Trade-offs between water-use related traits, yield components and mineral nutrition of wheat under Free-Air CO₂ Enrichment (FACE) in a semi-arid environment

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Abstract

This study investigated trade-offs between parameters determining water use efficiency of wheat under elevated CO₂ in contrasting growing seasons and a semi-arid environment. We also evaluated whether previously reported negative relationships between nutrient content and transpiration efficiency among wheat genotypes will be maintained under elevated CO₂ conditions. Two cultivars of wheat (*Triticum aestivum* L.), Scout and Yitpi, purportedly differing in water use efficiency related traits (e.g. transpiration efficiency) but with common genetic backgrounds were studied in a high yielding, high rainfall (2013), and in a low yielding, very dry growing season (2014) under Free-Air CO₂ Enrichment (FACE, CO₂ concentration of approximately 550 μmol mol⁻¹) and ambient (approximately 390 μmol mol⁻¹) CO₂. Gas exchange measurements were collected diurnally between stem elongation and anthesis. Aboveground biomass and nutrient content (sum of Ca, K, S, P, Cu, Fe, Zn, Mn and Mg) were determined at anthesis. Yield, yield components and harvest index were measured at physiological maturity. Cultivar Scout showed transiently greater transpiration efficiency

(measured by gas exchange) over cultivar Yitpi under both ambient and elevated CO₂ conditions, mainly expressed in the high yielding but not in the low yielding season. Nutrient content was on average 13% greater for the lower transpiration efficiency cultivar Yitpi than the cultivar with higher transpiration efficiency (Scout) in the high yielding season across both CO₂ concentrations. Elevated CO₂ stimulated grain yield to a greater extent in the high yielding season than in the low yielding season where increased aboveground biomass earlier in the season did not translate into fertile tillers in cultivar Yitpi. Yield increased 27 and 33% in the high yielding and 0 and 19% in the low yielding season for cultivars Yitpi and Scout, respectively. Intraspecific variation in CO₂ responsiveness related mechanisms of grain yield were observed. These results suggest CO₂-driven trade-offs between traits governing water use efficiency are related to both growing season and intraspecific variations, and under very dry finishes, the trade-offs may even reverse. The negative relationship between nutrient content and transpiration efficiency among wheat genotypes will be maintained under elevated CO₂ conditions.

Keywords: haying-off, stomatal conductance, transpiration efficiency, water use efficiency

1. Introduction

Atmospheric CO₂ concentrations and global climate are steadily impacting on agricultural production (Meehl and Stocker, 2007). Elevated CO₂ enhances photosynthetic rates and subsequently growth in C₃ plants (Amthor, 2001; Kimball et al., 2002; Leakey et al., 2009), but this "CO₂ fertilisation effect" may be counteracted by other climate change derived phenomena, e.g., more extreme and more frequent drought events (Mpelasoka et al., 2008; Kirono and Kent, 2011) and higher temperatures (Long, 1991; Houghton, 1996). Such changes raise many challenges for sustainable food production necessary to meet demands of a growing and more affluent global population (Tubiello et al., 2007).

Therefore, in addition to improving crop and resource management, plant breeding aims to develop more efficient and productive cultivars possessing new combinations of traits that are better adapted to future climate conditions (Semenov and Halford, 2009).

Given the predicted decrease in water availability, traits that improve crop yield under both high CO₂ and water-restricted situations, i.e., delivering high grain yields with high water use efficiency, will be especially important (Richards et al., 2010). This is most important for wheat, a dominant food crop which contributes to approximately 20% of the total human calorie consumption globally, and is commonly grown in water-limited environments (Shewry, 2009; Tester and Langridge, 2010).

In wheat, beside traits associated with high grain yield under elevated CO₂ such as greater tillering potential (Fischer, 2007; Reynolds et al., 2011), a number of other traits that enhance the amount of available water to a plant including increasing the proportion of water transpired by the crop rather than it being wasted, improving leaf level transpiration efficiency, and raising harvest index are key strategies which can be targeted to improve the agronomic productivity of water-use (Passioura and Angus, 2010) in a high CO₂ world.

Increasing tiller number is valuable for superior vegetative growth under elevated CO₂, probably because it achieves high sink capacity suited for enhanced photosynthetic production (Tausz et al., 2013). Under water-limited conditions, however, traits increasing leaf area index (LAI), such as high tillering potential, may be detrimental to yield because they can cause excessive water-use, especially during early growing stages. Whilst this increases the proportion of water transpired by crop, it also depletes soil water reserves needed for post-anthesis grain filling (Richards and Townleysmith, 1987).

Lower tillering potential was suggested as a promising trait, especially in drought environments (Duggan et al., 2005). This reduced tillering potential, of course, needs to be compensated by other sink strength associated traits, e.g., greater per tiller grain number and/or grain weight to provide an appropriate sink capacity to respond to a high CO₂ environments.

