Influence of nitrogen supply and variety on the grain yield and protein content of wheat under elevated carbon-dioxide

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Abstract
Previous research in Free Air CO_2 Enrichment (FACE) facilities has indicated a significant decline in grain protein levels of wheat grown under elevated carbon dioxide (eCO_2), raising the question whether nitrogen (N) management can be used to reverse this decline. Two experiments, one under controlled environment (CE) conditions, the other in a FACE array ("SoilFACE"), were conducted to examine the nature of interactions between genotype, N supply, eCO_2 (and soil type in SoilFACE) on grain yield and protein in wheat. The first experiment consisted of 9 wheat cultivars/lines by 3 rates of N application (corresponding to deficient, adequate and luxury) and either ambient (ca. 390 ppm) or eCO_2 (700 ppm). The cultivars tested putatively varied in aspects of 'nitrogen use efficiency' (NUE) and 'vigour': Gladius (low NUE), Wyalkatchem (high NUE), Mace (high NUE), Scout (high vigour), Yipti (low vigour), Spitfire (high protein), EGA Gregory (low protein/high yield), and two experimental lines varying in protein content (WB4-1-12 and WB4-1-16). Although there was a significant main effect for each of CO_2, variety and N on a range of physiological and morphological variables measured (although importantly not on either grain yield or protein) there was no significant interaction between CO_2 and variety. Increasing N rate increased grain number ($P < 0.05$) and this effect was greater under eCO_2. Increasing N rate tended ($P = 0.055$) to increase kernel weight at ambient CO_2 but had no significant effect at eCO_2. Results from SoilFACE generally verified those of the CE study, suggesting that there may be only limited opportunity to reverse grain protein decline under eCO_2 by 'genetic solutions'.

Key words
SoilFACE, nitrogen, nitrogen use efficiency

Introduction
One major concern for future increases in atmospheric CO_2 concentration (eCO_2) is consistent declines in grain protein found in both overseas (Högy et al 2013) and Australian (AGFACE- Australian Grains Free Air CO_2 Enrichment) studies (Fernando et al 2012). Grain protein is a critical market attribute for Australian wheat exports so strategies are needed to reverse this protein decline.

Two potential strategies for reversing protein decline under eCO_2 are identification of genetic variation for trait (and subsequent use in breeding programs) and nitrogen (N) management. Recent experimentation in Australian National Variety trials has indicated significant varietal variation in grain protein as well as from N management, although this relationship is also strongly linked to grain yield responses and soil water availability (McDonald 1989).This paper reports results of two experiments, one conducted under controlled environment conditions and the other under Free Air CO_2 Enrichment (FACE), that assessed interactions between wheat genotype, N supply and eCO_2. We tested the hypothesis that a combination of an appropriate cultivar when supplied with adequate N, will maintain grain protein whilst realising increased grain yield under eCO_2.

Methods
A controlled environment (CE) experiment was conducted in a naturally lighted glasshouse comprising 6 sealed units that were maintained at either ambient (390) or eCO_2 (700 ppm). The experimental design consisted of 9 wheat cultivars x 3 rates of N fertiliser application x either ambient or eCO_2 in a randomised complete block design with 3 replicates. The 9 wheat cultivars (or experimental lines) were selected on the basis of putative variations in aspects of N use efficiency, such as grain yield response at high N rate versus low N rate (NUE) using results of GRDC NVT field trials, and known differences in vigour or grain protein. Selected lines were Gladius (low NUE), Wyalkatchem (high NUE), Mace (High NUE), Spitfire (high protein), EGA Gregory (low protein/high yield), Scout (high vigour), Yipti (low vigour), WB4-1-12 and WB4-1-16. The three N rates were 0, 40 and 80 kg N/ha applied as a solution of NH_4NO_3 at the 2 leaf stage

(designed to represent full N response range). An additional 40 kg N/ha was applied to the 80 kg N/ha pots at stem elongation. Plants were grown in 180 mm square pots containing 5 kg of an N-deficient Vertosol soil. Basal nutrients (P, K, S, Cu, Zn, Mn, Mo and B) were applied to ensure that only N was limiting. Soil in the pots was maintained at field capacity by watering to weight twice weekly with reverse osmosis water. Six seeds per pot were sown in early June 2014 and then thinned to 4 at the 2 leaf stage. Plants were harvested at anthesis (GS 65 – decimal growth stage) and grain maturity (GS 92) before drying (60^{o}C), weighing and processing. Tissue N was determined by Dumas combustion (Leco).

