330	0.5953	0.8077	<b>0.8578</b>	0.7295	0.9790	0.9240
3	0.7141	0.6765	0.6921	0.6261	0.7286	-	0.8786	-	0.9420	0.9664
4	-	-	-	-	-	-	-	-	-	-
5	0.6658	0.5949	-	-	0.6465	-	-	-	-	-

6	-	-	0.633 9	-	-	-	0.892 8	-	-	0.980 3
7	0.735 7	0.6731	0.711 6	<b>0.618</b> 2	0.730 4	-	-	-	<b>0.920</b> 0	0.959 6
8	-	-	-	-	-	-	-	-	-	-
9	<b>0.732</b> 1	<b>0.6745</b>	0.728 2	-	<b>0.735</b> 2	-	-	0.946 9	-	0.957 2
10	0.708 0	0.6608	<b>0.731</b> 3	-	0.724 2	-	0.906 0	-	-	0.980 3
11	-	-	-	-	-	0.959 5	0.910 1	0.947 2	-	<b>0.984</b> 2
12	0.713 9	0.6768	0.692 1	0.631 3	0.733 6	0.923 6	0.860 3	0.920 1	0.979 9	0.966 1
13	Nc	Nc	Nc	Nc	Nc	Nc	-	Nc	Nc	Nc

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<sup>2</sup>: coefficient of determination; R<sup>2</sup><sub>adj</sub>: adjusted coefficient of determination; D: basal diameter; Ht: total height.

Species	Model	SSE	MSE	RMS E	R <sup>2</sup>	R <sup>2</sup> <sub>adj</sub>
<i>P. halepensis</i>	W <sub>thin</sub> branches=0.160399*D <sup>2</sup> +0.025503 *D*Ht	14529 46	23818 .8	154.3	0.671 7	0.671 7
	W <sub>stem</sub> =0.000598* D <sup>2</sup> *Ht+0.037706*D*Ht	10669 37	17490 .8	132.3	0.727 1	0.727 1
	W <sub>roots</sub> =0.13254* D <sup>2</sup> +0.529169*Ht	76804 0	12590 .8	112.2	0.613 0	0.613 0
<i>P. pinaster</i>	W <sub>needles</sub> =0.207597* D <sup>2</sup> +0.039945*D*Ht	20222 94	33152 .4	182.1	0.731 2	0.731 2
	W <sub>thin</sub> branches=0.000679* D <sup>2</sup> *Ht	43.829 1	1.593 8	1.262 5	0.812 8	0.816 2
	W <sub>stem</sub> =0.014081*D*Ht	61.825 5	2.248 2	1.499 4	0.873 8	0.876 1
	W <sub>roots</sub> =0.059415* D <sup>2</sup> +0.040243*Ht	60.620 6	2.245 2	1.498 4	0.862 9	0.862 9

	$W_{\text{needles}}=0.032261 \cdot D \cdot Ht$	191.2	6.529	2.636	0.927	0.929
				8	9	2

