Responses of native broadleaved woody species to elevated ozone in subtropical China

Weiwei Zhang^{a,b}, Zhaozhong Feng^{a,*}, Xiaoke Wang^{a,*}, Junfeng Niu^a

^aState Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, 2871 Mailbox, Shuangqing Road 18, Haidian District, Beijing 100085, China

^bKey Laboratory of Black Soil Ecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin 150081, China

ARTICLE INFO

Article history:

Received 12 August 2011

Received in revised form

10 December 2011

Accepted 15 December 2011

Keywords:

Ozone

Subtropical species

Sensitivity

Visible symptom

Gas exchange

Antioxidant defense

ABSTRACT

To assess ozone sensitivity of subtropical broadleaved tree species and explore possible underlying mechanisms, six evergreen and two deciduous native species were exposed to either charcoal-filtered air or elevated O₃ (E-O₃, ~150 ppb) for one growing season. Initial visible symptoms in deciduous species appeared much earlier than those in evergreen species. The species which first showed visible symptoms also had the largest reductions in biomass. E-O₃ induced significant decreases in photosynthesis rate, chlorophyll content and antioxidant capacity but a significant increase in malondialdehyde content in two deciduous species and two evergreen species (*Cinnamomum camphora* and *Cyclobalanopsis glauca*). Except *C. glauca*, however, E-O₃ had no significant effects on stomatal conductance (*g_s*), total phenols and ascorbate contents. Difference in O₃ sensitivity among all species was strongly attributed to specific leaf mass rather than *g_s*. It suggests that some subtropical tree species will be threatened by rising O₃ concentrations in the near future.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Tropospheric ozone (O₃) is considered as one of the most critical air pollutants for forests (Paoletti et al., 2010) due to its detrimental effects on carbon sequestration (Augustaitis and Bytnerowicz, 2008; McLaughlin et al., 2007), forest health (Paoletti, 2009; Tkacz et al., 2008) and biodiversity (Matussek et al., 2010; Pretzsch et al., 2010). A recent quantitative meta-analysis found that current ambient [O₃] (40 ppb, on average) and elevated [O₃] (97 ppb) significantly reduced the total biomass of trees by 7% and 17% compared with those grown in charcoal-filtered controls, respectively (Wittig et al., 2009). Furthermore, O₃ induced a variety of physiological and biochemical alterations at the leaf level, including reduction in photosynthesis, stomata closure, chlorophyll degradation, and premature senescence, with or without visible foliar injury (Matussek et al., 2007; Pina and Moraes, 2010; Tausz et al., 2007).

On the other hand, tree species can actively defend against O₃ through two main pathways. One is the avoidance strategy, i.e. the ability of leaves to partially close stoma to exclude O₃ from leaf

intercellular space (Kitao et al., 2009; Paoletti et al. 2009); the other is the repair strategy, i.e. the capacity to activate detoxifying systems (Castagna and Ranieri, 2009), especially cellular phenolic compounds and apoplastic ascorbate (AsA) although the relationship between the O₃ sensitivity of a plant and its apoplastic AsA concentration remains controversial (D'Haese et al., 2005; Di Baccio et al., 2008). Many Mediterranean evergreen species were tolerant to high O₃ levels in the summertime, due partly to their low stomatal conductance (*g_s*) (Paoletti, 2006). However, *Phillyrea latifolia* was the most tolerant to O₃ among three species due to higher antioxidant level despite having the highest *g_s* (Nali et al., 2004). Di Baccio et al. (2008) compared two poplar clones with different sensitivity to O₃ and found that O₃-induced increase in antioxidant level in the sensitive poplar clone is not sufficient to counteract the oxidative stress due to its larger *g_s*. Hence, it can be inferred that the balance between O₃ uptake and cellular antioxidant potential is very important in determining the sensitivity of plants to O₃.

The negative impact of O₃ on woody species has been well documented in the North America and Europe for their locally important trees like *Pinus halepensis* (e.g. Inclán et al., 2011), *Populus tremuloides* (e.g. McGrath et al., 2010), hybrid poplar (*Populus maximowiczii* × *trichocarpa*) (e.g. Orendovici-Best et al., 2008), *Betula pendula* (Kontunen-Soppela et al., 2010; Oksanen et al., 2005), *Fagus sylvatica* (Gerosa et al., 2008; Haberer et al., 2007) and *Quercus ilex*

* Corresponding authors.

E-mail addresses: zhzhfeng201@hotmail.com, fzz@rcees.ac.cn (Z. Feng), wangxk@rcees.ac.cn (X. Wang).

¹ Present address: Department of Plant and Environmental Sciences, University of Gothenburg, Göteborg SE-405 30, Sweden.

(e.g. Calatayud et al., 2011). However, very little is known about effect of O₃ on forests in Asia (Bytnerowicz et al., 2007; Yamaguchi et al., 2007). In China, only a few species have been investigated in elevated ozone, such as *Ginkgo biloba* (He et al., 2007), *Quercus mongolica* (Yan et al., 2010), *Cinnamomum camphora* (Feng et al., 2011), *Liriodendron chinense* (Zhang et al., 2011) and *Metasequoia glyptostroboides* (Feng et al., 2008). Furthermore, among all investigated species, most were the boreal and temperate tree species and there were also a few Mediterranean evergreen tree species. However, as one of the most important types of vegetations flora, evergreen/deciduous broadleaved species in subtropical terrestrial ecosystem have been rarely investigated.

Due to subtropical climate features and large amounts of NO_x and VOC emissions (main precursors to O₃ formation), the ambient O₃ in eastern China has reached such a high level that induced significant yield loss in food crops (Wang et al., 2007). Based on the China forest dataset of 1961–2005, a recent model has indicated that ambient O₃ induced a much greater loss of net carbon storage in (sub)-tropical broadleaf evergreen (9.6%) and deciduous (43.1%) forests than that in temperate broadleaf evergreen (7.6%) and deciduous (21.1%) forest, respectively (Ren et al., 2011). Therefore, it is imperative to investigate the response of subtropical broadleaved species to O₃ through field observation in order to protect biodiversity and enhance carbon sequestration in the subtropical forest ecosystem.

In this study, eight native broadleaved woody species, including six evergreen and two deciduous tree species, were exposed to relatively high O₃ concentration (~150 ppb) under local subtropical climate conditions. The objectives were (1) to compare the O₃ sensitivity among the tested woody species based on the AOT40 value at initial visible injury and their growth response; (2) to explore the possible mechanisms on differential response to O₃ among species in terms of foliar traits, g_s, antioxidant capacity and the combination of these parameters.

2. Materials and methods

2.1. Experimental site

The experimental site was located at the Tiantong National Field Observation and Research Station for Subtropical Forest Ecosystems (29°48'N, 121°47'E), Ningbo, Zhejiang province, China. The site is typical of the humid subtropical monsoon climate with cold dry winters and warm wet summers. The annual mean temperature is 16.2 °C and the warmest month is July with a mean temperature of 28.1 °C. Average annual precipitation is 1375 mm, concentrated from June to August.

2.2. Ozone fumigation

O₃ was generated from pure oxygen by an electrical discharge O₃ generator (HY003, Chuangcheng Co. Jinan, China) and then mixed with charcoal-filtered ambient air (CF) to achieve the target O₃ concentration in three open-top chambers (OTCs, octagonal base, 7 m² of growth space and 2.6 m in height). The averaged air velocity in the chambers corresponded to approximately two complete air changes per minute. The concentration of O₃ in the OTCs was continuously monitored at approximately 10 cm above plant canopy using a UV absorption O₃ analyzer (Model 49i-Thermo, USA).

In this study, there were two treatments: CF and elevated O₃ (~150 ppb, E-O₃) with three chambers replicates, respectively. O₃ fumigation started from 6 July 2008 and lasted to 28 September with a daily maximum of 8 h (from 08:00 to 16:00) when there was no rain, fog, mist or dew. There were in total 47 effective days during the experiment because of the interruptions in rainy and cloudy days, including three typhoons (from 21 August to 26 August, from 31 August to 7 September and from 9 September to 17 September). The cumulative O₃ exposure over a threshold of the 1-h average [O₃] of 40 ppb (AOT40) was 38.24 ppm h in E-O₃ by the end of the experiment.

