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7 **CO<sub>2</sub> and inorganic N supply modify competition for N between co-occurring grass**  
8 **plants, tree seedlings and soil microorganisms**

9

10 Juliette M. G. Bloor\*, Audrey Niboyet, Paul W. Leadley and Laure Barthes

11

12 Université Paris-Sud, Laboratoire d'Ecologie, Systématique et Evolution, UMR CNRS 8079,

13 F-91405 Orsay Cedex, France

14

15

16 \* Corresponding author. Tel.: +33 4 73 62 44 25; fax: +33 4 73 62 44 57.

17 *E-mail address:* [juliette.bloor@clermont.inra.fr](mailto:juliette.bloor@clermont.inra.fr)

18 Present address: INRA, UR874-Grassland Ecosystem Research Unit, 234 Avenue du Brézet,

19 F-63100 Clermont Ferrand, France

20 **ABSTRACT**

21

22 Plant-plant and plant-soil interactions play a key role in determining plant community  
23 structure and ecosystem function. However, the effects of global change on the interplay  
24 between co-occurring plants and soil microbes in successional communities are poorly  
25 understood. In this study, we investigated competition for nitrogen (N) between soil  
26 microorganisms, grass plants and establishing tree seedlings under factorial carbon dioxide  
27 (CO<sub>2</sub>) and N treatments. *Fraxinus excelsior* seedlings were germinated in the presence or  
28 absence of grass competition (*Dactylis glomerata*) at low (380 μmol mol<sup>-1</sup>) or high (645 μmol  
29 mol<sup>-1</sup>) CO<sub>2</sub> and at two levels of N nutrition in a mesocosm experiment. Pulse <sup>15</sup>N labelling  
30 was used to examine N partitioning among plant and soil compartments. *Dactylis* exerted a  
31 strong negative effect on *Fraxinus* biomass, N capture and <sup>15</sup>N recovery irrespective of N and  
32 CO<sub>2</sub> treatment. In contrast, the presence of *Dactylis* had a positive effect on the microbial N  
33 pool. Plant and soil responses to N treatment were of a greater magnitude compared with  
34 responses to elevated CO<sub>2</sub>, but the pattern of *Fraxinus*- and microbial-N pool response to N  
35 and CO<sub>2</sub> varied depending on grass competition treatment. Within the *Dactylis* competition  
36 treatment, decreases in *Fraxinus* biomass in response to N were not mirrored by decreases in  
37 tree seedling N content, suggesting a shift from below- to above-ground competition. In the  
38 *Dactylis*-sown pots, <sup>15</sup>N recovery could be ranked *Dactylis* > microbial pool > *Fraxinus* in all  
39 N and CO<sub>2</sub> treatment combinations. Inequalities between *Fraxinus* and soil microorganisms in  
40 terms of <sup>15</sup>N recovery were exacerbated by N addition. Contrary to expectations, elevated CO<sub>2</sub>  
41 did not increase plant-microbe competition. Nevertheless, microbial <sup>15</sup>N recovery showed a  
42 small positive increase in the high CO<sub>2</sub> treatment. Overall, elevated CO<sub>2</sub> and N supply did not  
43 interact on plant/soil N partitioning. Our data suggest that the competitive balance between

44 establishing tree seedlings and grass plants in an undisturbed sward is relatively insensitive to  
45 CO<sub>2</sub> or N-induced modifications in N competition between plant and soil compartments.

46

47 *Keywords:* Global change; *Dactylis glomerata*, Elevated CO<sub>2</sub>; *Fraxinus excelsior*; Nitrogen  
48 addition; Nitrogen uptake; Plant-microbial competition; <sup>15</sup>N stable isotopes; Tree-grass  
49 interactions.

## 50 **1. Introduction**

51

52 Substantial evidence suggests that global climate change and increasing levels of  
53 atmospheric carbon dioxide (CO<sub>2</sub>) may lead to significant changes in biodiversity and plant  
54 species distributions, with cascading effects on ecosystem function and carbon sequestration  
55 (Sala et al., 2000; Schroter et al., 2005; Potvin et al., 2007). Consequently, accurate  
56 projections of species' responses to future environmental change are crucial for the  
57 assessment of global change-related risks to ecosystem services. However, despite the wealth  
58 of literature on plant responses to CO<sub>2</sub> enrichment (see reviews by Ceulemans and Mousseau,  
59 1994; Poorter and Perez-Soba, 2001; Korner, 2003; Ainsworth and Long, 2005), predicting  
60 the effects of future CO<sub>2</sub> levels on plant community structure and biodiversity dynamics  
61 remains a major challenge for ecologists (Poorter and Navas 2003; Bradley and Pregitzer  
62 2007).

63 Previous work has shown that the net effect of CO<sub>2</sub> enrichment on plant community  
64 structure may depend on complex interactions between atmospheric changes and climate  
65 (Niklaus and Korner, 2004), soil nutrient availability (Maestre et al., 2005) or management  
66 (Harmens et al., 2004). There is growing recognition that plant-plant interactions also play a  
67 key role in mediating the effects of environmental change on plant community structure;  
68 given that elevated CO<sub>2</sub> can have a direct effect on plant physiology and growth, intra- and  
69 interspecific variation in plant responses to CO<sub>2</sub> may modify the outcome of plant interactions  
70 (Brooker, 2006; Bradley and Pregitzer, 2007). In contrast, few studies have explored the  
71 influence of soil micro-organisms on plant-plant interactions and species assemblages in a  
72 changing CO<sub>2</sub> environment. Plant and microbial communities are clearly interdependent;  
73 plant production is often limited by quantities of nitrogen (N) made available during the  
74 decomposition of fresh litter and organic matter in soil. At the same time, the growth and

75 maintenance of soil micro-organisms is controlled by the quality and quantity of organic  
76 compounds entering the soil via root exudation and above/belowground litter production  
77 (Schmidt et al., 1997; Zak et al., 2000). Microbe-driven resource partitioning and soil  
78 community feedback could have important implications for plant species diversity at the local  
79 scale (Reynolds et al., 2003).

80         The intensity of plant-microbial competition for N is known to vary depending on  
81 microbial activity, resource availability or ecosystem productivity (Kaye and Hart 1997;  
82 Hodge et al., 2000; Dunn et al., 2006). In theory, high levels of CO<sub>2</sub> may increase root  
83 exudation and pools of available C, leading to increased microbial demand for N and  
84 modified competition for nutrients between plants and microorganisms (Diaz et al., 1993).  
85 Plant-microbe competition may further be amplified by enhanced plant growth, and thus N  
86 demand, under elevated CO<sub>2</sub> (Williams et al., 2001). In practice, however, findings from  
87 experimental systems are inconsistent. For example, results from <sup>15</sup>N pulse-labelling studies  
88 carried out in multispecies grassland systems under elevated CO<sub>2</sub> show no clear pattern in the  
89 relative N recovery between plants and soil microbes (reviewed by Barnard et al., 2006). One  
90 of the complications in determining CO<sub>2</sub> effects on plant-soil interactions is that CO<sub>2</sub>-induced  
91 changes in plant physiology and growth can modify water and nutrient availability as well as  
92 C supply, resulting in multiple effects that both enhance and suppress microbial processes  
93 (Hungate, 1999; Hu et al., 1999; Barnard et al., 2005). In addition, overall patterns of plant-  
94 soil partitioning may be confounded by different levels of competition for N exerted by soil  
95 microbes on individual plant species within a mixed species community (Harrison et al.,  
96 2008).

97         Here we use a model tree-grass system (the early successional tree, *Fraxinus excelsior*  
98 and the grass, *Dactylis glomerata*) to investigate the interactive effects of CO<sub>2</sub> and N supply  
99 on competition for nitrogen between soil microorganisms and co-occurring plants. Tree-grass

100 interactions are of particular interest because i) there is a global trend towards increased  
101 woody plant encroachment into grasslands, causing well-documented changes in nutrient  
102 cycling and ecosystem productivity (Dickie et al., 2007; Knapp et al., 2008), ii) the role of  
103 plant-soil interactions in successful woody plant establishment in grassland communities is  
104 unclear (Cheng and Bledsoe, 2004) and, iii) it has been suggested that grassland invasion by  
105 woody species may accelerate under elevated CO<sub>2</sub> (Bond et al., 2003; Zavaleta, 2006). In a  
106 previous paper we showed that increasing N inputs tend to reinforce the competitive  
107 inequality between *Fraxinus excelsior* seedlings growing with *Dactylis glomerata*, whereas  
108 elevated CO<sub>2</sub> may have indirect benefits for *Fraxinus* seedling establishment in experimental  
109 grass mesocosms (Bloor et al., 2008a). In the present study we measure total N pools and use  
110 a <sup>15</sup>N pulse-labelling approach to determine how CO<sub>2</sub> and N supply affect N partitioning  
111 among different plant and soil compartments. Use of <sup>15</sup>N tracer techniques can greatly  
112 increase the ability to detect small CO<sub>2</sub>-enhancements in plant and soil N retention against a  
113 background of large N stocks in soil organic matter and vegetation (Hu et al., 2006). We test  
114 the hypothesis that plant-microbe competition for N increases under elevated CO<sub>2</sub>. We also  
115 predict that newly-germinated *Fraxinus* seedlings with small biomass will be less able to  
116 compete for soil N with soil microorganisms than established *Dactylis* plants.

