Growth and photosynthetic responses of four landscape shrub species to elevated ozone

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Abstract

Attention should be paid to ozone (O_3) sensitivity of greening plant since ground-level O_3 concentrations are increasing especially in urban and suburban area. We studied the ecophysiological responses to elevated O_3 of four shrub species [*Euonymus bungeanus* Maxim. (EB), *Photinia × fraseri* (PF), *Chionanthus retusus* Lindl. & Paxt. (CR) and *Cornus alba* L. (CA)], which are often used for garden greening in China. Saplings of those species were exposed to high O_3 concentration (70 nmol mol^{-1}, 7 h d^{-1} for 65 d) in open-top growth chambers. Responses to O_3 were assessed by gas exchanges, chlorophyll (Chl) fluorescence and dry mass. We found that elevated O_3 significantly decreased light-saturated net photosynthetic rate (P_{Nsat}), transpiration rate (E) and stomatal conductance (g_s). The ratio of intercellular CO_2 to ambient CO_2 concentration (C_i/C_a) did not reduce under O_3 fumigation which suggested that the O_3-induced depressions of P_{Nsat} under O_3 fumigation were probably due to limitation of mesophyll processes rather than stomatal limitation. High O_3 exposure also significantly depressed the maximum efficiency of photosystem II (PSII) photochemistry in the dark-adapted state (F_v/F_m) which meant the O_3-induced photoinhibition. Both root dry mass and root/shoot ratios were significantly decreased under ozone fumigation, but the total mass was unchanged. The responses of gas exchange such as P_{Nsat} in these four shrubs to O_3 exposure were species-specific. Highest loss of P_{Nsat} was observed in EB (–49.6%), while the CR had the lowest loss (–36.5%). Moreover, the O_3-exposed CR showed similar g_s as CF, reflecting that its O_3 flux might be unchanged under elevated O_3 environment. Ozone drastically decreased actual quantum yield of PSII (Φ_{PSII}) and electron transport rate (ETR) in EB while increased Φ_{PSII} and ETR in CR. Furthermore, the relative losses in P_{Nsat} positively correlated with the relative decreases in Φ_{PSII} and ETR which indicated that the impairment of photosynthesis was probably affected by the light reaction process. The light reaction of EB was impaired most seriously but that of CR was not damaged. All results indicated that EB was probably the most sensitive shrub species to O_3 while CR the most tolerant one. Therefore, CR might be an ideal choice for greening in ozone-polluted areas.

Additional key words: biomass; *Chionanthus retusus* Lindl. & Paxt.; chlorophyll *a* fluorescence; *Cornus alba*; *Euonymus bungeanus*; gas exchange; ozone; *Photinia × fraseri*.

Introduction

Ground-level ozone (O_3) has been a major air pollutant and a minor greenhouse gas that created by chemical reaction between nitrogen oxides (NO_x) and volatile organic compounds (VOCs) in the presence of sunlight (Seinfeld 1989). As concentrations of NO_x and VOCs increase with industrialization and vehicle exhaust,

the daily surface O_3 levels in industrialized countries have increased remarkably from 10 nmol mol^{-1} prior to the industrial revolution to 60 nmol mol^{-1} in current summer seasons, and are predicted to increase by 20% until 2050 (IPCC 2007). Cities with concentrated industrial plants and heavy traffic are prone to high O_3 concentrations. The hourly maximum O_3 concentration has already reached 215 nmol mol^{-1} in Beijing in August, 1998 (Ma and Zhang 2000). The daily maximum O_3 concentration of urban Beijing in summer has increased from 40 nmol mol^{-1} in 1982 to 125 nmol mol^{-1} in 2003 (Shao *et al.* 2006). The increasing O_3 concentration may impact on human beings' health.

Landscape plants in cities play very important roles in reducing dust and removing some air pollutant such as SO_2, NO_2, and suspended particulates (Jim and Chen 2008). Moreover, many modeling studies have simulated that urban trees could have great capabilities to absorb O_3 and to protect the health of human beings in cities (Nowak *et al.* 2000, Kim *et al.* 2008). However, the information about responses of landscape plants to O_3 is limited (Lumis and Ormrod 1978, Paoletti *et al.* 2009, Szantoi *et al.* 2009). Especially, much less is known about effects of O_3 on landscape plants in China (He *et al.* 2007, Feng *et al.* 2008).

Ozone diffuses into plant leaves through stomata and dissolves in the water in the apoplast, then rapidly degrades and yields various reactive oxygen species (ROS) (Heath 1980, Heath 1987). The ROS can attack lipid and protein components of plasma membrane causing leakage of cell membranes. It also leads to programmed cell death (Overmyer *et al.* 2003). Therefore the stomatal conductance is an important index to determine the ozone absorption, while the antioxidant system affects the degradation of ROS and then influence

plant tolerance (Ryang *et al.* 2009). Visible injury and decline in growth are often considered as indices of plant sensitivity to ozone (Ashmore *et al.* 1987, Guidi *et al.* 2000, Bussotti *et al.* 2003). However, leaf injury symptoms do not always correlate with the decrease of growth and the loss of biomass or yield (Bergmann *et al.* 1995, Pleijel and Danielsson 1997, Booker *et al.* 2009). As useful nondestructive tools for stress detection (Owens 1994), gas exchange and Chl *a* fluorescence have been used to analyze species-specific sensitivities to O_3, which can in part explain the O_3 effects on plants growth (Seppo and Wang 1997, Calatayud *et al.* 2006, Biswas *et al.* 2008, Contran *et al.* 2009).

