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Original Article

Transcriptional reprogramming and stimulation of leaf respiration by elevated CO_2 concentration is diminished, but not eliminated, under limiting nitrogen supply

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ABSTRACT

Plant respiration responses to elevated CO_2 concentration ($[CO_2]$) have been studied for three decades without consensus about the mechanism of response. Positive effects of elevated $[CO_2]$ on leaf respiration have been attributed to greater substrate supply resulting from stimulated photosynthesis. Negative effects of elevated $[CO_2]$ on leaf respiration have been attributed to reduced demand for energy for protein turnover assumed to result from lower leaf N content. *Arabidopsis thaliana* was grown in ambient (370 ppm) and elevated (750 ppm) $[CO_2]$ with limiting and ample N availabilities. The stimulation of leaf dark respiration was attenuated in limiting N (+12%) compared with ample N supply (+30%). This response was associated with smaller stimulation of photosynthetic CO_2 uptake, but not interactive effects of elevated CO_2 and N supply on leaf protein, amino acids or specific leaf area. Elevated $[CO_2]$ also resulted in greater abundance of transcripts for many components of the respiratory pathway. A greater transcriptional response to elevated $[CO_2]$ was observed in ample N supply at midday versus midnight, consistent with reports that protein synthesis is greatest during the day. Greater foliar expression of respiratory genes under elevated $[CO_2]$ has now been observed in diverse herbaceous species, suggesting a widely conserved response.

Key-words: *Arabidopsis*; climate change; genomic; respiratory metabolism; transcriptome.

INTRODUCTION

Atmospheric CO_2 concentration ($[CO_2]$) is increasing because of anthropogenic emissions of ~10 Pg of carbon each year (Canadell *et al*. 2007). The amount of anthropogenic CO_2 released into the atmosphere is relatively small compared with the 50–60 Pg carbon that is released each year through terrestrial plant respiration (Prentice *et al*. 2001). Plant respiration can re-release 30–80% of carbon fixed through photosynthesis while providing the C skeletons and energy needed to support plant growth and maintenance (Atkin & Tjoelker 2003). Plant requirements for C skeletons and energy can vary spatially across tissues (Reich *et al*. 1998), daily between light and dark cycles (Hurry *et al*. 2005), developmentally (Armstrong *et al*. 2006) and in response to changing environmental conditions (Amthor 2000). Because of its importance at the plant, ecosystem and global scales, there has been much debate about the magnitude and direction of plant respiratory responses to elevated $[CO_2]$ (Drake *et al*. 1997; Amthor 2000; Leakey *et al*. 2009a,b), and key synthesis papers have variously concluded that leaf respiration at elevated $[CO_2]$ increases, decreases or does not change (Drake *et al*. 1999; Wang & Curtis 2002; Gifford 2003; Davey *et al*. 2004; Gonzalez-Meler *et al*. 2004). In this body of literature there are primarily two mechanisms discussed by which night-time leaf respiration could change under elevated growth $[CO_2]$: (1) photosynthesis is stimulated in elevated growth $[CO_2]$, which leads to greater carbohydrate content that could stimulate dark respiration because of greater supply of respiratory substrate, and (2) growth in elevated $[CO_2]$ can reduce leaf nitrogen (N) concentration, which is often accepted as a proxy for reduced demand on dark respiration to support protein turnover at night (Amthor 1991; Ryan 1991; Gonzalez-Meler *et al*. 2004). These opposing, but not mutually exclusive, influences on dark respiration make it very difficult to predict leaf dark respiratory responses to elevated $[CO_2]$, making dark respiration one of the largest knowledge gaps in climate change modelling (Atkin *et al*. 2010).

Crosstalk between C and N metabolism at the biochemical and transcriptional level is essential for supporting maximal growth on limited N resources (Hirel *et al*. 2007; Lea & Azevedo 2007; Tschoep *et al*. 2009), and is a well-recognized driver of photosynthetic and biomass responses to elevated $[CO_2]$. Limiting N availability reduces the stimulation of photosynthesis by elevated $[CO_2]$ because excess photoassimilate availability triggers a sugar-signalling feedback that reduces expression of photosynthetic genes, especially for Rubisco, reallocating photosynthetic N reserves to other sinks where they are needed for biosynthesis (Moore *et al*. 1999; Rolland *et al*. 2002; Stitt & Krapp 1999; Ainsworth & Long 2005; Ainsworth & Rogers 2007; Leakey *et al*. 2009b). Elevated $[CO_2]$ decreases leaf N concentration, partly

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because of dilution by larger carbohydrate pools and partly as a result of changes in N acquisition and allocation, with the effect being greater as the N supply becomes increasingly limiting (Ainsworth & Long 2005; Taub & Wang 2008). Many studies have examined plants growing under varied elevated [CO_2] levels and N availabilities, and have discovered much about the mechanistic basis of photosynthetic, biomass and yield responses to elevated [CO_2] and varied N supply (Conroy & Hocking 1993; Webber, Nie & Long 1994; Lloyd & Farquhar 1996; Rogers *et al*. 1996a,b; Farage *et al*. 1998; Geiger *et al*. 1999). However, the role of N supply in determining respiratory responses to elevated CO_2 remains unclear (Gifford 2003; Gonzalez-Meler *et al*. 2004). A number of studies have examined the relationship between N and respiration using correlative approaches to link leaf N to respiration rates across species (Ryan 1991; Wullschleger *et al*. 1992; Thomas *et al*. 1993; Ziska & Bunce 1994; Will & Ceulemans 1997; Tjoelker *et al*. 1999). However, few studies have quantified dark respiration responses to elevated CO_2 under varying levels of N supply. Those studies that have examined the interaction have focused on trees and produced conflicting results (Curtis *et al*. 1995; Volin & Reich 1996).

Substrate supply is proposed to control respiratory capacity in the long term, whereas demand for energy and carbon skeletons determines respiration rates in the short term (Williams & Farrar 1990). Recent molecular and physiological evidence from plants grown at elevated [CO_2] in the field lends support to the Williams and Farrar hypothesis by showing that greater photoassimilate supply was linked with transcriptional reprogramming of respiratory metabolism, that is, a regulated increase of respiratory capacity and flux associated with altered transcript abundance for a significant fraction of the respiratory pathway components in soybean and rice grown under elevated [CO_2] (Leakey *et al*. 2009a; Fukayama *et al*. 2011). Greater photosynthetic carbon gain could also be associated with greater demand for the energy necessary to support phloem loading as additional photoassimilates are exported to sink tissues to support enhanced growth (Korner *et al*. 1995; Komor 2000). This would represent a significant modification to the leaf energy budget as phloem loading is estimated to account for ~30% of night-time energy demand (Bouma *et al*. 1995). However, soybean is a legume, and rice is grown with heavy N inputs. These two studies are examples of plant growth under ample N conditions where both the greatest stimulations in photosynthesis and small or no reductions in leaf N concentration in elevated [CO_2] are observed (Stitt & Krapp 1999; Ainsworth & Long 2005). N metabolism and protein turnover are intrinsically linked to respiration because C skeletons are needed to incorporate inorganic N into organic amino acids (Fernie *et al*. 2004; Palenchar *et al*. 2004; Plaxton & Podestá 2006), and respiration-derived energy is needed for protein turnover (Bouma *et al*. 1994; Amthor 2000; Gifford 2003). Therefore it has been proposed that some of the reported variability in respiratory responses to elevated [CO_2] may then relate to plant N status, where plants growing with limiting N supply may have reduced protein turnover at elevated [CO_2] (Amthor 1989; Drake *et al*. 1999; Gonzalez-Meler *et al*. 2004). This would reduce the demand for respiratory products and attenuate or eliminate changes in respiratory flux, despite greater photoassimilate availability. Under such circumstances, transcriptional up-regulation of the respiratory pathway leading to greater respiratory capacity would be of no adaptive benefit.

Poplar has been grown without significant N fertilization at elevated [CO_2] in two Free Air Concentration Enrichment (FACE) experiments. In both cases, there was no evidence of transcriptional reprogramming of respiration in developing or mature leaves prior to the onset of senescence (Gupta *et al*. 2005; Taylor *et al*. 2005; Cseke *et al*. 2009; Tallis *et al*. 2010). Additionally, significant effects of elevated [CO_2] on poplar leaf dark respiration rates were not detected (Davey *et al*. 2004; Loreto *et al*. 2007). The contrasting responses of soybean and rice versus poplar suggest that a direct comparison of the genome-wide transcriptional response in leaves to elevated [CO_2] under ample and limiting N coupled to biochemical and physiological analysis could provide a valuable initial step towards understanding the complex signalling and metabolic responses regulating leaf respiration in elevated [CO_2]. The current study tested leaf dark respiratory responses to elevated CO_2 in *Arabidopsis thaliana* under ample versus limiting N availability. The use of *A. thaliana* is advantageous for asking mechanistic questions regarding [CO_2] and N interactions because of the availability of: (1) genomic tools and existing knowledge of transcriptional and biochemical regulation of C and N metabolism (Scheible *et al*. 2004); (2) experimental data on the thresholds at which low N becomes limiting for growth (Tschoep *et al*. 2009); and (3) detailed previous work regarding whole-plant responses to elevated CO_2 (Teng *et al*. 2006; Li *et al*. 2008). A majority of work on *A. thaliana* focuses on entire rosette tissue, instead of individual leaves, and an individual leaf approach has been demonstrated to better resolve molecular responses to mild treatments that might have been otherwise masked using whole rosettes (Skirycz *et al*. 2010). The individual leaf approach was used to test the hypotheses that elevated CO_2 and N supply interact in mature leaves so that (1) under ample N supply, greater photoassimilate availability and no change in leaf N and protein content at elevated [CO_2] will be associated with transcriptional up-regulation of the respiratory to support greater respiratory flux, and (2) under limiting N supply, a reduction in leaf N and protein content at elevated [CO_2] will counteract greater photoassimilate availability such that there will be no significant transcriptional changes observed for genes in the respiratory pathway and no stimulation of respiration rates.

