Daytime and Phenological Characteristics of O₃ and CO₂ Fluxes of Winter Wheat Canopy Under Short-Term O₃ Exposure

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Abstract To analyze the daytime and phenological variations of canopy O₃ and CO₂ uptake of winter wheat, the canopy fluxes of wheat plants were measured using a chamber system with four different O₃ levels (0, 40, 80, and 120 nmol mol⁻¹) being applied. During the daytime (7:30–18:00 hours), canopy fluxes usually peaked around noon in early growing stages, while a generally decreasing trend from morning to afternoon was observed in the later stages. O₃ and CO₂ fluxes were positively and negatively correlated with O₃ concentration, respectively. Significant differences were observed in O₃ fluxes but CO₂ fluxes among O₃ treatments. Photosynthetically active radiation (PAR) and vapor pressure deficit (VPD) could affect canopy gas uptake in opposite ways. On the phenological timescale, both O₃ and CO₂ fluxes followed the variation of leaf

area index (LAI) with the maximum occurring simultaneously at the booting stage. The daytime mean fluxes varied from -10.6 to -17.2 nmol m⁻² s⁻¹ for O₃ and from -5.9 to -19.6 μmol m⁻² s⁻¹ for CO₂. Quantitatively important O₃ deposition (-3.1~ -11.6 nmol m⁻² s⁻¹) was also observed at night with the ratios being about 40~70 % relative to the daytime O₃ fluxes for most measuring days, which indicates a significant contribution from non-stomatal components to canopy O₃ removal. This study confirms that environmental variables and plant phenology are important factors in regulating canopy O₃ and CO₂ uptake. O₃ exposure (≤120 nmol mol⁻¹) could not significantly affect the CO₂ uptake of wheat canopy in a short time (ca. 10 min).

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1 Introduction

As a strong oxidative greenhouse gas, tropospheric O₃ may cause a great loss on crop production by inducing a variety of physiological and biochemical alterations (Pleijel et al. 2000; Castagna et al. 2001; Wang et al. 2007). With the increase of O₃ precursor emissions due to anthropogenic activities, current O₃ background concentration has doubled compared with that of a century ago (Vingarzan 2004). Many kinds of crops worldwide are experiencing a high level of O₃ with concentrations

over the critical values for crop protection (Meehl et al. 2007).

The degree of O₃ stress is directly related to its access to the reactive sites inside plants and plant antioxidative ability. Determination of O₃ uptake by plants (i.e., O₃ flux) is essential to quantitatively assess its potential risks (Pleijel et al. 2007). The chamber method is a useful way in direct measuring of O₃ fluxes (Ennis et al. 1990; Altimir et al. 2002). By enclosing plants in a transparent chamber built with inert materials (e.g., Teflon), the variations of O₃ concentrations caused by plant can be monitored, and then the O₃ fluxes can be calculated based on mass balance equations (Amiro et al. 1984).

Stomatal uptake is the main pathway for O₃ entering plant while plant cuticle is the main area where O₃ deposition occurs by chemical reactions (Kerstiens et al. 1992). As both stomatal movements and surface chemical reactions are affected by environmental factors (e.g., light intensity, temperature) and plant phenology, plant O₃ flux often varies with weather conditions and growth periods (Grulke et al. 2007; Fares et al. 2010). A single-peak pattern was often observed for both daytime and phenological variations of plant O₃ fluxes, with the maximum values appearing at noon and in the middle of the growing season, respectively (Fares et al. 2010; Zhu et al. 2015).

Actually, O₃ uptake is mainly an accompanying process of plant exchanges of CO₂ and H₂O. However, it can significantly inhibit CO₂ sequestration and other physiological functions of plant (Sild et al. 2002; Feng et al. 2008). Although the responses of single-leaf photosynthesis to O₃ has been studied intensively so far, changes in canopy CO₂ exchanges under O₃ stress have not received so much attention. Simultaneous measurements of O₃ and CO₂ fluxes of plant canopy could give us more insights into the O₃ effects on photosynthetic capacity at the whole plant level. However, only a few researches have been carried out on this subject (e.g., Grimm and Fuhrer 1992a; Kanerva et al. 2007; Tong et al. 2011).