We selected four species of shrubs, *i.e.* winterberry euonymus (*Euonymus bungeanus*, EB), fraser's photinia (*Photinia × fraseri*, PF), chinese fringetree (*Chionanthus retusus*, CR), tatarian dogwood (*Cornus alba*, CA), which are of high ornamental value and are widely used as greening plants in urban and suburban areas to study responses of greening shrubs to elevated O_3. There are some available information about these shrub species in biology (Tu *et al.* 1992, Spivey *et al.* 2001), propagation (Dirr 1990, Ramírez-Malagón *et al.* 1997, Larraburu *et al.* 2007) and genetic variations (Soejima *et al.* 1998, Chien *et al.* 2004), however, to our knowledge, there are few reports on the ecophysiological responses of these four shrubs to elevated O_3. Since these species have different foliage and growth characters, their sensitivities to O_3 pollution might be diverse.

The objectives of this research therefore were: (*1*) to assess ozone effects on these four shrub species, (*2*) to compare physiological responses to ozone among these species, and (*3*) to reveal any potential mechanism that causes their different sensitivities.

Materials and methods

Plants: Four garden shrub species (EB, PF, CR, and CA) were obtained from *Shandong Guanghe Landscape Science & Technology Company, Ltd*. (Jinan, Shandong province, China). Saplings of these shrubs (50–60 cm high) were planted in PVC pots (20 cm in diameter) filled with a mixture of vermiculite, peat, and field soil (1:3:6, v:v:v) on May 1st, 2007. Water was supplied once a week throughout the experiment.

Experimental design: The experiment was conducted in four open-top chambers (OTCs, 1.8 m in diameter and 2.4 m in height) at the Botanical Garden, Institute of Botany, the Chinese Academy of Sciences, Beijing, China (39°92'N, 116°46'E). The OTCs were made of transparent polymethylmethacrylate plates. The max/min temperature and relative humidity in the OTCs were 35/20°C and 60/45%, respectively. The photosynthetic photon flux density (PPFD) inside the OTCs was averaged at 800 µmol m^{-2} s^{-1}. The O_3-treatment method

was referred to Biswas *et al.* (2008) and the ventilation tube was adjusted 20 cm higher considering the heights of shrubs. On August 1st, 2007, six saplings of each species were randomly placed in each OTC. All plants were firstly received charcoal-filtered air (CF, < 5 nmol mol^{-1} O_3) for adaptation for one week. Then, two chambers were ventilated with O_3 and maintained O_3 concentration at 70 ± 5 nmol mol^{-1} (09:00–16:00 h) for 65 d and the other two were treated with CF air. Ozone was generated by electrical discharge using charcoal-filtered ambient oxygen with an O_3 generator (*CF-KG1, Beijing Sumsun EP Hi-Tech.*, Beijing, China) and bubbled through distilled water before entering the elevated O_3 chambers (Zhang *et al.* 2010). Manual mass flow controllers regulated the flow of injected O_3 to the tubes. Ozone concentrations in the OTCs were monitored once an hour at approximately 10 cm above the plant canopy using an O_3 analyzer (*Model 205, 2B Technologies Inc.*, Boulder, Colorado, USA). In order to diminish the chamber

effects, pots were rotated between the chambers every week and randomized within the chambers every day.

Gas exchange: On the 60th d after fumigation, instant gas exchange was measured on the latest fully expanded leaves using a gas-exchange and fluorescence systems (*GFS3000, Heinz Walz*, Effeltrich, Germany). Six plants of each species in O_3 or in CF were randomly selected for measurement. The *GFS3000* system was connected to a PC with data acquisition software (*GFS-Win*, Germany) and calibrated to zero point prior to measurements. The cuvette condition was set with a light intensity of 1,500 µmol m^{-2} s^{-1} provided by the special artificial light (*LED-Array/PAM- Fluorometer 3055-Fl, Heinz Walz,* Effeltrich, Germany), with relative humidity and air temperature being set at $70 \pm 5\%$ and $25 \pm 0.5°C$, respectively. Gas exchange was measured using ambient CO_2 concentration (380 ± 10 µmol mol^{-1}). P_{Nsat}, g_s, C_a, and C_i were calculated according to von Caemmerer and Farquhar (1981).

