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1 **Above- and below-ground carbon accumulation and biomass allocation in poplar short**
2 **rotation plantations under Mediterranean conditions**

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11

12 **Abstract**

13 Beside the production of biomass, short rotation coppice (SRC) poplar plantations can also
14 contribute to carbon sequestration in the soil through their below-ground biomass. The present
15 study evaluated the allocation of above and below-ground biomass at the end of the first rotation
16 of four SRC plantations under Mediterranean conditions. The genotypes evaluated are commonly
17 used for biomass plantations, i.e. genotypes ‘AF2’ and ‘I-214’ (*Populus* × *canadensis* Mönch),
18 and ‘Monviso’ (*P.* × *generosa* Henry × *P. nigra* L.). No significant differences among genotypes
19 were found with regard to below-ground biomass yield. The root:shoot ratio decreased in line
20 with the growth in shoot basal diameter, with values ranging from 0.15-0.26. The accumulation
21 of carbon in the below-ground fraction of the biomass ranged from 0.86 to 0.91 Mg C ha⁻¹ yr⁻¹,
22 whereas the above-ground carbon accumulation ranged from 3.89 to 6.48 Mg C ha⁻¹ yr⁻¹. A
23 general as well as a genotype-specific allometric model allowed to accurately predict the below-
24 ground biomass yield using shoot basal diameter as the predictor variable. Both models provide
25 an important tool to quantify the carbon accumulated in the below-ground fraction of the biomass.
26 Keywords: Root biomass; Below-ground allometry models; *Populus* spp.; Root:Shoot ratio; Short
27 Rotation Coppice (SRC).

28 **1. Introduction**

29 Research concerning poplar plantations under a short rotation regime has recently become
30 prominent with the aim of maximizing the raw material both for bioenergy and more recently for
31 bioproducts. Additionally, these plantations can play an important role in maintaining or
32 sequestering carbon in the soil (Berhongaray and Ceulemans, 2015; Block *et al.*, 2006) as well as
33 in contributing towards the reduction of greenhouse gas emissions thereby helping to mitigate
34 climate change (Agostini *et al.*, 2015). Studies focusing on differences in yield based on the
35 genotypes or on the management have led to much progress in this area, even though variation
36 due to genotype-environment interactions (plasticity) makes it more difficult to attain the
37 anticipated progress. Furthermore, the final tally in terms of carbon sequestration has also been
38 the subject of research since the capacity of short rotation coppice (SRC) to sequester carbon
39 remains unclear (Hillier *et al.*, 2009; Walter *et al.*, 2015). Some studies suggest a neutral carbon
40 balance without emissions (Hansen, 1993; Johnson *et al.*, 2007), although some recent studies
41 reported that after successive rotations a point is reached at which the carbon balance becomes
42 positive (Arevalo *et al.*, 2011; Verlinden *et al.*, 2013).

43 SRC crops are defined as high-density plantations of fast growing trees (such as poplars or
44 willows), managed in rotations of 2 to 8 years (Berhongaray *et al.*, 2017; Ferré and Comolli,
45 2018). This implies a different stage of development of the shoots (S) which regrow from the
46 stool and the root (R) in successive rotations. Plant roots are an essential component of net primary
47 productivity (NPP), which is the sum of visible growth and litter production of above- and below-
48 ground components (Roy *et al.*, 2001). Poplar root systems can account for around 25-35% of the
49 total plant biomass (Heilman *et al.*, 1994; Pregitzer and Friend, 1996), this percentage being
50 higher (up to 63%) in juvenile trees (Block *et al.*, 2006; King *et al.*, 1999; Yin *et al.*, 2004). In
51 SRC plantations the roots can be of even higher importance given the expected rotations, which
52 will imply that the above-ground and the below-ground parts are of different ages over the
53 duration of the crop. Due to the difficulties in studying the root system, advancing the knowledge
54 in this area is far from simple (Jha, 2017; Mokany *et al.*, 2006). Despite this, some studies have
55 focused on this fraction of the stool in relation to the whole SRC system (Berhongaray *et al.*,

56 2015; Block *et al.*, 2006). Although studies on poplar root systems are somewhat scarce, there are
57 more that focus on fine roots given the ease of their extraction, as well as their importance in the
58 assimilation of water and nutrients (Dickmann *et al.*, 1996) and their important influence on
59 carbon turnover (Mulia and Dupraz, 2006), despite the fact that fine roots only represent a small
60 fraction of total tree biomass (Afas *et al.*, 2008; Berhongaray *et al.*, 2013). There has not been
61 much progress in the study of roots under irrigated short rotation plantations in Mediterranean
62 areas, neither as regards fine roots nor as regards the radical system as a whole.

63 Root biomass can be directly measured using excavation techniques (Levillain *et al.*, 2011)
64 although these methods are costly and time consuming (Addo-Danso *et al.*, 2016). A common
65 approach for estimating not only above-ground biomass in highly diverse forests but also below-
66 ground biomass, is to use allometric relationships based on an easily measurable variable (Clark
67 *et al.*, 2001; Kenzo *et al.*, 2009; Saint-André *et al.*, 2005). The use of allometric equations to
68 predict root biomass is a generalized method (Cairns *et al.*, 1997; Kurz *et al.*, 1996; Snowdon *et*
69 *al.*, 2000; Vogt *et al.*, 1995), since these relationships provide a simpler and more accurate means
70 for estimating root biomass (Domenicano *et al.*, 2011). However, few allometric relationships are
71 available for below-ground biomass estimations in poplar SRC (Berhongaray *et al.*, 2015; Coyle
72 and Coleman, 2005; Fang *et al.*, 2007), particularly as regards those growing under Mediterranean
73 conditions where irrigation determines root development.

74 Poplar SRC plantations for the production of biomass provide an additional benefit in the form of
75 carbon sequestration in the soil (Smith, 2004). To quantify the potential of carbon sequestration
76 in the soil in SRC plantations, it is important to take both litterfall and fine root turnover into
77 account (Walter *et al.*, 2015), as well as the carbon accumulated in the below-ground woody
78 biomass which remains in the soil during the successive coppices (Pacaldo *et al.*, 2014). Hence,
79 the accuracy of the allometric equations used to quantify the below ground biomass is of major
80 importance.

