Science of the Total Environment
2019, 651:2641-2647
https://doi.org/10.1016/j.scitotenv.2018.10.170

Impact of elevated CO_2 on grain nutrient concentration varies with crops and soils – a long-term FACE study

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Abstract
The impact of elevated CO_2 (eCO_2) on grain nutrient concentration is becoming a global concern in terms of future human nutrition. Previous research has shown that eCO_2 can alter the availability and uptake of nutrients in crops. However, the interactive effects of long-term eCO_2 and soil types on the concentrations of nutrients in grain are poorly understood. By understanding such effects, we are able to develop management practices that maintain grain nutritional quality while improving crop yield in response to future climatic conditions. We conducted a seven-year experiment of free air CO_2 enrichment (FACE) with a rotation of wheat, field pea and canola grown in a Chromosol (Luvisol), Vertosol (Vertisol) and Calcarosol (Calcic Xerosol) under ambient CO_2 (aCO_2) (390 ± 10 μmol mol^{-1}) or eCO_2 (550 ± 30 μmol mol^{-1}). The concentration and amount of five macro- and four micro-nutrients in grain over the seven years were determined. Compared to aCO_2, the concentrations of N, P and Zn decreased by 6%, 5% and 10% under eCO_2, respectively, irrespective of soil, crop and year. A greater decrease in N concentration was found in canola and wheat compared to field pea. The reduction in P and Mg concentration of canola was significant in Chromosol, but not in the Vertosol nor Calcarosol soils. The concentrations of K, Fe, Mn and Cu were not affected by eCO_2 in any crop grown in the soils tested. Furthermore, eCO_2 significantly decreased soil labile N and P and exchangeable Mg and Cu due to greater nutrient uptake, which was in part ascribed to the decreased nutrient accumulation in crop grains. It appears that eCO_2 lowers the nutritional quality (nutrient concentration) in grains of non-legume crops, and that the extent of this decrease was greater in relatively fertile than infertile soils.

Keywords: Climate change; Crop rotation; free air CO_2 enrichment; Grain quality; High atmospheric CO_2; Soil types

1. Introduction
Atmospheric CO_2 concentration has passed 400 μmol mol^{-1} (www.co2.earth) in 2017, and continues to increase (Eggleton, 2013). Under the scenario of climate change, the atmospheric

CO_2 is predicted to reach approximately 550 μmol mol^{-1} by 2050 and 700 μmol mol^{-1} by 2100 (IPCC, 2013). Elevated atmospheric CO_2 (eCO_2) can affect both grain yields and quality (Dietterich et al., 2015). Although crop growth and yields under eCO_2 have been well investigated, relatively less attention has been paid to the nutritional quality of grains (Ainsworth and Long, 2005; Thomas et al., 2009; Saha et al., 2015). In the grain, nutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), iron (Fe), copper (Cu), manganese (Mn) and selenium (Se) are essential mineral elements for human and animal nutrition (Smith et al., 2018). Previous short-term controlled environment studies reported that eCO_2 led to a decrease in the concentrations of Zn and Fe in the grains of wheat (La Puente et al., 2000), barley (Manderscheid et al., 1995), and rice plants (Seneweera and Conroy, 1997). More recently, the decrease in concentrations of grain nutrients such as N, Ca, Zn and Fe were observed in soybean, sorghum, potatoes, wheat and barley grown in the free-air CO_2 enrichment (FACE) facilities with 546–586 μmol mol^{-1} of CO_2 concentration (Myers et al., 2014; Dietterich et al., 2015). However, the effect of eCO_2 on the concentrations of nutrients in grain are still unclear especially over longer time frames and in in dryland cropping regions. The adverse effect of eCO_2 on grain quality reduces both nutritional (Wang et al., 2011) and economic benefits (Lyman et al., 2013). As some nutrients such as Fe and Zn are already deficient in the diets of major developing counties (Brown et al., 2001; Nielsen, 2015), the continuous increase of atmospheric CO_2 concentration is a potential challenge for global human nutrition (Ingram et al., 2008; Erbs et al., 2010).

The mechanisms for the eCO_2-induced decrease in nutrient concentrations remain unclear. The 'carbohydrate dilution effect' is one possible mechanism whereby a stimulation of plant carbohydrate production by eCO_2 dilutes the rest of the grain components (Gifford et al., 2000; Myers et al., 2014). However, the CO_2 effect differed among N, P, K, Zn, Ca, Mn and Sulphur (S) in wheat (Fangmeier et al., 1999), suggesting that the dilution effect alone cannot produce these various responses. Effects on the nutrient concentrations varied among crop species, and even among cultivars of the same species, suggesting that the mechanisms associated with these changes may be species specific (Dietterich et al., 2015). Varying responses of grain nutrient concentration to eCO_2 among species reflect both differing physiological responses including transpirational efficiency and rates of photosynthesis, resulting in yield differences as well as inherent differences between species in their ability to access soil nutrients. This is because eCO_2 may stimulate root exudation that likely facilitates plants to access soil nutrients in many species (Haase et al., 2008). Moreover, the nutritional quality of grains under eCO_2 is likely to vary between soils as well, because soil properties influence nutrient availability to plant growth (Jin et al., 2017). Although the application of fertilizers can assist plants to meet their demands for nutrients, plants still rely on the nutrient reserves in the soil for nutrient uptake to some extent (White and Brown, 2010). When crops are grown under eCO_2, the availability of soil nutrients may decrease over longer time periods (years) due to greater yields which result in a greater quantity of nutrients being exported from the system. Decreases in soil nutrient supply may lower the concentration of nutrients in grain (Tausz et al., 2017). Nevertheless, the impacts of eCO_2 on grain nutritional quality may differ markedly between soils contrasting in chemical and physical properties. When plants are grown in different soils, plant response to eCO_2 may be reflected on changes of root morphology and root exudation depending on soil type (Jin et al., 2015), leading to likely changes in nutrient uptake and translocation to grains. Furthermore, under field conditions, environmental factors such as rainfall and temperature are also likely to influence interactions between atmospheric CO_2, soil and the crop, and therefore the pattern and amount of nutrients absorbed and ultimately grain nutritional quality. Under eCO_2 environments, longer-term field trails where crops are grown

