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

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Abstract

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

Keywords: Root biomass; Below-ground allometry models; *Populus* spp.; Root:Shoot ratio; Short Rotation Coppice (SRC).

1. Introduction

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

SRC crops are defined as high-density plantations of fast growing trees (such as poplars or willows), managed in rotations of 2 to 8 years (Berhongaray *et al.*, 2017; Ferré and Comolli, 2018). This implies a different stage of development of the shoots (S) which regrow from the stool and the root (R) in successive rotations. Plant roots are an essential component of net primary productivity (NPP), which is the sum of visible growth and litter production of above- and below-ground components (Roy *et al.*, 2001). Poplar root systems can account for around 25-35% of the total plant biomass (Heilman *et al.*, 1994; Pregitzer and Friend, 1996), this percentage being higher (up to 63%) in juvenile trees (Block *et al.*, 2006; King *et al.*, 1999; Yin *et al.*, 2004). In SRC plantations the roots can be of even higher importance given the expected rotations, which will imply that the above-ground and the below-ground parts are of different ages over the duration of the crop. Due to the difficulties in studying the root system, advancing the knowledge in this area is far from simple (Jha, 2017; Mokany *et al.*, 2006). Despite this, some studies have focused on this fraction of the stool in relation to the whole SRC system (Berhongaray *et al.*, 2015; Block *et al.*, 2006). Although studies on poplar root systems are somewhat scarce, there are more that focus on fine roots given the ease of their extraction, as well as their importance in the assimilation of water and nutrients (Dickmann *et al.*, 1996) and their important influence on carbon turnover (Mulia and Dupraz, 2006), despite the fact that fine roots only represent a small fraction of total tree biomass (Afas *et al.*, 2008; Berhongaray *et al.*, 2013). There has not been much progress in the study of roots under irrigated short rotation plantations in Mediterranean areas, neither as regards fine roots nor as regards the radical system as a whole.

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

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

The present study evaluated the allocation of the above and below-ground fraction in different short rotation poplar plantations under Mediterranean conditions at the end of the first rotation. Our main objective was to contribute towards furthering our understanding of the root system, providing accurate knowledge of the root system allocation in the first rotation of this type of plantations under Mediterranean conditions, which necessitate irrigation due to the severe summer drought. Therefore four plantations in Spain comprising three commonly used genotypes and with a range of different ages were evaluated. The specific objectives were: (i) to quantify the above and below-ground biomass, as well as the root:shoot relationship at genotype level, (ii) to develop allometric relationships based on shoot basal diameter or shoot basal area, from which root biomass could be estimated, and finally (iii) to compare the total and disaggregated amount of carbon accumulated in the biomass (above and below-ground) at plantation level, considering the genotype used.

2. Material and Methods

2.1. Experimental field sites

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

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

2.2. Above- and below-ground biomass

The sampling unit was considered the stool, which is the plant consisting of a stump, base or root from which one or several shoots are produced. Measurements of the above-ground fraction were taken at the end of each rotation on 16-25 stools per site, replicate and genotype. Shoot diameters over bark at 10 cm above ground (d_{basal}, mm), accounting for the size of the shoots at the usual harvesting height, were measured using a digital calliper (Absolute, Vogel Germany, Kevelaer, Germany) (accuracy to 1 mm). The basal area (BA_{basal}, mm^2) of the entire stool was calculated from basal diameters of all shoots. Total height of the highest shoot (H_{total}, cm) was also measured with a measuring pole to an accuracy of 1 cm. The number of shoots and the density of living stools were also recorded to quantify mortality. The above-ground woody biomass was assessed in all stools through destructive sampling after leaf fall. We considered above-ground woody biomass as the whole biomass above the harvesting height of these stools, which were cut back at 10 cm above the soil. Total above-ground dry woody biomass ($W_{above\text{-}ground}$, g DM (dry matter)) was determined for each sampling unit from the fresh weight and the estimated above-ground wood moisture by randomly selecting one entire stool from each of the plots and then oven-drying a randomly selected sample of each genotype to constant weight at 105 ºC.

Five stools for each site, replicate and genotype were selected for below-ground biomass measurements. They were selected by covering the different diameter classes observed for each replicate. The root systems were extracted using a backhoe with manual assistance, exploring around 1 m^2 of soil for each stool (the dimensions were adapted to the different planting design in proportion to the density). All roots that we were able to retrieve within this area (up to a diameter of 0.10 mm) were manually collected, those roots belonging to the adjacent stools were excluded, although in some cases identification was not possible. The diameter of each broken root tip in each sample was measured to estimate the remaining biomass in the soil. We considered below-ground biomass as the whole biomass below the harvesting height of these stools, so it included the stump below the harvesting height, as well as the coarse roots and fine roots. Total dry biomass of the roots ($W_{below\text{-}ground}$, g DM) was determined for each sample from the fresh weight and the estimated below-ground wood moisture that was obtained using the same procedure as for the above-ground wood moisture.

