# Interactive effect of elevated $CO_2$ and supplemental N on above- and belowground growth and water use of dryland wheat

**Shihab Uddin**[1 2 3 *], Shahnaj Parvin[3 4], Markus Löw[2], Sabine Tausz-Posch[5], Roger Armstrong[6 7], Garry O'Leary[6], Glenn Fitzgerald[2 6], Michael Tausz[5]

[1]NSW Department of Primary Industries, Wagga Wagga Agricultural Institute, Wagga Wagga, NSW 2650, Australia.
[2]Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Creswick, VIC, Australia.
[3]Department of Agronomy, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.
[4]School of Ecosystem and Forest Sciences, The University of Melbourne, Creswick, VIC, Australia.
[5]Department of Agriculture, Science and Environment, School of Health and Applied Sciences, CQUniversity Australia, Rockhampton, QLD, Australia.
[6]Agriculture Victoria, 110 Natimuk Road, Horsham, VIC 3400, Australia.
[7]Department of Animal, Plant and Soil Sciences, La Trobe University, Bundoora, VIC, Australia
[*]Corresponding author: email: shihab.uddin@dpi.nsw.gov.au

## Abstract
Elevated atmospheric $CO_2$ concentration (e[$CO_2$]) stimulates biomass and yield of crops through the '$CO_2$ fertilisation effect'. Stimulation of biomass with supplemental nitrogen (N) under e[$CO_2$] may influence water use dynamics, which is particularly important in relatively low yielding dryland Mediterranean regions where timing of water limitations interacts with N availability and intra-seasonal variability is high. This study investigated the interactive effect of N supply (with and without supplemental N) and [$CO_2$] (ambient [$CO_2$] and e[$CO_2$]~550 µmol mol$^{-1}$) on aboveground biomass, root length and water use of two wheat cultivars putatively differing in N use efficiency using a Free Air $CO_2$ Enrichment (FACE) facility in Horsham, Victoria. Elevated [$CO_2$] stimulated biomass and grain yield, and this stimulation was influenced by both cultivar and N supplementation. The observed differential response of cultivars to e[$CO_2$] and N rates suggests that there is potential to select germplasm that maximises the benefit from $CO_2$ fertilisation under a wide range of soil N availability.

## Keywords
Climate change, FACE, dryland agriculture, wheat, nitrogen, water use

## Introduction
Atmospheric $CO_2$ concentration ([$CO_2$]) is rising due to anthropogenic activities and is expected to reach ~550 µmol mol$^{-1}$ by 2050. This rising [$CO_2$] can increase crop water use efficiency by increasing net assimilation rate and reduce stomatal conductance (Leakey et al., 2009). Alternatively, elevated [$CO_2$] (e[$CO_2$]) can increase water use demand by increasing the transpiring leaf area and/or biomass (Wu et al., 2004). The overall effect of e[$CO_2$] on crop water use is not straightforward and depends on the balance between these two abovementioned effects (Uddin et al., 2018b). Therefore, crop water use under e[$CO_2$] would be of particular interest for dryland agroecosystems in Australia where crop growth is often limited by water availability and intra-seasonal rainfall variability is very high (Angus, 2001).

As the stimulation of plant biomass under e[$CO_2$] depends on soil N availability (Reich et al., 2014), therefore crop water use in future will be further complicated by the interactive effect of e[$CO_2$] and N fertilisation. To investigate the interactive effect of e[$CO_2$] and supplemental N on biomass production and water use of dryland wheat an experiment was conducted in the Australian Grains Free Air $CO_2$ Enrichment (AGFACE) facility in Horsham, Victoria, representative of Mediterranean or semi-arid, water limited, low yielding wheat cropping systems worldwide (Fitzgerald et al., 2016). As our experimental plots were characterised as low in background soil N, additional environmental variation was achieved by adding supplemental N. The plant root system is the most important component of capturing water and nutrient resources and acquisition of subsoil water under dryland conditions can have a strong effect on grain yield (Kirkegaard et al., 2007). We, therefore, monitored the root growth at different depths of the soil profile.