MATERIALS AND METHODS

Plant growth conditions

A. thaliana (Col) seeds were surface sterilized with 70% ethanol solution for 2 min and a 15% Clorox solution for 15 min with occasional shaking, before being rinsed five times

in sterile deionized (DI) water. Seeds were plated on sterilized 0.5% gellan gum (Sigma, St Louis, MO, USA) containing 0.5× Murashige and Skoog salts (Sigma) and 0.3% sucrose (pH 5.7) in a sterile hood where the plates were wrapped in aluminium foil and stored at 4 °C for 48 h to synchronize emergence. Plates were removed from foil and placed in growth chambers vertically to allow for downward root growth. Five days after emergence, seedlings were transplanted to 514 cm^3 pots containing LC1 Sunshine Mix (Sun Gro Horticulture, Agawam, MA, USA) mixed homogeneously with 20% v/v of small-grain vermiculite. Two identical growth chambers (PGR14; Conviron, Winnipeg, Canada) were used to provide growing conditions of 10/14 h day/night cycle at 21/18 °C, 70% relative humidity (RH) and 250 μmol m^{-2} s^{-1} of photosynthetically active radiation. Trays of 18 pots were rotated within chambers every other day to reduce in-chamber variance in light levels and between chambers every 5 d to reduce any between chamber bias. Independent dataloggers (HOBO; Onset, Cape Cod, MA, USA) were placed within each chamber, and confirmed environmental conditions were consistent with chamber settings. Pots were watered by adding 1 L of 40% Long Ashton solution (Hewitt & Smith 1975) per tray once per week until week 4 when trays were watered every 5 d. NH_4NO_3 concentration was varied in the Long Ashton solution to establish the N treatments as limiting (0.25 mM NH_4NO_3) and ample (6.0 mM NH_4NO_3). Chamber CO_2 concentration was maintained at ambient (370 ppm) or elevated (750 ppm) using a custom retrofitted chamber CO_2 scrubbing and delivery system. Briefly, [CO_2] in each chamber was sampled continuously every second using an infrared gas analyser. Ambient [CO_2] was maintained at 370 ppm by routing the growth chamber exhaust and intake through a sealed box containing soda lime (CarboLime; Allied Healthcare, St. Louis, MO, USA) and then adding pure [CO_2] back into the line to get a constant 370 ppm. Elevated CO_2 was maintained at 750 ppm by adding pure [CO_2] to the chamber air intake line using the same delivery system as the ambient chamber. With the exception of final biomass, which involved all aboveground tissue, the following analyses were performed on the youngest mature leaves 35 days after germination (DAG).

Leaf-level physiology

Photosynthetic CO_2 assimilation at growth CO_2 concentration, saturating light intensities [900 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD)], and 21 °C was measured at dawn using a LI-6400 portable infrared gas analyser (n = 8; Li-Cor, Lincoln, NE, USA). In order to avoid significant measurement artefacts identified when using open-path gas analysers to measure small respiratory fluxes of CO_2 (Jahnke 2001; Gifford 2003), midnight dark respiratory CO_2 efflux was measured using a custom-designed closed-gas exchange system built around a LI-840 infrared gas analyser (Li-Cor). The custom system consisted of an inline, DC brushless pump (Brailsford, Antrim, NH, USA) circulating air at 0.5 L min^{-1} to a leaf chamber (5.7 × 2.0 × 0.5 cm, L × W × H). The chamber was custom machined out of aluminium with rounded corners to maximize chamber mixing. The chambers were coated with nickel infused polytetrafluoroethylene (PTFE; Teflon™; Wilmington, DE, USA), which along with the use of stainless steel tubing and fittings, minimized water condensation on the internal surfaces of the system, thereby reducing the likelihood of CO_2 going into solution and being released between measurements. The leaf chamber contained a thermocouple to monitor leaf temperature and a custom machined water jacket to control the temperature from a circulating water bath. Whole plants were kept in the dark whereas entire, mature, attached leaves were sealed into the chamber around the base of the leaf blade by application of non-stick putty (Qubitac sealant; Qubit Systems, Kingston, Canada). An O-ring between the chamber base and lid was sealed by pressure from two spring-loaded clips. A two-way valve was used inline on the system to vent excess CO_2 while the leaves were equilibrating to the chamber (1–2 min) and leaf temperature was stable at night-time growth temperature 18 °C. Once measurements commenced, the system was sealed and CO_2 concentration increase over time was recorded through a CR1000 datalogger (Campbell Scientific, Logan, UT, USA). After 1 min of recording linear CO_2 increase (at least a 50 ppm rise in [CO_2] from the start of the measurement) the chamber was opened, the leaf was excised and photographed for leaf area. Leaf area was determined using Image J (http://rsbweb.nih.gov/ij/). CO_2 increase per unit time was calculated for each replicate using a linear regression model (PROC REG; SAS, Cary, NC, USA). Five of these independent respiration systems running simultaneously allowed for measurement of ~25 individuals in 1 h with a replication of 10 individual leaves per treatment. Rates were measured at subjective midnight so as to reduce potential experimental artefacts by post-illumination respiratory bursts (Atkin *et al*. 1998), and preliminary data demonstrated that the middle 4 h of the dark period had the greatest and most stable respiration rates under these conditions.

Gene expression

The youngest mature leaves were excised from individual replicate plants (n = 5) at midday and midnight on day 35, wrapped in aluminium foil, immediately plunged into liquid N_2 and stored at −80 °C until total RNA was isolated using a Spectra Plant RNA Isolation Kit (Sigma) following manufacturer's instructions. The cRNA labelling and subsequent steps leading to hybridization and scanning of the GeneChip Arabidopsis ATH1 Genome Array (Affymetrix, Santa Clara, CA, USA) were performed by the Keck Center for Comparative and Functional Genomics at the University of Illinois (http://www.biotech.uiuc.edu/) following manufacture's protocols. The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE50966.

Leaf carbohydrates, soluble proteins and amino acids

Leaf disks (1.2 cm^2) were collected from the youngest mature leaves at midnight on day 35 (n = 8), wrapped in aluminium

foil, immediately plunged into liquid N and stored at –80 °C until carbohydrates, proteins and amino acids were extracted and analysed as described by Ainsworth *et al*. (2007).

Specific leaf area (SLA), leaf N content and biomass

Leaves excised after respiration measurements were oven dried at 70 °C and weighed ($n = 8$). Subsequently, the dried leaf material was powdered and analysed for N content using an elemental combustion system (model 4010; Costech Analytical Technologies, Valencia, CA, USA) as described by Leakey *et al*. (2006). Thirty-five DAG whole rosette tissue from each treatment were excised at the soil surface, oven dried at 70 °C and weighed for final aboveground biomass.

Statistics

All leaf physiological and biochemical parameters were tested using an analysis of variance (ANOVA; PROC GLM, SAS 9.1; SAS). Following the detailed protocols of Leakey *et al*. (2009a) for microarray analysis, the transcriptional dataset was analysed using an ANOVA (JMP Genomics 5.1; SAS). In brief, CO_2, N and time of day (TOD) were each considered fixed effects in the model. Individual transcripts were not tested if they were not present in at least three of the replicated chips for each $CO_2 \times N \times TOD$ treatment combination. Regression analysis was performed on genes responding significantly to elevated [CO_2] within each level of N using the PROC REG function in SAS.

