



Ozone-induced changes in physiological and biochemical traits in *Elaeocarpus sylvestris* and *Michelia chapensis* in South China

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ABSTRACT

The influences of elevated ozone (O_3) concentrations on biomass, leaf gas exchange, fluorescence parameters, and leaf physiological and biochemical traits were examined in *Elaeocarpus sylvestris* and *Michelia chapensis* seedlings under four O_3 conditions for a growing season in open-top chambers (OTCs). The four O_3 concentrations were charcoal-filtered air (CF) (20 ppb), $1 \times O_3$ air (40 ppb), $2 \times O_3$ air (80 ppb), and $4 \times O_3$ air (160 ppb), respectively. The significant decrease in the root/shoot (R/S) ratios of both species indicated that under O_3 stress root biomass was more negatively affected than shoot biomass for both tree seedlings. Along with the loss of chlorophyll and carotenoid contents, decrease in the superoxide dismutase (SOD), and enhanced level of lipid peroxidant, the light-saturated net photosynthesis rate (P_{nmax}) of *E. sylvestris*, the effective quantum yield of PSII photochemistry (Y(II)) and the electron transport rate (ETR) of both species decreased, suggesting that impaired photosynthesis occurred. The negative effect of O_3 on physiological and biochemical parameters was greater for *M. chapensis* than for *E. sylvestris*. As the O_3 concentration increased, the leaf mass per area (LMA) of *E. sylvestris* decreased, while that of *M. chapensis* increased. Therefore, the increased SOD activity as a hormetic-compensatory response, the increases in LMA and longer vegetative period contributed to the good adaptability and high tolerance of *M. chapensis*.

1. Introduction

Ozone (O_3) in the troposphere is a potent phytotoxic air pollutant, which is a pressing concern for forests due to phytotoxic effects (Matyssek et al., 2007; Jolivet et al., 2016; Cailleret et al., 2018). The ground-level concentration of O_3 has continuously increased since the last century (Derwent et al., 2007). With rapid industrialization and urbanization in the Pearl River Delta of China, the annual mean O_3 concentrations of 23 stations in the network were 17–40 ppb in 2015. Twenty-two of these stations recorded average O_3 concentrations that exceeded the 1-h limit (GB3095-2012; 200 $\mu\text{g}/\text{m}^3$, i.e., 93 ppb) (PRDAIR, 2016). Therefore, the increasing trend of the tropospheric O_3 concentration is expected to continue and further threaten the function and productivity of terrestrial ecosystems (Vingarzan, 2004; Dai et al., 2017).

Generally, an increased ground-level O_3 concentration causes growth reduction (Díaz-de-Quijano et al., 2012) and impairs plant physiological traits, such as leaf gas exchange (Gao et al., 2017), which ultimately leads to a decrease in biomass and net primary productivity

(Díaz-de-Quijano et al., 2012). Elevated ground-level O_3 changes leaf structural traits, nutrient allocation and absorption, and alters photosynthetic responses and reduces plant biomass (Pleijel et al., 2006). Moreover, some researchers reported that the activities of reactive oxygen species (ROS)-scavenging enzymes increased with increasing ozone (Nali et al., 2004), whereas other authors found that enzyme activities decreased (Polle et al., 1993; Then et al., 2009). The changes of antioxidants induced by O_3 exposure was largely due to the varied dose and the duration of O_3 exposure (Nali et al., 2004). Therefore, the dose-responses of leaf structural and physiological traits and leaf nutrient status to O_3 and their role in the O_3 sensitivity among woody plants species is still unclear.

Although the O_3 effect on some deciduous broadleaved species (e.g., Li et al., 2016) and needle leaf species (Polle et al., 1993; Then et al., 2009) has been widely studied, little research has been conducted on subtropical evergreen species in China (Zhang et al., 2012; Yu et al., 2017; Feng et al., 2019), which creates a knowledge gap that limits the understanding of this important part. Further studies are needed to determine the responses of O_3 on broadleaf evergreen tree species (e.g.,

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Elaeocarpus sylvestris (Lour.) Poir and *Michelia chapensis* Dandy) that are endemic to particular regions where background O₃ concentrations exceed the thresholds (GB3095-2012) for forest protection in South China. *E. sylvestris* and *M. chapensis* are two dominant tree species in secondary evergreen broadleaf forests of the subtropical zones of China. With the increasing background O₃ concentration in China, the responses of elevated O₃ concentrations on the forests in the subtropical zone have received considerable attention from scientists, especially the dominant species.

