Effects of elevated O₃ exposure on nutrient elements and quality of winter wheat and rice grain in Yangtze River Delta, China

Feixiang Zheng^{a,b}, Xiaoke Wang^{b,*}, Weiwei Zhang^{b,c}, Peiqiang Hou^b, Fei Lu^b,
Keming Du^a, Zhongfu Sun^a

^a Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, Beijing 100081, China

^b State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

^c Key Laboratory of Black Soil Ecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin 150081, China

ARTICLE INFO

Article history:

Received 2 February 2013

Received in revised form

28 March 2013

Accepted 30 March 2013

Keywords:

Ozone

Winter wheat

Rice

Nutrient elements

Grain quality

ABSTRACT

With the open-top chambers (OTCs) *in situ* in Yangtze River Delta, China in 2007 and 2008, the effects of elevated O₃ exposure on nutrient elements and quality of winter wheat and rice grain were investigated. Grain yield per plant of winter wheat and rice declined in both years. The N and S concentrations increased under elevated O₃ exposure in both years and C–N ratios decreased significantly. The concentrations of K, Ca, Mg, P, Mn, Cu and Zn in winter wheat and the concentrations of Mg, K, Mn and Cu in rice increased. The concentrations of protein, amino acid and lysine in winter wheat and rice increased and the concentration of amylose decreased. The increase in the nutrient concentration was less than the reduction of grain yield in both winter wheat and rice, and, hence, the absolute amount of the nutrients was reduced by elevated O₃.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Ozone (O₃), a highly reactive and oxidative gas from photochemical reactions of precursor gases such as nitrous oxides, carbon monoxide, or volatile organic compounds, is the basis for photochemical smog and secondary pollutants in the Earth's troposphere. O₃ concentrations have dramatically increased over the last century in some areas and it has been suggested that the ground-level O₃ concentration is increasing at a rate of about 0.3%–2% per year (Vingarzan, 2004). A pessimistic estimate showed that the tropospheric O₃ concentration will go up to 0.08 ppm in 2100 (Fiscus et al., 2005). Although the increasing trend in O₃ concentration has leveled off or been slightly negative in the USA and Europe (IPCC, 2007), the tropospheric O₃ is still rising in developing countries, where environmental standards have not been adequately established, due to the rapid industrialization and urbanization (Ariyaphanphitak et al., 2005). In China, it is reported that higher O₃ concentration has attained more than 150 ppb in some developed regions (Shao et al., 2006).

Near surface O₃ levels greater than 40 ppb may cause visible leaf injury, decreased photosynthesis and increased senescence, which has significant influence on the yield of major agricultural crops (Morgan et al., 2003; Fiscus et al., 2005; Ashmore, 2005; Feng et al., 2008; Feng and Kobayashi, 2009; Wilkinson et al., 2011). Many regions of the globe are now suffering from these crops yield loss, with associated economic costs having reached several billion dollars per annum in such regions as the US, EU and East Asia (Sitch et al., 2007). The available ambient O₃ monitoring data have indicated that O₃ concentration were high enough to significantly reduce rice and wheat yield in some developed regions of China (Wang and Mauzerall, 2004) including the Yangtze River Delta (Zhou, 2004; Wang et al., 2005) and the Pearl River Delta (Zhang et al., 2004).

Wheat and rice are the two most important staple foods in the world. Wheat, the most popular grain crops grown on about 200 million hectares in a range of environments, can provide the world with an annual production of more than 619 million metric tons (FAO, 2007). Rice is listed as the second largest grain crops which is consumed by over half of the world's current population (IRRI, 2002). As a major grain in Asia, rice supports the most populous regions including China, India, Japan, and other tropical Asian countries, and among all the countries, China's sown area of rice ranks the second largest but the total rice yield is the largest. Under

* Corresponding author.

E-mail address: wangxk@rcees.ac.cn (X. Wang).

these circumstances, it is important to investigate the effect of elevated O₃ on crop in order to ensure the food security.

Although many studies have been conducted on the impacts of elevated O₃ concentrations on crops yield, such as rice, wheat, bean, soybean, barley, potato (Feng and Kobayashi, 2009; Mills et al., 2007; Fiscus et al., 2005; Fuhrer, 2009), the study on the effects on grain quality traits have been mainly focused on a limited number of crops (Booker et al., 2009), particularly the spring wheat (Fuhrer et al., 1990; Fangmeier et al., 1997; Pleijel et al., 1997, 1999; Piikki et al., 2008; Feng et al., 2008). The protein and starch concentrations changes under elevated O₃ exposure were included in most of these studies but the nutrient elements changes were rarely studied. Frei et al.'s (2012) study has showed that protein yield and iron yield of rice grain were negatively affected by ozone.

Since 2003, open-top chambers (OTCs) have been used to investigate the effects of elevated O₃ exposure on winter wheat and rice in physiological processes (Zheng, 2006; Zheng et al., 2007), yield (Wang et al., 2007, 2012), soil microorganism (Chen et al., 2008, 2010), agroecosystem (Zheng et al., 2011), in Yangtze River Delta, China. The objectives of this study is to investigate the changes of nutrient elements and quality of winter wheat and rice grain under the elevated O₃ exposure.

