Title:

The effect of elevated atmospheric carbon dioxide concentration on the contribution of residual legume and fertilizer nitrogen to a subsequent wheat crop

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Abstract

Purpose

This study investigated the residual contribution of legume and fertilizer nitrogen (N) to a subsequent crop under the effect of elevated carbon dioxide concentration ($[CO_2]$).

Methods

Field pea (*Pisum sativum* L.) was labeled *in situ* with ^{15}N (by absorption of a ^{15}N-labeled urea solution through cut tendrils) under ambient and elevated (700 μmol mol^{-1}) $[CO_2]$ in controlled environment glasshouse chambers. Barley (*Hordeum vulgare* L.) and its soil were also labeled under the same conditions by addition of ^{15}N-enriched urea to the soil. Wheat (*Triticum aestivum* L.) was subsequently grown to physiological maturity on the soil containing either ^{15}N-labeled field pea residues (including ^{15}N-labeled rhizodeposits) or ^{15}N-labeled barley plus fertilizer ^{15}N residues.

Results

Elevated $[CO_2]$ increased the total biomass of field pea (21%) and N-fertilized barley (23%), but did not significantly affect the biomass of unfertilized barley. Elevated $[CO_2]$ increased the C:N ratio of residues of field pea (18%) and N-fertilized barley (19%), but had no significant effect on that of unfertilized barley. Elevated $[CO_2]$ increased total biomass (11%) and grain yield (40%) of subsequent wheat crop

regardless of rotation type in the first phase. Irrespective of [CO_2], the grain yield and total N uptake by wheat following field pea were 24% and 11%, respectively, higher than those of the wheat following N-fertilized barley. The residual N contribution from field pea to wheat was 20% under ambient [CO_2], but dropped to 11% under elevated [CO_2], while that from fertilizer did not differ significantly between ambient [CO_2] (4%) and elevated [CO_2] (5%).

Conclusions

The relative value of legume derived N to subsequent cereals may be reduced under elevated [CO_2]. However, compared to N fertilizer application, legume incorporation will be more beneficial to grain yield and N supply to subsequent cereals under future (elevated [CO_2]) climates.

Keywords

Elevated [CO_2], ^{15}N labeling, below-ground legume N, residual legume N, residual fertilizer N

Introduction

Atmospheric carbon dioxide concentration ([CO_2]) has increased from 280 μmol mol^{-1} at the beginning of the Industrial Revolution to the current level of 392 μmol

56 mol^{-1} (NOAA 2012). If atmospheric CO_2 emissions continue at their present rate the
57 CO_2 concentration is estimated to reach about 700 μmol mol^{-1} by 2100 (IPCC 2007).
58 Elevated [CO_2] generally stimulates plant photosynthetic processes, often resulting in
59 increased crop growth and yield (Kimball 1983; Drake et al. 1997; Ainsworth and
60 Long 2005; Long et al. 2006) if progressive nitrogen (N) limitation (Luo et al. 2004;
61 Reich et al. 2006) does not occur. Total N uptake by crops and N removal in grain
62 also generally increase under elevated [CO_2] (Kimball et al. 2002; Miyagi et al. 2007),
63 and this increase in N demand in cropping systems would be expected to gradually
64 reduce soil N reserves unless replenished.

65 Depletion of soil N in agroecosystems can be compensated by applying fertilizer
66 and N_2 fixation by legumes. Legume or fertilizer residues can contribute to
67 subsequent crops over time (Ladd and Amato 1986), and alleviate N limitation under
68 ambient [CO_2]. While elevated [CO_2] may affect the quantity, quality and
69 decomposition rate of crop residues (Torbert et al. 2000; Kimball et al. 2002), the
70 availability of residual legume and fertilizer N to subsequent crops under elevated
71 [CO_2] remains unclear.

72 The use of *in situ* ^{15}N feeding techniques provides an estimate of the total legume
73 below-ground N accumulating in a growing season, and allows the quantification of
74 this N to successive crops (Russell and Fillery 1996a, b; McNeill et al. 1997, 1998;

75 Khan et al. 2002a, b). Nitrogen rhizodeposited by legumes accounts for 4–71% of the
76 plant N under ambient [CO_2] (Fustec et al. 2010). While an increase in N
77 rhizodeposition (29–31%) has been observed under elevated [CO_2] in wheat and
78 perennial ryegrass (de Graaff et al. 2007; Bazot et al. 2008; Schulze and Merbach
79 2008), the effect of elevated [CO_2] on N rhizodeposition by legumes has rarely been
80 studied. Symbiotic N_2 fixation is generally increased under elevated [CO_2] (Rogers et
81 al. 2009). This, together with [CO_2]-induced increase in legume above-ground and
82 below-ground biomass (Kimball et al. 2002; Ainsworth and Long 2005), suggests that
83 the availability of N in legume residue for subsequent crop growth will likely be
84 higher under future elevated CO_2 atmospheres.

