

Mixed short rotation plantations of *Populus alba* and *Robinia pseudoacacia* for biomass yield

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Article info

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Abstract

The possibility of using mixtures of species in an attempt to increase biomass yield in plantations managed in short rotation coppice (SRC) has received little attention in scientific literature. The hypothesis is that mixtures of two complementary species under SRC are more productive than monocultures. The objective of this study was to compare the growth and yield of mixtures and monocultures at the tree and stand levels. A mixed plantation of *Populus alba* L. genotype ‘111’ (P) and the nitrogen-fixing species *Robinia pseudoacacia* L. genotype ‘Nyirsegi’ (R) was established in 2012 in the centre of Spain, at a density of 10,000 trees per hectare (spacing 0.4m×2.5 m). The experimental design included pure as well as mixtures with different proportions of the species (100P:0R; 75P:25R; 50P:50R; 25P:75R and 0P:100R) following a completely randomized block design with 64 trees per plot. There was a positive effect of the mixture 75P:25R in terms of biomass yield, achieving a total yield of 13.66 Mg ha⁻¹ yr⁻¹ (26.83% higher than that of the *P. alba* monoculture and 89.99% higher than the *R. pseudoacacia* monoculture), with both species achieving a greater biomass yield per tree. In the other mixture treatments there was no significant positive effect of the nitrogen (N) fixing species on the non-N-fixing species. Within-row interactions were more important than between row-interactions. There was a positive effect on the yield of *R. pseudoacacia* when the neighbouring trees (within a row) on both sides were *P. alba*, while *P. alba* responded better with a *P. alba* tree on one side and a *R. pseudoacacia* tree on the other. The results point to the presence of competitive reduction or facilitation as the mechanisms for increased biomass yield under certain mixture ratios, and indicates the importance of mixing within the same row rather than row-by-row, in this type of plantation.

1. Introduction

The need to address the consequences of climate change (COP21) as well as to generate economic wealth from biological resources (bioeconomy) is becoming an important global challenge (European Commission, 2012). One approach to achieving this is to optimize the production of lignocellulosic biomass as feedstock for multiple uses (energy and bioproducts) (Zhang et al., 2011). Typical forest management cannot meet the global demand for biomass. Hence, forest plantations constitute an essential complement in the biomass market, providing an alternative use for abandoned land and contributing to rural development (BIC, 2013). Short Rotation Coppice (SRC) plantations, consisting of woody cultivation in short cycles (3–7 years), provide a suitable means to obtain this raw material in short periods of time, and therefore SRC cultivation is becoming more widespread globally (Srirangan et al., 2012).

43 Species belonging to the Salicaceae family (*Populus* spp. and *Salix* spp.) have proved to be one of the most suitable
44 for SRC management in Europe (Nassi O Di Nasso et al., 2010). Monoclonal (Verlinden et al., 2015) as well as
45 multi-clonal willow and poplar plantations (Aylott et al., 2008) are common in many countries (Yemshanov and
46 McKenney, 2008; O'Neill et al., 2010), both in northern and central Europe (Aust et al., 2014), as well as in the
47 Mediterranean region of Europe (Weih, 2004; Paris et al., 2005).

48 In forestry, many studies have shown that mixtures of species are more productive than monocultures because
49 complementary traits and species interactions increase resource availability and enable them to use a higher
50 proportion of the site resources or to use them more efficiently (Kelty, 1992; Forrester, 2014). These processes
51 and interactions are often described as facilitative, where the growing conditions are improved; or competitive
52 reduction, where intense intra-specific competition is replaced with a less intense inter-specific competition (Kelty,
53 1992; Brooker et al., 2008; Forrester and Bauhus, 2016). Competitive reduction and facilitation can occur
54 simultaneously and their effects are difficult to separate, therefore, both effects, as well as competition, are often
55 collectively described as complementarity (Forrester, 2014). A classic example of facilitation is the effect of
56 nitrogen-fixing species on the growth of non-nitrogen-fixing species, this effect increases as soil nitrogen
57 becomes more limiting (Bouillet et al., 2013; Forrester, 2014). The response of mixtures containing fastgrowing
58 species such as *Eucalyptus* and nitrogen-fixing species, such as *Acacia*, has received a lot of attention (Forrester
59 et al., 2006; Bouillet et al., 2008; Laclau et al., 2008; Nouvellon et al., 2012; Zhang et al., 2012). Similarly, Sayyad
60 et al. (2006), evaluated plantations of *Populus deltoides* Bartram ex. Marshall and the nitrogen-fixing species
61 *Alnus subcordata* L. using different mixing ratios. They found that poplar was positively affected by the presence
62 of alder.

63 The mixtures containing *Eucalyptus* or *Populus* described above were planted at relatively low densities (< 2500
64 trees ha⁻¹), whereas SRC plantations are typically planted at much higher densities (> 5000 trees ha⁻¹). Many
65 studies have shown that stand density can modify the size of mixing effects, with some studies reporting that the
66 mixing effect was greater as density increased (Boyden et al., 2005; Amoroso and Turnblom, 2006; Condés et al.,
67 2013; Forrester et al., 2013) while others point to reduced effects (Garber and Maguire, 2004; del Río and Sterba,
68 2009) depending on which resources were limiting growth and how the change in density influenced the
69 interactions for those resources (Forrester, 2014). It remains unclear, whether the balance between facilitation and
70 competition might shift more towards competition in high density SRC plantations. For example, while many
71 studies have shown facilitative effects of *Acacia* in *Eucalyptus* plantations, high densities of *Acacia* weeds (1300–
72 20,000 trees ha⁻¹), in *Eucalyptus* plantations (with 1000 *Eucalyptus* trees ha⁻¹), can cause net competitive effects
73 on *Eucalyptus* growth, even though soil nitrogen increases with *Acacia* density (Hunt et al., 1999).

