1 **Title:**

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

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16

17

18 **Abstract**

19 *Purpose*

20 This study investigated the residual contribution of legume and fertilizer nitrogen (N)
21 to a subsequent crop under the effect of elevated carbon dioxide concentration
22 ([CO₂]).

23 *Methods*

24 Field pea (*Pisum sativum* L.) was labeled *in situ* with ¹⁵N (by absorption of a
25 ¹⁵N-labeled urea solution through cut tendrils) under ambient and elevated (700 μmol
26 mol⁻¹) [CO₂] in controlled environment glasshouse chambers. Barley (*Hordeum*
27 *vulgare* L.) and its soil were also labeled under the same conditions by addition of
28 ¹⁵N-enriched urea to the soil. Wheat (*Triticum aestivum* L.) was subsequently grown
29 to physiological maturity on the soil containing either ¹⁵N-labeled field pea residues
30 (including ¹⁵N-labeled rhizodeposits) or ¹⁵N-labeled barley plus fertilizer ¹⁵N residues.

31 *Results*

32 Elevated [CO₂] increased the total biomass of field pea (21%) and N-fertilized barley
33 (23%), but did not significantly affect the biomass of unfertilized barley. Elevated
34 [CO₂] increased the C:N ratio of residues of field pea (18%) and N-fertilized barley
35 (19%), but had no significant effect on that of unfertilized barley. Elevated [CO₂]
36 increased total biomass (11%) and grain yield (40%) of subsequent wheat crop

37 regardless of rotation type in the first phase. Irrespective of [CO₂], the grain yield and
38 total N uptake by wheat following field pea were 24% and 11%, respectively, higher
39 than those of the wheat following N-fertilized barley. The residual N contribution
40 from field pea to wheat was 20% under ambient [CO₂], but dropped to 11% under
41 elevated [CO₂], while that from fertilizer did not differ significantly between ambient
42 [CO₂] (4%) and elevated [CO₂] (5%).

43 *Conclusions*

44 The relative value of legume derived N to subsequent cereals may be reduced under
45 elevated [CO₂]. However, compared to N fertilizer application, legume incorporation
46 will be more beneficial to grain yield and N supply to subsequent cereals under future
47 (elevated [CO₂]) climates.

48

49 **Keywords**

50 Elevated [CO₂], ¹⁵N labeling, below-ground legume N, residual legume N, residual
51 fertilizer N

52

53 **Introduction**

54 Atmospheric carbon dioxide concentration ([CO₂]) has increased from 280 μmol
55 mol⁻¹ at the beginning of the Industrial Revolution to the current level of 392 μmol

56 mol⁻¹ (NOAA 2012). If atmospheric CO₂ emissions continue at their present rate the
57 CO₂ concentration is estimated to reach about 700 μmol mol⁻¹ by 2100 (IPCC 2007).
58 Elevated [CO₂] 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 [CO₂] (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 N₂ 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 [CO₂]. While elevated [CO₂] 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 [CO₂] remains unclear.

72 The use of *in situ* ¹⁵N 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 [CO₂] (Fustec et al. 2010). While an increase in N
77 rhizodeposition (29–31%) has been observed under elevated [CO₂] in wheat and
78 perennial ryegrass (de Graaff et al. 2007; Bazot et al. 2008; Schulze and Merbach
79 2008), the effect of elevated [CO₂] on N rhizodeposition by legumes has rarely been
80 studied. Symbiotic N₂ fixation is generally increased under elevated [CO₂] (Rogers et
81 al. 2009). This, together with [CO₂]-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 CO₂ atmospheres.

85 Fertilizer N recovery by crops rarely exceeds 40% under ambient [CO₂] (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 [CO₂] 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 [CO₂] (Kim
92 et al. 2011). The contribution of fertilizer residue to subsequent crops may therefore
93 be lower under future CO₂ climates.

To our knowledge, no previous study has been conducted to compare the contribution of legume and fertilizer residue N to a subsequent crop under elevated [CO₂]. Such information is critical for N management practice for soil N replenishment under elevated [CO₂]. The objective of this study was to investigate the interactive effects of elevated [CO₂] and residual N (legume or fertilizer N) on subsequent wheat growth and N uptake. We hypothesized that elevated [CO₂] increases crop growth (crop residues input to subsequent rotation phase), and that [CO₂]-induced changes in the quantity and quality of crop residues affect the growth and N uptake of a subsequent crop. We predict that the relative impact of legume residues should increase under elevated [CO₂] whereas that of fertilizer residues should decrease under elevated [CO₂].