Chl *a* fluorescence: On the 61st d after exposure, Chl *a* fluorescence was measured on the latest fully expanded leaves using gas exchange and fluorescence systems (*GFS3000, Heinz Walz*, Effeltrich, Germany). After 40 min of dark adaptation, the minimal (F_o) and maximal fluorescence (F_m) in the dark-adapted state were determined with a modulated irradiation (<0.1 µmol m^{-2} s^{-1}) and a 0.8-s saturating pulse (>8,000 µmol m^{-2} s^{-1}) respectively. The variable fluorescence (F_v) was calculated as $F_v = F_m - F_o$. Following 2 min of dark readaptation, actinic white light (400 µmol m^{-2} s^{-1}) was switched on. The steady-state fluorescence (F_s) was reached within 5 min and a second saturating pulse was imposed to determine the maximum fluorescence in the irradiation-

adapted state (F_m'). The minimum fluorescence in the irradiation-adapted state (F_o') was determined during a brief interruption of actinic illumination in the presence of far-red light. F_v/F_m and the nonphotochemical quenching (NPQ) were calculated by $F_v/F_m = (F_m - F_o)/F_m$ and $NPQ = F_m/F_m' - 1$, respectively (Bilger and Björkman 1990, Schreiber 2004). Φ_{PSII} and ETR were calculated as $\Phi_{PSII} = (F_m' - F_s)/F_m'$ and $ETR = \Phi_{PSII} \times PAR \times 0.5 \times 0.84$, respectively (Genty *et al.* 1989).

Biomass accumulation and biomass partition: Destructive harvests were conducted by the end of fumigation. Six plants per species in O_3 or in CF treatments were harvested. Stems, leaves and roots were separated and oven-dried at 80°C for 72 h, then the stem dry mass (SDM), leaf dry mass (LDM) and root dry mass (RDM) were measured with an electronic balance (*BP221S, Sartorius*, Göttingen, Germany). Root/shoot ratio was calculated as the RDM divided by the sum of LDM and SDM, and Biomass was calculated as the sum of SDM, LDM and RDM.

Statistical analysis: The experiment consisted of two randomized blocks of two treatments with six plants per replicate. Data were analyzed using the *General Linear Models Procedure* of *SPSS* (*version 12, SPSS*, Chicago, IL, USA). Means of each parameter were analyzed using the Student's *t*-test to compare values between treatments in each species. Interactions between treatment and species were identified using two-way factorial analysis of variance (*ANOVA*) in the *General Linear Model*. *Pearson*'s correlation among relative changes in gas exchange, Chl *a* fluorescence and biomass parameters in the four shrubs exposed to elevated O_3 were analyzed.

Results

Gas exchange: The ozone fumigation significantly decreased P_{Nsat} ($P<0.0001$), E ($P=0.0004$), g_s ($P=0.0002$) and C_i/C_a ($P=0.0082$) of the shrubs, however, there were significant interactions between treatments and species on the P_{Nsat} ($P=0.0013$), E ($P=0.003$), g_s ($P=0.0019$) and C_i/C_a ($P=0.0077$) (Table 1). P_{Nsat}, E and g_s of EB, PF and CA decreased notably under O_3 fumigation compared to CF (Fig. 1). CR also had lower P_{Nsat} in O_3-exposed plant than that in CF, however, its E and g_s were not significantly affected by the O_3 exposure. C_i/C_a of EB and PS were not significantly affected by O_3 fumigation, while CR and CA had significantly higher C_i/C_a in O_3 than in CF. Highest relative loss in P_{Nsat} (−49.6%) and g_s (−45.1%) were observed in EB while lowest relative loss in P_{Nsat} (−36.5%) was found in CR (Fig. 1).

Chl *a* fluorescence: Ozone exposure significantly decreased F_o ($P=0.0481$), F_m ($P=0.0015$) and F_v/F_m ($P=0.0165$), but the effects on Φ_{PSII}, ETR, and NPQ did

not show a significance ($P>0.05$) (Table 1). There were significant interactions between treatments and species on F_o ($P=0.0256$), F_m ($P=0.0152$), Φ_{PSII} ($P=0.0007$), ETR ($P=0.0007$), and NPQ ($P=0.0001$) (Table 1). The F_o of CA was significantly depressed by O_3, while the other three species were not notably affected (Fig. 2). Both EB and CA had lower F_m under O_3 exposure compared with CF, however, the F_m of both PF and CR were not significantly different between treatments. Φ_{PSII} and ETR decreased drastically after 61 d of O_3 fumigation in EB, while these parameters rose under O_3 exposure in CR. Ozone did not change the Φ_{PSII} and ETR in PF or CA. After 61 d of O_3 fumigation, the NPQ of both CR and CA significantly decreased, while EB had higher NPQ in O_3-treated plants compared to CF-treated plants. It was noted that F_v/F_m ratio was significantly decreased under O_3 exposure although the relative loss in each species was small (Table 1, Fig. 2).