Wheat is an O₃-sensitive plant (LRTAP Convention 2010) and one of the most important crops in China as well. Recently, with the increase of tropospheric O₃ concentration in China, wheat production has been severely decreased by O₃ resulting in a great economic loss (Wang and Mauzerall 2004). Although the relationships between wheat yield and O₃ dose have been widely studied in China recently (Feng et al. 2003;

Zhu et al. 2012; Tang et al. 2013). most results of them were derived from the index of O₃ concentration instead of O₃ flux, which is more related to plant growth. In this study, both O₃ and CO₂ fluxes of wheat canopy were measured simultaneously using a chamber system. The objectives of this study are to (1) characterize the day-time and phenological variations of O₃ and CO₂ fluxes and (2) analyze the relationships between different influencing factors and canopy fluxes. We hypothesized that the short-term exposure to elevated O₃ will significantly (1) increase plant O₃ flux and (2) decrease CO₂ fluxes and (3) on the phenological time scale, both O₃ and CO₂ fluxes will increase first and then decrease with plant thrive and aging.

2 Materials and Methods

2.1 Experimental Site

The experimental site is located at the Seed Administration Station (40° 12' N, 116° 8' E), Changping District, Beijing City. The site is dominated by the warm temperate continental monsoon climate with four distinctive seasons. The average annual temperature and precipitation are 11.8 °C and 550.3 mm, respectively. The soil type is alluvial soil with 16.4 g kg⁻¹ organic matter, 0.9 g kg⁻¹ total nitrogen, 38.1 mg kg⁻¹ available phosphorus, 102.1 mg kg⁻¹ available potassium, pH of 8.3, and field water capacity of 18.5 %.

2.2 Plant Material

Winter wheat (*Triticum aestivum* L. Beinong 9549) seeds were sown on 28 August, 2009, after applying compost to the farmland. On 26 April, 2010, 225 kg ha⁻² urea was top dressed. Winter wheat was harvested on 23 June, 2010. During the whole growing season, the field management was kept in line with local regulations.

2.3 Chamber System Setup

A crop chamber system was developed in reference to Garcia et al. (1990) to determine the canopy fluxes to winter wheat. Only a set of system was applied during the experimental period. The system consisted of a transparent chamber, a blower, a flow meter, a ball valve, ozonators,

gas analyzers, and plastic pipes (Fig. 1). The chamber was built with aluminum square tubes (20×20 mm) and covered with Teflon film (0.1 mm in thickness) on the top and sides. The bottom was open, so that the plants could be inserted into the chamber during flux measurements. Two chamber sizes with length $\times$ width $\times$ height of $50 \times 50 \times 50$ cm (volume 125 l) and $50 \times 50 \times 70$ cm (volume 175 l) were designed to adapt to wheat growth. During the first three measurements (Table 1) when wheat plants were relatively short (height < 50 cm), the small chamber was used to minimize background noise of O_3 flux, which was mainly resulted from surface reactions of O_3 with chamber material. A centrifugal blower was connected to the chamber with an aluminum corrugated pipe. Ambient air was drawn into the system by the blower from about 4 m above ground to avoid disturbance of CO_2 fluctuation near wheat canopy and was then emitted outside from the chamber outlet. The flow rate was regulated with a ball valve and monitored with a flow meter (LWQ-40D, Sure, Tianjin). Two flow rates of 15 and $21 \text{ m}^3 \text{ h}^{-1}$ were set for the 125- and 175-l chambers, respectively. The air inside the chamber was homogenized by two small electric fans installed on the aluminum frames. A PVC pipe filled with activated carbon was mounted upstream of

the chamber inlet to filter ambient O_3 . To achieve the target O_3 concentrations, a small flow ($200\text{--}400 \text{ ml min}^{-1}$) of concentrated O_3 , generated from dry O_2 by ozonators, was injected to the filtered air before it entered the chamber. The ozonator was customized with a pen-like ultraviolet lamp sealed in an aluminum cylinder (length/diameter 12 cm/5 cm), in which O_3 could be generated from O_2 under the effect of ultraviolet radiation. Rotary flow meters were used to regulate the flow rate of O_2 into the ozonators.

O_3 and CO_2/H_2O concentrations of the chamber were monitored simultaneously using an O_3 analyzer (model 49i, Thermo Electron Co., Franklin, MA, USA) and a photosynthesis analyzer (CIRAS-1, PP Systems, Hitchin, UK), respectively. Photosynthetically active radiation (PAR) and vapor pressure (VP) inside the chamber were monitored by the light and water vapor detectors of CIRAS-1. Air temperature (T) was measured with a thermometer. The vapor pressure deficit (VPD) could thus be calculated from T and VP according to Buck (1981).