in different soils are needed to clarify the eCO_2 effect on grain nutritional quality and to explore relevant mechanisms.

The aim of this study was to investigate the interactive effect of eCO_2 and soil type on the concentration of key nutrients in grains of crops grown in a wheat-field pea-canola-rotation system. We hypothesized that the eCO_2-induced changes in grain nutrient concentrations depended on soils and crop species because the investigated crops differ in nutrient acquisition capability under eCO_2 and nutrient availability differs between soils. The grains were collected from a seven-year field trial under the FACE so that accurate and reliable information regarding the long-term impact of climate change on grain quality would be provided.

2. Materials and Methods

2.1. Experimental design

We conducted an FACE experiment from 2009 to 2016 in a dryland cropping region (36°44′57″S, 142°06′50″E). Eight FACE bunkers were established; four were treated for eCO_2 (550 ± 30 μmol mol^{-1}) and the other four for aCO_2 (390 ± 30 μmol mol^{-1}), representing four replicates for each treatment. The design and management of the FACE system were specified in Mollah et al. (2009).

Three soils, i.e. Chromosol, Vertosol and Calcarosol (Isbell 1996) or Luvisol, Vertisol and Calcisol (WRB, 2014) were involved in this FACE study (SoilFACE). These soils represented three major soil types in dryland cropping systems of south-eastern Australia. Intact soil cores were collected from paddocks, and placed in in large PVC cylinders or mesocosms (0.3 m in diameter; 1.0 m in depth), weighing between 135 and 150 kg each depending on the soil type. The soil profile of 1 m is considered to be sufficient to allow normal root development as root depths of wheat were generally in the range of 50-110 cm in some soils (Dracup et al., 1992; Kirkegaard and Lilley, 2007**).** The soil properties in the top 10-cm soil were as followed. The Chromosol had total C, 48.9 g kg^{-1}; total N, 4.0 g kg^{-1}; pH, 4.5; clay, 18.3% and sand, 15.9%. The Vertosol had total C, 9.4 g kg^{-1}; total N, 0.8 g kg^{-1}; pH, 7.3; clay, 51.1% and sand, 12%. The Calcarosol had total C 4.4 g kg^{-1}; total N, 0.4 g kg^{-1}; pH, 5.7; clay, 5.5% and sand 84.5% (Jin et al., 2017). The intact soil cores were put into the bunkers levelled with the ground (Butterly et al., 2016). The physicochemical properties of soil in the mesocosms were maintained the same as the soil profile in paddocks. Twelve mesocosms in each bunker were used in this study (four for each soil), and these mesocosms were next to each other to allow plants to establish canopy in a similar environmental conditions to surrounding crops in the field. The plants were rain-fed without any extra irrigation. The climate at the experiment location belongs to a Mediterranean type of which winter is cool and wet, while summer is dry and hot. The details of rainfall and temperature information during the experimental period were provided in Jin et al. (2017).

This study had a wheat-field pea-canola rotation: field pea (*Pisum sativum* L. cv. PBA Twilight) in 2009, wheat (*Triticum aestivum* L. cv. Yitpi) in 2010, field pea in 2011, wheat in 2012, canola (*Brassica napus* L. cv. Hyola 50) in 2013, wheat in 2014, and canola in 2015. Phosphorus was annually applied as triple superphosphate at 15 kg P ha^{-1}. Nitrogen was applied in urea at 75 kg N ha^{-1} for wheat and canola, while no N fertilizer was applied to field pea. The same types and amounts of fertilizers were applied to all three soils.

2.2. Grain harvest, soil sampling and measurements

At crop maturity, grains were harvested each year and grain weight measured after manually threshing the grain from the straw. In each bunker, grains collected from four mesocosms of individual soil type were bulked to form one replicate, dried, weighed and kept in air-tight

plastic containers at room temperature. Subsamples of grains were further dried at 70°C for 72 h, and ground into fine powder using a ball mill (Retsch MM400, Germany). The ground samples were digested in a nitric and perchloric acid mixture (4:1) (Yuen and Pollard, 1954), and the concentrations of P, K, Ca, Mg, Fe, Mn, Zn and Cu in digests were analysed using ICP-OES (Perkin Elmer Optima 8000, MA, USA). Nutrient concentration was presented on a dry-weight basis. The concentration of N in grains was determined by dry combustion using a CHNS/O analyser (Perkin Elmer 2400 Series II, USA).

