

N dynamics under elevated carbon dioxide in the Australian FACE experiment

Robert M Norton^{1,2}, S K Lam^{2,3}, D Chen³, G. Fitzgerald⁴, R Armstrong⁴

¹ International Plant Nutrition Institute, 54 Florence St, Horsham, Victoria, Australia. Email: rnorton@ipni.net

² Department of Agriculture and Food Systems, The University of Melbourne, Private Box 260, Horsham, Victoria 3401, Australia.

³ Department of Resource Management and Geography, The University of Melbourne, Victoria, 3010, Australia.

⁴ Victorian Department of Primary Industries, Private Bag 260, Horsham, Victoria, 3401, Australia.

Abstract

The Australian Grains Free Air Carbon Dioxide Enrichment (AGFACE) facility was established to compare wheat growth, yield and development under ambient (~380 $\mu\text{mol/mol}$) and elevated (~550 $\mu\text{mol/mol}$) carbon dioxide ($a[\text{CO}_2]$ and $e[\text{CO}_2]$, respectively). Experiments on N uptake and location have been undertaken to estimate how $e[\text{CO}_2]$ and a changing climate could affect N supply and demand for annual crop production systems. These experiments were undertaken at Horsham in the Victorian Wimmera in 2007, 2008 and 2009 with two cultivars (Yitpi and Janz), two sowing times and two irrigation treatments, although results from only 2008 and 2009 are presented.

When grown under $e[\text{CO}_2]$, wheat crops showed higher crop biomass at the end of tillering, anthesis and maturity. Although plant and grain N contents declined, crop N uptake was 24% higher with $e[\text{CO}_2]$. Stubble C:N ratio was not affected by $e[\text{CO}_2]$. Generally, total plant N and leaf specific N levels were lower under $e[\text{CO}_2]$ at stem elongation and anthesis although at maturity straw C:N ratio was not altered. Grain N content decline significantly in all years under $e[\text{CO}_2]$.

Introduction

Present day atmospheric CO_2 levels have risen from 295 $\mu\text{mol/mol}$ in 1900 to about 386 $\mu\text{mol/mol}$ now and by 2100 these levels could reach between 490 and 1260 $\mu\text{mol/mol}$ (Carter et al. 2007), forcing a rise in global temperature of up to 4.5°C as well as shifts in rainfall amount, intensity and distribution (Whetton 2001). Such changes will impact on agricultural land management and food production.

These atmospheric changes will affect a range of processes that cycle nutrients within and between the soil, plant and atmospheric systems. Elevated $[\text{CO}_2]$ increases plant growth, termed the "fertilization effect". This extra growth requires additional N even though the amount of N in plant tissue grown for long periods of time declines, due to dilution by the expanding canopy and also probably due as Rubisco biosynthesis is down regulated (Ainsworth and Rogers 2007).

Studies of the effects of $e[\text{CO}_2]$ on soil N processes have indicated that the increased level of plant demand is not always able to be met by soil processes and as a result, over time N becomes more limiting, an effect termed progressive nitrogen limitation. Progressive N limitation (PNL) is closely linked to potential C sequestration under $e[\text{CO}_2]$ (Schlesinger & Litcher 2001) and this occurs when the availability of mineral N declines over time at $e[\text{CO}_2]$ in comparison to ambient $[\text{CO}_2]$ ($a[\text{CO}_2]$) and if there is no new N input or higher N losses. The result is a gradual decrease in the $[\text{CO}_2]$ -induced increment in ecosystem C storage (Luo et al. 2004) so that the actual response of these systems based on carbon dioxide response is significantly less than if N was not limiting.

To develop data to assist with understanding the effects of $e[\text{CO}_2]$ on wheat growth and yield in Australia, the Australian Grains FACE (AGFACE) facility was commissioned (Mollah et al. 2009). This paper reports the effects of $e[\text{CO}_2]$ on two particular aspects of N dynamics using data collected from the AGFACE experiment, in particular what is the effect of elevated CO_2 on N demand by wheat and do these changes impact on the efficiency with which soil and fertiliser N is accessed?

