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Nitrogen nutrition and aspects of root growth and function of two wheat cultivars under elevated [CO₂]

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Highlights

- Wheat cultivars increased either root growth or specific root N uptake at low soil N
- Wheat can utilise nitrate, ammonium, or glutamine as N source in the field
- Specific root uptake capacity for ammonium was different between cultivars
- Root growth but not specific root N uptake capacity was linked to greater grain N
- Elevated CO₂ decreased grain N of both cultivars and did not affect uptake capacities

Abstract

Nitrogen (N) input into food production is environmentally sensitive and economically significant, making efficient N use an important goal in agricultural practice and in plant breeding. In cereals, grain N concentration [N] is an important component of grain quality and nutritional value. Increasing atmospheric CO₂ concentration [CO₂] will not only stimulate growth and yield but also diminish grain [N], raising concerns about product quality and nutritional value.

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In this study we investigated how differences in root structure and function and agronomic properties between two bread wheat (*Triticum aestivum* L.) cultivars affect N uptake and allocation to grains in a low rainfall environment, and whether such differences can indicate strategies to mitigate grain [N] decreases under increased [CO_2]. Two cultivars ('Silverstar' and 'Yitpi') were chosen for their similar phenology and yields, but 'Silverstar' often has lower grain [N]. A glasshouse experiment showed contrasting root structure and function strategies between the two cultivars in response to soil N: 'Yitpi' but not 'Silverstar' responded to lower soil N with increased root growth, whereas 'Silverstar' but not 'Yitpi' showed increased N uptake per unit root mass in response to lower N.

When grown in the Australian Grains Free Air CO_2 Enrichment facility over multiple seasons both cultivars produced similar yields, but 'Silverstar' had consistently lower grain [N], smaller grains and greater harvest index. *In situ* N uptake measurements with ^{15}N label showed that wheat roots can take up nitrate, ammonium and glutamine, and also confirmed differences in uptake strategies between cultivars: 'Silverstar' roots had significantly greater uptake capacity than 'Yitpi' roots for ammonium. Whilst these results suggest that different strategies in response to variations in soil N supply could be related to grain N outcomes at this field site, there was no interaction with atmospheric [CO_2] for any of the measured parameters. Regardless of cultivar, elevated [CO_2] (550 μmol mol^{-1}) increased yields and decreased grain [N], but did not affect root uptake capacities for either N form. Contrasting root uptake strategies seem unrelated to grain [N] decrease under elevated [CO_2], at least for this site.

Keywords

wheat, elevated CO_2, grain protein, nitrogen uptake, roots, FACE

1. Introduction

Improved nitrogen (N) use efficiency of crop production is a priority goal (Parry and Hawkesford, 2010; Tilman et al., 2011; for definitions of N use efficency see Hawkesford, 2011) if sustained intensification of agriculture and maintenance of functional environmental quality is to be achieved (Reeves, 1999). Crop N use is determined by many interacting and complex mechanisms that can broadly be assigned to one of two components: N uptake and N utilisation (Hawkesford, 2011). Root growth and architecture as well as root function are the main determinants of N uptake (Palta and Watt, 2009). Nitrogen utilisation is related to biomass and yield production, and in the context of grain quality, also to mechanisms of remobilising N to grains (Gregersen, 2011), with protein concentration determining grain quality (Shewry, 2009). In cereals such as wheat, an inverse relationship between yield and grain protein concentration is commonly observed, the so-called 'yield quality conundrum' (Hawkesford, 2011).

This conundrum is further accentuated by the increase in atmospheric CO_2 concentration [CO_2], which has been rising from pre-industrial 280 to 400 ppm in 2014, and on current trend will increase by nearly 40% more over its current value by 2050 (Cubasch, 2013). In its role as a principal plant nutrient, rising [CO_2] increases crop growth and yield, but decreases plant and grain N

concentrations [N] resulting in lower grain protein especially in cereal crops (Fangmeier et al., 1999; Myers et al., 2014). A simple hypothesis attests that decreases in plant and grain [N] are the result of a 'biomass dilution effect', where N uptake is unable to meet the rising demand by increased biomass growth (Stitt and Krapp, 1999). However, a recent meta-analysis showed that decreases in plant [N] under elevated [CO_2] occur even without 'biomass dilution' and suggested a link to decreased access of plants to N, rather than decreased N demand (Feng et al., 2015). Improved N uptake capacity by larger or more efficient root systems could therefore help to mitigate the decrease in grain protein due to rising [CO_2].

Because root systems are designed to explore the soil for water and nutrients, root growth traits are already of great interest for their potential in crop improvement, especially to optimise water and nutrient acquisition (Gregory, 2011; Palta and Watt, 2009). Size and architecture of root systems is known to vary among wheat cultivars, and large root systems, especially where they show vigorous early growth, can contribute to better N nutrition of the crop (Palta et al., 2011).