2.4 Experimental Design

Three wheat plots and a bare plot ($0.5 \text{ m} \times 0.5 \text{ m}$) were enclosed by stainless steel pedestals in the field. Each pedestal has a trough on the top. During flux measurements, the plot was covered

Fig. 1 Scheme of the flux measuring system

Table 1 Dates of measurements for canopy fluxes and LAI of winter wheat in 2010

Daytime variation measurements		Phenological variation measurements		LAI	
Date	Growth stage	Date	Growth stage	Date	Growth stage
April 13	Erecting stage	April 12	Erecting stage	April 11	Erecting stage
April 28	Jointing stage	April 26	Jointing stage	April 25	Jointing stage
May 11	Booting stage	May 10	Booting stage	May 13	Booting stage
June 2	Filling stage	May 29	Filling stage	June 1	Filling stage
June 15	Mature stage	June 12	Mature stage	June 13	Mature stage

LAI leaf area index

with chamber on the pedestal with the trough being filled with water to make the chamber airtight. To minimize the experimental disturbance to plant growth, wheat plants were exposed to natural environment most of the time and enclosed in the chamber only when measurements were taken. When the environmental condition is relatively stable, it will take several minutes for the O_3 concentration inside the chamber to reach a steady state (Altimir et al. 2002). Therefore, the duration for each chamber closure in this study was set for 10 min, with the O_3 concentrations of both inlet and outlet being measured for 5 min.

O_3 and CO_2 fluxes were measured during five growth stages, namely, erecting stage, jointing stage, booting stage, filling stage, and mature stage (Table 1). Two patterns of O_3 exposure were designed to investigate the daytime and phenological variations of canopy fluxes. In the daytime measurements, three inlet O_3 concentrations of 40, 80, and 120 $nmol\ mol^{-1}$ were applied to the three wheat plots, respectively. Each level of O_3 concentration was set constant through the day and was applied sequentially once every 1 to 2 h. About six measurements were taken for each treatment during the day. In the phenological measurements, daily dynamics of ambient O_3 concentration were simulated with the inlet O_3 concentrations being set at four levels, that is, 0 $nmol\ mol^{-1}$ (8:00–9:00 hours), 40 $nmol\ mol^{-1}$ (9:00–11:00 and 17:00–18:00 hours), 80 $nmol\ mol^{-1}$ (11:00–12:00 and 16:00–17:00 hours), and 120 $nmol\ mol^{-1}$ (13:30–15:30 hours). Each time period contains five–nine replicative measurements. In order to determine the O_3 flux at night, measurements with inlet O_3 concentrations of 40 and 80 $nmol\ mol^{-1}$ were also taken from 20:00 to 22:00 hours.

The O_3 and CO_2 fluxes of the bare plot were also measured and subtracted from the fluxes derived from the wheat chambers. A total of 160 leaves were sampled near the wheat plots during our experiment, with leaf area (A), length (L), and maximum width (W) being measured using a leaf area meter (LI-3000, Licor, USA). In reference to Palaniswamy and Gomez (1974), the relationship between A and $L \times W$ was linearly fitted. Based on the fitting result and values of L and W measured manually from the wheat plots, the leaf area index (LAI) was determined nondestructively.

2.5 Flux Calculations

Canopy flux (J) for O_3 ($nmol\ m^{-2}\ s^{-1}$) or CO_2 ($\mu mol\ m^{-2}\ s^{-1}$) are calculated as Eq. (1) (Amiro et al. 1984):

$$J = F\Delta C / ART \quad (1)$$

where F is the volume flow rate ($m^3\ s^{-1}$), ΔC is the molar concentration differences caused by wheat for O_3 ($nmol\ mol^{-1}$) or CO_2 ($\mu mol\ mol^{-1}$), P is the air pressure (Pa), A is the plot area (m^2), R is the universal gas constant ($J\ mol^{-1}\ K^{-1}$), and T is the absolute temperature (K). ΔC was calculated from the mass balance equation, which can be expressed as Eq. (2) (Ennis et al. 1990):

$$FC_{in} - FC_{out} + F\Delta C - K_{chamber}C_{out}V = 0 \quad (2)$$

where C_{in} and C_{out} are the gas concentrations of the chamber inlet and outlet, respectively, $K_{chamber}$ is the first-order rate constants (s^{-1}) describing the reactions between gas species and chamber surface, and V is the chamber volume (m^3). $K_{chamber}$ is calculated based on

the flux measurements of bare plot. More detailed descriptions about the theory on flux calculations exist elsewhere (Ennis et al. 1990).

