Effects of Elevated Near-surface Ozone Concentration on Rice Quality: FACE Study Postprint

Authors: Shen Shibo, Zhang Dinghe, Yang Kaifang, Wang Yunxia, Zhu Jianguo, Yang Lianxin, Wang Yulong

Date: 2017-10-20T00:00:00+00:00

Abstract

Using a Free Air gas Concentration Enrichment (FACE) platform in paddy fields, with conventional japonica rice 'Wujing 15'and hybrid japonica rice 'Lingfengyou 18' as test materials, two levels were set: ambient ozone concentration (Ambient) and elevated ozone concentration (21% higher than Ambient, simulating ozone concentration in the mid-21st century), to investigate the effects of ozone stress on processing, appearance, cooking/eating quality, and nutritional quality of rice at maturity in field-grown rice and their interspecific differences. The results showed that elevated near-surface ozone concentration caused varying degrees of decline in brown rice rate, milled rice rate, and head rice rate, among which the decrease in milled rice rate reached a significant level. Compared with Ambient, ozone stress increased chalky rice rate, chalkiness size, and chalkiness degree of the two varieties by an average of 15.0% ($P=0.10$), 42.0% ($P<0.05$), and 60.5% ($P<0.05$), respectively. Ozone stress decreased rice gel consistency by an average of 7.1% ($P<0.05$), but had no significant effect on amylose content and gelatinization temperature of the two varieties. RVA profile analysis showed that ozone stress had no significant effect on peak viscosity, breakdown, cool paste viscosity, setback, and consistency of rice. Ozone stress showed an increasing trend in protein concentration of the two varieties, but neither reached a significant level. Analysis of variance showed that in most cases, there were significant differences in various rice quality traits between the two varieties, but the variety × ozone interaction had no significant effect on all measured indicators, indicating that there was no significant difference in the response of rice quality to ozone stress between the two varieties. This experiment was conducted under open paddy field conditions; moderate ozone stress significantly increased rice chalkiness and significantly decreased gel consistency, but had relatively minor effects on other rice quality indicators, with consistent trends between the two varieties.

Full Text

Preamble

Chinese Journal of Eco-Agriculture, Sep. 2016, Vol. 24, No. 9: 1231–1238
DOI: 10.13930/j.cnki.cjea.160319

Effect of Elevated Surface Layer Ozone Concentration on Rice Grain Quality: A FACE Study

SHEN Shibo[1], ZHANG Dinghe[1], YANG Kaifang[1], WANG Yunxia[2], ZHU Jianguo[3], YANG Lianxin[1]**, WANG Yulong[1]

[1] Yangzhou University / State Key Laboratory of Crop Genetics & Physiology of Jiangsu Province / Co-Innovation Center for Modern Production Technology of Grain Crops, Yangzhou 225009, China
[2] College of Environmental Science and Engineering, Yangzhou University, Yangzhou 225009, China
[3] State Key Laboratory of Soil and Sustainable Agriculture / Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China

Abstract

Tropospheric ozone has been assumed to be the most phytotoxic air pollutant, which has created severe concern for environmental pollution due to its negative impact on crop production. However, high ozone concentration also affects crop quality, which has so far not been treated in sufficient detail. Rice (*Oryza sativa* L.) is one of the most important food crops in the world, providing a significant proportion of human daily dietary needs. The accurate assessment of the impact of elevated surface layer ozone concentration on rice quality is critical for reducing uncertainties in predicting future global food security. Using a Free-Air gas Concentration Enrichment (FACE) facility in Xiaoji Town, Jiangdu County, Jiangsu Province, China, we conducted a field experiment to investigate the impacts of ozone stress on rice grain quality, including processing quality, appearance quality, cooking/eating quality, and nutritional quality. Two rice cultivars (a conventional japonica cultivar 'Wujing 15' and a hybrid japonica cultivar 'Lingfengyou 18') were exposed to either ambient or elevated ozone concentration (ca. 21% above ambient values) from tillering to harvest. The results showed that elevated ozone resulted in a small decrease in brown rice percentage, milled rice percentage, and head rice percentage, with the effect on milled rice percentage significant at the 0.05 level. Averaged across the two cultivars, elevated ozone concentration increased chalky grain percentage, area of chalkiness, and degree of chalkiness by 15.0% ($P = 0.10$), 42.0% ($P < 0.05$), and 60.5% ($P < 0.05$), respectively. On average, elevated ozone concentration decreased gel consistency by 7.1% ($P < 0.05$), while no significant effect was observed on amylose concentration and gelatinization temperature for both cultivars. Measurements of RVA profile (Rapid Visco Analyser) indicated no significant changes in maximum viscosity, breakdown, cold viscosity, setback, and return due to elevated ozone. Elevated ozone concentration caused a non-significant increase in grain protein concentration for both cultivars. Analysis of variance indicated that in most cases, the effect of cultivar differences was significant. However, the interaction of ozone with cultivar was not detected for the tested traits of grain quality, suggesting that the response to ozone stress was similar for the two rice cultivars. The open-air field experiment suggested that moderate high ozone environment greatly increased grain chalkiness and significantly decreased gel consistency, but had little effect on other quality parameters of both cultivars.

