## Chapter 12 Effects of Ozone on Crops in China

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**Abstract** Current ambient  $O_3$  concentrations in China are high, as shown by observations of typical  $O_3$  symptoms in some plant species and crop yield losses, as detected by the use of chemical protectants against  $O_3$ . Experiments with artificially elevated  $O_3$  concentrations have shown the effects of  $O_3$  on growth processes, grain yield, grain quality,  $CH_4$  emissions, and soil microbiology. The experiments have facilitated estimations of the yield losses in wheat and rice caused by current and future  $O_3$  concentrations at the national scale. Further studies are warranted on the interactions between  $O_3$  and other environmental changes, such as increasing  $CO_2$  concentrations, increased nitrogen deposition, aerosol loading, and climatic changes. Future needs for research include improvement of  $O_3$  impact models and the development of an  $O_3$  monitoring network to cover the vast areas of crop production in China. The establishment of an air quality standard for protecting crops from  $O_3$  damage is of critical importance for food security in China.

**Keywords** China •  $CH_4$  emission • Rice • Wheat •  $O_3$  • Yield

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### **12.1** Current O<sub>3</sub> Concentrations in China

Until 1990, ozone  $(O_3)$  concentrations  $([O_3])$  in China were lower than those in the United States and European cities, but they have increased quite rapidly since then, due to the increased emissions of  $O_3$  precursors arising from automobile transportation, electricity generation, and other industrial activities. Current  $[O_3]$ is rising at a higher rate in China than in other countries, and the daily 24 h mean  $[O_3]$  often exceeds 50 ppb on average across the crop growing season in some regions (Tang et al. 2013; Zhao et al. 2009). Regional  $O_3$  pollution has become one of the top environmental concerns in China, especially in economically vibrant and densely populated regions. Some major cities in China, such as Beijing, Shanghai, Jinan, Hong Kong, and Guangzhou, are faced with photochemical threat (Feng et al. 2015a). In recent years, ambient  $[O_3]$  has often reached 150 ppb in the afternoons from May to August in farmlands near Beijing city.

The variability of  $[O_3]$  is a function of geographic location. In the central and northern parts of China,  $[O_3]$  reaches a maximum in summer. In southern China, by comparison,  $[O_3]$  is generally characterized by a peak in fall and a trough in summer (Feng et al. 2015a). On a monthly-mean basis, surface  $[O_3]$  peaks in May in the Yangtze River Delta, in June in the North China Plain, and in October in the Pearl River Delta (Wang et al. 2011). The peak hourly  $[O_3]$ reaches as high as 316 ppb in the North China Plain (Feng et al. 2015a). The high surface  $[O_3]$  can therefore be regarded as a present threat to food security.

## **12.2** O<sub>3</sub> Injuries Observed in the Field

 $O_3$  concentrations in the Beijing area are high enough to induce foliar symptoms in plants. During our survey around Beijing in 2013, we found 12 species, including food crops, vegetables, and fruit trees, showing typical  $O_3$  symptoms (Table 12.1). Among the crops, different types of beans belonging to the genera *Canavalia, Vigna*, and *Phaseolus* showed distinctive and severe  $O_3$  symptoms at most of the places examined. These species have strong potential to be used as  $O_3$ bio-indicators in China, given that they are  $O_3$ -sensitive and commonly planted. The observed  $O_3$  symptoms are described in Table 12.1. During the survey in 2014, however, we found fewer plant species showing  $O_3$  injury in the field, presumably due to the weather conditions not being conducive to photochemical  $O_3$ production. Furthermore, no more plant species, other than those shown in the list in Table 12.1, were found to show  $O_3$  injury at sites situated in Hebei Province or Tianjin.

Species	Common name	Symptom description
Abelmoschus esculentus	Okra plant	Fine light brown and purple interveinal stippling on the adaxial side of older leaves
Abutilon theophrasti	Velvetleaf, China jute, Buttonweed	Fine yellow to light brown stippling between main and secondary veins, which remain green
Arachis hypogaea	Peanut	Very fine light brown interveinal stippling, finally also with dark purple stippling
Benincasa pruriens	Winter gourd	Fine light brown diffuse stippling between main and secondary veins, which remain green
Canavalia gladiata	Sword bean	Distinctive purple-brown or dark purple stippling that can cover a large portion of the interveinal areas of leaves. Sometimes with associated chlorosis
Citrullus lanatus	Watermelon	Dark brown stippling on interveinal parts of older leaves. Stippling evolves to form very distinctive brown necrotic areas, sometimes with associated white flecking. Chlorosis is frequently observed together with stippling
Humulus scandens	Hops	Very fine light brown interveinal stippling
Luffa cylindrica	Courgette	Fine yellow to light brown stippling between main and secondary veins, which remain green
Phaseolus vulgaris	Common bean	Distinctive purple-brown or dark purple stippling that can cover a large portion of the interveinal areas of leaves. Sometimes with associated chlorosis. Severely affected leaves finally become dry
Prunus persica f. duplex	Peach f. duplex	Dark purple stippling, usually more intense towards the leaf margins
Vigna unguiculata subsp. sesquipedalis	Chinese long bean, cowpea	Distinctive purple-brown or dark purple stippling that can cover a large portion of the interveinal areas of leaves. Sometimes with associated chlorosis. Severely affected leaves finally become dry
Vitis vinifera	Grapevine	Brown and purple-brown interveinal stippling on the adaxial side of older leaves

Table 12.1  $O_3$ -sensitive species and their common names, and description of the  $O_3$  symptoms observed in the field (Feng et al. 2014)

# **12.3** Effects of Ambient O<sub>3</sub> on Crops as Detected by Using a Plant Protectant

Ethylenediurea (N-[2-(2-oxo-1-imidazolidinyl)ethyl]-N-phenylurea), abbreviated as EDU, has protective effects against O<sub>3</sub> damage, and, hence, has been used extensively to assess the O<sub>3</sub>-induced impact on many crops, e.g., wheat, potato, bean, and

tomato (Feng et al. 2010a). The application of EDU as a 'control' for ambient  $O_3$  is useful to determine ambient  $O_3$  effects on field-grown plants, particularly in remote and under-developed regions where the availability of electricity and funding is limited (Feng et al. 2010a; Manning et al. 2011). So far, only two EDU studies have been conducted in China, focusing on the effects of ambient  $O_3$  on one cultivar of wheat and rice (Wang et al. 2007) and various cultivars of snap bean (Yuan et al. 2015).