85 Fertilizer N recovery by crops rarely exceeds 40% under ambient [CO_2] (Chen et
86 al. 2008; Gardner and Drinkwater 2009), and its contribution to the first succeeding
87 cereal crop typically ranges from 2 to 5% (Ladha et al. 2005). The few published
88 studies indicate that the effect of elevated [CO_2] on fertilizer N recovery in cereals
89 was either positive (Martín-Olmedo et al. 2002; Weerakoon et al. 2005) or neutral
90 (Torbert et al. 2004; Kim et al. 2011; Lam et al. 2012). The remobilization of
91 fertilizer-derived N into grain has been shown to increase under elevated [CO_2] (Kim
92 et al. 2011). The contribution of fertilizer residue to subsequent crops may therefore
93 be lower under future CO_2 climates.

94 To our knowledge, no previous study has been conducted to compare the
95 contribution of legume and fertilizer residue N to a subsequent crop under elevated
96 [CO_2]. Such information is critical for N management practice for soil N
97 replenishment under elevated [CO_2]. The objective of this study was to investigate the
98 interactive effects of elevated [CO_2] and residual N (legume or fertilizer N) on
99 subsequent wheat growth and N uptake. We hypothesized that elevated [CO_2]
100 increases crop growth (crop residues input to subsequent rotation phase), and that
101 [CO_2]-induced changes in the quantity and quality of crop residues affect the growth
102 and N uptake of a subsequent crop. We predict that the relative impact of legume
103 residues should increase under elevated [CO_2] whereas that of fertilizer residues should
104 decrease under elevated [CO_2].
105
106 **Materials and methods**
107 *Glasshouse chambers*
108 This study was conducted between September 2010 and April 2011, in soil in pots in a
109 set of four naturally lighted glasshouse chambers (3.1 m long × 2.4 m wide × 2.6 m
110 high) located at the Department of Primary Industries Grains Innovation Park in
111 Horsham (36°43’ S, 142°10’ E), Victoria, Australia. Two glasshouse chambers had
112 ambient [CO_2] (390 μmol mol^{-1}), and two had elevated [CO_2] (700 μmol mol^{-1}). Each

pair of [CO_2] treatment chambers was considered to be a block. The elevated [CO_2] treatment was created by adding pure CO_2 to a mixing fan within the chambers, and the [CO_2] was monitored using an infra red gas analyser (Guardian SP 97301, Edinburgh Instruments Ltd). Air temperature in each chamber was measured by a temperature sensor (DS1923 Hygrochron iButton, Thermodata Pty Ltd) that was mounted 15 cm above the plant canopy. The average day/night temperature of the chamber throughout the growing season was 24/21°C.

Experimental design

The effect of elevated [CO_2] on the contribution of residual legume N and residual fertilizer N from a previous barley crop to subsequent wheat crops was investigated in pots in a glasshouse under three different 2-phase rotations, viz. field pea-wheat, N-fertilized barley-wheat, and barley (no fertilizer)-wheat (control). In rotation phase 1 we determined the effect of elevated [CO_2] on the accumulation of legume N and the partitioning of N derived from fertilizer in the crop-soil system. In rotation phase 2 we examined the recovery of residual legume N and fertilizer N by a following wheat crop under ambient or elevated [CO_2]. Residual legume N refers to N derived from field pea residues. Residual fertilizer N refers to N derived from fertilizer residues and barley residues i.e. direct soil-derived fertilizer residues plus indirect

132 barley-derived fertilizer residues, which will be referred to as fertilizer residues
133 throughout the text. The experimental design was two CO_2 concentrations × three
134 rotation types × two rotation phases, with four replications, totaling 48 pots (PVC
135 tubes, 15 cm diameter, 45 cm deep, sealed at the bottom). The four replicates in both
136 experiments were subdivided into two groups of duplicates and put to each block of
137 [CO_2]. The PVC pots within each chamber were completely randomized.
138
139 *Soil and PVC pot preparation*
140 A Vertosol soil (Isbell 1996) was collected from the plough layer (top 20 cm) of an
141 undisturbed area 8 km south-west of Horsham. The soil had a pH (soil:water ratio of
142 1:5) of 8.10, and contained 1.10% organic C, 0.12% total N, 2.0 mg ammonium-N
143 kg^{-1}, 4.1 mg nitrate-N kg^{-1}, 7 mg Colwell P kg^{-1}, 630 mg available K kg^{-1} and 37%
144 clay. The soil was air dried, crushed into < 1 cm fractions, and mixed thoroughly. Any
145 visible plant residues were picked out manually and discarded.
146 The 48 PVC pots were filled with 7.4 kg (dry weight) of the Vertosol. Field
147 capacity of the soil was determined and the soil was re-wetted to 80% of field
148 capacity before sowing. To ensure the soil was wet evenly throughout the column, the
149 7.4 kg soil was added in three portions of 1.8 kg (equivalent to 9 cm depth of soil) and
150 a top portion of 2.0 kg (equivalent to 10 cm depth of soil), and each portion was

