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CO_2 and inorganic N supply modify competition for N between co-occurring grass plants, tree seedlings and soil microorganisms

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ABSTRACT

Plant-plant and plant-soil interactions play a key role in determining plant community structure and ecosystem function. However, the effects of global change on the interplay between co-occurring plants and soil microbes in successional communities are poorly understood. In this study, we investigated competition for nitrogen (N) between soil microorganisms, grass plants and establishing tree seedlings under factorial carbon dioxide (CO_2) and N treatments. *Fraxinus excelsior* seedlings were germinated in the presence or absence of grass competition (*Dactylis glomerata*) at low (380 μmol mol^{-1}) or high (645 μmol mol^{-1}) CO_2 and at two levels of N nutrition in a mesocosm experiment. Pulse ^{15}N labelling was used to examine N partitioning among plant and soil compartments. *Dactylis* exerted a strong negative effect on *Fraxinus* biomass, N capture and ^{15}N recovery irrespective of N and CO_2 treatment. In contrast, the presence of *Dactylis* had a positive effect on the microbial N pool. Plant and soil responses to N treatment were of a greater magnitude compared with responses to elevated CO_2, but the pattern of *Fraxinus*- and microbial-N pool response to N and CO_2 varied depending on grass competition treatment. Within the *Dactylis* competition treatment, decreases in *Fraxinus* biomass in response to N were not mirrored by decreases in tree seedling N content, suggesting a shift from below- to above-ground competition. In the *Dactylis*-sown pots, ^{15}N recovery could be ranked *Dactylis* > microbial pool > *Fraxinus* in all N and CO_2 treatment combinations. Inequalities between *Fraxinus* and soil microorganisms in terms of ^{15}N recovery were exacerbated by N addition. Contrary to expectations, elevated CO_2 did not increase plant-microbe competition. Nevertheless, microbial ^{15}N recovery showed a small positive increase in the high CO_2 treatment. Overall, elevated CO_2 and N supply did not 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_2 or N-induced modifications in N competition between plant and soil compartments.
46
47 *Keywords*: Global change; *Dactylis glomerata*, Elevated CO_2; *Fraxinus excelsior*; Nitrogen
48 addition; Nitrogen uptake; Plant-microbial competition; ^{15}N stable isotopes; Tree-grass
49 interactions.

1. Introduction

Substantial evidence suggests that global climate change and increasing levels of atmospheric carbon dioxide (CO_2) may lead to significant changes in biodiversity and plant species distributions, with cascading effects on ecosystem function and carbon sequestration (Sala et al., 2000; Schroter et al., 2005; Potvin et al., 2007). Consequently, accurate projections of species' responses to future environmental change are crucial for the assessment of global change-related risks to ecosystem services. However, despite the wealth of literature on plant responses to CO_2 enrichment (see reviews by Ceulemans and Mousseau, 1994; Poorter and Perez-Soba, 2001; Korner, 2003; Ainsworth and Long, 2005), predicting the effects of future CO_2 levels on plant community structure and biodiversity dynamics remains a major challenge for ecologists (Poorter and Navas 2003; Bradley and Pregitzer 2007).

Previous work has shown that the net effect of CO_2 enrichment on plant community structure may depend on complex interactions between atmospheric changes and climate (Niklaus and Korner, 2004), soil nutrient availability (Maestre et al., 2005) or management (Harmens et al., 2004). There is growing recognition that plant-plant interactions also play a key role in mediating the effects of environmental change on plant community structure; given that elevated CO_2 can have a direct effect on plant physiology and growth, intra- and interspecific variation in plant responses to CO_2 may modify the outcome of plant interactions (Brooker, 2006; Bradley and Pregitzer, 2007). In contrast, few studies have explored the influence of soil micro-organisms on plant-plant interactions and species assemblages in a changing CO_2 environment. Plant and microbial communities are clearly interdependent; plant production is often limited by quantities of nitrogen (N) made available during the 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_2 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_2 (Williams et al., 2001). In practice, however, findings from
87 experimental systems are inconsistent. For example, results from ^{15}N pulse-labelling studies
88 carried out in multispecies grassland systems under elevated CO_2 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_2 effects on plant-soil interactions is that CO_2-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_2 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_2 (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_2 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 ^{15}N pulse-labelling approach to determine how CO_2 and N supply affect N partitioning
111 among different plant and soil compartments. Use of ^{15}N tracer techniques can greatly
112 increase the ability to detect small CO_2-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_2. 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

