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1 **Root growth dynamics of Aleppo pine (*Pinus halepensis* Mill.) seedlings in relation to**  
2 **shoot elongation, plant size and tissue nitrogen concentration**

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11 **Abstract**

12 Large and high nitrogen (N) concentration seedlings frequently have higher survival and  
13 growth in Mediterranean forest plantations than seedlings with the opposite traits, which has  
14 been linked to the production of deeper and larger root systems in the former type of  
15 seedlings. This study assessed the influence of seedling size and N concentration on root  
16 growth dynamics and its relation to shoot elongation in Aleppo pine (*Pinus halepensis* Mill.)  
17 seedlings. We cultivated seedlings that differed in size and N concentration that were  
18 subsequently transplanted into transparent methacrylate tubes in the field. The number of  
19 roots, root depth, and the root and shoot elongation rate (length increase per unit time) were  
20 periodically measured for 10 weeks. At the end of the study, we also measured the twig water  
21 potential ( $\psi$ ) and the mass of plant organs. New root mass at the end of the study increased  
22 with seedling size, which was linked to the production of a greater number of new roots of  
23 lower specific length rather than to higher elongation rate of individual roots. Neither plant  
24 size nor N concentration affected root depth. New root mass per leaf mass unit, shoot  
25 elongation rate, and pre-dawn  $\psi$  were reduced with reduction in seedling size, while mid-day  
26  $\psi$  and the root relative growth rate was not affected by seedling size. N concentration had an  
27 additive effect on plant size on root growth, but its overall effect was less important than  
28 seedling size. Shoot and roots had an antagonistic elongation pattern through time in small  
29 seedlings, indicating that the growth of both organs depressed each other and that they  
30 competed for the same resources. Antagonisms between shoot and root elongation decreased  
31 with plant size, disappearing in large and medium seedlings, and it was independent of  
32 seedling N concentration. We conclude that root and shoot growth but not rooting depth  
33 increased with plant size and tissue N concentration in Aleppo pine seedlings. Since  
34 production of new roots is critical for the establishment of planted seedlings, higher absolute  
35 root growth in large seedlings may increase their transplanting performance relative to small

36 seedlings. The lack of antagonism between root and shoot growth in large seedlings suggests  
37 that these plants can provide resources to sustain simultaneous growth of both organs.

38

39 **Key words:** Nitrogen content, rhizotron, root elongation, shoot growth, sink-source relations,  
40 water potential.

## 41 **Introduction**

42 Abiotic and biotic factors have a profound influence on root growth and structure  
43 (Alvarez-Uria and Korner 2007; Andersen, et al. 1986; Lopushinsky and Max 1990; Lyr  
44 1996; Munro, et al. 1999). Root growth and structure also varies across plant species and are  
45 linked to species functional and ecological characteristics (Comas and Eissenstat 2004;  
46 Schenk and Jackson 2002). By contrast, fewer studies have analysed how plant functional  
47 characteristics affect root growth and structure within a given species. Nursery cultivation  
48 conditions may determine future root growth and architecture. For instance, root restriction by  
49 containers in nursery-cultivated plants can affect root morphology for many years after  
50 transplanting (Halter and Chanway 1993; Lindström and Rune 1999). Similarly, new root  
51 growth capacity in greenhouse or growth chamber experiments is positively related to shoot  
52 size and nitrogen (N) concentration in forest species (van den Driessche 1992; Villar-  
53 Salvador, et al. 2004).

54 New root growth after transplanting is essential to ensure seedling survival in forest  
55 plantations (Burdett 1990; Burdett, et al. 1983; Grossnickle 2005; Ritchie and Dunlap 1980)  
56 as new roots allow seedlings to access soil water and mineral nutrients in the surrounding soil  
57 (Grossnickle 2005; Lyr and Hoffmann 1967; Padilla and Pugnaire 2007). Villar-Salvador  
58 (2003) suggested that higher root growth after transplanting in containerized plants might  
59 explain the frequently improved survival and growth of large, N-rich seedlings relative to  
60 seedlings with the opposite traits in Mediterranean plantations (Luis, et al. 2009; Oliet, et al.  
61 2009; Puértolas, et al. 2003; Tsakalimi, et al. 2005; Villar-Salvador, et al. 2004; Villar-  
62 Salvador, et al. 2008). Cuesta et al. (2010) observed that large Aleppo pine (*Pinus halepensis*  
63 Mill.) seedlings had greater new root biomass in the field than small seedlings at the onset of  
64 summer drought. However, they did not examine the effect of seedling size and N  
65 concentration in root growth dynamics and rooting depth.

66 Plants usually have limited resources to simultaneously support their main  
67 physiological processes (growth, defence, maintenance, storage, and reproduction) (Chapin  
68 1990; Herms and Mattson 1992; Obeso 2002). Trade-off in resource allocation among organs  
69 or functions increases with limiting environmental conditions and varies with organ type  
70 (Obeso 2002; Thaler and Pagès 1996b). In some studies, roots and shoots alternate their  
71 growth through time suggesting that both organs compete for the same pool of resources and,  
72 therefore, occur at the expense of the other (Langlois, et al. 1983; Thaler and Pagès 1996a;  
73 Willaume and Pagès 2006). Other studies, however, have not reported an alternating growth  
74 between roots and shoots (Corchero-de la Torre, et al. 2002; Harmer 1990; Lyr and Hoffmann  
75 1967). Differences among studies suggest different sink/source relations among species or  
76 individuals within a species. Consistent with this argument, defoliation of *Quercus pubescens*  
77 seedlings amplified the decrease in root growth concomitant with leaf expansion (Willaume  
78 and Pagès 2006). Therefore, it can be expected that antagonist growth between organs in a  
79 given species will be lower in individuals with high photosynthetic capacity and / or amount  
80 of nutrients stored for remobilization.