151 followed by the addition of reverse osmosis water. A basal nutrient application of
152 35.3 mg P pot^{-1} (as $NaH_2PO_4 \cdot 2H_2O$, equivalent to 20 kg P ha^{-1}), 4.4 mg Cu pot^{-1} (as
153 $CuSO_4 \cdot 5H_2O$, equivalent to 2.5 kg Cu ha^{-1}) and 8.8 mg Zn pot^{-1} (as $ZnSO_4 \cdot 7H_2O$,
154 equivalent to 5 kg Zn ha^{-1}) was added to the top 2.0 kg portion, and the contents of
155 the top portion were thoroughly mixed.
156
157 *Rotation phase 1—Plant cultivation and ^{15}N-labeling*
158 In the first phase, five seeds of field pea (*Pisum sativum* L. cv. Kaspa; a semi-leafless
159 field pea cultivar) were sown to the PVC pots of field pea-wheat rotation on 6
160 September 2009. The seeds of field pea were inoculated with a commercial inoculant
161 (Becker Underwood Pty Ltd) following the manufacturer's instructions. Ten days
162 after emergence, field pea seedlings were thinned to two per pot based on sowing
163 density in the field. The thinned plants were returned to the soil. All the pots were
164 watered with reverse osmosis water to constant weight (80% of field capacity) every
165 two to three days. Field pea plants were labeled with ^{15}N urea (3 mL, 0.5% w/w urea,
166 98.26 atom% ^{15}N) on 34 days (growth stage: 12th node), 42 days (16th node) and 49
167 days (20th node) after emergence. The tip (2 mm) of two tendrils per field pea plant
168 was cut off and the remainder of the tendrils was immersed in the labeling solution in
169 sealed ziplock bags (4 cm by 6 cm) for 24 hours, by which time the solution was

mostly absorbed (de Graaff et al. 2007). The ziplock bags were attached to the petiole of the tendrils by Blutack. The quantity of labeling solution absorbed by the plants (93–96%) was determined by weighing the solution in the ziplock bags before and after the labeling process using a 5-decimal digital balance. No loss of solution due to evaporation was detected from control ziplock bags containing 3 mL of solution without tendrils.

Ten seeds of barley (*Hordeum vulgare* L. cv. Gairdner) were sown to the PVC pots of barley-wheat rotation on the same day when field pea seeds were sown. ^{15}N-labeled urea (10.22 atom% ^{15}N) was applied to the PVC pots at 50 or 0 kg N ha^{-1} as a band with the seeds to minimize losses by volatilization. Ten days after emergence, barley seedlings were thinned to four per pot. The thinned plants were returned to the soil. All the pots were watered with reverse osmosis water to constant weight (80% of field capacity) every two to three days.

Sample collection and preparation

All plants in each pot were harvested when leaves were completely senesced and grain was fully developed and ripened, approximately 90 days after emergence. The plants were cut at ground level to separate the above-ground parts from the bulk soil, and the above-ground parts were separated into grain and shoot (stem and leaf). The

189 entire 0–10 cm and 10–37 cm soil layers from half of the 48 PVC pots were removed.
190 Soil samples were weighed and ground to less than 2 mm diameter. For both soil
191 layers, root materials were collected and separated into clean roots (after brushing
192 away the soil particles) and very fine roots which were mixed with the bulk soil. To
193 reduce sampling error due to large bulk soil weight, triplicate subsamples of the bulk
194 soil were taken for subsequent analysis. The plant and soil samples were oven dried at
195 60°C and 40°C, respectively, for 48 hours and weighed. The dried plant and soil
196 material was ground (in a ball mill; TissueLyser II, QIAGEN) to yield a fine powder
197 of ~100 μm. The control pots (barley) were treated as described above except the
198 plants were not ^{15}N-labeled. These pots provided background atom% ^{15}N values for
199 calculating excess ^{15}N of the labeled plant and soil material.
200
201 *Rotation phase 2—Wheat cultivation*
202 The dried shoot material obtained from the harvest of the first phase was cut into
203 segments of 2–3 cm length. Around 90% of this material was added on 16 December
204 to the 24 PVC pots that had not been destructively harvested. The remaining 10% of
205 the plant material was retained for chemical analysis to determine the amount of ^{15}N
206 added to rotation phase 2. The amount of residues incorporated corresponded to 4.1,
207 5.9 and 4.1 t ha^{-1} (on an area basis) for field pea-wheat rotation, ^{15}N-fertilized

208 barley-wheat rotation and unfertilized barley-wheat rotation, respectively, under
209 ambient [CO_2], and 5.1, 6.3 and 4.3 t ha^{-1} under elevated [CO_2]. The soil and root
210 (0–10 cm) was lightly 'ploughed' to incorporate the residue material into the top 10
211 cm of soil to simulate field practice. Reverse osmosis water was added to each pot to
212 constant weight (80% of field capacity) every week for one month before sowing of a
213 subsequent crop. This simulated the fallow period for residue decomposition between
214 crop harvest and the sowing of a subsequent crop. After a wetting / drying cycle of
215 one month, ten seeds of wheat (*Triticum aestivum* L. cv. Yitpi) were sown on the soil
216 on 15 January 2011. No fertilizers were applied in this 2nd phase. Ten days after
217 emergence, plant seedlings were thinned to four per pot. All the pots were watered
218 with reverse osmosis water to constant weight (80% of field capacity) every two to
219 three days.
220
221 *Sample collection and preparation*
222 All plants in each pot were harvested when leaves were completely senesced and
223 grain was fully developed and ripened (approximately 80 days after emergence). The
224 plants were cut at ground level to separate the above-ground parts from the bulk soil,
225 and the above-ground parts were separated into grain and shoot. The entire 0–10 cm
226 and 10–37 cm soil layers were removed from the pots. Soil samples were weighed,