Table 1. Effects of O_3 treatments, species and their interactions on gas-exchange parameters (P_{Nsat} – light-saturated net photosynthetic rate; E – transpiration rate; g_s – stomatal conductance; C_i/C_a – intercellular CO_2 concentration/ambient CO_2 concentration), chlorophyll *a* fluorescence parameters (F_o – minimal fluorescence of the dark-adapted state; F_m – maximal fluorescence of the dark-adapted state; F_v/F_m – the maximum efficiency of photosystem II photochemistry in the dark-adapted state; Φ_{PSII} – the actual quantum yield of PSII; ETR – electron transport rate; NPQ – nonphotochemical quenching) and biomass parameters [SDM – stem dry mass; LDM – leaf dry mass; RDM – root dry mass; root/shoot – root dry mass/(stem dry mass + leaf dry mass); biomass – stem dry mass + leaf dry mass + root dry mass] after more than 60-d fumigation. The values in the table are the *P* values by the two way *ANOVA* test.

	P_{Nsat}	E	g_s	C_i/C_a	F_o	F_m	F_v/F_m	Φ_{PSII}	ETR	NPQ	SDM	LDM	RDM	Root/shoot	Biomass
Treatment	<0.0001	0.0004	0.0002	0.0082	0.0481	0.0015	0.0165	0.4996	0.4925	0.5869	0.1527	0.8435	0.0383	0.0032	0.2607
Species	<0.0001	<0.0001	0.0001	<0.0001	0.0318	0.0096	0.0208	0.0011	0.0011	0.0004	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Treatment × species	0.0013	0.0030	0.0019	0.0077	0.0256	0.0152	0.4906	0.0007	0.0007	0.0001	0.2612	0.0645	0.7182	0.0262	0.6182

Table 2. Correlation among relative changes in gas-exchange parameters (P_{Nsat} – light-saturated net photosynthetic rate; E – transpiration rate; g_s – stomatal conductance; C_i/C_a – intercellular CO_2 concentration/ambient CO_2 concentration), chlorophyll *a* fluorescence parameters (F_o – minimal fluorescence of the dark-adapted state; F_m – maximal fluorescence of the dark-adapted state; F_v/F_m – the maximum efficiency of photosystem II photochemistry in the dark-adapted state; Φ_{PSII} – the actual quantum yield of PSII; ETR – electron transport rate; NPQ – nonphotochemical quenching) and biomass parameters [SDM – stem dry mass; LDM – leaf dry mass; RDM – root dry mass; root/shoot – root dry mass/(stem dry mass + leaf dry mass); biomass – stem dry mass + leaf dry mass + root dry mass] in four shrub species exposed to elevated O_3. Changes are expressed as percent differences between O_3-exposed (O_3) plants and CF plants – (O_3-CF)/CF – before *Pearson*'s correlation test. Asterisks denote significant correlation – * <0.05 – ** <0.01.