2.6 Statistical Analysis

An analysis of variance (ANOVA) was used to test for mean differences among treatments. The Fisher's protected LSD test was used for post hoc examination of treatment differences. Pearson correlation analysis was used to check the relationships among variables. The statistics was performed by the statistical package SPSS (SPSS Inc., Chicago, IL, USA).

3 Results

3.1 LAI

LAI is an important index related to wheat growth and canopy gas exchanges. For the five measurements, LAI increased from April 11 to maximum in May 13, and then decreased to wheat maturity on June 13 (Fig. 2). Little difference was observed among the three plots for each measurement.

3.2 O₃ and CO₂ Fluxes from Bare Plot

Obvious O₃ removal from bare plot was observed (Fig. 3) with its O₃ fluxes (means the absolute value of gas exchanges here and hereafter) increasing with elevated O₃ concentrations. Significant differences in O₃ fluxes from bare plot were observed among treatments

Fig. 2 Phenological variation of leaf area index (LAI) of the three wheat plots

($p < 0.01$). Small CO₂ fluxes were observed on bare plot (Fig. 3) with no significant differences being observed among treatments ($p > 0.05$).

3.3 Daytime Variations of Environmental Factors and Canopy Fluxes

A single-peak pattern of variation was observed for PAR and VPD for each day of measurement (Figs. 4 and 5). The changes of both factors synchronized with each other for different O₃ concentrations (40, 80, and 120 nmol mol⁻¹). No significant differences ($p > 0.05$) were observed among treatments for both factors.

The daytime variation of canopy O₃ flux differed among the five measurements (Fig. 6). In early measuring stages on April 13 and 28, O₃ flux often increased from morning to noon when it reached the peak value. In the later periods, O₃ flux was usually higher in the morning and then decreased continuously to noon. A slight upturn of O₃ flux was also observed in the afternoon on June 2 and 15. O₃ fluxes were positively correlated with O₃ concentrations (Table 2). Significant differences in O₃ fluxes were observed among treatments on all measuring days, except for June 15 when wheat was senescent with less O₃ uptake ($p < 0.05$; Table 3).

A significant correlation between CO₂ flux and environmental variables was observed (Table 2). On April 13, CO₂ flux generally followed the variation of environmental variables with maximum values appearing around noon (Fig. 7). In the later three measurements on April 28, May 11, and June 2, CO₂ fluxes usually reached the peak values in the morning around 9:00 hours and then decreased. Small midday depressions in CO₂ uptake were observed on May 11, June 2, and June 15. CO₂ uptake was near zero or positive on June 15 when wheat was mature. CO₂ fluxes differed significantly ($p < 0.05$) among the three O₃ treatments on April 13 while no significant differences ($p > 0.05$) were found in later measurements.

3.4 Phenological Variations of Daytime Canopy Fluxes

The daytime mean O₃ flux increased from April 12 to maximum on May 10, and then decreased to minimum on June 12 (Table 4). Similar pattern of phenological variation was also found for each O₃ treatment. Canopy O₃ fluxes were positively correlated with O₃ concentrations with the maximum and minimum values occurring

Fig. 3 Diurnal variations of average **a** O_3 and **b** CO_2 fluxes of bare plot for the whole experimental period

at 120 and 40 $nmol\ mol^{-1}$ for all five measurements. Similar to O_3 fluxes, CO_2 fluxes also increased first with wheat growth and then decreased to wheat maturity. The maximum daytime mean CO_2 flux occurred at booting stage on May 10, and the minimum occurred at mature stage on June 12 (Table 4). This pattern of variation was observed in nearly all the six daytime periods.

3.5 Phenological Variations of Nocturnal Canopy Fluxes

Nocturnal O_3 flux of wheat increased from April 12 to May 29, and then decreased to the end of wheat growth (Table 4). Higher O_3 concentrations resulted in higher O_3 fluxes. Quantitatively important CO_2 emission was found for wheat plants at night (Tables 4 and 5). Nocturnal CO_2 fluxes increased continuously with wheat development, with the maximum values occurring at the mature stage. No significant differences ($p > 0.05$) were observed in nocturnal CO_2 fluxes between 40 and 80 $nmol\ mol^{-1}$ treatments.