Yuan et al. (2015) investigated the effects of ambient O<sub>3</sub> (accumulated hourly O<sub>3</sub> concentration over a threshold of 40 ppb during daytime (AOT40) of 29.0 ppm·h) on four genotypes of snap bean (*Phaseolus vulgaris* L.) in a cropland area around Beijing by using 450 ppm of EDU as a protectant. All genotypes showed foliar injuries, but O<sub>3</sub>-sensitive genotypes exhibited much more injury than O<sub>3</sub>-tolerant ones. In the O<sub>3</sub>-sensitive genotypes, EDU significantly alleviated foliar injuries, increased the photosynthesis rate and chlorophyll *a* fluorescence, and alleviated the O<sub>3</sub> effects on photosynthetic parameters (maximum carboxylation efficiency ( $V_{cmax}$ ) and maximum rate of electron transport ( $J_{max}$ )) and seed and pod weights. These effects of EDU were not observed in the O<sub>3</sub>-tolerant genotypes. EDU did not, however, significantly affect antioxidant content in any of the genotypes.

Wang et al. (2007) applied EDU, at four concentrations of 0, 150, 300, or 450 ppm, as a foliar spray to field-grown rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) in the Yangtze Delta in China. They found that rice and wheat responded differently to ambient  $O_3$  and EDU applications. In wheat, some growth characteristics, such as yield, seed number per plant, seed set rate, and harvest index, were increased significantly with 300 ppm EDU treatment. In rice, by comparison, no parameters measured were significantly different among the various EDU application levels. It is, therefore, suggested that EDU is effective in demonstrating  $O_3$  effects on wheat, but not on rice. The different response to EDU between wheat and rice could be attributed to the facts that the wheat cultivar used is more sensitive to  $O_3$  than the rice cultivar and that  $[O_3]$  was lower during the rice season than during the wheat season.

From these two studies, it can be concluded that EDU can be regarded as a useful tool in the risk assessment of ambient  $O_3$  on  $O_3$ -sensitive crop species. Currently, high ambient  $[O_3]$  occurs in summer throughout China, which suggests the threat of ground-level  $O_3$  to food security. Breeding  $O_3$ -tolerant cultivars will be needed to protect the crops from the threat, considering the significant varietal difference in  $O_3$  sensitivity, as found in the  $O_3$  exposure studies noted later in this chapter.

## **12.4** Effects of Elevated O<sub>3</sub> Concentration on Crops Studied with Artificial O<sub>3</sub> Exposures

Artificial  $O_3$  exposure experiments with agricultural crops: winter wheat, rice, and oil-rape (*Brassica napus* L.) have been conducted at five different sites in China, using open-top chamber (OTC) or free-air  $O_3$  concentration enrichment (FACE- $O_3$ ) facilities (Tables 12.2 and 12.3). Experiments in both Jiaxing and Jiangdu, which

Table 12.2 Summary of O <sub>3</sub> impac	ts on wheat y	ield reported in s	studies condu	Icted in China compared with fin	dings in European studies (1	Feng et al. 2015a)
Site	Rooting	Duration	Facility	O <sub>3</sub> treatment <sup>a</sup>	Dose-response <sup>b</sup>	Reference
Gucheng (39°08'N, 115°48'E)	Pot	1999	OTC	CF, NF, 50, 100, 200 ppb	RY = 100 - 1.30 AOT40	Feng et al. (2003)
Jiaxing (31°53'N, 121°18'E)	Field	2004-2008	OTC	CF, NF, 75/100, 150/200 ppb	RY = 100 – 2.28 AOT40	Wang et al. (2012)
Jiangdu (32°35'N, 119°42'E)	Field	2007-2011	FACE	Ambient air (AA) and	RY = 96.1 – 2.5 AOT40	Feng et al. (2012)
				$1.5*AA (E-O_3)$	$RY = 100 - 8.2 POD_{12}$	
Changping (40°12'N, 116°08'E)	Field	2010	OTC	NF, NF + 30, NF + 60, NF +	RY = 100 - 2.2 AOT40	Tong (2011)
				90 ppb	$RY = 100 - 3.7 POD_4$	
Synthesis of European studies	Pot and		OTC		RY = 99 – 1.6 AOT40	Mills et al. (2007)
	field				$RY = 100 - 3.8 POD_6$	Mills et al. (2011)
FACE free-air O <sub>3</sub> concentration en	richment, OT	C open-top cham	ber			

<sup>a</sup>CF charcoal-filtered ambient air, NF non-charcoal-filtered ambient air, E-O<sub>3</sub> elevated O<sub>3</sub> concentration

<sup>b</sup>RY relative yield (%), AOT40 (ppm-h), accumulated hourly O<sub>3</sub> concentration over a threshold of 40 ppb during daytime; PODy (nmol m<sup>-2</sup>), phytotoxic O<sub>3</sub> dose (POD), is used for explaining what POD is, the accumulated stomatal O<sub>3</sub> flux above a flux threshold of y nmol O<sub>3</sub> m<sup>-2</sup> s<sup>-1</sup> (LRTAP Convention 2010)