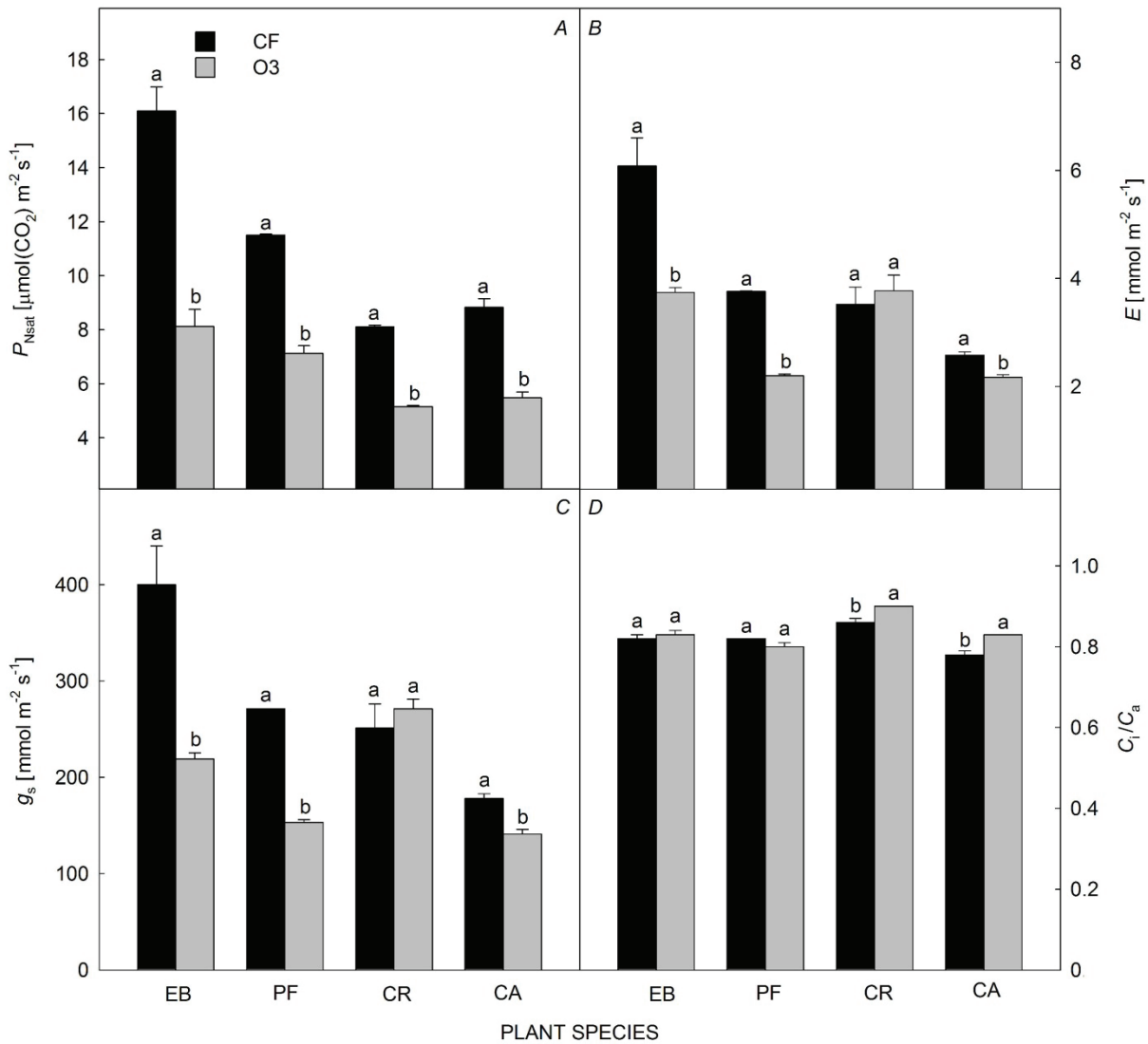|	E	g_s	P_{Nsat}	C_i/C_a	F_o	F_m	F_v/F_m	Φ_{PSII}	ETR	NPQ	SDM	LDM	RDM	Root/shoot	Biomass
E	1.000														
g_s	0.994**	1.000													
P_{Nsat}	0.564	0.614	1.000												
C_i/C_a	0.849	0.787	0.228	1.000											
F_o	0.661	0.732	0.540	0.187	1.000										
F_m	0.531	0.617	0.637	0.004	0.970*	1.000									
F_v/F_m	-0.619	-0.566	0.300	-0.757	-0.261	-0.018	1.000								
Φ_{PSII}	0.837	0.869	0.924*	0.539	0.674	0.677	-0.089	1.000							
ETR	0.839	0.871	0.922*	0.540	0.678	0.680	-0.093	1.000**	1.000						
NPQ	-0.692	-0.739	-0.984*	-0.344	-0.645	-0.704	-0.136	-0.974*	-0.973*	1.000					
SDM	0.074	0.021	0.360	0.346	-0.537	-0.486	0.269	0.261	0.256	-0.276	1.000				
LDM	0.880	0.920*	0.854	0.532	0.809	0.787	-0.208	0.976*	0.978*	-0.932*	0.055	1.000			
RDM	-0.108	-0.001	0.404	-0.617	0.635	0.782	0.497	0.229	0.229	-0.378	-0.545	0.307	1.000		
Root/shoot	-0.580	-0.532	-0.581	-0.726	0.099	0.127	0.106	-0.644	-0.641	0.579	-0.856	-0.498	0.507	1.000	
Biomass	0.465	0.510	0.987**	0.179	0.402	0.520	0.402	0.868	0.865	-0.947*	0.479	0.767	0.349	-0.627	1.000

Fig. 1. *A*: Light-saturated net photosynthesis (P_{Nsat}), *B*: stomatal conductance (g_s), *C*: transpiration rate (*E*), and *D*: ratio of inter-cellular CO_2 to ambient CO_2 concentration (C_i/C_a), of four shrub species (*Euonymus bungeanus*, EB; *Photinia × fraseri*, PF; *Chionanthus retusus*, CR; *Cornus alba*, CA) under charcoal-filtered air (CF) and O_3 fumigation (O_3) on the 60th d after treatments. Error bars show SE, *n* = 6. Means of each parameter were analyzed using the Student's *t*-test to compare values between treatments in each species. *Letters* are comparable within treatments in each species. Values with *different letters* are significantly different ($P<0.05$).

Biomass accumulation and partition: The RDM ($P=0.0383$) and root/shoot ratio ($P=0.0032$) of the shrubs were significantly reduced by ozone fumigation (Table 1). Significant interaction between treatments and species was only found on root/shoot ratio (Table 1). Ozone significantly suppressed the root/shoot ratio in CA, while the other three species were not affected significantly. There were no significant differences in biomass between CF and O_3-treated plants in all species. The SDM, LDM, RDM, and biomass were significantly different among species. The CR had the highest SDM, RDM, and biomass while the EB had the lowest LDM and biomass.

Correlations among gas exchanges, Chl *a* fluorescence and biomass parameters as affected by O_3: The relative loss of P_{Nsat} positively correlated with relative loss in Φ_{PSII}, ETR and biomass, but adversely with O_3-caused increase in NPQ (Table 2). Relative changes in *E* and LDM exhibited significantly positive relationships with g_s. Φ_{PSII} and ETR significantly negatively correlated with NPQ, but positively with LDM. Moreover, NPQ significantly negatively correlated with LDM. Biomass loss negatively correlated with NPQ change besides positively with P_{Nsat} (Table 2).

