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2 Mind the gap among patches in arid plant communities: Rapid root proliferation in
3 response to N addition.

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11 Running title: Rapid root proliferation in bare soil patches

12

13 ABSRACT

14 Aims. It has been proposed that in arid communities, individual plants can extend their
15 roots beyond their canopy exploring neighbouring bare ground areas. This becomes
16 relevant in systems where the vegetation is distributed in patches surrounded by bare soil.
17 However, whether roots of different species may be overlapping under bare ground areas is
18 still controversial. What factors control root responses when no plants appear to be directly
19 influencing the gap among patches is still unclear. The aim of our study was to detect
20 perennial grasses responses to an N enrichment pulse.

21 Methods. In a semi-arid steppe (Patagonia, Argentina) we buried root traps filled with
22 sieved soil with and without N addition, under bare soil patches. Traps were harvested after
23 4 and 6 months. Trap neighbourhoods (30 cm in diameter) included at least three of the
24 dominant tussock species. After harvests, we identified species in the traps by root traits,
25 and quantified diversity, biomass and specific relative growth rates.

26 Important Findings. Bare ground areas show simultaneous growth of root of different
27 species. Diversity of perennial grass roots was higher with N addition than without it in the
28 first harvest (4 months), but this difference disappeared in the second harvest (6 months).
29 Root biomass was maximal after 6 months in + N traps. Species preferred by herbivores
30 (*Bromus pictus* and *Poa ligularis*) showed rapid growth and responses to N addition.
31 Differences between harvests may be indicative that N pulse interacts with rising
32 temperatures and soil water content as growing season progress.

33 Keywords: belowground community ecology; grass species roots; Patagonian steppe;
34 patchiness; root growth rates; zone of influence.

35

36 INTRODUCTION

37 In natural environments, plants interact above and belowground, sharing space and
38 influencing each other through soil occupation and resources utilisation (Caldwell et al.
39 1987; McConnaughay and Bazzaz 1992; Cahill *et al.* 2010). Plant influence in the soil is
40 maximum under its canopy (zone of high influence, Reyes and Aguiar 2017a) and may
41 decrease with distance (zone of low influence). This influence would be defined by relative
42 growth rates, competitive abilities of interacting plants and resources availability, among

43 other factors (Casper *et al.* 2003; Berger *et al.* 2006; Armas and Pugnaire 2011; Reyes and
44 Aguiar 2017a,b). Nevertheless, shoot and root overlapping could be broadly different
45 depending on kind of system. It has been proposed, for humid systems in general, that root
46 of several species overlap because their roots laterally extend more widely than their
47 canopy, e.g. herbs and grasses (Pecháčková *et al.* 1999; Schenk and Jackson 2002; Casper
48 *et al.* 2003). In ecosystems with sparse vegetation (< 50% plant cover) bare ground is a
49 major feature but root proliferation under these areas remains poorly studied. Specially,
50 which factors control spatial organization of roots of species with different growth rates in
51 response to nutrient distribution is still unclear (Hutchings *et al.* 2003; Hodge 2009; de
52 Kroon *et al.* 2012, McNickle *et al.* 2016). Furthermore, because most knowledge derives
53 from pot experiments, there is no information about what factors control root organisation
54 when no plants appear to directly influence above the ground, as it happens under a gap
55 among patches (called here: a zone of minimal influence).

56 Responses to nutrient distribution in a competitive environment depend on how
57 competitive each species is (Mommer *et al.* 2012) and this interaction could define root
58 distribution (Cahill *et al.* 2010; McNickle *et al.* 2016). Under natural conditions, roots are
59 exposed to multiple environmental cues, in addition to heterogeneous distribution of
60 nutrients and presence of neighbour roots (Cahill *et al.* 2010; Pierik *et al.* 2012).
61 Insufficient information on the spatial distribution of root biomass could be the result of the
62 difficulty in distinguishing species by their roots. This difficulty leads to the inference that
63 aboveground presence of a species is an appropriate indicator of its belowground biomass
64 (Soriano *et al.* 1987; de Kroon *et al.* 2012). But this conclusion can lead to mistakes such as

65 underestimation of root community diversity under bare soil, where neighbouring species
66 are distant (see Schenk *et al.* 1999, for a more general case).