Keywords
Rice; Ozone stress; FACE (Free Air gas Concentration Enrichment); Processing quality; Appearance quality; Cooking/eating quality; Nutritional quality

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Introduction

In many Asian countries, rapid economic development in recent years has increased emissions of air pollutants such as nitrogen oxides (NOx) and volatile organic compounds (VOC). These ozone precursors are converted to ozone through photochemical reactions under high temperature and strong radiation conditions, leading to rapidly rising surface (near-surface or tropospheric) ozone concentrations in many countries, particularly in populous nations like China and India [1-2]. In addition to high population density and active economic development, the tropical and subtropical climate conditions in these regions also favor ozone formation [3-5]. Recent modeling studies have shown that even with strict pollution control (keeping emission levels at 2006 levels), surface ozone concentrations in southern China will continue to increase by the middle of the 21st century [4]. It is predicted that over the next 40 years, the average surface ozone concentration in Southeast Asia will be 25 $\mathrm{nL} \cdot \mathrm{L}^{1}$ higher than present levels [1]. Ozone is a strong oxidant, and the continuously increasing surface ozone concentration has already caused damage to the productivity of major food crops, and this impact will be even greater in the future [6-9].

Rice (*Oryza sativa*) is one of the most important crops in the world, providing calories and nutrition for more than half of the global population [10], and is also a crop sensitive to ozone stress [6-7]. With rapid population growth and declining cultivated land area and quality, the world’ s demand for rice, particularly high-quality rice, will continue to increase in the coming decades [9,11]. This surge in demand poses a major challenge to rice production, which is made more severe by increasing surface ozone concentration because major rice-producing regions are typically also areas with high surface ozone concentrations. In recent years, numerous studies have been conducted on the effects of ozone stress on rice, but most have focused on growth, development, and yield formation, with few addressing responses in rice grain quality [7,9,12]. Moreover, existing literature usually only involves responses of a few quality indicators (mainly nutritional quality) [13-16], with comprehensive reports on quality traits being rare [17-18]. Similar situations exist for wheat (*Triticum aestivum*) [19].

Rice grain quality is a multi-indicator comprehensive concept, generally including processing, appearance, cooking/eating, and nutritional quality aspects. Wang et al. [17-18] first systematically reported the effects of ozone stress on the comprehensive quality of hybrid indica rice ‘Shanyou 63’ , finding that a 25% increase in ozone concentration significantly deteriorated the appearance and cooking/eating quality of this variety [17], but this change was significantly reduced under high CO concentration environments [18]. This demonstrates that the response of rice grain quality to ozone is also affected by external environmental conditions, but whether it varies with tested varieties remains unclear.