Depending on growing conditions, greater early seedling vigour can contribute to increased water use efficiency by shading the soil surface from direct solar radiation and minimising evaporative water loss from the soil surface. This advantage, however, is more prominent where the soil surface is frequently rewetted (Richards and Lukacs, 2002).

83 Increasing leaf level transpiration efficiency, *per se*, or in combination with other associated traits is
 84 another important option to improve crop water use efficiency (Condon et al., 2004). Leaf level
 85 transpiration efficiency is defined as photosynthetic rate divided by transpiration rate, which is in turn
 86 controlled by stomatal conductance. Stomatal conductance is partly determined by genetic factors,
 87 e.g., as a result of different stomatal density, and partly determined by microclimate parameters
 88 controlling stomatal opening, e.g., soil water potential and vapour pressure deficit (VPD). Higher
 89 transpiration efficiency could be due to decreased stomatal conductance and/or an increase in
 90 photosynthetic rate (Farquhar and Richards, 1984; Condon et al., 2004). In C₃ plants, including wheat,
 91 transpiration efficiency is positively correlated with CO₂ because rising CO₂ is likely to decrease
 92 stomatal conductivity while promoting photosynthetic rate (Wall et al., 2000; Kimball et al., 2002;
 93 Ainsworth and Rogers, 2007; Leakey et al., 2009).
 94 Harvest index measures the allocation of aboveground biomass to grain yield (Passioura, 1977;
 95 Takeda and Frey, 1987) which is a vital factor for both achieving high grain yield and high water use
 96 efficiency. The maximum possible value for wheat harvest index has been shown to be 0.62 (Fischer,
 97 2011). In Australia, typical harvest index values are mostly between 0.30 and 0.45 depending on
 98 growing conditions and genetic potential.
 99 Whilst each of the aforementioned characteristics can be individually used in a breeding strategy to
 100 achieve a genotype with appropriate traits, e.g., superior transpiration efficiency (Condon et al.,
 101 2004), their trade-offs also have to be optimized. This is important especially for targeting future
 102 conditions, because earlier studies have shown that elevated CO₂ does not only increase grain yield
 103 (Hogy et al., 2009) and transpiration efficiency (Leakey et al., 2009) but also tillering and therefore
 104 LAI (Tausz-Posch et al., 2012). The CO₂-driven changes are also dependent on both growing
 105 conditions and intraspecific variation: grain yield increases of between 8 and 20% were reported by
 106 Kimball et al. (1995) depending on watering regimes, and LAI increases of between 14 and 20% were
 107 reported by Tausz-Posch et al. (2012) depending on intraspecific variation. In addition, some wheat
 108 cultivars are likely to differ in the way they respond to elevated CO₂: grain yield stimulation under
 109 elevated CO₂ has been shown to be linked to increased numbers of fertile tillers in a freely tillering
 110 cultivar, whereas, for a restricted tillering cultivar, increased kernel weight and kernel number per

spike were additional associated mechanisms (Tausz-Posch et al., 2015). To what extent these potential changes may affect trait combinations (or “trade-off”) in a cultivar with water use efficiency incorporated traits is not clear. Climate change related preparation for crop production is about identifying and then optimising these trade-offs to maximise both grain yield and water use efficiency. In addition, plant nutrient content (kg^{-1} dry matter, DM) has been shown to be negatively correlated with transpiration efficiency among genotypes in several C_3 species including wheat. Thus, assessment of nutrient content has been proposed to be a useful criteria in selection programs for superior transpiration efficiency (Masle et al., 1992; Mayland et al., 1993; Voltas et al., 1998; Araus et al., 2002; Richards et al., 2002). The relationship was reported to be partly related to differences in passive nutrient uptake (driven by the transpiration flow) *per se*, but other mechanisms were also suggested to be proportionally involved (Masle et al., 1992). Limited information is available on potential changes in this relationship under elevated CO_2 where passive nutrient uptake is likely to be suppressed (McGrath and Lobell, 2013; Houshmandfar et al., 2015).