Little is known regarding the species-specific tolerance of the two dominant species to O₃. Furthermore, comprehensive studies on assessment of dose-relationship for these two species are currently little. We studied the effects of ozone fumigation on the biochemical, physiological and morphological traits of *E. sylvestris* and *M. chapensis* in field-established open-top chambers (OTCs). The objectives were to 1) reveal the effects of O₃ fumigation on the biomass allocation, photosynthetic and physiological characteristics, and nutrient availability of *E. sylvestris* and *M. chapensis* and 2) compare the pleiotropic adaptive responses, dose-responses and O₃ sensitivity of the two dominant tree species in the subtropical mountainous regions of China. Our first hypothesis was that the seedlings with higher leaf mass per area (LMA) would be more tolerant to O₃. Second, we hypothesized that different changes in antioxidants would also affect the tolerance to O₃.

2. Material and methods

2.1. Ozone fumigation and plant materials

The study site was located at the campus of the South China Agricultural University, Guangzhou, China (N 23°8', E 113°17'). The experimental area has a humid subtropical monsoon climate. The annual mean temperature was 22.3 °C and the annual precipitation was 2015.4 mm (Guangzhou Central Meteorological Observatory, 2015). The OTCs for the four O₃ conditions was hexagonal. The size was 11.7 m² (side length 1.5 m and height 2.0 m). The CFY5 oxygen concentrator (Shanmeishuimei Co., Ltd) and COM-AD-01 O₃ generator (Anseros, Germany) were used for producing O₃. In addition, the charcoal-filtered ambient air was also used for mixing the four O₃ condition in the OTCs. Chamber O₃ conditions were monitored at 10 min intervals for every OTC using a GT903-O₃ monitor (Keernuo Co., China). The whole-plot treatment comprised four O₃ concentrations: charcoal-filtered air (CF) (20 ppb), 1 × O₃ air (40 ppb), 2 × O₃ air (80 ppb), and 4 × O₃ air (160 ppb), respectively. The four conditions were randomly assigned and applied to each OTC. The 8-h mean O₃ concentration of the CF, 1 × O₃, 2 × O₃ and 4 × O₃ treatment groups during the fumigation period were 19.2, 38.7, 77.6 and 156.3 ppb, respectively. The AOT40 (accumulated O₃ exposure over a threshold of 40 ppb) by the end of the fumigation in these treatments were 3.4, 25.8, 74.9 and 144.8 ppm h, respectively. The 40 one-year-old seedlings (around 49 and 44 cm height for *Elaeocarpus sylvestris* and *Michelia chapensis*, respectively) were individually planted in 40 pots (diameter 16 cm and height 17 cm) filled with peat and yellow forest soil and grown in the OTCs. The seedlings were under well-watered condition during the experiment. Plants were fumigated from March 1 to December 30, 2013 (a ten month experimental period) with a daily maximum exposure of 8 h (from 09:00 to 17:00) when there was no rainfall.

2.2. Plant biomass

At the end of the experiment (December 30, 2013), fifteen plants per species were randomly collected from each OTC, and the leaves, stems and roots were sampled. The dry weight was measured after drying the seedlings in a forced-air dryer at 70 °C to a constant weight. The dry weights of the stem, leaves and roots were used to calculate the root/shoot (R/S) (stem + leaves) ratio.

2.3. Gas exchange and chlorophyll *a* fluorescence

Gas exchange measurements and chlorophyll *a* fluorescence measurement were performed on the plants treated with different O₃ concentrations at the end of the experimental period (December 30, 2013). The five seedlings of every treatment were randomly chosen, and the three fully expanded current-year leaves of every seedling were measured. Data on the light-saturated net photosynthesis rate (P_{nmax}), stomata conductance (G_s) and internal CO₂ concentration (C_i) were collected using LI-COR LI-6400 photosynthesis system (Lincoln, America). All parameters were measured under a photosynthetically active radiation of 1000 μmol (photon) m⁻² s⁻¹, leaf temperature of 20–25 °C and 400 μmol (CO₂) mol⁻¹ (C_a).