Discussion

Under high O_3 fumigation (the AOT40 value was approximately 13,650 nmol mol^{-1} h), the adaxial surface of old leaves of all shrubs showed visible symptoms as red spots in the tissue between veins showed in CA and PF, while brown spots occurred in CR and EB. On the backside of leaves, no visible O_3 damage symptoms were observed in CR and CA, while visible O_3 injury symptoms also showed in EB and PF. This result indicated that EB and PF might be more sensitive to O_3 than CR and CA.

Many researches have found that photosynthetic processes are very sensitive to high O_3 (Xu *et al.* 2009, Zhang *et al.* 2010). In our experiment, O_3 exposure caused significant reductions in P_{Nsat} of the four shrubs, but C_i/C_a were increased or unchanged, indicating that the depressions of P_{Nsat} were caused by limitations of mesophyll processes rather than stomatal limitation (Reich 1987, Weber *et al.* 1993). Our results are generally in agreement with the findings of other researchers (Mulholland *et al.* 1997, Biswas *et al.* 2008). The losses of P_{Nsat} were significantly different between species. Among the four shrub species, P_{Nsat} of EB was the most sensitive, while that of CR was the least. E and g_s were the most negatively affected in EB, while unchanged in CR. The maintenance of g_s in CR under ozone fumigation meant that its O_3 flux might be unchanged and O_3 damage might be reduced probably through antioxidant system rather than stomatal closure (Biswas *et al.* 2008). These results indicated that EB might be the most sensitive to O_3, and CR the most tolerant species in terms of gas exchanges.

The ratio of F_v/F_m ratio is often regarded as an indicator of photoinhibition. The significant decrease in F_v/F_m caused by high O_3 exposure indicated that the O_3 fumigation induced photoinhibition to all the shrub species, and this effect was found non species-specific as reflected by the nonsignificant interaction between treatment and species. Effects of O_3 on Φ_{PSII}, ETR, and NPQ were not significant, however, there were significant interactions between treatment and species in these parameters. Ozone drastically decreased Φ_{PSII} and ETR in EB, but enhanced these two parameters in CR. It indicated that the photochemical efficiency of open PSII reaction centers during irradiation was depressed, and the noncyclic electron transport from PSII and PSI was impaired by O_3 significantly in EB. Higher NPQ in O_3 than in CF demonstrated that the nonradiative energy dissipation was developed in EB under O_3 exposure. However, CR had significantly higher Φ_{PSII} and ETR under O_3 fumigation which meant that the linear electron transport was stimulated. Moreover, the NPQ of CR decreased after O_3 treatment compared to CF which probably indicated that more energy was distributed to

photochemical quenching than thermal dissipation in PSII in CR to avoid photodamage (Calatayud and Barreno 2004). It is reported that the reduced demand from the Calvin cycle can downregulate the electron supply (Bussotti *et al.* 2010), thus in this study the activity and/or content of Rubisco in Calvin cycle was probably not affected in CR as reflected by higher electron transport. Therefore, the loss of P_{Nsat} in CR was probably caused by the reaction beyond Calvin cycle, such as photorespiration. The correlation analysis showed that the loss of P_{Nsat} positively correlated with the relative loss of Φ_{PSII} and that of ETR. It exhibited again that the decrease of P_{Nsat} was mainly caused by nonstomatal process such as light reaction.