67 In arid and semi-arid ecosystems, resources as soil water and nutrients typically go
68 through periods of high and low abundance. Short periods of high resource abundance are
69 triggered by rainfall events, which can temporarily saturate the resource demand for some
70 biological processes (Schwinning and Sala 2004). In these ecosystems, where vegetation is
71 usually distributed in patches interspersed in bare soil matrix, overlap among rooting zones
72 might be more common than in the humid ones (Frank *et al.* 2010; de Kroon *et al.* 2012).
73 Understanding these factors becomes highly relevant, as soil resources are limiting for plant
74 communities (Schenk and Jackson 2002; Chesson *et al.* 2004) and it is simpler and better
75 for investigating what controls overlapping. A recent study detected some degree of
76 territoriality of fast-growing species in a zone of high influence of grass species, but it also
77 revealed root intermingling of several species (Reyes and Aguiar 2017a). Root proliferation
78 appears to be the result of the interaction between individual density in the community and
79 specific root growth rates (Reyes and Aguiar 2017b).

80 The concept of the belowground zone of influence has been used to model and
81 understand plant competition for soil resources ([Biondini 2001; Casper *et al.* 2003] in
82 Berger *et al.* 2006). There are already several studies that provide insights into the
83 likelihood of belowground competition occurring among plants based on root systems
84 overlapping and exploring the same soil volume (Berger *et al.* 2006; Reyes and Aguiar
85 2017a). The aim of our study was to detect a short term belowground response of grasses to
86 N enrichment conditions. In order to do this, we conducted a relatively short-term field
87 experiment in a semi-arid steppe to reduce the incidence of root mortality. It was

88 hypothesized that root overlapping (measured as high diversity) is explained by high
89 nutrient availability that allows the coexistence of a high number of species. We expect to
90 find a higher diversity and root proliferation in the enriched microsites than in those non-
91 enriched. We also expect to explain the increase in root overlapping with an increase of
92 root biomass of the fast-growing species under the same condition.

93

94 METHODS

95 STUDY SITE AND GRASS SPECIES

96 The study was conducted in the semi-arid Patagonian steppe ecosystem (Chubut, Argentina
97 45° 25'S, 70° 20'W). The climate is arid, with an intense summer drought. In June and July,
98 average temperatures are the lowest of the year (between 2 and 3 °C), while in August and
99 September, temperatures start to increase (between 5 and 7 °C) reaching 16 °C in January.
100 Mean annual precipitation is 130 ±29 mm (mean ±SD) and most precipitation occurs
101 during winter and early spring (May-September) (INTA 2016). Vegetation is sparse (48%
102 plant cover) and species richness is commonly 26 species (Golluscio *et al.* 1982) dominated
103 by five perennial grasses, since shrubs cover <18% of the total surface. Grasses have most
104 of their roots in the upper soil layer. Thus, 54% of their root biomass occupies the first 10
105 cm of the profile (Soriano *et al.* 1987). Grass species develop horizontal roots, while shrubs
106 mainly explore deeper soil layers (Sala *et al.* 1989).

107 The grass community includes eight perennial-graminoid species. The five
108 dominant species are: *Pappostipa speciosa* (Trin. & Rupr.) Romasch; *Pappostipa humilis*
109 (Cav.) Romasch; *Poa ligularis* Nees ex Steud; *Bromus pictus* Hook (Poaceae species) and

110 *Carex* sp. (Cyperaceae), and another three representing less than 3% frequency in the field:
111 *Bromus setifolius* J. Presl *setifolius* (Hook. f.) Skottsbo; *Festuca argentina* (Speg.) Parodi
112 and *Hordeum comosum* J. Presl (all *Poaceae* species). Leva *et al.* (2009) studied root
113 morphology of dominant graminoid species and built a taxonomic key using the main
114 features of roots harvested in the field, such as: diameter, colour and hairiness. Reyes and
115 Aguiar (2017a) tested the taxonomic key and obtained a 95% specific biomass recovery
116 rate on blind samples. In this steppe, grasses present a sparse distribution, as tussock plants.
117 This feature allowed us to work with isolated individuals under field conditions without
118 causing major disturbances, such as removal of individuals or placing barriers or pots to
119 measure root growth. The soil texture is sandy which facilitates the extraction of soil
120 samples, root separation and identification without damaging them (Leva *et al.* 2009; Reyes
121 and Aguiar 2017a).