4 Discussion

4.1 Relationships Between Canopy Fluxes and O_3 Concentrations

O_3 concentration gradient between leaf inside and outside is the driving force for O_3 uptake by plants (Roshchina and Roshchina 2003). As the O_3 concentration within leaf is close to zero (Laisk et al. 1989), the O_3 level in ambient air becomes the major factor in affecting

O_3 uptake. Under a stable condition when canopy conductance is relatively constant, O_3 fluxes will correlate positively with ambient O_3 concentrations. Grimm and Fuhrer (1992a) have found a significant positive correlation ($r \geq 0.83$) between solar radiation-weighted O_3 flux and O_3 concentration in spring wheat. Fuentes and Gillespie (1992) has also reported an obvious increase of leaf O_3 flux (from -1.7 to $-5.2\ nmol\ m^{-2}\ s^{-1}$) with elevated O_3 (from 28.4 to 91.2 $nmol\ mol^{-1}$) under a constant light intensity on red maple. Similar results were also observed in our study, with higher levels of O_3 concentrations resulting in significantly higher O_3 fluxes (Table 3) (hypothesis 1).

As a strong oxidative gas species, O_3 could significantly inhibit leaf photosynthesis by changing e.g., chlorophyll content, enzyme activities, and electron transfer rate (Grimm and Fuhrer 1992b; Mishra et al. 2013). Net CO_2 uptake will decrease if the photosynthetic capacity is damaged by O_3 . In this study, a significantly negative correlation between canopy CO_2 fluxes and O_3 concentration was observed (Table 2), which suggested the stress effect of O_3 on canopy CO_2 uptake. However, the correlation was relatively weak ($r = 0.27$), which means that other factors (e.g., PAR and VPD) might also play roles in affecting canopy CO_2 uptake (see Table 2). In this study, no significant differences in canopy CO_2 fluxes were observed among O_3 treatments (negating hypothesis 2). This indicates that the exposure intensity (duration $\times$ concentration) of O_3 was not high enough to seriously damage the canopy photosynthesis of winter wheat. Injury will not occur until the antioxidative ability was diminished by O_3 exposure over a critical dose (LRTAP Convention 2010), which might

Fig. 4 Daytime variations of photosynthetically active radiation (PAR) inside flux chamber of different O_3 treatments

not be exceeded for wheat in this study due to the short exposure time (ca. 10 min) and relatively low O_3 concentration ($<120 \text{ nmol mol}^{-1}$).

4.2 Daytime Variations of Canopy Fluxes

O_3 flux is the product of O_3 concentration and leaf conductance for O_3 (Pleijel et al. 2000). As the O_3

concentration in this study was constant in each treatment all day, the daytime variation of O_3 flux indicates the variation of leaf conductance, which results from its stomatal and non-stomatal components. It has been observed that the stomatal conductance of winter wheat usually increased to its peak in the morning (9:00–11:00 hours) and then decreased generally in the evening (Rai et al. 2011; Xu et al. 2013). In contrast, non-

Fig. 5 Daytime variations of vapor pressure deficit (VPD) inside flux chamber of different O_3 treatments

stomatal conductance usually reached maximum in the afternoon when the environmental conditions were favorable to surface O_3 deposition (Gerosa et al. 2003). In this study, the daytime variation of O_3 flux, which was characterized by a decreasing trend, was more similar to that of the stomatal conductance than that of the non-stomatal conductance. This

indicated the prevailing effect of stomatal movement over non-stomatal limitation on O_3 uptake. The slight depression of canopy O_3 flux in the afternoon on June 2 and June 15 (Fig. 6), which was probably due to the stomatal closure caused by the high VPD (Fig. 5), may also indicate the stomatal limitation on O_3 uptake.

Fig. 6 Daytime variations of O_3 fluxes with three levels of inlet O_3 concentrations (40, 80, and $120 \text{ nmol mol}^{-1}$) for the wheat chamber on 13 April, 28 April, 11 May, 2 June, and 15 June, 2010

Table 2 Correlation coefficients between O_3 flux, CO_2 flux, O_3 concentration, photosynthetic active radiation (PAR), and vapor pressure deficit (VPD) during the experimental period

	O_3 flux	CO_2 flux	O_3 concentration	PAR	VPD
O_3 flux	1	0.45**	0.46**	0.10	-0.17
CO_2 flux	0.45**	1	-0.27*	0.39**	-0.31**

**Correlation is significant at 0.01 level (two-tailed) ($n=87$); *correlation is significant at 0.05 level (two-tailed) ($n=87$)

Table 3 Daytime average O₃ and CO₂ fluxes ($\pm$ SDs) of winter wheat in the daytime variation experiments ($n=5-6$)

