Water use dynamics of dryland wheat grown under elevated CO₂ with supplemental nitrogen

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ABSTRACT

Context. Elevated atmospheric CO_2 (e[CO_2]) and nitrogen (N) fertilisation stimulate biomass and yield of crops. However, their interactions depend on crop growth stages and may affect water use dynamics. **Aims and methods.** This study investigated the interactive effects of two N rates, 0 and 100 kg N ha^{-1}, and two CO_2 concentrations, ambient (a[CO_2], ~400 μmol mol^{-1}) and e[CO_2] (~550 μmol mol^{-1}), on biomass, yield and water use of two wheat cultivars, Wyalkatchem (N-use efficient) and Yitpi (local), using a free air CO_2 enrichment facility. **Key results.** Elevated [CO_2] stimulated leaf area (10%, $P = 0.003$) and aboveground biomass (11%, $P = 0.03$). In addition, e[CO_2] reduced stomatal conductance (25%, $P < 0.001$) and increased net assimilation rates (12%, $P < 0.001$), resulting in greater (40%, $P < 0.001$) intrinsic water use efficiency. During early growth stages, e[CO_2] resulted in higher water use than a[CO_2]; however, this difference disappeared later in the season, resulting in similar cumulative water use under both CO_2 concentrations. Supplemental N stimulated grain yield of Yitpi by 14% while decreasing that of Wyalkatchem by 7% (N × cultivar, $P = 0.063$). With supplemental N, Yitpi maintained greater post-anthesis leaf N, chlorophyll content, canopy cover and net assimilation rate than Wyalkatchem. **Conclusions.** During early growth stages, the e[CO_2]-induced stimulation of leaf-level water use efficiency was offset by greater biomass, resulting in higher water use. By the end of the season, similar cumulative water use under both CO_2 concentrations indicates the dominating effect of the prevailing seasonal conditions in the study area. Observed yield responses of the studied cultivars to supplemental N were associated with their ability to maintain post-anthesis photosynthetic capabilities. **Implications.** Our findings suggest that N-use efficiency traits and responsiveness need to be considered independently to optimise benefits from the 'CO₂ fertilisation effect' through breeding.

Keywords: AGFACE, climate change, dryland agriculture, FACE, leaf gas exchange, N-use efficiency, root length, water use.

Introduction

Atmospheric carbon dioxide concentration ([CO_2]) has risen steadily from ~280 μmol mol^{-1} prior to the Industrial Revolution to >421 μmol mol^{-1} in June 2022 (NOAA 2022) measured at Mauna Loa. If effective mitigation strategies are not implemented, atmospheric [CO_2] is projected to continue increasing exponentially in the coming decades, reaching ~550 μmol mol^{-1} by the mid-21st Century (IPCC 2013). As the main substrate of photosynthesis, increasing atmospheric [CO_2] is known to stimulate growth and grain yield of crops, particularly those with the C₃ photosynthetic pathway, through the so-called 'CO₂ fertilisation effect' (Ainsworth and Long 2005). This CO₂ fertilisation effect may offset some of the negative effects of extreme climatic events (e.g. heat waves and/or droughts) on crop production (Hatfield *et al.* 2011) but impacts on crop water use and N dynamics are less clear (Li *et al.* 2004; Manderscheid *et al.* 2018).

Elevated atmospheric [CO_2] (e[CO_2]) has been extensively investigated in relation to effects on crop performance. Studies have consistently shown that an increase of ~150 μmol mol^{-1} above ambient [CO_2] (a[CO_2]) can significantly enhance both biomass

and grain yield in wheat, resulting in improvements ranging from 6% to 79% (Ainsworth and Long 2005; Kimball 2016; Fitzgerald *et al.* 2022). However, the magnitude of the observed CO$_2$ fertilisation effect is contingent upon various growing conditions, including water and nitrogen (N) availability (Reich *et al.* 2014; Gray *et al.* 2016). The relative CO$_2$ fertilisation effect has been reported to be stronger when water availability is limited (Leakey *et al.* 2009; Kimball 2016). Reduced transpiration under e[CO$_2$] (Leakey *et al.* 2009; Kimball 2016) may improve the soil-water status (Burkart *et al.* 2011; Hussain *et al.* 2013). Under drier conditions, these water savings potentially extend well into the grain-filling stages during which carbon gain can be stimulated by e[CO$_2$], thus mitigating the effect of drought on crop productivity (Wall 2001; Manderscheid and Weigel 2007). However, some recent experimental (Gray *et al.* 2016; Houshmandfar *et al.* 2016; Parvin *et al.* 2018), meta-analytical (van der Kooi *et al.* 2016) and modelling (O'Leary *et al.* 2015; Jin *et al.* 2018) analyses have not supported this view.

This complexity arises because e[CO$_2$] does not always lead to water savings, and water use at the canopy level is dependent on the balance of gains in leaf-level water use efficiency (WUE) and expansion of leaf area (Tausz-Posch *et al.* 2020). Furthermore, water savings or soil-water depletion also vary because of differential response of stomatal conductance (g_s) to e[CO$_2$] depending on soil-water availability and canopy microclimate. Increases in canopy temperature due to e[CO$_2$] (Kimball 2016) would increase leaf-to-air vapour pressure deficit and therefore evaporative demand (Ainsworth and Rogers 2007). Studies conducted at the Australian Grains Free Air CO$_2$ Enrichment facility (AGFACE; in a dryland Mediterranean region) (Tausz-Posch *et al.* 2013; Houshmandfar *et al.* 2016) have reported lower relative reductions of g_s in response to e[CO$_2$] than studies conducted in fully irrigated systems (Garcia *et al.* 1998; Wall *et al.* 2000). These variations in g_s response may contribute to regional disparities in changes in water use under e[CO$_2$] (Deryng *et al.* 2016). Therefore, it is crucial to consider the context-specific nature of water use dynamics when examining the effects of e[CO$_2$] on crop–water interactions.

The CO$_2$ fertilisation effect is reported to be weaker when soil N supply limits plant growth (Weigel and Manderscheid 2012; Reich *et al.* 2014; Ainsworth and Long 2021; Lenka *et al.* 2021). In N-limited soil, e[CO$_2$]-induced stimulation of green leaf area was reduced relative to non-limiting N supply (Wang *et al.* 2013). In high-yielding, high-rainfall or continuously irrigated agroecosystems where growth and yield often are N-limited, several studies reported a stronger response to e[CO$_2$] with supplemental N (Ziska *et al.* 1996; Weigel and Manderscheid 2012; Pleijel *et al.* 2019). Stimulation of biomass with supplemental N under e[CO$_2$] influences cumulative water use and WUE (Hunsaker *et al.* 2000; Manderscheid *et al.* 2018). The interactive effect of e[CO$_2$] and N on biomass also depends on the crop growth stage (Ziska *et al.* 1996; Yang *et al.* 2006), which may affect the water use dynamics

during the growing season. This will be particularly important in relatively low-yielding dryland Mediterranean regions where water limitations interact with N availability and where intra-seasonal variability is high (Angus 2001; Li *et al.* 2009). If N amplifies the e[CO$_2$]-induced stimulation of biomass early in the season, soil water will be depleted faster, which in combination with the typical end-of-season terminal drought in Mediterranean environments may increase the risk of 'haying-off', a situation where the rapid depletion of soil water curtails grain filling prematurely (van Herwaarden *et al.* 1998; Nuttall *et al.* 2012).

The root system plays a crucial role as the interface between plants and the soil, facilitating the uptake of essential resources such as water and nutrients (Palta and Watt 2009). In dryland Mediterranean regions, the effect of e[CO$_2$] on root characteristics holds significant implications for crop adaptation. Although there are a few exceptions (Benlloch-Gonzalez *et al.* 2014*a*), it is generally reported that e[CO$_2$] stimulates root growth (Wechsung *et al.* 1999; Madhu and Hatfield 2013; Uddin *et al.* 2018*a*). Increased root length under e[CO$_2$] can enhance rooting depth and improve access to subsoil water (Chaudhuri *et al.* 1990; Uddin *et al.* 2018*b*, 2018*c*), providing a valuable buffer against unpredictable rainfall patterns in water-limited conditions. Utilisation of subsoil water during the grain-filling period, which often occurs later in the season, is more efficient in converting water into grain than in-season rainfall (Kirkegaard *et al.* 2007; Wasson *et al.* 2012). The response of root growth to e[CO$_2$] varies depending on the availability of soil water and N (Stulen and den Hertog 1993; Wechsung *et al.* 1999) as well as the growth stage of the crop (Weigel and Manderscheid 2012). Thus, it is crucial to investigate the extent and timing of interactions between [CO$_2$] and N rates on root growth under dryland conditions, particularly during terminal drought periods.

