N DYNAMICS UNDER ELEVATED CARBON DIOXIDE IN THE AUSTRALIAN FACE EXPERIMENT

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Abstract

The Australian Grains Free Air Carbon Dioxide Enrichment (AGFACE) facility was established to compare wheat growth, yield and development under ambient (~380 (μmol/mol) and elevated (~550 (μmol/mol) carbon dioxide (a[CO_2] and e[CO_2]). Experiments on fertilizer N recovery and straw decomposition have been undertaken to estimate how e[CO_2] and a changing climate could affect N supply and demand for annual crop production systems.

When grown under e[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[CO_2]. Stubble C:N ratio was not affected by e[CO_2].

Wheat was grown with ^{15}N enriched urea in PVC microplots in the AGFACE facility. Harvest biomass increased by 23% and N uptake increased by 17% under e[CO_2]. Like the main experiment, stubble C:N ratio was not affected by e[CO_2] and it had no significant effect on the proportion of N derived from fertilizer (%Ndff) for grain, stem and root. There were no significant effects of e[CO_2] on ^{15}N recoveries in soil and total fertilizer N losses.

The effects of e[CO_2] and irrigation on straw decomposition and soil respiration was also undertaken within the AGFACE experiment. Pure cotton cloth, wheat straw and pea straw were decomposed using litter-bag method for 140 days. The mass remaining was the highest for cotton cloth (90%), then wheat (73%) and pea (50%). Total C content of wheat and pea straw and total N content of pea straw were reduced only under e[CO_2] and irrigated conditions. Soil CO_2 emissions were increased by e[CO_2] only under irrigation.

In these experiments, the C:N ratio and degradation of organic residues in the wheat crop was not affected by carbon dioxide levels, although larger quantities of residue would enter soil nutrient cycles. These data indicate that e[CO_2] increases plant N demand but does not increase the efficiency with which fertilizers are used nor the likely supply of N from residues. Further research is planned to investigate mineralization and N fixation under e[CO_2] and these data will be used to develop N strategies for future cropping systems.

Introduction

Present day atmospheric CO_2 levels have risen from 295 (μmol/mol) in 1900 to about 386 μmol/mol now and by 2100 these levels could reach between 490 and 1260 μmol/mol (Carter

et al. 2007). It has been estimated that this CO_2 rise produce will result in global temperature rises of up to 4.5^oC, and shifts in rainfall amount, intensity and distribution (Whetton 2001). Such changes will impact on agricultural land management and food production.

Carbon dioxide, as well as being a greenhouse gas, is a primary input into photosynthesis and as a consequence increases in its concentration could be expected to increase plant growth. A review of results from 50 experiments showed that increasing [CO_2] from 350 μmol/mol to 700 μmol/mol increased wheat yield about 31% on average, although the actual increases were moderated by temperature, water supply and nutrient supply (Amthor 2001).

Ainsworth and Long (2005) used a meta-analysis of data from 12 large scale Free Air Carbon Dioxide Enrichment (FACE) experiments to predict a 17% yield increase in response to e[CO_2] and also concluded that water stress generally increases the response, while the response under N stress was highly variable.

A key aspect of this rise is the impact elevated [CO_2] (e[CO_2]) will have on the soil and the various processes that cycle nutrients within and between the soil, plant and atmosphere systems. Elevated [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, probably due as Rubisco biosynthesis is down regulated. This process of acclimation occurs as less enzyme is required to maintain photosynthetic rates (Ainsworth and Rogers 2007).

Studies of the effects of e[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[CO_2] (Schlesinger & Litcher 2001; Gill *et al*. 2002) and this occurs when the availability of mineral N declines over time at e[CO_2] in comparison to ambient [CO_2] (a[CO_2]) and if there is no new N input or higher N losses. The result is a gradual decrease in the [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. PNL has been observed in woodland (Hungate *et al*. 2006) and grassland ecosystems where the stimulation of biomass accumulation by e[CO_2] was constrained by N limitation (Newton et al. 2006, Reich *et al*. 2006, Hovenden et al. 2008), but there are few studies reported for cropping systems despite the implications for rates of N fertilizer application as well as the incorporation of legumes into crop rotations.

