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Yield responses of wild C_3 and C_4 crop progenitors to sub-ambient CO_2: A test for the role of CO_2 limitation in the origin of agriculture.

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Abstract

Limitation of plant productivity by the low partial pressure of atmospheric CO_2 (C_a) experienced during the last glacial period is hypothesised to have been an important constraint on the origins of agriculture. In support of this hypothesis, previous work has shown that glacial C_a limits vegetative growth in the wild progenitors of both C_3 and C_4 founder crops. Here we present data showing that glacial C_a also reduces grain yield in both crop types. We grew four wild progenitors of C_3 (einkorn wheat and barley) and C_4 crops (foxtail and broomcorn millets) at glacial and post-glacial C_a, measuring grain yield, and the morphological and physiological components contributing to these yield changes. The C_3 species showed a significant increase in unthreshed grain yield of ~50% with the increase in C_a, which matched the stimulation of photosynthesis, suggesting that increases in photosynthesis are directly translated into yield at sub-ambient levels of C_a. Increased yield was controlled by a higher rate of tillering, leading to a larger number of tillers bearing fertile spikes, and increases in seed number and size. The C_4 species showed smaller, but significant, increases in grain yield of 10-15%, arising from larger seed numbers and sizes. Photosynthesis was enhanced by C_a in only one C_4 species and the effect diminished during development, suggesting that an indirect mechanism mediated by plant water relations could also be playing a role in the yield increase. Interestingly, the C_4 species at glacial C_a showed some evidence that photosynthetic capacity was upregulated to enhance carbon capture. Development under glacial C_a also impacted negatively on the subsequent germination and viability of seeds. These results suggest that the grain production of both C_3 and C_4 crop progenitors was limited by the atmospheric conditions of the last glacial period, with important implications for the origins of agriculture.

Introduction

Cultivation of wild grasses and grain legumes by people began ~12,000 years ago in Southwest Asia (Meyer & Purugganan, 2013; Willcox *et al.*, 2008), leading to the evolution of domesticated cereals and pulses dependent on humans for their dispersal (Harlan, 1992). Within five millennia, cultivation leading to domestication had occurred in multiple independent regions in the both the Old and New World (Blumler, 1996; Fuller *et al.*, 2012; Larson *et al.*, 2014; Piperno, 2006; Piperno, 2011; Purugganan & Fuller, 2009). Numerous lines of evidence now suggest a prolonged period during which certain species were cultivated but had not yet acquired the morphological changes associated with domestication (Colledge, 1998; Fuller, 2007; Larson *et al.*, 2014; Piperno, 2006; Piperno & Dillehay, 2008; Purugganan & Fuller, 2011; Weiss *et al.*, 2006; Willcox, 2005; Willcox *et al.*, 2008). Debate in this area currently centres around whether the process of domestication was an unconscious product of cultivation and harvesting (Darwin, 1875; Purugganan & Fuller, 2011; Rindos, 1984) or the result of deliberate breeding by early farmers (Abbo *et al.*, 2011; 2014a; 2014b).

Plant cultivation may be practiced by hunter-gatherer communities, and only becomes agriculture when it forms the basis of subsistence economies (Harlan, 1992). Why people made this transition during the Neolithic remains one of the most important questions in archaeology. Numerous explanations have been suggested, including demographic (Cohen, 1977; 2009) and social pressures (Bender, 1978), and environmental change (Bettinger *et al.*, 2009; Byrne, 1987; Richerson *et al.*, 2001). However, none can account for the synchronicity in the start of agriculture in distinct regions across the globe. Sage (1995) proposed a global factor, hypothesising that low partial pressures of atmospheric CO_2 (C_a) during the last glacial period may have been insufficient to support the level of plant productivity required for the successful establishment of agriculture. However, deglaciation at the end of the Pleistocene period was coupled to a rapid rise in atmospheric C_a from below 18 Pa to 27 Pa between 15,000 and 12,000 years ago (Jouzel *et al.*, 1993; Petit *et al.*, 1999), and soon afterwards plant cultivation began.

Previous experiments have shown that the wild progenitors of the primary C_3 and C_4 cereals from different continental centres where agriculture originated independently exhibited significant increases in vegetative biomass with an increase in C_a equivalent to the end glacial change (Cunniff *et al.*, 2008). Biomass of a single C_3 species in these experiments nearly doubled, whilst the C_4 species showed a smaller, but significant, increase of up to 40%. This increase in the C_4 species is unexpected, since plants using C_4 photosynthesis are not expected to respond to C_a because a series of biochemical and structural modifications raise CO_2 at the sites of carbon fixation to three to ten times that of ambient air (Hatch, 1987). These increases in biomass, particularly for the C_3 species, are caused by a direct effect of low C_a on photosynthesis (A) (Cunniff *et al.*, 2008), whilst for the C_4 species, indirect effects of C_a mediated via improved water relations may be more important (Cunniff *et al.*, 2016).

Previous research has shown that C_3 plants show significant yield enhancements with an increase in the level of atmospheric CO_2. Studies considering rising atmospheric C_a over the coming 50 years have shown that yields could increase as much as 35%, or as little as 13%, in modern C_3 crop cultivars grown with ample water and nutrients (Ainsworth & Long, 2005; Kimball, 1983; Kimball *et al.*, 2002; Long *et al.*, 2006), whilst grain increases in C_4 species only occur when plants experience intermittent water deficits (Leakey, 2009; Leakey *et al.*, 2006; Manderscheid *et al.*, 2014; Ottman *et al.*, 2001).

The grain yields of modern C_3 crop cultivars are significantly more sensitive to variation in sub-ambient than elevated C_a (Allen *et al.*, 1991; Baker *et al.*, 1990; Campbell *et al.*, 2005; Gifford, 1977; Mayeux *et al.*, 1997), and there is recent evidence that yields of C_4 species are also substantially reduced by glacial levels of C_a (Piperno *et al.*, 2015). Mayeux *et al.* (1997) found that a rise in C_a from 20 to 35 Pa led to a 200% increase in grain yield in a modern spring wheat cultivar, and that even a 27% increase in C_a from pre-industrial levels of 28 Pa to near-ambient levels of 35 Pa increased yields by 55%. Yield increases in the sub-ambient C_a range are controlled by a variety of components including: a greater number of seeds per spike (Mayeux *et al.*, 1997), increased tiller production (Gifford, 1977; Sionit *et al.*, 1981; Wand *et al.*, 1999), greater seed number and mass (Campbell *et al.*, 2005; Sionit *et al.*, 1981) and a greater percentage of filled spikelets (Ziska *et al.*, 1997).

Long-term exposure to sub-ambient C_a can also impact on plant fitness. Growth at very low C_a levels of 15 Pa led to seed abortion in *Abutilon theophrasti* (Dippery *et al.*, 1995), and at 20 Pa fitness (survival and seed production) was significantly reduced in *Arabidopsis thaliana* (Ward & Kelly, 2004; Ward & Strain, 1997). Furthermore, the germination rate of seeds developed under low C_a can be reduced (Campbell *et al.*, 2005), leading to intergenerational effects.

Physiological regulation may go some way towards offsetting the limiting effects of sub-ambient C_a. For example, increasing stomatal conductance (g_s) improves CO_2 supply, and up-regulation of photosynthetic enzymes improves CO_2-capture (Anderson *et al.*, 2001; Gesch *et al.*, 2000; Sage & Reid, 1992). However, such acclimation is less frequent in C_4 species, since their carbon concentrating mechanism makes photosynthesis less limited by

CO_2 supply, although small changes in leaf nitrogen, g_s and photosynthetic enzymes have been reported in some species (Anderson *et al.*, 2001; Pinto *et al.*, 2014; Ripley *et al.*, 2013).

Following on from our previous experiments on vegetative biomass reported in Cunniff *et al.* (2008), here we measure the yield and physiological responses of the modern day representatives of two wild C_3 and C_4 crop progenitors to glacial and post glacial C_a. The C_3 crop progenitors are from the Southwest Asia centre of domestication and the C_4 crop progenitors are from the North China domestication centre. We aimed to test the hypotheses that: (i) a rise in C_a equivalent to the end glacial change increases grain yield in the wild progenitors of both C_3 and C_4 progenitors, but the yield enhancement is greater in the C_3 species; (ii) yield increases are controlled by several components e.g. increased tiller production, seed size and number; (iii) in C_3 species, the increased yield is due to direct effects on photosynthesis; (iv) acclimation (if it occurs) is greater in C_3 than C_4 species; and (v) grains developed under low C_a have reduced germination potential.

