

# Effect of elevated carbon dioxide on <sup>15</sup>N-fertilizer recovery under wheat in Australia

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## Abstract

The effect of elevated [CO<sub>2</sub>] on the recovery of <sup>15</sup>N-labeled urea by wheat was investigated under ambient (380 μmol/mol) and elevated (550 μmol/mol) [CO<sub>2</sub>] at the Free-Air Carbon dioxide Enrichment (FACE) experiment in western Victoria, Australia. <sup>15</sup>N-enriched (c. 10% atom excess) granular urea was applied to PVC microplots at 50 kg N/ha at rainfed and irrigated condition and two times of sowing. At physiological maturity, total biomass was increased by 23% under elevated [CO<sub>2</sub>], as a result of a 25% and 22% increase in stem and root biomass, respectively, but not grain yield. Total N uptake of wheat was increased by 17% under elevated [CO<sub>2</sub>], irrespective of irrigation and sowing time. Elevated [CO<sub>2</sub>] increased grain C/N ratio by 11% only under irrigation, but not for stem and root. Elevated [CO<sub>2</sub>] had no significant effect on percentage of N derived from fertilizer (%Ndff) for grain, stem and root but irrigation increased %Ndff for all wheat parts only in late sowing. Elevated [CO<sub>2</sub>] did not alter the percentage of <sup>15</sup>N recovered in grain, stem and root, but marginally increased the total recovery by 30% at late sowing time under irrigation. There were no significant effects of elevated [CO<sub>2</sub>] on <sup>15</sup>N recoveries in soil and total fertilizer N losses, indicating potentially similar availability of fertilizer N residues to subsequent crops in the longer term.

## Key Words

Free-air carbon dioxide enrichment, climate change, nitrogen fertilizer recovery, irrigation, sowing time, wheat.

## Introduction

Meeting crop nutrient demand is crucial to the sustainability of any crop production system (Torbert *et al.* 2004). Nitrogen (N) fertilizers are commonly applied in agricultural fields to optimize crop yield. However, the N-use efficiency of N-fertilizers by crop plants worldwide remains low, rarely more than 40% (Chen *et al.* 2008) while up to 20-50% of annual applications can remain unaccounted for at harvest (Azam *et al.* 1990; Pilbeam 1996). As atmospheric carbon dioxide (CO<sub>2</sub>) levels rise due to the burning of fossil fuels, elevated [CO<sub>2</sub>] generally results in higher crop biomass and yield but tissue N content is lower (Kimball *et al.* 2002). It is not clear whether higher fertilizer-N input is required or capable of meeting the increased demand of N under elevated [CO<sub>2</sub>]. For example, elevated [CO<sub>2</sub>] had no significant effect on recovery of fertilizer N in both the soil and grain sorghum (Torbert *et al.* 2004), but increased the recovery in spring barley (Martín-Olmedo *et al.* 2002) and rice (Weerakoon *et al.* 2005). The effect of elevated [CO<sub>2</sub>] on N fertilizer recovery in crop, especially by wheat grown on low fertility sites, is unclear. This information is important to not only improving crop productivity, but also addressing Progressive N Limitation (PNL) (Luo *et al.* 2004) under elevated [CO<sub>2</sub>] in the long term. The objective of this study is to investigate the interactions of elevated [CO<sub>2</sub>], irrigation and sowing time on the recovery of <sup>15</sup>N-labelled urea on a wheat field in Victoria, Australia using Free-Air Carbon dioxide Enrichment (FACE) facility.

## Methods

### *Experimental site and design*

Field experiments were conducted from early June to mid December in 2008 at Horsham, Victoria, Australia (36°45'S, 142°07'E) on a Vertosol used for a range of crops. The climate is temperate with an average rainfall and maximum temperature of 316 mm and 17.5°C during wheat growing season. The trial was a factorial combinations of two [CO<sub>2</sub>]s, two irrigation scenarios and two times of sowing with four replicates in a randomized complete block design.

### *Carbon dioxide elevation*

The elevation of atmospheric [CO<sub>2</sub>] was achieved using a FACE system, consisting of 16 12 m diameter experimental areas, eight ambient and eight elevated. The two target CO<sub>2</sub> concentrations were 380 (ambient) and 550 μmol/mol (elevated). Carbon dioxide exposure commenced at sowing and terminated at harvest.

