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 \mu mol/mol) and elevated (~550 \mu mol/mol) carbon dioxide (a[CO\textsubscript{2}] and e[CO\textsubscript{2}]). Experiments on N uptake and fertilizer N recovery have been undertaken to estimate how e[CO\textsubscript{2}] and a changing climate could affect N supply and demand for annual crop production systems.

When grown under e[CO\textsubscript{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 higher in all three years with e[CO\textsubscript{2}]. Root samples taken at anthesis showed that in two of three years, root length density was higher for crops grown under e[CO\textsubscript{2}]. Both root biomass and root length density at anthesis also increased in two of the three years of the experiment, although there was no consistent relationship between root length density and N uptake.

Wheat was grown with \textsuperscript{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\textsubscript{2}]. Elevated [CO\textsubscript{2}] 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\textsubscript{2}] on \textsuperscript{15}N recoveries in soil and total fertilizer N losses.

These data indicate that e[CO\textsubscript{2}] increases growth and plant N demand but there does not seem to be a consistent relationship between N uptake and root density. Wheat under high [CO\textsubscript{2}] showed no increase in uptake efficiency from fertilizers or from the soil. Given those two factors, it would appear that the added growth and yield which is a consequence of better C supply will need to be balanced with extra N either from fertilizer or biological sources in future cropping systems.

Key Words
Free air carbon dioxide enrichment, wheat, nitrogen fertilizer, root length density.

Introduction
It is predicted that by 2100 that atmospheric CO\textsubscript{2} levels could reach between 490 and 1260 \mu mol/mol from 295 \mu mol/mol in 1900 and about 386 \mu mol/mol now (Carter et al. 2007). It has been estimated that this CO\textsubscript{2} rise produce will result in global temperature rises of up to 4.5\(^\circ\)C, and shifts in rainfall amount, intensity and distribution (Whetton 2001). Such changes will impact on agricultural land management and food production.

Ainsworth & 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\textsubscript{2}] and also concluded that water stress generally increases the response, while the response under N stress was highly variable. This prediction is somewhat lower than the 31\% on average reported by Amthor (2001) although the actual increases are likely to be moderated by temperature, water supply and nutrient availability.
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. Because CO$_2$ is a primary input into photosynthesis, higher [CO$_2$] should increase 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 fewer enzymes are required to maintain photosynthetic rates (Ainsworth & Rogers 2007).

The effects of e[CO$_2$] on soil N processes is to increase the plant demand for N, and other nutrients, and this may not always be able to be met by soil processes. As a result, over time N becomes more limiting, an effect termed progressive nitrogen limitation (PNL). PNL is closely linked to potential C sequestration under e[CO$_2$] (Schlesinger & Litcher 2001; Gill et al. 2002) and 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 implication that N demand would change and would need to be met by altering the rates of N fertilizer and/or changing the frequency of legumes into crop rotations.

To develop data to assist with understanding the effects of e[CO$_2$] on wheat growth and yield, the Australian Grains FACE (AGFACE) facility was commissioned in 2006. In addition to investigating biotic and abiotic effects on growth, yield and water use responses to climate change, the AGFACE is investigating if PNL is a likely consequence of rising [CO$_2$] for modern rainfed cropping systems, and if so, what interventions would be appropriate. This paper reports on the ability of wheat to access N when grown under e[CO$_2$], in particular the effect of e[CO$_2$] on N demand by wheat and the impact of these changes on the efficiency with which soil and fertilizer N is accessed. Other research is investigating C and N cycling, N fixation, N$_2$O production and mineralisation under high CO$_2$.

**Materials and Methods**

Field experiments were conducted from June to December in 2007, 2008 and 2009 at Horsham, Victoria, Australia (36°45’S, 142°07’E) on a vertisol that is commonly used for winter grain crop production. The elevation of atmospheric [CO$_2$] was achieved using a FACE system, consisting of sixteen 12 m (2007, 2008) or 16 m (2009) diameter experimental areas, eight ambient and eight elevated. The target CO$_2$ concentrations were ambient (~380) and 550±28 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 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. In each year, the experiment was designed with factorial combination 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 either early (2007 03 June, 2008 07 June, 2009 23 June) or late (2007 22 August, 2008 6 August, 2009 19 August). In each year, half the experimental areas received between 50 and 80 mm of additional water from around anthesis to maturity. These treatments provided a range of hydrothermal environments under
which the response of wheat to e\(\text{CO}_2\) can be assessed. Four replications were used with \(\text{CO}_2\) rings split for either sowing time (2007) or irrigation (2008, 2009) and then within each sowing time or irrigation split, two varieties (Janz and Yitpi) were sown, with the latter having two levels of applied N. Individual experimental plots were 1.6 m by 4 m.