Uptake of N into roots occurs through both, passive and active mechanisms. Water flux with the transpiration stream is considered passive, whereas the accumulation of N against a concentration gradient from the soil solution into the roots is an active mechanism (White, 2012). Elevated [CO_2] causes stomatal closure and therefore decreases transpiration, which may affect the passive uptake mechanism (McDonald et al., 2002). For active uptake of N in its different forms, the activities of specialised uptake systems (transporters) play important roles. Transporters have been characterised for nitrate, ammonium and organic forms of N in many plants (Hawkesford, 2011; Miller and Cramer, 2005). In wheat, for example, nitrate transporters have been related to intra-specific differences in nitrate uptake capacity of roots, a trait that has been shown to contribute to genetic variation in N acquisition among wheat lines (Melino et al., 2015).

Measurements of *in-situ* root uptake capacities in natural ecosystems have demonstrated that roots can have high uptake capacity for nitrate, ammonium and amino acids, and that uptake preference and acquisition strategies can vary according to environmental factors and competition (Simon et al., 2014). Less is known about differences in uptake preferences among wheat cultivars, especially in the field, where nitrate is often considered the predominant N source for crops (Masclaux-Daubresse et al., 2010). Whilst higher plants, including cereals, can absorb organic N, ammonium and nitrate proportionally to their relative abundance (Bloom, 2015; Näsholm et al., 2009), conditions in agricultural soils often favour nitrification and soil microorganisms generally outcompete plant roots for organic N as well as for ammonium (Bloom, 2015; Jones et al., 2013; Masclaux-Daubresse et al., 2010).

Increased plant uptake of reduced forms of N, such as ammonium and organic N forms such as urea or amino acids, could be beneficial for a variety of reasons. For example, nitrate assimilation is a major energy cost in higher plants (Bloom, 2015). Furthermore, because nitrate is prone to losses from the system through leaching, greater proportion of N acquired in other forms could improve crop N use efficiency and minimise environmental impacts, such as pollution of ground and surface waters by leached nitrate. In addition, as the negative effect of elevated [CO_2] on plant N concentrations is associated with an inhibition of plant nitrate assimilation (Bloom et al., 2010), N management could be adapted to provide plants directly with greater proportions of reduced N, which would circumvent nitrate assimilation (Bloom, 2009).

To investigate the potential importance of differences in N acquisition strategies between wheat cultivars for plant N nutrition and grain N under elevated [CO_2], we selected two commercial wheat cultivars with similar yields and phenological development, but contrasting agronomic characteristics (differences in harvest index, grain size and grain N concentrations). Experiments were conducted in the Australian Grains Free Air CO_2 Enrichment (AGFACE) facility, which allowed us to study crops in the field and assess changes upon increased atmospheric [CO_2]. As destructive root sampling was not feasible at this facility, we investigated responses of root growth to variation in N supply using the same cultivars in an accompanying glasshouse experiment. We tested the following hypotheses: (1) The two cultivars show consistent differences in N uptake and tissue and grain [N] in the field. (2) The cultivar with greater N uptake has greater root biomass and greater specific root N uptake rates. (3) Root N uptake rates will decrease under elevated [CO_2] in line with decreased tissue and grain N concentrations, and (4) cultivar differences mitigate this decrease.

2. Material and Methods

2.1 Plant material

All investigations were made on two bread wheat (*Triticum aestivum* L.) cultivars 'Yitpi' and 'Silverstar'. These cultivars were chosen because both are well adapted to low to medium rainfall areas, have similar phenology at the field site used for this experiment and are classified in similar grain quality classes, but show differences in grain parameters, with farmers reporting of small grains and low grain [N] in 'Silverstar' (McClelland and Fay, 2001).

2.2 Glasshouse study

To address differences between these two cultivars in root growth, they were compared at two different soil N levels in a glasshouse trial. Plants were grown in deep pots (100mm diameter, 1000mm depth) containing sandy loam (0.38% (w/w) C, 0.006% N, pH 6, all nutrients except N added in non-limiting quantities). Pots were assigned to either low N (30 mg NH_4NO_3 kg^{-1} soil), or high N (estimated as sufficient N, 95 mg kg^{-1} NH_4NO_3) with n=4 replications per cultivar and N treatment. Four seeds per pot were sown at 28 May 2013, and thinned to the most vigorous single plant on 12 June (two leaf stage). In the naturally lit glasshouse, plants were maintained at a day/night temperature of $20^0C/5^0C$ until sampling at booting stage (DC 45 according to the decimal code growth stages (Zadoks et al., 1974)). Plants were watered regularly through pre-buried perforated watering pipes to maintain the soil close to field capacity throughout the experiment. At sampling, biomass was separated into aboveground (stem and leaves) and roots, the roots washed in a sieve system, and biomass was then dried at 70 °C for 72 h to determine dry weight. After drying, biomass was ground and aliquots used to determine tissue [N].