81 The present study evaluated the allocation of the above and below-ground fraction in different
82 short rotation poplar plantations under Mediterranean conditions at the end of the first rotation.
83 Our main objective was to contribute towards furthering our understanding of the root system,

84 providing accurate knowledge of the root system allocation in the first rotation of this type of
85 plantations under Mediterranean conditions, which necessitate irrigation due to the severe
86 summer drought. Therefore four plantations in Spain comprising three commonly used genotypes
87 and with a range of different ages were evaluated. The specific objectives were: (i) to quantify
88 the above and below-ground biomass, as well as the root:shoot relationship at genotype level, (ii)
89 to develop allometric relationships based on shoot basal diameter or shoot basal area, from which
90 root biomass could be estimated, and finally (iii) to compare the total and disaggregated amount
91 of carbon accumulated in the biomass (above and below-ground) at plantation level, considering
92 the genotype used.

93 **2. Material and Methods**

94 **2.1. Experimental field sites**

95 Four experimental field sites in Spain (S1 to S4) were used in this study, covering different site
96 conditions in the Mediterranean climate (Table 1). Three high-yielding genotypes were selected
97 from the trial network. These were: ‘AF2’ (*Populus* × *canadensis* Mönch), ‘I-214’ (*P.* ×
98 *canadensis* Mönch) and ‘Monviso’ (*P.* × *generosa* Henry × *P. nigra* L.). The plantations were
99 established in early spring using cuttings of 20-30 cm in length. Densities ranged from 5555
100 cuttings ha⁻¹ to 13,333 cuttings ha⁻¹ in a design consisting of single rows 2.5 or 3 m apart. Three
101 to four randomized replications were established for each genotype within each plantation. A
102 similar management was applied in all plantations as regards fertilization during soil tillage
103 according to the specific soil characteristics, weed control and irrigation (to field capacity) during
104 the summer months (Sixto *et al.*, 2013). A drip irrigation system was established to optimize water
105 application.

106 Different rotation lengths were applied depending on the developmental status. The rotation
107 length established at sites S2 and S4 was three years, while at sites S1 and S3 it was four years.
108 Hence, the stages of development covered in this study at the end of the first rotation were: R3S3
109 or R4S4, denoting root age as R and shoot age as S. None of the stands were coppiced at the
110 studied time; so the age in the above- and below-ground fraction was the same.

111 **2.2. Above- and below-ground biomass**

112 The sampling unit was considered the stool, which is the plant consisting of a stump, base or root
113 from which one or several shoots are produced. Measurements of the above-ground fraction were
114 taken at the end of each rotation on 16-25 stools per site, replicate and genotype. Shoot diameters
115 over bark at 10 cm above ground (d_{basal} , mm), accounting for the size of the shoots at the usual
116 harvesting height, were measured using a digital calliper (Absolute, Vogel Germany, Kevelaer,
117 Germany) (accuracy to 1 mm). The basal area (BA_{basal} , mm²) of the entire stool was calculated
118 from basal diameters of all shoots. Total height of the highest shoot (H_{total} , cm) was also measured
119 with a measuring pole to an accuracy of 1 cm. The number of shoots and the density of living
120 stools were also recorded to quantify mortality. The above-ground woody biomass was assessed
121 in all stools through destructive sampling after leaf fall. We considered above-ground woody
122 biomass as the whole biomass above the harvesting height of these stools, which were cut back
123 at 10 cm above the soil. Total above-ground dry woody biomass ($W_{above-ground}$, g DM (dry matter))
124 was determined for each sampling unit from the fresh weight and the estimated above-ground
125 wood moisture by randomly selecting one entire stool from each of the plots and then oven-drying
126 a randomly selected sample of each genotype to constant weight at 105 °C.

127 Five stools for each site, replicate and genotype were selected for below-ground biomass
128 measurements. They were selected by covering the different diameter classes observed for each
129 replicate. The root systems were extracted using a backhoe with manual assistance, exploring
130 around 1 m² of soil for each stool (the dimensions were adapted to the different planting design
131 in proportion to the density). All roots that we were able to retrieve within this area (up to a
132 diameter of 0.10 mm) were manually collected, those roots belonging to the adjacent stools were
133 excluded, although in some cases identification was not possible. The diameter of each broken
134 root tip in each sample was measured to estimate the remaining biomass in the soil. We considered
135 below-ground biomass as the whole biomass below the harvesting height of these stools, so it
136 included the stump below the harvesting height, as well as the coarse roots and fine roots. Total
137 dry biomass of the roots ($W_{below-ground}$, g DM) was determined for each sample from the fresh
138 weight and the estimated below-ground wood moisture that was obtained using the same
139 procedure as for the above-ground wood moisture.

140 The root:shoot ratio (defined as the below-ground biomass divided by the above-ground biomass)
141 was calculated using the mean of the individual ratio of all samples (five stools per genotype and
142 site). Below-ground (root) was considered as all biomass below the harvesting height and above-
143 ground as all woody biomass above the harvesting height, i.e. all shoots produced by each stool.

$$144 \quad \text{root:shoot ratio} = \frac{W_{\text{below-ground}}}{W_{\text{above-ground}}} \quad (1)$$

145 Dried above- and below-ground biomass was separated for subsequent carbon analyses through
146 dry combustion using a CNS-2000 (TruSpec® CHNS, LECO, St Joseph, Michigan, USA).
147 Above- and below-ground biomass values were converted to carbon accumulations using the
148 average above- and below-ground carbon accumulations fractions, respectively.

149 **2.3. Data analysis**

150 **2.3.1. Exploratory analysis of the data**

151 The influence of the genotype, site and their interaction (G*S) was examined using a mixed model
152 of analysis of variance (ANOVA) for each of the studied variables (above-ground biomass,
153 below-ground biomass and root:shoot ratio). The λ value of the Box-Cox procedure was used to
154 transform the non-normal data. The values shown in the test, as well as in the tables and figures
155 correspond to the non-transformed data.

$$156 \quad X_{ijkl} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + C_{k(j)} + \varepsilon_{ijkl} \quad (2)$$

157 where X_{ijkl} is the dependent variable, μ is the intercept of the model or overall mean, α_i is the
158 genotype effect, β_j is the site effect, $\alpha\beta_{ij}$ is the interaction between genotype and site, $C_{k(j)}$ is the
159 random effect of the replicate within the site and ε_{ijkl} is the residual error.

160 Each mixed model was developed with two crossed fixed factors (genotype and site), their
161 interaction (G*S) and a third random factor nested in one of the fixed factors (in this case the
162 replication nested in the site).

