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Elevated CO_2 and temperature increase grain oil concentration but their impacts on grain yield differ between soybean and maize grown in a temperate region

Yunfa Qiao[a, b] , Shujie Miao[a], Qi Li[a], Jian Jin[c], Xiaosan Luo[a] and Caixian Tang[c]

[a.] Nanjing University of Information Sciences & Technology, No. 219 Ningliu Road, Nanjing, 210044, China
[b.] Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin 150081, China
[c.] Department of Animal, Plant & Soil Sciences, Centre for AgriBioscience, La Trobe University (Melbourne Campus), Bundoora, Vic 3086, Australia

ABSTRACT
The increases in CO_2 concentration and attendant temperature are likely to impact agricultural production. This study investigated the effects of elevated temperature alone and in combination with CO_2 enrichment on grain yield and quality of soybean (*Glycine max*) and maize (*Zea mays*) grown in a Mollisol over five-year growing seasons. Plants were grown in open-top chambers with the ambient control, 2.1 °C increase in air temperature (eT) and eT together with 700 ppm atmospheric CO_2 concentration ($eTeCO_2$). While $eTeCO_2$ but not eT increased the mean grain yield of soybean by 31%, $eTeCO_2$ and eT increased the yield of maize similarly by around 25% compared to the ambient control. Furthermore, eT and $eTeCO_2$ did not significantly affect grain protein of either species but consistently increased oil concentrations in grains of both species with $eTeCO_2$ increasing more. The eT increased grain Fe concentration relative to the control treatment but decreased Ca concentration, while the relative concentrations of P, K, Mn and Zn varied with crop species. The elevated CO_2 enlarged the eT effect on Fe concentration, but decreased the effect on Ca concentration. The results suggest that crop selection is important to maximize yield benefits while maintain grain quality to cope with elevated CO_2 and temperature of future climate change in this temperate region where the temperature is near or below the optimal temperature for crop production.

Keywords: high CO_2; grain component; oil; mineral nutrients; open-top chambers; warming

1. Introduction

The changing climate has been mostly attributed to the increasing atmosphere CO_2 concentration and attendant increases in temperature (Vanaja et al., 2015). The atmospheric CO_2 concentration is projected to reach 850 ppm at 2100 with the Representative Concentration Pathway (RCP 6.0) (van Vuuren et al., 2011), correspondingly with 1.4-3.1 ℃ increase in temperature (IPCC, 2014, AR5) if no effective strategy is applied to reduce CO_2 emission. These changes directly or indirectly affect plant growth, grain yield and grain quality in agricultural systems (Fernando et al., 2012; Ruiz-Vera et al., 2013; Myers et al., 2014; Kimball, 2016; Köhler et al., 2019). Many studies have been carried out on the effect of increased temperature (Mochizuki et al., 2005; Hatfield et al., 2011; Tacarindua et al., 2012; Choi et al., 2016; Wang et al., 2016; Jumrani et al., 2017; Palacios et al., 2019) or CO_2 rising on crop production (Högy et al., 2013; Vanaja et al., 2015; Abebe et al., 2016; Kimball, 2016; Lenka et al., 2017; Köhler et al., 2019). In general, the increase of temperature benefits crop yield if the increased temperature is below the optimal growth temperature, otherwise the elevated temperature exerted negative impact on crop yield. Elevated CO_2 is broadly beneficial for seed yield if nutrient supply is not limited (Lemonnier and Ainsworth, 2018). Based on these results, it is speculated if the increased mean temperature below the optimal temperature level for growth, additional CO_2 enrichment might accentuate the impact of elevated temperature.

The effect of elevated temperature and CO_2 rising on crop growth varies between crop species. This is because different optimal temperatures are required for maximum yields across crop species, such as 20-30 ℃ for soybean (Norman, 1978; Kumagai and Sameshima et al., 2014; Li et al., 2018), and 23-32 ℃ for maize (Naveed et al., 2014; SÁnchez et al., 2014; Vanaja et al., 2017). Hatfield et al. (2011) reported that the increase of 0.8 ℃ in temperature caused a 2.4% reduction in soybean yield when the plants were grown at the initial temperature of 26.7 ℃ for the southern US, but 1.7% increase in yield at 22.5 ℃ in the Midwest US with the optimal temperature of 22-24℃. Responses to elevated CO_2 are different between C3 and C4 plants due to the C saturation in C4 plant, but not in C3 plant at the current CO_2 level. For example, increasing CO_2 concentration from 390 ppm to 585 ppm did not stimulate the yield of the C4 crop maize (Rui-vera et al., 2015). In Soy-FACE experiment, 30-year of elevated CO_2 resulted in 10% increase in soybean yield (Twine et al., 2013). Thus, the additional CO_2 enrichment based on moderately temperature increase would differently influence on the yield of C3 and C4 plants.