Compared with chambers, FACE (Free Air gas Concentration Enrichment) experiments use standard local crop management techniques to study crops under free-air flow field conditions, featuring large experimental spaces and environments closer to natural farmland conditions [20]. In 2007, China established the world’ s first rice/wheat rotation ozone FACE platform [21], providing an opportunity to study interactive effects between ozone and variety [22-23] as well as cultivation factors [16,23-25]. Using this platform, this study simulated surface ozone concentrations expected in the mid-21st century, with conventional japonica rice ‘Wujing 15’ and hybrid japonica rice ‘Lingfengyou 18’ as test materials, to investigate the effects of ozone stress on rice processing, appearance, cooking/eating, and nutritional quality and their inter-varietal differences, aiming to provide a basis for developing adaptation strategies for rice production in high ozone concentration environments.

1.1 Experimental Platform

This experiment was conducted in 2009 at the China Rice Ozone FACE Research Technology Platform, located at the Liangzhong Farm Experimental Field in Xiaoji Town, Jiangdu City, Jiangsu Province (119°42 0 E, 32°35 5 N). The experimental field soil type was Qingnitu (a local soil type). Annual precipitation was 918-978 mm, annual evaporation exceeded 1,100 mm, mean annual temperature was 14-16 °C, annual sunshine hours exceeded 2,000 h, and the average annual frost-free period was about 220 days. The cropping system was rice-winter wheat rotation. Soil physicochemical properties were: organic matter content 18.39 $\mathrm{g \cdot kg^{1}}$, total nitrogen 1.51 $\mathrm{g \cdot kg^{1}}$, total phosphorus 0.63 $\mathrm{g \cdot kg^{1}}$, total potassium 14.02 $\mathrm{g \cdot kg^{1}}$, pH 7.9, clay content (<0.002 mm) 13.6%, and bulk density 1.16 $\mathrm{g \cdot cm^{3}}$ [26].

The platform consisted of three FACE rings and three control (Ambient) rings. The distance between FACE rings and between FACE and control rings was greater than 90 m to reduce ozone release effects on other rings. FACE rings were designed as regular octagons with a diameter of 12.5 m. Pure ozone gas was injected toward the center of each FACE ring through pipes surrounding the ring. A computer network system monitored and controlled platform ozone concentrations, automatically adjusting ozone release rate and direction based on atmospheric ozone concentration, wind direction, wind speed, ozone concentration at crop canopy height, and diurnal variation, maintaining ozone concentration within FACE rings at 50% higher than atmospheric ozone concentration throughout the rice growth period. Ozone release was suspended when control ring ozone concentration was below 20 nL • L ^{1}, when rain or dew caused leaf wetness, and during ozone analyzer calibration or equipment maintenance (i.e., ozone release was suspended on August 14 and September 25). Ozone treatment in this experiment began on July 1 and ended on October 15, with effective control days of 50 d, accounting for 48% of total control days and 42% of total growth period days. Therefore, the actual average ozone concentration in FACE rings during the rice growing season was only 21% higher than in control rings, with actual increased ozone concentration also 21% from panicle initiation (July 18) to maturity [Figure 1: see original paper]. Control plots had no FACE pipes installed, and other environmental conditions were consistent with natural conditions. Treatment period was from July 1 (tillering stage) to maturity, with ozone emitted daily from 9:00–18:00 on sunny or cloudy days, and emission stopped on rainy days.

1.2 Plant Material and Cultivation

The tested varieties were conventional japonica rice 'Wujing 15' and hybrid japonica rice 'Lingfengyou 18', which have similar whole growth periods and main growth stages. Dry nursery seedlings were raised in the field, sown on May 22 and transplanted on June 17. Row spacing was 25 cm, hill spacing was 16.7 cm, with 3 seedlings per hill for 'Wujing 15' and 1 seedling per hill for 'Lingfengyou 18'. Total nitrogen application was 20 g • m ^{2}, with basal fertilizer applied on June 17 (accounting for 60% of total nitrogen) and panicle fertilizer applied on August 1 (accounting for 40% of total nitrogen). Phosphorus and potassium fertilizers were both applied as basal fertilizer entirely. Phosphorus and potassium application rates were both 7 g • m ^{2}. Water management involved maintaining a shallow water layer (about 5 cm) from June 18 to July 18, conducting multiple mild midseason drainages from July 4 to August 5, implementing intermittent irrigation after August 6, maintaining shallow water during heading and flowering, and draining water 10 days before harvest. Diseases, pests, and weeds were controlled timely to ensure normal rice growth and development.