2.3. Plant materials

Eight native broadleaved species were selected considering their dominance in subtropical forest or their potential for urban greening in eastern China. The characteristics of these tree species are described in Table 1. There were two deciduous

Table 1

The characteristics of eight native broadleaved woody species in subtropical China.

Scientific name	Family	Life type	Functional leaf trait
<i>Liriodendron chinense</i>	Magnoliaceae	Deciduous tree	Green, thin and soft
<i>Liquidambar formosana</i>	Hamalidaceae	Deciduous tree	Green and thinly leathery
<i>Ilex integra</i>	Aquifoliaceae	Evergreen shrub	Dark green, glossy leathery, stiff and thick
<i>Photinia × fraseri</i>	Rosaceae	Evergreen shrub	Dark green, glossy leathery, stiff and thick
<i>Cinnamomum camphora</i>	Lauraceae	Evergreen tree	Dark green and glossy leathery
<i>Cyclobalanopsis glauca</i>	Fagaceae	Evergreen tree	Dark green, glossy leathery and stiff
<i>Neolitsea sericea</i>	Lauraceae	Evergreen tree	Green, glossy leathery, and golden densely fine hairy on both surfaces of expanding leaf
<i>Schima superba</i>	Theaceae	Evergreen tree	Dark green and glossy leathery

(*L. chinense* and *Liquidambar formosana*) and six evergreen species (*C. camphora*, *Cyclobalanopsis glauca*, *Schima superba*, *Ilex integra*, *Photinia × fraseri* and *Neolitsea sericea*).

At December 2007, six-month-old seedlings of the selected species were individually planted into 6 L circular plastic pots in a temperature controlled and double glazed greenhouse (25 ± 2 °C, air relative humidity 70–90%). Pots were filled with native yellowish brown lateritic soil (soil organic C 0.98%, total N 1.36 g kg⁻¹, total P 0.27 g kg⁻¹, total K 1.41%) in mixture with litter collected under firry forest at the ratio of 1:1 (v/v). After 4 months' cultivation, these seedlings were moved outside for field acclimation and then grew in OTCs for one month. Twenty four seedlings with similar height and basal diameter were selected for each species (with 10–20 fully expanded leaves) and randomly assigned to six chambers with four plants per OTC before O₃ fumigation started. The growth status (stem height and basal diameter) in each species did not show any significant difference between CF and E-O₃, suggesting that the original difference of seedlings between treatments can be neglected for each species.

The position of plants was changed every 3–5 days within each OTC to eliminate positional effects. During the growth of seedlings, average air temperature, average relative air humidity and total radiation density were 25.6 °C, 90% and 13.8 MJ m⁻² d⁻¹, respectively. Plants were well watered with tap water to avoid drought stress.

2.4. Sampling, physiological and biochemical measurements

For each seedling, stem height and stem basal diameter in two directions at 5 cm of stem height were measured at the beginning of the experiment. At the end of the experiment, the upper fully expanded leaves (3rd to 7th leaf position from the apex) were used for gas exchange measurement and biochemical analysis. Finally, all plants were harvested and separated into leaves, stems and roots. Dry mass was determined after oven-drying at 80 °C until a constant weight.

2.4.1. Visible O₃ injury

Current-year leaves of each seedling were observed every two days until symptoms first appeared and they were checked twice a week thereafter. The date of first foliar injury onset was defined as plants showed the same symptoms in three replicated chambers. Visible foliar injury as well as its onset date was recorded, and the AOT40 at initial visible injury onset (AOT40_{injury}) was calculated as well.

2.4.2. Gas exchange measurement

Two fully expanded upper leaves per seedling and three different plants per chamber were randomly selected. Gas exchange was measured with a portable photosynthesis system fitted with a 6400-40 leaf chamber fluorometer (LCF) (LI-6400, LI-COR Inc. Lincoln, NE, USA). The system controlled saturating PPFD at 1000 μmol m⁻² s⁻¹, block temperature at 27 ± 0.5 °C, CO₂ at 380 ppm with a supply from pure CO₂ cylinder, and relative humidity (RH) at 50%–65%. All measurements were conducted during 09:00–14:00 h, after which the leaves for gas exchange and one additional leaf were punched for sampling, avoiding the mid-vein. Leaf discs (~0.8 cm²) were plunged immediately into liquid N and then stored in –80 °C for physiological and biochemical analysis. Of all, 8 leaf discs in each plant were oven-dried for 48 h at 80 °C to calculate specific leaf mass (SLM) as dry mass per unit of leaf area.

2.4.3. Leaf chlorophyll content

Two leaf discs per plant from three plants in each chamber were extracted with 4 ml 95% ethanol in the dark for 48 h at 4 °C. The absorbance of leaf

pigment extracts was measured at 646 and 663 nm. Chlorophyll content was calculated according to the specific absorption coefficients provided by Lichtenthaler (1987).

2.4.4. Malondialdehyde (MDA) and ascorbate (AsA) content

Three leaf discs per plant (~80 mg) were homogenized in a pre-chilled mortar and extracted in 2 ml ice-cold 6% (w/v) trichloroacetic acid, and then centrifuged at 4000 rpm for 10 min. The absorbance was read at 450 nm in addition to 532 and 600 nm, and MDA content was calculated using its extinction coefficient $155 \text{ mM}^{-1} \text{ cm}^{-1}$ (Heath and Packer, 1968). The total ascorbate (AsA) content was determined following the protocol of Gillespie and Ainsworth (2007).

2.4.5. Total antioxidant capacity (TAC) and total phenol content

Samples (~30 mg) were added to 2 ml cold 70% (v/v) ethanol and homogenized in darkness. The mixture was incubated for 20 min in darkness at 4 °C, then centrifuged at 3000 rpm for 20 min. 100 μL supernatant were taken for the ferric reducing antioxidant power (FRAP) assay to express the total antioxidant activity. The principle of the assay was conducted following the method of Benzie and Strain (1999), expressed as Fe^{3+} equivalents ($\mu\text{mol Fe}^{2+}/\text{g}$ fresh mass).

The rest of supernatant was shaken and incubated at 4 °C for 12 h in darkness. After the incubation, the mixture was centrifuged at 10,000 g for 20 min in darkness. The supernatant was analyzed for the total phenols content according to Kähkönen et al. (1999) as estimated by the Folin-Ciocalteu reagent, expressed as mg of Gallic acid equivalents (GAE) per unit of fresh mass.

2.4.6. Potential available detoxification capacity

The potential available detoxification capacity (A) can be expressed as: $A = \text{AA}_{\text{area}}/F_{\text{O}_3}$, where AA_{area} is the area-based content of detoxification capacity or antioxidant content, and F_{O_3} ($\text{nmol m}^{-2} \text{ s}^{-1}$) is stomatal O_3 flux. We calculated AA_{area} as: $\text{AA}_{\text{area}} = \text{AA}_{\text{dw}} \times \text{SLM}$, where AA_{dw} represents mass-based content of detoxification capacity or antioxidant measured above (Wieser et al., 2002). We calculated F_{O_3} as: $F_{\text{O}_3} = \text{O}_a (g_{\text{O}_3})$, where g_{O_3} is g_s to water vapor multiplied by 0.613 and O_a is exposure O_3 concentration (ppb) (Di Baccio et al., 2008).

2.5. Statistical analysis

In this study, the OTC was treated as an independent experimental unit for either treatment, which means that the mean of each chamber was obtained first from three to four replicated plants. The data for each variable was checked for normality and homogeneity of variance by Kolmogorov–Smirnov and Levenes' tests, respectively. The effects of O_3 , species and their interaction were tested with generalized linear model by using SPSS 16.0 software package for Windows. The difference of means between CF and E- O_3 was compared by Tukey's tests and considered significant at $P \leq 0.05$. Data shown in tables and figures were means $\pm$ SD ($n = 3$). The relationship between the measured parameters was assessed by linear regression (SPSS 16.0) with a significant correlation when $P \leq 0.05$ and a tendency toward significance when $0.05 < P < 0.1$.