117

## 118 **2. Materials and methods**

119

### 120 *2.1. Study species*

121 The tree *Fraxinus excelsior* L. (common ash) is an important pioneer species which  
122 occurs widely across Europe; expansion of *F. excelsior* populations has been greatly favoured  
123 by agricultural abandonment in recent times (Kerr and Cahalan 2004). *Dactylis glomerata* L.  
124 (cocksfoot) is a vigorously growing, strongly-competitive perennial grass common to a wide

125 variety of habitats worldwide. Stratified tree seeds for this experiment were obtained from  
126 Forestart Ltd. (Hadnall, UK) and grass seed was obtained from Arbiotech (St Gilles, France).

127

## 128 2.2. *Experimental design*

129

130 Two plant competition treatments (*Fraxinus* seedlings alone, *Fraxinus* seedlings  
131 grown with *Dactylis*) were established in either ambient or elevated atmospheric CO<sub>2</sub>  
132 treatments under glasshouse conditions. In order to investigate the interactive effects of CO<sub>2</sub>  
133 and N supply on plant and microbial competition for nutrients, two N treatments (low/high)  
134 were crossed with each CO<sub>2</sub> and competition treatment (two competition treatments x two  
135 CO<sub>2</sub> treatments x two N treatments x 6 replicates = 48 pots).

136

## 137 2.3. *Soil, plant material and growing conditions*

138

139 Loamy topsoil was collected on the grounds of the University of Paris XI (Orsay,  
140 France) at the start of February 2006. The soil was sieved (1 cm), diluted with locally-  
141 obtained river sand in a 50:50 mix, and packed in deep PVC pots (20 x 15 x 40 cm) as  
142 described in Bloor et al. (2008a). The soil/ sand mix contained 0.23 g N kg<sup>-1</sup> soil, 2.46 g C  
143 kg<sup>-1</sup> soil and had a pH of 8.5. Experimental pots were assigned to one of twelve naturally-lit  
144 growth chambers (wooden frame and clear plastic walls, 65 x 65 x 100 cm high) set up inside  
145 a large glasshouse at the University of Paris XI and ventilated with air taken from outside the  
146 glasshouse. Six chambers were ventilated with ambient atmospheric CO<sub>2</sub> concentrations and  
147 the remaining six chambers were ventilated with elevated CO<sub>2</sub>; elevated atmospheric CO<sub>2</sub>  
148 concentrations were adjusted to a differential of 265 μmol mol<sup>-1</sup> ± 2% compared with ambient  
149 chambers by injection of pure CO<sub>2</sub> in each enriched chamber (see Bloor et al., 2008a for full



150 details). CO<sub>2</sub> concentrations were monitored throughout the experiment using a portable  
151 carbon dioxide analyser (Carbocap GM 70, Vaisala, Helsinki, Finland), indicating an average  
152 CO<sub>2</sub> concentration of 380 μmol mol<sup>-1</sup> (standard error = 6 μmol mol<sup>-1</sup>) and 645 μmol mol<sup>-1</sup>  
153 (standard error = 9 μmol mol<sup>-1</sup>) in the ambient and elevated CO<sub>2</sub> chambers respectively. Over  
154 the course of the experimental period, no temperature difference was observed between the  
155 ambient and elevated CO<sub>2</sub> chambers (mean daily temperatures based on hourly measurements  
156 ranged between 11.7°C and 23.1°C).

157 On 23<sup>rd</sup> February 2006, seeds of *Dactylis glomerata* were sown into half of the pots at  
158 a density of 2000 seeds m<sup>-2</sup>, leaving clear a central 5 x 5 cm zone per pot. One month later  
159 when the *Dactylis* seedlings had fully emerged, a high-nutrient treatment was established by  
160 supplementing half the pots with 200 ml of 7.9 mM NH<sub>4</sub>NO<sub>3</sub> solution at two-week intervals.  
161 Pots in the low-nutrient treatment received the equivalent amount of distilled water alone. The  
162 high nutrient treatment was intended to ensure that soil N was non-limiting; over the course of  
163 the experiment, high-nutrient pots received the equivalent of 100 kg N ha<sup>-1</sup>.

164 On 11 April 2006, evenly-sized germinating *Fraxinus excelsior* seeds were planted in  
165 the centre of all pots (radicle < 0.5 cm long, one seed per pot). At the time of *Fraxinus*  
166 planting, each grass-sown pot had 30-35 grass plants and average grass height ranged from  
167 18-25 cm in the different N/CO<sub>2</sub> treatments. *Fraxinus* seedlings and *Dactylis* plants were left  
168 to grow in the experimental treatments for ten weeks prior to <sup>15</sup>N labelling, and all pots were  
169 watered regularly.

170

#### 171 2.4. <sup>15</sup>N plant labelling

172

173 In June 2006, 5mg <sup>15</sup>N was injected per experimental pot in the form of ammonium  
174 solution (<sup>15</sup>NH<sub>4</sub>Cl at 99% <sup>15</sup>N, 0.01 M). A <sup>15</sup>NH<sub>4</sub><sup>+</sup> marker was chosen because ammonium is

175 first inorganic N form made available to plants and previous studies have shown that soil  
176 microbes compete effectively with plants for this resource (Hodge et al., 2000). In order to  
177 minimise the time between first and last pot injection and to ensure that all pots were exposed  
178 to the same  $^{15}\text{N}$  labelling period, labelling was carried out in two stages; half of the pots in  
179 each experimental treatment were injected on 12<sup>th</sup> June (harvested 13<sup>th</sup> June) whereas the  
180 remainder were injected on 13<sup>th</sup> June (harvested 14<sup>th</sup> June). One ml of solution was slowly  
181 injected (needle length 9 cm, diameter 0.9 mm) at 36 injection locations in the 0-10 cm soil  
182 layer of each pot following Barnard et al. (2006). Injection locations were arranged in a three  
183 dimensional grid with three injection depths (1.5, 5 and 7.5 cm) to maximise  $^{15}\text{N}$  mixing with  
184 the native  $\text{NH}_4^+$  pool. Pots were not watered after injection to minimize leaching loss.

185

## 186 2.5. Harvest and analyses

187

188 Plants and soil were harvested 24 h after  $^{15}\text{N}$  injection as preliminary work on  
189 *Fraxinus* seedlings and *Dactylis* indicated rapid plant uptake of  $^{15}\text{NH}_4^+$ . Pots were harvested  
190 in the order in which they were injected. At harvest, aboveground plant material was clipped  
191 at the soil surface and separated into *Dactylis* leaves, *Fraxinus* leaves+cotyledons and  
192 *Fraxinus* stems. Roots were separated from the soil manually and washed; *Fraxinus* roots  
193 could be clearly distinguished from grass roots and were carefully disentangled from the  
194 *Dactylis* belowground material. All plant material was oven-dried (60°C for at least 72 h),  
195 weighed, ground and analysed for total N and atom%  $^{15}\text{N}$  (mass spectrometers, CNRS,  
196 Service central d'Analyse, Solaize, France).

197 Soil in the 0-10 cm layer of each pot was separated from the deeper soil layers, well-  
198 mixed and sieved (2 mm). Soil and microbial properties in the top 10 cm of grass-sown pots  
199 have been shown to respond more strongly to experimental treatments than those of deeper

200 soil layers over short-term studies (Barnard et al., 2004). Analysis of the 0-10 cm soil layer  
201 rather than mixed soil from the entire profile also ensured an undiluted, more detectable  
202 microbial  $^{15}\text{N}$  signal. Microbial biomass N,  $^{15}\text{N}$  and C were measured on 5 g subsamples of  
203 the sieved 0-10 cm soil layer using the chloroform fumigation-incubation method (Brookes et  
204 al., 1985). Soil samples were extracted with 20 mL of 30 mM  $\text{K}_2\text{SO}_4$  solution following  
205 Fontaine et al. (2004). Microbial C ( $C_{\text{mic}}$ ) was calculated as the difference in total C extracted  
206 in fumigated and unfumigated soils, with  $k_{\text{C}} = 0.45$  as the adjustment factor (Wu et al., 1990).  
207 Microbial N ( $N_{\text{mic}}$ ) was calculated as the difference in total N extracted in fumigated and  
208 unfumigated soils, with  $k_{\text{N}} = 0.54$  as the adjustment factor (Brookes et al., 1985). Soil  
209 extractable N and  $^{15}\text{N}$  were measured in the unfumigated soil extracts following Barnard et al.  
210 (2006). Correction for the natural abundance of  $^{15}\text{N}$  was based on the atomic ratio of  
211 atmospheric N. Additional soil sub-samples were oven-dried (105°C, 24 h) to determine soil  
212 water content per pot.

213 All soil and microbial extracts were analysed using continuous-flow gas isotope-ratio  
214 mass spectrometry (BIOMCO, INRA-INAPG, Thiverval Grignon, France). Values for  $\delta^{15}\text{N}$  in  
215 microbial extracts in the pots with *Fraxinus* alone were extremely heterogeneous and could  
216 not be used for calculations of  $^{15}\text{N}$  uptake by the microbial biomass for this treatment.  
217 Consequently, microbial  $^{15}\text{N}$  uptake data is only reported for the *Dactylis-Fraxinus*  
218 competition treatment.

