# Can Nitrogen Management maintain Grain Protein Content of wheat under elevated $CO_2$? A FACE study

Cassandra Walker, Roger Armstrong, Joe Panozzo, Glenn Fitzgerald

Department of Economic Development, Jobs, Transport and Resources, Horsham, Victoria, 3400, cassandra.walker@ecodev.vic.gov.au

**Abstract**
The impact of different nitrogen (N) management strategies (rate, split-N application, foliar-N application, legume pre-cropping) were assessed for their effectiveness in reversing the reduction of grain protein content in order to maintain grain quality of wheat (cv. Yitpi) under elevated $CO_2$ ($eCO_2$) using Free Air Carbon Dioxide Enrichment (FACE).

Preliminary results show that under $eCO_2$ conditions the plant biomass, grain yield and grain size increased and that grain protein content decreased when no N fertiliser was applied (N0). Significant grain yield responses to increasing rates of N fertiliser (applied at sowing) were observed in Yitpi, with the grain yield under $eCO_2$ conditions increasing by 63% when 100 kg N/ha (N100) was applied compared with N0; an increased response (59%) was also observed under ambient $CO_2$ conditions. The largest grain yield response for a N management strategy compared with N0 under $eCO_2$ conditions was when Yitpi was grown after prior rotation with annual medic pasture (M), with an increase of 74%. Grain protein increased significantly (31%) under $eCO_2$ conditions at N100 compared with N0, however it seems that vegetative growth demand 'took preference' with an increase in dry matter (DC90) of 101% under $eCO_2$ conditions at N100 compared with N0. Under ambient $CO_2$ only 50 kg N/ha at sowing was required to gain a similar increase in grain protein content. When comparing the grain protein content of Yitpi sown into medic stubble (M) to that at N0, there was a significant increase ($P = 0.05$) at ambient $CO_2$ conditions, however, under $eCO_2$ conditions no significant response observed.

**Key Words**
AGFACE, grain protein content, nitrogen management, elevated carbon dioxide

## Introduction

Over the last 100 years, atmospheric carbon dioxide ($CO_2$) levels have risen from 280 to 390 $\mu$mol mol$^{-1}$. It is estimated that in the next 40-50 years, $CO_2$ levels will increase by a further 40%. An Australian Grains Free Air Carbon dioxide Enriched (AGFACE) experiment was set up at Horsham, Victoria to understand crop responses to future elevated $CO_2$ in semi-arid cropping systems (Fitzgerald et al. 2016). Previous experimentation in AGFACE has identified that typically the plant biomass, grain yield and grain size increases, but that grain protein content and baking quality decreases in wheat cultivars under elevated carbon dioxide ($eCO_2$) environments (Panozzo et al. 2014; Fernando et al. 2012; Fitzgerald et al. 2016). For grain protein content one key hypothesis proposed to account for the decrease observed is that there is a 'dilution effect' where enhanced biomass and grain yield production under $eCO_2$ results in inadequate N availability. Previous research has demonstrated that root growth is potentially increased under $eCO_2$ and that soil N reserves, especially that of mineral N ($NO_3$ and $NH_4$), may be inadequate to meet the increased crop demand. Under current cultivation practices, growers have a variety of options available to supply extra N, including increasing the rate of fertiliser N, use of biologically fixed N (via legume $N_2$ fixation) and altering the timing of N supply to meet grain demand without affecting the vegetative supply. This latter strategy can be achieved by either delaying the timing of fertiliser N supply (topdressing later in the growing season) or the direct application of N to the crop itself (via foliar application). The main objective of this study was to use the AGFACE facility to assess the ability of different Nitrogen (N) management strategies so as to maintain grain protein content whilst increasing grain yields in an $eCO_2$ environment.

## Methods

A bread wheat cultivar, Yitpi, was grown in 2015 under ambient $CO_2$ ($aCO_2$, ~390 $\mu$mol /mol) and elevated $CO_2$ ($eCO_2$, ~550 $\mu$mol /mol) at Horsham, Victoria, Australia. The site was characterised by a Vertisol clay soil with long history of cropping, where the previous crop in 2015 was barley. The plot size was 6 rows (25 cm spacing) x 6 metre length.