2.3 Free Air CO_2 Enrichment (FACE) study

The two wheat cultivars were grown side by side in the AGFACE facility during the growing seasons 2009 – 2012. The AGFACE facility is in a low rainfall cropping area (long term average annual rainfall 435 mm) with Mediterranean climate, on a 7.5 ha site west of Horsham, Victoria, Australia (36◦45'07''S, 142◦06'52''E, 127m a.s.l.). The soil is Murtoa Clay, a cracking Vertosol (Isbell, 2002). More details on the site are given in (Fitzgerald et al., 2016).

Eight plots of 16 m diameter were assigned to either elevated (target 550 µmol mol^{-1}) or ambient [CO_2] (~390 µmol mol^{-1}) in four replications, each plot split in half for irrigation treatment: one half received only rainfall, and the other received supplemental irrigation to create greater variability in growing environments (Table 1). Within each half plot, cultivars were grown at 0.195 m row spacing on randomly allocated subplots (4×1.7 m) with a sowing rate targeted at 150 plants m^{-2}. At sowing, granular phosphorus and sulphur (as 'superphosphate') were incorporated into the soil at rates of 7–9 kg P ha^{-1} and 8–11 kg S ha^{-1} (variation between seasons; lowest and highest rates given). As the soil contained relatively high amounts of N sufficient for achieving typical yields for the region, no N fertiliser was applied (Fitzgerald et al., 2016). Pre-sowing soil ammonium levels over the years of experimentation ranged from 1.8 to 2.9 mg kg^{-1} and nitrate levels from 13 to 26 mg kg^{-1} for 0-60 cm depth (averages of replicate plots for each growing season; range indicates variation between seasons).

Details on the design and performance of the CO_2 delivery system are given in (Mollah et al., 2009). In brief, the elevated [CO_2] plots were surrounded by rings (octagons) of horizontal stainless steel tubes injecting pure CO_2 into the wind. [CO_2], wind speed and direction were monitored at the centre of each plot and used to control CO_2-delivery to maintain the concentration at 550 µmol mol^{-1}. CO_2 was injected from sunrise to sunset starting from germination to maturity. Fumigation tubes were raised periodically to keep them about 0.1 to 0.15 m above the crop canopy.

The experiment was repeated from 2009 to 2012, and once more in 2009 for a later date of sowing, resulting in five different growing environments (Table 1).

2.3.1 Biomass, yield and [N] measurements

Measurements in the AGFACE experiments were taken at anthesis and maturity (growth stages DC65 and DC90 according to Zadoks et al., 1974) following procedures as described in (Fitzgerald et al., 2016; Tausz-Posch et al., 2015). At DC65, aboveground biomass was hand harvested from 0.4 m^2 of each sub-plot and oven dried at 70°C for 72 h. At DC90, aboveground biomass was hand harvested from 0.78 m^2 of each sub-plot, dried at 40 ∘C for 72 h and separated into heads and straw. Heads were threshed and grain yield, total above root biomass (=grains + chaff + straw) and thousand kernel weights were determined from the whole sample, and expressed per m^2 plot area. Harvest Index (HI) was calculated as grain yield divided by total aboveground biomass at maturity. Total nitrogen concentrations [N] of aboveground biomass and grains were determined on dried and ground tissue aliquots by Dumas combustion in an elemental analyser (LECO, TruMac, MI).

2.3.2 *In-situ* N uptake measurements

In 2012, net ^{15}N uptake capacity in fine roots was quantified according to (Gessler et al., 1998) and (Simon et al., 2010) during the wheat growth stages DC55-60 (Zadoks et al., 1974). Fine roots were carefully dug out, but kept attached to the plants, cleaned of adherent soil material with tap water, and incubated in 4 ml of an artificial nutrient solution. The solution contained 100 µM KNO_3, 90 µM $CaCl_2 \cdot 2H_2O$, 70 µM $MgCl_2 \cdot 6H_2O$, 50 µM KCl, 24 µM $MnCl_2 \cdot 4 H_2O$, 20 µM NaCl, 10 µM $AlCl_3$, 7 µM $FeSO_4 \cdot 7 H_2O$, 6 µM K_2HPO_4 and 1 µM NH_4Cl as well as the amino acid glutamine (Gln, 25 µM). Solutions contained either a label for one of the three different N sources (glutamine double-labelled with ^{15}N/^{13}C) or no ^{15}N label (control to account for natural abundance). ^{15}N uptake experiments were conducted between 10 am and 2 pm to avoid potential diurnal variation in N

uptake (Gessler et al., 2002). Roots were incubated for 2h, then cut off, washed twice in 0.5 M $CaCl_2$ and dried with cellulose tissue. Fresh weight was determined, after which the samples were oven-dried (48h, 60°C) and dry weight was measured. Dry root material was finely ground in a ball mill and prepared for isotope ratio mass spectrometry.