Materials and methods

Plant material and growth conditions

The C_a treatments were applied in controlled environment (CE) chambers (Conviron BDR16, Conviron, Winnipeg, Manitoba, Canada) at two levels throughout the full period of plant growth: glacial (18 Pa) and postglacial (27 Pa). The chambers were operated in a closed configuration, by connecting the outlet vent to the air inlet via a filter packed with a layer of activated charcoal and a layer of sodalime (Sofnolime 1.0 - 2.5 mm granules, Molecular Products Ltd, Mill End, Essex). Activated charcoal was employed to filter the air and remove any trace gases such as ethylene which could be emitted by plants or soil, and have the potential to affect plant development. The C_a in each chamber was controlled using a CO_2 sensor (CARBOCAP® Carbon Dioxide Probe GMP343, Vaisala, Finland) that was linked to a feedback system regulating the circulation of chamber air through the soda-lime scrubber. C_a was recorded every minute, giving overall mean values over the full growth period of 18.2 Pa (±SD 0.48) and 27.1 Pa (± SD 0.49). To maintain this tight control, the soda lime was changed as soon as C_a started to drift above the target level, which was approximately every four weeks. Treatment and plants were exchanged between the two controlled environment chambers every week from germination to harvest to help minimise the confounding effect of the chamber on the growth environment.

Seeds of the wild progenitors of C_3 and C_4 crops were obtained from germplasm holdings or commercial sources. They included two C_4 species, *Setaria viridis* (L.) P. Beauv (Herbiseed, Twyford, UK. Cat no. 9602) and *Panicum miliaceum* var. *ruderale* (Kitag.) (Herbiseed, Cat no. 9507) from North China, and two C_3 species, *Hordeum spontaneum* K. Koch (Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany, Accession number: HOR 13798) and *Triticum boeoticum* Boiss. (IPK, Accession number: TRI 17093) from Southwest Asia. All were important as founder crops in the two regions, upon which Neolithic agricultural economies were first established (Evans, 1993; Zohary *et al.*, 2012). Batches of ~30 seeds of each species were sown into trays containing a 1:1 sand: John Innes no. 2 compost (7 parts loam, 3 parts peat, 2 parts sand, 20:10:10 N:P:K) mix (Ivandic *et al.*, 2000). This mix was chosen in an attempt to replicate an unimproved soil.

Seeds were germinated at 20/10 °C (day/night) for the C_3 species and 30/25 °C for the C_4 species, with an 8 hour photoperiod and a photosynthetic photon flux density (PPFD) of 300 μmol photons m^{-2} s^{-1} in the CE chambers. Once established, sixteen seedlings of a similar size for each species were selected and planted into 10 litre containers (0.16×0.2×0.35m, Wilkinson Hardware Store Limited, Manton Wood, Worksop, UK) containing the same growth medium. For the C_3 species, an 8-week vernalization period was then imposed to enable flowering. The same CE chambers were used, except that conditions were set at 4 °C (day and night) with PPFD and photoperiod as during germination. After vernalization of the C_3 species, temperature was returned to 20/10 °C (day/night), photoperiod to 16 hours and PPFD to a maximum level of 650 µmol photons $m^{-2}s^{-1}$ measured at canopy height. Vapour pressure deficit (VPD) had a minimum value of 0.2 kPa at night and a maximum value of 0.7 kPa during the day until grain filling, when it was increased to 0.5 kPa at night and 0.9 kPa during the day to facilitate maturation. The C_4 species were grown at 30/25 °C (day/night), with a 14 hour photoperiod, and PPFD of 622 µmol photons $m^{-2}s^{-1}$ measured at canopy height. VPD had a minimum value of 0.7 kPa at night and a maximum value of 0.9 kPa during the day until grain filling when it was increased to 1 kPa at night and 1.3 kPa during the day to facilitate ripening. For both the C_3 and C_4 species the total integrated daily photon flux is equivalent to averages for the growing seasons for these crops in Southwest Asia and Northern China, although the daily maximum was lower than that experienced under clear skies in the field (Duzen & Aydin, 2012; Wang *et al.*, 2013). Plants were watered 3-4 times per week in the early stages of the experiment; but this was reduced at grain filling as the water demands of the plants decreased. When grain filling was complete and senescence had commenced, watering was terminated to promote drying of the plant material. Plants were fertilised with granular fertiliser (Osmocote exact standard 1:1:3 N:P:K) after two months of growth. The chambers were of adequate internal height for all of the plant species tested to grow to maturity without significant shading.

Gas exchange measurements

CO_2 and H_2O exchange were measured at three stages during the experiment; tillering, flowering and grain filling, using a portable open gas exchange system (LI-6400P, LI-COR Biosciences, Lincoln, Nebraska, USA), for the youngest fully expanded leaf. PPFD within the chamber was set to 1800 µmol $m^{-2}s^{-1}$ for the C_3 species and 2000 µmol $m^{-2}s^{-1}$ for the C_4 species (6400-02B LED Light Source chamber, LICOR), since preliminary measurements of the photosynthetic response to light showed that these saturated photosynthesis (A_{sat}). Leaf temperature was matched to the growth environment; 20 °C for the C_3 species and 30 °C for the C_4 species throughout all of the measurements, and incoming air was maintained at a constant humidity to keep the leaf-air vapor pressure deficit at less than 1 kPa. Gas exchange parameters were calculated using the equations of von Caemmerer and Farquhar (1981) and photosynthetic responses to variation in intercellular CO_2 (A/C_i curves) determined following Ainsworth *et al.* (2002). Photosynthesis was induced under the growth C_a level, either 18 Pa or 27 Pa, until A_{sat} and g_s reached steady state, typically after 20 mins. Values of A_{sat} and C_i were then recorded at C_a of (27, 18), 15, 12, 10, 8, and 5 Pa for the C_3 species and (27, 18), 15, 12, 10, 8, 5 and 2 Pa for the C_4 species, with a time interval of 2-3 mins between C_a levels. The growth C_a (27 or 18 Pa) was then repeated to verify that the original value of A_{sat} could be restored, and then C_a was increased in steps to (18, 27), 38, 45, 65, 80, 100, 130, 160, 180 and 200 Pa. Curves were fitted as described in Cunniff *et al.*(2008). Stomatal limitation (l_s) was calculated following Farquhar & Sharkey(1982) as:

$$l_s = \frac{A_o - A}{A_o} \qquad \text{Equation 1,}$$

where A is the photosynthetic rate at growth C_a, i.e 18 Pa or 27 Pa, and A_o is the photosynthetic rate which would occur as g_s approaches infinity, assuming that C_i is 18 Pa or 27 Pa. Instantaneous WUE was calculated as:

$$\mathrm{WUE} = \frac{A_{sat}}{g_s} \qquad \text{Equation 2.}$$

Components of yield

Due to the shattering mechanism (brittle rachis) employed as a dispersal mechanism by wild cereal inflorescences, each was enclosed in a muslin bag during grain filling to keep seeds with the parent plant. Total yield was determined by a destructive harvest when plants had completely dried. Final plant height, measured from soil level to the collar of the leading spike, and the total number of tillers (fertile and sterile) was recorded. Fertile tillers were classed as those which bore seed heads, whilst sterile tillers as those which had no inflorescence, or an inflorescence with spikelets containing no seeds. The C_3 plants were then divided into leaves, culms, and unthreshed spikelets; the long awns were removed from the spikelets of *H. spontaneum*. The C_4 plants were divided into leaves, culms, inflorescence heads and seeds. The total number of filled and empty spikelets was counted for the C_3 species, but this could not be determined for the C_4 species, as seeds are not retained within the glumes upon shattering. All of the viable spikelets/seeds for each plant were weighed immediately after harvest, then random subsets of 100 spikelets/seeds were weighed separately. Finally, the spikelets/seeds subset, leaves, culms and awns were dried at 70 °C for 7 days, and weighed.