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

2.2. Experimental design

Two plant competition treatments (*Fraxinus* seedlings alone, *Fraxinus* seedlings grown with *Dactylis*) were established in either ambient or elevated atmospheric CO_2 treatments under glasshouse conditions. In order to investigate the interactive effects of CO_2 and N supply on plant and microbial competition for nutrients, two N treatments (low/high) were crossed with each CO_2 and competition treatment (two competition treatments x two CO_2 treatments x two N treatments x 6 replicates = 48 pots).

2.3. Soil, plant material and growing conditions

Loamy topsoil was collected on the grounds of the University of Paris XI (Orsay, France) at the start of February 2006. The soil was sieved (1 cm), diluted with locally-obtained river sand in a 50:50 mix, and packed in deep PVC pots (20 x 15 x 40 cm) as described in Bloor et al. (2008a). The soil/ sand mix contained 0.23 g N kg^{-1} soil, 2.46 g C kg^{-1} soil and had a pH of 8.5. Experimental pots were assigned to one of twelve naturally-lit growth chambers (wooden frame and clear plastic walls, 65 x 65 x 100 cm high) set up inside a large glasshouse at the University of Paris XI and ventilated with air taken from outside the glasshouse. Six chambers were ventilated with ambient atmospheric CO_2 concentrations and the remaining six chambers were ventilated with elevated CO_2; elevated atmospheric CO_2 concentrations were adjusted to a differential of 265 μmol mol^{-1} ± 2% compared with ambient chambers by injection of pure CO_2 in each enriched chamber (see Bloor et al., 2008a for full

150 details). CO_2 concentrations were monitored throughout the experiment using a portable
151 carbon dioxide analyser (Carbocap GM 70, Vaisala, Helsinki, Finland), indicating an average
152 CO_2 concentration of 380 μmol mol^{-1} (standard error = 6 μmol mol^{-1}) and 645 μmol mol^{-1}
153 (standard error = 9 μmol mol^{-1}) in the ambient and elevated CO_2 chambers respectively. Over
154 the course of the experimental period, no temperature difference was observed between the
155 ambient and elevated CO_2 chambers (mean daily temperatures based on hourly measurements
156 ranged between 11.7°C and 23.1°C).
157 On 23rd February 2006, seeds of *Dactylis glomerata* were sown into half of the pots at
158 a density of 2000 seeds m^{-2}, 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_4NO_3 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^{-1}.
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_2 treatments. *Fraxinus* seedlings and *Dactylis* plants were left
168 to grow in the experimental treatments for ten weeks prior to ^{15}N labelling, and all pots were
169 watered regularly.
170
171 *2.4. ^{15}N plant labelling*
172
173 In June 2006, 5mg ^{15}N was injected per experimental pot in the form of ammonium
174 solution ($^{15}NH_4Cl$ at 99% ^{15}N, 0.01 M). A $^{15}NH_4^+$ marker was chosen because ammonium is

first inorganic N form made available to plants and previous studies have shown that soil microbes compete effectively with plants for this resource (Hodge et al., 2000). In order to minimise the time between first and last pot injection and to ensure that all pots were exposed to the same ^{15}N labelling period, labelling was carried out in two stages; half of the pots in each experimental treatment were injected on 12th June (harvested 13th June) whereas the remainder were injected on 13th June (harvested 14th June). One ml of solution was slowly injected (needle length 9 cm, diameter 0.9 mm) at 36 injection locations in the 0-10 cm soil layer of each pot following Barnard et al. (2006). Injection locations were arranged in a three dimensional grid with three injection depths (1.5, 5 and 7.5 cm) to maximise ^{15}N mixing with the native NH_4^+ pool. Pots were not watered after injection to minimize leaching loss.

