Chronic drought stress reduced but not protected Shantung maple (*Acer truncatum* Bunge) from adverse effects of ozone (O_3) on growth and physiology in the suburb of Beijing, China

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ABSTRACT

A two-year experiment exposing *Acer truncatum* Bunge seedlings to elevated ozone (O_3) concentrations above ambient air (AO) and drought stress (DS) was carried out using open-top chambers (OTCs) in a suburb of Beijing in north China in 2012–2013. The results suggested that AO and DS had both significantly reduced leaf mass area (LMA), stomatal conductance (Gs), light saturated photosynthetic rate (A_{sat}) as well as above and below ground biomass at the end of the experiment. It appeared that while drought stress mitigated the expression of foliar injury, LMA, leaf photosynthetic pigments, height growth and basal diameter, due to limited carbon fixation, the O_3 – induced reductions in A_{sat} , Gs and total biomass were enhanced 23.7%, 15.5% and 8.1% respectively. These data suggest that when the whole plant was considered that drought under the conditions of this experiment did not protect the Shantung maple seedlings from the effects of O_3 .

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1. Introduction

Ozone (O_3) is the most important phytotoxic pollutant threatening forests productivity and health in many parts of the world (Matyssek et al., 2014; Ferretti et al., 2007; Matyssek and Sandermann, 2003; Ollinger et al., 1997). Due to fossil fuel and biomass burning, O_3 concentrations in east Asia are predicted to increase in the future, with background possibly reaching 70 ppb by 2100 (Sitch et al., 2007). China, as an important country in East Asia, has experienced phenomenally rapid economic expansion in the past several decades, but it also resulted in more emissions of O_3 precursors such as NO_x due to increased consumption of fossil fuels (Wang et al., 2007). High levels of O_3 had been observed in many areas and cities of China (Tang et al., 2009; Wang et al., 2009, 2012; Zhang et al., 2014; Zheng et al., 2011), especially in Beijing. According to Ding et al. (2008) from aircraft observations over Beijing, the O_3 concentration was increasing approximately at a rate of 2%

per year from 1995 to 2005. Also, through ozonesonde measurements from 2002 to 2010, the summer O_3 maximum concentration in Beijing frequently exceed 120 ppb and exceed 160 ppb in some cases (Wang et al., 2012b). Recently, Wan et al. (2014) reported that the average O_3 levels in Beijing ranged from 22.5 to 48.1 ppb with a maximum value of 195.8 ppb during July to August in 2012. Due to high O_3 concentration, plants injury including native and cultivated trees, shrubs, and herbs have been found widespread in and around Beijing now (Feng et al., 2014; Wan et al., 2014). Therefore, there is an increasing concern about O_3 damage to forest trees. Besides O_3 , trees are often also subjected to periodic drought in North China during summer. Global climate change is expected to enhance elevated temperatures, affect incidence and intensity of precipitation, which may increase the probability of regional drought. The frequency and the severity of drought events in several regions and particularly in the northern hemisphere have been predicted (Saxe et al., 2001).

Drought incidence in summer can coincide with episodes of elevated O_3 concentrations, which are high enough to cause plant injury and growth reduction. Many investigations have focused on interactions to determine possible ways in which drought would affect O_3 injury and growth effects. Possible interactions have been

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determined to be antagonistic, neutral (additive) or synergistic.

Some interactions were antagonistic. Drought stress appeared to protect plants from O₃ injury and loss mainly through its influence on reducing stomata aperture (Beyers et al., 1992; Temple et al., 1992; Wieser and Havranek, 1995); some interactions were neutral (additive). Drought didn't change O₃ damage and the effects of drought and ozone were independent in the mechanism (Pearson and Mansfield, 1994); some were synergistic and even negative. Drought did not protect trees from O₃ effects but further enhanced O₃ damage. It was reported that drought increase the sensitivity to O₃ (Pollastrini et al., 2014; Alonso et al., 2013) and slowed or even stopped stomata response to abrupt stresses (Paoletti, 2005), which may cause a stronger reduction and may actually increase the overall oxidative risk as well due to limited carbon fixation (Matyssek et al., 2006; Tausz et al., 2007).