The responses of grain quality including nutrient concentrations to both climate factors remain largely unknown because various studies have shown inconsistent changes in nutrient concentrations in grains under either elevated temperature or elevated CO_2 conditions. A number of short-term studies showed that elevated CO_2 decreased the concentrations of nutrients such as Zn, Fe, Ca and Mn in wheat grains (Fernando et al., 2012; Högy et al., 2013), Zn and Fe in soybean grains (Myers et al., 2014; Köhler et al., 2019), and Ca, Zn and Mn in oilseed rape (Högy et al., 2010). In a 7-year FACE study, elevated CO_2 decreased the concentrations of N, P and Zn by 6%, 5% and 10%, respectively, when averaged across various soil types, crop species and seasons (Jin et al., 2019). In contrast, elevated temperature increased the concentrations of N (or protein), P and K in grains of maize grown in a subtropical region (Abebe et al., 2016). Studies on rice showed that eCO_2 or increasing temperature had different effects on grain formation and quality characters (Madan et al., 2012; Roy et al., 2015; Jing et al., 2016; Liu et al., 2017). However, it is not clear how the combined climate factors impact grain quality. Such investigations are essential to global public health as the deficiency of nutrients such as Zn, Fe, Ca and Mn in diet may be exacerbated due to climate change (Loladze, 2002; Miraglia et al., 2009; Myers et al., 2014). For example, Myers et al. (2014) reviewed that the concentrations of Fe and Zn in grains of most crops decreased due to elevated CO_2. In addition, the oil concentration of soybean and maize grains was also important quality indicator, because it accounts for about 30% of the world's human consumption (USDA 2016/2017). Some reports on the effect of elevated CO_2 on oil percentage in seeds of sunflower (Pal et al. 2014) and soybean (Hao et al., 2014; Singh et al., 2016; Li et al., 2018; Köhler et al., 2019), indicated that elevated CO_2 might increase seed oil production. However, little information is available on the impact of both elevated CO_2 and temperature on seed oil concentration.

The objective of this study was to evaluate the impact of elevated CO_2 and temperature on yield and grain quality of soybean and maize grown in a temperate Mollisol. We hypothesized that the increase in both temperature and CO_2 concentration would improve soybean growth and grain yield, but maize only responded to temperature increase. Furthermore, the concentrations of mineral nutrients in grains might decrease due to carbohydrate accumulation under elevated CO_2, but oil concentration increased because of direct photosynthesis stimulation.

2. Materials and methods

2.1. Site description and experimental design

To investigate the impact of eT with and without eCO_2, a factorial experiment was designed. The experiment consisted of three environmental treatments and two crop species (maize and soybean) in three replicates. Three environmental treatments were 1) ambient control, 2) elevated air temperature (by 2.1 °C), and 3) a combination of elevated air temperature and CO_2 (by 2.1 °C plus 700 ppm CO_2) to mimic the CO_2 level by the end of this century according to RCP 6.0 (van Vuuren et al., 2011) and IPCC AR5. The experiment was a split-plot design with environmental treatments as main plots and crop species as sub-plots. It was conducted during growing seasons from 2012 to 2016. Soybean and maize were grown in open-top chambers (OTC) located in Hailun County, Heilongjiang, China (47°26'N, 126°38'E). The soil at the experimental site was Mollisols with 40% of clay, 34% of sand and 26% of silt. The region has a temperate monsoon climate with mean precipitation of 530 mm during growing season across five years. The highest precipitation during the growing season (from 1st May to 30th September) was 817 mm in 2013, and the temperature was lower than any of other years. The lowest precipitation during the growing season was 338 mm in 2015 which was lower than the long-term average. The temperature in this year was relatively higher compared to other years. The rainfall and temperature of air and soil surface, and temperature difference during the growing seasons in five years are shown in Figure 1. During the experimental period, photosynthetic active radiation ranged from 401 to 447 μmol m^{-2} s^{-1} with averaged value of 425 μmol m^{-2} s^{-1}. The average air humidity was 77%, 56% and 74% at 8:00, 14:00 and 20:00, respectively, with an average of 69%. The photosynthetically active radiation and air temperatures outside OTCs are provided in Figure 2.

Nested within the 900 m^2 field were three ambient CO_2 and temperature (control), three elevated temperature (eT), three combined elevated CO_2 and temperature ($eTeCO_2$) octagonal OTCs (3-m diameter), each 3-m height and 1.2-m side length joined with stainless steel frame structure with 3-mm thick colorless transparent glass. Light transmission of the glass was >80%. There was a 50-mm gap between the soil surface and the glass to aid air circulation. The total volume of each OTC was 20 m^3. In the $eTeCO_2$ treatment, the concentration of CO_2 was maintained at 700 ± 25 ppm inside the OTC during 6:00 am to 6:00 pm by regulating the release of compressed CO_2 gas from cylinders. The gas was supplied with the finger ring through tubes at 10 cm above crop canopy.

Along the gas tube, a copper-finger condenser pipe used to cool the air in the chamber through circulation of groundwater from 30-m depth to keep the temperature as outside (control treatment) and designed treatment (eT and $eTeCO_2$). Air circulation in the chamber was done via 1-m fan blowers located at the center of the top chamber for all OTCs. A CO_2 detector was used to monitor the actual concentration and maintained it by computer-aided regulation of inlet values (Vaisala GMM220) with the mean value of 705, 689, 697, 685, and 710 ppm during 2012, 2013, 2014, 2015 and 2016 growing seasons, respectively. Average air-temperature at 1 m above the soil surface and soil-surface temperature in OTC was 2.14±0.47°C, and 1.45 ±0.02°C higher in the eT and $eTeCO_2$ treatments than the ambient control, respectively. The designed temperature was maintained by an automatic management system through adjusting fan speed and water flow in the condenser pipe. The seasonal average air temperatures (°C) were 18.0 and 20.1, and those of soil surface were 21.0 and 22.4 for the control and eT (eCO_2) treatment, respectively, across the five-year experimental period.

