

# The availability of residual legume and fertilizer nitrogen to a subsequent wheat crop under elevated CO<sub>2</sub> concentration

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## Abstract

The effect of elevated carbon dioxide concentrations ([CO<sub>2</sub>]) on the residual contribution of legume and fertilizer nitrogen (N) to a subsequent crop was investigated. Field pea was labeled *in situ* with <sup>15</sup>N under ambient and elevated (700 μmol mol<sup>-1</sup>) [CO<sub>2</sub>] in controlled environment glasshouse chambers. Barley was also labeled under the same conditions by addition of <sup>15</sup>N-enriched urea to the soil. Wheat was subsequently grown to physiological maturity in soil containing either <sup>15</sup>N-labeled field pea residues or <sup>15</sup>N-labeled barley plus fertilizer <sup>15</sup>N residues. Elevated [CO<sub>2</sub>] 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<sub>2</sub>] increased total biomass (11%) and grain yield (40%) of subsequent wheat crop regardless of rotation type in the first phase. Irrespective of [CO<sub>2</sub>], 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<sub>2</sub>], but dropped to 11% under elevated [CO<sub>2</sub>], while that from fertilizer did not differ significantly between ambient [CO<sub>2</sub>] (4%) and elevated [CO<sub>2</sub>] (5%).

## Key Words

Elevated [CO<sub>2</sub>], <sup>15</sup>N labeling, below-ground legume N, residual legume N, residual fertilizer N

## Introduction

The concentration of atmospheric carbon dioxide (CO<sub>2</sub>) has increased from 280 μmol mol<sup>-1</sup> in 1800 to around 390 μmol mol<sup>-1</sup> now, and is expected to reach about 550 μmol mol<sup>-1</sup> in 2050 (Houghton *et al.* 2001). Total N uptake by crops and N removal in grain also generally increase under elevated [CO<sub>2</sub>] (Kimball *et al.* 2002; Miyagi *et al.* 2007), and this increase in N demand in cropping systems would be expected to gradually reduce soil N reserves unless replenished.

Legume or fertilizer residues can contribute to subsequent N supply of crops (Ladd and Amato 1986), and alleviate N limitation under ambient [CO<sub>2</sub>]. While elevated [CO<sub>2</sub>] may affect the quantity, quality and decomposition rate of crop residues (Torbert *et al.* 2000; Kimball *et al.* 2002), the availability of residual legume and fertilizer N to subsequent crops under elevated [CO<sub>2</sub>] remains unclear. Such information is critical for N management practice for soil N replenishment under elevated [CO<sub>2</sub>]. The objective of this study was to investigate the interactive effects of elevated [CO<sub>2</sub>] and residual N (legume or fertilizer N) on subsequent wheat growth and N uptake.

## Materials and Methods

### *Glasshouse chambers*

This study was conducted in potted soil in a set of four naturally light 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 [CO<sub>2</sub>] (390 μmol mol<sup>-1</sup>), and two had elevated [CO<sub>2</sub>] (700 μmol mol<sup>-1</sup>). The average day/night temperature of the chamber throughout the growing season was 24°C/21°C.

### *Experimental design*

The effect of elevated [CO<sub>2</sub>] on the contribution of residual legume N and residual fertilizer N from a previous barley crop to subsequent wheat crops was investigated in pots 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<sub>2</sub>] 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<sub>2</sub>]. The experimental design was two CO<sub>2</sub> concentrations × three rotation types × two rotation phases, with four replications, totaling 48 pots (PVC tubes, 15 cm diameter, 45 cm long, sealed at the bottom). 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 (0–20 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<sup>-1</sup>, 4.1 mg nitrate-N kg<sup>-1</sup>, 7 mg Colwell P kg<sup>-1</sup>, 630 mg available K kg<sup>-1</sup> and 37% clay. The soil was air dried, crushed into < 1 cm fractions, and mixed thoroughly. The 48 PVC pots were filled with 7.4 kg (dry weight) of the Vertosol. A basal nutrient application of 20 kg P ha<sup>-1</sup>, 2.5 kg Cu ha<sup>-1</sup> and 5 kg Zn ha<sup>-1</sup> was added to the top 2.0 kg portion, and the contents of this portion were thoroughly mixed.