2. Materials and methods

2.1. Experimental site

The experimental site is located at the Shuangqiao Farm (31°53'N, 121°18'E) in Xiuzhou District, Jiaxing City, Zhejiang Province, about 100 km from Shanghai, China and in the center of Hang-Jia-Hu Plain of the Yangtze River Delta (YRD) in China. The YRD, which is a highly productive region in terms of both agriculture and industry in China, has witnessed a rapid industrialization and urbanization in recent years. The ozone concentration is rising year by year in YRD: the average monitoring concentration is up to 50–60 ppb (Feng et al., 2003) and the peak ozone concentration was as high as 196 ppb (Zhou, 2004). Ozone pollution has been one of the prime reasons for the crop yield loss in this area (Feng et al., 2003).

The experimental site is influenced by the Asian monsoon climate system, which is noted for its cold dry winter and warm wet summer. The annual mean temperature is 15.9 °C; the annual precipitation is 1169 mm and the annual sunshine-duration is 2017 h (Zheng et al., 2011). The prevailing cropping pattern is wheat-rice or rape-rice rotation. Generally, rice and winter wheat were grown in rotation, with rice being grown from July to November and winter wheat from November to May of the next year.

2.2. Crop management

Winter wheat (*Triticum aestivum* L., Jia 002) and rice (*Oryza sativa* L., Fan 3694) seeds were provided by Jiaxing Academy of Agriculture according to local condition. All crop managements, such as sowing, transplanting (rice), harvest, fertilizing, were adopted according to the local agronomic customs so that water, fertilizer, pathogen, insect and weed were not limiting factors (Tables 1 and 2). 375 g (1325 kg hm⁻²) compound fertilizer (N:P₂O₅:K₂O = 15:15:15, total fertility ≥ 45%) was applied before transplanting (rice) or sowing (winter wheat) and 100 g (353 kg hm⁻²) urea (N ≥ 46.5%) was applied during the jointing stage and the booting stage (rice) or tillering stage (winter wheat) in each block.

2.3. Ozone exposure

The regular octagon open-top chamber (OTCs, 2.2 m in height and 2 m in diameter) was made of aluminum alloy frame covered with polyethylene plastic film (Zheng et al., 2011). The ambient air or O₃ added air delivered by a centrifugal blower was released from down holes in automatically-rotating pipes. The OTCs were 3 m apart from each other and the water-proof barriers were established for blocking surface water exchange between OTCs. O₃ concentration monitoring in previous

Table 2

The crop management and O₃ exposure of rice.

Years	Crop management			Exposure	
	Sowing	Transplanting	Harvest	Beginning	End
2007	May 25	Jun. 29	Nov. 2	Jul. 29	Oct. 11
2008	May 26	Jul. 4	Nov. 12	Jul. 25	Oct. 19

studies had showed that O₃ concentrations were evenly distributed, both vertically and horizontally, within the OTC (Zheng et al., 2007). O₃ was generated from pure oxygen by the ozone generator with high voltage discharge method (Yuyao Shenglete Company, Zhejiang, China). O₃ concentrations within the OTCs were monitored by an ozone analyzer (Model 9810B, Monitor Labs, Australia). A data logger (CR1000, Campbell Scientific Inc., UT, USA) stored O₃ concentration data by connecting with the O₃ analyzer and the air temperature within the OTCs by connecting with constantan-copper thermocouples (Type-T, Omega Engineering, CT, USA). The periods of ozone exposure in 2007 and 2008 were showed in Tables 1 and 2.

2.4. Experimental design

Four treatments were applied, i.e. charcoal-filtered air (CF), non-filtered air (NF), charcoal-filtered air with lower O₃ (O₃-1) and higher O₃ (O₃-2). The mean O₃ concentration and the AOT40 value (the accumulated ozone concentration above 40 ppb over the growing period for daylight hours) during fumigation in each treatment were showed in Table 3. There were three replicates (i.e. OTCs) for each treatment and 12 OTCs in total. The O₃ exposure began at 9:00 and ended at 17:00 except when it was raining.

2.5. Sample collection and analysis

After harvest, grain yield per plant was analyzed and grain samples were dried at 75 °C for 48 h and then milled to a fine flour-like powder. The powder was stored in a refrigerator in the dark at 4 °C and oven-dried again before analysis.

The concentrations of C, N and S and the C–N ratio in the grain powder were determined by an elemental analyzer (Vario EL III, Elementar Inc., Germany).

The powder samples (0.25 g) were digested into solutions using the nitric-perchloric acid digestion and then the concentrations of P, K, Mg, Ca, Fe, Mn, Na, Zn and Cu in the solutions were determined by ICP-OES (Pro DiGY, Lee MANS Inc, USA).