Chlorophyll *a* fluorescence parameters were assessed using Heinz Walz PAM-2500 fluorimeter (Effeltrich, Germany) at the end of the experiment. The sun-lit leaves were covered with an opaque paper bag for 1 h, and then, the minimal fluorescence (F_0) in the dark-adapted state was measured. A saturating pulse of irradiation of 2000 μmol m⁻² s⁻¹ was applied for 3 s to measure the maximal fluorescence (F_m) in the dark-adapted state. Then, the leaves were illuminated with actinic light (300 μmol m⁻² s⁻¹) for 10 min in order to measure minimum fluorescence (F_0'), maximal fluorescence (F_m'), and steady-state fluorescence (F_s). The following parameters were determined: The maximum quantum efficiency of PSII (F_v/F_m) = ($F_m - F_0$)/ F_m), the maximum quantum yield of PSII in the light-adapted state (F_v'/F_m') = ($F_m' - F_0'$)/ F_m'), the effective quantum yield of PSII photochemistry [$Y(\text{II})$] = ($F_m' - F_s$)/ F_m'], electron transport rate ($\text{ETR} = Y(\text{II}) \times 0.5 \times \text{absorbance} \times \text{PPFD}$, PPFD: 300 μmol m⁻² s⁻¹ absorbance was measured by Heinz Walz PAM-2500 fluorimeter directly) and non-photochemical dissipation of absorbed light energy [$\text{NPQ} = F_m/F_m' - 1$].

2.4. Leaf traits

The leaf traits of the mature fresh leaves of seedlings, such as chlorophyll, carotenoid, superoxide dismutase (SOD) activity, malondialdehyde (MDA), soluble protein, leaf carbon (C), nitrogen (N), phosphorus (P) and leaf mass per area (LMA), were determined at the end of the experimental period (December 30, 2013).

The chlorophyll and carotenoid contents were determined by the method of Lichtenthaler (1987).

SOD activity was determined according to the method provided by the nitroblue tetrazolium (NBT) photoreduction method (Chen and Wang, 2002). MDA was determined by the thiobarbituric acid method of determination (Chen and Wang, 2002). Soluble protein content was determined by the Coomassie Brilliant Blue G-250 method (Li, 2000).

Plant C concentrations were determined with a combustion/non-dispersive infrared gas analyzer (Shimadzu TOC 5000 A Total Organic Carbon Analyzer, Kyoto, Japan). The leaf N and P were determined by the alkali hydrolysis method and molybdenum-antimony-D-isoascorbic-acid-colorimetry, respectively (ISSCAS, 1978). Fifteen leaves were collected to determine the leaf mass per area (LMA) and the N content in the leaves (December 30, 2013). The LMA was calculated from the area and dry mass of the leaves (g m⁻²). We calculated the area-based N content (N_{area}) as the product of the leaf N and LMA and the photosynthetic nitrogen-use efficiency (PNUE) as P_{nmax} divided by N_{area} .

2.5. Statistical analysis

A completely randomized experimental design was adopted. All data analyses were performed using the statistical software IBM SPSS Statistics 20. For each biomass parameter and physiological and biochemical variables, data were analyzed using a two-way ANOVA. Here, O₃ and tree species served as the main fixed factors plus an O₃ × tree species interaction term. Comparisons between the O₃ treatment groups were analyzed using one-way ANOVA followed by the Tukey HSD post hoc test (different letters denote a significant difference among the four

Table 1

Effects of O₃, tree species, and their interactions on biomass allocation, leaf gas exchange, chlorophyll fluorescence parameters, and physiological and biochemical traits.

	Parameters	O ₃	Tree species	O ₃ × Tree species
1	Total biomass	< 0.001	0.033	0.21
2	Leaf biomass	< 0.001	0.30	0.28
3	Stem biomass	< 0.001	0.97	0.81
4	Root biomass	0.003	< 0.001	0.62
5	LWR	0.029	0.78	0.093
6	SWR	< 0.001	< 0.001	0.40
7	RWR	< 0.001	< 0.001	0.16
8	R/S ratio	< 0.001	< 0.001	0.071
9	P _{mas}	0.22	0.018	0.78
10	G _s	0.009	< 0.001	< 0.001
11	C _i	0.44	< 0.001	< 0.001
12	F _v /F _m	< 0.001	0.035	0.14
13	F _v '/F _m '	< 0.001	0.085	0.39
14	Y(II)	< 0.001	< 0.001	< 0.001
15	ETR	< 0.001	< 0.001	< 0.001
16	NPQ	< 0.001	0.072	0.48
17	Chlorophyll content	< 0.001	< 0.001	< 0.001
18	Carotenoid content	< 0.001	< 0.001	< 0.001
19	MDA	< 0.001	< 0.001	< 0.001
20	SOD	< 0.001	< 0.001	< 0.001
21	Soluble protein	< 0.001	< 0.001	0.011
22	Leaf C content	< 0.001	< 0.001	< 0.001
23	Leaf N content	< 0.001	< 0.001	< 0.001
24	Leaf P content	< 0.001	< 0.001	< 0.001
25	Leaf C/N ratio	< 0.001	< 0.001	< 0.001
26	LMA	< 0.001	< 0.001	< 0.001
27	N _{area}	< 0.001	< 0.001	< 0.001

treatment groups at $P < 0.05$).