Materials and methods

Glasshouse chambers

This study was conducted between September 2010 and April 2011, in soil in pots in a set of four naturally lighted glasshouse chambers (3.1 m long × 2.4 m wide × 2.6 m high) located at the Department of Primary Industries Grains Innovation Park in Horsham (36°43' S, 142°10' E), Victoria, Australia. Two glasshouse chambers had ambient [CO₂] (390 μmol mol⁻¹), and two had elevated [CO₂] (700 μmol mol⁻¹). Each

pair of [CO₂] treatment chambers was considered to be a block. The elevated [CO₂] treatment was created by adding pure CO₂ to a mixing fan within the chambers, and the [CO₂] 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 [CO₂] 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 [CO₂] 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 [CO₂]. 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

barley-derived fertilizer residues, which will be referred to as fertilizer residues throughout the text. The experimental design was two CO₂ concentrations × three rotation types × two rotation phases, with four replications, totaling 48 pots (PVC tubes, 15 cm diameter, 45 cm deep, sealed at the bottom). The four replicates in both experiments were subdivided into two groups of duplicates and put to each block of [CO₂]. The PVC pots within each chamber were completely randomized.

Soil and PVC pot preparation

A Vertosol soil (Isbell 1996) was collected from the plough layer (top 20 cm) of an undisturbed area 8 km south-west of Horsham. The soil had a pH (soil:water ratio of 1:5) of 8.10, and contained 1.10% organic C, 0.12% total N, 2.0 mg ammonium-N kg⁻¹, 4.1 mg nitrate-N kg⁻¹, 7 mg Colwell P kg⁻¹, 630 mg available K kg⁻¹ and 37% clay. The soil was air dried, crushed into < 1 cm fractions, and mixed thoroughly. Any visible plant residues were picked out manually and discarded.

The 48 PVC pots were filled with 7.4 kg (dry weight) of the Vertosol. Field capacity of the soil was determined and the soil was re-wetted to 80% of field capacity before sowing. To ensure the soil was wet evenly throughout the column, the 7.4 kg soil was added in three portions of 1.8 kg (equivalent to 9 cm depth of soil) and a top portion of 2.0 kg (equivalent to 10 cm depth of soil), and each portion was

followed by the addition of reverse osmosis water. A basal nutrient application of 35.3 mg P pot⁻¹ (as NaH₂PO₄·2H₂O, equivalent to 20 kg P ha⁻¹), 4.4 mg Cu pot⁻¹ (as CuSO₄·5H₂O, equivalent to 2.5 kg Cu ha⁻¹) and 8.8 mg Zn pot⁻¹ (as ZnSO₄·7H₂O, equivalent to 5 kg Zn ha⁻¹) was added to the top 2.0 kg portion, and the contents of the top portion were thoroughly mixed.

Rotation phase I—Plant cultivation and ¹⁵N-labeling

In the first phase, five seeds of field pea (*Pisum sativum* L. cv. Kaspia; a semi-leafless field pea cultivar) were sown to the PVC pots of field pea-wheat rotation on 6 September 2009. The seeds of field pea were inoculated with a commercial inoculant (Becker Underwood Pty Ltd) following the manufacturer's instructions. Ten days after emergence, field pea seedlings were thinned to two per pot based on sowing density in the field. 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. Field pea plants were labeled with ¹⁵N urea (3 mL, 0.5% w/w urea, 98.26 atom% ¹⁵N) on 34 days (growth stage: 12th node), 42 days (16th node) and 49 days (20th node) after emergence. The tip (2 mm) of two tendrils per field pea plant was cut off and the remainder of the tendrils was immersed in the labeling solution in 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. ¹⁵N-labeled urea (10.22 atom% ¹⁵N) 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

entire 0–10 cm and 10–37 cm soil layers from half of the 48 PVC pots were removed. Soil samples were weighed and ground to less than 2 mm diameter. For both soil layers, root materials were collected and separated into clean roots (after brushing away the soil particles) and very fine roots which were mixed with the bulk soil. To reduce sampling error due to large bulk soil weight, triplicate subsamples of the bulk soil were taken for subsequent analysis. The plant and soil samples were 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. The control pots (barley) were treated as described above except the plants were not ¹⁵N-labeled. These pots provided background atom% ¹⁵N values for calculating excess ¹⁵N of the labeled plant and soil material.