Maple species, as an important component of temperate forests, have been reported frequently on the response to elevated O₃. Some studies have shown that maple species with a relatively high sensitivity to elevated O₃, such as sycamore maple (*Acer opalus* Mill. and *Acer pseudoplatanus* L.) in Mediterranean region (Calatayud et al., 2007); Red maple (*Acer rubrum* L.) in North America (Davis and Skelly, 1992); Sugar maple (*Acer saccharum* Marsh) in France (Gaucher et al., 2003) and eastern North America (Topa et al., 2001). But some studies also showed that the more tolerance in some species resulting in low O₃ uptake whether under laboratory or field conditions, such as Montpellier maple (*Acer monspessulanum*) in Mediterranean region (Calatayud et al., 2007); Red maple (*A. rubrum* L.) in Pennsylvania, USA (Schaub et al., 2005; Wei et al., 2004); Sugar maple (*A. saccharum* Marsh) in north (Karnosky et al., 2005) and east of America (Rebbeck, 1996); Sycamore Maple (*A. pseudoplatanus* L.) in Switzerland (VanderHeyden et al., 2001). So far, information regarding the response of Asia maple species to O₃ in Asia under ozone and interactive with global change is rather limited or absent.

Shantung maple (*Acer truncatum* Bunge), a particular *Acer* species in China, is an important widespread prominent species in the hardwood forests from north to west China, and widely planted as landscape trees for its brilliant leaf color in autumn. It's also used for timber, medicine and food due to sturdy texture and high contents of chlorogenic acid, tannin, flavonoids (Ma et al., 2005) and oil in seeds. We selected Shantung maple as a representative maple tree species in north China. So far, no research about *A. truncatum* Bunge response to O₃ has been reported, especially interactive effects with drought stress. In this paper, the response of *A. truncatum* Bunge to ozone and drought alone and in combination in foliar injury, physiological, gas exchange and growth responses will be studied. Our hypothesis is: (1) Both O₃ and drought will influence the growth and physiology of Shantung maple; (2) Synergistic interaction can be expected but drought will aggravate the adverse effects from O₃ due to limited carbon fixation. However, in a natural environment the roots of trees are not constrained in a small flowerpot. So we planted the seedlings directly in the ground.

2. Materials and methods

2.1. Experimental site and plant material

The experimental site was located in the Chang Ping district, a suburb of Beijing, China (40°12'N, 116°08'E). Beijing has a typical temperate and monsoonal climate with four clearly distinct seasons. The annual mean precipitation is 550.3 mm and the mean air temperature is 11.8 °C (Tong et al., 2012). The soil is generally characterized as moist with moderate fertility with an organic content of 16.4 g kg⁻¹, total nitrogen content of 0.9 g kg⁻¹, rapidly available phosphorus content of 102.1 mg kg⁻¹ and a PH of 8.3. The

soil texture of the OTC site has no limits to the growth of Shantung maple (*A. truncatum* Bunge) compared with their requirements at natural conditions.

One year old Shantung maple (*A. truncatum* Bunge) seedlings were obtained from a nearby nursery on April 2nd and then were well-planted in the ground outside the chambers until May 30th. Then 120 uniform seedlings were transplanted into the ground of 12 open-top chambers (OTCs) from May 30th to acclimate the OTCs environment until July 28th.

2.2. OTCs

The design of OTCs was improved on the one we used before (Zheng et al., 2011). The improvements include larger size, more durable material, and a rain cap. The twelve cylindrical OTCs (2.5 m in height and 2 m in diameter, with an inside area of 3.14 m²), 2 m apart from each other, were made of an aluminum alloy frame covered with 2 mm-transparent plastic (85% transparency). A rain cap was located 25 cm above the frustum to prevent rain from intruding into the chamber yet allowed ambient air to freely circulate. For each of the open-top chambers selected for drought treatments, there was a 70 cm deep by 25 cm wide trench dug around the inside of the chamber and covered with impermeable plastic sheeting. These trenches served as a barrier to both reduce the transfer of surface water entering the chamber as well as further prevent rain water from entering the chambers. The difference of average air temperature from 8:30 am to 17:30 pm inside and outside of the chamber was no more than 2 °C.