In addition to the influence of soil water and N availability, crop responses to e[CO$_2$] can also be influenced by genotypic variability. Identification of traits responsible for genetic variation in CO$_2$ responsiveness is essential for effectively harnessing the benefits of rising [CO$_2$] through breeding approaches (Tausz *et al.* 2013; Bishop *et al.* 2015). Wheat cultivars exhibit differences in their response to e[CO$_2$] (Ziska 2008; Tausz-Posch *et al.* 2012); however, limited information is available on how supplemental N may affect intra-specific variability in response to e[CO$_2$]. Furthermore, there is a need to comprehend the underlying processes governing this response, particularly under water-limited conditions where natural soil N content is insufficient to achieve maximum yield potential.

In this study, we investigated the seasonal dynamics of biomass production and water use of wheat (*Triticum aestivum* L.) in semi-arid, water-limited, wheat cropping systems of south-eastern Australia, using the AGFACE facility. One of the typical features of the study area is that water availability is generally non-limiting during early in the winter-wheat growing season,

either from stored soil water or early-season rainfall. However, crops often experience water deficits during the flowering and grain-filling periods, referred to as terminal drought, which is considered the major cause of grain yield variability in these regions (Turner 2004). Under such water-limited conditions, the impact of e[CO_2] on wheat yield can be attributed to several factors. First, the reduction in transpiration under e[CO_2] can improve soil-water status during the early growth stages (Leakey et al. 2009; Kimball 2016). Second, early-season crop growth can provide soil shading, further contributing to improved water availability (Christy et al. 2018). Third, under e[CO_2], wheat exhibits a deeper and denser root proliferation, enabling enhanced acquisition of subsoil water (Madhu and Hatfield 2013). These combined effects have the potential to impact wheat yield positively (Kirkegaard et al. 2007).

Our experimental site was characterised by low background soil N (Walker et al. 2017) compared with much higher levels of soil mineral N (>250 kg NO_3^--N ha^{-1}) measured in previous AGFACE studies (O'Leary et al. 2015; Bahrami et al. 2017), where N supplementation did not show any significant biomass and yield responses (Tausz et al. 2017b). This new site facilitates investigation of how supplemental N will affect growth and biomass production and thereby water use dynamics of wheat under e[CO_2]. We chose two wheat cultivars for their putative differences in N acquisition and utilisation patterns: cv. Wyalkatchem, N-use efficient; Alhabbar et al. (2018); and cv. Yitpi, local practice. The experimental design allowed testing of the following hypotheses:

1. Supplemental N will stimulate the CO_2 fertilisation effect, leading to increased water use under e[CO_2] relative to a[CO_2], primarily driven by aboveground biomass and leaf area growth.
2. Supplemental N under e[CO_2] will stimulate root length growth, thereby improving subsoil water acquisition and subsequently increasing water use.
3. The N-use efficient cv. Wyalkatchem will exhibit a stronger response to e[CO_2] than cv. Yitpi when supplemented with additional N.

Materials and methods

Plant materials

Wheat cvv. Yitpi and Wyalkatchem were selected for their similar grain yield potential but contrasting agronomic features. Yitpi is a free-tillering cultivar with long coleoptile and broad adaptation to most soil types and rainfall regions across Australia's wheatbelt (Seednet 2005), whereas Wyalkatchem is characterised as having poor early vigour, short coleoptiles, high harvest index and adaption to low-rainfall regions (Intergrain 2001). Under low background soil N, cv. Wyalkatchem has been found to maintain higher grain protein than cv. Yitpi (Malik et al. 2016), most likely due to superior N acquisition and utilisation efficiencies as shown

by its performance on a sandy, non-alkaline soil in Western Australia (Alhabbar et al. 2018).

Experimental design

This experiment was conducted in 2016 within the AGFACE facility in Horsham (36°44′57″S, 142°06′50″E; 127 m a.m.s.l.), Victoria, Australia. The study site has been described in detail by Mollah et al. (2009) and Fitzgerald et al. (2022), but the present experiment was conducted on an adjacent site with low background soil N (Walker et al. 2017). Soil type at the site is a Vertosol (Isbell 1996) clay soil (~35% clay at the surface and 60% at 1.4 m depth). The soil at this site has an alkaline pH throughout the profile with moderate sodicity (mainly >20 cm depth) and high levels of boron and salt (>40 cm depth) (Table 1). The site has a Mediterranean climate with cool, wet winters and dry summers (Hutchinson et al. 2005). Long-term (30-year, 1981–2010) average annual rainfall is 445 mm, with 314 mm typically falling during the wheat growing season (May–November) (Bureau of Meteorology 2017, Polkemmet Road #079023). For the same time period, average maximum and minimum temperatures were 17.8°C and 5.3°C, respectively. During the study year, total annual rainfall was 550 mm, of which 406 mm fell during May–November. Average daily maximum (17.6°C) and minimum (5.3°C) temperatures were similar to the long-term average. A summary of the environmental variables recorded by an on-site weather station (MEA Premium Weather Station 103; Measurement Engineering Australia, Magill, SA, Australia) during the studied growing season is given in Fig. 1.

The experimental was a nested split-plot design with six replicate plots (octagonal 'rings') of 16 m diameter for a[CO_2] (~400 μmol mol^{-1}) and four rings for e[CO_2] (~550 μmol mol^{-1}) spaced at 60 m centre-to-centre. Normalised differences vegetation index (NDVI), profile probe, aboveground biomass and final harvest measurement were collected from the six a[CO_2] rings and the four e[CO_2] rings, whereas other periodic measurements (e.g. leaf gas exchange, green leaf area, N-Pen, SPAD and root growth) were acquired in four rings only for both a[CO_2] and e[CO_2] treatments. Within each ring, cvv. Yitpi and Wyalkatchem were randomly allocated to two subplots each, and each subplot was treated without (−N, 0 kg N ha^{-1}) or with (+N, 100 kg N ha^{-1}) supplemental N (see Supplementary Fig. S1) as granular urea lightly incorporated into the soil at sowing. Each subplot was 4 m long by 1.46 m wide and consisted of six rows with 0.244 m spacing. Plots were dry seeded (by drilling into 3–5 cm depth) in north–south orientation on 31 May 2016. At sowing, granular fertiliser (8.8% phosphorus (P), 11% sulfur (S)) was incorporated into the soil at rates of 8 kg P ha^{-1} and 10 kg S ha^{-1}. Initial soil mineral N was 90.3 kg NO_3^--N ha^{-1} (0–120 cm depth of soil profile).

At the perimeter of the e[CO_2] octagonal rings, horizontal stainless-steel tubes were positioned about 15 cm above the

Table 1. Physicochemical properties at different soil depths of the experimental site at Horsham, Victoria.

Soil depth (cm)	pH (1:5 water)	EC (dS m^{-1})	CEC (cmol(+) kg^{-1})	ESP (%)	Colwell P (mg kg^{-1})	Organic C (%)	NO$_3^-$–N (mg kg^{-1})	NH$_4^+$–N (mg kg^{-1})	Boron (mg kg^{-1})	BD (g cm^{-3})
0–10	8.4 ± 0.1	0.16 ± 0.03	36.9 ± 0.7	3.2 ± 0.2	24.9 ± 2.8	0.81 ± 0.03	15.9 ± 1.3	2.1 ± 0.2	3.4 ± 0.1	1.27 ± 0.02
10–20	8.5 ± 0.1	0.22 ± 0.02	36.3 ± 1.1	6.8 ± 0.5	5.8 ± 0.8	0.52 ± 0.01	14.5 ± 1.1	1.6 ± 0.1	4.3 ± 0.4	1.45 ± 0.03
20–40	8.7 ± 0.1	0.25 ± 0.02	36.2 ± 1.0	13.3 ± 0.7	3.5 ± 0.3	0.43 ± 0.02	5.9 ± 0.5	1.4 ± 0.1	6.8 ± 0.9	1.44 ± 0.03
40–80	8.8 ± 0.1	0.59 ± 0.08	37.4 ± 1.2	25.7 ± 0.9	3.3 ± 0.3	0.25 ± 0.02	3.1 ± 0.4	0.8 ± 0.1	21.3 ± 2.3	1.44 ± 0.02
80–120	8.8 ± 0.1	0.96 ± 0.10	39.6 ± 0.8	33.2 ± 0.9	2.2 ± 0.1	0.12 ± 0.01	2.5 ± 0.4	1.0 ± 0.1	33.1 ± 2.4	1.43 ± 0.01