## 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.

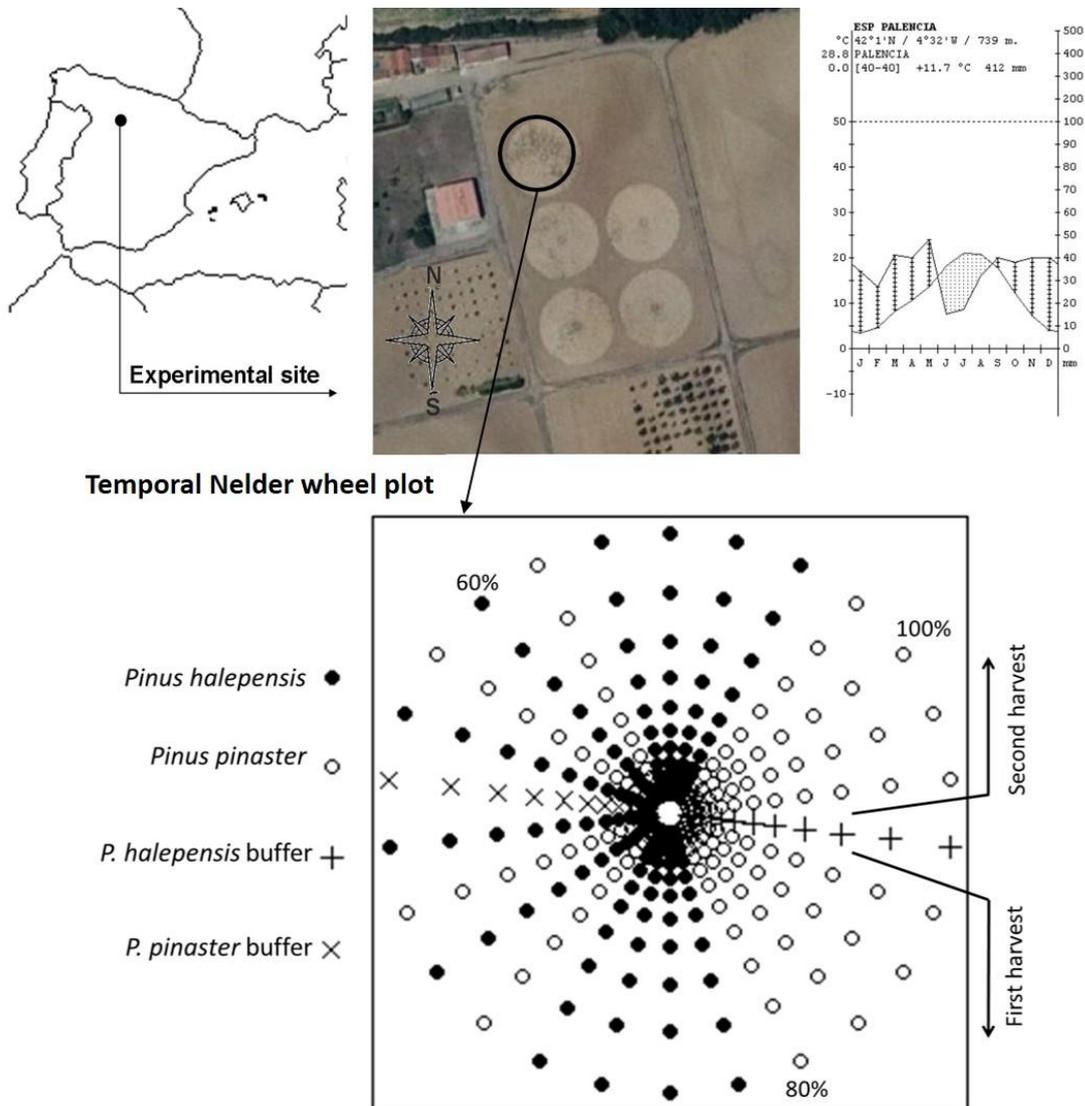


Figure 2. Relative compartment biomass composition per species.