2.3.3 Quantification of ^{15}N, ^{13}C, and total N and C amounts in the fine roots

^{15}N and ^{13}C abundance and total N and C were determined in 1.2-2.5 mg aliquots of finely ground root material which was transferred into tin capsules (4-6 mm, IVA Analysentechnik, Meerbusch, Germany). Analyses were conducted using an elemental analyser (NA2500, CE Instruments, Milan, Italy) coupled via a Conflo II interface to an isotope ratio mass spectrometer (Delta Plus, Thermo Finnigan MAT GmbH, Bremen, Germany). Glutamic acid was analysed as a working standard every 10 samples to detect potential instrument drift over time. The standard was calibrated against the primary standards USGS 25 (ammonium sulphate, $\delta^{15}N_{Air}$ = -30.4) and USGS 41 (δ $^{15}N_{Air}$ = 41.6).), and USGS 40 (glutamic acid, $\delta^{13}C_{PDB}$ = -26.39) and USGS 41 (glutamic acid, $\delta^{13}C_{PDB}$ = 37.63) for $\delta^{13}C$. Net N and C uptake capacity was calculated based on the incorporation of ^{15}N or ^{13}C into the roots according to the equation used by Kreuzwieser et al. (2002). Apparent glutamine uptake was generally lower when calculated from ^{13}C incorporation into the root than when calculated from ^{15}N incorporation. Respiration of amino acid-derived C inside the roots or degradation of amino acids in the incubation solution or on the root surface are considered responsible for such differences (Simon et al., 2011).

2.4 Statistical analyses

All statistical evaluations were done in R (R Core Team, 2015) using ANOVAs with the "aov" function. For the glasshouse trial, two-way ANOVAs were used with cultivar and N-levels as independent factors. For biomass, yield, and nitrogen measurements from the FACE study, the design was defined as split-split-plot with growing environment (five levels, two sowing dates in season 2009, seasons 2010 to 2012), [CO_2], irrigation, and cultivar as factors, using FACE rings as random variable. Levene's test (R package DeskTools; Signorell et al., 2016) was used to test each variable for homogeneity of variances across groups, and data were ln transformed where needed. By this criterion all variables except nitrogen harvest index (NHI) conformed to homogeneity of variance criteria. For NHI, variances and means were not correlated across treatment groups, and in this case ANOVA procedures are considered relatively robust (StatSoft, 2013). Graphic analysis of residual distribution did not reveal systematic deviations from normality in any of the resulting models.

3. Results

3.1 Glasshouse experiment

The two cultivars showed significantly different responses to variation in soil N supply. Cultivar 'Yitpi' but not 'Silverstar' increased root growth, and increased root/shoot ratio in response to low N (Fig. 1 a, b). In contrast, specific N uptake (per g root mass) increased upon low N supply in 'Silverstar' but not 'Yitpi', pointing to different N uptake strategies between the two cultivars (Fig. 1c). Biomass N content was affected by N supply, but did not differ between the two cultivars (Fig. 1d).

3.2 Free Air CO_2 Enrichment (FACE)

When grown under AGFACE conditions, there were significant effects of season and supplemental irrigation on most parameters, and these effects were mainly responsible for the large variability in the data (as indicated by SDs in 'Base values' in Table 2). These effects are not surprising, given the wide range of seasonal conditions and resulting low to high yielding crops for the region (Table 1). As there are many studies on seasonal variation and water supply effects on the parameters investigated in the present study, these effects were only examined further where significant interactions with the main factors of interest, cultivar and [CO_2], were observed (Table 2).

After accounting for seasonal variability and irrigation effects, at maturity there were generally small, but consistent differences between the two cultivars in grain [N] ('Yitpi'>'Silverstar'), above ground biomass ('Yitpi'>'Silverstar'), N content in above ground biomass (= N uptake, 'Yitpi'>'Silverstar'), as well as single grain weight ('Yitpi'>'Silverstar'), harvest index ('Yitpi'<'Silverstar'), and N harvest index ('Yitpi'<'Silverstar'). Grain yields, on the other hand, did not differ between cultivars (Table 2). Similarly at anthesis, 'Yitpi' had greater aboveground biomass and N content compared to 'Silverstar' but there was no difference in shoot [N] between the two cultivars.

Elevated [CO_2] significantly increased grain yield, above ground biomass, N content and single grain weight, independent of season and irrigation effects (Table 2). There were significant interactions between [CO_2] and irrigation for harvest index and N harvest index: Harvest index increased slightly under elevated [CO_2] from 0.35±0.07 to 0.37±0.07 (means±SD for ambient and elevated [CO_2] across all cultivars and seasons, n=40), but not under rainfed conditions (0.35±0.07 versus 0.34±0.07); N harvest index increased slightly under elevated [CO_2] from 0.62±0.09 to 0.66±0.10, but decreased under rainfed conditions (0.64±0.08 versus 0.61±0.11). Aboveground biomass, N uptake and grain weight increases under elevated [CO_2] were also relatively greater under irrigated conditions: Aboveground biomass at maturity increased by about 31% from 1229±484 to 1613±604 g dry weight m^{-2} under irrigation, but only by 22% from 1127±447 to 1375±559 under rainfed conditions. Corresponding increases under elevated [CO_2] for N uptake were 17.2±6.1 to 21.0±7.3 g N m^{-2} (22% with supplemental irrigation) and 15.3±5.5 to 18.5±6.5 (21% under rainfed conditions), and for single grain weight 29.6±7.3 to 32.2±7.2 mg per grain (9%) with supplemental irrigation and 31.0±7.8 versus 32.2±7.2 (4%) under rainfed conditions.