The protein concentrations were determined with coomassie brilliant blue G-250 staining. Each 0.1 mL protein extract was homogenized with 5 mL reaction solution containing 100 mg/mL coomassie brilliant blue G-250, 4.7% (w/v) ethanol, and 8.5% (w/v) phosphoric acid. The absorbance at 595 nm was measured after 2 min reaction. Protein concentration was calculated by comparing the standard curve utilizing bovine serum albumin as standard.

The concentrations of amino acid were determined with double indicator formol-titration. A sample of solution with 3 drops of 0.1% (w/v) neutral red indicator was titrated with 0.1 mL/L NaOH. The other sample of solution with 3 drops of 0.1% (w/v) thymolphthalein indicator and 20 mL 40% (w/v) neutral buffered formalin was titrated with 0.1 mL/L NaOH after 1 min reaction. The consumptions of NaOH in the twice titration were recorded. The same experiment was performed with glycine and the recovery rate was obtained. The concentration of amino acid was calculated by the following formula (1).

$$C_a = [C \times (V_1 - V_2) \times 0.014 \times 100] / (m \times R) \quad (1)$$

where C_a is the concentration of amino acid; C is the concentration of NaOH (mol L⁻¹); V_1 is the volume of NaOH with neutral red indicator (mL); V_2 is the volume of NaOH with thymolphthalein indicator (mL); m is the quality of the sample in the solution (g); R is the recovery rate; 0.014 is the millimole quantity of N (g mmol⁻¹).

The concentrations of lysine were determined with colorimetric method. The standard curve was produced by leucine standard solution. Each 2 mL lysine extract with 2 mL ninhydrin reagent was treated with 80 °C water bath heating for 30 min, and the cooled solution with 5 mL 95% ethanol (w/v) was filtered. The filtrate was measured in the absorbance at 530 nm. The concentration of lysine was calculated by comparing the standard curve utilizing leucine as standard.

The concentrations of amylose were also determined by colorimetric method. The standard curve was produced by amylose standard solution. The powder sample (0.10 g) with 1 mL 95% ethanol (w/v) and 9 mL NaOH (1 mol L⁻¹) was treated with boiling water bath for 10 min and was then mixed into sample solution in a constant volume at 100 mL. 5 mL sample solution with 1 mL acetic acid solution (1 mol L⁻¹) and 1.5 mL iodine solution was mixed in a constant volume at 50 mL. The blank solution was made with 5 mL NaOH (0.09 mol L⁻¹). The spectrophotometer was under zero adjustment with the blank solution in the absorbance at 620 nm and then the absorbance value of sample solution was measured. The amylose was

Table 1

The crop management and O₃ exposure of winter wheat.

Years	Crop management		Exposure	
	Sowing	Harvesting	Beginning	End
2007	Nov. 15	May 28	Mar. 26	May 19
2008	Nov. 18	May 31	Mar. 20	May 13

Table 3The mean ($\pm$ SD) O₃ concentration (ppb) during fumigation and AOT40 values (ppm h) for winter wheat and rice in the growing seasons for each treatment.

	Index	Years	O ₃ treatments			
			CF	NF	O ₃ -1	O ₃ -2
Winter wheat	Mean O ₃	2007	11.64(±5.06)	22.70(±6.35)	51.53(±3.48)	98.44(±3.48)
		2008	20.32(±9.46)	27.66(±2.81)	96.48(±4.14)	142.66(±8.23)
	AOT40	2007	0	0.21(±0.05)	1.58(±0.17)	9.17(±0.46)
		2008	0.10(±0.02)	0.15(±0.01)	15.32(±1.15)	27.67(±2.18)
Rice	Mean O ₃	2007	19.69(±4.63)	22.57(±1.29)	69.60(±4.92)	118.62(±5.33)
		2008	6.98(±3.65)	17.44(±4.49)	82.23(±4.29)	138.30(±6.58)
	AOT40	2007	0.40(±0.20)	0.51(±0.12)	13.83(±2.02)	32.08(±1.66)
		2008	0.01(±0.00)	0.21(±0.12)	22.77(±2.40)	52.84(±3.52)

calculated by comparing the standard curve utilizing amylose standard solution as standard.

The reference materials in this study were provided by the Chinese CRM/RM Information Center.

2.6. Data analysis

An analysis of variance (ANOVA) was used to test for mean differences between treatments (CF, NF, O₃-1 and O₃-2). The statistics was performed by the statistical package SPSS (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Yield

Grain yield per plant of winter wheat and rice were both reduced under elevated O₃ exposure (Fig. 1). Relative to CF treatment, the grain yield per plant of winter wheat in NF, O₃-1, O₃-2 treatments were 97.6%, 91.5% and 60.0% respectively in 2007 and 98.7%, 54.8% and 36.9% in 2008, and there were significant differences between O₃-2 and the other treatments ($P < 0.05$). The differences in the grain yield per plant of rice between O₃-2 treatment and CF or NF treatments in both 2007 and 2008 were significant ($P < 0.05$); however, the differences between O₃-1 and the other treatments, between CF and NF treatments and between O₃-1 and O₃-2 treatments were not clear.