To develop data to assist with understanding the effects of e[CO2] on wheat growth and yield in Australia, the Australian Grains FACE (AGFACE) facility was commissioned (Norton et al. 2008a). The facility consists of 8 elevated carbon dioxide (eCO_2) rings each 12 m in diameter, with equivalent ambient carbon dioxide (aCO_2) experimental areas spread over a 5 ha site. Treatments imposed aimed to develop a range of temperature and water regimes during crop growth under aCO_2 and eCO_2 conditions. More details of the experimental design and equipment specifications are reported in Norton et al. (2008a) and Mollah et al. (2009).

A key part of the ongoing research at the AGFACE site is to determine if PNL is a likely consequence of rising CO_2 levels for modern rainfed cropping systems, and if so, investigate what interventions would be appropriate to meet that change. This paper reports on three 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, do these changes impact on the efficiency with which soil and fertilizer N is accessed, and what is the impact of crop

residues on N and C cycling. Other research is investigating N fixation, N_2O production and mineralisation under high CO_2.

Materials and Methods

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 winter grain crops. The elevation of atmospheric [CO_2] was achieved using a FACE system, consisting of sixteen 12 m diameter experimental areas, eight ambient and eight elevated. The two target CO_2 concentrations were 380 (ambient) and 550 μmol/mol (elevated). Carbon dioxide exposure commenced at sowing and terminated at harvest. More details of the experimental design and equipment specifications are reported in Norton et al. (2008a) and 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. The trial was a factorial combinations of two [CO_2], two irrigation scenarios and two times of sowing with four replicates in a randomized complete block design.

Biomass production and N uptake

Spring wheat (*Triticum aestivum* L. cv. Yitpi) was sown on 3 June (early sowing) and 6 August (late sowing) in 2008. The ^{15}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. Four replications were used with CO_2 and sowing time combined factorially at the ring level. Each ring was split for irrigation and then within each irrigation treatment, two varieties were sown one with two levels of applied N.

Growth and plant N content were measured at stem elongation (growth stage (GS) 30), anthesis (GS65) and maturity (GS90) for all treatments. Plant N content was estimated by Leco combustion method and grain protein content (at 0% moisture) by NIR. Data were analysed using a general linear model with factors of carbon dioxide, sowing time and irrigation. Values presented are the means for three half ring level treatments (Janz N0, Yitpi N0 and Yitpi N+). Nitrogen uptake is the product of biomass and plant N content and was estimated on a plot basis rather than as the product of the means. Because of some missing values in the data set, N uptake is not the perfect product of mean biomass and mean plant N content.

^{15}N labelling and fertilizer N recovery

Within each half ring, one circular PVC microplot (internal diameter of 24 cm and height 25 cm) was inserted to 20 cm depth. ^{15}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. At harvest plants were cut to 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. Major wheat roots were collected by digging out the top (0-10 cm) soil. Reference plant and soil samples were taken approximately 1 m from the microplot to determine the background enrichments.

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 ^{15}N enrichment by isotope ratio mass spectrometry following combustion. The recovery of ^{15}N applied and percentage of plant N

derived from fertilizer (%Ndff) were calculated by the equations described by Malhi *et al*. (2004).

Crop residue breakdown

Three straw types were included, pure cotton cloth, wheat straw and pea straw, representing respectively straw type of high, medium and low C:N ratios. Pure cotton cloth (100% C) was used as to indicate the presence of N in residue is the prerequisite of microbial composition. Wheat straw and pea straw grown under ambient [CO_2] were collected from a farm near the study site. Cotton cloth and the two straw were washed, oven-dried at 60°C and cut into pieces of around 3-5 cm long. Three grams of each straw type were put into polyester bag of 0.1 m by 0.15 m, with 1 mm mesh size. Five bags of each straw type were buried 5 cm deep, and one of these five bags was collected at 30, 60, 90, 120 and 140 days. The straw remaining in the bag was washed with tap water to remove adhered soil, dried at 60°C for 48 h and weighed to give the percentage mass remaining.