Rotation phase 2—Wheat cultivation

The dried shoot material obtained from the harvest of the first phase was cut into segments of 2–3 cm length. Around 90% of this material was added on 16 December to the 24 PVC pots that had not been destructively harvested. The remaining 10% of the plant material was retained for chemical analysis to determine the amount of ¹⁵N added to rotation phase 2. The amount of residues incorporated corresponded to 4.1, 5.9 and 4.1 t ha⁻¹ (on an area basis) for field pea-wheat rotation, ¹⁵N-fertilized

barley-wheat rotation and unfertilized barley-wheat rotation, respectively, under ambient [CO₂], and 5.1, 6.3 and 4.3 t ha⁻¹ under elevated [CO₂]. The soil and root (0–10 cm) was lightly ‘ploughed’ to incorporate the residue material into the top 10 cm of soil to simulate field practice. Reverse osmosis water was added to each pot to constant weight (80% of field capacity) every week for one month before sowing of a subsequent crop. This simulated the fallow period for residue decomposition between crop harvest and the sowing of a subsequent crop. After a wetting / drying cycle of one month, ten seeds of wheat (*Triticum aestivum* L. cv. Yitpi) were sown on the soil on 15 January 2011. No fertilizers were applied in this 2nd phase. Ten days after emergence, plant seedlings were thinned to four per pot. 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 80 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. The entire 0–10 cm 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 $\delta^{15}\text{N}$ 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 ($\text{N}_{\text{bulk soil}}$) in the first rotation phase was estimated by mass of $^{15}\text{N}_{\text{excess bulk soil}}$ divided by specific enrichment of clean roots ($\mu\text{g } ^{15}\text{N}_{\text{excess clean root}} / \text{mg root N}$) (Khan et al. 2002a). Total below-ground legume N ($\text{N}_{\text{below-ground}}$) was calculated as the sum of measured clean root N ($\text{N}_{\text{clean root}}$), and

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

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

Statistical analysis

Data were analyzed with MINITAB 16 statistical package using a General Linear 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 I

Total biomass

Elevated $[\text{CO}_2]$ increased ($p < 0.001$) the total biomass of field pea and N-fertilized barley by 21% and 23%, respectively, but the $[\text{CO}_2]$ -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 $[\text{CO}_2]$ 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

2). The estimated root-derived N at the corresponding soil depth was 40–56 and 34–39 mg N pot⁻¹. Elevated [CO₂] had no significant effect on these parameters (Table 2). The total below-ground N of field pea represented 18% and 19% of the total plant N under ambient and elevated [CO₂], respectively. These values were not significantly different (Table 2).

For N-fertilized barley, the recovery of fertilizer N in the plant was in the range of 48–49% while that in the soil ranged from 31–33% (25% in the 0–10 cm soil layer; 6–8% in the 10–37 cm soil layer). Elevated [CO₂] was associated with a marginally significant increase in the N recovery in grain from 33.8 to 35.3% ($p = 0.07$), but did not significantly affect the recovery of fertilizer N in other plant parts or in the soil.

>> Insert Table 2 near here>>

Residue C:N ratio

Elevated [CO₂] increased the C:N ratio of field pea and ¹⁵N-fertilized barley residues by 18% and 19%, respectively, but had no significant effect on that of unfertilized barley residues (Table 1).

Rotation phase 2

303

304 *Wheat biomass*

305 Elevated [CO₂] 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 [CO₂],
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 [CO₂] and
318 previous rotation phase on total N uptake by wheat: elevated [CO₂] 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 [CO₂] 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 [CO₂] 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 [CO₂] and previous rotation type had a significant effect on grain protein concentration of wheat (Table 3). Irrespective of rotation type, elevated [CO₂] had a significant negative effect on grain protein concentration but [CO₂]-induced reductions were greater for wheat following unfertilized barley than for wheat following field pea and N-fertilized barley (significant [CO₂] × species interaction, Table 3). When averaged across [CO₂], 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 $[\text{CO}_2]$ than under ambient $[\text{CO}_2]$, whereas
344 excess ^{15}N added from fertilizer residues did not differ significantly between $[\text{CO}_2]$
345 treatments (Table 4). The amount of ^{15}N recovered in wheat parts followed the order
346 grain > shoot > root, irrespective of $[\text{CO}_2]$ 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 $[\text{CO}_2]$ 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 $[\text{CO}_2]$ had no significant effect ($p > 0.05$) on the residual N

contribution from fertilizer to wheat parts (Table 4). The above-ground parts of wheat following field pea represented 20% of the ^{15}N present at sowing under ambient $[\text{CO}_2]$ and 11% under elevated $[\text{CO}_2]$. These percent recoveries were significantly greater than those of the wheat following N-fertilized barley under ambient $[\text{CO}_2]$ (4%) and elevated $[\text{CO}_2]$ (5%) (Table 4).