163 To get a better understanding of below-ground biomass distribution we explored the relationship
164 between the below- and above-ground biomass using the root:shoot ratio. The similarities in
165 allometric relationships among genotypes were evaluated by cross-predicting with the proposed
166 root:shoot ratio theoretical trend and comparing this trend at the different genotype levels ('AF2',

167 ‘I-214’ and ‘Monviso’) with the observed values. Two levels were assumed to share the same
 168 allometry when the model fitted for a given level produced non-deviated predictions for the other.
 169 The α -trimmed non-parametric validation tests were used to evaluate the similarity in biomass
 170 allometry among genotypes (Pérez-Cruzado et al., 2015). The null hypothesis for the test was that
 171 both samples belonged to the same population, so small p-values led us to reject the null
 172 hypothesis that both samples shared the same model. We simulated the theoretical root:shoot ratio
 173 trend using models to estimate the above and the below-ground biomass and calculating the
 174 root:shoot ratio derived from these estimations. For the below-ground biomass estimation, we
 175 used the specific genotype-models fitted in the current study, whereas for the above-ground
 176 biomass estimation we used the *ad hoc* specific genotype-models previously described (Oliveira
 177 *et al.* 2017), that were fitted for Mediterranean conditions. Both models are specific to short
 178 rotation, high density plantations, so the estimations of the below- and above-ground biomass
 179 ranged from 0 to 200 mm basal diameter.

180 **2.3.2. Allometric relationships for below-ground biomass**

181 The total below-ground biomass for each selected stool was carefully measured, but losses in the
 182 root extraction procedure could not be completely avoided had to be corrected, e.g. broken root
 183 tips. To estimate the remaining root biomass ($W_{under-estimated\ below-ground}$, g DM) beyond the broken
 184 root tip, we used the data obtained for a single root from one of each five stools selected per
 185 replicate, genotype and site. For each single root, segments of 10 cm were cut, and their diameter
 186 ($d_{broken\ tip}$, mm) and dry biomass ($W_{under-estimated\ below-ground}$) were measured. Based on this
 187 information, an allometric relationship was established between the broken tip diameter and the
 188 remaining root biomass up to the full length of the root for each genotype (Table 2). A non-linear
 189 allometric relationship was fitted by ordinary least squares using the statistical programme *R* (*R*
 190 Development Core Team, 2017).

$$191 \quad W_{under-estimated\ belowground} = a * d_{broken\ tip}^b + \epsilon \quad (3)$$

192 where $W_{under-estimated\ below-ground}$ (g) is the remaining below-ground biomass beyond the broken root
 193 tip, $d_{broken\ tip}$ (mm) is the diameter of the broken root tip and, a and b are regression parameters to
 194 be estimated and ϵ is the error term.

195 Total below-ground biomass was determined from the sum of the root biomass extracted and the
 196 remaining root biomass estimated with the broken tip diameter. We fitted different allometric
 197 relationships between below-ground biomass and the dominant basal diameter as well as between
 198 below-ground biomass and basal area including the basal diameter of all shoots from a stool.

$$199 \quad W_{below-ground} = a * D_{basal}^b + \epsilon \quad (4)$$

$$200 \quad W_{below-ground} = a * BA_{basal}^b + \epsilon \quad (5)$$

201 where $W_{below-ground}$ (g) is the below-ground biomass, D_{basal} (mm) is the dominant basal
 202 diameter per stool, BA_{basal} (mm²) is the basal area obtained for the total basal diameters of all the
 203 shoots per stool and a and b are regression parameters to be estimated and ϵ is the error term.

204 Different hierarchical levels were considered: (i) general level using all information (196 data
 205 points) and (ii) a model at genotype level.

206 To evaluate the goodness-of-fit of the models, we used the proportion of variance explained by
 207 the models (pseudo coefficient of determination, $pse-R^2$) and the root of mean square error
 208 ($RMSE$).

$$209 \quad pse-R^2 = r_{y_i \hat{y}_i}^2 \quad (6)$$

$$210 \quad RMSE(g) = \sqrt{\frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{n-p}} \quad (7)$$

211 where $r_{y_i \hat{y}_i}^2$ is the correlation coefficient for a linear regression between the observed and the
 212 predicted values of the dependent variable (Ryan, 1997), y_i is the observed value, \hat{y}_i is the
 213 estimated value, n is the number of data used in the fitting and p is the number of parameters to
 214 be estimated.

215 The similarities in allometric relationships among genotypes were evaluated by cross-predicting
 216 with the proposed models and comparing the predictions at the different levels – i.e. at the general
 217 level using all information (196 data points) and at the genotype level (‘AF2’, ‘I-214’ and
 218 ‘Monviso’) – with the observed values. The α -trimmed non-parametric validation tests were used
 219 for evaluating the similarity in biomass allometry among genotypes (Pérez-Cruzado *et al.*, 2015).

220 2.3.3. Stand extrapolation and carbon accumulation

221 The below-ground biomass of each stool in the plantation was estimated using the best models
222 for each genotype provided in this study. From the above-ground biomass data collected at the
223 end of the rotation (16-25 stools per site, replicate and genotype) and the estimated below-ground
224 biomass data calculated with the models, the carbon accumulation in each above- and below-
225 ground fraction was calculated using the carbon concentration percentages that were obtained by
226 dried combustion, analyzing samples of each genotype (Table 3).

227 3. Results

228 3.1. Exploratory analysis of the data

229 In the case of the above-ground biomass there were significant differences among the genotypes
230 (p -value=0.040), sites (p -value=0.001), and the interactions between them (p -value<0.045). The
231 interactions among genotypes and sites are shown in Table 4. In every site, ‘AF2’ was the most
232 productive genotype with a $W_{above-ground}$ of 2,441 g stool⁻¹ yr⁻¹, and ‘I-214’ was the least productive
233 with 1,683 g stool⁻¹ yr⁻¹, with the exception of S3, where ‘I-214’ exhibited a special behaviour and
234 was the most productive genotype (Table 4). The differences among sites were even more evident
235 (Fig. 1) and the $W_{above-ground}$ ranged between 610.9 and 3,123 g stool⁻¹ yr⁻¹, being the S3 the most
236 productive and S2 the least productive in all cases.

237 The below-ground biomass showed neither differences among the genotypes (p -value=0.818) nor
238 G*S interactions (p -value=0.766), but there were significant differences among the sites (p -
239 value<0.009) (Fig. 1). The below-ground biomass ranged between 330.6 and 360.3 g stool⁻¹ yr⁻¹
240 for genotypes ‘Monviso’ and ‘AF2’, respectively, and between 161.6 g stool⁻¹ yr⁻¹ and 514.1 g
241 stool⁻¹ yr⁻¹ for the different sites (S2 and S3, respectively).