2.5. Harvest and analyses

Plants and soil were harvested 24 h after ^{15}N injection as preliminary work on *Fraxinus* seedlings and *Dactylis* indicated rapid plant uptake of $^{15}NH_4^+$. Pots were harvested in the order in which they were injected. At harvest, aboveground plant material was clipped at the soil surface and separated into *Dactylis* leaves, *Fraxinus* leaves+cotyledons and *Fraxinus* stems. Roots were separated from the soil manually and washed; *Fraxinus* roots could be clearly distinguished from grass roots and were carefully disentangled from the *Dactylis* belowground material. All plant material was oven-dried (60°C for at least 72 h), weighed, ground and analysed for total N and atom% ^{15}N (mass spectrometers, CNRS, Service central d'Analyse, Solaize, France).

Soil in the 0-10 cm layer of each pot was separated from the deeper soil layers, well-mixed and sieved (2 mm). Soil and microbial properties in the top 10 cm of grass-sown pots 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}N signal. Microbial biomass N, ^{15}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 K_2SO_4 solution following
205 Fontaine et al. (2004). Microbial C (C_{mic}) was calculated as the difference in total C extracted
206 in fumigated and unfumigated soils, with $k_C = 0.45$ as the adjustment factor (Wu et al., 1990).
207 Microbial N (N_{mic}) was calculated as the difference in total N extracted in fumigated and
208 unfumigated soils, with $k_N = 0.54$ as the adjustment factor (Brookes et al., 1985). Soil
209 extractable N and ^{15}N were measured in the unfumigated soil extracts following Barnard et al.
210 (2006). Correction for the natural abundance of ^{15}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 $\delta^{15}N$ in
215 microbial extracts in the pots with *Fraxinus* alone were extremely heterogeneous and could
216 not be used for calculations of ^{15}N uptake by the microbial biomass for this treatment.
217 Consequently, microbial ^{15}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 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_2 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_2 (Table 1). Under high N supply,
236 *Dactylis* root and shoot biomass showed a positive response to elevated CO_2; the magnitude
237 of dry mass increase to CO_2 under high N was greater in roots than in shoots (+47% and
238 +20% respectively). However, elevated CO_2 had little effect on either root or shoot biomass
239 under low N conditions (significant N x CO_2 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_2 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_2 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_2 treatment (Fig.
248 1). Furthermore, grass competition modified *Fraxinus* responses to N and CO_2 (significant
249 competition x N and competition x CO_2 interactions, Table 2). In the absence of grass

competition, increasing N supply was associated with a significant increase in *Fraxinus* seedling shoot mass, N content and shoot N concentration (Fig. 1, Table 2). Increasing N supply had no significant effect on *Fraxinus* root biomass or N content, but there was a tendency towards greater root mass in the high N treatments and an increase in root N content under high N, high CO_2 conditions for seedlings grown alone (Fig. 1, Table 2). In the presence of grass competition, both *Fraxinus* shoot and root mass showed a significant decrease in response to increasing N (Fig. 1, Table 2). Neither above- nor belowground N content showed any significant response to N supply for *Fraxinus* seedlings grown with grass, but tissue N concentrations were significantly greater in the high N treatment (Fig. 1). On average, the shoot N concentration of *Fraxinus* seedlings grown with *Dactylis* under high N conditions was similar to that of *Fraxinus* seedlings grown alone. Patterns of variation in *Fraxinus* root and shoot N concentrations were further modified by CO_2 treatment since tissue N concentration showed a significant decrease in high N, high CO_2 conditions for seedlings grown with grass (Fig. 1).

The presence of *Dactylis* had contrasting effects on soil microbes and the soil extractable N pool (Fig. 2). Overall, grass competition treatment had a positive effect on C_{mic} and N_{mic} ($F_{1,24} = 22.35$ and 17.73 respectively, $P < 0.001$). Furthermore, both C_{mic} and N_{mic} showed a significant positive correlation with *Dactylis* dry mass (all N and CO_2 treatments combined, Pearson's R = 0.70, $P < 0.001$ and 0.48, $P < 0.05$ respectively). At the same time, presence of *Dactylis* was associated with a significant decrease in soil extractable N (Fig. 2, $F_{1,24} = 62.35$, $P < 0.001$). As with *Fraxinus* seedling traits, microbial and soil N responses to N and CO_2 treatment varied depending on plant competition treatment (Fig. 2). In the absence of *Dactylis*, neither N supply nor CO_2 had any significant effect on C_{mic} , N_{mic} or the soil extractable N pool ($P > 0.1$ in all cases). However, in pots with *Fraxinus* and *Dactylis* grown together, C_{mic} showed a significant increase in response to increasing N and CO_2 (Fig 2., $F_{1,9} =$