Materials and Methods

The Australian Grains FACE project at Horsham, Victoria was designed to simulate atmospheric carbon dioxide levels in the year 2050 and then to use those data to model and predict spring wheat responses (Mollah et al. 2009). The climate is temperate with an average rainfall and maximum temperature of 316 mm and 17.5°C during wheat growing season, respectively.

The experiment aims to measure the interacting effects of carbon dioxide (ambient $a\text{CO}_2$ ~380 $\mu\text{mol/mol}$, elevated $e\text{CO}_2$ ~550 $\mu\text{mol/mol}$) with variations in water supply (by irrigation), temperature during grain fill (by altering sowing time), nitrogen (by fertiliser addition) and cultivar on wheat growth and productivity. Carbon dioxide was injected over the crop in open-air 12 m rings from emergence until maturity in 2007, 2008 and 2009. In 2007, 2008 and 2009, two cultivars Janz and Yitpi were compared and Yitpi was also tested with added fertiliser nitrogen. In 2009, additional cultivars were added but this paper reports the

results for the same treatments only in 2008 and 2009. Sowing times were late May/Early June (TOS1) and late July/Early August (TOS2). The two watering regimes were designed to provide an average (~290 mm) and above average (~350 mm) growing season (May to November) rainfall for the region.

Growth, tissue N content and partitioning among tissues were measured at stem elongation (GS30), anthesis (GS65) and maturity (GS90) for all treatments, and yield components, screenings (<2 mm) and grain protein content (at 0% moisture) were also measured. Results were analysed using ReML mixed models where some data were missing, or by a split-plot Analysis of Variance for balanced data sets. This paper presents growth and N uptake data over the two of the three years of the experiment.

15N fertiliser recovery

In 2008, within each half ring, one circular PVC microplot (internal diameter of 24 cm and height 25 cm) was inserted to 20 cm depth. 15N-enriched granular urea(10.22 atom%) was applied at the same rate (50 kg N/ha) as non-labelled urea was applied to the larger plots. In each microplot, plants were taken to ground level and soil sampled to 40 cm. 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 15N enrichment by isotope ratio mass spectrometry following combustion. The recovery of 15N applied and percentage of plant N derived from fertiliser (%Ndff) were calculated by the equations described by Malhi *et al.* (2004).

Results and Discussion

Biomass production and N uptake

There were significant main plot effect for sowing time and watering regime on biomass at GS30, GS65 and GS90, as well as grain yields (data not presented) but there were very few significant interactions with these factors and [CO₂]. In all three years of the FACE experiment, e[CO₂] increased crop biomass at tillering, anthesis and maturity except at tillering 2009 (Table 1). Irrigation usually commenced near anthesis so had little effect on biomass before then, while later season biomass was greater with the earlier sowing. The data did show significant interactions between sowing time and water supply, and in 2008, there was a significant interaction between sowing time and carbon dioxide level ($p<0.05$) for growth at anthesis and maturity, with no response to e[CO₂] at the second sowing time. Similarly, in 2009, supplementary water had no effect on biomass at maturity.

The results for plant growth, N concentration and N uptake showed very few interactions between [CO₂] and sowing time, watering regime or cultivar treatment.

Table 1 The effect of elevated CO₂ on the growth N content and N uptake of wheat from the AGFACE experiment 2008 and 2009. Values presented are the means of all N, water, TOS and cultivar treatments. Means of CO₂ treatments in bold are significantly different ($p<0.05$).

Factor	[CO ₂] (μmol/mol)	2008			2009		
		GS30	GS65	GS90	GS30	GS65	GS90
Biomass (g/m ²)	380	166	700	791	89	571	557
	550	208	915	1043	100	651	715
Plant N (%)	380	3.77	2.05	1.63	4.80	2.89	2.05
	550	3.69	1.90	1.56	4.67	2.62	1.97
*N Uptake (g/m ²)	380	6.1	14.3	12.7	4.2	16.4	11.5
	550	7.5	17.2	15.7	4.7	16.8	14.4

* Note that N uptake is not the perfect product of biomass and plant N content because of some missing values in the data sets used.