Gas sampling and flux determination

Within selected treatments in the main experiment, gas samples for CO_2 analysis were taken from closed flux chamber (0.15 m height by 0.16 m diameter) on 24 September, 7 and 30 October, 18 November and 10 December between 1300 and 1500 h. The chambers were inserted between crop drill rows on 23 September to a depth of 7 cm, and remained *in situ* throughout the experimental period. There were no wheat plants or any crop residue inside the chamber, although the chambers would have had roots under the chamber. Fluxes measured from this experiment reflected soil respiration from both root and microbial activity. On each sampling day, the chamber was closed for 0.5 h prior to the first gas sampling and five gas samples each of 15 cm^3 were then collected from the closed chambers at 7 minute intervals using a gas tight syringe through a rubber bung. The gas samples were transferred into evacuated glass vials and transported to the laboratory for analysis by gas chromatography equipped with TCD. Flux rate of CO_2 was calculated from the linear change in gas concentrations in the chamber.

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. However, the results for the growth, N concentration and N uptake showed very few interactions between [CO_2] and sowing time, watering regime or cultivar treatment.

Table 1 shows the effects of e[CO_2] on wheat growth and grain yield from the main AGFACE experiment. Because there were few significant statistical interactions among the treatments, the results presented are for the main CO_2 effect from the experiment, which is the mean of two sowing times, two irrigation treatments and three plot level treatments.

Across the three sampling times, the higher level of CO_2 increased above ground biomass by 25%, 31% and 32%. However, the plant N contents were significantly lower with the higher CO_2 in all except the first sampling. This decline was also seen in leaf N concentration at DC65 where the leaf N declined from 3.78% to 3.54% (p<0.000), and at DC90 the grain protein content declined from 18.0% to 17.3% (p=0.004). We reported a similar decline in grain protein in 2007 for these treatments (Norton et al. 2008b) and lower grain protein content under high CO_2 has been noted in other studies (Blumenthal *et al*. 1996). It is likely that this effect is a consequence of lower N contents in the plant during growth, which leads to a lower labile N pool for N translocation during grain filling.

Table 1 The effect of elevated CO_2 on the growth N content and N uptake of wheat from the AGFACE experiment 2008. P values are from the analysis of variance tables.

Factor	[CO_2] (μmol/mol)		GS30	GS65	GS90
Biomass (g/m^2)	550		208	915	1043
	380		166	700	791
		p	*0.000*	*0.000*	*0.000*
Plant N (%)	550		3.69	1.90	1.56
	3800		3.77	2.05	1.63
		p	*0.274*	*0.000*	*0.006*
*N Uptake (g/m^2)	550		7.47	17.24	15.73
	380		6.11	14.28	12.73
		p	*0.000*	*0.000*	*0.000*

** 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 above ground crop residue C:N ratio is an important determinant of the likely N demand to decompose stubble in modern stubble retained cropping systems. Higher C:N ratios require soil N to break down crop residues leading to N immobilization and in this experiment the mean straw C:N ratio was 50±1 and it was not significantly affected by the carbon dioxide level. Even though this ratio was not affected, there was more stubble with e[CO_2] 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.

It is generally recognized that this lower N supply 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. 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 CO_2.

Crop biomass, total N uptake and C/N ratio

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[CO_2] compared to a[CO_2] 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 2). Total N uptake of wheat was increased by 17% (p<0.05) under e[CO_2], 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 [CO_2] 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.

Elevated [CO_2] 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).

Table 2. Dry weight and total N uptake at maturity of crops grown in microplots under ambient and elevated [CO_2] in 2008.

[CO_2]	Biomass (g/m^2)				Total N uptake
(μmol/mol)	Grain	Stem	Root	Total	(g/m^2)
550	314	713	61	1088	15.6
380	264	572	50	886	13.3
% change	+19 ns	+25**	+22**	+23**	+17*

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

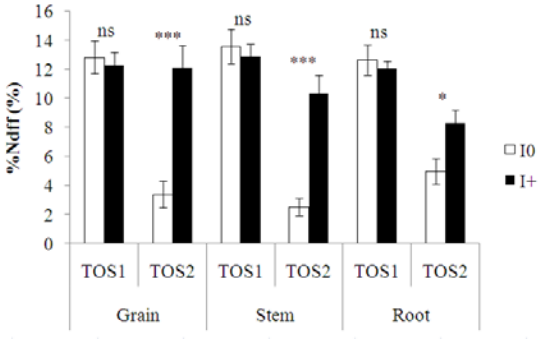

*Figure 1. The effect of irrigation and sowing time on %Ndff of grain, stem and root of wheat crops. Bars indicate standard errors. ns, no significant difference, * p<0.05, *** p< 0.001. I0: rainfed; I+ irrigated; TOS1: early sowing; TOS2: late sowing*