3. Results

3.1. Visible injury

At the end of the experiment, no visible O_3 injury was found in any of eight species grown under CF conditions. However, typical visible symptoms were observed in E- O_3 , including foliar stipple, reddish stipple, necrotic spots, chlorotic mottling and brown spots (Table 2). *L. chinense* and *L. formosana* displayed visible symptoms on the upper surface of leaves when the AOT40 was about 10 ppm h (Table 2). Evergreen species *C. camphora*, *C. glauca*, *S. superba* and *I. integra* displayed symptoms when the AOT40 was higher than 20 ppm h (Table 2). Also, symptoms developed with the increase in AOT40, and injuries in evergreen species were far less severe than those in two deciduous species along with leaf premature leaf senescence and severe defoliation at the end of the experiment. However, *Photinia* $\times$ *fraseri* and *N. sericea* did not display visible symptoms on current-year leaves. Table 2 shows the specific symptoms and the AOT40_injury for each species.

3.2. Dry matter accumulation

Across all species, E- O_3 significantly decreased shoot mass, root mass, total biomass and SLM by 8%, 10%, 11% and 6%, respectively (Table 3). Significant $\text{O}_3 \times$ species interactions were also observed in these variables ($P < 0.001$). In the E- O_3 treatment, two deciduous species, *L. chinense* and *L. formosana*, showed significant reductions in shoot mass, root mass and total biomass (20%–30%), whereas evergreen species showed a decrease in biomass of less than 15% (Table 3). SLM of *L. formosana*, *C. camphora* and *S. superba* in E- O_3 were significantly decreased by 20%, 12% and 11%, respectively, whereas no significant effects were observed in other species.

3.3. Gas exchange and leaf pigment content

There were significant differences in gas exchange parameters and total chlorophyll content among the eight species (Table 4). Across all species, E- O_3 significantly decreased A_{sat} by 24%, increased C_i by 8% and reduced total chlorophyll content by 19%, but

Table 2
AOT40 at the initial visible O_3 symptom onset and the description of the visible symptoms in eight native species exposed to elevated ozone (E- O_3).

Species	Symptom onset date (days after fumigation)	Ozone dose (AOT40, ppm h)	Description of symptoms
<i>Liriodendron chinense</i>	13	9.40	Necrotic spots appeared at the edges of adaxial surface of fully expanded leaves, and expanding leaves turned light-brown. With the increase in AOT40, the light-brown coloration turned dark-brown and necrotic spots formed patches. Finally, pre-senescence and earlier defoliation occurred in developed leaves.
<i>Liquidambar formosana</i>	19	13.52	Light-brown foliar stipple and necrotic spots interspersed on the adaxial surface of fully developed leaves. Expanding leaves turned light-green and/or yellow. Light-brown necrotic spots turned to dark-brown necrotic patch with AOT40 increasing. Finally, early leaf senescence with pronounced leaf abscission occurred after reddening.
<i>Cinnamomum camphora</i>	29	20.06	Chlorosis firstly appeared in fully developed leaves and then leaves turned yellow with pale white stipple widespread on the surface of both developed and expanding leaves. Young leaves were small, yellow, thin and weak.
<i>Cyclobalanopsis glauca</i>	40	27.85	Chlorotic mottling, light-green and/or yellow coloration and water-logging spots were widespread on the adaxial surface of both developed and expanding leaves. Young leaves turned light-green or yellow.
<i>Schima superba</i>	42	29.35	Brown spots were interspersed among the abaxial surface of leaves in some individuals with no symptoms on adaxial surface of developed leaves. Expanding leaves turned yellow, thin and easy to curl as well.
<i>Ilex integra</i>	50	33.36	Chlorosis firstly appeared in the middle of adaxial surface of fully developed leaves along with red-brown and/or dark-brown spots. Dark-brown spots formed necrotic patches interspersed on the adaxial surface.
<i>Photinia</i> $\times$ <i>fraseri</i>	84	38.24	No visible symptoms occurred in developed leaves, but the expanding leaves showed curling.
<i>Neolitsea sericea</i>	84	38.24	No visible symptoms occurred in both developed and expanding leaves.

Table 3
Shoot mass, root mass, total biomass and specific leaf mass (SLM) in eight native woody species exposed to charcoal-filtered air (CF) and elevated O₃ (E-O₃). Data are shown as Mean ± 1S.D (n = 3), and percentage change in each variable was calculated as (O₃-CF) * 100/CF. *, ** indicated significant difference between CF and E-O₃ at P ≤ 0.05 and P ≤ 0.01, respectively.

	Shoot mass (g, DM)			Root mass (g, DM)			Total biomass (g, DM)			SLM (g, DM/m ²)		
	CF	E-O ₃	%	CF	E-O ₃	%	CF	E-O ₃	%	CF	E-O ₃	%
<i>Liriodendron chinense</i>	43.5 ± 3.01	33.5 ± 4.53	-23*	26.1 ± 3.02	17.8 ± 1.53	-32*	69.5 ± 4.51	51.3 ± 6.06	-26*	33.0 ± 2.04	31.9 ± 2.11	-4
<i>Liquidambar formosana</i>	32.1 ± 0.71	26.1 ± 1.67	-19**	19.1 ± 1.79	14.5 ± 1.00	-24*	52.2 ± 2.79	41.1 ± 2.44	-21**	64.4 ± 5.97	51.6 ± 1.75	-20*
<i>Cinnamomum camphora</i>	29.6 ± 1.48	29.0 ± 3.56	-2	17.3 ± 1.08	16.3 ± 1.07	-6	50.1 ± 0.59	43.8 ± 1.60	-13**	77.2 ± 1.31	68.2 ± 4.37	-12*
<i>Cyclobalanopsis glauca</i>	36.9 ± 2.60	36.0 ± 2.81	-2	14.6 ± 1.14	13.2 ± 1.11	-10	51.9 ± 4.28	48.5 ± 2.36	-6	117.2 ± 6.14	115.4 ± 3.24	-2
<i>Schima superba</i>	43.9 ± 2.71	40.6 ± 3.11	-7	20.0 ± 3.85	18.0 ± 3.54	-10	65.3 ± 2.39	58.1 ± 6.16	-11	89.2 ± 3.49	79.0 ± 2.11	-11*
<i>Ilex integra</i>	47.7 ± 2.81	42.5 ± 7.22	-11	20.7 ± 1.22	21.1 ± 4.19	2	68.4 ± 3.43	63.6 ± 2.91	-7	141.8 ± 1.85	143.2 ± 3.76	1
<i>Photinia × fraseri</i>	53.9 ± 0.72	51.0 ± 2.82	-5	15.7 ± 2.58	16.6 ± 3.23	6	67.6 ± 3.77	65.9 ± 3.31	-3	148.6 ± 4.17	155.6 ± 7.95	5
<i>Neolitsea sericea</i>	27.6 ± 1.29	29.6 ± 1.93	7	15.7 ± 1.18	14.7 ± 0.98	-7	44.0 ± 0.70	43.1 ± 2.08	-2	117.1 ± 2.67	109.4 ± 4.11	-7
Overall mean (+/-)		-8			-10			-11			-6	
ANOVA (P-values)												
O ₃		0.001			0.003			<0.001			<0.001	
Species		<0.001			<0.001			<0.001			<0.001	
O ₃ * Species		0.032			0.040			0.003			0.001	

had no significant effect on g_s. E-O₃ induced larger decreases in A_{sat} (36%~42%) and total chlorophyll content (39%~48%) of two deciduous species in comparison with evergreen tree species although *C. camphora* and *C. glauca* also showed significant reduction in A_{sat} and chlorophyll content.

3.4. MDA, TAC and total antioxidant level

Averaged across the eight species, E-O₃ significantly increased MDA content by 28% and decreased TAC by 13%, but it had no effect on total phenols and total AsA level (Table 5). Significant increases in MDA by E-O₃ were observed in both deciduous species *L. chinense* and *L. formosana* and some evergreen species (*C. camphora*, *S. superba* and *Photinia × fraseri*). Relative to CF, E-O₃ significantly decreased TAC in deciduous species *L. chinense* and *L. formosana* by 33% and 28%, respectively, whereas TAC increased in *C. glauca* by 32%. E-O₃ had no significant effects on the total phenols level of any species and total AsA level with the exception of a significant increase in *S. superba*.