Because the timeline from testing functional traits to incorporating them into a commercially released cultivar can be 20 years (Chapman et al., 2012), crop improvement must include an appraisal of the current strategies under future high CO_2 conditions, i.e. the atmospheric environment under which new cultivars will have to perform (Ainsworth et al., 2008; Hatfield et al., 2011). Free-Air CO_2 Enrichment (FACE) facilities are the best option to test crops under actual future field conditions, particularly where the experiments are conducted directly in representative cropping areas (Ainsworth et al., 2008). In dryland cropping systems such as those in the Mega-environment 4 as defined by the International Maize and Wheat Improvement Centre (CIMMYT) (Braun et al., 1996), and many Australian wheatbelt areas, breeding strategies targeting water use efficiency associated traits have led to the development of commercial bread wheat varieties that incorporate high transpiration efficiency traits (Condon et al., 2004), often in combination with other aforementioned traits. One recent example is cultivar Scout, released in 2008 (Wheeler, 2010; Shackley et al., 2012), which incorporates a high transpiration efficiency trait as developed by CSIRO (Condon et al. 2004) and good early vigour. Cultivar Scout was developed from the same parentage as cultivar Yitpi using carbon isotope discrimination ($\Delta^{13}\text{C}$) technique previously employed to identify genetic variation for superior

transpiration efficiency in wheat (Farquhar and Richards, 1984; Condon et al., 2004). The cultivar Yitpi is an established variety with superior grain size that has been widely grown in dryland cropping systems in southeast Australia.

In this study, we investigated water use efficiency associated traits of wheat cultivars Scout and Yitpi under ambient (approximately 390 $\mu\text{mol mol}^{-1}$) and elevated (approximately 550 $\mu\text{mol mol}^{-1}$) CO_2 , in the Australian Grains Free-Air CO_2 Enrichment (AGFACE) facility (Mollah et al., 2009), which is the only FACE currently in the world examining dryland crop production in a semi-arid environment. The two growing seasons studied were contrasting in water inputs: 2013 was high yielding with high rainfall and 2014 was low yielding and very dry. We particularly aimed to (i) evaluate the CO_2 -driven changes to parameters determining water use efficiency under contrasting growing conditions, and (ii) understand how such changes (or potentially “trade-offs”) might affect the relative performance of cultivars differing in water use efficiency associated traits. In addition, we tested (iii) whether the previously reported negative association between nutrient content and transpiration efficiency among wheat genotypes will be maintained under elevated CO_2 conditions.

2. Materials and methods

2.1. Experimental setup and plant material

Two field experiments were conducted during 2013 (a high yielding, high rainfall) and 2014 (a low yielding, very dry) wheat growing seasons at the AGFACE site (Mollah et al., 2009) located in Horsham, Victoria, Australia (36°45'07"S, 142°06'52"E; 127 m above mean sea level). The soil type is a Vertosol clay with non-dispersive and pedal surface (Isbell, 2002), approximately 35% clay at the top increasing to 60% at 1.4 m depth. The soil had pH of 8.2, EC of 0.1 $\mu\text{S cm}^{-1}$, Mehlich 3 extractable (Mehlich, 1984) Ca of 6.4 g kg^{-1} , K of 501.8 mg kg^{-1} , Mg of 1.1 g kg^{-1} , Cu of 1.6 mg kg^{-1} , Fe of 120.6 mg kg^{-1} , Mn of 111.6 mg kg^{-1} , Zn of 2.2 mg kg^{-1} , and Bray extractable (Bray and Kurtz, 1945) PO_4 of 19.2 mg kg^{-1} . These values are average of 32 soil samples collected from 0 to 25 cm depth in various areas in the AGFACE site.

The AGFACE site has a Mediterranean type climate but with cooler and drier winters (Hutchinson et al., 2005). Long-term (30-year) average annual rainfall of the area is 435 mm (274 mm rainfall during

wheat growing season). Long-term average maximum and minimum temperatures are 21.5 °C and 8.2 °C, respectively (Australian Bureau of Meteorology). Rainfall was extremely low in 2014 season (Table 1) to the extent that many local farmers had to abandon their crops. Therefore, a total of 153 mm irrigation in four splits was applied from September to October to keep the wheat alive (all before the first gas exchange measurement was undertaken). Summary of the environmental conditions outlining differences between the two studied growing seasons is given in Table 1.

Wheat (*Triticum aestivum* L.) cultivars Scout and Yitpi were selected for their contrasting water use efficiency traits. The cultivar Scout was developed from cultivar Yitpi genetic background, but has higher grain yield and improved transpiration efficiency, as well as medium to long coleoptiles allowing deeper planting with good early seedling vigour (Hunt and Brill, 2012).

The experimental design was fully randomized complete block with four replications, including eight plots (16 and 12 m in diameter in 2013 and 2014, respectively) of which four were ambient CO₂ (approximately 390 µmol mol⁻¹, average daytime CO₂ concentration) and four elevated CO₂ (centre concentration set at 550 µmol mol⁻¹). In 2013, each cultivar was sown into two randomly allocated subplots which were treated similarly (1.5 × 4 m; one each in opposing halves of the ring). Measurements from the two subplots were averaged for each replicate plot. In 2014, each cultivar was sown into one randomly allocated subplot (1.5 × 4 m) within the entire ring. Sowing date for both growing seasons was 29 May.