Table 12.3 Summary of O <sub>3</sub> impact	ts on rice yield n	eported in studi	es conducte	d in China compared with finding	gs in European studies (Feng	g et al. 2015a)
Site	Rooting	Duration	Facility	O <sub>3</sub> treatment	Dose-response	Reference
Gucheng (39°08'N, 115°48'E)	Pot	2000	OTC	CF, NF, 50, 100, 200 ppb	RY = 100 – 0.53 AOT40	Feng et al. (2003)
Jiaxing (31°53'N, 121°18'E)	Field	2004-2008	OTC	CF, NF, 75/100, 150/200 ppb	RY = 100 - 0.95 AOT40	Wang et al. (2012)
Jiangdu (32°35'N 119°42'E)	Field	2007-2011	FACE	Ambient air (AA) and 1.5*AA (E-O <sub>3</sub> )	1	
Dongguan (23°01'N, 113°45'E)	Field	2010	OTC	NF, NF + 40, NF + 80, NF +	RY = 100 - 3.9 AOT40;	Tong (2011)
				120 ppb	$RY = 100 - 2.3 POD_2$	
Synthesis of European studies	Pot and field		OTC		RY = 94 - 0.39 AOT40	Mills et al. (2007)
The abbreviations are as defined in	Table 12.2					

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ined in Table 12.2 e B alc lasted for 5 years, aimed to study the impacts of elevated  $[O_3]$  on the growth, physiological characteristics, and yield components of rice and winter wheat. Experiments at both sites serve as rich sources of information for the regional assessment of ambient and elevated  $[O_3]$  effects on the food crop yield loss in the Yangtze River Delta. The experiment at the Jiangdu site also investigated four cultivars of wheat and rice (including Japonica, Indica, and hybrid cultivars) in response to elevated  $[O_3]$ .

These experiments elucidated the effects of elevated  $[O_3]$  on the following aspects: crop growth processes, crop yield, crop quality, and soil processes. These effects are described in more detail in the subsequent sections of this chapter.

## 12.4.1 Effects of $O_3$ on Growth Processes in Crop Cultivars

A meta-analysis showed that elevated  $[O_3]$ , compared with charcoal-filtered air, decreased the leaf photosynthetic rate by 20% in wheat and by 28% in rice, and decreased grain yield by 29% in wheat and by 14% in rice plants (Feng et al. 2008; Ainsworth 2008), suggesting that decreased photosynthesis was a key factor driving the yield loss in crops exposed to elevated [O<sub>3</sub>]. In China, many crop species have been investigated for the effects of O<sub>3</sub>. The species studied were winter wheat (e.g. Feng et al. 2011a; Zheng et al. 2005, 2010; Zhu et al. 2011), rice (e.g. Shi et al 2009; Pang et al. 2009; Shao et al. 2014; Zhang et al. 2008), soybean (e.g. Zhang et al. 2014a, b; Zhao et al. 2012), oil rape (Feng et al. 2006; Zheng et al. 2006), maize (Sun et al. 2008), and spinach (Yao et al.2007). Most studies focused on winter wheat and rice. Similar to the results of other experiments outside of China, chronic exposure to elevated [O<sub>3</sub>] caused a range of adverse effects on plants, including enhanced lipid peroxidation, reduced or enhanced antioxidant system activity, reduced photosynthetic activity, altered carbon allocation, diminished biomass accumulation, reduced yield, and accelerated senescence, with or without visible injury. Ozone-induced grain yield loss in winter wheat was mainly caused by a reduction in grain mass (Wang et al. 2012; Zhu et al. 2011). On the other hand, a reduction in panicle numbers contributed to the yield loss in rice when exposed to elevated [O<sub>3</sub>] (Shi et al. 2009; Pang et al. 2009).

It has also been reported that the effects of  $O_3$  vary by cultivars. Modern wheat cultivars are reported to be more sensitive to  $O_3$  than older accessions, and this was largely attributed to higher stomatal conductance ( $g_s$ ) in modern cultivars allowing for greater  $O_3$  uptake (Barnes et al. 1990; Pleijel et al. 2006; Biswas et al. 2008). The FACE- $O_3$  experiment in China was therefore conducted with multiple cultivars to determine their responses to elevated [ $O_3$ ].

For wheat, two modern cultivars [Yangmai16 (Y16) and Yangfumai 2 (Y2)] of winter wheat with almost identical phenology were investigated. In cultivar Y2, elevated  $[O_3]$  significantly accelerated leaf senescence, as indicated by increased lipid oxidation, as well as by faster declines in pigment amounts and photosynthetic rates. The lower photosynthetic rates were mainly due to non-stomatal factors, e.g., lower maximum carboxylation capacity, electron transport rates, and light energy distribu-

tion. In cultivar Y16, by contrast, the effects of elevated  $[O_3]$  were observed only at the very last stage of flag leaf aging (Feng et al. 2011a). The less sensitive variety Y16 had 33.5% and 12.0% higher concentrations of reduced ascorbate in the apoplast and leaf tissue, respectively, than those in the more sensitive variety Y2, whereas no varietal difference was detected in the decline of reduced ascorbate concentration in response to elevated  $[O_3]$ . No effects of  $O_3$  or variety were detected in either oxidized ascorbate or the redox state of ascorbate in the apoplast and leaf tissue (Feng et al. 2010b). Since the two cultivars had almost identical phenology and very similar leaf stomatal conductance before senescence, the greater impacts of elevated  $[O_3]$  on cultivar Y2 than on cultivar Y16 cannot be explained by differential  $O_3$  uptake, but by apoplast ascorbate contents. Our findings will be useful for scientists to select wheat cultivars that will be tolerant to rising surface  $[O_3]$  in East and South Asia.