Values are means ± s.e. of $n = 10$ (rings), and for each ring, samples from four cores were pooled.
EC, electrical conductivity; CEC, cation exchange capacity; ESP, exchangeable sodium percentage; BD, bulk density.
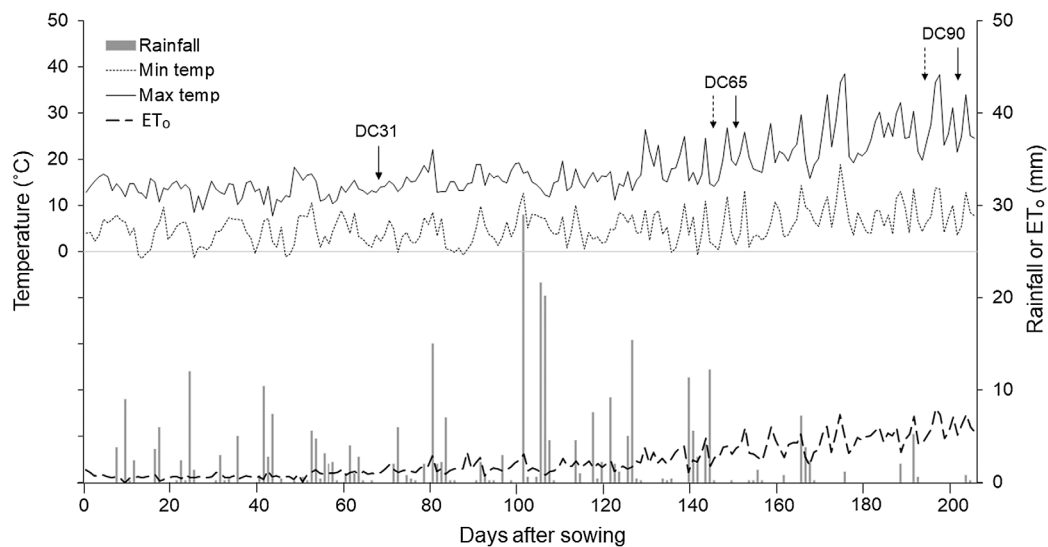

Fig. 1. Daily maximum and minimum temperatures, rainfall and reference evapotranspiration (ET) recorded on-site at the Australian Grains FACE (AGFACE) facility in Horsham, Victoria, Australia in 2016. Arrows indicate stem-elongation (growth stage DC31; Zadoks *et al.* 1974), anthesis (DC65) and physiological maturity (DC90) of wheat cvv. Yitpi (—) and Wyalkatchem (....).

canopy at any developmental stage. From sunrise to sunset, enrichment of the [CO$_2$] of ambient air to the target of 550 μmol mol^{-1} was achieved by injecting pure CO$_2$ into the air through 0.3-mm laser-drilled holes (20 holes m^{-1}) on the upwind-side tubes. CO$_2$ then quickly mixed with the air and was blown throughout the plot by prevailing wind. CO$_2$ concentrations were monitored and recorded every minute with an infrared gas analyser (SBA-4; PP Systems, Amesbury, MA, USA) positioned at the centre of each ring. The performance of the CO$_2$ delivery system is characterised in Mollah *et al.* (2009) and spatial variations of [CO$_2$] within e[CO$_2$] rings are described by Mollah *et al.* (2011).

Soil-water content and water use

Soil water was measured weekly with a PR2/6 Profile Probe (Delta-T Devices, Cambridge, UK) with six sensors positioned at 10, 20, 30, 40, 60 and 100 cm depth. A 100-cm-long, thin-walled fibreglass access tube (ATS1/ATL1; Delta-T Devices) was inserted into the soil in each plot and left *in situ* for the entire growing season (Fig. S1). To take a measurement, the PR2/6 capacitance probe was inserted into the access tube. It measures the dielectric constant (ε) via emission of an electromagnetic field 100 mm into the surrounding soil at each sensor depth. The output from each sensor is a DC voltage (in mV), which is converted to volumetric water content (m^3 m^{-3}) via a site-specific calibration curve (Whalley *et al.* 2004) developed for each soil depth of the AGFACE facilities in the earlier years. Soil-water content (mm) for each depth was calculated from the volumetric soil water multiplied by the corresponding depth increment and summed up to 100 cm depth. No irrigation was applied; therefore, water use between physiologically important growth stages was determined by the following equation:

$$\text{Water use (mm)} = P + SWD \quad (1)$$

where *P* is precipitation (mm) and SWD (mm) is the change in water content (mm) of the soil profile between the two measurements. The final measurement of soil-water content coincided with maturity of cv. Wyalkatchem, which occurred several days earlier than that of cv. Yitpi, possibly leading to an underestimate of Yitpi water use. Cumulative water use over the season was calculated by summing water use estimates at different growth stages.

Normalised difference vegetation index

Non-destructive measurements of canopy reflectance were done in all treatment subplots by using a handheld CropCircle ACS-210 with GeoSCOUT GLS-400 data logger (Holland Scientific, Lincoln, NE, USA), from which the NDVI was calculated. Measurements were taken from 51 to 189 days after sowing (DAS) at approximately weekly intervals. The sensor was held ~0.5 m above the crop canopy and oriented to measure a foot-print across the middle rows of the plot. The NDVI is calculated directly by the sensor, and mean values of transects from non-disturbed areas within the subplot were used as a surrogate for canopy cover (Perry *et al.* 2012).

Root length

Root length was measured at ~2-week intervals with a mini-rhizotron (CI-600; CID Bio-Science, Camas, WA, USA), which enables non-destructive monitoring of the same individual roots over the selected time intervals (Andersson and Majdi 2005). Detailed description of installing acrylic clear rhizo-tubes and acquiring the images of the root system for a similar experimental set-up in the AGFACE facility is given in Bahrami *et al.* (2017). In short, one 1.05-m-long rhizo-tube with internal diameter 7 cm was pre-installed at a 45° angle in each of the four subplots per ring (Fig. S1). For each rhizo-tube, four images were captured at four depths (1, 0–16 cm; 2, 17–32 cm; 3, 33–48 cm; 4, 49–64 cm) below the soil surface. The images were analysed for the captured root length per image using the software RootSnap! version 1.2.8.23 (CID Bio-Science). Total root length was calculated as the sum of root length across all four depths. The ratios of root length between the deeper (sum of Depths 3 and 4) and upper (sum of Depths 1 and 2) soil layers are reported.

Leaf gas exchange measurements

Stomatal conductance (g_s) and net photosynthetic assimilation rate (A_{net}) of fully expanded flag leaves (or uppermost leaf when flag leaves were not yet fully expanded) were measured weekly from 90 to 170 DAS. An open-path infrared gas analyser (LI-6400; LI-COR, Lincoln, NE, USA) with a standard leaf chamber (clear-top with a maximum measurement area of 2 cm by 3 cm) was used to measure instantaneous gas exchange of a single plant per plot. The cuvette air flow rate was set to 500 μmol s^{-1} with [CO_2] of either 400 or 550 μmol mol^{-1} depending on the [CO_2] growth conditions.

Measurements were performed on sunny days from 09:00 to 11:30, and photosynthetically active radiation ranged between 900 and 1250 μmol m^{-2} s^{-1} during measurement periods (Houshmandfar *et al.* 2015). Values were recorded after first stabilisation (generally after 90 s), and three measurements were recorded at an interval of 5 s and averaged. This allowed conditions in the cuvette to reach a steady state but did not allow stomata to adjust to the cuvette conditions. Vapour pressure deficit was between 0.9 and 2.2 kPa depending on measurement dates and was not different among samples and treatments on each date (Houshmandfar *et al.* 2015). After completion of the gas exchange measurements, the leaf surface area was calculated from the length and width of the part of the leaf enclosed in the cuvette. Values for all gas exchange parameters were calculated based on the surface area of the leaf inside the cuvette. Intrinsic WUE (iWUE) was calculated as A_{net} divided by g_s.

Chlorophyll and N content

Surrogate measurements of chlorophyll and N content of flag leaves were taken non-destructively by chlorophyll meter (SPAD-502; Konica Minolta Sensing, Tokyo, Japan) and N analyser (N-Pen N 100; Photon Systems Instruments, Drásov, Czech Republic), at approximately weekly intervals. Both SPAD and N-Pen readings were taken on one side of the midrib and around the midpoint of the topmost leaf or flag leaf (Xiong *et al.* 2015). For each replicate subplot, SPAD and N-Pen values were averaged from the representative measurements of 10 and six leaves, respectively.

Growth, yield and morpho-physiological parameters

For determination of green leaf area, destructive samples were collected at approximately weekly to 2-week intervals from one week before stem elongation (DC31; Zadoks *et al.* 1974) until physiological maturity (DC90). Uniform individual plants were initially tagged at 40 DAS at the soil surface level to identify them clearly as single plants in later stages. Then at each sampling date, three predetermined and representative plants per plot were harvested (excluding edge rows). Green leaf area was measured with a leaf area meter (LI-3100C Area Meter; LI-COR).