3.5. Potential antioxidant availability and amount of antioxidants per unit of stomatal O₃ flux

Significant differences among species were observed in potential antioxidant capacity (A_{TAC}), potential available amount of total phenol (A_{phenol}) and AsA (A_{AsA}) with the lowest in *L. chinense* in comparison with other species (Table 6). E-O₃ had a significant effect on A_{TAC} but no effect on A_{phenol} and A_{AsA}. A significant

interaction between O₃ and species was found in A_{TAC} and A_{phenol}. Relative to CF, E-O₃ significantly decreased A_{TAC} by 33% in *L. formosana*, but increased A_{TAC} by 60% in *C. glauca*. E-O₃ significantly decreased A_{phenol} in *C. camphora* by 28%, but increased A_{phenol} in *Photinia × fraseri* by 26%. On the other hand, A_{AsA} in all species was not affected significantly by E-O₃, although a large increase (42%) was found in *L. chinense*.

3.6. Correlations between variables

The AOT40_injury showed a significant negative correlation with the degree of reduction in total biomass, root biomass, A_{sat} and total chlorophyll content (Fig. 1). As shown in Table 7, SLM in CF plants was positively correlated with AOT40_injury, and negatively correlated with E-O₃-induced reductions in total biomass and A_{sat}. In contrast, g_s or antioxidant level (TAC, t-AsA and t-phenols) in CF plants measured at the end of fumigation was not found to be significantly correlated with the AOT40_injury, reduction in total biomass and A_{sat} (Table 7). However, there was significant or slightly significant (P < 0.06) correlation between both AOT40_injury and biomass reduction and potential antioxidant availability or potential amount of antioxidants (A_{TAC} or A_{phenols}) measured at the end of fumigation in CF plants.

4. Discussion

In this study, impacts of O₃ on subtropical evergreen and deciduous broadleaved tree species were compared under the

Table 4
Gas exchange and total chlorophyll content in eight native woody species exposed to charcoal-filtered air (CF) and elevated O₃ (E-O₃). Data are shown as mean ± 1S.D (n = 3), and percentage change in each variable was calculated as (O₃-CF) * 100/CF. *, ** indicated significant difference between CF and E-O₃ at P ≤ 0.05 and P ≤ 0.01, respectively.

	A _{sat} (μmol CO ₂ m ⁻² s ⁻¹)			g _s (mol H ₂ O m ⁻² s ⁻¹)			C _i (μmol mol ⁻¹)			Total chlorophyll (mg/g FM)		
	CF	E-O ₃	%	CF	E-O ₃	%	CF	E-O ₃	%	CF	E-O ₃	%
<i>Liriodendron chinense</i>	7.65 ± 1.56	4.47 ± 1.07	-42*	0.102 ± 0.020	0.076 ± 0.026	-25	235 ± 12	252 ± 19	7	1.93 ± 0.17	1.19 ± 0.26	-39*
<i>Liquidambar formosana</i>	5.54 ± 0.84	3.53 ± 0.24	-36*	0.068 ± 0.012	0.078 ± 0.004	15	231 ± 14	288 ± 12	24**	1.20 ± 0.11	0.63 ± 0.11	-48**
<i>Cinnamomum camphora</i>	4.44 ± 0.91	3.26 ± 0.19	-27	0.064 ± 0.004	0.067 ± 0.009	6	258 ± 14	294 ± 2	14*	0.75 ± 0.08	0.57 ± 0.06	-25*
<i>Cyclobalanopsis glauca</i>	5.87 ± 0.80	4.18 ± 0.31	-29*	0.072 ± 0.006	0.058 ± 0.006	-19*	224 ± 9	244 ± 20	9	3.11 ± 0.09	3.02 ± 0.12	-3
<i>Schima superba</i>	6.40 ± 0.64	4.32 ± 1.38	-32	0.063 ± 0.005	0.071 ± 0.014	14	226 ± 15	236 ± 15	5	3.02 ± 0.23	3.01 ± 0.23	0
<i>Ilex integra</i>	5.86 ± 0.28	5.66 ± 1.30	-3	0.066 ± 0.011	0.076 ± 0.033	15	224 ± 22	228 ± 45	2	2.89 ± 0.11	2.64 ± 0.08	-9*
<i>Photinia × fraseri</i>	6.58 ± 0.21	6.17 ± 1.15	-6	0.083 ± 0.004	0.084 ± 0.014	1	238 ± 9	240 ± 11	1	2.85 ± 0.14	2.78 ± 0.07	-2
<i>Neolitsea sericea</i>	6.49 ± 0.28	5.47 ± 1.05	-16	0.086 ± 0.011	0.076 ± 0.014	-12	232 ± 12	237 ± 8	2	2.92 ± 0.11	2.92 ± 0.10	0
Overall mean (+/-)		-24			-1			8			-16	
ANOVA (P-values)												
O ₃		<0.001			0.573			<0.001			<0.001	
Species		<0.001			0.013			0.001			<0.001	
O ₃ * Species		0.128			0.135			0.135			<0.001	

Table 5

Leaf MDA content, total antioxidant activity, total phenols and total ascorbate contents in eight native woody species exposed to charcoal-filtered air (CF) and elevated O₃ (E-O₃). Data are shown as Mean ± 1S.D (n = 3), and percentage change in each variable was calculated as (O₃-CF) * 100/CF. *, ** indicated significant difference between CF and E-O₃ at P ≤ 0.05 and P ≤ 0.01, respectively.

	MDA content (nmol g ⁻¹ FM)			Total antioxidant capacity (μmol Fe ²⁺ g ⁻¹ FM)			Total phenols (mg gallic acid equivalents g ⁻¹ FM)			Total ascorbate (μmol g ⁻¹ FM)		
	CF	E-O ₃	%	CF	E-O ₃	%	CF	E-O ₃	%	CF	E-O ₃	%
<i>Liriodendron chinense</i>	75.9 ± 2.8	95.0 ± 1.6	25**	20.9 ± 1.2	14.1 ± 3.2	-33*	7.6 ± 1.3	9.5 ± 0.7	25	9.4 ± 1.1	9.9 ± 0.6	6
<i>Liquidambar formosana</i>	77.3 ± 13.7	128.6 ± 10.3	66**	28.1 ± 1.3	20.3 ± 4.9	-28*	13.1 ± 2.6	13.5 ± 0.7	3	24.9 ± 1.9	30.7 ± 3.1	23
<i>Cinnamomum camphora</i>	123.2 ± 9.2	156.8 ± 10.1	27*	24.8 ± 7.2	18.4 ± 1.2	-26	12.4 ± 0.7	10.8 ± 2.4	-13	14.6 ± 1.2	16.6 ± 3.9	14
<i>Cyclobalanopsis glauca</i>	94.9 ± 17.6	128.7 ± 14.5	36	20.6 ± 1.6	27.3 ± 3.6	32*	12.5 ± 1.0	9.8 ± 1.3	-21	13.9 ± 2.7	14.7 ± 1.5	5
<i>Schima superba</i>	90.9 ± 2.8	103.9 ± 6.8	14*	32.7 ± 5.9	26.5 ± 4.1	-19	9.0 ± 1.4	11.1 ± 2.3	22	20.5 ± 1.3	23.4 ± 0.4	14*
<i>Ilex integra</i>	35.0 ± 9.0	43.2 ± 10.2	23	15.2 ± 3.1	13.0 ± 1.2	-15	7.3 ± 0.5	5.2 ± 1.3	-28	5.2 ± 1.3	4.9 ± 1.6	-7
<i>Photinia × fraseri</i>	87.2 ± 11.3	110.6 ± 6.5	27*	29.2 ± 0.7	26.5 ± 2.1	-9	10.9 ± 0.6	13.1 ± 1.3	21	10.5 ± 0.6	10.7 ± 0.8	1
<i>Neolitsea sericea</i>	85.1 ± 14.0	86.0 ± 7.1	1	20.7 ± 3.1	19.5 ± 2.4	-6	10.5 ± 3.0	8.8 ± 1.5	-16	22.4 ± 5.1	19.1 ± 4.0	-15
Overall mean (+/-)		28			-13			-1			5	
ANOVA (P-values)												
O ₃		<0.001			0.001			0.688			0.300	
Species		<0.001			<0.001			<0.001			<0.001	
O ₃ * Species		0.004			0.007			0.040			0.474	

same growth and exposure conditions, and most tree species were investigated for the very first time in the literature. Significant O₃ × species interaction was found in most variables, thereby indicating that the responses to E-O₃ were variable among species.