81 This study examines the root growth dynamics of Aleppo pine (*Pinus halepensis*) and  
82 how it is influenced by shoot growth, seedling size and tissue N concentration. We  
83 assessed the hypotheses that large seedlings and plants with high tissue N concentration 1)  
84 produce larger and deeper root systems because they produce more roots and individual roots  
85 elongate faster, and 2) have lower antagonism between root and shoot growth relative to  
86 plants with the opposite attributes. To test these hypotheses, we transplanted into transparent  
87 methacrylate tubes seedlings that differed in size and N concentration and periodically  
88 measured root and shoot growth. We selected *P. halepensis* because it is a structural species  
89 in many types of woodland throughout the Mediterranean basin and it is commonly used in  
90 reforestation projects.

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## Materials and Methods

### Seedling nursery cultivation

Seeds from an inland Spain provenance were sown in Plasnor 190/300-45 trays (45 cavities of 300 ml per tray; Plasnor, Spain) with unfertilized *Sphagnum* peat (Kekkilä B0, Finland). We cultivated 135 seedlings of six phenotypes that differed in size (small, medium and large types) and N concentration (high and low) (Table 1). To achieve differences in plant size we varied seeding date, which determined the length of the growing season. Seeds were sown on December 15, 2005 (large seedlings), March 13, 2006 (medium seedlings) and May 23, 2006 (small seedlings). After emergence, seedlings were fertilized weekly with a 100-ppm N fertilizer solution until September 27, 2006. Fertilization started in May 10, June 2 and August 2 for the seedlings sown in December, March and May, respectively. To achieve differences in tissue N concentration, half of the seedlings of each seeding date were fertilized weekly with 200 ppm N from early October until mid December 2006 (high-fertilized seedlings, N<sup>+</sup>). The remainder half was not fertilized any more (low-fertilized seedlings, N<sup>-</sup>). Fertilization was done with a Peters Professional® 20-7-19: N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O fertilizer (Scotts Professional, The Netherlands). Seedlings were grown in a glasshouse, which temperature ranged from 4 to 25 °C and radiation was approximately 50% of that outside, until mid May 2006. Then plants were moved outside and kept under full sun during the rest of the experiment. Seedlings were kept well watered according to their requirements by irrigating them every 1–3 days. Cultivation finished when plants stopped their growth due to low winter temperature in late December 2006.

116 Morphology and N concentration measurements following cultivation

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118 Fifteen plants of each seedling phenotype were randomly sampled in mid January 2007 for  
119 morphology and N concentration determinations. Shoots were cut at the cotyledon insertion  
120 point and root plugs were cleaned from the peat. Shoot height was measured as the length of  
121 the stem while shoot diameter was measured as the stem thickness at the cotyledon insertion  
122 point. Then, all plant parts were washed with tap water, rinsed in distilled water and dried in a  
123 ventilated oven at 60°C for 48 h to measure their mass (Table 1). Plants of each seedling  
124 phenotype were randomly distributed into three groups and finely ground to assess tissue N  
125 concentration. N concentration was determined by Kjeldahl analysis with K-SeSO<sub>4</sub>-Se<sub>2</sub>Cu<sub>4</sub> as  
126 catalyst in a Tecator DS-40 digestion system (FOSS Tecator, Sweden) and a SAN ++ auto-  
127 analyser (Skalar, Netherlands).

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129 Experimental design and field measurements

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131 The experiment was carried out in Guadalajara, central Spain (38°38' N, 3°28' W) at 650 m  
132 a.s.l. on a flat plot. The climate is Mediterranean continental with mean annual precipitation  
133 and temperature of 400 mm and 13.4 °C, respectively. A pronounced dry and hot season  
134 occurs from June to late September and frosts in winter are frequent.

135 Six seedlings per seedling phenotype were transplanted into transparent methacrylate  
136 tubes (here after rhizotrons) on April 18, 2007. One seedling was planted in each rhizotron.  
137 Rhizotrons were 1 m in length, 13.5 cm in exterior diameter, and had a wall thickness of 0.5  
138 cm. Rhizotron bottom end was closed with a perforated PVC lid, which drainage holes were  
139 covered with a mesh to prevent substrate loss. The bottom of the rhizotron was filled with  
140 gravel (*ca.* 10 cm in height) and the remainder was filled with washed and sieved sand.



141 Subsequently, we planted the seedlings placing the root plug against the rhizotron wall.  
142 Rhizotrons were inserted into the soil to maintain roots as close as the plot soil temperature.  
143 To facilitate periodic extraction, each rhizotron was inserted into a second opaque plastic tube  
144 1.1 m in length and 15 cm in interior diameter that was buried in the soil with a 30°  
145 inclination to force root growth against the rhizotron wall. As the top of the opaque tube and  
146 of the rhizotron protruded 10-15 cm from the ground, we wrapped an insulating sheet around  
147 the protruding portion of the opaque tube to prevent rhizotron overheating. Seedlings were  
148 thoroughly irrigated at planting and then again on April 23, May 10 and May 18 with 200 ml  
149 per plant to simulate standard spring rainfall at the experimental site. During the rest of the  
150 experiment, seedlings were not irrigated to simulate the summer drought typical of  
151 Mediterranean climate.

