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Title:

CARBON SOURCE-SINK LIMITATIONS DIFFER BETWEEN TWO SPECIES WITH CONTRASTING GROWTH STRATEGIES

Running title:

Carbon source-sink limitations vary with growth strategy

Authors

Angela C Burnett[1], Alistair Rogers[2], Mark Rees[1], Colin P Osborne[1]

[1] Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK

[2] Environmental and Climate Sciences Department, Brookhaven National Laboratory, Upton, NY 11973, USA

Corresponding Authors

Angela C Burnett, Colin P Osborne

Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK

Email: avenetianmorning@gmail.com, c.p.osborne@sheffield.ac.uk

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ABSTRACT

Understanding how carbon source and sink strengths limit plant growth is a critical knowledge gap that hinders efforts to maximise crop yield. We investigated how differences in growth rate arise from source-sink limitations, using a model system comparing a fast-growing domesticated annual barley (*Hordeum vulgare* cv. NFC Tipple) with a slow-growing wild perennial relative (*Hordeum bulbosum*). Source strength was manipulated by growing plants at sub-ambient and elevated CO_2 concentrations ($[CO_2]$). Limitations on vegetative growth imposed by source and sink were diagnosed by measuring relative growth rate, developmental plasticity, photosynthesis and major carbon and nitrogen metabolite pools. Growth was sink limited in the annual but source limited in the perennial. RGR and carbon acquisition were higher in the annual, but photosynthesis responded weakly to elevated $[CO_2]$ indicating that source strength was near maximal at current $[CO_2]$. In contrast, photosynthetic rate and sink development responded strongly to elevated $[CO_2]$ in the perennial, indicating significant source limitation. Sink limitation was avoided in the perennial by high sink plasticity: a marked increase in tillering and root:shoot ratio at elevated $[CO_2]$, and lower non-structural carbohydrate accumulation. Alleviating sink limitation during vegetative development could be important for maximising growth of elite cereals under future elevated $[CO_2]$.

INTRODUCTION

Global population growth, economic development and climate change are exerting increasing pressure on our global food supply, raising demand that must be met, in part, by improving crop yields (Ainsworth *et al.*, 2008*b*; Godfray *et al.*, 2010; Foley *et al.*, 2011; von Caemmerer *et al.*, 2012; Reynolds *et al.*, 2012; FAO *et al.*, 2014; Ort *et al.*, 2015). Increasing yield depends critically on a firm understanding of plant growth, which is in turn underpinned by the interactions between carbon and nitrogen sources and sinks (White *et al.*, 2016). Sources provide net uptake of resources from the external environment whilst sinks cause a net internal drawdown of these resources. For carbon, mature leaves are sources and roots are sinks, and the balance between them is achieved by well-characterised molecular crosstalk mechanisms (Smith and Stitt, 2007; Lawlor and Paul, 2014; White *et al.*, 2016). Decades of research into the effects of elevated CO_2 have demonstrated that increasing source activity through a stimulation of photosynthesis often does not translate into corresponding yield increases (Long *et al.*, 2006; Ainsworth *et al.*, 2008*a*; Leakey *et al.*, 2009), although this depends on the species (Yamori *et al.*, 2016). Similarly, increasing sink capacity does not always translate into greater yield under field conditions (Weichert *et al.*, 2010). A holistic approach to growth and yield considering both source and sink capacities is therefore essential for developing higher yielding crop varieties (White *et al.*, 2016). In this context, source strength is the product of source activity and size, with an equivalent definition for sinks (Geiger and Shieh, 1993; White et al., 2016).

One strategy for understanding the fundamental limitations on growth is to investigate the natural diversity of growth rates in wild plants. In wild species, one of the major causes of growth rate variation is life-history (Grime and Hunt, 1975; Garnier, 1992). Annual and perennial growth strategies enable plants to allocate resources in a way that is appropriate for their environment: annuals grow quickly and invest everything in reproduction in the first year before they die, whilst perennials grow more slowly and conserve resources for the following season (Garnier, 1992; Iwasa, 2000; Bennett *et al.*, 2012). Annuals are typically seen as having flexible growth strategies for exploiting fluctuating environments, whereas perennials have more conservative growth strategies – i.e. lower allocation to reproduction and slower growth (Atkinson *et al.*, 2012, 2014). Although perennials with large storage organs may never be sink limited, annuals generally transition from sink to source limitation during development when they switch from vegetative to reproductive growth (Arp, 1991), and perennials lacking large storage organs are likely to undergo this transition as well. Because perennials grow more slowly than annuals and transition to the reproductive growth stage later, they are therefore likely to be sink limited for a longer period of time.

Despite this well developed ecological theory, we do not currently know the extent to which slower growth in perennials than annuals arises from greater source or sink limitation. Experimental manipulations of the source:sink ratio provide insights into the relative contributions of source and sink processes to growth rate, and may be achieved through a variety of techniques including: sink removal (Arp, 1991); genetic modification (Ainsworth *et al.*, 2004; Weichert *et al.*, 2010; Zuther *et al.*, 2011); source removal (von Caemmerer and Farquhar, 1984; Bryant et al., 1998; Rogers et

al., 1998; Eyles et al., 2013); inhibiting resource export from the source (Ainsworth and Bush, 2011); and increasing source activity using elevated CO_2 (Kinsman *et al.*, 1997; Masle, 2000), reviewed by White et al. (2016). Here, we alter the atmospheric CO_2 concentration ($[CO_2]$) to non-invasively manipulate the source:sink ratio in barley – elevated $[CO_2]$ to increase the source strength and sub-ambient $[CO_2]$ to decrease it – with current $[CO_2]$ as a reference against which to compare the source manipulations. This approach enables analysis of source and sink limitation under current $[CO_2]$, and strong CO_2 treatments are applied in order to produce marked perturbations of the system. In C_3 plants, $[CO_2]$ affects carbon source strength directly through one well-understood process i.e. carbon assimilation by Rubisco, and therefore avoids wounding responses and other confounding effects, which may arise from alternative approaches for source:sink manipulation. We took a holistic approach to investigating source-sink interactions, measuring the responses of development, growth, allocation, photosynthesis and key carbon and nitrogen metabolite pools on the same plants. Together, these simultaneous measurements of growth, carbon uptake and carbon utilization allowed us to diagnose source and sink limitation in our model system. For example, a high concentration of free amino acids indicates carbon source limitation (Paul and Driscoll, 1997; Stitt and Krapp, 1999; Isopp *et al.*, 2000; Rogers *et al.*, 2006), whilst a build-up of non-structural carbohydrates in leaves indicates carbon sink limitation (Rogers and Ainsworth, 2006; Ainsworth and Bush, 2011).

In order to elucidate physiological mechanisms underpinning differences in growth rate, this study compared domesticated annual barley (*Hordeum vulgare* cv. NFC Tipple) and a wild perennial relative (*Hordeum bulbosum*). Annual barley is sink

limited during grain filling (Schnyder, 1993; Bingham *et al.*, 2007; Serrago *et al.*, 2013), yet to our knowledge no study of source- and sink limitation during the vegetative growth stage has been made in this species. The annual barley used here is an elite agricultural spring barley from the HGCA recommended list (HGCA, 2014) and has a fast-growing life-history strategy. The perennial is a wild species from Turkey, which is able to grow in diverse habitats but generally occupies nutrient-rich environments (von Bothmer, 1996). CO_2 treatments were applied at germination and maintained until harvest, which occurred during the vegetative growth phase of the life cycle. We predicted that annual barley, which grows faster than perennial barley, would be sink limited during vegetative growth, and the perennial would be more strongly sink limited (Jaikumar *et al.*, 2014). Based on this hypothesis we would expect the fast-growing annual to show a greater increase in growth and photosynthesis in response to elevated [CO_2] than the perennial (Poorter, 1993; Roumet and Roy, 1996). This is because the elevated [CO_2] alleviates source limitation and will therefore have a greater effect in the plants which are less sink limited (Bryant *et al.*, 1998; Rogers *et al.*, 1998; Ainsworth *et al.*, 2003). In contrast, we expected that the more strongly sink limited, slower-growing perennial would show a greater increase in the storage of carbon-rich metabolites under elevated [CO_2].

MATERIALS AND METHODS

Plant material and growth conditions

Seeds of *Hordeum vulgare* cv. NFC Tipple and *Hordeum bulbosum* (Accessions GRA1031 and GRA947) were obtained from Syngenta and IPK Gatersleben respectively. Seeds were germinated on wet filter paper and transplanted to 4-litre pots filled with 1:10 sand:vermiculite and topped with an additional layer of sand to aid root development of the seedlings. Plants were grown in controlled environment growth chambers (BDR 16, Conviron, Isleham, UK) at the University of Sheffield, two of which had been modified to scrub CO_2 using soda lime. Plants were grown in three chambers with fixed CO_2 levels of 180 µmol mol^{-1}, 400 µmol mol^{-1} and 1500 µmol mol^{-1} for 61 days. 180 and 1500 µmol mol^{-1} were chosen in order to impose strong carbon source and sink manipulations. All chambers had a 12-hour photoperiod with day/night temperatures of 20/18℃, 65% humidity, and daytime light levels of 600 µmol photons m^{-2} s^{-1} at plant height resulting in a daily light integral of 25.92 mols m^{-2} day^{-1}. Plants were kept adequately watered with 20% Long Ashton's nutrient solution. During seedling establishment, plants were watered daily – with 150ml Reverse Osmosis water for 8 days and with 150ml Long Ashton's solution thereafter. After 17 days, plants were watered three times per week with 150ml Long Ashton's solution until 29 days old, 225ml until 45 days old, and 450ml thereafter.

Photosynthesis measurements and metabolite harvests were carried out three times in consecutive weeks, between 46 and 61 days after germination (DAG). In each of these harvest weeks, six annuals from each CO_2 level were harvested (three at dawn

and three at dusk), giving a total of 54 individuals across three weeks. In the first and third of these harvest weeks, six perennials from each CO_2 level were harvested (three at dawn and three at dusk), giving a total of 36 individuals.

Relative growth rate, root:shoot ratio and tillering

RGR was calculated based on the plant mass estimated from weekly imaging of above ground biomass, beginning when plants were two weeks old. Plants were photographed (PowerShot G9, Canon, Tokyo, Japan) six times, from the side against a white background, with the plant rotated 60 degrees between successive photographs. A scale bar of known length was included for calibration. Leaf area in pixels was obtained for each photograph using Image J (U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/), and converted to mm^2 using the area of the scale bar. A batch of 29 additional plants, not used in the main study, was also photographed weekly. At nine time points between 33 and 60 DAG, individuals from this batch were separated into leaf, leaf sheath and root, and oven-dried to provide a calibration curve for leaf area and dry mass for each species. These curves were then used to predict dry mass for each plant for each set of six photographs (Fig. S1; r^2 = 0.97 for species-based calibration). Individual growth curves showing predicted dry mass over time were obtained for each plant using a nonlinear mixed-effects model obtained by stepwise selection and used to estimate RGR at multiple timepoints by differentiation, where more than three timepoints had been measured.

The calibration was also used to predict root:shoot ratio for the plants in the main study. For each of the oven-dried individuals, the relative contribution of shoot and root to whole plant mass was recorded and the mean fraction of root and shoot was calculated. This was then applied to the plant mass predicted for the plants in the main study using calibration of image data, to give an estimate of root:shoot ratio for each individual.

Tillers were counted the day before metabolite harvests were carried out.

Photosynthesis

Diurnal measurements of photosynthesis were made the day before plants were harvested for metabolite assays. 51 annual and 26 perennial individuals were measured (the remaining plants were too small for gas exchange measurements to be performed). Instantaneous net photosynthetic rate was measured every 3.5 hours between 30 minutes after dawn and 30 minutes before dusk, using the LI-6400XT Portable Photosynthesis System (LI-COR Biosciences, Lincoln, NE, USA). Net photosynthetic rate was measured *in situ* within growth chambers, under the ambient environmental conditions of each chamber described above. These measurements were used to obtain a curve of photosynthesis during the photoperiod for each plant, and the area underneath was integrated to give a daily rate of net carbon fixation per unit area – i.e. the carbon source activity. This value was multiplied by the projected shoot area to estimate the total daily photosynthesis in the whole shoot – i.e. the carbon source strength.