1.3 Measurements and Methods

At maturity, 50 hills were continuously harvested (without disturbance), threshed, and actual yield was measured. From these, 300 g samples were taken, packed in mesh bags, and hung in the laboratory for 3 months. One week before testing, samples were hung in a dry and ventilated laboratory to maintain moisture content at 13% ± 1%. Tested rice grains were uniformly processed using an NP-4350 winnower to remove empty and shriveled grains, and measurements were conducted according to the People' s Republic of China High-Quality Paddy GB1350–1999 standards.

A dehusker (OHYA-25, Japan) was used to produce brown rice from 100 g of paddy, which was then processed for about 1.2 min using a CPC-3 type milling machine to remove bran and embryo from 60 g of brown rice. After milling, milled rice grains that still reached or exceeded 2/3 of the average length of whole milled rice grains were selected as head rice. Brown rice percentage, milled rice percentage, and head rice percentage were calculated accordingly. From head rice samples, 100 grains were randomly taken and placed on a chalkiness meter. Grains with chalkiness (including white core, white belly, and white back) were picked out to calculate chalky grain percentage. Ten chalky grains were randomly taken, placed flat on the chalkiness meter, and the percentage of chalkiness area relative to the whole grain area was visually estimated for each grain to obtain the average chalkiness area. Chalkiness degree was calculated by multiplying chalky grain percentage by chalkiness area.

Amylose content and gel consistency were measured according to the People' s Republic of China High-Quality Paddy GB/T15683–1995 and GB/T17891–1999 standards, respectively. Gel consistency was determined using the gel extension method, measuring the length of rice gel after cooling in millimeters. Amylose content was determined using the iodine blue colorimetric method [27]. Gelatinization temperature was measured simultaneously with rice starch viscosity profile (RVA profile) [27] using a 3-D type visco-analyzer produced by Newport Scientific Instruments, Australia.

Protein concentration was measured using milled rice as the test sample with a 1241 type near-infrared rapid quality analyzer (Foss Tecator, Sweden). The amount of milled rice for measurement should be no less than 20 g and no more than 65 g.

1.4 Statistical Analysis Methods

All data in this experiment were processed and graphed using Microsoft Excel 2013. Analysis of variance was performed using SPSS 22.0 with a general linear model. Comparisons among treatments used the least significant difference (LSD) method. Significance levels were set at $P < 0.01$, $P < 0.05$, $P < 0.1$, and $P > 0.1$, denoted by **, *, +, and ns, respectively.

2.1 Effects of Increased Ozone Concentration on Rice Processing Quality

Brown rice percentage refers to the percentage of brown rice weight relative to paddy weight. The response of this parameter to high ozone concentration is shown in [Figure 2: see original paper]a. The average brown rice percentages of 'Wujing 15' and 'Lingfengyou 18' were 84.9% and 83.5%, respectively, with the difference reaching a highly significant level. Neither ozone treatment nor its interaction with cultivar had significant effects on brown rice percentage.

Milled rice percentage refers to the percentage of milled rice weight relative to paddy weight when brown rice is milled into milled rice. As shown in [Figure 2: see original paper]b, the average milled rice percentages of ‘Wujing 15’ and ‘Lingfengyou 18’ were 75.9% and 75.6%, respectively, with no significant difference between them. Compared with the control, high ozone concentration decreased the milled rice percentage of both varieties by an average of 0.54% (P = 0.03), with decreases of 0.83% (P = 0.04) and 0.25% (P = 0.07) for ‘Wujing 15’ and ‘Lingfengyou 18’ , respectively. The interaction between ozone and cultivar had no significant effect on milled rice percentage.

Head rice percentage refers to the percentage of head rice grains relative to total milled rice grains. As shown in [Figure 2: see original paper]c, the average head rice percentages of ‘Wujing 15’ and ‘Lingfengyou 18’ were 73.1% and 72.5%, respectively, with no significant difference between them. Neither ozone treatment nor its interaction with cultivar had significant effects on head rice percentage.

