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Nitrate assimilation is inhibited by elevated CO_2 in field-grown wheat

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Total protein and nitrogen concentrations in plants generally decline under elevated CO_2 atmospheres[1,2]. Explanations for this decline include that plants under elevated CO_2 grow larger, diluting the protein within their tissues[3,4]; that carbohydrates accumulate within leaves, downregulating the amount of the most prevalent protein Rubisco[2]; that carbon enrichment of the rhizosphere leads to progressively greater limitations of the nitrogen available to plants[4]; and that elevated CO_2 directly inhibits plant nitrogen metabolism, especially the assimilation of nitrate into proteins in leaves of C_3 plants[5]. Recently, several meta-analyses have indicated that CO_2 inhibition of nitrate assimilation is the explanation most consistent with observations[6–8]. Here, we present the first direct field test of this explanation. We analysed wheat (*Triticum aestivum* L.) grown under elevated and ambient CO_2 concentrations in the free-air CO_2 enrichment experiment at Maricopa, Arizona. In leaf tissue, the ratio of nitrate to total nitrogen concentration and the stable isotope ratios of organic nitrogen and free nitrate showed that nitrate assimilation was slower under elevated than ambient CO_2. These findings imply that food quality will suffer under the CO_2 levels anticipated during this century unless more sophisticated approaches to nitrogen fertilization are employed.

Many lines of evidence from laboratory studies demonstrate that elevated CO_2 concentrations in the atmosphere inhibit leaf nitrate (NO_3^-) assimilation in C_3 plants. These include: plants receiving NO_3^- as their sole source of nitrogen (N) accumulate less organic N under elevated than ambient CO_2 (refs 7,9–11); plants subjected to a pulse of ^{15}N–NO_3^- incorporate less ^{15}N into organic N compounds under elevated than ambient CO_2 (ref. 10); plant growth is slower under elevated than ambient CO_2 when NO_3^- serves as the sole N source and faster when NH_4^+ serves as the sole N source[5,12]; ΔAQ (changes in the ratio of net CO_2 consumption to net O_2 evolution after shifting N nutrition from NH_4^+ to NO_3^-), a real-time measure of leaf NO_3^- assimilation, decreases with increasing leaf internal CO_2 concentration[9,12]; and maximum NO_3^- reductase activity *in vitro* is usually less under elevated than ambient CO_2 (refs. 11–13). Verification of CO_2 inhibition of NO_3^- assimilation in the field, however, is still lacking.

Here, we conducted chemical analyses of wheat (*Triticum aestivum* L.) grown in 1996 and 1997 under elevated or ambient atmospheric CO_2 concentrations in the free-air CO_2 enrichment (FACE) experiment at Maricopa, Arizona. This experiment originally assessed grain yield[14], total N of green leaves[15], grain protein[16] and soil N dynamics[17]. Leaf material collected from this experiment was stored on ice, transported to the laboratory, oven dried at 70 °C, stored in evacuated plastic bags that were sealed in paint cans, and kept in a storeroom at the US Water Conservation Laboratory in Phoenix, Arizona, USA until air freighted to UC Davis for the chemical analyses described below. This preparation and storage of samples minimized changes over time in total N, nitrate and nitrogen isotope ratios[18]. What prompted these additional analyses of the Maricopa leaf material was the development of a new technique to assess the N isotope signature of NO_3^- (ref. 19) as well as a new perspective about the interactions between elevated CO_2 and NO_3^- assimilation[5,9,10,12].

The values for leaf total N (Fig. 1) did not differ from those reported over a decade earlier[15], supporting the assumption that the samples were well preserved. Plants in the low-N treatment of either CO_2 treatment contained no detectable leaf NO_3^- on most sampling dates (data not shown). In contrast, a significant percentage of leaf N remained as unassimilated NO_3^- (ratio of NO_3^- to total N) in plants subjected to the high-N treatment (Fig. 2). The first fertilization, applied 4 weeks after plant emergence, increased leaf NO_3^- concentration in the short term an average of fivefold and twofold in 1996 and 1997, respectively (Figs 1 and 2). Therefore, we focused our analysis on the high-N treatment from week 6 onwards.

