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4 Elevated CO₂ (FACE) increases grain yield of aluminium-resistant but not aluminium-sensitive wheat (*Triticum aestivum* L.) grown in an acid soil

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Running title: Elevated CO₂ increases yield of aluminium-resistant wheat in acid soil

22 Abstract

- Background and Aims: Soil acidity currently limits root growth and crop production in
- 24 many regions whilst climate changes raise many uncertainties regarding future food supply. However, it is unknown how elevated CO_2 (eCO₂) affects the performance of wheat crops in
- acid soils under field conditions. We investigated the effects of eCO₂ on plant growth and yield of three pairs of near-isogenic hexaploid wheat lines differing in alleles of aluminium-
- 28 resistant genes-*TaALMT1* (conferring root malate efflux) and *TaMATE1B* (conferring citrate efflux).
- Methods: Plants were grown till maturity in an acid soil under ambient CO₂ (400 μmol mol⁻¹) and eCO₂ (550 μmol mol⁻¹) in a soil free-air CO₂ enrichment facility (SoilFACE).
- 32 Growth parameters and grain yields were measured.
 - Key Results: Elevated CO₂ increased grain yield of lines carrying *TaMATE1B* by 22%
- 34 and lines carrying only *TaALMT1* by 31%, but did not increase the grain yield of Al^{3+} sensitive lines. Although eCO₂ promoted tiller formation, coarse root length and root biomass
- 36 of lines carrying *TaMATE1B*, it did not affect ear number, and thus limited the yield potential. By contrast, eCO₂ decreased or did not change these parameters for lines carrying
- 38 only *TaALMT1*, and enhanced biomass allocation to grains resulting in increased grain yield. Despite *TaMATE1B* is less effective than *TaALMT1* at conferring Al^{3+} resistance based on
- 40 root growth, the gene promoted grain yield to a similar level to *TaALMT1* when grown in the acid soil. Furthermore, *TaALMT1* and *TaMATE1B* were not additive in their effects.
- **Conclusions:** As atmospheric CO₂ increases, it is critical that both Al^{3+} -resistance genes (particularly *TaALMT1*) should be maintained in hexaploid wheat germplasm in order for
- 44 yield increases from CO₂ fertilisation to be realised in acid soils.

46 Key words:

Acid-soil tolerance, biomass allocation, genotypic variation, near-isogenic lines, phosphorus deficiency, *TaALMT1*, *TaMATE1B*

INTRODUCTION

- 50 Climate change is a serious threat to agricultural production and food security (IPCC, 2014; Long et al., 2004). Fortunately, the causative increase in atmospheric CO₂ concentration
- 52 promotes plant photosynthesis and crop yield, and counteracts some of the negative impacts on food supply (Long et al., 2004; Wang et al., 2013). However, the magnitude of yield
- 54 increase due to elevated CO₂ (eCO₂) differs among species, cultivars, and environmental factors (Ainsworth, 2008; Wang et al., 2015; Wang et al., 2013). Yield gain under eCO₂
- 56 decreases for wheat cultivars with a reduced vigor during the vegetative growth (Manderscheid and Weigel, 1997) and for rice grown under stressed conditions such as in
- 58 cadmium-polluted soils (Wu et al., 2016).
- 60 Acid soils comprise around 50% of the area of the world's arable lands (Kochian et al., 2015). Crops often fail to realise their full yield potential on acid soils because of poor root
- 62 growth, mainly resulting from aluminium (Al^{3+}) toxicity or P deficiency or a combination of both (Kochian et al., 2015). To date, the efflux of organic anions and the genes that underlie
- 64 these mechanisms are the most thoroughly described Al³⁺ resistance mechanisms in hexaploid wheat (*Triticum aestivum* L.) (Ryan et al., 2011). A resistant allele of *TaALMT1*
- 66 encodes a transporter protein conferring malate efflux, which is specifically activated by the presence of Al^{3+} and forms a malate-Al complex that is thought to detoxify Al^{3+} in
- 68 rhizosphere or apoplast (Delhaize et al., 1993b; Ryan et al., 1995). Another gene that confers Al^{3+} -resistance is *TaMATE1B* which encodes a transporter protein conferring citrate efflux
- ⁷⁰ but is less effective than *TaALMT1* in hexaploid wheat in protecting root growth from Al^{3+} toxicity (Dong et al., 2018; Han et al., 2016; Ryan et al., 2009). By conferring improved root
- 72 elongation in acid soils, the *TaALMT1* gene has been shown to increase plant productivity and grain yield of hexaploid wheat grown in acid soils (Tang et al., 2001; 2002). There are no
- 74 reports of similar field experiments with hexaploid wheat lines that possess Al^{3+} -resistant alleles of the *TaMATE1B* gene grown in acid soils, although field trials on soils without Al^{3+}
- 76 toxicity did not show improved grain yield (Ryan et al., 2014).
- 78 To date, the effect of eCO_2 on grain yield of wheat lines carrying resistant alleles of *TaALMT1* and *TaMATE1B* grown in acid soils has not been reported. Improved root growth
- 80 under eCO₂ could promote nutrient uptake, thus plant productivity and yield (Kochian et al., 2004; Norby and Zak, 2011). Our previous short-term (24 d) study conducted on wheat
- 82 seedlings grown in acid soils showed that eCO_2 promoted root elongation of wheat lines carrying *TaALMT1* and/or *TaMATE1B* to a greater extent than Al³⁺-sensitive wheat lines that
- had Al³⁺-sensitive alleles of both genes (Dong et al., 2018). This result is likely due to Al³⁺ resistance conferring improved root elongation on acid soils (Delhaize et al., 2012), and
- 86 improved root growth allowing a greater yield response to eCO₂ as observed for wheat grown in non-acid soils (Kirschbaum, 2011; Rogers et al., 1995). For *Stipa krylovii* L. grown in a
- 88 Calcic-orthic Aridisol (P deficient), eCO₂ decreased malate exudation of the intact roots but did not affect citrate exudation (Liu et al., 2016). In another study, malate addition slightly
- 90 decreased root elongation of wild-type Arabidopsis grown in P-deficient solution, whilst citrate addition promoted the root elongation (Mora-Macías et al., 2017). From these
- 92 observations, it is expected that eCO_2 might decrease Al^{3+} resistance or root elongation, and thus reduce grain yield of hexaploid wheat lines carrying *TaALMT1*, whilst the reverse could
- 94 be true for wheat that carrying *TaMATE1B*, although one needs to be cautious in extrapolating findings across species.
- 96

This study was conducted in a free-air CO₂ enrichment systems (FACE) using three nearisogenic pairs of hexaploid wheat lines varying in alleles of *TaALMT1* and *TaMATE1B*. The

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lines were grown to maturity in an Al^{3+} -toxic and P-deficient acid soil. We hypothesized that eCO₂ would promote root growth, and thus plant growth and grain yield of Al^{3+} -resistant

- 100 eCO₂ would promote root growth, and thus plant growth and grain yield of Al³⁺-resistant lines to a greater extent than sensitive lines when grown in acid soils. Amongst the Al³⁺-
- 102 resistant lines, the growth and yield response would be greater in wheat lines carrying *TaMATE1B* compared to those carrying *TaALMT1*.
- 104