Yield components were calculated, as follows:

$$S = \frac{SF}{(SF_{\mathrm{subset}}/100)} \qquad \text{Equation 3,}$$

$$Y = S \times \left(\frac{SD_{\mathrm{subset}}}{100}\right) \qquad \text{Equation 4,}$$

$$TDM = (LDM + CDM + Y + ADM) \qquad \text{Equation 5,}$$

$$HI = (Y/TDM) \qquad \text{Equation 6,}$$

where *S* is the total number of spikelets/seeds, *SF* is the air-dried fresh mass of all harvested spikelets/seeds at maturity, SF_{subset} is the fresh mass of the 100 spikelets/seeds subset, *Y* is the total yield (g), SD_{subset} is the dry mass of the 100 spikelets/seeds subset, *TDM* the total dry matter (g), *LDM* the leaf dry mass (g), *CDM* the culm dry mass (g), *ADM* the awn dry mass (g), and *HI* the harvest index.

Germination trial

Forty seeds were randomly selected from each plant at the end of the experiment to give a total of 320 seeds from each species and each C_a. Seeds were sown equally between two trays (160 in each) containing the sand:compost mix described previously, returned to the controlled environment chambers and supplied with either the original growth C_a, or the alternative C_a. Carrying out this trial at both C_a levels allowed the direct effects of C_a to be distinguished from the indirect effects mediated via seed quality. Conditions in the CE chambers were the same as described for seed germination at the beginning of the experiment. For ten days, the number of germinated seeds from each species at each C_a was recorded, to determine the rate of germination and viability of seeds which had developed under the two different C_a levels.

Experimental design and statistical analysis

Because only two growth cabinets were available with a C_a scrubbing system, the trials for the C_3 and C_4 species were run in succession to give the desired growth conditions and level of replication. The two C_3 species were used in the first experiment and the two C_4 species in the second, and there were 16 representatives of each species, which gave eight replicates at each C_a level. All yield, phenology and germination measurements included all eight replicates, and six replicates were measured for gas exchange.

All statistical analysis was carried out using the statistical computing package R (version 3.0.1, The R Foundation for Statistical Computing) with $P = 0.05$ as the critical level of significance. In all cases, the results show a minimum adequate model with all non-significant interaction terms eliminated (Crawley, 2005). For the components of yield, statistical significance of the C_a treatments was tested using a linear mixed effects model (lme) (Pinheiro & Bates, 2000), with species as a random effect, and tested for the effects of C_a, photosynthetic type and the interacting effects of C_a and photosynthetic type. Percentage data were arcsine-transformed before analysis. Although photosynthetic type was confounded with experiment (trial one vs. trial two) and growth conditions (photoperiod, temperature and VPD differences between experiments), it was included in the analysis to test the expectation that the yield response to C_a is smaller in C_4 than C_3 species, with the caveat that differences could also arise from environmental and random effects. Student's t-test was also used to test for the effect of C_a on the components of yield for individual species.

For photosynthetic parameters (A, g_s, $V_{c,max}$, J_{max}, k, V_T, l_s, WUE, C_i/C_a), ANOVA (aov) was used to test for the effect of C_a and growth stage and any interaction between the two in each species. Data were transformed if they did not meet the assumptions of ANOVA. The estimable function [(library(gmodels))] was then used to apply a contrast matrix to the data, which computes a significance value between the C_a treatments at each growth stage.

For the germination trials, data during the initial exponential phase of germination were log transformed and initial slopes calculated. Student's t-test was then used to compare the slopes for each species to investigate the effects of C_a on germination rate. To test the effects of C_a on viability, Student's t-test was performed for each species on the percentage of seeds which had germinated at day ten under the two levels of C_a, after arcsine-transformation to ensure a normal distribution of errors.

Results

Components of yield

Yield of the C_3 and C_4 crop progenitors was affected by the C_a during growth (Fig. 1; $F_{1,58}$ = 157.5, P = <.001). Comparing the two photosynthetic types, yield increases were greater in the two C_3 species (CO_2× type: $F_{1,58}$ = 73.1, P = <.001). Between glacial (18 Pa) and postglacial (27 Pa) C_a, the unthreshed yield of *T. boeoticum* increased by 48%, and *H. spontaneum* by 51% (Fig. 1a,b). In comparison, the two C_4 species showed small but still significant increases in seed yield of 10% for *P. miliaceum* and 15% for *S. viridis* (Fig. 1c,d).

In the C_3 crop progenitors, yields were partially influenced by the number of tillers produced by the plant and partially by the percentage which were fertile (Table 1). Between glacial and postglacial C_a, *T. boeoticum* showed a 35% increase in the number of tillers and, although *H. spontaneum* followed the same trend, the increase was not significant. Instead, the percentage of fertile tillers increased between 18 and 27 Pa C_a (Table 1). Neither the number of tillers nor the percentage that were fertile was affected by growth C_a in either of the C_4 crop progenitors.

Seed number responded significantly to growth C_a in both photosynthetic types (Table 1; $F_{1,58}$ = 7.2, P = <.01). In the C_3 species, seed number increased by up to 45% between 18 Pa and 27 Pa C_a. Furthermore, there was a significant effect of growth C_a on the percentage of seeds that were viable (Table 1). In comparison, the two C_4 crop progenitors showed a lesser response of total seed number to C_a (Table 1; CO_2 × type: $F_{1,58}$ = 3.8, P = <.05). For *S.*

viridis the increase was 15%, matching the change in yield, whilst for *P. miliaceum* there was no significant change in seed number (Table 1). For the C_3 progenitors, the disparity between increased yields and seed numbers can be explained by changes in seed size. Both showed a significant increase in seed size between 18 and 27 Pa C_a (Table 1). For the C_4 progenitors, increases in seed size between the two levels of C_a were smaller (CO_2 × type: $F_{1,58} = 13.8$, $P = <.001$), and only significant for *P. miliaceum* (Table 1).

Total dry matter (TDM) responded strongly to growth C_a ($F_{1,58} = 148.1$, $P = <.001$), especially in the C_3 types, increasing by up to 44% in *H. spontaneum* (Table 1). *P. miliaceum* showed a smaller but significant increase in TDM. The ratio of harvested grain to total dry matter was positively affected by growth C_a (Table 1; $F_{1,58} = 5.1$, $P = <.05$). In the C_3 species harvest index increased by a maximum of 11% in *T. boeoticum*, whereas, for the two C_4 progenitors, there was no significant change (Table 1).

Gas exchange

Light-saturated rates of photosynthesis (A_{sat}) were affected by growth C_a at all developmental stages in the C_3 and, to a lesser extent, the C_4 species (Fig. 2a-d; Table 2). For the C_3 species at tillering, A_{sat} was up to 48% greater at 27 Pa compared 18 Pa C_a. Values of A_{sat} declined significantly at both C_a levels as the crops matured, and the decline at C_a 27 Pa was steeper than at 18 Pa, especially between flowering and grain filling (Fig. 2a,b; Table 2). Overall the average difference in A_{sat} between 18 and 27 Pa C_a over the three growth stages was 48%, and 55% for *T. boeoticum* and *H. spontaneum* respectively.

The two C_4 species showed a smaller response of A_{sat} to growth C_a (Fig. 2c,d; Table 2). At tillering in *P. miliaceum*, A_{sat} was 41% greater at 27 Pa compared to 18 Pa C_a. A_{sat} declined from tillering to grain filling, and the decline was greater at 27 Pa (Fig. 2c; Table 2).

Overall, the average C_a-mediated increase in A_{sat} from tillering to grain filling was 26% (Fig. 2c). *S. viridis* showed a markedly different response of A_{sat} to growth C_a (Fig. 2d). At tillering and flowering there was no significant difference in A_{sat} between 18 Pa and 27 Pa C_a. A_{sat} then declined, and the decline was faster at 27 Pa, leading to a significant difference in A_{sat} (Fig. 2d; Table 2). Overall, A_{sat} was on average 8% less in 27 compared with 18 Pa C_a, over the three developmental stages.