#### *Rotation phase 1*

Field pea (*Pisum sativum* L. cv. Kaspera) plants (two per pot) were labeled with <sup>15</sup>N urea (3 mL, 0.5% w/w urea, 98.26 atom% <sup>15</sup>N) at growth stages of 12<sup>th</sup>, 16<sup>th</sup> and 20<sup>th</sup> nodes 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). Barley (*Hordeum vulgare* L. cv. Gairdner) plants (four per pot) were grown in the PVC pots of the barley-wheat rotation. <sup>15</sup>N-labeled urea (10.22 atom% <sup>15</sup>N) was applied to the PVC pots at 50 or 0 kg N ha<sup>-1</sup> as a band with the seeds to minimize losses by volatilization. All the pots were watered with reverse osmosis water to constant weight (80% of field capacity) every two to three days.

#### *Rotation phase 2*

The dried shoot material obtained from the harvest of the first phase was cut into segments of 2–3 cm length, and added to the 24 PVC pots that had not been destructively harvested. 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 the subsequent wheat crop. After a wetting / drying cycle of one month, wheat (*Triticum aestivum* L. cv. Yitpi) plants (four per pot) were grown in the soil to maturity.

#### *Chemical analysis and <sup>15</sup>N calculations*

The finely ground plant and soil samples in both phases were analyzed for total C, total N and δ<sup>15</sup>N values by isotope ratio mass spectrometry. Total below-ground legume N was calculated as described in Khan *et al.* (2002). The percentages of <sup>15</sup>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<sub>wheat</sub>) was calculated according to 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.

## **Results and Discussion**

#### *Rotation phase 1*

Elevated [CO<sub>2</sub>] increased ( $p < 0.001$ ) the total biomass of field pea and N-fertilized barley by 21% and 23%, respectively, but the [CO<sub>2</sub>]-induced increase (9%) in the total biomass of barley receiving no N fertilizer was not significant (Table 1). A similar N-dependent growth response of barley to elevated [CO<sub>2</sub>] 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 [CO<sub>2</sub>] was associated with plant N uptake. Increasing [CO<sub>2</sub>] 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 reduction in plant N uptake by unfertilized barley under elevated [CO<sub>2</sub>] indicates that soil N availability was insufficient to meet plant demand under elevated [CO<sub>2</sub>]. A declining N availability was shown to

eliminate the [CO<sub>2</sub>]-induced enhancement in leaf area index of cereal (Kim *et al.* 2003), thereby restricting the CO<sub>2</sub> fertilization effect on plant growth. In contrast, we found that this limitation of CO<sub>2</sub> fertilization effect on growth did not occur when N-fertilizer was applied to barley, or for field pea, which could source its N via N<sub>2</sub> fixation.

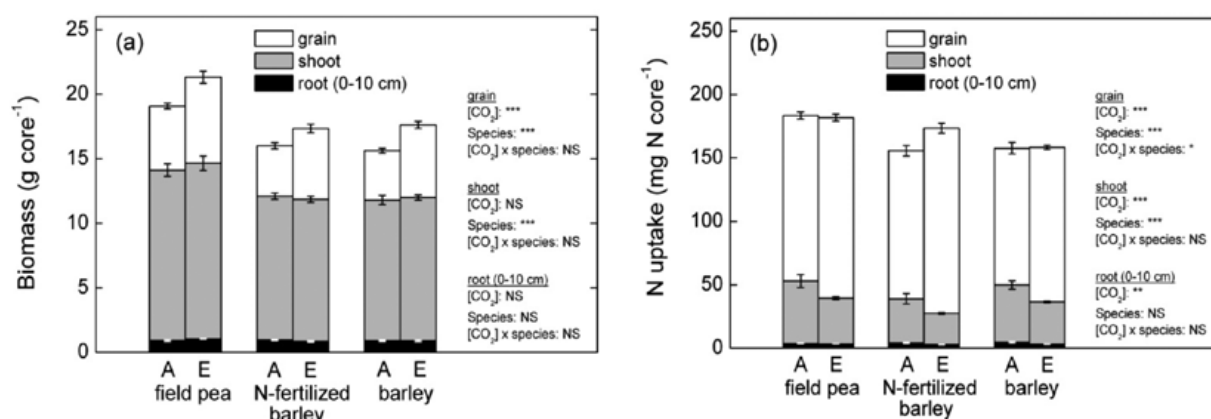
**Table 1: The effect of elevated [CO<sub>2</sub>] on total biomass, total plant N and residue C:N ratio of field pea, N-fertilized barley and unfertilized barley. Values are means of the four replicates for each treatment.**