74 Despite their potential, less information exists with regard to mixed species plantations in SRC, an example of
75 high density SRC plantations include *Populus* and *Alnus* (Dawson and Hansen, 1983; Radwan and DeBell, 1988).
76 A potentially useful mixture in the Mediterranean is that of *Populus* with *Robinia*, planted at high density and in
77 short rotation (Gana et al., 2014). Poplars and their hybrids are generally suitable for high density cultivation and
78 also for coppicing (Dickmann, 2006). Among these, *Populus alba* L. (white poplar) is a species with a broad
79 natural distribution, a strong presence in the Mediterranean basin and it is suitable for SRC (Rosso et al., 2013).
80 The hardiness and plasticity of *Populus alba* justify its use in marginal areas affected by dry climates, high
81 temperatures or salinity (Sixto et al., 2005; Di Lonardo et al., 2011). It is also an important species for improving
82 adaptive traits in poplar cultivation (Alba and Agundez, 2000). *Robinia pseudoacacia* L. (black locust) is also
83 considered a fast growing species of interest for biomass yield in SRC (Annighöfer et al., 2012; Crosti et al., 2016).
84 It displays good resprouting capacity after coppicing (Luken et al., 1991) and is a nitrogen fixing species
85 (Grunewald et al., 2009), which makes it a particularly good candidate for mixed-species plantations. Both poplars
86 and black locust are considered of interest from the point of view of lignocellulosic biomass production in the
87 south of Europe.

88 The objective of this study was to compare the growth and yield of mixtures and monocultures of *Populus alba*
89 and *Robinia pseudoacacia* at the tree level, species level and stand level using a replacement series experiment
90 with five mixture compositions. The main hypothesis was that mixtures of these two species under SRC are more
91 productive than their monocultures as a result of the theoretically expected positive effect of nitrogen-fixing on

92 non-nitrogen species and of competitive reduction between the two species. The specific objectives were therefore
93 to answer the following questions: (1) Does the mixture composition in SRC influence stand growth and yield?
94 and (2) Does it influence growth and yield of the individual trees of the two species?

95 2. Materials and methods

96 2.1. Trial site and experimental design

97 Short-rotation (3 years) mono-specific and mixed-species plantations established at high density (10,000 cuttings
98 ha⁻¹ spacing 0.4m×2.5 m) were evaluated in an experimental trial in central Spain (40°28'N, 3°22'W). The land,
99 which was previously used for cereal production, is situated at an elevation of 595m asl. The annual mean
100 temperature is 14 °C and the soil has a silty loam texture with a pH of 8.1.

101 The plantation was manually established in the spring of 2012. Immediately after the cuttings were planted,
102 Oxifluorfen herbicide (4 L ha⁻¹) was applied over the whole area in order to avoid weed competition during the
103 establishment period. The whole area was watered regularly to field capacity using a drip system between June
104 and August of each year. Under Mediterranean conditions, SRC plantations need to be watered due to the severe
105 summer drought. By irrigating all plots to field capacity, it is assumed that irrigation will influence all treatments
106 in the same way.

107 The plantation included two species, the genotype *Populus alba* '111' and the genotype *Robinia pseudoacacia*
108 'Nyirsegi'. We used unrooted cuttings for the *P. alba* (0.40m long) and bare rooted cuttings of the same length
109 for *R. pseudoacacia*. The plant material was previously hydrated in water for 48 h to facilitate rooting.

110 A randomized complete block design (3 blocks) was applied, including five different proportions of the two
111 species in each one (Fig. 1). Species were systematically mixed following a similar replacement series design to
112 that proposed by Kelty and Cameron (1995). The combinations of the five different species compositions were:

113 (1) 100% *Populus alba* (100P:0R)

114 (2) 75% *Populus alba*+25% *Robinia pseudoacacia* (75P:25R)

115 (3) 50% *Populus alba*+50% *Robinia pseudoacacia* (50P:50R)

116 (4) 25% *Populus alba*+75% *Robinia pseudoacacia* (25P:75R)