122

123 EXPERIMENTAL DESIGN

124 In an area closed to sheep grazing, we buried root traps under bare soil patches (5-11 cm
125 depth) in early winter (June). The root trap consisted of a plastic tube with 10 mm²
126 perforations all along and around, 4 cm in diameter and 6 cm high (a common hair curler,
127 see Supplementary material in Reyes and Aguiar 2017a). Before burial of traps we
128 controlled that neighbourhoods included individual plants from at least three of the
129 dominant graminoid species, and the exact location of the traps was 15 cm away from (Fig
130 1). Traps were filled before burial with: i. sieved soil free of stones and roots (control
131 treatment, C); and ii. sieved soil with 4 g N m⁻² (nitrogen addition, N+). Nitrogen was
132 added as ammonium nitrate (NH₄NO₃) granules with a slow nitrogen release rate

133 appropriate for the site. This was performed following the method proposed by Austin and
134 Vivanco (2006). The amount of added nitrogen was greater than the annual rate of
135 mineralisation estimated in the steppe (around $0.1 \text{ g N m}^{-2} \text{ year}^{-1}$, Yahdjian and Sala 2008)
136 and would eliminate the limitation for this nutrient. Trap harvests were at 4 (120 days) and
137 6 months (180 days) from burial. The harvests intended to register growth at the start of the
138 growing season (June to September) and at the peak of root growth (October to December,
139 Soriano *et al.* 1987). After December plant growth tends to decrease because of an abrupt
140 decrease in soil water availability (January to May, Paruelo and Sala 1995). A factorial
141 design was conducted with two factors, soil substrate inside the traps (treatment) and time
142 of harvest. We distributed traps in a randomized block design ($n=10$ per treatment and time,
143 a total of 40 traps) to assure that root composition and proliferation in traps were not
144 determined by possible differences among aboveground neighbourhood. Distance among
145 buried traps was >1 m in each block.

146 Root trap exact location was indicated by a wire and a label indicating treatment and
147 number of replication. In each harvest, we removed the soil above the trap and used a sharp
148 knife to cut the roots following the outside border of the trap. We removed a total of 20
149 traps per harvest (10 with N addition and 10 without). In the laboratory, the roots found in
150 traps were separated by species following the morphological identification key (Leva *et al.*
151 2009), oven-dried at $70 \text{ }^\circ\text{C}$ for 48 hours and weighed on a precision balance. Treatment and
152 harvest effects over diversity (Simpson index⁻¹) as well as root biomass of grass community
153 and relative growth rates (RGRs) of graminoid species that reappeared in traps were
154 characterized. We estimated RGRs as $(\ln W_2 - \ln W_1) T^{-1}$, where W_2 : root biomass weight at
155 the end of the study (g x m^{-2}) + 0.5; W_1 : initial root biomass weight (0 g) + 0.5; T: 120 days.

156 We transformed W_1 and W_2 by adding 0.5 to both weights in order to avoid zero values in
157 W_1 and to be able to calculate $\ln W_1$ because the sieved soil contained in traps was initially
158 free of roots (Reyes and Aguiar 2017a).

159

160 STATISTICAL ANALYSIS

161 The effect of blocks, factors (nitrogen treatment and time), and the treatment*time
162 interaction on diversity and root biomass was compared using ANOVA. The effect of
163 factors on species was compared with MANOVA (because the species presence inside the
164 traps was not independent), considering individual species RGRs as a variable response.
165 Previously, homogeneity of variances and normality assumptions were tested. Variables
166 were transformed as $X' = \text{Log}(X + 1)$ or $X' = X^{1/2}$ when needed. A nonparametric test
167 (Kruskal Wallis) was used when residual distribution was not normal. Analyses were
168 conducted with Statistica 7.0 software (Stat Soft Inc., Tulsa, Oklahoma, USA).

169

170 RESULTS

171 Fine roots of grasses colonized all traps. Treatment effect on diversity and root biomass
172 depended significantly on the harvest time (treatment*time $F = 12.18$, $df_t = 1$, $df_e = 35$, $P <$
173 0.001 and $F = 8.37$, $df_t = 1$, $df_e = 35$, $P < 0.001$, respectively). After 4 months, diversity was
174 significantly lower in control traps (C) than in N+ traps ($F = 38.31$, $df_t = 1$, $df_e = 19$, $P < 0.01$,
175 lowercase letters, Fig 2a). After 6 months, diversity was similar in both treatments ($F <$
176 0.01 , $df_t = 1$, $df_e = 18$, $P = 0.93$). In C traps, diversity was significantly higher after 6 months
177 than after 4 months ($F = 9.72$, $df_t = 1$, $df_e = 18$, $P < 0.01$, capital letters, Fig 2a), while N+