3.2. Nutrient elements

Concentrations of C, N, C–N ratio and concentrations of S in winter wheat and rice grain from different treatments were showed in Figs. 2 and 3 respectively. There were no significant difference in the concentrations of C in the two crops grain between the treatments (CF, NF, O₃-1, O₃-2) in both 2007 and 2008, but the concentrations of C in the treatments (CF, NF, O₃-1, O₃-2) in 2008 were lower than those in 2007. The concentrations of N in O₃-2 in

2007 and 2008 were both higher than those in the non-ozone treatments (CF, NF) ($P < 0.05$). As a result, the C–N ratios in winter wheat grain in O₃-2 in both 2007 and 2008 were lower than those in the non-ozone treatments (CF, NF) ($P < 0.05$). No obvious differences were found in concentrations of N in rice grain between CF and NF in both 2007 and 2008, however, there were significant differences in the concentrations of N between non-ozone treatments (CF, NF) and ozone treatments (O₃-1, O₃-2) ($P < 0.05$), and between O₃-1 and O₃-2 ($P < 0.05$) treatments in both 2007 and 2008. There were significant differences in the concentrations of N in rice grain between the treatments (CF, NF, O₃-1, O₃-2) ($P < 0.05$) in 2007 but no significant difference between CF and NF in 2008.

The concentrations of S in winter wheat grain in the treatments (CF, NF, O₃-1, O₃-2) were 0.17%, 0.19%, 0.19% and 0.20%, respectively in 2007 and 0.23%, 0.25%, 0.34% and 0.28% respectively in 2008. Although there were no significant differences in the concentrations of S in the rice grain between the treatments (CF, NF, O₃-1, O₃-2) in 2008, the differences between ozone (O₃-1, O₃-2) and non-ozone treatments (CF, NF) and between O₃-1 and O₃-2 treatments were clear in 2007 (both $P < 0.05$).

Concentrations of P, K, Mg, Ca, Fe, Mn, Na, Zn and Cu in winter wheat and rice grain were showed in Figs. 4 and 5, respectively. The change trends of these nine elements concentrations in winter wheat and rice grain were inconsistent under elevated O₃ exposure. No significant differences between the treatments (CF, NF, O₃-1, O₃-2) were found in the concentrations of Fe and Na in winter wheat grain in 2007 and the concentrations of Ca, Fe, Na and Zn in rice grain in both years. Although the increment varied with the elements and years, the concentrations of the other elements in the two crops increased with the increasing O₃ exposure. The significant differences in the elements of winter wheat between CF and NF were only found in Mg and Mn in 2007 and the differences between O₃-1 and O₃-2 were only found in P, K and Mg in 2007 ($P < 0.05$). The differences in the concentrations of P, Mg, Mn and Cu in rice grain between the non-ozone treatments (CF, NF) and

Fig. 1. Grain yield per plant of winter wheat and rice after exposure to different O₃ treatments. Different letters within the column stand for significant difference at $p < 0.05$ (measured with one-way ANOVA, $N = 3$).

Fig. 2. Concentrations of C and N, C–N ratio and concentrations of S in winter wheat grain after exposure to different O₃ treatments. Different letters within the column stand for significant difference at $p < 0.05$ (measured with one-way ANOVA, $N = 3$).

ozone treatments (O₃-1, O₃-2) were clear in both years ($P < 0.05$). The differences in the concentrations of K in rice grain between non-ozone treatments (CF, NF) and ozone treatments (O₃-1, O₃-2) as well as the differences between CF and NF were significant in 2007 (both $P < 0.05$).

3.3. Grain quality

No significant differences in concentrations of protein, amino acid, lysine and amylose were found between CF and NF treatments in winter wheat and rice grain in 2007 and 2008 (Figs. 6 and 7). The concentrations of protein, amino acid, lysine in both crops grain increased clearly while the concentration of amylose decreased under elevated O₃ exposure in 2007 and 2008 (except lysine in rice grain in 2008).

The concentrations of protein, amino acid, lysine in winter wheat grain in O₃-2 treatment in 2007 and 2008 were higher than those in the other treatments (CF, NF, O₃-1) ($P < 0.05$), but there were no clear differences in protein and amino acid in 2007 and 2008 between O₃-1 and non-ozone treatments (CF, NF). The concentrations of lysine in winter wheat grain in the treatments (CF, NF, O₃-1, O₃-2) in 2008 were 0.47%, 0.53%, 0.87%, 0.90%, respectively and the concentrations in ozone treatments (O₃-1, O₃-2) was significantly higher than those in the non-ozone treatments (NF, CF) ($P < 0.05$). The concentrations of amylose in winter wheat grain decreased by 41.97% (O₃-1) and 44.82% compared to that in the CF treatments in 2008, but there were no significant differences in amylose between O₃-1 and non-ozone treatments (CF, NF) in 2007.

There were no significant differences among the non-ozone and O₃-1 treatments in the concentrations of protein in rice grain in

Fig. 3. Concentrations of C and N, C–N ratio and concentrations of S in rice grain after exposure to different O₃ treatments. Different letters within the column stand for significant difference at $p < 0.05$ (measured with one-way ANOVA, $N = 3$).