152 When most seedlings had visible roots, we measured root elongation and depth as well  
153 as shoot height every 6-12 days from April 30 to July 11. On each date, all new roots were  
154 drawn on the same acetate sheet, which was placed over the rhizotron wall on identical  
155 position, and their length was measured after correcting for root curvatures. We counted the  
156 number of new growing roots. The roots that stopped their growth for at least 15 days were  
157 not counted. The mean root elongation rate was calculated as the mean elongation of each  
158 individual root per time unit. We also calculated the total root elongation rate of a plant as the  
159 sum of elongations of all individual roots per time unit, which estimates plant effort to expand  
160 its root system. Root depth was measured as the vertical distance from the plug bottom to the  
161 tip of each drawn root. Shoot height was measured as the distance between the cotyledon  
162 insertion point and the shoot apex, and shoot elongation rate was calculated as the differences  
163 in seedling height between two dates. We also measured the stem diameter at the cotyledon  
164 insertion point with a calliper in the first day of the field experiment to calculate the initial

165 stem volume assuming the stem to be a cone. Stem volume was highly correlated with plant  
166 mass ( $r = 0.92$ ,  $P < 0.001$ ,  $n = 36$ ) and we used it as a proxy of initial seedling size.

167 On the last day of the field experiment, we measured the twig water potential at  
168 predawn ( $\psi_{pd}$ ) and at midday ( $\psi_{md}$ ) in all seedlings with a pressure chamber.

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170 Morphological analyses following the field experiment

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172 At the end of the field experiment, seedlings were extracted from the rhizotrons and  
173 separated into leaves, stems and old and new roots, which were washed with tap water and  
174 dried in a ventilated oven at 60 °C for 48 h to measure dry mass. Roots protruding out of the  
175 root plug were designated as new roots while those in the root plug were classified as old  
176 roots. Absolute increments in shoot and old and new root mass, and the root relative grow  
177 rate ( $RGR_{root}$ ) were calculated.  $RGR_{root}$  was calculated as:

$$178 \quad RGR = (\log_e m_2 - \log_e m_1) / (t_2 - t_1)$$

179 where  $m_1$  and  $m_2$  are the total root mass at the end of the nursery cultivation and at the end of  
180 the field experiment, respectively.

181 Standardization of new root mass by leaf mass (NR/L) was used as a proxy of the  
182 plant's water balance potential. We also calculated the production of new root mass per total  
183 plant mass (NR/P). Before drying the new roots, we measured new root length according to  
184 methodology proposed in Marsh (1971). We calculated the specific root length (SRL) as the  
185 new root length to mass ratio.

186

187 Data analysis

188

189 Differences in seedling attributes following nursery cultivation were analysed by two-factorial  
190 ANOVA, with sowing date and fertilization as main factors. Root and shoot mass increment,  
191  $RGR_{\text{root}}$ , NR/L and NR/P at the end of the field experiment was analysed by ANCOVA. Mean  
192 and total root elongation rate and depth, the number of roots and  $\psi$  were analysed by repeated  
193 measure ANCOVA. In these analyses, fertilization was the categorical predictor and seedling  
194 stem volume at the beginning of the field experiment was the covariable.

195 Antagonism between root and shoot growth was measured by calculating the slope of  
196 the linear regressions between the standardized shoot and total root elongation rate. Negative  
197 slopes mean that roots and shoot have an antagonistic growth pattern, while regressions with  
198 zero or positive slope values mean no antagonism between roots and shoot growth. The effect  
199 of fertilization and plant size on the slope values between root and shoot elongation rate was  
200 analysed by ANCOVA, where plants size was the covariable. To assess if the slope values in  
201 each seedling type were different from zero, we carried out a t-test for single samples. Data  
202 were checked for normality and homogeneity of variances and were transformed when  
203 necessary to correct deviations from these assumptions. In ANCOVA, we also checked the  
204 homogeneity of slopes across fertilization treatments and in all cases treatment slopes were  
205 similar (no significant covariable  $\times$  Fertilization interaction). All statistical analyses were  
206 performed with the Statistica 6.1. Package (StatSoft, Inc., Tulsa, OK, USA). For simplicity  
207 we classified plants into three size categories according to sowing date in the nursery (small,  
208 medium and large; see Table 1), to show the results of the effect of the stem volume  
209 covariable on studied variables.

210

## 211 **Results**

212

213 Seedling attributes following nursery cultivation

214

215 Seedling height increased with the delay in sowing date ( $F = 214, P < 0.001$ ), and increased  
216 with fertilization, but only in seedling sown in March (sowing date  $\times$  fertilization interaction,  
217  $F = 5.3, P = 0.011$ ). Delay in sowing date and fertilization increased seedling diameter ( $F =$   
218  $190, P < 0.001$  and  $F = 7.5, P = 0.01$ , respectively). Shoot and root mass decreased with the  
219 delay in sowing date ( $F=116, P<0.001$  and  $F = 103, P < 0.001$ , respectively), while  
220 fertilization had no effect. Seedling total mass in plants sown in December were 1.5 and more  
221 than six times larger than plants sown in March and in May, respectively, while plants sown  
222 in March were more than four times larger than those sown in May. By contrast, seedling  
223 mass did not differ between fertilization treatments (Table 1). Seedling shoot/root ratio was  
224 close to 1 and did not differ among sowing dates, but it was lower in  $N^+$  than in  $N^-$  seedlings  
225 ( $F = 7.09, P = 0.009$ ).

226 Fertilization increased seedling N concentration ( $F = 53.4, P < 0.001$ ), which was on  
227 average 35% higher in  $N^+$  than in  $N^-$  seedlings. Delay in sowing date also increased seedling  
228 N concentration ( $F = 15.3, P < 0.001$ ); seedlings sown in May had 26 and 30% higher N  
229 concentration than those sown either in March or in December, respectively. Both sowing  
230 date ( $F = 81.5, P < 0.001$ ) and fertilization ( $F = 23.5, P < 0.001$ ) affected seedling N content,  
231 with seedlings sown in December and May having the highest and lowest N content,  
232 respectively, while seedlings sown in March had intermediate values. N content was 30%  
233 higher in  $N^+$  than in  $N^-$  seedlings.