MATERIALS AND METHODS

- 106 Experimental design
- The experiment was a split-plot design conducted in soil columns in the soil free-air CO₂ enrichment systems (SoilFACE) in Horsham, Victoria Australia (36°44′57″ S, 142°06′50″ E)
- for 203 d from 2 June to 21 December 2016. The details of SoilFACE systems can be found in Mollah et al. (2009). The experiment consisted of two CO₂ levels as the main plot (rings)
- and six wheat lines as the sub-plot. There were four rings as four replicates for each CO₂
 concentration (eight rings in total) maintained at either 400 μmol mol⁻¹ (ambient CO₂, aCO₂)
- or 550 μ mol mol⁻¹ (eCO₂). The position of each of these subplots within each ring was fully randomized. The six wheat lines were three near-isogenic pairs varying in Al³⁺ resistance due
- to differences in organic anion efflux from root tips. The three pairs of near-isogenic lines (NILs) comprised of: (a) ES8 (carrying Al^{3+} -sensitive alleles of both *TaALMT1* and
- TaMATE1B genes; Al^{3+} -sensitive line) and ET8 (carrying an Al^{3+} -resistant allele of
- 118 TaALMTI gene; Al³⁺-resistant line), (b) Egret (carrying Al³⁺-sensitive alleles of both TaALMTI and TaMATEIB genes; Al³⁺-sensitive line) and Egret TaMATEIB (carrying an
- 120 Al³⁺-resistant allele of *TaMATE1B* gene; Al³⁺-resistant line), (c) EGA-Burke (carrying an Al³⁺-resistant allele *TaALMT1* gene; Al³⁺-resistant line) and EGA-Burke *TaMATE1B*
- 122 (carrying an Al³⁺-resistant allele of both *TaALMT1* and *TaMATE1B* genes, Al³⁺-resistant line) (Delhaize et al., 1993a; Han et al., 2016). For simplicity, here we refer to Al³⁺-resistant lines
- 124 that carry Al^{3+} -resistant alleles as lines carrying *TaALMT1* or *TaMATE1B* unless otherwise specified. Previous short-term experiments have shown that *TaALMT1* is a more effective
- 126 Al³⁺-resistant gene than *TaMATE1B* (Han et al. 2016). The experimental soil was a composite acid soil made by combining a Dermosol and a Ferrosol (Isbell, 1996), both
- 128 collected from Kinglake, Victoria (Table 1). ES8 and ET8 were also grown in limed acid
- soils to establish the effect of eCO_2 on wheat growth in the absence of Al^{3+} toxicity. In
- 130 addition, "control" columns without plants were also included in these treatments.

132 Soil preparation and plant growth

The soils were air-dried, passed through a 4-mm sieve and stored in plastic bins before use.

- Each soil column (0.60 m in height and 0.15 m in diameter) consisted of 2.2 kg of the Ferrosol mixed with 1 g $CaCO_3 kg^{-1}$ soil as a topsoil (0-0.10 m) and 8.8 kg mixture of the
- 136 Dermosol and the Ferrosol (20/80, w/w) as a subsoil (0.10-0.50 m). A preliminary experiment indicated the soil column gave good separation of growth parameters of the
- 138 various lines in response to the soil acidity. The limed soils used the same mixtures but 5 g of $CaCO_3 kg^{-1}$ soil was mixed into both the topsoils and subsoils to remove Al^{3+} toxicity. Basal
- 140 nutrients were applied as follows (mg kg⁻¹ soil): K_2SO_4 , 441; MgSO₄.7H₂O, 122;
- CaCl₂.2H₂O, 186; KH₂PO₄, 112.5; CO(NH₂)₂, 60; CuSO₄.5H₂O, 6; ZnSO₄.7H₂O, 8;
- 142 NaMoO₄.2H₂O, 0.4; and H₃BO₃, 1.04, and mixed into both topsoil and subsoil. After the columns were filled with soils, they were watered with reverse osmosis (RO) water to 100%
- 144 field capacity and allowed to equilibrate for one week before seeds were sown. The bottoms of columns were perforated to allow drainage during the winter and early spring. The
- 146 columns were sealed from Day 144 and watered by weighing over the dry half of spring.

148 Twenty pre-germinated seeds (1 d at 25 °C) were sown at 2-cm depth into each column and the columns were then placed into the FACE rings with the top of each column at the soil

150 surface of the surrounding field. The seedlings were thinned twice to allow a final number of 4 uniform plants per column. To minimise the potential deficiencies of N and P, 1125 mg

- 152 KH₂PO₄ (256 mg P) and 1320 mg CO(NH₂)₂ (616 mg N) were added to each column as five separate top-dressing applications prior to anthesis.
- 154

The site has a Mediterranean climate with a cool wet winter and a hot dry summer. Soils were rain-fed during the wet season, and were watered to 100%-120% field capacity by weighing the columns twice a week during grain filling. Polyethylene beads were added to

- 158 the soil surface to minimise evaporation after the first watering. CO₂ treatments were stopped at the firm-dough stage of development and the plants were kept in the rings until they
- 160 reached grain maturity. Over the experimental period, the weather record was taken from the Horsham Aerodrome Climatological Station (079100) which is 10 km from the experimental

162 site (Figure S1). The total rainfall was 302 mm ranging from 9 mm to 91 mm monthly. The maximum monthly mean temperature ranged from 13.2 °C to 27.2 °C, minimum monthly

- 164 mean temperature from 3.6 °C to 8.3 °C, and monthly mean solar radiation from 2.2 to 6.7 kWh m⁻².
- 166

Measurements

- 168 Net photosynthetic rates, transpiration rates and stomatal conductance of flag leaves were measured at flowering in the field using a portable photosynthesis system (Li-Cor, Lincoln,
- 170 US). Measurements were conducted between 0900 and 1200 hrs on sunny days, with the temperature ranging from 22 to 30 °C. Three flag leaves with moderate growth vigor in each
- 172 column were used for measurements from pair to pair of the wheat NILs and from replicate to replicate to minimize the effect of environmental variations. Instantaneous transpiration

174 efficiency was calculated by dividing net photosynthetic rates by transpiration rates. As the columns were sealed from Day 144 to maturity, the water input per column in this period was

176 estimated as a combination of each water addition and rainfall, and weight loss of the column. Water-use efficiency was calculated as a division of total biomass by the total of

- 178 water addition and rainfall over the entire growing season.
- 180 Shoot biomass was measured at Days 67 and 85 and at crop maturity (Day 203). The tiller numbers were recorded at Days 97 and 107 and at maturity. At maturity, total tiller and ear
- 182 numbers were counted, shoots were cut at ground level, and soil columns were temporarily stored at 4 °C to avoid root decomposition. The columns were disassembled and divided into
- 184 three soil layers: 0-0.1 m, 0.1-0.2 m and 0.2-0.5 m. Roots of each layer were separated from
- soil by washing with tap water, and then floated in RO water in a clear plastic tray and
 scanned using a flatbed scanner (Epson EU-35, Seiko Epson Corp., Japan) at a resolution of
 600 dots per inch. Root length was determined using WinRhizo Pro version 2003B software

188 (Régent Instruments Inc., CA). The roots with diameter <0.3 mm are defined as fine roots and those >0.3 mm as coarse roots. All the plant materials were washed thoroughly with RO

- 190 water and oven-dried at 70 °C for 3 d prior to weighing. The dried heads were threshed,
- separated from chaff, and total grain and thousand-grain weights were recorded.
- 192

Grains were finely ground using a mixer mill (MM400, Retsch GmbH, Germany). A 0.3 g

- 194 subsample of ground plant material was digested with 8 ml concentrated nitric acid (HNO₃) in Eppendorf reaction vials using a microwave reactive system (Multiwave 3000, Anton Paar
- 196 GmbH, Austria). All digests were diluted into 50-ml tubes and analysed for P, Ca, Mg, S, Fe, Mn, and Zn using an inductively coupled plasma atomic emission spectrometer (ICP-AES,

- 198 Optima 8000, PerkinElmer, US). The ground grains were also used for N determination using a CHNS/O analyser (EA2400, PerkinElmer, US), and the protein concentration was
- 200 calculated by multiplying N concentration by 5.7 (Högy et al., 2009).
- 202 Statistical analysis

The data were analysed with linear mixed models to determine the main effect of CO₂ and

204 wheat line and their interactions in GenStat user interface (Version 17.1, VSN International, UK). CO₂ and line were used as fixed effects and whole plot (ring) as a random effect. Since

- 206 least significant difference tests (LSD) multiple range tests may not have detected CO₂
 effects due to large differences when all lines were analysed together, Student's two-tailed *t*-
- 208 test was specifically performed to determine the CO₂-effect of individual lines or within pairs of NILs using Microsoft Excel 2016 rather than the LSD values. The correlation between
- 210 total tiller number and total root biomass/shoot biomass was done using Microsoft Excel 2016. The effects were considered significant at 5% confidence levels (p < 0.05) and near-
- 212 significant at 10% confidence levels (p < 0.1).