Leaf total N in the high-N treatment did not differ significantly between the CO_2 treatments ($P = 0.12$; Fig. 1), but overall the ratio of NO_3^- to total N was greater under elevated than ambient CO_2 from week 6 onwards ($P < 0.0001$; Fig. 2). Total N and the ratio of NO_3^- to total N were lower in 1996 than in 1997 ($P < 0.003$; Figs 1 and 2). The analysis of variance tables are available in Supplementary Tables 2–5.

In leaves, both organic N and unassimilated NO_3^- in 1996 were less enriched in ^{15}N under elevated than ambient CO_2 from week 6 onwards ($P < 0.0001$; Figs 3 and 4). The $\delta^{15}N$ of leaf organic N and NO_3^- declined as the plants matured under both CO_2 treatments ($P < 0.0001$; Figs 3 and 4).

All three measures of NO_3^- assimilation assessed in this study confirm that elevated CO_2 inhibited leaf NO_3^- assimilation in field-grown wheat. The first measure was the proportion of leaf N that remained as free NO_3^-. Leaf total N in the high-N treatment did not differ significantly between the CO_2 treatments (Fig. 1), as reported earlier[15]. The percentage of leaf total N that remained as unassimilated NO_3^- was higher under elevated than ambient CO_2 from week 6 onwards in both years (Fig. 2 and Supplementary Tables 3–5). Higher free NO_3^- relative to total N suggests that NO_3^- assimilation was slower under elevated CO_2.

The second measure was the $\delta^{15}N$ of leaf organic N. It was more depleted in ^{15}N under elevated than ambient CO_2 from 6 weeks onwards (Fig. 3). If NO_3^- availability does not limit assimilation, leaves preferentially assimilate ^{14}N–NO_3^- (ref. 20). Therefore, the lower leaf $\delta^{15}N_{organic}$ signatures under elevated than ambient CO_2

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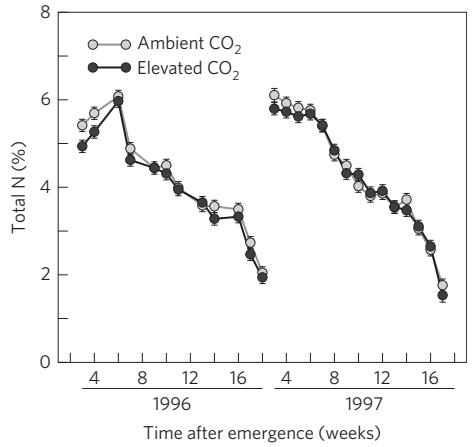

Figure 1 | Total nitrogen (percentage of dry matter) in wheat leaves as a function of time after emergence (weeks). Shown are data from the 1996 and 1997 field seasons for plants grown under ambient (363 μmol mol^{-1} in 1996 and 370 μmol mol^{-1} in 1997) or elevated (548 μmol mol^{-1} in 1996 and 559 μmol mol^{-1} in 1997) CO_2 atmospheres in the high-N fertilization treatment at Maricopa, Arizona. Plotted are the estimated means and standard errors (SAS Proc Mixed version 9.3).

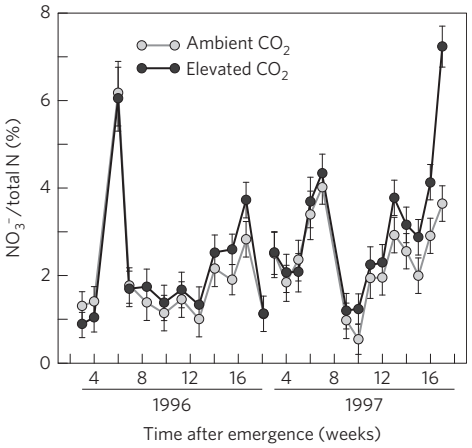

Figure 2 | Nitrate as a percentage of total N in wheat leaves as a function of time after emergence (weeks). Shown are data from the 1996 and 1997 field seasons for plants grown under ambient (363 μmol mol^{-1} in 1996 and 370 μmol mol^{-1} in 1997) or elevated (548 μmol mol^{-1} in 1996 and 559 μmol mol^{-1} in 1997) CO_2 atmospheric conditions in the high-N fertilization treatment at Maricopa, Arizona. Plotted are the estimated means and standard errors (SAS Proc Mixed version 9.3).