RESULTS

Biomass, photosynthesis, respiration and leaf biochemistry

The stimulation of biomass by elevated [CO_2] was significantly smaller under limiting N (47%) compared with the ample N supply (63%; Fig. 1a). Likewise, the stimulation of light-saturated photosynthetic CO_2 assimilation (A_{sat}) by elevated [CO_2] was significantly smaller under limiting N (61%) compared with the ample N supply (82%; Fig. 1b), consistent with a large number of previous experiments. There was a detectable stimulation of night-time leaf respiration by elevated [CO_2] under both ample and limiting N supplies, but the effect was smaller under limiting N (+12%) than ample N (+30%; Fig. 1c). In contrast to the interactive effects of CO_2 and N supply on A_{sat}, respiration and biomass, the responses of SLA as well as leaf carbohydrate, protein and amino acid pools to elevated [CO_2] did not vary with the level of N supply (Fig. 2 & Supporting Information Fig. S1). At midnight, elevated [CO_2] led to 50% greater leaf starch content and 24% greater sugar content on average across limiting N and ample N treatments (Fig. 2a,b). At the same time, there was no significant effect of elevated [CO_2] on leaf soluble protein or free amino acid contents per unit leaf area in limiting N or ample N treatments (Fig. 2c,d). Elevated [CO_2] led to a lower leaf protein and amino concentrations

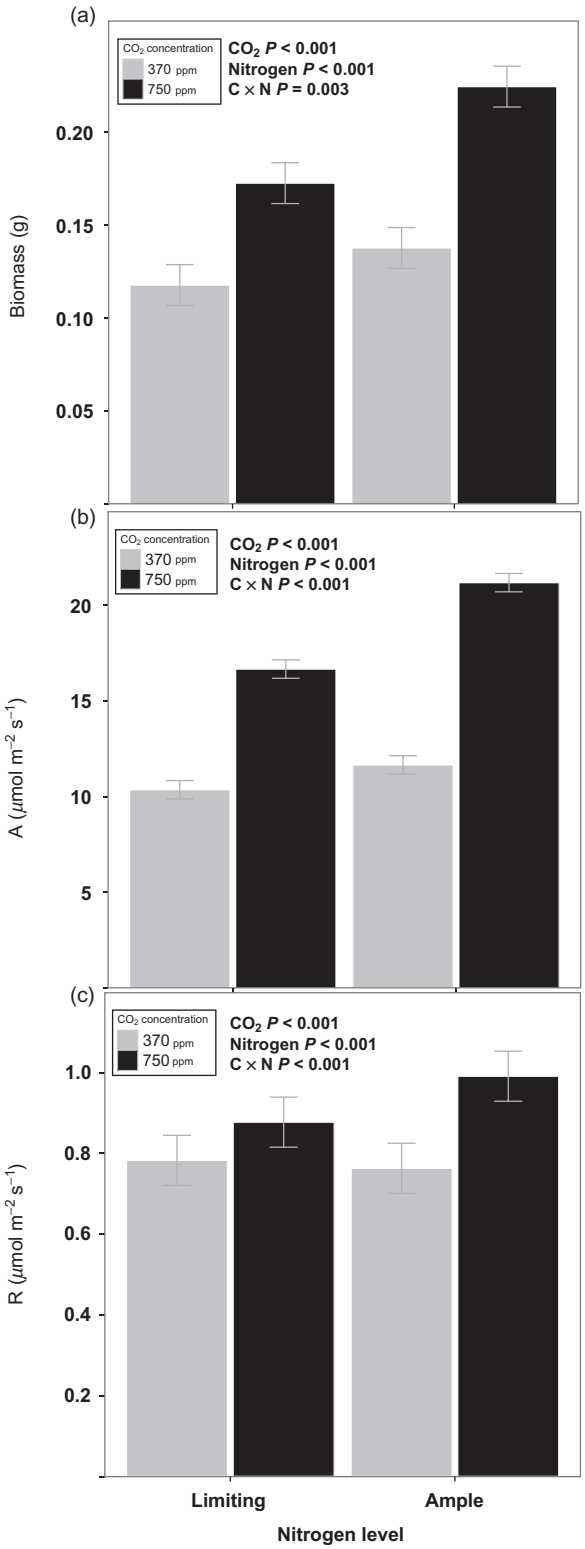

Figure 1. Panels are as follows: aboveground dry biomass (a), light-saturated CO_2 assimilation (A) at growth [CO_2] (b), and dark respiration (R) rates taken at subjective midnight (c). Mean values (±SE) of physiological parameters of plants growing in ambient (370 ppm) or elevated (750 ppm) [CO_2] and limiting or ample N conditions. In addition, plotted are the *P*-values from the statistical model each of the parameters.

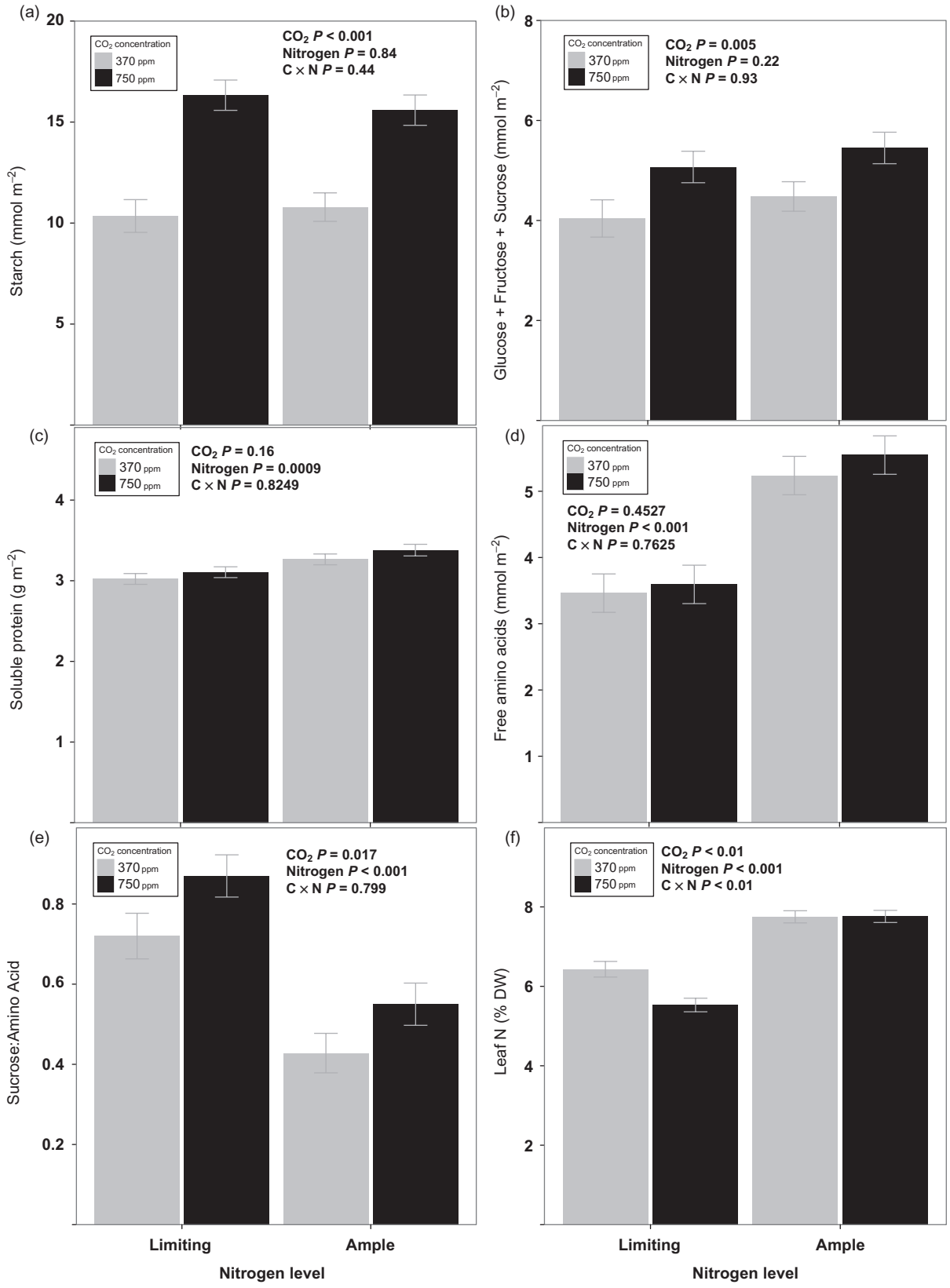

Figure 2. Panels are starch content (a); combined glucose, fructose and sucrose content (b); soluble protein content (c); free amino acids (d); sucrose to amino acid ratio (e); and leaf N concentration percentage (f). Mean values (±SE) of biochemical parameters in the youngest mature leaves growing in ambient (370 ppm) or elevated (750 ppm) [CO_2] and limiting or ample N conditions collected at subjective midnight. In addition, plotted are the *P*-values from the statistical model for each of the parameters.

Table 1. Number of transcripts responding significantly ($P < 0.05$) to each of the main effects and/or interactions in the ANOVA model of the 12 826 genes tested in at least three biological replicates

Factor in ANOVA model	Number of significant transcripts
CO_2 (C)	4439
Nitrogen (N)	1708
Time of day (TOD)	8640
C × N	258
C × TOD	678
N × TOD	812
C × N × TOD	376

ANOVA, analysis of variance.

on a dry mass basis to a similar degree in the limiting and ample N treatments, respectively (Supporting Information Fig. S1). The decrease in protein and amino acid concentrations at elevated [CO_2] were approximately in proportion to changes in SLA, which also did not differ in magnitude between ample N and limiting N treatments (Supporting Information Fig. S1). The limiting N treatment caused a significant increase in the sucrose to amino acid ratio within each level of N, whereas elevated CO_2 caused a significant increase in the ratio relative to the ambient treatment (Fig. 2e). Leaf protein and amino acid contents per unit leaf area were greater under ample N compared with limiting N supply (Fig. 2c,d), but this was not associated with any N supply effects on SLA (Supporting Information Fig. S1). Distinct from leaf protein and the other leaf chemistry parameters assessed, only leaf N concentration showed a significant elevated CO_2 by N supply interaction response (Fig. 2f). Elevated [CO_2] led to 20% lower leaf N content in the limiting N treatment, but had no effect in the ample N treatment (Fig. 2f).