The N content of crop residue was not affected by [CO₂] level in 2008, but was slightly – but significantly ($p=0.04$) reduced from 1.09% to 1.02% under e[CO₂]. Even though the C:N ratio of the residues was only slightly affected, there was more stubble with e[CO₂] and this may lead to a larger demand for soil N to breakdown the stubbles. The duration and extent of the immobilization is not known at present.

As well as plant N concentration, specific leaf N content also declines under e[CO₂] and it is proposed that this is a consequence of down-regulation of plant photosynthetic proteins (Ainsworth and Rogers 2007) and not necessarily a consequence of a reduction in N supply from the soil during growth due to changed soil supply processes. A direct result of the lower plant N concentration is a decrease in grain protein, a common

feature seen in cereals grown under e[CO₂] (Blumenthal et al. 1996). These changes in the AGFACE experiment are summarised in Table 2.

Table 2 Grain protein and green leaf N content (GS30 and GS65) in 2008 and 2009 in response to elevated carbon dioxide in the AGFACE experiment. Values presented are the means of all N, water, TOS and cultivar treatments. Means of CO₂ treatments in bold are significantly different (p<0.05).

[CO ₂] (μmol/mol)	Grain Protein (%)		Leaf N GS30 (%)		Leaf N GS65 (%)	
	2008	2009	2008	2009	2008	2009
380	18.0	18.0	4.48	5.03	3.78	1.78
550	17.3	16.5	4.38	4.88	3.54	1.64

A recent report by Bloom et al. (2010) indicates the involvement of Nitrate Reductase down regulation under eCO₂. Therefore, selection for wheat genotypes that do not down-regulate could be a strategy to improve grain protein levels as well as capture the added growth benefits that are lost due to reduced leaf photosynthetic rates under elevated CO₂.

15N fertiliser recovery

The results in the micro-plots were similar results to those from the main experiment, with crop biomass increased by 23% ($p < 0.01$) under the e[CO₂] compared to a[CO₂] and this was associated with a 25% ($p < 0.01$) and 22% ($p < 0.01$) increase in stem and root biomass. However, the grain yield was not significantly increased under these conditions ($p > 0.05$) (Table 3). Total N uptake of wheat was increased by 17% ($p < 0.05$) under e[CO₂], irrespective of irrigation and sowing time and irrigation increased ($p < 0.05$) total N uptake by 86% only in late sowing, but not in early sowing. Elevated [CO₂] 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 3 Dry weight and total N uptake at maturity of crops grown in microplots under ambient and elevated [CO₂] in 2008.

[CO ₂] (μmol/mol)	Biomass (g/m ²)				Total N uptake (g/m ²)
	Grain	Stem	Root	Total	
380	264	572	50	886	13.3
550	314	713	61	1088	15.6
% change	ns	+25**	+22**	+23**	+17*

ns, no significant difference at $p=0.05$, * $p < 0.05$, ** $p < 0.01$

The percentage of ¹⁵N recovered in the crops averaged 42-48% and 4-31% for early and late sowing, respectively. Elevated [CO₂] did not alter the percentage of ¹⁵N 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 ¹⁵N recovered in the soil averaged 24-28% and 52-80% for early and late sowing, respectively. The percentage recovered was not significantly different between a[CO₂] and e[CO₂] for soil depths of 0-10 cm and 10-20 cm except less (46%, $p < 0.01$) ¹⁵N was recovered in the lower soil depth (20-40 cm) under e[CO₂] at early sowing. When averaged across soil depths, e[CO₂] had no significant effect on soil ¹⁵N recovery.