Soil samples were collected from surface soils (0-10 cm) in December 2015 following crop harvest. Soil cores from 4 mesocosms of each soil type within a bunker were bulked and treated as one replicate. Fifteen grams of fresh soil samples were extracted with 15 ml of 2 M KCl and the concentrations of NH_4^+ and NO_3^- in the extracts were then determined using a flow injection analyser (Lachat QuickChem 8500 Series II, USA). The remaining soil samples was air-dried and passed through a 0.5-mm sieve for other chemical analyses. Forty million litre of Mehlich 3 extractant that contained ammonium fluoride, ethylene diamine tetra acetic acid (EDTA) and ammonium nitrate was added to 4 g of soil to extract exchangeable nutrients including K, Ca, Mg, Fe, Mn, Zn and Cu (Rayment and Lyons, 2011). The concentrations of these nutrients in extracts were then determined by ICP-OES.

2.3. Statistical analyses

A split-plot design was deployed in this experiment with CO_2 as the main plots, and soil type and species nested within the CO_2 (Uddin et al., 2018). Analysis of variance (ANOVA) was processed with a linear-mixed model (Steel and Torrie, 1980) fit by residual maximum likelihood (REML) in Genstat 13 (VSN International, Hemel Hemspstead, UK). In this model, the effects of CO_2, soil type, and plant species were considered as fixed effects. As the grain nutrient concentrations in each crop species were repeatedly measured across years, year was used as a random effect (Parvin et al., 2018). As the FACE rings were randomly allocated in the field, the ring numbers were treated as a random effect in this model as well. The data set of each variable was normally distributed. The statistical analyses were performed using 4 replicates for each treatment. The least significant difference (LSD) at $p \leq 0.05$ was applied to compare the difference between treatment means. The comparative response to eCO_2 (as % aCO_2) of grain N, P, K, Ca, Mg, Fe, Mn, Zn and Cu concentrations was presented with a box plot (Fig. 1), displaying the distribution of data in minimum, first quartile, median, third quartile, and maximum across crops, soils and years.

3. Results

Elevated CO_2 decreased the concentrations of N, P and Zn in grains by 6%, 5% and 10% ($p < 0.05$), respectively, when averaged across the soils, crops and years (Fig. 1, Table 1). In particular, under eCO_2, a relative greater decrease in N concentration was found in canola and wheat, compared to field pea (Table 2), contributing to a CO_2 × crop interaction ($p < 0.05$) (Table 1). The effect of eCO_2 on grain P concentration depended on soil and crop with a decrease of 23% in canola grown in the Chromosol in 2015, while no CO_2 effect was detected in wheat and field pea in the two other soils (Tables 1, 2). The Mg concentration decreased only in the Chromosol ($p < 0.05$) but not in the Vertisol or Calcarosol (Table 2), resulting in a CO_2 × soil interaction ($p < 0.01$) (Table 1). The negative effect of eCO_2 on grain Zn concentration was consistent across both crop and soil type (Table 3).

Interestingly, the CO_2 effect on grain Ca concentration varied with soil type, resulting in a 18% increase in canola in Vertisol in 2015, but 12% decrease in Calcarosol (Tables 1, 2). There was no CO_2 effect on Ca concentration observed in other crops. The remaining nutrients tested

including K, Fe, Mn and Cu did not differ in response to eCO_2, irrespective of crop and soil types (Tables 2, 3). In addition, eCO_2 greatly increased the total contents of investigated nutrients in grain (mg per mesocosm) compared to aCO_2, which was attributed to the increase in grain yield in response to eCO_2 (Tables S1, S2).

After 7 years of cropping rotation under eCO_2, the availability of several nutrients in the topsoil changed relative to aCO_2. The available N decreased by 22%, 9% and 34% in Chromosol, Vertisol and Calcarosol, respectively (Figure 2), contributing to the significant CO_2 effect ($p < 0.05$). Similar trends were found in Colwell P and exchangeable Mg, resulting in eCO_2-induced decrease ($p < 0.05$) of, on average, 20% and 12%, respectively. However, there was no CO_2 effect on exchangeable K and Ca. Among the micro-nutrients measured, eCO_2 decreased extractable Cu in soil ($p < 0.05$) but not other micro-nutrients (Figure 2).

4. Discussion

This is the first study to the best of our knowledge to report the long-term effect of eCO_2 on the concentrations of nutrients in grains in a dryland wheat-pulse-canola rotation in different soils. Elevated CO_2 decreased grain N in canola and wheat but not in field pea. Similarly, protein concentration decreased from 15.3% to 13.4% when wheat plants were grown under eCO_2 with sufficient N supply (Fernando et al., 2012). It is expected that the soil N supply might not satisfy the increasing N demand by eCO_2-stimulated biomass growth in the two crops (Table 2). This view was further supported by the eCO_2-induced decrease in the concentration of available N in soils in this study (Figure 2). Thus, the lower soil N availability might contribute to the lower concentrations of N in tissue and likely decreased protein concentrations in grain of non-legume crops, lowering potential food and feed quality (Wroblewitz et al., 2013).

In field pea, a pulse crop, the lack of any significant difference in N concentration between aCO_2 and eCO_2 might be attributed to the eCO_2-induced stimulation on N_2 fixation. Li et al. (2017) reported that the grain N was dominantly derived from the symbiotically-fixed N in N_2-fixing soybean under eCO_2. Moreover, our previous study found that the number of nodules and shoot biomass of field pea significantly increased in response to eCO_2, which might contribute to the increase of N uptake (Jin et al., 2012). Thus, the impact of eCO_2 on grain N is likely to be greater in the non-legume crops with high N demands. Nevertheless, 2% of decrease in grain N concentration in lentils was observed under eCO_2, indicating that eCO_2-induced stimulation on N_2 fixation might not meet the photosynthetic efficiency to avoid the dilution effect (Moore et al., 1999; Bourgault et al., 2017).