Transcript profiles

The *A. thaliana* ATH1 microarray used to quantify gene expression represents ~24 000 *A. thaliana* genes. Of the 12 826 gene transcript, probe sets were present in at least three replicate samples from every treatment, 4439 had significant differences in abundance between ambient [CO_2] and elevated [CO_2], 1708 transcripts differed significantly in abundance between limited N and ample N supplies, 8640 transcripts differed significantly in abundance between midday and midnight (TOD), and 258 transcripts had a significant CO_2 by N interaction (Table 1). Transcripts that significantly responded to elevated CO_2 during the day tended to respond more in the ample N treatment compared with the limiting N treatment, and this trend was not apparent at night (Fig. 3). The stimulation of respiration in elevated [CO_2] was associated with greater abundance of transcript-encoding components of glycolysis, the tricarboxylic acid (TCA) cycle and mitochondrial electron transport chain including three of the four CO_2-producing steps of the TCA cycle, and genes encoding mitochondrial protein import complexes during both the midday and midnight time points in both ample N and limiting N treatments (Figs 4 & 5).

DISCUSSION

This experiment reproduced the interactive effects of elevated [CO_2] and N supply observed in many previous studies, where the stimulation of photosynthesis and biomass accumulation by elevated [CO_2] was attenuated by limiting N

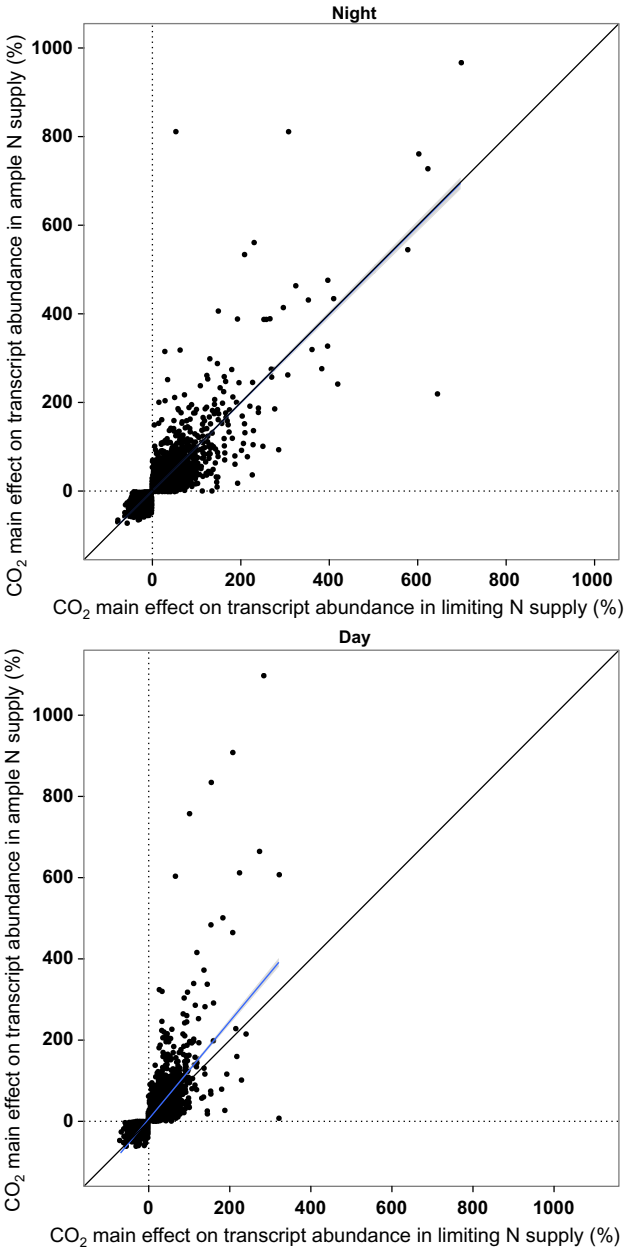

Figure 3. A regression plot of the relationship of genes responding significantly and in the same direction to elevated [CO_2] in limiting (*x*-axis) or ample (*y*-axis) N supply during midnight (top) and midday (bottom). The blue line is the line of best fit with grey 95% confidence intervals whereas the black line is a 1:1 line.

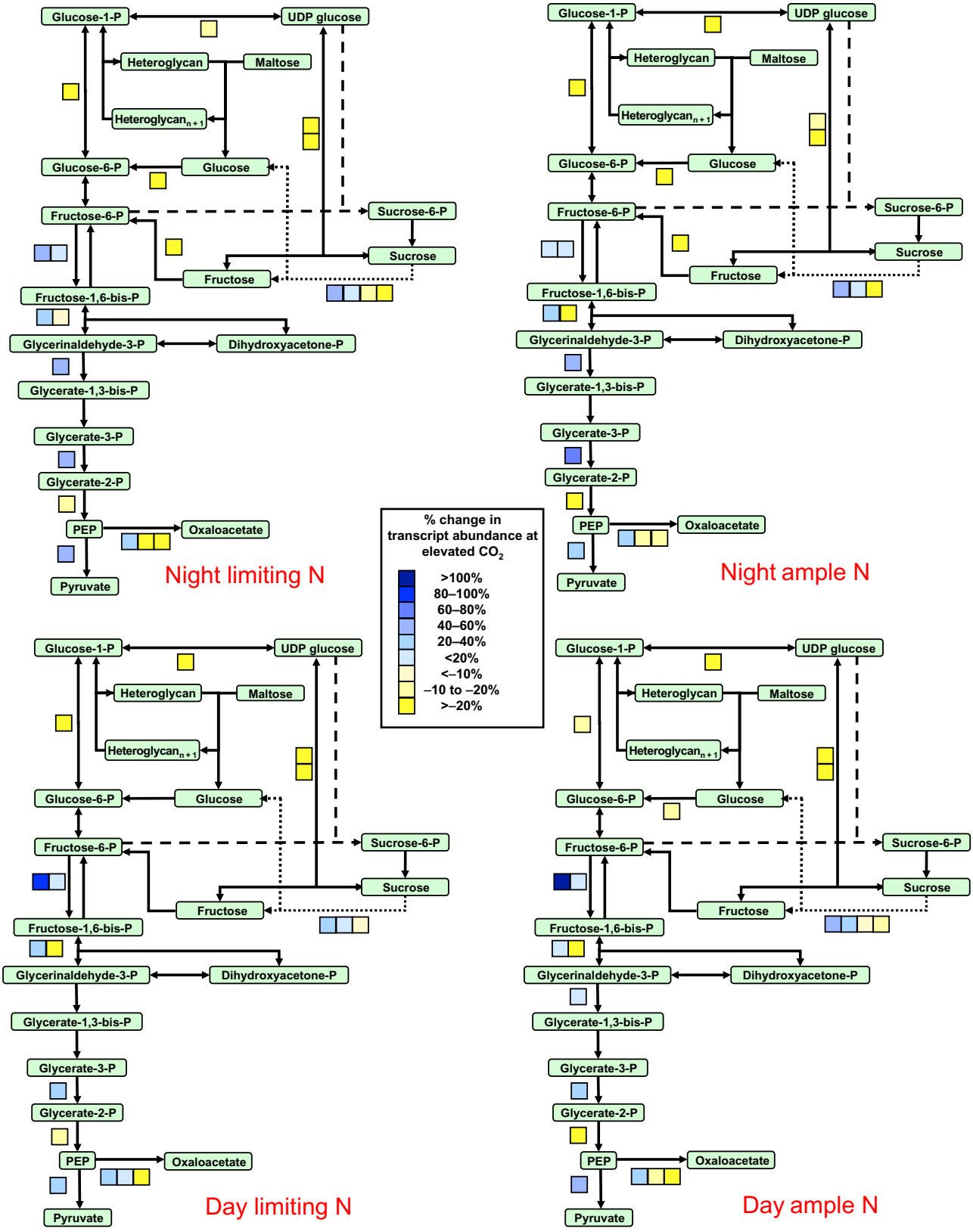

Figure 4. Graphical representation of genes encoding components of sugar transformations reactions and glycolysis that responded to elevated [CO_2] during midnight (top) or midday (bottom) and limiting N (left) or ample N (right). Each blue (positive percentage change) and yellow (negative percentage change) represents the mean percentage change of a unique transcript that responded significantly ($P < 0.05$) to elevated [CO_2]. Details about individual transcripts can be found in Supporting Information Table S1.