219

## 220 2.6. Statistical analysis

221

222 The experiment was analysed as a split-plot design following Zar (1999), with  $\text{CO}_2$   
223 treatment as the whole-plot factor, fixed and among growth chambers, and both N and  
224 competition treatments as fixed sub-plot factors within growth chambers. All statistical

225 analysis was carried out using the PROC MIXED procedure in SAS 9.1. (SAS Institute Inc.,  
226 Cary, NC, USA). Where necessary, data were log transformed prior to analysis to conform  
227 with assumptions of normality and homogeneity of variances.

228

### 229 **3. Results**

230

#### 231 *3.1. Effects of competition, N and CO<sub>2</sub> on plant and microbial N pools*

232

233         Shoot and root biomass of *Dactylis* plants showed a significant increase in response to  
234 N addition (Table 1). The biomass response to increased N supply of aboveground organs was  
235 stronger than that of roots, particularly under ambient CO<sub>2</sub> (Table 1). Under high N supply,  
236 *Dactylis* root and shoot biomass showed a positive response to elevated CO<sub>2</sub>; the magnitude  
237 of dry mass increase to CO<sub>2</sub> under high N was greater in roots than in shoots (+47% and  
238 +20% respectively). However, elevated CO<sub>2</sub> had little effect on either root or shoot biomass  
239 under low N conditions (significant N x CO<sub>2</sub> interaction in both cases). Overall, increasing N  
240 supply was associated with a significant increase in *Dactylis* above- and belowground N  
241 content (Table 1). In contrast, *Dactylis* root N concentration had a significant negative  
242 response to increasing N. Moreover, *Dactylis* shoot N concentration showed no significant  
243 response to N supply (Table 1). Elevated CO<sub>2</sub> was associated with a decrease in both shoot N  
244 content and concentration but had no significant effect on root nutritional status (Table 1).

245         *Fraxinus* seedlings were generally more sensitive to plant competition treatment than  
246 to N supply or CO<sub>2</sub> level (Table 2). Presence of *Dactylis* had a strong negative effect on  
247 *Fraxinus* seedling biomass and N content, irrespective of N supply and CO<sub>2</sub> treatment (Fig.  
248 1). Furthermore, grass competition modified *Fraxinus* responses to N and CO<sub>2</sub> (significant  
249 competition x N and competition x CO<sub>2</sub> interactions, Table 2). In the absence of grass

250 competition, increasing N supply was associated with a significant increase in *Fraxinus*  
251 seedling shoot mass, N content and shoot N concentration (Fig. 1, Table 2). Increasing N  
252 supply had no significant effect on *Fraxinus* root biomass or N content, but there was a  
253 tendency towards greater root mass in the high N treatments and an increase in root N content  
254 under high N, high CO<sub>2</sub> conditions for seedlings grown alone (Fig. 1, Table 2). In the  
255 presence of grass competition, both *Fraxinus* shoot and root mass showed a significant  
256 decrease in response to increasing N (Fig. 1, Table 2). Neither above- nor belowground N  
257 content showed any significant response to N supply for *Fraxinus* seedlings grown with grass,  
258 but tissue N concentrations were significantly greater in the high N treatment (Fig. 1). On  
259 average, the shoot N concentration of *Fraxinus* seedlings grown with *Dactylis* under high N  
260 conditions was similar to that of *Fraxinus* seedlings grown alone. Patterns of variation in  
261 *Fraxinus* root and shoot N concentrations were further modified by CO<sub>2</sub> treatment since tissue  
262 N concentration showed a significant decrease in high N, high CO<sub>2</sub> conditions for seedlings  
263 grown with grass (Fig. 1).

264         The presence of *Dactylis* had contrasting effects on soil microbes and the soil  
265 extractable N pool (Fig. 2). Overall, grass competition treatment had a positive effect on C<sub>mic</sub>  
266 and N<sub>mic</sub> (F<sub>1,24</sub> = 22.35 and 17.73 respectively, *P* < 0.001). Furthermore, both C<sub>mic</sub> and N<sub>mic</sub>  
267 showed a significant positive correlation with *Dactylis* dry mass (all N and CO<sub>2</sub> treatments  
268 combined, Pearson's R = 0.70, *P* < 0.001 and 0.48, *P* < 0.05 respectively). At the same time,  
269 presence of *Dactylis* was associated with a significant decrease in soil extractable N (Fig. 2,  
270 F<sub>1,24</sub> = 62.35, *P* < 0.001). As with *Fraxinus* seedling traits, microbial and soil N responses to  
271 N and CO<sub>2</sub> treatment varied depending on plant competition treatment (Fig. 2). In the absence  
272 of *Dactylis*, neither N supply nor CO<sub>2</sub> had any significant effect on C<sub>mic</sub>, N<sub>mic</sub> or the soil  
273 extractable N pool (*P* > 0.1 in all cases). However, in pots with *Fraxinus* and *Dactylis* grown  
274 together, C<sub>mic</sub> showed a significant increase in response to increasing N and CO<sub>2</sub> (Fig 2., F<sub>1,9</sub> =

275 10.98 and  $F_{1,10} = 6.68$  respectively,  $P < 0.05$  in both cases). This pattern was mirrored by  $N_{mic}$   
276 responses to increasing N supply and elevated  $CO_2$  (Fig 2.,  $F_{1,9} = 15.19$ ,  $P < 0.01$  and  $F_{1,10}$   
277  $= 10.25$ ,  $P < 0.01$  respectively). In contrast, soil extractable N showed a marginally significant  
278 decrease in response to elevated  $CO_2$  (Fig 2.,  $F_{1,10} = 3.67$ ,  $P < 0.09$ ). Microbial C:N ratios were  
279 not affected by N or  $CO_2$  in any plant treatments (data not shown). Moreover,  $C_{mic}$  and  $N_{mic}$   
280 showed no correlation with *Fraxinus* dry mass across N and  $CO_2$  treatments.

281

### 282 3.2. Effects of competition, N and $CO_2$ on $^{15}N$ uptake by plants

283

284 *Dactylis* showed extremely rapid uptake of  $^{15}N$ ; after only 24 h, recovery of  $^{15}N$  in  
285 *Dactylis* tissue averaged 84% of the total amount of  $^{15}N$  added across all N and  $CO_2$   
286 treatments (Table 3). Recovery of  $^{15}N$  in *Fraxinus* seedlings was considerably more limited;  
287 in the absence of grass competition, *Fraxinus*  $^{15}N$  uptake represented 15-20% of the total  
288 amount of  $^{15}N$  recovered (Table 3). Species differences in  $^{15}N$  uptake reflected differences in  
289 biomass rather than uptake efficiency since *Dactylis* exhibited significantly lower  $^{15}N$  uptake  
290 on a root mass basis compared to *Fraxinus* seedlings grown alone (Table 3).

291 Presence of *Dactylis* had a significant negative effect on total *Fraxinus*  $^{15}N$  uptake and  
292 *Fraxinus*  $^{15}N$  uptake on a root dry mass basis ( $F_{1,25} = 126.65$  and  $110.78$  respectively,  $P$   
293  $< 0.001$ , Table 3). The magnitude of the grass-induced reduction in total *Fraxinus*  $^{15}N$  uptake  
294 varied from 91-98% across N and  $CO_2$  treatment combinations, and was significantly greater  
295 under high N conditions ( $F_{1,9} = 10.53$ ,  $P < 0.001$ ).

296 Plant  $^{15}N$  uptake showed significant responses to N for both *Dactylis* and *Fraxinus*,  
297 but the effects of N treatment varied depending on the species considered (Table 3).  
298 Increasing N supply was associated with an increase in total *Dactylis*  $^{15}N$  uptake although  
299 *Dactylis*  $^{15}N$  uptake expressed on a root dry mass basis showed a significant decrease with N

300 addition (Table 3). In contrast, total *Fraxinus* <sup>15</sup>N uptake showed a negative response to N  
301 addition, irrespective of the level of competition experienced by *Fraxinus* seedlings. Patterns  
302 of total <sup>15</sup>N uptake were mirrored by <sup>15</sup>N uptake on a root dry mass basis for *Fraxinus*  
303 seedlings grown alone. However, *Fraxinus* seedlings showed no significant response to N  
304 supply in terms of <sup>15</sup>N uptake on a root mass basis in the presence of grass competition.  
305 Elevated CO<sub>2</sub> had no effect on <sup>15</sup>N uptake for either *Dactylis* or *Fraxinus* in any N or  
306 competition treatment (Table 3).

307 In general, *Dactylis* allocated more <sup>15</sup>N to shoots rather than roots compared to  
308 *Fraxinus* seedlings across treatments (Table 3). Neither N supply nor CO<sub>2</sub> had a significant  
309 effect on the <sup>15</sup>N distribution pattern (roots versus shoots) of *Dactylis* plants or *Fraxinus*  
310 seedlings grown alone (Table 3). Patterns of <sup>15</sup>N distribution in *Fraxinus* seedlings grown  
311 with grass varied depending on N and CO<sub>2</sub> treatment; less <sup>15</sup>N accumulated in roots in  
312 response to an increase in N supply while more <sup>15</sup>N accumulated in roots under elevated CO<sub>2</sub>  
313 (Table 3).