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

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

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

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

Plant ^{15}N uptake showed significant responses to N for both *Dactylis* and *Fraxinus*, but the effects of N treatment varied depending on the species considered (Table 3). Increasing N supply was associated with an increase in total *Dactylis* ^{15}N uptake although *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* ^{15}N uptake showed a negative response to N
301 addition, irrespective of the level of competiton experienced by *Fraxinus* seedlings. Patterns
302 of total ^{15}N uptake were mirrored by ^{15}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 ^{15}N uptake on a root mass basis in the presence of grass competition.
305 Elevated CO_2 had no effect on ^{15}N uptake for either *Dactylis* or *Fraxinus* in any N or
306 competition treatment (Table 3).
307 In general, *Dactylis* allocated more ^{15}N to shoots rather than roots compared to
308 *Fraxinus* seedlings across treatments (Table 3). Neither N supply nor CO_2 had a significant
309 effect on the ^{15}N distribution pattern (roots versus shoots) of *Dactylis* plants or *Fraxinus*
310 seedlings grown alone (Table 3). Patterns of ^{15}N distribution in *Fraxinus* seedlings grown
311 with grass varied depending on N and CO_2 treatment; less ^{15}N accumulated in roots in
312 response to an increase in N supply while more ^{15}N accumulated in roots under elevated CO_2
313 (Table 3).
314
315 *3.3. ^{15}N distribution among plant and soil pools in the Dactylis-Fraxinus competition*
316 *treatment*
317
318 Total recovery of ^{15}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 ^{15}N recovery was due to an increase in *Dactylis* ^{15}N uptake (previously described); N
321 treatment had no significant effect on microbial ^{15}N uptake and was associated with a
322 decrease in ^{15}N recovery by both *Fraxinus* seedlings and the soil extractable N pool (Fig. 3).
323 Elevated CO_2 did not significantly affect total ^{15}N recovery but was associated with a
324 marginally significant increase in microbial ^{15}N uptake ($F_{1,10} = 4.84$, $P = 0.054$, Fig. 3).

325 Overall, ^{15}N uptake could be ranked *Dactylis* > microbial biomass > *Fraxinus* seedlings (Fig.
326 3). Recovery of ^{15}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}N
328 uptake between *Fraxinus* seedlings and the microbial pool were unaffected by elevated CO_2.
329
330 **4. Discussion**
331
332 *4.1. Effects of N and CO_2 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}N recovery in all treatment combinations. Furthermore, *Dactylis*
341 was associated with a significant reduction in *Fraxinus* N content and ^{15}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}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_2 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_2 enrichment on total plant N content or ^{15}N uptake for either *Dactylis* or *Fraxinus*.
392 Decreases in plant nutrient concentration in response to short-term CO_2 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_2 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_2 treatment
397 in terms of N uptake and plant nutrient status, *Fraxinus* and *Dactylis* displayed clear
398 differences in biomass response to elevated CO_2; unlike *Fraxinus*, *Dactylis* biomass showed
399 significant CO_2 x N interactions and a positive biomass response to CO_2 enrichment in the

high N treatment alone, suggesting N limitation effects under low N conditions. Positive responses of biomass to elevated CO_2 are expected to become progressively weaker in low N conditions, particularly for fast-growing species with high N demand (Arp et al., 1998; Lee et al., 2001; Hu et al., 2006). Given the importance of plant size for resource acquisition and competitive ability (Weiner, 1990), CO_2 enrichment may therefore reduce competitive differences between woody seedlings and herbaceous species in the longer-term.