### Wheat cultivation, fertilization and irrigation

Spring wheat (*Triticum aestivum* L. cv. Yitpi) was sown on 3 June (early sowing) & 6 August (late sowing) in 2008. The <sup>15</sup>N labelled granular urea was topdressed at 50 kg N/ha at 3-5 leaf stage for both sowing times. The two irrigation scenarios were decile 5 (225 mm April to November) and decile 7 (275 mm April to November) rainfall conditions.

### <sup>15</sup>N labelling

At each site, one circular PVC microplot (internal diameter of 24 cm and height 25 cm) was inserted to 20 cm depth. <sup>15</sup>N-enriched granular urea with an abundance of 10.22 atom % was applied at the same rate (50 kg N/ha) as non-labelled urea was applied to the larger plots.

### Sample collection

At harvest on 27 November and 16 December for early and late sowing time, respectively, plants were cut at ground level from within each microplot and separated into grain and aboveground biomass. In each microplot soil was sampled from 0-10, 10-20 and 20-40 cm depths. For the 0-10 and 10-20 cm depths, all the soil within the microplot was removed and a representative subsample was taken after the thorough mixing. For the 20-40 cm depth, one soil core was collected using a 5 cm diameter auger in each microplot. Major wheat roots were collected by digging out the top (0-10 cm) soil. Reference plant and soil samples were taken around 1 m away from the microplot to determine the background enrichments.

### Chemical analysis

The plant and soil samples were dried at 60°C and 40°C, respectively, for 48 h, weighed, finely-ground and analysed for total C, total N and <sup>15</sup>N enrichment by isotope ratio mass spectrometry following combustion. The recovery of <sup>15</sup>N applied and percentage of plant N derived from fertilizer (%Ndff) were calculated by the equations described by Malhi *et al.* (2004):

$$\text{Recovery of applied N in plant (\%)} = \frac{[(\%N \text{ plant}) \times (\text{kg dry yield/ha}) \times (\text{atom}\%^{15}\text{N excess of total N in plant})]}{[(\text{Rate of applied N in kg N/ha}) \times (\text{atom}\%^{15}\text{N excess of total N in fertilizer})]} \quad (1)$$

$$\text{Recovery of applied N in soil (\%)} = \frac{[(\%N \text{ soil}) \times (\text{kg dry soil/ha}) \times (\text{atom}\%^{15}\text{N excess of total N in soil})]}{[(\text{Rate of applied N in kg N/ha}) \times (\text{atom}\%^{15}\text{N excess of total N in fertilizer})]} \quad (2)$$

$$\%Ndff = (\text{atom}\%^{15}\text{N excess of total N in plant} / \text{atom}\%^{15}\text{N excess of total N in fertilizer}) \times 100 \quad (3)$$

### Statistical analysis

All data were analysed using the MINITAB 14 statistical package using a factorial model analysis of variance with main effects as [CO<sub>2</sub>], irrigation and time of sowing.

## Results

### Crop biomass, total N uptake and C/N ratio

When averaged across all treatments, total biomass was 23% ( $p < 0.01$ ) higher from elevated than from ambient [CO<sub>2</sub>] microplots, regardless of irrigation and sowing time, which was associated with a 25% ( $p < 0.01$ ) and 22% ( $p < 0.01$ ) increase in stem and root biomass, respectively, but not grain yield ( $p > 0.05$ ) (Table 1). There was a trend for the interaction between irrigation and sowing time to be significant ( $p = 0.066$ ), with higher (82%) biomass observed under irrigation treatment in late sowing, but not in early sowing, irrespective of [CO<sub>2</sub>]. Total N uptake of wheat was increased by 17% ( $p < 0.05$ ) under elevated [CO<sub>2</sub>], irrespective of irrigation and sowing time. Irrigation increased ( $p < 0.05$ ) total N uptake by 86% only in late sowing, but not in early sowing. Elevated [CO<sub>2</sub>] increased grain C/N ratio by 11% only under irrigation, owing to a slight decrease in N concentration, rather than a change in C concentration; no change in C/N ratio was observed for stem and root.

