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Photosynthesis and yield responses of ozone-polluted winter wheat to drought

H. XU[*], D.K. BISWAS[*], W.-D. LI[*], S.-B. CHEN[*], L. ZHANG[*], G.-M. JIANG[*,**,***], and Y.-G. LI[*,***]

Key Laboratory of Vegetation and Environmental Change, Institute of Botany, The Chinese Academy of Sciences, 20 Nanxincun, 100093, Beijing, P.R. China[*]
School of Crop Sciences, Shandong Agricultural University, No. 61, Daizong Avenue, 271018, Tai'an, P.R. China[**]

Abstract

Winter wheat (*Triticum aestivum* L. cv. Jingdong 8) was exposed to short-term high ozone treatment after anthesis and then was either well irrigated with soil water content (SWC) of 80–85 % (O_3+W) or drought treated (SWC 35–40 %, O_3+D). Short-term ozone exposure significantly decreased irradiance-saturated net photosynthetic rate (P_N) of winter wheat. Under good SWC, P_N of the O_3-treated plant was similar to that of control on 2 d after O_3-exposure (6 DAA), but decreased significantly after 13 DAA, indicating that O_3 exposure accelerated leaf senescence. Meanwhile, green flag leaf area was reduced faster than that of control. As a result, grain yield of O_3+W was significantly decreased. P_N of O_3+D was further notably decreased and green flag leaf area was reduced more than that in O_3+W. Consequently, substantial yield loss of O_3+D was observed compared to that of O_3+W. Although P_N was significantly positively correlated with stomatal conductance, it also had notable positive correlation with the maximum photochemical efficiency in the dark adapted leaves (F_v/F_m), electron transport rate (ETR), photochemical quenching (q_P), as well as content of chlorophyll, suggesting that the depression of P_N was mainly caused by non-stomatal limitation. Hence optimal soil water condition should be considered in order to reduce the yield loss caused by O_3 pollution.

Additional key words: air pollution; chlorophyll *a* fluorescence; gas exchange; intercellular CO_2 concentration; stomatal conductance; transpiration rate; *Triticum aestivum* L.; water management.

Introduction

Wheat (*Triticum aestivum* L.) is particularly sensitive to O_3 (Fuhrer *et al*. 1992, Selldén and Pleijel 1995). High O_3 exposure from anthesis to maturity causes more severe yield reduction of wheat (Lee *et al*. 1988, Slaughter *et al*. 1989, Pleijel *et al*. 1998, 2000). High O_3 causes severe detrimental effects such as visible foliar injury (Barnes *et al*. 1990), accelerated senescence (Grandjean and Fuhrer 1989, Ojanpera *et al*. 1998), growth reduction and yield loss (Grandjean and Fuhrer 1989, Ollerenshaw and Lyons 1999). Photosynthetic performance including guard cell homeostasis, electron transport, carbon fixation, and the translocation of photosynthates are the main targets among the negative effects of O_3 (Meyer *et al*. 1997, Grantz and Farrar 1999, Zheng *et al*. 2002). O_3-induced yield loss may be correlated with a reduction in flag leaf duration (Pleijel *et al*. 1998) as well as perturbation of assimilate allocation (Meyer *et al*. 1997).

Water stress can reduce stomatal conductance (g_s) resulting in lower ozone uptake rate and thus positively affect ozone damage and yield decrease of wheat (Mortensen 1990, Herbinger *et al*. 2002, Khan and Soja 2003). No O_3 effects existed only at the severe water stress (Khan and Soja 2003). However, it is very complicated to determine or separate drought effect and O_3 damage, because both stresses together may disturb the stomatal control (Maier-Maercker and Koch 1995). To our knowledge, there exist only a few researches about the drought effects on yield and physiology of ozone-exposed winter wheat.