242 There were significant differences in the root:shoot ratio among the sites (p -value=0.001),
243 genotypes (p -value<0.001) and the G*S interactions (p -value<0.001) (Fig. 2). The root-shoot
244 ratio of ‘I-214’ (i.e. a value of 0.29) was significantly different from the two other genotypes,
245 which showed ratios of 0.21 and 0.18 for ‘Monviso’ and ‘AF2’, respectively. Analyzing the G*S
246 interaction, it is observed that the root:shoot ratio of ‘I-214’ (i.e. a value of 0.29) is higher than the
247 two other genotypes (averages of 0.21 and 0.18 for ‘Monviso’ and ‘AF2’, respectively), with the
248 exception of the site S3. In this location, ‘I-214’ showed a low root:shoot ration due to the

249 notable imbalance between the above- and below-ground yield. Even so, this genotype also stands
250 out as regards the below-ground fraction in site S3 (Table 4).

251 The relation between the root:shoot ratio and D_{basal} for each genotype is shown in Fig. 3. The
252 root:shoot ratio was simulated using the specific genotype-models fitted in this study (Table 4)
253 for the below-ground biomass and the specific genotype-models for the above-ground biomass
254 explained in Oliveira *et al.* (2017). The lines represent the trends of the ratios and the symbols
255 represent the real values calculated in this study. We found significant differences among
256 genotypes using the α -trimmed non-parametric validation tests (Pérez-Cruzado *et al.*, 2015). As
257 can be seen from Fig. 3 the trends obtained from the models explained how the different genotypes
258 behaved. The three genotypes included in this study showed a greater decline in the root:shoot
259 ratio at smaller diameters, achieving a constant value as the diameter increases. Whereas ‘AF2’
260 and ‘I-214’ showed higher root:shoot ratios for small diameters, ‘Monviso’ showed similar
261 root:shoot ratios regardless of the diameter.

262 **3.2. Allometric relationships for below-ground biomass**

263 From the similarities in the allometric parameters between both below-ground biomass estimation
264 models (general model and model at genotype level), it became clear that a specific model should
265 be used for each genotype, at least for the first rotation that was the rotation analysed in this study
266 (data not shown). The environmental effect included in the model derived from the four study
267 sites made the below-ground biomass allometry different for each genotype. Whereas there were
268 no significant differences in below-ground biomass among the genotypes for the four sites nor
269 G*S interaction, differences were observed among genotypes in some particular sites.

270 The below-ground biomass estimation models with shoot diameter as the predictor variable were
271 as good as those which used the basal area, at least for this first rotation. Therefore, given their
272 simplicity, we selected the below-ground biomass estimation models with shoot diameter as the
273 predictor variable for this study (Table 5). The genotype specific below-ground biomass
274 estimation models accounted for more than 80% of the total variability in root biomass. All
275 considered equations converged and the \hat{b} parameter estimates significantly improved the quality
276 of the model fit to the data at p -value = 0.05. The general below-ground biomass estimation model

277 explained 78% of the total variability in root biomass. It included all the variability of the different
278 genotypes and site conditions typical of the Mediterranean climate.

279 **3.3. Above- and below-ground carbon accumulation**

280 The carbon accumulated per year in the above-ground fraction of the biomass was four to seven
281 times larger than the carbon accumulated in the below-ground fraction. The highest amount of
282 carbon in the first rotation was stored in the above-ground woody biomass, although between
283 12.38 and 17.31 % of the total carbon pool is stored in the below-ground fraction, and will remain
284 there regardless of the fate of the above-ground biomass.

285 The range of genotypic variation was smaller in the below-ground fraction than in the above-
286 ground fraction. In both fractions (above- and below-ground) genotype 'AF2' showed the highest
287 amount of carbon accumulation (6.44 and 0.91 Mg C ha⁻¹ yr⁻¹, respectively). In contrast carbon
288 accumulation values of 'I-214' (4.28 Mg C ha⁻¹ yr⁻¹) were the lowest in the above-ground fraction,
289 while carbon accumulation values of 'Monviso' (0.89 Mg C ha⁻¹ yr⁻¹) were the lowest in the
290 below-ground fraction (Table 6).

291 **4. Discussion**

292 **4.1. Exploratory analysis of the data**

293 Genetic and environmental effects on the above-ground biomass of SRC have been previously
294 documented in Europe (Benetka *et al.*, 2014; Dillen *et al.*, 2007), including under Mediterranean
295 conditions (Bergante *et al.*, 2010; Sixto *et al.*, 2015). Nevertheless, the results of the present study
296 show that the differences in the above-ground biomass due to the genotype are not always
297 reflected in the below-ground part. The factors linked to the site had a larger influence. It seems
298 that at least under the conditions described, differences among genotypes as well as the G*S
299 interaction in the below-ground fraction are not evident. This finding contradicts some studies
300 that reported a strong genetic control of allometric biomass partitioning to roots (Al Afas *et al.*,
301 2008; King *et al.*, 1999), although other studies reported results similar to the present ones
302 (Dickmann *et al.*, 1996; Verlinden *et al.*, 2013). As regards fine roots a study on poplar SRC
303 without irrigation, reported that in spite of the genotypic differences in above-ground biomass,
304 annual fine root productivity was similar (Verlinden *et al.*, 2013).

305 The range of above-ground yield (1,683-2,441 g stool⁻¹ yr⁻¹) in our plantations at the end of the
306 first rotation was larger than that reported for other poplar SRC plantations (e.g. 380-870 g stool⁻¹
307 yr⁻¹ in Belgium (Berhongaray *et al.* 2015) or 118-438 g stool⁻¹ yr⁻¹ under conditions with plenty
308 of water and nitrogen at the end of the first growing season in the USA (Pregitzer *et al.* 1990)).
309 However, the below-ground biomass values found in this study (331-360 g stool⁻¹ yr⁻¹) were very
310 similar to those previously reported (e.g. Berhongaray *et al.* (2015): 270-400 g stool⁻¹ yr⁻¹; or
311 Pregitzer *et al.* (1990): 132-375 g stool⁻¹ yr⁻¹). This may be highly affected by the fact that
312 irrigation is applied, as well as by the climatic conditions of the area. As poplar is an opportunistic
313 rooter (Hallgren, 1989), the availability of water through irrigation means that it does not need to
314 explore a large ground area or to develop an extensive root system. This might, however, also be
315 explained by the limited space for development due to the high planting density. Furthermore, the
316 guaranteed water availability, together with the high average temperature and irradiance in the
317 Mediterranean region (Faria *et al.*, 1998), has led to a high above-ground development.