The decrease in grain P concentration under eCO_2 was greater in canola than in field pea and wheat, especially when the crops were grown in the Chromosol. The greater decrease in P concentration in canola was likely to contribute to the greater demand for P in canola than the other crops (Table 2), and higher grain yield in the Chromosol might lead to a stronger dilution effect on the P concentration compared to other soils (data not shown). Several studies showed that the greater accumulation of carbohydrates than nutrients might cause the reduction of nutrient concentration in plants, i.e. a 'dilution effect' (Taub and Wang, 2008, Taub et al., 2008; Fernando et al., 2014). Apparently, whether this dilution effect occurs or not depends on the inherent capability of nutrient acquisition of crop species and their interaction with soil. Under eCO_2, plant P acquisition is associated with the C status of plants, i.e. photosynthetic C allocation to roots and subsequent effects on root length and morphology and the composition of root exudates (Pandey et al., 2015; Jakobsen et al., 2016). The increase in C efflux from roots under eCO_2 may facilitate the mobilization of insoluble P fractions over time (Jin et al., 2017), but this mobilized P may not satisfy the increased P demand by plants under eCO_2.

Consequently, eCO_2-induced decrease in labile P may still limit the P uptake and utilization. Therefore, eCO_2-induced changes in plant C metabolisms play a key role in P uptake and translocation to grain. Moreover, since P is predominantly stored as phytate in grain and phytate is hydrolyzed to supply P for seed germination (Bewley and Black, 1978; Yang et al., 2017), eCO_2-induced decrease in phytate concentration may limit seedling growth due to insufficient supply of nutrients including P. In addition, as the Mg is also combined with phytate (Yang et al., 2017) and a similar decreasing trend was found in this study, Mg may also become a liming nutrient for germination.

Grain Zn concentration decreased in response to eCO_2 irrespective of soil type. The reduction of Zn concentration was consistent with the study by Fernando et al. (2014) who found that the Zn concentration in wheat grain decreased by 6% under eCO_2 when the plants were grown in a Vertisol. However, the exchangeable Zn in top-10 cm of soil was not affected by eCO_2 (Figure 2), indicating that eCO_2 did not cause depletion of Zn in soil profile where major roots distributed. The likely reasons for the decrease in grain Zn concentration would be related to Zn distribution in grains and the carbohydrate accumulation in grains. First, eCO_2 may lead to greater proportion of endosperm to grain weight in wheat as grain Zn was mainly distributed in the outer layers of the kernels (Petersen et al., 1983; Pleijel and Danielsson, 2009). Second, the Zn concentration was mainly lower in the distal grains from rachis on spike where a larger number of grains might develop under eCO_2 (Calderini and Ortiz-Monasterio, 2003). However, mechanisms for the grain Zn decrease in field pea and canola need further investigation.

Unlike the above nutrients, the concentrations of Fe, Mn and Cu in grains did not significantly respond to eCO_2 in any crop grown in the three soils studied nor was any increase in Ca concentration observed in different soils in response to eCO_2 (Tables 2, 3). This indicates that these nutrients did not experience a dilution effect, and the accumulation of these nutrients in grain could keep pace with increased C assimilation. Although eCO_2 decreased photorespiration and transpiration-driven mass flow of nutrients (Gifford et al., 2000; Leakey et al., 2009; Bloom et al., 2012), these nutrients are considered phloem immobile and can only be transported in the chelate form in the phloem (Wang et al., 2017). Thus, the pattern of their mobility in plants would much differ from other ions. Moreover, the maintenance of cation-anion balance in plants may contribute to the differences in nutrient concentrations. A large amount of nitrate might be primarily taken up by plants under eCO_2, which required the equivalent cation uptake to maintain the charge balance (Marschner et al. 1996). These three nutrients may be associated with the cation-anion balance. Additionally, the lack of change in concentrations of these nutrients in grains may be associated with rhizosphere processes. Significant changes in biochemical properties in the rhizosphere under eCO_2 have been shown to occur in crops grown in soils (Jin et al., 2012, 2013; Yu et al., 2016). The changes in enzymes activities, rhizosphere pH, and ion transporters in roots may stimulate the mobilization of these ions in the rhizosphere and subsequent uptake by plants. However, except for Fe and Mn, the exchangeable Cu in soils decreased under eCO_2 (Figure 2), indicating that the depletion of Cu may reach a point limiting grain nutrition if crops keep growing under eCO_2. Thus, the eCO_2-induced increase in grain nutrient contents (Tables S1, S2) may lead to the depletion of some nutrients in soil solutions, but the potential decrease in soil nutrient availability depends on the rhizosphere effect on the nutrient mobilization under eCO_2.