Each elevated CO₂ plot was encircled by horizontal CO₂-release-tubes in an octagonal shape which were progressively raised as the crop grew so that the CO₂ was injected about 15 cm above the canopy. A plot centre CO₂ concentration of 550 µmol mol⁻¹ was maintained for the elevated CO₂ treatment from sunrise to sunset starting from germination (Mollah et al., 2009). Average plot central CO₂ concentrations were recorded every minute with an infrared gas analysers (IRGA, SBA-4, PP Systems, Amesbury, MA, USA) located at the central part of each plot. The spatial variations in the CO₂ of the site were described by Mollah et al. (2011).

Table 1. Summary of environmental conditions outlining differences between the two studied growing seasons: 2013, high yielding, high rainfall, and 2014, low yielding, very dry growing

seasons. Climatic parameters are expressed as total or average during each growing season from sowing until physiological maturity. Grain yield is averaged for all experimental units for each season. GSR: global solar radiation.

	Growing seasons	
	High yielding 2013	Low yielding 2014
Average air temperature (°C)	11.0	11.4
Average Humidity (%)	80.2	72.7
Growing season rainfall (mm)	322	108.2
Total irrigation (mm)	0	153
Average soil temperature (°C)	13.1	15.6
Average GSR (W m ⁻²)	144.8	160.3
Average daylight (h)	10.8	10.9
Average grain yield (g m ⁻²)	718.1	188.7

2.2. Gas exchange measurement

Approximately 1000 gas exchange measurements were collected during several diurnal courses, commencing at approximately stem elongation (fourth node detectable, decimal code (DC) 34 – according to Zadoks et al. (1974) cereal growth scale) until after anthesis (DC 69), using an open infrared gas analyser with the default clear-top window chamber and a maximum measurement area of $2 \times 3 \text{ cm}^2$ (IRGA, Li-6400, Li-Cor, Lincoln, NE, USA). Gas exchange measurements were collected on four replicates per cultivar per CO₂ treatment on randomly selected healthy, youngest fully expanded leaves on 90, 96, 105, 118, 132 and 139 days after sowing (DAS) in 2013, and on 125, 126, and 138 DAS in 2014. The central portion of most of the leaves was approximately horizontal, and the leaf cuvette was clamped onto this portion of the leaf and maintained in the horizontal aspect (Osborne et al., 1998). Ambient temperatures as well as a chamber air flow rate of 500 $\mu\text{mol s}^{-1}$ were used with either 390 or 550 $\mu\text{mol mol}^{-1}$ of CO₂ concentrations for ambient and elevated CO₂ treatments, respectively. Chamber conditions were continuously matched with outside temperature, and water vapour in the incoming airstream was scrubbed to an extent that kept chamber relative humidity close to ambient relative humidity. Measurements were recorded after gas exchange rates first stabilised (after about 60 seconds). Transpiration efficiency was calculated as net assimilation rates divided by stomatal conductance. Ambient microclimate variables including temperature, humidity, and solar radiation were collected on each gas exchange measurement day using continuously logging meteorological stations located at the site (Figure 1). In all cases, daily averages were calculated.

Figure 1. Average daily air temperature, relative humidity, and global radiation recorded using continuously logging meteorological station located in the study site on days when leaf gas exchange was measured. Average day after sowing (DAS) changes ($\pm$ SE) of temperature ($^{\circ}$ C) (A and B), relative humidity (RH, %) (C and D), and global solar radiation (GSR, W m^{-2}) (E and F) recorded diurnally on 90, 96, 105, 118, 132 and 139 DAS in 2013, a high yielding, high rainfall (A, C and E), and on 125, 126 and 138 DAS in 2014, a low yielding, very dry (B, D and F) wheat cultivated season in the Australian Grains Free-Air CO_2 Enrichment (AGFACE) facility in Horsham, Victoria, Australia.

2.3. Plant sampling and analysis

Aboveground biomass, number of tillers, number of spikes, number of kernels, and kernel weight within a labelled area of $1.35 \times 0.5 \text{ m}^2$ in 2013, and $0.98 \times 0.75 \text{ m}^2$ in 2014 were recorded for each experimental series at physiological maturity (DC 90) (Calderini et al., 2000). Harvest index was calculated as grain yield divided by aboveground biomass (dried for 72 h in a 70°C of oven temperature) at physiological maturity. Aboveground biomass was harvested at anthesis (DC 65) (three or more plants were harvested and dried for 72 h at 70°C) to measure the concentration of nutrients including calcium (Ca), potassium (K), sulphur (S), phosphorus (P), copper (Cu), iron (Fe),

zinc (Zn), manganese (Mn), and magnesium (Mg) using inductively coupled plasma atomic emission spectrometry (ICP-AES) (Applied Research Laboratories, 3580B, Switzerland) after digestion in concentrated nitric acid (HNO₃) following the procedure described by Zarcinas et al. (1987). Nutrient content was expressed as sum of measured nutrients in g per kg dry matter (DM). The sum of measured nutrients was studied because total nutrient content has consistently shown a better correlation and lower coefficient of variation than nutrients individually (Masle et al., 1992; Mayland et al., 1993).