In rice, varietal difference in the effects of elevated  $[O_3]$  was clearer than in wheat. Shi et al. (2009) studied four Chinese rice cultivars: Wujing 15 (WJ15, inbred Japonica cultivar), Yangdao 6 (YD6, inbred Indica cultivar), Shanyou 63 (SY63, three-line hybrid rice cultivar), and Liangyoupeijiu (LYPJ, two-line hybrid rice cultivar) at the FACE-O<sub>3</sub> site. The elevated  $[O_3]$  (a mean 25% enhancement above the ambient  $[O_3]$  strongly accelerated the phenological development of WJ15 and SY63, with maturity being reached by 4 and 8 days earlier, respectively, compared with findings in ambient  $[O_3]$ , with maturity being reached only 1 day earlier for YD6 and LYPJ. SY63 and LYPJ exhibited significant yield losses by exposure to elevated  $[O_3]$ (17.5% and 15%, respectively), while WJ15 and YD6 showed no responses to the elevated  $[O_3]$ . For all cultivars, no  $O_3$  effect was observed on panicle number per unit area, as a result of there being no changes in either maximum tiller number or productive tiller ratio. However, the number of spikelets per panicle in SY63 and LYPJ showed a significant reduction due to O<sub>3</sub> exposure, while these numbers remained unaffected in WJ15 and YD6. O<sub>3</sub> exposure also caused minor reductions in both filled spikelet percentage and individual grain mass in all tested cultivars. The results of these experiments indicated that yield loss due to O<sub>3</sub> exposure differs among rice cultivars, with the hybrid cultivars (i.e., SY63 and LYPJ) exhibiting greater yield loss than the inbred cultivars (i.e., WJ15 and YD6), a finding which could be attributed to the suppression of spikelet formation in the hybrid cultivars under  $O_3$  stress (Shi et al. 2009). In regard to photosynthetic characteristics, flag leaves in SY63 exhibited an earlier and stronger response to elevated  $[O_3]$  than flag leaves in WYJ3. Depression of the light-saturated photosynthetic rate (Asat) was first observed at 237 day of year (DOY) in SY63, and the seasonal mean Asat was reduced by 23.1%. In contrast, in WYJ3, a conventional inbred cultivar, the impact of elevated [O<sub>3</sub>] on A<sub>sat</sub> was negligible until 266 DOY, and the seasonal mean decrease of Asat was only 9.4%. The same trend was found in chlorophyll a fluorescence parameters. In SY63, the actual quantum yields of photosynthesis system II (PSII) and photochemical quenching (qP) were significantly decreased at 248 DOY, and the downward trends persisted throughout the rest of the life span of flag leaves. No such changes were observed in WYJ3. In the ozonated flag leaves of SY63, necrotic damage occurred and chlorophyll contents declined significantly, by 12.6-43.6%, throughout the entire functional duration of the leaf. As for WYJ3, chlorophyll remained unaffected until a 25.7 % decrease appeared at 278 DOY under elevated  $[O_3]$  (Pang et al. 2009).

### 12.4.2 Effects of $O_3$ on Crop Yield

In China, the effects of elevated  $[O_3]$  on crops were studied with plants rooted in the field at only two experimental sites. One is Jiaxing, where wheat and rice were investigated for four and five seasons, respectively, in OTCs for the period from 2004 to 2008. The other site is Jiangdu, where four cultivars of winter wheat and five rice cultivars were investigated for five growing seasons (2007–2012) in the FACE-O<sub>3</sub> facility.

At the Jiaxing site, four different levels of  $[O_3]$  were set up to build the  $O_3$  doseresponse relationship. Results indicated that elevated  $[O_3]$  significantly reduced winter wheat yield, by 8.5–58% and 40–73% compared with the charcoal-filtered air (CF) control for  $O_3$ -1 (AOT40 of 14.3–22.6 ppm·h) and  $O_3$ -2 (AOT40 of 24.2– 61.9 ppm·h) treatments, respectively. As compared with the subambient  $[O_3]$  control, the mean yield losses in rice were 10–34% and 16–43%, respectively, when plants were exposed to  $O_3$ -1 (AOT40 of 13.8–29.5 ppm·h) and  $O_3$ -2 (AOT40 of 32.1– 82.6 ppm·h) treatments, respectively. Winter wheat appeared to be more sensitive to  $O_3$  than rice. The  $O_3$ -induced yield declines for winter wheat were attributed primarily to the 1,000-grain weight and the harvest index, and the declines for rice were attributed primarily to grain number per panicle and the harvest index (Wang et al. 2012).

At the Jiangdu FACE-O<sub>3</sub> site, a mean 25% enhancement above the ambient  $[O_3]$  (45.7 ppb) significantly reduced the grain yield, by 20%, with significant variation, in the range from 10 to 35%, among the combinations of four cultivars and three growth seasons. The reduction of individual grain mass mostly accounted for the yield loss induced by O<sub>3</sub>, and showed significant difference between the cultivars (Zhu et al. 2011). The response of relative yield to elevated  $[O_3]$  was not significantly different from those reported in China, Europe, and India on the basis of experiments in OTCs.

Elevated  $[O_3]$  significantly reduced the grain yield by 12%, when averaged across all the tested cultivars (hybrid rice cultivars SY63 and LYPJ, and inbred cultivars WJ15 and YD6). However, the hybrid rice cultivars SY63 and LYPJ exhibited greater yield losses, by 17.5% and 15.0%, respectively, than the inbred cultivars WJ15 and YD6, which showed no significant yield loss. The different responses between cultivars were attributed to the suppression of spikelet formation in the hybrid cultivars under O<sub>3</sub> stress (Shi et al. 2009).

Our results thus confirmed the rising threat of surface  $[O_3]$  on wheat production in China and, indeed, in other parts of the developing world in the near future. Various countermeasures are urgently needed against the crop losses due to  $O_3$ , such as mitigation of the increase in surface  $[O_3]$  with stricter pollution controls, and enhancement of wheat tolerance to  $O_3$  by breeding and management.