234

235 Root and shoot growth in the field

236

237 Shoot elongation rate decreased with seedling size. Small seedlings had two shoot elongation  
238 rate peaks through time while medium and large seedling had only one peak, which did not

239 coincide in time with those of small seedlings (time  $\times$  stem volume interaction; Table 2). Over  
240 all dates, mean elongation rate of individual roots was not affected by seedling size (Table 2,  
241 Fig. 1). This occurred because seedling size increased mean root elongation rate during the  
242 first weeks but it reduced it at the end of the experiment (time  $\times$  stem volume interaction;  
243 Table 2). Fertilization significantly reduced the mean elongation rate of individual roots. Total  
244 root elongation rate increased with seedling stem volume, but there were no differences  
245 between large and medium plants at the end of the study (time  $\times$  stem volume interaction;  
246 Table 2). Fertilization did not affect total root elongation rate. The number of new roots  
247 increased with initial seedling stem volume, except in the first measurement date (time  $\times$   
248 initial stem volume interaction; Table 2). Nursery fertilization significantly increased the  
249 number of roots, N<sup>+</sup> and N<sup>-</sup> plants having  $20.2 \pm 1.3$  and  $14.5 \pm 1.4$  roots (mean  $\pm$  1SE),  
250 respectively. Mean and maximum root depth increased through time, ranging from 32 to 37  
251 cm and from 51 to 61 cm, respectively, at the end of the experiment. Neither initial seedling  
252 size nor fertilization significantly affected mean or maximum root depth.

253 At the end of the experiment, both initial seedling stem volume and nursery  
254 fertilization significantly increased new root mass and length (Table 2, Fig. 2). New root mass  
255 was 29% higher in N<sup>+</sup> seedlings than in N<sup>-</sup> seedlings. Large and medium seedlings had 52 and  
256 48% higher new root mass than small seedlings, respectively. New root length was  $7.9 \pm 0.92$   
257 and  $5.6 \pm 0.54$  m (mean  $\pm$  1 SE) in N<sup>+</sup> and N<sup>-</sup> seedlings, respectively, whereas large, medium  
258 and small seedlings had  $8.0 \pm 1.03$ ,  $8.1 \pm 0.89$  and  $4.2 \pm 0.52$  m of new roots, respectively.  
259 Seedling stem volume and fertilization significantly enhanced growth of both shoot and old  
260 root mass. Root to shoot mass ratio at the end of the experiment and  $RGR_{\text{root}}$  were not affected  
261 either by initial seedling stem volume or nursery fertilization (Table 2, means not shown).  
262 Increase in seedling stem volume significantly diminished NR/L, NR/P and SRL, although the  
263 effect was marginal in the latter ( $P = 0.065$ ). Medium and large seedlings had 22% and 30%

264 lower NR/L, respectively, than small seedlings. Nursery fertilization did not significantly  
265 affect either NR/L, NR/TP or SRL.

266 Mass of new roots was positively related to the seedling N content (Fig. 3) and the  
267 number of roots ( $r^2 = 0.32$ ,  $P < 0.001$ ,  $n=36$ ) at the end of the experiment, but not with either  
268 the mean elongation rate of individual roots ( $r^2 = 0.05$ ,  $P = 0.19$ ,  $n=36$ ) or the plant N  
269 concentration at the beginning of the study ( $r^2 = 0.05$ ,  $P = 0.67$ ,  $n=6$ ).

270  
271 Seedling water potential and relation between root and shoot elongation rate

272  
273 Seedling  $\psi_{pd}$  was higher than  $\psi_{md}$ . Stem volume reduced  $\psi_{pd}$  whereas it did not affect  $\psi_{md}$   
274 (time  $\times$  initial stem volume interaction, Table 2).  $\psi_{pd}$  for large, medium and small seedlings  
275 was  $-1.21 \pm 0.04$ ,  $-1.05 \pm 0.04$  and  $-0.96 \pm 0.04$  MPa, respectively, whereas  $\psi_{md}$  was  $-2.53 \pm$   
276  $0.08$ ,  $-2.53 \pm 0.08$  and  $-2.48 \pm 0.08$  MPa, respectively. Nursery fertilization did not affect  $\psi$ .

277 The slope of the regression of total root elongation rate against shoot elongation rate  
278 was significantly and positively related to stem volume while fertilization had no effect  
279 (Table 2 and Fig. 4). Similarly, no interaction between fertilization and stem volume on the  
280 slope of the regression between the elongation rates of both organs was observed ( $F = 0.18$ ,  $P$   
281  $= 0.68$ ). Slopes of small seedlings were significantly lower from zero ( $-0.26 \pm 0.046$  [mean  $\pm$   
282 1SE],  $t = -5.63$ ,  $P < 0.001$ ). On the contrary, the slope of large plants was significantly greater  
283 than zero ( $0.20 \pm 0.089$ ,  $t = 2.25$ ,  $P = 0.045$ ), whereas the slope of medium seedlings did not  
284 significantly differ from zero ( $0.12 \pm 0.09$ ,  $t = 1.30$ ,  $P = 0.22$ ).

285

286

287 **Discussion**

288

289 Root growth and structure

290

291 Large Aleppo pine seedlings produced larger new root systems than small seedlings, which is  
292 in agreement with results in field experiments in *P. halepensis* and *Juniperus thurifera*  
293 (Cuesta et al. 2010; Martínez-Sanz 2006) and in root growth capacity tests performed in  
294 controlled environments in other forest species (van den Driessche 1992; Villar-Salvador, et  
295 al. 2004). In agreement with our first hypothesis, large seedlings produced larger new root  
296 systems because they produced higher number of roots than small seedlings. Furthermore,  
297 new roots in large seedlings had lower SRL than in small seedlings. However, contrary to our  
298 hypothesis individual roots did not grow faster in larger than in smaller plants. Unlike Aleppo  
299 pine, elongation rate of individual roots increased with seedling size in *J. thurifera* (Martínez–  
300 Sanz 2006).