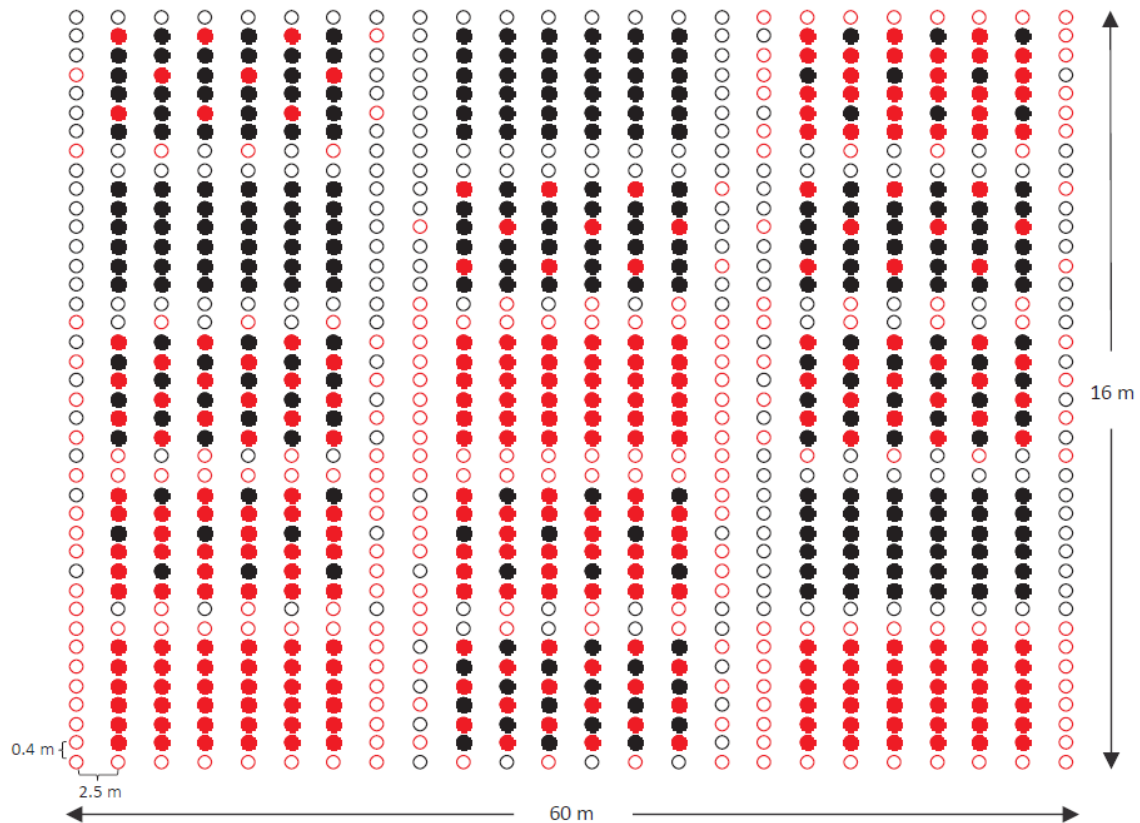
117 (5) 100% *Robinia pseudoacacia* (0P:100R)

118 The number of trees per plot was 64 but only the inner 36 trees were evaluated to avoid border effects. The design
119 of the experimental plot was consistent with commercial plantations in terms of the spacing.

120 2.2. Variables measured

121 The number of shoots per tree (n), the number of branches on the dominant shoot of the tree (br), stem diameter
122 of each shoot at basal height (d₁₀, mm) and at breast height (d₁₃₀, mm) as well as total height of the dominant shoot
123 (h, cm) were recorded for 36 trees for each plot at the end of each of the three growing seasons, comprising a
124 single rotation. The basal area of each tree (ba, cm² tree⁻¹) was calculated from the diameter at basal height (d₁₀)
125 of all the shoots, the current annual basal area increment (ba_{yr}, cm² tree⁻¹ yr⁻¹) was also calculated to evaluate the
126 effect of age on growth. These variables were also expressed per hectare (BA, m² ha⁻¹), and per hectare and year
127 (BA_{yr}, m² ha⁻¹ yr⁻¹).

128 Fresh biomass data were collected at the end of the rotation (third year) after the leaves had fallen. A subsample
129 of each species in each block and composition was oven-dried to constant weight at 100 °C to obtain the dry
130 weight (DM). All biomass information is presented as dry biomass per tree at the end of the rotation (bi, kg DM
131 tree⁻¹), as mean annual biomass increment per tree (bi_{yr}, kg DM tree⁻¹ yr⁻¹) or as the mean annual biomass
132 increment per hectare (BI_{yr}, Mg DMha⁻¹ yr⁻¹).



133

134 **Fig. 1.** Experimental design scheme. Red dots represent *Robinia pseudoacacia* trees and black ones the *Populus*
 135 *alba* trees. Solid dots correspond to the measured trees and the empty dots are the buffer trees of each treatment.
 136 The three columns represent the blocks 1, 2 and 3, respectively.

137 2.3. Statistical analysis

138 The data were analysed at the stand and individual tree levels considering the different species compositions
 139 (100P:0R, 75P:25R, 50P:50R, 25P:75R and 0P:100R) as levels of the treatment in an analysis of variance.

140 To study the response on growth and yield, data were analysed using analyses of variance (ANOVA) at both tree-
 141 and stand-levels. The Student-Newman-Keuls-test (SNK) was used to identify the means which were significantly
 142 affected by treatment. The time effect (three growing seasons) on growth variables was evaluated using an analysis
 143 of variance for repeated measures using mixed models.