2.4. Statistical analyses

Analysis of variance for the effects of growing season, CO₂, cultivar, and their interactions were evaluated using GenStat software (v 16.1) (Payne, 2009). Gas exchange parameters were examined separately for each measurement day by running the Split-Plot module with CO₂ × cultivar treatment structure (subplot: cultivar). For yield, yield components, harvest index and nutrient content, Split-Split-Plot module with growing season × CO₂ × cultivar treatment structure were used (subplot: CO₂, sub-subplots: cultivar). Graphs were fitted using “ggplot2” package (Wickham, 2009) of R software (v 3.0.3) (R Core Team, 2008). Statistical effects were regarded significant at $P \leq 0.05$.

3. Results

3.1. Leaf level transpiration efficiency

Greater net assimilation rates were generally observed throughout the measurement period in plants grown under elevated CO₂ as compared with ambient CO₂ (Figure 2A and B). The effect was statistically significant ($P \leq 0.01$) on 96, 105, 118, and 132 DAS in the high yielding season and on 138 DAS in the low yielding season. Cultivar effect and its interaction with CO₂ were significant only on 138 DAS in the low yielding season when cultivar Scout had significantly greater net assimilation rates than cultivar Yitpi (Figure 2B).

Figure 2. Net assimilation rates (A_{net}) (A and B), stomatal conductance (g_s) (C and D), and transpiration efficiency (A_{net}/g_s) (E and F) of last fully expanded leaves of *Triticum aestivum* cultivar Yitpi (Δ and ▲) and cultivar Scout (□ and ■) grown under ambient CO₂ (a[CO₂] of approximately 390 μmol mol⁻¹; Δ and □) and elevated CO₂ (e[CO₂] of approximately 550 μmol mol⁻¹; ▲ and ■). Daily averages for 90, 96, 105, 118, 132 and 139 DAS (days after sowing) in 2013, a high yielding, high rainfall (A, C and E), and on 125, 126 and 138 DAS in 2014, a low yielding, very dry (B, D and

F) wheat cultivated season in the Australian Grains Free-Air CO₂ Enrichment (AGFACE) facility in Horsham, Victoria, Australia. Cv, CO₂, and CO₂ × Cv denote *P*-values for the effects of cultivar, CO₂, and their interaction on each DAS. Data points are means ±SE of 4 replicate plots.

The effect of CO₂ on stomatal conductance was significant ($P \leq 0.01$) for all measurement days up to 132 DAS in the high yielding season (Figure 2C), except for 105 DAS when both average daily light intensity and temperature were lower than on all other investigated days (Figure 1E and A). No significant effect of CO₂ on stomatal conductance was observed within the first two days of measurements (125 and 126 DAS) in the low yielding season (Figure 2D). On the third measurement day (138 DAS), however, stomatal conductance was higher in plants grown under elevated than under ambient CO₂ ($P \leq 0.01$). No significant effect of cultivar or cultivar × CO₂ interaction on stomatal conductance was observed (Figure 2C and D).

Transpiration efficiency was greater in plants grown under elevated as compared with ambient CO₂ on all measurement days for both growing seasons (Figure 2E and F). There were significant cultivar effects on 96 and 115 DAS in the high yielding season, when Scout showed greater transpiration efficiency (Figure 2E). A cultivar × CO₂ interaction effect on transpiration efficiency was observed on 96 DAS in the high yielding season, when transpiration efficiency of cultivar Scout was greater than that of cultivar Yitpi under elevated but not ambient CO₂ ($P \leq 0.05$) (Figure 2E). No significant cultivar or cultivar × CO₂ interaction effect was observed under the growing conditions of the low yielding season (Figure 2F).

3.2. Aboveground biomass and nutrient content

A three-way interaction effect between growing season, CO₂ and cultivar was significant ($P \leq 0.05$) for aboveground biomass (g m⁻²) measured at anthesis (DC 65) (Figure 3). Growth under elevated CO₂ stimulated aboveground biomass to a similar extent for both cultivars in the high yielding season but had stronger effect on Yitpi in the low yielding season, where the overall average aboveground biomass was 54% lower than the high yielding season (Figure 3).