Stomatal conductance (g_s) was generally depressed at the higher growth C_a and the C_4 species responded more strongly than the C_3 species (Fig. 2e-h). The g_s declined with growth stage, but showed little response to growth C_a in either C_3 species, except during grain filling in *T. boeoticum* (Fig. 2e,f; Table 2). Furthermore, C_i/C_a did not differ between the growth C_a treatments in either of the C_3 species (Fig. 2i-j). In contrast, g_s in the C_4 species responded significantly to growth C_a and declined as the plants matured (Fig. 2g-h). In *P. miliaceum*, g_s remained 20-24% higher at 27 Pa C_a throughout the full growth period (Fig. 2g; Table 2). *S. viridis* demonstrated a differential response of g_s to C_a; the g_s was higher at 18 Pa C_a and declined with plant age whilst, at 27 Pa, g_s did not alter from tillering to grain filling (Fig. 2h; Table 2). The C_i/C_a was less at 18 Pa than 27 Pa C_a in both C_4 species. In *P. miliaceum* C_i/C_a increased as the plant matured at both levels of C_a whilst, in *S. viridis*, C_i/C_a increased at 27 Pa, but remained uniform across all developmental stages at 18 Pa (Fig. 2k,l; Table 2).

These changes in g_s and A_{sat} combined to give large improvements in WUE (A_{sat}/g_s) with an increase in the growth C_a (Fig. 2m-p). In the C_3 species the improved WUE at 27 Pa was maintained throughout development and did not decline significantly at either C_a from tillering to grain filling (Fig. 2m,n; Table 2). The changes in WUE closely tracked the changes in A_{sat}, because g_s was constant throughout the full growth period and not influenced by C_a. The two C_4 species had a greater WUE than the C_3 species due to the higher photosynthetic efficiency of C_4 leaves (Fig. 2o,p). In *P. miliaceum*, WUE was significantly higher at 27 Pa C_a and did not decline with maturity at either level of C_a (Fig. 2o; Table 2). *S. viridis* showed the largest variation in the response of WUE to C_a; WUE was significantly greater at 27 Pa, then declined rapidly at anthesis (Fig. 2p; Table 2).

A/C_i responses

To further decipher whether acclimation was occurring in either photosynthetic type, the response of A to C_i was measured in all species at three developmental stages (Figs. 3&4). For both C_3 species, there was no evidence of acclimation (Fig. 3). The V_{cmax} declined significantly with developmental stage, but there was no response to growth C_a (Table 3). Similarly, J_{max} declined significantly during development, yet did not differ between 18 and 27 Pa C_a from tillering to grain filling (Table 3). The operating points at 18 Pa and 27 Pa C_a both sat on the initial slope of the A/C_i curve, explaining the strong response of A_{sat} to growth C_a (Figs. 2&3). The supply functions (l_s), which represent the limitation on A imposed by the stomata, differed between growth C_a, apart from at grain filling (Table 3).

The C_4 subtypes demonstrated some evidence of acclimation. *P. miliaceum* showed a small but significant response of the initial slope (k) of the A/C_i curve to growth C_a. The k was 14% and 25% greater at 18 Pa compared to 27 Pa C_a at tillering and anthesis respectively (Fig. 4; Table 3). However, the saturated rate (V_T) of the A/C_i curve did not respond to growth C_a at any developmental stage (Table 3). The operating point at 18 Pa lay on the initial slope, showing that A was not saturated at the lower C_a, also causing a significant increase in stomatal limitation (Fig. 4; Table 3). Over time, the operating points moved towards the inflexion point of the curve, explaining the diminished response of A to C_a at later developmental stages (Fig. 2). *S. viridis* displayed a stronger acclimation response, with both k and V_T affected by growth C_a at most developmental stages (Fig. 4d-e; Table 3). The k responded to C_a with increasing strength as the plant aged, differing by 20% at tillering and 66% at grain filling between 27 and 18 Pa (Fig. 4d-e; Table 3). V_T also showed a maximum difference at grain filling, increasing by 26% between 18 and 27 Pa C_a. Differences in l_s were only seen at tillering, with values of 6.2 % at 27 Pa and 16.1 % at 18 Pa (Table 3).

Germination rate and seed viability

Germination rate and viability of seeds were significantly lower in the C_3 species grown under glacial C_a (Fig. 5a,b). For both C_3 species, the rate of seed germination was reduced in seeds collected from plants grown in 18 Pa C_a. In *T. boeoticum*, the reduced rate of germination at 18 Pa meant that, at the end of the germination trial, approximately 25% more seeds had germinated when collected from plants grown at postglacial C_a than seeds collected from plants grown under glacial C_a (Fig. 5a; t_{14}=4.6, P=<.001). Similarly, for *H. spontaneum*, the viability of seeds was significantly reduced at the 18 Pa C_a, with 37% more seeds germinating by the end of the trial from plants developed at postglacial C_a (Fig. 5b; t_{14}=2.1, P=<.05).

In comparison, plants of the C_4 photosynthetic type showed little or no effect of C_a on germination rate and seed viability (Fig. 5c,d). In *P. miliaceum*, the initial rate of germination was significantly slower in seeds collected from plants grown at 18 Pa than 27 Pa C_a (Fig. 5c; t_{14}=2.5, P=<.05), but there was no overall effect of C_a on seed viability.

Discussion

Postglacial C_a increased grain yield in the wild progenitors of C_3 and C_4 crops.

An increase in C_a from glacial to post-glacial levels significantly enhanced the yield of both C_3 and C_4 crop progenitors, the response being much larger in the C_3 species. This supports the CO_2 limitation hypothesis of Sage (1995), suggesting that plant yields were significantly reduced during the last glacial period. Our data for C_3 species are in agreement with Sage (1995) who predicted 30-50% increases in seed yield with the post-glacial rise in C_a, and with experimental evidence from other C_3 plants grown at sub-ambient levels of C_a (Dippery *et al.*, 1995; Gifford, 1977; Mayeux *et al.*, 1997).

A recent study by Piperno *et al.* (2015), the first to measure the effects of glacial CO_2 on seed yield of a C_4 crop progenitor, found substantial reductions in productivity in the wild ancestor of maize (*Zea mays* ssp. *parviglumis*) when grown at late-glacial (~20 Pa) compared to early Holocene (~26 Pa) and modern day levels (up to 41 Pa) of C_a. Specifically, in early Holocene conditions, seed yield increased by 180% over that in late-glacial conditions. These changes in seed yield are more pronounced than those measured in our study. However, the authors simultaneously altered temperatures alongside the different C_a treatments, as temperatures in the late-glacial period were typically 4-5 °C cooler than today (Hodell *et al.*, 2008, Piperno *et al.*, 2007). For this reason, the effects of C_a on yield in our experiment are likely to be conservative.

Increases in the grain yield of C_3 species resulted from a greater seed number and seed mass, an increase in tiller number and a greater proportion of fertile tillers. These data agree with previous studies which showed that increases in grain number (Mayeux *et al.*, 1997), tillering (Gifford, 1977; Polley *et al.*, 1992; Sionit *et al.*, 1981) and grain size

(Campbell *et al.*, 2005; Sionit *et al.*, 1981) may all be involved in the yield responses of C_3 cereals to increased C_a at both sub-ambient and future levels. In the C_4 species, grain yield was controlled by small increases in seed number (*S. viridis*) and seed size (*P. miliaceum*) between glacial and post-glacial C_a, with no changes in tiller number. Increased kernel number per head has been recognised in C_4 sorghum at elevated C_a (Ottman *et al.*, 2001) and this may explain the yield increases in *S. viridis*. In agreement with the data for *P. miliaceum*, kernel weight also increased in sorghum under elevated C_a (Ottman *et al.*, 2001). However, the 2.5% increase in individual seed weight we found does not match the total yield increase (10%). It is possible that small but non-significant increases in tiller and grain number explain this disparity.

Effect of glacial C_a on seed germination and viability

Seed quality and germination are critical factors for sustainable yield production and species persistence. In our study we found diminished germination and viability in seeds which developed under glacial C_a. Earlier studies have predominately looked at the germination of seeds collected from plants which completed their lifecycle under elevated C_a. Results are variable; often germination is both slower and lower (Andalo *et al.*, 1996; Bezemer & Jones, 2012), but sometimes it increases (Barnes *et al.*, 1997; Edwards *et al.*, 2001), or shows no change (Huxman *et al.*, 1998; Steinger *et al.*, 2000). It appears that the response of seed traits to elevated C_a differs between functional groups and species; the effect is stronger in non-legumes and generally absent in C_4 species (Jablonski *et al.*, 2002).