301           Seedling survival in dry ecosystems depends on the development of large and deep  
302 root systems (Burdett, et al. 1983; Grossnickle 2005; Padilla and Pugnaire 2007). Greater root  
303 systems of large seedlings could explain their frequently improved post-transplanting  
304 performance relative to small seedlings in Mediterranean plantations (Luis, et al. 2009; Oliet,  
305 et al. 2009; Tsakalidimi, et al. 2005; Villar-Salvador, et al. 2008). Contrary to our  
306 expectations, we did not find differences in root depth among seedlings phenotypes, which  
307 differs with the result found for *J. thurifera* seedlings, where roots of large seedlings grew  
308 deeper than those of small seedlings (Martínez-Sanz 2006). Absence of differences in root  
309 depth among seedling types is consistent with their lack of differences in the mean elongation  
310 rate of individual roots. Padilla and Pugnaire (2007) did not find any relationship between  
311 initial seedling size and either root extension rate or maximum root depth in a comparison of  
312 several Mediterranean woody species. This suggests that functional processes occurring  
313 across species may not coincide with those occurring at the intraspecific level. Accordingly

314 with our first hypothesis, high-fertilized seedlings also produced greater root systems than  
315 low-fertilized plants. Nevertheless, root elongation rate of individual roots was just slightly  
316 higher in low fertilized seedlings than in high-fertilized seedlings, which can explain the  
317 similar total root elongation rate between fertilization regimes.

318         Specific root length depends on root thickness and/or density and varies widely among  
319 species, although it usually has low variation in response to environmental conditions such as  
320 temperature or nutrient availability (Alvarez-Uria and Korner 2007; George, et al. 1997;  
321 Pregitzer, et al. 2002; Reich, et al. 1998). We found that, at the intraespecific level, small  
322 seedlings tended to have higher SRL than large seedlings; this suggests that the former  
323 produced thinner and/or less dense roots, which have lower construction and maintenance  
324 cost than low SRL roots (Pregitzer, et al. 2002).

325         NR/L provides an idea of the potential balance between the water transpiration and  
326 water uptake capacity in a plant. High NR/L may confer greater capacity of plants to maintain  
327 high water potential under drought conditions. In spite of the large differences in seedling size  
328 in our study, large seedlings were similarly water balanced than small seedlings. This  
329 argument is supported by the lack of differences in mid-day  $\psi$  among seedlings of different  
330 size. Higher root density (mass of new roots per soil volume unit) and enhanced root  
331 hydraulic conductance in larger plants (Chirino, et al. 2008; Wan, et al. 1996) in comparison  
332 to smaller plants could explain why large seedlings, in spite of having lower NL/R and NR/P,  
333 had similar mid-day  $\psi$  than small seedlings. Small seedlings had lower pre-dawn  $\psi$  than large  
334 seedlings, suggesting that the former rehydrate faster than the latter at night or that night  
335 transpiration increased with seedling size.

336         Seedling size had greater effect on root growth than nursery fertilization as much more  
337 variables were affected by seedling size than fertilization (see Table 2). Differences in size  
338 among seedling phenotypes were higher than differences in N concentration, which probably



339 explains the greater effect of seedling size on root growth. As root growth in *P. halepensis*  
340 and other conifers strongly relies on current photosynthesis (Moreno 2003; van den Driessche  
341 1987), an increase in root growth with seedling size might be attributed to higher  
342 photosynthesis in larger plants than in small plants (Cuesta, et al. 2010). Early growth of new  
343 organs in spring is also supported by remobilization of stored N and growth is usually  
344 positively linked to the amount of remobilised N (Dyckmans and Flessa 2001; Malik and  
345 Timmer 1996; Millard 1996; Salifu and Timmer 2003). In our study, large plants had higher  
346 N content than small seedlings, which potentially increased the amount of remobilizable N.  
347 This might explain the positive relationship between the new root mass at the end of the  
348 experiment and seedling N content at planting (Fig. 3). In addition, nursery fertilization,  
349 which increased plant N concentration without promoting significant growth, had an additive  
350 effect on the effect of plant size on root growth. Increase in N concentration can stimulate  
351 growth by increasing the amount of remobilizable N and / or by enhancing photosynthetic rate  
352 (Field and Mooney 1986).

353

#### 354 Relationship between root and shoot growth

355

356 As resources become limited, investment of resources to root growth can reduce shoot growth  
357 or reproduction and *vice versa* (Bloom, et al. 1985; Chapin 1990). The relationship between  
358 root and shoot elongation through time differs among species and if lateral or taproots are  
359 considered (Harris, et al. 1995; Lyr and Hoffmann 1967; Reich, et al. 1980; Riedacker 1976;  
360 Thaler and Pagès 1996a). Our study demonstrates that antagonism between root and shoot  
361 elongation also varies among individuals of contrasted size at a within species scale. In  
362 agreement with our second hypothesis, root and shoot growth did not have an antagonistic  
363 growth pattern in large and medium seedlings as the slope of the regression between shoot

364 and root elongation rate were positive and zero, respectively. On the contrary, the slope was  
365 negative in small seedlings indicating the growth of both organs depressed each other  
366 probably because they competed for the same resources. Willaume and Pagès (2006) found  
367 that reduction of photosynthesis by defoliation increased the alternating growth pattern of  
368 roots and shoots in *Quercus pubescens*, highlighting the importance of carbohydrate  
369 sink/source relations in this process. Decrease in root and shoot growth antagonism with  
370 increase in seedling size suggests that large seedlings can provide resources to sustain high  
371 and simultaneous growth of both organs. Because increase in seedling N concentration did not  
372 have an additive effect over the plant size effect on the antagonism between shoot and root  
373 growth, we suggest that differences in carbon economy rather than N remobilization might be  
374 the mechanism underlying the differences in the shoot-root growth relationship in Aleppo  
375 pine seedlings. Future studies should investigate this hypothesis.