2.2 Effects of Increased Ozone Concentration on Rice Appearance Quality

Chalky grain percentage refers to the percentage of chalky grains relative to total sample grains. As shown in [Figure 3: see original paper]a, the average chalky grain percentages of ‘Wujing 15’ and ‘Lingfengyou 18’ were 47.7% and 39.6%, respectively, with the former being 20.6% higher than the latter, reaching a significant difference. Neither ozone treatment nor its interaction with cultivar had significant effects on chalky grain percentage.

Chalkiness area refers to the percentage of chalkiness area relative to the projected area of the whole grain. As shown in [Figure 3: see original paper]b, the average chalkiness areas of ‘Wujing 15’ and ‘Lingfengyou 18’ were 32.3% and 26.2%, respectively, with no significant difference between them. Compared with the control, high ozone concentration increased the chalkiness area of both varieties by an average of 42.0% (P = 0.03), with increases of 40.1% and 44.3% for ‘Wujing 15’ and ‘Lingfengyou 18’ , respectively, reaching significance levels of 0.1 and 0.01. The interaction between ozone and cultivar had no significant effect on chalkiness area.

Chalkiness degree refers to the percentage of chalkiness area of chalky grains relative to the total area of sample grains. As shown in [Figure 3: see original paper]c, the chalkiness degrees of ‘Wujing 15’and ‘Lingfengyou 18’were 15.9% and 10.6%, respectively, with the former being 49.9% higher than the latter, reaching a significant difference. Compared with the control, high ozone concentration increased the chalkiness degree of both varieties by an average of 60.5% (P = 0.04), with increases of 62.4% and 57.8% for ‘Wujing 15’ and ‘Lingfengyou 18’ , respectively, reaching significance levels of 0.1 and 0.05. The interaction between ozone and cultivar had no significant effect on chalkiness degree.

2.3 Effects of Increased Ozone Concentration on Rice Cooking/Eating Quality

Gel consistency refers to the viscosity of rice gel in endosperm, reflecting the extensibility of rice starch gel after cooling. As shown in [Figure 4: see original paper]a, the average gel consistency of ‘Wujing 15’ and ‘Lingfengyou 18’ was 92.6 mm and 75.0 mm, respectively, with the former being 19.0% higher than the latter, reaching a highly significant difference. Compared with the control, high ozone concentration decreased the gel consistency of both varieties by an average of 7.1%, reaching a significant level. From different cultivar perspectives, ozone stress decreased ‘Wujing 15’ and ‘Lingfengyou 18’ by 8.0% and 5.8%, respectively, with the former reaching a significant level. Analysis of variance indicated that the interaction between ozone and cultivar had no significant effect on gel consistency.

Amylose content directly affects the physicochemical properties and palatability of cooked rice. As shown in [Figure 4: see original paper]b, the average amylose contents of ‘Wujing 15’ and ‘Lingfengyou 18’ were 16.2% and 16.9%, respectively, with the former being 4.1% lower than the latter, reaching a significant difference. Neither ozone treatment nor its interaction with cultivar had significant effects on amylose content.

Gelatinization temperature refers to the temperature at which rice starch granules absorb water in hot water and undergo irreversible swelling. As shown in [Figure 4: see original paper]c, the average gelatinization temperatures of ‘Wujing 15’ and ‘Lingfengyou 18’ were 78.5 °C and 70.9 °C, respectively, with the former being 9.6% higher than the latter, reaching a highly significant difference. Neither ozone treatment nor its interaction with cultivar had significant effects on gelatinization temperature.

2.4 Effects of Increased Ozone Concentration on Rice Protein Concentration

Protein concentration is an important indicator of rice nutritional quality and one of the main components of rice endosperm. As shown in [Figure 5: see original paper], the average protein concentrations of ‘Wujing 15’ and ‘Lingfengyou 18’ were 7.7 mg • g ^{1} and 8.0 mg • g ^{1}, respectively, with no significant difference between them. Neither ozone treatment nor its interaction with cultivar had significant effects on rice protein concentration.