There were no significant interactions between cultivar and [CO_2] for any parameter.

3.3 *In-situ* root N uptake rates in FACE

Root N uptake rates for nitrate, ammonium, and glutamine are commonly proportional to the relative availability of the different N sources. Therefore the measured uptake rates for the different N-forms are specific to the composition of the solution used in the experiments. We focus on differences in uptake rates between cultivars and [CO_2]: Root N uptake rate of 'Silverstar' was greater than that of 'Yitpi' for ammonium, but similar for nitrate and glutamine. There was no significant effect of [CO_2] on N uptake rates (Figure 2).

4. Discussion

Our results from *in-situ* uptake measurements of ^{15}N labelled N compounds show that wheat roots have the capacity to absorb all three forms of N offered, viz. nitrate, ammonium, and glutamine

(Figure 2). Plants generally take up N sources in proportion to their relative abundance in soil solution (Bloom 2015), but uptake rates can also be subject to inhibitory effects by other N sources. For example, nitrate uptake may be inhibited in the presence of higher concentrations of ammonium or amino acids (Näsholm et al., 2009; Stoelken et al., 2010). In agro-ecosystems more broadly (Garnett et al., 2009), and also at the field site used in this study particularly, nitrate is the dominant available form of N. The solution chosen for the uptake experiments reflects typical proportions of nitrate and ammonia, but large (up to 1000x) variability of N source concentrations are common at small spatial (cm) and in the short-term (h; Jackson and Bloom 1990). Hence, whilst the artificial soil solution used to measured uptake rates is broadly representative for typical field conditions, direct comparisons between the rates measured for the different N compounds must be treated with caution. Nevertheless, the chosen method is well suited to compare N uptake rates between cultivars and [CO_2] treatments in this present study.

Contrary to natural (forest) ecosystems (Simon et al., 2010) the significance of direct uptake of organic N into crop roots in agro-ecosystems is unclear (Jones et al., 2013). Wheat breeding in high input, nitrate-rich systems may have selected towards a preference for nitrate and against amino acid uptake, although this was not directly confirmed in a pot study comparing old and new wheat varieties (Reeve et al., 2009). Even if measured uptake rates are specific to the measurement conditions, our results show that wheat roots have considerable capacity to take up glutamine, and this capacity was similar in both wheat cultivars. Our results confirm previous studies that demonstrate uptake of intact amino acids into wheat roots from soil (Näsholm et al., 2001) even though we can only speculate about the practical significance of this capacity. Because soil microbes are strong competitors for amino acids, and amino acids are mineralised quickly, the capture of organic N by wheat roots in the fields is considered to be slower than that of inorganic N (Jones et al., 2013).

Both root architecture and root functions, such as specific uptake capacities, contribute to N capture and N use efficiency (more specifically, N uptake efficiency) in agricultural crop plants (Foulkes et al., 2009; Garnett et al., 2009). Genotypic differences in both root architecture and root uptake capacity have been established for wheat (Melino et al., 2015), and are potential targets for improving nitrogen use efficiency (Garnett et al., 2009; Palta and Watt, 2009). The two cultivars investigated in our study showed significant differences in N uptake capacities and root growth response to N supply, indicating different N uptake strategies. The cultivar 'Yitpi' had lower specific uptake rates for ammonium, and responded with enhanced root growth and increased root/shoot ratio to the low N treatment in the glasshouse trial. The cultivar 'Silverstar' showed no such plasticity in root growth upon variation in N supply, but had increased specific N uptake per root weight in the glasshouse. Specific N uptake per total root weight is an imprecise measure of root functional uptake, because only a small proportion of roots are absorbing nutrients, but this glasshouse result was corroborated by greater specific ammonium uptake rates of 'Silverstar' measured with the ^{15}N labelling method in the field. Enhanced root growth (and, possibly, architecture) – as in the cultivar 'Yitpi' in this study – are considered relatively more important in conditions of low N supply, or where mass flow of N is limited by drought (Laperche et al., 2006; Liao et al., 2004; Melino et al., 2015). In contrast, increased root uptake rates – the strategy implemented in 'Silverstar' - determines maximum uptake under conditions of greater soil N availability (Garnett et al., 2009), and can improve the ability of crops to compete for soil N.