In summary, although eCO_2 can often increase grain yield of crops, it had the negative effect on nutrient concentration in the grain but this effect varies with nutrient, crop and soil type. The eCO_2-induced decrease in N concentration mainly occurred in non-legume crops such as wheat and canola rather than the pulse field pea. The reduction of P and Mg under eCO_2 was

likely to occur in the acidic Chromosol that may have a higher productivity compared to the neutral/alkaline Vertisol and Calcarosol. Elevated CO_2 resulted in the decrease in grain Zn concentration rather than other micronutrients such as Fe, Mn and Cu. The potential benefits of increasing grain yields that may occur under eCO_2 (Thomas et al., 2009; Saha et al., 2015), other factors such as rainfall and temperature effects aside, may be offset by reduced nutritional value of grains of non-legume crops. The decrease in grain quality in term of concentrations of essential nutrients under eCO_2 is likely to be greater in relatively fertile than infertile soils although absolute concentrations may be higher in fertile soils. In addition, low nutrient concentrations in grains also constrain seed germination and seedling establishment, leading to low yields at harvest (Muhammad et al., 2015). A number of studies have demonstrated that seed nutrient concentrations can strongly affect seedling vigour and development (Singh and Bharti, 1985; Ros et al., 1997; Genc et al., 2000). Comprehensive management choices such as cultivar selection for greater nutrient-use-efficiency and increasing fertilization or developing new more efficient fertilization methods are recommended to mitigate the negative impacts of eCO_2 on nutrient concentrations in grains.

Acknowledgements

The Australian Grains Free Air CO_2 Enrichment program including SoilFACE is jointly run by Agriculture Victoria Research (Victorian State Department of Economic Development, Jobs, Transport and Resources) with the University of Melbourne and funding from the Grains Research and Development Corporation (GRDC) and the Australian Government Department of Agriculture and Water Resources. We gratefully acknowledge Mel Munn, Roger Perris, Liana Warren and Russel Argall and team (Agriculture Victoria Research) for management the SoilFACE experiment and Mahabubur Mollah (Research Engineer, Agriculture Victoria) for running SoilFACE. This study was also supported by a fellowship of Research Officer in the School of Life Sciences at La Trobe University.

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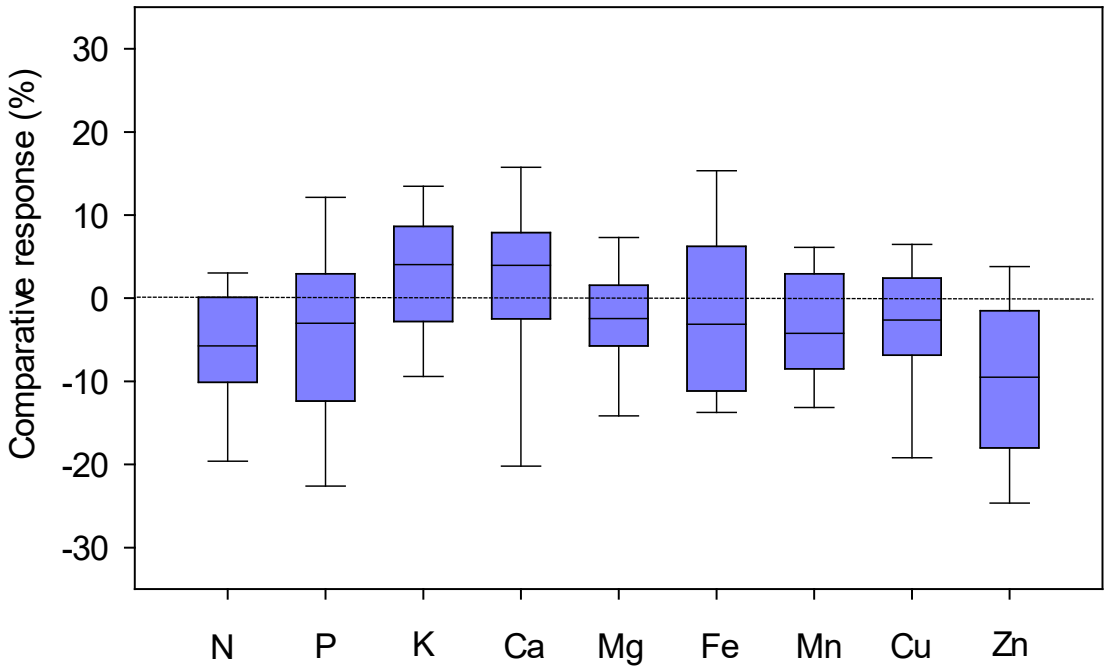

Fig. 1. Comparative response to eCO_2 (as % aCO_2) of N, P, K, Ca, Mg, Fe, Mn, Cu and Zn concentrations in grains. Crops were grown in the Chromosol, Vertosol and Calcarosol soils and exposed to either aCO_2 (390 ppm) or eCO_2 (550 ppm) from 2009 to 2015. Bars show the maximum (top edge) and minimum (lower edge) percentiles, and boxes the 25% and 75% percentiles across crops, soils and years. The median (50%) percentile is represented by the horizontal line within the box.