(Fig. 3) indicate that leaf NO_3^- assimilation was slower relative to replenishment of leaf NO_3^- from roots under elevated than ambient CO_2.

The third measure was $\delta^{15}N$ of free NO_3^- in the leaves. If leaf NO_3^- assimilation is slower relative to replenishment of leaf NO_3^- from roots under elevated than ambient CO_2, NO_3^- assimilation more slowly depletes leaf tissues of ^{14}N–NO_3^- and $\delta^{15}N_{nitrate}$ becomes less enriched in ^{15}N. Here, unassimilated NO_3^- in wheat leaves was less enriched in ^{15}N under elevated than ambient CO_2 from 6 weeks onwards (Fig. 4), indicating that leaf NO_3^- assimilation was slower under elevated than ambient CO_2.

The isotopic signature of free NO_3^- in leaves also depends on the $\delta^{15}N$ of NO_3^- translocated from the roots. For example, if NO_3^- assimilation rates in the roots are faster under elevated than ambient CO_2 (ref. 21), isotope discrimination by nitrate reductase will enrich the root NO_3^- pool in ^{15}N, and so NO_3^- translocated to the leaves will be more ^{15}N enriched. The $\delta^{15}N$ of leaf NO_3^-, however, was lower under elevated than ambient CO_2 (Fig. 4), indicating that the isotopic signature of NO_3^- derived primarily from leaf NO_3^- assimilation being slower under elevated than ambient CO_2.

These field results are consistent with those of previous laboratory studies showing that several physiological mechanisms are responsible for CO_2 inhibition of leaf NO_3^- assimilation in C_3 plants[5,9,10,12]. One mechanism involves the first biochemical step of NO_3^- assimilation, the conversion of NO_3^- to NO_2^- in the cytoplasm of leaf mesophyll cells. Photorespiration stimulates the export of malic acid from chloroplasts[22] and increases the availability of NADH in the cytoplasm[23] that powers this first step[24,25]. Elevated CO_2 decreases photorespiration and thereby decreases the amount of reductant available for NO_3^- reduction. Another physiological mechanism is that elevated CO_2 inhibits NO_2^- influx into chloroplasts, and this decreases NO_3^- assimilation[12]. A third physiological mechanism is that processes in the chloroplast stroma compete for reduced ferredoxin: because ferredoxin–NADP reductase has a higher affinity for reduced ferredoxin than nitrite reductase[26], NO_3^- assimilation proceeds only if the availability of reduced ferredoxin exceeds that needed for NADPH formation[24,27]. For most plants, this occurs when CO_2 availability limits C_3 carbon fixation[10].

Several earlier studies at the Maricopa FACE site examined soil N in wheat plots that received irrigation, fertilizer and CO_2 treatments similar to the high-N treatment here. Total inorganic N through the soil profile was similar in the ambient and elevated CO_2 treatments from 6 weeks onwards[28]. Nitrogen mineralization was unaffected by CO_2 treatment[17], and soil NO_3^- constituted between 90 and 98% of inorganic N extractable by 2 M KCl at harvest (S. A. Prior and H. A. Torbert personal communication, 2013). Therefore, these soil N data support the conclusion that the leaf N differences that we observed between the elevated and ambient CO_2 treatments derived from altered plant responses and not altered soil N availability.

Several recent meta-analyses of the literature on plant responses to elevated CO_2 support that CO_2 inhibits leaf NO_3^- assimilation. One[7] based on 43 studies of wheat protein and grain yield under ambient and elevated CO_2 concluded that 'elevated CO_2 has a direct negative effect on grain protein accumulation independent of the yield effect, supporting recent evidence of CO_2-induced impairment of nitrate uptake/assimilation'. Another meta-analysis[6] based on 38 studies of soil NH_4^+ and NO_3^- concentrations and plant NH_4^+ and NO_3^- uptake in 58 species concluded that 'differential CO_2 effects on soil NH_4^+ and NO_3^-…were consistent qualitatively with recent discoveries of eCO_2 effects on plant N utilization', citing our laboratory studies on CO_2 inhibition of leaf NO_3^- assimilation.