314

### 315 3.3. <sup>15</sup>N distribution among plant and soil pools in the *Dactylis*-*Fraxinus* competition 316 treatment

317

318 Total recovery of <sup>15</sup>N in plant and soil pools averaged 87% across treatments and  
319 showed a significant increase in response to N supply ( $F_{1,9} = 15.41$ ,  $P < 0.01$ ). This increase in  
320 <sup>15</sup>N recovery was due to an increase in *Dactylis* <sup>15</sup>N uptake (previously described); N  
321 treatment had no significant effect on microbial <sup>15</sup>N uptake and was associated with a  
322 decrease in <sup>15</sup>N recovery by both *Fraxinus* seedlings and the soil extractable N pool (Fig. 3).  
323 Elevated CO<sub>2</sub> did not significantly affect total <sup>15</sup>N recovery but was associated with a  
324 marginally significant increase in microbial <sup>15</sup>N uptake ( $F_{1,10} = 4.84$ ,  $P = 0.054$ , Fig. 3).

325 Overall,  $^{15}\text{N}$  uptake could be ranked *Dactylis* > microbial biomass > *Fraxinus* seedlings (Fig.  
326 3). Recovery of  $^{15}\text{N}$  by the microbial pool was fivefold that of *Fraxinus* under low N  
327 conditions and roughly 15 times greater under high N conditions. Relative differences in  $^{15}\text{N}$   
328 uptake between *Fraxinus* seedlings and the microbial pool were unaffected by elevated  $\text{CO}_2$ .  
329

## 330 **4. Discussion**

331

### 332 *4.1. Effects of N and CO<sub>2</sub> on tree-grass competition for N*

333

334 Seedling establishment is the major bottleneck for plant regeneration and successful  
335 tree recruitment in grasslands (Van Auken and Bush, 1997; Wilson, 1998). Nevertheless, little  
336 information is available on the growth responses of newly-germinated temperate tree  
337 seedlings to interactive biotic and abiotic factors. Competition for N is of particular interest  
338 since it is the nutrient most commonly limiting to plant growth in temperate ecosystems  
339 (Vitousek and Howarth, 1991). In the present study, *Dactylis* dominated *Fraxinus* in terms of  
340 biomass, N capture and  $^{15}\text{N}$  recovery in all treatment combinations. Furthermore, *Dactylis*  
341 was associated with a significant reduction in *Fraxinus* N content and  $^{15}\text{N}$  uptake, in  
342 agreement with previous studies on tree-grass interactions (Coll et al., 2004; Cheng and  
343 Bledsoe, 2004).

344 Several lines of evidence lead us to believe that our results indicate competition for  
345 soil N between *Dactylis* and *Fraxinus*. Firstly,  $^{15}\text{N}$  labelling demonstrates mutual N resource  
346 use between the two plant species. Secondly, *Fraxinus* shows N limitation (*sensu* Kaye and  
347 Hart, 1997) in low N conditions; we found increased N absorption and growth in *Fraxinus*  
348 seedlings subjected to N addition in the absence of *Dactylis*. Finally, field and greenhouse  
349 studies suggest that competition between woody seedlings and herbaceous plants is generally



350 driven by belowground, rather than aboveground interactions (Aerts et al., 1991; Van Auken  
351 and Bush, 1997; Bloor et al., 2008b). In this study, belowground interactions were governed  
352 by competition for soil nutrients since regular watering ensured that plants were not water-  
353 limited. However, we do not rule out the possibility that competition for light contributed to  
354 the biomass reductions observed in *Fraxinus* seedlings growing with *Dactylis*, particularly  
355 under high N conditions. Morphological responses reported for *Dactylis* and *Fraxinus* in a  
356 companion paper (Bloor et al., 2008a) suggest that a shift from root to shoot competition may  
357 exacerbate the competitive intensity experienced by young tree seedlings growing with grass  
358 under high N conditions.

359 Different plant species are known to differ in their ability to use both inorganic and  
360 organic forms of N (Weigelt et al., 2005; Dunn et al., 2006), and shifts in short-term resource  
361 use have been demonstrated in response to competition from neighbouring plants (Miller et  
362 al., 2007). In view of the strong negative effects imposed by *Dactylis* on *Fraxinus* seedlings,  
363 it seems unlikely that flexibility in *Fraxinus* N use plays a significant role in buffering the  
364 effects of grass neighbours. The strong competitive effects of *Dactylis* must in part reflect the  
365 fast growth rates and high biomass of this species compared to the young *Fraxinus* seedlings;  
366 it has long been suggested that rapidly growing species have a greater capacity to acquire  
367 nutrients compared with slower-growing species in a given habitat (Grime, 1979). Of course,  
368 plant nutrient uptake may be driven by root morphology, physiology and/or root turnover as  
369 well as root mass (Bassirirad et al., 2001; Raynaud and Leadley, 2005). Whilst we did not  
370 measure *Dactylis* root morphology in this study, the high root density and finely-branched  
371 root architecture reported elsewhere for *D.glomerata* plants (Ryser and Lambers, 1995)  
372 undoubtedly added to the competitive dominance of *Dactylis* over *Fraxinus*.

373 Overall, we found that the *Dactylis* N pool was significantly more responsive to N  
374 addition compared with the *Fraxinus* N pool. These data corroborate previous work which

375 indicates that increasing N supply amplifies the competitive dominance of *Dactylis* in tree-  
376 grass mixtures (Bloor et al., 2008a). However, our results also indicate that competition-  
377 induced decreases in *Fraxinus* biomass under high N conditions are not mirrored by decreases  
378 in seedling N content or tissue concentration. The uncoupling of *Fraxinus* biomass responses  
379 and N content suggests that competition for soil N is less limiting to growth under high N  
380 conditions, and is consistent with the idea of a shift from root to shoot competition along  
381 nutrient gradients (Tilman, 1990; Cahill, 1999). This agrees with the increased biomass  
382 investment in leaves and higher specific leaf area observed in response to N addition for  
383 *Fraxinus* seedlings growing with *Dactylis* (Bloor et al., 2008a). The ability of *Fraxinus* to  
384 maintain a constant seedling N content in the face of increased shoot competition (and  
385 inhibited seedling growth) may also indicate interactions between *Dactylis* root and shoot  
386 competitive effects under high N conditions (Cahill, 1999); N uptake capacity and seedling N  
387 content are expected to be proportional to *Fraxinus* seedling size if *Dactylis* root and shoot  
388 competition are independent.

389         In the present study, elevated CO<sub>2</sub> had a significant negative effect on shoot N  
390 concentrations, irrespective of plant species and competition treatment, but we found no effect  
391 of CO<sub>2</sub> enrichment on total plant N content or <sup>15</sup>N uptake for either *Dactylis* or *Fraxinus*.  
392 Decreases in plant nutrient concentration in response to short-term CO<sub>2</sub> enrichment are well  
393 documented (Ceulemans and Mousseau, 1994; Korner, 2003; Reich et al., 2006), and may be  
394 partially offset by concomittant increases in N use efficiency. In contrast, patterns of N uptake  
395 under elevated CO<sub>2</sub> are less predictable and may vary depending on nutrient form, plant  
396 species and growing conditions (Luo et al., 1999). Despite similar responses to CO<sub>2</sub> treatment  
397 in terms of N uptake and plant nutrient status, *Fraxinus* and *Dactylis* displayed clear  
398 differences in biomass response to elevated CO<sub>2</sub>; unlike *Fraxinus*, *Dactylis* biomass showed  
399 significant CO<sub>2</sub> x N interactions and a positive biomass response to CO<sub>2</sub> enrichment in the

400 high N treatment alone, suggesting N limitation effects under low N conditions. Positive  
401 responses of biomass to elevated CO<sub>2</sub> are expected to become progressively weaker in low N  
402 conditions, particularly for fast-growing species with high N demand (Arp et al., 1998; Lee et  
403 al., 2001; Hu et al., 2006). Given the importance of plant size for resource acquisition and  
404 competitive ability (Weiner, 1990), CO<sub>2</sub> enrichment may therefore reduce competitive  
405 differences between woody seedlings and herbaceous species in the longer-term.

406

#### 407 4.2. Plant-microbial N partitioning

408

409 Soil microorganisms are generally thought to be strong short-term competitors for soil  
410 N due to their high surface area to volume ratio, wide-spread spatial distribution in the soil  
411 and rapid growth rates compared with plant roots (Kaye and Hart, 1997; Hodge et al., 2000).  
412 However, previous studies which have used <sup>15</sup>N labelling techniques to examine short-term  
413 inorganic N partitioning between plants and microbes have generated conflicting results  
414 (Hungate et al., 1996; Burger and Jackson, 2004; Cheng and Bledsoe, 2004; Barnard et al.,  
415 2006; Harrison et al., 2008). We found that the outcome of plant-soil competition for <sup>15</sup>N-  
416 NH<sub>4</sub><sup>+</sup> was plant species-dependent; *Dactylis* had a consistently greater capacity for <sup>15</sup>N uptake  
417 compared with the soil microbial pool, but microorganisms were more efficient in <sup>15</sup>N capture  
418 than *Fraxinus* seedlings. These results support the hypothesis that small, germinating  
419 *Fraxinus* seedlings are less able to compete with soil microorganisms for N compared to  
420 large, established *Dactylis* plants. Competitive inequalities between microorganisms and  
421 plants may become less pronounced over longer time periods as a result of rapid microbial  
422 turnover which releases N to the soil, combined with the capacity of plants to sequester N for  
423 longer (Hodge et al., 2000; Barnard et al., 2006). The balance of plant-microbe N competition  
424 may also change due to temporal asynchrony in plant and microbial N-limitation (Hodge et

425 al., 2000; Augustine and McNaughton, 2004). Nonetheless, we found that patterns in  $^{15}\text{N}$   
426 uptake in the different plant and microbial compartments were broadly similar to  
427 measurements of total N content after ten weeks growth. This confirms that short-term  
428 competitive interactions may have longer-lasting effects on plant/ microbial resource  
429 acquisition (Miller et al., 2007).