2.3. Ozone (O₃) concentration control and distribution

Ozone (O₃) concentrations in 12 chambers were monitored sequentially using an automated time-sharing system connected to an ozone monitor (ML 9810, Teledyne Monitor Labs, Englewood, USA) and all data were stored using a data logger (CR1000, Campbell Scientific Inc., Logan, UT, USA). Target O₃ concentration was generated from pure medical oxygen using the high-voltage discharge method (CFG-140, Jinan Sankang Envi-tech, Jinan, China) and the flow of oxygen was manually controlled by a glass

Fig. 1. Soil moisture contents and O₃ concentrations during experiment in 2012 (A and C) and 2013 (B and D) under two O₃ treatments (AA and AO) and two water stress treatments (WW and DS).

rotameter (LZB-2, Suzhou Chemical Instrument, Suzhou, China). Ambient air or O₃-added air delivered by a 750 W centrifugal blower was released from a 13 cm diameter two-way connector which was placed in the middle ground of each chamber with an aluminum tube. The ML 9810 O₃ analyzer was calibrated every month with a Model 49i-PS O₃ analyzer. When the O₃ concentration within OTC is beyond 10% of target O₃ concentration, rotameter is tuned to achieve target O₃ concentration. Pre tests showed that O₃ concentrations were evenly distributed, both vertically and horizontally, within the OTCs.

2.4. Soil moisture level control

Water levels were set among twelve chambers by means of fixed quantity watering. When drought stress treatments started, seedlings were stopping watering and then different water volumes were supplied for different water stress treatments until the water moisture contents reduced to nearly the same low level (Fig. 1). Well-watered treatments were provided almost 18 L of water weekly per chamber while drought stress treatments were provided 9 L. Irrigation frequency and volume of water varied a small amount during the hottest summer which depended on soil moisture contents and soil water potential measured. Ten soil moisture sensors (EC-5, Decagon Device, UK) and five soil moisture potential sensors (MPS-2, Decagon Device, UK) were allocated to 12 chambers. All soil sensors were placed at 15 cm depth of the roots area and data was collected every 15 min with a data logger (EM 50, Decagon Device, Campbell Scientific, UK).

2.5. Experimental design and sampling

The distribution of the 12 chambers with treatments in the field was randomized block design. Two separate water treatments (well watered-WW and drought stress-DS) and two different ozone (O₃) concentrations (ambient air-AA and elevated O₃ above ambient air-AO) were arranged among 12 OTCs. The target soil moisture contents (SMC) of WW was set as 75% of field water capacity while DS was 60%–70% compared to WW. The target O₃ concentration of AO was 60 ppb above ambient air in 2012. Since July 26th of 2013, the O₃ concentration of AO was set as 100 ppb above ambient air because no significant O₃-induced effects were found on most of the indicators measured under AO treatment of the first year. The set of O₃ target concentration was based on the present regional concentration situation in Beijing and possible O₃ trends in future. So four treatments were administered with three replicates among chambers: 1): AA-WW (the control) = ambient air (AA) + well watered (WW). 2): AO-WW (elevated O₃) = AO + WW. 3): AA-DS (Drought) = AA + DS. 4): AO-DS (Elevated O₃ and drought) = AO + DS. The experiment treatments lasted two growth seasons from 2012 to 2013. The cumulative O₃ exposure was described as AOT40 (Kärenlampi and Skärby, 1996), which was calculated as the

Table 1

Basic experimental date (Day-Month) information on the seedlings transplanting time (STT), beginning time of ozone treatments (BOT), beginning time of drought treatments (BDT), ending time of all treatments (ET), harvesting time (HT) sampling date (S-date) and sampling number (SN) from 2012 to 2013.

Year	STT	BOT	BDT	ET	HT	S-date ^b	SN
2012	30-May ^a	28-Jul.	3-Aug.	25-Oct.		15-Sept.	S1
						10-Oct.	S2
2013		28-Apr.	16-May.	15-Oct.		7-Jul.	S3
						22-Aug.	S4
						16-Oct.	S5

^a The date transplanted inside the chambers.

^b The day of gas exchange and sampling time was the same.

Table 2

Mean O₃ concentrations, AOT40 and soil moisture contents under two O₃ treatments (AA and AO) and two water stresses (WW and DS) during the sampling time (S1–S5).