Under elevated CO_2, protein concentrations in wheat grain[16,29], rice grain[8], potato tuber[8] and barley grain[29,30] decline an average of around 8%. Wheat, rice, potato and barley, respectively, provide 21, 13, 2 and 0.3% of the protein in the human diet[31]. Consequently, protein available for human consumption may diminish by about 3% as atmospheric CO_2 reaches the levels anticipated during the next few decades.

Increased yields under CO_2 enrichment and heavy N fertilization may partially compensate for the decrease in food quality resulting from elevated CO_2. In the low-N treatment at Maricopa, elevated CO_2 increased grain yields by 9% (ref. 32), but decreased grain protein concentrations by 11% (ref. 16), and so grain protein yields decreased by about 2%. In the high-N treatment, elevated CO_2 increased grain yields about 16% (ref. 32), but had an insignificant effect on grain protein concentrations[16], and so grain protein yields increased about 16%. In the high-N treatment, however, the fertilizer and soil supplied 430 and 490 kg N ha^{-1} during 1996 and 1997, respectively, but crop biomass N under elevated CO_2 was only

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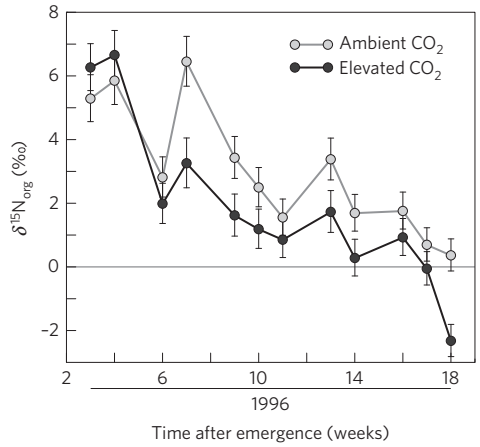

Figure 3 | Isotopic signature of organic N ($\delta^{15}N_{org}$) in wheat leaves as a function of time after emergence (weeks). Shown are data from the 1996 field season for plants grown under ambient (363 μmol mol^{-1}) and elevated (548 μmol mol^{-1}) CO_2 atmospheres in the high-N fertilization treatment at Maricopa, Arizona. Plotted are the estimated means and standard errors (SAS Proc Mixed version 9.3).

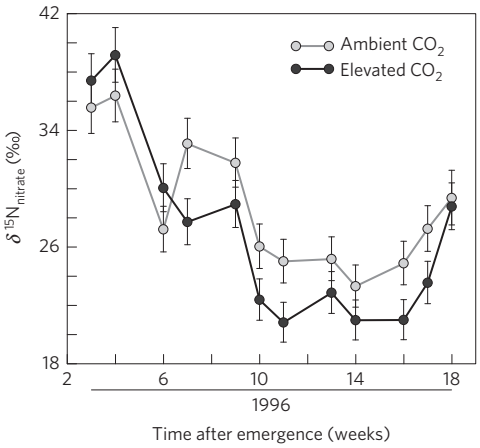

Figure 4 | Isotopic signature of nitrate ($\delta^{15}N_{nitrate}$) in wheat leaves as a function of time after emergence (weeks). Shown are data from the 1996 field season for plants grown under ambient (363 μmol mol^{-1}) and elevated (548 μmol mol^{-1}) CO_2 atmospheres in the high-N fertilization treatment at Maricopa, Arizona. Plotted are the estimated means and standard errors (SAS Proc Mixed version 9.3).

270 kg N ha^{-1}, indicating that nearly half of the N applied was not retained in the crop[16,33]. Thus, such high fertilization rates would be undesirable because of higher costs, greater NO_3^- leaching into groundwater, and greater N_2O emissions.

Obviously, plants have alternative strategies for acquiring organic N. One such strategy is the use NH_4^+ as a N source. Undoubtedly, the wheat at Maricopa absorbed NH_4^+ as part of its mineral N supply, but nitrification at this site was rapid[17], and the soils contained relatively little NH_4^+ as the growing season progressed (S. A. Prior and H. A. Torbert personal communication, 2013). Another strategy is root NO_3^- assimilation, which may be enhanced under elevated CO_2[21]. Unfortunately, relatively little is known about the extent to which the balance between root and leaf NO_3^- assimilation varies within and among species[34]. Breeding crops for enhanced root NO_3^- and NH_4^+ assimilation has the potential to compensate for lower shoot NO_3^- assimilation rates and likely losses in food quality as atmospheric CO_2 rises, but this approach is yet untapped.