Lower germination at elevated C_a is often attributed to an increased C/N ratio, which leads to a decrease in seed protein and a reduction in the ability of the seed to supply the amino acids required for protein synthesis during embryo growth in the germinating seed

(Andalo *et al.*, 1996; Hampton *et al.*, 2013; Jablonski *et al.*, 2002; Steinger *et al.*, 2000). In our study we did not carry out chemical analyses of the seeds. However, Grünzweig & Dumbur (2012) showed that the seeds of grasses grown at sub-ambient levels C_a had higher protein content than those grown at elevated levels, so it is possible that there is a negative relationship between protein content and C_a. We did however see significant effects on seed size, with seeds developed under glacial C_a generally being of reduced weight

Lower seed weight may have contributed in part to the slower germination rates and reduced viability of seeds from both C_3 species which had developed under glacial C_a. Campbell *et al.* (2005) reported similar effects in *Nicotiana tabacum* seeds from plants grown at 10 Pa and 15 Pa, finding that seeds were 14% smaller and had 22% lower germination rates at the lower level of C_a. Dippery *et al.* (1995) found the most extreme response; in the C_3 annual *A. theophrasti*, reproductive output was reduced to zero at a C_a of 15 Pa due to the abortion of all floral buds. In the C_4 species, only *P. miliaceum* showed any impact of glacial C_a, and the viability of seeds was not reduced, whereas the rate of seed germination was slower. Slower seed germination expands the period of seedling establishment, which is one of the most vulnerable points of a plant's life cycle. The lengthening of this period under low C_a would greatly increase the time during which an episodic stress event could impact the seedling (Campbell *et al.*, 2005; Sage & Coleman, 2001).

Atmospheric C_a as a selective agent on plant populations

One caveat to our study is that plants were only grown for a single generation. Given the long duration of sub-ambient levels of C_a over geological time, strong selection pressures must have been exerted. Ward *et al.*(2000) found that biomass production was increased by 35% in *A. thaliana* after only 5 generations of growth at a C_a of 20 Pa, due to an increase in the length of the lifecycle, resulting in a longer period of biomass accumulation before senescence. The modern day genotypes used in our study may therefore be more sensitive to sub-ambient levels of C_a and may not truly reflect the historical conditions where plants adapted and underwent genetic changes to deal with the reduced availability of atmospheric C_a (Gerhart & Ward, 2010; Ward, 2005; Ward *et al.*, 2000).

Contribution of photosynthesis to the yield response

Values of A_{sat} were limited by glacial C_a in both the C_3 and C_4 species, with a more pronounced effect in C_3 leaves, as expected. Values of A_{sat} were approximately 50% greater at post-glacial C_a in both *H. spontaneum* and *T. boeoticum*. The enhancement of A_{sat} is consistent with other studies at sub-ambient C_a (Polley *et al.*, 1992; Tissue *et al.*, 1995) and was closely correlated with the increase in yield, showing that increases in A are directly translated into yield at sub-ambient levels of C_a (Sage, 1995).

Of the C_4 species, only *P. miliaceum* showed a significant positive response of A_{sat} to C_a at sub-ambient levels. During tillering, the difference was substantial, with a 40% increase in A_{sat} between 18 and 27 Pa, showing that C_4 plants can respond very sensitively to changes in C_a at sub-ambient levels because their photosynthetic system may not be CO_2-saturated (Anderson *et al.*, 2001, Cunniff *et al.*2008; Johnson *et al.*, 1993; Pinto *et al.*, 2014; Wand *et al.*, 1999; Ziska & Bunce, 1997).

No acclimation responses occurred in either of the C_3 species growing at glacial C_a, in agreement with earlier studies finding no evidence of photosynthetic adjustment (Maherali *et al.*, 2002; Sage, 1994; Sage & Coleman, 2001; Sage & Reid, 1992; Tissue *et al.*, 1995). However, this finding conflicts with that of Ripley *et al.* (2013) who showed that the C_3

subspecies of *Alloteropsis semialata* acclimated to glacial C_a via the physiological up-regulation of $V_{c,max}$, J_{max}, and g_s linked to increased nitrogen concentration in leaves. Our experiment was performed in large pots (10 l), which may impose some sink limitation due to root restriction (Arp, 1991), whilst experiments in a field system have demonstrated upregulation of A at sub-ambient levels of C_a (Anderson *et al.*, 2001). Furthermore, our experiment did not include an ambient or elevated level of C_a so it is unknown whether the two sub-ambient levels (18 Pa and 27 Pa) would show an acclimatory response if compared to higher levels of C_a.

The lack of acclimation could be related to the light levels of the controlled environment chambers, which were lower than the maxima these crops would have experience in the field. However, photosynthesis has been shown to acclimate to elevated C_a even when plants are grown under light-limited conditions (Osborne *et al.*, 1997; Osborne *et al.*, 1998). Generally, plants are stimulated more by an increase in C_a if grown under light limiting conditions (Kirschbaum & Lambie, 2015) and this response is also recognised in vines grown at sub-ambient levels of C_a (Granados & Körner, 2002).

Interestingly, the response of the C_4 species suggested that some acclimation was occurring at the glacial level of C_a. Previous work has shown that acclimation to C_a in C_4 plants is largely absent unless resources are limited, and this is true for both sub-ambient and elevated C_a studies (Ainsworth & Rogers, 2007; Sage, 1994; Wand *et al.*, 1999). However, some small adjustments in photosynthetic capacities have been previously reported at sub-ambient C_a. For example, Anderson *et al.* (2001) observed greater PEP carboxylase capacity in the C_4 perennial grass *Bothriochola ischaemum* across a sub-ambient gradient of C_a. Similarly, Pinto *et al.* (2014) recognised the up-regulation of C_4 decarboxylase enzymes in several C_4 grasses grown at 18 Pa compared to 40 Pa C_a. Stomatal conductance was significantly higher at glacial C_a, for both C_4 crops in our study, however this did not increase the C_i/C_a ratio. In fact, the opposite pattern was seen, suggesting that stomatal acclimation did not occur (Sage, 1994). In this study, A was C_a-limited in the C_4 grasses at glacial levels, whilst at the postglacial level it was more C_a-saturated, consistent with previous work which showed no increase in A between postglacial and ambient C_a (Cunniff *et al.*, 2008).

Effect of glacial C_a on plant water relations

Growth at post-glacial C_a caused significant reductions in g_s in the C_4 species at all developmental stages, signifying a decrease in the use of water at the leaf scale. However, the same response was not seen in the C_3 species. As photosynthesis is not CO_2-saturated at either the glacial or postglacial levels, then g_s may remain unchanged to ensure that carbon is not limiting for growth.

Increases in WUE were found in both C_3 and C_4 crop species. Improved WUE is one of the most consistent effects of rising C_a in both C_3 and C_4 species at both sub-ambient and future levels (e.g. Conley *et al.*, 2001; Garcia *et al.*, 1998; Maroco *et al.*, 1999; Polley *et al.*, 1993, 1996). Lower g_s and increased WUE at post-glacial C_a can extend the period for positive carbon gain and contribute to increased yield, and this has been demonstrated in these crops previously. Cunniff *et al.* (2016) showed that, over a normal soil drying cycle between watering events, higher g_s and increased leaf transpiration (E_{leaf}) at glacial C_a led to decreased plant water status, which then fed-back to cause stomatal closure that negatively impacted A. Conversely, at post-glacial C_a, g_s and A were maintained through the full drying cycle. This indirect response of g_s to C_a mediated by plant water status has been reported in a large range of field and pot studies in both C_3 and C_4 species, and is an important mechanism by which plants growing at elevated C_a can attain significant biomass during periods of episodic water deficit (Leakey *et al.*, 2004; Samarakoon & Gifford, 1996; Vu & Allen 2009; Wall, 2001; Wall *et al.*, 2001). Though these stimulations of A may be infrequent, they can accumulate to give significant growth enhancements by the end of the growth season. Furthermore, it has been demonstrated that in natural grasslands, especially those of C_4 species, water deficits occur frequently (Owensby *et al.*, 1997). These results have added significance when set against the globally drier climate of the last glacial period (Robinson *et al.*, 2006, Yung *et al.*, 1996).