4.1. Effects of E-O₃ differed among species

In this study, E-O₃ (AOT40 of 38.2 ppm h) induced visible foliar injuries in all species except *Photinia × fraseri* and *N. sericea*, but the symptoms were species-specific. However, the AOT40_injury differed significantly among species, as shown by lower AOT40_injury in two deciduous species compared to those in evergreen species. For deciduous species *L. chinense* and *L. formosana*, the visible injury was first observed at AOT40 of about 10 ppm h, indicating that they were less sensitive than beech and birch seedlings (with a critical AOT40 of 5 ppm h) (UNECE, 2004), but more sensitive than oak (with an AOT40 of 18 ppm h) (Karlsson et al., 2004). In contrast, most evergreen species except *C. camphora* did not display visible injury until the AOT40 reached above 27 ppm h, indicating that the investigated deciduous species in this study were more sensitive to O₃ than the evergreen species.

From the response of biomass to E-O₃, only two deciduous species and *C. camphora* in E-O₃ showed significant reductions (13–26%) compared to CF plants, whereas most evergreen species showed little change (Table 3). During our experiment, there were

three typhoons with a 6-day rain period from 21 August to 26 August, an 8-day rain period from 31 August to 7 September and a 9-day rain period from 9 September to 17 September (Zhang et al., 2011), which could have aided the trees's recovery from O₃ damage. Heath (2004) indicated that the longer the time between ozone episodes, the greater the ability of the plant to maintain normal growth and development. Hence, it can be inferred that the climate in subtropical regions curtails the negative effects of O₃ on most tree species due to possible recovery to some degree during long stretches of rainy days.

From Fig. 1, there was a significantly negative relationship between AOT40_injury and the percentage reduction in both total biomass and root biomass across all eight species, indicating that the species which first showed visible foliar symptoms also had the largest reductions in biomass. Furthermore, the AOT40_injury was significantly negatively correlated with losses in A_{sat} or total chlorophyll content. All of this suggests that the AOT40 value for the occurrence of initial foliar symptoms can be used as a valuable criterion to rank the sensitivity of these broadleaf species to O₃. Similar results were also reported in VanderHeyden et al. (2001) who investigated the visible O₃ injury in 16 forest species and found that the species which show the earliest foliar injury were also the most sensitive to O₃ throughout the growing season. There was an exception, however, in that some species like maple (*Acer campestre*) showed symptoms at first but were not clearly the

Table 6

Potentially available amount of total antioxidant activity, total phenols and total ascorbate per unit of stomatal O₃ uptake in eight native woody species exposed to charcoal-filtered air (CF) and elevated O₃ (E-O₃). Data are shown as Mean ± 1S.D (n = 3), and percentage change in each variable was calculated as (O₃-CF)*100/CF. *, ** indicated significant difference between CF and E-O₃ at P ≤ 0.05 and P ≤ 0.01, respectively.

	Potential total antioxidant capacity (mmol Fe ²⁺ nmol ⁻¹ s O ₃ ⁻¹)			Potential total phenols (g gallic acid equivalents nmol ⁻¹ s O ₃ ⁻¹)			Potential total ascorbate (mmol ASC nmol ⁻¹ s O ₃ ⁻¹)		
	CF	E-O ₃	%	CF	E-O ₃	%	CF	E-O ₃	%
<i>Liriodendron chinense</i>	0.30 ± 0.06	0.27 ± 0.08	-11	0.11 ± 0.02	0.19 ± 0.06	70	0.14 ± 0.02	0.19 ± 0.05	42
<i>Liquidambar formosana</i>	1.18 ± 0.17	0.59 ± 0.15	-50*	0.55 ± 0.12	0.39 ± 0.05	-29	1.05 ± 0.23	0.88 ± 0.12	-16
<i>Cinnamomum camphora</i>	1.30 ± 0.30	0.83 ± 0.16	-36	0.66 ± 0.03	0.47 ± 0.04	-28**	0.77 ± 0.09	0.76 ± 0.28	-2
<i>Cyclobalanopsis glauca</i>	1.48 ± 0.20	2.36 ± 0.02	60*	0.90 ± 0.18	0.85 ± 0.12	-5	1.00 ± 0.26	1.27 ± 0.14	27
<i>Schima superba</i>	2.04 ± 0.48	1.33 ± 0.41	-35	0.57 ± 0.14	0.54 ± 0.12	-5	1.27 ± 0.14	1.15 ± 0.19	-10
<i>Ilex integra</i>	1.42 ± 0.37	1.22 ± 0.60	-14	0.69 ± 0.12	0.46 ± 0.17	-33	0.50 ± 0.17	0.46 ± 0.24	-7
<i>Photinia × fraseri</i>	2.28 ± 0.25	2.18 ± 0.41	-5	0.85 ± 0.07	1.06 ± 0.10	26*	0.82 ± 0.10	0.88 ± 0.22	7
<i>Neolitsea sericea</i>	1.25 ± 0.37	1.28 ± 0.44	2	0.61 ± 0.11	0.57 ± 0.16	-7	1.31 ± 0.14	1.22 ± 0.26	-7
Overall mean (+/-)		-11			-1			4	
ANOVA (P-values)									
O ₃		0.043			0.139			0.892	
Species		<0.001			<0.001			<0.001	
O ₃ * Species		0.009			0.034			0.535

Fig. 1. Correlations between the AOT40 at initial visible O_3 symptom onset and (a) the reduction in total biomass, (b) the reduction in root biomass, (c) the reduction in A_{sat} , and (d) the reduction in total chlorophyll content expressed as $(CF - O_3) \times 100 / CF$ ($n = 8$).

most greatly injured by the end of O_3 exposure (Calatayud et al., 2007). The present study indicated that the two investigated deciduous species were more sensitive to O_3 than most evergreen broadleaf species in terms of both the timing of injury onset and O_3 -induced reduction in biomass. Calatayud et al. (2011) compared deciduous and evergreen *Quercus* species in response to O_3 and found that deciduous *Quercus* species was more sensitive than evergreen *Q. ilex* based on visible injury, gas exchange, chlorophyll content and biomass responses. Similar results were also found in two deciduous shrubs, which were more sensitive to O_3 than two evergreen shrubs (Calatayud et al., 2010).

O_3 -induced decrease in photosynthesis and premature senescence have been well-documented in many tree species, including deciduous conifer species (e.g. *M. glyptostroboides*) (Feng et al., 2008), deciduous broadleaf species (e.g. *Q. mongolica*, *F. sylvatica*, *Quercus pyrenaica*) (Calatayud et al., 2011; Matyssek et al., 2010; Yan et al., 2010), evergreen needle species (e.g. *Picea abies*, *Pinus sylvestris*) (Emberson et al., 2007; Pretzsch et al., 2010), and evergreen broadleaf species (e.g. *C. camphora*, *Fraxinus ornus*) (Feng et al., 2011; Paoletti et al., 2009). In this study, E- O_3 induced

a large degree of reduction in A_{sat} in most plants with an uncoupling of the relationship between A_{sat} and g_s . The response of g_s to E- O_3 was not consistent among species as shown by an increase in some species and a decrease in others. Moreover, O_3 -induced increase or no change in C_i indicated that the decrease in the photosynthetic capacity in mesophyll cells (Reich, 1987), rather than stomatal closure, appeared to be responsible for the decrease in photosynthetic rate observed in this study.