Figure 3. Aboveground biomass (g DM m⁻²) of *Triticum aestivum* cultivar Yitpi and cultivar Scout grown under ambient CO₂ concentrations (a[CO₂]) of approximately 390 μmol mol⁻¹ and elevated CO₂ concentrations (e[CO₂]) of approximately of 550 μmol mol⁻¹ measured at anthesis (DC 65) in 2013, a high yielding, high rainfall, and 2014, a low yielding, very dry wheat cultivated season in the Australian Grains Free-Air CO₂ Enrichment (AGFACE) facility in Horsham, Victoria, Australia. DM: dry matter, Cv: cultivar, and Se: growing season. Data points are means ±SE of 4 replicate plots.

Figure 4 shows nutrient content for cultivars Yitpi and Scout under elevated CO₂ and contrasting seasons. In the high yielding season, nutrient content for Yitpi biomass was approximately 13% greater than for Scout ($P \leq 0.01$), while no significant CO₂ effect was observed (Figure 4A). In the low yielding season, no differences in nutrient content between cultivars or CO₂ treatments were observed (Figure 4B).

3.3. Tillering and harvest index

Plants grown under elevated CO₂ had significantly greater number of tillers as compared with ambient-CO₂-grown plants in the high yielding season. In this season, cultivar Scout produced fewer tillers, although CO₂ stimulation on tiller number was about twice as great for Scout than for Yitpi ($P \leq 0.05$). In the low yielding season, cultivar Yitpi produced fewer tillers under elevated CO₂ while Scout produced more compared with ambient CO₂ ($P \leq 0.05$) (Table 2).

286 Harvest index was affected by cultivar ($P \leq 0.01$): harvest index was lower in cultivar Yitpi than
 287 cultivar Scout (Table 2).

288

Figure 4. Nutrient content (sum of Ca, K, S, P, Cu, Fe, Zn, Mn, and Mg) ($\text{g kg}^{-1} \text{DM}$) in aboveground biomass of *Triticum aestivum* cultivar Yitpi and cultivar Scout grown under ambient CO_2 concentrations ($a[\text{CO}_2]$) of approximately $390 \mu\text{mol mol}^{-1}$ and elevated CO_2 concentrations ($e[\text{CO}_2]$) of approximately of $550 \mu\text{mol mol}^{-1}$ measured at anthesis (DC 65) in 2013, a high yielding, high rainfall (A) and 2014, a low yielding, very dry (B) wheat cultivated season in the Australian Grains Free-Air CO_2 Enrichment (AGFACE) facility in Horsham, Victoria, Australia. Cv: cultivar. Data points are means $\pm$ SE of 4 replicate plots. DM: dry matter.

289 3.4. Yield and yield components

Yield and yield components of the studied cultivars under elevated CO₂ and contrasting growing seasons are presented in Table 2. Growth under elevated CO₂ stimulated grain yield to a greater extent in the high yielding season than in the low yielding season ($P \leq 0.01$). In respect to cultivar specific responses, Scout had fewer spikes ($P \leq 0.05$) but more kernels ($P \leq 0.001$) and higher kernel weight ($P \leq 0.01$), which resulted in greater yields and yield stimulation for Scout than Yitpi under elevated CO₂ ($P \leq 0.01$).

4. Discussion

Net assimilation rates were on average 20% greater under elevated CO₂ as compared with ambient CO₂ and approximately 28% greater in the high yielding than in the low yielding season. This result is in agreement with earlier FACE experiments in wheat, where net assimilation rates were stimulated between 15 to 28% (Garcia et al., 1998; Wall et al., 2006; Tausz-Posch et al., 2012) depending on variations in the growing conditions directly affecting photosynthesis rates (e.g. light intensity), or indirectly affecting stomatal conductance (e.g. soil water potential). Net assimilation rates correlate with stomatal conductivity as a general principle for plants (Wong et al., 1979).

Stomatal conductance was on average 18% less under elevated than under ambient CO₂ and approximately 55% greater in the high yielding than in the low yielding season. Previous FACE experiments reported decreases of between 25 to 30% for stomatal conductance under elevated CO₂ (Garcia et al., 1998; Wall et al., 2000; Tausz-Posch et al., 2012). Stomatal conductance was consistently lower under elevated CO₂ on all measurement days over the two growing seasons (albeit not always significant) except on the last measurement day in the low yielding season when stomatal conductance was significantly greater under elevated than under ambient CO₂ (Figure 2D). Drying soil stimulates abscisic acid (ABA) signalling from the root to the shoot which induces stomatal closure (Davies et al., 1994). Because evapotranspiration is consistently smaller, it is likely that growth under elevated CO₂ decreases soil water depletion between rainfall (or irrigation) events (Leakey et al., 2006). Approximately three weeks before the last measurement day in the low yielding season, 24 mm of irrigation was applied. Therefore, we speculate that soil moisture would have been

317 less depleted during this dry period under elevated CO₂, potentially minimizing drought-induced
318 decreases in stomatal conductance (Leakey et al., 2006; Bernacchi et al., 2007).