Metabolites

Plants were harvested from 47 to 61 DAG, within one hour before dawn and one hour before dusk. Samples were flash frozen in liquid nitrogen, stored at -80°C, and freeze-dried prior to analysis. For small plants, the entire plant was harvested; for larger plants, representative samples of leaf, leaf sheath and root from both young and old tissue were harvested. In the first week, plants were harvested at 47, 48 and 49 DAG. In the second week, plants were harvested at 52, 53 and 54 DAG. In the third week, plants were harvested at 59, 60 and 61 DAG. Three replicates from each species from one chamber were harvested on each date, with the exception of the second week when only annuals were harvested. The order of chambers was randomised in each of the three harvest weeks.

Metabolite analysis was carried out at Brookhaven National Laboratory. Metabolites were extracted from the freeze-dried ground tissue using sequential ethanol extractions.

Ethanol soluble carbohydrates (glucose, fructose, sucrose, low degree of polymerisation (LDP) fructan) were analysed using a continuous enzymatic substrate assay as described previously (Ainsworth *et al.*, 2007) adapted for measuring sucrose in the presence of LDP fructans (Harrison *et al.*, 1997). All biochemical analysis was conducted in standard 96-well microplates (Microtest Plate 96-Well Flat Bottom, Sarstedt, Nümbrecht, Germany), using a robotic liquid handling system (Evolution P^3 Precision Pipetting Platform, Perkin Elmer, Waltham, MA, USA).

The pellets from the ethanol extraction were heated to 95℃ in 0.1M NaOH, to solubilise protein. A commercially available protein assay kit (Pierce BCA protein assay kit, Thermoscientific, Rockford, IL, USA) based on the Lowry method was used to measure protein content (Lowry et al., 1951) using BSA as a standard. Following the protein assay, samples were neutralised with HCl.

For the starch and high degree of polymerisation (HDP) fructan assay, starch and HDP fructans from 40 µl aliquots of the suspended pellet material were digested using enzymes in 60 µl 0.05M acetate buffer as follows. Starch: 0.17U $well^{-1}$ amyloglucosidase (EC 3.2.1.3) and 0.1U $well^{-1}$ α-amylase (EC 3.2.1.1); starch and HDP fructan: 0.1U $well^{-1}$ exo-inulinase (EC 3.2.1.80), 0.1U $well^{-1}$ endo-inulinase (EC 3.2.1.7), 0.17U $well^{-1}$ amyloglucosidase and 0.1U $well^{-1}$ α-amylase. Plates were incubated overnight at 37℃. 40 µl of the supernatant from the overnight digest was transferred to each well of a 96-well microplate. 262µg ATP $well^{-1}$, 349µg NADP $well^{-1}$ and 3.6U $well^{-1}$ glucose-6-phosphate dehydrogenase (EC 1.1.1.49, grade II) were added in a buffer of 0.1M HEPES/KOH, 3mM $MgCl_2$, ph7.0 to initiate the reaction. Microplates were centrifuged for 1 minute to remove bubbles then inserted into a plate reader and the NADPH associated with the carbohydrates in the sample was measured at A_{340} (ELx808, BioTek, Winooski, VT, USA).

Starch and HDP fructans were assayed by sequentially adding 1U enzyme in HEPES buffer to each well as follows, using the rationale for the soluble carbohydrates assay (Ainsworth *et al.*, 2007). Starch: hexokinase (EC 2.7.1.1); starch and HDP fructan: hexokinase, phosphoglucose-isomerase (EC 5.3.1.9). HDP fructan values were obtained by subtracting the starch assay values for starch from the starch and fructan

values for combined starch and fructan. This approach was necessary because preliminary recovery experiments had shown that digesting fructan using exoinulinase and endoinulinase also degraded a small amount of starch, leading to an artificially high value for fructan content which was corrected using the approach described here. Starch and HDP fructan content was measured as nmol hexose equivalents using a standard glucose curve loaded on each plate.

Total free amino acids were quantified using fluorescamine. 15µl 0.1M sodium borate buffer, 90µl fluorescamine and 100µl water were combined with 2µl of ethanol extract in a black 96-well microplate (Nunc MicroWell, Thermo Fisher Scientific, Waltham, MA, USA). Following a 5 minute dark incubation, fluorescence (360nm excitation, 460nm emission, 40 nm bandwidth) was measured (Synergy HT, BioTex, Winooski, VT, USA) and converted into nmol amino groups using a standard glutamate curve loaded on each plate.

The Griess reaction was used to quantify free nitrate. First, 0.005U well^{-1} nitrate reductase (EC 1.7.1.3) and 50 nmol NADPH in 0.11M potassium phosphate buffer were added to a 10µl aliquot of the ethanol extract. Each microplate was shaken. Following a 30 minute incubation (dark, room temperature), 20µl 0.25mM phenazine methosulfate was added to each well. Plates were shaken again and incubated for a further 20 minutes. 45µl 1% w/v sulfanilamide in 5% phosphoric acid followed by 45µl 0.02% N(1-Napthyl)ethylendiamine dihydrochloride was added to each well. Following another shake and a 5 minute incubation, A_{540} was measured (ELx808, BioTex, Winooski, VT, USA) and converted into nmol nitrate using a standard nitrate curve loaded on each plate.

Metabolite data were expressed per g carbohydrate-corrected dry weight, obtained by subtracting the mass of total non-structural carbohydrate (the sum of glucose, fructose, sucrose, LDP and HDP fructan, and starch) from the dry mass of each sample. Technical and analytical replicates were run for all assays (Fernie *et al.*, 2011).

Statistical Methods

Analysis was performed using R (2015). Analysis of variance models incorporating error terms reflecting the split-split plot design of the experiment were carried out for each variable measured. Logarithmic transformations were performed on all data prior to analysis to improve the fit of the models.

For photosynthesis, TNC, amino acids and amino acid:sucrose (Figs. 2, 4, 6, 7), small error bars are present but are obscured by symbols.

RESULTS

Perennial barley shows greater developmental plasticity in response to elevated [CO_2] than the annual species

Relative growth rate (RGR; the efficiency of whole plant dry mass increase obtained from calibration of shoot area, and measured in g g^{-1} day^{-1}) was obtained from individual growth curves by differentiation, and represents the sink activity of plant growth. RGR was higher in the annual than perennial plants, and greater at higher CO_2 levels (Fig. 1). Stepwise model selection was used to choose fixed effects, and the effects of species and [CO_2] on the maximum plant size and the time to reach half size were each highly significant ($p<0.001$) – although these are additive effects with no significant interaction between [CO_2] and species. In both species, the increase in RGR was greater between 180 and 400 µmol mol^{-1} CO_2 than between 400 and 1500 µmol mol^{-1} CO_2. Peak RGR in the annual increased by 17.1% between 180 and 400 µmol mol^{-1} CO_2, but only 5.0% between 400 and 1500 µmol mol^{-1} CO_2. Peak RGR in the perennial increased by 20.5% between 180 and 400 µmol mol^{-1} CO_2, but only 6.0% between 400 and 1500 µmol mol^{-1} CO_2. However the difference between annual and perennial remained relatively consistent: peak RGR in the annual was 21.1%, 17.7% and 16.7% higher than in the perennial, at 180, 400 and 1500 µmol mol^{-1} CO_2 respectively.

The modular nature of plant body plans means that, in order for RGR to increase, plants must either increase the biomass of existing organs, or initiate new structures through branching (tillering, in the case of grasses). Tillering in the perennial

increased by 163% between 180 and 1500 µmol mol^{-1} CO_2, whereas the number of tillers in the annual increased by just 15% across the same CO_2 range (Table 1). This highly significant species x [CO_2] interaction ($F_{(1,80)}$ = 56, p<0.001) indicates greater developmental plasticity in the perennial.

Root:shoot ratio also showed a larger response to increasing [CO_2] in perennial than annual barley. In the annual, the root:shoot ratio increased by only 2.8% between 180 and 400 µmol mol^{-1} CO_2, whilst in the perennial it increased 11.6% between 180 and 400 µmol mol^{-1}, and 4.2% between 400 and 1500 µmol mol^{-1} (Table 1). This highly significant species x [CO_2] interaction ($F_{(1,77)}$ = 24, p<0.001) provides further evidence of greater developmental plasticity in the perennial.

Perennial barley also shows a greater photosynthetic response to elevated [CO_2] than the annual species

Annual barley generally has a higher photosynthetic rate than the perennial, but the photosynthetic rate in the perennial shows a much stronger response to [CO_2] (Fig. 2B). In the annual plants, the daily photosynthetic rate increased by 87% between 180 and 400 µmol mol^{-1} CO_2, but only by 13% between 400 and 1500 µmol mol^{-1}. In contrast, in the perennial it increased 58% between 180 and 400 µmol mol^{-1}, but 75% between 400 and 1500 µmol mol^{-1}. This led to a significant species x [CO_2] interaction: $F_{(1,67)}$ = 4.9, p<0.05; Fig. 2B. Because the annual is a larger plant than the perennial, the difference in whole shoot photosynthetic rate, i.e. carbon source strength (Fig. 2C) is greater than the difference in the rate per unit area, i.e. carbon source activity (Fig. 2B). In the annual, the whole shoot daily photosynthetic rate

360 increased by 177% between 180 and 400 µmol mol^{-1} CO_2, but only 25% between
361 400 and 1500 µmol mol^{-1}. In contrast, in the perennial it increased 528% between
362 180 and 400 µmol mol^{-1}, and 123% between 400 and 1500 µmol mol^{-1}. There was a
363 highly significant species x [CO_2] interaction: $F_{(1,66)} = 18$, $p<0.001$; Fig. 2C.
364
365 The ratio of photosynthesis to growth is higher in the annual than the perennial (Fig.
366 3), seen in the plots of individuals (Fig. 3A,B) and means (Fig. 3C,D) with a highly
367 significant effect of species: $F_{(1,61)} = 25$, $p<0.001$. When expressed in g C g^{-1} day^{-1}
368 (Fig. 3A), growth shows three clusters corresponding to the decreasing values of
369 RGR as time progresses over the three harvests. When expressed in g C plant^{-1}
370 day^{-1} (Fig. 3B), these clusters are no longer present, and a positive correlation
371 between photosynthesis and growth is seen. The ratio increases with [CO_2] (Fig. 3C)
372 and with plant age at harvest (Fig. 3D). There was a highly significant interaction of
373 the harvest week x [CO_2] ($F_{(4,61)} = 16$, $p<0.001$), such that the photosynthesis:growth
374 ratio is greater at higher [CO_2], but this trend becomes less pronounced at later
375 harvests.
376
377 **Annual barley accumulates more non-structural carbohydrates than the**
378 **perennial species**
379
380 Pre-dawn measurements indicate the basal level of carbohydrates in plant organs,
381 when metabolites accumulated during the previous photoperiod have been utilised
382 for respiration, exported or consumed by growth at night. Before dawn, annual barley
383 had a higher concentration of total non-structural carbohydrates (TNC, the sum of
384 glucose, fructose, sucrose, fructan and starch) than the perennial, and showed a

385 greater accumulation of TNC in leaf sheaths and roots when [CO_2] was increased
386 from 180 to 1500 µmol mol^{-1} (Fig. 4). However, in the leaves, perennial barley
387 showed a stronger TNC response than the annual when [CO_2] was increased from
388 180 to 1500 µmol mol^{-1} (Fig. 4). Across all TNC data, there was a significant organ
389 type x time of day x species interaction ($F_{(2,120)}$ = 9.2, $p<0.001$); a significant organ
390 type x time of day x [CO_2] interaction ($F_{(4,120)}$ = 18, $p<0.001$); a significant organ x
391 species x [CO_2] interaction ($F_{(4,120)}$ = 22, $p<0.001$); and a significant organ x harvest
392 week x [CO_2] interaction ($F_{(8,120)}$ = 4.5, $p<0.001$). In the leaf, TNC was 114% greater
393 in the annual than the perennial at 180 µmol mol^{-1} CO_2, 57% greater in the annual at
394 400 µmol mol^{-1} CO_2, but approximately equal at 1500 µmol mol^{-1} CO_2 (Fig. 4A). In
395 the leaf sheath, TNC was 29% greater in the annual than the perennial at 180 µmol
396 mol^{-1} CO_2, 57% greater in the annual at 400 µmol mol^{-1} CO_2, and 25% greater in the
397 annual at 1500 µmol mol^{-1} CO_2 (Fig. 4B). In the root, TNC was 35% greater in the
398 annual than the perennial at 180 µmol mol^{-1} CO_2, 56% greater in the annual at 400
399 µmol mol^{-1} CO_2, and 97% greater in the annual at 1500 µmol mol^{-1} CO_2 (Fig. 4C). In
400 both species, TNC concentration is highest at 1500 µmol mol^{-1} CO_2 suggesting that
401 sinks are replete under these conditions.
402
403 Subtracting the mean pre-dawn values from the mean pre-dusk values provides a
404 differential of TNC (Fig. 5), which represents the amount of carbon accumulated
405 during the photoperiod, and is equivalent to the amount of carbon available for
406 respiration, export or growth at night. These differentials are much greater in the leaf
407 than in leaf sheath or root (Fig. 5), since diurnal fluctuations in leaves are more tightly
408 coupled to the diurnal activity of photosynthesis than the distal sinks of leaf sheath
409 and root. The perennial shows a greater TNC differential than the annual in leaves at

400 and 1500 µmol mol^{-1} CO_2, yet there is little difference in TNC differentials in leaf sheath and root, across the CO_2 concentrations (Fig. 5). Therefore, whilst the basal pre-dawn level of TNC is higher in annuals (Fig. 4), the diurnal accumulation of TNC is greater in perennials for leaves at 400 and 1500 µmol mol^{-1} CO_2 (Fig. 5).