At our field site, the strategy used by 'Yitpi' – of greater plasticity in growth and putatively better exploration of soil volumes – was reflected in consistently greater grain [N] at similar yields, indicating the potential to achieve greater grain protein without yield penalty. Greater grain [N] in this cultivar was associated with greater biomass and greater N uptake into biomass, at least up to anthesis. In the water limited environment of this study, wheat crops mature under terminal drought, and N uptake is commonly severely limited after anthesis. Grain N is therefore supplied chiefly by remobilisation from N taken up into vegetative biomass earlier in the growing season, with this remobilisation supplying 80 % or more of the grain N (Palta et al., 1994). However, nitrogen harvest index, one indicator of remobilisation efficiency (the proportion of N in aboveground biomass that ends up in the grains), was less for 'Yitpi' than 'Silverstar'. Nevertheless, variability in N acquisition strategies among bread wheat cultivars, such as shown in this study, are an important prerequisite to identify functional traits for breeding programs (Foulkes et al., 2009).

Regardless of these cultivar differences, growth under elevated [CO_2] consistently increased biomass and grain yield, N uptake into biomass, and decreased [N] in vegetative parts (at anthesis) as well as decreased grain [N]. These changes and their magnitude are in line with previous results from this site (Fitzgerald et al., 2016; Tausz-Posch et al., 2015) and global analyses (Ainsworth and Long, 2005; Högy and Fangmeier, 2008; Kimball, 2016). A widely held view contends that responses to elevated [CO_2] are proportionally greater under drought conditions, because CO_2-induced stomatal closure reduces water loss and increases water use efficiency, and this is most important in dry environments (Kimball, 2016). In apparent contrast, our data showed that when there were significant interactions between [CO_2] and supplemental irrigation (such as for biomass or yield), the [CO_2] effect on the parameter in question was greater with supplemental irrigation. Simulation studies with six crop models also showed such increased growth response to elevated [CO_2] under unstressed conditions at this site (O'Leary et al., 2015). This may be specific to our site conditions where terminal drought dominates and the main effect of irrigation was to extend the period of post-anthesis carbon assimilation, which would prolong the period during which carbon and biomass gain can be stimulated by elevated [CO_2] (Fitzgerald et al., 2016).

Effects on root function, such as down-regulation of N uptake rates, may help to explain the decrease in tissue [N] under elevated [CO_2]. Previous results, mainly from natural ecosystems and tree species, reported highly variable effects with both upregulation or downregulation of uptake capacities possible; dependent on species, N-form and experimental conditions (Dong et al., 2015; Zerihun and Bassirirad, 2001). Our measurements of field uptake capacity gave no evidence that growth under elevated [CO_2] down-regulated or affected specific root N uptake capacities directly, but such results may vary with growth stage or season (Shimono and Bunce 2009). Our field measurements were done in the lead up to flowering, a very active period for N uptake of wheat in this dryland agro-ecosystem (Bahrami et al., 2017), where uptake after flowering is low due to seasonal drought (Palta et al. 1994, Table 2). In a chamber study on hydroponically grown rice, nitrate and ammonium uptake per g root dry weight were decreased by elevated [CO_2] only at or after flowering, but not at an earlier growth stage (Shimono and Bunce, 2009). Although we cannot exclude that elevated [CO_2] had transient effects on root uptake capacity earlier in the season, and we have no information on between-season variability of uptake capacities, it appears that root growth and architecture, rather than uptake capacity, may be affected by elevated [CO_2]. Root growth and architecture were not directly measured in AGFACE in this study, but results from a recent glasshouse study and from AGFACE on different cultivars showed that root growth responses

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to elevated [CO_2] can also be highly variable, even among bread wheat cultivars (Bahrami et al., 2017, Benlloch-Gonzalez et al., 2014).

Despite the consistent differences between the two wheat cultivars in N uptake and allocation strategies, as well as in grain [N], our results provided no evidence that grain [N] depression under elevated [CO_2] is affected by these cultivar differences, as there were no cultivar x [CO_2] interactions for any measured parameter, and elevated [CO_2] decreased grain [N] in both cultivars to the same extent. Our results indicate that effects other than limitations to N acquisition are more important for tissue and grain [N] decreases under elevated [CO_2] (such as e.g. direct effects of elevated [CO_2] on N assimilation as suggested by Bloom et al., 2014), at least for the cultivars studied here.

In response to our research hypotheses, we could confirm that (1) the two cultivars showed consistent differences in N uptake into biomass and grain N concentrations in the field, with 'Yitpi' consistently outperforming 'Silverstar' in these parameters. In response to hypothesis 2, we observed cultivar-specific differences in root growth response to variation in N supply and specific root N uptake rates (for ammonium), whereby greater N uptake and grain N of 'Yitpi' was associated with greater plasticity in root growth, but not with greater specific root N uptake rates. Such cultivar-specific differences are of interest for crop improvement, especially where they are associated with increased grain [N] at similar yields. Our field data add to previous laboratory studies on a range of wheat lines. (3) We found no evidence that growth under elevated [CO_2] decreases specific root N uptake capacities in the field. Whilst cultivar-specific differences in N acquisition strategies were linked to different outcomes in grain [N] (at similar yields), there were no interactions with effects of elevated [CO_2] on tissue and grain [N]. (4) This study therefore gives no indication that uptake strategies can counteract tissue and grain [N] decrease under elevated [CO_2].