## Methods
The experiment was conducted in 2016 in the Australian Grains Free Air $CO_2$ Enrichment (AGFACE) facility in Horsham (36°44'57"S, 142°06'50"E; 127 m elevation), Victoria, Australia. A detailed description of this study site and AGFACE facility is given in Mollah et al. (2009) and Fitzgerald et al. (2016), but this present experiment unlike the original AGFACE site, was conducted on an adjacent paddock with low

background soil N (~90 kg N/ha soil mineral N in the 1.2 m root zone). The experimental design was a nested split-plot design with four replicate plots ('rings') of 16 m diameter for a[$CO_2$] (~400 µmol $mol^{-1}$) and four rings for e[$CO_2$] (~550 µmol $mol^{-1}$). Within each ring two wheat cultivars 'Yitpi' and 'Wyalkatchem' with putative higher N use efficiency were randomly allocated to sub-plots and treated without (N0) or with (N100; 100 kg N $ha^{-1}$) supplemental N. Each sub-plot was 4 m long by 1.4 m wide. Plots were sown on 31 May 2016 with 0.244 m row spacing. Enrichment of [$CO_2$] in the elevated rings was done from sunrise to sunset with a target of 550 µmol $mol^{-1}$, starting within a few days of 50% seedling emergence until harvest. The engineering and performance of the $CO_2$ fumigation systems within the AGFACE facility are described in detail by Mollah et al. (2009).

At anthesis, canopy reflectance was measured non-destructively using a handheld CropCircle ACS-210 with GeoSCOUT GLS-400 data logger (Holland Scientific, Lincoln, NE, USA), from which the normalised difference vegetation index (NDVI) was calculated. At the same growth stage, root length at four depths (depth 1: 0–16 cm; depth 2: 17–32 cm; depth 3: 33–48 cm and depth 4: 49–64 cm) of the soil profile was measured using a mini-rhizotron (CI-600™, CID Bio-Science Inc., Camas, WA, USA) system. Detailed description of installing acrylic clear rhizo-tubes, acquiring the images of root system in the field and analysing the root length from the images for a similar experimental set-up within AGFACE facility is given in Uddin et al. (2018a). Chlorophyll and N content of flag leaves were measured non-destructively by SPAD (SPAD-502, Konica Minolta, sensing, Inc. Japan).

Volumetric soil water content was monitored weekly with a PR2 profile probe (PR2/6, Delta-T Devices Ltd., Cambridge, UK) at six different depths (10, 20, 30, 40, 60 and 100 cm) of the soil profile. Soil water (mm) for each depth was calculated from the volumetric soil water content multiplied by their corresponding depth increment and summed up for the whole soil profile. Weekly water use was calculated using a water balance equation and summed up for the whole season to calculate cumulative water use (see; Uddin et al., 2019). Both at anthesis and physiological maturity, a predetermined sub-plot was harvested that included the middle 4 of 6 rows and measurements were converted to t $ha^{-1}$ units.

**Results**

At anthesis, NDVI was 7% greater for wheat grown with supplemental N than without (Tables 1 & 2). At the same growth stage, both NDVI and SPAD values of cultivar Yitpi were 13% and 15% respectively, greater than those of Wyalkatchem. At anthesis, e[$CO_2$] stimulated the aboveground biomass of cultivar Yitpi by 27% when grown with supplemental N, but reduced it by 13% when grown without supplemental N. The e[$CO_2$] stimulation of anthesis biomass of cultivar Wyalkatchem was similar (~11%) regardless of whether grown with or without supplemental N (Tables 1 & 2). Captured root length was not significantly ($p > 0.05$) affected by $CO_2$ and cultivars. Supplemental N however stimulated the root length at depth 2 by 60% (Figure 1). Biomass at final harvest was about 13-15% greater under a[$CO_2$] than e[$CO_2$], with supplemental N than without supplemental N and for Yitpi than Wyalkatchem. Grain yield of wheat grown under e[$CO_2$] was 14% greater than wheat grown a[$CO_2$]. Yitpi produced 29% more grain yield than Wyalkatchem (Tables 1 & 2). Cumulative water use across the growing season was not significantly affected by any of the treatments.