	<sup>15</sup> N-fed field pea		<sup>15</sup> N-fertilized barley		unfertilized barley		[CO <sub>2</sub> ]	Species	[CO <sub>2</sub> ] <sup>1</sup> species
	Ambient [CO <sub>2</sub> ]	Elevated [CO <sub>2</sub> ]	Ambient [CO <sub>2</sub> ]	Elevated [CO <sub>2</sub> ]	Ambient [CO <sub>2</sub> ]	Elevated [CO <sub>2</sub> ]			
Total biomass (g core <sup>-1</sup> )	16.9	20.4	18.2	22.3	13.3	14.5	***	***	**
Total plant N (mg N core <sup>-1</sup> )	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

### Rotation phase 2

The grain yield and shoot biomass of wheat following field pea were 24% (*p* < 0.001) and 21% greater (*p* < 0.001), respectively, than those of wheat following N-fertilized barley, regardless of [CO<sub>2</sub>] (Fig. 1a). These results suggest that N fertilizer application does not have a substantial residual effect (Ladd and Amato 1986; Ladha *et al.* 2005), whereas that derived from legume residue N is a more accessible N source for subsequent crops, especially over the longer term (Ladd and Amato 1986). The supply of residual N to the 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 [CO<sub>2</sub>] (Reich *et al.* 2006). Elevated [CO<sub>2</sub>] increased the amount of residues produced from the preceding field pea resulting in a greater mass of N being present for the following crop (Table 2), however this residue also had a greater C:N ratio (Table 1) favouring immobilization. This suggests that soil N availability to the subsequent wheat crop was reduced under elevated [CO<sub>2</sub>] which partly explains why whole plant N uptake of the subsequent wheat was not higher under elevated [CO<sub>2</sub>] (Fig. 1b). Thus manipulation of post legume N management at higher [CO<sub>2</sub>] may produce substantial agronomic gains and warrants future research.



**Figure 1. Biomass (a) and N uptake (b) in different wheat parts following field pea, N-fertilized barley, and unfertilized barley under ambient [CO<sub>2</sub>] (A) and elevated [CO<sub>2</sub>] (E) in rotation phase 2. Values are means of the four replicates for each treatment. Vertical bars indicate standard errors. Significant effects are indicated as \**p* < 0.05, \*\**p* < 0.01 and \*\*\**p* < 0.001. NS, not significant**

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 [CO<sub>2</sub>], the contribution of field pea residue N to subsequent wheat (11%) was significantly greater than that of fertilizer residue N (5%) in our study (Table 2). While soil microbes prefer higher quality substrates (Kuzakov 2002), the lower C:N ratio of the field pea residues than that of barley residues may explain the difference in residual contribution between field pea N and fertilizer N.

Unaffected by elevated [CO<sub>2</sub>], the recovery of residual fertilizer N by wheat in the present study was within the range of the generally low fertilizer N recovery (2–5%) in the first succeeding crop under various N application methods and residue management practices reported by Ladha *et al.* (2005). These results suggest that N uptake from legume residue was not enhanced under elevated [CO<sub>2</sub>] even though biomass was. Therefore manipulation of post legume N management may yield significant agronomic gains (yield and protein) under future CO<sub>2</sub> environments.

**Table 2: The amount of <sup>15</sup>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.**

	<sup>15</sup> N-fed field pea		<sup>15</sup> N-fertilized barley		[CO <sub>2</sub> ]	Species	[CO <sub>2</sub> ] <sup>†</sup> species
	Ambient [CO <sub>2</sub> ]	Elevated [CO <sub>2</sub> ]	Ambient [CO <sub>2</sub> ]	Elevated [CO <sub>2</sub> ]			
Total excess <sup>15</sup> N added from phase 1 (mg)	9.30	12.33	4.01	3.75	0.08	***	*
Grain <sup>15</sup> N (mg)	1.37	1.05	0.11	0.15	0.07	***	*
Shoot <sup>15</sup> N (mg)	0.475	0.260	0.039	0.024	*	***	*
Root (0–10 cm) <sup>15</sup> N (μg)	48.9	37.8	6.4	5.0	0.06	***	NS
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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