Four different N management strategies were applied: Rate (N0, N25, N50, N100), Timing (N50S), Placement (N25F, N25T), and Form (M), Table 1.

**Table 1. The experimental design applied to Yitpi of ten treatments at ambient and elevated $CO_2$**

| Code | Treatment |
|---|---|
| N0 | No fertiliser input |
| N25 | 25 kg N/ha urea equivalent incorporated into soil at sowing |
| N50 | 50 kg N/ha urea equivalent incorporated into soil at sowing |
| N100 | 100 kg N/ha urea equivalent incorporated into soil at sowing |
| M | Medic sown previous season and stubble incorporated pre-sowing |
| N25F | 25 kg N/ha urea equivalent applied at sowing plus 6.25 kg N/ha applied as foliar spray at 4 dates around anthesis |
| N25T | 25 kg N/ha urea equivalent applied at sowing plus 6.25 kg N/ha N (as granular urea) topdressed on 4 dates urea around anthesis |
| N50S | 50 kg N/ha Polymer Coated (slow release) Urea incorporated into soil at sowing |

Soil tests were taken before sowing and at harvest according to Fitzgerald et al. (2016). The AGFACE facility at Horsham was arranged in a completely randomised unbalanced design with 4 elevated ($eCO_2$) reps and 6 ambient ($aCO_2$) replications. The 2015 season was characterized by dry annual (total = 214 mm) and poor growing season rainfall (128 mm), requiring the use of supplementary irrigation (applied by drip irrigation to ensure accurate placement) across all plots to ensure 'adequate' crop growth, this equated to 247 mm of water during the growing season.

Above ground biomass was measured at DC31, DC65, and DC90, and grain yield components at DC 90, including tiller number/dry weight, head number/dry weight, and 1000 grain weight, according to Fitzgerald et al. (2016). After harvest, grain protein content was determined by NIR (Walker and Panozzo 2014), and are expressed on an 11% moisture basis.

## Results

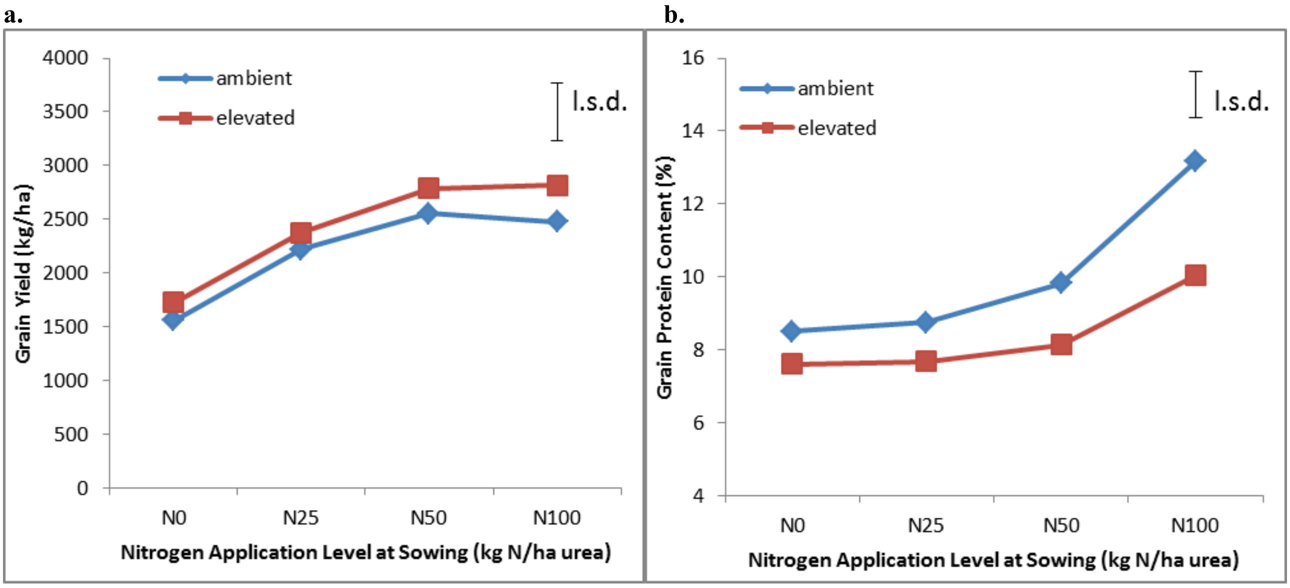



**Figure 1. a) Grain yield of Yitpi grown under different N levels applied at sowing (kg N/ha urea) under ambient and elevated $CO_2$ in 2015, and b) Grain protein content of Yitpi grown under different N levels applied at sowing (kg N/ha urea) under ambient and elevated $CO_2$ in 2015.**