Perennial barley accumulates more free amino acids than the annual species

Free amino acids are an indicator of source limitation (Paul and Driscoll, 1997; Stitt and Krapp, 1999; Isopp *et al.*, 2000). A high free amino acid concentration or high amino acid:sucrose ratio reflects a surplus of available nitrogen for biosynthesis, since source limited plants lack sufficient carbon to use along with this nitrogen for growth and development. The perennial has a higher concentration of free amino acids than the annual (Fig. 6). In both annual and perennial, free amino acid concentration is highest at 180 µmol mol^{-1} CO_2, which implies a carbon source limitation, and decreases as [CO_2] increases (Fig. 6). Before dawn, amino acid concentration is 41% greater in the perennial than the annual at 180 µmol mol^{-1} CO_2, 127% greater in the perennial at 400 µmol mol^{-1} CO_2, and 12% greater in the perennial at 1500 µmol mol^{-1} CO_2 (Fig. 6A). Before dusk, amino acid concentration is 67% greater in the perennial than the annual at 180 µmol mol^{-1} CO_2, 47% greater in the perennial at 400 µmol mol^{-1} CO_2, and 64% greater in the perennial at 1500 µmol mol^{-1} CO_2 (Fig. 6B).

There was a highly significant organ x species x [CO_2] interaction for free amino acid concentration: $F_{(4,117)} = 9.3$, $p<0.001$. A similar trend for the two species and three CO_2 levels is seen for free nitrate (data shown in summary form in Fig. 8) and there

was also a significant organ x species x [CO_2] interaction for these data: $F_{(4,116)} = 6.9$, $p<0.001$. The perennial also has a higher free amino acid:sucrose ratio than the annual (Fig. 7), indicative of carbon source limitation. This ratio is higher pre-dawn since sucrose accumulates during the day, and decreases with [CO_2]; for leaves, there is a significant species x [CO_2] x time of day interaction: $F_{(1,70)} = 13$, $p<0.001$.

Metabolite data reveal source limitation in the perennial and sink limitation in the annual

Figure 8 synthesises the metabolite data, expressed as ratios relative to 400 µmol mol^{-1} CO_2, in each compartment (leaf, sheath and root), for each species and time of day. In general, the amount of each non-structural carbohydrate was lower at 180 µmol mol^{-1} and higher at 1500 µmol mol^{-1}, compared to 400 µmol mol^{-1} CO_2 (Fig. 8), with short- and long-chain fructans representing the major stores for carbon at elevated CO_2 (Fig. 8). In contrast, free nitrate, free amino acid and protein levels tended to show the opposite trend (especially for the annual, Fig. 8A,B). At 400 µmol mol^{-1}, growth in the annual shows strong evidence of sink limitation, shown by a high rate of photosynthesis (Fig. 2), high TNC accumulation (Fig. 4) and low amino acid concentration and amino acid:sucrose ratio – indicating that sufficient carbon skeletons are available for utilising available amino acids (Fig. 6, 7). At 180 µmol mol^{-1} CO_2, growth becomes more source limited, with lower carbohydrate and higher nitrate and amino acid concentrations compared to 400 µmol mol^{-1} (Figs. 4, 6, 8A,B), whilst at 1500 µmol mol^{-1} CO_2, growth becomes more sink limited, with higher carbohydrate and lower nitrate and lower amino acid concentrations (Figs. 4, 6, 8A,B). This trend is seen at both times of day, but is most pronounced before dawn

460 (Figs. 8A,B), since carbon skeletons and reductants from photosynthesis are
461 required to incorporate free nitrate into amino acids and to assimilate amino acids
462 into proteins. As a result, the levels of these metabolites decrease during the day as
463 carbohydrates build up. Although this trend is seen in all organ types, it is most
464 pronounced in leaves, where photosynthesis is strongly coupled to changes in
465 carbon and nitrogen metabolism.
466
467 In contrast to the annual, at 400 µmol mol^{-1} the perennial shows strong evidence of
468 source limitation, having a lower rate of photosynthesis than the annual (Fig. 2), low
469 TNC accumulation (Fig. 4) and high amino acid concentrations and amino
470 acid:sucrose ratio (Figs. 6, 7). At 180 µmol mol^{-1} CO_2, the perennial remains source
471 limited, so levels of free nitrate and amino acids generally do not increase relative to
472 400 µmol mol^{-1} (Fig. 8C,D). Just as the perennial shows a greater response of
473 tillering and root allocation (Table 1) and photosynthesis (Fig. 2B) than the annual
474 between 400 and 1500 µmol mol^{-1} CO_2, as this alleviates source limitation, it also
475 shows a more dramatic decrease in free amino acids and amino acid:sucrose (Figs.
476 6, 7) as it is better able than the annual to pair additional sugars from photosynthesis
477 with existing free amino acids to bring about a growth response. However at 1500
478 µmol mol^{-1}, growth in the perennial transitions to become sink limited, and the plants
479 have a high carbohydrate content, and low nitrate and low amino acid concentrations
480 (Figs. 4, 6, 8A,B). Thus the treatments imposed are sufficiently strong that even the
481 annual becomes more source limited at low [CO_2], and even the perennial becomes
482 more sink limited at elevated [CO_2].
483
484

DISCUSSION

Developmental plasticity in the perennial enables extra CO_2 to be utilised in growth, suggesting source limitation

Increasing [CO_2] increases the availability of photosynthetic substrate and suppresses photorespiration (Farquhar *et al.*, 1980). This increases the potential rate of carbon uptake into the plant, increasing source strength, alleviating source limitation, and increasing the source:sink ratio. Conversely, decreasing [CO_2] has the opposite effects. The stronger photosynthetic, tillering and root partitioning responses of perennial than annual barley to increasing [CO_2] (Table 1; Fig. 2) suggest that the source is more limiting for growth than the sink in this species during the vegetative stage. This response is not seen to such a great extent in the annual, suggesting that its growth is primarily sink limited and constrained by developmental potential; as a consequence the annual is operating at near-maximum source activity under current ambient conditions (400 µmol mol^{-1} CO_2). The ratio of photosynthesis to growth is higher in annual barley (Fig. 3), a further indication of sink limitation, and increases at higher [CO_2] and as plants become older and leave the exponential phase of growth. Furthermore, the developmental plasticity seen in the perennial, via its ability to increase tillering and root partitioning in response to greater carbon source strength, suggests that it is better able than the domesticated annual crop to adapt to fluctuating environmental conditions. In general, selective breeding of crops has resulted in plants with fewer tillers because, although additional non-flowering tillers provide a selective advantage through competition in wild plants, they reduce the yield of crop stands by diverting resources away from flowering tillers. To an extent,

510 domesticated barley has retained its tillering capacity (Doust, 2007; Sang, 2009).
511 However, under experimental conditions, the perennial barley was far readier to
512 increase tillering in response to increased [CO_2] than the annual crop.
513
514 Altering the root:shoot ratio enables plants to increase access to the most limiting
515 resources by adjusting allocation to nitrogen- or carbon-acquiring tissues (Stitt and
516 Krapp, 1999; Freschet *et al.*, 2015). Under elevated [CO_2], nitrogen becomes more
517 limiting for growth, making an increase in root:shoot ratio advantageous. The
518 perennial was better able to make this plastic adjustment to growth (Table 1).
519 However, its greater relative increase in allocation to roots (Table 1) would have also
520 tended to offset its growth response, since roots are heterotrophic and root
521 respiration represents a significant carbon sink. This greater allocation to a
522 respiratory carbon sink may explain why the perennial still showed a similar increase
523 in RGR to the annual at higher CO_2 levels (Fig. 1). In combination, these results
524 suggest that the combined response of sink strength (growth and respiration) to
525 [CO_2] was stronger in the perennial than annual. Increasing root allocation enabled
526 the perennial to take up more nitrogen, further increasing its ability to match carbon
527 skeletons with amino acids for growth.
528
529 Our findings suggest a more opportunistic growth strategy in the perennial than
530 annual, whereby the use of additional resources is maximised via partitioning into
531 more branches above ground and roots below ground. In contrast, the annual
532 appears to be highly constrained in its ability to develop larger sinks at 400 µmol mol^{-1}
533 CO_2 (Table 1; Fig. 2), and unable to increase these to the same extent as the
534 perennial. It thus seems that the strategy of the annual is for maximal growth under

current [CO_2] – and as a result it is sink limited. The annual has been subjected to intense selective breeding that has maximised growth under current ambient CO_2 conditions, but suppressed its developmental plasticity, and growth during the vegetative phase is largely unresponsive to increased [CO_2].

The annual accumulates carbohydrates whilst having low amino acids, suggesting carbon sink limitation

The metabolite data reinforce the pattern of source limitation in the perennial and sink limitation in the annual seen in the growth and photosynthesis data. The annual has higher TNC concentration, and lower amino acid concentration and amino acid:sucrose ratio than the perennial, indicating an excess of carbon that cannot be invested in growth (Figs. 4, 6, 7, 8). Although many studies into the relationship between amino acid accumulation and carbon source limitation have focused on a single species (Paul and Driscoll, 1997; Isopp *et al.*, 2000), the use of the amino acid:sucrose ratio, which is a more robust measurement, confirms the trend seen for free amino acids, and is one of several lines of evidence pointing towards greater carbon source limitation in the perennial. The lower basal level of TNC in the perennial (Fig. 4) suggests that this species is highly efficient at utilising the carbon acquired each day – by developing new sinks or enlarging existing ones, seen in the strong tillering response to elevated [CO_2] (Table 1), or by increasing TNC storage in the leaf sheath (Fig. 4). Developing new sinks such as tillers increases sink size, whilst increasing storage in existing sink organs increases sink activity; both enable the plant to upregulate its sink capacity (Geiger and Shieh, 1993; White *et al.*, 2016). The high rate of tillering and root allocation in the perennial translates to a higher sink

capacity and high demand for photosynthate which could explain the high accumulation of carbohydrates in these organs. As a consequence, the large quantity of leaf carbohydrates accumulated during the day are likely to be exported to developing tillers or other sinks, in addition to the carbon sink of maintenance respiration at night; in future work the use of isotopic CO_2 in a series of staged harvests could enable diurnal carbon utilisation to be tracked (e.g. Ferrieri *et al.*, 2013).

Both species are carbon sink limited at elevated [CO_2]; leaf sucrose is a key driver of phloem loading for photosynthate export (Ainsworth and Bush, 2011), yet the increase in TNC at elevated [CO_2] seen here is primarily driven by increases in storage carbohydrates (fructans and starch, Fig. 8) and not transport carbohydrates (sucrose). This provides evidence that the carbohydrate accumulation at elevated [CO_2] arises from sink limitation rather than reflecting the increased phloem loading of recent photosynthate. Indeed, the increased accumulation of carbohydrates will feed back on phloem transport throughout the plant and phloem loading in the leaf (Ainsworth and Bush, 2011), and high foliar TNC concentration is thus an indicator of sink limitation in both species. The fact that TNC does not accumulate in roots of the perennial under elevated CO_2 suggests that carbon transport may be more limiting in this species.