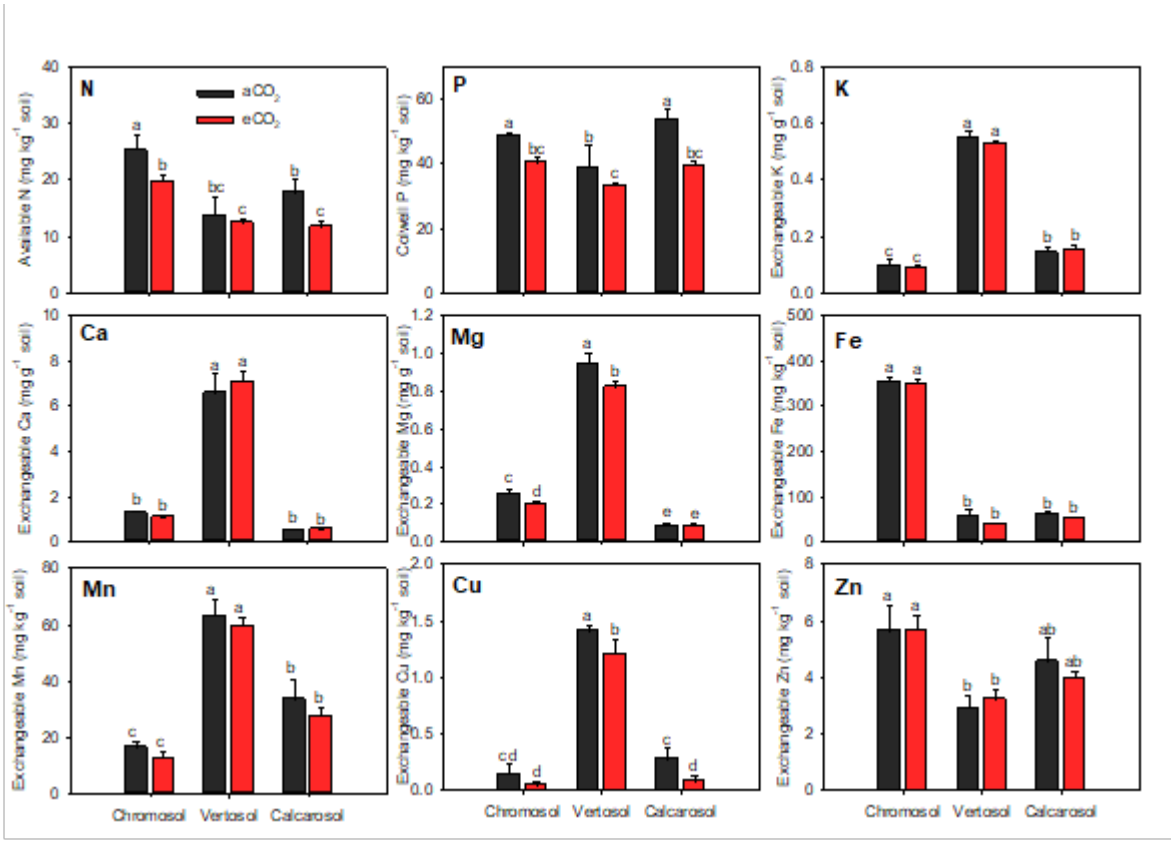

Fig. 2. Effect of eCO_2 on labile N (NH_4^+ and NO_3^-), Colwell P, exchangeable K, Ca, Mg, Fe, Mn, Cu and Zn concentrations in the Chromosol, Vertosol and Calcarosol soils (0-10 cm). Crops were exposed to either aCO_2 (390 ppm) or eCO_2 (550 ppm) from 2009 to 2015. Values were means ± SE (n=4). Values with a common letter are not significantly different between treatments ($p = 0.05$). The Colwell P data were obtained from Jin et al. (2017). The CO_2 effect was significant ($p < 0.05$) for labile N, Colwell P, exchangeable Mg and Cu concentrations and the soil effect was significant ($p < 0.01$) for all of nutrient concentration in soil.

Table 1. Significant levels of main effects and interactions of CO_2, crop and soil type on concentrations of N, P, K, Ca, Mg, Fe, Mn, Cu and Zn in grains.

	N	P	K	Ca	Mg	Fe	Mn	Cu	Zn
CO_2	0.005	0.035	0.558	0.019	0.068	0.689	0.174	0.999	<0.001
Crop	0.010	0.003	0.827	<0.001	<0.001	<0.001	0.004	0.061	0.299
Soil	<0.001	<0.001	<0.001	<0.001	<0.001	0.011	<0.001	0.093	<0.001
CO_2 × Crop	0.043	0.951	0.538	0.377	0.906	0.656	0.114	0.838	0.723
CO_2 × Soil	0.123	0.149	0.985	0.095	0.006	0.711	0.820	0.545	0.492
Crop × Soil	<0.001	<0.001	0.153	<0.001	<0.001	0.244	0.297	<0.001	<0.001
CO_2 × Crop × Soil	<0.889	0.014	0.063	0.030	0.174	0.994	0.925	0.707	0.113

Table 2. The effect of eCO_2 on concentrations of macronutrients of N, P, K, Ca and Mg in grains of field pea, wheat and canola grown in Chromosol, Vertosol and Calcarosol. Crops were exposed to either aCO_2 (390 ppm) or eCO_2 (550 ppm) from 2009 to 2015.