2.2. *Plant cultivation*

Uniform seeds of soybean (*Glycine max* (L.) Merr. cv. Dongsheng 2) and maize (*Zea mays* (L.) cv. Demeiya 1) were grown during the period from May to September from 2012 to 2016. The plant densities were 6.0 and 27 plants m^{-2} for maize and soybean, respectively. The fertilizer application was the same as that of farm paddocks in the region in the following composition: 63.0 kg N ha^{-1} as urea, 24.1 kg P ha^{-1} as superphosphate, and 29.8 kg K ha^{-1} as K_2SO_4 for soybean; and the same type of fertilizers as 174 kg N ha^{-1}, 40.2 kg P ha^{-1}, and 44.8 kg K ha^{-1} was applied to maize. The supply of water during plant growth was reliant on natural precipitation. Weeding was carried out manually when necessary from sowing to harvest. After harvest, all shoot residues and maize roots were removed from the field as local farming practices.

2.3. *Measurement of growth, yield and mineral nutrients*

At maturity, the above-ground part of randomly-selected 10 plants each plot was harvested, and shoot biomass and grain yield recorded. Yield components were determined in 2014 and 2016 because the precipitations of these two years were similar to long-term average. Numbers of pod and seed per pod of soybean, and numbers of cob and seed per cob of maize were counted. All the above-ground parts were oven-dried at 70 °C for 48 h to obtain dry biomass. Harvest index was calculated as grain yield divided by above-ground biomass. The grain sub-samples were ground and digested with H_2SO_4-H_2O_2. The digests were used to analyze the concentrations of N, P, K, Ca, Fe, Zn and Mn. The concentration of N was determined with Kjeldahl apparatus (Hongji TAN-100, Yoshida et al., 1976). P concentration was measured with a spectrometer using the vanado-molybdate method (Jackson, 1973). K concentration was measured with atomic absorption emission spectrometry (Austria Sens AA Dual). Ca, Fe, Zn and Mn were measured with inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 8×00, USA). Concentrations of all nutrients were presented on a dry-weight basis. The protein concentration of grains was calculated as the N concentration multiplied by 6.25 (Jones, 1931). The concentrations of oil in grains were determined with Infrared 1241 Analyser (Foss, Denmark) by averaging 10 measurements per sample.

2.4. *Statistical analysis*

The yield, grain weight, shoot biomass, harvest index, protein and oil concentrations were analyzed using two-way ANOVA with a split-plot design using climatic treatments as main plots and crop species as sub-plots for individual years. One-way ANOVA was used to analyze the yield components for each crop species because the parameters measured were not the same for the two species. The responses of grain nutrient concentrations to eT and $eTeCO_2$ (% increment relative to the ambient control, Fig. 3) were compared using Student's two-tailed t-test. Average grain yields, mean concentrations of protein and oil in grains across five years were statistically analyzed with a linear-mixed model, which was fit by residual maximum likelihood (REML). Treatments and species were treated as fixed effects and year as a random effect (Parvin et al., 2018), because these variables were repeatedly measured across years. All the analyses were performed using using Genstat 13 (VSN International, Hemel Hemspstead, UK).

3. Results

3.1. Shoot biomass and grain yield

The grain yield of soybean was generally more responsive to elevated CO_2 while maize yield was more responsive to elevated temperature, leading to significant interactions between climatic factors and crop species in most years (Table 1). Compared to the control, $eTeCO_2$ but not eT increased the average yield of soybean by 31% while both eT and $eTeCO_2$ increased maize yield by 25% across five years.

For soybean, eT did not significantly affect pod number while $eTeCO_2$ increased it (Table 2, $P < 0.05$). Both eT and $eTeCO_2$ increased seed number per pod compared to the control in 2014 but not in 2016 (Table 2, $P < 0.01$). Neither eT nor $eTeCO_2$ altered the number of cobs in maize (Table 2). However, the seed number per cob in 2016 increased by 25% and 34% under eT and $eTeCO_2$ compared to the control. The different responses between 2014 and 2016 indicated that the effect of eT or $eTeCO_2$ on yield components was probably also related to other environmental factors, such as photosynthetically active radiation (Fig. 2).

The grain weights of two crops were not affected by climate treatments (Table 3). In 2014, $eTeCO_2$ increased shoot biomass of soybean by 48% compared to the ambient control, while climate treatment did not affect that of maize. In 2016, compared to the ambient control, shoot biomass of soybean was not affected by climate factors while that of maize increased by 31% and 29% under eT and $eTeCO_2$, respectively, leading to a significant treatment × species interaction (Table 3). There was no climate factor effect on the harvest index, except that 53% greater in soybean than maize in 2016 (Table 3).

3.2. Protein and oil concentrations in grains

The protein concentrations in grains of both crops were not affected by eT or $eTeCO_2$ in any year, but were, on average, 3.8 fold higher in soybean than in maize across five years ($P < 0.001$, Table 4). In comparison with the control, the concentration of oil in soybean grains was 9% higher under eT and 14% higher under $eTeCO_2$, and that of maize grains increased by 12% and 20% under eT and $eTeCO_2$, respectively, across five years, leading to a significant treatment × species interaction (Table 5). As expected, the oil concentration in soybean grain was higher than in maize grain ($P < 0.001$).

3.3. Nutrient concentrations in grains

When the nutrient concentrations were averaged across five years, eT relatively increased the Fe concentration by 10.5% and 7.3% in maize and soybean grains, and the concentrations of P and Mn by 7.3% and 6.5% in maize grains compared to the control, but decreased P and Mn by 6.5% and 3.4% in soybean grains, and Ca by 9.0% and 11.6% in maize and soybean grains, respectively (Fig. 3). Additional eCO_2 further enhanced the eT effect on Fe and Mn concentrations, but offset its effect on Ca. Compared to the control treatment, the effect of $eTeCO_2$ on P and K concentrations in gains depended on crop species. Genaerally, Zn concentration in grains was not significantly affected by eT or $eTeCO_2$ compared to the control.