214 **RESULTS**

Grain yield and biomass

- Elevated CO₂ increased grain yield of all wheat lines by an average of 20% (p=0.027). Specifically, eCO₂ increased the yield of lines ET8 (Al³⁺-resistant allele of *TaALMT1*) by
- 218 32% (p=0.016) and EGA-Burke (Al³⁺-resistant allele of *TaALMT1*) by 30% (p=0.017), and showed a similar trend for a line that carried only Al³⁺-resistant allele of *TaMATE1B* (Egret
- 220 TaMATE1B; 23%, p=0.099) and a line that carried both Al³⁺-resistant allele of TaALMT1 and TaMATE1B genes (EGA-Burke TaMATE1B; 21%, p=0.025), whilst it did not significantly
- 222 affect the grain yield of sensitive lines ES8 and Egret (Figure 1). Elevated CO_2 increased the harvest index of ET8 by 22% (p=0.014) and tended to increase that of EGA-Burke by 7%
- 224 (*p*=0.080), with no effect on that of other lines (Table S1). On average, EGA-Burke had the greatest yield, followed by EGA-Burke *TaMATE1B*, ET8, Egret *TaMATE1B*, Egret and ES8
- 226 the least. Compared to their sensitive NILs, the effects of both *TaALMT1* (ET8) and *TaMATE1B* (Egret *TaMATE1B*) genes on grain yield of the lines grown in the acid soil (data
- of CO₂ treatments combined) were significant, with the increases of 163% and 109%, respectively. However, there was no significant effect of *TaMATE1B* in addition to
- 230 *TaALMT1* (Figure 1).
- 232 Compared with aCO_2 , eCO_2 did not affect total root biomass (p=0.427). Elevated CO₂ had variable effects on root biomass that were not consistent across lines. Specifically, eCO_2
- tended to decrease root biomass of Egret by 25% (Figure 2; p=0.061), and ET8 by 27% (p=0.039). However, eCO₂ increased root biomass in lines carrying Al³⁺-resistant allele of
- 236 TaMATE1B- Egret TaMATE1B by 24% (p=0.037) and EGA-Burke TaMATE1B by 26% (p=0.029). Elevated CO₂ tended to increase shoot biomass of Egret TaMATE1B by 38%
- 238 (p=0.013) and EGA-Burke *TaMATE1B* by 21% (p=0.080), and EGA-Burke by 21% (p=0.093), with no significant effect on that of other lines. As a result, eCO₂ decreased root-
- 240 to-shoot biomass ratio (p=0.015) across all lines. Elevated CO₂ decreased the root-to-shoot ratio of ET8 (p=0.015) and tended to decrease that of EGA-Burke (p=0.067), whilst it had no
- effect on sensitive and *TaMATE1B* lines (Table S1). The total tiller number was related to total root biomass (r=0.83, p<0.001) and shoot biomass (r=0.91, p<0.001). Elevated CO₂
- increased the total tiller number by an average of 11% (*p*=0.012). Specifically, eCO₂ increased the tiller number of Egret *TaMATE1B* by 37% (*p*=0.041) and EGA-Burke
- 246 *TaMATE1B* by 21% (p=0.037), with no significant effect on other lines. As found for grain yield, Al³⁺-resistant lines harbouring either the *TaALMT1* or *TaMATE1B* gene had greater

- total root biomass, shoot biomass, and total tiller number when compared to their nearisogenic sister lines (Figure 2). When compared to ES8, ET8 had 161%, 168% and 80% more
- 250 total root biomass, shoot biomass, and total tiller number, respectively. The effect on Egret *TaMATE1B* was less dramatic for these parameters when compared with Egret, showing
- 252 increases of 129%, 91%, and 47%, respectively. The effect of the *TaMATE1B* gene did not appear to be additive with *TaALMT1* since EGA-Burke *TaMATE1B* showed no increase in
- these parameters relative to its near isogenic pair EGA-Burke.
- 256 Root length
- The total root length followed a similar pattern as total root biomass in response to eCO₂
- 258 (Figure 3). Elevated CO₂ decreased total root length of the lines only carrying *TaALMT1* gene-ET8 by 27% (p=0.089) and EGA-Burke by 15% (p=0.010) with no significant effect on
- sensitive lines and lines carrying *TaMATE1B* gene. Elevated CO₂ tended to decrease the total fine root length of lines only carrying *TaALMT1* gene ET8 (*p*=0.076) and EGA-Burke
- 262 (p=0.083). By contrast, eCO₂ increased total coarse root length of lines carrying *TaMATE1B* gene-Egret *TaMATE1B* (p=0.011) and EGA-Burke *TaMATE1B* (p=0.033), but not of other
- 264 lines. Again, the lines harbouring the Al³⁺-resistant genes conferred greater root growth on the acid soil compared to their near-isogenic pairs. When combing data for the CO₂
- treatments, ET8 had 253%, 290% and 201% greater total root length, fine and coarse root length, respectively, than ES8. Similarly, Egret *TaMATE1B* had improved root growth with
- 268 increases in these parameters of 104%, 68% and 143%, respectively, compared to Egret. As observed for root mass, the effect of the resistant genes was not additive on root length with
- 270 EGA-Burke showing similar root growth to EGA-Burke *TaMATE1B*.
- 272 *Relative growth*
- Growth parameters of ET8 and ES8 in the acid soil were also expressed as percentages of growth parameters in the limed soil and were referred to as relative values. Figure 4 shows
- that eCO₂ decreased the relative root length of ES8 by 20% (p=0.016). Similarly, eCO₂ tended to decrease relative root biomass of ES8 by 32% (p=0.096) and ET8 by 18%
- (*p*=0.004). The relative grain yield of both lines remained stable under eCO₂. The relative
 data show the large benefit of wheat having *TaALMT1* when grown in acid soil. Table S2 shows the absolute data for the various parameters and includes measurements of shoot
- 280 biomass and tiller number at Day 97, 107 and at maturity. Table S2 shows that eCO₂ increased grain yield of both ET8 and ES8 on limed soil whereas only ET8 had an increased
- 282 grain yield on unlimed acid soil.

284 **DISCUSSION**

- Elevated CO₂ increased the grain yield of Al^{3+} -resistant lines but not of Al^{3+} -sensitive lines when grown in the acid soil. More specifically, although there were no CO₂ × line interactions on the components of yield due to the large variation inherent in field studies (Table S1), the
- 288 greater yield increase of Al³⁺-resistant compared to Al³⁺-sensitive lines can be largely attributed to the greater ear number. This conclusion is consistent with studies showing that
- an increased ear number contributes to a yield increase (Dijkstra et al., 1999; Hakala, 1998;
 Wang et al., 2015). The average 20% increase in grain yield across all lines due to eCO₂ is
- well within the range of a previous meta-analysis that found an average increase of 24% in grain yield under eCO₂ for wheat grown on a range of soils (Wang et al., 2013). Similarly, a
- FACE study with a similar atmospheric environment as our study showed a grain yield increase of 24% for wheat grown in the field under dryland conditions (Fitzgerald et al.,
- 296 2016). *TaALMT1* and *TaMATE1B* genes confer the Al^{3+} -resistant lines a greater ability to promote root growth and thus shoot growth including potentially increased leaf area which

- allows plants to respond to eCO_2 to a greater extent than Al^{3+} -sensitive lines. Our results also showed that both *TaALMT1* and *TaMATE1B* genes frequently increased concentrations of
- nutrients in shoots, particularly P which was below the critical value (Ryan et al., 2014) at the vegetative stage when compared the resistant lines with their sensitive isogenic pairs (Table S3).
- 304 Amongst the Al³⁺-resistant lines, the increased grain yield due to eCO_2 of lines carrying Al³⁺-resistant allele of *TaMATE1B* (Egret *TaMATE1B* and EGA-Burke *TaMATE1B*) could be
- 306 largely attributed to their increased growth as indicated by plant shoot biomass and number of tillers (Figure 2). By contrast, the increases in harvest index of lines only carrying Al³⁺-
- 308 resistant allele of *TaALMT1* (ET8 and EGA-Burke) might contribute to the yield increase to an extent (Table S1). The difference in growth between the lines carrying *TaMATE1B* gene
- 310 and those carrying only *TaALMT1* gene could stem from the differences in biomass allocation. Amongst the Al^{3+} -resistant lines, eCO₂ maintained biomass allocation to roots of
- 312 lines carrying *TaMATE1B* gene (Egret *TaMATE1B* and EGA-Burke *TaMATE1B*) but decreased that of lines carrying only *TaALMT1* gene (ET8 and EGA-Burke) (Table S1).
- 314 More specifically, when the various root types were analysed, eCO₂ consistently increased only coarse root length (likely nodal roots) in lines carrying *TaMATE1B* gene (Figures 3).
- 316 The greater improvement of net photosynthetic rates and stomatal conductance in flag leaves and water-use efficiency of lines carrying *TaMATE1B* gene under eCO₂ (Table S4) are
- 318 consistent with the greater promotion of root growth from eCO_2 relative to lines carrying only *TaALMT1*. Therefore, it appears that the yield increase of the resistant lines under eCO_2
- 320 could not all be attributed to improved root growth.
- 322 This study thus did not support our speculation that the more Al^{3+} -resistant a line was, the more root elongation there would be under eCO₂. This argues against our hypothesis that
- 324 improved grain yield due to eCO_2 would be driven by improved root growth although it appears to be the case when Al^{3+} -resistant lines as a group are compared to Al^{3+} -sensitive
- 326 lines. The main reason for the discrepancy could be that Al³⁺-toxicity in the acid soil used inhibited fine root growth (Figure 3 and Table S3), which might impair the root function for
- 328 nutrient uptake. We speculate that in highly Al^{3+} -toxic conditions, the increased carbon availability under eCO₂ is more likely used for biomass accumulation in shoots and coarse
- 330 roots or to form grain yield instead of improving fine root growth as occurred under nonstressed conditions. Therefore, more biomass can be translocated to grains of the lines
- 332 carrying only *TaALMT1* when less biomass was allocated to the roots.
- Lines carrying *TaMATE1B* gene also formed more tillers under eCO₂ than lines carrying only *TaALMT1* gene (Tables 1 and S5). However, the increased tillers were unable to develop to
- 336 effective tillers (Table S1) resulting in a greater number of infertile tillers (haying off). The greater mass of nodal roots associated with greater tiller initiation might limit biomass
- 338 allocation to grain to some extent as well (Watt et al., 2008). Tausz-Posch et al. (2015) observed similar carbon allocation to non-effective tillers in wheat cv. Silverstar and
- 340 speculated that the greater early vegetative growth decreased the amount of water available for grain filling later in the growing season (Herwaarden et al., 1998). However, eCO₂
- 342 decreased the water consumption and slightly increased soil water content (Table S4 and Figure S2). It is therefore unlikely that the tiller abortion resulted from water deficit. Since
- 344 lines carrying *TaMATE1B* gene enhanced carbon allocation to roots under eCO₂, the early tillers possibly could not receive enough carbon to complete their development, resulting in
- 346 the tiller abortion. Alternatively, *TaMATE1B* gene might respond to other soil factors apart from Al^{3+} toxicity, which favoured biomass allocation to the root under eCO₂.