Soil	Year	Crop	N (mg g^{-1})		P (mg g^{-1})		K (mg g^{-1})		Ca (mg g^{-1})		Mg (mg g^{-1})	
			aCO_2	eCO_2	aCO_2	eCO_2	aCO_2	eCO_2	aCO_2	eCO_2	aCO_2	eCO_2
Chromosol	2009	Field pea	32.3	31.3	2.47	2.42	6.12	6.02	0.67	0.65	1.14	1.04
	2010	Wheat	24.1	21.1*	2.77	3.13	4.84	5.04	0.35	0.37	1.36	1.36
	2011	Field pea	32.7	33.2	2.28	2.30	3.68	3.69	0.75	0.71	1.22	1.16
	2012	Wheat	22.0	20.5	2.31	2.04	3.72	3.87	0.29	0.31	1.21	1.17
	2013	Canola	28.5	28.5	6.60	6.91	5.70	6.48	3.22	3.48	3.29	3.34
	2014	Wheat	23.3	18.5*	2.73	2.11	4.21	3.35	0.33	0.33	1.37	1.12
	2015	Canola	45.8	40.4*	6.53	4.51	3.54	3.74	3.67	3.83	3.09	2.65*
Vertisol	2009	Field pea	38.3	36.2	3.58	3.13	6.81	6.71	0.62	0.72	1.12	1.13
	2010	Wheat	15.7	15.6	3.91	4.05	4.97	4.81	0.37	0.39	1.36	1.39
	2011	Field pea	35.5	37.1	2.52	2.13	3.66	3.94	0.72	0.74	1.19	1.18
	2012	Wheat	15.4	14.5	2.70	2.70	3.68	3.69	0.30	0.31	1.23	1.19
	2013	Canola	28.6	28.5	8.44	8.83	5.26	5.59	4.58	4.76	3.35	3.41
	2014	Wheat	13.9	11.0	3.55	3.18	4.68	4.23	0.49	0.38	1.25	1.23
	2015	Canola	39.1	34.9*	6.59	7.62*	4.17	4.61	3.78	4.45*	2.92	3.21
Calcarosol	2009	Field pea	35.7	36.8	2.65	2.63	6.43	7.00	0.80	0.63	1.21	1.13
	2010	Wheat	14.2	13.0	4.70	3.87	6.90	6.66	0.42	0.43	1.61	1.40
	2011	Field pea	35.8	35.2	2.29	2.04	3.70	4.04	0.63	0.71	1.24	1.19
	2012	-	-	-	-	-	-	-	-	-	-	-
	2013	Canola	27.4	27.9	9.71	9.15	5.89	6.73	4.16	4.13	3.74	3.57
	2014	Wheat	14.8	13.5	2.77	2.80	4.09	4.42	0.35	0.40	1.12	1.21
	2015	Canola	37.4	34.3*	8.77	8.40	3.99	3.63	4.17	3.68*	3.71	3.71
LSD ($p = 0.05$)			5.13		1.04		1.28		0.44		0.30	

Values were means of four replicates. * indicates significant difference (*t* test) between aCO_2 and eCO_2 at $p \leq 0.05$, for individual years. LSD values correspond to the CO_2 × soil × species interaction (three-way ANOVA). -, data not available.

Table 3. The effect of eCO_2 on concentrations of micronutrients of Fe, Mn, Cu and Zn in grains of field pea, wheat and canola grown in Chromosol, Vertosol and Calcarosol. Crops were exposed to either aCO_2 (390 ppm) or eCO_2 (550 ppm) from 2009 to 2015.

Values were means of four replicates. * indicates significant difference (*t* test) between aCO_2 and eCO_2 at $p \leq 0.05$, for individual years. LSD

Soil	Year	Crop	Fe (μg g^{-1})		Mn (μg g^{-1})		Cu (μg g^{-1})		Zn (μg g^{-1})	
			aCO_2	eCO_2	aCO_2	eCO_2	aCO_2	eCO_2	aCO_2	eCO_2
Chromosol	2009	Field pea	40.3	39.2	7.64	6.9	7.1	6.6	43.2	38.3*
	2010	Wheat	77.6	86.9	40.1	41.8	5.1	5.4	37.6	31.6*
	2011	Field pea	55.7	51.8	9.64	9.3	7.7	7.2	47.7	46.7
	2012	Wheat	47.7	58.5	44.3	41.7	3.2	3.4	41.8	34.0*
	2013	Canola	34.9	32.5	29.8	31.2	2.6	2.7	32.1	33.4
	2014	Wheat	31.5	28.6	50.2	40.7	5.4	4.5	39.8	30.6*
	2015	Canola	36.1	37.6	35.2	37.4	2.6	2.5	45.2	39.4*
Vertisol	2009	Field pea	26.2	27.2	6.5	6.1	5.7	5.8	21.0	19.6
	2010	Wheat	66.4	71.0	48.0	43.7	5.6	5.3	17.4	17.4
	2011	Field pea	38.1	34.3	7.55	7.5	7.1	6.9	29.7	27.4
	2012	Wheat	37.6	43.5	35.6	33.6	4.7	4.6	18.3	18.8
	2013	Canola	36.8	32.3	27.0	27.3	2.5	2.6	24.9	24.5
	2014	Wheat	22.6	19.4	32.0	28.7	6.0	4.5	22.0	16.2*
	2015	Canola	36.8	35.7	27.5	28.3	2.4	2.4	37.6	29.1*
Calcarosol	2009	Field pea	33.7	33.3	7.82	6.8	4.7	3.8	25.9	19.5
	2010	Wheat	85.0	74.7	56.6	53.0	8.8	7.8	32.8	32.3
	2011	Field pea	43.2	38.2	9.09	9.1	4.8	5.4	25.8	28.9
	2012	Wheat	-	-	-	-	-	-	-	-
	2013	Canola	37.8	36.6	30.6	29.1	2.7	2.8	28.8	24.1*
	2014	Wheat	22.4	25.1	26.0	26.7	5.7	5.7	13.5	13.1
	2015	Canola	56.8	43.4	29.1	32.9	2.6	2.5	35.1	30.0*
LSD ($p = 0.05$)			18.0		5.95		1.69		8.31	

values correspond to the CO_2 × soil × species interaction (three-way ANOVA).

Supplementary Table S1. The effect of eCO_2 on contents of macronutrients of N, P, K, Ca and Mg in grains of field pea, wheat and canola grown in Chromosol, Vertosol and Calcarosol. Crops were exposed to either aCO_2 (390 ppm) or eCO_2 (550 ppm) from 2009 to 2015.