E- O_3 caused a large reduction in chlorophyll content and TAC, accompanied by a significant increase in MDA content (Tables 4 and 5), confirming that there was oxidative stress in most E- O_3 plants. Many studies have reported an increased concentration of antioxidants or elevated defense enzyme activities in response to O_3 exposure, which interacts with O_3 to neutralize its effect on plant tissues (Löw et al., 2006). Furthermore, the generation and accumulation of superoxide and/or peroxide may differ among species (Wohlgemuth et al., 2002). In this study, however, all plants showed lower total antioxidant capacity in E- O_3 relative to CF at the end of fumigation, with an exception of *C. glauca*, although E- O_3 caused considerable increases of total phenol content in *L. chinense*, *S. superba* and *Photinia × fraseri* (Table 5). The results suggested that increased phenol contents, and other possible related antioxidant contents and antioxidant enzymes activities were not enough to detoxify the high concentration of O_3 in the E- O_3 treatment.

4.2. Possible causes for the different sensitivities to O_3

Inherent SLM can distinguish roughly O_3 -sensitive and O_3 -tolerant species (Bussotti, 2008). Higher O_3 -tolerant species had a lower SLA i.e. higher SLM (Calatayud et al., 2011; Franzaring et al., 2000), which can be a consequence of a high density of mesophyll tissues or of great leaf thickness (Bussotti, 2008; Wieser et al., 2002). Thick leaves with a palisade mesophyll have a greater quantity of chlorophyll molecular per unit of leaf surface, thus enhancing the photosynthesis capacity of this species, which is capable of feeding its detoxification processes more intensely (Bussotti et al., 2007). From Table 7, SLM in CF had a significant

Table 7

Pearson coefficient (r) and levels of significance (P value) in the correlations between parameters in CF plants at the end of the experiment and the AOT40 at initial O_3 symptom onset (AOT40_injury), reduction in total biomass and reduction in A_{sat} , respectively ($n = 8$).

	AOT40_injury		Reduction in total biomass		Reduction in A_{sat}	
	r	P	r	P	r	P
SLM	0.922	0.001	-0.922	0.001	-0.914	0.002
g_s	-0.153	0.717	0.230	0.583	0.142	0.737
TAC	-0.053	0.901	0.105	0.8305	0.294	0.480
t-phenols	-0.099	0.816	-0.094	0.824	0.199	0.636
t-AsA	-0.092	0.829	0.067	0.875	0.415	0.306
A_{TAC}	0.724	0.042	-0.702	0.052	-0.549	0.159
$A_{phenols}$	0.689	0.059	-0.810	0.015	-0.607	0.111
A_{AsA}	0.470	0.240	-0.511	0.196	-0.059	0.890

positive correlation with AOT40_injury and a significant negative relationship with the loss in biomass and net photosynthesis, indicating that O₃ sensitivity is likely to be higher for species with an inherently lower SLM. Thus, it can be inferred that inherent SLM may be one key parameter to determine the onset of visible injury and plant sensitivity to O₃. Similar results were also found in different clones of silver birch, where the thickness of leaves was an important factor influencing the development of foliar visible O₃ injury (Karlsson et al., 2007).

The harmful effects of O₃ on plants directly depend on the stomatal O₃ flux (Gerosa et al., 2009), largely controlled by the degree of stomatal opening (Castagna and Ranieri, 2009). Zhang et al. (2001) reported that species with intrinsically higher g_s have a higher potential O₃ uptake and thus exhibit a greater negative growth response to a given level of O₃ than species with lower g_s . During the experiment, the g_s in each species was measured three times in total, and there was no significant interaction of dates $\times$ species $\times$ O₃ ($P=0.829$), suggesting that the response of each species to E-O₃ was similar at different measurement dates. Therefore, the g_s measured at the last time was used to make regression relationships with other parameters. From the analysis, higher g_s only partially explained the earlier occurrence of visible symptom and greater reduction in A_{sat} in one deciduous tree *L. chinense*. However, the other five broadleaved evergreen species, with intrinsically higher g_s , showed larger AOT40_injury and lower losses in biomass, suggesting that g_s did not contribute to the different sensitivity to O₃ among selected species (Table 7). Similar findings were reported in inter- or intra-specific differences in O₃ sensitivity for aspen and Mediterranean shrubs (Calatayud et al., 2010; Nikula et al., 2009).

It can be inferred that the negative effects of O₃ on plants were dependent not only on the O₃ flux (Matyssek et al., 2007), but on the balance between the flux and detoxification to cope with oxidative stress (Castagna and Ranieri, 2009). The antioxidant capacity per unit of O₃ influx was assumed to be the best indicator to combine these two factors. From our results, both AOT40_injury and O₃-induced reduction in total biomass were strongly related with A_{TAC} or $A_{phenols}$, but not with TAC or phenol or AsA content, indicating that potential detoxification capacity, rather than antioxidant capacity, contributed to the different response to O₃ among the eight subtropical broadleaved species. Similar results were also found in Di Baccio et al. (2008), in which lower potential detoxification capacity in the poplar clone Eridano contributed to the greater sensitivity to O₃ than clone I-214, although foliar antioxidant defense capacities were increased in the clone Eridano. The present results further confirmed that the effective O₃ flux (i.e., the balance between the flux and detoxification in plants) was more important for assessing the sensitivity of species to O₃ than g_s or antioxidant (Musselman et al., 2006). Admittedly, g_s or antioxidant capacity evaluated by the activity of radical scavenging enzymes and concentration of antioxidants changes with time during the O₃ fumigation. The analysis above based on the biochemical measurements at the end of the fumigation was able to provide some clues to determine the biochemical mechanisms of sensitivity of plants to O₃.

Notably, *N. sericea* had similar SLM, g_s and intrinsic antioxidant capacity to *C. glauca*, but showed a totally different O₃ sensitivity. Compared with the CF treatment, E-O₃ had no overall effects on carbon assimilation rate and biomass accumulation in *N. sericea*. The resistance of *N. sericea* could be related to its leaf morphology, e.g. golden densely fine hair, on both surfaces of young leaves or some anatomy features. Hayes et al. (2007) investigated the sensitivity of 83 semi-natural vegetation species to O₃ and found that plants of the therophyte lifeform were particularly sensitive to O₃ and that species with higher mature leaf N concentration were

more sensitive to O₃ than those with lower leaf N concentration. It was suggested that other plants factors like leaf longevity, foliar N concentration and BVOC (especially ethylene) emission rate are also necessary to further identify the response of species to O₃.

The O₃ concentration used in this study was much higher than the mean daytime O₃ concentration in the surrounding regions, but it is similar to the 1-h peak concentration (Wang et al., 2006). However, the purpose of this study was to determine the O₃ sensitivity among eight woody species; hence, the simplest way is that small seedlings were exposed to high O₃ concentration for a relatively short term until almost all species showed visible injury. Admittedly, the conclusions obtained in this study remain to be verified in other experiments due to some uncertainties. The seedlings were grown in pots with ample water and nutrients, and therefore had more dynamic carbon economies than mature trees which are less allocationally plastic and must compete for light, water, and other resources (Ollinger et al., 1997). Moreover, the degree of increase or decrease in the gas exchange and antioxidant capacity varied significantly from day to day and from one season to another, suggesting that a multi-year experiment with many measurements of plant structure and functions related to the sensitivity to O₃ and data analysis based on the accumulated stomatal O₃ uptake is needed to clarify the effects of O₃ on Chinese tree species, the difference in the sensitivity of tree species to O₃ and its mechanisms.

5. Conclusions

After exposure to E-O₃ for one growing season, visible injury and significant reduction in carbon assimilation and biomass accumulation were found in some native subtropical broadleaved woody species in Eastern China. The species which first showed visible foliar symptoms also had the largest reductions in biomass. The evergreen broadleaf species that were investigated showed less sensitivity to O₃ than the two deciduous species in terms of AOT40 value at initial visible O₃ injury onset and biomass losses. From the variables investigated, specific leaf mass, rather than g_s , contributed significantly to the different ozone sensitivity between eight broadleaf species. Notably, foliar morphology (e.g. densely fine hair) could not be neglected as possible reasons to explain O₃ tolerance. It remains to be verified whether the observed responses to O₃ in these species changes with the extended experimental seasons or in natural forest ecosystems.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (No. 30700086, 31170424) and Tiantong National Station of Forest Ecosystem of China (No. TT200707). We gratefully acknowledge Dr. Xihua Wang, Ms. Fangfang Yao and Dr. Yuan Tian for offering the location and ozone Monitor, respectively. We also acknowledge Mr. Wenzhi Song for his technical support in the open-top chambers system. Special appreciation is given to Professor Zongwei Feng for his constructive suggestions on the experimental design. We also express our appreciation to Dr. Charles Chen for English improvement throughout the manuscript.