**Table 1. Growth, yield, morphological parameters and water use of wheat (*Triticum aestivum* L.) cvs. 'Yitpi' and 'Wyalkatchem' grown under ambient [$CO_2$] (a[$CO_2$], ~400 µmol $mol^{-1}$) or elevated [$CO_2$] (e[$CO_2$], ~550 µmol $mol^{-1}$) with (N100: 100 kg N $ha^{-1}$) or without (N0: 0 kg N $ha^{-1}$) supplemental N.**

| Response parameters | Yitpi | | | | Wyalkatchem | | | |
|---|---|---|---|---|---|---|---|---|
| | N0 | | N100 | | N0 | | N100 | |
| | a[$CO_2$] | e[$CO_2$] | a[$CO_2$] | e[$CO_2$] | a[$CO_2$] | e[$CO_2$] | a[$CO_2$] | e[$CO_2$] |
| NDVI | 0.72±0.02 | 0.69±0.02 | 0.75±0.01 | 0.73±0.02 | 0.61±0.00 | 0.62±0.01 | 0.65±0.00 | 0.67±0.01 |
| SPAD unit | 43.2±1.03 | 43.0±1.3 | 45.7±1.8 | 46.6±0.8 | 39.1±0.9 | 39.9±1.1 | 39.0±1.5 | 38.3±2.0 |
| Anthesis biomass (t $ha^{-1}$) | 10.1±0.9 | 8.9±0.8 | 10.5±0.1 | 13.4±0.3 | 8.9±0.6 | 9.9±0.5 | 10.0±0.5 | 10.9±0.9 |
| Biomass (t $ha^{-1}$)) | 13.1±0.7 | 14.1±1.4 | 14.1±0.8 | 16.5±0.9 | 10.7±0.8 | 12.9±0.7 | 13.1±1.0 | 14.5±0.5 |
| Yield (t $ha^{-1}$)) | 5.1±0.2 | 5.4±0.6 | 5.5±0.5 | 6.2±0.4 | 3.9±0.3 | 5.0±0.2 | 3.9±0.3 | 4.4±0.5 |
| Water use (mm) | 328.5±9.8 | 306.1±7.9 | 314.7±9.6 | 314.3±4.1 | 317.7±11.6 | 296.2±5.0 | 318.1±9.6 | 310.5±7.0 |

**Table 2. Mixed effect model P-values of the main effects $CO_2$ treatments ($CO_2$), nitrogen rates (N) and cultivars (Cv) as well as their interactions for reported parameters in Table 1. Statistically significant ($P \leq 0.05$) effects are shown in bold numbers.**

| Effects | Parameters | | | | | |
|---|---|---|---|---|---|---|
| | NDVI | SPAD unit | Anthesis biomass | Biomass | Yield | Water use |
| $CO_2$ | 0.875 | 0.975 | 0.156 | **0.029** | **0.050** | 0.072 |
| N | **0.001** | 0.285 | **<0.001** | **0.008** | 0.622 | 0.707 |
| Cv | **<0.001** | **<0.001** | 0.086 | **0.016** | **<0.001** | 0.390 |
| $CO_2$ × N | 0.706 | 0.975 | **0.028** | 0.762 | 0.863 | 0.149 |
| $CO_2$ × Cv | 0.129 | 0.991 | 0.842 | 0.958 | 0.533 | 0.796 |
| N ×x Cv | 0.628 | **0.050** | 0.120 | 0.827 | 0.147 | 0.407 |
| $CO_2$ × N x× Cv | 0.787 | 0.366 | **0.018** | 0.396 | 0.382 | 0.739 |