144 The effect of complementarity at the species level was calculated using the following equation, which uses mean
 145 growth or yield values for the given species:

$$\text{Complementarity (\%)} = \left(\frac{\text{growth or yield in mixture} - \text{growth or yield in monoculture}}{\text{growth or yield in monoculture}} \right) * 100 \quad (1)$$

146

147 One characteristic of SRC plantations is that the high density is mainly achieved by narrow spacing within rows
 148 (0.4 m), since a much higher minimum distance between rows (2.5 m) is necessary to optimize biomass harvesting.
 149 Preliminary analyses, where the growth of individual trees was described as a function of the basal area of
 150 neighbouring trees, showed that most of the neighbour effects on growth were described when the neighbourhood
 151 size was a circle with a radius of 0.4m from the target tree (data not shown), and therefore that within row
 152 competition was much more important than between row competition. Therefore, in order to analyse whether the

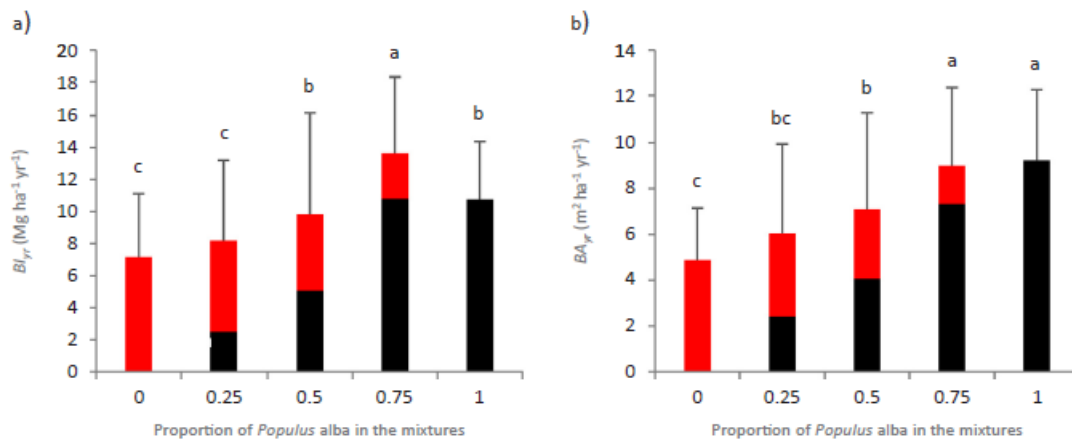
153 spatial distribution of species influences overyielding, we also explored complementarity, considering the species
 154 distribution within rows regardless of the species composition of the stand. Therefore, taking into account the
 155 influence of the surrounding trees within the row, three neighbourhood situations exist (Fig. 1): trees surrounded
 156 by the same species (neighbourhood situation 1), trees surrounded by both species (neighbourhood situation 2) or
 157 trees surrounded by the admixed species (neighbourhood situation 3).

158 3. Results

159 3.1. Does the mixture composition in SRC influence stand growth and yield?

160 Survival for both species (*P. alba* ‘111’ and *R. pseudoacacia* ‘Nyirsegi’) in monocultures or mixed plantations
 161 was 100%, revealing that competition between these two species did not influence their mortality rates during the
 162 first rotation. Mean annual biomass increment (BI_{yr}) and current annual basal area increment (BA_{yr}), at the end of
 163 the rotation (Fig. 2) were, however, significantly different for the different species compositions (p-value<0.0001),
 164 while no difference was detected among blocks (p-value=0.1299 and 0.7490, respectively).

165 The most productive mixture in terms of biomass (BI_{yr}) was the proportion 75P:25R (p-value<0.0001) with a yield
 166 of 13.66 Mg ha⁻¹ yr⁻¹ (10.80 Mg ha⁻¹ yr⁻¹ of *P. alba* and 2.86 Mg ha⁻¹ yr⁻¹ of *R. pseudoacacia*). The least productive
 167 was the *R. pseudoacacia* monoculture (0P:100R) with 7.19 Mg ha⁻¹ yr⁻¹ (Fig. 2a). It is worthy of note that there
 168 were no differences in basal area between the 75P:25R and *P. alba* monoculture 100P:0R, the rest of compositions
 169 showing significantly lower basal areas (Fig. 2b).



170

171 **Fig. 2.** Growth at stand level by species at the end of the rotation in terms of a) mean annual biomass increment
 172 (BI_{yr}) and b) current annual basal area increment (BA_{yr}). *Populus alba* is represented by black bars and *Robinia*
 173 *pseudoacacia* by red bars. The letters represent the results of SNK post-hoc comparisons of group means (p-
 174 value<0.05). The proportions of *P. alba* are as follows: 0 corresponds to the species composition 0P:100R, 0.25
 175 to 25P:75R, 0.5 to 50P:50R, 0.75 to 75P:25R and 1 to 100P:0R.

176 The complementarity in relation to basal area for *P. alba* was positive in 25P:75R and 75P:25R, while the
 177 complementarity for *R. pseudoacacia* increased with the percentage of *P. alba* in the mixture (Fig. 3b). This trend
 178 was also observed for the biomass (Fig. 3a). While the complementarity in terms of biomass for *R. pseudoacacia*
 179 increased with a greater presence of *P. alba*, the latter species only benefited in the mixture with the lowest
 180 proportion of *R. pseudoacacia* (75P:25R).

181 3.2. Does the mixture composition in SRC influence growth and yield of the individual trees of the two species?