For estimation of the area-based aboveground biomass, a predetermined subplot of 0.29, 0.49 and 0.73 m^2 including the middle four rows was harvested at DC31, DC65 and DC90, respectively. At DC31 and DC65, samples were oven-dried for 72 h at 70°C, and at DC90, samples were oven-dried for the same time but at 40°C. Dry weights were recorded at each stage. At DC90, plant height was also recorded. Spikes were threshed to separate the grains, and both grain yield and 1000-grain weight were recorded. Harvest Index (HI) was calculated as grain yield divided by the aboveground biomass. WUE of biomass (WUEb) and grain yield (WUEy)

were calculated by dividing aboveground biomass and grain yield with cumulative water use.

Statistical analyses

The experiment was a split-plot design with two $[CO_2]$ treatments as main-plots (rings) and two cultivars and two N rates in full factorial combination as subplots, with four $e[CO_2]$ and six $a[CO_2]$ replications. The effects of CO_2, cultivars and N rates were considered fixed effects and ring and plot as random effects in a linear mixed effects model analysed with R package 'nlme' (Pinheiro *et al.* 2022). For repeatedly measured parameters, DAS was used as an additional fixed effect. Homogeneity of variances was evaluated visually, and the model was adjusted when stepwise evaluation of the model indicated an improvement after correction. Statistical effects were regarded as significant where $P < 0.1$. Data analyses were performed in R version 3.4.3 (R Core Team 2022) and mixed effects model *P*-values are reported in Supplementary Tables S1, S2 and S3.

Results

Plant density and phenological development

Plant density was significantly affected by cultivar ($P = 0.056$) and N rate ($P = 0.040$), where cv. Yitpi had 10% more seedlings established than cv. Wyalkatchem, and under supplemental N, 11% fewer seedlings were established than without supplemental N (Table 2). Wyalkatchem started flowering earlier and senesced faster, resulting in harvest 10 days earlier than Yitpi (Fig. 1); however, the other treatment variables had no significant effects on phenological development of either cultivar.

Gas exchange parameters

Net assimilation rate (A_{net}) of wheat grown under $e[CO_2]$ was 12% greater than under $a[CO_2]$ averaged across all measurements (Fig. 2a, Table S1). With supplemental N, cv. Yitpi maintained higher A_{net} than cv. Wyalkatchem later in the season, but without supplemental N, differences between cultivars diminished as the season progressed (DAS × N × Cv, $P = 0.002$). Stomatal conductance was 25% lower under $e[CO_2]$ than $a[CO_2]$ averaged across all measurements, but this difference diminished as the growing season progressed (Fig. 2b). Yitpi maintained greater g_s with supplemental N throughout the growing season, whereas this trend was reversed for Wyalkatchem so that, later in the season, g_s was greater for wheat grown without supplemental N (DAS × N × Cv, $P = 0.012$). Elevated $[CO_2]$ increased iWUE, and differences between $a[CO_2]$ and $e[CO_2]$ increased later in the season with decreasing g_s (Fig. 2c; DAS × CO_2, $P < 0.001$). Early in the season, iWUE was similar for the two cultivars,

but as the season progressed Yitpi had greater iWUE than Wyalkatchem (DAS × Cv, $P = 0.005$).

Green leaf area, chlorophyll and N content

Averaged across all measurement dates and treatments, green leaf area under $e[CO_2]$ was 10% greater than under $a[CO_2]$ (Fig. 3a, Table S1). Cv. Yitpi had greater green leaf area than cv. Wyalkatchem, with the difference increasing as the season progressed (DAS × Cv, $P < 0.001$). Supplemental N increased green leaf area and maintained greater leaf area for longer than when wheat was grown without supplemental N (DAS × N, $P = 0.004$). Supplemental N stimulated both leaf N and chlorophyll content, but to a greater extent for Yitpi than for Wyalkatchem (Fig. 3b, c). As the season progressed, Yitpi grown with supplemental N maintained greater leaf N and chlorophyll content than Wyalkatchem; however, these differences disappeared where no supplemental N was applied (DAS × N × Cv, $P = 0.004$ and <0.001 for leaf N and chlorophyll content, respectively).

Normalised difference vegetation index

Both early and later in the season, NDVI of cv. Yitpi was greater than for cv. Wyalkatchem; however, this difference was not evident during canopy closure (maximum NDVI; Fig. 4, Table S1; DAS × Cv, $P < 0.001$). Wheat grown with supplemental N had higher NDVI values than wheat grown without supplemental N, and these differences increased throughout the season until maturity (DAS × N, $P < 0.001$).

Root length

Effects of CO_2, N rate and cultivar on root length depended on rooting depth (Fig. 5; CO_2 × N × Cv × depth, $P < 0.001$). At Depth 1 (0–16 cm), root length of cv. Yitpi was smaller under $e[CO_2]$ than $a[CO_2]$ regardless of N supply (Fig. 5a, Table S2). Root length of cv. Wyalkatchem with supplemental N was greater under $e[CO_2]$ than $a[CO_2]$; without supplemental N, $[CO_2]$ had no effect. At this depth, root length of Yitpi was greater than that of Wyalkatchem. The difference was greatest when maximum root length was achieved but was diminished before and after this time.

At Depth 2 (17–32 cm), root length was smaller under $e[CO_2]$ than $a[CO_2]$ in the absence of supplemental N, but with supplemental N, root length was greater under $e[CO_2]$ than $a[CO_2]$ (Fig. 5b, Table S2). At this depth, stimulation of root length by supplemental N was most pronounced during the period of maximum root length but the difference was less both before and after this time. Three-way interactions CO_2 × N × Cv were significant ($P < 0.001$) for root length of wheat at both Depth 3 (33–48 cm) and Depth 4 (49–64 cm; Fig. 5c, d, Table S2). At Depth 3, root length of cv. Yitpi grown without supplemental N was 25% smaller under $e[CO_2]$ than $a[CO_2]$, but when N was applied, it was

Table 2. Plant emergence and other growth and yield variables at final harvest of wheat cvv. 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 (100 kg N ha^{-1}) or without (0 kg N ha^{-1}) supplemental N.

Response parameters	Yitpi				Wyalkatchem				P-value						
	−N		+N		−N		+N		CO_2	N	Cv	$CO_2 \times N$	$CO_2 \times Cv$	$N \times Cv$	$CO_2 \times N \times Cv$
	a$[CO_2]$	e$[CO_2]$	a$[CO_2]$	e$[CO_2]$	a$[CO_2]$	e$[CO_2]$	a$[CO_2]$	e$[CO_2]$							
Plant density (no. m^{-2})	130.9 ± 5.4	130.1 ± 11.8	115.9 ± 8.9	111.0 ± 9.8	109.5 ± 7.3	123.0 ± 8.6	112.5 ± 3.5	97.7 ± 11.8	0.772	**0.040**	**0.056**	0.181	0.856	0.476	0.314
Plant height (cm)	108.5 ± 1.3	106.0 ± 1.3	111.8 ± 0.5	107.6 ± 0.7	87.0 ± 1.9	90.8 ± 2.1	87.3 ± 1.5	90.0 ± 2.5	0.892	0.449	**<0.001**	0.511	**0.011**	0.340	0.799
Aboveground biomass (g m^{-2})	1280 ± 50.1	1409 ± 139.7	1470 ± 67.6	1654 ± 92.8	1133 ± 77.9	1286 ± 66.8	1320 ± 72.0	1450 ± 52.7	**0.030**	**0.002**	**0.010**	0.888	0.900	0.761	0.731
Grain yield (g m^{-2})	499.9 ± 15.9	538.5 ± 56.4	571.0 ± 33.9	615.1 ± 39.9	420.9 ± 34.4	500.5 ± 23.8	416.1 ± 35.3	442.1 ± 49.2	0.108	0.366	**<0.001**	0.649	0.829	**0.063**	0.576
Harvest index	0.39 ± 0.01	0.38 ± 0.01	0.39 ± 0.01	0.37 ± 0.01	0.37 ± 0.01	0.39 ± 0.00	0.31 ± 0.01	0.30 ± 0.03	0.699	**<0.001**	**<0.001**	0.343	0.320	**0.002**	0.561
1000-grain weight (g)	46.8 ± 0.4	46.9 ± 0.6	41.1 ± 1.1	44.8 ± 1.9	43.4 ± 0.9	44.5 ± 0.9	39.4 ± 0.8	37.5 ± 3.4	0.448	**<0.001**	**<0.001**	0.872	0.240	0.604	**0.098**
Cumulative water use (mm)	272.6 ± 9.5	278.8 ± 10.6	300.7 ± 12.6	307.5 ± 4.5	274.8 ± 16.5	369.6 ± 12.1	280.4 ± 12.6	273.5 ± 3.5	0.789	**0.099**	**0.079**	0.776	0.343	0.264	0.851
WUEy (kg yield ha^{-1} mm^{-1})	18.5 ± 1.0	18.7 ± 1.8	19.3 ± 1.6	20.0 ± 1.5	15.4 ± 1.0	18.6 ± 1.0	15.1 ± 1.7	16.2 ± 1.9	0.242	0.967	**0.008**	0.706	0.425	0.309	0.517
WUEb (kg biomass ha^{-1} mm^{-1})	47.3 ± 2.6	48.8 ± 4.1	49.7 ± 3.9	53.9 ± 3.5	41.4 ± 2.1	47.9 ± 2.8	48.0 ± 4.3	53.1 ± 2.4	0.114	**0.059**	0.287	0.892	0.552	0.590	0.675