Acknowledgements

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Table 1. Summary of growing conditions in the Australian Grains Free Air CO_2 Enrichment (AGFACE) facility in Horsham, Victoria, Australia from 2009 to 2012. In-season (sowing to harvest) rainfall only or rainfall plus supplemental irrigation (+Sup) treatments, replicated four times (n=4) in each environment (Env). Temperatures are reported as seasonal means for the growing season from sowing to harvest for each year. Yield is the mean yield of the bread wheat cultivar *Triticum aestivum* 'Yitpi' under ambient [CO_2] in that environment (without supplementary irrigation).

Env	Year	Sowing date	Days to harvest	In-season rainfall [mm]	(+Sup) [mm]	Mean growing season air T [C]	Yield [t ha^{-1}]
1	2009	Local practice (23 Jun)	164	223	70	12.1	2.7
2	2009	Late (19 Aug)	116	170	60	14.1	1.2
3	2010	Local practice (27 May)	196	299	80	11.2	5.5
4	2011	Local practice (25 May)	194	218	100	11.3	6.2
5	2012	Local practice (30 May)	189	180	120	11.1	6.5

Table 2. Effects of $[CO_2]$ (ambient at 390 µmol mol^{-1} versus elevated at 550 µmol mol^{-1}) and cultivar (*Triticum aestivum* L. 'Silverstar' versus *T. aestivum* 'Yitpi') on wheat growth, yield, and nitrogen parameters in the Australian Grains Free Air CO_2 Enrichment facility during the growing seasons 2009 (two dates of sowing), 2010, 2011, and 2012 (factor "season"). In each season, plots were additionally split for irrigation, with the treatments rainfed (no irrigation) or supplemental irrigation (factor "irrigation"). Four replicates in each treatment, growing condition and cultivar combination. Two growth stages (anthesis and maturity, DC65 and DC90 according to (Zadoks et al., 1974)). See Material and Methods for detailed experimental design.

Raw mean differences (SDs of raw mean difference) for significant $[CO_2]$ and cultivar effects, with positive values indicating increase from ambient to elevated $[CO_2]$, and from 'Silverstar' to 'Yitpi'. Significance levels of the F-tests: *** $P<0.001$, ** $P<0.01$, * $P<0.05$, ns $P≥0.05$

Maturity (DC90)

Parameter	Base value[1]	Cultivar	$[CO_2]$	Other significant effects
Grain yield [g m^{-2} plot area]	437 (213)	-21 (39)^{ns}	+113 (38) ***	season***, irrigation***, season x $[CO_2]$ x irrigation*
Aboveground biomass [g m^{-2} plot area]	1140 (438)	+86 (88)*	+317 (84) ***	season***, irrigation***, irrigation x season**, $[CO_2]$ x irrigation*
Grain [N] [mg N g^{-1} grain dwt^{-1}]	21.2 (3.3)	+0.8 (0.5)***	-1.1 (0.5) **	season***, irrigation*, cultivar x season***
N in aboveground biomass [g N m^{-2} plot area]	15.5 (5.7)	+1.5 (1.0)**	+3.5 (1.0)***	season***, irrigation***, season x irrigation*, season x irrigation x $[CO_2]$*
Single grain weight [mg grain^{-1}]	27.7 (6.9)	+5.8 (1.1)***	+1.3 (1.2) *	season***, $[CO_2]$ x irrigation***, cultivar x season***, irrigation x cultivar**
Harvest index	0.37 (0.08)	-0.04 (0.01)***	0.00 (0.01)^{ns}	season***, $[CO_2]$ x irrigation*
Nitrogen Harvest Index	0.66 (0.09)	-0.07 (0.01)***	0.00 (0.02)^{ns}	season***, $[CO_2]$ x irrigation***

Parameter	Base value[1]	Cultivar	$[CO_2]$	Other significant effects
Anthesis (DC65)				
Aboveground biomass [g m^{-2} plot area]	725 (194)	+234 (52)***	+179 (53)***	season***, season x irrigation*, season x cultivar***
N in aboveground biomass [g N m^{-2} plot area]	15.0 (4.4)	+4.9 (1.1)**	+2.6 (1.1)***	season***, cultivar x season **, season x irrigation**
[N] in aboveground biomass [mg N g^{-1}]	20.7 (2.2)	+0.3 (0.4)^{ns}	-1.1 (0.4)***	season***, cultivar x season***