430 *Dactylis*  $^{15}\text{N}$  recovery measured in this study was high compared to values reported for  
431 grass species elsewhere (Hungate et al., 1996; Hu et al., 2001; Weigelt et al., 2005; Barnard et  
432 al., 2006), whereas microbial uptake was somewhat lower than expected. High *Dactylis*  $^{15}\text{N}$   
433 uptake must reflect the large root biomass and rapid plant growth under favorable growing  
434 conditions at the time of labelling; plant N uptake is driven by demand which fluctuates over  
435 the course of the growing season depending on ontogenetic or phenological state (Bassirirad  
436 et al., 2001). *Dactylis* growth and  $^{15}\text{N}$  uptake capacity may also have been favoured by the  
437 improved drainage and aeration associated with a soil/sand mix rather than *in situ* grassland  
438 soils. In contrast, the high sand content (and low microbial C) in our growing medium might  
439 partly explain the low microbial  $^{15}\text{N}$  uptake observed here compared with true grassland soils  
440 (Hungate et al., 1996; Hodge et al., 2000). Despite the high  $^{15}\text{N}$  recovery in *Dactylis*, we  
441 found no evidence for a trade-off in N acquisition between *Dactylis* and soil microorganisms  
442 i.e. N uptake in *Dactylis* occurring at the expense of microbial N acquisition. Such a lack of  
443 trade-off between  $^{15}\text{N}$  uptake in plants and microorganisms corroborates the results of other  
444 studies on grass species (Hungate et al., 1996; Barnard et al., 2006). Furthermore, the  
445 microbial carbon (C) pool showed a significant positive relationship with *Dactylis* biomass  
446 across treatments in the present study. These results are consistent with the idea that microbial  
447 activity is C limited and benefits from greater root C inputs to the soil at high plant biomass  
448 (Williams et al., 2001). Reduced microbial N uptake as a result of C limitation may have

449 contributed to the inequality in  $^{15}\text{N}$  acquisition observed here between *Dactylis* and soil  
450 microorganisms.

451 In line with Diaz et al. (1993), we predicted that elevated  $\text{CO}_2$  would lead to increased  
452 microbial demand for N and constraints on plant N acquisition. Increases in microbial N and  
453  $^{15}\text{N}$  uptake in response to  $\text{CO}_2$  in the presence of *Dactylis* provide support for the idea of  
454 increased microbial N demand under  $\text{CO}_2$  enrichment. However, changes in microbial N  
455 acquisition under elevated  $\text{CO}_2$  were unrelated to plant N or  $^{15}\text{N}$  uptake, irrespective of plant  
456 species. This is perhaps unsurprising given the discrepancy between *Dactylis* and microbial N  
457 uptake in the present study; microbial N acquisition was simply too small to drive plant N  
458 uptake. The discrepancy between plant and microbial responses could also reflect  $\text{CO}_2$ -  
459 induced changes in the balance between N immobilisation and N mineralisation (Hungate,  
460 1999). Although it is clear from the literature that elevated  $\text{CO}_2$  increases C substrate  
461 availability and microbial metabolism, the effect of such increases in substrate availability on  
462 the rate at which N becomes available to plants remains poorly understood (Zak et al., 2000).

463

## 464 **5. Conclusion**

465 Our results show that grass-induced competition for N has a strong negative influence  
466 on the early tree seedling establishment phase, particularly under low N conditions. In  
467 contrast, plant-soil competition for N appears to have limited effects on early tree seedling  
468 growth and nutritional status in habitats dominated by actively growing, aggressive grass  
469 plants. Under high N conditions, decreases in tree-grass competition for N do not translate  
470 into increased tree seedling biomass, suggesting a switch from below- to above-ground plant  
471 competition. Furthermore,  $\text{CO}_2$  enrichment appears to have little effect on the interplay  
472 between microorganisms, tree seedlings and highly-competitive grass plants. Taken together,  
473 our data suggest that the competitive balance between establishing tree seedlings and grass

474 plants in an undisturbed sward is relatively insensitive to CO<sub>2</sub> or N-induced modifications in  
475 N competition between plant and soil compartments. Additional experiments are required to  
476 determine under which sward conditions (height, density, gap size) young tree seedlings may  
477 benefit from the effects of global change on plant-soil interactions. Future research should  
478 also address how responses of germinating seedlings translate into longer term seedling  
479 growth and survival under elevated atmospheric CO<sub>2</sub>.

480

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482

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488

## 489 **References**

490

491 Aerts, R., Boot, R.G.A., van der Aart, P.J.M., 1991. The relation between above- and  
492 belowground biomass allocation patterns and competitive ability. *Oecologia* 87, 551-  
493 559.

494

495 Ainsworth, E.A., Long, S.P., 2005. What have we learned from 15 years of free-air CO<sub>2</sub>  
496 enrichment (FACE)? A meta-analytic review of the responses photosynthesis, canopy  
497 properties and plant production to rising CO<sub>2</sub>. *New Phytologist* 165, 351-371.

498

499 Arp, W.J., Van Miierlo, J.E.M., Berendse, F., Snijders, W., 1998. Interactions between  
500 elevated CO<sub>2</sub> concentration, nitrogen and water: effects on growth and water use of six  
501 perennial plant species. *Plant, Cell and Environment* 21, 1-11.

502

503 Augustine, D.J., McNaughton, S.J., 2004. Temporal asynchrony in soil nutrient dynamics and  
504 plant production in a semiarid ecosystem. *Ecosystems* 7, 829-840.

505

506 Barnard, R., Barthes, L., Le Roux, X., Leadley, P.W., 2004. Dynamics of nitrifying activities,  
507 denitrifying activities and nitrogen in grassland mesocosms as altered by elevated CO<sub>2</sub>.  
508 *New Phytologist* 162, 365-376.

- 509 Barnard, R., Leadley, P.W., Lensi, R., Barthes, L., 2005. Plant, soil microbial and soil  
510 inorganic nitrogen responses to elevated CO<sub>2</sub>: a study in microcosms of *Holcus lanatus*.  
511 Acta Oecologia 27, 171-178.  
512
- 513 Barnard, R., Barthes, L., Leadley, P.W., 2006. Short-term uptake of <sup>15</sup>N by a grass and soil  
514 micro-organisms after long-term exposure to elevated CO<sub>2</sub>. Plant and Soil 280, 91-99.  
515
- 516 Bassirirad, H., Gutschick, V.P., Lussenhop, J., 2001. Root system adjustments: regulation of  
517 plant nutrient uptake and growth responses to elevated CO<sub>2</sub>. Oecologia 126, 305-320.  
518
- 519 Bloor, J.M.G., Barthes, L., Leadley, P.W., 2008a. Effects of elevated CO<sub>2</sub> and N on tree-grass  
520 interactions: an experimental test using *Fraxinus excelsior* and *Dactylis glomerata*.  
521 Functional Ecology 22, 537-546.  
522
- 523 Bloor, J.M.G., Leadley, P.W., Barthes, L., 2008b. Responses of *Fraxinus excelsior* seedlings  
524 to grass-induced above- and below-ground competition. Plant Ecology 194, 293-304.  
525
- 526 Bond, W.J., Midgley, G.F., Woodward, F.I., 2003. The importance of low atmospheric CO<sub>2</sub>  
527 and fire in promoting the spread of grasslands and savannas. Global Change Biology 9,  
528 973-982.  
529
- 530 Bradley, K.L., Pregitzer, K.S., 2007. Ecosystem assembly and terrestrial carbon balance under  
531 elevated CO<sub>2</sub>. Trends in Ecology and Evolution 22, 538-547.  
532
- 533 Brooker, R.W., 2006. Plant-plant interactions and environmental change. New Phytologist  
534 171, 271-284.  
535
- 536 Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and  
537 the release of soil nitrogen: a rapid direct extraction method to measure microbial  
538 biomass nitrogen in soil. Soil Biology & Biochemistry 17, 837-842.  
539
- 540 Burger, M., Jackson, L.E., 2004. Plant and microbial nitrogen use and turnover, rapid  
541 conversion of nitrate to ammonium in soil with plant roots. Plant and Soil 266, 289-301.  
542
- 543 Cahill, J.F., 1999. Fertilization effects on interactions between above- and belowground  
544 competition in an old field. Ecology 80,466-480.  
545
- 546 Ceulemans, R., Mousseau, M., 1994. Effects of elevated atmospheric CO<sub>2</sub> on woody plants.  
547 New Phytologist 127, 425-446.  
548
- 549 Cheng, X., Bledsoe, C.S., 2004. Competition for inorganic and organic N by blue oak  
550 (*Quercus douglasii*) seedlings, an annual grass and soil microorganisms in a pot study.  
551 Soil Biology & Biochemistry 36, 135-144.  
552
- 553 Coll, L., Balandier, P., Picon-Cochard, C., 2004. Morphological and physiological responses  
554 of beech (*Fagus sylvatica*) seedlings to grass-induced belowground competition. Tree  
555 Physiology 24, 45-54.  
556
- 557 Diaz, S., Grime, J.P., Harris, J., McPherson, E., 1993. Evidence of a feedback mechanism  
558 limiting plant response to elevated carbon dioxide. Nature 364, 616-617.