It is interesting to note that the negative correlation between starch and biomass observed in a range of accessions of *Arabidopsis* (Sulpice *et al.*, 2009) is not borne out by the data of this study – rather, the fast-growing annual species has a higher rate of carbohydrate accumulation despite having greater biomass. However, the

585 physiology and metabolism of *Arabidopsis* do not always map onto those of crop
586 plants (White *et al*., 2016), for example the relationship between protein and starch
587 found by Sulpice *et al*. (2009) was uncoupled in these data. Growth in plants with
588 different life forms and life histories may be subject to different constraints; in slow
589 growing *Arabidopsis* accessions, growth is slow because it is sink limited, whereas in
590 perennial barley, growth is slower than the annual because it is source limited and
591 therefore uncorrelated with carbohydrate content.
592
593 **Ecological strategies and intrinsic limits to growth**
594
595 The typical growth strategy of wild annual plants can be caricatured as 'live fast, die
596 young', leading to the expectation of a growth strategy that is primarily source limited
597 during the lifetime of the plant, and that enables the annual to maximise the use of
598 available CO_2 for growth. We therefore expected the annual to be less sink limited
599 than the perennial during vegetative growth, especially since it is adapted for
600 fertilised soils. In contrast, we expected the perennial to have a more conservative
601 growth habit, 'live slow, live long', which limits photosynthesis and growth but is
602 opportunistic, being better adapted for the possibility of low nutrients in a variable
603 environment yet able to capitalise on rising [CO_2] by increasing storage when
604 substrates are available. Although plants are typically sink limited during the
605 vegetative stage and transition to source limitation at reproduction (Arp, 1991), many
606 crops are co-limited by sinks and sources during grain-filling (Álvaro *et al*., 2008;
607 Acreche and Slafer, 2009; Peterhansel and Offermann, 2012; Slewinski, 2012). We
608 anticipated that during the vegetative stage, the 'live slow' perennial would be more

609 sink limited than the ‘live fast’ annual (Jaikumar *et al.*, 2014). The results confounded
610 these expectations.
611
612 The perennial adopts more of a ‘live fast’ strategy than anticipated; perennials
613 generally store carbon for future use (Atkinson *et al.*, 2012), yet here the perennial
614 showed a dramatic increase in growth under elevated [CO_2] rather than an increase
615 in storage, indicating source limitation. Coming from a fluctuating natural
616 environment, and being able to grow in a variety of habitats including roadsides,
617 ditches and rich grassy meadows and at varying altitudes (von Bothmer, 1996), this
618 species has the plasticity to maximise growth when CO_2 is abundant. However, a
619 perennial confined to unproductive habitats might be expected to display slower
620 growth and greater sink limitation.
621
622 Although the perennial displays a greater response to [CO_2] for photosynthetic rate
623 per unit leaf area and leaf TNC concentration, even the maximal values at 1500 µmol
624 mol^{-1} CO_2 never exceed those of the annual, implying intrinsic physiological or
625 developmental limits that are common to both species. The annual is unable to
626 utilise more photosynthate than it acquires at 400 µmol mol^{-1} CO_2 by increasing
627 partitioning to tillers and roots; the perennial has greater developmental flexibility and
628 is able to utilise the additional photosynthate acquired at the highest CO_2
629 concentration, but never exceeds the maximum rates of growth and photosynthesis
630 seen in the annual (Figs. 1, 2).
631
632 The developmental plasticity of the annual species appears to have been altered
633 through selective breeding such that it cannot adapt to live faster when conditions

allow, and the results of this study show that it is sink limited during vegetative growth even under elevated [CO_2], in addition to being sink limited during reproduction (Schnyder, 1993; Bingham *et al.*, 2007; Serrago *et al.*, 2013). It thus seems that the sink strength of barley will limit yield of this important crop in the current global context of rising atmospheric [CO_2], and a concerted effort to increase sink strength would be a vital part of breeding programmes in order to increase yield.

CONCLUSIONS

Contrary to expectations these results indicate that annual barley is more sink limited and perennial barley is more source limited during the vegetative growth stage. Our findings show that annual barley germplasm is optimised for growth at current [CO_2] and that future elevated [CO_2] may be unlikely to facilitate yield increases in this species; the lack of developmental plasticity in the annual means that new sinks are not readily initiated, which could result in a critical lack of flexibility for developing additional grain sinks and thus increasing yield under elevated [CO_2]. The holistic approach taken here enables a broad view of source-sink balance to be taken, encompassing measurements of resource acquisition, storage, allocation to growth, and plant development, in a model system of congeneric species. In order to draw firm conclusions of agricultural relevance, it will be vital to extend such research: including nitrogen as well as carbon source-sink manipulations, following source-sink processes throughout crop development to their impact on yield; investigating these processes in a wider range of cereal varieties and wild species; and carrying out agronomically relevant experiments in the field.

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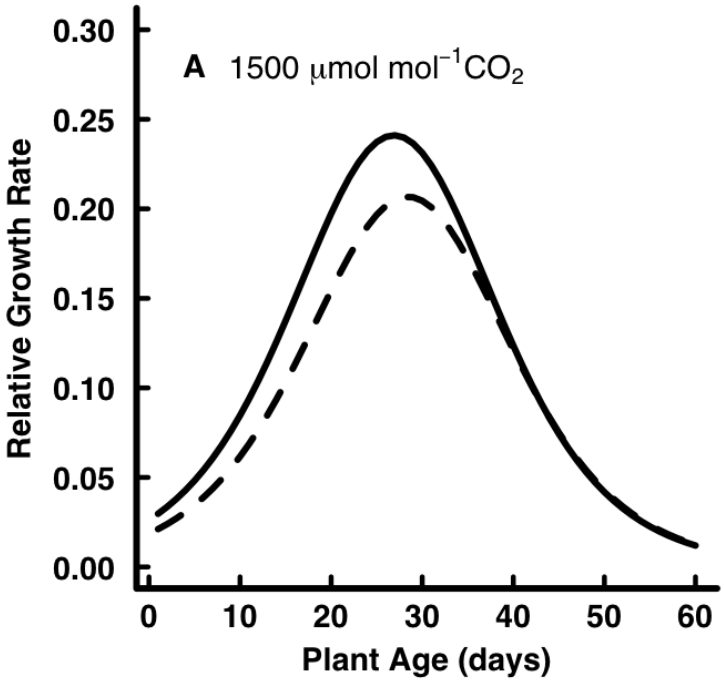
FIGURES & TABLES

Figure 1. Relative growth rate is higher in annual (solid line) than perennial (dashed line) barley, and greater at higher [CO_2]. Relative growth rate (RGR) is daily gain in dry mass relative to whole plant dry mass, g g^{-1} day^{-1}. A, elevated [CO_2]: 1500 µmol mol^{-1}; B, current [CO_2]: 400 µmol mol^{-1}; C, sub-ambient [CO_2]: 180 µmol mol^{-1}.



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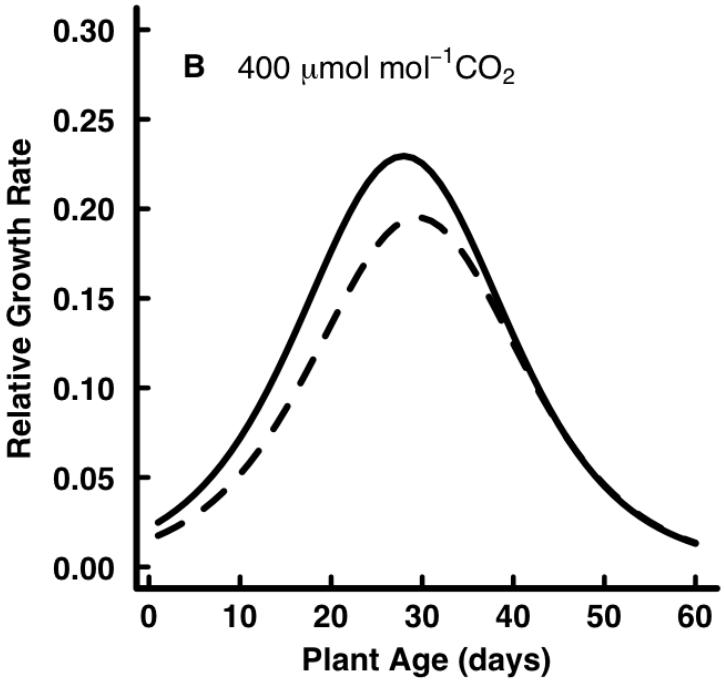

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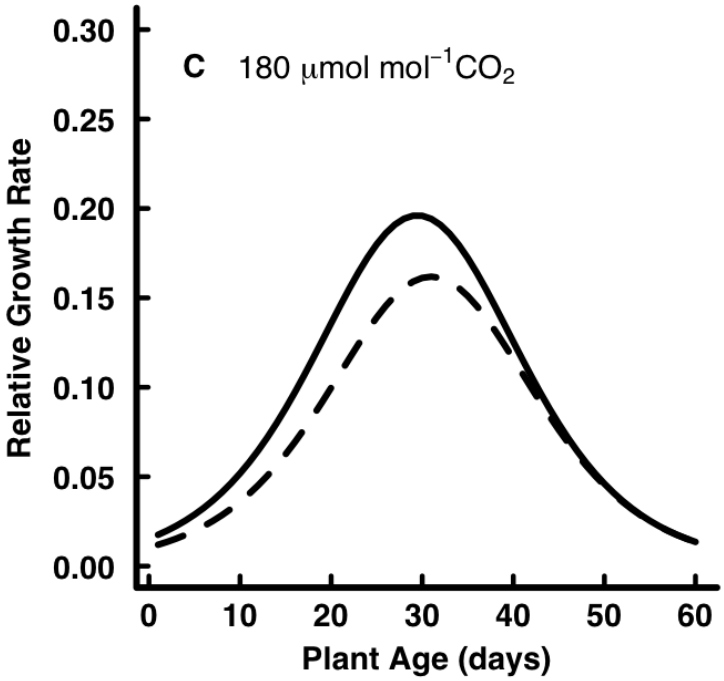