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 N content (at 0\% moisture) by NIR. Data were analysed using a general linear model analysis of variance with factors of carbon dioxide, sowing time and irrigation. Nitrogen uptake is the product of biomass and plant N content and was estimated on a plot basis. Because of some missing values in the data set, N uptake is not the perfect product of mean biomass and mean plant N content. Due to poor establishment, no data is presented for the second sowing time from 2007.

**Root Sampling**

At GS65 plant roots were sampled from the experiment by taking two volumetric cores per plot from a Yitpi N0 treatment to 160 cm. One core was taken in the drill row and the other between the drill rows, and these were sectioned and the sections composited. The roots were washed to remove the soil and then the root length measured using a WinRhizo imaging root scanner. Following scanning, roots were dried and weighed. Root length density was derived for each profile level using the sampling core volume and the measured root length.

**\(^{15}\text{N}\) labelling and fertilizer N recovery**

In 2008, within each half ring, one circular PVC microplot (24 cm diam. by 25 cm) was inserted to 20 cm depth. \(^{15}\text{N}\)-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 10 cm of soil. Reference plant and soil samples were taken approximately 1 m from the microplot to determine the background enrichments.

Dried, ground plant and soil samples were analysed for total N and \(^{15}\text{N}\) enrichment by isotope ratio mass spectrometry following combustion. The recovery of \(^{15}\text{N}\) applied and percentage of plant N derived from fertilizer (%Ndff) after Malhi et al. (2004).

**Results and discussion**

**Biomass production and N uptake**

The effects of [\(\text{CO}_2\)] on biomass, N concentration and N uptake are shown in Table 1 for the three years of the experiment. Although there were also significant main plot effects of sowing time and watering regime, there were very few significant interactions among these factors. The values shown are means from one (2007) or two sowing times (2008, 2009), two irrigation treatments and three plot level treatments.

The higher [\(\text{CO}_2\)] increased above ground biomass by 17\%, 20\% and 28\% for the GS30, GS65 and GS90 samplings respectively on average across the three years. However, the plant N contents were significantly lower with the higher \(\text{CO}_2\) in all except the first sampling of 2008. This decline was also seen in leaf N concentration at DC65 where, for example, the leaf N declined from 3.78\% to 3.54\% (\(p<0.000\)) in 2008. However, the increase in biomass more than compensated for the decline in plant N, so that the amount of N extracted in each

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of the three years was significantly higher at maturity by around 22%. This increased demand was noted during early growth in 2008, but only after GS65 in 2007 and 2009.

Table 1 The effect of [CO$_2$] on growth, plant N content and N uptake of wheat in 2007, 2008 and 2009. Values are the mean two irrigation treatments and three plot level treatments for 2007, and for two sowing times, two irrigations and three plot level treatments for 2008 and 2009. Pairs of means in bold are significantly different (p<0.05).

<table>
<thead>
<tr>
<th>Factor</th>
<th>[CO$_2$] (µmol/mol)</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass (g/m$^2$)</td>
<td>380</td>
<td>51</td>
<td>732</td>
<td>739</td>
</tr>
<tr>
<td></td>
<td>550</td>
<td>58</td>
<td>852</td>
<td>910</td>
</tr>
<tr>
<td>Plant N (%)</td>
<td>380</td>
<td>3.86</td>
<td>2.26</td>
<td>1.45</td>
</tr>
<tr>
<td></td>
<td>550</td>
<td>3.63</td>
<td>2.12</td>
<td>1.34</td>
</tr>
<tr>
<td>N Uptake (g/m$^2$)</td>
<td>380</td>
<td>1.97</td>
<td>14.86</td>
<td>10.64</td>
</tr>
<tr>
<td></td>
<td>550</td>
<td>2.11</td>
<td>14.91</td>
<td>12.36</td>
</tr>
</tbody>
</table>

In all three years, grain nitrogen content declined on average around 6% (Table 2). Conditions in 2008 and 2009 were characterised by very hot and dry conditions during grain filling so grain sizes were small, and grain N contents high. Lower grain N (protein) content under e[CO$_2$] has been noted in other studies (e.g. 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, rather than dilution with extra starch supplied as a result of an enhanced carbon assimilation capacity.