Data are means ± s.e. of n = 6 and 4 replicates for a$[CO_2]$ and e$[CO_2]$, respectively. *P*-values of mixed effects model with CO_2 treatment (CO_2), nitrogen rate (N) and cultivar (Cv) as well as their interactions are presented. Statistically significant ($P < 0.1$) effects are shown in bold.
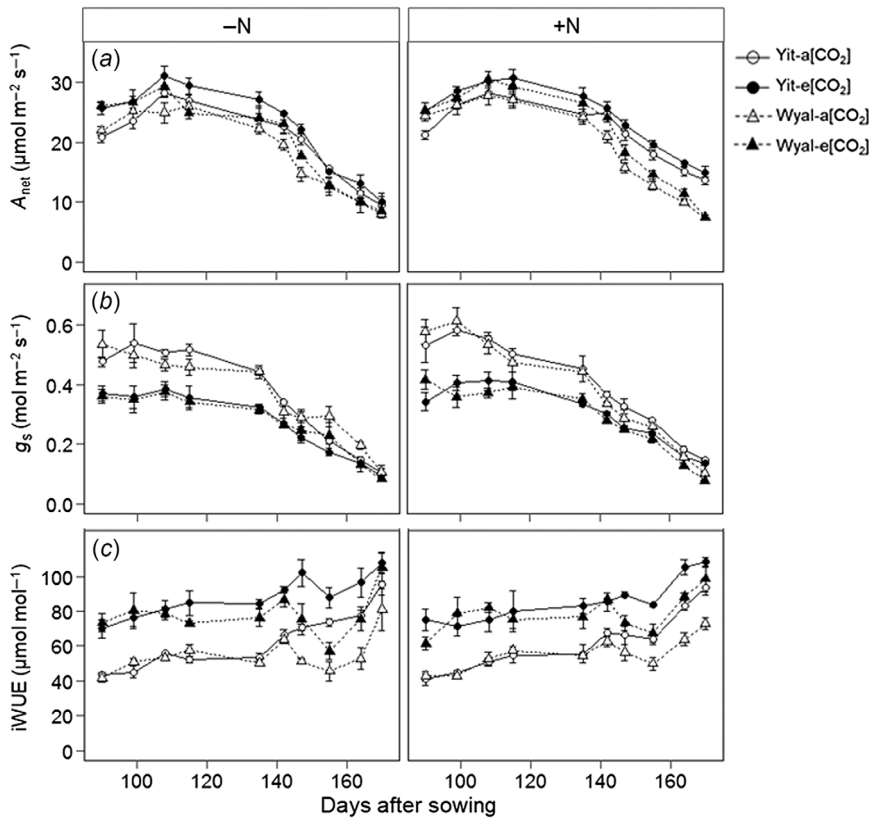
Fig. 2. (*a*) Net photosynthetic assimilation rate (A_{net}), (*b*) stomatal conductance (g_s), and (*c*) intrinsic water use efficiency (iWUE) measured weekly during the growing season of wheat cvv. Yitpi (Yit) and Wyalkatchem (Wyal) grown under ambient [CO_2] (a[CO_2], ~400 µmol mol^{-1}) or elevated [CO_2] (e[CO_2], ~550 µmol mol^{-1}) with (100 kg N ha^{-1}) or without (0 kg N ha^{-1}) supplemental N. Data are means and standard errors of $n = 4$ replicates.
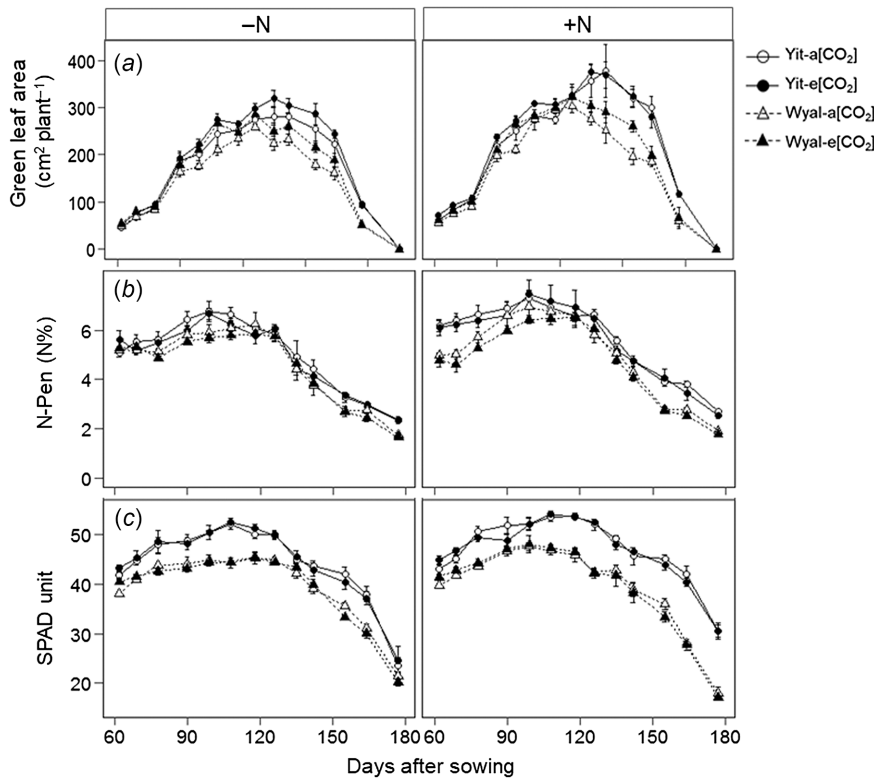

Fig. 3. (*a*) Green leaf area, (*b*) N concentration (N-Pen), and (*c*) chlorophyll content (SPAD) measured weekly during the growing season of wheat cvv. Yitpi (Yit) and Wyalkatchem (Wyal) grown under ambient [CO_2] (a[CO_2], ~400 µmol mol^{-1}) or elevated [CO_2] (e[CO_2], ~550 µmol mol^{-1}) with (100 kg N ha^{-1}) or without (0 kg N ha^{-1}) supplemental N. Data are means and standard errors of $n = 4$ replicates; each replicate of leaf area is an average of three plants; for N concentration and chlorophyll content, each replicate is an average of 6 and 10 measurements, respectively.
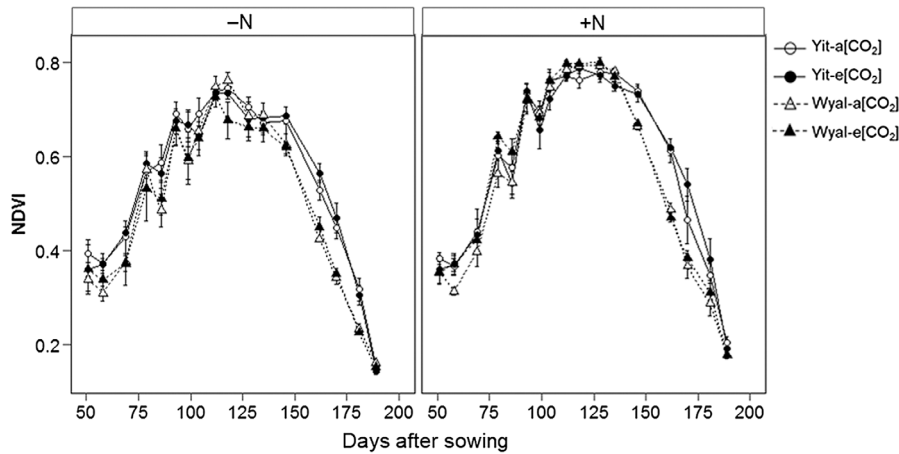
Fig. 4. Normalised differences vegetation index (NDVI) measured at weekly to 2-week intervals during the growing season of wheat cvv. Yitpi and Wyalkatchem grown under ambient $[CO_2]$ (a$[CO_2]$, ~400 μmol mol⁻¹) or elevated $[CO_2]$ (e$[CO_2]$, ~550 μmol mol⁻¹) with (100 kg N ha⁻¹) or without (0 kg N ha⁻¹) supplemental N. Data are means and standard errors of $n = 6$ and 4 replicates for a$[CO_2]$ and e$[CO_2]$, respectively.