3. Results

3.1. Plant biomass allocation

Across all measurements, levels of total biomass, leaf biomass, stem biomass and leaf dry weight ratio (LWR) differed significant among O₃ treatments with no significant differences in the species and the interactions (Table 1, Figs. 1 and 2). The root biomass, stem plus branch dry weight ratio (SWR), root dry weight ratio (RWR) and R/S ratio were significantly affected by O₃ and species but not in interactions between O₃ and species (Table 1). The SWRs of both species showed a significantly increasing trend, while the root biomass, total biomass, RWR and R/S ratio significantly decreased (Figs. 1 and 2).

3.2. Leaf gas exchange and fluorescence parameters

Significant differences in G_s, Y(II), ETR, chlorophyll and carotenoid contents were detected in response to O₃, species and their interaction

(Table 1, Fig. 3). For *E. sylvestris*, exposure to elevated O₃ levels significantly decreased G_s and C_i at the end of the experimental period (Fig. 3). Exposure to the 4 × O₃ concentration (160 ppb) significantly decreased the P_{max} of *E. sylvestris*. For both species, the F_v'/F_m', Y(II) and ETR decreased with increasing O₃ concentration, while NPQ increased (Fig. 3). Relative to CF, the chlorophyll and carotenoid contents of the 4 × O₃ concentration group significantly decreased 58.7% and 40.7%, respectively, in *E. sylvestris* and 38.7% and 24.1%, respectively, in *M. chapensis* (Fig. 3).

3.3. Leaf physiological and biochemical traits

Significant differences in physiological and chemical parameters were demonstrated in response to O₃, tree species and the interaction between them (Table 1, Fig. 4). Relative to CF, the MDA of the 4 × O₃ concentration group significantly increased 65.9% and 117.4% in *E. sylvestris* and *M. chapensis*, respectively (Fig. 4). SOD levels in the CF treatment group of *E. sylvestris* significantly increased compared with other treatments, while that of *M. chapensis* significantly increased and then decreased. Relative to CF, SOD levels in the 4 × O₃ concentration group significantly decreased by 36.0% and 25.4% in *E. sylvestris* and *M. chapensis*, respectively. O₃ caused a significant reduction in the soluble protein of *E. sylvestris*. For both species, O₃ caused a significant reduction in leaf C/N ratio and an increase in leaf N, P and N_{area} by the end of the experimental period. Exposure to elevated O₃ conditions significantly decreased LMA of *E. sylvestris* but increased that of *M. chapensis*.

4. Discussion

4.1. Biomass allocation

Several previous studies have demonstrated a significant reduction in root biomass (King et al., 2005; Vollsnes et al., 2010) and whole plant biomass under elevated ambient O₃ concentration (Díaz-de-Quijano et al., 2012; Yu et al., 2017), which is consistent with the results of this study (Table 1, Fig. 1). Exposure to elevated O₃ concentration reduced the root biomass of *E. sylvestris* and *M. chapensis* by 28–40% and 12–25%, respectively, which led to a significant reduction in the R/S ratio and RWR and an increase in the SWR (Table 1, Fig. 2). Typically, exposure to O₃ leads to a shift in biomass allocation in the above-ground part of a tree species with an indeterminate growth pattern (Hoshika et al., 2013; Matyssek et al., 2010; Hoshika et al., 2013; Agathokleous et al., 2015). Shorter and less branched roots in exposed plants cause an alternation of source-sink balance, which can be attributed to a decrease in the carbon translocation to roots and maintains a certain net photosynthesis in the shoots (Gimeno et al., 2004; Agathokleous et al., 2015). As noted in Table 1, the lack of interaction between O₃ and species suggested that the influences of O₃ on these