4.2. Plant-microbial N partitioning

Soil microorganisms are generally thought to be strong short-term competitors for soil N due to their high surface area to volume ratio, wide-spread spatial distribution in the soil and rapid growth rates compared with plant roots (Kaye and Hart, 1997; Hodge et al., 2000). However, previous studies which have used ^{15}N labelling techniques to examine short-term inorganic N partitioning between plants and microbes have generated conflicting results (Hungate et al., 1996; Burger and Jackson, 2004; Cheng and Bledsoe, 2004; Barnard et al., 2006; Harrison et al., 2008). We found that the outcome of plant-soil competition for ^{15}N-NH_4^+ was plant species-dependent; *Dactylis* had a consistently greater capacity for ^{15}N uptake compared with the soil microbial pool, but microorganisms were more efficient in ^{15}N capture than *Fraxinus* seedlings. These results support the hypothesis that small, germinating *Fraxinus* seedlings are less able to compete with soil microorganisms for N compared to large, established *Dactylis* plants. Competitive inequalities between microorganisms and plants may become less pronounced over longer time periods as a result of rapid microbial turnover which releases N to the soil, combined with the capacity of plants to sequester N for longer (Hodge et al., 2000; Barnard et al., 2006). The balance of plant-microbe N competition may also change due to temporal asynchrony in plant and microbial N-limitation (Hodge et

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

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

449 contributed to the inequality in ^{15}N acquisition observed here between *Dactylis* and soil
450 microorganisms.
451 In line with Diaz et al. (1993), we predicted that elevated CO_2 would lead to increased
452 microbial demand for N and constraints on plant N acquisition. Increases in microbial N and
453 ^{15}N uptake in response to CO_2 in the presence of *Dactylis* provide support for the idea of
454 increased microbial N demand under CO_2 enrichment. However, changes in microbial N
455 acquisition under elevated CO_2 were unrelated to plant N or ^{15}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 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 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, 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

plants in an undisturbed sward is relatively insensitive to CO_2 or N-induced modifications in N competition between plant and soil compartments. Additional experiments are required to determine under which sward conditions (height, density, gap size) young tree seedlings may benefit from the effects of global change on plant-soil interactions. Future research should also address how responses of germinating seedlings translate into longer term seedling growth and survival under elevated atmospheric CO_2.

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705 Table 1

706 Plant dry mass and N nutritional status of *Dactylis* grown under interactive N and CO_2 treatments

	Treatments				ANOVA results (P values)		
	cn	cN	Cn	CN	N	CO_2	N x CO_2
Root mass (g)	6.60 ± 0.73	16.17 ± 1.42	6.90 ± 0.60	23.78 ± 2.39	**<0.001**	**0.017**	**0.026**
Shoot mass (g)	6.00 ± 0.46	21.56 ± 0.82	6.37 ± 0.43	25.85 ± 0.58	**<0.001**	**0.018**	**0.046**
Root N content (mg)	48.8 ± 3.72	112.8 ± 12.4	51.3 ± 4.12	138.6 ± 14.5	**<0.001**	0.147	0.248
Shoot N content (mg)	50.2 ± 2.27	183.8 ± 3.68	45.0 ± 2.27	166.4 ± 5.01	**<0.001**	**0.010**	0.091
Root [N] (mg g^{-1} dry mass)	7.52 ± 0.27	6.95 ± 0.29	7.52 ± 0.23	5.84 ± 0.24	**0.004**	0.061	0.073
Shoot [N] (mg g^{-1} dry mass)	8.47 ± 0.35	8.58 ± 0.35	7.15 ± 0.30	6.44 ± 0.21	0.406	**<0.001**	0.268

707

708 Treatment codes are given by: c = ambient CO_2, 380 μmol mol^{-1}; C = elevated CO_2, 645 μmol mol^{-1}; 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).
711

712

713

714

715

Table 2

Results of ANOVA showing effects of plant competition, N supply, CO_2 and all interactions on *Fraxinus* seedling biomass and plant nutritional 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^{-1} shoot dry mass)	Root [N] (mg g^{-1} root dry mass)
Competition (Comp)	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.033**	0.061
N supply (N)	0.877	0.558	**0.014**	0.191	**<0.001**	**<0.001**
CO_2	0.281	0.350	0.859	0.303	**0.025**	0.229
Comp x N	**0.005**	**0.014**	**0.029**	0.076	**<0.001**	**0.016**
Comp x CO_2	0.851	0.844	0.935	0.415	**0.021**	**0.024**
N x CO_2	0.554	0.512	0.283	0.300	0.095	0.093
Comp x N x CO_2	0.605	0.999	0.368	0.179	**0.003**	**0.002**

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

Table 3.