Fig. 4. Elements concentrations in winter wheat grain after exposure to different O₃ treatments. Different letters within the column stand for significant difference at $p < 0.05$ (measured with one-way ANOVA, $N = 3$).

2007 and the concentrations of amino acid in both 2007 and 2008. The concentrations of lysine in rice grain in the treatments (CF, NF, O₃-1, O₃-2) in 2007 were 0.30%, 0.31%, 0.41%, 0.37%, respectively, but the change trend in 2008 was not clear. The differences in the concentrations of amylose in rice grain between non-ozone (CF, NF) and O₃-1 treatments were not clear in both 2007 and 2008.

4. Discussion

Previous studies have showed that the negative effects of long-term higher O₃ exposure on crops were caused by the cumulative

effects of O₃ (Nussbaum et al., 1995; Fuhrer et al., 1997). In this study, the cumulative effects of O₃ on the grains of winter wheat and rice were significantly exhibited at the end of the long-term higher O₃ exposure when the crops were harvested, and the yield, nutrient elements concentrations and quality of grain were all influenced. The crops growth and yield were related to the translocation and distribution of photosynthate. Under higher O₃ exposure, the leaves of crops are injured seriously: browning spot appeared in the leaves surface after two (winter wheat) or four (rice) weeks and the leaves were necrotic in the end of the experiment (Zheng, 2006); the photosynthetic rate of leaves is reduced

Fig. 5. Elements concentrations in rice grain after exposure to different O₃ treatments. Different letters within the column stand for significant difference at $p < 0.05$ (measured with one-way ANOVA, $N = 3$).

Fig. 6. Concentrations of protein, amino acid, lysine and amylose in winter wheat grain after exposure to different O₃ treatments. Different letters within the column stand for significant difference at $p < 0.05$ (measured with one-way ANOVA, $N = 3$).

and translocation of photosynthate could be inhibited (Cooley and Manning, 1988; Fiscus et al., 2005). In this case, the crops yields were clearly reduced.

We found that there were no significant differences in the C concentrations of winter wheat and rice grains between the treatments in both years, but the N concentrations has increased and the C–N ratios decreased under the treatments. Higher N concentrations in plant organs are useful for plant to resist O₃ damage (Maurer and Matyssek, 1997). The increased N concentrations under O₃ exposure were also observed in spring wheat grain when the yield was reduced (Fangmeier et al., 1997; Pleijel et al., 1998). The C–N ratio is an important index in the coordination degree for carbon and nitrogen metabolism, and it could reflect the relative abundance of each sink–source of carbon and nitrogen and

the influence to the plant growth and development. In this study, the reduced C–N ratio of grain was an indirect effect of the reduction in carbohydrate accumulation to the elevated O₃ exposure. Sulfur is an essential macronutrient in plant and sulfur-containing defence compounds are crucial for the survival of plants under biotic and abiotic stress, e.g., elemental sulfur, H₂S, glutathione, phytochelutins, various secondary metabolites and sulfur-rich proteins (Rausch and Wachter, 2005), which are related with antioxidant system of plant. The O₃ stress resistance capacity of winter wheat and rice was enhanced due to the increased S concentrations under O₃ exposure.

K, Ca, Mg, P are essential macronutrient and Mn, Fe, Na, Cu, Zn are essential micronutrient in plant. Although the concentrations of these two series of elements in plant vary with the plant

Fig. 7. Concentrations of protein, amino acid, lysine and amylose in rice grain after exposure to different O₃ treatments. Different letters within the column stand for significant difference at $p < 0.05$ (measured with one-way ANOVA, $N = 3$).

physiological needs, they both play different but important roles. At the end of the elevated O₃ exposure, most of the element concentrations in winter wheat grain increased while only the concentrations of Mg, P, Mn and Cu of rice grain increased in both years. The sensitivity of winter wheat and rice (Wang et al., 2012) to O₃ may lead to the different degrees of changed element concentration between winter wheat and rice under elevated O₃ exposure in this study. It revealed that elevated O₃ exposure could influence the plant nutrient cycling and element metabolism and increasing concentrations of the elements were the responses of the plants to elevated O₃. The increasing concentrations of essential elements in winter wheat and rice grain were the indirect effect of the transformation in material metabolism to the elevated O₃ exposure. This is also observed in spring wheat (Fuhrer et al., 1990) and beans (Tingey et al., 1986), but in Frei et al.'s (2012) study about rice grain, the Fe concentration decreased with the increasing O₃ level and the Zn concentration was not consistently affected by O₃.