crushed to particles less than 2 mm in diameter, and sieved through a 2 mm sieve to remove undecomposed residue from the first phase. For the top 0–10 cm soil layer, coarse root material was separated manually, cleaned by brushing away the soil particles, and a sample was taken for analysis. The fine roots were mixed thoroughly with the soil, and sample of the mixture was taken for analysis. No wheat roots were picked from the 10–37 cm layer because wheat roots and roots from the previous crop were not easily differentiated. The plant and soil material was oven dried at 60°C and 40°C, respectively, for 48 hours and weighed. The dried plant and soil material was ground (in a ball mill; TissueLyser II, QIAGEN) to yield a fine powder of ~100 μm.

Chemical analysis and ^{15}N calculations

The finely ground plant and soil samples in both phases were analyzed for total C, total N and $\delta^{15}N$ values by isotope ratio mass spectrometry (IRMS) (Hydra 20-20, SerCon). Grain protein concentration of wheat was estimated by multiplying grain N concentration by 5.7 (Jones 1941).

Legume root-derived N in the bulk soil ($N_{bulk\ soil}$) in the first rotation phase was estimated by mass of $^{15}N_{excess\ bulk\ soil}$ divided by specific enrichment of clean roots (μg $^{15}N_{excess\ clean\ root}$ / mg root N) (Khan et al. 2002a). Total below-ground legume N ($N_{below\text{-}ground}$) was calculated as the sum of measured clean root N ($N_{clean\ root}$), and

246 estimated legume root-derived N in soil, i.e. $N_{below\text{-}ground} = N_{clean\ root} + N_{bulk\ soil}$ (Russell
247 and Fillery 1996b; Khan et al. 2002a). The major assumptions of these calculations
248 are that any excess ^{15}N in the soil is derived only from the ^{15}N-enriched legume root
249 and that any root-derived N in the below-ground pools has the same specific ^{15}N
250 enrichment as the clean root material (Khan et al. 2002a; McNeill and Fillery 2008).
251 Enrichments (atom% ^{15}N excess) were calculated by correcting measured atom% ^{15}N
252 values with background atom% ^{15}N values of corresponding non-labeled barley plant
253 and soil fractions. The percentages of ^{15}N applied that were recovered in crop and soil
254 were calculated according to Hauck and Bremner (1976).

255 The percentage of residual field pea N or fertilizer N in the first phase recovered
256 by the subsequent wheat ($\%N_{wheat}$) was calculated as: $\%N_{wheat} = \mu g\ ^{15}N_{excess\ wheat} / \mu g$
257 $^{15}N_{excess\ residual\ N} \times 100$, where $\mu g\ ^{15}N_{excess\ wheat}$ is the total excess ^{15}N content of the
258 subsequent wheat, and $\mu g\ ^{15}N_{excess\ residual\ N}$ the excess ^{15}N content of estimated total
259 residual legume N or fertilizer N in the first phase (Rees et al. 1993; Williams et al.
260 2000; McNeill 2001).

261

262 *Statistical analysis*

263 Data were analyzed with MINITAB 16 statistical package using a General Linear
264 Model analysis of variance with a level of significance of $p < 0.05$ unless otherwise

stated. Data were tested for normality and $\log_e$-transformed where necessary to equalize variances between treatments.

Results

Rotation phase 1

Total biomass

Elevated [CO_2] increased ($p < 0.001$) the total biomass of field pea and N-fertilized barley by 21% and 23%, respectively, but the [CO_2]-induced increase (9%) in the total biomass of barley receiving no N fertilizer was not significant (Table 1).

>> Insert Table 1 near here>>

N uptake

Increasing [CO_2] resulted in a 25% increase in total N uptake by field pea ($p < 0.001$), but had no effect on total N uptake by N-fertilized barley and reduced that of unfertilized barley by 10% ($p < 0.001$) (Table 1).

The specific enrichment of clean root fraction for ^{15}N-fed field pea was in the range of 7.4–8.4 and 6.3–7.2 at 0–10 cm and 10–37 cm soil depth, respectively (Table

284 2). The estimated root-derived N at the corresponding soil depth was 40–56 and
285 34–39 mg N pot^{-1}. Elevated [CO_2] had no significant effect on these parameters
286 (Table 2). The total below-ground N of field pea represented 18% and 19% of the
287 total plant N under ambient and elevated [CO_2], respectively. These values were not
288 significantly different (Table 2).