4. Discussion

4.1. Shoot biomass and yield under eT

Elevated temperature resulted in greater yield of maize, but not soybean, which was likely attributed to higher optimal temperatures for maize than soybean growth (Sage and Kubien, 2007). The effect of elevated temperature on maize yield was consistent with some previous studies (Vanaja et al., 2017; Tigchelaar et al., 2018), but inconsistent with other studies (Abebe et al., 2016; Bacon et al., 2016; Shim et al., 2017). The difference might be attributed to the background temperature and the increased temperature. The background temperature (17.9 °C) in the present study was lower than other studies, such as 34-35 °C in Abebe et al. (2016). The high temperature reduced photosynthesis and shifted carbon away to reproduction, thus resulted in lower yield (Ruiz-Vera et al., 2015). Moreover, once temperature above 32℃, the decrease in maize pollen germination might severely decline kernel number per plant and apical dominance (Uribelarrea et al., 2002; 2008). However, the elevated temperature in the present study was still within the optimal temperature range of 22-32 °C for maize growth (Naveed et al., 2014). Thus, the greater photosynthetic rate than respiration induced biomass accumulation (Naveed et al., 2014), and contributed to greater grain yield.

The lack of eT effect on shoot biomass or yield of soybean in this study might be attributed to the insensitivity of our early-maturing soybean variety to temperature, though the eT was within the optimal temperature range for soybean growth. In another study, Kumagai and Sameshima (2014) found that the increase of 4.8-5.7 °C temperature, but still within the optimal temperature, did not change the seed number, pod number and yield of an early-maturing cultivar (Yukihomare) but increased these of late-maturing cultivars in Morioka, Japan. A possible explanation for the result of early-maturing soybean was that the length of flowering and maturity period, and opened flower number, which related to pod and seed numbers, did not respond to elevated temperature (Kumagai and Sameshima, 2014). The result was also supported by the seed size and number in eT in the present study. On the contrary, yield of late-maturing soybean var. Enrei decreased with elevated temperature (1-3 ºC) at a hot region (average daily temperature 25.9-27.1 ºC) of Japan (Tacarindua et al., 2013). The yield of cv. Thorne was decreased by 2.7-3.4 ºC increase with background temperature of 23.3-25.7 ºC (Köhler et al., 2019) because high temperature decreased photosynthetic carbon assimilation (Ruiz-Vera et al., 2013). All these indicated that the potential impact of elevated temperature on soybean yield depends on background temperature and genotypes.

4.2. Shoot biomass and yield under $eTeCO_2$

Similar shoot biomass and yield of C4 maize between $eTeCO_2$ and eT indicated additional CO_2 enrichment had no further beneficial effect on maize production under elevated temperature conditions. Our results are consistent to the findings of Hunt et al. (1991), Kim et al. (2007) and

Ruiz-vera et al. (2015). All these could be attributed to the higher native CO_2 concentration in bundle sheath cells of maize (Furbank and Hatch, 1987; von Caemmerer and Furbank, 2003) which could meet the requirement of CO_2 for improved growth at elevated temperatures. In addition, the stimulated effect of eCO_2 on maize yield was reported by Long et al. (2004) due to reduction in stomatal conductance to water saving. In the present study, although we could not separate the effect of eCO_2 on maize yield due to lack of eCO_2 alone treatment, the similar yield between eT and $eTeCO_2$ indicated that the greater effect of elevated temperature than eCO_2.

Greater soybean yield under $eTeCO_2$ than eT indicated that soybean production benefited from CO_2 rising. The result supported our hypothesis that eCO_2 would improve soybean grain yield. The increase in grain yield might be attributed to the improved development of flowers, pollens and grains (Jablonski et al., 2002; Ziska and Bunce, 2006), due to decreased photorespiration (Long, 1991; Long et al., 2004), increased photosynthetic rate (Ruiz-Vera et al., 2013), increased canopy C assimilation and water-use efficiency due to declined stomatal conductance (review of Aninsworth et al., 2002) in response to eCO_2. The 6-49% increase in shoot biomass in this present study was consistent with the value of 19% at 592 ppm CO_2 (Kumagai et al., 2012) and 16-18% at 550 ppm CO_2 (Lam et al., 2012). Moreover, soybean grown under eCO_2 could have an advantage to increase the C-sink capacity from forming nodules (Jin et al., 2017), which in turn improved N_2 fixation and N nutrition for a higher yield (Sulieman et al., 2015).

4.3. Protein and oil in grains

The similar concentrations of protein in grains between $eTeCO_2$ and eT in this study suggested that the N supply was adequate to maintain the demand for grain development under eCO_2. The result could not be explained only by a dilution effect due to an increased carbohydrate accumulation under eCO_2 as shown by a meta-analysis (Pleijel and Udding, 2012). The lack of eCO_2 effect on the concentration of grain protein has been reported in many leguminous plants (Jablonski et al., 2002; Hao et al., 2014; Myers et al., 2014; Jin et al., 2017). This could be attributed to the stimulation on N_2-fixation to counteract the carbohydrate dilution effect (Bourgault et al., 2017). However, for some non-leguminous crops, previous studies have shown the negative effect of eCO_2 on grain protein concentrations in canola and wheat with sufficient N application (Fernando et al., 2012; Pleijel and Uddling, 2012; Jin et al., 2019). They attributed the negative effect to the translocation of carbohydrates to grains more efficiently than N (Taub and Wang, 2008) and impaired nitrate uptake/assimilation induced by eCO_2 (Pleijel and Uddling, 2012).