318 As regards the root:shoot ratio values, we found genotypic differences similar to those reported
319 in previous studies with different poplar genotypes (King *et al.*, 1999; Yin *et al.*, 2005). The
320 root:shoot ratios in our study (0.18-0.29) were lower than those obtained in two other studies,
321 both with younger plantations (2 years) (0.27-0.33 or 0.45-0.70) (Berhongaray *et al.*, 2015; Friend
322 *et al.*, 1991). However, our root:shoot ratios are consistent with those (0.16-0.23) reported for
323 another irrigated Mediterranean plantation (Calfapietra *et al.*, 2003). These findings emphasize
324 the difference in above- and below-ground development under irrigation, with higher growth of
325 the above-ground fraction in relation to other plantations although with similar growth of the
326 below-ground fraction.

327 The root:shoot ratio reflects – in physiological terms – the differential investment of
328 photosynthates between the above- and below-ground fraction of the tree (Titlyanova *et al.*,
329 1999). The ratio may, therefore, reflect the cumulative response to biotic, abiotic and management
330 influences, although it only represents the net effects of carbon allocation, without considering
331 carbon losses (Mokany *et al.*, 2006).

332 Our results showed an exponential decrease in the root:shoot ratio values as basal diameter
333 increased, which agrees with the findings of other authors who reported that this ratio declined
334 significantly with stand age (Berhongaray *et al.*, 2015; Coleman *et al.*, 2004; Coyle and Coleman,
335 2005). The genetic background of the two groups of genotypes ('AF2' and 'I 214', versus
336 'Monviso') is different, but there were insufficient genotypes to support this hypothesis. The
337 differences in the root:shoot ratio value reported by other authors (e.g. Berhongaray *et al.*, 2015)
338 are probably explained by the differences in age (3 or 4 years old *versus* 2 years old), although
339 the higher above-ground biomass in our plantations was also due to the better growing conditions.
340 When developing an eco-physiological based model (as e.g. 3PG) the root:shoot ratio can be very
341 useful for estimating the root biomass allocation (Landsberg and Waring, 1997). The maximum
342 fraction of NPP allocated to roots has been considered as 0.7, and the minimum as 0.17 (Headlee
343 *et al.*, 2013). The latter authors argued that different genotypes may have different parameter
344 values, particularly in the case of maximum allocation. In this sense, the relationship shown by
345 this root:shoot ratio under Mediterranean conditions could provide a useful estimation parameter
346 for the root biomass allocation.

347 **4.2. Allometric relationships for below-ground biomass**

348 Given the differences among sites but not among genotypes nor G*S interaction, it would seem
349 rational to fit site-specific below-ground biomass models with no differentiation at genotype
350 level. However, site-specific models have been ruled out, at least in this study, due to their limited
351 applicability (Oliveira *et al.*, 2017). When fitting general models it is important to consider that
352 the inclusion of all sites would result in model errors due to the significant differences associated
353 with the site. Therefore, both a general model and genotype-specific models were developed in
354 this study, despite the absence of significant differences among genotypes. Other authors,
355 however, have opted for a single, general model which included all genotypes (Verlinden *et al.*,
356 2013), given that no differences were found among them. In our case, the models used data from
357 the four study sites and therefore this variability has been included in the model.
358 The availability of modeling tools to estimate the below-ground fraction is important when
359 quantifying the distribution and the amount of carbon. Models for predicting below-ground

360 biomass in poplar trees have been developed also using shoot diameter or basal area as
361 independent variables (Berhongaray *et al.*, 2015; Domenicano *et al.*, 2011; Heilman *et al.*, 1994).
362 However, none of them has been developed specifically for short rotation, high density poplar
363 plantations under Mediterranean conditions. Furthermore, both methodologies (direct digging or
364 estimations based on models) have their shortcomings due to root breakage and incomplete
365 sampling associated with excavations of root systems (Clark *et al.*, 2001).

366 **4.3. Carbon accumulation**

367 The accumulation of carbon by SRC occurs in the soil, not only in the litterfall and fine root
368 turnover, but also in the below-ground biomass fraction (Pacaldo *et al.*, 2014). Hence it is
369 important to take this fraction into account in the final carbon balance. The carbon concentrations
370 found in our plantations were consistent with previously reported values (Fang *et al.*, 2007), that
371 ranged from 42-50% for all biomass components, with the highest carbon concentrations in
372 shoots.

373 As a result of the higher biomass yield found in our studies, the values for above-ground carbon
374 accumulation were higher (4.3-6.4 Mg C ha⁻¹ yr⁻¹) than the values obtained in conditions of lower
375 productivity (e.g. Verlinden *et al.*, 2013: 2.5 Mg C ha⁻¹ yr⁻¹). Other studies in which biomass yield
376 were more similar to those obtained here reported similar values (e.g. Fang *et al.*, 2007: 3.1-5.75
377 Mg C ha⁻¹ yr⁻¹). However, under Mediterranean conditions with irrigation (Central Italy), the
378 above-ground carbon accumulation were notably higher (6.1-8.6 Mg C ha⁻¹ yr⁻¹; Gielen *et al.*,
379 2005) than the values obtained in our study. The below-ground carbon accumulations (0.89-0.91
380 Mg C ha⁻¹ yr⁻¹) were very similar to those reported under non-Mediterranean conditions
381 (Verlinden *et al.*, 2013: 1.01 Mg C ha⁻¹ yr⁻¹), although a wider range of total below-ground
382 biomass (0.85-1.5 Mg C ha⁻¹ yr⁻¹) was reported for lower density plantations (1,111 trees ha⁻¹;
383 Fang *et al.*, 2007). Under Mediterranean conditions with irrigation in Central Italy the below-
384 ground carbon accumulations reported were also similar (0.87-1.21 Mg C ha⁻¹ yr⁻¹; Gielen *et al.*,
385 2005) to the values obtained in our study.

386 The below-ground carbon accumulation would surely be larger if not only the carbon accumulated
387 in the below-ground biomass but also in the fine roots turnover had been taken into account.
388 However these accumulations are difficult to account for because of the high turnover rates.

389 **5. Conclusions**

390 This study provided models which may be used to estimate the biomass yield in poplar root
391 systems in Mediterranean SRC during the first rotation. The root:shoot ratio provided useful
392 information with regard to the allocation of the biomass in the presence of irrigation, which could
393 be useful for the development of future ecophysiological models. Whereas the above-ground
394 biomass yield was notably higher than that reported in other studies, the below-ground biomass
395 yield was more similar. The accumulation of carbon in the below-ground fraction was therefore
396 similar to other scenarios and was approximately 1 Mg C ha⁻¹ yr⁻¹.