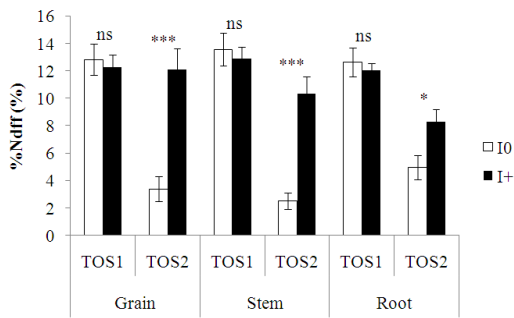
**Table 1. Dry weight and total N uptake of crops grown from microplots under ambient and elevated [CO<sub>2</sub>]**

[CO <sub>2</sub> ] (μmol/mol)	Biomass (g/m <sup>2</sup> )			Total N uptake (g/m <sup>2</sup> )
	Grain	Stem	Root	
380	264.1	571.8	50.2	886.1
550	314.2	712.8	61.3	1088.3
% change	+19 ns	+25**	+22**	+23**

ns, no significant difference, \* $p < 0.05$ , \*\* $p < 0.01$

### Percentage of plant N derived from fertilizer

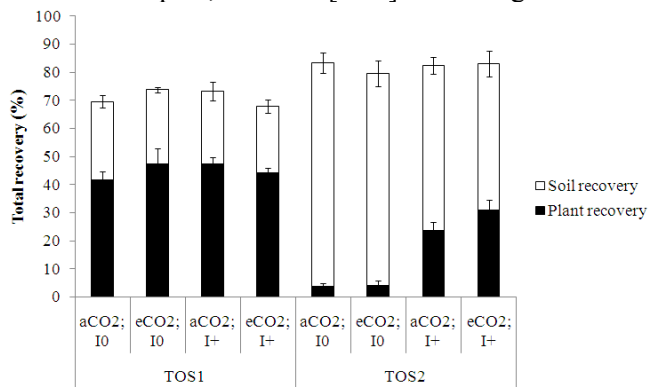
Elevated [CO<sub>2</sub>] had no significant effect on %Ndff for grain, stem and root, regardless of irrigation regime and sowing time. Irrigation increased %Ndff by 260% ( $p < 0.001$ ), 313% ( $p < 0.001$ ) and 66% ( $p < 0.05$ ) for grain, stem and root, respectively, but only in late sowing (Figure 1).



**Figure 1. The effect of irrigation and sowing time on %Ndff of grain, stem and root of wheat crops. Bars indicate standard errors. ns, no significant difference, \*  $p < 0.05$ , \*\*\*  $p < 0.001$ . I0: rainfed; I+ irrigated; TOS1: early sowing; TOS2: late sowing**

#### Recovery of fertilizer in the crop and soil

The percentage of  $^{15}\text{N}$  recovered in the crops averaged 42-48% and 4-31% for early and late sowing, respectively (Figure 2). Elevated  $[\text{CO}_2]$  did not alter the percentage of  $^{15}\text{N}$  recovered in grain, stem and root irrespective of irrigation regime and sowing time, but increased the total recovery by 30% ( $p = 0.066$ ) at late sowing time under irrigation (Figure 2). The percentage of  $^{15}\text{N}$  recovered in the soil averaged 24-28% and 52-80% for early and late sowing, respectively. The percentage recovered was not significantly different between ambient and elevated  $[\text{CO}_2]$  for soil depths of 0-10 cm and 10-20 cm except less (46%,  $p < 0.01$ )  $^{15}\text{N}$  was recovered in the lower soil depth (20-40 cm) under elevated  $[\text{CO}_2]$  at early sowing. When averaged across soil depths, elevated  $[\text{CO}_2]$  had no significant effect on soil  $^{15}\text{N}$  recovery (Figure 2).



**Figure 2. The effect of elevated  $[\text{CO}_2]$ , irrigation and sowing time on  $^{15}\text{N}$  fertilizer recovery of plant (■) and soil (□). Bars indicate standard errors. aCO<sub>2</sub>: ambient  $[\text{CO}_2]$ ; eCO<sub>2</sub>: elevated  $[\text{CO}_2]$  I0: rainfed; I+ irrigated; TOS1: early sowing; TOS2: late sowing**

#### Fertilizer unaccounted for

At harvest, the percentage of the  $^{15}\text{N}$ -labelled fertilizer unaccounted for was reduced ( $p < 0.001$ ) from 29% in early sowing time to 18% in late sowing, but was not affected by either elevated  $[\text{CO}_2]$  or irrigation.