Methods

Wheat (*Triticum aestivum* L.) leaves were obtained from the 1996 and 1997 FACE experiment at the Maricopa Agricultural Center near Phoenix, Arizona[15]. Briefly, high- and low-N treatments at this site were assigned in four replicates under ambient and FACE rings 25 m in diameter. Within the rings, ambient and elevated CO_2 were controlled at 363 and 548 μmol CO_2 mol^{-1} in 1996 and 370 and 559 μmol CO_2 mol^{-1} in 1997 by releasing air containing different CO_2 concentrations from 2.5-m-high vertical pipes spaced every 2 m around the periphery. Certified seed of Yecora Roho, a cultivar still widely used in the region[33], was planted on 15 December 1995 or 1996 and seedlings emerged 1 January 1996 or 1997. The soil at the experimental site is classified as Trix clay loam, fine-loamy, mixed (calcareous), hyperthermic Typic Torrifluvents. Nitrogen fertilizer in the form of NH_4NO_3 was applied in the drip irrigation water: the high-N treatment received four applications (50 kg N ha^{-1} at 4 weeks after emergence, 125 kg N ha^{-1} at 8 weeks, 125 kg N ha^{-1} at 12 weeks, and 50 kg N ha^{-1} at 16 weeks) for a total rate of 350 kg N ha^{-1}; the low-N treatment received a total of 70 and 15 kg N ha^{-1} for 1996 and 1997, respectively, in three increments[16,35]. In addition to the fertilizer applied, substantial residual inorganic N was present at sowing (80 kg N ha^{-1} in 1996 and 145 kg N ha^{-1} in 1997).

Plant harvests were made at 10–14 day intervals through the season[15]. At each harvest, 24 plants were sampled within each replicate of a treatment. The plants were stored on ice and transported to the laboratory. Green leaf tissue was oven dried at 70 °C and stored in evacuated plastic bags that were sealed in paint cans. Subsequently, this leaf tissue was ball-milled at UC Davis, and total N and total N isotope ratios were determined using an elemental analyser interfaced to an isotope ratio mass spectrometer (Sercon) at the UC Davis Stable Isotope Facility. During analysis, samples were interspersed with two or more different $\delta^{15}N$ standards.

Nitrate was extracted with 1 mM $CaSO_4$ from subsamples of the pulverized leaves by using an orbital shaker, followed by centrifugation. The NO_3^- concentration of the diluted extracts was determined spectrophotometrically[36]. The nitrogen isotopic composition of plant NO_3^- extracts was analysed from N_2O generated by denitrifying bacteria lacking N_2O reductase[19]. Briefly, 2 ml aliquots of *Pseudomonas chlororaphis* culture were sealed into 20 ml headspace vials that were purged for 2 h with N_2 gas to remove N_2O and O_2. Samples containing 0.1 μmol NO_3^-–N of the plant tissue extracts or standards were injected through the septae of the vials. The N_2O was flushed from the vials with He, trapped cryogenically, and then released into the isotope ratio mass spectrometer (Sercon) at the UC Davis Stable Isotope Facility. Samples were interspersed with $\delta^{15}N$ KNO_3 standards that were processed like the plant tissue extracts.

The leaf samples collected in the second year (1997) seemed to have become contaminated with the heavy nitrogen isotope because the $\delta^{15}N$ values were highly variable and reached up to 250 ‰ in individual samples. Therefore, we report N isotope ratios for only the first year (1996).

Leaf organic N was estimated from the difference between leaf total N and leaf unassimilated NO_3^- because NH_4^+ concentrations in wheat leaves are low and do not vary significantly with CO_2 treatment[12]. The isotope ratio of the leaf organic nitrogen ($\delta^{15}N_{organic}$) was thus calculated by dividing the mass of ($^{15}N_{total}$–^{15}N–NO_3^-) by the mass of ($^{14+15}N_{total}$–$^{14+15}N$–NO_3^-).