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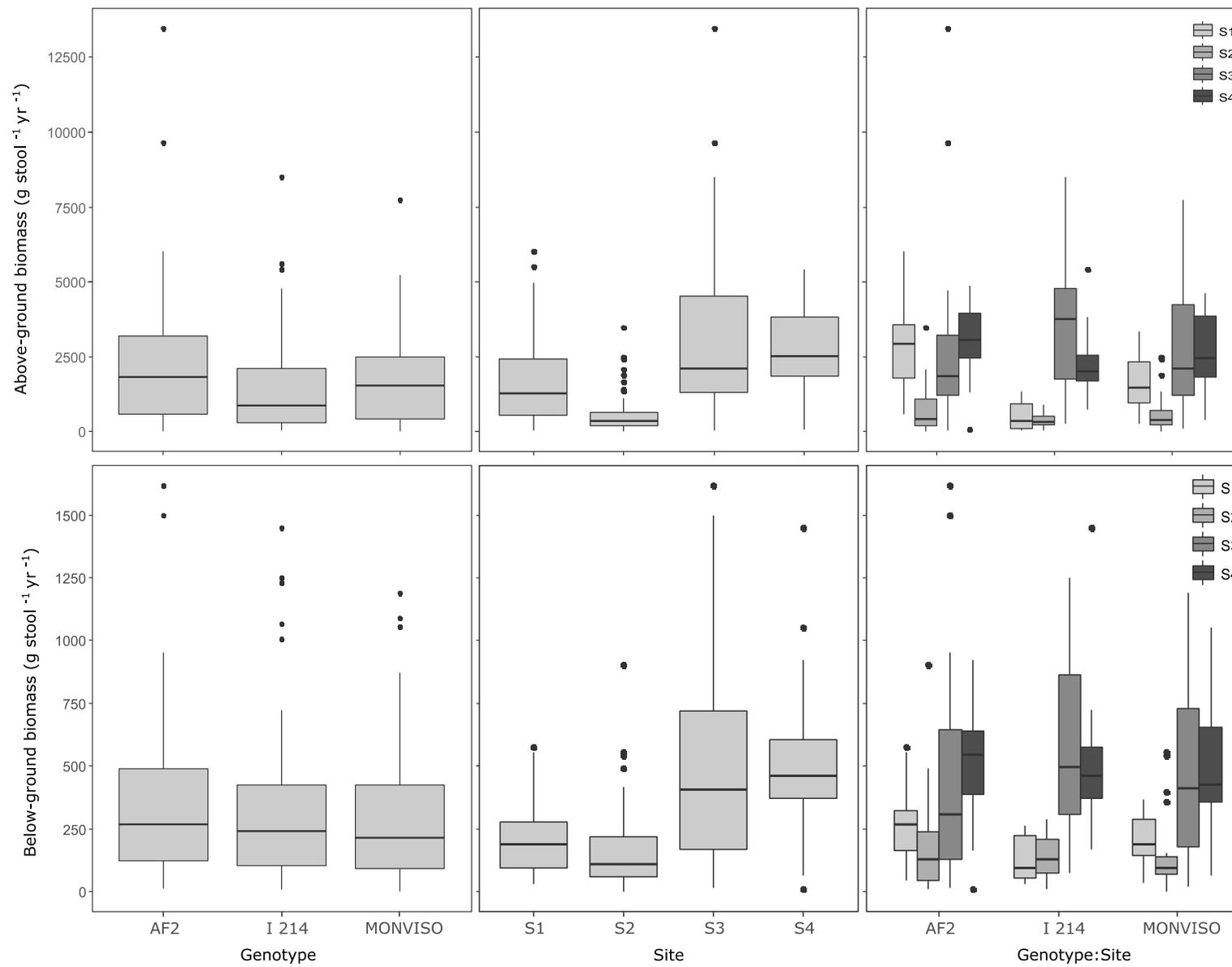
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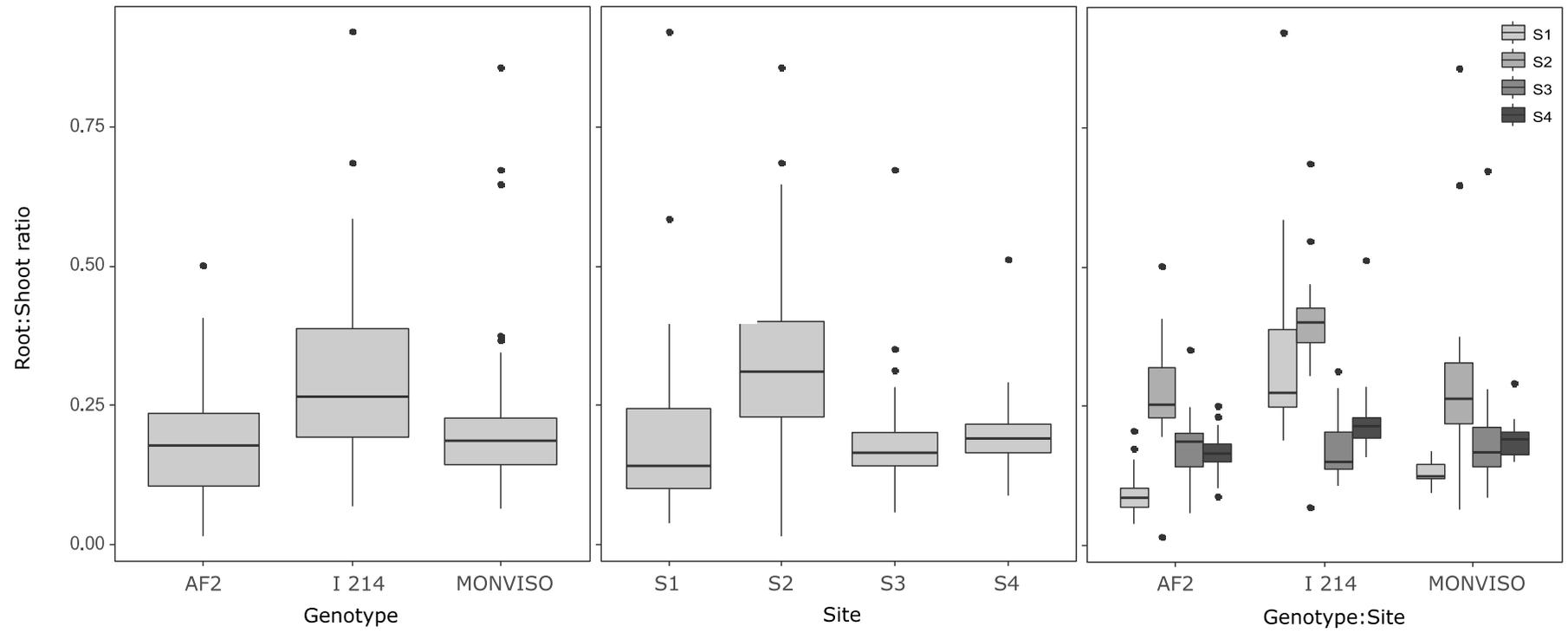
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Fig. 1. Above-ground (top) and below-ground (bottom) biomass per stool and per year at end of the first rotation (three genotypes, four sites and G*S interactions). The values have been represented by boxplots.