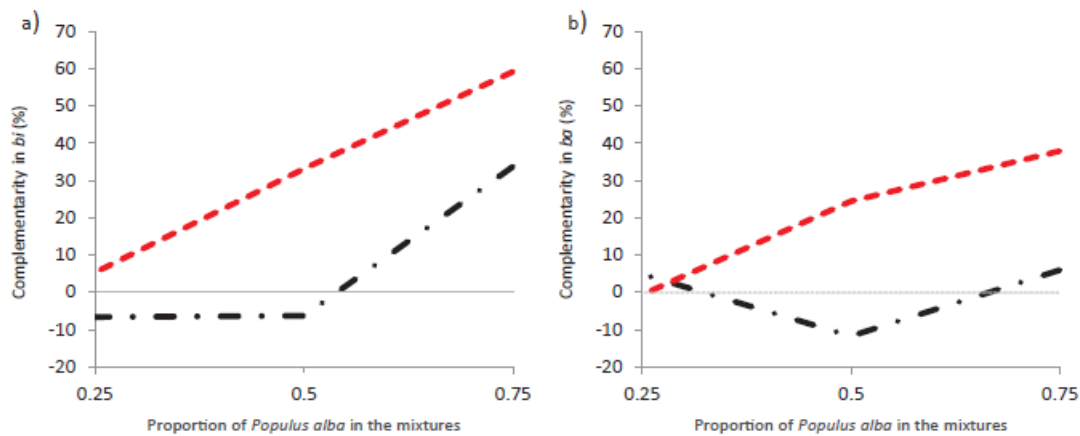
182 The biomass yield per tree (bi) ranged from 3.02 to 4.32 kg tree⁻¹ for *P. alba* and from 2.16 to 3.43 kg tree⁻¹ for
 183 *R. pseudoacacia*, presenting significant differences (p-value<0.0001) in both species among species compositions
 184 (Fig. 4a). The basal area at the end of the rotation (ba) also presented significant differences between the different
 185 species compositions (mixtures and monocultures) for *R. pseudoacacia* (p-value=0.0002). The only exception

186 was that the individual tree basal area (ba) of *P. alba* did not differ between mixtures and monocultures (p-
187 value=0.0944) (Fig. 4b).

188 A more in depth analysis of the behaviour of the different species and species compositions (mixtures and
189 monocultures) was performed to evaluate the temporal development during the rotation.

190 By the end of the rotation, *P. alba* had higher values than *R. pseudoacacia* for all variables except for the number
191 of branches (Table 1). For all variables, the differences between species increased over time, except for the number
192 of shoots per tree, for which the relative difference between species remained relatively constant.

193 At the tree level, there was no significant difference in ba_{yr} for *P. alba* between the mixtures for the second year
194 (p-value=0.6810), but there were significant differences in the first year (p-value=0.0288) and at the end of the
195 rotation (p-value=0.0485). For *R. pseudoacacia*, there were significant differences in ba_{yr} between mixtures only
196 for the first year of the rotation (p-value<0.0001). In terms of height, there were significant differences in each
197 year of rotation for *P. alba* (p-value<0.0001), with the monoculture (100P:0R) and the 75P:25R mixture showing
198 greater height growth than the 50P:50R and 25P:75R mixtures. For *R. pseudoacacia*, the first and the last years
199 of the rotation presented significantly higher growth in the 75P:25R mixture (p-value=0.0003 and p-
200 value<0.0001). In the second year however, all the mixtures grew more than the monoculture (0P:100R) (p-value=
201 0.0003) (Table 1).



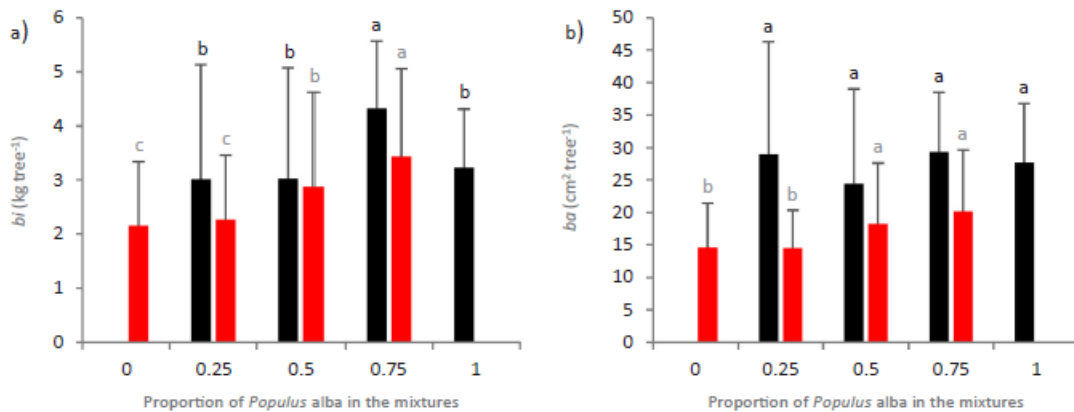
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203 **Fig. 3.** Relationship between complementarity (in %) and proportion of species in the mixture at the end of the
204 rotation, in terms of (a) biomass and (b) basal area. Complementarity (%) was calculated using Eq. (1), with the
205 mean value per species and mixture. *Populus alba* is represented by the black dashed line and *Robinia*
206 *pseudoacacia* by the red dashed line. The proportions of *P. alba* are as follows: 0.25 corresponds to the species
207 composition 25P:75R, 0.5 to 50P:50R and 0.75 to 75P:25R.

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211

212 **Fig. 4.** Growth at individual tree level, by species, at the end of the rotation in terms of a) biomass (b_i) and b)
 213 basal area (b_a) per tree. *Populus alba* is represented by black bars and *Robinia pseudoacacia* by red bars. The
 214 letters represent the results of SNK post-hoc comparisons of group means (p -value<0.05). The proportions of *P.*
 215 *alba* are as follows: 0 corresponds to the species composition 0P:100R, 0.25 to 25P:75R, 0.5 to 50P:50R, 0.75 to
 216 75P:25R and 1 to 100P:0R.