This is the first study to report that the introgression of TaMATE1B into an Al³⁺-sensitive

- 350 hexaploid wheat cultivar promoted grain yield of wheat in the field in an acid soil (Figure 1). The introgression of *TaMATE1B* into a sensitive hexaploid wheat cultivar was far less
- 352 effective than *TaALMT1* in enhancing acid-soil tolerance when considering root and shoot growth parameters at maturity (Figures 2 and 3). Despite *TaMATE1B* being less effective
- than *TaALMT1* in conferring improved shoot and root biomass in an acid soil, *TaMATE1B* was as effective as *TaALMT1* in increasing grain yield (Figure 1). The greater harvest index
- 356 and reduced biomass allocation to roots resulting from *TaMATE1B* gene (Table S1) might have resulted in greater relative biomass allocation to grain and thus grain yield while still
- 358 maintaining sufficient root growth. Despite the benefit of each gene acting independently they did not appear to be additive since in the EGA-Burke background which has *TaALMT1*,
- 360 the plant growth and grain yield were not increased by the inclusion of *TaMATE1B*. Similarly, there was no additive effect of the gene in a short-term study using the lines grown
- 362 in a Ferrosol (Han et al., 2016). However, a previous short-term chamber study using a very Al^{3+} -toxic Dermosol showed that there was an additive effect on root length (Dong et al.,
- 2018), suggesting that an additive effect might only be realised in highly Al³⁺-toxic soils.
- The increases in shoot biomass, tiller number, and root biomass of ES8 and ET8 at maturity when grown in limed soils (Table S2, absolute data) indicates that eCO₂ increased plant
- 368 performance, which is consistent with previous studies for wheat grown on non-acid soils (Long et al., 2004; Wang et al., 2013). The relative data (Figure 4) shows the benefit of
- having *TaALMT1* on various plant growth parameters including grain yield when ET8 and ES8 were grown on an acid soil as previously found (Tang et al., 2002). However, eCO₂
- 372 decreased the acid-soil tolerance of sensitive line-ES8 and tended to decrease that of resistant line-ET8 when grown in acid soils as indicated by the general decreases in relative root
- 374 length, relative root biomass and relative total biomass accordingly (Figure 4). This finding is consistent with a previous short-term study using a controlled-environment chamber showing
- 376 that eCO₂ decreased acid-soil tolerance of ES8 (Dong et al., 2018). Reduced relative root biomass of ET8 might be due to the reduced rates of carbon fixation in this study (*t*-test,
- 378 p=0.033, Table S4), which probably resulted from the photosynthetic acclimation by longerterm eCO₂ exposure or lower CO₂ level, resulting in less carbon input into root elongation
- 380 (Kirschbaum, 2011) or less antioxidants to counteract Al^{3+} toxicity (Pietrini et al., 2016).
- 382 In our study, eCO₂ did not affect grain quality in terms of protein and mineral concentrations (Table S6). This contrasts with findings that eCO₂ decreases grain concentrations of protein
- 384 (Taub et al., 2008) and minerals in various crops (Loladze, 2014; Myers et al., 2014) as well as wheat (Högy and Fangmeier, 2008). Environmental or species variations may have
- 386 contributed to these differences (Fernando et al., 2014; Högy and Fangmeier, 2008).

388 CONCLUSIONS

This FACE study demonstrated that eCO₂ increased the grain yield of wheat lines carrying only *TaALMT1* gene (ET8 and EGA-Burke) or *TaMATE1B* (Egret *TaMATE1B* and EGA-Burke *TaMATE1B*) but not of sensitive lines (ES8 and Egret) when grown in an Al³⁺-toxic

- 392 soil. The increased grain yield could not be attributed to improved root growth since eCO₂ reduced or did not affect the total root length and biomass of lines carrying only *TaALMT1*
- 394 gene-ET8 and EGA-Burke. By contrast, eCO_2 improved the coarse root length and tiller formation in lines carrying *TaMATE1B*, which may have resulted in a greater abortion of
- 396 tillers and limited the biomass allocation to grains relative to lines carrying only *TaALMT1*. The introgression of *TaMATE1B* gene into cv. Egret increased the grain yield in the acid soil

348

- 398 to a similar level as ET8 which has *TaALMT1*. This was despite *TaMATE1B* being a less effective Al^{3+} -resistance gene than *TaALMT1* based on the ability to protect root growth.
- 400 However, despite the Al^{3+} -resistance genes conferring improved grain yield when present individually, their effects were not additive. As the atmospheric CO₂ inevitably increases in
- 402 the future, wheat breeders should maintain the resistant genes (*TaALMT1* and *TaMATE1B*) in their germplasm, but be prudent in using *TaMATE1B* as a source of Al^{3+} resistance in Al^{3+} -
- 404 toxic soils. Further studies are needed to ascertain the reliability of the respective resistant genes and to assess the lines grown in the field on various acid soil sites over multiple
- 406 seasons under a range of climatic conditions.

408 SUPPLEMENTARY DATA

Table S1. Root-to-shoot biomass ratio (root/shoot), harvest index and the yield components of six wheat lines grown to maturity in an acid soil under two CO₂ concentrations (aCO₂, 400 μ mol mol⁻¹ and eCO₂, 550 μ mol mol⁻¹).

- 412 **Table S2.** Shoot biomass at Days 67 and 85 and maturity, tiller number at Days 97 and 107 and maturity, and growth parameters (root biomass, root length, root-to-shoot biomass ratio
- 414 (root/shoot) and grain yield) at maturity of two wheat lines (ES8 and ET8) grown in an acid soil with or without lime under two CO₂ concentrations (aCO₂, 400 µmol mol⁻¹ and eCO₂,

416 550 μmol mol⁻¹).

Table S3. Concentration of nutrients in shoots of plants (67 d and 85 d from sowing) of six 418 wheat genotypes grown in an acid soil under two CO₂ concentrations (aCO₂, 400 μ mol mol⁻¹ and eCO₂, 550 μ mol mol⁻¹).

- 420 **Table S4**. Net photosynthetic rates, stomatal conductance, instantaneous transpiration efficiency of the flag leaf at the flowering stage, and water input and water-use efficiency of
- 422 six wheat lines grown in an acid soil under two CO_2 concentrations (a CO_2 , 400 µmol mol⁻¹ and e CO_2 , 550 µmol mol⁻¹).
- Table S5. Shoot biomass (Days 67 and 85) and tiller number (Days 97 and 107) of six wheat lines grown in an acid soil at various growth stages under two CO₂ concentrations (aCO₂, 400
 weat weat¹ and aCO₂, 550 weat weat¹.