Table 2. Yield, yield components and harvest index at physiological maturity (DC 90) for *Triticum aestivum* cultivars Yitpi and Scout grown under ambient CO₂ concentrations (a[CO₂]) of approximately 390 µmol mol⁻¹ and elevated CO₂ concentrations (e[CO₂]) of approximately 550 µmol mol⁻¹ in the Australian Grains Free-Air Carbon dioxide Enrichment (AGFACE) facility. Data for two contrasting wheat cultivated seasons: 2013, high yielding, high rainfall, and 2014, low yielding, very dry seasons. Statistical effects were regarded significant at $P \leq 0.05$. Values within parentheses indicate percentage difference of elevated CO₂ compared to ambient conditions. Se: growing season, Cv: cultivar, and ns: not significant. Three-way interactions (Se × CO₂ × Cv) were not significant. All values are means ±SE of 4 replicate plots.

	High yielding season 2013						Low yielding season 2014						P-values					
	Yitpi			Scout			Yitpi			Scout								
	a[CO ₂]	e[CO ₂]		a[CO ₂]	e[CO ₂]		a[CO ₂]	e[CO ₂]		a[CO ₂]	e[CO ₂]							
													Se	CO ₂	Cv	Se × CO ₂	Se × Cv	CO ₂ × Cv
Grain yield (g m ⁻²)	595.9 ±36.68	759.1 ±16.32	(+27)	653.0 ±33.26	866.5 ±24.95	(+33)	181.8 ±36.96	182.3 ±19.41	(0)	178.3 ±32.07	212.6 ±38.68	(+19)	≤0.001	0.010	0.002	0.024	0.016	ns
Tillers m ⁻²	449.0 ±18.72	486.6 ±7.99	(+8)	372.0 ±15.71	438.4 ±21.48	(+18)	321.0 ±52.08	271.5 ±22.08	(-15)	273.9 ±33.68	324.1 ±18.28	(+18)	≤0.001	ns	0.074	ns	0.053	0.057
Spikes m ⁻²	442.0 ±16.44	477.9 ±18.79	(+8)	363.0 ±51.03	430.1 ±16.28	(+18)	293.0 ±51.17	240.8 ±22.10	(-18)	254.1 ±34.30	285.5 ±15.80	(+12)	≤0.001	ns	0.049	ns	0.034	0.060
Kernels spike ⁻¹	32.89 ±2.14	37.64 ±0.49	(+14)	42.99 ±1.05	44.80 ±2.15	(+4)	16.15 ±1.33	19.18 ±2.91	(+19)	17.75 ±1.18	18.03 ±2.84	(+2)	≤0.001	ns	≤0.001	ns	≤0.001	ns
1000 kernel weight (g)	39.17 ±0.48	42.48 ±0.44	(+8)	42.05 ±0.62	46.09 ±0.17	(+10)	38.76 ±1.76	41.14 ±3.26	(+6)	39.14 ±1.82	40.87 ±2.57	(+4)	ns	0.089	0.006	ns	0.008	ns
Harvest index	0.410 ±0.013	0.411 ±0.011	(0)	0.442 ±0.005	0.456 ±0.008	(+3)	0.365 ±0.008	0.347 ±0.034	(-4)	0.387 ±0.006	0.397 ±0.078	(+3)	0.080	ns	0.013	ns	ns	ns

Previous FACE studies have suggested there are increased leaf and canopy transpiration efficiencies under elevated CO₂ (Leakey et al., 2009). We observed an average increase of approximately 30% for leaf level transpiration efficiency over the two studied growing seasons (71% for the high yielding and 11% for the low yielding season). Tausz-Posch et al. (2012) reported an average increase of 57% for two different AGFACE grown wheat cultivars in a season with approximately 300 mm rainfall.

Intraspecific differences in parameters governing transpiration efficiency showed transiently greater transpiration efficiency of Scout over Yitpi under both ambient and elevated CO₂ conditions. This is in agreement with earlier work comparing varieties carrying the “CSIRO transpiration efficiency gene”, where transpiration efficiency measured by gas exchange showed only temporary differences (Tausz-Posch et al., 2012). In this study, such differences were mainly expressed in the season where water stress was less pronounced. The relatively higher transpiration efficiency of cultivar Scout observed in the high yielding season resulted from both small increases in net assimilation rates and small decreases in stomatal conductivity (Figure 2). This is consistent with Condon et al. (2004) who suggested differences in $\Delta^{13}\text{C}$ may arise from both small decreases in stomatal conductance and small increases in net assimilation rates because the low $\Delta^{13}\text{C}$ (=high transpiration efficiency) lines carried no yield penalties, even under high yielding growing conditions.