In an attempt to compare yield response of winter

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[***]Corresponding authors; fax: +861082590815, e-mail: liyonggeng@ibcas.ac.cn, jianggm@126.com
Abbreviations: C_i – intercellular CO_2 concentration; Chl – chlorophyll; DAA – days after anthesis; E – transpiration rate; ETR – electron transport rate; F_v/F_m – photochemical capacity of photosystem 2 in the dark adapted state; g_s – stomatal conductance; HI – harvest index; P_N – net photosynthetic rate per unit leaf area at saturation irradiance; PAR – photosynthetically active radiation; PS2 – photosystem 2; q_P – photochemical quenching; SWC – soil water content.
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wheat and its physiological reactions after short-term high O_3 pollution to soil water supply, two irrigation strategies were applied. Parameters connected with photosynthesis as well as final yield components were studied. Such investigation may inform what kind of measure can be taken to reduce further yield loss after O_3 pollution; on the other hand, it may help forecast the yield loss caused by O_3 exposure and other following stresses.

Materials and methods

Plants: The experiment was conducted at the research station of the Institute of Botany, the Chinese Academy of Sciences (39°92'N, 116°46'E). Winter wheat (*Triticum aestivum* L. cv. Jingdong 8) seeds were sown in 20 cm (diameter) × 30 cm (height) pots filled with 10 kg clay loamy soil. Five healthy plants were kept. Nutrients and water were supplied sufficiently to avoid potential nutrient and drought stresses. Wheat reached anthesis stage when more than 50 % main-stem ears flowered on May 5, 2005.

Ozone exposure: When ears emerged, 48 pots of wheat were randomly separated into four open-top chambers (OTCs, 1.8 m in diameter and 2.4 m in height) for pre-adaptation till anthesis with charcoal-filtered air [<5 g $kg^{-1}(O_3)$]. The gas dispensing system of the OTC was constructed according to Uprety (1998). During O_3 exposure, two chambers were injected with O_3 generated using ambient oxygen by electrical discharge with an ozone generator (*JQ-6A*, *Telijie Co.*, Beijing, China). O_3 concentration was maintained at 0.125±0.012 g kg^{-1} (09:00–16:00) for 4 d, and the other two OTCs were not. The concentration was monitored by an ambient ozone monitor (*APOA-360*, *Horiba*, Japan) continuously using a cross flow modulated ultraviolet absorption method. In order to diminish chamber effects, pots were rotated between the chambers and randomized within the chambers every other day. Before and during O_3 exposure, all plants were well irrigated. The max/min temperature and relative humidity in the OTCs were 32/17 °C and 60/47 %, respectively. The photosynthetic photon flux density (PPFD) inside the OTCs was averaged at 600 μmol m^{-2} s^{-1}.

Drought treatment: After O_3 exposure, plants were moved out of the greenhouse. Those exposed to elevated O_3 were divided into 2 groups. One group was well watered each day to maintain soil water content (SWC) at 80–85 % (O_3+W) and the other was watered less to exert drought stress (SWC 35–40 %; O_3+D). The charcoal-filtered plants during O_3 exposure were well watered and taken as control. The plants were watered at 18:00 everyday. During the experimental period, the weather was typical for late spring in Beijing, with a mean daily air temperature from 17 to 32 °C and daily maximum photosynthetic photon flux density (PPFD) of about 2 000 μmol m^{-2} s^{-1} at midday.

Visible injury and green flag leaf area were measured using a leaf area meter (*AM100*, *ADC*, UK) on 4, 6, 13, 20, 25, and 27 d after anthesis (DAA).

Gas exchange was measured on the green portion of flag leaves using an open infra-red gas-exchange system (*GFS-3000*, *H. Walz*, Germany) between 09:30 and 11:30 on 0, 2, 9, and 16 d after the end of O_3 exposure, *i.e.* on 4, 6, 13, and 20 DAA, using ambient CO_2 (380±5 mg kg^{-1}). Leaf cuvette environment was controlled at PAR of 1 500 μmol m^{-2} s^{-1}, relative humidity of 60 %, and temperature of 25 °C. Leaf sample was adapted to the cuvette conditions for 5 min before readings were recorded. Irradiance saturated net photosynthetic rate (P_N), g_s, intercellular CO_2 concentration (C_i), and transpiration rate (E) were calculated according to Caemmerer and Farquhar (1981).