2.5 Effects of Increased Ozone Concentration on Rice RVA Profile

RVA (Rapid Visco-Analyser) can measure the viscous force of rice starch slurry during heating, sustained high temperature, and cooling processes. As shown in [Figure 6: see original paper], RVA parameters including hot paste viscosity, breakdown, cold paste viscosity, setback, and return value showed obvious cultivar differences. Compared with the control, high ozone concentration significantly increased hot paste viscosity, while changes in maximum viscosity, cold paste viscosity, setback, return value, and breakdown were relatively small and did not reach significant levels. Analysis of variance indicated that the interaction between ozone and cultivar had no significant effect on all RVA profile parameters.

3 Discussion and Conclusion

Rice processing quality indicators include brown rice percentage, milled rice percentage, and head rice percentage. Previous studies have shown that a 25% increase in ozone concentration caused consistent decreasing trends in brown rice rate and milled rice rate of hybrid indica rice ‘Shanyou 63’, although the decreases were small [17-18]. This study set the same ozone concentration and found that ozone treatment had no significant effect on brown rice percentage of conventional japonica rice ‘Wujing 15’ and hybrid japonica rice ‘Lingfengyou 18’, but significantly decreased milled rice percentage, with both varieties showing consistent trends. Similarly, ozone stress also caused consistent decreasing trends in head rice percentage of both varieties, although not reaching significant levels. Combined with previous studies, it can be concluded that after ozone fumigation, higher proportions of outer layers are removed during rice milling.

Unlike most other cereals, rice grain appearance quality, particularly chalkiness, is an important market quality indicator because rice is typically consumed as whole grains without further processing. Chalkiness is the trait most sensitive to environmental changes in rice quality [28]. Previous studies found that a 25% increase in ozone concentration significantly increased chalkiness in hybrid indica rice ‘Shanyou 63’, with chamber studies [18] showing average increases of 39%, 56%, and 114% in chalky grain percentage, chalkiness degree, and chalkiness area, respectively, while only chalky grain percentage increased significantly in FACE studies [17]. This study showed that compared with Ambient, ozone stress increased chalky grain percentage, chalkiness area, and chalkiness degree of both varieties by averages of 15%, 42%, and 61%, respectively, with the latter two reaching significant levels. Studies on other stresses such as high temperature have found that increased chalkiness may be due to poor starch grain filling, which may be related to plant premature senescence and shortened grain filling time under stress conditions [12,22,29]. Rice with more chalkiness under ozone fumigation is prone to broken grains during processing, so the decreased processing quality and increased chalkiness caused by ozone fumigation are actually interrelated [30]. Such changes caused by ozone stress will directly affect the commercial value of rice and farmers’ income.

Rice cooking/eating quality is generally evaluated indirectly through measurement of physicochemical properties. Previous studies have shown that ozone stress had no significant effect on amylose content and gel consistency of ‘Shanyou 63’, but results from starch viscosity profile (RVA profile) [17-18] and taste meter measurements [15] indicated that ozone stress would reduce rice viscosity and deteriorate eating quality (palatability). This study showed that the same ozone treatment had no significant effect on amylose content of the two japonica rice varieties, consistent with the response of 'Shanyou 63' [17-18]. The difference was that this study found ozone stress significantly decreased rice flour gel consistency (-7.1%), but all RVA profile parameters except hot paste viscosity, including breakdown, setback, and gelatinization temperature, showed no significant changes, with consistent trends in both varieties. This difference may be related to different tested varieties: this experiment used japonica varieties, while previous studies used indica rice [17-18]. Gel consistency reflects a colloidal property of rice starch gel, directly related to rice softness. The significant decrease in rice gel consistency caused by ozone treatment indicates that rice quality may become harder under ozone stress environments, consistent with the results of Song et al. [15] using a texture analyzer for japonica rice.