426 μ mol mol⁻¹ and eCO₂, 550 μ mol mol⁻¹).

Table S6. Concentrations of protein and nutrients in grains of six wheat lines grown in an acid soil under two CO₂ concentrations (aCO_2 , 400 µmol mol⁻¹ and eCO_2 , 550 µmol mol⁻¹).

- Figure S1. Rainfall, daily solar radiation, daily maximum temperature (Tmax) and daily
- minimum temperature (Tmin) over the experimental period. Data were obtained from Horsham Aerodrome Climatological Station, Bureau of Meteorology 10 km from the
- 432 experimental site.Figure S2. Water content (average of the entire soil column) of the acid soils in which six
- 434 wheat lines were grown in an acid soil under two CO₂ concentrations (aCO₂, 400 μmol mol⁻¹ and eCO₂, 550 μmol mol⁻¹) from Day 138 to Day 192 of growth. Vertical error bars denote the
- 436 least significant difference (p=0.05) values to compared any two means among treatments at individual times. The thin arrows indicate the times for watering events and the thick arrows
- 438 indicate the time to start flowering for each pair.

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- 448 Department of Agriculture and Water Resources.

LITERATURE CITED

- 450 Ainsworth EA. 2008. Rice production in a changing climate: a meta-analysis of responses to elevated carbon dioxide and elevated ozone concentration. *Global Change Biology* 452 14: 1642-1650.
- 454 Delhaize E, Craig S, Beaton CD, Bennet RJ, Jagadish VC, Randall PJ. 1993a. Aluminum
 454 tolerance in wheat (*Triticum aestivum* L.) (I. uptake and distribution of aluminum in root apices). *Plant Physiology* 103: 685-693.
- 456 **Delhaize E, Ma JF, Ryan PR. 2012**. Transcriptional regulation of aluminium tolerance genes. *Trends in Plant Science* **17**: 341-348.
- 458 Delhaize E, Ryan PR, Randall PJ. 1993b. Aluminum tolerance in wheat (*Triticum aestivum* L.) (II. Aluminum-stimulated excretion of malic acid from root apices). *Plant* 460 *Physiology* 103: 695-702.
- Dijkstra P, Schapendonk AHMC, Groenwold KO, Jansen M, Van De Geijn SC. 1999.
 Seasonal changes in the response of winter wheat to elevated atmospheric CO₂ concentration grown in Open-Top Chambers and field tracking enclosures. *Global Change Biology* 5: 563-576.
- 466
 466 Dong J, Hunt J, Delhaize E, Tang C. 2018. The impact of elevated CO₂ on acid-soil tolerance of hexaploid wheat (*Triticum aestivum* L.) genotypes varying in organic anion efflux. *Plant and Soil* 428: 401-413.
- Fernando N, Panozzo J, Tausz M, Norton RM, Neumann N, Fitzgerald GJ, Seneweera S. 2014. Elevated CO₂ alters grain quality of two bread wheat cultivars grown under different environmental conditions. *Agriculture, Ecosystems and Environment* 185: 24-33.
- Fitzgerald GJ, Tausz M, O'Leary G, Mollah MR, Tausz-Posch S, Seneweera S, Mock I, Löw M, Partington DL, McNeil D, Norton RM. 2016. Elevated atmospheric [CO₂] can dramatically increase wheat yields in semi-arid environments and buffer against heat waves. *Global Change Biology* 22: 2269-2284.
- 476 **Hakala K. 1998**. Growth and yield potential of spring wheat in a simulated changed climate with increased CO₂ and higher temperature. *European Journal of Agronomy* **9**: 41-52.
- 478 Han C, Zhang P, Ryan PR, Rathjen TM, Yan Z, Delhaize E. 2016. Introgression of genes from bread wheat enhances the aluminium tolerance of durum wheat. *Theoretical and* 480 *Applied Genetics* 129: 1-11.
- Herwaarden AFv, Farquhar GD, Angus JF, Richards RA, Howe GN. 1998. 'Haying-off',
 the negative grain yield response of dryland wheat to nitrogen fertiliser. I. Biomass,
 grain yield, and water use. Crop and Pasture Science 49: 1067-1082.

484 **Högy P, Fangmeier A. 2008**. Effects of elevated atmospheric CO₂ on grain quality of wheat. *Journal of Cereal Science* **48**: 580-591.

- 486 Högy P, Wieser H, Köhler P, Schwadorf K, Breuer J, Franzaring J, Muntifering R, Fangmeier A. 2009. Effects of elevated CO₂ on grain yield and quality of wheat:
 488 results from a 3-year free-air CO₂ enrichment experiment. *Plant Biology* 11: 60-69.
- **IPCC. 2014**. Climate change 2014: synthesis report. Contribution of working groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate

Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (Eds.)]. IPCC, Geneva. Isbell R. 1996. The Australian soil classification. Melbourne: CSIRO publishing.

492 Isbell R. 1996. *The Australian soil classification*. Melbourne: CSIRO publishing.
 Kirschbaum MU. 2011. Does enhanced photosynthesis enhance growth? Lessons learned
 494 from CO₂ enrichment studies. *Plant Physiology* 155: 117-124.