62% greater under e$[CO_2]$ than a$[CO_2]$. However, root length of cv. Wyalkatchem when grown without supplemental N was 32% greater under e$[CO_2]$ than a$[CO_2]$ but was similar for both CO_2 treatments when grown with supplemental N. The trend of root length at Depth 4 was similar to that of Depth 3.

Supplementation with N stimulated total root length, with greater effect later in the season than at the early measurement dates (DAS × N, $P = 0.033$; Fig. 5e, Table S2). A four-way interaction DAS × CO_2 × N × Cv was significant ($P < 0.001$) for the ratio of root length in the deeper (Depth 3 + 4) versus the upper (Depth 1 + 2) soil layers (Fig. 5f, Table S2). Elevated $[CO_2]$ increased this ratio for cv. Wyalkatchem when grown without supplemental N, but with supplemental N the ratio was greater under a$[CO_2]$ than e$[CO_2]$. Yitpi showed an opposite trend with negligible difference between a$[CO_2]$ and e$[CO_2]$ treatments when grown without supplemental N, but with supplemental N, the ratio was greater under e$[CO_2]$ than a$[CO_2]$. All those differences were more prominent later in the growing season.

Aboveground biomass at different growth stages

At DC31, e$[CO_2]$ stimulated aboveground biomass by 28% when wheat was grown with supplemental N, whereas this effect was negligible (<5%) when N was not applied (CO_2 × N, $P = 0.073$; Fig. 6a). At the same growth stage, supplemental N stimulated the aboveground biomass of cv. Yitpi to a greater extent than cv. Wyalkatchem (58% vs 32%, N × Cv, $P = 0.096$). At DC65, aboveground biomass showed a significant three-way interaction (CO_2 × N × Cv, $P = 0.030$; Fig. 6b). Elevated $[CO_2]$ stimulated the aboveground biomass of Yitpi by 28% when grown with supplemental N but decreased it by 9% when N was not

applied. At the same growth stage, e$[CO_2]$ stimulated the aboveground biomass of Wyalkatchem to a similar extent for both N rates. At DC90, e$[CO_2]$ and supplemental N stimulated aboveground biomass by 11% ($P = 0.03$) and 15% ($P = 0.002$), respectively (Table 2, Fig. 6c). Yitpi had 12% greater ($P = 0.01$) aboveground biomass than Wyalkatchem.

Grain yield, 1000-grain weight and harvest index

At harvest (DC90), e$[CO_2]$ increased the plant height of cv. Wyalkatchem by 4%, whereas it reduced that of cv. 'Yitpi' by 3% (CO_2 × Cv, $P = 0.011$; Table 2). Supplemental N stimulated the grain yield of Yitpi by 14% while decreasing that of Wyalkatchem by 7% (N × Cv, $P = 0.063$; Table 2). Supplemental N decreased the HI of Wyalkatchem by 19% but did not affect that of Yitpi (N × Cv, $P = 0.002$). Elevated $[CO_2]$ stimulated 1000-grain weight of Yitpi grown with supplemental N (9%) but had no effect when N was not applied. For Wyalkatchem, e$[CO_2]$ decreased 1000-grain weight by 5% when grown with supplemental N and increased it by 3% when grown without supplemental N (CO_2 × N × Cv, $P = 0.098$; Table 2).

Water use at different growth stages and water use efficiency

During early growth stages (i.e. sowing until DC31), e$[CO_2]$ resulted in 45% higher water use than a$[CO_2]$ ($P = 0.009$; Fig. 7a). However, during this stage, e$[CO_2]$-induced stimulation of water use was greater for cv. Yitpi than cv. Wyalkatchem (74% vs 21%, CO_2 × Cv; $P = 0.073$). From DC65 to DC90, Yitpi used 34% more water than Wyalkatchem ($P = 0.029$; Fig. 7c). Cumulative water use of Yitpi was 6%
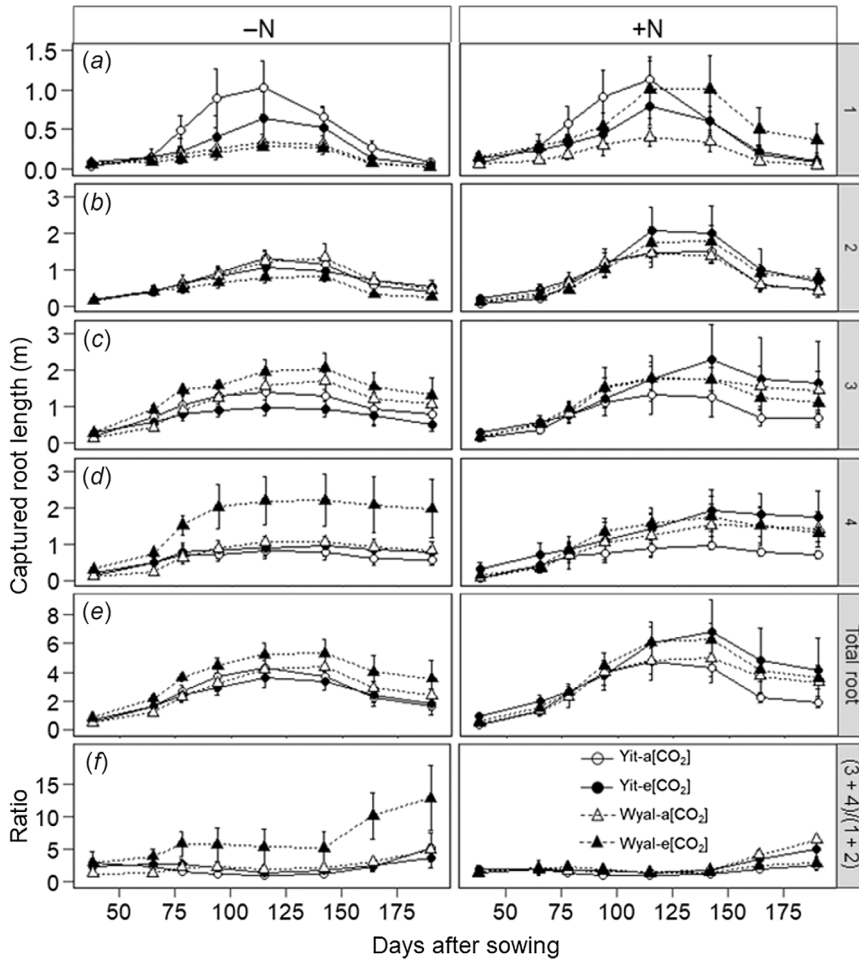
Fig. 5. Captured root length at four depths of the soil profile: (*a*) 1, 0–16 cm; (*b*) 2, 17–32 cm; (*c*) 3, 33–48 cm; and (*d*) 4, 49–64 cm. (*e*) Total root length (sum of all depths); (*f*) ratio of root length between Depths 1 + 2 and Depths 3 + 4. Measurements taken at ~2-week intervals during the growing season of wheat cvv. 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 (100 kg N ha^{-1}) or without (0 kg N ha^{-1}) supplemental N. Data are means and standard errors of *n* = 4 replicates.
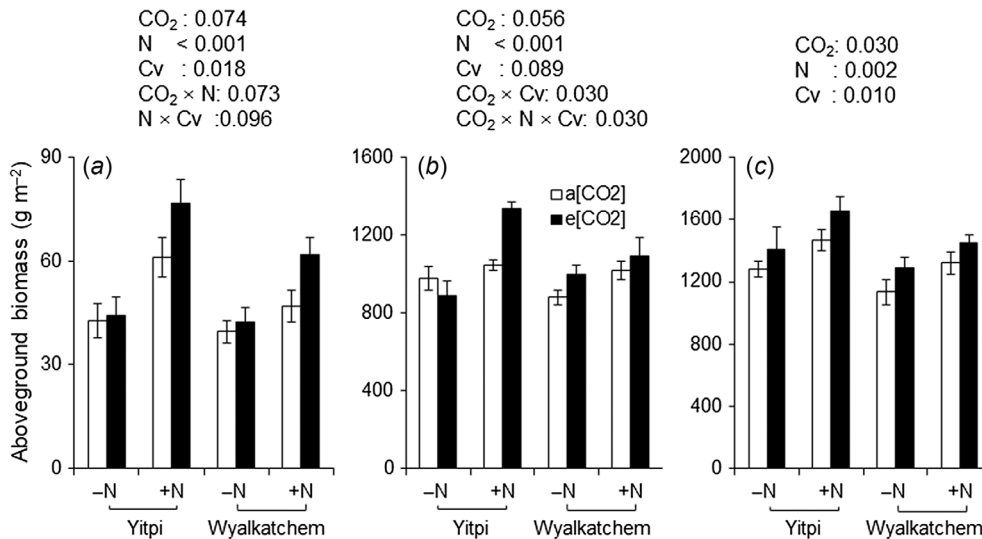