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

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

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

2.3. Data analysis

2.3.1. Exploratory analysis of the data

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

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

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

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

To get a better understanding of below-ground biomass distribution we explored the relationship between the below- and above-ground biomass using the root:shoot ratio. The similarities in allometric relationships among genotypes were evaluated by cross-predicting with the proposed root:shoot ratio theoretical trend and comparing this trend at the different genotype levels (‘AF2’, ‘I-214’ and ‘Monviso’) with the observed values. Two levels were assumed to share the same allometry when the model fitted for a given level produced non-deviated predictions for the other. The α-trimmed non-parametric validation tests were used to evaluate the similarity in biomass allometry among genotypes (Pérez-Cruzado et al., 2015). The null hypothesis for the test was that both samples belonged to the same population, so small p-values led us to reject the null hypothesis that both samples shared the same model. We simulated the theoretical root:shoot ratio trend using models to estimate the above and the below-ground biomass and calculating the root:shoot ratio derived from these estimations. For the below-ground biomass estimation, we used the specific genotype-models fitted in the current study, whereas for the above-ground biomass estimation we used the *ad hoc* specific genotype-models previously described (Oliveira *et al.* 2017), that were fitted for Mediterranean conditions. Both models are specific to short rotation, high density plantations, so the estimations of the below- and above-ground biomass ranged from 0 to 200 mm basal diameter.

2.3.2. Allometric relationships for below-ground biomass

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

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

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

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

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

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

where $W_{below-ground}$ (g) is the below-ground biomass, D_{basal} (mm) is the dominant basal diameter per stool, BA_{basal} (mm^2) is the basal area obtained for the total basal diameters of all the shoots per stool and a and b are regression parameters to be estimated and ϵ is the error term. Different hierarchical levels were considered: (i) general level using all information (196 data points) and (ii) a model at genotype level.

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

$$pse\text{-}R^2 = r^2_{y_i\hat{y}_i} \quad (6)$$

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

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

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

2.3.3. Stand extrapolation and carbon accumulation

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

3. Results

3.1. Exploratory analysis of the data

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

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

There were significant differences in the root:shoot ratio among the sites (*p*-value=0.001), genotypes (*p*-value<0.001) and the G*S interactions (*p*-value<0.001) (Fig. 2). The root-shoot ratio of 'I-214' (i.e. a value of 0.29) was significantly different from the two other genotypes, which showed ratios of 0.21 and 0.18 for 'Monviso' and 'AF2', respectively. Analyzing the G*S interaction, it is observed that the root:shoot ratio of 'I-214' (i.e. a value of 0.29) is higher than the two other genotypes (averages of 0.21 and 0.18 for 'Monviso' and 'AF2', respectively), with the exception of the site S3. In this location, 'I-214' showed a low root:shoot ration due to the notable imbalance between the above- and below-ground yield. Even so, this genotype also stands out as regards the below-ground fraction in site S3 (Table 4).

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

3.2. Allometric relationships for below-ground biomass

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

The below-ground biomass estimation models with shoot diameter as the predictor variable were as good as those which used the basal area, at least for this first rotation. Therefore, given their simplicity, we selected the below-ground biomass estimation models with shoot diameter as the predictor variable for this study (Table 5). The genotype specific below-ground biomass estimation models accounted for more than 80% of the total variability in root biomass. All considered equations converged and the $\hat{b}$ parameter estimates significantly improved the quality of the model fit to the data at p-value = 0.05. The general below-ground biomass estimation model explained 78% of the total variability in root biomass. It included all the variability of the different genotypes and site conditions typical of the Mediterranean climate.

3.3. Above- and below-ground carbon accumulation

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

The range of genotypic variation was smaller in the below-ground fraction than in the above-ground fraction. In both fractions (above- and below-ground) genotype ‘AF2’ showed the highest amount of carbon accumulation (6.44 and 0.91 Mg C ha^{-1} yr^{-1}, respectively). In contrast carbon accumulation values of ‘I-214’ (4.28 Mg C ha^{-1} yr^{-1}) were the lowest in the above-ground fraction, while carbon accumulation values of ‘Monviso’ (0.89 Mg C ha^{-1} yr^{-1}) were the lowest in the below-ground fraction (Table 6).