Since the accumulation of biomass represents the net effect of carbon assimilation and maintenance, detrimental effects on biomass have often been considered as the most important indicators of O_3 damage (Skärby *et al.* 1998). In this experiment, the biomass loss positively correlated with the relative loss in P_{Nsat} which can be easily understood since the net photosynthesis was the only resource of the biomass. The interspecific variations were significant in biomass and biomass partition. However, the responses of all species to ozone were similar in biomass. Root/shoot ratios were lower in elevated O_3 fumigation, indicating that O_3 exposure resulted in the change of biomass partition. There were significantly different responses to ozone in root/shoot ratio among species with only CA showing significantly lower root/shoot ratio under ozone fumigation. The increased SDM in CA indicated that more biomass was partitioned to the stem. This result is in accordance with other studies (Cooley and Manning 1987). It is reported that the content of anthocyanin, an antioxidant, in the stem of CA is high (Gitelson *et al.* 2009). Whether this is the possible reason for the increased stem biomass in CA under high O_3 stress, needs further researches. As to CR, no significant changes in root/shoot ratio, but the higher LDM indicating more biomass partitioned into leaves, which might compensate for the loss of P_{Nsat} to some extent. The characteristic of carbon allocation might be another reason for the tolerance to O_3 of CR besides the relative less reduction in net CO_2 assimilation as demonstrated above. The almost unchanged g_s further demonstrated that CR could be an excellent landscape plant whose physiological processes would be less negatively affected but also its absorbance of O_3 might be unchanged so as to purify the air. The change of biomass was negatively correlated with the change of NPQ, which indicated that the strategy of thermal dissipation to protect photosynthetic apparatus from ozone stress might be at the expense of biomass accumulation.
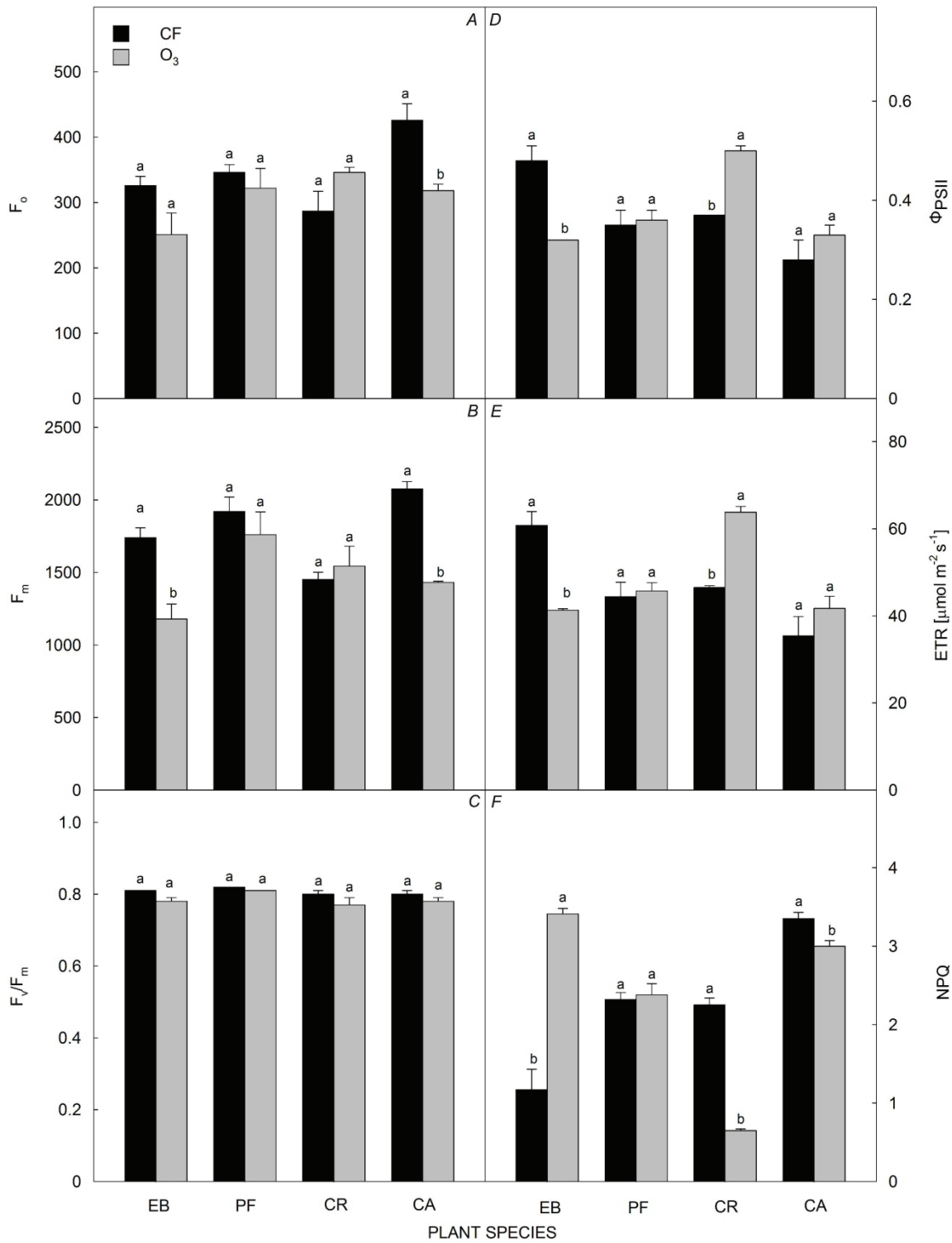