Fig. 6. Aboveground biomass at (*a*) DC31, (*b*) DC65, and (*c*) DC90 of wheat cvv. Yitpi and Wyalkatchem grown under ambient $[CO_2]$ (a$[CO_2]$, ~400 μmol mol^{-1}) or elevated $[CO_2]$ (e$[CO_2]$, ~550 μmol mol^{-1}) with (100 kg N ha^{-1}) or without (0 kg N ha^{-1}) supplemental N. Data are means and standard errors of *n* = 6 and 4 replicates for a$[CO_2]$ and e$[CO_2]$, respectively. Only significant (*P* < 0.1) *P*-values of the mixed effects model with main effects CO_2, N rate and cultivar (Cv) as well as their interactions are presented.
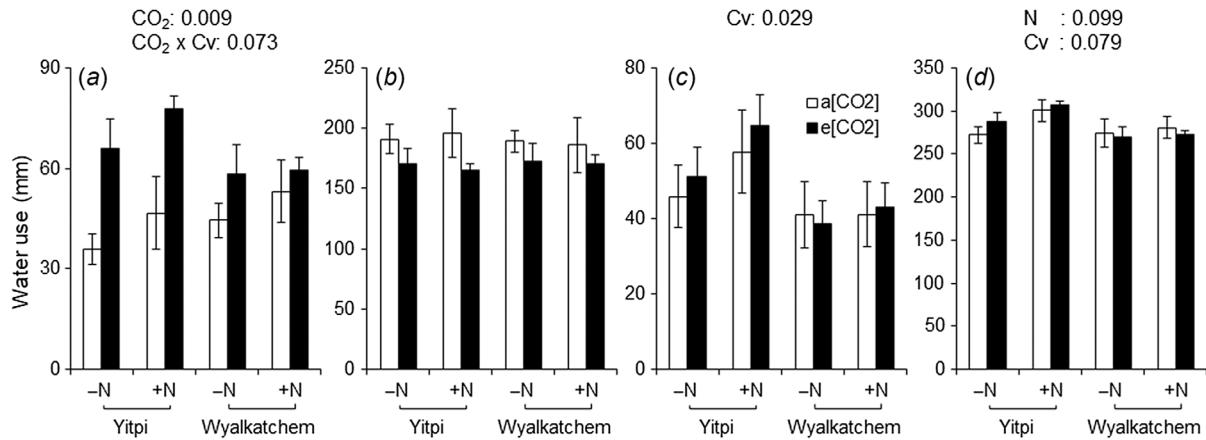
Fig. 7. Water use (*a*) from 21 DAS to DC31, (*b*) from DC31 to DC65, (*c*) from DC65 to DC90, and (*d*) cumulative through all growth stages of wheat cvv. Yitpi and Wyalkatchem grown under ambient [CO_2] (a[CO_2], ~400 µmol mol^{-1}) or elevated [CO_2] (e[CO_2], ~550 µmol mol^{-1}) with (100 kg N ha^{-1}) or without (0 kg N ha^{-1}) supplemental N. Data are means and standard errors of *n* = 6 and 4 replicates for a[CO_2] and e[CO_2], respectively. Only significant (*P* < 0.1) *P*-values of the mixed effects model with main effects CO_2, N rate and cultivar (Cv) as well as their interactions are presented.

higher than that of Wyalkatchem (*P* = 0.079; Fig. 7*d*). Wheat grown with supplemental N had 5% higher cumulative water use than wheat grown without supplemental N (*P* = 0.099). Supplemental N increased the WUEb by 10% (*P* = 0.059), and Wyalkatchem had 17% lower WUEy than Yitpi (*P* = 0.008; Table 2).

Discussion

Increased early biomass and leaf area under e[CO_2] led to increased water use, offsetting leaf level intrinsic water use efficiency

Plants grown under e[CO_2] conditions commonly exhibit a reduction in g_s (Ainsworth and Long 2005). Our study quantified a 25% decrease in g_s under e[CO_2], which, in combination with a 12% increase in A_{net}, resulted in a 40% enhancement in iWUE compared with a[CO_2] conditions. Although this indicates an improvement in leaf-level WUE, it is crucial to consider the implications at larger scales such as the plant, field or regional level. The well-known CO_2 fertilisation effect stimulates plant biomass, including leaf area, which can counteract the water savings achieved at the leaf level (Ainsworth and Long 2005; Bunce 2016). Previous studies (Wu *et al.* 2004; Ukkola *et al.* 2016) support this notion. Our investigation, specifically during the early growth stage (from sowing until DC31), revealed that the increased biomass and leaf area offset the gains in leaf-level WUE, resulting in higher water use under e[CO_2] than a[CO_2]. Consequently, greater canopy transpiration likely contributed to soil water depletion, aligning with recent studies conducted in both controlled environment (Uddin *et al.* 2018*d*) and field (Gray *et al.* 2016) conditions. The

elevated water use during the early growth stages under e[CO_2] may subsequently lead to reduced water availability during critical stages (i.e. flowering and grain filling). This, combined with terminal drought events during low-rainfall years, can result in the phenomenon of haying-off, significantly impacting grain yield (van Herwaarden *et al.* 1998; Nuttall *et al.* 2012). To ensure optimal soil-water availability under e[CO_2] for grain filling (and to capitalise on the CO_2 fertilisation effect), multiple adaptations are likely necessary. These adaptations may involve advancements in genetics for enhanced transpiration efficiency (Christy *et al.* 2018), early-season vigour to facilitate rapid soil coverage (Bourgault *et al.* 2013), and strategic soil-water management practices to minimise soil evaporation (Lascano and Baumhardt 1996).

Differences in water use between a[CO_2] and e[CO_2] conditions during the early growth stages were no longer evident in the later growth stages, resulting in similar cumulative water use. The study area is characterised by low and unpredictable rainfall during the grain-filling period, which coupled with high evaporative demand (Fig. 1) driven by elevated temperatures and strong winds can mean that all available soil water is transpired (Sadras and Rodriguez 2007; Uddin *et al.* 2018*a*). The greater canopy cover under e[CO_2] during anthesis may have mitigated the impact of evaporative water loss, leading to lower water use (although not statistically significant, *P* = 0.138). Despite the study period experiencing above-average rainfall primarily driven by a single large event (DAS 100–105), there were only a few small rainfall events (<5 mm) accompanied by a steady increase in reference evapotranspiration from anthesis onward, which likely masked any minor differences in water use between a[CO_2] and e[CO_2].

Although the increase in water use due to supplemental N was marginal during each growth stage, it led to greater

cumulative water use. This observation can be primarily attributed to the significant increase in various growth parameters, including green leaf area, biomass and canopy cover, resulting from N supplementation. Of the two cultivars tested, Yitpi consistently outperformed Wyalkatchem in these growth parameters, ultimately exhibiting higher cumulative water use. Notably, during the grain-filling period, Yitpi exhibited higher water use than Wyalkatchem (Fig. 6c). Water use during this critical stage has a higher conversion efficiency into grain production (Kirkegaard *et al.* 2007; Wasson *et al.* 2012), which likely contributed to the grain yield and WUEy advantage of Yitpi over Wyalkatchem.

Stimulation of root length under e[CO_2] increased crop water use through improved soil-water acquisition

The stimulation of root length observed under e[CO_2] conditions in our study is consistent with earlier findings from an adjacent site with high background soil N (Bahrami *et al.* 2017; Uddin *et al.* 2018a). This consistency is further supported by reports from chamber studies (Chaudhuri *et al.* 1990; Benlloch-Gonzalez *et al.* 2014a) and other FACE experiments (Pacholski *et al.* 2015; Gray *et al.* 2016). Averaged across all measurement dates and treatments, the stimulation of root length under e[CO_2] was greater (22%) than the stimulation of aboveground biomass (11%) and grain yield, which aligns with trends reported in previous studies investigating CO_2 enrichment effects (Kimball *et al.* 2002; Benlloch-Gonzalez *et al.* 2014b).