4. Discussion

4.1. Exploratory analysis of the data

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

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

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

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

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

4.2. Allometric relationships for below-ground biomass

Given the differences among sites but not among genotypes nor G*S interaction, it would seem rational to fit site-specific below-ground biomass models with no differentiation at genotype level. However, site- specific models have been ruled out, at least in this study, due to their limited applicability (Oliveira *et al.*, 2017). When fitting general models it is important to consider that the inclusion of all sites would result in model errors due to the significant differences associated with the site. Therefore, both a general model and genotype-specific models were developed in this study, despite the absence of significant differences among genotypes. Other authors, however, have opted for a single, general model which included all genotypes (Verlinden *et al.*, 2013), given that no differences were found among them. In our case, the models used data from the four study sites and therefore this variability has been included in the model.

The availability of modeling tools to estimate the below-ground fraction is important when quantifying the distribution and the amount of carbon. Models for predicting below-ground biomass in poplar trees have been developed also using shoot diameter or basal area as independent variables (Berhongaray *et al.*, 2015; Domenicano *et al.*, 2011; Heilman *et al.*, 1994). However, none of them has been developed specifically for short rotation, high density poplar plantations under Mediterranean conditions. Furthermore, both methodologies (direct digging or estimations based on models) have their shortcomings due to root breakage and incomplete sampling associated with excavations of root systems (Clark *et al.*, 2001).