289 For N-fertilized barley, the recovery of fertilizer N in the plant was in the range
290 of 48–49% while that in the soil ranged from 31–33% (25% in the 0–10 cm soil layer;
291 6–8% in the 10–37 cm soil layer). Elevated [CO_2] was associated with a marginally
292 significant increase in the N recovery in grain from 33.8 to 35.3% (p = 0.07), but did
293 not significantly affect the recovery of fertilizer N in other plant parts or in the soil.

294

295 >> Insert Table 2 near here>>

296

297 *Residue C:N ratio*

298 Elevated [CO_2] increased the C:N ratio of field pea and ^{15}N-fertilized barley residues
299 by 18% and 19%, respectively, but had no significant effect on that of unfertilized
300 barley residues (Table 1).

301

302 *Rotation phase 2*

303

304 *Wheat biomass*

305 Elevated [CO_2] increased total biomass (11%, $p < 0.001$) and grain yield (40%, $p <$

306 0.001) of wheat regardless of rotation type in the first phase, but had no significant

307 effect on the shoot and root biomass of wheat (Fig. 1). When averaged across [CO_2],

308 the grain yield and shoot biomass of the wheat following field pea were 24% ($p <$

309 0.001) and 21% greater ($p < 0.001$), respectively, than those of the wheat following

310 N-fertilized barley. When compared to the wheat following barley which received no

311 N fertilizer, N application to barley in the first phase did not significantly affect the

312 grain yield and shoot biomass of the subsequent wheat (Fig. 1).

313

314 >> Insert Fig. 1 near here>>

315

316 *Wheat N uptake*

317 There was a marginally significant ($p = 0.1$) interaction between elevated [CO_2] and

318 previous rotation phase on total N uptake by wheat: elevated [CO_2] increased the total

319 N uptake by wheat following N-fertilized barley (11%), but had no significant effect

320 on the N uptake by wheat following field pea or unfertilized barley (Fig. 2). When

321 averaged across the rotation treatments, elevated [CO_2] increased wheat grain N

content by 15% ($p < 0.001$), but reduced the shoot N content by 27% ($p < 0.001$) and root N content 26% ($p < 0.01$). When averaged across [CO_2] treatments, total N uptake by wheat following field pea was 11% higher ($p < 0.001$) than that grown after N-fertilized barley (Fig. 2).

>> Insert Fig. 2 near here>>

Grain protein concentration

Both elevated [CO_2] and previous rotation type had a significant effect on grain protein concentration of wheat (Table 3). Irrespective of rotation type, elevated [CO_2] had a significant negative effect on grain protein concentration but [CO_2]-induced reductions were greater for wheat following unfertilized barley than for wheat following field pea and N-fertilized barley (significant [CO_2] × species interaction, Table 3). When averaged across [CO_2], the grain protein concentration of the wheat crop grown after N-fertilized barley (16.2%) was higher than that grown after field pea (13.7%) and unfertilized barley (14.3%) (Table 3).

>> Insert Table 3 near here>>

341 *Recovery and distribution of ^{15}N in wheat*

342 The amount of excess ^{15}N added from field pea residues in the previous phase
343 was 33% greater ($p < 0.05$) under elevated [CO_2] than under ambient [CO_2], whereas
344 excess ^{15}N added from fertilizer residues did not differ significantly between [CO_2]
345 treatments (Table 4). The amount of ^{15}N recovered in wheat parts followed the order
346 grain > shoot > root, irrespective of [CO_2] treatment (Table 4).

347

348 >> Insert Table 4 near here>>

349

350 *Contribution of residual legume and fertilizer N to wheat*

351 For soils which had been amended with legume material, wheat grain, shoot and
352 upper root contained 9–15%, 2–5% and 0.3–0.5%, respectively, of the total residual
353 ^{15}N that was in the soil at sowing. For soils which had received fertilizer ^{15}N for the
354 previous barley crop, ^{15}N in wheat grain, shoot and upper root accounted for 3–4%,
355 0.7–1.0% and 0.1–0.2%, respectively, of the residual ^{15}N remaining in the soil when
356 the wheat was planted (Table 4).

357 Elevated [CO_2] reduced the proportion of residual legume N recovered by grain,
358 shoot and upper root of the wheat crop by 40, 56 and 38%, respectively (Table 4).
359 However, increasing [CO_2] had no significant effect ($p > 0.05$) on the residual N

360 contribution from fertilizer to wheat parts (Table 4). The above-ground parts of wheat
361 following field pea represented 20% of the ^{15}N present at sowing under ambient [CO_2]
362 and 11% under elevated [CO_2]. These percent recoveries were significantly greater
363 than those of the wheat following N-fertilized barley under ambient [CO_2] (4%) and
364 elevated [CO_2] (5%) (Table 4).
365
366 ***Discussion***
367 In the first phase of the rotation, the [CO_2] fertilization effects on total biomass of
368 field pea and N-fertilized barley were within the range of growth responses of various
369 crops to elevated [CO_2] (Kimball et al. 1983; Cure and Acock 1986; Jablonski et al.
370 2002). However, the total biomass of barley grown did not respond to elevated [CO_2]
371 without the application of fertilizer N. Similar N-dependent growth response of barley
372 to elevated [CO_2] was also observed by Thompson and Woodward (1994) and
373 Fangmeier et al. (2000). In our study, the N-dependent effect of growth response to
374 elevated [CO_2] was associated with plant N uptake. The reduction in plant N uptake
375 by unfertilized barley under elevated [CO_2] indicates that soil N availability was
376 insufficient to meet plant demand under elevated [CO_2]. A declining N availability
377 has previously been shown to eliminate the [CO_2]-induced enhancement in leaf area
378 index of cereal (Kim et al. 2003), thereby restricting the CO_2 fertilization effect on