Date	O ₃ flux (nmol m ⁻² s ⁻¹)			CO ₂ flux (μ mol m ⁻² s ⁻¹)		
	40 nmol mol ⁻¹	80 nmol mol ⁻¹	120 nmol mol ⁻¹	40 nmol mol ⁻¹	80 nmol mol ⁻¹	120 nmol mol ⁻¹
13/4/2010	-4.2 $\pm$ 1.1 ^a	-7.7 $\pm$ 2.7 ^b	-12.5 $\pm$ 3.1 ^c	-11.0 $\pm$ 1.9 ^a	-6.9 $\pm$ 0.9 ^b	-7.8 $\pm$ 3.0 ^b
28/4/2010	-7.9 $\pm$ 1.9 ^a	-14.0 $\pm$ 0.9 ^b	-21.1 $\pm$ 4.1 ^c	-15.1 $\pm$ 4.4	-14.4 $\pm$ 2.7	-13.4 $\pm$ 5.2
11/5/2010	-8.8 $\pm$ 1.4 ^a	-15.5 $\pm$ 3.9 ^b	-21.2 $\pm$ 2.5 ^c	-16.9 $\pm$ 4.4	-12.5 $\pm$ 5.6	-13.2 $\pm$ 5.2
2/6/2010	-6.2 $\pm$ 3.4 ^a	-12.4 $\pm$ 4.7 ^b	-16.9 $\pm$ 6.2 ^c	-13.0 $\pm$ 3.9	-13.8 $\pm$ 3.3	-12.1 $\pm$ 3.3
15/6/2010	-4.2 $\pm$ 3.1	-7.0 $\pm$ 3.8	-8.4 $\pm$ 4.0	0.9 $\pm$ 1.5	1.9 $\pm$ 1.4	-0.2 $\pm$ 0.6

Different superscripts within rows for O₃ flux stand for significant differences ($p<0.05$). SDs are standard deviations of daytime fluxes for O₃ and CO₂ of wheat canopy

Light is the driving force for plant CO₂ sequestration. A close relationship between canopy CO₂ flux and PAR was often observed on wheat, with the correlation coefficient bigger than 0.54 (Wang et al. 2013; Kupisch et al. 2015). However, in this study, the correlation between CO₂ flux and PAR was found to be weak ($r=0.39$) (Table 2). In the daytime measurements, higher CO₂ fluxes (i.e., higher absolute values of CO₂ uptake) were often observed in the morning (9:00–11:00 hours) instead of at noon, when the light intensity reached its maximum (Figs. 4 and 7, Table 5). The light saturation point of winter wheat was reported to be less than 1200 μ mol m⁻² s⁻¹ (Shi et al. 2011; Zheng et al. 2011), which was lower than the midday light intensity in most measurements of this study (Fig. 4). Saturation of wheat photosynthetic capacity might occur in the afternoon, which had led to the decline of CO₂ fluxes before light intensity decrease. Besides PAR, VPD is another important factor in affecting plant gas exchanges (LRTAP Convention 2010). Winter wheat might close its stomata under high VPD in the afternoon (Fig. 5), especially when the wheat field was relatively arid (42.6 % of field water capacity on average in this study). Therefore, the uptake of CO₂ was inhibited in the afternoon, which might account for the asynchrony between canopy CO₂ flux and PAR. A similar effect of VPD on CO₂ flux was also observed on the rain-fed winter wheat ecosystem of the Loess Plateau, China (Wang et al. 2013).

4.3 Phenological Variations of Canopy Fluxes

Single-peak patterns of phenological variations were found for both daytime O₃ and CO₂ fluxes (Table 4)

(hypothesis 3), both of which correlated well with LAI (O₃: $r^2=0.996$, CO₂: $r^2=0.988$, $p<0.001$). However, the difference in CO₂ fluxes among measuring stages was slightly bigger than that in O₃ fluxes, which indicates different absorption mechanisms for the two gas species. The stomata of green leaves are the main access for ambient CO₂ to leaf inside, while both stomatal and non-stomatal pathways could contribute to canopy O₃ uptake (Stella et al. 2013). When wheat plants were senescent with relatively small stomatal conductance and weak photosynthetic capacity, more O₃ removal could occur by non-stomatal pathway (Potier et al. 2015). This might account for the difference in phenological variations of daytime O₃ and CO₂ fluxes.