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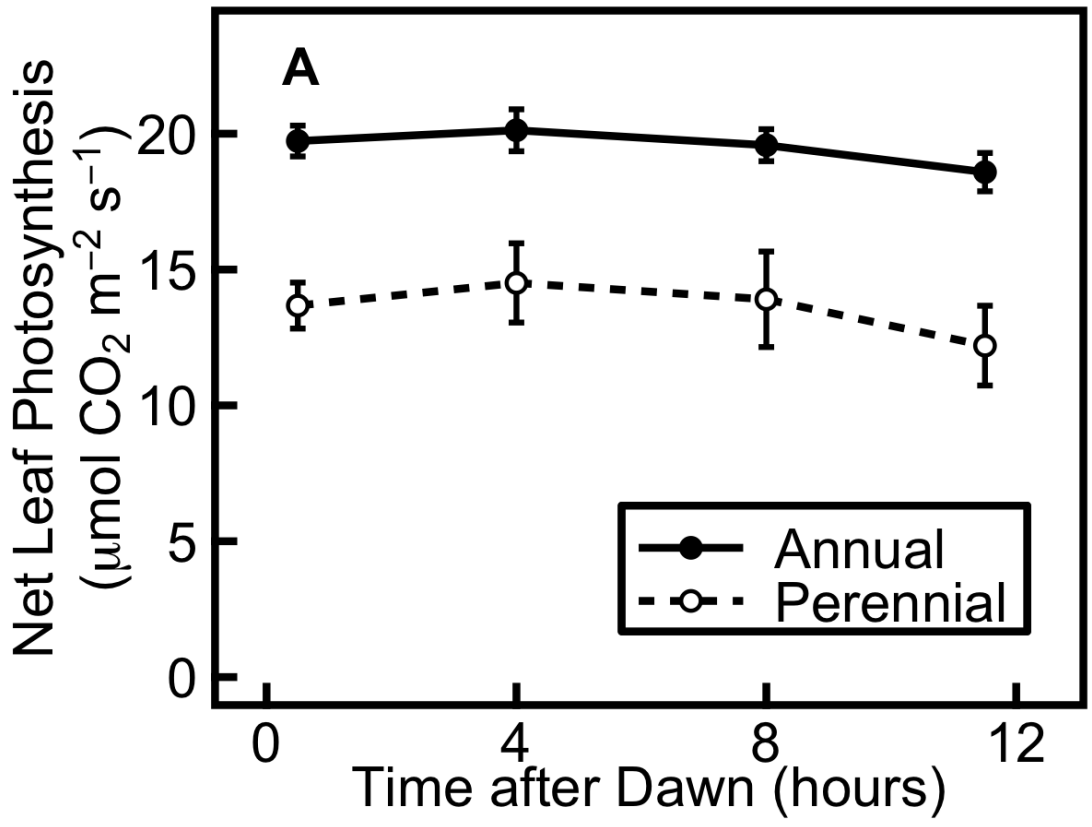
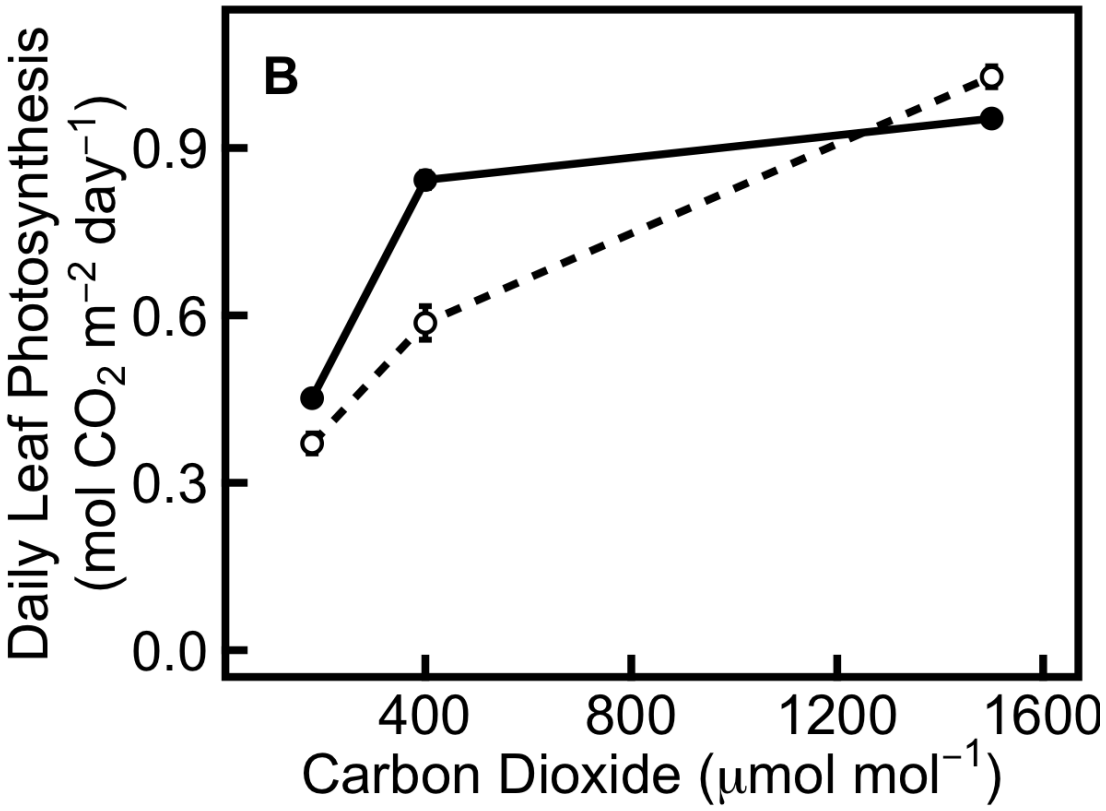
Table 1. Responses of tillering and root:shoot ratio to increasing [CO_2]. Annual barley shows very limited tillering and root:shoot ratio responses to increasing CO_2 concentration, whilst the perennial shows a dramatic increase in tillering and a significant increase in root:shoot ratio. Data shown are obtained from 54 annual and 36 perennial individuals across the three treatments. Tillers were counted directly, whilst root:shoot ratio was estimated non-destructively using imaging. Means and their associated standard errors (S.E.) are reported to three significant figures (annual n=18, perennial n=12).

	Annual, 180 µmol mol^{-1} CO_2	Annual, 400 µmol mol^{-1} CO_2	Annual, 1500 µmol mol^{-1} CO_2	Perennial, 180 µmol mol^{-1} CO_2	Perennial, 400 µmol mol^{-1} CO_2	Perennial, 1500 µmol mol^{-1} CO_2
Tillers (mean)	**13.3**	**13.4**	**15.3**	**12.4**	**17.4**	**32.6**
Tillers (S.E.)	0.753	0.506	0.676	1.275	1.341	2.464
Root:Shoot Ratio (mean)	**0.529**	**0.544**	**0.547**	**0.466**	**0.520**	**0.542**
Root:Shoot Ratio (S.E.)	0.00321	0.00190	0.00187	0.0174	0.00768	0.00309

Figure 2. Perennial barley (dashed line) has a more pronounced photosynthetic response to elevated [CO_2] than annual barley (solid line). A, diurnal timecourse of net leaf photosynthesis in annuals and perennials grown at 400 µmol mol^{-1} CO_2. B, daily rate of net leaf photosynthesis per unit area obtained from integrating curves (e.g. A); C, total daily photosynthesis in the whole shoot, obtained by multiplying the daily rate (B) by projected shoot area. Data show mean ± SE (A: annual n=18, perennial n=9; B: at 180, 400, 1500 µmol mol^{-1} CO_2, annual n=15, 18, 18, perennial n=5, 9, 12; C: at 180, 400, 1500 µmol mol^{-1} CO_2, annual n=15, 18, 18, perennial n=4, 9, 12).




B
Daily Leaf Photosynthesis
(mol CO2 m−2 day−1)
0.0
0.3
0.6
0.9
400
800
1200
1600
Carbon Dioxide (μmol mol−1)

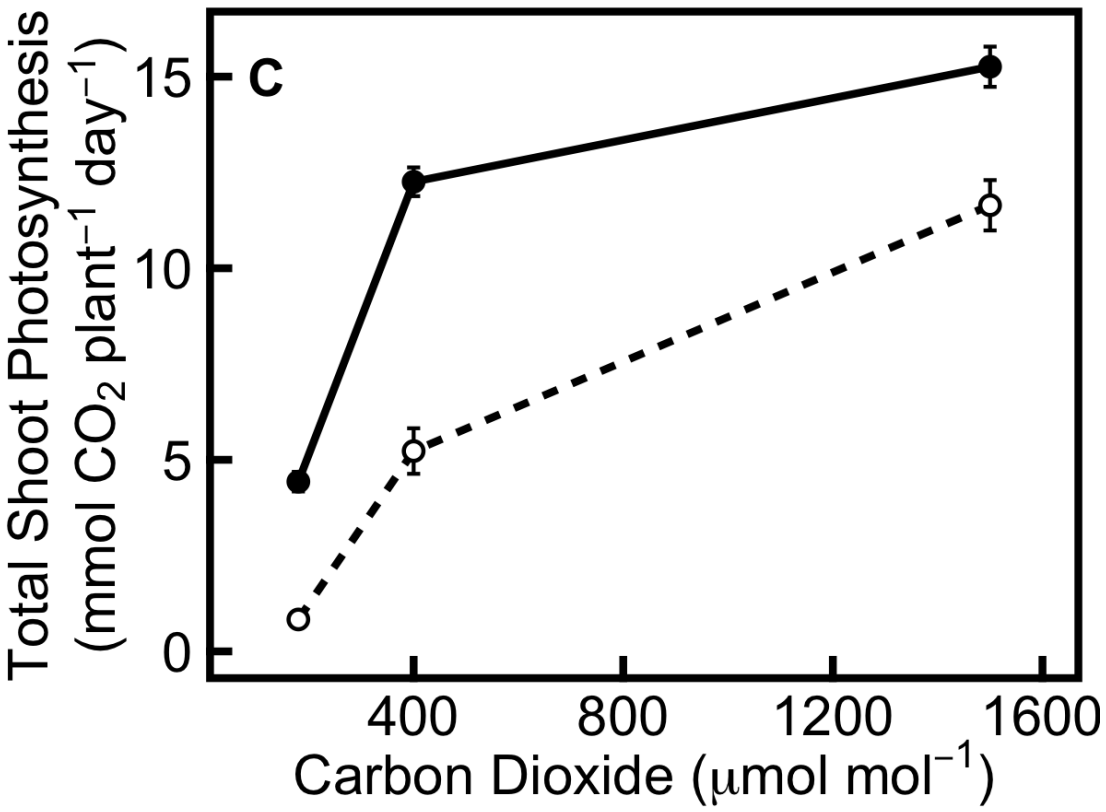
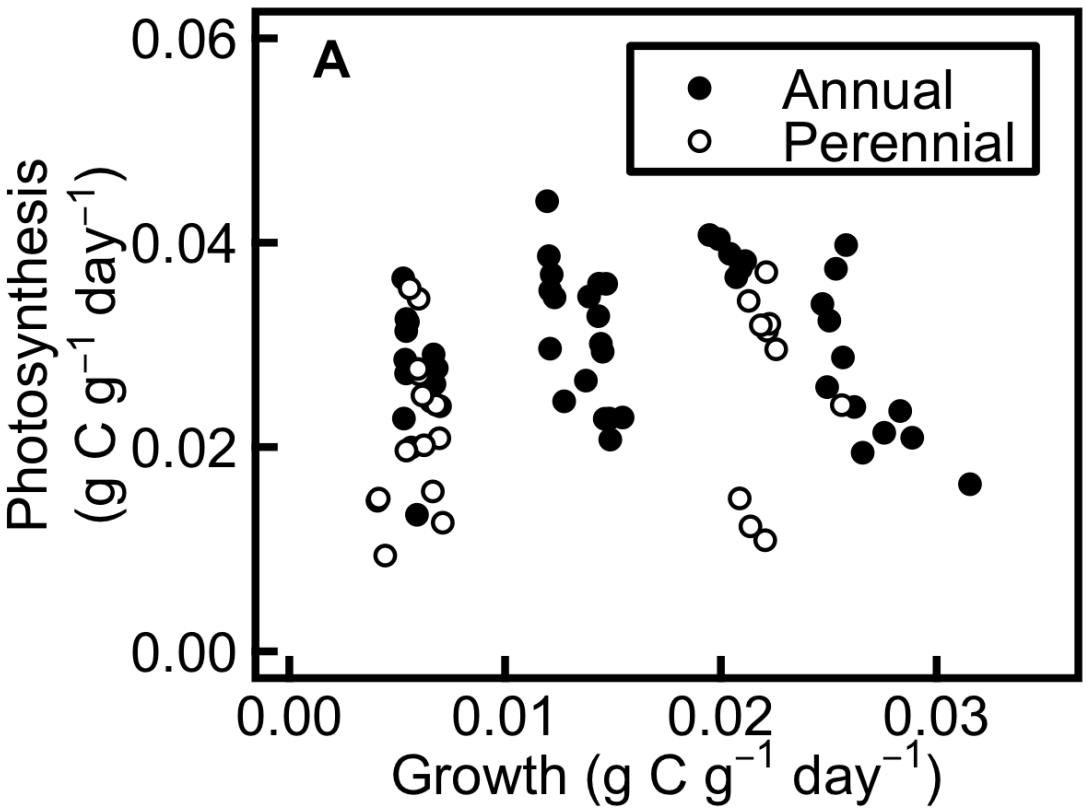
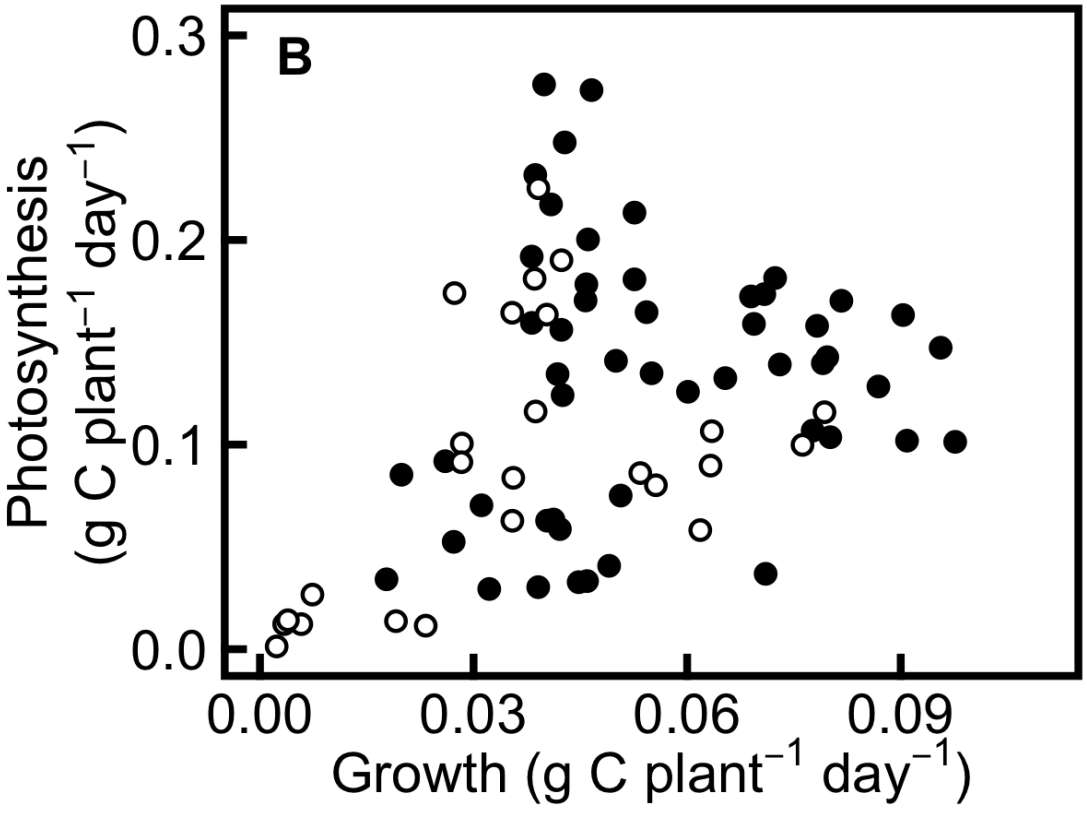