The increased concentrations of protein, amino acid and lysine of winter wheat and rice grain under elevated O₃ exposure showed that O₃ could influence the nitrogen metabolism. There is a negative relationship between cereal crops yield and its grain protein concentration, which is called “growth dilution” (Pleijel et al., 1999; Piikki et al., 2008; Pleijel and Uddling, 2011), and the increase in protein concentration was likely the consequence of reduced carbohydrate levels (Slaughter et al., 1989). In this study, the grain yield of winter wheat and rice decreased while the grain protein concentrations increased under elevated O₃ exposure and the phenomenon was verified. Similar results were also obtained in winter wheat (Slaughter et al., 1989; Feng et al., 2008), spring wheat (Fuhrer et al., 1990; Pleijel et al., 1997, 1999; Piikki et al., 2008; Feng et al., 2008) and rice with six genotypes (Frei et al., 2012), but the opposite result was achieved in maize (Fu et al., 2008), and no treatment effects were observed on peanut seed (Burkey et al., 2007). Amino acid is a substance that makes up protein and the change trend was similar with protein under elevated O₃ exposure in this study. Lysine is a limiting amino acid and its concentration in cereal grain is very low. For this reason, the lysine concentration change is more important to grain quality. Guo et al.'s (2001) study using pot culture test showed that the protein and amino acid concentrations of rice grain increased under elevated O₃ exposure despite the decreased yield, but there was also one study that showed the amino acid and lysine concentrations of spring wheat were not affected by O₃ (Fuhrer et al., 1990).

There are two kinds of starch in crop grain, amylose and amylopectin, and the change in the ratio between amylose and amylopectin contents would change many food quality traits (e.g. taste, whiteness, volume and cooking quality). Amylose is more easily digested by humankind and its concentration is an important evaluating index of the grain quality. Previous studies have showed that the starch in winter wheat (Fuhrer et al., 1990) and rice (Frei et al., 2012) grain decreased under elevated O₃ exposure. Although there were no differences of C concentration between the treatments, the amylose concentrations were clearly reduced under elevated O₃ exposure and. The crop leaves are directly affected by O₃ and leaf chemistry responses revealed that protein concentration has decreased while the starch concentration increased under elevated O₃ exposure (Feng et al., 2008). The ozone-induced reduction in net production and translocation of photosynthates in the leaves could lead to the amylose concentration decrease and it revealed that less carbohydrate was translocated from the leaves (source) to the grain (sink).

In this study, there were significant differences between different species in the effect of elevated O₃ exposure on grain. Although besides the Lysine concentration, the change trends of yield, C, N, S concentrations, C–N ratios, protein, amino acid, lysine

and amylose concentrations of winter wheat grain and S concentration of rice grain in 2008 remained similar, the descent range or rising ranges were different. After long-term O₃ exposure, most of the nutrient element concentrations of winter wheat grain which were determined in this study increased, but there were no differences in Fe, Na, Zn and Ca concentrations of rice grain between the treatments. This result indicated that the sensitivity of the two crops to O₃ were different and the winter wheat we planted is more sensitive to O₃ than the rice. Furthermore, there were also inter-annual variations of the measuring indexes in winter wheat or rice. The reason may be that the growth of the crops was also affected by some other factors than the elevated O₃ exposure.

5. Conclusions

Under the elevated O₃ exposure, all measured nutrient concentrations except C in winter wheat grain and the concentration of N, S, Mn, Cu, K, Mg, P in rice grain increased, and the C–N ratios were reduced in both the two crops. The concentrations of protein, amino acid and lysine in winter wheat and rice grain increased and the concentrations of amylose decreased in this study. The alterations in nutrient elements concentrations and grain quality of winter wheat and rice were adaptive responses to elevated O₃.

Although there was a very modest increase in the nutrient elements and quality of winter wheat and rice grains under elevated O₃ exposure, the increase in the measured indexes was not large enough to compensate for the yield loss in both winter wheat and rice and the effects of elevated O₃ exposure on winter wheat and rice remain negative. Therefore, these changes in crops grains under elevated atmospheric O₃ should be of greater concern to guarantee the food security.

Acknowledgments

The research has been supported by the National Natural Science Foundation of China (No. 31200295), the China Postdoctoral Science Foundation (No. 20100480488), the National Natural Science Foundation of China (No. 31170424), Chinese Academy of Sciences for Strategic Priority Research Program (XDA05050602).