	Kochian LV, Hoekenga OA, Piñeros MA. 2004. How do crop plants tolerate acid soils?
496	mechanisms of aluminum tolerance and phosphorous efficiency. <i>Annual Review of Plant Biology</i> 55 : 459-493.
498	Kochian LV, Piñeros MA, Liu J, Magalhaes JV. 2015. Plant adaptation to acid soils: The
	molecular basis for crop aluminum resistance. Annual Review of Plant Biology 66:
500	571-598.
	Liu N, Tian Q, Zhang WH. 2016. Artemisia frigida and Stipa krylovii, two dominant
502	species in Inner Mongolia steppe, differed in their responses to elevated atmospheric CO ₂ concentration. <i>Plant and Soil</i> 409 : 117-129.
504	Loladze I. 2014. Hidden shift of the ionome of plants exposed to elevated CO ₂ depletes
	minerals at the base of human nutrition. <i>eLife</i> 3 : e02245.
506	Long SP, Ainsworth EA, Rogers A, Ort DR. 2004. Rising atmospheric carbon dioxide:
5 00	Plants FACE the Future. Annual Review of Plant Biology 55: 591-628.
508	Manderscheid R, Weigel HJ. 1997. Photosynthetic and growth responses of old and modern
510	spring wheat cultivars to atmospheric CO_2 enrichment. Agriculture, Ecosystems and
510	Environment 64: 65-73.
510	Mollan M, Norton R, Huzzey J. 2009. Australian grains free-air carbon dioxide enrichment
512	(AGFACE) facility: design and performance. Crop and Pasture Science 60: 697-707. More Maxies I. Oroda Pivora IO. Cutiérroz Alanís D. Vong Villalobes I. Oronoza
514	Aburto A Baya Conzáloz I Jimánoz Domínguoz C Chávoz Calvillo C Bollán
514	Álvarez R. Herrera-Estrella I. 2017 Malate-dependent Fe accumulation is a
516	critical checkpoint in the root developmental response to low phosphate <i>Proceedings</i>
010	of the National Academy of Sciences of the United States of America 114 : E3563-
518	E3572.
	Myers SS, Zanobetti A, Kloog I, Huybers P, Leakey ADB, Bloom AJ, Carlisle E,
520	Dietterich LH, Fitzgerald G, Hasegawa T, Holbrook NM, Nelson RL, Ottman
	MJ, Raboy V, Sakai H, Sartor KA, Schwartz J, Seneweera S, Tausz M, Usui Y.
522	2014 . Increasing CO ₂ threatens human nutrition. <i>Nature</i> 510 : 139-142.
	Norby RJ, Zak DR. 2011. Ecological lessons from Free-Air CO ₂ enrichment (FACE)
524	experiments. Annual Review of Ecology, Evolution, and Systematics 42: 181-203.
	Pietrini F, Bianconi D, Massacci A, Iannelli MA. 2016. Combined effects of elevated CO ₂
526	and Cd-contaminated water on growth, photosynthetic response, Cd accumulation and
	thiolic components status in <i>Lemna minor</i> L. <i>Journal of Hazardous Materials</i> 309 :
528	
520	Rayment GE, Lyons DJ. 2011 . Colwell-P and P buffer index. In: Rayment GE, Lyons DJ
530	(eas). Soil chemical methods - Australasia. Melbourne: CSIRO Publishing.
522	influenced by CO ₂ . Diant and Soil 197 : 220-248
332	Dyan DD Delhaize F Dandell DI 1005 Characterisation of A1 stimulated offlux of malate
534	from the anices of Al-tolerant wheat roots <i>Planta</i> 196 : 103-110
554	from the appees of Al-tolerant wheat roots. <i>I tuntu</i> 190 . 103-110.
526	Ryan PR Raman H Gunta S Horst WJ Delhaize F. 2009 A second mechanism for
, ,,,,	Ryan PR, Raman H, Gupta S, Horst WJ, Delhaize E. 2009. A second mechanism for aluminum resistance in wheat relies on the constitutive efflux of citrate from roots
550	Ryan PR, Raman H, Gupta S, Horst WJ, Delhaize E. 2009. A second mechanism for aluminum resistance in wheat relies on the constitutive efflux of citrate from roots. <i>Plant Physiology</i> 149 : 340-351.
538	 Ryan PR, Raman H, Gupta S, Horst WJ, Delhaize E. 2009. A second mechanism for aluminum resistance in wheat relies on the constitutive efflux of citrate from roots. <i>Plant Physiology</i> 149: 340-351. Ryan PR, Tverman SD, Sasaki T, Furuichi T, Yamamoto Y, Zhang WH, Delhaize E.
538	 Ryan PR, Raman H, Gupta S, Horst WJ, Delhaize E. 2009. A second mechanism for aluminum resistance in wheat relies on the constitutive efflux of citrate from roots. <i>Plant Physiology</i> 149: 340-351. Ryan PR, Tyerman SD, Sasaki T, Furuichi T, Yamamoto Y, Zhang WH, Delhaize E. 2011. The identification of aluminium-resistance genes provides opportunities for
538 540	 Ryan PR, Raman H, Gupta S, Horst WJ, Delhaize E. 2009. A second mechanism for aluminum resistance in wheat relies on the constitutive efflux of citrate from roots. <i>Plant Physiology</i> 149: 340-351. Ryan PR, Tyerman SD, Sasaki T, Furuichi T, Yamamoto Y, Zhang WH, Delhaize E. 2011. The identification of aluminium-resistance genes provides opportunities for enhancing crop production on acid soils. <i>Journal of Experimental Botany</i> 62: 9-20.
538 540	 Ryan PR, Raman H, Gupta S, Horst WJ, Delhaize E. 2009. A second mechanism for aluminum resistance in wheat relies on the constitutive efflux of citrate from roots. <i>Plant Physiology</i> 149: 340-351. Ryan PR, Tyerman SD, Sasaki T, Furuichi T, Yamamoto Y, Zhang WH, Delhaize E. 2011. The identification of aluminium-resistance genes provides opportunities for enhancing crop production on acid soils. <i>Journal of Experimental Botany</i> 62: 9-20. Ryan PR, James RA, Weligama C, Delhaize E, Rattey A, Lewis DC, Bovill WD,
530538540542	 Ryan PR, Raman H, Gupta S, Horst WJ, Delhaize E. 2009. A second mechanism for aluminum resistance in wheat relies on the constitutive efflux of citrate from roots. <i>Plant Physiology</i> 149: 340-351. Ryan PR, Tyerman SD, Sasaki T, Furuichi T, Yamamoto Y, Zhang WH, Delhaize E. 2011. The identification of aluminium-resistance genes provides opportunities for enhancing crop production on acid soils. <i>Journal of Experimental Botany</i> 62: 9-20. Ryan PR, James RA, Weligama C, Delhaize E, Rattey A, Lewis DC, Bovill WD, McDonald G, Rathjen TM, Wang E, Fettell NA and Richardson AE. 2014. Can
538540542	 Ryan PR, Raman H, Gupta S, Horst WJ, Delhaize E. 2009. A second mechanism for aluminum resistance in wheat relies on the constitutive efflux of citrate from roots. <i>Plant Physiology</i> 149: 340-351. Ryan PR, Tyerman SD, Sasaki T, Furuichi T, Yamamoto Y, Zhang WH, Delhaize E. 2011. The identification of aluminium-resistance genes provides opportunities for enhancing crop production on acid soils. <i>Journal of Experimental Botany</i> 62: 9-20. Ryan PR, James RA, Weligama C, Delhaize E, Rattey A, Lewis DC, Bovill WD, McDonald G, Rathjen TM, Wang E, Fettell NA and Richardson AE. 2014. Can citrate efflux from roots improve phosphorus uptake by plants? Testing the hypothesis

	Tang C, Diatloff E, Rengel Z, McGann B. 2001. Growth response to subsurface soil acidity
546	of wheat genotypes differing in aluminium tolerance. Plant and Soil 236: 1-10.
	Tang C, Rengel Z, Abrecht D, Tennant D. 2002. Aluminium-tolerant wheat uses more
548	water and yields higher than aluminium-sensitive one on a sandy soil with subsurface
	acidity. Field Crops Research 78: 93-103.
550	Taub DR, Miller B, Allen H. 2008. Effects of elevated CO ₂ on the protein concentration of
	food crops: a meta-analysis. Global Change Biology 14: 565-575.
552	Tausz-Posch S, Dempsey RW, Seneweera S, Norton RM, Fitzgerald G, Tausz M. 2015.
	Does a freely tillering wheat cultivar benefit more from elevated CO ₂ than a restricted
554	tillering cultivar in a water-limited environment? <i>European Journal of Agronomy</i> 64:
	21-28.
556	Wang J, Wang C, Chen N, Xiong Z, Wolfe D, Zou J. 2015. Response of rice production to
	elevated [CO ₂] and its interaction with rising temperature or nitrogen supply: a meta-
558	analysis. <i>Climate Change</i> 130 : 529-543.
	Wang L, Feng Z, Schjoerring JK. 2013. Effects of elevated atmospheric CO ₂ on
560	physiology and yield of wheat (Triticum aestivum L.): A meta-analytic test of current
	hypotheses. Agriculture, Ecosystems and Environment 178: 57-63.
562	Watt M, Magee LJ, McCully ME. 2008. Types, structure and potential for axial water flow
	in the deepest roots of field-grown cereals. New Phytologists 178: 135-146.
564	Wu H, Song Z, Wang X, Liu Z, Tang S. 2016. Increasing CO ₂ differentially affects
	essential and non-essential amino acid concentration of rice grains grown in
566	cadmium-contaminated soils. Environmental Pollution 216: 86-94.

CaCl₂-Collection pН Total C Total N Olsen-P[‡] Colwell-P PBI extractable Al† Soil (CaCl₂) (g kg⁻¹) $(mg kg^{-1}) (mg kg^{-1})$ (mg P kg⁻¹) sites $(g kg^{-1})$ (mg kg⁻¹) 37.462S, 4.12 38.8 739 Dermosol 1.74 54.3 2.4 3.2 145.263E 37.474S, Ferrosol 4.55 41.9 1.92 21.6 1.2 1.8 1095 145.257E

Table 1. Sampling location and chemical properties of two experimental soils used in this study.

569 [†]. Soil extracted with 0.01 M CaCl₂ were measured by ICP-AES.

570 ‡. Measurements of Olsen-P, Colwell-P and PBI (phosphorus buffer index) according to Rayment and

571 Lyons (2011).

568



Figure 1. Grain yield of six wheat lines grown in an acid soil under two CO₂ concentrations (aCO₂, 400 µmol mol⁻¹ and eCO₂, 550 µmol mol⁻¹). NS, *, and *** denote the levels of significance at p > 0.1, p < 0.05, and p < 0.001 between CO₂ levels of a single line or within a near-isogenic pair using a *t*-test. Means are presented and an LSD bar is also presented to compare any two means at $p \le 0.05$ within a panel.



Figure 2. Root biomass (a) and shoot biomass (b) and total tiller number (c) of six wheat lines grown to maturity in an acid soil under two CO₂ concentrations (aCO₂, 400 µmol mol⁻¹ and eCO₂, 550 µmol mol⁻¹). NS, *, and *** denote the levels of significance at p>0.1, p<0.05, and p<0.001 between CO₂ levels of a single line or within a near-isogenic pair using a *t*-test. Means are presented and an LSD bar is also presented to compare any two means at $p\leq0.05$ within a panel.