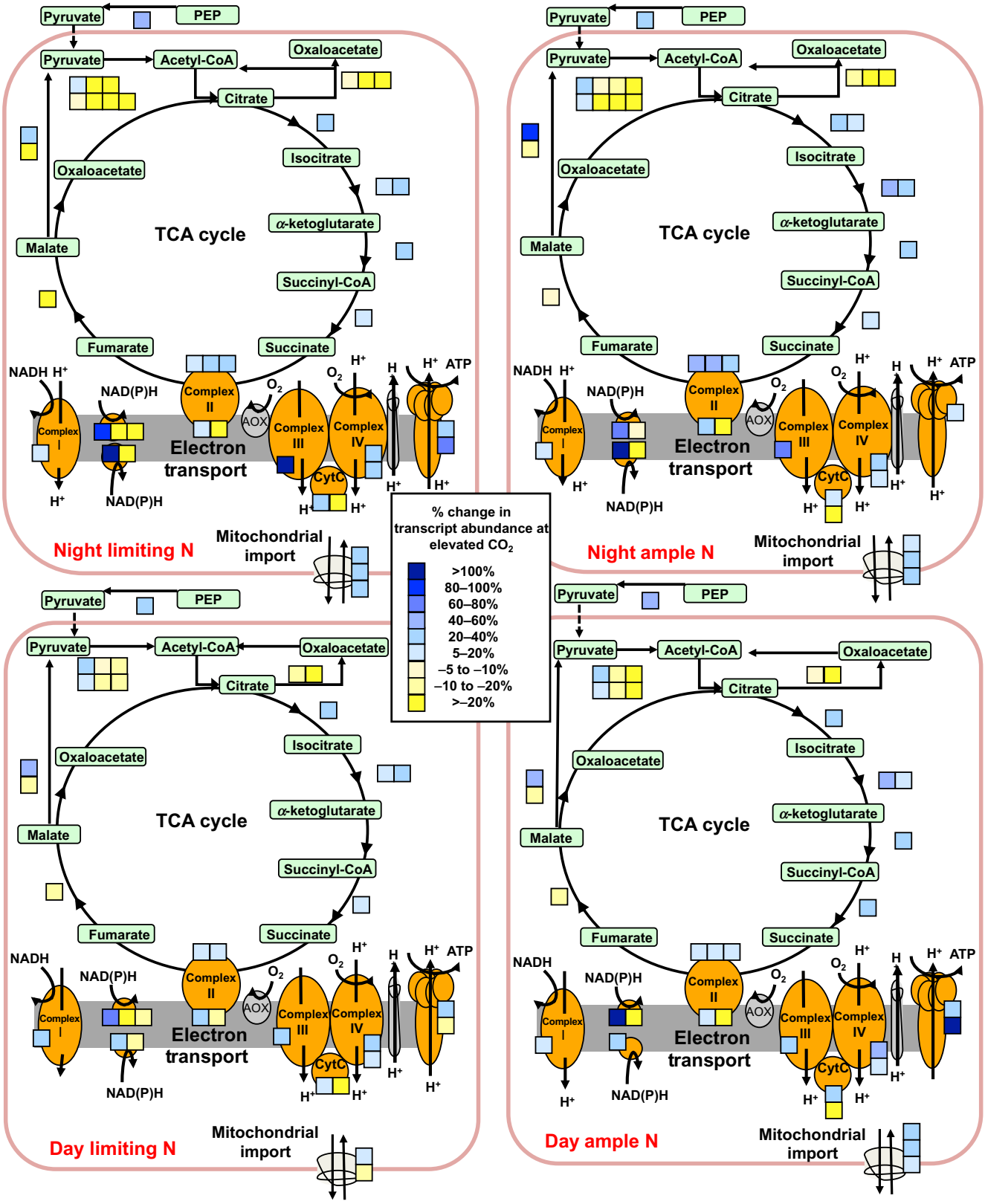

Figure 5. Graphical representation of genes encoding components of the TCA cycle and mitochondrial electron transport chain that responded to elevated [CO_2] during midnight (top) or midday (bottom) and limiting N (left) or ample N (right). Each blue (positive percentage change) and yellow (negative percentage change) represents the mean percentage change of a unique transcript that responded significantly ($P < 0.05$) to elevated [CO_2]. Details about individual transcripts can be found in Supporting Information Table S1.

supply (Stitt & Krapp 1999; Ainsworth & Long 2005; Reich *et al*. 2006). Leaf N concentration was reduced by elevated [CO_2] in plants grown with a limiting N supply, but not in plants grown with ample N supply, providing an appropriate context for investigating how N supply impacts respiratory responses to elevated [CO_2]. Elevated [CO_2] stimulated leaf respiration at night, and the response was attenuated with limiting N supply (+12%) compared with ample N supply (+30%). This provides new evidence that variation in plant N status is likely to have contributed to the substantial variability in respiratory responses to elevated [CO_2] previously described in the literature (Drake *et al*. 1999; Wang & Curtis 2002; Gifford 2003; Davey *et al*. 2004; Gonzalez-Meler *et al*. 2004). With limiting N supply, the response of leaf respiration at night to elevated [CO_2] was modest in relative terms (+12%) and very small in absolute terms (0.1 μmol m^{-2} s^{-1}). This was detectable through the use of a custom-built gas exchange system. This CO_2 effect might not have been statistically resolved using the commercially available gas exchange systems used in most prior studies, which has probably compounded the challenge of understanding biological variation in respiration responses to elevated [CO_2]. Nevertheless, once integrated over the leaf canopy and time, small changes in respiration have the potential to significantly impact leaf, plant and ecosystem carbon balance (Poorter, Remkes & Lambers 1990; Drake *et al*. 1999; Atkin & Tjoelker 2003; Gifford 2003). The abundance of transcripts encoding many components of the respiratory pathway were greater at elevated [CO_2] under both ample N and limiting N supplies in *A. thaliana*. This extends the evidence of transcriptional up-regulation of respiration under elevated [CO_2] to include a non-leguminous dicot species in addition to a legume (Leakey *et al*. 2009b; soybean) and a monocot (Fukayama *et al*. 2011; rice). Together these findings suggest the existence of a conserved transcriptional mechanism across a wide range of herbaceous species that helps to maintain sink–source balance within leaves in the manner proposed by Williams & Farrar (1990). This study takes an initial step towards resolving the details of this mechanism and addressing the major uncertainty surrounding the role of respiration in driving plant and ecosystem responses to global environmental change (Amthor 2000; Atkin *et al*. 2010).

In accordance with the first hypothesis, under ample N supply, growth at elevated [CO_2] led to greater photoassimilate availability, no change in N concentration, no change in leaf protein content per unit leaf area, greater abundance of transcript-encoding components of the respiratory machinery and greater rates of leaf respiration at night. However, the second hypothesis related to the limiting N condition was not fully supported by the data. There was an interaction effect of elevated [CO_2] and N supply on respiration, where the stimulation of respiration at elevated [CO_2] was reduced with limiting N supply (+12%) compared with ample N supply (+30%). This attenuated respiration response to elevated [CO_2] was unlikely to be caused by reduced plant N status counteracting the influence of greater photoassimilate availability, as was predicted. These findings are evaluated with consideration of treatment effects on (1) substrate supply for respiration; (2) demand for C skeletons and energy from respiration; (3) leaf N as a proxy for leaf protein status; and (4) transcriptional regulation of the respiratory machinery.

It is widely accepted that the stimulation of photosynthetic CO_2 uptake by elevated [CO_2] will generate a greater supply of carbohydrate substrate for respiration and that this response is observed across a wide range of species and environmental conditions (Drake *et al*. 1997, 1999; Gifford 2003; Gonzalez-Meler *et al*. 2004). In addition, studies have observed an attenuation of the photosynthetic response to elevated [CO_2] as N supply declines (Drake *et al*. 1997; Ainsworth & Long 2005). This study is consistent with these previous findings. The smaller stimulation of photoassimilate supply for respiration by elevated [CO_2] under limiting N supply compared with ample N supply provides a direct mechanism to explain the interactive effects of elevated [CO_2] and N supply on respiration. Under limited N supply, the smaller stimulation of A_{sat} by elevated [CO_2] potentially alters both the substrate supply to respiration and the demand on respiration for energy. Phloem loading can account for an estimated ~30% of night-time energy demand (Bouma *et al*. 1995). Because greater whole-plant growth at elevated [CO_2] can only result from stimulated photosynthesis if photoassimilate export from leaves is greater, this provides a potential explanation for variation in supply and demand control of respiration in response to interacting CO_2 and N supply.

The nature of altered demand at night for C skeletons and energy from leaf respiration when plants are grown at elevated [CO_2] is difficult to assess. This study focused on mature leaves where respiration supplies 'maintenance' processes, without the additional complication of growth processes found in developing leaves or whole-plant analyses. In addition to phloem loading, there are a number of significant sinks for respiratory products/energy in mature leaves, including protein turnover and maintenance of ion concentration and pH gradients (Penning De Vries 1975; Cannell & Thornley 2000). Protein turnover is estimated to account for approximately 20–30% of energy demand at night (Barneix *et al*. 1988; Bouma *et al*. 1994). It has been frequently asserted that lower leaf protein status, and thereby protein turnover, at elevated [CO_2] could exert a negative effect on demand for respiratory products and therefore reduce respiration rates (Ryan 1991; Amthor 1991; Ziska & Bunce 1994; Poorter *et al*. 1997; Curtis & Wang 1998; Drake *et al*. 1999; Gonzalez-Meler *et al*. 2004). A reduction in leaf protein status in response to elevated [CO_2] is more likely as N supply decreases (Conroy & Hocking 1993; Drake *et al*. 1997; Taub & Wang 2008). Contrary to this expectation, leaf protein status responded to elevated [CO_2] equally under limiting N supply and ample N supply. There was no significant effect of elevated [CO_2] on protein content per unit leaf area, at either level of N supply. Protein concentration per unit dry mass decreased at elevated [CO_2], likely because of dilution by greater carbohydrate and cell wall contents (Teng *et al*. 2006; Taub & Wang 2008). Changes in N acquisition and allocation to protein

could also have contributed to the effect (Taub & Wang 2008). However, if reduced demand for protein turnover did cause reduced demand for respiratory products at elevated [CO_2], it did so equally in ample N and limited N treatments, and therefore could not have been the basis for a smaller stimulation of respiration by elevated [CO_2] under limiting N supply. In addition, the sucrose to amino acid ratio was greater in elevated [CO_2] and lower in ample N supply, but again there was no interaction effect of elevated [CO_2] and N supply. This ratio indicates that elevated [CO_2] perturbed the relative pool sizes of reduced carbon and reduced N available to biosynthetic pathways to similar degrees under ample N and limiting N supplies. Furthermore, the global transcriptional response to elevated [CO_2] also reinforces this notion because the identity of transcripts responding to the elevated [CO_2] treatment was similar under limiting N and ample N supply. This is consistent with a common set of gene networks responding to signals associated with greater photoassimilate availability at both ample and limiting N supply.