The first fertilization, applied 4 weeks after plant emergence, increased leaf NO_3^- concentration an average of fivefold and twofold in 1996 and 1997, respectively, and therefore, we considered only data collected after week 6 in our statistical analysis. An analysis of variance using the MIXED procedure with repeated measures in SAS (version 9.3, SAS Institute) assessed the effects of year (1 or 2 years), CO_2 treatment (2 treatments), week after emergence (11 weeks), blocks (4 blocks), and their interactions on total N, the ratio of NO_3^- to total N, $\delta^{15}N_{nitrate}$ and $\delta^{15}N_{organic}$ (see Supplementary Tables for the SAS program used and the resulting analysis of variance tables). All of the data met the assumptions of normality and homogeneity of variance as evaluated using the Shapiro–Wilks and Levene's tests, respectively. The dependent variables (total N, ratio of NO_3^- to total N, $\delta^{15}N_{nitrate}$ and $\delta^{15}N_{organic}$) were repeated for each experimental block. The independent variables and their interactions were considered significant when $P \leq 0.05$.

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References

1. Cotrufo, M. F., Ineson, P. & Scott, A. Elevated CO_2 reduces the nitrogen concentration of plant tissues. *Glob. Change Biol.* **4,** 43–54 (1998).
2. Long, S. P., Ainsworth, E. A., Rogers, A. & Ort, D. R. Rising atmospheric carbon dioxide: Plants face the future. *Annu. Rev. Plant Biol.* **55,** 591–628 (2004).
3. Ellsworth, D. S. *et al.* Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO_2 across four free-air CO_2 enrichment experiments in forest, grassland and desert. *Glob. Change Biol.* **10,** 2121–2138 (2004).