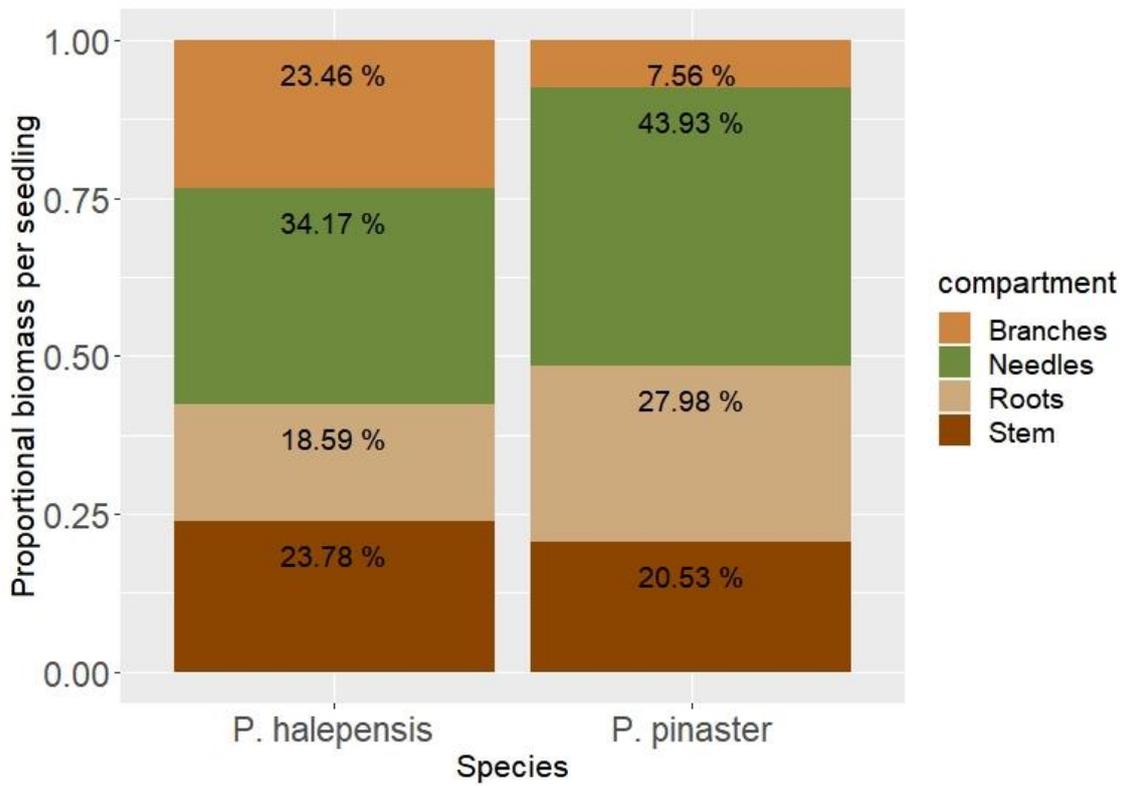


Figure 3. Belowground/aboveground biomass ratio of each species

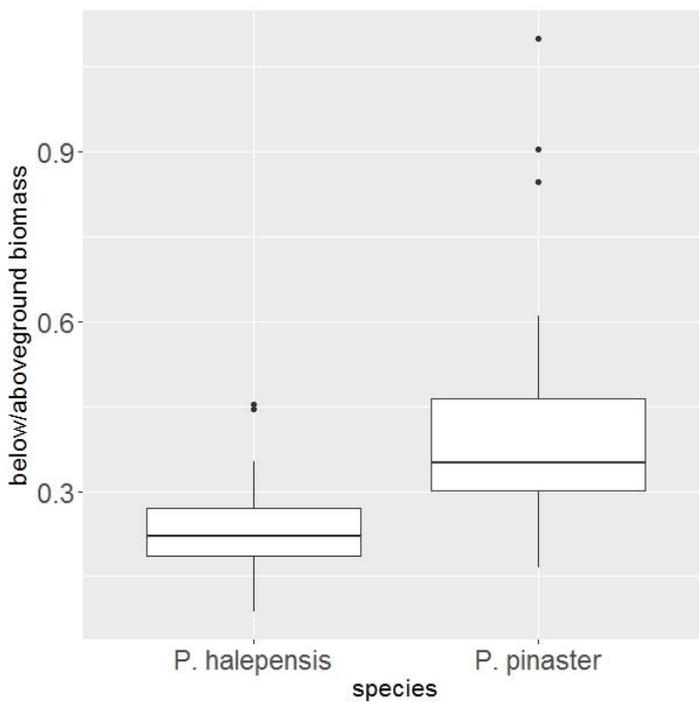


Figure 4. Fitted values of the Dirichlet regression model for each component of *Pinus halepensis*. Fitted values for the minimum basal diameter of harvested *Pinus halepensis* seedlings are represented by dashed lines and fitted values for the maximum basal diameter of harvested *Pinus halepensis* seedlings are represented by solid lines.