As to the nocturnal canopy fluxes, quantitatively important O₃ deposition ($-3.1\sim-11.6$ nmol m⁻² s⁻¹) was observed, with the ratios being about 40–70 % relative to the daytime (9:00–12:00 hours) O₃ fluxes for most measuring days (Table 4). This was similar to the result found over wheat field by Gerosa et al. (2003). The contact reactions between O₃ and plant surface might account for this strong O₃ removals through the non-stomatal pathway.

In comparison to the diurnal maximum O₃ flux, the nocturnal peak values appeared a little later (Table 4). Due to the stomatal closure at night, non-stomatal deposition played the dominant role in nocturnal O₃ removal. With the increase of wheat surface area after ear emergence, non-stomatal O₃ removal might grow stronger by surface reactions (Fowler et al. 2001) while the stomatal O₃ uptake began to decrease with leaf senescence. This might result in the asynchrony in phenological variations between the diurnal and nocturnal O₃ fluxes.

Fig. 7 Daytime variations of CO_2 fluxes with three levels of inlet O_3 concentrations (40, 80, and 120 nmol mol^{-1}) for the wheat chamber on 13 April, 28 April, 11 May, 2 June, and 15 June, 2010

During the experimental period, nocturnal CO_2 emission increased continuously as wheat growth with the maximum value being observed at mature stage. This is different from other chamber-based results that found higher ecosystem respiration before aging of wheat plants (Zhang et al. 2014; Ohkubo et al. 2015). Based on meta-analysis, Feng et al. (2008) found that O_3 could

significantly increase the dark respiration of wheat. The short-term O_3 exposure in this study might also have stimulated the canopy respiration of wheat during the later part of experimental period, when the cumulative exposure of O_3 might be in effect. However, this is uncertain so far and needs further confirmation.

Table 4 Mean O₃ and CO₂ fluxes ($\pm$ SDs) during different phenological stages of winter wheat ($n=5-9$)

Gas fluxes	Time periods (hours)	O ₃ concentrations (nmol mol ⁻¹)	Date				
			12/4/2010	26/4/2010	10/5/2010	29/5/2010	12/6/2010
O ₃ (nmol m ⁻² s ⁻¹)	9:00–11:00	40	-7.5 $\pm$ 1.5	-11.0 $\pm$ 1.3	-11.2 $\pm$ 1.6	-7.7 $\pm$ 1.0	-8.0 $\pm$ 0.5
	11:00–12:00	80	-13.2 $\pm$ 1.8	-15.2 $\pm$ 1.7	-19.8 $\pm$ 2.7	-15.5 $\pm$ 4.5	-14.9 $\pm$ 2.1
	13:30–15:30	120	-19.1 $\pm$ 1.8	-25.4 $\pm$ 5.0	-27.8 $\pm$ 4.1	-20.8 $\pm$ 2.9	-16.2 $\pm$ 5.1
	16:00–17:00	80	-9.7 $\pm$ 1.2	-14.1 $\pm$ 1.9	-14.3 $\pm$ 1.8	-13.1 $\pm$ 6.1	-8.5 $\pm$ 3.6
	17:00–18:00	40	-3.8 $\pm$ 1.2	-5.8 $\pm$ 1.4	-8.9 $\pm$ 0.3	-3.9 $\pm$ 0.1	-2.1 $\pm$ 0.6
	9:00–18:00		-11.4 $\pm$ 6.0	-15.4 $\pm$ 7.4	-17.2 $\pm$ 8.0	-12.8 $\pm$ 6.7	-10.6 $\pm$ 5.4
	20:00–22:00	40	-3.1 $\pm$ 2.0	-5.5 $\pm$ 0.9	-6.3 $\pm$ 2.1	-7.3 $\pm$ 0.1	-4.7 $\pm$ 0.4
	20:00–22:00	80	-5.5 $\pm$ 0.8	-8.8 $\pm$ 2.8	-10.8 $\pm$ 1.2	-11.6 $\pm$ 2.3	-10.6 $\pm$ 1.7
CO ₂ (μ mol m ⁻² s ⁻¹)	8:00–9:00	0	-6.8 $\pm$ 2.9	-16.3 $\pm$ 1.4	-25.4 $\pm$ 2.2	-16.3 $\pm$ 0.8	-3.6 $\pm$ 1.0
	9:00–11:00	40	-10.1 $\pm$ 2.1	-20.3 $\pm$ 2.3	-23.8 $\pm$ 2.1	-13.0 $\pm$ 1.5	-8.5 $\pm$ 1.4
	11:00–12:00	80	-11.1 $\pm$ 2.4	-19.2 $\pm$ 4.1	-23.9 $\pm$ 3.6	-11.4 $\pm$ 1.6	-8.8 $\pm$ 2.1
	13:30–15:30	120	-9.1 $\pm$ 2.3	-16.4 $\pm$ 1.9	-20.8 $\pm$ 3.5	-12.1 $\pm$ 1.2	-6.9 $\pm$ 1.7
	16:00–17:00	80	-7.1 $\pm$ 2.3	-9.7 $\pm$ 3.0	-9.2 $\pm$ 8.3	-10.0 $\pm$ 2.5	-3.9 $\pm$ 1.7
	17:00–18:00	40	-2.4 $\pm$ 2.4	-3.7 $\pm$ 3.6	-8.8 $\pm$ 2.3	-5.9 $\pm$ 1.2	0.0 $\pm$ 2.6
	9:00–18:00		-8.2 $\pm$ 2.8	-15.3 $\pm$ 5.8	-19.6 $\pm$ 6.7	-11.7 $\pm$ 3.0	-5.9 $\pm$ 3.1
	20:00–22:00	40	1.5 $\pm$ 0.0	3.7 $\pm$ 0.2	3.8 $\pm$ 0.8	5.9 $\pm$ 0.8	6.0 $\pm$ 1.1
	20:00–22:00	80	1.5 $\pm$ 0.5	3.0 $\pm$ 0.2	3.7 $\pm$ 0.6	5.2 $\pm$ 0.5	5.9 $\pm$ 0.9