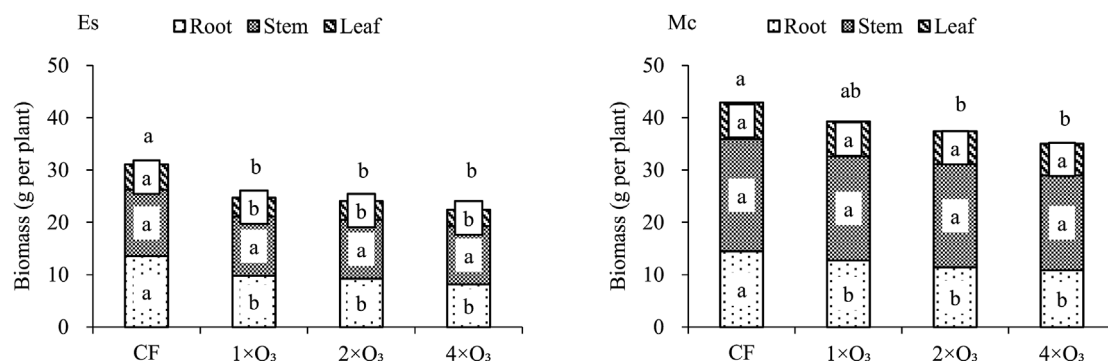


Fig. 1. Biomass of *E. sylvestris* (Es) and *M. chapensis* (Mc) grown under ozone stress in December 2013. Different letters indicate that the means are significantly different between ozone treatments. Error bars represent standard deviations of means ($n = 15$).

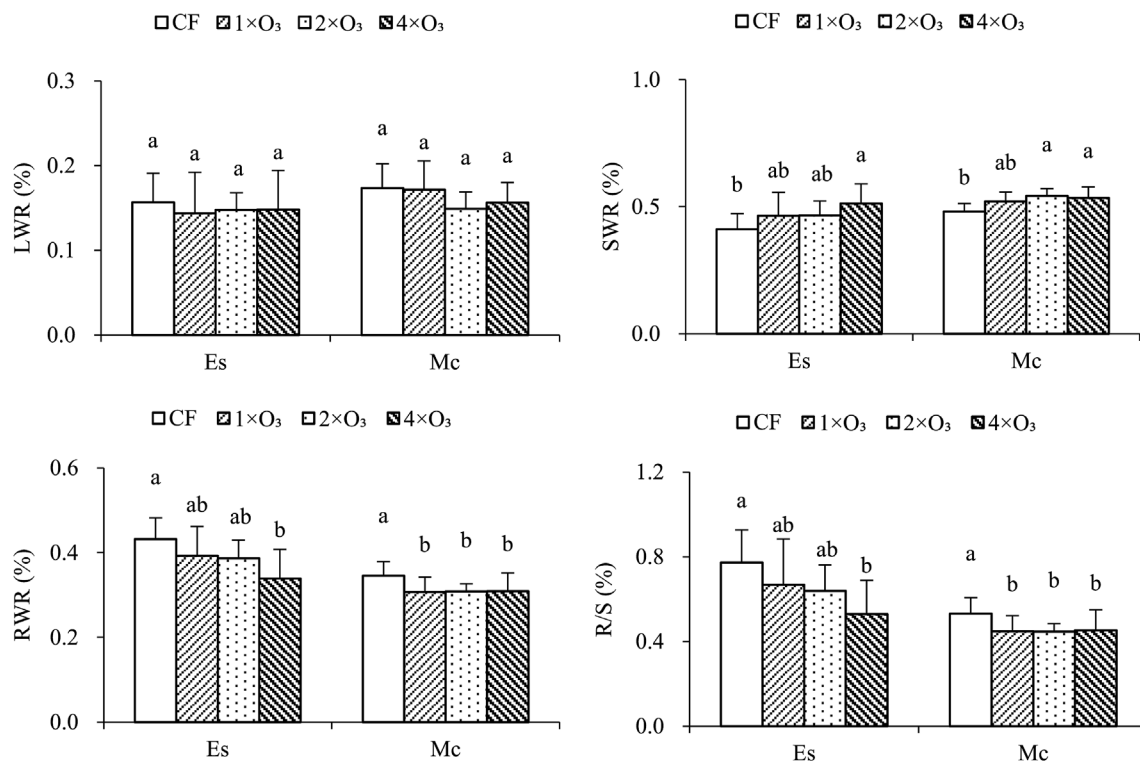


Fig. 2. Leaf dry weight ratio (LWR), stem plus branch dry weight ratio (SWR), and root dry weight ratio (RWR) relative to the whole dry plant weight and R/S ratio of *E. sylvestris* (Es) and *M. chapensis* (Mc) grown under ozone stress in December 2013. Error bars represent standard deviations of means ($n = 15$).

parameters were fairly constant for tree species seedlings, i.e., the relative impacts of O₃ on biomass accumulation and allocation did not change with different tree species. The decrease in root accumulation induced by O₃ fumigation might affect the efficient use of water and nutrients to the aboveground portion of the plant. The changed root/shoot balance might negatively affect the productivity and function of terrestrial ecosystems, especially when the trees are exposed to elevated background O₃ for growing seasons.