376           We conclude that large seedlings developed denser but not deeper root systems than  
377 small seedlings due to greater proliferation of new roots with lower specific root length.  
378 Seedling size had greater influence on root growth than N concentration, which had an  
379 additive effect over plant size. Root and shoot growth did not depress each other in large and  
380 medium seedlings, whereas it did not occur in small seedlings. These findings provide  
381 insights for understanding root growth differences and transplanting performance among  
382 Aleppo pine stock-types in Mediterranean woodland plantations.

383

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538 Table 1. Morphology and N concentration content of *Pinus halepensis* seedlings that were  
 539 cultivated by sowing seeds on three distinct dates (December, March and May) and fertilized  
 540 with nitrogen at two distinct rates (N<sup>+</sup> and N<sup>-</sup>). Data are means  $\pm$  one SE. n=15 except for N  
 541 concentration where n=3. Seedling types with different letter indicate significant differences  
 542 at P<0.05 after Tukey's post-hoc test.

543

	December		March		May	
	N <sup>-</sup>	N <sup>+</sup>	N <sup>-</sup>	N <sup>+</sup>	N <sup>-</sup>	N <sup>+</sup>
Plant height (cm)	19.8 $\pm$ 0.6 <sup>a</sup>	19.1 $\pm$ 0.8 <sup>a</sup>	15.2 $\pm$ 0.4 <sup>b</sup>	16.9 $\pm$ 0.4 <sup>ab</sup>	8.0 $\pm$ 0.5 <sup>c</sup>	8.4 $\pm$ 0.3 <sup>c</sup>
Plant diameter (mm)	4.0 $\pm$ 0.1 <sup>a</sup>	4.5 $\pm$ 0.1 <sup>a</sup>	3.4 $\pm$ 0.1 <sup>b</sup>	3.8 $\pm$ 0.1 <sup>b</sup>	2.1 $\pm$ 0.1 <sup>c</sup>	2.1 $\pm$ 0.1 <sup>c</sup>
Root mass (g)	3.0 $\pm$ 0.34 <sup>a</sup>	2.9 $\pm$ 0.16 <sup>ab</sup>	2.0 $\pm$ 0.09 <sup>c</sup>	2.2 $\pm$ 0.08 <sup>bc</sup>	0.5 $\pm$ 0.07 <sup>d</sup>	0.6 $\pm$ 0.06 <sup>d</sup>
Shoot mass (g)	3.2 $\pm$ 0.35 <sup>a</sup>	3.0 $\pm$ 0.25 <sup>ab</sup>	2.3 $\pm$ 0.18 <sup>bc</sup>	2.2 $\pm$ 0.14 <sup>c</sup>	0.5 $\pm$ 0.04 <sup>d</sup>	0.5 $\pm$ 0.05 <sup>d</sup>
Shoot / Root mass ratio	1.12 $\pm$ 0.1 <sup>a</sup>	1.07 $\pm$ 0.1 <sup>ab</sup>	1.11 $\pm$ 0.05 <sup>a</sup>	1.01 $\pm$ 0.1 <sup>ab</sup>	1.12 $\pm$ 0.1 <sup>a</sup>	0.77 $\pm$ 0.1 <sup>b</sup>
Plant N concentration (mg g <sup>-1</sup> )	6 $\pm$ 0.3 <sup>d</sup>	10 $\pm$ 1.1 <sup>bc</sup>	7 $\pm$ 0.2 <sup>cd</sup>	10 $\pm$ 0.9 <sup>b</sup>	9 $\pm$ 0.6 <sup>bcd</sup>	14 $\pm$ 0.6 <sup>a</sup>
Plant N content (mg)	42 $\pm$ 2.5 <sup>bc</sup>	56 $\pm$ 3.6 <sup>a</sup>	30 $\pm$ 1.9 <sup>c</sup>	45 $\pm$ 4.7 <sup>ab</sup>	9 $\pm$ 2.1 <sup>d</sup>	15 $\pm$ 1.8 <sup>d</sup>

544

545 Table 2. Effects of initial stem volume (covariable), fertilization and time (within effect in  
 546 repeated measure ANCOVA) on root and shoot growth variables and twig water potential ( $\psi$ )  
 547 in *P. halepensis* seedlings. Data are F ratios.