Implications of the glacial environment for agriculture

These results add significant support to Sage's (1995) hypothesis that the low level of C_a in the Pleistocene was a limiting factor in the successful establishment of agriculture. The low level of C_a not only affects the vegetative biomass, but translates to significantly smaller grain yields in C_4 as well as C_3 species, albeit to a lesser extent in the former. Further climatic interactions, including lower temperatures and less rainfall during the glacial period would have likely exacerbated these yield reductions. Climate change records for western Asia from 25,000 to 5,000 years ago show that the last glacial maximum (LGM) [23,000-19,000 calendar years before present (cal yrs BP)] was colder (in some records predicted to be 5 °C less) and more arid (with some sources predicting 50% less rainfall) (Robinson *et al.*, 2006). Furthermore, it is predicted that, globally, the strength of the hydrological cycle in the late Pleistocene was about half of that at present (Yung *et al.*, 1996). Primary production may have been unstable and haphazard, making foraging difficult, and attempts to specialise on a limited crop base would have been risky (Cowling & Sage, 1998; Sage, 1995). However, once C_a increased and the climate ameliorated as the glacial period waned, a suite of physiological improvements would have increased productivity and reduced the risk of a failed harvest, making specialization a viable alternative to broad-spectrum exploitation, thereby enabling the development of agriculture (Richerson *et al.*, 2001).

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Figure legends

Figure 1. Total yield of the C_3 species (a) *T. boeoticum* and (b) *H. spontaneum*, and the C_4 species (c) *P. miliaceum* and (d) *S .viridis*, grown at C_a of 18 Pa (filled bar) and 27 Pa (open bar). Data are means +SE of eight replicates. Significance codes are ***= <.001, *=<.05.

Figure2. Light-saturated rates of photosynthesis (A_{sat} [a-d]), Stomatal conductance (g_s [e-h]), C_i/C_a (i-l) and water-use efficiency (WUE [m-p]), at three developmental stages; tillering, anthesis and grain filling for the C_3 species (a, e, i, m) *T. boeoticum* and (b, f, j, n) *H. spontaneum*, and the C_4 species (c, g, k, o) *P. miliaceum* and (d, h, l, p) *S .viridis*, grown at C_a of 18 Pa (closed circles) and 27 Pa (open circles). Data are means +SE of six replicates. Significance codes are ***= <.001, **=<.01 and *=<.05.

Figure 3. A/C_i responses for the C_3 species, *T. boeoticum* at (a) tillering, (b) anthesis, and (c) grain filling and *H. spontaneum* at (d) tillering, (e) anthesis, and (f) grain filling, grown at C_a of 18 Pa (closed circles) and 27 Pa (open circles). The square symbols (18 Pa closed, and 27 Pa open) are the photosynthetic rates when C_a was equal to the value used for growth and the dotted lines are the supply functions. Data are means ±SE of six fitted curves, and error bars are smaller than symbols in many cases.

Figure 4. A/C_i responses for the C_4 species, *P. miliaceum* at (a) tillering, (b) anthesis, and (c) grain filling and *S. viridis* at (d) tillering, (e) anthesis, and (f) grain filling, grown at C_a of 18 Pa (closed circles) and 27 Pa (open circles). The square symbols (18 Pa closed, and 27 Pa open) are the photosynthetic rates when C_a was equal to the value used for growth and the dotted lines are the supply functions. Data are means ±SE of six fitted curves, and error bars are smaller than symbols in many cases.

Figure 5. Germination rate and viability of seeds collected from plants grown at C_a of 18 Pa (closed circles) and 27 Pa (open circles) for the C_3 species (a) *T. boeoticum* and (b) *H. spontaneum*, and the C_4 species (c) *P. miliaceum* and (d) *S. viridis*. Percentage germination was measured daily over a 10-day period. Data are means ±SE of eight replicates.

	H. spontaneum (C_3)			*T. boeoticum* (C_3)		
	Growth CO_2 (Pa)			Growth CO_2 (Pa)		
Component	18	27	*P*	18	27	*P*
No. tillers	96.3 ± 3.0	107 ± 5.0	n.s	49 ± 1.0	66±1.0	***
% fertile tillers	79.8 ± 0.9	92.0 ± 0.9	***	89.3 ±1.1	92.6±1.0	*
Seed number	1237 ± 50	1673 ± 54	***	1177 ± 41	1540±34	***
% viable seeds	70.4 ± 2.2	77.6 ± 1.7	*	91.1 ± 1.3	93.3±0.4	n.s
Seed size (mg)	40.9 ± 0.6	44.2 ± 1.7	*	38.8 ±1.1	43.3±0.5	**
TDM (g)	100.8 ± 3.1	145.3 ± 3.0	***	95.8 ± 3.3	136.5±2.4	***
Harvest index	0.35 ± 0.01	0.39 ± 0.01	***	0.43 ± 0.004	0.46±0.003	***

	P. miliaceum (C_4)			*S. viridis* (C_4)		
	Growth CO_2 (Pa)			Growth CO_2 (Pa)		
Component	18	27	*P*	18	27	*P*
No. tillers	13.1 ± 1	16.0 ±1	n.s	192 ± 24	164 ± 39	n.s
% fertile tillers	87.9 ± 2.5	91.4 ± 2.9	n.s	100	100	n.s
Seed number	5223 ± 188	5749 ± 307	n.s	11472 ± 1279	14294 ± 974	*
Seed size (mg)	5.0 ± 0.03	51.4 ± 0.03	*	1.45 ± 0.03	1.49 ± 0.04	n.s
TDM (g)	71.9 ± 1.3	79.3 ± 2.6	*	35.8 ± 2.7	41.2 ± 4.0	n.s
Harvest index	0.36 ± 0.01	0.37 ± 0.01	n.s	0.47 ± 0.04	0.53 ± 0.03	n.s

Table 1. Components of yield of the C_3 and C_4 crop progenitors grown at C_a of 18 Pa or 27 Pa. Values are means ±SE of 8 replicates. Significance codes are *=<.05, **=<.01 and ***=<.001.

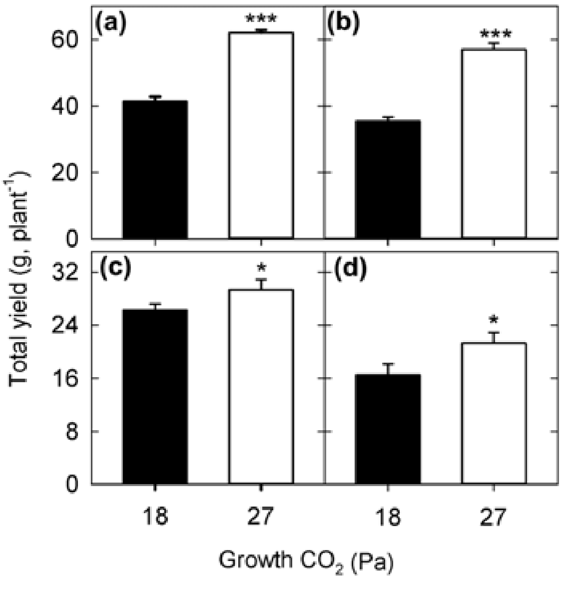
		CO_2			Stage			CO_2 × Stage		
Species	Parameter	*F*	d.f	*P*	*F*	d.f	*P*	*F*	d.f	*P*
T. boeoticum	A_{sat}	143.1	1	***	97.8	2	***	8.1	2	**
	g_s	21.1	1	***	31.1	2	***	-	2	n.s
	C_i/C_a	-	1	n.s	23.4	2	***	-	2	n.s
	WUE	131.5	1	***	3.7	2	*	-	2	n.s
H. spontaneum	A_{sat}	171.3	1	***	93.3	2	***	5.6	2	**
	g_s	-	1	n.s	-	-	n.s	-	2	n.s
	C_i/C_a	-	1	n.s	91.9	2	***	-	2	n.s
	WUE	136.2	1	***	65.8	2	***	5.6	2	**
P. miliaceum	A_{sat}	150.7	1	***	152.4	2	***	19.4	2	***
	g_s	93.6	1	***	92.1	2	***	-	2	n.s
	C_i/C_a	17.7	1	***	11.2	2	***	-	2	n.s
	WUE	176.5	1	***	-	2	n.s	-	2	n.s
S. viridis	A_{sat}	8.2	1	**	47.2	2	***	4.6	2	*
	g_s	95.9	1	***	17.9	2	***	5.9	2	**
	C_i/C_a	64.4	1	***	15.6	2	***	5.9	2	*
	WUE	60.2	1	***	-	2	n.s	6.5	2	**