[1] Base values are measured means (SD) across all seasons and irrigation levels for the combination 'Silverstar' and ambient $[CO_2]$ (n=40).
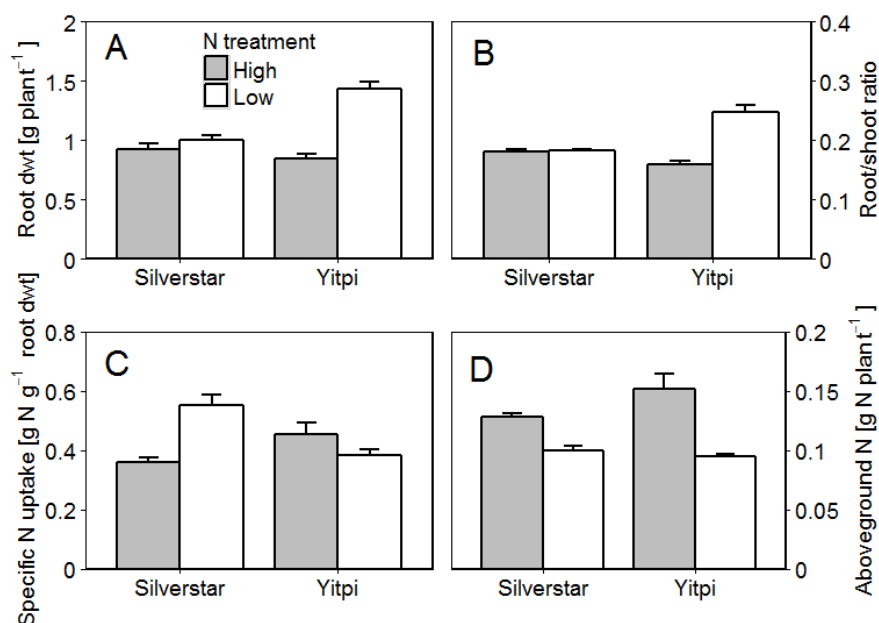
Figure 1. Root dry weight (A), root/shoot dry weight ratio (B), N uptake per root dry weight (C), and N uptake into aboveground biomass (D) of two glasshouse grown wheat cultivars (*Triticum aestivum* 'Silverstar' and *T. aestivum* 'Yitpi') under high (sufficient) and low soil N supply. Means and standard error of means for n=4 replicates in each data point. Effects of N treatment, cultivar and their interaction were tested. For panels A, B, and C the interaction effects N treatment x cultivar were significant at p<0.001; for D, N treatment was significant at p<0.001; all other effects were not significant (P>0.05).
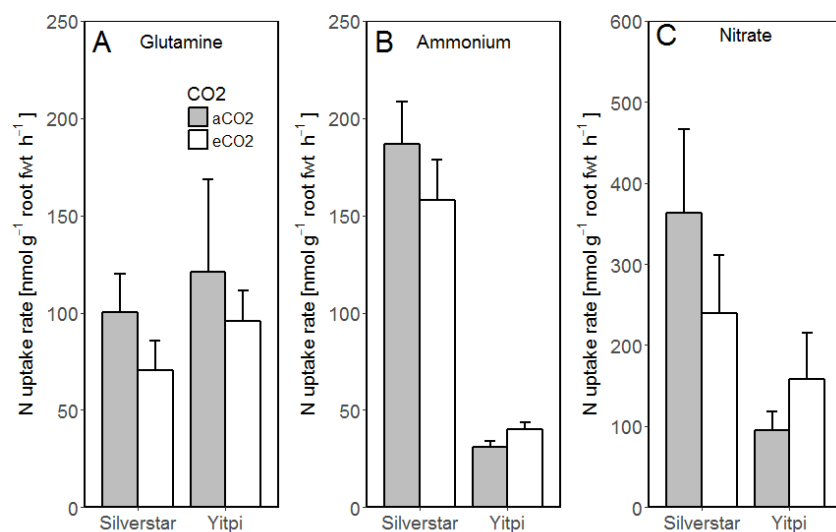
Figure 2. Root uptake rates of glutamine-N (A), ammonium-N (B) and nitrate-N (C) measured *in situ* on two wheat cultivars (*Triticum aestivum* 'Yitpi' and *T. aestivum* 'Silverstar') in the Australian Grains Free Air CO_2 Enrichment facility. aCO2 ambient [CO_2] (390 µmol mol^{-1}); eCO2 elevated [CO_2] (550 µmol mol^{-1}) Measurements were taken 13-29 Sep 2012. Means and standard errors of means of n=4 replicate plots with results from 5 individually measured roots averaged per plot. fwt root fresh weight. Effects of cultivar, [CO_2] and their interaction cultivar x [CO_2] were evaluated for each panel separately. For panel B the effect of cultivar ('Silverstar' versus 'Yitpi') was significant at $p<0.001$, all other tested effects were not significant ($p>0.05$).