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Increasing CO_2 threatens human nutrition

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Dietary deficiencies of zinc and iron are a substantial global public health problem. An estimated two billion people suffer these deficiencies[1], causing a loss of 63 million life-years annually[2,3]. Most of these people depend on C_3 grains and legumes as their primary dietary source of zinc and iron. Here we report that C_3 grains and legumes have lower concentrations of zinc and iron when grown under field conditions at the elevated atmospheric CO_2 concentration predicted for the middle of this century. C_3 crops other than legumes also have lower concentrations of protein, whereas C_4 crops seem to be less affected. Differences between cultivars of a single crop suggest that breeding for decreased sensitivity to atmospheric CO_2 concentration could partly address these new challenges to global health.

In the 1990s, several investigators found that elevated atmospheric CO_2 concentration (hereafter abbreviated to [CO_2]) decreased the concentrations of zinc, iron and protein in grains of wheat[4–7], barley[5] and rice[8] grown in controlled-environment chambers. However, subsequent studies failed to replicate these results when plants were grown in open-top chambers and free-air CO_2 enrichment (FACE) experiments. A previous study[9] found no effect of [CO_2] on the concentrations of zinc or iron in rice grains grown under FACE and suggested that the earlier findings had been influenced by 'pot effects', by which a small rooting volume led to nutrient dilution at the root–soil interface. Of the more recent studies[10–13], most have indicated lower elemental concentrations in soybeans[10], sorghum[10], potatoes[11], wheat[12] or barley[13] grown at elevated [CO_2], but with the exception of iron in one study on wheat[12], these results were statistically insignificant, perhaps because of small sample sizes.

Small sample sizes have limited the statistical power of individual studies of many aspects of plant responses to elevated [CO_2], and meta-analyses involving larger samples of genotypes, environmental conditions and experimental locations have been important in resolving which elements of plant function respond reliably to altered [CO_2][14,15]. A recent meta-analysis of published data concluded that only sulphur is decreased in grains grown at elevated [CO_2][16].

Here we report findings from a meta-analysis of newly acquired data from 143 comparisons of the edible portions of crops grown at ambient and elevated [CO_2] from seven different FACE experimental locations in Japan, Australia and the United States involving six food crops (see Table 1). We tested the nutrient concentrations of the edible portions of rice (*Oryza sativa*, 18 cultivars), wheat (*Triticum aestivum*, 8 cultivars), maize (*Zea mays*, 2 cultivars), soybeans (*Glycine max*, 7 cultivars), field peas (*Pisum sativum*, 5 cultivars) and sorghum (*Sorghum bicolor*, 1 cultivar). In all, forty-one genotypes were tested over one to six growing seasons at ambient and elevated [CO_2], where the latter was in the range 546–586 p.p.m. across all seven study sites. Collectively, these experiments contribute more than tenfold more data regarding both the zinc and iron content of the edible portions of crops grown under FACE conditions than is currently available in the literature. Consistent with earlier meta-analyses of other aspects of plant function under FACE conditions[14,15], we considered the response comparisons observed from different species, cultivars and stress treatments and from different years to be independent. The natural logarithm of the mean response ratio (r = response in elevated [CO_2]/response in ambient [CO_2]) was used as the metric for all analyses. Meta-analysis was used to estimate the overall effect of elevated [CO_2] on the concentration of each nutrient in a particular crop and to determine the significance of this effect (see Methods).

We found that elevated [CO_2] was associated with significant decreases in the concentrations of zinc and iron in all C_3 grasses and legumes (Fig. 1 and Extended Data Table 1). For example, wheat grains grown at elevated [CO_2] had 9.3% lower zinc (95% confidence interval (CI) −12.7% to −5.9%) and 5.1% lower iron (95% CI −6.5% to −3.7%) than those grown at ambient [CO_2]. We also found that elevated [CO_2] was associated with lower protein content in C_3 grasses, with a 6.3% decrease (95% CI −7.5% to −5.2%) in wheat grains and a 7.8% decrease (95% CI −8.9% to −6.8%) in rice grains. Elevated [CO_2] was associated with a small decrease in protein in field peas, and there was no significant effect in soybeans or C_4 crops (Fig. 1 and Extended Data Table 1).

In addition to our own observations, we obtained data from 10 of 11 previously published studies investigating nutrient changes in the edible portion of food crops (Extended Data Table 6) and combined these data with our own observations in a larger meta-analysis. Analysis of our results combined with previously published FACE data (Extended Data Table 2), or combined with previously published data from both FACE and chamber experiments (Extended Data Table 3), was consistent with the results obtained using only our new data. Combining our data with previously published data did not alter the significance or substantially alter the effect size of the nutrient changes for any crop or any nutrient.

In addition to nutrient concentrations, we also measured phytate, a phosphate storage molecule present in most plants that inhibits the absorption of dietary zinc in the human gut[17]. We had no a priori reason to assume that phytate concentrations would change in response to rising [CO_2]. However, formulae for calculating absorbed, or bioavailable, zinc depend on both the amount of dietary zinc and the amount of dietary phytate consumed[17], making it important to interpret changes in zinc concentration in the context of possible changes in phytate. Phytate content decreased significantly at elevated [CO_2] only in wheat ($P < 0.01$). This decrease might offset some of the declines in zinc for this particular crop, although the decrease was slightly less than half of the decrease in zinc. For other crops examined, however, the lack of a concurrent decrease in phytate may further exacerbate problems of zinc deficiency.

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Table 1 | Characteristics of agricultural experiments

Crops	Country	Treatments used	Years grown	Number of replicates	Number of cultivars	CO_2 ambient/elevated (p.p.m.)
Wheat						
Site 1	Australia	2 water levels, 2 nitrogen treatments, 2 sowing times	2007–2010	4	8	382/546–550
Site 2	Australia	1 water level, 1 nitrogen treatment, 2 sowing times	2007–2009	4	1	382/546–550
Field peas	Australia	2 water levels	2010	4	5	382/546–550
Rice						
Site 1	Japan	1 nitrogen treatment, 2 warming treatments	2007–2008	3	3	376–379/570–576
Site 2	Japan	3 nitrogen treatments, 2 warming treatments	2010	4	18	386/584
Maize	United States	2 nitrogen treatments	2008	4	2	385/550
Soybeans	United States	1 treatment	2001, 2002, 2004, 2006–2008	4	7	372–385/550
Sorghum	United States	2 water levels	1998–1999	4	1	363–373/556–579

'Number of replicates' refers to the number of identical cultivars grown under identical conditions in the same year and location but in separate FACE rings.