SN	S1	S2	S3	S4	S5
AA (ppb)	41.9	34	45.8	52.7	42.6
AO (ppb)	109	102	113	147	141
AOT40-AA (ppm h)	2.65	2.65	3.9	8.72	9.27
AOT40-AO (ppm h)	21.1	28.2	22.9	48.6	75.6
WW (%)	21.2	20.4	15.8	17.1	16.5
DS (%)	14.7	13.6	10.5	11.6	10.7

Sampling number – SN. The accumulation elevated O₃-exposure periods for the AOT40 see Table 1.

accumulated hourly mean O₃ concentrations over 40 ppb during O₃ fumigation period (Table 2). The experiment treatments lasted two growth seasons from 2012 to 2013. The O₃ fumigation period began from July 28th to October 15th in 2012 and April 28th to October 15th in 2013 (Table 1). The daily O₃ fumigation time was from 8:30am to 17:30 pm. The drought stress treatments began from August 3rd in 2012 and May 16th in 2013 and the ending time was same as O₃ treatment.

Samples were taken two times in 2012(S1 and S2) and three times (S3–S5) in 2013 (Table 1). Four trees were selected from ten trees per chamber avoiding edge effects per chamber and the medium-aged, sun leaves were taken as samples in a sunny day at intervals of more than one month. Irrigation was stopped before growth measurements and samplings. Except for the biomass measurements, all remaining samplings were taken after the gas exchange measurements were made. Four additional leaves were punched for sampling, avoiding the mid-vein. One part of the samples was immediately put into 4 °C incubator for chlorophyll content analysis for 2–3 days while the other leaf parts were set aside in an oven for the determination of leaf mass per area (LMA).

2.6. Measurements

2.6.1. Foliar visible injury

All seedlings were evaluated daily until symptoms first appeared and then twice weekly thereafter. Dates of first foliar injury appearance were recorded. Comparisons of the plants within AA and AO treatments were made to confirm elevated O₃ as the cause of the visible injury.

2.6.2. Growth and biomass measurements

Stem diameter, height and the size of leaves were measured five times in accordance with sampling time. All plants in chambers were harvested at the end of two years experiment (October, 2013). Root, stem and leaves were separated and the dry weights were determined after drying at 70 °C to constant weight.

2.6.3. Leaf water potential

Leaf water potential was measured after gas exchange measurement by using a PSYPRO water potential measurement system (PSYPRO, Wescor, USA).

2.6.4. Gas exchange measurement

We measured light-saturated net photosynthetic (A_{sat}) and stomatal conductance (G_s). Two fully expanded upper sun leaves per seedling from three different marked plants per chamber were selected. Leaf gas exchange was measured using a portable Li-Cor 6400 photosynthesis system-6400-40 leaf chamber fluorometer (LCF) (Li-Cor Inc., Lincoln NE, USA). The system controlled saturating PPFD at 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, CO₂ at 400 ppm with a supply from pure CO₂ cylinder. All measurements were conducted during

Fig. 2. O_3 foliar injury symptoms of *A. truncatum* under AO-WW treatment (A) and AO-DS treatment (B) on September 15th, 2013.

09:00–11:30 am and 14:00–15:00 pm.

2.6.5. Leaf chlorophyll contents and leaf mass per area (LMA)

50 mg of leaf tissue per tree seedling from three trees in each chamber were extracted with 4 ml 95% ethanol in the dark for 48 h

Fig. 3. Mean value (mean $\pm$ S.E.) of chloroplast pigments (Chl a: chlorophyll a, Chl b: Chlorophyll b, Car: Caroten) contents under four treatments (AA-WW: the control, AO-WW: elevated O_3 , AA-DS: drought stress, AO-DS: elevated O_3 + drought stress) in 2012 (A) and 2013 (B). Letters above bars indicate significant differences between treatments when $P < 0.05$. Letters above bars indicate significant interactive effects between drought and elevated O_3 . ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$. S1–S5 represent the sampling time (see Table 1).

at room temperature. The absorbance of leaf pigment extracts was measured at 646, 450 and 663 nm using a spectrophotometer (UV2101PC, Shimadzu, Kyoto, Japan) for chlorophyll a (Chla), chlorophyll b (Chlb) and carotene (Car). Chlorophyll contents were calculated according to the specific absorption coefficients provided by Lichtenthaler (1987). 24 leaf discs from each plant were dried for 48 h at 70 °C to constant weight then calculate LMA as dry mass per area.