## Discussion

#### Effect of $[\text{CO}_2]$ , irrigation and sowing time on N demand

Elevated  $[\text{CO}_2]$  significantly increased total biomass by 23%, which is consistent with the 20% stimulation of biomass at maturity reported in the main FACE experiment (Norton *et al.* 2008), with total N uptake by the crop 17% higher under elevated than ambient  $[\text{CO}_2]$  microplots. These results indicate that N demand was increased, and PNL is possible in this wheat field if additional N input (or reduction in N losses) is not made to compensate for the greater demand in the longer term (Luo *et al.* 2004). There was no change in C/N ratio (other than for grain) in contrast to other studies where elevated  $[\text{CO}_2]$  increased the C/N ratio of plant residues (Kimball *et al.* 2002). Nonetheless, the present study suggests that PNL may eventually occur as a result of increased biomass and residue production, rather than changes in C/N ratio of crop tissues, as grain is generally removed from the field. Irrigation was critical for the greater increase in crop biomass and N uptake, and therefore demands for N, in drier and warmer climate.

#### Effect of $[\text{CO}_2]$ , irrigation and sowing time on fertilizer uptake

Recovery of  $^{15}\text{N}$ -labelled fertilizer in the early sown crop (42-48%) was higher than late sowing (4-31%), and was slightly higher than the range of 22-40% recovery of  $^{15}\text{N}$  fertilizer observed by Carranca *et al.*

(1999) for wheat, but within the range (22-59%) reviewed by Chen *et al.* (2008). Despite the increase in N demand under elevated [CO<sub>2</sub>], the contribution of fertilizer N to the uptake of N in plant was not significantly different from the ambient, as evidenced by the values of %Ndff. Moreover, no significant effect of elevated [CO<sub>2</sub>] was observed on the percentage of fertilizer recovery by the crop itself, which agrees with Torbert *et al.* (2004)'s study on <sup>15</sup>N fertilizer recovery by sorghum. Furthermore, the recovery of the fertilizer N remaining in the soil at crop maturity did not differ between [CO<sub>2</sub>] treatments. In the short term, PNL is unlikely because of relatively high soil N supply at this site. Nevertheless, the marginally significant increase in total plant recovery N percentage under elevated [CO<sub>2</sub>] and irrigation in late sowing suggests water availability plays profound role when the climate becomes drier and warmer under future atmospheric [CO<sub>2</sub>]. Early sowing resulted in higher recovery of fertilizer N in the crop than that remaining in the soil in this semi-arid environment, but the opposite was observed in late sowing with a drier climate. This is consistent with a meta-analysis of the recovery of <sup>15</sup>N-labelled fertilizer applied to wheat (Pilbeam 1996), which shows greater recovery of fertilizer N by crops in humid environments, but the opposite happened in dry environments. This has major implications on fertilizer recovery of crops grown in regions which are predicted to be drier in the future. A higher percentage of unrecovered <sup>15</sup>N was observed under early sowing than late sowing, which may be attributed to greater denitrification in early sowing when soil moisture was higher (Bolan *et al.* 2004) which may lead to high N<sub>2</sub>O flux. N loss via leaching was unlikely as evidenced by the small percentage of <sup>15</sup>N recovered in soil depth of 20-40 cm (data not shown). Ammonia volatilization was likely increased with air temperature (Bolan *et al.* 2004) (late sowing in the present study), yet the greater N loss in early sowing suggests denitrification contributed more than ammonia volatilization in the percentage <sup>15</sup>N unaccounted for.

## Conclusions

Wheat total biomass, total N uptake and grain C/N ratio were increased under elevated [CO<sub>2</sub>]. Progressive N Limitation, if any, is likely a result of greater biomass and residue production, rather than change in C/N ratio of crop residue. Elevated [CO<sub>2</sub>] had no effect on the relative contribution of fertilizer N to the uptake of N in plant, indicating soil N was not limiting in the present study, and higher fertilizer-N application is not necessary. There was an indication however of higher fertilizer recovery in the potentially drier and warmer climates predicted under future [CO<sub>2</sub>], if irrigation is applied. Higher inputs of N may be required to satisfy greater N demand and to address PNL, if it happens. Irrigation was also important to crop biomass, total N uptake and %Ndff in late sowings. Longer term experiments, in combination with process based plant-soil modeling, are required to assess the likelihood of PNL occurring in this system.

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