The combination of elevated temperature and CO_2 favored the accumulation of grain oil. The greater concentration of oil in grains under $eTeCO_2$ compared to eT could confirm the positive influence of CO_2 rising on oil production. The beneficial effect of elevated CO_2 on oil concentration has also been reported on soybean cultivars Essex, Holladay, and NK6955 (Heagle et al., 1998) and Williams 82 (Bellaloui et al., 2016). Elevated CO_2 enhanced the photosynthesis, and would relocate more sucrose and raffinose to grains, favoring oil formation (Hymowitz et al., 1972). Similar results have been reported in soybean by Hao et al. (2014) who indicated that the increase in the concentration of linoleic acid and palmitic acid induced by high CO_2 favored total oil production.

Elevated temperature also increased the accumulation of grain oil in the present study. This increase in oil accumulation might be attributed to the temperature of eT treatments was still lower than the optimal temperature for oil production in soybean (28 °C, Dornbos and Mullen, 1992) and maize (30 °C, Commuri and Jones, 2001). Zuil et al. (2012) showed that the concentration of oleic acid, a major component of oil, in soybean and maize linearly increased with increasing temperature in the range of 12-28 ºC. In other studies, increasing temperature to exceed the optimal temperature decreased the concentration of oil in grains of soybean (Dornbos and Mullen, 1992) and flax (Green, 1986).

4.4. Mineral nutrient concentrations in grains

Greater P accumulation in maize grains at eT was likely to attribute to two reasons. First, the increased C efflux from roots facilitates the mobilization of insoluble P fraction in soil (Jin et al., 2017). However, the mobilized P could not offset the eCO_2-induced decline in mineral nutrient movement due to the reduction of stomatal conductance (Oliveria et al., 2010; Köhler et al., 2019). Second, the improved P uptake from soil or fertilizer by enhanced sap flow at eT (Pregitzer and King, 2005). In contrast, the decrease in P concentration relative to the control in soybean grains at eT was likely due to the insufficient C flux to mobilize P at eT, and greater P demand for soybean growth than maize (Lavado et al., 2001). Additional eCO_2 counteracted the low P concentration in soybean grains at eT. It is explained by the fact that the stimulation of eCO_2 on soybean growth and N_2 fixation transferred additional C and N to favor mycorrhizal associations, and increase chelating agents and extracellular phosphatases to mobilize more soil P (reviewed by Rogers et al., 2009). All these attributed to the improvement of P uptake through P mobilization and root development at eCO_2 (Kuzyakov et al., 2019).

Four immobile elements of Ca, Fe, Zn and Mn showed different response to eT and $eTeCO_2$, indicating that different mechanisms were responsible for their accumulation in grains to adapt to climate change. The decrease in grain Ca concentration at eT relative to the control could not be explained fully by carbohydrate dilution effect (Myers et al., 2014) as the same was not applied to other immobile nutrients. It is expected that eT might indirectly decreased the Ca nutrition in grains. This was likely to attribute to the negative correlation between Ca and oil concentration in grains (Gibson and Mullen, 2001). In the present study, the eT-induced increase of oil concentration might decrease grain Ca concentration. Additional eCO_2 offset the negative effect of eT on grain Ca concentration. The result was consistent with the eCO_2-induced increase of grain Ca in soybean (Prior et al., 2008; Li et al., 2018), inconsistent with the negative effect in soybean (Köhler et al., 2019) and wheat (Beleggia et al., 2018). All these indicated that the exact mechanisms controlling the response of Ca concentration in grains to eT and eCO_2 are not fully understood.

The relative increase of grain Fe concentration at eT in the present study was reported by Köhler et al. (2019) who found the increase in grain Fe concentration was mainly derived from the eT-enhanced Fe diffusion into the roots (Oliveira et al., 2010). Additional eCO_2 further enhanced the effect of eT. Previous studies showed positive (Singh et al., 2016) and negative (Rodriguez et al., 2011; Myers et al., 2014; Köhler et al., 2019; Li et al., 2018) effects of eCO_2 on Fe concentration in soybean grains. Two possible reasons could be used to explain the increase. First, eCO_2 enhances the rhizosphere acidification due to the preferential uptake of NH_4^+-N by plant (Zhang et al., 2018), which benefits Fe uptake. Second, the increase of NADPH-ferric chelate reductase activity and expression of the LeIRT1 gene under eCO_2 contributed to the increased Fe concentration in plants (Jin et al., 2009).

The positive influence of elevated temperature on maize yield depended on the lower baseline temperature in the study region. This is because the increase of 2.1 °C above the 18.0 °C of the baseline temperature in this study was still in the optimal temperature range of 23-32°C for maize growth (Naveed et al., 2014; SÁnchez et al., 2014; Vanaja et al., 2017). Although the temperature increase was less than 3.8 °C increase in correspondence to 700 ppm of CO_2 projected using the

LDAS (Law Dome ice-core together with air samples collected at the South Pole) model (Florides and Christodoulides, 2009), the greater temperature increase was still within the optimal temperature range for crop growth (Naveed et al., 2014; SÁnchez et al., 2014; Vanaja et al., 2017). Therefore, the effect of 2.1 °C of temperature increase was likely to be similar to or smaller than the effect of the 3.8 °C increase.