Background soil N concentration were low (0-10 cm total N: 0.091%; organic C: 0.73%), resulting in low profile (0-120 cm) mineral N levels at sowing (47.9 kg/ha) but significantly higher levels in the plots sown to annual medic in 2014 (71.6 kg/ha). Nitrate–N tended to be concentrated in the topsoil (0-10 cm) of the medic plots whereas in the 'bulk area' it was highest in lower parts of the soil profile (> 40 cm). There were large growth responses of Yitpi to urea N incorporated at sowing at both ambient and $eCO_2$ conditions (Figure 1a); however there were no significant differences in between equivalent treatments grown under ambient and $eCO_2$ conditions (Table 2, Figure 1a). Large biomass responses ($P < 0.001$) to N fertiliser were observed in the early growth stages and these continued to grain maturity, and there was evidence that dry matter production was still limited at the highest rate of N application (N100) applied (Table 2). The biomass responses to N applied at sowing translated to significant grain yield responses to N fertiliser application with yields at N100 under ambient $CO_2$ conditions 59% greater than those at N0, a larger response was observed under $eCO_2$ of 63% (Table 2, Figure 1a). These grain yield responses were primarily due to greater tiller number (and associated grain number) rather than increased grain size or harvest index (Table 2). The highest grain yield response was observed with prior rotation with annual medic pasture (M), where yields were 74% greater than those at N0 under $eCO_2$ conditions (Table 2).

**Table 2. Dry matter, yield components and grain protein response of wheat (cv. Yitpi) to different N management strategies and elevated $CO_2$ in 2015.**

| Treatment | $CO_2$[a] | DC31 dry matter (g/m$^2$) | DC65 dry matter (g/m$^2$) | DC90 dry matter (g/m$^2$) | Grain yield (kg/ha) | 1000 grain wt (g) | Tiller (no./m$^2$) | Heads (no./m$^2$) | Grains (no./m$^2$) | Grain Protein (%) | Grain N (kg/ha) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **N0**[b] | $aCO_2$ | 42.8 | 361 | 498 | 1560 | 45.3 | 258 | 239 | 4806 | 8.50 | 23.3 |
| | $eCO_2$ | 38.8 | 349 | 458 | 1728 | 48.6 | 211 | 197 | 3877 | 7.60 | 22.9 |
| **N25** | $aCO_2$ | 67.4 | 434 | 749 | 2222 | 43.2 | 345 | 325 | 6978 | 8.75 | 34.6 |
| | $eCO_2$ | 53.2 | 581 | 644 | 2375 | 47.1 | 289 | 259 | 5249 | 7.68 | 32.0 |
| **N50** | $aCO_2$ | 66.3 | 617 | 711 | 2553 | 39.2 | 326 | 329 | 6038 | 9.83 | 45.8 |
| | $eCO_2$ | 73.2 | 650 | 859 | 2788 | 43.5 | 276 | 351 | 5375 | 8.13 | 34.4 |
| **N100** | $aCO_2$ | 69.2 | 649 | 749 | 2479 | 34.1 | 377 | 335 | 7190 | 13.17 | 57.0 |
| | $eCO_2$ | 82.3 | 806 | 920 | 2814 | 37.3 | 432 | 375 | 8739 | 10.03 | 49.0 |
| **M** | $aCO_2$ | 56.8 | 577 | 771 | 2536 | 40.9 | 426 | 370 | 7594 | 9.80 | 43.7 |
| | $eCO_2$ | 74.2 | 683 | 873 | 3017 | 42.6 | 395 | 359 | 7986 | 8.03 | 42.5 |
| **N25F** | $aCO_2$ | 45.0 | 396 | 570 | 2248 | 46.0 | 262 | 249 | 5455 | 8.80 | 35.1 |
| | $eCO_2$ | 41.8 | 468 | 601 | 2121 | 48.1 | 259 | 243 | 5274 | 7.75 | 28.8 |
| **N25T** | $aCO_2$ | 36.3 | 390 | 507 | 1993 | 44.3 | 238 | 229 | 4714 | 8.48 | 29.5 |
| | $eCO_2$ | 35.8 | 471 | 544 | 2030 | 46.7 | 251 | 233 | 5083 | 7.68 | 27.4 |
| **N50S** | $aCO_2$ | 53.5 | 548 | 748 | 2539 | 43.2 | 358 | 337 | 7310 | 9.12 | 40.6 |
| | $eCO_2$ | 49.8 | 649 | 676 | 2516 | 44.5 | 299 | 272 | 6137 | 7.85 | 34.5 |
| l.s.d. (P = 0.05) | | 17.8 | 155 | 189 | 548 | 4.64 | 79.8 | 75.9 | 1983 | 1.27 | 5.1 |
| Significance for N treatment[c] | | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| Significance for $CO_2$ treatment | | 0.95 | ** | 0.34 | 0.077 | * | 0.34 | 0.253 | 0.91 | *** | 0.25 |
| Significance for N x $CO_2$ treatment | | 0.8 | 0.6 | 0.18 | 0.26 | 0.94 | 0.36 | 0.22 | 0.18 | 0.093 | 0.15 |