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4. Reich, P. B. *et al.* Nitrogen limitation constrains sustainability of ecosystem response to CO_2. *Nature* **440,** 922–925 (2006).
5. Bloom, A. J. *et al.* CO_2 enrichment inhibits shoot nitrate assimilation in C_3 but not C_4 plants and slows growth under nitrate in C_3 plants. *Ecology* **93,** 355–367 (2012).
6. Cheng, L. *et al.* Arbuscular mycorrhizal fungi increase organic carbon decomposition under elevated CO_2. *Science* **337,** 1084–1087 (2012).
7. Pleijel, H. & Uddling, J. Yield vs quality trade-offs for wheat in response to carbon dioxide and ozone. *Glob. Change Biol.* **18,** 596–605 (2012).
8. Myers, S. S. *et al.* Rising CO_2 threatens food quality. *Nature* (in the press; 2014).
9. Rachmilevitch, S., Cousins, A. B. & Bloom, A. J. Nitrate assimilation in plant shoots depends on photorespiration. *Proc. Natl Acad. Sci. USA* **101,** 11506–11510 (2004).
10. Bloom, A. J., Burger, M., Asensio, J. S. R. & Cousins, A. B. Carbon dioxide enrichment inhibits nitrate assimilation in wheat and *Arabidopsis*. *Science* **328,** 899–903 (2010).
11. Lekshmy, S., Jain, V., Khetarpal, S. & Pandey, R. Inhibition of nitrate uptake and assimilation in wheat seedlings grown under elevated CO_2. *Indian J. Plant Physiol.* 1–7 (2013).
12. Bloom, A. J., Smart, D. R., Nguyen, D. T. & Searles, P. S. Nitrogen assimilation and growth of wheat under elevated carbon dioxide. *Proc. Natl Acad. Sci. USA* **99,** 1730–1735 (2002).
13. Matt, P. *et al.* Elevated carbon dioxide increases nitrate uptake and nitrate reductase activity when tobacco is growing on nitrate, but increases ammonium uptake and inhibits nitrate reductase activity when tobacco is growing on ammonium nitrate. *Plant Cell Environ.* **24,** 1119–1137 (2001).
14. Jamieson, P. D. *et al.* Modelling CO_2 effects on wheat with varying nitrogen supplies. *Agr. Ecosyst. Environ.* **82,** 27–37 (2000).
15. Sinclair, T. R. *et al.* Leaf nitrogen concentration of wheat subjected to elevated (CO_2 and either water or N deficits. *Agr. Ecosyst. Environ.* **79,** 53–60 (2000).
16. Kimball, B. A. *et al.* Elevated CO_2, drought and soil nitrogen effects on wheat grain quality. *New Phytol.* **150,** 295–303 (2001).
17. Prior, S. A. *et al.* Free-air carbon dioxide enrichment of wheat: Soil carbon and nitrogen dynamics. *J. Environ. Qual.* **26,** 1161–1166 (1997).
18. Harborne, A. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis* (Springer, 1998).
19. Sigman, D. M. *et al.* A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. *Anal. Chem.* **73,** 4145–4153 (2001).
20. Tcherkez, G. & Farquhar, G. D. Isotopic fractionation by plant nitrate reductase, twenty years later. *Funct. Plant Biol.* **33,** 531–537 (2006).
21. Kruse, J. *et al.* Elevated pCO_2 favours nitrate reduction in the roots of wild-type tobacco (*Nicotiana tabacum* cv. Gat.) and significantly alters N-metabolism in transformants lacking functional nitrate reductase in the roots. *J. Exp. Bot.* **53,** 2351–2367 (2002).
22. Backhausen, J. E. *et al.* Transgenic potato plants with altered expression levels of chloroplast NADP-malate dehydrogenase: interactions between photosynthetic electron transport and malate metabolism in leaves and in isolated intact chloroplasts. *Planta* **207,** 105–114 (1998).
23. Igamberdiev, A. U., Bykova, N. V., Lea, P. J. & Gardestrom, P. The role of photorespiration in redox and energy balance of photosynthetic plant cells: A study with a barley mutant deficient in glycine decarboxylase. *Physiol. Plant.* **111,** 427–438 (2001).
24. Robinson, J. M. *Models in Plant Physiology and Biochemistry* 25–35 (CRC Press, 1987).
25. Quesada, A., Gomez-Garcia, I. & Fernandez, E. Involvement of chloroplast and mitochondria redox valves in nitrate assimilation. *Trends Plant Sci.* **5,** 463–464 (2000).
26. Knaff, D. B. *Oxygenic Photosynthesis: The Light Reactions* 333–361 (Kluwer Academic, 1996).
27. Backhausen, J. E., Kitzmann, C., Horton, P. & Scheibe, R. Electron acceptors in isolated intact spinach chloroplasts act hierarchically to prevent over-reduction and competition for electrons. *Photosynth. Res.* **64,** 1–13 (2000).
28. Adamsen, F. J. *et al.* Temporal changes in soil and biomass nitrogen for irrigated wheat grown under free-air carbon dioxide enrichment (FACE). *Agron. J.* **97,** 160–168 (2005).
29. Erbs, M. *et al.* Effects of free-air CO_2 enrichment and nitrogen supply on grain quality parameters and elemental composition of wheat and barley grown in a crop rotation. *Agr. Ecosyst. Environ.* **136,** 59–68 (2010).
30. Manderscheid, R., Bender, J., Jäger, H-J. & Weigel, H. J. Effects of season long CO_2 enrichment on cereals II Nutrient concentrations and grain quality. *Agr. Ecosyst. Environ.* **54,** 175–185 (1995).
31. FAOSTAT *Food Supply, Crops Primary Equivalent*, http://faostat3.fao.org/faostat-gateway/go/to/download/C/CC/E (2013).
32. Pinter, P. J. Jr *et al. Annual Research Report* 71–74 (USDA Water Conservation Laboratory, 1997).
33. Ko, J. *et al.* Simulation of free air CO_2 enriched wheat growth and interactions with water, nitrogen, and temperature. *Agric. Forest Meteorol.* **150,** 1331–1346 (2010).
34. Nunes–Nesi, A., Fernie, A. R. & Stitt, M. Metabolic and signaling aspects underpinning the regulation of plant carbon nitrogen interactions. *Mol. Plant* **3,** 973–996 (2010).
35. Hunsaker, D. J. *et al.* CO_2 enrichment and soil nitrogen effects on wheat evapotranspiration and water use efficiency. *Agric. Forest Meteorol.* **104,** 85–105 (2000).
36. Doane, T. A. & Horwath, W. R. Spectrophotometric determination of nitrate with a single reagent. *Anal. Lett.* **36,** 2713–2722 (2003).

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Author contributions

All authors contributed to the data set, discussed the results and commented on the manuscript. A.J.B. and M.B. designed the study. M.B. conducted the chemical analyses. A.J.B. carried out the statistical analysis and wrote the paper.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to A.J.B.

Competing financial interests

The authors declare no competing financial interests.

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