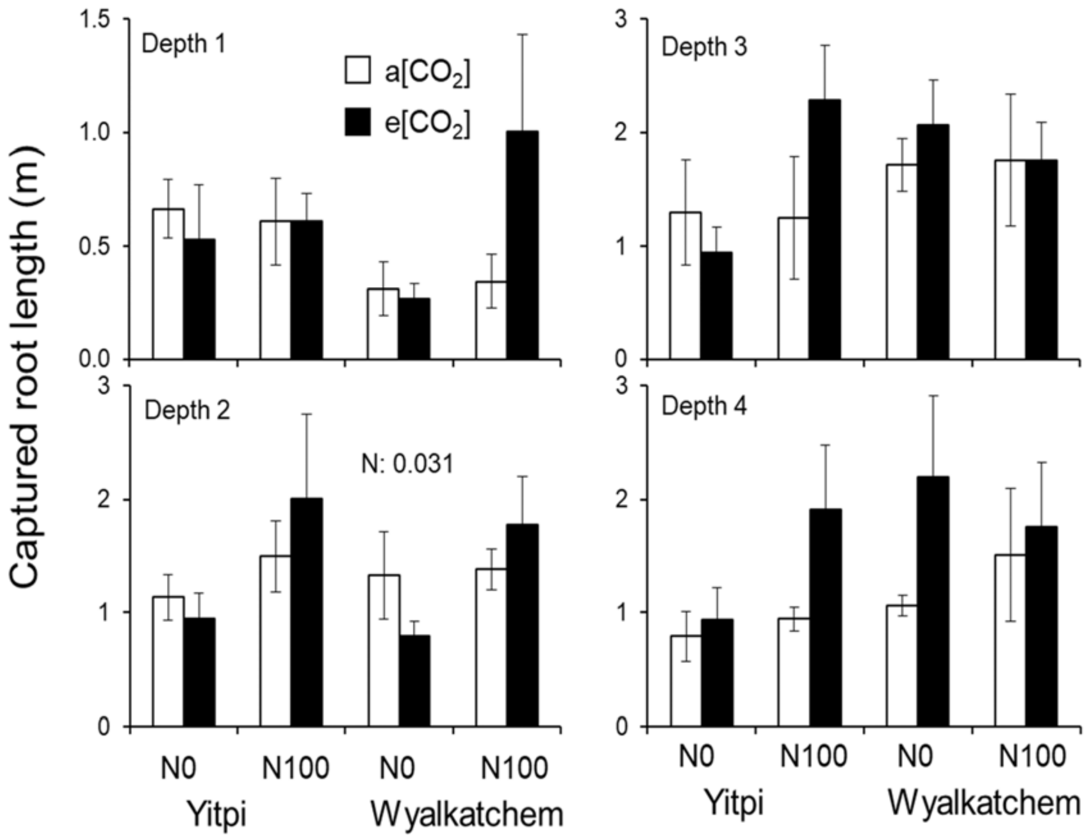


**Figure 1. Captured root length at four depths (depth 1: 0 – 16 cm, depth 2: 17 – 32 cm, depth 3: 33 - 48 cm and depth 4: 49 – 64 cm depth of the soil profile) measured at anthesis of wheat (*Triticum aestivum* L.) cvs. 'Yitpi' and 'Wyalkatchem' grown under ambient [$CO_2$] (a[$CO_2$], ~400 µmol mol$^{-1}$) or elevated [$CO_2$] (e[$CO_2$], ~550 µmol mol$^{-1}$) with (N100: 100 kg N ha$^{-1}$) or without (N0: 0 kg N ha$^{-1}$) supplemental N.**

## Discussion

The stimulation of both aboveground biomass and grain yield by e[$CO_2$] in the current study is in consistent with earlier findings in both overseas (Leakey et al., 2009) and AGFACE (Fitzgerald et al., 2016) studies. This stimulation of biomass and grain yield with or without lowering the water use resulted in greater water use efficiency under e[$CO_2$] than a[$CO_2$] (Leakey et al., 2009). Greater canopy cover of cultivar Yitpi coupled with greater $A_{net}$ (data not shown) improved the assimilate supply to developing grains and resulted in greater biomass and grain yield over Wyalkatchem.