The effect of elevated [CO_2] on leaf N concentration has been studied extensively. In plants grown at elevated [CO_2] with limiting N supply, N concentration is typically reduced. If N is readily available, little or no change in N concentration occurs (Conroy & Hocking 1993; Webber *et al*. 1994; Lloyd & Farquhar 1996; Rogers *et al*. 1996a,b; Farage *et al*. 1998; Ainsworth & Long 2005; Taub & Wang 2008). The same pattern of response was observed in this study, but leaf N was not a consistently reliable proxy for leaf soluble protein content. Under limiting N supply, the impacts of elevated [CO_2] on leaf N and protein were consistent. However, under ample N supply, elevated [CO_2] decreased protein concentration proportionally to changes in SLA although there was no change in N concentration. This is consistent with plants grown in ample N supply at elevated [CO_2] being limited by the availability of an alternative nutrient and storing excess N as nitrate. Nitrate can represent 20% of the leaf N pool in herbaceous species (Millard 1988) and responds much more strongly than either protein or amino acid contents to N supply treatments similar to those used in this study (Tschoep *et al*. 2009).

Although the concept that reduced leaf N may drive decreases in respiration at elevated [CO_2] has proven very popular in the literature, it is worth noting that there is not always a significant relationship between leaf N concentration and respiration rate (Barneix *et al*. 1988; Amthor 1989; Byrd *et al*. 1992). Consequently, Gifford (2003) concluded that there was enough variability in the respiration–N relationship that the case was not strong for building mechanistic models for maintenance respiration based solely on N content. By comparison with N analyses, far fewer papers have directly assessed leaf soluble protein content responses to elevated [CO_2]. Although reduced leaf protein content per unit leaf area has been observed in a number of cases (Rogers *et al*. 1996a,b; Sicher & Bunce 1997; Rogers and Ellsworth 2002), there is clear precedent for the finding in this study of no change in protein content on an area or fresh weight basis (Isopp *et al*. 2000; Vu *et al*. 2002; Bae & Sicher 2004; Ainsworth *et al*. 2007). Nevertheless, attempts to quantify the impact of elevated [CO_2] on the demand for C skeletons and energy for protein turnover from respiration would be valuable.

The stimulation in leaf respiration at night was associated with greater abundance of transcript-encoding respiratory genes including components of glycolysis, TCA cycle, mitochondrial electron transport chain and mitochondrial import proteins (Figs 4 & 5). This response included greater transcript abundance for phosphofructokinase (Fig. 4), which is generally considered the first committed step to the glycolytic pathway under non-stressful conditions (Plaxton 1996) and the enzymes catalysing the CO_2-producing steps of the TCA cycle (Fig. 5; pyruvate dehydrogenase complex, isocitrate dehydrogenase, alpha-ketoglutarate dehydrogenase, NADP–malic enzyme; Plaxton and Podestá 2006). Although transcript abundance does not necessarily correlate with encoded protein abundance because of post-transcriptional and translational regulation, protein abundance for some mitochondrial proteins is highly correlated with transcript abundance across multiple tissue types (Lee *et al*. 2012). Importantly, some transcripts that had significantly greater abundance under elevated [CO_2] in this study, for example, succinate dehydrogenase 1 (SDH1-1), translocase of the outer mitochondrial membrane 40 (TOM40-1), succinyl-coenzyme A (CoA) ligase and aconitate hydratase 2 (ACO2), were shown by Lee *et al*. (2012) to have strong correlations ($r > 0.8$) with protein abundance. Additionally, transcripts coding for fumarase (FUM1) and electron transfer flavoprotein : ubiquinone oxidoreductase, which both had significantly reduced transcript abundance in elevated CO_2 in the current study, were shown to not be significantly correlated with protein levels (Lee *et al*. 2012).

Examining the transcriptional dataset as a whole shows that genes that have significantly greater abundance in elevated [CO_2] responded more in ample N versus limiting N at midday, but the response to elevated [CO_2] was similar in ample and limiting N at midnight. Although the functional significance of this finding is not currently known, there is circadian control of transcriptional and enzymatic activity for primary and secondary metabolism (Graf *et al*. 2010; Graf & Smith 2011; Kerwin *et al*. 2011), and the general transcriptional response to elevated [CO_2] is dependent on day length (Queval *et al*. 2012). Furthermore, the majority of *A. thaliana* rosette protein turnover occurs during the light period when more energy is available compared with during the dark when growth and maintenance processes must be maintained on starch reserves alone (Piques *et al*. 2009). A stronger transcriptional response to elevated [CO_2] in the ample N treatment during the day may reflect that this is when the majority of protein synthesis takes place in *A. thaliana*. If so, this would diminish the significance of leaf protein status as a driver of leaf respiration responses to elevated [CO_2] at night.

CONCLUSIONS

This study demonstrates that the effect of elevated [CO_2] on leaf photosynthesis and respiration is attenuated by limiting

N supply in *A. thaliana*. There was no interaction between the effect of elevated [CO_2] and N supply on leaf protein status. Therefore, smaller stimulations of substrate supply and demand for energy from phloem loading by elevated [CO_2] appear to be the most parsimonious explanation for the attenuated respiratory response to elevated [CO_2] under limiting N versus ample N supply. Variation in N supply may therefore be an important contributing factor to the variable responses of respiration to elevated [CO_2] that have been previously reported. Future studies should be designed to reflect that the small relative and absolute differences in leaf respiratory CO_2 fluxes between ambient and elevated [CO_2] observed in this study would be challenging to detect without the use of a custom-built gas exchange system. The finding of a conserved transcriptional response to elevated [CO_2] across soybean, rice and *A. thaliana* suggests that common regulatory mechanisms exist to control sink–source balance across diverse herbaceous species. Finally, the effects of elevated [CO_2] and N supply on transcript profiles were observed to be dependent on the TOD, demonstrating a commonality between the response of carbon metabolism to both feast and famine (Blasing *et al*. 2005; Usadel *et al*. 2008; Gibon *et al*. 2009; Queval *et al*. 2012).

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REFERENCES

Ainsworth E.A. & Long S.P. (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *The New Phytologist* **165,** 351–371.

Ainsworth E.A. & Rogers A. (2007) The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. *Plant, Cell & Environment* **30,** 258–270.

Ainsworth E.A., Rogers A., Leakey A.D.B., Heady L.E., Gibon Y., Stitt M. & Schurr U. (2007) Does elevated atmospheric [CO2] alter diurnal C uptake and the balance of C and N metabolites in growing and fully expanded soybean leaves? *Journal of Experimental Botany* **58,** 579–591.

Amthor J.S. (1989) *Respiration and Crop Productivity*, Springer-Verlag, New York, NY, USA.

Amthor J.S. (1991) Respiration in a future, higher-CO_2 world. *Plant, Cell & Environment* **14,** 13–20.

Amthor J.S. (2000) The McCree–de Wit–Penning de Vries–Thornley Respiration Paradigms: 30 years later. *Annals of Botany* **86,** 1–20.

Armstrong A.F., Logan D.C. & Atkin O.K. (2006) On the developmental dependence of leaf respiration: responses to short- and long-term changes in growth temperature. *American Journal of Botany* **93,** 1633–1639.

Atkin O.K. & Tjoelker M.G. (2003) Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends in Plant Science* **8,** 343–351.

Atkin O.K., Evans J.R. & Siebke K. (1998) Relationship between the inhibition of leaf respiration by light and enhancement of leaf dark respiration following light treatment. *Australian Journal of Plant Physiology* **25,** 437–443.

Atkin O.K., Millar H.A. & Turnbull M.H. (2010) Plant respiration in a changing world. *The New Phytologist* **187,** 268–272.

Bae H. & Sicher R. (2004) Changes of soluble protein expression and leaf metabolite levels in *Arabidopsis thaliana* grown in elevated atmospheric carbon dioxide. *Field Crops Research* **90,** 61–73.

Barneix A.J., Cooper H.D., Stulen I. & Lambers H. (1988) Metabolism and translocation of nitrogen in two *Lolium perenne* populations with contrasting rates of mature leaf respiration and yield. *Physiologia Plantarum* **72,** 631–636.

Blasing O.E., Gibon Y., Gunther M., Hohne M., Morcuende R., Osuna D., . . . Stitt M. (2005) Sugars and circadian regulation make major contributions to the global regulation of diurnal gene expression in *Arabidopsis*. *The Plant Cell* **17,** 3257–3281.