2.7. Statistical analyses

Differences and interactive effects between the O_3 and drought treatments among parameters were measured by two-way ANOVA, with drought and O_3 levels as independent factors. Post-hoc comparisons were completed using the LSD-test. Prior to analysis, data were checked for normality (Kolmogorov–Smirnov test) and homogeneity of variance (Levene's test). Results were considered

Fig. 4. Mean value (mean $\pm$ S.E.) of net photosynthesis rates (A_{sat}), stomatal conductance (G_s) under four treatments (AA-WW: the control, AO-WW: elevated O_3 , AA-DS: drought stress, AO-DS: elevated O_3 + drought stress) in 2012 (A) and 2013 (B). Letters above bars indicate significant differences between treatments when $P < 0.05$. Letters above bars indicate significant differences between drought and elevated O_3 . ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$. S1–S5 represent the sampling time (see Table 1).

Fig. 5. Mean value (mean ± S.E.) of leaf water potential (LP), Leaf mass per unit area (LMA) under four treatments (AA-WW: the control, AO-WW: elevated O₃, AA-DS: drought stress, AO-DS: elevated O₃ + drought stress) in 2012 (A) and 2013 (B). There was no LP data in S1. Letters above bars indicate significant differences between treatments when P < 0.05. Different letters above between treatments. D × O indicates Interactive effects between drought and elevated O₃. ***: P < 0.001; **: P < 0.01; *: P < 0.05. S1–S5 represent the sampling time (see Table 1).

significant at $p < 0.05$. All the analyses were performed with SPSS statistics software (Version 17.0, SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Ozone (O₃) exposure and soil moisture

Ozone (O₃) fumigation was stopped several times for rainy and

Fig. 6. Mean value (mean ± S.E.) of height and diameter of seedlings under four treatments (AA-WW: the control, AO-WW: elevated O₃, AA-DS: drought stress, AO-DS: elevated O₃ + drought stress) in 2012 (A) and 2013 (B). Letters above bars indicate significant differences between treatments when P < 0.05. S1–S5 represent the sampling time (see Table 1).

cloudy weather. The actual fumigation periods, however, were 57 days in 2012 and 110 days in 2013. The seasonal average O₃ concentration of ambient air was 34 ppb in 2012 and 42.6 ppb in 2013 during the 9 h of the experiment (see Table 2, Fig. 1). AOT40 during the O₃-exposure periods was 28.2 ppm h in 2012 (from late July to October, 2012) and 75.60 ppm h in 2013 (from May to October, 2013), respectively (Table 2).

Average Soil moisture contents (SMC) of WW and DS were 16.4% and 10.8% in 2013 while which were 18.9% and 14.2% in 2012 (Fig. 1). The soil moisture potential (SWP) of WW was kept nearly 0 while DS was nearly −0.75 Mpa (data not shown).

3.2. Foliar injury and physiology

Visible foliar injury typically progressed on upper leaf surfaces with chlorotic stippling, reddening, and premature leaf senescence. O₃-like symptoms first started to appear on older middle-aged leaves on August 8th exposed to AO-WW, when AOT40 was 39.9 ppm h (since April 28th, 2013). Treatments under AO-DS displayed foliar injury later on September 2nd when AOT40 was 51 ppm h (since April 28th, 2013). Visible symptoms were typically punctate purple-red stipples on the interveinal areas of upper leaf surfaces (Fig. 2).

Chlorophyll a (Chla), chlorophyll b (Chlb) and carotene (Car) in AO-WW seedlings were significantly reduced by 38.2%, 43.2% and 36.9% at the fifth sampling (S5) (Fig. 3). No significant effects were found in chlorophyll contents of AA-DS seedlings, although the drought stress was maintained throughout the experiment. Significant interactive effects were detected at the fourth sampling (S4) and the fifth sampling (S5) in AO-DS treatment. The data of S5 suggested that the drought stress protected chloroplast pigments from decreasing under elevated O₃ because the reductions of Chla, Chlb and Car in AO-DS seedlings were 16.9, 18.6, and 22.7% less than AO-WW.

A_{sat} significantly reduced in AA-DS seedlings in S2 and reduced both in AO-WW and AA-DS seedlings during the second year. Gs had mostly the similar trends with A_{sat}. Interactive effects in A_{sat} and Gs were found in AO-DS seedlings in S4 (Fig. 4). However, except for S4, A_{sat} and Gs under AO-DS treatments all showed more reduction during the second year.

Leaf water potential in DS treatments was significantly reduced (Fig. 5). LMA in AO-WW and AA-DS seedlings were both reduced at the end of second year. Interactive effect of LMA was found at the end of experiment in AO-DS, which showed the interactive effects alleviated a 7.5% reduction compared to AO-WW (Fig. 5).