- 559 Dickie, I.A., Schnitzer, S.A., Reich, P.B., Hobbie, S.E., 2007. Is oak establishment in old-  
560 fields and savanna openings context dependent? *Journal of Ecology* 95, 309-320.  
561
- 562 Dunn, R.M., Mikola, J., Bol, R., Bardgett, R.D., 2006. Influence of microbial activity on plant-  
563 microbial competition for organic and inorganic nitrogen. *Plant and Soil* 289, 321-334.  
564
- 565 Fontaine, S., Bardoux, G., Benest, D., Verdier, B., Mariotti, A., Abbadie, L., 2004.  
566 Mechanisms of the priming effect in a savannah soil amended with cellulose. *Soil*  
567 *Science Society of America Journal* 68, 125-131.  
568
- 569 Grime, J.P., 1979. *Plant Strategies and Vegetation Processes*. Wiley, Chichester, UK, 222 pp.  
570
- 571 Harmens, H., Williams, P.D., Peters, S.L., Bambrick, M.T., Hopkins, A., Ashenden, T.W.,  
572 2004. Impacts of elevated atmospheric CO<sub>2</sub> and temperature on plant community  
573 structure of a temperate grassland are modulated by cutting frequency. *Grass and Forage*  
574 *Science* 59, 144-156.  
575
- 576 Harrison, K.A., Bol, R., Bardgett, R.D., 2008. Do plant species with different growth  
577 strategies vary in their ability to compete with soil microbes for chemical forms of  
578 nitrogen? *Soil Biology & Biochemistry* 40, 228-237.  
579
- 580 Hodge, A., Robinson, D., Fitter, A.H., 2000. Are microorganisms more effective than plants  
581 at competing for nitrogen? *Trends in Plant Science* 5, 304-308.  
582
- 583 Hu, S., Firestone, M.K., Chapin, F.S., 1999. Soil microbial feedbacks to atmospheric CO<sub>2</sub>  
584 enrichment. *Trends in Ecology and Evolution* 14, 433-437.  
585
- 586 Hu, S., Chapin, F.S., Firestone, M.K., Field, C.B., Chiariello, N.R., 2001. Nitrogen limitation  
587 of microbial decomposition in a grassland under elevated CO<sub>2</sub>. *Nature* 409, 188-191.  
588
- 589 Hu, S., Tu, C., Chen, X., Gruver, J.B., 2006. Progressive N limitation of plant response to  
590 elevated CO<sub>2</sub>: a microbiological perspective. *Plant and Soil* 289, 47-58.  
591
- 592 Hungate, B.A., 1999. Ecosystem responses to rising atmospheric CO<sub>2</sub>: feedbacks through the  
593 nitrogen cycle. In: Luo, Y., Mooney, H.A. (Eds.), *Carbon Dioxide and Environmental*  
594 *Stress*. Academic Press, San Diego, USA, pp. 265-285.  
595
- 596 Hungate, B.A., Canadell, J., Chapin, F.S. 1996. Plant species mediate changes in soil  
597 microbial N in response to elevated CO<sub>2</sub>. *Ecology* 77, 2505-2515.  
598
- 599 Kaye, J.P., Hart, S.C., 1997. Competition for nitrogen between plants and soil  
600 microorganisms. *Trends in Ecology and Evolution* 12, 139-143.  
601
- 602 Kerr, G., Cahalan, C., 2004. A review of the site factors affecting the early growth of ash  
603 (*Fraxinus excelsior* L.). *Forest Ecology and Management* 188, 225-234.  
604
- 605 Knapp, A.K., Briggs, J.M., Collins, S.L., Archer, S.R., Bret-Harte, M.S., Ewers, B.E., Peters,  
606 D.P., Young, D.R., Shaver, G.R., Pendall, E., Cleary, M.B., 2008. Shrub encroachment  
607 in North American grasslands: shifts in growth form dominance rapidly alters control of  
608 ecosystem carbon inputs. *Global Change Biology* 14, 615-623.



- 609 Korner, C., 2003. Ecological impacts of atmospheric CO<sub>2</sub> enrichment on terrestrial  
610 ecosystems. *Philosophical Transactions of the Royal Society London, Series A* 361,  
611 2023-2041.  
612
- 613 Lee, T.D., Tjoelker, M.G., Ellsworth, D.S., Reich, P.B., 2001. Leaf gas exchange responses of  
614 13 prairie grassland species to elevated CO<sub>2</sub> and increased nitrogen supply. *New*  
615 *Phytologist* 150, 405-418.  
616
- 617 Luo, Y., Reynolds, J., Wang, Y., Wolfe D., 1999. A search for predictive understanding of  
618 plant responses to elevated CO<sub>2</sub>. *Global Change Biology* 5, 143-156.  
619
- 620 Maestre, F.T., Bradford, M.A., Reynolds, J.F., 2005. Soil nutrient heterogeneity interacts with  
621 elevated CO<sub>2</sub> and nutrient availability to determine species and assemblage responses in  
622 a model grassland community. *New Phytologist* 168, 637-649.  
623
- 624 Miller, A.E., Bowman, W.D., Suding, K.N., 2007. Plant uptake of inorganic and organic  
625 nitrogen: neighbour identity matters. *Ecology* 88, 1832-1840.  
626
- 627 Niklaus, P.A., Korner C., 2004. Synthesis of a six-year study of calcareous grassland  
628 responses to in situ CO<sub>2</sub> enrichment. *Ecological Monographs* 74, 491-511.  
629
- 630 Poorter, H., Perez-Soba, M., 2001. The growth response of plants to elevated CO<sub>2</sub> under non-  
631 optimal environmental conditions. *Oecologia* 129, 1-20.  
632
- 633 Poorter, H., Navas, M.L., 2003. Plant growth and competition at elevated CO<sub>2</sub>: on winners,  
634 losers and functional groups. *New Phytologist* 157, 175-198.  
635
- 636 Potvin, C., Chapin, F.S., Gonzalez, A., Leadley, P.W., Reich, P., Roy, J., 2007. Plant  
637 biodiversity and responses to elevated carbon dioxide. In: Canadell, J., Pataki, D.,  
638 Pitelka, L. (Eds), *Terrestrial Ecosystems in a Changing World*. Springer Verlag, Berlin-  
639 Heidelberg, Germany, pp. 103-112.  
640
- 641 Raynaud, X., Leadley, P.W., 2005. Symmetry of belowground competition in a spatially  
642 explicit model of nutrient competition. *Ecological Modelling* 189, 447-453.  
643
- 644 Reich, P.B., Hobbie, S.E., Lee, T., Ellsworth, D.S., West, J.B., Tilman, D., Knops, J.M.H.,  
645 Naeem, S., Trost, J., 2006. Nitrogen limitation constrains sustainability of ecosystem  
646 response to CO<sub>2</sub>. *Nature* 440, 922-924.  
647
- 648 Reynolds, H.L., Packer, A., Bever, J.D., Clay, K., 2003. Grassroots ecology: plant-microbe-  
649 soil interactions as drivers of plant community structure and dynamics. *Ecology* 84,  
650 2281-2291.  
651
- 652 Ryser, P., Lambers, H., 1995. Root and leaf attributes accounting for the performance of fast-  
653 and slow-growing grasses at different nutrient supply. *Plant and Soil* 170, 251-265.  
654
- 655 Sala, O.E., Chapin, F.S., Armesto, J.J., Berlow, E., BloomFeld, J., Dirzo, R., Huber-Sanwald,  
656 E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney,  
657 H.A., Oesterheld, M., Poff, N.L., Sykes M.T., Walker, B.H., Walker, M., Wall, D.H.,  
658 2000. Global biodiversity scenarios for the year 2100. *Science* 287, 1770-1774.

- 659 Schmidt, I.K., Michelsen, A., Jonasson, S., 1997. Effects of labile soil carbon on nutrient  
660 partitioning between an arctic graminoid and microbes. *Oecologia* 112, 557-565.  
661
- 662 Schroter, D., Cramer, W., Leemans, R., Prentice, I.C., Araujo, M.B., Arnell, N.W., Bondeau,  
663 A., Bugmann, H., Carter, T.R., Gracia, C.A., C. de la Vega-Leinert, A., Erhard, M.,  
664 Ewert, F., Glendining, M., House, J.I., Kankaanpaa, S., Klein, R.J.T., Lavorel, S.,  
665 Lindner, M., Metzger, M.J., Meyer, J., Mitchell, T.D., Reginster, I., Rounsevell, M.,  
666 Sabaté, S., Sitch, S., Smith, B., Smith, J., Smith, P., Sykes, M.T., Thonicke, K., Thuiller,  
667 W., Tuck, G., Zaehle, S., Zierl, B., 2005. Ecosystem service supply and vulnerability to  
668 global change in Europe. *Science* 310, 1333-1337.  
669
- 670 Tilman, D., 1990. Constraints and tradeoffs: towards a predictive theory of competition and  
671 succession. *Oikos* 58, 3-15.  
672
- 673 Van Auken, O.W., Bush, J.K., 1997. Shrub invasions of North American semiarid grasslands.  
674 *Annual Review of Ecology and Systematics* 31, 197-215.  
675
- 676 Vitousek, P.M., Howarth, R.W., 1991. Nitrogen limitation on land and in the sea, how can it  
677 occur? *Biogeochemistry* 13, 87-115.  
678
- 679 Weigelt, A., Bol, R., Bardgett, R.D., 2005. Preferential uptake of soil nitrogen forms by  
680 grassland plant species. *Oecologia* 142, 627-635.  
681
- 682 Weiner, J., 1990. Asymmetric competition in plant populations. *Trends in Ecology and*  
683 *Evolution* 5, 360-364.  
684
- 685 Williams, M.A., Rice, C.W., Owensby, C.E., 2001. Nitrogen competition in a tallgrass prairie  
686 ecosystem exposed to elevated carbon dioxide. *Soil Science Society of America Journal*  
687 65, 340-346.  
688
- 689 Wilson, S.D., 1998. Competition between grasses and woody plants. In: Cheplick, G.P. (Ed.),  
690 *Population Biology of Grasses*. Cambridge University Press, Cambridge, UK, pp. 231-  
691 254.  
692
- 693 Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement  
694 of soil microbial biomass C by fumigation-extraction – an automated procedure. *Soil*  
695 *Biology & Biochemistry* 22, 1167-1169.  
696
- 697 Zak, D.R., Pregitzer, K.S., King, J.S., Holmes, W.E., 2000. Elevated atmospheric CO<sub>2</sub>, fine  
698 roots and the response of soil microorganisms: a review and hypothesis. *New*  
699 *Phytologist* 147, 201-222.  
700
- 701 Zar, J.H., 1999. *Biostatistical Analysis*. Prentice-Hall International, London, UK, 929 pp.  
702
- 703 Zavaleta, E.S., 2006. Shrub establishment under experimental global changes in a California  
704 grassland. *Plant Ecology* 184, 53-63.