217 To evaluate whether the neighbourhood composition explained the overyielding better than the species
 218 composition, the complementarity was evaluated considering the three different within-row neighbourhood
 219 situations that were presented under this replacement series experiment. At this tree neighbourhood level within
 220 a given row, the positive complementarity effect of *R. pseudoacacia* on *P. alba* only occurred when there was an
 221 individual of each species on each side within the same row (neighbourhood situation 2), and as the proportion of
 222 *R. pseudoacacia* increased, this complementarity effect disappeared (Fig. 5). Notice that neighbourhood situation
 223 2 is only presented when the percentage of the target species is 25%. However, in the case of *R. pseudoacacia*,
 224 the complementarity increases with the presence of *P. alba*. For both species, these patterns of complementarity
 225 were stronger for biomass than for basal area.

226 4. Discussion

227 Despite the greater growth often found in mixtures compared to monoculture plantations (Forrester et al., 2005;
 228 Pretzsch and Schütze, 2009), there are also some studies in which this benefit has not been detected (Pretzsch et
 229 al., 2010; Bouillet et al., 2013). Whether the greater biomass yield can be attained by mixing species in very short
 230 rotations and at very high densities, such as in Short Rotation Coppice plantations, is of great interest with regard
 231 to satisfying the demand for biomass. Our results point to a benefit for both species in terms of stand biomass (BI)
 232 in the case of the 75P:25R mixture at the end of the first rotation, which resulted in a 26.83% greater yield than
 233 that achieved with the more productive species, i.e. *P. alba*, or transgressive overyielding (Pretzsch and Schütze,
 234 2009). This corroborates our main hypothesis that mixtures under high density short rotation plantations can
 235 improve productivity.

236 In the presence of facilitation, as a consequence of nitrogen fixation by *R. pseudoacacia*, a constant or increasingly
 237 positive response of *P. alba* would be expected as the proportion of *R. pseudoacacia* is increased (Binkley, 1992;
 238 Bouillet et al., 2013). However, while a positive response was observed for the 75P:25R mixture, it was not found
 239 for the other mixing proportions. This could be due to confounding effects of competitive interactions associated
 240 with *R. pseudoacacia*, which change disproportionately with the nitrogen fixation effect. Competition,
 241 competitive reduction, and facilitation mechanisms frequently occur simultaneously (Forrester, 2017) and their
 242 relative importance often varies not only with abiotic conditions (Callaway, 1998; Forrester, 2014; Río et al.,
 243 2014), but also with species proportions (Binkley, 1992). However, no data were available to help decipher the
 244 underlying mechanisms.

245 Therefore, the greater yield obtained in the 75P:25R mixture may not be due exclusively to the influence of the
 246 nitrogen fixing species on the non-fixing species, but also to the different competitive interactions occurring
 247 between the two species in that mixture. Certain studies have

248 shown that N in the roots of N fixing species can be transferred to the non-N-fixing species in the first or second
249 year (Van Kessel et al., 1994; Parrotta et al., 1996; Khanna, 1997). However, other studies report that the
250 facilitation effect produced by the nitrogen fixing species may not be effective until some years after planting, as
251 suggested in studies concerning tropical *Acacia* and *Eucalyptus* (Bouillet et al., 2008), or *Alnus* and *Pseudotsuga*
252 (Binkley, 1992).

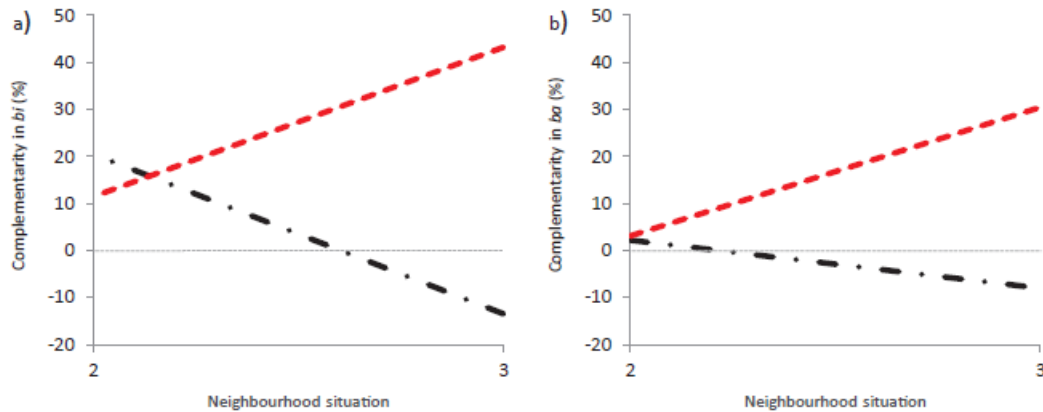
253 The biomass yield in *P. alba* was only significantly higher than expected, based on monoculture yield, when
254 growing with a small proportion of *R. pseudoacacia*. By contrast, *R. pseudoacacia* always benefited as the
255 proportion of *P. alba* increased, which points to competitive reduction for this species in mixed plantations. A
256 greater presence of *R. pseudoacacia* leads to less height growth in *P. alba* as well as a lower number of branches
257 for all the mixtures except 75P:25R, suggesting that competition for aboveground resources may occur between
258 the two species, with a positive net effect for *R. pseudoacacia*. However, at the end of the rotation, the height of
259 *P. alba* was still higher due to its greater growth rates, as can be seen in the monocultures. Some studies have also
260 found that, on the one hand, height growth of *P. alba* is faster than that of *R. pseudoacacia* when these two species
261 grow together and on the other, that *R. pseudoacacia* is unlikely overgrow *P. alba* in mixed stands (Rédei et al.,
262 2006).