Bouma J., Janssen J.A., Kock M.J.D., Leeuwen P.H.V. & Lambers H. (1994) Respiratory energy requirements and rate of protein turnover *in vivo* determined by the use of an inhibitor of protein synthesis and a probe to assess its effect. *Physiologia Plantarum* **92,** 585–594.

Bouma T.J., Visser R.D., Leeuwen P.H.V., Kock M.J.D. & Lambers H. (1995) The respiratory energy requirements involved in nocturnal carbohydrate export from starch-storing mature source leaves and their contribution to leaf dark respiration. *Journal of Experimental Botany* **46,** 1185–1194.

Byrd G.T., Sage R.F. & Brown R.H. (1992) A comparison of dark respiration between C_3 and C_4 plants. *Plant Physiology* **100,** 191–198.

Canadell J.G., Le Quéré C., Raupach M.R., Field C.B., Buitenhuis E.T., Ciais P., . . . Marland G. (2007) Contributions to accelerating atmospheric CO_2 growth from economic activity, carbon intensity, and efficiency of natural sinks. *Proceedings of the National Academy of Sciences of the United States of America* **104,** 18866–18870.

Cannell M.G.R. & Thornley J.H.M. (2000) Modeling the components of plant respiration: some guiding principles. *Annals of Botany* **85,** 45–54.

Conroy J. & Hocking P. (1993) Nitrogen nutrition of C_3 plants at elevated atmospheric CO_2 concentrations. *Physiologia Plantarum* **89,** 570–576.

Cseke L.J., Tsai C.J., Rogers A., Nelsen M.P., White H.L., Karnosky D.F. & Podila G.K. (2009) Transcriptomic comparison in the leaves of two aspen genotypes having similar carbon assimilation rates but different partitioning patterns under elevated [CO_2]. *The New Phytologist* **182,** 891–911.

Curtis P.S. & Wang X. (1998) A meta-analysis of elevated CO2 effects on woody plant mass, form and physiology. *Oecologia* **113,** 299–313.

Curtis P.S., Vogel C.S., Pregitzer K.S., Zak D.R. & Teeri J.A. (1995) Interacting effects of soil fertility and atmospheric CO_2 on leaf area growth and carbon gain physiology in *Populus x euramericana* (Dode) Guinier. *The New Phytologist* **129,** 253–263.

Davey P., Hunt S., Hymus G., DeLucia E., Drake B.G., Karnosky D.F. & Long S.P. (2004) Respiratory oxygen uptake is not decreased by an instantaneous elevation of [CO_2], but is increased with long-term growth in the field at elevated [CO_2]. *Plant Physiology* **134,** 520–527.

Drake B.G., Gonzalez-Meler M.A. & Long S.P. (1997) More efficient plants: a consequence of rising atmospheric CO_2? *Annual Review of Plant Physiology and Plant Molecular Biology* **48,** 609–639.

Drake B.G., Berry J., Bunce J., Dijkstra P., Farrar J. & Gifford R.M. (1999) Does elevated atmospheric CO_2 concentration inhibit mitochondrial respiration in green plants? *Plant, Cell & Environment* **22,** 649–657.

Farage P., McKee I. & Long S.P. (1998) Does a low nitrogen supply necessarily lead to acclimation of photosynthesis to elevated CO_2? *Plant Physiology* **118,** 573–580.

Fernie A.R., Carrari F. & Sweetlove L.J. (2004) Respiratory metabolism: glycolysis, the TCA cycle and mitochondrial electron transport. *Current Opinion in Plant Biology* **7,** 254–261.

Fukayama H., Sugino M., Fukuda T., Masumoto C., Taniguchi Y., Okada M., . . . Miyao M. (2011) Gene expression profiling of rice grown in free air CO_2 enrichment (FACE) and elevated soil temperature. *Field Crops Research* **121,** 195–199.

Geiger M., Haake V., Ludewig F., Sonnewald U. & Stitt M. (1999) The nitrate and ammonium nitrate supply have a major influence on the response of photosynthesis, carbon metabolism, nitrogen metabolism and growth to elevated carbon dioxide in tobacco. *Plant, Cell & Environment* **22,** 1177–1199.

Gibon Y., Pyl E.T., Sulpice R., Lunn J.E., Höhne M., Günther M. & Stitt M. (2009) Adjustment of growth, starch turnover, protein content and central metabolism to a decrease of the carbon supply when *Arabidopsis* is grown in very short photoperiods. *Plant, Cell & Environment* **32,** 859–874.

Gifford R.M. (2003) Plant respiration in productivity models: conceptualisation, representation and issues for global terrestrial carbon-cycle research. *Functional Plant Biology* **30,** 171–186.

Gonzalez-Meler M.A., Taneva L. & Trueman R.J. (2004) Plant respiration and elevated atmospheric CO_2 concentration: cellular responses and global significance. *Annals of Botany* **94,** 647–656.

Graf A. & Smith A.M. (2011) Starch and the clock: the dark side of plant productivity. *Trends in Plant Science* **16,** 169–175.

Graf A., Schlereth A., Stitt M. & Smith A.M. (2010) Circadian control of carbohydrate availability for growth in *Arabidopsis* plants at night. *Proceedings of the National Academy of Sciences of the United States of America* **107,** 9458–9463.

Gupta P., Duplessis S., White H., Karnosky D.F., Martin F. & Podila G.K. (2005) Gene expression patterns of trembling aspen trees following long-term exposure to interacting elevated CO_2 and tropospheric O_3. *The New Phytologist* **167,** 129–141.

Hewitt E.J. & Smith T.A. (1975) *Plant Mineral Nutrition*. John Wiley and Sons, New York, NY, USA.

Hirel B., Le Gouis J., Ney B. & Gallais A. (2007) The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *Journal of Experimental Botany* **58,** 2369–2387.

Hurry V., Igamberdiev A.U., Keerberg O., Pärnik T., Atkin O.K., Zaragoza-Castells J. & Gardeström P. (2005) Respiration in photosynthetic cells: gas exchange components, interactions with photorespiration and the operation of mitochondria in the light. In *Plant Respiration: From Cell to Ecosystem. Advances in Photosynthesis and Respiration* (eds H. Lambers & M. Ribas-Carbo), vol. 18, pp. 43–61. Springer, Dordrecht.

Isopp H., Frehner M., Long S.P. & Nösberger J. (2000) Sucrose–phosphate synthase responds differently to source–sink relations and to photosynthetic rates: *Lolium perenne* L. growing at elevated pCO_2 in the field. *Plant, Cell & Environment* **23,** 597–607.

Jahnke S. (2001) Atmospheric CO_2 concentration does not directly affect leaf respiration in bean or poplar. *Plant, Cell & Environment* **24,** 1139–1151.

Kerwin R.E., Jimenez-Gomez J.M., Fulop D., Harmer S.L., Maloof J.N. & Kliebenstein D.J. (2011) Network quantitative trait loci mapping of circadian clock outputs identifies metabolic pathway-to-clock linkages in *Arabidopsis*. *The Plant Cell* **23,** 471–485.

Komor E. (2000) Source physiology and assimilate transport: the interaction of sucrose metabolism, starch storage and phloem export in source leaves and the effects on sugar status in phloem. *Australian Journal of Plant Physiology* **27,** 497–505.

Korner C., Pelaez-Riedl S. & Van Bel J.E. (1995) CO_2 responsiveness of plants: a possible link to phloem loading. *Plant, Cell & Environment* **18,** 595–600.

Lea P.J. & Azevedo R.A. (2007) Nitrogen use efficiency. 2. Amino acid metabolism. *Annals of Applied Biology* **151,** 269–275.

Leakey A.D.B., Uribelarrea M., Ainsworth E.A., Naidu S.L., Rogers A., Ort D.R. & Long S.P. (2006) Photosynthesis, productivity, and yield of maize are not affected by open-air elevation of CO_2 concentration in the absence of drought. *Plant Physiology* **140,** 779–790.

Leakey A.D.B., Ainsworth E.A., Bernacchi C.J., Rogers A., Long S.P. & Ort D.R. (2009a) Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. *Journal of Experimental Botany* **60,** 2859–2876.

Leakey A.D.B., Xu F., Gillespie K.M., McGrath J.M., Ainsworth E.A. & Ort D.R. (2009b) Genomic basis for stimulated respiration by plants growing under elevated carbon dioxide. *Proceedings of the National Academy of Sciences of the United States of America* **106,** 3597–3602.

Lee C.P., Eubel H., Solheim C. & Millar A.H. (2012) Mitochondrial proteome heterogeneity between tissues from the vegetative and reproductive stages of *Arabidopsis thaliana* development. *Journal of Proteome Research* **11,** 3326–3343.

Li P., Ainsworth E.A., Leakey A.D.B., Ulanov A., Lozovaya V., Ort D.R. & Bohnert H.J. (2008) *Arabidopsis* transcript and metabolite profiles: ecotype-specific responses to open-air elevated [CO_2]. *Plant, Cell & Environment* **31,** 1673–1687.

Lloyd J. & Farquhar G.D. (1996) The CO_2 dependence of photosynthesis, plant growth responses to elevated atmospheric CO_2 concentrations and their interaction with soil nutrient status. I. General principles and forest ecosystems. *Functional Ecology* **10,** 4–32.

Loreto F., Centritto M., Barta C., Calfapietra C., Fares S. & Monson R.K. (2007) The relationship between isoprene emission rate and dark respiration rate in white poplar (*Populus alba* L.) leaves. *Plant, Cell & Environment* **30,** 662–669.