The value of carbon isotope discrimination has been shown to have limitations in some growing conditions (Condon et al., 1990). Particularly, environmental factors such as VPD may lead to decreased stomatal conductance, decreased intercellular CO₂ concentrations and therefore limited $\Delta^{13}\text{C}$ (Sinclair, 2012). If stomatal conductance were highly restricted by water stress in the low yielding season, it may well be likely that any cultivar differences in transpiration efficiency were not expressed. This is in close agreement with the methodology used for the selection of high transpiration efficiency genotypes, where ^{13}C signatures were determined early in the growing season when soil moisture conditions were quite favourable (Condon and Richards, 1992). The rationale was that higher transpiration efficiency early in the growing season increases the soil moisture conservation which can contribute to crop growth and development during late-season dry periods (Sinclair, 2012). Simulation models have shown this to be beneficial for sorghum (Sinclair et al., 2005) and soybean (Sinclair et al., 2010), but not in all growing seasons.

The cultivar with lower transpiration efficiency (Yitpi) had greater nutrient content than the higher transpiration efficiency cultivar (Scout) under both ambient and elevated CO₂ in the high yielding season. This result is in agreement with earlier reports indicating nutrient content is negatively related to transpiration efficiency among genotypes of C₃ species (Masle et al., 1992; Mayland et al., 1993; Voltas et al., 1998; Araus et al., 2002; Richards et al., 2002). The relationship was consistent with nutrient data obtained from well-irrigated treatments using the same cultivars as in the 2014 AGFACE trial (data not shown), but not with the growing conditions of the low yielding season (2013) where no intraspecific variation was observed (Figure 4B). Differences in nutrient content between cultivars confirm the negative relationship between transpiration efficiency and nutrient content among wheat genotypes in the literature; but if it was mainly this relationship resulting from decreases in transpiration-driven mass flow of nutrients (passive nutrient uptake), the difference in nutrient content should have been even greater between ambient and elevated CO₂. Therefore, we conclude that other quantitatively important mechanisms other than mass flow of nutrients are involved; some of which may be associated with control of plant water content as suggested by Masle et al. (1992).

Elevated CO₂ had no significant effect on nutrient content. In general, lower nutrient content may have been expected (Fernando et al., 2014), but results were not always consistent for all of the nutrients in the literature, e.g., for K (Loladze, 2002). Potassium quantitatively contributed most to the sum of nutrients evaluated in this study (approximately 70%). Masle et al. (1992) reported a 57% contribution of K to a similar relationship in wheat.

Elevated CO₂ stimulated grain yield of both cultivars with a greater increase for Scout than for Yitpi in the high yielding season. In this season, grain yield stimulation of cultivar Scout mainly resulted from an increase in the number of tillers and therefore, the number of spikes. This result supports the importance of tillering potential as a contributing factor to grain yield response to elevated CO₂ (Ziska, 2008; Shimono, 2011). In contrast, for cultivar Yitpi, grain yield stimulation mainly resulted from an increase in the number of kernels per spike. Intraspecific variation in CO₂ responsive mechanisms have been previously proposed in the literature (Tausz-Posch et al., 2015). In the low yielding season, elevated CO₂ stimulated grain yield of cultivar Scout but had no effect on cultivar Yitpi. In this season, the CO₂-stimulation of the number of tillers for Scout was to some extent offset

by a lower “tiller economy” (fewer tillers were translated to fertile spike). Overall, grain yield was 19% greater under elevated as compared with ambient CO₂. Similarly, the CO₂ responsiveness mechanism for cultivar Yitpi (increase in the number of kernels) was traded off with 18% decrease in the number of fertile tillers resulted in a similar grain yield under ambient and elevated CO₂. These results are consistent with the phenomenon of “haying-off”, where in a very dry season’s finish stimulation of vegetative growth does not fully translate into grains. Haying-off is observed in response to excessive application of nitrogen (N) fertiliser early in the growing season (van Herwaarden et al., 1998), but it has also been suggested that CO₂-stimulation of early season growth could increase the haying-off risk (Nuttall et al., 2012). AGFACE data from previous years showed that at this site such an increased hay-off risk under elevated CO₂ is observed only in very low yielding and dry seasons, a condition which occurred during the dry growing season studied in this paper.

Conclusion

The CO₂-driven trade-offs in water use efficiency associated traits are related to both intraspecific variation and growing seasons in this dry-land cropping system. When the growing season is characterised by a very dry finish, the trade-offs may even reverse, i.e. more aboveground biomass earlier in the season may lead to a decrease in tiller economy (surviving tillers) and an inability to complete grain filling. Furthermore, our results suggest that a previously reported negative relationship between nutrient content and transpiration efficiency among wheat genotypes will be maintained under elevated CO₂ conditions.

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