705 Table 1

706 Plant dry mass and N nutritional status of *Dactylis* grown under interactive N and CO<sub>2</sub> treatments

	Treatments				ANOVA results (P values)		
	cn	cN	Cn	CN	N	CO <sub>2</sub>	N x CO <sub>2</sub>
Root mass (g)	6.60 ± 0.73	16.17 ± 1.42	6.90 ± 0.60	23.78 ± 2.39	<b>&lt;0.001</b>	<b>0.017</b>	<b>0.026</b>
Shoot mass (g)	6.00 ± 0.46	21.56 ± 0.82	6.37 ± 0.43	25.85 ± 0.58	<b>&lt;0.001</b>	<b>0.018</b>	<b>0.046</b>
Root N content (mg)	48.8 ± 3.72	112.8 ± 12.4	51.3 ± 4.12	138.6 ± 14.5	<b>&lt;0.001</b>	0.147	0.248
Shoot N content (mg)	50.2 ± 2.27	183.8 ± 3.68	45.0 ± 2.27	166.4 ± 5.01	<b>&lt;0.001</b>	<b>0.010</b>	0.091
Root [N] (mg g <sup>-1</sup> dry mass)	7.52 ± 0.27	6.95 ± 0.29	7.52 ± 0.23	5.84 ± 0.24	<b>0.004</b>	0.061	0.073
Shoot [N] (mg g <sup>-1</sup> dry mass)	8.47 ± 0.35	8.58 ± 0.35	7.15 ± 0.30	6.44 ± 0.21	0.406	<b>&lt;0.001</b>	0.268

707

708 Treatment codes are given by: c = ambient CO<sub>2</sub>, 380 μmol mol<sup>-1</sup>; C = elevated CO<sub>2</sub>, 645 μmol mol<sup>-1</sup>; n = low nitrogen; N = high nitrogen  
709 treatment. Values are means ± standard errors (n = 6). Significance of F values is shown: significant effects (P < 0.05) are shown in bold type.

710 Additional biomass data are available in Bloor et al. (2008a).

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716 Table 2

717 Results of ANOVA showing effects of plant competition, N supply, CO<sub>2</sub> and all interactions on *Fraxinus* seedling biomass and plant nutritional  
718 status (N content, N concentration)

Effect	Variables					
	Shoot dry mass (g)	Root dry mass (g)	Shoot N content (mg)	Root N content (mg)	Shoot [N] (mg g <sup>-1</sup> shoot dry mass)	Root [N] (mg g <sup>-1</sup> root dry mass)
Competition (Comp)	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.033</b>	0.061
N supply (N)	0.877	0.558	<b>0.014</b>	0.191	<b>&lt;0.001</b>	<b>&lt;0.001</b>
CO <sub>2</sub>	0.281	0.350	0.859	0.303	<b>0.025</b>	0.229
Comp x N	<b>0.005</b>	<b>0.014</b>	<b>0.029</b>	0.076	<b>&lt;0.001</b>	<b>0.016</b>
Comp x CO <sub>2</sub>	0.851	0.844	0.935	0.415	<b>0.021</b>	<b>0.024</b>
N x CO <sub>2</sub>	0.554	0.512	0.283	0.300	0.095	0.093
Comp x N x CO <sub>2</sub>	0.605	0.999	0.368	0.179	<b>0.003</b>	<b>0.002</b>

719

720 Values shown are probabilities associated with the F ratio; significant effects (P < 0.05) are shown in bold type

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725 Table 3.

726 Total <sup>15</sup>N recovery (expressed as absolute values and on a root dry mass basis) and distribution of added <sup>15</sup>N in root versus shoot fractions for

727 *Dactylis* and *Fraxinus* seedlings grown under interactive N and CO<sub>2</sub> treatments, either alone or in competition with each other

	Treatments				ANOVA results (P values)		
	cn	cN	Cn	CN	N	CO <sub>2</sub>	N x CO <sub>2</sub>
a) <i>Fraxinus</i> grown alone							
Total <sup>15</sup> N (mg)	0.29 ± 0.06	0.17 ± 0.03	0.31 ± 0.05	0.22 ± 0.02	<b>0.023</b>	0.469	0.699
Total <sup>15</sup> N (mg g <sup>-1</sup> root dry mass)	2.09 ± 0.02	1.35 ± 0.26	2.23 ± 0.43	1.33 ± 0.11	<b>0.007</b>	0.537	0.705
<sup>15</sup> N in roots (%)	53.3 ± 9.2	51.2 ± 3.1	45.3 ± 2.0	47.8 ± 4.1	0.822	0.752	0.246
b) <i>Fraxinus</i> grown in competition treatment							
Total <sup>15</sup> N (mg)	0.014 ± 0.002	0.005 ± 0.001	0.017 ± 0.004	0.006 ± 0.002	<b>0.004</b>	0.446	0.936
Total <sup>15</sup> N (mg g <sup>-1</sup> root dry mass)	0.35 ± 0.06	0.45 ± 0.14	0.36 ± 0.06	0.32 ± 0.10	0.762	0.420	0.334
<sup>15</sup> N in roots (%)	52.0 ± 3.8	29.5 ± 5.2	71.6 ± 3.4	34.4 ± 9.0	<b>&lt;0.001</b>	<b>0.021</b>	0.353
c) <i>Dactylis</i> grown in competition treatment							
Total <sup>15</sup> N (mg)	3.8 ± 0.16	4.6 ± 0.23	4.0 ± 0.23	4.7 ± 0.22	<b>0.004</b>	0.830	0.517
Total <sup>15</sup> N (mg g <sup>-1</sup> root dry mass)	0.60 ± 0.05	0.31 ± 0.03	0.60 ± 0.05	0.20 ± 0.01	<b>&lt;0.001</b>	0.262	0.322
<sup>15</sup> N in roots (%)	38.4 ± 0.9	42.0 ± 4.8	40.3 ± 1.6	47.1 ± 4.4	0.123	0.262	0.602

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729 Treatment codes are given by: c = ambient CO<sub>2</sub>, 380 μmol mol<sup>-1</sup>; C = elevated CO<sub>2</sub>, 645 μmol mol<sup>-1</sup>; n = low nitrogen; N = high nitrogen

730 treatment. Values are means ± standard errors (n = 6). Significance of F values is shown: significant effects (P < 0.05) are shown in bold type.

731 Figure captions

732

733 **Fig. 1.** *Fraxinus* biomass and N nutritional status for seedlings grown in treatment  
734 combinations of CO<sub>2</sub> and N in the presence (+comp) and absence (-comp) of grass  
735 competition. Treatment codes are given by: c = ambient CO<sub>2</sub>, 380 μmol mol<sup>-1</sup>; C = elevated  
736 CO<sub>2</sub>, 645 μmol mol<sup>-1</sup>; n = low nitrogen; N = high nitrogen treatment. Means and standard  
737 errors are presented (n = 6).

738

739 **Fig. 2.** Microbial biomass C, microbial biomass N and soil extractable N in the 0-10 cm soil  
740 layer of soils under interactive CO<sub>2</sub>, N and grass competition treatments. Treatment codes are  
741 given by: -comp = absence of grass competition; +comp = presence of grass competition; c =  
742 ambient CO<sub>2</sub>, 380 μmol mol<sup>-1</sup>; C = elevated CO<sub>2</sub>, 645 μmol mol<sup>-1</sup>; n = low nitrogen; N = high  
743 nitrogen treatment. Means and standard errors are presented (n = 6).