C
Total Shoot Photosynthesis
(mmol CO2 plant−1 day−1)
0
5
10
15
400
800
1200
1600
Carbon Dioxide (μmol mol−1)

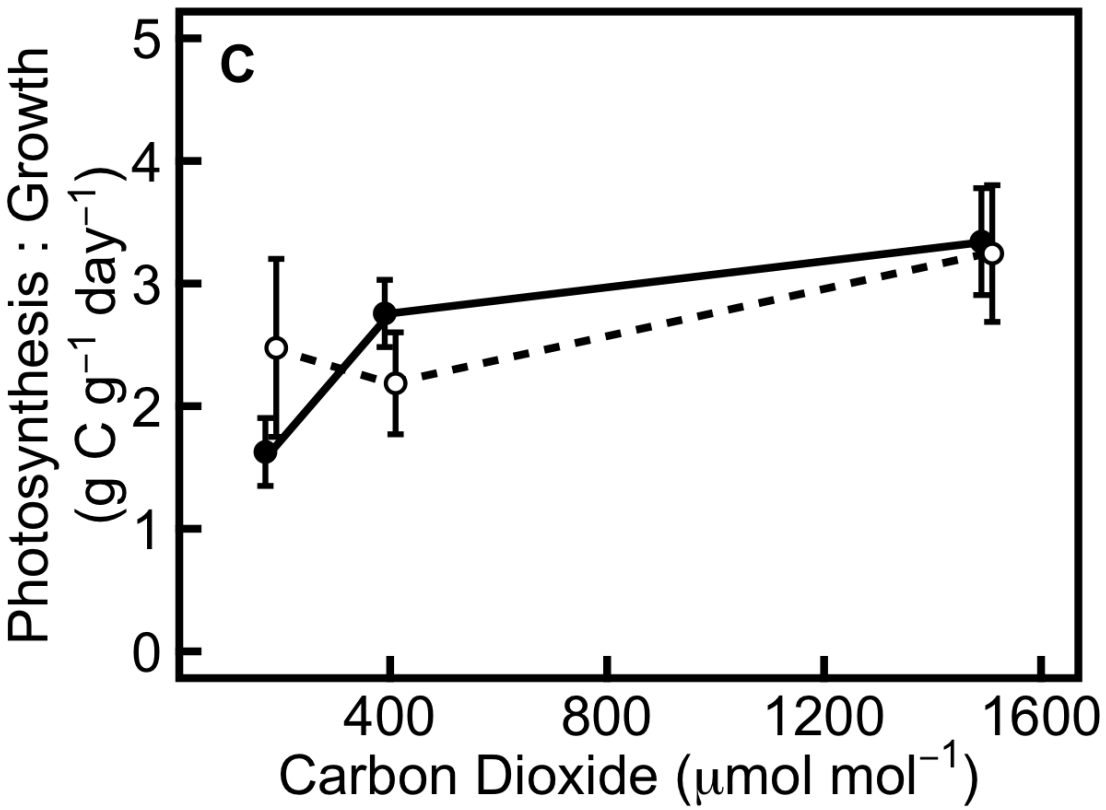
Figure 3. The ratio of photosynthesis to growth is higher in annual than perennial barley and greater at higher [CO_2] and in older plants. A, source activity vs sink activity, plotted as photosynthesis and growth for individual plants at all times and CO_2 levels, expressed in g C g^{-1} day^{-1}, showing three clusters along the x-axis corresponding to the three harvest times with RGR decreasing as time progresses; B, source strength vs sink strength, plotted as photosynthesis and growth for individual plants at all times and CO_2 levels expressed in g C $plant^{-1}$ day^{-1}; C, photosynthesis:growth ratio in the three [CO_2] treatments; D, changes in the photosynthesis:growth ratio with respect to the mean plant age at harvest. Data show mean ± SE (C and D: at 180, 400, 1500 µmol mol^{-1} CO_2, annual n=15, 18, 18, perennial n=4, 9, 12).


A
Annual
Perennial
Photosynthesis
(g C g^{-1} day^{-1})
0.06
0.04
0.02
0.00
0.00
0.01
0.02
0.03
Growth (g C g^{-1} day^{-1})

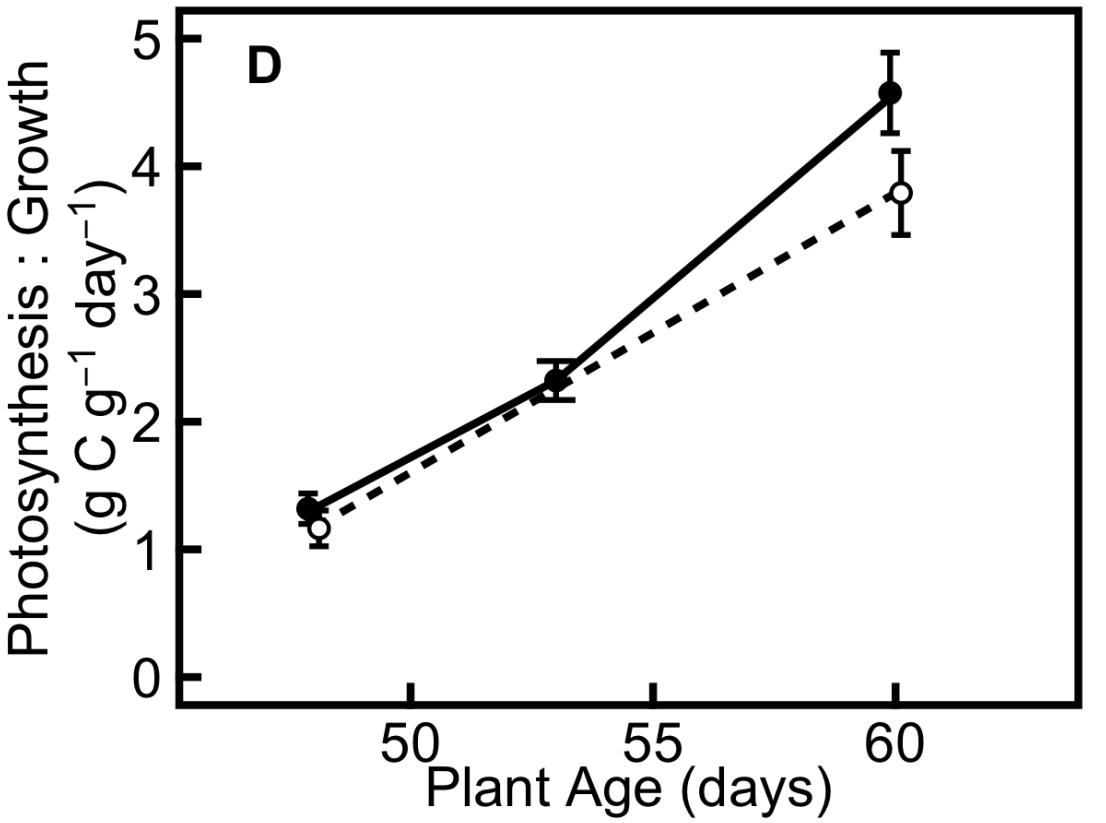
908


B
Photosynthesis
(g C plant^{-1} day^{-1})
0.3
0.2
0.1
0.0
0.00
0.03
0.06
0.09
Growth (g C plant^{-1} day^{-1})

909

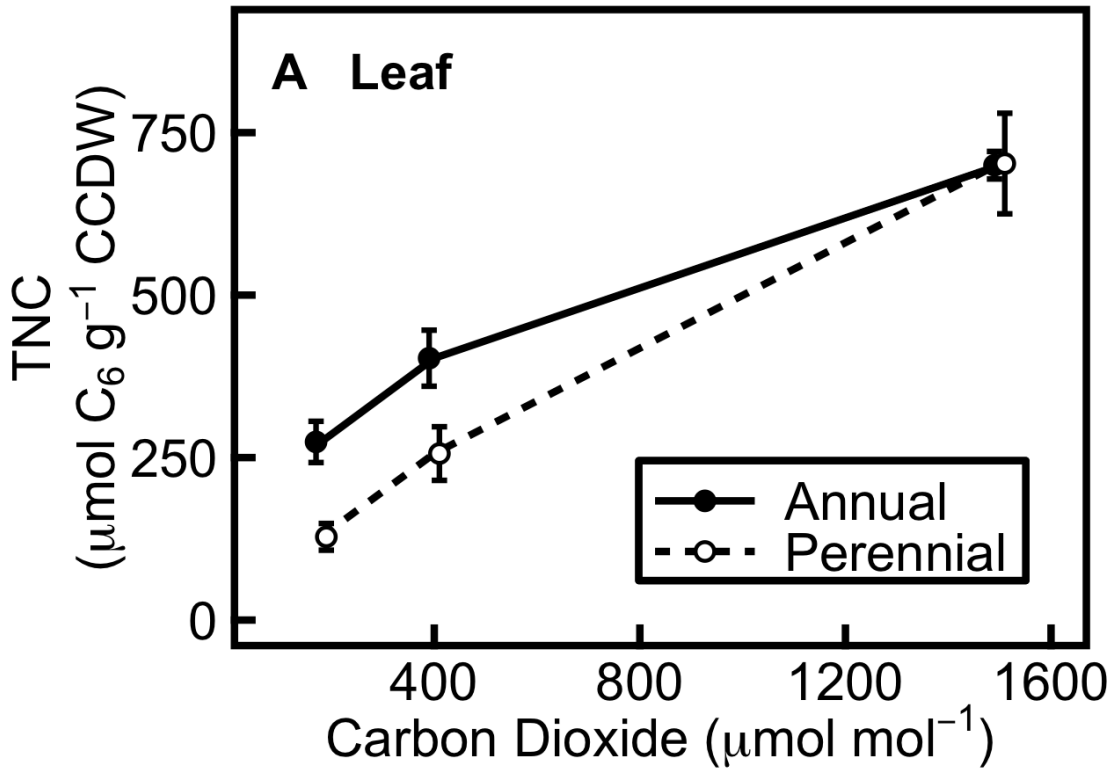

C
Photosynthesis : Growth
(g C g^{-1} day^{-1})
0
1
2
3
4
5
400
800
1200
1600
Carbon Dioxide (μmol mol^{-1})