263 We verified that the design of these plantations (high density but different planting distances between rows and
264 within the rows) had a notable influence on the observed overyielding effects, indicating the importance of
265 analysing mixing effects at different levels, from tree to stand levels (Forrester and Pretzsch, 2015). For example,
266 the neighbourhood level analyses indicate the importance of the within row (nearest neighbour) interactions as
267 opposed to the between row interactions in these high density mixtures. In the 50P:50R mixture, the neighbours
268 on each side of all the trees are of the other species (neighbourhood situation 3), this being the best neighbourhood
269 situation for *R. pseudoacacia* and the worst for *P. alba*. In this mixture, the higher mortality of shoots per tree in
270 the case of *P. alba* along with the lower diameter and height growth points to a clear negative interaction for this
271 species. For the 75P:25R mixture, 25% of the trees are *P. alba* with other *P. alba* trees on both sides
272 (neighbourhood situation 1), 50% of the trees are *P. alba* with another *P. alba* on one side and a *R. pseudoacacia*
273 on the other side (neighbourhood situation 2) and the last 25% are *R. pseudoacacia* with *P. alba* trees on both
274 sides (neighbourhood situation 3), in accordance with the best situation for each species.

Table 1

Analysis of variance (ANOVA), mean and standard deviation at tree level for the number of shoots per tree, the number of branches per tree, the dominant height (H , cm), the current annual basal area increment (I_{ba} , $\text{cm}^2 \text{tree}^{-1} \text{yr}^{-1}$) and the mean annual biomass increment per tree (I_{ba} , $\text{kg DM tree}^{-1} \text{yr}^{-1}$). The letters represent the results of SNK post-hoc comparisons of group means (p -value < 0.05). The proportion of F , I_{ba} are as follows: 0 corresponds to the species composition 0P:100R, 0.25 to 25P:75R, 0.5 to 50P:50R, 0.75 to 75P:25R and 1 to 100P:0R. No data was available for the biomass growth during the first two years.

Variable	Species	p -value	100P:0R	75P:25R	50P:50R	25P:75R	0P:100R	100P:0R	75P:25R	50P:50R	25P:75R	0P:100R
Year 1	No Shoots	0.6790	-	-	-	-	-	3 ± 1	3 ± 1	3 ± 1	3 ± 1	3 ± 1
	<i>R. pseudoacacia</i>	0.0696	-	-	-	-	-	3 ± 1	3 ± 1	3 ± 1	3 ± 1	3 ± 1
	No Branches	<0.0001	a	a	b	b	-	16 ± 8	14 ± 7	8 ± 6	8 ± 10	3 ± 1
H	<i>P. alba</i>	0.1450	-	-	-	-	-	9 ± 7	9 ± 7	9 ± 6	9 ± 8	7 ± 5
	<i>R. pseudoacacia</i>	<0.0001	a	a	b	b	-	271.81 ± 35.30	279.26 ± 43.81	248.00 ± 50.97	239.07 ± 60.18	190.41 ± 34.98
	I_{ba}	0.0003	a	a	b	b	-	399.49 ± 140.58	213.85 ± 31.76	198.19 ± 40.63	324.25 ± 161.61	182.47 ± 34.21
Year 2	No Shoots	0.0288	a	a	ab	b	-	550.83 ± 201.78	396.67 ± 128.90	352.67 ± 156.47	376.58 ± 135.36	352.06 ± 116.10
	<i>R. pseudoacacia</i>	0.0504	-	-	-	-	-	3 ± 1	3 ± 1	2 ± 1	3 ± 1	2 ± 1
	No Branches	0.0015	a	a	b	b	-	82 ± 36	69 ± 34	53 ± 30	63 ± 39	40 ± 17
H	<i>P. alba</i>	<0.0001	a	ab	c	bc	-	467.61 ± 51.04	54 ± 22	42 ± 22	38 ± 19	419.00 ± 87.91
	<i>R. pseudoacacia</i>	0.0023	a	a	b	b	-	631.94 ± 244.38	477.40 ± 61.88	424.07 ± 73.91	419.00 ± 87.91	306.12 ± 55.63
	I_{ba}	0.0003	a	a	a	a	-	697.75 ± 292.98	344.93 ± 53.04	327.70 ± 54.72	333.20 ± 46.54	647.08 ± 309.93
Year 3	No Shoots	0.6810	-	-	-	-	-	3 ± 1	3 ± 1	2 ± 1	3 ± 1	2 ± 1
	<i>R. pseudoacacia</i>	0.6850	-	-	-	-	-	3 ± 1	3 ± 1	2 ± 1	3 ± 1	2 ± 1
	No Branches	0.0336	a	a	ab	a	-	43 ± 9	40 ± 10	33 ± 11	35 ± 11	36 ± 11
H	<i>P. alba</i>	0.0008	a	a	b	b	-	773.15 ± 102.85	777.48 ± 124.08	666.69 ± 166.07	680.04 ± 169.96	460.62 ± 90.94
	<i>R. pseudoacacia</i>	<0.0001	a	a	b	b	-	1592.92 ± 590.59	541.93 ± 80.09	497.39 ± 102.13	497.05 ± 69.10	1946.62 ± 1235.82
	I_{ba}	0.2490	a	a	b	b	-	1077.11 ± 359.41	1790.37 ± 680.94	1513.23 ± 1058.23	524.40 ± 528.50	666.50 ± 451.10
I_{ba}	<i>P. alba</i>	<0.0001	a	ab	b	b	-	1144.34 ± 540.27	789.65 ± 619.65	743.56 ± 708.15	1005.59 ± 705.03	718.89 ± 394.00
	<i>R. pseudoacacia</i>	0.0485	ab	ab	-	-	-	-	-	-	-	-
	I_{ba}	0.0615	b	b	b	c	-	-	-	-	-	-
I_{ba}	<i>P. alba</i>	<0.0001	a	a	b	b	-	-	-	-	-	-
	<i>R. pseudoacacia</i>	<0.0001	b	b	b	c	-	-	-	-	-	-
	I_{ba}	<0.0001	a	a	b	c	-	-	-	-	-	-