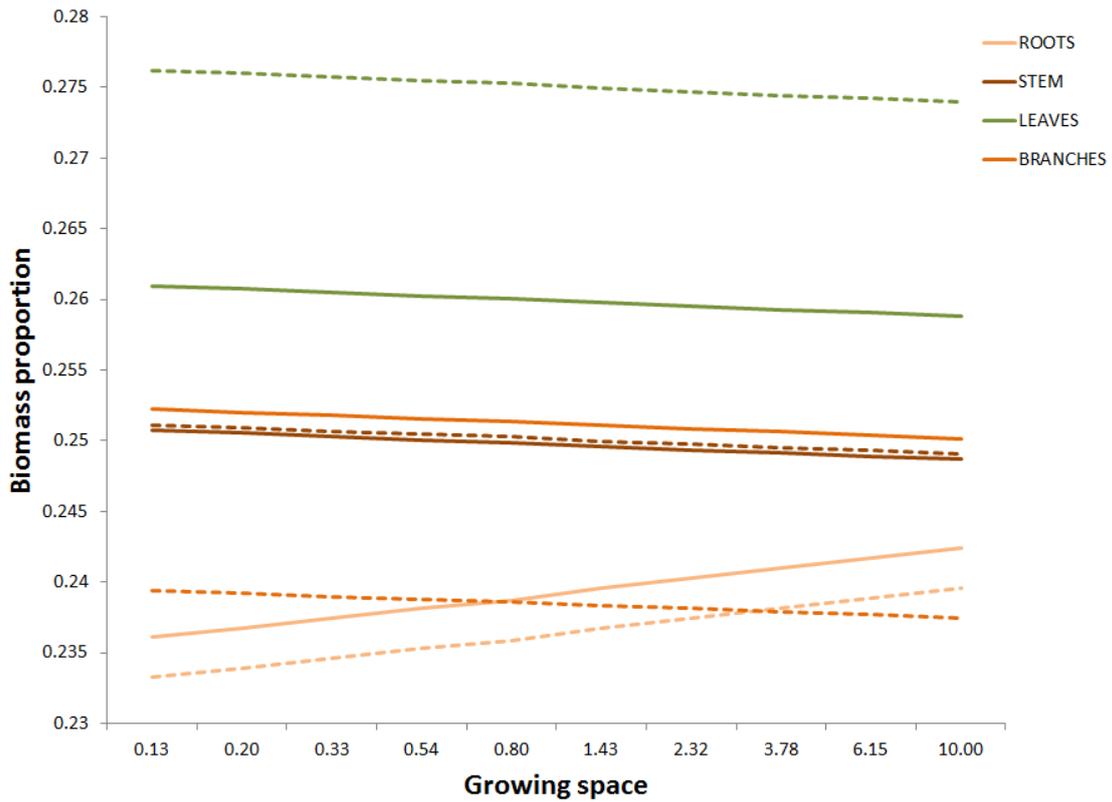
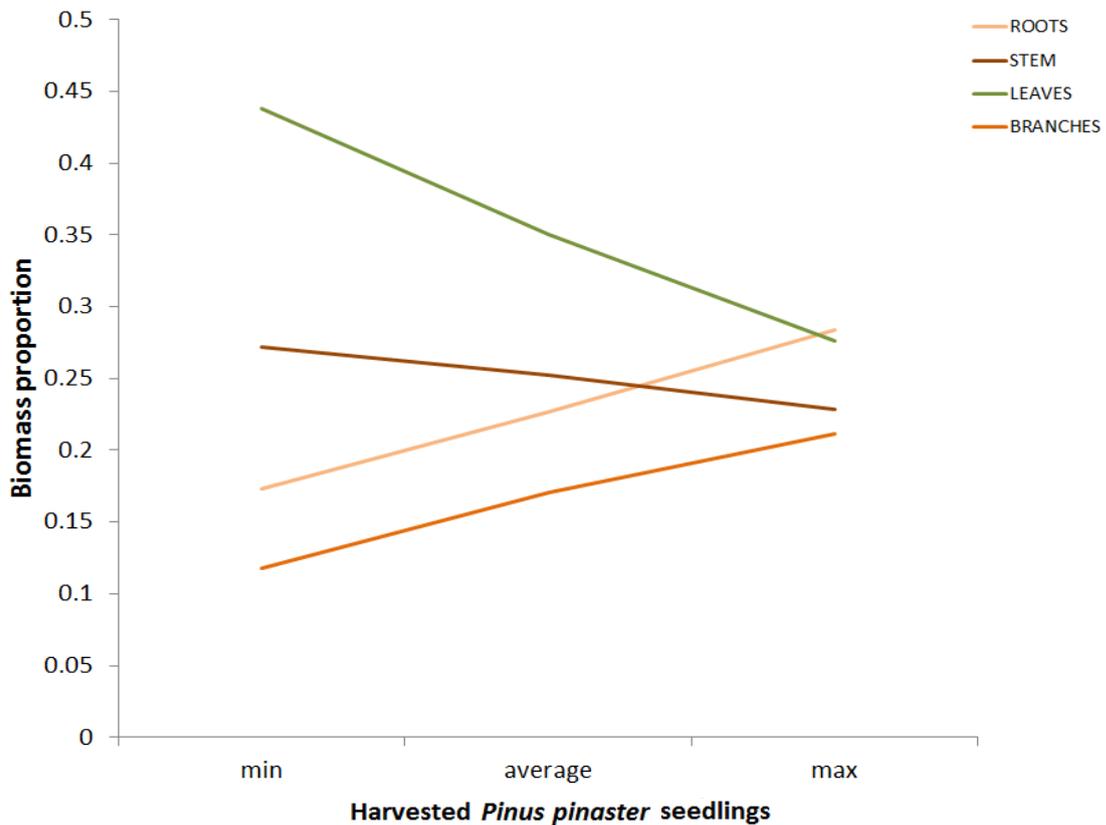