Protein concentration is an important indicator of rice nutritional quality and greatly affects rice eating quality. Consistent with other crops [9], chamber studies have found that ozone stress significantly increased rice protein concentration, with the magnitude varying with ozone concentration [13-14] and tested variety [14]. Chinese FACE studies also found that ozone stress increased protein concentration of indica rice 'Shanyou 63' by about 10% [16-18], corresponding to previously reported results that ozone-fumigated rice became harder and had deteriorated taste after cooking [15,17-18]. This study showed that ozone stress caused an increasing trend in protein concentration of the two japonica varieties, but neither reached significant levels, which is also consistent with the non-significant response of rice RVA profile observed in this study. Thus, although rice protein concentration showed an increasing trend under ozone stress, this increase may vary greatly among varieties. It is generally believed that increased protein concentration in ozone-fumigated rice is related to a "concentration effect," where carbon metabolism pathways may be more affected by ozone stress than nitrogen metabolism [9], but recent studies have shown that changes in element concentration under ozone stress may be related to factors other than "concentration" effects, such as changes in transpiration and root growth.

This study showed that although the two tested varieties had significant differences in many rice quality traits, the interaction between variety and ozone was not significant for all measured indicators, indicating that the effects of ozone stress on rice quality of 'Wujing 15'and 'Lingfengyou 18'showed consistent trends: moderate ozone stress significantly increased rice chalkiness and decreased gel consistency, but had little effect on other rice quality indicators. Since only two varieties were used in this experiment, the universality of these results needs verification through experiments with more varieties. Additionally, recent FACE studies have found that cultivation regulation can reduce ozone stress effects on rice growth and yield to some extent [23-25], but how this affects responses of different rice quality indicators remains to be determined. Clarifying this issue is important for developing adaptation strategies for rice production in high ozone concentration environments to minimize ozone damage.

Acknowledgments

We thank Mr. Liu Gang and Mr. Tang Haoye from the Institute of Soil Science, Chinese Academy of Sciences, for their daily maintenance of the ozone FACE system, which provided hardware support for the smooth implementation of this experiment.

References

[1] Fowler D, Amann M, Anderson F, et al. *Ground-level Ozone in the 21st Century: Future Trends, Impacts and Policy Implications*. London: The Royal Society, 2008
[2] Xing J, Wang S X, Chatani S, et al. Projections of air pollutant emissions and its impacts on regional air quality in China in 2020. *Atmospheric Chemistry and Physics*, 2011, 11(7): 3119-3136
[3] Lal D M, Ghude S D, Patil S D, et al. Tropospheric ozone and aerosol long-term trends over the Indo-Gangetic Plain (IGP), India. *Atmospheric Research*, 2012, 116: 82-92
[4] Liu Q, Lam K S, Jiang F, et al. A numerical study of the impact of climate and emission changes on surface ozone over South China in autumn time in 2000-2050. *Atmospheric Environment*, 2013, 76: 227-237
[5] Wang Y X, Shen L L, Wu S L, et al. Sensitivity of surface ozone over China to 2000-2050 global changes of climate and emissions. *Atmospheric Environment*, 2013, 75:
[6] Feng Z Z, Kobayashi K. Assessing the impacts of current and future concentrations of surface ozone on crop yield with meta-analysis. *Atmospheric Environment*, 2009, 43(8):
[7] Ainsworth E A, McGrath J M. Direct effects of rising atmospheric carbon dioxide and ozone on crop yields. In: Lobell D, Burke M. *Climate Change and Food Security: Advances in Global Change Research*. Germany: Springer Netherlands, 2010, 37: 109-130
[8] Yang L X, Wang Y X, Zhao Y P, et al. Responses of soybean to free-air ozone concentration enrichment: A research review. *Acta Ecologica Sinica*, 2010, 30(23): 6635-6645
[9] Wang Y X, Frei M. Stressed food —The impact of abiotic environmental stresses on crop quality. *Agriculture, Ecosystems & Environment*, 2011, 141(3/4): 271-286
[10] Timmer C P, Block S, Dawe D. Long-run dynamics of rice consumption, 1960-2050. In: Pandey S, Byerlee D, Dawe D, et al. *Rice in the Global Economy: Strategic Research and Policy Issues for Food Security*. Los Baños (Philippines): International Rice Research Institute, 2010: 139-174
[11] Lal R. Food security in a changing climate. *Ecohydrology & Hydrobiology*, 2013, 13: 8-21