SDs are standard deviations of O₃ and CO₂ fluxes during different time periods of the measuring days

5 Conclusions

The present study showed that (1) higher canopy uptake of O₃ and CO₂ usually occurred in the morning and at booting stage of wheat on the diurnal and seasonal timescales, respectively. Environmental variables such as PAR and VPD were important factors affecting the diurnal variations of canopy fluxes, while phenology was predominant on the seasonal timescale; (2) short-term O₃ exposure could significantly increase O₃

deposition to wheat canopy, while no significant effect was found on canopy CO₂ flux. This indicated that O₃ exposure (≤ 120 nmol mol⁻¹) could not damage the ability of canopy CO₂ uptake in a short time (ca. 10 min). Non-stomatal pathway played an important role in canopy O₃ deposition, which can be confirmed by the considerable amount of nocturnal O₃ flux. Nocturnal CO₂ emission increased continuously with plant growth to maturity, which suggested an enhanced dark respiration with wheat aging. As the exposure

Table 5 Seasonal average O₃ and CO₂ fluxes ($\pm$ SEs) of winter wheat for different time periods ($n=5$)

Time	Treatment (nmol mol ⁻¹)	O ₃ flux (nmol m ⁻² s ⁻¹)	CO ₂ flux (μ mol m ⁻² s ⁻¹)	PAR (μ mol m ⁻² s ⁻¹)	VPD (kPa)
8:00–9:00	0		-13.7 $\pm$ 8.7	530.4 $\pm$ 155.2	1.2 $\pm$ 0.4
9:00–11:00	40	-9.1 $\pm$ 1.8	-15.1 $\pm$ 6.6	882.8 $\pm$ 212.4	1.7 $\pm$ 0.6
11:00–12:00	80	-15.7 $\pm$ 2.4	-14.9 $\pm$ 6.4	1200.0 $\pm$ 195.0	2.4 $\pm$ 0.9
13:30–15:30	120	-21.8 $\pm$ 4.7	-13.1 $\pm$ 5.6	1010.2 $\pm$ 194.7	2.5 $\pm$ 1.0
16:00–17:00	80	-12.0 $\pm$ 2.7	-8.0 $\pm$ 2.6	472.0 $\pm$ 200.0	1.9 $\pm$ 0.9
17:00–18:00	40	-4.9 $\pm$ 2.6	-4.2 $\pm$ 3.4	207.8 $\pm$ 104.7	1.5 $\pm$ 0.8
20:00–22:00	40	-5.4 $\pm$ 1.6	4.2 $\pm$ 1.9	3.90.6	0.9 $\pm$ 0.3
20:00–22:00	80	-9.5 $\pm$ 2.4	3.9 $\pm$ 1.7	4.0 $\pm$ 0.7	0.8 $\pm$ 0.3

SEs are standard errors of O₃ and CO₂ fluxes for different time periods of the whole seasonal measurements

intensity is limited in this study, the O₃ effects on canopy CO₂ exchanges were not confirmed. More flux measurements with long-term O₃ fumigation are still necessary in the future experiment.

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