379 plant growth. In contrast, we found that this limitation of CO_2 fertilization effect on
380 growth did not occur when N fertilizer was applied to barley or for field pea, which
381 could source its N via N_2 fixation. A review by Reich et al. (2006) suggests that a
382 general N limitation of biomass accumulation to elevated [CO_2] effect is common,
383 although not ubiquitous. Intense competition between plants and microbes for soil N
384 can restrict plant growth responses to elevated [CO_2] (Gill et al. 2002). Improved N
385 management strategies will therefore be needed to sustain crop growth (and residue
386 input) and account for the additional grain N removal under future elevated CO_2
387 atmospheres.

388 Many cropping systems, especially those where fertilizer N inputs are relatively
389 low by international standards due to low and unreliable rainfall that dominate much
390 of Australia, rely heavily on legume N_2 fixation to supply N to subsequent cereal
391 cropping phases. The supply of residual N to subsequent crop depends on various
392 biotic and abiotic factors including the quantity and quality of residue, and the
393 interaction between plants and microbes under elevated [CO_2] (Reich et al. 2006).
394 Previous work, often focusing on soybeans and pasture legumes, showed that legume
395 biomass and N_2 fixation increase under elevated [CO_2] (e.g. Soussana and Hartwig
396 1996; Kimball et al. 2002; Rogers et al. 2009). We found that elevated [CO_2]
397 increased the amount of residues produced (and incorporated) from the preceding

398 field pea but that this residue also had a greater C:N ratio. This suggests that soil N
399 availability to the subsequent wheat crop was reduced under elevated [CO_2], similar
400 to that observed by others (Hungate et al. 1997; de Graaff et al. 2006). This increase
401 in N immobilization partly explains why whole plant N uptake of the subsequent
402 wheat was not higher under elevated [CO_2], despite a potentially greater amount of N
403 in the soil system originating from the field pea residues. This phenomenon could be
404 also be related to a decrease in the uptake rates of N by unit mass or length of roots
405 under elevated [CO_2] (Taub and Wang 2008). Another possible explanation is a
406 reduced rate of decomposition of crop residues (including legumes) observed in other
407 studies under elevated [CO_2] (Torbert et al. 2000). As a result, the contribution of field
408 pea N to subsequent wheat, at least in the short term, was lower under elevated [CO_2]
409 in our study.

410 The contribution of field pea N to subsequent wheat N (11–20%) was within the
411 range of the recovery of N contained in grain legume residues (2–27%) by various
412 first succeeding crops (Fillery 2001). Although reduced under elevated [CO_2], the
413 contribution of field pea residue N to subsequent wheat (11%) was significantly
414 greater than that of fertilizer residue N (5%) in our study. While soil microbes prefer
415 higher quality substrates (Kuzyakov 2002), the lower C:N ratio of the field pea
416 residues than that of barley residues may explain the difference in residual

417 contribution between field pea N and fertilizer N. Unaffected by elevated [CO_2], the
418 recovery of residual fertilizer N by wheat in the present study was within the range of
419 the generally low fertilizer N recovery (2–5%) in the first succeeding crop under
420 various N application methods and residue management practices reported by Ladha
421 et al. (2005). Furthermore, we found that the grain yield of wheat following field pea
422 was greater than that of wheat following N-fertilized barley, regardless of [CO_2].
423 These results suggest that N fertilizer application does not have a substantial residual
424 effect, whereas that derived from legume residue N is a more accessible N source for
425 subsequent crops, especially over the longer term (Ladd and Amato 1986).

426 The negative effect of elevated [CO_2] on grain protein concentration, consistent
427 with the quantitative reviews by Jablonski et al. (2002) and Taub et al. (2008), may
428 represent a 'dilution effect' (Loladze 2002) and/or the adverse effect of elevated [CO_2]
429 on nitrate assimilation in wheat (Bloom et al. 2010); this warrants further study.
430 Although the grain protein concentration of wheat grown after field pea was lower
431 than that of wheat grown after N-fertilized barley, grain protein concentration of all
432 treatments was greater than the market requirement of 12% protein in Australia
433 (Department of Primary Industries 2008). This suggests that residual legume N and
434 fertilizer N will be sufficient for at least the first succeeding wheat crop to be
435 marketable under future CO_2 climates.