The global [CO_2] in the atmosphere is expected to reach 550 p.p.m. in the next 40–60 years, even if further actions are taken to decrease emissions[18]. At these concentrations, we find that the edible portions of many of the key crops for human nutrition have decreased nutritional value when compared with the same plants grown under identical conditions but at the present ambient [CO_2]. Analysis of the United Nations' Food and Agriculture Organization food balance sheets reveals that in 2010 roughly 2.3 billion people were living in countries whose populations received at least 60% of their dietary zinc and/or iron from C_3 grains and legumes, and 1.9 billion lived in countries that received at least 70% of one or both of these nutrients from these crops (Extended Data Table 5). Reductions in the zinc and iron content of the edible portion of these food crops will increase the risk of zinc and iron deficiencies across these populations and will add to the already considerable burden of disease associated with them.

The implications of decreased protein concentrations in non-leguminous C_3 crops are less clear. From a study of adult men and women in the United States, there is strong evidence that the substitution of dietary carbohydrate for dietary protein increased the risk of hypertension, lipid disorders, and 10-year coronary heart disease risk[19]. For the developing world, minimum protein requirements for different demographic groups are an area of active research and debate[20]. For countries such as India, however, in which up to one-third of the rural population is thought to be at risk of not meeting protein requirements[21] and in which most protein comes in the form of C_3 grains[21], decreased protein content in non-leguminous C_3 crops may have serious consequences for public health.

Whereas zinc and iron were significantly decreased in all C_3 crops tested, only iron in maize was observed to decrease among the C_4 crops. No changes were found in sorghum. That zinc and iron declines were notable in C_3 crops but less so in C_4 crops is consistent with differences in physiology. C_4 crops concentrate CO_2 internally, which results in photosynthesis being CO_2-saturated even under ambient [CO_2] conditions, leading to no stimulation of photosynthetic carbon assimilation at elevated [CO_2] levels under mesic growing conditions[22]. Our finding that protein content was less affected in legumes than in other C_3 crops is also physiologically consistent with the general ability of leguminous crops to match the stimulation of photosynthetic carbon gain at elevated [CO_2] with greater nitrogen fixation, to maintain tissue carbon:nitrogen (C:N) ratios[23]. In contrast, most temperate non-legume C_3 crops are generally unable to extract and assimilate sufficient nitrogen from soils to maintain tissue C:N ratios[24,25].

Little is known about the mechanism(s) responsible for the decline in nutrient concentrations associated with elevated [CO_2]. Some authors have proposed 'carbohydrate dilution', by which CO_2-stimulated carbohydrate production by plants dilutes the rest of the grain components[26]. To test this hypothesis, we measured concentrations of additional elements for all crops except wheat (Extended Data Table 4). Our findings were inconsistent with carbohydrate dilution operating alone. If only

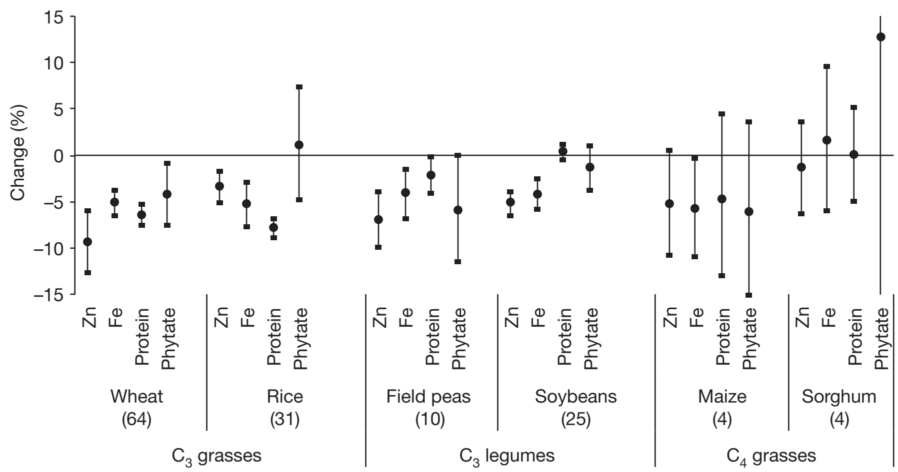

Figure 1 | Percentage change in nutrients at elevated [CO_2] relative to ambient [CO_2]. Numbers in parentheses refer to the number of comparisons in which replicates of a particular cultivar grown at a specific site under one set of growing conditions in one year at elevated [CO_2] have been pooled and for which mean nutrient values for these replicates are compared with mean values for identical cultivars under identical growing conditions except grown at ambient [CO_2]. In most instances, data from four replicates were pooled for each value, meaning that eight experiments were combined for each comparison (see Table 1 for details of experiments). Error bars represent 95% confidence intervals of the estimates.