4.3. Carbon accumulation

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

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

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

5. Conclusions

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

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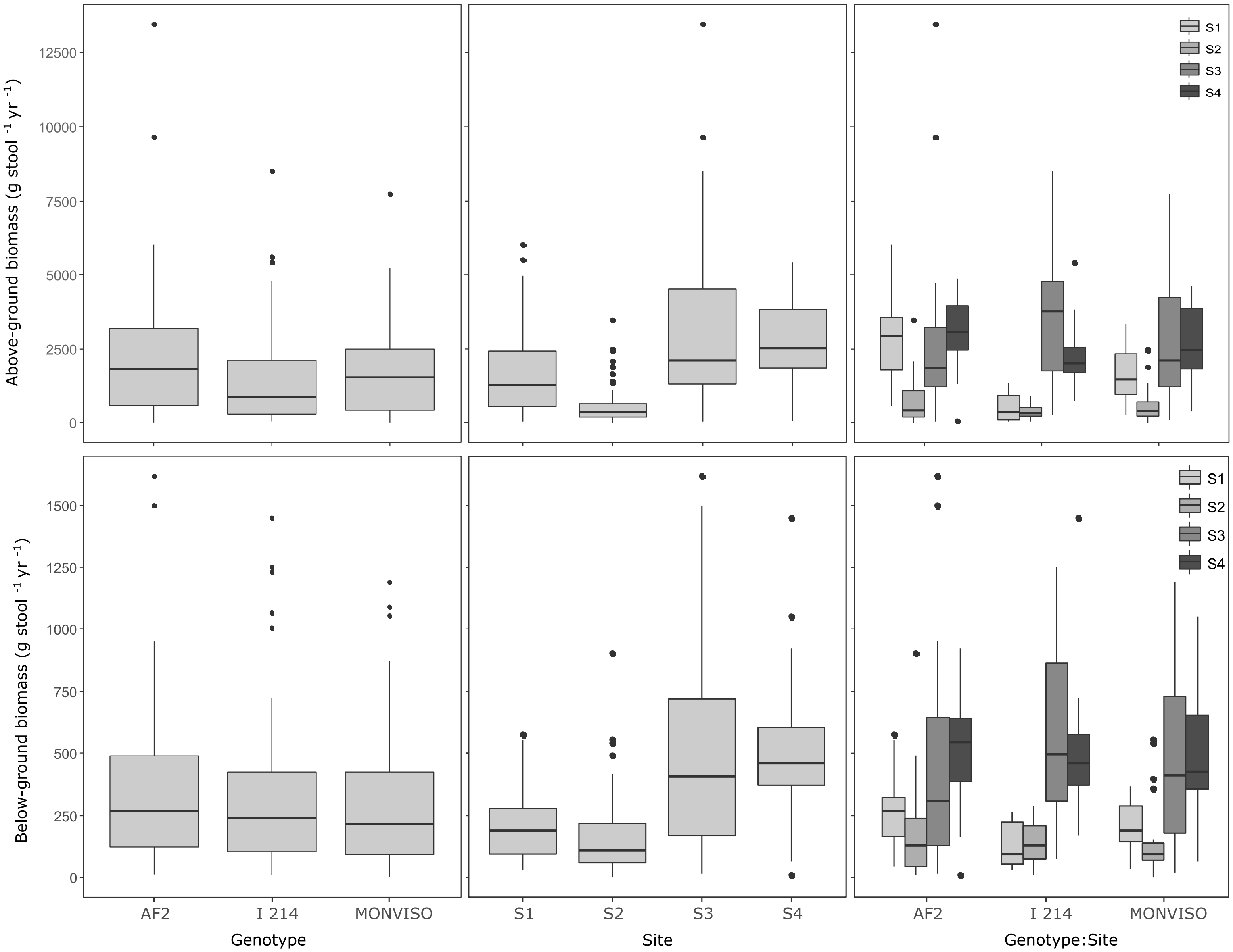
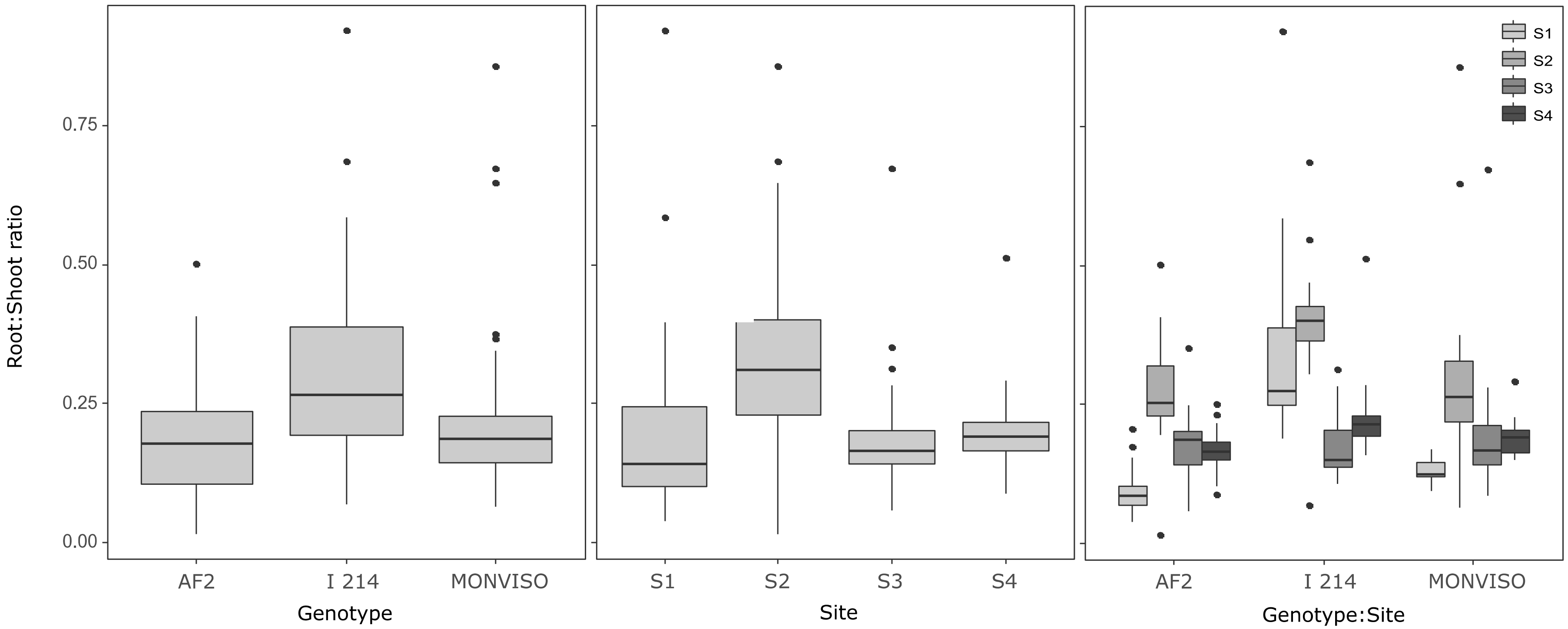
Fig. 1. Above-ground (top) and below-ground (bottom) biomass per stool and per year at end of the first rotation (three genotypes, four sites and G*S interactions). The values have been represented by boxplots.

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.