548

	Initial stem volume	Fertilization	Time	Initial stem volume $\times$ Time	Fertilization $\times$ Time
Shoot elongation rate	9.98 <sup>***</sup>	0.074	4.24 <sup>***</sup>	6.64 <sup>***</sup>	0.79
Mean root elongation rate	0.32	3.49 <sup>†</sup>	16.4 <sup>***</sup>	2.66 <sup>*</sup>	0.73
Total root elongation rate	17.5 <sup>***</sup>	0.09	6.44 <sup>***</sup>	4.44 <sup>***</sup>	1.65
New root number	29.7 <sup>***</sup>	4.91 <sup>*</sup>	2.32 <sup>*</sup>	6.40 <sup>***</sup>	1.24
Average root depth	1.76	0.01	116 <sup>***</sup>	0.42	0.18
Maximum root depth	2.00	1.57	137 <sup>***</sup>	1.18	1.46
New root mass	23.1 <sup>***</sup>	4.42 <sup>*</sup>	—	—	—
New root mass / leaf mass	4.44 <sup>*</sup>	0.41	—	—	—
New root mass / total plant mass	13.7 <sup>***</sup>	1.48	—	—	—
Shoot mass to root mass	0.06	0.19	—	—	—
RGR <sub>root</sub>	0.0002	2.02	—	—	—
New root length	11.4 <sup>**</sup>	4.67 <sup>*</sup>	—	—	—
New root specific length	3.51 <sup>†</sup>	0.14	—	—	—
Root-shoot growth interference	19.5 <sup>***</sup>	0.01	—	—	—
Old root increment	25.3 <sup>***</sup>	3.99 <sup>†</sup>	—	—	—
Shoot increment	25.5 <sup>***</sup>	10.28 <sup>**</sup>	—	—	—
$\psi$	5.08 <sup>*</sup>	3.64 <sup>†</sup>	580 <sup>***</sup>	5.61 <sup>*</sup>	0.04

549

550 <sup>\*\*\*</sup>  $P \leq 0.001$ , <sup>\*\*</sup>  $P \leq 0.01$ , <sup>\*</sup>  $P \leq 0.05$ , <sup>†</sup>  $P < 0.07$ .

551

552 Fig. 1. Variation of shoot elongation rate, mean elongation rate of individual roots, total root  
553 elongation rate and number of roots in *Pinus halepensis* seedlings of three size types along the  
554 experiment. Data are means  $\pm$  1 SE. n=6

555

556 Fig. 2. Shoot and old and new root mass increment (left), and new root mass to leaf mass ratio  
557 and new root mass to total plant mass ratio (right) at the end of the rhizotron experiment in  
558 *Pinus halepensis* seedlings that differed in size at transplanting and were cultivated with  
559 contrasting fertilization rate in the nursery. Data are means  $\pm$  1 SE. n=6. S (small seedling), M  
560 (medium seedling), L (large seedling)

561

562 Fig. 3. Relationship between new root mass and N content in *Pinus halepensis* seedlings. Data  
563 are means  $\pm$  one SE. S (small seedling), M (medium seedling), L (large seedling), N<sup>-</sup> (low  
564 fertilization) and N<sup>+</sup> (high fertilization)

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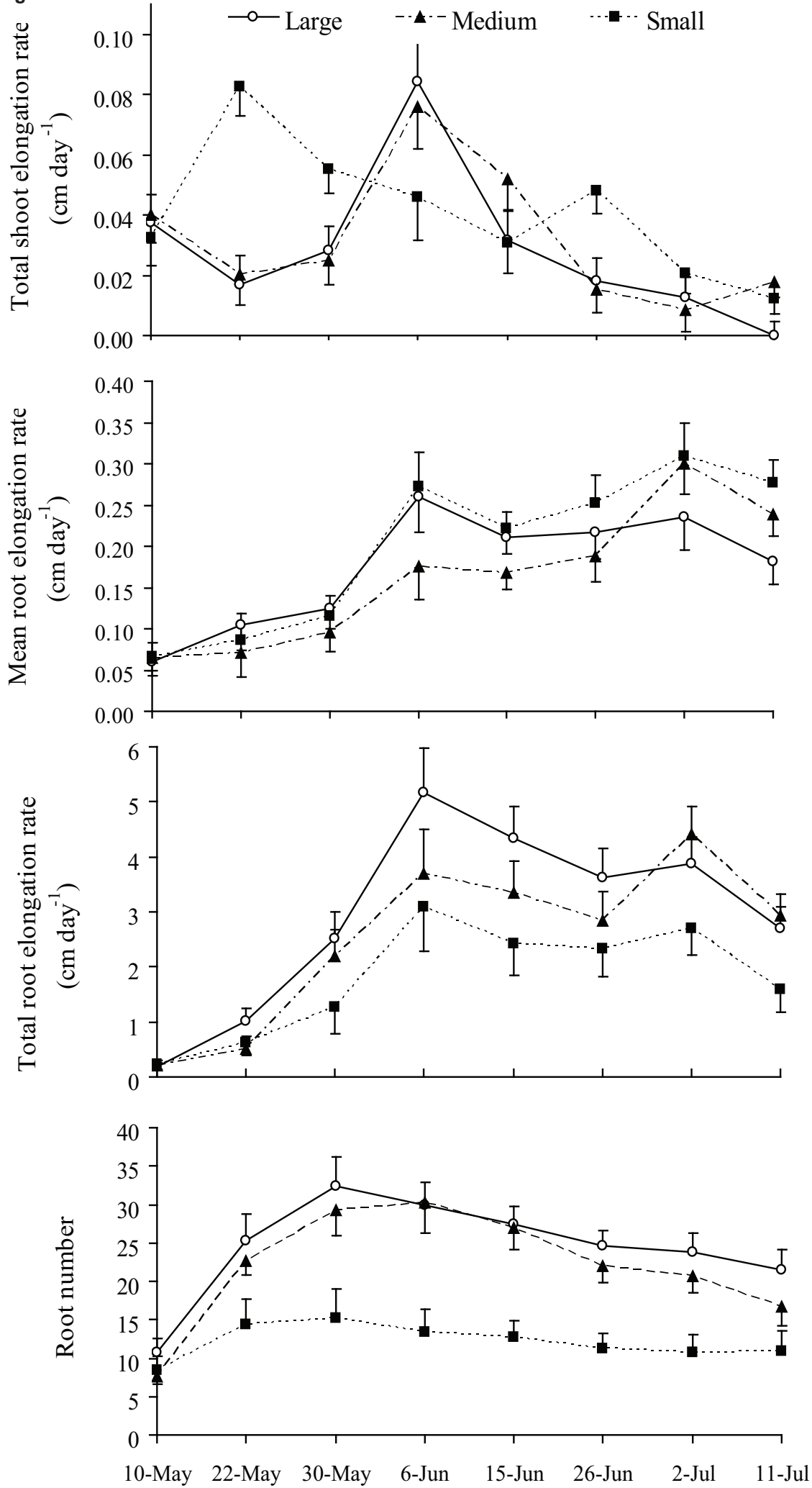
566 Fig. 4. Relationship between the slope of the regressions of shoot against root elongation rates  
567 and seedling size in high and low N fertilized plants. Seedling size was measured as the initial  
568 stem volume at transplanting. Each point represents an individual seedling. S- (small seedling  
569 with low fertilization), S+ (small seedling with high fertilization), M- (medium seedling with  
570 low fertilization), M+ (medium seedling with high fertilization), L- (large seedling with low  
571 fertilization), L+ (large seedling with high fertilization).