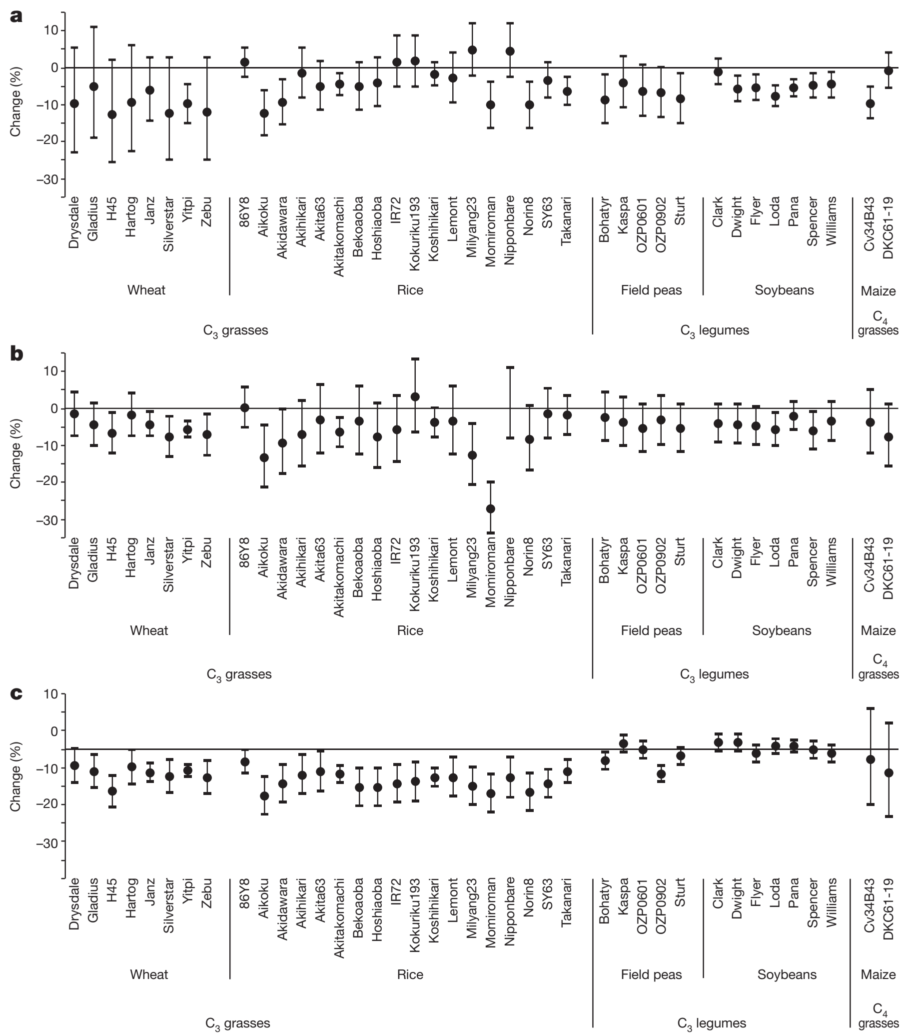

Figure 2 | Percentage change (with 95% confidence intervals) in nutrients at elevated [CO_2] relative to ambient [CO_2], by cultivar. **a**, Zinc; **b**, iron; **c**, protein.

passive dilution of nutrients were occurring, we would have expected to see very similar changes in the concentration of each nutrient tested for a given crop. In contrast, we found that elemental changes in the individual crops are distinct from each other. For example, in rice grains (Extended Data Table 4) the decrease in zinc concentrations associated with elevated [CO_2] was significantly different from the decreases in the concentrations of copper ($P \leq 0.001$), calcium ($P \leq 0.001$), boron ($P \leq 0.001$) and phosphate ($P = 0.010$). This heterogeneous response was also observed in recent analyses reviewing possible mechanisms for nutrient changes in both edible and non-edible plant tissues grown at elevated [CO_2][27]. It also seems that the mechanism(s) causing these changes operate distinctly in different species. In one instance, for example, we found boron to be significantly decreased in soybeans ($P \leq 0.001$), whereas it was significantly elevated in rice grains ($P \leq 0.001$). Although these differences may, in part, have derived from different environmental conditions, they suggest that the mechanism is more complex than carbohydrate dilution alone. Of all the elements, changes in nitrogen content at elevated [CO_2] have been the most studied, and inhibition of photorespiration and malate production[24], carbohydrate dilution[26], slower uptake of nitrogen in roots[25] and decreased transpiration-driven mass flow of nitrogen[7] may all be significant.

We also examined the effects of elevated [CO_2] on zinc, iron and protein content as a function of cultivar when data were available (Fig. 2). Whereas most crops showed negligible differences across cultivars, concentrations of zinc and iron across rice cultivars varied substantially ($P = 0.04$ and $P = 0.03$, respectively; Fig. 2a, b). Such differences between cultivars suggest a basis for breeding rice cultivars whose micronutrient levels are less vulnerable to increasing [CO_2]. Similar effects may occur in other crops, given that the statistical power of many of our other intercultivar tests was limited by sample size. We note, however, that such breeding programmes will not be a panacea for many reasons including the affordability of improved seeds and the numerous criteria used by farmers in making planting decisions that include taste, tradition, marketability, growing requirements and yield. In addition, as has been noted previously, there are likely to be trade-offs with respect to yield and other performance characteristics when breeding for increased zinc and iron content[28].

The public health implications of global climate change are difficult to predict, and we expect many surprises. The finding that raising atmospheric [CO_2] lowers the nutritional value of C_3 food crops is one such surprise that we can now better predict and prepare for. In addition to efforts to limit increases in [CO_2], it may be important to develop breeding programmes designed to decrease the vulnerability of key crops to these changes. Nutritional analysis of which human populations are most vulnerable to decreased dietary availability of zinc, iron and protein from C_3 crops could help to target response efforts, including breeding decreased sensitivity to elevated [CO_2], biofortification, and supplementation.

METHODS SUMMARY

We examined the response of nutrient levels to elevated atmospheric [CO_2] for the edible portions of rice (*Oryza sativa*, 18 cultivars), wheat (*Triticum aestivum*, 8 cultivars), maize (*Zea mays*, 2 cultivars), soybeans (*Glycine max*, 7 cultivars), field peas (*Pisum sativum*, 5 cultivars) and sorghum (*Sorghum bicolor*, 1 cultivar). The six crops were grown under FACE conditions; in all six experiments the elevated [CO_2] was in the range 546–586 p.p.m.

In accordance with methods described previously[14,15], the natural logarithm of the response ratio (r = response in elevated [CO_2]/response in ambient [CO_2]) was used as the metric for analyses and is reported as the mean percentage change ($100 \times (r - 1)$) at elevated [CO_2]. Consistent with these earlier analyses of multiple species grown under FACE conditions, the responses of different species, cultivars and stress treatments and from different years of the FACE experiments were considered to be independent and suited to meta-analytic analysis[14].

The meta-analysis was designed to estimate the effect of elevated [CO_2] on the concentration of each nutrient in a particular crop and to determine the significance of this effect relative to a null hypothesis of no change. All tests were conducted as two-sided; that is, not specifying which direction the nutrient concentrations were expected to change under elevated [CO_2]. Meta-analysis was conducted with a linear mixed model.

Parameter estimates were obtained by the restricted maximum-likelihood method, a standard approach for analysing repeated measurements[29] that, in our case, were of nutrient concentrations at the time of harvest. Results for all analyses are reported as the best estimate of percentage changes in the concentration of nutrients along with the 95% confidence intervals associated with each estimate. Two-tailed P values are also reported.