References

- Ariyaphanphitak, W., Chidthaisong, A., Sarobol, E., Bashkin, V.N., Towprayoon, S., 2005. Effects of elevated ozone concentrations on Thai Jasmine rice cultivar (*Oryza sativa* L.). *Water, Air and Soil Pollution* 167, 179–200.
- Ashmore, M., 2005. Assessing the future global impacts of ozone on vegetation. *Plant, Cell and Environment* 28, 949–964.
- Booker, F., Muntifering, R., McGrath, M., Burkey, K., Decoteau, D., Fiscus, D., Manning, W., Krupa, S., Chappelka, A., Grantz, D., 2009. The ozone component of global change: potential effects on agricultural and horticultural plant yield, product quality and interactions with invasive species. *Journal of Integrative Plant Biology* 51 (4), 337–351.
- Burkey, K.O., Booker, F.L., Pursley, W.A., Heagle, A.S., 2007. Elevated carbon and dioxide and ozone effects on peanut: II. Seed yield and quality. *Crop Science* 47, 1488–1497.
- Chen, Z., Wang, X.K., Feng, Z.Z., Zheng, F.X., Duan, X.N., Yang, W.R., 2008. Effects of elevated ozone on growth and yield of field-grown rice in Yangtze River Delta, China. *Journal of Environmental Science* 20, 320–325.
- Chen, Z., Wang, X.K., Yao, F.F., Zheng, F.X., Feng, Z.Z., 2010. Elevated ozone induced changes in soil microbial community structure and function in a rice (*Oryza sativa* L.) paddy. *Soil Science Society of America Journal* 74 (3). <http://dx.doi.org/10.2136/sssaj2009.0258>.
- Cooley, D.R., Manning, W.J., 1988. Effects on ozone growth and assimilate partition in alfalfa, *medicago sativa* L. *Environmental Pollution* 49, 19–36.
- Fangmeier, A., Grütters, U., Högy, P., Vermehren, B., Jäger, H.J., 1997. Effects of elevated CO₂, nitrogen supply and tropospheric ozone on spring wheat-II. Nutrients (N, P, K, S, Ca, Mg, Fe, Mn, Zn). *Environmental Pollution* 96, 43–59.
- FAO, July 2007. Statistical database. No. 4. <http://www.fao.org>.
- Feng, Z.W., Jin, M.H., Zhang, F.Z., Huang, Y.Z., 2003. Effects of ground-level ozone (O₃) pollution on the yield of rice and winter wheat in the Yangtze River Delta. *Journal of Environmental Science* 15, 360–362.