Previous studies in AGFACE found a relatively greater stimulation of root growth than biomass and yield by e[$CO_2$] (Uddin et al., 2018a). In the current study we did not observe such significant root growth stimulation at anthesis, which might be associated with the rainfall variability. The studied year was a high rainfall wet year and the site received higher than average growing season rainfall. In earlier AGFACE study, Uddin et al (2018a) reported e[$CO_2$]-induced stimulation of deeper root growth of cv. Yitpi under rainfed conditions but not under well-watered conditions. However, during the whole growing season the dynamics of root growth was complex with significant three-way ($CO_2 \times N \times Cv$) interactions in most of the soil depths measured (data not shown).

The lack of water savings under e[$CO_2$] observed in the current study might be due to the strong effect of the prevailing terminal drought during grain filling, which might have mask the small treatment effects (Uddin et al., 2018a). However, e[$CO_2$] can affect the water use dynamics at different growth stages without showing significant effect on cumulative water use at the end of the season (Hunsaker et al., 2000, Uddin et al., 2018b). Therefore, the dynamics of water use needs to be addressed in conjunction with the leaf and root growth throughout the whole crop growing season. Furthermore, the observed differential responses of cultivars to the interactive effect of e[$CO_2$] and N rates, suggest the potential for breeding lines that maximise the benefit from '$CO_2$ fertilisation effect' under a wide range of growing conditions.

**Acknowledgements**
Research at the Australian Grains Free Air $CO_2$ Enrichment (AGFACE) project was jointly run by the University of Melbourne and Agriculture Victoria Research (Department of Economic Development, Jobs, Transport and Resources) with substantial funding from the Grains Research and Development Corporation and the Australian Department of Agriculture and Water Resources. The authors gratefully acknowledge the contributions of the AGFACE field team lead by Russel Argall, Mel Munn and Roger Perris (all Agriculture Victoria) for agronomic management of the experiment and collecting soil water and CropCircle data. Special thanks to Helale Bahrami for tracing root images and Mahabubur Mollah (Agriculture Victoria) for operating and maintaining the $CO_2$ enrichment technology. SU was supported by a Melbourne International Research Scholarship.

**References**
Angus JF (2001). Nitrogen supply and demand in Australian agriculture. Australian Journal of Experimental Agriculture 41, 277-288.

Fitzgerald GJ, Tausz M, O'Leary G, et al. (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.

Hunsaker DJ, Kimball BA, Pinter PJ, et al. (2000). $CO_2$ enrichment and soil nitrogen effects on wheat evapotranspiration and water use efficiency. Agricultural and Forest Meteorology 104, 85-105.

Kirkegaard JA, Lilley JM, Howe GN, et al. (2007). Impact of subsoil water use on wheat yield. Australian Journal of Agricultural Research 58, 303-315.

Leakey ADB, Ainsworth EA, Bernacchi CJ, et al. (2009). Elevated $CO_2$ effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. Journal of Experimental Botany 60, 2859-2876.

Mollah M, Norton R and Huzzey J. (2009). Australian grains free-air carbon dioxide enrichment (AGFACE) facility: design and performance. Crop & Pasture Science 60,: 697-707.

Reich PB, Hobbie SE and Lee TD. (2014). Plant growth enhancement by elevated $CO_2$ eliminated by joint water and nitrogen limitation. Nature Geoscience 7, 920-924.

Uddin S, Löw M, Parvin S, et al. (2018a). Water use and growth responses of dryland wheat grown under elevated $CO_2$ are associated with root length in deeper, but not upper soil layer. Field Crops Research 224, 170-181.

Uddin S, Parvin S, Löw M, et al. (2018b). The water use dynamics of canola cultivars grown under elevated $CO_2$ are linked to their leaf area development. Journal of Plant Physiology 229, 164-169.

Uddin S, Parvin S, Löw M, et al. (2019). Water use dynamics of dryland canola (*Brassica napus* L.) grown on contrasting soils under elevated $CO_2$. Plant and Soil. 10.1007/s11104-019-03987-1

Wu DX, Wang GX, Bai YF, et al. (2004). Effects of elevated $CO_2$ concentration on growth, water use, yield and grain quality of wheat under two soil water levels. Agriculture Ecosystems & Environment 104, 493-507.