Chlorophyll (Chl) *a* fluorescence was measured using a fluorometer (*PAM 2100*, *H. Walz*, Germany) on the green portion of the same flag leaf whose gas exchange was measured. After a 40 min dark adaptation, the minimum (F_0) and maximum fluorescence (F_m) were determined with modulated irradiation (<0.1 μmol m^{-2} s^{-1}) and 0.8 s saturating pulse (>8 000 μmol m^{-2} s^{-1}), respectively. For measurement of quenching components, the sample was continuously irradiated with "white actinic light" of 400 μmol m^{-2} s^{-1}. The steady state fluorescence (F_s) was reached within 5 min after a second saturating pulse was imposed to determine maximum fluorescence in the irradiation-adapted state (F_m'). Minimum fluorescence in the irradiation-adapted state (F_0') was determined at far-red irradiation during a short interruption of actinic irradiation. The maximum photochemical efficiency in the dark adapted state (F_v/F_m), electron transport rate (ETR), photochemical quenching coefficients (q_P), and non-photochemical quenching coefficients (q_N) were calculated as $F_v/F_m = (F_m - F_0)/F_m$, ETR = Yield×PAR/2× ETR-factor, where Yield = $(F_m' - F)/F_m'$; PAR/2, the absorbed photon energy, is assumed equally distributed between photosystems (PS) 1 and PS2; ETR-factor, fraction of incident radiation absorbed by the leaf sample, is approximately 84 %; $q_P = (F_m' - F_s)/(F_m' - F_0')$ and $q_N = 1 - (F_m' - F_0')/(F_m - F_0)$, respectively (van Kooten and Snel 1990, Krause and Weis 1991, Schreiber 2004).

Chl contents were measured on 6, 13, and 20 DAA just after gas exchange and fluorescence measurements. Green sections were sampled and homogenized with 20 cm^3 of 95 % ethanol and the amounts were calculated according to Arnon (1949).

Yield: The above-ground main stems were harvested and dried at 70 °C to constant mass. Grains of each ear were threshed by hand. The number of grains per ear was counted and total dry mass as well as 1 000-grain mass were determined. Harvest index (HI) was calculated as the ratio of grain dry mass to total above-ground dry mass per stem.

Statistics: Each plant from individual treatment was taken as replicate. Replicates were 4 for all the measurements except yield (20). Means of each parameter were compared among treatments using a one-way analysis of variance (ANOVA) in the General Linear Model Procedure of SPSS (version *12*, *SPSS*, Chicago, IL, USA). Least significant differences (LSD) were considered between individual treatments when the *F*-test of the ANOVA was significant at $p<0.05$. Pearson's correlation test determined the relationships between the photon-saturated P_N and other measured physiological parameters.

Results

Visible injury and green flag leaf area: O_3 exposure caused visible injury in the form of necrotic stipples at the inter-veinal areas of the end sections of leaves. Around 10 % of a flag leaf was damaged. Green flag leaf area of O_3+W was reduced, but the reduction was lower than in O_3+D. Significant ($p<0.05$) difference of green flag leaf area between O_3+W and O_3+D occurred after 13 DAA, *i.e.* 9 d after the end of O_3 exposure (Fig. 1).

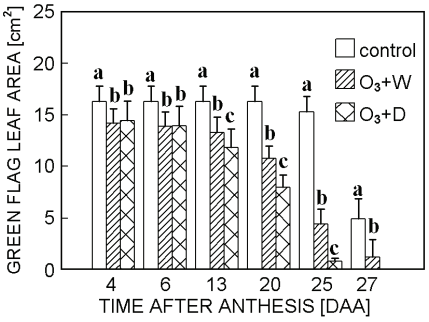

Fig. 1. Changes of green flag leaf area of winter wheat (*Triticum aestivum* L. cv. Jingdong 8) in control, O_3+W, and O_3+D plotted against days after anthesis (DAA). *Error bars* show S.E., $n = 10$. Significant differences (ANOVA and LSD-test at the 95 % level) among the treatments are indicated by *different letters*.

Gas exchange: P_N was considerably reduced (–23.4 %, $p<0.001$) by high O_3 exposure, but under O_3+W the P_N was similar to that of control on 2 d after the end of O_3 exposure (6 DAA). However, significant reductions occurred after 13 DAA (Fig. 2*A*). Drought after O_3 treatment caused considerable reductions in P_N. On 20 DAA, P_N of O_3+D was only 20 % while that of O_3+W was 80 % of the control values (Fig. 2*A*).