178 traps diversity was similar in both harvests ($F= 0.87$, $df_t= 1$, $df_e= 19$, $P= 0.36$). Root
179 biomass in traps was different between treatments and times. After 4 months, N addition
180 did not significantly affect root biomass ($F= 1.41$, $df_t= 1$, $df_e= 19$, $P= 0.27$, lowercase
181 letters, Fig 2b). After 6 months, root biomass was significantly lower in C traps than in N+
182 traps ($F= 8.18$, $df_t= 1$, $df_e= 18$, $P= 0.02$). Root biomass was similar in C traps ($F= 3.96$, $df_t=$
183 1 , $df_e= 19$, $P= 0.06$, lowercase letters, Fig 2b) and was significantly lower at 4 months than
184 at 6 months harvests in N+ traps ($F= 10.49$, $df_t= 1$, $df_e= 19$, $P< 0.01$).

185 Among grass species, *B. pictus*, *P. ligularis* and *P. speciosa* were the most frequent
186 in traps (between 60% and 90% of traps, on average, contained roots of these species),
187 while *P. humilis* and *Carex* sp. were rare or absent (between 3% and 15% on average).
188 Nitrogen effect on RGRs of *B. pictus* significantly depended on harvest (treatment*time $F=$
189 7.48 , $df_t= 1$, $df_e= 35$, $P<0.01$, Fig 3) and it was lower in C than in N+ traps after 4 months
190 ($F= 13.43$, $df_t= 1$, $df_e= 19$, $P< 0.01$, lowercase letters), while there were no differences after
191 6 months ($F= 1.73$, $df_t= 1$, $df_e= 18$, $P= 0.22$). In C traps, RGRs of *B. pictus* were
192 significantly lower after 4 than after 6 months ($F= 24.01$, $df_t= 1$, $df_e= 18$, $P< 0.001$, capital
193 letters, Fig 3) and it was similar between harvests in N+ traps ($F= 1.59$, $df_t= 1$, $df_e= 19$, $P=$
194 0.24). Nitrogen effect on the RGRs of *P. ligularis* and *P. speciosa* did not depend on
195 harvest (treatment*time $F= 2.16$, $df_t= 1$, $df_e= 35$, $P= 0.15$ and $F= 0.21$, $df_t= 1$, $df_e= 35$, $P=$
196 0.65 , respectively, Fig 3). Relative growth rates of *P. ligularis* were similar in both N
197 treatment and control ($F= 0.53$, $df_t= 1$, $df_e= 35$, $P= 0.47$, lowercase letters, Fig 3) and
198 between harvests ($F< 0.01$, $df_t= 1$, $df_e= 35$, $P= 0.94$). Relative growth rates of *P. speciosa*
199 were lower in C traps than in N+ traps ($F= 14.44$, $df_t= 1$, $df_e= 35$, $P< 0.001$, lowercase
200 letters) in both harvests, and there were no differences among harvests ($F= 2.66$, $df_t= 1$,

201 $df_e= 35$, $P= 0.11$, lowercase letters, Fig 3). Comparing among species after 4 months, RGRs
202 of *P. ligularis* were significantly higher than RGRs of *P. speciosa*, while *B. pictus* and the
203 rest of grass species were almost absent in C traps ($F= 25.68$, $df_t= 2$, $df_e= 51$, $P< 0.001$).
204 These differences disappeared in C traps after 6 months ($F= 1.35$, $df_t= 2$, $df_e= 51$, $P= 0.28$)
205 and RGRs of species were similar in both harvests in N+ traps ($F= 2.52$, $df_t= 2$, $df_e= 51$, $P=$
206 0.11 and $F= 2.41$, $df_t= 2$, $df_e= 51$, $P= 0.12$, after 4 and 6 months respectively).

207

208 DISCUSSION

209 Several authors, based on studies under controlled conditions, suggest that soil occupation
210 by several species (root overlapping) increases with nutrient availability (Casper *et al.*
211 2003; Cahill *et al.* 2010; Rajaniemi 2011). The overall objective of our study was to
212 estimate under field conditions the rate of soil occupation by roots of different species in
213 the short term. In addition, we were also interested in exploring the specific differences in
214 growth strategies in response to a pulse of N addition under bare soil among vegetation
215 patches where plant influences are low, in a system where bare soil is an important feature.
216 Our results showed that the maximum diversity of grass roots was reached in less time with
217 N addition than without it. But, after two months these differences in the diversity
218 disappeared. In other words, the roots of the grass species responded to N pulses and
219 promoted a rapid overlapping as our hypothesis suggested. Our study is original because
220 we worked under field conditions, at the community level and estimating root biomass by
221 species.