3.3. Plant height, basal diameter and biomass

Significant reductions on height and basal diameter were detected in AO-WW seedlings since S4, which showed a 30.9% and 25.8% reduction in height and basal diameter, respectively (Fig. 6). No significant reductions were found in AA-DS seedlings.

At the end of second year, total, aboveground, leaf, stem and root biomass were all significantly reduced under AO-WW and AA-DS treatments (Table 3). Compared with all the parts biomass reductions under AO-WW treatment, reductions in root biomass had a minimal of 44.8% while reductions in leaf biomass had a maximum of 61.3%. Reductions caused by AA-DS treatment were from 29.8 to 31.3%. However, root/shoot ratio (R/S) was significantly increased by 42.1% in AO-WW seedlings but essentially no change was found in AA-DS seedlings (Table 3). Significant interaction effects were detected with total and aboveground biomass in AO-DS seedlings, which suggested that drought, caused reduction by 64.9 and 68.9%, much greater than AO-WW or AA-DS treatment (Table 3).

Table 3

Total biomass (TB), aboveground biomass (AGB), leaf biomass (LB) and stem biomass (SB), root biomass (RB), root/shoot (R/S) under four treatments (AA-WW: Ambient O₃ + Well watered, AO-WW: elevated O₃ + Well watered, AA-DS: Ambient O₃ + Drought stress, AO-DS: Elevated O₃ + Drought stress) at the end of second year.

Biomass	AA-WW	AO-WW	AA-DS	AO-DS	Significant effects		
					O	D	O × D
TB (kg)	1.28 ± 0.15a	0.56 ± 0.02c	0.89 ± 0.02b	0.45 ± 0.04d	***	***	*
AGB (kg)	0.93 ± 0.14a	0.36 ± 0.01c	0.64 ± 0.01b	0.29 ± 0.02d	***	**	*
LB (kg)	0.35 ± 0.01a	0.14 ± 0.01c	0.25 ± 0.04b	0.12 ± 0.01c	***	*	—
SB (kg)	0.58 ± 0.10a	0.22 ± 0.01c	0.40 ± 0.04b	0.17 ± 0.02c	***	**	—
RB (kg)	0.35 ± 0.04a	0.20 ± 0.02bc	0.25 ± 0.02b	0.16 ± 0.03c	***	**	—
R/S	0.38 ± 0.06a	0.54 ± 0.07b	0.39 ± 0.03a	0.56 ± 0.09b	***	—	—

Different letters followed by values indicate significant differences between treatments when $P < 0.05$. Significant effects are the statistical results of a two-way ANOVA, O-Elevated O₃ effects; D-Drought stress. ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; — indicates no significant results.

4. Discussion

4.1. Foliar injury

In our experiment, foliar injury first appeared on the upper side of older leaves under AO-WW treatments, then after nearly one month similar but slight symptoms appeared under AO-DS treatments. This suggests that drought may reduce foliar O₃ injury due to lower stomata conductance (Fig. 4). Various studies have also shown that water-stressed plants are more tolerant to O₃ than unstressed plants because of stomata closure and anatomical changes (Guerrero et al., 2013; Wittig et al., 2007).

Until now, there are few reports about Shantung maple (*A. truncatum* Bunge) sensitivity to O₃ in Asia. However, there have been similar research studies using OTCs addressing sensitivity of other maple species such as *A. pseudoplatanus* L. in Switzerland (VanderHeyden et al., 2001). The AOT40 threshold was found to be 25 ppm h and was considered the least sensitive species compared to other 14 native tree and shrub species. *A. Monspessulanum* was thought to be a resistant species in Spain, displayed the first injury symptoms when AOT40 was 28.9 ppm h after 79 days O₃ fumigation (Calatayud et al., 2007). According to Kohno et al. (2005), for moderate and low sensitive (tolerant) species in East Asia, AOT40 of O₃ in a growing season corresponding to 10% reduction of whole-plant dry mass was estimated to be greater than 31 ppm h for 6 months. Although the one year old seedlings we were using should be more sensitive to environmental stress than mature trees, as suggested by Heagle et al. (1989), compared with these results above, Shantung maple (*A. truncatum* Bunge) is more like an O₃ insensitive or tolerant species since the threshold for onset of the first foliar injury symptom was 39.9 ppm h during the second year while the 10% reduction of whole-plant dry mass occurred when the AOT40 was 36.8 ppm h (data from dose response on total AOT40 values and biomass reduction percentage). However, our control treatment was ambient air, not a charcoal filtered air treatment. The ambient air in this area was an O₃ polluted atmosphere with the AOT40 values were over plant protection thresholds according to the EU Directive (3-month AOT40 = 6 ppm h) or the CLRTAP critical level for forest (6-month AOT40 = 5 ppm h) in 2013 (Table 2), therefore, the species sensitivity to elevated O₃ would be accelerated due to such high background O₃ concentration.