The g_s showed similar response trend under the different treatments (Fig. 2*B*). Shortly after O_3-treatment, C_i of O_3-exposed wheat was significantly ($p<0.01$) lower than that of control. Under O_3+W, C_i was marginally ($p>0.05$) or significantly ($p<0.05$) higher than that of control, while C_i of O_3+D was similar on 6 and 13 DAA but significantly higher ($p<0.05$) on 20 DAA in relation to control (Fig. 2*C*). There was no significant difference of E between O_3+W and control, but E of O_3+D was dramatically ($p<0.001$) suppressed (Fig. 2*D*).

Chl *a* fluorescence: Short-term high O_3 exposure caused significant ($p<0.05$) decrease in maximum efficiency of PS2 photochemistry (F_v/F_m). After O_3 exposure, F_v/F_m of O_3+W was marginally ($p>0.05$) lower on 6 DAA, but later it decreased significantly ($p<0.05$) over that of control. Drought after O_3 exposure (O_3+D) resulted in considerable ($p<0.01$) decrease in F_v/F_m, especially after

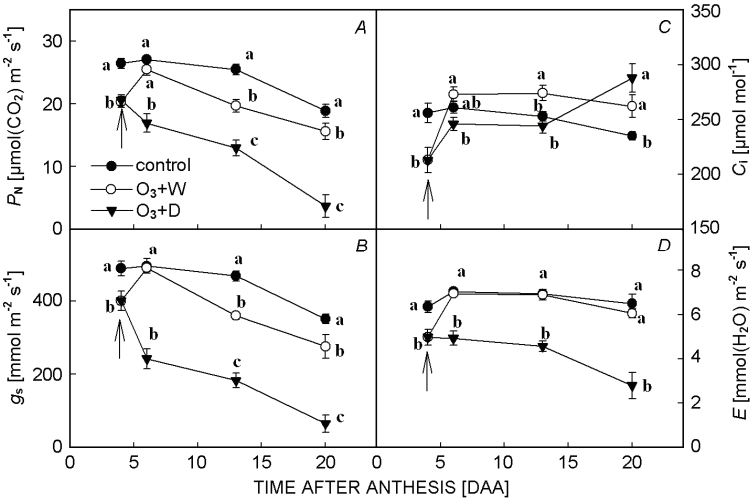

Fig. 2. Irradiance-saturated net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and transpiration rate (E) of winter wheat (*Triticum aestivum* L. cv. Jingdong 8) in control, O_3+W, and O_3+D plotted against days after anthesis (DAA). *The arrow* indicates the removal of O_3-exposure and the beginning of drought treatment. *Error bars* show S.E., $n = 4$. Significant differences (ANOVA and LSD-test at the 95 % level) among the treatments are indicated by *different letters*.

13 DAA (Fig. 3*A*). Similar trends but larger reductions were found in ETR and q_P of the O_3-exposed wheat under both water treatments (Fig. 3*B*,*C*), while q_N was significantly (p<0.05) higher in O_3+W than that of control, and it was considerably increased in O_3+D (Fig. 3*D*).

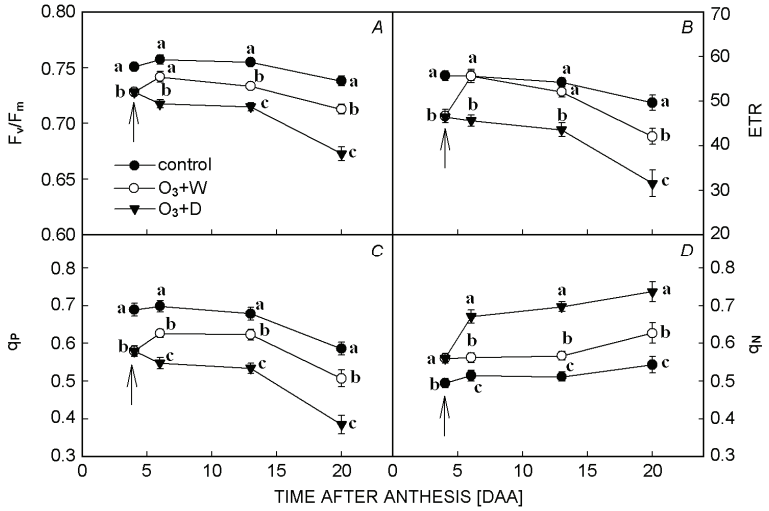

Fig. 3. The maximum photochemical efficiency in the dark adapted state (F_v/F_m), electron transport rate (ETR), and photochemical (q_P) and non-photochemical (q_N) quenching coefficients of winter wheat (*Triticum aestivum* L. cv. Jingdong 8) in control, O_3+W, and O_3+D plotted against days after anthesis (DAA). *The arrow* indicates the removal of O_3-exposure and the beginning of drought treatment. *Error bars* show S.E., n = 4. Significant differences (ANOVA and LSD-test at the 95 % level) among the treatments are indicated by *different letters*.