Figure 3. Total root length (a), fine root length (diameter <0.3 mm) (b) and coarse root length (diameter >0.3 mm) (c) of six wheat lines grown in an acid soil in the whole columns under two CO₂ concentrations (aCO₂, 400 µmol mol⁻¹ and eCO₂, 550 µmol mol⁻¹). NS, *, ** and *** denote the levels of significance at p>0.1, p<0.05, p<0.01 and p<0.001 between CO₂ levels of a single line or within a near-isogenic pair using a *t*-test. Means are shown and an LSD bar is also presented to compare any two means at $p\leq0.05$ within a panel.



Figure 4. Relative root length (a), relative root biomass (b), relative grain yield (c) and relative total shoot biomass (d) (as % of the limed soil) of ES8 and ET8 grown in an acid soil with or without lime application under two CO₂ concentrations (aCO₂, 400 µmol mol⁻¹ and eCO₂, 550 µmol mol⁻¹). NS and * denote the levels of significance at p>0.1 and p<0.05 between CO₂ levels of a single line using a *t*-test. Data are means ± se. The genotypic variation was significant on all these parameters (p<0.001).

Supplementary information

Elevated CO₂ (FACE) increases grain yield of aluminium-resistant but not aluminium-sensitive wheat (*Triticum aestivum* L.) grown in an acid soil

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	Harves	t index		Root/sh	noot		Compo	onents of y	vield						
Wheat lines	That ves	n maex		1000/31	1001		Ears (n	o. plant ⁻¹)		Kernel	s (no. ear	¹)	Thousa	ınd grair	n weight (g)
	aCO ₂	eCO ₂	Means	aCO ₂	eCO ₂	Means	aCO ₂	eCO ₂	Means	aCO ₂	eCO ₂	Means	aCO ₂	eCO ₂	Means
ES8	0.33	0.30	0.31	0.136	0.119	0.128	2.38	2.44	2.41	23.5	21.2	22.4	30.4	31.5	31.0
ET8	0.28	0.34	0.31	0.151	0.100	0.126	3.88	4.56	4.22	24.6	26.7	25.7	39.5	40.3	39.9
Egret	0.41	0.38	0.40	0.083	0.065	0.074	2.00	2.25	2.13	34.4	27.4	30.9	31.9	31.3	31.6
Egret TaMATE1B	0.43	0.40	0.41	0.088	0.083	0.086	2.50	3.19	2.85	36.9	36.8	36.9	41.3	40.4	40.9
EGA-Burke	0.44	0.47	0.45	0.111	0.087	0.099	3.75	4.58	4.17	39.0	41.6	40.3	39.4	38.8	39.1
EGA-Burke TaMATE1B	0.43	0.43	0.43	0.091	0.095	0.093	3.81	4.25	4.03	34.6	38.6	36.6	41.6	40.8	41.2
Means	0.39	0.39		0.110	0.092		3.05	3.55		32.2	32.1		37.4	37.2	
<i>p</i> -value (LSD, $p=0.03$	5)														
CO_2	0.742			0.015 (0.011)		0.016 ((0.27)		0.766			0.778		
Line	< 0.001	(0.05)		< 0.001	(0.015)		< 0.001	(0.66)		< 0.001	(6.0)		<0001	1 (2.7)	
$CO_2 \times Line$	0.288			0.019 (0.022)		0.855			0.499			0.961		

Table S1. Harvest index, root-to-shoot biomass ratio (root/shoot), and the yield components of six wheat lines grown to maturity in an acid soil under two CO₂ concentrations (aCO₂, 400 μ mol mol⁻¹ and eCO₂, 550 μ mol mol⁻¹).

Least significant difference tests (LSD) values are presented in parentheses where a main effect or an interaction is significant at $p \le 0.05$.

Table S2. Shoot biomass at Days 67 and 85 and maturity, tiller number at Days 97 and 107 and maturity, and growth parameters (root biomass, root length, root-to-shoot biomass ratio (root/shoot) and grain yield at maturity of two wheat lines (ES8 and ET8) grown in an acid soil with or without lime under two CO₂ concentrations (aCO₂, 400 μ mol mol⁻¹ and eCO₂, 550 μ mol mol⁻¹).

Linaa	Lima	CO	Shoot bioma	ass (g plant ⁻	¹)	Tiller numbe	r (no. plant ⁻¹	¹)	Root	Total root	Root/	Grain
Lines	Linte	CO_2	Day 67	Day 85	Maturity	Day 97	Day 107	Maturity	(g plant ⁻¹)	(m plant ⁻¹)	shoot	(g plant ⁻¹)
ES8	+	aCO ₂	0.181	0.726	11.0	6.75	7.75	5.8	1.73	223	0.110	4.8
		eCO ₂	0.207	0.763	14.2	8.44	8.81	7.3	1.96	272	0.101	5.7
ET8	+	aCO_2	0.206	0.679	11.5	6.81	7.94	6.3	1.80	214	0.106	5.6
		eCO ₂	0.219	0.727	15.9	8.88	10.31	8.9	1.94	266	0.096	6.3
ES8	-	aCO_2	0.086	0.159	3.4	3.13	3.88	2.8	0.69	43	0.136	1.7
		eCO ₂	0.074	0.161	3.7	3.25	4.19	2.9	0.62	42	0.119	1.5
ET8	-	aCO_2	0.088	0.170	9.6	4.63	6.69	5.1	1.99	165	0.151	3.7
		eCO ₂	0.111	0.182	9.6	3.94	5.88	5.1	1.46	140	0.100	4.9
<i>p</i> -value												
$\rm CO_2$			0.790	0.721	0.021	0.161	0.184	0.019	0.592	0.361	0.003	0.300
Line			< 0.001	0.232	< 0.001	0.063	< 0.001	< 0.001	< 0.001	< 0.001	0.562	< 0.001
Lime			< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
$CO_2 \times I$	Line		0.270	0.933	0.550	0.417	0.903	0.228	0.037	0.657	0.135	0.179
$CO_2 \times I$	Lime		0.301	0.690	< 0.001	0.013	0.019	< 0.001	0.001	0.036	0.039	0.492
Line×	Lime		0.708	0.470	< 0.001	0.105	0.081	0.006	< 0.001	< 0.001	0.764	0.009
$CO_2 \times I$	Line× Lime		0.092	0.942	0.389	0.178	0.127	0.136	0.151	0.481	0.150	0.235

Genotypes $P(mg g^{-1})$ $Ca (mg g^{-1})$ $Mg (mg g^{-1})$ $S (mg g^{-1})$ Fe ($\mu g g^{-1}$) $Mn (\mu g g^{-1})$ $Zn (\mu g g^{-1})$ aCO₂ eCO_2 aCO_2 eCO_2 eCO_2 eCO₂ aCO_2 eCO₂ aCO_2 aCO_2 aCO_2 eCO_2 aCO_2 eCO_2 Day 67 ES8 1.10 0.89 1.87 0.94 2.03 55 1.51 1.23 1.59 151 141 73 69 61 ET8 79 1.31 1.35 2.05 2.01 1.31 1.27 1.88 2.29 160 230 127 70 102 1.07 1.00 1.32 1.25 0.97 0.90 149 142 68 64 69 63 Egret 1.46 1.71 Egret TaMATE1B 1.97 1.58 0.99 2.30 2.33 175 85 68 1.74 1.58 1.09 213 131 112 EGA-Burke 1.79 1.59 1.78 1.59 1.36 1.15 2.13 2.33 186 177 144 119 83 57 EGA-Burke TaMATE1B 1.59 1.11 2.27 3.30 132 119 104 60 1.67 1.51 1.21 1.13 190 161 *p*-value (LSD, *p*=0.05) 0.611 0.015 (0.09) 0.215 0.206 CO_2 0.009 (0.11) 0.832 0.015 (12) Genotype < 0.001(0.33) < 0.001(0.19) 0.005 (0.16) 0.336 0.398 < 0.001 (25) 0.445 $CO_2 \times Genotype$ 0.716 0.288 0.474 0.948 0.544 0.360 0.150 Day 85 ES8 1.28 1.88 1.06 0.76 2.61 1.94 182 30 41 1.67 1.31 126 79 49 44 ET8 1.72 1.66 1.95 1.88 1.09 0.98 3.28 2.62 163 227 121 114 36 Egret 2.41 1.76 1.54 1.27 0.94 0.81 2.73 2.36 162 111 86 34 47 65 Egret TaMATE1B 1.95 2.19 1.51 1.35 0.82 2.82 2.86 168 120 113 97 33 39 1.00 EGA-Burke 2.65 2.62 1.96 1.55 1.05 3.18 2.84 178 143 123 34 46 1.36 116 EGA-Burke TaMATE1B 142 35 57 3.54 2.28 1.92 0.99 1.34 0.75 4.07 2.73 158 101 96 *p*-value (LSD, *p*=0.05) CO_2 0.028 (0.44) 0.011 (0.29) 0.015 (0.21) 0.018 (28) 0.036 (22) < 0.001 (4) 0.011 (0.40) 0.015 (0.76) 0.394 0.057 0.122 0.011(37) 0.074 0.250 Genotype $CO_2 \times Genotype$ 0.313 0.506 0.723 0.433 0.098 0.906 0.237

Table S3. Concentration of nutrients in shoots of plants (67 d and 85 d from sowing) of six wheat lines grown in an acid soil under two CO_2 concentrations (a CO_2 , 400 µmol mol⁻¹ and e CO_2 , 550 µmol mol⁻¹).