Table 2 The effect of [CO$_2$] on grain yield and grain yield and grain N content of wheat in 2007, 2008 and 2009. Values are the mean of two sowing times, two irrigation treatments and three plot level treatments. Pairs of means in bold are significantly different (p<0.05).

<table>
<thead>
<tr>
<th>Factor</th>
<th>[CO$_2$] (µmol/mol)</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain yield (g/m$^2$)</td>
<td>380</td>
<td>258</td>
<td>247</td>
<td>252</td>
</tr>
<tr>
<td></td>
<td>550</td>
<td>323</td>
<td>310</td>
<td>332</td>
</tr>
<tr>
<td>Grain N content (%)</td>
<td>380</td>
<td>2.44</td>
<td>3.16</td>
<td>3.06</td>
</tr>
<tr>
<td></td>
<td>550</td>
<td>2.33</td>
<td>3.04</td>
<td>2.81</td>
</tr>
</tbody>
</table>

Root Growth
Unlike to top growth, root biomass at GS65 increased in response to e[CO$_2$] in only one of the three years evaluated and Table 3 shows the root biomass to 60 cm, which is where 85 to 90% of the roots were located. On the other hand, root length density (RLD), a measure of degree of exploration of the soil, was significantly increased under e[CO$_2$] by 60% in 2007 and 22% in 2008, but there was no significant difference in 2009. Despite that RLD, there seemed to be no relationship between higher densities and more N uptake at GS65. In 2007,
N uptake did not increase even though RLD increased by 60%. Further, in 2009, neither RLD nor N uptake were affected by e[CO₂], although in 2008 these two parameters increased by 22%. It is proposed that water supply, rather than N supply was driving root growth in these situations, although a detailed examination of water extraction pattern may provide further data to test this hypothesis.

**Uptake of fertilizer N**

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 (Table 4). 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, but not in early sowing. Elevated [CO₂] 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>
<thead>
<tr>
<th>[CO₂] (µmol/mol)</th>
<th>Biomass (g/m²)</th>
<th>Total N uptake (g/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grain</td>
<td>Stem</td>
</tr>
<tr>
<td>550</td>
<td>314</td>
<td>713</td>
</tr>
<tr>
<td>380</td>
<td>264</td>
<td>572</td>
</tr>
<tr>
<td>% change</td>
<td>+19 ns</td>
<td>+25**</td>
</tr>
</tbody>
</table>

Table 4. Dry weight and total N uptake at maturity of wheat (cv Yitpi) grown in microplots under ambient and elevated [CO₂] in 2008.

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<0.01

Figure 2. The effect of elevated [CO₂], irrigation and sowing time on °N fertilizer recovery of plant (■) and soil (○). Bars indicate standard errors. a[CO₂]: ambient [CO₂]; e[CO₂]: elevated [CO₂] I0: rainfed; I+: irrigated; TOS1: early sowing; TOS2: late sowing

The percentage of °N recovered in the crops averaged 42-48% and 4-31% for early and late sowing, respectively (Figure 2). Elevated [CO₂] did not alter the percentage of °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 under irrigation (Figure 2). The percentage of °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[CO₂] and e[CO₂] for soil depths of 0-10 cm and 10-20 cm except less (46%, *p <0.01*) °N 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 °N recovery (Figure 2).
Conclusion
These data indicate that wheat grown under e[CO$_2$] will have a higher N demand due to the stimulation of C gain, even though plant and grain N levels may be lower. It appears that there is no increase in the efficiency with which N is accessed from either fertilizer or soil resources under e[CO$_2$] so that higher inputs of fertilizer N will be required, or more frequent pulse crops deployed within farming systems to meet this N demand.

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