Above-ground biomass (g stool $^{-1}$ yr $^{-1}$)
12500
10000
7500
5000
2500
0
Below-ground biomass (g stool $^{-1}$ yr $^{-1}$)
1500
1250
1000
750
500
250
0
S1
S2
S3
S4
AF2
I 214
MONVISO
Genotype
S1
S2
S3
S4
Site
AF2
I 214
MONVISO
Genotype:Site


Root:Shoot ratio
0.00
0.25
0.50
0.75
AF2
I 214
MONVISO
Genotype
S1
S2
S3
S4
Site
AF2
I 214
MONVISO
Genotype:Site
S1
S2
S3
S4

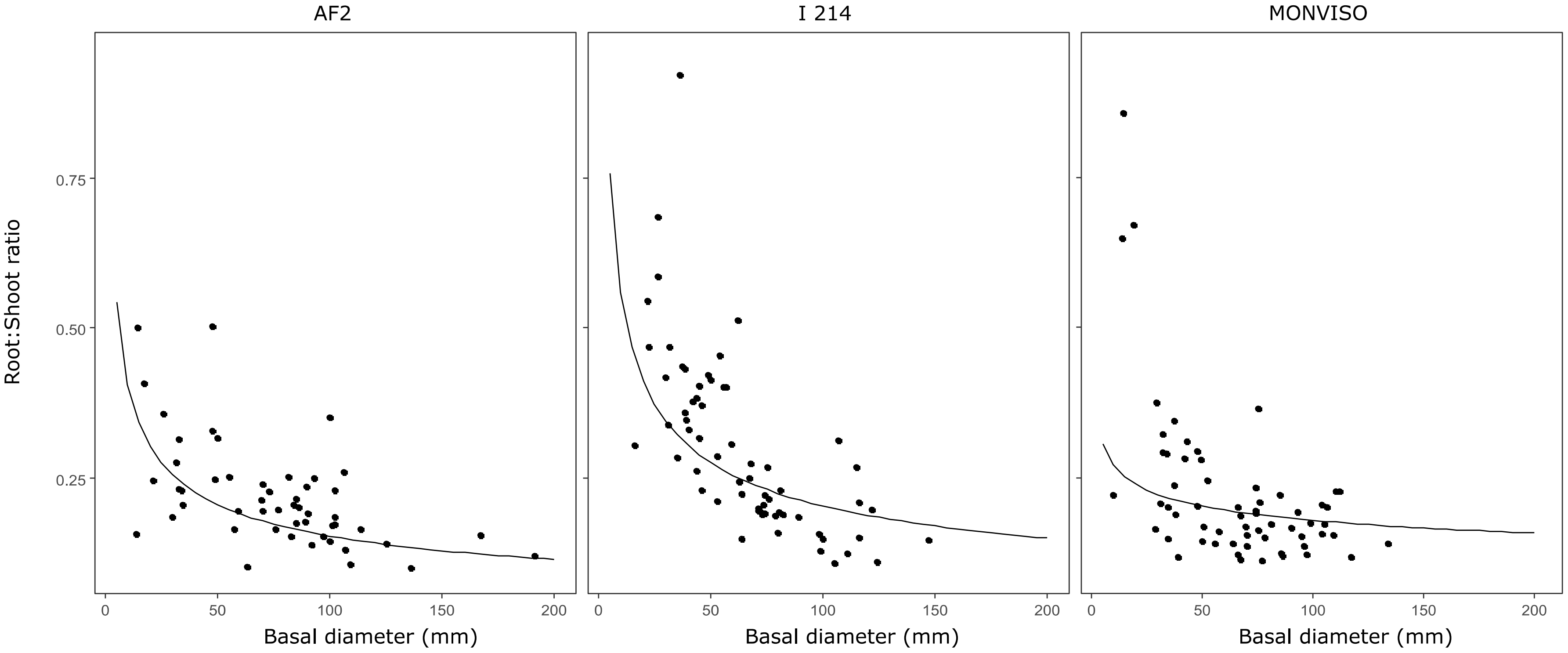

AF2
I 214
MONVISO
Root:Shoot ratio
0.75
0.50
0.25
0
50
100
150
200
Basal diameter (mm)

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 DEVELOMENT**	**DESING (m)**	**DENSITY (cuttings ha^{-1})**	**REPLICATE**
	LATITUDE	**LONGITUDE**									
S1	42^{o} 9´ 6.24´´N	5^{o} 33´ 42.37´´W	Temperate Mediterranean climate	254	11.8	6.84	0.62	R4S4	3 x 0.5	6,666	3
S2	42^{o} 37´ 25´´N	6^{o} 38´ 54´´W	Temperate Mediterranean climate	556	12.7	6.21	0.85	R3S3	2.5 x 0.3	13,333	4
S3	39^{o} 14´ 42.75´´N	4^{o} 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^{o} 15´ 24.74´´N	4^{o} 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-R*^{2}	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*-R^2: 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-R*^{2}	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
	1.981	0.080	24.90	<0.001			

where $W_{below\text{-}ground}$ (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*-R^2: 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