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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1. Tulchinsky, T. H. Micronutrient deficiency conditions: global health issues. *Public Health Rev.* **32,** 243–255 (2010).
2. Caulfield, L. E. & Black, R. E. in *Comparative Quantification of Health Risks: Global and Regional Burden of Disease Attribution to Selected Major Risk Factors* (eds Ezzati, M., Lopez, A. D., Rodgers, A. & Murray, C. J. L.) Vol. 1, Ch. 5 (World Health Organization, 2004).
3. Stoltzfus, R. J., Mullany, L. & Black, R. E. in *Comparative Quantification of Health Risks: Global and Regional Burden of Disease Attribution to Selected Major Risk Factors* (eds Ezzati, M., Lopez, A. D., Rodgers, A. & Murray, C. J. L.) Vol. 1, Ch. 3 (World Health Organization, 2004).
4. De la Puente, L. S., Pérez, P. P., Martinez-Carrasco, R., Morcuende, R. M. & Del Molino, I. M. M. Action of elevated CO_2 and high temperatures on the mineral chemical composition of two varieties of wheat. *Agrochimica* **44,** 221–230 (2000).
5. 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. *Agric. Ecosyst. Environ.* **54,** 175–185 (1995).
6. Fangmeier, A., Grüters, U., Högy, P., Vermehren, B. & Jäger, H.-J. Effects of elevated CO_2, nitrogen supply and tropospheric ozone on spring wheat. II. Nutrients (N, P, K, S, Ca, Mg, Fe, Mn, Zn). *Environ. Pollut.* **96,** 43–59 (1997).
7. Pleijel, H. *et al.* Effects of elevated carbon dioxide, ozone and water availability on spring wheat growth and yield. *Physiol. Plant.* **108,** 61–70 (2000).
8. Seneweera, S. P. & Conroy, J. P. Growth, grain yield and quality of rice (*Oryza sativa* L.) in response to elevated CO_2 and phosphorus nutrition. *Soil Sci. Plant Nutr.* **43,** 1131–1136 (1997).
9. Lieffering, M., Kim, H.-Y., Kobayashi, K. & Okada, M. The impact of elevated CO_2 on the elemental concentrations of field-grown rice grains. *Field Crops Res.* **88,** 279–286 (2004).
10. Prior, S. A., Runion, G. B., Rogers, H. H. & Torbert, H. A. Effects of atmospheric CO_2 enrichment on crop nutrient dynamics under no-till conditions. *J. Plant Nutr.* **31,** 758–773 (2008).
11. Högy, P. & Fangmeier, A. Atmospheric CO_2 enrichment affects potatoes. 2. Tuber quality traits. *Eur. J. Agron.* **30,** 85–94 (2009).
12. Högy, P. *et al.* Effects of elevated CO_2 on grain yield and quality of wheat: results from a 3-year free-air CO_2 enrichment experiment. *Plant Biol.* **11,** 60–69 (2009).
13. 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. *Agric. Ecosyst. Environ.* **136,** 59–68 (2010).
14. Ainsworth, E. A. & Long, S. P. 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. *New Phytol.* **165,** 351–372 (2005).
15. Curtis, P. S. & Wang, X. A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. *Oecologia* **113,** 299–313 (1998).
16. Duval, B. D., Blankinship, J. C., Dijkstra, P. & Hungate, B. A. CO_2 effects on plant nutrient concentration depend on plant functional group and available nitrogen: a meta-analysis. *Plant Ecol.* **213,** 505–521 (2012).
17. Miller, L. V., Krebs, N. F. & Hambidge, M. K. A mathematical model of zinc absorption in humans as a function of dietary zinc and phytate. *J. Nutr.* **137,** 135–141 (2007).
18. Fisher, B. S. *et al.* in *Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Inter-governmental Panel on Climate Change* (eds Metz, B. *et al.*) 169–250 (Cambridge Univ. Press, 2007).
19. Appel, L. J. *et al.* Effects of protein, monounsaturated fat, and carbohydrate intake on blood pressure and serum lipids: results of the OmniHeart randomized trial. *J. Am. Med. Assoc.* **294,** 2455–2464 (2005).
20. Millward, D. Joe. Identifying recommended dietary allowances for protein and amino acids: a critique of the 2007 WHO/FAO/UNU report. *Br. J. Nutr.* **108,** S3–S21 (2012).
21. Swaminathan, S., Vaz, M. & Kurpad, A. V. Protein intakes in India. *Br. J. Nutr.* **108,** S50–S58 (2012).
22. Leakey, A. Rising atmospheric carbon dioxide concentration and the future of C_4 crops for food and fuel. *Proc. R. Soc. Lond. B* **276,** 2333–2343 (2009).
23. Rogers, A., Ainsworth, E. A. & Leakey, A. D. Will elevated carbon dioxide concentration amplify the benefits of nitrogen fixation in legumes? *Plant Physiol.* **151,** 1009–1016 (2009).
24. 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).
25. Leakey, A. D. *et al.* Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. *J. Exp. Bot.* **60,** 2859–2876 (2009).
26. Gifford, R., Barrett, D. & Lutze, J. The effects of elevated [CO_2] on the C:N and C:P mass ratios of plant tissues. *Plant Soil* **224,** 1–14, 10.1023/A:1004790612630 (2000).
27. McGrath, J. M. & Lobell, D. B. Reduction of transpiration and altered nutrient allocation contribute to nutrient decline of crops grown in elevated CO_2 concentrations. *Plant Cell Environ.* **36,** 697–705, 10.1111/pce.12007 (2013).
28. Monasterio, I. & Graham, R. D. Breeding for trace minerals in wheat. *Food Nutr. Bull.* **21,** 392–396 (2000).
29. Searle, S. R., Casella, G. & McCulloch, C. E. *Variance Components* (Wiley, 1992).

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Author Contributions S.S.M. conceived the overall project and drafted the manuscript. A.Z., I.K., J.S. and P.H. performed statistical analyses. P.H. and A.D.B.L. provided substantial input into methods descriptions. A.J.B., E.C. and V.R. analysed grain samples for nutrient content. G.F., T.H., A.D.B.L., R.L.N., M.J.O., H.S., S.S., M.T. and Y.U. conducted FACE experiments and supplied grain for analysis. N.M.H. and P.H. assisted with elements of experimental design. K.A.S. and L.H.D. assisted with data collection and analysis. All authors contributed to manuscript preparation.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to S.S.M. (smyers@hsph.harvard.edu).

METHODS

We examined the response of nutrient levels to elevated atmospheric [CO_2] for the edible portions of rice (*Oryza sativa*, 18 cultivars), wheat (*Triticum aestivum*, 8 cultivars), maize (*Zea mays*, 2 cultivars), soybeans (*Glycine max*, 7 cultivars), field peas (*Pisum sativum*, 5 cultivars) and sorghum (*Sorghum bicolor*, 1 cultivar). The six crops were grown under FACE conditions; in all six experiments, the elevated [CO_2] was in the range 546–586 p.p.m. (see the Agricultural Methods section below for details associated with individual trials).

Statistics. In accordance with methods described previously[14,15], the natural logarithm of the response ratio (r = response in elevated [CO_2]/response in ambient [CO_2]) was used as the metric for analyses and is reported as the mean percentage change ($100 \times (r - 1)$) at elevated [CO_2]. Consistent with these earlier analyses of multiple species grown under FACE conditions, the responses of different species, cultivars and stress treatments and from different years of the FACE experiments were considered to be independent and suited to meta-analytic analysis[14].