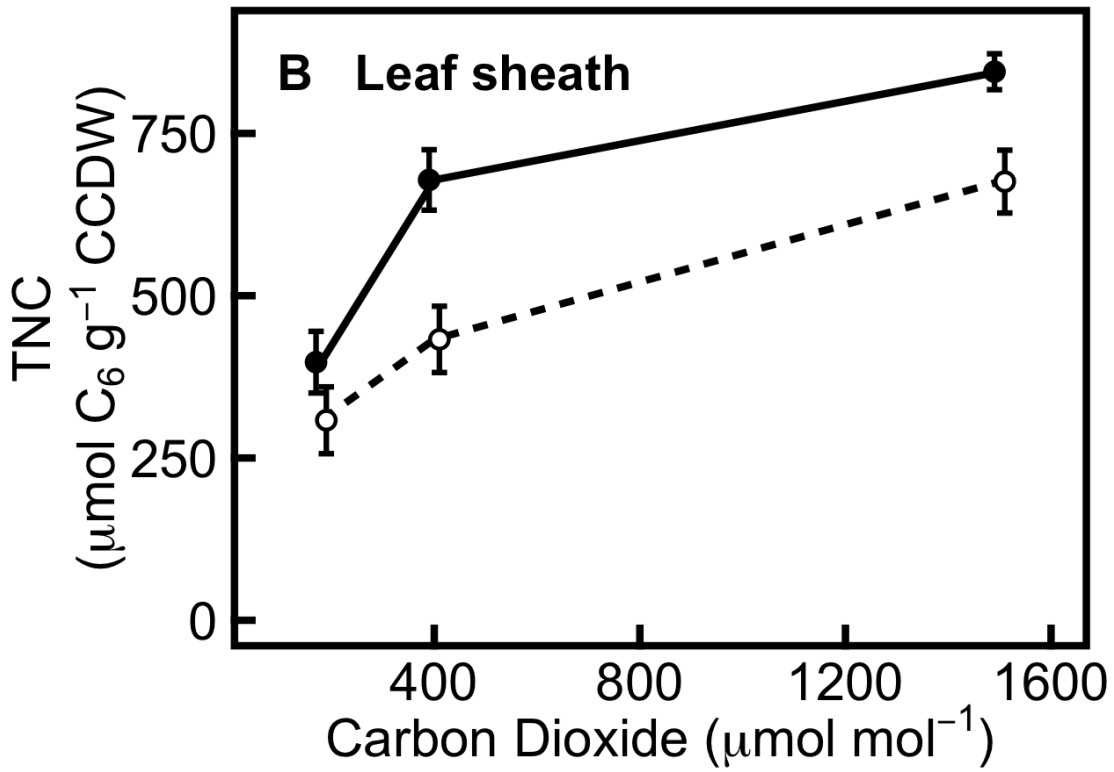
910


D
Photosynthesis : Growth
(g C g^{-1} day^{-1})
0
1
2
3
4
5
50
55
60
Plant Age (days)

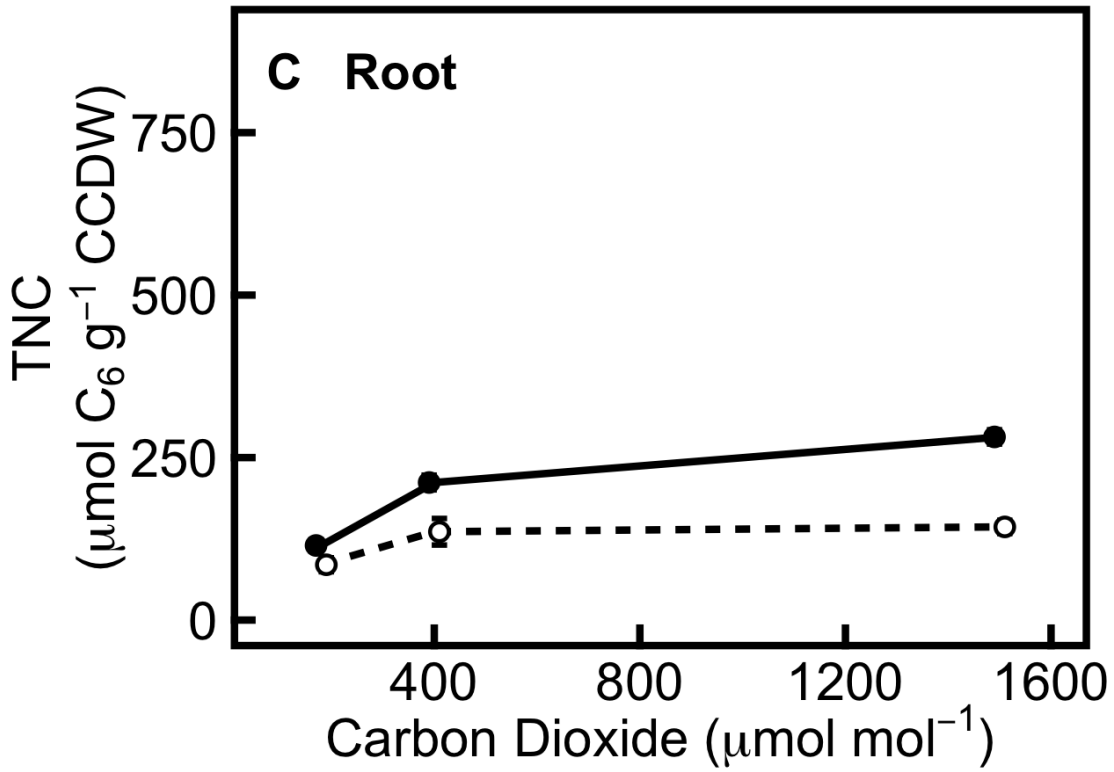
911

Figure 4. Pre-dawn concentrations of total non-structural carbohydrates (TNC) are higher in the annual (solid line) than perennial (dashed line) barley. A, leaf; B, leaf sheath; C, root. The overall CO_2 response is greater for perennials in the leaf, but greater for annuals in the leaf sheath and root. Data are expressed in µmol glucose equivalents per g carbohydrate-corrected dry weight (CCDW). Data show mean ± SE (annual n=9, perennial n=6).

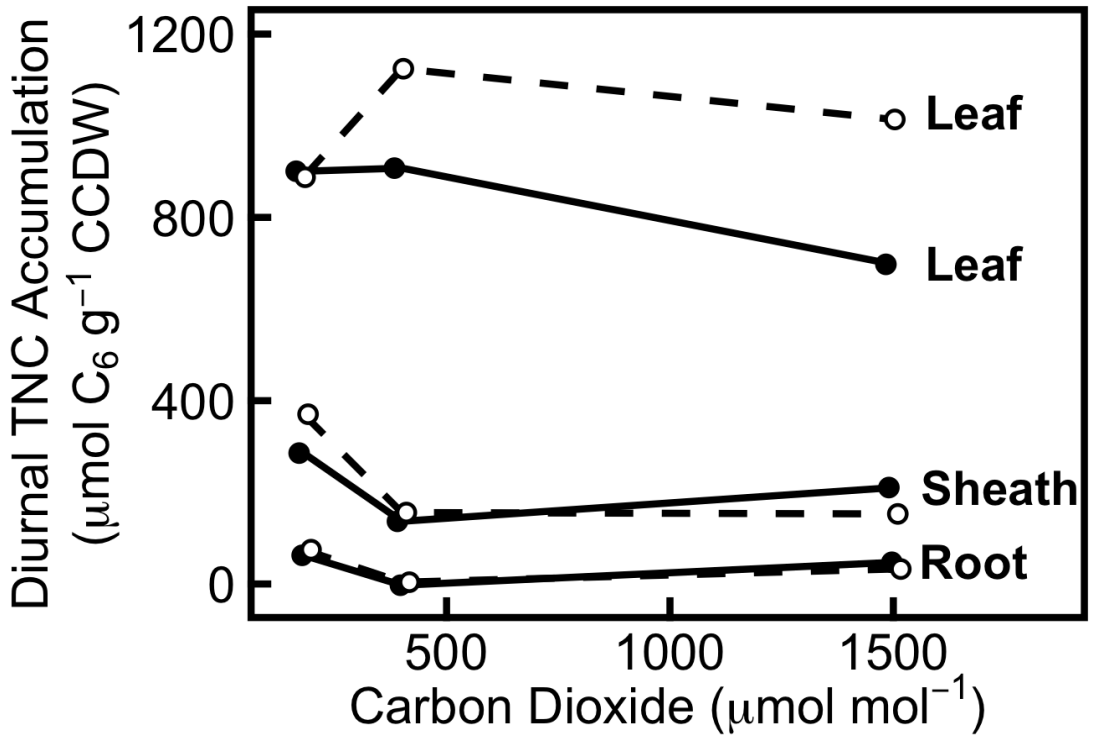



B Leaf sheath
TNC
(μmol C6 g−1 CCDW)
750
500
250
0
400
800
1200
1600
Carbon Dioxide (μmol mol−1)

927

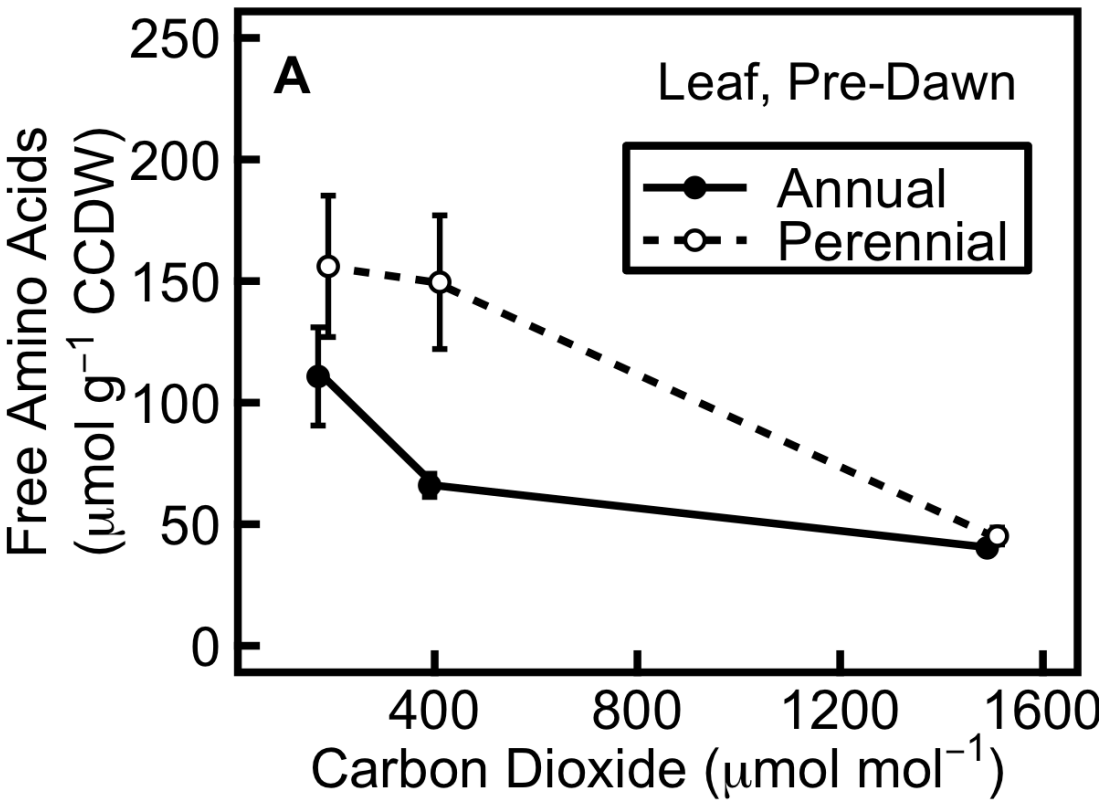

C Root
TNC
(μmol C6 g−1 CCDW)
750
500
250
0
400
800
1200
1600
Carbon Dioxide (μmol mol−1)

928 **Figure 5.** The diurnal accumulation of total non-structural carbohydrates (TNC),
929 equivalent to the carbon pool available for nocturnal use or export in the leaf, leaf
930 sheath and root, in annual barley (solid line) and perennial barley (dashed line). Data
931 show the mean pre-dawn concentrations subtracted from mean pre-dusk
932 concentrations, expressed in µmol glucose equivalents per g carbohydrate-corrected
933 dry weight (CCDW). Different plants were harvested at dawn and dusk, so standard
934 errors cannot be calculated for these data (raw data presented in Table S1).
935
936
937
938
939