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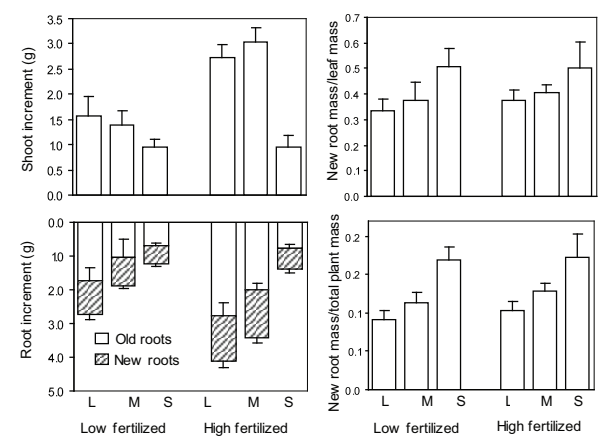
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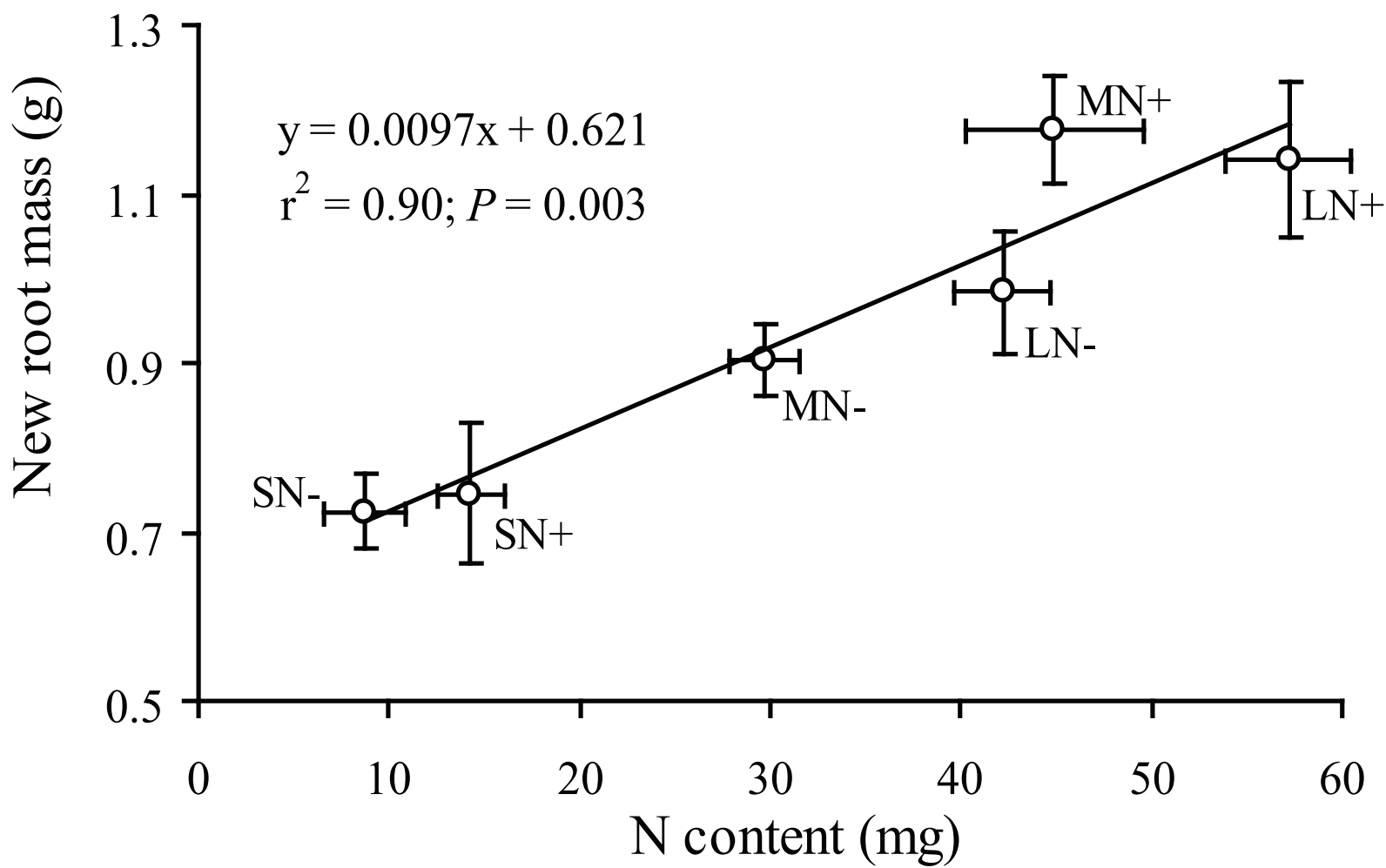
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