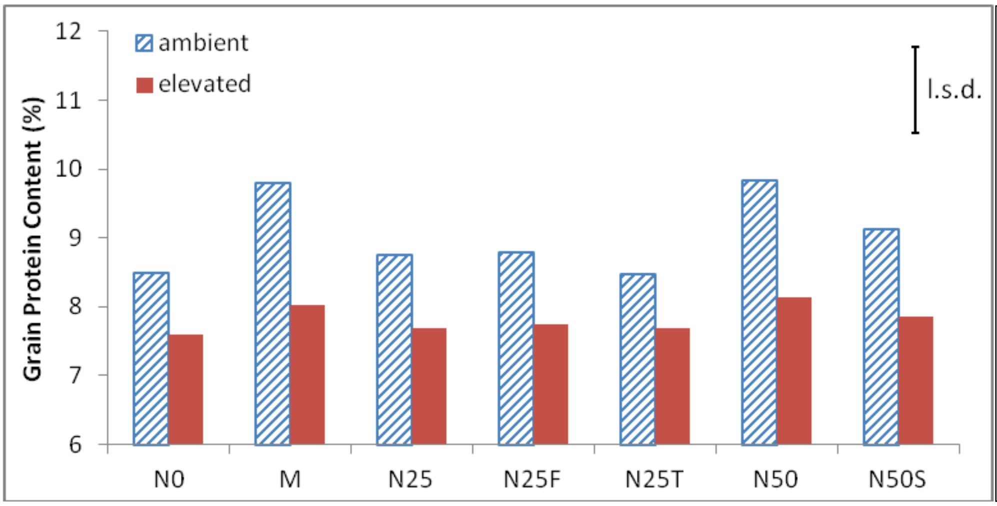
[a] $aCO_2$, ambient $CO_2$; and $eCO_2$, elevated $CO_2$

[b] N0:No fertiliser; N25: 25 kg N/ha urea; N50: 50 kg N/ha urea; N100: 100 kg N/ha urea; M: Medic sown previous season; N25F: 25 kg N/ha urea plus 4 x 6.25 kg N/ha applied as foliar spray around anthesis; N25T: 25 kg N/ha plus 4 x 6.25kg N/ha N topdressed around anthesis; N50S: 50 kg N/ha Polymer coated (slow release) urea

[c] *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

The grain protein content of Yitpi in the absence of applied N was low (< 8.6% w/w). Similar to previous observations in AGFACE, $CO_2$ fertilisation reduced grain protein under $eCO_2$ conditions (to < 7.6%, w/w) (Table 2, Figure 1b). The grain protein content responses of Yitpi to N applied at sowing under ambient $CO_2$ conditions translated to a significant increase in grain protein content of 54% at N100 when compared with the level observed at N0 (Table 2, Figure 1a). Under $eCO_2$ conditions adding N at sowing did increase grain protein content but vegetative growth demand 'took preference' with grain protein content only increasing significantly by 31% at the highest rate of N application (N100). In contrast, at ambient $CO_2$ conditions only 50 kg N/ha at sowing was required for a significant response in grain protein content.