222 The total root biomass only responded to N addition after 6 months (spring months),
223 when it was 5 to 10 times higher than after 4 months (winter months growth). Soriano *et al.*

224 (1987) estimated that fine root growth occurs mainly during spring months. The 6 months
225 harvest showed the effect of N addition under increasing temperatures (mean day average
226 3°C until the first harvest and 7.5°C until the second one) and accumulation of rainfall
227 (which accumulated 44 mm before the first harvest and accumulated 74 mm before the
228 second one, INTA 2016). Rodríguez and Bertiller (2014) proposed that fine root
229 proliferation increases as a result of a synergistic combination of higher temperatures and
230 cumulative rainfalls. Our study indicated that in spring months, when water availability was
231 not limiting and temperatures were higher, N was responsible for this increase. The
232 magnitude of the response to N shown in spring months may denote an interactive effect of
233 N and humidity, that could determine root proliferation (Schwinning and Sala 2004;
234 Rodríguez *et al.* 2007).

235 Plant strategies have been based, mostly, on aboveground responses to
236 environmental variability. Our study generates data about belowground responses under
237 field conditions. *Bromus pictus*, *P. ligularis* and *P. speciosa* were the species that rapidly
238 dominated the sieved soil in the traps, both in terms of frequency (i.e. number of traps) and
239 biomass. The RGRs of these species showed different responses to the interaction of the
240 factors (N addition and time of harvest). In the case of *B. pictus*, its RGRs were limited by
241 N addition, but also by cumulative rainfall and temperatures. We propose that moisture and
242 temperature control root proliferation of *B. pictus* as much as N availability. This
243 behaviour, combined with the high root RGRs that species showed in a zone of high
244 influence (Reyes and Aguiar 2017a), supports the view that *B. pictus* is a species with
245 precise nutrient foraging (Campbell *et al.* 1991; Reyes and Aguiar 2017b), responsive to
246 humidity (Couso and Fernández 2012) and the most competitive in the grass community

247 (Graff 2009). Instead, *P. ligularis* showed high root RGRs independently of the
248 environmental cues (i.e. N addition, cumulative rainfall and temperatures). Accordingly, *P.*
249 *ligularis* was the most abundant species of the grass community in control traps in the first
250 harvest, whereas in the second harvest, differences among species disappeared. This pattern
251 could indicate a significant difference of this species over the others in terms of root rate
252 proliferation, which could explain the dominance of *P. ligularis* roots under bare soil
253 patches and of its individuals in the aboveground portion (Oñatibia 2013). *Pappostipa*
254 *speciosa* showed a consistent increase in root RGRs with N addition and this response also
255 increased from the first to the second harvest. This response was similar to *B. pictus* root
256 dynamics and could explain the increase in the total root biomass in the traps. This analysis
257 of root proliferation at species level is based only on resource dynamics and, therefore,
258 incomplete. The first two species are highly palatable to wild and domestic herbivores,
259 whereas *P. speciosa* is a non-palatable species. As rangelands co-evolved with grazers, our
260 ability to understand natural root communities could improve if we add the herbivory effect
261 to the species growth rates, which determines their abundance and competitive abilities
262 (Reyes and Aguiar 2017b).

263 Our short term study contributes to the understanding of belowground ecology, in
264 particular what factors may control root organisation under natural conditions. Recently, a
265 pot study reported that neighbour presence is more important than soil nutrient content for
266 predicting root organisation in soil (McNickle *et al.* 2016). However, these findings leave
267 out bare ground patches that represent at least 50% of total area in arid steppes. Our study
268 in the Patagonian steppe indicates that grass roots of different species may concurrently
269 grow in the same soil micro-volume, independently of N addition. And therefore there is no

270 pre-emption. These results partially agree with de Kroon *et al.* (2012) that suggest the
271 independence between root growth and high nutrient availability. In this Patagonian steppe,
272 a sole pulse of nutrient availability (such a urine o faecal pat deposition by sheep or
273 guanacos) may play a minor role directing root biomass accumulation. Whereas nutrients in
274 addition to soil water availability and temperatures appear to define a synergism over root
275 community. Nevertheless, it is worth mentioning that the responses of the dominant
276 perennial grasses were diverse and related to their growth rates, their aboveground
277 abundance and their response to grazing, in addition to the three mentioned variables. We
278 expect to find differences in nutrient absorption at a specific level. Further exploration of
279 this issue using tracers may shed light on the crucial role of spatial organisation of plant
280 community.