4.2. The physiological and growth responses to elevated O₃

Our results showed that gas exchange, growth, biomass and related parameters were significantly decreased under O₃ fumigation by the end of experiments. Decreases in photosynthetic assimilation rates (Tausz et al., 2007), chlorophyll destruction (Wallin et al., 2002), growth and biomass (Topa et al., 2001), LMA

and leaf area (Wittig et al., 2009) are well known responses to enhanced O₃ levels.

Our results showed that compared with control, R/S ratio was significantly increased by 42.1% under AO-WW treatment but not affected by drought at all (Table 3). Although some research found that R/S ratio decreased under O₃ exposure (Landolt et al., 2000; Yamaji et al., 2003), the similar results of increased R/S ratio were also found, such as some clones of birch trees (*Betula pendula* Roth) (Yamaji et al., 2003) and red maple allocated greater biomass to roots (Ashton et al., 1999). The explanation for increased R/S ratio under AO-WW treatment of our results might be: 1.) *A. truncatum* may naturally have high levels of antioxidants (our unpublished data) so they can use limited availability of C in leaves to produce antioxidants and improve O₃ tolerance. 2.) O₃ avoidance capacity by reduction of leaf area and stomata closure reduced the use of carbon in leaves and more leaf senescence and abscission were accelerated under Elevated O₃ concentration, so the more leaf biomass reduction due to the increasing R/S ratio. 3.) Another possible reason is root growth space. We planted seedlings on the field directly which avoided the root growth limitation while most other OTC experiments planted the seedlings in pots. Our results also showed that the R/S ratio had a significantly liner relationship with all parts of biomass, which means the size of the seedlings mainly affected the R/S ratio.

4.3. Drought treatments effects

Root/shoot ratio was not affected by drought stress, although at the end of experiment the SMC in the DS treatment was nearly half of field water capacity (Table 2). This suggests that carbon allocation was not changed after intermediate and late drought. Normally drought stress is thought to cause a shift in carbon allocation in plants since drought stress leads to a deeper rooting system so that the trees could get water supply from deeper water pools (Eilmann and Rigling, 2012).

4.4. Combination effects of elevated O₃ and drought stress

For the significant interactive effects on foliar injury, chlorophyll pigments and leaf mass per area, drought showed significant protective effects from O₃ injury (Figs. 1, 3 and 5). The main possible reason was the reduction of stomatal conductance (Fig. 4) which protected the leaf from O₃ injury as reported in other research reports (Beyers et al., 1992; Wieser and Havranek, 1995). A_{sat} was protected by drought from O₃ effects only in S4 when it was the rainy season in Beijing and high air moisture mitigated the effect of drought stress during the gas exchange measurement. However, when it came to the biomass results and gas exchange (Table 3, Fig. 4), the results were varied. Total biomass reduction percents under interaction (AO-DS treatment) were higher (64%) than

individual O₃ (AO-WW treatment, 56%) and drought (AA-DS treatment, 30%) effect. A_{sat} and G_s under AO-DS treatments all showed more reduction at the second year except S4. A possible explanation may be that due to the extended elevated O₃ fumigation coupled with the drought stress with less leaf biomass and reduced A_{sat}, there was not enough carbohydrate available for plants to carry out defense and detoxification processes. Further the defense processes may have been weakened by elevated O₃ concentration to an extent that a greater reduction in growth resulted (Matussek et al., 2006; Tausz et al., 2007). There were similar reports about drought increases tree sensitivity to O₃ and the overall oxidative risk as well, such as *Quercus ilex* L. (Alonso et al., 2013), *Pinus halepensis* (Alonso et al., 2001) and poplar clones (Pollastrini et al., 2014). Although the results indicated that drought didn't protect biomass as well as gas exchange from adverse O₃ injury at the end because the effects of interaction were higher than any individual factor, ozone or drought. However, the interaction also showed that ozone and drought effects were synergistic but not additive (less than the sum of the factors individually), although drought may not totally avoid the ozone effects, thus any of the effects were being reduced (mitigated) by the other. Therefore, considering all of these results, the role "drought" may play should be in relation to the relative intensity of each stress or how the drought stress was applied which was also indicated by Pollastrini et al. (2014), the relative low intensity would be more effective in reducing the ozone effects without causing a high depletion of the yield.