These data indicate that fertiliser N is not likely to be proportionately more or less available to crops under e[CO₂] despite the higher absolute demand for N due the growth stimulating effect of carbon dioxide. Of course, the proportion available and recovered is greatly influenced by soil N status, and if N status declines with e[CO₂] then fertiliser recovery (%Ndff) is likely to increase. In the main experiment in 2007, there was 46% higher root biomass and higher root-length density (top 60 cm) for wheat under e[CO₂] than under a[CO₂] (Norton et al. 2008). The increase in root-length density could enable the increased N acquisition under e[CO₂] by increasing the intensity of exploitation of the soil, although other factors including changing rhizosphere biology may be implicated. These issues are currently under investigation in the AGFACE experiment.

Conclusions

Higher atmospheric [CO₂] increased plant growth, even though plant N content was somewhat reduced, and there was an increase in the demand for N under those conditions. In these experiments, plant N uptake increased by 20 to 30%. It appears that the extra N sourced by the crop came in the same proportions from soil and fertiliser, as there was no increase in the percentage of N derived from fertiliser. Extra root-length density in the topsoil under e[CO₂] could provide part of the explanation for this extra uptake.

The C:N ratio of crop residues was not altered by e[CO₂] and even under e[CO₂] the rate of breakdown was similar for materials with the same C:N ratio. Even though the rates remain the same, the extra amount of crop residue produced due to the fertiliser effect of carbon dioxide would require added N from the soil, suggesting that immobilization of N could be higher with e[CO₂]. The implications for these changes on the development of PNL require additional information on soil N mineralisation rates, greenhouse gas production and N fixation rates, and experiments to address these issues is progressing.

Acknowledgements

This research is supported by the Grains Research and Development Corporation (UM00027) and the Victorian Department of Primary Industries. The authors acknowledge the technical support of Mr Peter Howie (The University of Melbourne), Mr Russel Argall and Ms Justine Ellis (both Victorian Department of Primary Industries). Mr Lam is supported by a University of Melbourne Postgraduate scholarship.

References

- Ainsworth EA, A Rogers (2007). The response of photosynthesis and stomatal conductance to rising [CO₂]: mechanisms and environmental interactions. *Plant Cell and Environment* 30, 258-270.
- Bloom, AJ, M Burger, JS Rubio-Asensio, et al. (2010) Carbon dioxide enrichment inhibits nitrate assimilation in wheat and arabidopsis. *Science* 328, 899-903.
- Blumenthal C, HM Rawson, E McKenzie, et al. (1996). Changes in wheat grain quality due to doubling the level of atmospheric CO₂. *Cereal Chemistry* 73, 762-766.
- Carter TR, RN Jones, X Lu, et al. (2007) New assessment methods and the characterisation of future conditions. In 'Climate Change 2007: Impacts, adaptation and vulnerability'. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. (Eds ML Parry, OF Canziani, JP Palutikof, PJ van der Linden, CE Hanson) pp. 133–171. (IPCC, Cambridge University Press: Cambridge, UK).
- Luo Y, B Su, WS Currie et al. (2004) Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. *Bioscience*, 54, 731–739.
- Malhi SS, AM Johnston, KS Gill, DJ Pennock (2004) Landscape position effects on the recovery of ¹⁵N-labelled urea applied to wheat on two soils in Saskatchewan, Canada. *Nutrient Cycling in Agroecosystems* 68, 85-93.
- Mollah M, RM Norton, JE Huzzey (2009) National free-air CO₂ enrichment facility at Horsham Australia for grain crops: design and performance *Crop and Pasture Science*, 60 (8), 697-707.
- Norton R, G Fitzgerald, C Korte (2008) The effect of elevated carbon dioxide on growth and yield of wheat in the Australian Grains Free Air Carbon dioxide Enrichment (AGFACE) experiment, In. "Global Issues, Paddock Action". Proceedings of the 14th Australian Agronomy Conference, 21-25 September 2008, Adelaide, South Australia. Australian Society of Agronomy.
- Schlesinger WH, J Lichter (2001) Limited carbon storage in soil and litter of experimental forest plots under increased atmospheric CO₂. *Nature*, 411, 466–469.
- Whetton P (2001) Climate change: projections for Australia. CSIRO Atmospheric Research Report, Aspendale, Victoria.