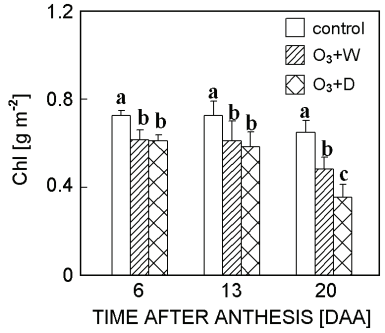

Fig. 4. Contents of total chlorophyll (Chl) of winter wheat (*Triticum aestivum* L. cv. Jingdong 8) in control, O_3+W, and O_3+D plotted against days after anthesis (DAA). *Error bars* show S.E., n = 4. Significant differences (ANOVA and LSD-test at the 95 % level) among the treatments are indicated by *different letters*.

Correlation between P_N and other physiological parameters: P_N had significant positive correlations (p<0.01) with g_s and E, but not with C_i. There were also significant positive correlations (p<0.01) between P_N and F_v/F_m, ETR, and q_P. Notable (p<0.001) negative correlation between P_N and q_N was found. The content of Chl was also markedly (p<0.001) correlated with P_N (Table 1).

Yield: Grain yield of O_3+W was 21 % lower than that of control and the difference was statistically significant (p<0.001). Grain yield of O_3+D was further significantly lower (–36 %, p<0.001) than that of control. There was no difference in amount of kernels per ear between O_3+W and control, but 15 % fewer kernels per ear of O_3+D than that of control were observed and the difference was statistically significant (p<0.01) (Table 2). Grain production as indicated by 1 000-grain mass was notably (–20 %, p<0.001) lower in O_3+W and O_3+D than in control, but there was no statistical difference of 1 000-grain mass between O_3+W and O_3+D. No significant differences in straw mass among the treatments were noted (data not shown). Nevertheless, remarkable (p<0.001) decreases in HI were observed in both O_3+W and O_3+D (Table 2).

Chl content: Two days after O_3 exposure (6 DAA) there was no significant difference in the contents of Chl between O_3+W and O_3+D, which were significantly lower by 15.3 and 16.1 %, respectively, than that of control. However, after 13 DAA, Chl content of O_3+D was notably lower than that of O_3+W (Fig. 4).

Table 1. Coefficient r^2 and p-values of linear relationships between P_N and other physiological variables. For abbreviations see the text.

	r^2	p
g_s	0.93	<0.001
C_i	–0.16	0.350
E	0.85	<0.010
F_v/F_m	0.909	<0.010
ETR	0.962	<0.001
q_P	0.965	<0.010
q_N	–0.917	<0.001
Chl	0.904	<0.001

Discussion

Plants at the reproductive stage are usually more sensitive to high ozone exposure than at vegetative stage (Lee *et al*. 1988, Slaughter *et al*. 1989, Pleijel *et al*. 1998). In our experiment, short-term O_3 exposure after anthesis caused a visible damage symptome, the still green portion of leaf showed accelerated senescence and shortened green leaf area duration, which are the typical effects of high O_3 exposure (Grandjean and Fuhrer 1989, Pell *et al*. 1992, Ojanpera *et al*. 1998).

Numerous studies have clearly documented that high O_3 exposure results in decreased photosynthetic carbon assimilation, as recently reviewed by Fiscus *et al*. (2005). We found that under good watering, P_N of O_3-exposed wheat was similar to that of control on 2 d after the cessation of O_3 exposure but later it decreased substantially. This indicated that acceleration of leaf senescence was triggered by short-term O_3-exposure, which is consistent with the results of Mulholland *et al*. (1997). Drought after O_3-exposure caused significant reduction in P_N. Although this reduction was parallel with decrease in g_s, the C_i of O_3+D was not much different or even significantly higher than that of control, indicating the depression of P_N caused by drought after O_3 pollution was a non-stomatal limitation (Cornic 2000, Calatayud *et al*. 2002).