Other N management strategies specifically designed to stimulate grain protein (foliar application-N25F and top-dressing-N25T) had no significant (P = 0.05) effect (compared to the equivalent rate of N applied, N25) for grain protein response or dry matter production and grain yield (Table 2). The lack of effect of the top-dressing and foliar application treatments reflected the very dry conditions experienced prior to anthesis and subsequent grain filling period. The 'slow release' urea formation (applied at sowing) increased grain yield compared to N50 treatment of urea applied at sowing, but this came at the expense of grain protein (Table 2). Dry matter production and grain yield of wheat sown into medic stubble (M) tended to match those of the higher rates of urea application (N100) throughout the growing season, whereas the grain protein response was similar to that of the N50 treatment (Table 2). When comparing the grain protein content of Yitpi sown into medic stubble (M) to N0, there was a significant increase ($P = 0.05$) at ambient $CO_2$ conditions, however under $eCO_2$ conditions there was no significant response observed when comparing M with N0. This lack of response in grain protein content under $eCO_2$ conditions was most likely due to the significant ($P = 0.05$) increase in biomass production and grain yield for the M treatment compared with N0 (Table 2).




N0:No fertiliser input
N25: 25 kg N/ha urea
N50: 50 kg N/ha urea
N100: 100 kg N/ha urea
M: Medic sown previous season
N25F: 25 kg N/ha urea plus 4 x 6.25 kg N/ha applied as foliar spray around anthesis
N25T: 25 kg N/ha plus 4 x 6.25kg N/ha N topdressed around anthesis
N50S: 50 kg N/ha Polymer coated (slow release) urea

**Figure 2. Grain protein content of wheat (cv. Yitpi) in AGFACE subjected to different N management strategies under ambient and elevated $CO_2$ conditions in 2015.**

## Conclusion

Under $eCO_2$, significant grain yield responses were observed for three of the four N management strategies assessed in this study: increasing rate of N fertiliser applied at sowing, delaying the timing of application ('slow release' urea, N50S), and form (N derived from previous legume phase, M). In contrast to ambient $CO_2$, grain protein content did not significantly increase under $eCO_2$ for most of the N management strategies assessed in this study. This suggests that a different strategy e.g. specific adapted germplasm, may be required if grain growers are to maintain grain protein (and quality) in future $eCO_2$ environments.

## Acknowledgements

Research within the AGFACE facility was supported by the Grains Research and Development Corporation (GRDC) in a joint collaboration between the Victoria Department of Economic Development, Jobs, Transport and Resources (DEDJTR) and the University of Melbourne (UM). We wish to acknowledge the contributions of Russel Argall , Nathan Neumann, Mel Munn and Roger Peris (DEDJTR) in running and maintaining the AGFACE facility, collecting key measurements and sample processing.

## References

Fernando N, Panozzo J, Tausz M, Norton RM, Fitzgerald GJ, Myers S, Walker C, Stangoulis J, Seneweera S (2012) Wheat grain quality under increasing atmospheric $CO_2$ concentrations in a semi-arid cropping system. Journal of Cereal Science 56:684-690.

Fitzgerald GJ, Tausz M, O'Leary G, Mollah MR, Tausz-Posch S, Seneweera S, Mock I, Löw M, Partington DL, McNeil D, Norton RM (2016) Elevated atmospheric [$CO_2$] can dramatically increase wheat yields in semi-arid environments and buffer against heat waves. Global Change Biology 22:2269-2284.

Panozzo JF, Walker CK, Partington DL, Neumann NC, Tausz M, Seneweera S, Fitzgerald GJ (2014) Elevated carbon dioxide changes grain protein concentration and composition and compromises baking quality. A FACE study. Journal of Cereal Science 60:461-470.

Walker C, Panozzo J (2014) Gathering reliable data on malting quality for genetic analysis from barley using near infrared spectroscopy. Journal of Near Infrared Spectroscopy 22:81-92