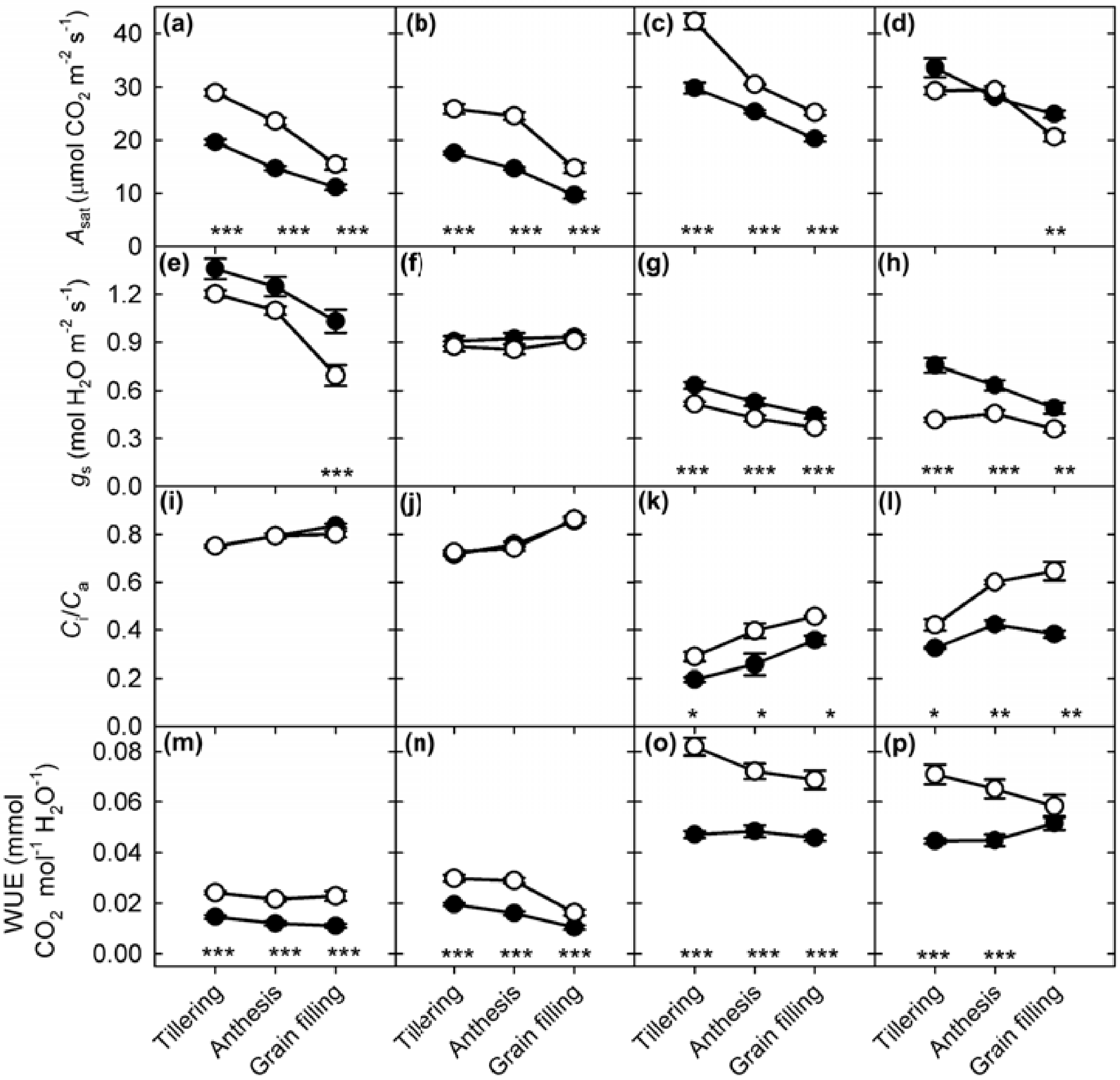
Table 2. ANOVA for gas exchange and WUE for the C_3 and C_4 crop progenitors, testing for the effects of CO_2 treatments, growth stage and the interacting effects of growth stage and CO_2. In each case, a minimal adequate model is presented, obtained by the removal of non-significant (n.s) interactions. Significance thresholds were set at 0.05. Significance codes are *=<0.05, **=<0.01 and ***=<0.001.

		T. boeoticum (C_3)			*H. spontaneum* (C_3)		
		Growth CO_2 (Pa)			Growth CO_2 (Pa)		
Stage	Parameter	18	27	*P*	18	27	*P*
Tillering	$V_{c,max}$ (µmol m^{-2} s^{-1})	129.2 ± 4.0	132.8 ± 1.6	n.s	116.5 ± 1.0	111.4 ± 4.6	n.s
Anthesis		92.8 ± 2.8	100.2 ± 3.7	n.s	92.4 ± 3.0	105.9 ± 4.4	n.s
Grain Filling		77.4 ± 4.8	70.5 ± 6.9	n.s	54.1 ± 3.7	54.2 ± 3.9	n.s
Tillering	J_{max} (µmol m^{-2} s^{-1})	321.5 ± 13.1	320.6 ± 9.0	n.s	332.1 ± 2.8	309.3 ± 17.5	n.s
Anthesis		197.1 ± 7.3	220.6 ± 5.9	n.s	192.4 ± 6.8	240.9 ± 12.0	n.s
Grain Filling		151.6 ± 5.9	131.4 ± 16.4	n.s	101.0 ± 9.7	107.2 ± 10.7	n.s
Tillering	l_s (%)	26.3± 1.5	16.5 ± 0.9	**	27.3 ± 2.4	21.7 ± 1.8	*
Anthesis		21.3± 1.3	12.9 ± 1.7	**	19.5 ± 2.2	12.7 ± 2.1	*
Grain Filling		18.0 ± 2.5	13.6 ± 2.4	n.s	7.5 ± 1.1	6.9 ± 0.7	n.s

		P. miliaceum (C_4)			*S. viridis* (C_4)		
		Growth CO_2 (Pa)			Growth CO_2 (Pa)		
Stage	Parameter	18	27	*P*	18	27	*P*
Tillering	*k* (mol m^{-2} s^{-1})	0.8 ± 0.01	0.7 ± 0.02	**	0.6 ± 0.04	0.5 ± 0.06	*
Anthesis		0.5 ± 0.04	0.4 ± 0.03	*	0.6 ± 0.04	0.4 ± 0.05	**
Grain Filling		0.4 ± 0.08	0.3 ± 0.02	n.s	0.5 ± 0.05	0.3 ± 0.04	***
Tillering	V_T (µmol m^{-2} s^{-1})	50.8 ± 0.4	49.6 ± 1.0	n.s	43.5 ± 2.6	34.9 ± 0.4	**
Anthesis		34.5 ± 0.3	36.2 ± 0.4	n.s	31.4 ± 0.2	32.9 ± 1.2	n.s
Grain Filling		31.1 ± 0.2	29.1 ± 0.6	n.s	30.9 ± 1.0	24.4 ± 1.03	***
Tillering	l_s (%)	36.7 ± 3.7	10.7 ± 1.2	**	16.1 ± 1.4	6.2 ± 1.6	**
Anthesis		25.5 ± 0.3	10.9 ± 1.7	**	1.8 ± 1.2	2.7 ± 0.6	n.s
Grain Filling		26.2 ± 1.8	9.6 ± 0.6	**	10.2 ± 1.2	6.2 ± 1.4	n.s

Table 3. Values of the A/C_i parameters of the C_3 and C_4 crop progenitors grown at C_a of 18 Pa or 28 Pa. Values are means ±SE of 6 replicates. Significance codes are *=<.05, **=<.01 and ***=<.001. Abbreviations for A/C_i parameters are: C_3 maximum rate of Rubisco carboxylation ($V_{c,max}$), the apparent C_3 maximum rate of photosynthetic electron transport (J_{max}), initial slope of the C_4 photosynthetic response (*k*), C_4 maximum photosynthetic capacity (V_T) and l_s (stomatal limitation).