[12] Yang L X, Wang Y L, Shi G Y, et al. Responses of rice growth and development to elevated near-surface layer ozone (O) concentration: A review. *Chinese Journal of Applied Ecology*, 2008, 19(4): 901-910

[13] Guo J P, Wang C Y, Wen M, et al. The experimental study on the impact of atmospheric O variation on rice. *Acta Agronomica Sinica*, 2001, 27(6): 822-826

[14] Frei M, Kohno Y, Tietze S, et al. The response of rice grain quality to ozone exposure during growth depends on ozone level and genotype. *Environmental Pollution*, 2012, 163:

[15] Song Q L, Qi Y T, Zhao Y P, et al. Effects of free-air ozone concentration enrichment on cooked rice (Wuyunjing 21) texture and palatability. *Chinese Journal of Eco-Agriculture*, 2013, 21(5): 566-571

[16] Zhou X D, Zhou J, Wang Y X, et al. Elevated tropospheric ozone increased grain protein and amino acid content of a hybrid rice without manipulation by planting density. *Journal of the Science of Food and Agriculture*, 2015, 95(1):

[17] Wang Y X, Yang L X, Han Y, et al. The impact of elevated tropospheric ozone on grain quality of hybrid rice: A free-air gas concentration enrichment (FACE) experiment. *Field Crops Research*, 2012, 129: 81-89

[18] Wang Y X, Song Q L, Frei M, et al. Effects of elevated ozone, carbon dioxide, and the combination of both on the grain quality of Chinese hybrid rice. *Environmental Pollution*, 2014, 189: 9-17

[19] Broberg M C, Feng Z Z, Xin Y, et al. Ozone effects on wheat grain quality —A summary. *Environmental Pollution*, 2015, 197: 203-213

[20] Long S P, Ainsworth E A, Leakey A D B, et al. Food for thought: Lower-than-expected crop yield stimulation with rising CO concentrations. *Science*, 2006, 312(5782): 1918-1921

[21] Tang H Y, Liu G, Han Y, et al. A system for free-air ozone concentration elevation with rice and wheat: Control performance and ozone exposure regime. *Atmospheric Environment*, 2011, 45(35): 6276-6282

[22] Shi G Y, Yang L X, Wang Y X, et al. Impact of elevated ozone concentration on yield of four Chinese rice cultivars under fully open-air field conditions. *Agriculture, Ecosystems & Environment*, 2009, 131(3/4): 178-184

[23] Luo K J, Zhu J G, Liu G, et al. Effects of elevated ozone on leaf photosynthesis of rice (*Oryza sativa* L.) and mitigation with high nitrogen supply. *Ecology and Environmental Sciences*, 2012, 21(3): 481-488

[24] Peng B, Li P L, Zhou N, et al. Effects of ozone stress on photosynthesis, dry matter production and yield of rice under different seedling quality and plant density. *Acta Ecologica Sinica*, 2013, 33(12): 3668-3675

[25] Peng B, Lai S K, Li P L, et al. Interactive effects of ozone concentration and planting density on growth, development and yield formation of Yangdao 6 —A FACE study. *Chinese Journal of Rice Science*, 2014, 28(4): 401-410

[26] Yu Y C, Lin X G, Feng Y Z, et al. Effects of elevated surface O concentration on functional diversity of soil microbial community in rice field. *Acta Pedologica Sinica*, 2011, 48(6): 1227-1234

[27] Supervising Department of Quality and Technology of China. GB/T 17891 —1999 *High Quality Paddy.* Beijing: China Standard Publishing House, 2000

[28] Fitzgerald M A, Resurrection A P. Maintaining the yield of edible rice in a warming world. *Functional Plant Biology*, 2009, 36(12): 1037-1045
[29] Yang L X, Wang Y L, Dong G C, et al. The impact of free-air CO enrichment (FACE) and nitrogen supply on grain quality of rice. *Field Crops Research*, 2007, 102(2): 128-140
[30] Juliano B O, Oñate L U, Del Mundo A M. Relation of starch composition, protein content, and gelatinization temperature to cooking and eating qualities of milled rice. *Food Technology*, 1965, 19(6): 116-121

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