### 12.4.3 Effects of $O_3$ on Grain Quality

Elevated  $[O_3]$  also significantly changed grain quality. The OTC experiments in 2007 and 2008 indicated that N and S concentrations were increased by exposure to elevated  $[O_3]$  (Zheng et al. 2013a). The experiments also showed increases in the

concentrations of K, Ca, Mg, P, Mn, Cu, and Zn for winter wheat and Mg, K, Mn, and Cu for rice. The concentrations of protein, amino acid, and lysine in winter wheat and rice were increased and the concentration of amylose was decreased. The increase in the nutrient concentrations was less than the reduction of the grain yield in both winter wheat and rice, and, hence, the absolute amount of the nutrients was reduced by elevated [O<sub>3</sub>] (Zheng et al. 2013a).

In the FACE-O<sub>3</sub> study on winter wheat, elevated  $[O_3]$  decreased the accumulation rates of amylose, amylopectin, and starch amylase, reduced the accumulation amounts of amylopectin and starch, and decreased the contents of amylopectin and starch, but increased the content of amylose. With the elevation of  $[O_3]$ , the enzyme activity of grain granule-bound starch synthase (GBSS), soluble starch synthase (SSS), and starch branching enzyme (SBE) decreased after anthesis. The activities of GBSS and SSS had highly significant correlations with amylose, amylopectin, and starch accumulation rates, and the activity of SBE had significant correlations with these rates (Zhang et al. 2013).

Elevated  $[O_3]$  significantly increased grain chalkiness and the concentrations of essential nutrients, which was particularly significant for Zn and Cu. The O<sub>3</sub>-induced changes in starch pasting properties (e.g., amylose concentration decreased by 15.1%) indicated a trend of deterioration in the cooking and eating quality of the grain (Wang et al. 2014). The contents of protein, total amino acids (TAAs), total essential AAs (TEAAs) and total non-essential AAs (TNEAAs) in rice grain were increased by 12–14% with elevated  $[O_3]$  (Wang et al. 2014; Zhou et al. 2015). A similar significant response to O<sub>3</sub> was observed for concentrations of the seven essential and eight non-essential AAs. In contrast, elevated  $[O_3]$  caused a small but significant decrease in the percentage of TEAAs within TAAs (Zhou et al. 2015).

### 12.4.4 Effects of $O_3$ on $CH_4$ Emission in Rice Paddies

Rice paddies are an important CH<sub>4</sub> source, accounting for nearly 20% of global anthropogenic CH<sub>4</sub> emission (Intergovernmental Panel on Climate Change; IPCC 2007) and they are also a major source of N<sub>2</sub>O, accounting for 22% of the total emission from croplands in China (Xing 1998). Using OTCs in situ with different [O<sub>3</sub>] treatments, CH<sub>4</sub> emissions were measured in a rice paddy in the Yangtze River Delta, China, in 2007 and 2008. The diurnal patterns of CH<sub>4</sub> emission varied temporally with treatments and there was inconsistence in the diurnal variations of CH<sub>4</sub> emissions from the paddy field. CH<sub>4</sub> emissions from the paddy field throughout the growing season were reduced by 46.5% and 50.6% in elevated [O<sub>3</sub>] of 69.6 ppb and 118.6 ppb, respectively, in 2007, and by 38.3% and 46.8% in elevated [O<sub>3</sub>] of 82.2 ppb and 138.3 ppb, respectively, in 2008, as compared with the subambient [O<sub>3</sub>] level. The seasonal mean CH<sub>4</sub> emissions were negatively correlated with AOT40 (P < 0.01 in both years), but were positively correlated with the relative rice yield, as well as with the above- and below-ground biomass (Zheng et al. 2011).

Tang et al. (2015) reported, in a FACE-O<sub>3</sub> experiment, that a mean 26.7%enhancement of  $[O_3]$  above ambient  $[O_3]$  significantly reduced CH<sub>4</sub> emission at the tillering and flowering stages, leading to a reduction of seasonal integral  $CH_4$  emission by 29.6% on average across two cultivars: an inbred Indica cultivar, Yangdao 6 (YD6), and a hybrid one, II-Y084. Also, the reduced  $CH_4$  emission was associated with  $O_3$ -induced reductions in whole-plant biomass (-13.2%), root biomass (-34.7%), and maximum tiller number (-10.3%). Furthermore, a larger decrease in CH<sub>4</sub> emission with II-Y084 (-33.2%) than that with YD6 (-7.0%) was observed at the tillering stage, which may have been due to the larger reduction in tiller number in II-Y084 in elevated  $[O_3]$ . Additionally, elevated  $[O_3]$  reduced the seasonal mean nitrogen oxides (NOx) flux by 5.7% and 11.8% with II-Y084 and YD6, respectively, but these effects were not statistically significant. The relative response of  $CH_4$  emission to elevated  $[O_3]$  in the FACE-O<sub>3</sub> experiment was not significantly different from those reported in OTC experiments. The two studies, i.e., the study by Tang et al. (2015) and the study by Zheng et al. (2011) have thus confirmed that increasing [O<sub>3</sub>] could mitigate the global warming potential of CH<sub>4</sub> and the findings suggest that the feedback mechanism between O<sub>3</sub> and its precursor emission should be considered in the projection of future O<sub>3</sub> effects on terrestrial ecosystems.

## 12.4.5 Effects of O<sub>3</sub> on Soil Microbiology

Knowledge is limited regarding the impact of elevated  $[O_3]$  on below-ground processes in agro-ecosystems. There are many reports, e.g. Andersen (2003), Grantz et al. (2006), and Feng et al. (2008), that exposure of plants to elevated  $[O_3]$  reduces carbon allocation to roots, reduces the root/shoot biomass ratio, and reduces root exudates. As plants serve as the main source of carbon and energy inputs to the plant-soil microbe web, a decrease in the carbon flux from plants due to elevated  $[O_3]$  could adversely influence the diversity of soil microbes.