Millard P. (1988) The accumulation and storage of nitrogen by herbaceous plants. *Plant, Cell & Environment* **11,** 1–8.

Moore B.D., Cheng S.H., Sims D. & Seemann J.R. (1999) The biochemical and molecular basis for photosynthetic acclimation to elevated atmospheric CO_2. *Plant, Cell & Environment* **22,** 567–582.

Palenchar P.M., Kouranov A., Lejay L.V. & Coruzzi G.M. (2004) Genome-wide patterns of carbon and nitrogen regulation of gene expression validate the combined carbon and nitrogen (CN)-signaling hypothesis in plants. *Genome Biol* **5**, R91.

Penning De Vries F.W.T. (1975) The cost of maintenance processes in plant cells. *Annals of Botany* **39,** 77–92.

Piques M., Schulze W.X., Höhne M., Usadel B., Gibon Y., Rohwer J. & Stitt M. (2009) Ribosome and transcript copy numbers, polysome occupancy and enzyme dynamics in Arabidopsis. *Molecular Systems Biology* **5,** 1–17.

Plaxton W.C. (1996) The organization and regulation of plant glycolysis. *Annual Review of Plant Physiology and Plant Molecular Biology* **47,** 185–214.

Plaxton W.C. & Podestá F.E. (2006) The functional organization and control of plant respiration. *Critical Reviews in Plant Sciences* **25,** 159–198.

Poorter H., Remkes C. & Lambers H. (1990) Carbon and nitrogen economy of 24 wild species differing in relative growth rate. *Plant Physiology* **94,** 621–627.

Poorter H., Van Berkel Y., Baxter R., Den Hertog J., Dijkstra P., Gifford R.M., . . . Wong S.C. (1997) The effect of elevated CO_2 on the chemical composition and construction costs of leaves of 27 C_3 species. *Plant, Cell & Environment* **20,** 472–482.

Prentice I.C., Farquhar G.D., Fasham M., Goulden M.L., Heimann M., Jaramillo V.J., . . . Yool A. (2001) The carbon cycle and atmospheric carbon dioxide. In *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change* (eds J. Houghton, Y. Ding, D. Griggs, *et al.*), pp. 185–237. Cambridge University Press, New York, NY, USA.

Queval G., Neukermans J., Vanderauwera S., Van Breusegem F. & Noctor G. (2012) Day length is a key regulator of transcriptomic responses to both CO_2 and H_2O_2 in *Arabidopsis*. *Plant, Cell & Environment* **35,** 374–387.

Reich P.B., Ellsworth D.S., Vose M. & Volin J.C. (1998) Relationships of leaf dark respiration to leaf nitrogen, specific leaf area and leaf life-span: a test across biomes and functional groups. *Oecologia* **114,** 471–482.

Reich P.B., Hungate B.A. & Luo Y. (2006) Carbon–nitrogen interactions in terrestrial ecosystems in response to rising atmospheric carbon dioxide. *Annual Review of Ecology, Evolution, and Systematics* **37,** 611–636.

Rogers A. & Ellsworth D.S. (2002) Photosynthetic acclimation of *Pinus taeda* (loblolly pine) to long-term growth in elevated pCO_2 (FACE). *Plant, Cell & Environment* **25,** 851–858.

Rogers G.S., Milham P.J., Gillings M. & Conroy J.P. (1996a) Sink strength may be the key to growth and nitrogen responses in N-deficient wheat at elevated CO_2. *Australian Journal of Plant Physiology* **23,** 253–264.

Rogers G.S., Milham P.J., Thibaud M.C. & Conroy J.P. (1996b) Interactions between rising CO_2, concentration and nitrogen supply in cotton. I. Growth and leaf nitrogen concentration. *Australian Journal of Plant Physiology* **23,** 119–125.

Rolland F., Moore B. & Sheen J. (2002) Sugar sensing and signaling in plants. *The Plant Cell* **14,** S185–S206.

Ryan M.G. (1991) Effects of climate change on plant respiration. *Ecological Applications* **1,** 157–167.

Scheible W.R., Morcuende R., Czechowski T., Fritz C., Osuna D., Palacios-rojas N., . . . Stitt M. (2004) Genome-wide reprogramming of primary and secondary metabolism, protein synthesis, cellular growth processes, and the regulatory infrastructure of *Arabidopsis* in response to nitrogen. *Plant Physiology* **136,** 2483–2499.

Sicher R.C. & Bunce J.A. (1997) Relationship of photosynthetic acclimation to changes of Rubisco activity in field-grown winter wheat and barley during growth in elevated carbon dioxide. *Photosynthesis Research* **52,** 27–38.

Skirycz A., De Bodt S., Obata T., De Clercq I., Claeys H., De Rycke R., . . . Inzé D. (2010) Developmental stage specificity and the role of mitochondrial metabolism in the response of *Arabidopsis* leaves to prolonged mild osmotic stress. *Plant Physiology* **152,** 226–244.

Stitt M. & Krapp A. (1999) The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant, Cell & Environment* **22,** 583–621.

Tallis M.J., Lin Y., Rogers A., Zhang J., Street N.R., Miglietta F., . . . Taylor G. (2010) The transcriptome of *Populus* in elevated CO_2 reveals increased

anthocyanin biosynthesis during delayed autumnal senescence. *The New Phytologist* **186,** 415–428.

Taub D.R. & Wang X. (2008) Why are nitrogen concentrations in plant tissues lower under elevated CO_2? A critical examination of the hypotheses. *Journal of Integrative Plant Biology* **50,** 1365–1374.

Taylor G., Street N.R., Tricker P.J., Sjödin A., Graham L., Skogström O., . . . Jansson S. (2005) The transcriptome of *Populus* in elevated CO_2. *The New Phytologist* **167,** 143–154.

Teng N., Wang J., Chen T., Wu X., Wang Y. & Lin J. (2006) Elevated CO_2 induces physiological, biochemical and structural changes in leaves of *Arabidopsis thaliana*. *The New Phytologist* **172,** 92–103.

Thomas R.B., Reid C.D., Ybema R. & Strain B.R. (1993) Growth and maintenance components of leaf respiration of cotton grown in elevated carbon dioxide partial pressure. *Plant, Cell & Environment* **16,** 539–546.

Tjoelker M.G., Reich P.B. & Oleksyn J. (1999) Changes in leaf nitrogen and carbohydrates underlie temperature and CO_2 acclimation of dark respiration in five boreal tree species. *Plant, Cell & Environment* **22,** 767–778.

Tschoep H., Gibon Y., Carillo P., Armengaud P., Szecowka M., Nunes-Nesi A., . . . Stitt M. (2009) Adjustment of growth and central metabolism to a mild but sustained nitrogen-limitation in *Arabidopsis*. *Plant, Cell & Environment* **32,** 300–318.

Usadel B., Bläsing O.E., Gibon Y., Retzlaff K., Höhne M., Günther M. & Stitt M. (2008) Global transcript levels respond to small changes of the carbon status during progressive exhaustion of carbohydrates in *Arabidopsis rosettes*. *Plant Physiology* **146,** 1834–1861.

Volin J.C. & Reich P.B. (1996) Interaction of elevated CO_2 and O_3 on growth, photosynthesis and respiration of three perennial species grown in low and high nitrogen. *Physiologia Plantarum* **97,** 674–684.

Vu J.C.V., Newman Y.C., Allen L.H., Gallo-meagher M. & Zhang M. (2002) Photosynthetic acclimation of young sweet orange trees to elevated growth CO_2 and temperature. *Journal of Plant Physiology* **157,** 147–157.

Wang X. & Curtis P. (2002) A meta-analytical test of elevated CO_2 effects on plant respiration. *Plant Ecology* **161,** 251–261.

Webber A.N., Nie G. & Long S.P. (1994) Acclimation of photosynthetic proteins to rising atmospheric CO_2. *Photosynthesis Research* **39,** 413–425.

Will R.E. & Ceulemans R. (1997) Effects of elevated CO_2 concentration on photosynthesis, respiration and carbohydrate status of coppice *Populus* hybrids. *Physiologia Plantarum* **100,** 933–939.

Williams J.H.H. & Farrar J.F. (1990) Control of barley root respiration. *Physiologia Plantarum* **79,** 259–266.

Wullschleger S.D., Norby R.J. & Gunderson C. (1992) Growth and maintenance respiration in leaves of *Liriodendron tulipifera* L. exposed to long-term carbon dioxide enrichment in the field. *The New Phytologist* **121,** 515–523.

Ziska L.H. & Bunce J.A. (1994) Direct and indirect inhibition of single leaf respiration by elevated CO_2 concentrations: interaction with temperature. *Physiologia Plantarum* **90,** 130–138.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Mean values (+/- standard errors) of specific leaf area (SLA), leaf protein (mass basis), and amino acids (mass basis) of fully expanded leaves grown in ambient (370 ppm) or elevated (750 ppm) [CO_2] and limiting or ample N condition.

Table S1. List of transcripts that were significant for the main effect of elevated [CO_2] that are displayed in Figures 4 and 5. AT locus IDs, functional description, and percent change in gene expression in elevated [CO_2] versus ambient [CO_2], with a negative percentage indicating a greater expression in ambient.