Figure 5. Fitted values of the Dirichlet regression model for each component of *Pinus pinaster*. Minimum (min), maximum (max) and average (average) values of harvested *Pinus pinaster* seedlings were considered as explanatory variables (basal diameter and total height) to calculate fitted values.



## 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	Compartment	$\alpha_{1c}$	$\alpha_{2c}$ (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	2
	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	2
	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	1
	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	6
	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	2
	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	2
	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;  $\Delta_i$  AIC difference; Significance values: \*\*\*: p-value <0.001; \*\*: p-value <0.01; \*: p-value < 0.05; ·: p-value < 0.1; ns: non-significant effect.

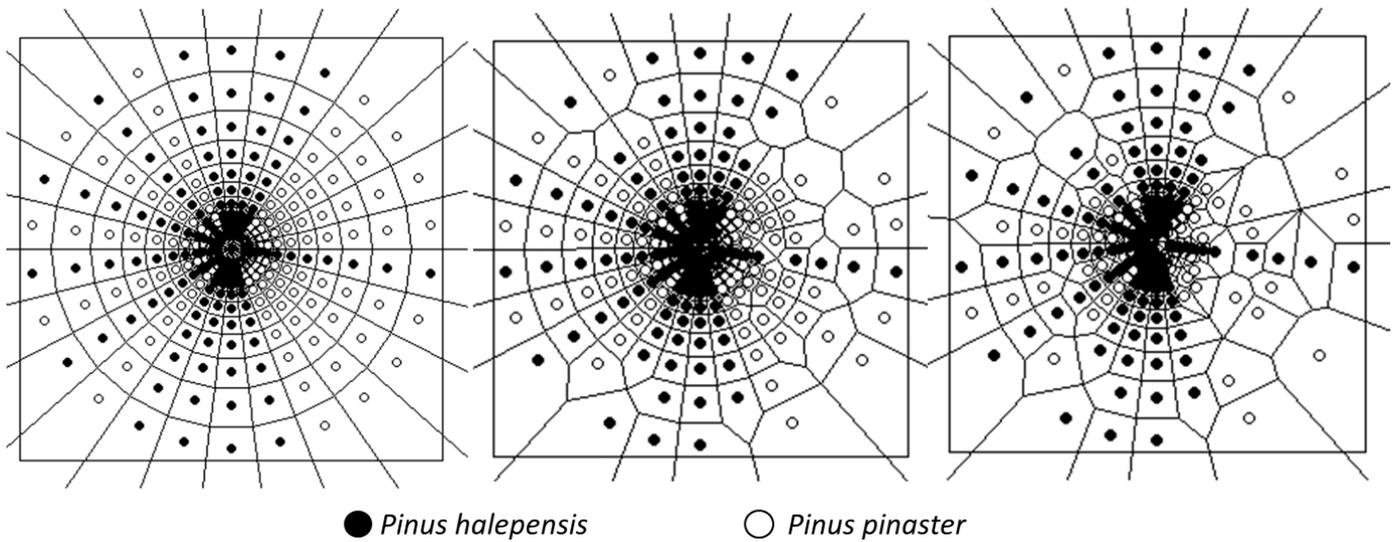
Supplementary Table 2. Alpha coefficients for different variables from Dirichlet regression of the models referring to Eqs. (5) to (12) for estimating relative biomass weight per compartment of *Pinus pinaster*.