Across the winter wheat growth period, elevated  $[O_3]$  significantly reduced soil microbial carbon and changed microbial community-level physiological profiles in rhizosphere soil, but not in non-rhizosphere soil. The relative abundances of fungal and actinomycetous indicator phospholipid fatty acids (PLFAs) were decreased in both rhizosphere and non-rhizosphere soils, while those of bacterial PLFAs were increased by elevated  $[O_3]$  (Chen et al. 2009, 2010, 2015).

However, the responses of the soil biota to  $O_3$  pollution are different between cultivars. In a FACE- $O_3$  experiment, Feng et al. (2015b) found that elevated  $[O_3]$  negatively influenced the bacterial community in an  $O_3$ -tolerant cultivar of rice, YD6, by decreasing bacterial phylogenetic diversities. In contrast, in the  $O_3$ -sensitive rice cultivar IIY-084, the bacterial community responded positively to elevated  $[O_3]$  at the tillering stage. However, several keystone bacterial guilds were consistently negatively affected by elevated  $[O_3]$  in both cultivars. These findings indicate that elevated  $[O_3]$  could negatively influence rice agro-ecosystems and that the crop cultivar is an important determinant of the soil biota responses to elevated  $[O_3]$ .

In our FACE-O<sub>3</sub> experiment, elevated  $[O_3]$  significantly reduced the abundance and percentage of anoxygenic phototrophic purple bacteria (AnPPB) in the total bacterial community in flooded rice soil, via decreasing their genotypic diversity and metabolic versatility (Feng et al. 2011b). Concomitantly, under elevated  $[O_3]$ , the community composition changed after the rice anthesis stage. These AnPPB responses imply that continuously elevated  $[O_3]$  in the future could eventually harm the health of paddy ecosystems through its negative effects on soil microorganisms (Feng et al. 2015b). In a parallel study, elevated  $[O_3]$  inhibited methanogenic activity and influenced the composition of paddy methanogenic communities, reducing the abundance and diversity of paddy methanogens by adversely affecting dominant groups, such as acetoclastic Methanosaeta, especially at the rice tillering stage (Feng et al. 2013). These results indicate that elevated  $[O_3]$  could negatively influence paddy methanogenic archaeal communities and their critical ecological function.

Both OTC and FACE-O<sub>3</sub> experiments have thus proven that elevated  $[O_3]$  significantly changes soil microbial community function and composition, which could influence soil nutrient supply and soil carbon metabolism.

## **12.5** Estimating the Impacts of Rising O<sub>3</sub> Concentrations on Crops in China

Both weighted-mean concentrations and flux-based approaches are widely used to assess  $O_3$  impacts on crops on regional, national, and global scales (Wang and Mauzerall 2004; Mills et al. 2011; Avnery et al. 2011). However, more and more studies have indicated that  $O_3$  flux is superior to concentration exposure indexes such as AOT40 for assessing  $O_3$  effects (Mills et al. 2011; Büker et al. 2015), because the flux-based approach considers biological and climatic factors that influence daytime stomatal  $O_3$  uptake. To obtain  $O_3$  flux-based assessments, multiplicative stomatal conductance models have been developed and parameterized to estimate  $O_3$  flux through stomata.

#### 12.5.1 Stomatal O<sub>3</sub> Flux Models

Emberson et al. (2000) and Pleijel et al. (2002) developed and parameterized Jarvis-type (Jarvis 1976) multiplicative stomatal conductance models for spring wheat and potato, respectively, and used them to derive relationships between yield loss and stomatal  $O_3$  flux for both species (Danielsson et al. 2003; Pleijel et al. 2002, 2004). These models predict stomatal conductance as a function of phenology and the short-term effects of environmental conditions such as radiation, temperature, vapor pressure deficit (VPD), and  $[O_3]$ , based on European field observations and conditions (Feng et al. 2015a).

In China, stomatal  $O_3$  flux and flux-response relationships were first derived for winter wheat grown in FACE-O<sub>3</sub> (Feng et al. 2012). A stomatal conductance ( $g_{sto}$ ) model developed for wheat in Europe was re-parameterized for the Chinese varieties.

Compared with European model parameterizations, the main changes were that the VPD and radiation response functions were made less and more restrictive, respectively, and the temperature function was omitted. The re-parameterized  $g_{sto}$  model performed well, with an r<sup>2</sup> value of 0.76. The slope and intercept of the regression between observed and predicted  $g_{sto}$  were not significantly different from 1 and 0, respectively.

Tang et al. (2014) parameterized a multiplicative model of  $g_{sto}$  for O<sub>3</sub> uptake by rice leaves with the field measurements in a FACE-O<sub>3</sub> experiment. In the  $g_{sto}$  model for rice, the entire accumulation period was 1,350 °C days, in contrast to 800–970 °C days for wheat (Pleijel et al. 2007; LRTAP 2010; Feng et al. 2012). The minimum fraction of VPD limitation for gs ( $f_{VPD}$ ) was set at VPD of 2.7 kPa and the maximum value of fraction of temperature limitation for gs ( $f_{temp}$ ) occurred at 28 °C. The estimated  $g_{sto}$  determined by the parameterized rice  $g_{sto}$  model compared well with the observed value ( $r^2$ =0.79). The regression line was close to and not significantly different from the line of equality, with the slope being 0.932 (no dimension) with a 95% confidence interval (CI) of 0.823–1.039 and the intercept being 21.66 (mmol m<sup>-2</sup> s<sup>-1</sup>), with a 95% CI of -2.74–46.06 (mmol m<sup>-2</sup> s<sup>-1</sup>) (Tang et al. 2014).