The response of root growth to e[CO_2] can be influenced by various factors including cultivar differences (Benlloch-Gonzalez *et al.* 2014a; Uddin *et al.* 2018a), growing conditions such as N and water availability (Stulen and den Hertog 1993; Wechsung *et al.* 1999; Burkart *et al.* 2004), and crop growth stage (Wechsung *et al.* 1999; Weigel and Manderscheid 2012). In the present study, the effect of supplemental N on the response of root length to e[CO_2] was dependent on the cultivar and growth stage. The pattern observed in root length (at soil Depths 3 and 4) of both cultivars in response to either supplemental N or e[CO_2] aligned with their respective biomass and grain yield responses. The root length in Depths 3 and 4, total root length, and ratio of root length in the deeper (Depths 3 and 4) versus the upper layers (Depths 1 and 2) were affected by complex interactions among all treatment variables. Yitpi exhibited greater stimulation of root growth with supplemental N, whereas Wyalkatchem showed the opposite trend, with greater e[CO_2]-induced stimulation of root growth, particularly in the lower soil profile, when grown without supplemental N. However, there was no interaction between N rate and [CO_2] in terms of grain yield, although supplemental N had a greater impact on increasing the grain yield of Yitpi than of Wyalkatchem. The stimulation of root growth by e[CO_2] and/or supplemental N may be attributed to indirect effects related to increased total plant biomass and

adaptive mechanisms for improved water and nutrient acquisition (Pacholski *et al.* 2015). Furthermore, the stimulation of root growth under e[CO_2] may be necessary to sustain the increase in biomass and grain yield (Contador *et al.* 2015).

Elevated [CO_2] stimulated the growth of roots at all measured soil depths, indicating a potential impact on the spatial pattern of soil-water and nutrient uptake within the soil profile (Fig. S2). The greater stimulation of root growth near the soil surface can be advantageous for dryland crops, because it enables them to utilise surface water that becomes temporarily available following small rainfall events (Sadras and Rodriguez 2007). Simultaneously, the stimulation of root length under e[CO_2] may allow extraction of water from deeper soil layers during the critical grain-filling periods, which is often associated with terminal drought in dryland Mediterranean agroecosystems (Passioura 1983). The uptake of subsoil water during grain filling has the potential to make a substantial contribution to the grain yield of dryland crops (Angus and van Herwaarden 2001; Kirkegaard *et al.* 2007).

A study conducted in the AGFACE facility, utilising similar CO_2 exposure treatments to those used in our study but with contrasting irrigation regimes on a soil with high background N levels, reported a positive correlation between seasonal water use and root length in deeper soil layers (Uddin *et al.* 2018a). Stimulation of root growth in the deeper soil layers plays a significant role in maintaining plant physiological processes (Gallardo *et al.* 1994; Uddin *et al.* 2018b) because these roots are younger and more efficient in extracting and supplying water than roots in the upper soil layers (Gregory *et al.* 1978). The higher water use under e[CO_2] during the early growth stages suggests that root growth stimulation improves water acquisition. However, these early-stage responses were subsequently overshadowed by the prevailing dryland condition at the study site as the growing season progressed (Uddin *et al.* 2018a). Although early depletion of soil water can have negative effects on grain yield, particularly during seasons with dry finishes, the small variation in cumulative water use observed in this study would have a negligible impact on stored soil moisture for the following season.

The effect of supplemental N on grain yield was cultivar-specific

Elevated CO_2 consistently stimulated aboveground biomass throughout the growing season, and significant interactions were observed among [CO_2], N rate and cultivar treatments during the early growth stages, with positive interactions observed between e[CO_2] and supplemental N. At maturity, e[CO_2] resulted in similar stimulation of both aboveground biomass and grain yield, leading to an insignificant HI response to [CO_2]. The lack of significance regarding the effect of [CO_2] on grain yield ($P = 0.108$) may be attributed to the smaller sample size and larger variability of the yield data in this study. Previous FACE studies, including those conducted over multiple seasons and with larger sample

sizes, have reported e[CO_2]-induced stimulation of biomass and grain yield within the AGFACE facility (Fitzgerald *et al.* 2016; Tausz *et al.* 2017a; Fitzgerald *et al.* 2022), as well as in other locations worldwide (Weigel and Manderscheid 2012; Ziska *et al.* 2012; Kimball 2016; Ainsworth and Long 2021). Similarly, no significant interaction was observed between [CO_2] and N rate or cultivar for grain yield, which aligns with findings from earlier FACE studies (Weigel and Manderscheid 2012; Bourgault *et al.* 2016; Fitzgerald *et al.* 2016; Tausz *et al.* 2017a, 2017b).

Previous research examining the interactive effect of e[CO_2] and N rates has primarily focused on high-yielding systems, such as those with high rainfall or continuous irrigation, where grain yields exceed 5 t ha^{-1} and N rates >250 kg ha^{-1} stimulate the CO_2 fertilisation effect (Kimball *et al.* 2001; Manderscheid *et al.* 2009). In a low-yielding, water-limited environment with high background soil N (~280 kg NO_3^--N ha^{-1}), the addition of supplemental N (50–60 kg N ha^{-1}) has been found to have no significant impact on biomass and grain yield, and it does not alter the CO_2 fertilisation effect (Tausz *et al.* 2017b). By contrast, in a similar environment with low background soil N, a significant positive interaction between supplemental N and e[CO_2] was observed affecting aboveground biomass and grain yield in wheat (Walker *et al.* 2017). In the present study, the addition of supplemental N influenced grain yield, with variations depending on the cultivar studied, but not CO_2 concentration.

Despite the initial expectation that cv. Wyalkatchem would outperform cv. Yitpi owing to its reputed greater N use efficiency (Alhabbar *et al.* 2018), the results of this study showed the opposite trend. Yitpi performed better than Wyalkatchem across various growth parameters and in grain yield, particularly when supplemental N was applied. One of the mechanisms underpinning the stimulation of grain yield by supplemental N in soil environments with low background N is the maintenance of higher A_{net} for an extended period (Ziska *et al.* 1996). During grain filling, wheat plants supplemented with N retained greater leaf N and chlorophyll contents, indicating greater photosynthetic capacity (Cannella *et al.* 2016). In this study, supplemental N maintained greater post-anthesis leaf N and chlorophyll content for cv. Yitpi under a low soil-N system. The combination of greater canopy cover (or green leaf area) and higher A_{net} in cv. Yitpi likely improved the supply of assimilates to developing grains. Late-season photosynthesis plays a crucial role in increasing grain yield under water-limited conditions (Liang *et al.* 2018), because most vegetative growth is completed and new photosynthates are primarily allocated to the growth and development of larger grains. This ultimately resulted in a greater yield benefit for cv. Yitpi in our study.

By contrast, application of supplemental N accelerated the post-anthesis senescence of cv. Wyalkatchem, resulting in a shorter critical grain-filling period. In dryland conditions, the remobilisation of stem water-soluble carbohydrate plays a significant role in wheat grain yield (Davidson and Chevalier 1992; van Herwaarden *et al.* 2003). In our study, Wyalkatchem showed early flowering and fast senescence compared with Yitpi, which is consistent with the findings of Nguyen *et al.* (2016). This early senescence adversely affects both the assimilate supply and complete remobilisation of stem water-soluble carbohydrate to the developing grains, leading to a substantial decrease in grain size and yield, and ultimately resulting in lower HI. The lack of a grain yield response to supplemental N in a cultivar with reputed high N use efficiency suggests that N use efficiency and responsiveness may need to be considered as separate factors (Ulas *et al.* 2013; Mahjourimajd *et al.* 2016; Ivić *et al.* 2021; Pujarula *et al.* 2021).

Conclusions

Our study demonstrates that early stimulation of biomass and leaf growth under e[CO_2] can offset the effect of increased leaf-level WUE on water use in the short term, resulting in higher crop-level water use under e[CO_2] than under a[CO_2]. Furthermore, higher water use under e[CO_2] during the early growth stages also indicates improved water acquisition through root growth stimulation. However, by the end of the season, the accumulated water use was similar under both a[CO_2] and e[CO_2], pointing to the dominating effect of the seasonal conditions. We observed genotypic variability in response to e[CO_2] and supplemental N for many growth and physiological parameters. Although supplemental N stimulated the grain yield of cv. Yitpi to a greater extent than cv. Wyalkatchem, there was no interaction with [CO_2]. Supplemental N resulted in greater post-anthesis leaf N, chlorophyll content and canopy cover for Yitpi, which in conjunction with the greater net assimilation rates would have improved assimilate supply to developing grains and thereby resulted in greater yield stimulation for this cultivar than for cv. Wyalkatchem. Our results did not show significant interactions between [CO_2] and cultivar for grain yield, indicating that for future breeding N efficiency traits and responsiveness need to be considered independently to maximise the benefit from the CO_2 fertilisation effect.

Supplementary material

Supplementary material is available online.

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