References

- Augustaitis, A., Bytnerowicz, A., 2008. Contribution of ambient ozone to Scots pine defoliation and reduced growth in the Central European forests: a Lithuanian case study. *Environmental Pollution* 155, 436–445.
- Benzie, I.F.F., Strain, J.J., 1999. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version

- for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology* 299, 15–27.
- Bussotti, F., 2008. Functional leaf traits, plant communities and acclimation processes in relation to oxidative stress in trees: a critical overview. *Global Change Biology* 14, 2727–2739.
- Bussotti, F., Desotgiu, R., Cascio, C., Strasser, R.J., Gerosa, G., Marzuoli, R., 2007. Photosynthesis responses to ozone in young trees of three species with different sensitivities, in a 2-year open-top chamber experiment (Curno, Italy). *Physiologia Plantarum* 130, 122–135.
- Bytnerowicz, A., Omasa, K., Paoletti, E., 2007. Integrated effects of air pollution and climate change on forests: a northern hemisphere perspective. *Environmental Pollution* 147, 438–445.
- Calatayud, V., Cerveró, J., Calvo, E., García-Breijo, F.J., Reig-Armiñana, J., Sanz, M.J., 2011. Responses of evergreen and deciduous *Quercus* species to enhanced ozone levels. *Environmental Pollution* 159, 55–63.
- Calatayud, V., Cerveró, J., Sanz, M.J., 2007. Foliar, physiological and growth responses of four maple species exposed to ozone. *Water, Air, and Soil Pollution* 185, 239–254.
- Calatayud, V., Marco, F., Cerveró, J., Sánchez-Peña, G., Sanz, M.J., 2010. Contrasting ozone sensitivity in related evergreen and deciduous shrubs. *Environmental Pollution* 158, 3580–3587.
- Castagna, A., Ranieri, A., 2009. Detoxification and repair process of ozone injury: from O₃ uptake to gene expression adjustment. *Environmental Pollution* 157, 1461–1469.
- D'Haese-Vandermeiren, K., Asard, H., Horemans, N., 2005. Other factors than apolipid ascorbate contribute to the differential ozone tolerance of two clones of *Trifolium repens* L. *Plant, Cell and Environment* 28, 623–632.
- Di Baccio, D., Castagna, A., Paoletti, E., Sebastiani, L., Ranieri, A., 2008. Could the differences in O₃ sensitivity between two poplar clones be related to a difference in antioxidant defense and secondary metabolic response to O₃ influx? *Tree Physiology* 28, 1761–1772.
- Emberson, L.D., Buker, P., Ashmore, M.R., 2007. Assessing the risk caused by ground level ozone to European forest trees: a case study in pine, beech and oak across different climate regions. *Environmental Pollution* 147, 454–466.
- Feng, Z.Z., Niu, J.F., Zhang, W.W., Wang, X.K., Yao, F.F., Tian, Y., 2011. Effects of ozone exposure on sub-tropical evergreen *Cinnamomum camphora* seedlings grown in different nitrogen loads. *Trees – Structure and Function* 25, 617–625.
- Feng, Z.Z., Zeng, H.Q., Wang, X.K., Zheng, Q.W., Feng, Z.W., 2008. Sensitivity of *Metasequoia glyptostroboides* to ozone stress. *Photosynthetica* 46, 463–465.
- Franzaring, J., Tonneijck, A.E.G., Kooijman, A.W.N., Dueck, Th.A., 2000. Growth responses to ozone in plant species from wetlands. *Environmental and Experimental Botany* 44, 39–48.
- Gerosa, G., Marzuoli, R., Desotgiu, R., Bussotti, F., Ballarin-Denti, A., 2008. Visible leaf injury in young trees of *Fagus sylvatica* L. and *Quercus robur* L. in relation to ozone uptake and ozone exposure. An Open-Top Chambers experiment in South Alpine environmental conditions. *Environmental Pollution* 152, 274–284.
- Gerosa, G., Marzuoli, R., Desotgiu, R., Bussotti, F., Ballarin-Denti, A., 2009. Validation of the stomatal flux approach for the assessment of ozone visible injury in young forest trees. Results from the TOP (transboundary ozone pollution) experiment at Curno, Italy. *Environmental Pollution* 157, 1497–1505.
- Gillespie, K.M., Ainsworth, E.A., 2007. Measurement of reduced, oxidized and total ascorbate content in plants. *Nature Protocols* 2, 871–874.
- Haberer, K., Herbinger, K., Alexou, M., Tausz, M., Rennenberg, H., 2007. Antioxidative defence of old growth beech (*Fagus sylvatica*) under double ambient O₃ concentrations in a free-air exposure system. *Plant Biology* 9, 215–226.
- Hayes, F., Jones, M.L.M., Mills, G., Ashmore, M., 2007. Meta-analysis of the relative sensitivity of semi-natural vegetation species to ozone. *Environmental Pollution* 146, 754–762.
- He, X.Y., Fu, S.L., Chen, W., Zhao, T.H., Xu, S., Tuba, Z., 2007. Changes in effects of ozone exposure on growth, photosynthesis, and respiration of *Ginkgo biloba* in Shenyang urban area. *Photosynthetica* 45, 555–561.
- Heath, R., 2004. The modification of photosynthetic capacity induced by ozone exposure. *Photosynthesis and the Environment* 5, 409–433.
- Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics* 125, 189–198.
- Inclán, R., Gimeno, B.S., Peñuelas, J., Gerant, D., Quejido, A., 2011. Carbon isotope composition, macronutrient concentrations, and carboxylating enzymes in relation to the growth of *Pinus halepensis* Mill. when subject to ozone stress. *Water, Air, and Soil Pollution* 214, 587–598.
- Kähkönen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S., Heinonen, M., 1999. Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry* 47, 3954–3962.
- Karlsson, P.E., Tang, L., Sundberg, J., Chen, D., Lindsog, A., Pleijel, H., 2007. Increasing risk for negative ozone impacts on vegetation in northern Sweden. *Environmental Pollution* 150, 96–106.
- Karlsson, P.E., Uddling, J., Braun, S., Broadmeadow, M., Elvira, S., Gimeno, B.S., Le Thiec, D., Oksanen, E., Vandermeiren, K., Wilkinson, M., Emberson, L., 2004. New critical levels for ozone effects on young trees based on AOT40 and simulated cumulative leaf uptake of ozone. *Atmospheric Environment* 38, 2283–2294.
- Kitao, M., Löw, M., Heerdt, C., Grams, T.E.E., Häberle, K.H., Matyssek, R., 2009. Effects of chronic elevated ozone exposure on gas exchange responses of adult beech trees (*Fagus sylvatica*) as related to the within-canopy light gradient. *Environmental Pollution* 157, 537–544.
- Kontunen-Soppela, S., Riikonen, J., Ruhanen, H., Brosché, M., Somervuo, P., Pelttonen, P., Kangasjärvi, J., Auvinen, P., Paulin, L., Keinänen, M., Oksanen, E., Vapaavuori, E., 2010. Differential gene expression in senescing leaves of two silver birch genotypes in response to elevated CO₂ and tropospheric ozone. *Plant, Cell and Environment* 33, 1016–1028.
- Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in Enzymology* 148, 350–382.
- Löw, M., Herbinger, K., Nunn, A.J., Häberle, K.H., Leuchner, M., Heerdt, C., Werner, H., Wipfler, P., Pretzsch, H., Tausz, M., 2006. Extraordinary drought of 2003 overrules ozone impact on adult beech trees (*Fagus sylvatica*). *Trees – Structure and Function* 20, 539–548.
- Matyssek, R., Bytnerowicz, A., Karlsson, P.E., Paoletti, E., Sanz, M., Schaub, M., Wieser, G., 2007. Promoting the O₃ flux concept for European forest trees. *Environmental Pollution* 146, 587–607.
- Matyssek, R., Wieser, G., Ceulemans, R., Rennenberg, H., Pretzsch, H., Haberer, K., Löw, M., Nunn, A., Werner, H., Wipfler, P., Oßwald, W., Nikolova, P., Hanke, D.E., Kaigher, H., Tausz, M., Bahnweg, G., Kitao, M., Dieler, J., Sandermann, H., Herbinger, K., Grebenc, M., Blumenröther, M., Deckmyn, G., Grams, T.E.E., Heerdt, C., Leuchner, M., Fabian, P., Häberle, K.H., 2010. Enhanced ozone strongly reduces carbon sink strength of adult beech (*Fagus sylvatica*) – resume from the free-air fumigation study at Kranzberg Forest. *Environmental Pollution* 158, 2527–2532.
- McGrath, J.M., Karnosky, D.F., Ainsworth, E.A., 2010. Spring leaf flush in aspen (*Populus tremuloides*) clones is altered by long-term growth at elevated carbon dioxide and elevated ozone concentration. *Environmental Pollution* 158, 1023–1028.
- McLaughlin, S.B., Nosal, M., Wullschlegel, S.D., Sun, G., 2007. Interactive effects of ozone and climate on tree growth and water use in a southern Appalachian forest in the USA. *New Phytologist* 174, 109–124.
- Musselman, R.C., Lefohn, A.S., Massman, W.J., Heath, R.L., 2006. A critical review and analysis of the use of exposure- and flux-based ozone indices for predicting vegetation effects. *Atmospheric Environment* 40, 1869–1888.
- Nali, C., Paoletti, E., Marabottini, R., Della Rocca, G., Lorenzini, G., Paolacci, A.R., Ciaffi, M., Badiani, M., 2004. Ecophysiological and biochemical strategies of response to ozone in Mediterranean evergreen broadleaf species. *Atmospheric Environment* 38, 2247–2257.
- Nikula, S., Percy, K., Oksanen, E., Holopainen, T., Manninen, S., 2009. Effects of elevated ozone on growth and foliar traits of European and hybrid aspen. *Boreal Environment Research* 14, 29–47.
- Oksanen, E., Riikonen, J., Kaakinen, S., Holopainen, T., Vapaavuori, E., 2005. Structural characteristics and chemical composition of birch (*Betula pendula*) leaves are modified by increasing CO₂ and ozone. *Global Change Biology* 11, 732–748.
- Ollinger, S.V., Aber, J.D., Reich, P.B., 1997. Simulating ozone effects on forest productivity: interactions among leaf-, canopy- and stand-level processes. *Ecological Applications* 7, 1237–1251.
- Orendovici-Best, T., Skelly, J.M., Davis, D.D., Ferdinand, J.A., Savag, J.E., Stevenson, R.E., 2008. Ozone uptake (flux) as it relates to ozone-induced foliar symptoms of *Prunus serotina* and *Populus maximowiczii* × *trichocarpa*. *Environmental Pollution* 151, 79–92.
- Paoletti, E., 2006. Impact of ozone on Mediterranean forests: a review. *Environmental Pollution* 144, 463–474.
- Paoletti, E., 2009. Ozone and urban forests in Italy. *Environmental Pollution* 157, 1506–1512.
- Paoletti, E., Contran, N., Bernasconi, P., Günthardt-Goerg, M.S., Vollenweider, P., 2009. Structural and physiological responses to ozone in Manna ash (*Fraxinus ornus* L.) leaves of seedlings and mature trees under controlled and ambient conditions. *Science of the Total Environment* 407, 1631–1643.
- Paoletti, E., Schaub, M., Matyssek, R., Wieser, G., Augustaitis, A., Bastrup-Birk, A.M., Bytnerowicz, A., Günthardt-Goerg, M.S., Müller-Starck, G., Serengil, Y., 2010. Advances of air pollution science: from forest decline to multiple-stress effects on forest ecosystem services. *Environmental Pollution* 158, 1986–1989.
- Pina, J.M., Moraes, R.M., 2010. Gas exchange, antioxidants and foliar injuries in saplings of a tropical woody species exposed to ozone. *Ecotoxicology and Environmental Safety* 73, 685–691.
- Pretzsch, H., Dieler, J., Matyssek, R., Wipfler, P., 2010. Tree and stand growth of mature Norway spruce and European beech under long-term ozone fumigation. *Environmental Pollution* 158, 1061–1070.
- Reich, P.B., 1987. Quantifying plant response to ozone: a unifying theory. *Tree Physiology* 3, 63–91.
- Ren, W., Tian, H.Q., Tao, B., Chappelka, A., Sun, G., Lu, C.Q., Liu, M.L., Chen, G.S., Xu, X.F., 2011. Impacts of tropospheric ozone and climate change on net primary productivity and net carbon exchange of China's forest ecosystems. *Global Ecology and Biogeography* 20, 391–406.
- Tausz, M., Grulke, N.E., Wieser, G., 2007. Defense and avoidance of ozone under global change. *Environmental Pollution* 147, 525–531.
- Tkacz, B., Moody, B., Castillo, J.V., Fenn, M.E., 2008. Forest health conditions in North America. *Environmental Pollution* 155, 409–425.
- UNECE, 2004. Manual on Methodologies and Criteria for Modelling and Mapping Critical Loads and Levels and Air Pollution Effects, Risks and Trends. UNECE. <http://www.icpmmapping.org>.
- VanderHeyden, D., Skelly, J., Innes, J., Hug, C., Zhang, J., Landolt, W., Bleuler, P., 2001. Ozone exposure thresholds and foliar injury on forest plants in Switzerland. *Environmental Pollution* 111, 321–331.
- Wang, H.X., Zhou, L.J., Tang, X.Y., 2006. Ozone concentrations in rural regions of the Yangtze Delta in China. *Journal of Atmospheric Chemistry* 54, 255–265.