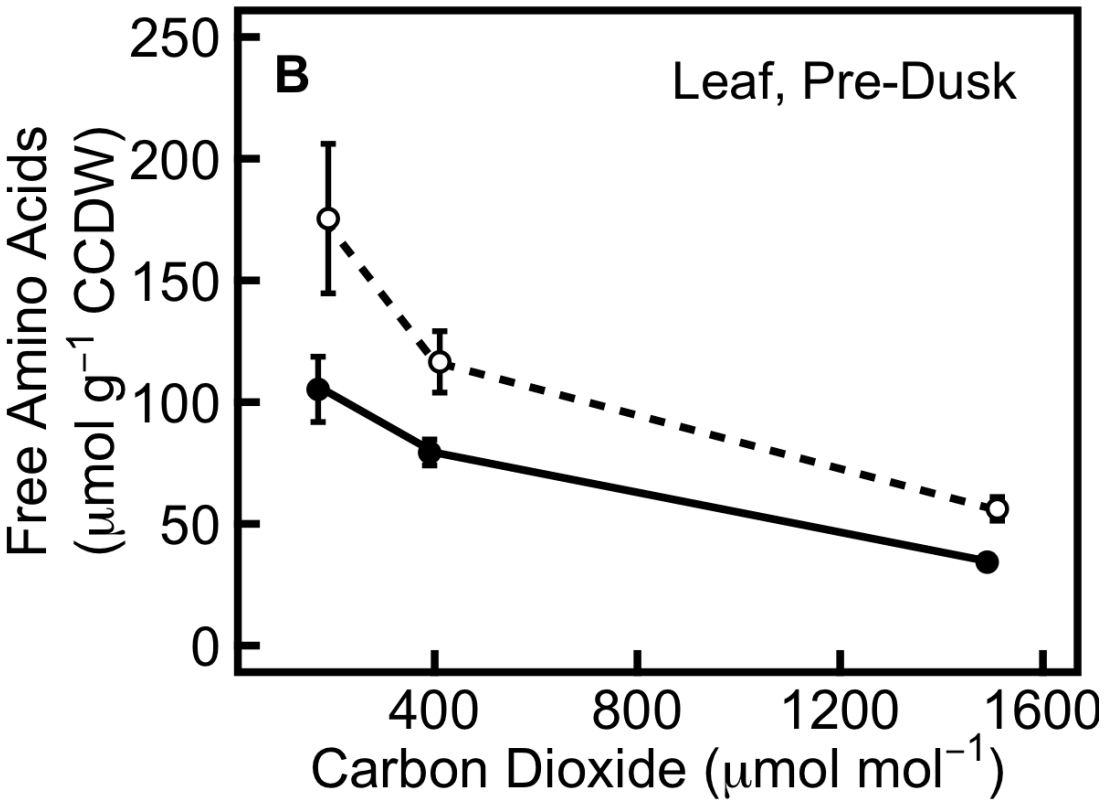


940
941
942
943

Figure 6. Free amino acid concentration is higher in perennial barley (dashed line) than annual barley (solid line). A, pre-dawn; B, pre-dusk. Data are expressed in µmol amino groups per g carbohydrate-corrected dry weight (CCDW). Data show mean ± SE (annual n=9, perennial n=6).



963

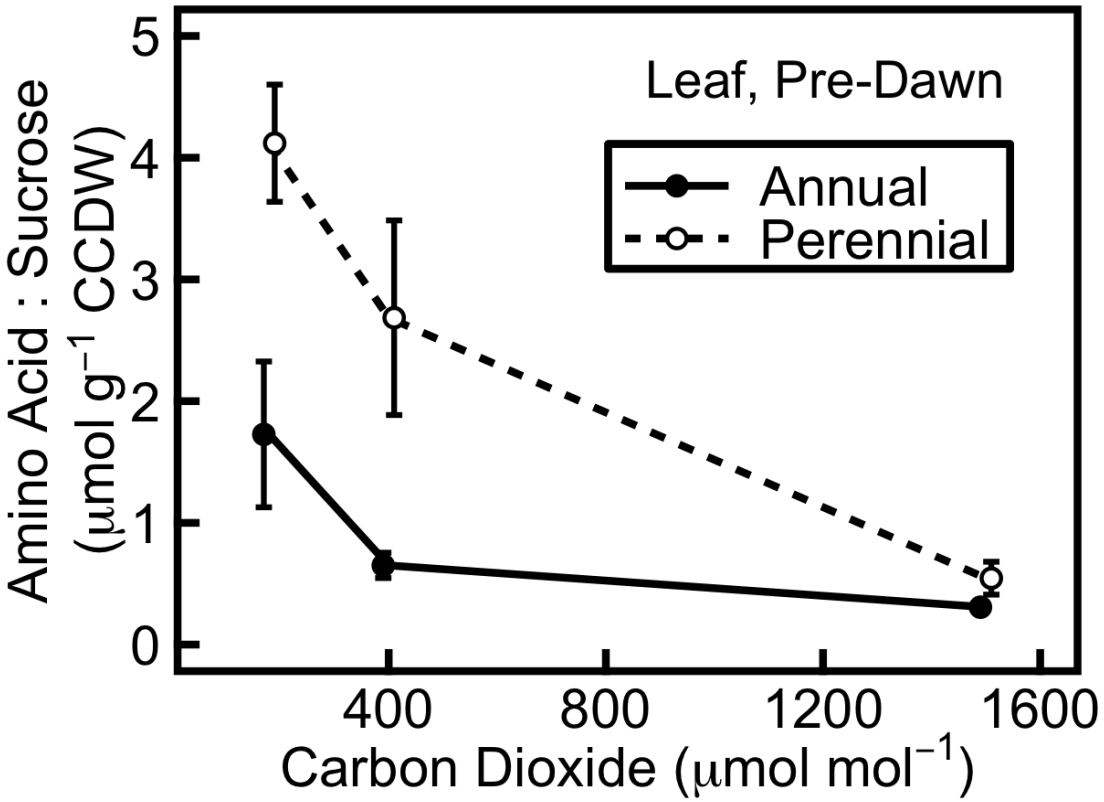


964

965

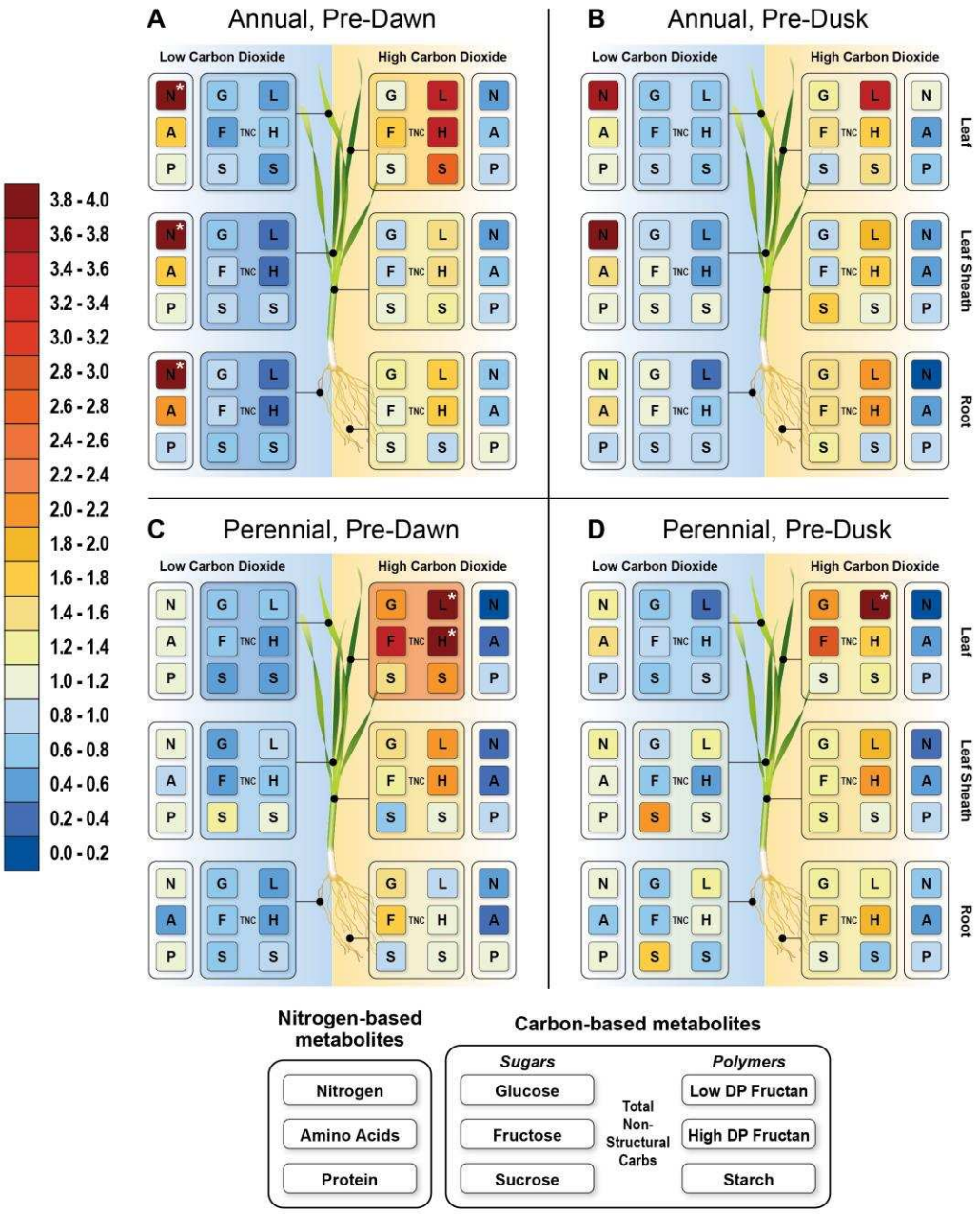
966

967 **Figure 7.** Ratio of free amino acids to free sucrose is higher in perennial barley
968 (dashed line) than annual barley (solid line) at 180 and 400 µmol mol^{-1} CO_2 in leaves
969 pre-dawn. This is an indicator of carbon source limitation. Metabolites are
970 expressed in µmol amino groups and µmol sucrose per g carbohydrate-corrected dry
971 weight (CCDW), respectively. The ratio is lower at higher [CO_2]. Data show mean ±
972 SE (annual n=9, perennial n=6).
973



974

Figure 8. Carbon- and nitrogen-based metabolites in annual and perennial barley at pre-dawn and pre-dusk harvests. Data are ratios of metabolite concentrations in leaf, leaf sheath and root of annual and perennial barley at 180 relative to 400 µmol mol^{-1} CO_2, and 1500 relative to 400 µmol mol^{-1} CO_2. Blue denotes a decrease and orange/red an increase compared to 400 µmol mol^{-1}, according to the colour scale on the left; a ratio of 1 signifies no change. Low and high DP refer to the degree of polymerisation in short- and long-chain fructans respectively. A, Annual pre-dawn; B, Annual pre-dusk; C, Perennial pre-dawn; D, Perennial pre-dusk. Exceptionally high ratios, indicated by asterisks, are as follows: annual pre-dawn (A) at 180 µmol mol^{-1} CO_2, nitrate in leaf is 6.5x concentration at 400 µmol mol^{-1}, nitrate in leaf sheath is 7.7x concentration at 400 µmol mol^{-1} and nitrate in root is 5.5x concentration at 400 µmol mol^{-1}; perennial pre-dawn (C), in leaf at 1500 µmol mol^{-1} CO_2, low DP fructan is 23.4x concentration at 400 µmol mol^{-1} and high DP fructan is 6.3x concentration at 400 µmol mol^{-1}; perennial pre-dusk (D), in leaf at 1500 µmol mol^{-1} CO_2, low DP fructan is 4.8x concentration at 400 µmol mol^{-1}.


A Annual, Pre-Dawn
B Annual, Pre-Dusk
C Perennial, Pre-Dawn
D Perennial, Pre-Dusk
Low Carbon Dioxide
High Carbon Dioxide
Leaf
Leaf Sheath
Root
3.8 - 4.0
3.6 - 3.8
3.4 - 3.6
3.2 - 3.4
3.0 - 3.2
2.8 - 3.0
2.6 - 2.8
2.4 - 2.6
2.2 - 2.4
2.0 - 2.2
1.8 - 2.0
1.6 - 1.8
1.4 - 1.6
1.2 - 1.4
1.0 - 1.2
0.8 - 1.0
0.6 - 0.8
0.4 - 0.6
0.2 - 0.4
0.0 - 0.2
Nitrogen-based metabolites
Nitrogen
Amino Acids
Protein
Carbon-based metabolites
Sugars
Glucose
Fructose
Sucrose
Total Non-Structural Carbs
Polymers
Low DP Fructan
High DP Fructan
Starch

995