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(b)
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(o)
(p)
A_{sat} (μmol CO$_2$ m^{-2} s^{-1})
g_s (mol H$_2$O m^{-2} s^{-1})
C_i/C_a
WUE (mmol CO$_2$ mol^{-1} H$_2$O^{-1})
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10
20
30
40
0.0
0.3
0.6
0.9
1.2
0.0
0.2
0.4
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0.02
0.04
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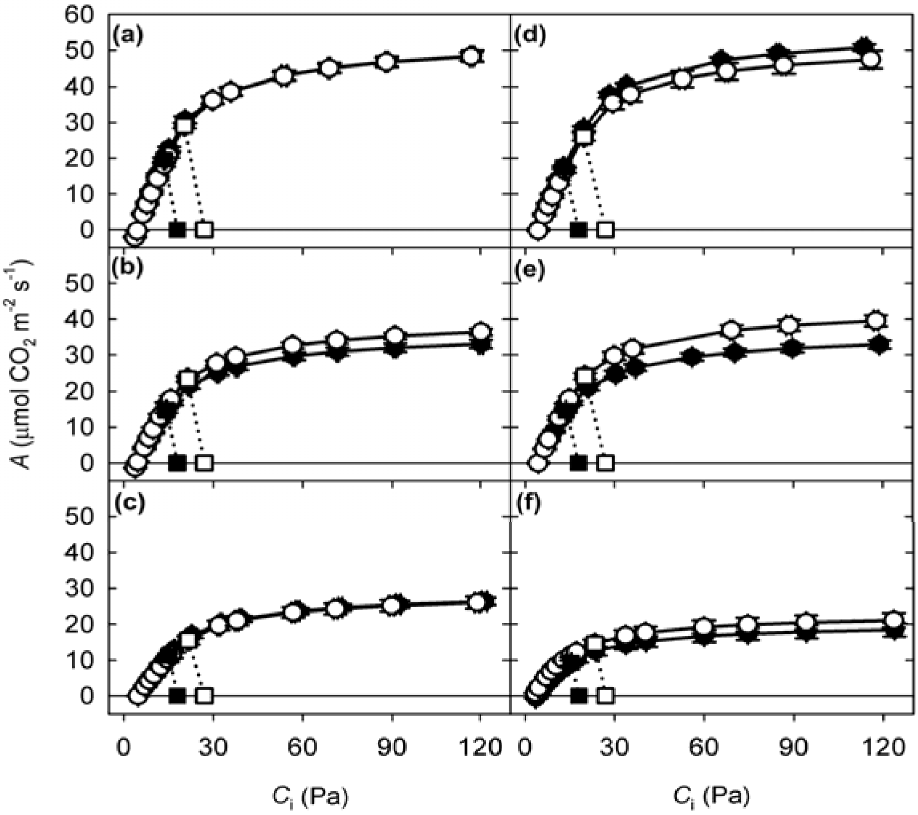
**
*
Tillering
Anthesis
Grain filling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(b)
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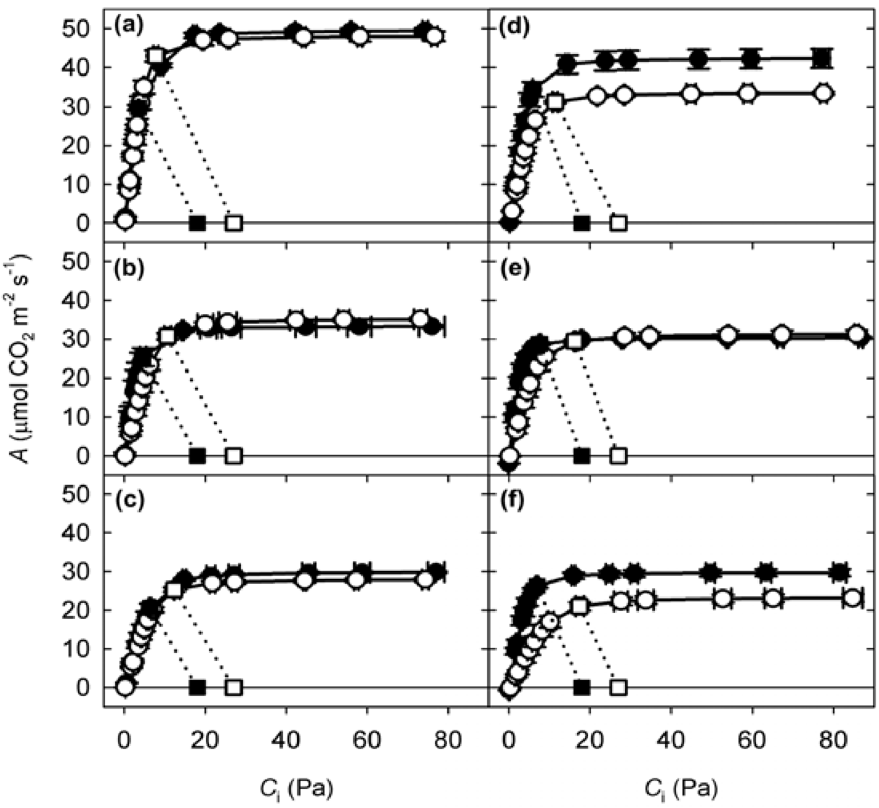
120
C_i (Pa)
C_i (Pa)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40
60
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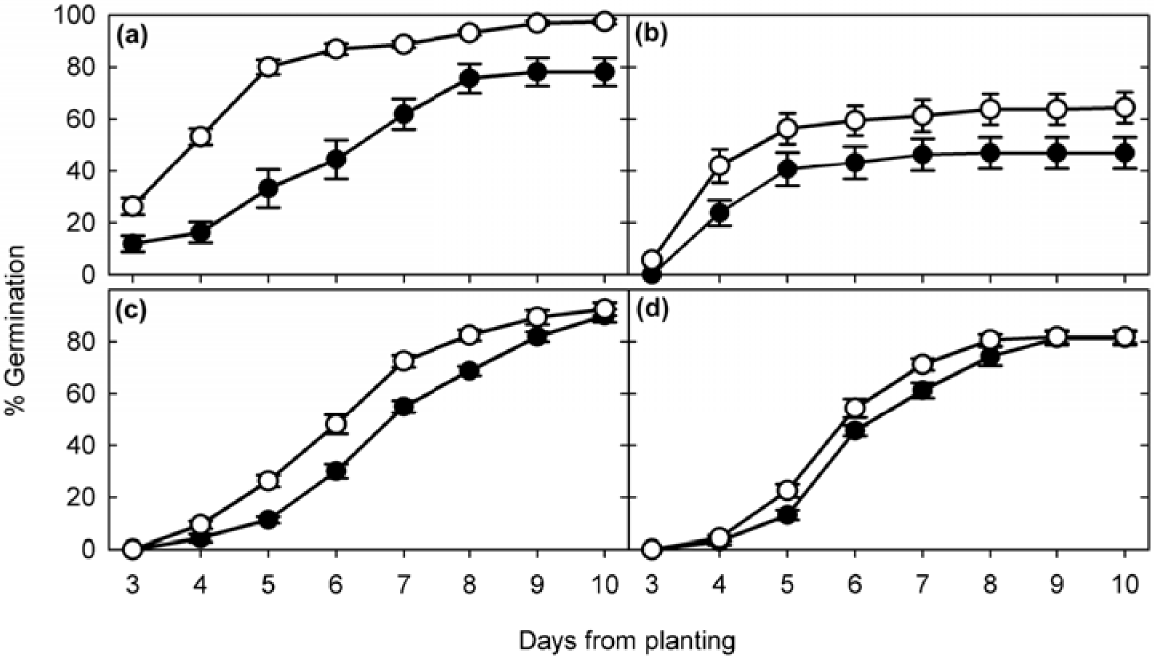
C_i (Pa)
C_i (Pa)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7
8
9
10
Days from planting