The meta-analysis was designed to estimate the overall effect of elevated [CO_2] on the concentration of each nutrient in a particular crop and to determine the significance of this effect relative to a null hypothesis of no change. All tests were conducted as two-sided—not specifying which direction the nutrient concentrations were expected to change under elevated [CO_2]—to make the analysis as general as possible. Meta-analysis was conducted with a linear mixed model. A random intercept was included for each comparison, representing nutrient level variability unrelated to [CO_2] that was common to both treatment groups. Additional analyses indicated that the effect of [CO_2] on zinc concentration in rice was modified by cultivar and amount of nitrogen application, suggesting systematic variations across the pooled analysis of rice, and for these samples it was shown that the effect on zinc concentration was still significant when including interactions terms for cultivar and nitrogen. No other significant modifications of the [CO_2] effect were identified. We tested whether changes in different nutrients for particular crops were statistically different from each other, as has been described[30]. To address the issue of multiple comparisons when testing for differences between cultivars within a crop, we multiplied the P value by the number of independent comparisons. This approach follows the so-called Bonferroni correction and is conservative in the sense of biasing the P values high, but still shows that individual test results are significant despite their having been selected from multiple tests.

Parameter estimates were obtained by the restricted maximum-likelihood method, a standard approach for analysing repeated measurement data[29] that, in our case, were of nutrient concentrations at time of harvest. Results for all analyses are reported as the best estimate of percentage changes in the concentration of nutrients along with the 95% confidence intervals associated with each estimate. Two-tailed P values are also reported.

When combining our data with previously published data, we defined outliers as pairs in which the difference between an observation at ambient [CO_2] and elevated [CO_2] was at least three times the standard deviation from the mean differences for that crop and nutrient type when calculated using all observations. Using this criterion, we excluded a total of two pairs of previously published data from all analyses; these included one observation of iron in rice and one observation of zinc in potato.

Agricultural methods. Rice (*Oryza sativa*, 18 cultivars), wheat (*Triticum aestivum*, 8 cultivars), maize (*Zea mays*, 2 cultivars), soybeans (*Glycine max*, 7 cultivars), field peas (*Pisum sativum*, 4 cultivars) and sorghum (*Sorghum bicolor*, 1 cultivar) were grown under FACE conditions during daylight hours. The experiments were conducted in Australia, Japan and the United States between 1998 and 2010. Ambient [CO_2] ranges were between 363 and 386 p.p.m.; elevated [CO_2] was between 546 and 584 p.p.m. With the exception of soybeans, each experiment involveed multiple cultivars of each crop and more than one set of growing conditions. Each experiment for each cultivar and set of treatments was replicated four times, with the exception of one of the rice sites, for which three replicates were performed. These data are summarized in Table 1, and additional details of the soil and growing conditions, FACE methods and experimental designs have been published for rice[31], wheat[32], maize[33], soybeans[34], field peas[32] and sorghum[35].

Minerals method. Samples were analysed for minerals by heated closed-vessel digestion/dissolution with nitric acid and hydrogen peroxide followed by quantification with an inductively coupled plasma atomic emission spectrometer[36]. Nitrogen content was measured by flash combustion of the sample coupled with thermal conductivity/infrared detection of the combustion gases (N_2, NO_x and CO_2) with a LECO TruSpec CN Analyzer[37]. Protein values are based on measurement of nitrogen and conversion to protein with the equation below, where $k = 5.36$ (ref. 38):

$$\text{protein (weight \%)} = k \times \text{nitrogen (weight \%)}$$

For phytic acid determination, a modified version of the method of ref. 39 was used. The accuracy of the method was monitored by the inclusion of tissue standards of known and varying levels of phytic acid[40].

Dietary calculations. The United Nations Food and Agriculture Organization (UNFAO) publishes annual Food Balance Sheets, which provide country-specific data on the quantities of 95 'standardized' food commodities available for human consumption. Data, expressed in terms of dietary energy (kilocalories per person per day) were downloaded for 210 countries and territories with available information for the period 2003–2007 (available at http://faostat.fao.org). The percentage of dietary energy available from C_3 grasses (wheat, barley, rye, oats, rice and 'cereals, other' (excluding *Eragrostis tef*)) was calculated globally with estimates weighted by national population size (188 countries available; UN 2011; 2012 revision available at http://esa.un.org/wpp/).

Dietary intake data from the UNFAO Food Balance Sheets (to year 2000) and food composition data from the United States Department of Agriculture National Nutrient Database for Standard Reference were used to calculate per-person nutrient intake for 95 food items; these were shared with us with permission[41]. This data set was used to calculate the contribution of each food item to total dietary zinc and iron intake, and the proportions of all food items derived from C_3 grains and legumes were summed to identify countries that are highly dependent on plant sources of iron and zinc (Extended Data Table 5).

30. Schenker, N. & Gentleman, J. F. On judging the significance of differences by examining the overlap between confidence intervals. *Am. Stat.* **55,** 182–186 (2001).
31. Hasegawa, T. A. *et al.* Rice cultivar responses to elevated CO_2 at two free-air CO_2 enrichment (FACE) sites in Japan. *Funct. Plant Biol.* **40,** 148–159 (2013).
32. Mollah, M., Norton, R. & Huzzey, J. Australian Grains Free Air Carbon dioxide Enrichment (AGFACE) facility: design and performance. *Crop Pasture Sci.* **60,** 697–707 (2009).
33. Markelz, R., Strellner, R. & Leakey, A. Impairment of C_4 photosynthesis by drought is exacerbated by limiting nitrogen and ameliorated by elevated CO_2 in maize. *J. Exp. Bot.* **62,** 3235–3246 (2011).
34. Gillespie, K. *et al.* Greater antioxidant and respiratory metabolism in field-grown soybean exposed to elevated O_3 under both ambient and elevated CO_2. *Plant Cell Environ.* **35,** 169–184 (2012).
35. Ottman, M. J. *et al.* Elevated CO_2 increases sorghum biomass under drought conditions. *New Phytol.* **150,** 261–273 (2001).
36. Sah, R. N. & Miller, R. O. Spontaneous reaction for acid dissolution of biological tissues in closed vessels. *Anal. Chem.* **64,** 230–233 (1992).
37. AOAC Official Method 972.43. in *Official Methods of Analysis of AOAC International, 18th edition, Revision 1, 2006* Ch. 12 5–6 (AOAC International, 2006).
38. Mosse, J. Nitrogen to protein conversion factor for ten cereals and six legumes or oilseeds. A reappraisal of its definition and determination. Variation according to species and to seed protein content. *J. Agric. Food Chem.* **38,** 18–24 (1990).
39. Haug, W. & Lantzsch, H. J. Sensitive method for the rapid determination of phytate in cereals and cereal products. *J. Sci. Food Agric.* **34,** 1423–1426 (1983).
40. Raboy, V. *et al.* Origin and seed phenotype of maize *low phytic acid 1-1* and *low phytic acid 2-1*. *Plant Physiol.* **124,** 355–368 (2000).
41. Wuehler, S. E., Peerson, J. M. & Brown, K. H. Use of national food balance data to estimate the adequacy of zinc in national food supplies: methodology and regional estimates. *Public Health Nutr.* **8,** 812–819 (2005).