Conclusion

This study showed that $eTeCO_2$ enhanced biomass production, grain yields and yield component formation of soybean and maize grown in a fertile Mollisol. The eT with or without eCO_2 increased oil concentration in grains, but no effect on protein in the temperate region where temperature was near or below the optimal temperature for two crops. Moreover, the eT increased and additional eCO_2 further enhanced the concentrations of Fe in grains of two crops. Additional eCO_2 offset the decrease in Ca concentration at eT. The results suggest that expanding cropping area to cold regions where elevated temperatures are still within or below the optimal range for plant growth might be potential to mitigate the negative impact of climate change on crop production and grain quality. Moreover, the heat-tolerant trait should be considered during cultivar selection to cope with future climate change.

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Table 1. Grain yield (g m^{-2}) of soybean and maize grown under ambient (control), elevated temperature (eT), and the elevated temperature and CO_2 concentration (eTeCO_2) in the period of 2012-2016

Significant levels are indicated as n.s., P>0.05; *, P<0.05; **, P<0.01; and ***, P<0.001.

Treatment	2012	2013	2014	2015	2016	***Mean***
Soybean						
Control	379cd	266cd	386d	233d	308cd	***314d***
eT	352d	235d	352d	237d	266d	***288d***
eTeCO_2	455c	356c	492c	409c	350c	***412c***
Maize						
Control	548b	722b	847b	881b	623b	***724b***
eT	733a	908a	1060a	1076a	745a	***904a***
eTeCO_2	705a	844a	1079a	1102a	805a	***907a***
LSD (P=0.05) and significance level						
Climate treatment (T)	49**	n.s.	79**	90**	49**	*53****
Species (S)	67***	38***	66***	69***	52***	*43****
T × S	n.s.	97**	97*	n.s.	70*	*75**

Table 2. Number of pod and seed number per pod of soybean, and number of cob and seed per cob of maize grown under ambient (control), elevated temperature (eT), and the elevated temperature and CO_2 concentration (eTeCO_2) in 2014 and 2016

Significant levels are indicated as n.s., *P*>0.05; *, *P*<0.05; and **, *P*<0.01.

Treatment	Soybean---------------------------------				Maize -------------------------------------			
	Number of pod (No. $plant^{-1}$)		Seed number per pod (No. pod^{-1})		Number of cob (No. $plant^{-1}$)		Seed number per cob (No. cob^{-1})	
	2014	2016	2014	2016	2014	2016	2014	2016
Control	35.4b	44.3ab	2.40b	2.33a	1.0a	1.0a	560a	332b
eT	34.5b	41.2b	2.64a	2.31a	1.1a	1.1a	644a	415a
eTeCO_2	42.9a	46.9a	2.68a	2.39a	1.0a	1.1a	650a	446a
LSD (*P*=0.05)	5.0*	4.2*	0.06**	n.s.	n.s.	n.s.	n.s.	34*

Table 3. The grain weight, shoot biomass and harvest index of soybean and maize grown under ambient (control), elevated temperature (eT), and the elevated temperature and CO_2 concentration (eTeCO_2) in 2014 and 2016

The data of shoot biomass are log_{10}-transformed for ANOVA, and the means followed by a common letter are not significantly different at $P \leq 0.05$. Significant levels are indicated as n.s., $P>0.05$; *, $P<0.05$; **, $P<0.01$; and ***, $P<0.001$.

Treatment	Grain weight (mg grain^{-1})		Shoot biomass (g m^{-2})		Harvest index	
	2014	2016	2014	2016	2014	2016
Soybean						
Control	210b	169b	553*c*	571*cd*	0.48a	0.54ab
eT	202b	161b	521*c*	525*d*	0.47a	0.50b
eTeCO_2	214b	184b	821*b*	*603c*	0.43a	0.58a
Maize						
Control	392a	397a	1642*a*	1705*b*	0.47a	0.37c
eT	382a	375a	1895*a*	2232*a*	0.48a	0.33c
eTeCO_2	386a	380a	1805*a*	2206*a*	0.47a	0.36c
LSD (P=0.05) and significance level						
Climate treatment (T)	n.s.	n.s.	*	*	n.s.	n.s.
Species (S)	26***	18***	***	***	n.s.	0.03***
T × S	n.s.	n.s.	n.s.	**	n.s.	n.s.

Table 4. Concentration of protein in grains (g kg^{-1}) of soybean and maize grown under ambient (control), elevated temperature (eT), and the elevated temperature and CO_2 concentration (eTeCO_2) during 2012-2016

Significant levels are indicated as n.s., P>0.05; and ***, P<0.001.

Treatment	2012	2013	2014	2015	2016	***Means***
Soybean						
Control	347a	273b	350a	336a	351a	***331a***
eT	345a	290a	348a	359a	368a	***342a***
eTeCO_2	347a	282ab	345a	361a	372a	***342a***
Maize						
Control	109b	67c	88b	88b	83b	***87b***
eT	110b	71c	99b	82b	86b	***90b***
eTeCO_2	112b	73c	99b	85b	85b	***91b***
LSD (P=0.05) and significance level						
Climate treatment (T)	n.s.	n.s.	n.s.	n.s.	n.s.	***n.s.***
Species (S)	9***	5***	16***	15***	13***	***7****
T × S	n.s.	n.s.	n.s.	n.s.	n.s.	***n.s.***

Table 5. Concentrations of oil in grains (g kg^{-1}) of soybean and maize grown under ambient (control), elevated temperature (eT), and the elevated temperature and CO_2 concentration ($eTeCO_2$) during 2012-2016

Significant levels are indicated as n.s., *P*>0.05; **, *P*<0.01; and ***, *P*<0.001.