Fig. 2. Root:shoot ratio (calculated per stool and year) at the end of the first rotation (three genotypes, four sites and G*S interactions).

Fig. 3. Root:shoot ratio estimated (lines) and real values (points) for genotypes 'AF2', 'I-214' and 'Monviso' respectively.





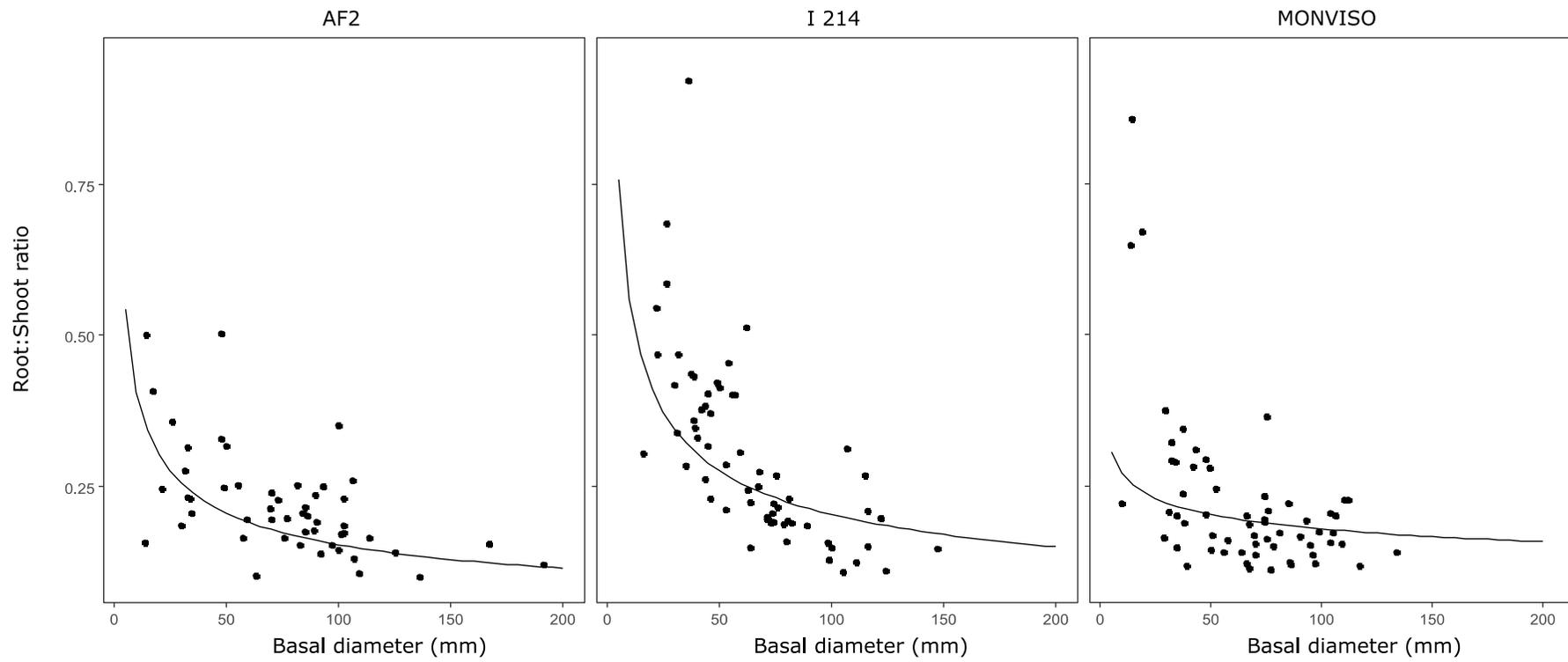


Table 1. Main characteristics of the four experimental sites (S1 to S4) in Spain including the Köppen climate classification.

SITES	COORDINATES		KÖPPEN CLIMATE CLASSIFICATION	ALTITUDE (masl)	TM (°C)	pH	OM (%)	STAGE OF DEVELOPMENT	DESING (m)	DENSITY (cuttings ha ⁻¹)	REPLICATE
	LATITUDE	LONGITUDE									
S1	42° 9' 6.24'' N	5° 33' 42.37'' W	Temperate Mediterranean climate	254	11.8	6.84	0.62	R4S4	3 x 0.5	6,666	3
S2	42° 37' 25'' N	6° 38' 54'' W	Temperate Mediterranean climate	556	12.7	6.21	0.85	R3S3	2.5 x 0.3	13,333	4
S3	39° 14' 42.75'' N	4° 13' 21.88'' W	Cold semi-arid climate	670	14.7	6.29	1.25	R4S4	3 x 0.5	6,666	3
S4	39° 15' 24.74'' N	4° 13' 36.87'' W	Warm Mediterranean climate	670	14.6	6.08	1.11	R3S3	3 x 0.6	5,555	3

TM: average annual mean temperature (°C); OM: soil organic matter (%); Stage of development: R4S4 indicates a root age of four years with four year old shoots, a.s.o. The number of replicates at each of the sites is also indicated.

Table 2. Parameter estimates and goodness-of-fit statistics for the remaining root biomass models with the equation $W_{under - estimated\ belowground} = a * d_{broken\ tip}^b + \epsilon$ for three genotypes during the first rotation.