Extended Data Table 1 | Percentage change in nutrient content at elevated [CO_2] relative to ambient [CO_2]

	N* (number of pairs)	Zn (µg/g) %	Zn 95% CI	Zn P-value	Fe (µg/g) %	Fe 95% CI	Fe P-value	Protein (mg/g) %	Protein 95% CI	Protein P-value	Phytate (g/100g) %	Phytate 95% CI	Phytate P-value
C3 grasses													
Wheat	64	-9.3	(-12.7,-5.9)	<.0001	-5.1	(-6.5,-3.7)	<.0001	-6.3	(-7.5,-5.2)	<.0001	-4.2	(-7.5,-0.8)	0.009
Rice	31	-3.3	(-5.0,-1.7)	<.0001	-5.2	(-7.6,-2.9)	<.0001	-7.8	(-8.9,-6.8)	<.0001	1.2	(-4.6,7.4)	0.697
C3 legumes													
Field peas	10	-6.8	(-9.8,-3.8)	0.002	-4.1	(-6.7,-1.4)	0.003	-2.1	(-4.0,-0.1)	0.039	-5.8	(-11.5,0.1)	0.055
Soybeans	25	-5.1	(-6.4,-3.9)	<.0001	-4.1	(-5.8,-2.5)	<.0001	0.5	(-0.4,1.3)	0.267	-1.3	(-3.7,1.2)	0.303
C4 grasses													
Maize	4	-5.2	(-10.7,0.6)	0.077	-5.8	(-10.9,-0.3)	0.038	-4.6	(-13.0,4.5)	0.312	-6.1	(-15.0,3.7)	0.215
Sorghum	4	-1.3	(-6.2,3.8)	0.603	1.6	(-5.8,9.7)	0.674	0.0	(-4.9,5.2)	0.993	12.8	(-15.8,51.1)	0.418

* 'Number of pairs' refers to the number of comparisons in which replicates of a particular cultivar grown at a specific site under one set of growing conditions in one year at elevated [CO_2] have been pooled and mean nutrient values for these replicates were compared with mean values for identical cultivars under identical growing conditions except grown at ambient [CO_2]. In most instances, data from four replicates were pooled for each value, meaning that eight experiments were combined for each comparison (see Table 1 for details of experiments).

Extended Data Table 2 | Original data combined with previously published FACE data from studies 3, 4, 6 and 7

	N* (number of pairs)	Zn (µg/g)			Fe (µg/g)			Protein (mg/g)		
		%	95% CI	P-value	%	95% CI	P-value	%	95% CI	P-value
C3 grasses										
Wheat	70	-8.8	(-11.9,-5.6)	<.0001	-5.5	(-6.8,-4.1)	<.0001	-6.5	(-7.5,-5.4)	<.0001
Rice	32	-3.1	(-4.8,-1.5)	<.0001	-4.9	(-7.3,-2.6)	<.0001	-8	(-9.0,-6.9)	<.0001
Barley	4	-11.4	(-19.3,-2.7)	0.012	-10.5	(-12.2,-8.7)	<.0001	-11.9	(-13.1,-10.7)	<.0001
C3 legumes										
Field peas	10	-6.8	(-9.8,-3.8)	0.002	-4.1	(-6.7,-1.4)	0.003	-2.1	(-4.0,-0.1)	0.039
Soybeans	25	-5.1	(-6.4,-3.9)	<.0001	-4.1	(-5.8,-2.5)	<.0001	0.5	(-0.4,1.3)	0.267
C_3 tubers										
Potato	2	-3.9	(-12.9,6.2)	0.440	2.3	(-3.8,8.7)	0.472	-4.6	(-7.7,-1.4)	<.0001
C4 grasses										
Maize	4	-5.2	(-10.7,0.6)	0.077	-5.8	(-10.9,-0.3)	0.038	-4.6	(-13.0,4.5)	0.312
Sorghum	4	-1.3	(-6.2,3.8)	0.603	1.6	(-5.8,9.7)	0.674	0.0	(-4.9,5.2)	0.993

See Extended Data Table 6 for a list of experiments. Percentage change in nutrient content at elevated [CO_2] relative to ambient [CO_2].
* 'Number of pairs' refers to the number of comparisons in which replicates of a particular cultivar grown at a specific site under one set of growing conditions in one year at elevated [CO_2] have been pooled and mean nutrient values for these replicates were compared with mean values for identical cultivars under identical growing conditions except grown at ambient [CO_2]. In most instances, data from four replicates were pooled for each value, meaning that eight experiments were combined for each comparison (see Table 1 for details of experiments).

Extended Data Table 3 | Original data combined with previously published FACE and chamber data from studies 1–10

	N* (number of pairs)	Zn (µg/g)			Fe (µg/g)			Protein (mg/g)		
		%	95% CI	P-value	%	95% CI	P-value	%	95% CI	P-value
C3 grasses										
Wheat	78	-9.1	(-12.1,-6.1)	<.0001	-5.9	(-7.8,-4.0)	<.0001	-7.2	(-8.6,-5.8)	<.0001
Rice	32	-3.1	(-4.8,-1.5)	<.0001	-4.9	(-7.3,-2.6)	<.0001	-8	(-9.0,-6.9)	<.0001
Barley	6	-13.6	(-19.3,-7.6)	<.0001	-10.0	(-12.4,-7.4)	<.0001	-15.0	(-19.1,-10.7)	<.0001
C3 legumes										
Field peas	10	-6.8	(-9.8,-3.8)	<.0001	-4.1	(-6.7,-1.4)	0.003	-2.1	(-4.0,-0.1)	0.039
Soybeans	28	-5.0	(-6.1,-3.9)	<.0001	-5.2	(-7.9,-2.5)	<.0001	0.1	(-0.8,0.9)	0.865
C_3 tubers										
Potato	5	-10.0	(-20.9,2.4)	0.110	-4.1	(-16.6,10.3)	0.555	-9.7	(-15.9,-3.1)	0.005
C4 grasses										
Maize	4	-5.2	(-10.7,0.6)	0.077	-5.8	(-10.9,-0.3)	0.038	-4.6	(-13.0,4.5)	0.312
Sorghum	7	-0.6	(-4.5,3.4)	0.764	33.8	(-10.2,99.3)	0.153	-5.6	(-12.7,2.1)	0.150

See Extended Data Table 6 for a list of experiments. Percentage change in nutrient content at elevated [CO_2] relative to ambient [CO_2].
* 'Number of pairs' refers to the number of comparisons in which replicates of a particular cultivar grown at a specific site under one set of growing conditions in one year at elevated [CO_2] have been pooled and mean nutrient values for these replicates were compared with mean values for identical cultivars under identical growing conditions except grown at ambient [CO_2]. In most instances, data from four replicates were pooled for each value, meaning that eight experiments were combined for each comparison (see Table 1 for details of experiments).