Discussion

In the first phase of the rotation, the $[\text{CO}_2]$ fertilization effects on total biomass of field pea and N-fertilized barley were within the range of growth responses of various crops to elevated $[\text{CO}_2]$ (Kimball et al. 1983; Cure and Acock 1986; Jablonski et al. 2002). However, the total biomass of barley grown did not respond to elevated $[\text{CO}_2]$ without the application of fertilizer N. Similar N-dependent growth response of barley to elevated $[\text{CO}_2]$ was also observed by Thompson and Woodward (1994) and Fangmeier et al. (2000). In our study, the N-dependent effect of growth response to elevated $[\text{CO}_2]$ was associated with plant N uptake. The reduction in plant N uptake by unfertilized barley under elevated $[\text{CO}_2]$ indicates that soil N availability was insufficient to meet plant demand under elevated $[\text{CO}_2]$. A declining N availability has previously been shown to eliminate the $[\text{CO}_2]$ -induced enhancement in leaf area index of cereal (Kim et al. 2003), thereby restricting the CO_2 fertilization effect on

plant growth. In contrast, we found that this limitation of CO₂ fertilization effect on growth did not occur when N fertilizer was applied to barley or for field pea, which could source its N via N₂ fixation. A review by Reich et al. (2006) suggests that a general N limitation of biomass accumulation to elevated [CO₂] effect is common, although not ubiquitous. Intense competition between plants and microbes for soil N can restrict plant growth responses to elevated [CO₂] (Gill et al. 2002). Improved N management strategies will therefore be needed to sustain crop growth (and residue input) and account for the additional grain N removal under future elevated CO₂ atmospheres.

Many cropping systems, especially those where fertilizer N inputs are relatively low by international standards due to low and unreliable rainfall that dominate much of Australia, rely heavily on legume N₂ fixation to supply N to subsequent cereal cropping phases. The supply of residual N to subsequent crop depends on various biotic and abiotic factors including the quantity and quality of residue, and the interaction between plants and microbes under elevated [CO₂] (Reich et al. 2006). Previous work, often focusing on soybeans and pasture legumes, showed that legume biomass and N₂ fixation increase under elevated [CO₂] (e.g. Soussana and Hartwig 1996; Kimball et al. 2002; Rogers et al. 2009). We found that elevated [CO₂] increased the amount of residues produced (and incorporated) from the preceding

field pea but that this residue also had a greater C:N ratio. This suggests that soil N availability to the subsequent wheat crop was reduced under elevated [CO₂], similar to that observed by others (Hungate et al. 1997; de Graaff et al. 2006). This increase in N immobilization partly explains why whole plant N uptake of the subsequent wheat was not higher under elevated [CO₂], despite a potentially greater amount of N in the soil system originating from the field pea residues. This phenomenon could be also be related to a decrease in the uptake rates of N by unit mass or length of roots under elevated [CO₂] (Taub and Wang 2008). Another possible explanation is a reduced rate of decomposition of crop residues (including legumes) observed in other studies under elevated [CO₂] (Torbert et al. 2000). As a result, the contribution of field pea N to subsequent wheat, at least in the short term, was lower under elevated [CO₂] in our study.

The contribution of field pea N to subsequent wheat N (11–20%) was within the range of the recovery of N contained in grain legume residues (2–27%) by various first succeeding crops (Fillery 2001). Although reduced under elevated [CO₂], the contribution of field pea residue N to subsequent wheat (11%) was significantly greater than that of fertilizer residue N (5%) in our study. While soil microbes prefer higher quality substrates (Kuzyakov 2002), the lower C:N ratio of the field pea residues than that of barley residues may explain the difference in residual

417 contribution between field pea N and fertilizer N. Unaffected by elevated [CO₂], 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 [CO₂].
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 [CO₂] 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 [CO₂]
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 CO₂ climates.

436

437 **Conclusions**

438 Elevated [CO₂] increased N accumulation by field pea but decreased fertilizer N
439 reserves in the soil after growing barley. Elevated [CO₂] 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 [CO₂]. 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 CO₂ environments.

447

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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 [CO₂] and elevated [CO₂] 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 [CO₂] and elevated [CO₂] 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 [CO₂] 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 × species]
	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	Elevated		Ambient	Elevated	
	[CO ₂]	[CO ₂]		[CO ₂]	[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

Table 3 Grain protein concentration of wheat following field pea, N-fertilized barley and 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
	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated		
	[CO ₂]	[CO ₂]	[CO ₂]	[CO ₂]	[CO ₂]	[CO ₂]		
Protein concentration (%)	15.1	12.3	17.2	15.3	16.1	12.4	***	***

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

Table 4 The amount of ^{15}N excess and percent recovery of residue N in various parts of wheat 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	**	***	***

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

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