Genotype	Estimate	Std. Error	t value	p-value (> t)	RMSE	pse-R²	DF
‘AF2’	0.009	0.002	4.300	<0.001	8.491	0.940	194
	2.476	0.056	43.88	<0.001			
‘I-214’	0.295	0.047	6.312	<0.001	5.414	0.831	348
	1.549	0.049	31.43	<0.001			
‘Monviso’	0.198	0.046	4.304	<0.001	8.231	0.832	256
	1.636	0.065	25.12	<0.001			

Std.Error: standard error; RMSE: root of mean square error; pse-R²: pseudo coefficient of determination; DF: degrees of freedom.

Table 3. Carbon (C) concentration (in %) of the above- and the below-ground woody biomass for the three genotypes. Values in brackets correspond to the standard deviation.

	ABOVE-GROUND CARBON (%)	BELOW-GROUND CARBON (%)
'AF2'	46.53 (± 0.18)	45.95 (± 0.17)
'I-214'	46.75 (± 0.15)	46.30 (± 0.05)
'Monviso'	49.92 (± 0.24)	46.36 (± 0.12)

Table 4. Genotype and total values (i.e. means and standard deviation) for the above- and below-ground biomass and the root:shoot ratio at the four experimental sites.

	SITE	AF2	I 214	MONVISO	TOTAL
ABOVE-GROUND BIOMASS (g yr ⁻¹)	S1	2894.3232 (1645.88)	534.1882 (454.66)	1650.0670 (966.90)	1692.8595 (1180.65)
	S2	779.3989 (862.18)	367.2629 (232.49)	685.9394 (763.67)	610.8671 (216.08)
	S3	3099.1010 (3725.13)	3553.2708 (2191.53)	2716.6280 (2173.30)	3122.9999 (418.83)
	S4	2992.0906 (1283.14)	2276.8529 (1160.19)	2593.5782 (1440.59)	2620.8406 (358.40)
	TOTAL	2441.2284 (1111.04)	1682.8937 (1516.74)	1911.5532 (945.83)	2011.8918 (1148.08)
BELOW-GROUND BIOMASS (g yr ⁻¹)	S1	264.1482 (153.71)	133.2521 (86.53)	208.9721 (107.22)	202.1241 (65.72)
	S2	186.4223 (217.88)	140.7222 (89.21)	157.6066 (165.90)	161.5837 (23.11)
	S3	487.9445 (506.62)	583.9806 (390.73)	470.2596 (367.14)	514.0616 (61.19)
	S4	502.6232 (247.55)	511.1325 (294.09)	485.3672 (290.92)	499.7076 (13.13)
	TOTAL	360.2846 (159.19)	342.2719 (238.92)	330.5514 (171.44)	344.3693 (175.10)
ROOT:SHOOT RATIO	S1	0.0978 (0.05)	0.3524 (0.19)	0.1334 (0.02)	0.1946 (0.14)
	S2	0.2783 (0.11)	0.3980 (0.12)	0.3055 (0.17)	0.3273 (0.06)
	S3	0.1798 (0.07)	0.1756 (0.06)	0.2042 (0.14)	0.1865 (0.02)
	S4	0.1676 (0.04)	0.2334 (0.08)	0.1912 (0.04)	0.1974 (0.03)
	TOTAL	0.1809 (0.07)	0.2899 (0.10)	0.2086 (0.07)	0.2264 (0.09)

Table 5. Parameter estimates and goodness-of-fit statistics for the root biomass models with the equation $W_{belowground} = a * D_{basal}^b + \epsilon$ (first rotation).

Genotype	Estimate	Std. Error	t value	p-value (> t)	RMSE	pse-R²	DF																																
AF2	0.080	0.048	1.669	0.010	519.1	0.825	63																																
	2.156	0.123	17.53	<0.001				I-214	0.141	0.094	1.505	0.137	476.3	0.838	64	2.107	0.142	14.81	<0.001	Monviso	0.071	0.060	1.183	0.241	450.9	0.812	63	2.246	0.185	12.24	<0.001	General	0.217	0.082	2.658	0.009	529.4	0.787	194
I-214	0.141	0.094	1.505	0.137	476.3	0.838	64																																
	2.107	0.142	14.81	<0.001				Monviso	0.071	0.060	1.183	0.241	450.9	0.812	63	2.246	0.185	12.24	<0.001	General	0.217	0.082	2.658	0.009	529.4	0.787	194	1.981	0.080	24.90	<0.001								
Monviso	0.071	0.060	1.183	0.241	450.9	0.812	63																																
	2.246	0.185	12.24	<0.001				General	0.217	0.082	2.658	0.009	529.4	0.787	194	1.981	0.080	24.90	<0.001																				
General	0.217	0.082	2.658	0.009	529.4	0.787	194																																
	1.981	0.080	24.90	<0.001																																			

where $W_{below-ground}$ (g) DM is total below-ground dry biomass and D_{basal} (mm) is diameter over bark at 10 cm for dominant shoots per stool. Std.Error: standard error; RMSE: root of mean square error; pse-R²: pseudo coefficient of determination; DF: degrees of freedom.

Table 6. Above- and below-ground biomass and accumulated amount of carbon per year in the three genotypes. Values in brackets correspond to the standard deviation.

GENOTYPE	ABOVE-GROUND BIOMASS (Mg ha⁻¹ yr⁻¹)	BELOW-GROUND BIOMASS (Mg ha⁻¹ yr⁻¹)	ABOVE-GROUND CARBON ACCUMULATION (Mg C ha⁻¹ yr⁻¹)	BELOW-GROUND CARBON ACCUMULATION (Mg C ha⁻¹ yr⁻¹)
AF2	13.83 (±5.10)	1.98 (±0.38)	6.44(±2.37)	0.91 (±0.18)
I-214	9.15 (±6.15)	1.93 (±0.85)	4.28 (±2.87)	0.90 (±0.39)
Monviso	11.43 (±1.76)	1.92 (±0.33)	5.36 (±0.83)	0.89 (±0.15)

Supplementary Table 1: Mean values of height (H_{total}) and basal diameter (D_{basal}) of the dominant shoot for each genotype and site.

SITE	DENSITY	STAGE OF DEVELOPMENT	GENOTYPE	MEAN D_{basal} (mm)	MEAN H_{total} (cm)
S1	6666	R4S4	AF2	79.64	918.91
			I 214	50.64	482.69
			MONVISO	65.70	675.56
S2	13333	R3S3	AF2	45.78	612.24
			I 214	37.54	505.23
			MONVISO	45.19	702.80
S3	6666	R4S4	AF2	82.89	1111.22
			I 214	87.17	1265.22
			MONVISO	65.52	1023.07
S4	5555	R3S3	AF2	75.04	1020.10
			I 214	85.98	1144.99
			MONVISO	66.32	956.94