281

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290

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380 Figure legends

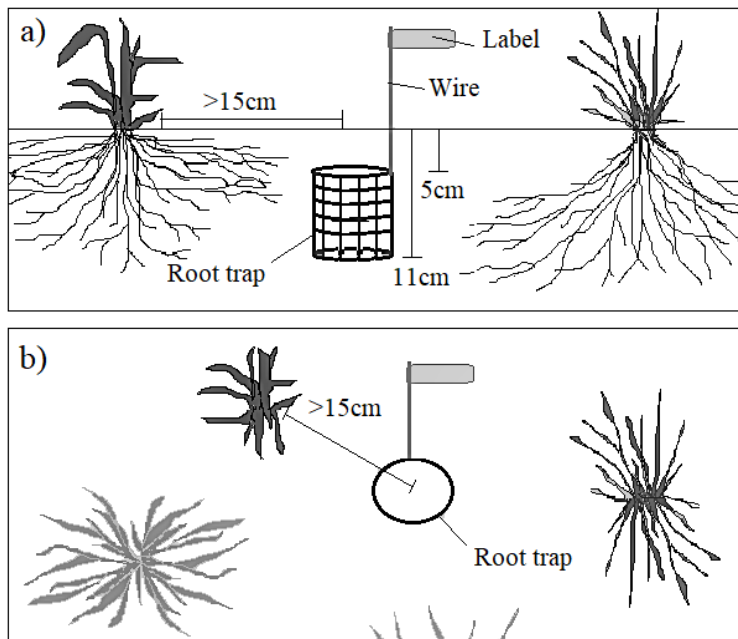
381 Fig 1. Diagrams of the experimental design including root trap and install location, in
382 horizontal (a) and zenithal (b) views. The radical systems in a) are illustrative.

383 Fig 2. Diversity (a) and root biomass (b) inside root traps with different treatments, after 4
384 and 6 months. Treatments are: C, control (sieved soil); N+, enriched (sieved soil +4 g N m⁻²).
385 Bars indicate main values and lines, standard errors. Lower case letters over bars
386 represent significant differences between treatments in each time. Capital letters represent
387 significant differences between times in the same treatment.

388 Fig 3. Belowground relative growth rates (RGRs) of grass species found inside roots traps
389 with different treatments, after 4 and 6 months. Species are: *Bromus pictus*, *Poa ligularis*
390 and *Pappostipa speciosa*. Treatments are: C, control (sieved soil); N+, enriched (sieved soil
391 + 4 g N m⁻²). Bars indicate main values and lines, standard errors. Lower case letters over
392 bars represent significant differences between treatments in each time. Capital letters
393 represent significant differences between times in the same treatment.

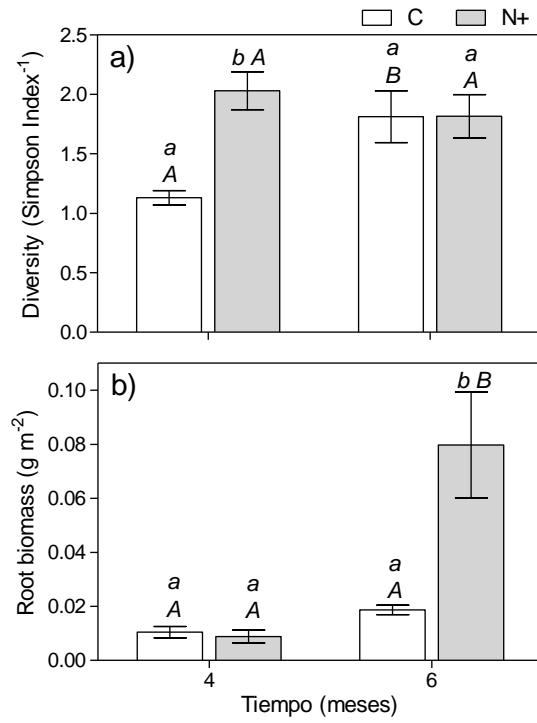
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395 Fig 1



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