Another interesting result was the inconsistency of stem size and biomass (Fig. 6 and Table 3). Although insignificant effects were found between O₃ treatments and interactive treatments no matter the stem size or biomass, the seedling height and basal diameter under interactive treatments were larger than under O₃ treatments, which was the inverse of the biomass results. The reasons could be mainly attributed to relative shortened growing seasons under elevated O₃ exposure and drought stress lead to reduction of leaf, branches and root biomass and wood density (Table 3). Many investigations had also reported accelerated leaf senescence in OTCs under elevated O₃ treatments as well as under drought stress treatments (Lim et al., 2007), as well as large twig and branch decline (Bréda et al., 2006) and altered wood density (Hacke et al., 2001).

Our results revealed few significant results under lower O₃ concentration in the first year while most significant O₃ induced reductions appeared with 40 ppb higher O₃ concentration during the second year, especially late in the growing season (Figs. 2–5). However, the reasons were to be expected due to the following. First, we spent a longer time in the first year making the seedlings acclimate to the chambers well so the O₃ fumigation and drought exposure duration was almost 3 months shorter than the second year. Second, O₃ fumigation after two months acclimation to OTCs may delay first year roots development and resulted in less aboveground growth in contrast to more fully developed seedlings during the second year (Fig. 3). Third, the O₃ concentration of the second year was 40 ppb higher since July 26th and due to higher temperature and bigger seedling size, the average soil moisture contents were also lower in the second year. Therefore, the shorter treatment time, less peak O₃ episodes, higher water moisture contents and the biological/physiological condition of the seedlings in 2012 were the reasons for less response to elevated O₃. The results were in accordance with other reports such as with *Ponderosa* pine (Andersen et al., 1997), birch (Oksanen, 2003), Norway spruce (Wallin et al., 2002) and sugar maple (Naidu and DeLucia, 1997).

Although our control treatment was ambient air and the OTC field site was located in the Beijing suburb, according to our former research (Wang et al., 2011), only O₃ is the most abundant air

pollutant in this area while the concentrations of other pollutants like NO_x and SO₂ are too low in summer to cause plant injury or interaction among pollutants. The results of this experiment were apparent and certify our hypothesis. The results can be more confidently applied to real nature because we planted the seedlings in the field in the OTCs directly which avoided the root growth limitation which has been a recognized problem in most other OTC experiments where the seedlings in the OTCs were grown in pots. However, one year old seedlings may be more sensitive to environmental stress than mature trees, so the results have limits to be applied totally to "well-growth" mature maple tree.

5. Conclusion

The results showed that elevated O₃ and drought stress had both significantly reduced growth and physiology of Shantung maple (*A. truncatum* Bunge) seedlings. Although drought mitigated the O₃-induced foliar injury and reductions of chloroplast pigments and LMA on leaf level, adverse O₃ impacts were not alleviated by drought when the whole plant was considered. More biomass and gas exchange reduction were detected at the end of experiment due to limited carbon fixation. However, the interaction also showed that ozone and drought effects were synergistic but not additive (less than the sum of the factors individually), which will provide an important case for understanding the complex of interactive effects of O₃ and drought.

The interactive effects of elevated O₃ and drought on plant are complicated, depending on O₃ dose, drought severity, plant species or its variance, and indicators concerned. It is not easy to reverse the negative effect of elevated O₃ by drought. The alleviative function of drought on O₃ damage might be effective when drought is mild. With the increasing O₃ exposure and drought due to human activity and climate change, the confounding effects of O₃ and drought would be more often faced by plants. New ambient O₃ standard protecting plant and nature should consider the influence of drought on O₃ effect. So it is pressing to investigate the interactive effects of O₃ and drought at different manipulated regimes.

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