Extended Data Table 4 | Percentage change in nutrient content at elevated [CO_2] compared with ambient [CO_2] for all nutrients

	C3 grasses						C3 legumes						C4 grasses					
	Wheat			Rice			Field Peas			Soybean			Maize			Sorghum		
	%	95% CI	P-value	%	95% CI	P-value	%	95% CI	P-value	%	95% CI	P-value	%	95% CI	P-value	%	95% CI	P-value
Zinc (ppm)	-9.3	(-12.7,-5.9)	<.0001	-3.3	(-5.0,-1.7)	<.0001	-6.8	(-9.8,-3.8)	<.0001	-5.1	(-6.4,-3.9)	<.0001	-5.2	(-10.7,0.6)	0.077	-1.3	(-6.2,3.8)	0.603
Iron (ppm)	-5.1	(-6.5,-3.7)	<.0001	-5.2	(-7.6,-2.9)	<.0001	-4.1	(-6.7,-1.4)	<.0001	-4.1	(-5.8,-2.5)	<.0001	-5.8	(-10.9,-0.3)	0.038	1.6	(-5.8,9.7)	0.674
Phytate (mg/g)	-4.2	(-7.5,-0.8)	0.009	1.2	(-4.6,7.4)	0.7	-5.8	(-11.5,0.1)	0.055	-1.3	(-3.7,1.2)	0.303	-6.1	(-15.0,3.7)	0.215	12.8	(-15.8,51.1)	0.418
Protein	-6.3	(-7.5,-5.2)	<.0001	-7.8	(-8.9,-6.8)	<.0001	-2.1	(-4.0,-0.1)	0.039	0.5	(-0.4,1.3)	0.267	-4.6	(-13.0,4.5)	0.312	0.0	(-4.9,5.2)	0.993
Mn (ppm)				-7.5	(-12.0,-2.8)	<.0001	-2.5	(-4.2,-0.8)	0.005	-1.4	(-3.5,0.8)	0.204	-4.2	(-10.5,2.5)	0.215	1.7	(-4.5,8.3)	0.596
Mg (%)				-0.9	(-2.3,0.6)	0.24	0.0	(-1.3,1.4)	0.960	-3.5	(-4.3,-2.8)	<.0001	-5.7	(-9.9,-1.3)	0.011	-0.2	(-5.1,4.9)	0.944
Cu (ppm)				-10.6	(-13.8,-7.1)	<.0001	-2.7	(-5.1,-0.3)	0.025	-5.7	(-8.0,-3.4)	<.0001	-9.9	(-19.3,0.7)	0.066	-2.9	(-7.1,1.5)	0.190
Ca (%)				2	(-0.8,4.9)	0.16	-0.5	(-4.2,3.3)	0.787	-5.8	(-7.3,-4.2)	<.0001	-2.7	(-16.9,13.9)	0.734	11.2	(-5.2,30.3)	0.190
S (ppm)				-7.8	(-8.8,-6.8)	<.0001	-2.2	(-3.6,-0.7)	0.003	-2.9	(-3.5,-2.2)	<.0001	2.1	(-2.2,6.7)	0.342	-0.2	(-5.4,5.2)	0.936
K (%)				1.1	(-0.3,2.5)	0.13	2.2	(0.6,3.8)	0.008	0.1	(-0.8,1.0)	0.857	-2.7	(-3.1,-2.2)	<.0001	3.0	(-2.7,9.1)	0.308
B (ppm)				5.1	(1.9,8.4)	0.002	-1.9	(-3.9,0.1)	0.057	-6.4	(-9.1,-3.6)	<.0001	4.9	(-1.0,11.1)	0.107	-0.3	(-9.3,9.6)	0.952
P (%)				-1.0	(-2.4,0.4)	0.160	-3.7	(-6.8,-0.5)	0.023	-0.7	(-2.2,0.9)	0.379	-7.1	(-9.0,-5.1)	<.0001	0.3	(-4.0,4.9)	0.881

Sample sizes for each crop type are identical to those listed in Table 1.

Extended Data Table 5 | Countries whose populations receive at least 60% of dietary iron and/or zinc from C_3 grains and legumes

Country	% Iron from C_3 grains & legumes	% Zinc from C_3 grains & legumes	Population (in thousands)
Afghanistan	78%	78%	31,412
Algeria	76%	79%	35,468
Iraq	74%	83%	31,672
Bangladesh	72%	88%	148,692
Iran, Islamic Rep of	72%	77%	73,974
Pakistan	70%	72%	173,593
Tunisia	70%	77%	10,481
Jordan	69%	73%	6,187
Morocco	69%	78%	31,951
Syrian Arab Republic	67%	71%	20,411
Libya	67%	71%	6,355
Yemen	66%	75%	24,053
Myanmar	65%	81%	47,963
Tajikistan	62%	56%	6,879
India	59%	71%	1,224,614
Egypt	54%	65%	81,121
Indonesia	52%	65%	239,871
Sierra Leone	51%	70%	5,868
Cambodia	49%	68%	14,138
Sri Lanka	46%	69%	20,860
Laos	44%	66%	6,201
Viet Nam	43%	61%	87,848
Total			2,329,612

Source: United Nations Food and Agriculture Organization food balance sheets and 2010 United Nations estimated population.