Least significant difference tests (LSD) values are presented in parentheses where a main effect or an interaction is significant at $p \le 0.05$.

Table S4. Net photosynthetic rates, stomatal conductance, instantaneous transpiration efficiency of the flag leaf at the flowering stage, and water input and water-use efficiency of six wheat lines grown in an acid soil under two CO_2 concentrations (a CO_2 , 400 µmol mol⁻¹ and e CO_2 , 550 µmol mol⁻¹).

Lines	Net photosynthetic rates (µmol m ⁻² s ⁻¹)			Stomatal conductance (mol m ⁻² s ⁻¹)			Instantaneous transpiration efficiency (µmol mmol ⁻¹)			Water input† (mm column ⁻¹)			Water-use efficiency (mg g ⁻¹)		
	aCO ₂	eCO ₂	Means	aCO ₂	eCO ₂	Means	aCO ₂	eCO ₂	Means	aCO_2	eCO ₂	Means	aCO ₂	eCO ₂	Means
ES8	8.2	8.7	8.5	0.149	0.188	0.169	2.60	2.10	2.35	138	129	134	3.23	3.33	3.28
ET8	14.8	13.8	14.3	0.198	0.172	0.185	3.57	3.03	3.30	648	583	615	3.76	4.25	4.01
Egret	9.2	6.8	8.0	0.165	0.120	0.143	3.72	2.50	3.11	127	120	124	3.26	3.10	3.18
Egret TaMATE1B	13.1	14.5	13.8	0.171	0.179	0.175	4.31	3.95	4.13	435	409	420	3.20	4.18	3.69
EGA-Burke	19.5	17.5	18.5	0.311	0.236	0.274	6.18	5.38	5.78	644	595	618	3.48	4.17	3.83
EGA-Burke TaMATE1B	17.9	18.5	18.2	0.246	0.272	0.259	6.59	6.21	6.40	678	579	627	3.37	4.44	3.91
Means	13.8	13.3		0.207	0.195		4.50	3.86		444	402		3.38	3.96	
<i>p</i> -value (LSD, <i>p</i> =0.05	5)														
CO_2	0.636			0.725			0.363			0.031 (25)		0.039 (0.42)	
Line	< 0.001	(1.5)		< 0.001	(0.032)		< 0.001	(0.47)		< 0.001	(29)		0.006 (0.50)	
$CO_2 \times Line$	0.048 (2	2.3)		0.004 (0.061)		0.424			0.021 (43)			0.079		

Least significant difference tests (LSD) values are presented in parentheses where a main effect or an interaction is significant at $p \le 0.05$.

† The total water input from Day 144 to maturity was calculated as a combination of water additions plus rainfall and weight loss of the column.

	Shoot bio	mass (g p	olant ⁻¹)				Tiller n	umber (no	. plant ⁻¹)			
Lines	Day 67			Day 85			Day 97			Day 107		
	aCO ₂	eCO ₂	Means	aCO ₂	eCO ₂	Means	aCO ₂	eCO ₂	Means	aCO ₂	eCO ₂	Means
ES8	0.086	0.074	0.080	0.159	0.161	0.160	2.50	3.25	2.88	3.88	4.19	4.04
ET8	0.088	0.111	0.099	0.170	0.182	0.176	4.63	3.94	4.29	6.69	5.88	6.29
Egret	0.103	0.082	0.093	0.242	0.185	0.214	2.81	1.75	2.28	2.75	2.44	2.60
Egret TaMATE1B	0.106	0.110	0.108	0.348	0.353	0.351	2.75	4.06	3.41	3.31	4.25	3.78
EGA-Burke	0.114	0.112	0.113	0.366	0.421	0.394	4.00	4.50	4.25	4.94	4.63	4.79
EGA-Burke TaMATE1B	0.104	0.190	0.147	0.387	0.540	0.464	3.42	4.94	4.18	4.33	5.88	5.11
Means	0.100	1.114		0.279	0.307		3.35	3.74		4.32	4.55	
<i>p</i> -value (LSD, <i>p</i> =0.05)												
CO_2	0.667			0.178			0.072			0.329		
Line	<0.001 (0	.020)		<0.001 (0).052)		<0.001((0.80)		<0.001 (0	.95)	
$CO_2 \times Line$	0.001 (0.0)33)		0.004 (0.	076)		0.013 (1	1.12)		0.061		

Table S5. Shoot biomass (Days 67 and 85) and tiller number (Days 97 and 107) of six wheat lines grown in an acid soil at various growth stages under two CO₂ concentrations (aCO₂, 400 μ mol mol⁻¹ and eCO₂, 550 μ mol mol⁻¹).

Least significant difference (LSD) values are presented in parentheses where a main effect or an interaction is significant at $p \le 0.05$.

Lines	Protein (mg g ⁻¹)		P (mg g ⁻¹)		Ca (mg g ⁻¹)		Mg (mg g^{-1})		S (mg g ⁻¹)		Fe (µg g ⁻¹)		Mn (µg g ⁻¹)		Zn (µg g ⁻¹)	
	aCO ₂	eCO ₂	aCO ₂	eCO ₂	aCO ₂	eCO ₂	aCO ₂	eCO ₂	aCO ₂	eCO ₂	aCO ₂	eCO ₂	aCO ₂	eCO ₂	aCO ₂	eCO ₂
ES8	202	195	1.91	2.04	0.69	0.68	1.43	1.43	1.99	2.11	20.9	23.2	62	57	42	46
ET8	183	175	2.61	2.70	0.50	0.45	1.51	1.49	2.02	2.08	29.2	27.5	103	109	54	54
Egret	193	190	1.58	1.94	0.58	0.61	1.37	1.34	1.88	1.81	21.9	21.4	51	53	31	34
Egret TaMATE1B	167	161	2.18	2.18	0.47	0.38	1.35	1.29	1.93	1.79	23.0	21.6	70	75	40	41
EGA-Burke	143	128	2.41	2.23	0.33	0.31	1.21	1.13	1.66	1.60	27.3	25.9	88	83	43	39
EGA-Burke	160	141	2.52	2.30	0.31	0.29	1.25	1.19	1.86	1.71	30.5	29.6	89	78	52	43
<i>p</i> -value (LSD, $p=0.05$)																
CO ₂	0.114		0.506		0.231		0.123		0.684		0.916		0.47 4		0.712	
Line	< 0.001	(16)	0.004 (0.38)		< 0.001 (0.06)		< 0.001 (0.11)		<0.001 (0.16)		<0.001 (4.4)		< 0.001 (9)		<0.001 (8)	
$CO_2 \times Line$	$CO_2 \times Line \qquad 0.596$		0.904	0.374		0.994		0.475		0.949		0.346		0.578		

Table S6. Concentrations of protein and nutrients in grains of six wheat lines grown in an acid soil under two CO₂ concentrations (aCO₂, 400 μ mol mol⁻¹ and eCO₂, 550 μ mol mol⁻¹).

Least significant difference tests (LSD) values are presented in parentheses where a main effect or an interaction is significant at $p \le 0.05$.



Figure S1. Rainfall, daily solar radiation, daily maximum temperature (Tmax) and daily minimum temperature (Tmin) over the experimental period. Data were obtained from Horsham Aerodrome Climatological Station, Bureau of Meteorology 10 km from the experimental site.



Figure S2. Water content (average of the entire soil column) of the acid soils in which six wheat lines were grown in an acid soil under two CO₂ concentrations (aCO₂, 400 μ mol mol⁻¹ and eCO₂, 550 μ mol mol⁻¹) from Day 138 to Day 192 of growth. Vertical error bars denote the least significant difference (*p*=0.05) values to compared any two means among treatments at individual times. The thin arrows indicate the times for watering events and the thick arrows indicate the time to start flowering for each pair.