436

437 **Conclusions**

438 Elevated [CO_2] increased N accumulation by field pea but decreased fertilizer N
439 reserves in the soil after growing barley. Elevated [CO_2] reduced the recovery in
440 succeeding wheat of N contained in field pea residue, but did not significantly affect
441 the recovery of fertilizer residue N. Field pea residue N contributed more to
442 subsequent wheat N than fertilizer residue N irrespective of [CO_2]. The grain yield
443 and N uptake by the wheat following field pea were greater than that for the wheat
444 following N-fertilized barley. These results suggest that N fertilizer application has a
445 minimal residual value, and that including a legume in a crop rotation will provide
446 more N and enhance yield of a subsequent crop under future CO_2 environments.

447

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611

612 **Figure captions**

613 **Fig. 1** Wheat biomass (total and different plant parts) following field pea,

614 N-fertilized barley, and unfertilized barley under ambient [CO_2] and elevated [CO_2] in

615 rotation phase 2. Values are means of the four replicates for each treatment. Vertical

616 bars indicate standard errors. Significant effects are indicated as ***$p < 0.001$. NS,

617 not significant

618

619 **Fig. 2** Wheat N uptake (total and different plant parts) following field pea,

620 N-fertilized barley, and unfertilized barley under ambient [CO_2] and elevated [CO_2] in

621 rotation phase 2. Values are means of the four replicates for each treatment. Vertical

622 bars indicate standard errors. Significant effects are indicated as ***$p < 0.001$, **$p <$

623 0.01 and *$p < 0.05$ and. NS, not significant

624

Table 1 The effect of elevated [CO_2] on total biomass, total plant N and residue C:N ratio of field pea, N-fertilized barley and unfertilized barley in rotation phase 1. Values are means of the four replicates for each treatment

	^{15}N-fed field pea		^{15}N-fertilized barley		unfertilized barley		[CO_2]	Species	[CO_2] × specie
	Ambient [CO_2]	Elevated [CO_2]	Ambient [CO_2]	Elevated [CO_2]	Ambient [CO_2]	Elevated [CO_2]			
Total biomass (g pot^{-1})	16.9	20.4	18.2	22.3	13.3	14.5	***	***	**
Total plant N (mg N pot^{-1})	421.3	525.5	146.8	151.6	90.2	81.3	**	***	***
C:N ratio	44.1	51.9	114.1	135.4	129.3	127.1	*	***	0.08

Significant effects are indicated as ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$

Table 2 The amount of N and excess ^{15}N in the root and the soil, and specific enrichment of clean root fraction for ^{15}N-fed field pea at two soil depths in rotation phase 1. Values are means of the four replicates for each treatment. All ^{15}N values expressed as atom excess

	0–10 cm			10–37 cm		
	Ambient $[CO_2]$	Elevated $[CO_2]$		Ambient $[CO_2]$	Elevated $[CO_2]$	
Clean root						
N (mg pot^{-1})	2.14	2.60	0.07	1.98	2.82	*
^{15}N (μg pot^{-1})	18.9	18.9	NS	14.8	17.4	NS
Specific enrichment (μg ^{15}N/mg N)	8.43	7.37	NS	7.20	6.26	NS
Soil						
N (mg pot^{-1})	1675	1631	NS	4603	4724	*
^{15}N (μg pot^{-1})	333.8	404.1	NS	240.7	245.7	NS
Root-derived N (mg pot^{-1})	39.9	55.7	NS	33.7	39.4	NS
Below-ground N (% of total N)	9.9	11.2	NS	8.4	8.0	NS

Significant effects are indicated as $*p < 0.05$. NS, not significant

636 **Table 3** Grain protein concentration of wheat following field pea, N-fertilized barley and
637 unfertilized barley. Values are means of the four replicates for each treatment

	^{15}N-fed field pea		^{15}N-fertilized barley		unfertilized barley		[CO_2]	Species	S
	Ambient [CO_2]	Elevated [CO_2]	Ambient [CO_2]	Elevated [CO_2]	Ambient [CO_2]	Elevated [CO_2]			
Protein concentration (%)	15.1	12.3	17.2	15.3	16.1	12.4	***	***	

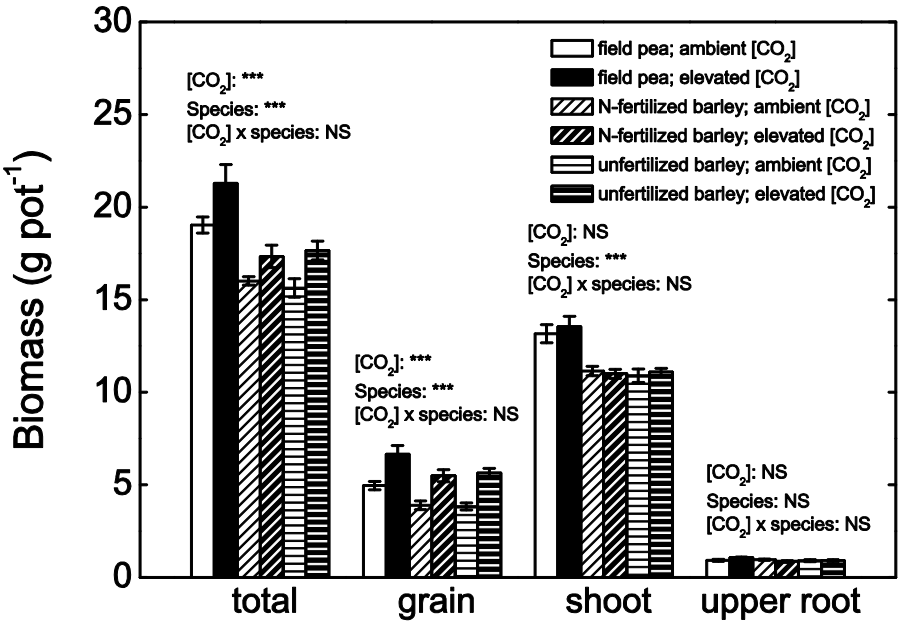
638 Significant effects are indicated as ***$p < 0.001$ and *$p < 0.05$