## 12.5.2 O<sub>3</sub> Dose-Response Relationships for Crop Yield in China

Tables 12.2 and 12.3 show the dose-response relationships for  $O_3$ . It seems that a similar  $O_3$  dose based on the AOT40 induces larger yield loss in winter wheat with FACE- $O_3$  than with OTC. Such a comparison is not feasible for the flux-based  $O_3$  dose, since an  $O_3$  flux model has not been established for the OTC experiments in Jiaxing, due to the limited number of observations. An  $O_3$  uptake threshold of 12 nmol m<sup>-2</sup> s<sup>-1</sup> was judged most reasonable for the wheat flux-response relationship in subtropical China (Feng et al. 2012). Judging from both flux- and concentration-based relationships, the cultivars investigated in China seem to be more sensitive to  $O_3$  than European cultivars (Feng et al. 2012). The new flux-response relationship can be applied to  $O_3$  risk assessment in wheat in subtropical regions.

For rice, the  $O_3$  dose-response relationship was established based on the AOT40, but not on the stomatal  $O_3$  flux dose. From the results to date, the  $O_3$  impact is estimated to double, based on  $O_3$  flux, or triple, based on  $O_3$  exposure, across the majority of rice-producing areas in the middle and lower reaches of the Yangtze River and in South China between the years 2000 and 2020 (Tang et al. 2014).

#### 12.5.3 Regional Estimates of O<sub>3</sub> Impacts on Crop Production

Past studies (Aunan et al. 2000; Wang and Mauzerall 2004; Tang et al. 2013) have estimated relative yield losses of food crops due to current and projected  $[O_3]$  across China, with wide margins of uncertainties in the estimates (Feng et al. 2015a).

Taking winter wheat as an example,  $[O_3]$  in 2020 is projected to induce a yield loss in the range of 2.9% to 7%, based on the mean  $[O_3]$ ; a yield loss of 2.3% to 63%, based on the seasonal sum of hourly  $[O_3]$  exceeding 60 ppb (SUM06); a yield loss of 13.4% to 16.6%, based on the AOT40; and a yield loss of 19.2% to 23.0%, based on stomatal O<sub>3</sub> flux (Aunan et al. 2000; Wang and Mauzerall 2004; Tang et al. 2013). The wide margins can be attributed to differences between and within the studies in the estimation of  $[O_3]$  at the plant canopy height, O<sub>3</sub> dose metrics, and the O<sub>3</sub> dose-yield loss relationships based on different sets of artificial exposure experiments.

Among the studies mentioned above, only Tang et al. (2013) used the  $O_3$  doseyield loss relationships established with experiments conducted in China, while the others used the relationships established for Europe and North America. The estimates of the crop losses varied by the dose-response relationships with different  $O_3$ dose metrics. The relative yield loss (RYL) of wheat for the whole of China in 2000, for example, was estimated to be in the range of 6.4% to 14.9% (Tang et al. 2013). The POD with the stomatal  $O_3$  flux threshold over 6 nmol m<sup>-2</sup> s<sup>-1</sup> (POD6) predicted greater RYL, whereas the 90-day AOT40 gave the lowest estimates. It is noteworthy, however, that the *increase* of RYL from 2000 to 2020 for China was estimated in a much narrower range, of 8.1–9.4%.

Tang et al. (2013) also showed that the  $O_3$  flux-based estimates of RYL were highly sensitive to perturbations in the meteorological inputs, but that the estimate of the *increase* in RYL from 2000 to 2020 was much more robust than the estimates of RYL per se. Thus, the projected *increase* in wheat production loss in China in the near future is substantial, beyond the uncertainties pointing to the obvious need to curb the rapid increase in surface  $[O_3]$  in China.

It must be noted, however, that the  $O_3$  impact estimated by Tang et al. (2013) is based on an experiment at a single site in the Yangze River Delta, whereas there is a vast range of plant varieties, agronomic practices, soil, and climate across the major crop-growing regions of China. Thus, the uncertainties due to the scaling-up from a single site to the entire country of China are yet to be quantified.

## **12.6** Combined Effects of O<sub>3</sub> and Other Environmental Changes on Chinese Crops

 $O_3$  is unlikely to be the only stressor that plants are subjected to during their growth and development. Previous experiments have demonstrated that plant response to  $O_3$  is altered under other environmental factors that stress crop systems, including atmospheric CO<sub>2</sub> concentration ([CO<sub>2</sub>]), temperature, solar radiation, soil moisture, and nitrogen availability. Changes in agricultural productivity can be the result of direct effects of these factors at the plant level, such as alterations in leaf stomatal  $O_3$  uptake, or indirect effects at the system level; for instance, through shifts in crop phenology, nutrient cycling, pest occurrence, and plant diseases. Furthermore, the combined effects of  $O_3$  and other environmental factors on crop systems and alterations to natural emissions of  $O_3$  precursors have the potential to feedback on tropospheric  $[O_3]$ , with implications for climate change. Nevertheless, in comparison with the studies on crop responses to each individual stressor, much less is known about the interactions of  $O_3$  and these other stressors on plant performance. Below, we outline the limited reports on the combined effects of  $O_3$  and other environmental factors on Chinese crops.

#### 12.6.1 Interaction Between $O_3$ and $CO_2$

Generally,  $O_3$  damages photosynthetic tissues and accelerates leaf senescence, whereas increased  $[CO_2]$  stimulates photosynthesis and biomass accumulation. The interactive effects of these two gases on plants have received much attention. By conducting a closed-top chamber experiment, Zhao et al. (2015) showed that the combined exposure to elevated  $[CO_2]$  (200 ppm above ambient) and  $[O_3]$  (60%) higher than ambient) had no significant effects on dry-matter production or nitrogen uptake in a hybrid rice cultivar, Shanyou 63. Similarly, in the same experiment, the combined effects of O<sub>3</sub> and CO<sub>2</sub> on leaf photosynthesis were not significant (Shao et al. 2014). As to the rice grain quality, O<sub>3</sub> affected many quality traits (chalkiness, protein nitrogen, Zn, Cu, hot viscosity, setback) significantly at ambient [CO<sub>2</sub>], but such effects became non-significant at elevated  $[CO_2]$  (Wang et al. 2014). These results indicate that elevated  $[CO_2]$  can modify the O<sub>3</sub> impacts on rice growth, leaf photosynthesis, and grain quality. The mechanisms underlying the amelioration of  $O_3$ -induced damage by elevated [CO<sub>2</sub>] are not well understood, but reduction in  $O_3$ uptake through stomatal closure induced by elevated [CO<sub>2</sub>] has been considered as the major factor responsible for the protection against  $O_3$  aggression. It can be inferred that decreased stomatal conductance due to elevated  $[CO_2]$  decreases  $O_3$ uptake; less  $O_3$  uptake will lead to less  $O_3$  damage to leaves, thus leading to more healthy green leaves for carbon assimilation, biomass growth, and grain filling.