Table 2. Kernel number per ear, 1 000-grain mass, yield, and harvest index (HI) of winter wheat (*Triticum aestivum* L. cv. Jingdong 8) under different treatments. *Different letters* (a–c) after mean±SE denote statistically significant differences between treatments (ANOVA and LSD test at the 95 % level, $n = 20$).

Treatment	Kernels per ear	1 000-grain mass [g]	Yield [g per ear]	HI [%]
control	44.78±1.42a	50.28±1.52a	2.238±0.050a	49.26±0.31a
O_3+W	44.20±1.71a	39.68±1.09b	1.759±0.110b	44.11±0.98b
O_3+D	38.25±1.65b	38.90±1.50b	1.487±0.100c	41.21±1.10b

The O_3 stress usually decreases maximum efficiency of PS2 photochemistry (F_v/F_m) of plant leaves (Meyer *et al*. 1997, Degl'Innocenti *et al*. 2003, Calatayud *et al.* 2006). We found that the reduction in F_v/F_m at the end of exposure was accompanied by a decrease in q_P and ETR and consequently by an increase in q_N, indicating an increased fraction of closed PS2 reaction centres and an enhancement of dissipation of excess excitation energy *via* non-radiative mechanisms due to an inhibition of CO_2 fixation (Guidi *et al*. 1997). Two days after the cessation of O_3 exposure, there was no significant difference in F_v/F_m between O_3+W and control, implying recoverable damage by O_3 stress (Grimm and Fuhrer 1992). However, faster decrease in F_v/F_m and q_P of O_3+W than those of control were observed on 13 DAA. These findings might indicate that high O_3 exposure exerted profound damage to the leaf tissue and therefore aroused acceleration of leaf senescence (Farage *et al*. 1991, Pell *et al*. 1992, Zheng *et al*. 2002). Drought after O_3 treatment reduced F_v/F_m, q_P, and ETR notably but increased q_N significantly, implying drought aroused an increased fraction of closed PS2 reaction centres and an enhancement of dissipation of excess excitation energy *via* non-radiative mechanisms due to an inhibition of CO_2 fixation (Guidi *et al*. 1997).

Although P_N had significant positive correlations with g_s, it also showed notable positive correlations with F_v/F_m, ETR, q_P, as well as with the content of Chl. Moreover, there was no significant correlation between P_N and C_i. This indicated that changes of photosynthesis caused by O_3-exposure and drought treatment resulted mainly from the reduced carboxylation.

The period of grain filling is strongly linked to (flag) leaf duration and the leaf area duration is of crucial importance for the final grain yield of wheat (Ojanpera *et al.* 1998). Ozone exposure induces the activation of plant leaf senescence-related processes (Ribas *et al*. 2005). In our experiment, the fast decreases in the measured physiological parameters of O_3-exposed wheat under good water conditions indicated short-term high O_3 exposure caused acceleration of leaf senescence, and as a consequence significant yield reduction of O_3+W was found. The kernel number per ear was not affected, but the grain size indicated by 1 000-grain mass was significantly decreased, which indicated that the main cause for the yield reduction might be the source limitation as pointed out by previous studies (Amundson *et al*. 1987, Slaughter *et al*. 1989, Pleijel *et al*. 1991, 1998, Gelang *et al*. 2000, Meyer *et al*. 2000). Under drought, the photosynthesis as well as flag leaf duration of the O_3-exposed wheat was further reduced, and consequently further significant decrease in grain yield of O_3+D was observed. This was mainly attributed to fewer kernels per ear due to an increase in the number of infertile florets per spikelet (data not shown). Therefore, attention should be paid to the effects of drought on crops polluted by high O_3.

Short-term high ozone exposure to winter wheat after anthesis significantly decreased P_N of winter wheat, enhanced leaf senescence, and finally decreased grain yield. Drought after O_3 pollution caused further serious yield loss by reducing photosynthesis and leaf area duration. Our findings suggest that optimal soil water condition reduces further yield loss of winter wheat after ozone pollution episodes.

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