639

640 **Table 4** The amount of ^{15}N excess and percent recovery of residue N in various parts of wheat
641 following field pea and N-fertilized barley. Values are means of the four replicates for each treatment

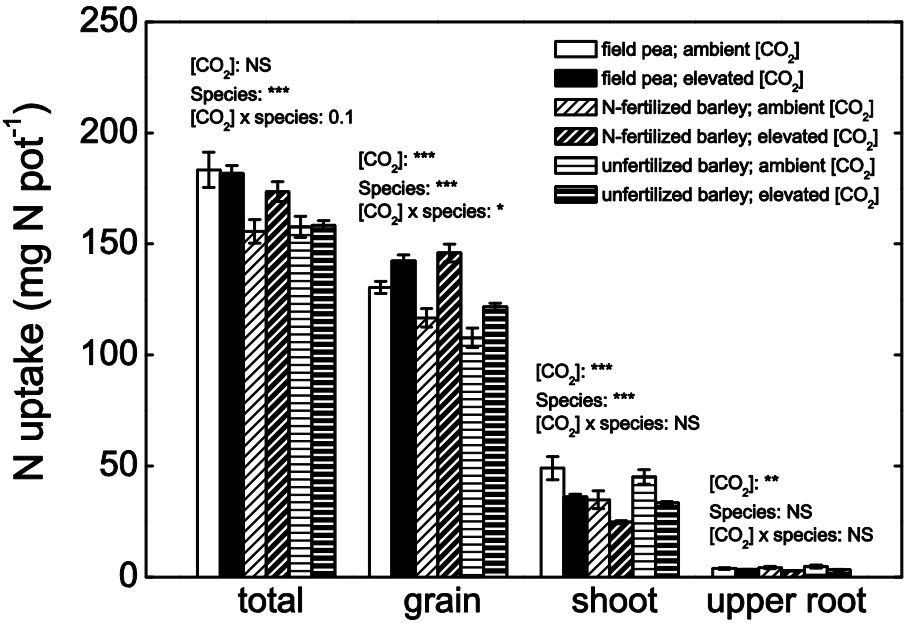
	^{15}N-fed field pea		^{15}N-fertilized barley		$[CO_2]$	Species	$[CO_2]$ × species
	Ambient $[CO_2]$	Elevated $[CO_2]$	Ambient $[CO_2]$	Elevated $[CO_2]$			
Total excess ^{15}N added from phase 1 (mg)	9.30	12.33	4.01	3.75	0.08	***	*
Grain							
^{15}N (mg)	1.37	1.05	0.11	0.15	0.07	***	*
% recovery	14.84	8.95	2.80	3.90	*	***	***
Shoot							
^{15}N (mg)	0.475	0.260	0.039	0.024	*	***	*
% recovery	5.01	2.22	0.97	0.65	***	***	**
Clean root (0–10 cm)							
^{15}N (μg)	48.9	37.8	6.4	5.0	0.06	***	NS
% recovery	0.52	0.32	0.16	0.13	***	***	**
Recovery in above-ground parts (%)	19.8	11.2	3.8	4.6	**	***	***

642 Significant effects are indicated as ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$. NS, not significant
643


Biomass (g pot-1)
30
25
20
15
10
5
0
field pea; ambient [CO2]
field pea; elevated [CO2]
N-fertilized barley; ambient [CO2]
N-fertilized barley; elevated [CO2]
unfertilized barley; ambient [CO2]
unfertilized barley; elevated [CO2]
[CO2]: ***
Species: ***
[CO2] x species: NS
[CO2]: ***
Species: ***
[CO2] x species: NS
[CO2]: NS
Species: ***
[CO2] x species: NS
[CO2]: NS
Species: NS
[CO2] x species: NS
total
grain
shoot
upper root

644

645


N uptake (mg N pot-1)
250
200
150
100
50
0
[CO2]: NS
Species: ***
[CO2] x species: 0.1
[CO2]: ***
Species: ***
[CO2] x species: *
[CO2]: ***
Species: ***
[CO2] x species: NS
[CO2]: **
Species: NS
[CO2] x species: NS
field pea; ambient [CO2]
field pea; elevated [CO2]
N-fertilized barley; ambient [CO2]
N-fertilized barley; elevated [CO2]
unfertilized barley; ambient [CO2]
unfertilized barley; elevated [CO2]
total
grain
shoot
upper root

646