Total ^{15}N recovery (expressed as absolute values and on a root dry mass basis) and distribution of added ^{15}N in root versus shoot fractions for *Dactylis* and *Fraxinus* seedlings grown under interactive N and CO_2 treatments, either alone or in competition with each other

	Treatments				ANOVA results (P values)		
	cn	cN	Cn	CN	N	CO_2	N x CO_2
a) *Fraxinus* grown alone							
Total ^{15}N (mg)	0.29 ± 0.06	0.17 ± 0.03	0.31 ± 0.05	0.22 ± 0.02	**0.023**	0.469	0.699
Total ^{15}N (mg g^{-1} root dry mass)	2.09 ± 0.02	1.35 ± 0.26	2.23 ± 0.43	1.33 ± 0.11	**0.007**	0.537	0.705
^{15}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) *Fraxinus* grown in competition treatment							
Total ^{15}N (mg)	0.014 ± 0.002	0.005 ± 0.001	0.017 ± 0.004	0.006 ± 0.002	**0.004**	0.446	0.936
Total ^{15}N (mg g^{-1} 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
^{15}N in roots (%)	52.0 ± 3.8	29.5 ± 5.2	71.6 ± 3.4	34.4 ± 9.0	**<0.001**	**0.021**	0.353
c) *Dactylis* grown in competition treatment							
Total ^{15}N (mg)	3.8 ± 0.16	4.6 ± 0.23	4.0 ± 0.23	4.7 ± 0.22	**0.004**	0.830	0.517
Total ^{15}N (mg g^{-1} root dry mass)	0.60 ± 0.05	0.31 ± 0.03	0.60 ± 0.05	0.20 ± 0.01	**<0.001**	0.262	0.322
^{15}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

Treatment codes are given by: c = ambient CO_2, 380 μmol mol^{-1}; C = elevated CO_2, 645 μmol mol^{-1}; n = low nitrogen; N = high nitrogen 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_2 and N in the presence (+comp) and absence (-comp) of grass

735 competition. Treatment codes are given by: c = ambient CO_2, 380 μmol mol^{-1}; C = elevated

736 CO_2, 645 μmol mol^{-1}; 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_2 , 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_2, 380 μmol mol^{-1}; C = elevated CO_2, 645 μmol mol^{-1}; n = low nitrogen; N = high

743 nitrogen treatment. Means and standard errors are presented (n = 6).

744

745 **Fig. 3**. ^{15}N recovery in *Dactylis* biomass, microbial biomass, *Fraxinus* biomass and the soil