Model	Compartment	$\alpha_{1c}$	$\alpha_{2c}$ (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			

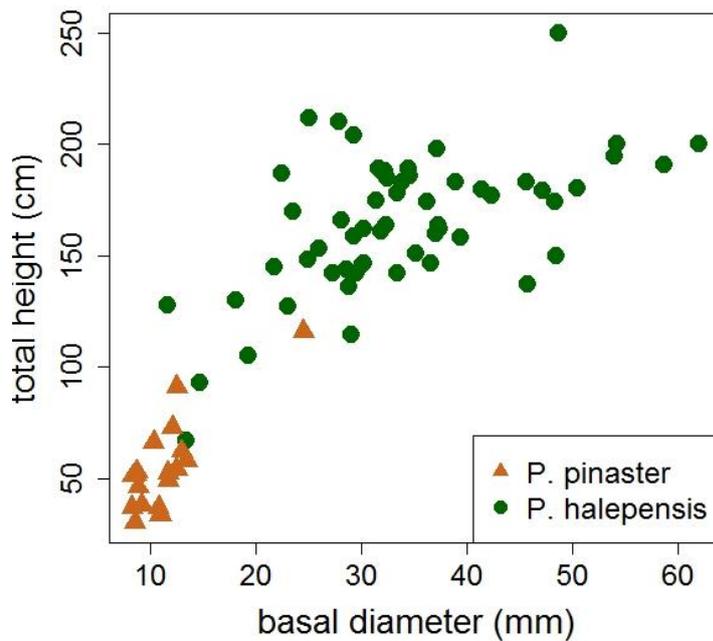
	Thin branches	-18.545***	9.017***			
12	Roots	-18.718***	9.235***		-194.3	11.6
	Stem	-17.933***	8.919***			
	Needles	-17.280***	8.783***			
	Thin branches	-19.022***	9.225***	-0.026 ns		
13	Roots	-17.410***	8.690***	-0.012 ns		
	Stem	-16.741***	8.415***	0.004 ns		
	Needles	-16.064***	8.271***			
	Thin branches	-18.018***	8.788***			
14	Roots	-18.417***	9.120***	0.007 ns	-190.9	15
	Stem	-17.738***	8.841***	0.022 ns		
	Needles	-17.129***	8.722***	0.028 ns		
	Thin branches	-18.862***	9.160***			
15	Roots	-17.933***	8.912***	-0.006 ns	-192.3	13.6
	Stem	-17.196***	8.613***			
	Needles	-16.647***	8.515***	0.016 ns		
	Thin branches	-18.480***	8.989***			
16	Roots	-18.931***	9.335***	-0.020 ns	-193.1	12.8
	Stem	-18.277***	9.066***			
	Needles	-17.622***	8.930***			
	Thin branches	-19.307***	9.352***	-0.032 ns		
17	Roots	-18.258***	9.054***		-192.9	13
	Stem	-17.599***	8.783***	0.018 ns		
	Needles	-16.989***	8.663***	0.025 ns		
	Thin branches	-18.752***	9.111***			
18	Roots	-18.685***	9.222***		-192.3	13.6
	Stem	-17.914***	8.910***	0.002 ns		
	Needles	-17.247***	8.770***			
	Thin branches	-18.995***	9.214***	-0.025 ns		
19	Roots	-18.849***	9.295***		-192.7	13.2
	Stem	-18.063***	8.978***			
	Needles	-17.497***	8.874***	0.013 ns		
	Thin branches	-19.183***	9.297***	-0.020 ns		
20	Roots	-19.089***	9.402***	-0.023 ns	-191.2	14.7
	Stem	-18.403***	9.121***	-0.008 ns		
	Needles	-17.800***	9.040***			
	Thin branches	-19.453***	9.415***	-0.036 ns		
21	Roots	-18.949***	9.343***	-0.017 ns	-191.2	14.7
	Stem	-18.276***	9.067***			
	Needles	-17.653***	8.942***	0.005 ns		
	Thin branches	-19.325***	9.360***	-0.029 ns		

D: basal diameter; Ht: total height; GS: growing space for the seedling; AIC: Akaike's information criterion;  $\Delta_i$  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.