## 12.6.2 $O_3$ and Aerosol Loading

 $O_3$  and other atmospheric pollutants, e.g.,  $O_3$  precursor gases and aerosol particles, usually have a common source from fossil fuel combustion and biomass burning; these species increase atmospheric aerosol loadings (Simpson et al. 2014), which could diminish solar irradiance at regional scales. Therefore, increased tropospheric  $[O_3]$  and reduced solar irradiance may occur concomitantly and have combined effects on crop growth. A recent OTC experiment concluded that a 40% shading of natural solar irradiance significantly exacerbated  $O_3$ -induced yield loss in winter wheat, but the extent of the yield loss was less than the additive effects of the individual  $O_3$  and shading effects (Zheng et al. 2013b). These results emphasize that, although reduction in solar irradiance could decrease stomatal conductance and lead

to less  $O_3$  uptake by plants, this mitigation may not be able to compensate for the negative effect of this interaction on leaf photosynthesis. Moreover, the interaction of  $O_3$  and solar irradiance could affect soil microbial functional diversity in the wheat rhizosphere, with implications for carbon cycling and sequestration below ground (Wu et al. 2015).

## 12.6.3 $O_3$ and Nitrogen Supply

 $O_3$  interactions with nitrogen may also occur with the increased use of nitrogen fertilizer, because  $O_3$ -induced acceleration in foliar senescence and reduced translocation of nitrogen from aged leaves may depend on nitrogen availability. In a FACE- $O_3$ experiment, yields of wheat with high nitrogen fertilizer input were increased significantly compared with yields of wheat with standard nitrogen input, both under elevated [ $O_3$ ] (Chen et al. 2011). Similar to findings in wheat, an increasing nitrogen supply also mitigated the  $O_3$ -induced yield loss in rice, and the application of additional nitrogen at the tillering stage was better than that at the panicle initiation stage (Luo et al. 2013) in terms of mitigating this  $O_3$ -induced yield loss. These mitigations of  $O_3$  stress were attributed to significant increases in the net photosynthetic rate and increases in the content of chlorophyll a and chlorophyll b in wheat and rice leaves with high nitrogen availability (Chen et al. 2011; Luo et al. 2012).

## 12.6.4 O<sub>3</sub>-Climate Interactions

Among weather variables, soil moisture is the one most often studied as a factor that interacts with the effects of  $O_3$ . This focuses on the soil moisture- $O_3$  interaction may have occurred because of concerns about the possible overestimation of O<sub>3</sub> impacts on crops.  $O_3$  impacts were estimated with the dose-response relationships based on OTC experiments, where supplementary irrigation was given to compensate for the partial exclusion of rain water and the higher evaporative demand due to the chamber effect. Crop yield losses induced by  $O_3$  were sometimes less in drought-stressed plants than in the well-watered plants. Stomatal closure due to water stress and, hence, reduced  $O_3$  influx has been assumed to be the main cause of the reduced  $O_3$ impacts. There is, however, another interpretation of the reduced  $O_3$  impacts under water stress (Kobayashi et al. 1993), where the drought stress was ameliorated by reduced water use in O<sub>3</sub>-stressed plants. In any case, the drought stress caused by O<sub>3</sub> interaction is important, since amelioration of water constraints by irrigation or increased rainfall due to climate change could increase the negative effects of  $O_3$  on the crop. The net effect would be a shift of the major environmental stressor from soil moisture to surface O<sub>3</sub>, resulting in a less-than-expected crop yield increase with the increased water availability.

Higher temperatures and altered precipitation can also affect  $O_3$  formation through alterations to natural emissions of  $O_3$  precursors. Finally, understanding how  $O_3$  acts in combination with other stressors, e.g., heat stress, excessive nitrogen

deposition, and high atmospheric aerosol loading, will also be important to fill the gaps in our knowledge how to target control efforts. As such, efforts to control  $O_3$  may benefit from coordinated hemispheric- or global-scale action that is closely integrated with efforts on regional and local scales.

### **12.7 Future Research Needs**

## 12.7.1 Establishing an $O_3$ Dose Model and Model Parameterization

It is difficult to assess the impact of ambient  $O_3$  on crop productivity over the vast territory of China using a unified dose-response model, since the crop varieties and climates differ greatly by region. In China, past research on wheat and rice has been limited to the Yangtze River Delta, the Pearl River Delta, and the regions of Beijing, Tianjin, and Hebei. A more comprehensive study covering the major agricultural regions and staple food crops is crucial for estimating the surface  $O_3$  impact on food production in China. A robust model of the relationship between crop productivity and  $O_3$  exposure under different conditions should be established and validated against the local field investigations.

## 12.7.2 Developing Air Quality Standards for Food Security in China

Currently there are no standards in China to protect crops from  $O_3$ . To develop a standard, we need to do more monitoring and carry out measurements in rural areas and major crop production regions, including Hebei, Inner Mongolia, Jilin, Heilongjiang, Jiangsu, Anhui, Shandong, Henan, Hunan, Sichuan, Liaoning, Jiangxi, and Hubei Provinces, all of which have a sowing area of more than 3 Mha and in combination account for approximately 76% of national food production. Combined with the controlled  $O_3$  experiments at different sites mentioned above,  $O_3$  standards for crop production should be developed for China.

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