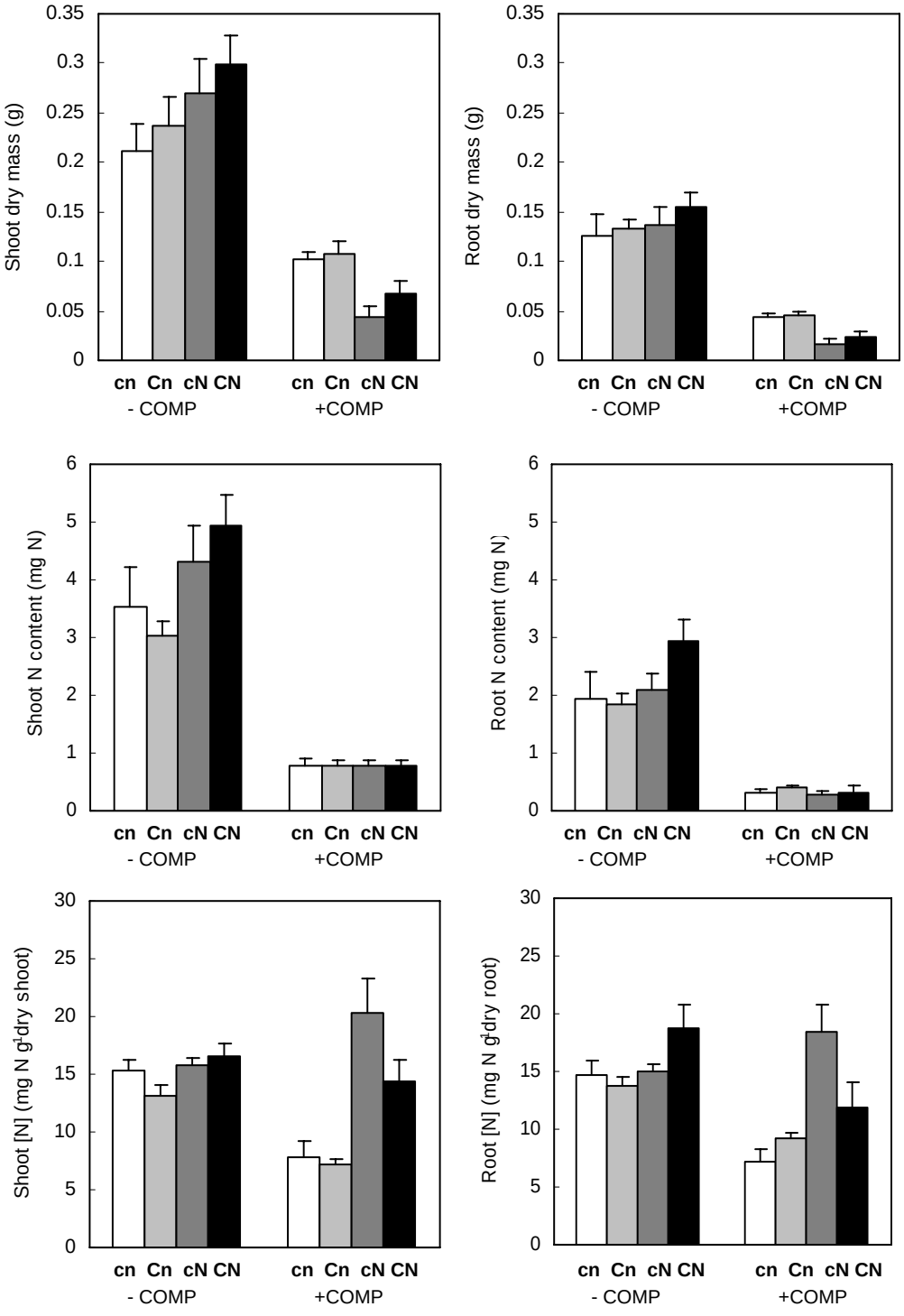
746 extractable N pool under interactive CO_2 and N treatments. Means and standard errors are

747 presented (n = 6).

748

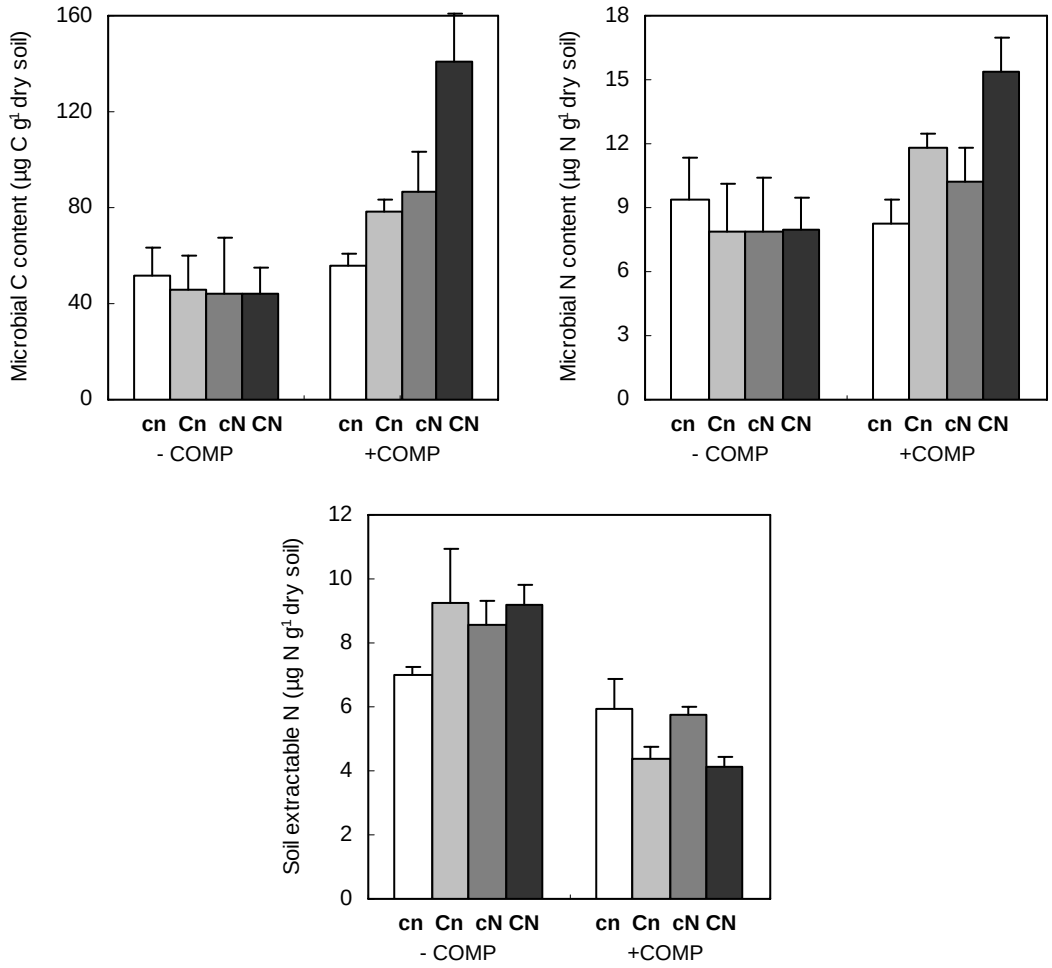
749 Fig. 1.