The field experiment was undertaken in SoilFACE, a FACE array based on the use of large intact cores (30 cm diameter x 100 cm deep cased in a PVC sleeve), that maintain the physicochemical integrity of the soil profile, to assess interactions between soil type and eCO_2 on crop growth. Cores were collected from the Victorian Mallee (Calcarosol), Wimmera (Vertosol) and High Rainfall Zone (Chromosol) (Table 1). The cores are placed in 8 bunkers (4M diameter) sunk into the ground (the top of the cores are at ground level). Four bunkers (replicates) are maintained at ambient (ca. 390 ppm) whilst four are maintained at 550 ppm atmospheric CO_2 (eCO_2) as per Mollah et al (2009). The experimental design consisted of 3 wheat varieties x 3 soil types x 2 levels of applied N (0, 75 kg N/ha) x 2 CO_2 levels x 4 reps (bunkers). The wheat varieties were Gladius, Wyalkatchem and Yipti. The N was topdressed (as granular urea) on 30 July. Monthly rainfall (April to November) was 42, 25, 35, 34, 11, 11, 7 and 17mm (total = 182 mm) compared to long-term average = 344 mm). Due to such low spring rainfall, supplementary irrigation (21mm/event) was applied to all cores on 5/9, 17/9, 19/9 and 3/10/2015. Wheat was sown on 4 June and harvesting commenced on 26 November. Plant samples were treated as per the CE experiment.

Table 1: Soil characteristics of 3 soils used in SoilFACE and profile (0-90 cm) soil nitrate (mg/core) and volumetric water (mm/core) prior to sowing in 2014.

Soil	pH ($CaCl_2$) 0-10 cm	EC(1:5) 80-100 cm (dS/m)	ESP 80-100 cm (%)	Total N 0-10 cm (%)	Total C 0-10 cm (%)	NO_3-N (mg/core)	Vol. H_2O (mm/core)
Chromosol	4.5	0.16	4.3	0.40	4.66	68	285
Vertosol	7.7	1.85	20.0	0.08	1.10	35	324
Calcarosol	5.9	0.53	7.5	0.05	0.64	34	179

Results

Under CE conditions, there was a significant main effect of CO_2, variety and N rate on a range of physiological and morphological variables measured (although not on grain yield) (Table 2). Highest protein content was recorded in Spitfire, WB4-1-12 and Wyalkatchem and lowest in Gregory and Mace (data not presented). There was however no interaction between CO_2 x variety, variety x cultivar and N x CO_2 (Table 2). Increasing N rate increased grain number ($P < 0.05$) and this effect was greater under eCO_2 whereas increasing N rate tended ($P = 0.055$) to increase kernel weight at ambient CO_2 but had no significant effect at eCO_2. Importantly, although there was a significant positive effect of N rate and variety on grain protein, eCO_2 had no significant effect nor did it interact with variety or N rate. There was a trend ($R^2 = 0.47$), when all varieties were pooled, for grain protein to decrease with increasing grain yield but there was no relationship ($R^2 = 0.15$) between grain protein and plant N uptake.

In SoilFACE, where experimentation was undertaken in large intact cores that varied markedly in background N supply but water availability was restricted, eCO_2 significantly increased grain yield of all varieties tested (average of 33%), reduced grain protein content (from 10.3 to 9.0%) and had no effect on total N uptake (Table 3). Gladius produced the lowest grain yield and total N uptake but had significantly higher protein (9.9%) than either Wyalkatchem (9.4%) with Yipti intermediate (9.7%). Grain yield was greatest on the Vertosol whereas wheat growing on this soil produced the lowest protein content. Applying N fertiliser increased grain yield, crop N uptake and protein across all 3 varieties. There was no significant interaction between soil type and eCO_2 or between variety and eCO_2.

Discussion

These two experiments, conducted under both CE and field (FACE) conditions, suggest that options for using current widely grown wheat cultivars to reverse the negative impact of eCO_2 on grain protein may be limited. Although there were strong variety (and N rate) effects on grain protein in both experiments, these individual responses were not affected by CO_2 treatment.

Doubts have been raised about use of small pots (such as used in the CE experiment) to study CO_2 effects on grain protein (Taub et al 2008). In our experiment there were initially significant biomass responses to eCO2 in the CE study but this did not translate to biomass or grain yield responses at maturity (Table 2), perhaps indicating pot limitations. The difficulty of generalising CE results on grain protein to field conditions is also underlined by the change of the relative ranking of protein content of the 3 varieties common to both experiment: Wyalkatchem was highest in the CE (data not shown) yet lowest (cf. Gladius and Yipti) in SoilFACE (Table 3).