276

277 **Fig. 5.** Relationship between complementarity (in %) and the neighbourhood situation at the end of the rotation,
 278 in terms of a) biomass and b) basal area. Complementarity (%) was calculated using Eq. (1), with the mean value
 279 per species and situation. *Populus alba* is represented by the black dashed line and *Robinia pseudoacacia* by the
 280 red dashed line. Situation 2 corresponds to a tree with a *P. alba* on one side and a *R. pseudoacacia* tree on the
 281 other and situation 3 to a tree with the opposite species neighbouring on both sides.

282 This pattern, with the 75P:25R presenting the greatest yield, coupled with the low yield and growth in the 50P:50R
 283 mixture, highlights the importance of the spatial pattern beyond the mixing ratio. Therefore, for short rotation
 284 mixed plantations for biomass production, it is desirable that the mixing is considered within the same row (where
 285 the distance between trees is smaller than that between rows). In any case, the complexity of this design, in which
 286 the distance between trees in the same row (0.4 m) is much less than the distance between rows (2.5 m), is
 287 increased by the presence of multiple shoots per tree, which makes it even more difficult to analyse the positive
 288 and negative effects of the different mixtures.

289 It is important to highlight the different behaviour observed between the biomass yield and the basal area growth.
 290 In contrast to the positive mixing effects on biomass for the 75P:25R mixture (considering together *Populus alba*
 291 and *Robinia pseudoacacia*), there was no such effect on the current annual basal area increment at individual tree
 292 level (bayr). Similarly, Sayyad et al. (2006) found no significant differences in basal area growth between planted
 293 monocultures and mixtures of *Populus* and *Alnus*. Biomass evaluation takes account of all the variables involved
 294 in the growth of the tree, whereas the analysis of basal area ignores the height and the number of branches. Species
 295 interactions can also trigger differences in the density of wood (Zeller et al., 2017), which would be ignored by
 296 basal area analyses. Our results show the importance of analysing biomass yield, since basal area provides biased
 297 estimations of productivity in these types of plantations. Accordingly, this suggests the need to develop specific
 298 biomass allometry equations for this kind of mixed plantations, which consider the effects of species interactions
 299 (Forrester and Pretzsch, 2015).

300 Different factors, such as the light efficiency derived from the canopy architecture for a given composition, the
 301 possible differences in root stratification in the soil profile or the N-effect on both the above-and belowground
 302 growth should be studied further in order to gain a better understanding of the behaviour of this mixture. This
 303 study illustrates the complexity of the interactions in mixtures in SRC plantations. Furthermore, an evaluation of
 304 progressive rotations and the effect of N-fixing species will be required to obtain a more complete impression of
 305 any changes in species after the roots are fully developed.

306 5. Conclusions

307 A clear benefit in terms of biomass per hectare was detected for the mixture 75P:25R at the end of the first rotation.
 308 This mixture was the most productive in terms of biomass yield at both tree- and stand-levels. The 50P:50R
 309 proportion was less productive. *P. alba* growth was lower for both basal area and biomass at the tree level when
 310 the neighbouring trees on both sides were *R. pseudoacacia*. *R. pseudoacacia* benefited from a higher presence of
 311 *P. alba*, which suggests that the inter-specific competition from *P. alba* was less intense than the intraspecific
 312 competition from other *R. pseudoacacia* trees.

313 We found a different trend when the response was evaluated using basal area growth compared with biomass
314 production, suggesting that basal area provides biased estimations of productivity in these types of plantations.

315 The distribution pattern of the species within the row seems to be very relevant. The growth response was positive
316 in the case of the 75P:25R mixture and negative for the 50P:50R mixture, showing that growth was largely affected
317 by competition within the same row and not between rows due to the specific design of these plantations. This
318 suggests that it would be more appropriate to consider the mixing of these species within rows rather than row by
319 row in the case of SRC plantations.

320 We concluded that SRC mixtures with certain proportions could provide an interesting alternative to SRC
321 monocultures in terms of biomass yield at the end of the first rotation. However, several aspects must be taken
322 into account when designing mixed plantations, not only the complexity of the species interactions, with positive
323 and negative effects acting simultaneously, but also the restrictions in the spatial distribution of trees as a result
324 of the complex establishment of the plantation and the harvesting requirements. Additionally, an evaluation of
325 subsequent rotations must be considered in order to gain a greater insight into the behaviour of the mixtures and
326 the final production which may be achieved with each one.

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