Fig. 2. Effects of O_3 on *A*: the minimal fluorescence (F_o), *B*: the maximal fluorescence (F_m), *C*: the maximum photochemical efficiency (F_v/F_m), *D*: the quantum yield of photosystem II (Φ_{PSII}), *E*: electron transport rate (ETR), and *F*: nonphotochemical quenching (NPQ), of four shrub species (*Euonymus bungeanus*, EB; *Photinia × fraseri*, PF; *Chionanthus retusus*, CR; *Cornus alba*, CA) on the 61st d after treatments. Error bars show SE, $n = 6$. Means of each parameter were analyzed using the Student's *t*-test to compare values between treatments in each species. *Letters* are comparable within treatments in each species. Values with *different letters* are significantly different (*P*<0.05).
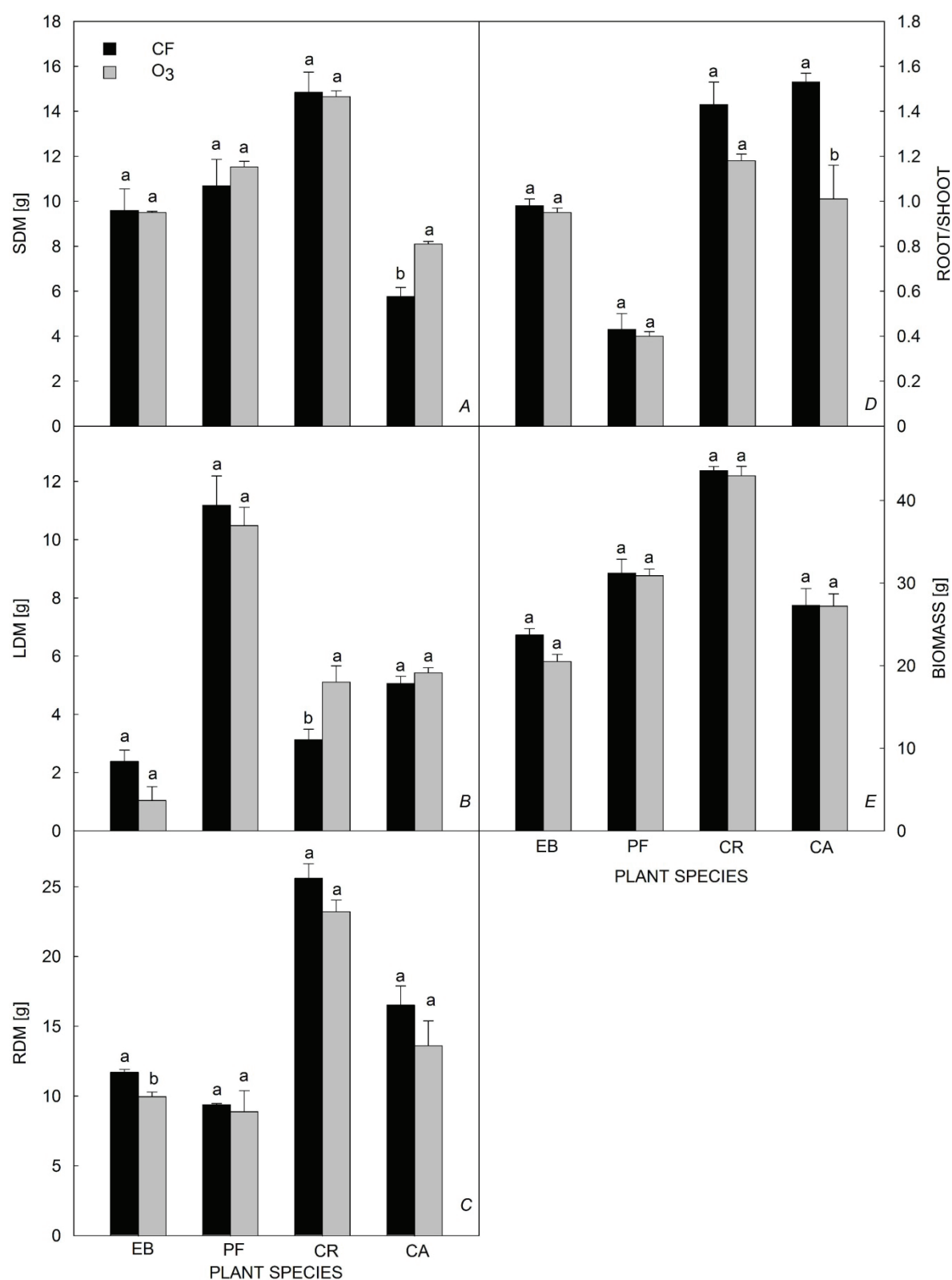
Fig. 3. Effects of O_3 on *A*: stem dry mass (SDM), *B*: leaf dry mass (LDM), *C*: root dry mass (RDM), *D*: root/shoot ratio and *E*: biomass of four shrub species (*Euonymus bungeanus*, EB; *Photinia × fraseri*, PF; *Chionanthus retusus*, CR; *Cornus alba*, CA) on the 65th d after treatments. Error bars show SE, *n* = 6. Means of each parameter were analyzed using the Student's *t*-test to compare values between treatments in each species. *Letters* are comparable within treatments in each species. Values with *different letters* are significantly different (*P*<0.05).

In conclusion, photosynthetic processes of the shrub species in our experiment were sensitive to elevated O_3, but species-specific responses existed. The assimilation ability of EB was the most sensitive in terms of P_{Nsat}, Φ_{PSII}, and ETR while that of CR was the most tolerant. Meanwhile, more biomass partitioned to foliage in CR which may help it to cope with O_3 stress by providing more assimilation carbon and energy through photosynthetic processes. It is suggested that ozone sensitivities are different in landscape species and should be considered to select plant species for garden greening in urban and suburban areas with potential high O_3 concentration. We here find that the CR might be an ideal choice for garden greening in the ozone polluted areas.

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