Treatment	2012	2013	2014	2015	2016	***Means***
Soybean						
Control	191c	186c	193c	196c	197c	***193c***
eT	202b	201b	216b	213b	218b	***210b***
$eTeCO_2$	213a	211a	227a	219a	229a	***220a***
Maize						
Control	48e	49e	49f	50f	51f	***49f***
eT	52de	54d	56e	58e	57e	***55e***
$eTeCO_2$	53d	58d	61d	63d	62d	***59d***
LSD (P=0.05) and significance level						
Climate treatment (T)	0.4***	0.5***	0.7***	0.6***	0.7***	***2.5****
Species (S)	3.5***	3.4***	3.6***	3.5***	3.7***	***2.0****
T × S	4.3**	4.1**	4.4**	n.s.	4.5**	***3.5****

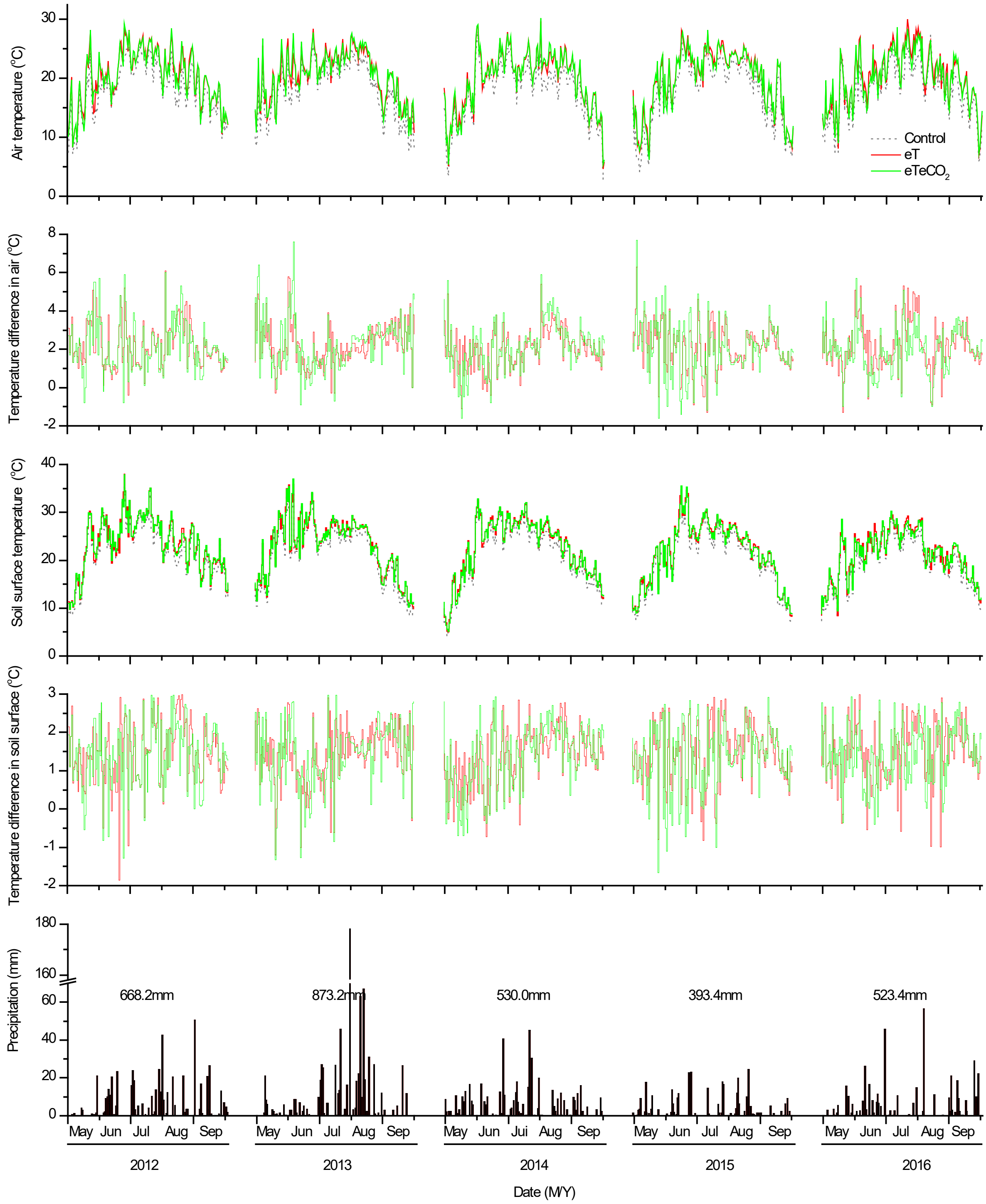

Figure 1. Daily change in the mean temperature of air and soil surface in OTC of the control, elevated temperature (eT), and the elevated temperature and CO_2 concentration ($eTeCO_2$) treatments, temperature differences of air and soil surface between the control (ambient) and eT and $eTeCO_2$, and precipitation during the experimental period. The single and double arrows indicate the sowing time and harvest time, respectively.