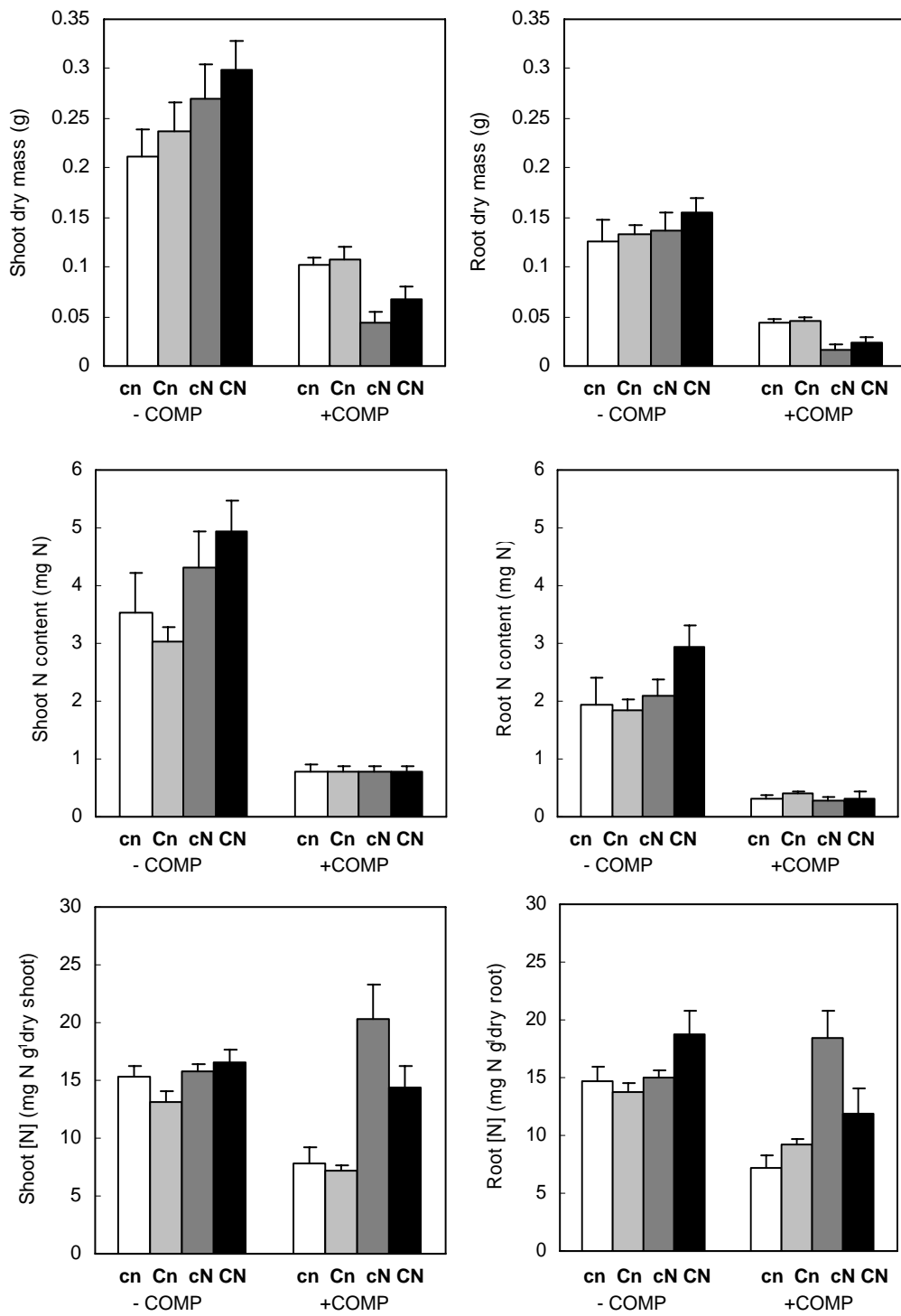
744

745 **Fig. 3.** <sup>15</sup>N recovery in *Dactylis* biomass, microbial biomass, *Fraxinus* biomass and the soil  
746 extractable N pool under interactive CO<sub>2</sub> and N treatments. Means and standard errors are  
747 presented (n = 6).

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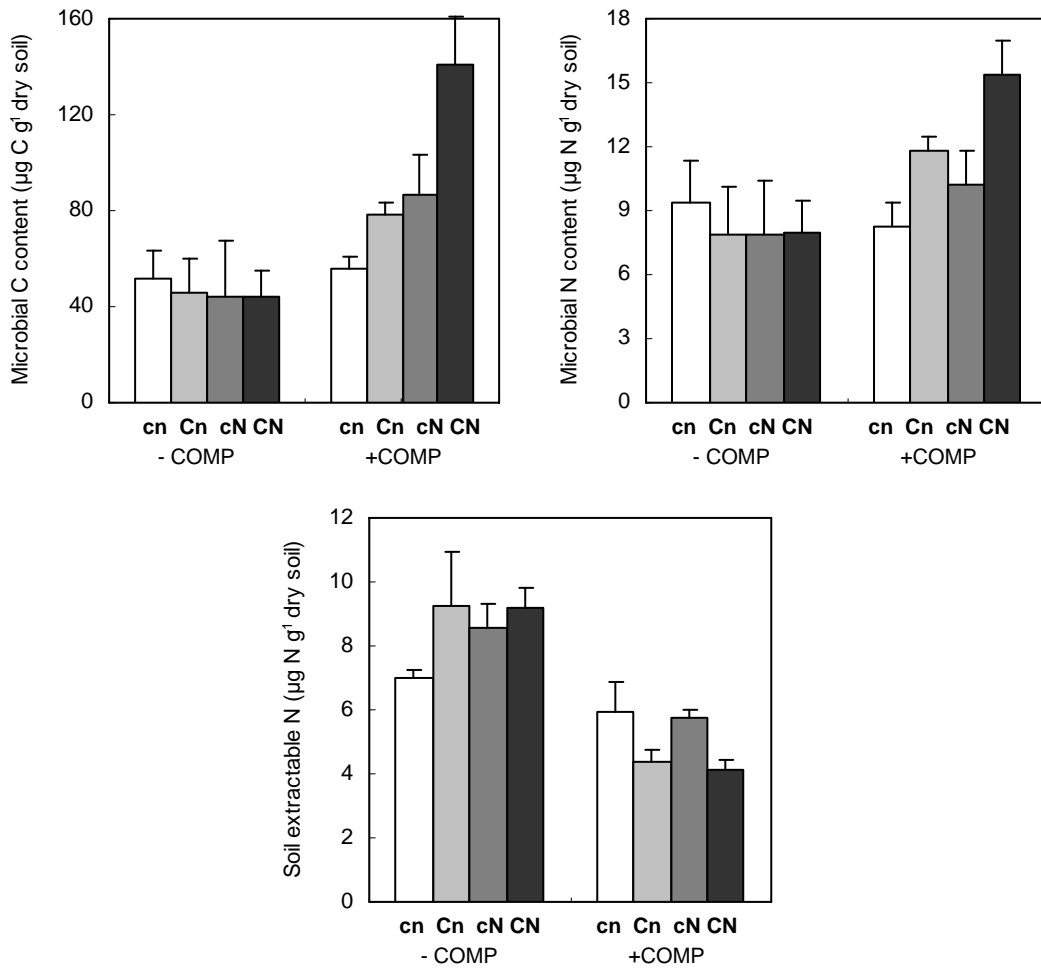
749 Fig. 1.

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752 Fig. 2.

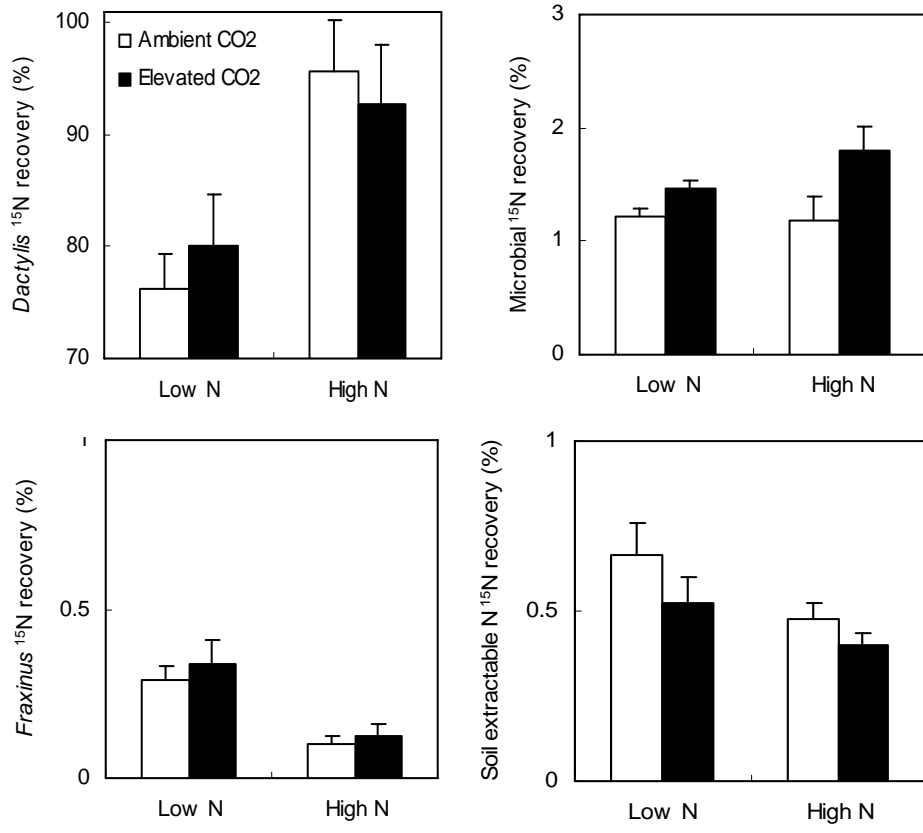


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755 Fig. 3.



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