- Wang, X.K., Manning, W., Feng, Z.W., Zhu, Y.G., 2007. Ground-level ozone in China: distribution and effects on crop yields. *Environmental Pollution* 147, 394–400.
- Wieser, G., Tegischer, K., Tausz, M., Häberle, K.H., Grams, T.E.E., Matyssek, R., 2002. Age effects on Norway spruce (*Picea abies*) susceptibility to ozone uptake: a novel approach relating stress avoidance to defense. *Tree Physiology* 22, 583–590.
- Wittig, V.E., Ainsworth, E.A., Naidu, S.L., Karnosky, D.F., Long, S.P., 2009. Quantifying the impact of current and future tropospheric ozone on tree biomass, growth, physiology and biochemistry: a quantitative meta-analysis. *Global Change Biology* 15, 396–424.
- Wohlgemuth, H., Mittelstrass, K., Kschieschan, S., Bender, J., Weigel, H.J., Overmyer, K., Kangasjavi, J., Sandermann, H., Langebartels, C., 2002. Activation of an oxidative burst is a general feature of sensitive plants exposed to the air pollutant ozone. *Plant, Cell and Environment* 25, 717–726.
- Yamaguchi, M., Watanabe, M., Iwasaki, M., Tabe, C., Matsumura, H., Kohno, Y., Izuta, T., 2007. Growth and photosynthetic responses of *Fagus crenata* seedlings to O₃ under different nitrogen loads. *Trees – Structure and Function* 21, 707–718.
- Yan, K., Chen, W., He, X.Y., Zhang, G.Y., Xu, S., Wang, L., 2010. Responses of photosynthesis, lipid peroxidation and antioxidant system in leaves of *Quercus mongolica* to elevated O₃. *Environmental and Experimental Botany* 69, 198–204.
- Zhang, J., Ferdinand, J.A., Vanderheyden, D.J., Skelly, J.M., Innes, J.L., 2001. Variation of gas exchange within native plant species of Switzerland and relationships with ozone injury: an open-top experiment. *Environmental Pollution* 113, 177–185.
- Zhang, W.W., Niu, J.F., Wang, X.K., Tian, Y., Yao, F.F., Feng, Z.Z., 2011. Effects of ozone exposure on growth and photosynthesis of the seedlings of *Liriodendron chinense* (Hemsl.) Sarg, a native tree species of subtropical China. *Photosynthetica* 49, 29–36.