Extended Data Table 6 | Literature reporting nutrient changes in the edible portion of crops grown at elevated and ambient [CO_2]

Study	Experimental Method	Associated Citations
1	Growth Chambers	Conroy , J., Seneweera, S. P., Basra, A., Rogers, G. & Nissen-Wooller, B. Influence of rising atmospheric CO_2 concentrations and temperature on growth, yield and grain quality of cereal crops. Australian Journal of Plant Physiology 21, 741-758 (1994).
		Seneweera , S., Milham, P. & Conroy, J. Influence of elevated CO_2 and phosphorus nutrition on the growth and yield of a short-duration rice. Australian Journal of Plant Physiology 21, 281-292 (1994).
		Seneweera, S. P. & Conroy, J. P. Growth, grain yield and quality of rice (Oryza sativa L.) in response to elevated CO_2 and phosphorus nutrition (Reprinted from Plant nutrition for sustainable food production and environment, 1997). Soil Sci. Plant Nutr. 43, 1131-1136 (1997).
2	Temperature Gradient Tunnels	De la Puente, L. S., Perez, P. P., Martinez-Carrasco, R., Morcuende, R. M. & Del Molino, I. M. M. Action of elevated CO_2 and high temperatures on the mineral chemical composition of two varieties of wheat. Agrochimica 44, 221-230 (2000).
3	Open Top Chambers & FACE	De Temmerman L et al. Effect of climatic conditions on tuber yield (Solanum tuberosum L.) in the European 'CHIP' experiments. European Journal of Agronomy 17, 243-255 (2002).
		De Temmerman, L., Hacour, A. & Guns, M. Changing climate and potential impacts on potato yields and quality 'CHIP': introduction, aims and methodology. European Journal of Agronomy 17, 233-242 (2002).
		Fangmeier, A., De Temmerman, L., Black, C., Persson, K. & Vorne, V. Effects of elevated CO_2 and/or ozone on nutrient concentrations and nutrient uptake of potatoes. European Journal of Agronomy 17, 353-368 (2002).
		Högy, P. & Fangmeier, A. Atmospheric CO_2 enrichment affects potatoes: 2. Tuber quality traits. European Journal of Agronomy 30, 85-94 (2009).
4	FACE	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. Agriculture, Ecosystems and Environment 136, 59-68 (2010).
5	Open Top Chambers	Fangmeier, A. et al. Effects of elevated CO_2, nitrogen supply and tropospheric ozone on spring wheat. I. Growth and yield. Environmental Pollution 91, 381-390 (1996).
		Fangmeier, A., Grüters, U., Högy, P., Vermehren, B. & Jäger, H.-J. Effects of elevated CO_2, nitrogen supply and tropospheric ozone on spring wheat – II. Nutrients (N, P, K, S, Ca, Mg, Fe, Mn, Zn). Environmental Pollution 96, 43-59 (1997).
		Fangmeier, A. et al. Effects on nutrients and on grain quality in spring wheat crops grown under elevated CO_2 concentrations and stress conditions in the European, multiple-site experiment 'ESPACE-wheat'. European Journal of Agronomy 10, 215-229 (1999).
		Jäger , H.-J., Hertstein, U. & Fangmeier, A. The European Stress Physiology and Climate Experiment – project 1: wheat (ESPACE-wheat): introduction, aims and methodology. European Journal of Agronomy 10, 155-162 (1999).
6	FACE	Högy, P. & Fangmeier, A. Effects of elevated atmospheric CO_2 on grain quality of wheat. Journal of Cereal Science 48, 580-591 (2008).
		Högy, P. et al. Does elevated atmospheric CO_2 allow for sufficient wheat grain quality in the future? . Journal of Applied Botany and Food Quality 82, 114-121 (2009).
		Högy, P. et al. Effects of elevated CO_2 on grain yield and quality of wheat: results from a 3-year free-air CO2 enrichment experiment. Plant Biology 11, 60-69 (2009).
		Högy, P., Zörb, C., Langenkämper, G., Betsche, T. & Fangmeier, A. Atmospheric CO_2 enrichment changes the wheat grain proteome. Journal of Cereal Science 50, 248-254 (2009).
7	FACE	Kim , H., Lieffering, M., Miura, S., Kobayashi, K. & Okada, M. Growth and nitrogen uptake of CO_2-enriched rice under field conditions. New Phytologist 150, 223-229 (2001).
		Kim , H. et al. Effects of free-air CO_2 enrichment and nitrogen supply on the yield of temperate paddy rice crops. Field Crops Research 83, 261-270 (2003).
		Lieffering, M., Kim, H.-Y., Kobayashi, K. & Okada, M. The impact of elevated CO_2 on the elemental concentrations of field-grown rice grains. Field Crops Research 88, 279-286 (2004).
8	Open Top Chambers	Pleijel, H. et al. Effects of elevated carbon dioxide, ozone and water availability on spring wheat growth and yield. Physiologia Plantarum 108, 61-70 (2000).
		Pleijel, H. & Danielsson, H. Yield dilution of grain Zn in wheat grown in open-top chamber experiments with elevated CO_2 and O3 exposure. Journal of Cereal Science 50, 278-282 (2009).
9	Open Top Chambers	Prior, S. A., Runion, G. B., Rogers, H. H., Torbert, H. A. Effects of atmospheric CO_2 enrichment on crop nutrient dynamics under no-till conditions. Journal of Plant Nutrition 31, 758-773 (2008).
10	Open Top Chambers	Weigel, H., Manderscheid, R., Jäger, H.-J. & Mejer, G. Effects of season-long CO_2 enrichment on cereals. I. Growth performance and yield. Agriculture, Ecosystems and Environment 48, 231-240 (1994).
		Manderscheid, R., Bender, J., Jager, H., J & Weigel, H., J. Effects of season long CO_2 enrichment on cereals. II. Nutrient concentrations and grain quality. Agriculture, Ecosystems & Environment 54, 175-185 (1995).
11	FACE	Yang, L., Wang, Y., Dong, G., Gu, H., Huang, J., Zhu, J., Yang, H., Liu, G., Han, Y. The impact of free-air CO_2 enrichment (FACE) and nitrogen supply on grain quality of rice. Field Crops Research 102, 128-140 (2007).
	Meta-Analyses	Loladze, I. Rising atmospheric CO_2 and human nutrition: toward globally imbalanced plant stoichiometry? Trends in Ecology and Evolution 17 (10), 457-461 (2002). [Uses data from studies 1, 2, 5, and 10 as well as numerous other studies on non-edible tissues and plants other than food crops].
		McGrath, J. M. and Lobell, D. B. Reduction of transpiration and altered nutrient allocation contribute to nutrient decline of crops grown in elevated CO_2 concentrations. Plant, Cell, & Environment 36, 697-705 (2013). [Uses data from studies 1, 5, and 10 as well as numerous other studies on non-edible tissues and plants other than food crops].
		Duval, B.D., Blankinship, J. C., Dijkstra, P., Hungate, B. A. CO2 effects on plant nutrient concentration depend on plant functional group and available nitrogen: a meta-analysis. Plant Ecology 213, 505-521 (2012). [Uses data from studies 1,2, 3, 5, 6, and 9 as well as numerous other studies on non-edible tissues and plants other than food crops].