750



751

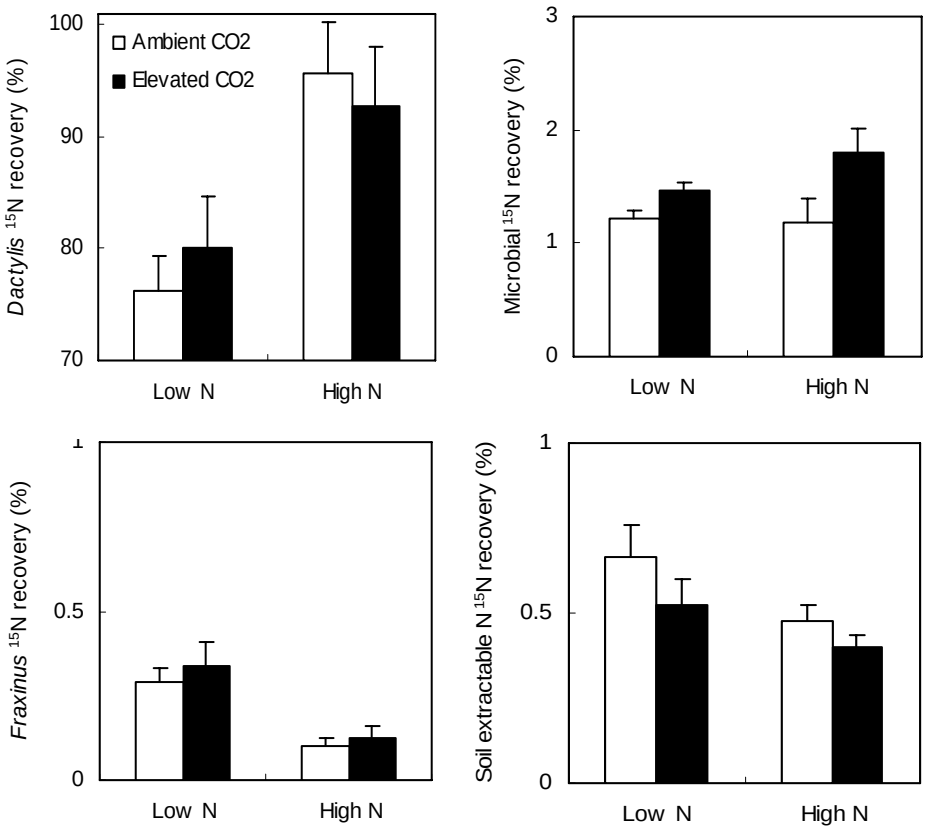
752 Fig. 2.



753

754

755 Fig. 3.


Ambient CO2
Elevated CO2
Dactylis 15N recovery (%)
Microbial 15N recovery (%)
Fraxinus 15N recovery (%)
Soil extractable N 15N recovery (%)
Low N
High N

756