Table 2. Average effect of eCO_2 on mean shoot and root dry matter (DM) at anthesis (g/pot), maturity shoot DM (g/pot), tiller number at maturity (tillers/pot), grain yield (g/pot), kernel weight (mg) and grain number (grains/pot) of nine wheat varieties grown under three levels of soil N in the glasshouse. Values in sub-table are l.s.d. (P = 0.05) n.s. not significant (P = 0.05); * F = 0.063 **F = 0.055

Ambient CO_2			Elevated CO_2		
0 kg N/ha	40 kg N/ha	120 kg N/ha	0 kg N/ha	40 kg N/ha	120 kg N/ha
Anthesis shoot DM (g/pot)					
9.6	14.7	17.8	9.9	15.4	19.4
Anthesis root DM (g/pot)					
1.3	2.0	2.2	9.9	1.6	2.0
Maturity shoot DM (g/pot)					
12.8	24.1	36.1	13.7	23.0	36.1
Maturity tiller number (tillers/pot)					
4.1	8.9	13.2	4.7	9.2	13.9
Grain yield (g/pot)					
6.2	12.4	19.3	6.8	11.4	19.6
Grain protein (%)					
8.7	8.3	10.0	8.8	8.6	9.8
Kernel weight (mg)					
44.6	48.1	47.2	45.5	42.7	43.3
Grain number (grains/pot)					
142.2	258.3	412.2	148.4	267.2	455.1

ANOVA (lsd)

Variable	CO_2	N rate	Variety	CO_2 x N rate	CO_2 x variety	N rate x variety
Anthesis shoot DM	0.53	0.65	n.s.	n.s.	n.s.	n.s.
Anthesis root DM	0.13	0.16	0.28	0.23*	n.s.	n.s.
Maturity shoot DM	n.s.	1.28	2.21	n.s.	n.s.	n.s.
Maturity tiller number	0.42	0.51	0.88	n.s.	n.s.	1.53
Grain yield	n.s.	0.97	n.s.	n.s.	n.s.	n.s.
Grain protein	n.s.	0.39	0.68	n.s.	n.s.	n.s.
Kernel weight	2.23	n.s.	4.72	n.s.	n.s.	n.s.
Grain number	11.9	14.6	25.3	20.6	n.s.	n.s.

Grain protein is also influenced by a range of other factors including water supply and temperature (Altenbach et al 2003). In contrast to the CE experiment, where water was kept non-limiting, rainfall was well below average throughout most of the growing season in SoilFACE and supplementary irrigation had to be applied near anthesis just to prevent crop failure. Under such conditions, crops would fill grains under water stress (terminal drought) where any post anthesis N uptake is very limited (Paltra and Fillery 1993). N supply to grains will then come mainly from remobilisation. Furthermore, protein responses in SoilFACE were affected by soil type, which not only varied in initial soil nitrate supply (and potential ability to mineralise N during the growing season) but also physicochemical conditions such as salinity and sodicity in the subsoil (Table 1) which may have influenced the ability of the different wheat varieties to access soil nitrate and water, and in turn, grain yield and protein responses to eCO_2.

Table 3: Influence of soil type and N rate (0 and 75 kg N/ha) on grain yield (g/core), protein (%) and total N uptake (mg/core) of Gladius, Wyalkatchem (Wyalk) and Yipti wheat varieties under Ambient (amb) and eCO_2 in SoilFACE in 2014. (ns: not significant (P=0.05) ; * F= 0.053; ** F = 0.069)

CO2	N rate kg/ha)	Calcarosol			Vertosol			Chromosol		
		Gladius	Wyalk.	Yipti	Gladius	Wyalk.	Yipti	Gladius	Wyalk.	Yipti
Grain yield (g/core)										
amb	0	10.1	10.8	8.8	13.9	15.5	15.2	10.0	12.1	14.2
amb	75	15.8	16.9	16.6	22.8	22.7	19.6	11.6	12.6	10.5
eCO_2	0	9.9	12.3	10.8	18.3	17.1	18.9	16.0	19.8	18.2
eCO_2	75	17.4	24.4	21.3	28.5	33.2	24.8	12.1	21.7	18.3
l.s.d. (5%): CO_2 2.70 ; N 1.45 ; Soil 1.79; Var 1.76 ; CO_2 x N 0.077 ; N x Soil 2.60 ; CO_2 x Var ns ; N x Var ns ; Soil x Var ns										
Grain protein (%)										
amb	0	9.1	8.9	8.4	8.1	7.1	7.5	12.8	12.5	11.8
amb	75	10.2	9.9	10.0	10.6	9.7	9.3	11.9	13.6	14.6
eCO_2	0	8.6	7.1	8.0	7.8	6.6	6.8	10.4	10.4	11.1
eCO_2	75	9.5	7.4	7.8	8.2	7.8	8.1	11.8	11.2	12.8
l.s.d. (5%): CO_2 0.50 ; N 0.37 ; Soil 0.46 ; Var 0.45*; CO_2 x N 0.69** ; CO_2 x Soil ns; N x Soil ns ; CO_2 x Var ns; N x Var ns										

In these experiments N, which was supplied early in the growth cycle, strongly influenced dry matter production. Grain protein, on the other hand, is stimulated by N application that does not increase biomass, when applied near anthesis. Experimentation is currently in progress at the AGFACE facility to assess if management strategies that delay N supply to latter in the crop cycle such as foliar application and novel fertiliser formulations can maintain or enhance protein content in future eCO_2 environments.

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