- Feng, Z.Z., Kobayashi, K., 2009. Assessing the impacts of current and future concentrations of surface ozone on crop yield with meta-analysis. *Atmospheric Environment* 43, 1510–1519.
- Feng, Z.Z., Kobayashi, K., Ainsworth, E.A., 2008. Impact of elevated ozone concentration on growth, physiology and yield of wheat (*Triticum aestivum* L.): a meta-analysis. *Global Change Biology* 14, 2696–2708.
- Fiscus, E.L., Booker, E.L., Burke, K.O., 2005. Crop responses to ozone: uptake, modes of action, carbon assimilation and partitioning. *Plant, Cell and Environment* 28, 997–1011.
- Frei, M., Kohno, Y., Tietze, S., Jekle, M., Husein, M.A., Becker, T., Becker, K., 2012. The response of rice grain quality to ozone exposure during growth depends on ozone level and genotype. *Environmental Pollution* 163, 199–206.
- Fu, Y., Zhao, T., Sun, J., Cao, Y., Hu, Y., Xu, L., Shi, Y., 2008. Effects of elevated ozone concentration on maize photosynthesis and grain quality. *Acta Agriculturae Boreali-Sinica* 23 (6), 120–124 (in Chinese with English abstract).
- Fuhrer, J., Lehnher, B., Moeri, P.B., Tschannen, W., Shariat-Madari, H., 1990. Effects of ozone on the grain composition of spring wheat grown in Open-top field chambers. *Environmental Pollution* 65, 181–192.
- Fuhrer, J., Skarby, L., Ashmore, M.R., 1997. Critical levels for ozone effects on vegetation in Europe. *Environmental Pollution* 97, 91–106.
- Fuhrer, J., 2009. Ozone risk for crops and pastures in present and future climates. *Naturwissenschaften* 96, 173–194.
- Guo, J.P., Wang, C.Y., Wen, M., Bai, Y.M., Huo, Z.G., 2001. The experimental study on the impact of atmospheric O₃ variation on rice. *Acta Agronomica Sinica* 27 (6), 822–826 (in Chinese with English abstract).
- IPCC (The Intergovernmental Panel on Climate Change), 2007. Climate change 2007: the physical science basis. <http://www.ipcc.ch/ipccreports/ar4-wg1.htm>.
- IRRI (International Rice Research Institute), 2002. Riceweb. <http://www.riceweb.org>.
- Maurer, S., Matyssek, R., 1997. Nutrition and the ozone sensitivity of birch (*Betula pendula*) II. Carbon balance, water-use efficiency and nutritional status of the whole plant. *Trees* 12, 11–20.
- Mills, G., Buse, A., Gimeno, B., Bermejo, V., Holland, M., Emberson, L., Pleijel, H., 2007. A synthesis of AOT40-based response functions and critical levels of ozone for agricultural and horticultural crops. *Atmospheric Environment* 41, 2630–2643.
- Morgan, P.B., Ainsworth, E.A., Long, S.P., 2003. How does elevated ozone impact soybean? A meta-analysis of photosynthesis, growth and yield. *Plant, Cell and Environment* 26, 1317–1328.
- Nussbaum, S., Geissmann, M., Fuhrer, J., 1995. Ozone exposure –response relationships for mixtures of perennial ryegrass and white clover depend on ozone exposure patterns. *Atmospheric Environment* 29, 989–995.
- Piikki, K., De Temmerman, L., Ojanperä, K., Danielsson, H., Pleijel, H., 2008. The grain quality of spring wheat (*Triticum aestivum* L.) in relation to elevated ozone uptake and carbon dioxide exposure. *European Journal of Agronomy* 28, 245–254.
- Pleijel, H., Danielsson, H., Gelang, J., Sild, E., Sellden, G., 1998. Growth stage dependence of the grain yield response to ozone in spring wheat (*Triticum aestivum* L.). *Agriculture, Ecosystems & Environment* 70, 61–68.
- Pleijel, H., Mortensen, L., Fuhrer, J., Ojanperä, K., Danielsson, H., 1999. Grain protein accumulation in relation to grain yield of spring wheat (*Triticum aestivum* L.) grown in open-top chambers with different concentrations of ozone, carbon dioxide and water availability. *Agriculture, Ecosystems & Environment* 72, 265–270.
- Pleijel, H., Ojanperä, K., Mortensen, L., 1997. Effects of tropospheric ozone on the yield and grain protein content of spring wheat (*Triticum aestivum* L.) in the Nordic countries. *Acta Agriculturae Scandinavica, Section B – Soil & Plant Science* 47 (1), 20–25.
- Pleijel, H., Uddling, J., 2011. Yield vs. quality trade-offs for wheat in response to carbon dioxide and ozone. *Global Change Biology* 18, 596–605.
- Rausch, T., Wachter, A., 2005. Sulfur metabolism: a versatile platform for launching defence operations. *Trends in Plant Science* 10, 503–509.
- Shao, M., Tang, X.Y., Zhang, Y.H., Li, W.J., 2006. City clusters in China: air and surface water pollution. *Frontiers in Ecology and the Environment* 4, 353–361.
- Sitch, S., Cox, P.M., Collins, W.J., Huntingford, C., 2007. Indirect radiative forcing of climate change through effects on the land-carbon sink. *Nature* 448, 791–794.
- Slaughter, L.H., Mulchi, C.L., Lee, E.H., Tuthill, K., 1989. Chronic ozone stress effects on yield and grain quality of soft red winter wheat. *Crop Science* 29, 1251–1255.
- Tingey, D.T., Rodecap, K.D., Lee, E.H., Moser, T.J., Hogsett, W.E., 1986. Ozone alters the concentrations of nutrients in bean tissue. *Angewandte Botanik* 60, 481–493.
- Vingarzan, R., 2004. A review of surface ozone background levels and trends. *Atmospheric Environment* 38, 3431–3442.
- Wang, X.K., Zhang, Q.Q., Zheng, F.X., Zheng, Q.W., Yao, F.F., Chen, Z., Zhang, W.W., Hou, P.Q., Feng, Z.Z., Song, W.Z., Lu, F., 2012. Effects of elevated O₃ concentration on winter wheat and rice yields in the Yangtze River Delta, China. *Environmental Pollution* 171, 118–125.
- Wang, X.K., Zheng, Q.W., Yao, F.F., Chen, Z., Feng, Z.W., Manning, W.J., 2007. Assessing the impact of ambient ozone on growth and yield of a rice (*Oryza sativa* L.) and a wheat (*Triticum aestivum* L.) cultivar grown in the Yangtze Delta, China, using three rates of application of ethylenediurea (EDU). *Environmental Pollution* 148, 390–395.
- Wang, H.X., Kiang, C.S., Tang, X.Y., Zhou, X.J., Chameides, W.L., 2005. Surface ozone: a likely threat to crops in Yangtze delta of China. *Atmospheric Environment* 39, 3843–3850.
- Wang, X.P., Mauzerall, D.L., 2004. Characterizing distributions of surface ozone and its impact on grain production in China, Japan, and South Korea: 1990 and 2020. *Atmospheric Environment* 38, 4383–4402.
- Wilkinson, S., Mills, G., Illidge, R., Davies, W.J., 2011. How is ozone pollution reducing our food supply? *Journal of Experimental Botany* <http://dx.doi.org/10.1093/jxb/err317>.
- Zhang, Y.H., Shao, M., Yu, K.H., 2004. Vehicle Emissions, Environmental Impact and Control: A Case Study of Guangzhou. Chemical Industry Press, Beijing.
- Zheng, F.X., Wang, X.K., Lu, F., Hou, P.Q., Zhang, W.W., Duan, X.N., Zhou, X.P., Ai, Y.P., Zheng, H., Ouyang, Z.Y., Feng, Z.W., 2011. Effects of elevated ozone concentration on methane emission from a rice paddy in Yangtze River Delta, China. *Global Change Biology* 17, 898–910.
- Zheng, Q.W., 2006. Effect of Elevated Ozone Level on Typical Crops in YangtzeDelta, China. PhD dissertation. Research Center for Eco-Environmental Science, Chinese Academy of Sciences, Beijing, China (in Chinese with English abstract).
- Zheng, Q.W., Wang, X.K., Feng, Z.W., Song, W.Z., Feng, Z.W., Ouyang, Z.Y., 2007. Effects of elevated ozone on biomass and yield of rice planted in open-top chamber with revolving ozone distribution. *Chinese Journal of Environmental Science* 28, 170–175 (in Chinese with English abstract).
- Zhou, X.J., 2004. The Interaction between the Atmosphere and Ecosystems in Yangtze Delta Region. Meteorological Press, Beijing.