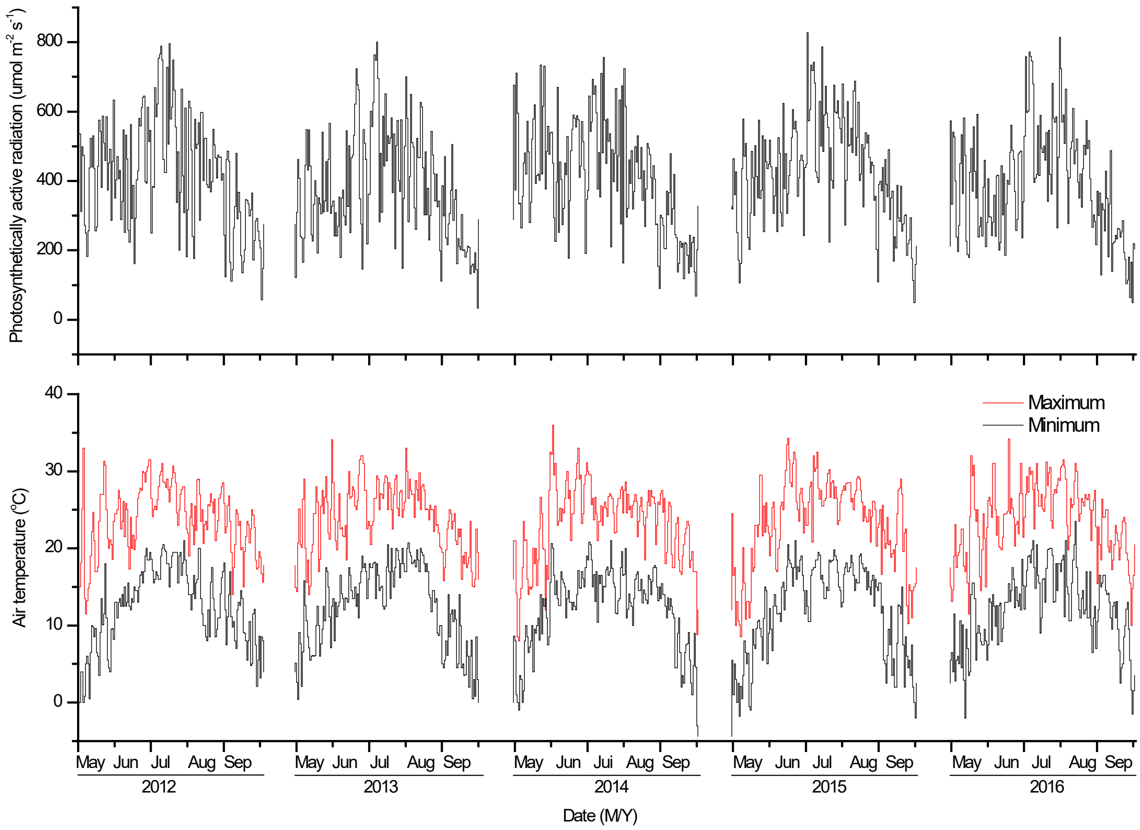

Figure 2. Daily changes in the photosynthetically active radiation, the maximum and minimum of air temperature (outside OTC's) during the experimental period.

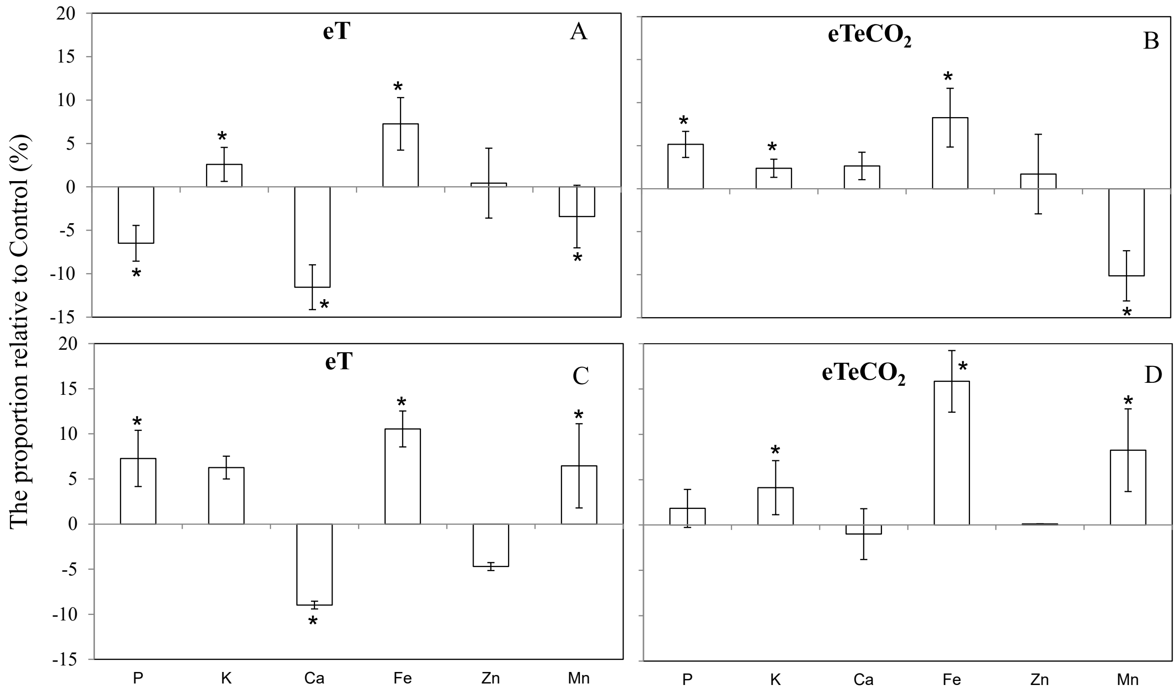

Figure 3. The proportions of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), iron (Fe), zinc (Zn) and manganese (Mn) in grains of soybean (A and B) and maize (C and D) grown under elevated temperature (eT) and the elevated temperature and CO_2 concentration (eTeCO_2) relative to control. The bars represent standard error (n=3). * indicated that the mineral nutrient was significant difference between eT or eTeCO_2 and control with two-way ANOVA analysis (p<0.05).