Soil	Year	Crop	N (mg mesocosm^{-1})		P (mg mesocosm^{-1})		K (mg mesocosm^{-1})		Ca (mg mesocosm^{-1})		Mg (mg mesocosm^{-1})	
			aCO_2	eCO_2	aCO_2	eCO_2	aCO_2	eCO_2	aCO_2	eCO_2	aCO_2	eCO_2
Chromosol	2009	Field pea	787	977*	60.1	75.6*	149	188*	16.3	20.3*	27.8	32.5*
	2010	Wheat	445	524	51.2	77.7*	89.6	125*	6.5	9.1*	25.1	33.8*
	2011	Field pea	809	959*	57.5	66.5	93.1	106	18.8	20.5	30.9	33.4
	2012	Wheat	662	808*	69.5	80.5*	112	152**	8.7	12.2**	36.3	46.0*
	2013	Canola	441	561	102	136	88.5	127*	49.9	68.6	51.0	65.7
	2014	Wheat	351	468	41.2	53.3	63.5	84.2	5.0	8.2*	20.6	28.2
	2015	Canola	66.3	188***	12.3	21.2*	5.2	17.6***	5.4	17.9***	4.6	12.5***
Vertosol	2009	Field pea	1061	1195	99.1	103	188	221	17.2	23.8*	30.9	24.7
	2010	Wheat	397	383	99.1	99.9	125	119	9.3	9.5	34.4	34.2
	2011	Field pea	1188	1552**	84.4	89.3	122	165**	24.1	31.1**	39.8	49.4**
	2012	Wheat	400	426	70.3	79.6*	95.7	109*	7.7	9.1*	32.0	35.0
	2013	Canola	158	218	46.6	67.5	39.0	42.7	25.3	36.4	18.6	26.1
	2014	Wheat	202	250*	51.6	72.0**	38.1	96.0**	7.1	8.5*	18.2	27.8**
	2015	Canola	72.9	171*	9.6	37.3**	7.8	22.5*	7.1	21.7*	5.4	15.7*
Calcarosol	2009	Field pea	731	827	54.2	59.0	118	157**	16.4	14.1	24.7	25.5
	2010	Wheat	98	129	35.7	40.3	47.6	66.2	2.9	4.2*	25.1	13.9
	2011	Field pea	1039	1252	66.5	72.7	107	144	18.2	25.4*	35.9	42.2
	2012	-										
	2013	Canola	102	126	36.5	41.4	22.1	30.5	15.6	18.7	14.0	16.2
	2014	Wheat	220	240	41.4	50.0	61.2	79.0	5.2	7.2	16.8	21.7
	2015	Canola	45.5	171**	10.7	42.1**	4.8	18.2**	5.1	18.4**	4.5	18.6**

Values were means of four replicates. *, ** and *** indicate significant differences (*t* test) between aCO_2 and eCO_2 at $p \leq 0.05$, $p \leq 0.05$ and $p \leq 0.05$, respectively, for individual years.

Supplementary Table S2. The effect of eCO_2 on contents of micronutrients of Fe, Mn, Cu and Zn in grains of field pea, wheat and canola grown in Chromosol, Vertosol and Calcarosol. Crops were exposed to either aCO_2 (390 ppm) or eCO_2 (550 ppm) from 2009 to 2015.

Soil	Year	Crop	Fe (μg mesocosm^{-1})		Mn (μg mesocosm^{-1})		Cu (μg mesocosm^{-1})		Zn (μg mesocosm^{-1})	
			aCO_2	eCO_2	aCO_2	eCO_2	aCO_2	eCO_2	aCO_2	eCO_2
Chromosol	2009	Field pea	982	1225	186	215*	173	207*	1050	1195
	2010	Wheat	1434	2154*	742	1036*	94.0	133*	696	785
	2011	Field pea	1407	1493	244	268	194	206	1205	1348
	2012	Wheat	1433	2031***	1332	1641*	95.0	132**	1256	1339
	2013	Canola	541	639	462	613	40.4	52.6	499	657
	2014	Wheat	475	723*	757	1027	82.0	112	601	771
	2015	Canola	53.1	176***	51.8	176***	3.8	11.5***	66.6	184***
Vertosol	2009	Field pea	725	690*	180	200	158	190	582	645
	2010	Wheat	1682	1748	1215	1076	142	131	440	429
	2011	Field pea	1273	1434*	253	315**	237	288**	993	1148*
	2012	Wheat	979	1282**	926	990	122	135	477	554*
	2013	Canola	203	247	149	209	13.8	19.5	138	187
	2014	Wheat	328	440**	465	650**	81.5	102*	320	366
	2015	Canola	68.6	175*	51.2	138*	4.5	11.5*	70.1	142*
Calcarosol	2009	Field pea	730	722	160	152	97.2	85.9	512	438
	2010	Wheat	586	743	390	527	60.5	77.6	225	321*
	2011	Field pea	1253	1358	264	324	140	193*	748	1027*
	2012	Wheat								
	2013	Canola	142	166	115	132	10.1	12.6	108	109
	2014	Wheat	334	448	388	477	84.8	102	201	234
	2015	Canola	69.0	217**	35.4	164**	3.2	12.4**	42.7	150**

Values were means of four replicates. *, ** and *** indicate significant differences (*t* test) between aCO_2 and eCO_2 at $p \leq 0.05$, $p \leq 0.05$ and $p \leq 0.05$, respectively, for individual years.