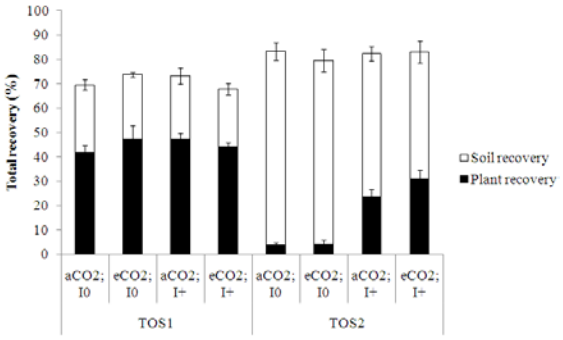

Figure 2. The effect of elevated [CO_2], irrigation and sowing time on ^{15}N fertilizer recovery of plant (■) and soil (□). Bars indicate standard errors. a[CO_2]: ambient [CO_2]; e[CO_2]: elevated [CO_2] I0: rainfed; I+ irrigated; TOS1: early sowing; TOS2: late sowing

The percentage of ^{15}N recovered in the crops averaged 42-48% and 4-31% for early and late sowing, respectively (Figure 2). Elevated [CO_2] did not alter the percentage of ^{15}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}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 a[CO_2] and e[CO_2] for soil depths of 0-10 cm and 10-20 cm except less (46%, p <0.01) ^{15}N was recovered in the lower soil depth (20-40 cm) under e[CO_2] at early sowing. When averaged across soil depths, e[CO_2] had no significant effect on soil ^{15}N recovery (Figure 2).

These data indicate that fertilizer N is not likely to be proportionately more or less available to crops under e[CO_2] 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[CO_2] then fertilizer 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[CO_2] than under a[CO_2] (Norton et al. 2008b). The increase in root-length density could enable the increased N acquisition under e[CO_2] 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.

Crop residue breakdown

The percentage mass remaining decreased with time for all the cotton cloth, wheat straw and pea straw for all treatments, and averaged 90±9, 73±8 and 50±5%, respectively, after 140 days of decomposition. Cotton was hardly decomposed throughout the course of the experiment, indicating the presence of N is crucial to microbial decomposition. The rate of residue breakdown was highest within the first three months of the study period, and levelled off as the soils dried and the temperatures increased. It is likely that the differences in breakdown rate was a consequence of the higher C:N ratio wheat straw (59±4) than pea straw (27±3). There was no significant effect of e[CO_2] on the rate of degradation irrespective of straw type.

Soil Respiration

During the experimental period, CO_2 fluxes were always positive. There was a marginally significant (p=0.095) interaction between e[CO_2] and irrigation with increased CO_2 emission by 78% under irrigation, but not under rainfed condition. This higher efflux was associated with increased biomass under e[CO_2] in this experiment is similar to reports from Jablonski et al. (2002) and Kimball et al. (2002). The results from the residue decomposition experiment indicated that there was no significant effect of e[CO_2] on decomposition rate regardless of irrigation, and the dry soil surface is not likely to host high microbial activity. It is proposed that the stimulation of CO_2 efflux resulted from higher microbial activity in subsurface soil and/or root respiration, as soil respiration comprises respiration by both autotrophs and heterotrophs (Kuzyakou et al. 2006). This is possible as root respiration was observed to increase under e[CO_2] as a result of increased root biomass and concomitant root activity (Søe et al. 2004).

Conclusions

It is apparent that higher atmospheric [CO_2] is likely to increase plant growth, even though plant N content is somewhat reduced, there is 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 fertilizer, as there was no increase in the percentage of N derived from fertilizer. Extra root-length density in the topsoil under e[CO_2] could provide part of the explanation for this extra uptake.

The C:N ratio of crop residues was not affected by e[CO_2] and even under e[CO_2] the rate of breakdown was similar for materials with the same C:N ratio. Even though the rates remain the same, the extra crop residue produced due to the fertilizer effect of carbon dioxide would require added N from the soil, suggesting that immobilization of N could be higher with e[CO_2]. 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.

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