4.2. Leaf photosynthetic and chlorophyll fluorescence parameters, and physiological and biochemical traits

Significant differences in G_s , Y(II), ETR, chlorophyll and carotenoid content were found in response to O₃, species and the interaction (Table 1, Fig. 3), indicating that negative effects of elevated O₃ were strongly dependent on tree species, as showed by greater effects of elevated O₃ on *E. sylvestris* than *M. chapensis*. The decreased G_s and C_i values of *E. sylvestris* (Hoshika et al., 2013) P_{nmax} declined under elevated O₃ conditions (Fig. 3), indicating that P_{nmax} downregulation was probably caused by a G_s reduction-induced C_i decrease. When the gas levels increased, the decrease in G_s may be a significant adaptive strategy, which hinders O₃ entering into the leaves via the stomatal apertures, which is consistent with previous studies in other plants (Guidi et al., 2001; He et al., 2007; Yan et al., 2010; Huntingford et al., 2018).

The general tendency of change observed in O₃-exposed seedlings involved reductions in F_v'/F_m' , Y(II) and ETR and an increase in NPQ (Fig. 3), reflecting the occurrence of more damage. Previous studies suggested that the decline of Y(II) might reflect a downregulatory process related to limitation of the Calvin cycle, which implies irreversible damage to the light harvesting system or noncyclic electron transport by O₃ (Calatayud et al., 2007; Li et al., 2017). But significant decreases in chlorophyll and carotenoid contents in both species in this study suggested that the chloroplast ultrastructure and photosynthetic pigments were impaired, especially in *E. sylvestris*, which could lead to

the decline of photosynthetic efficiency and relative electron transport rate (Alonso et al., 2001; Calatayud et al., 2003). Therefore, these results indicate that CO₂ assimilation decline mainly attributes to the occurrence of fluorescence impairment, instead of photoinhibitory damage in PSII reaction centers.

O₃ enters in plants and generates reactive oxygen species (ROS). Antioxidative compounds localized in the apoplast are potential scavengers of ROS and considered as critical components in plant responses against O₃ stress (Chaudhary and Agrawal, 2015; Vainonen and Kangasjärvi, 2015). The increase in MDA indicates enhanced levels of lipid peroxidant in O₃ fumigation seedlings of both species (Fig. 4). Some studies reported an O₃-induced increase in the synthesis of ROS-scavenging enzymes to mop up the highly reactive and membrane-damage molecules (Conklin and Barth, 2004; Kangasjärvi et al., 2005; Vainonen and Kangasjärvi, 2015), while a study found decreasing ROS-scavenging enzymes with increasing O₃ (Biswas et al., 2010). In this study, the significant decrease in SOD activity of *E. sylvestris* seedlings under ozone fumigation and the 4 × O₃ treatment groups of *M. chapensis* seedlings (Fig. 4), which suggested higher oxidative stress in the seedlings potentially beyond the capacity of antioxidants to detoxify O₃-induced ROS sufficiently to protect photosynthetic apparatus. This could lead to aggregation of the O₃-induced increase in ROS production and potential leaf senescence (Yan et al., 2010).

N is an important component of the chlorophyll structure and stromatic enzymes, mainly Rubisco (Calatayud et al., 2007). Some studies suggest that in the presence of O₃, the maintenance of basic plant functions increases proteolysis activity and necessitates a faster protein turnover rate, which subsequently imposes an elevated demand for energy and nutrients (Manderscheid et al., 1992; Inclán et al., 2011). Thus, the improvement of internal nutrient translocation related to repair or compensatory processes in the prematurely senescing foliage of the declining trees induced by O₃ would enhance the availability of N for redistribution to the newly flushed leaves (Temple and Riechers, 1995; Guan and Wen, 2011). Therefore, higher leaf N and P in both species (Fig. 4), which were induced by the cumulative effects of

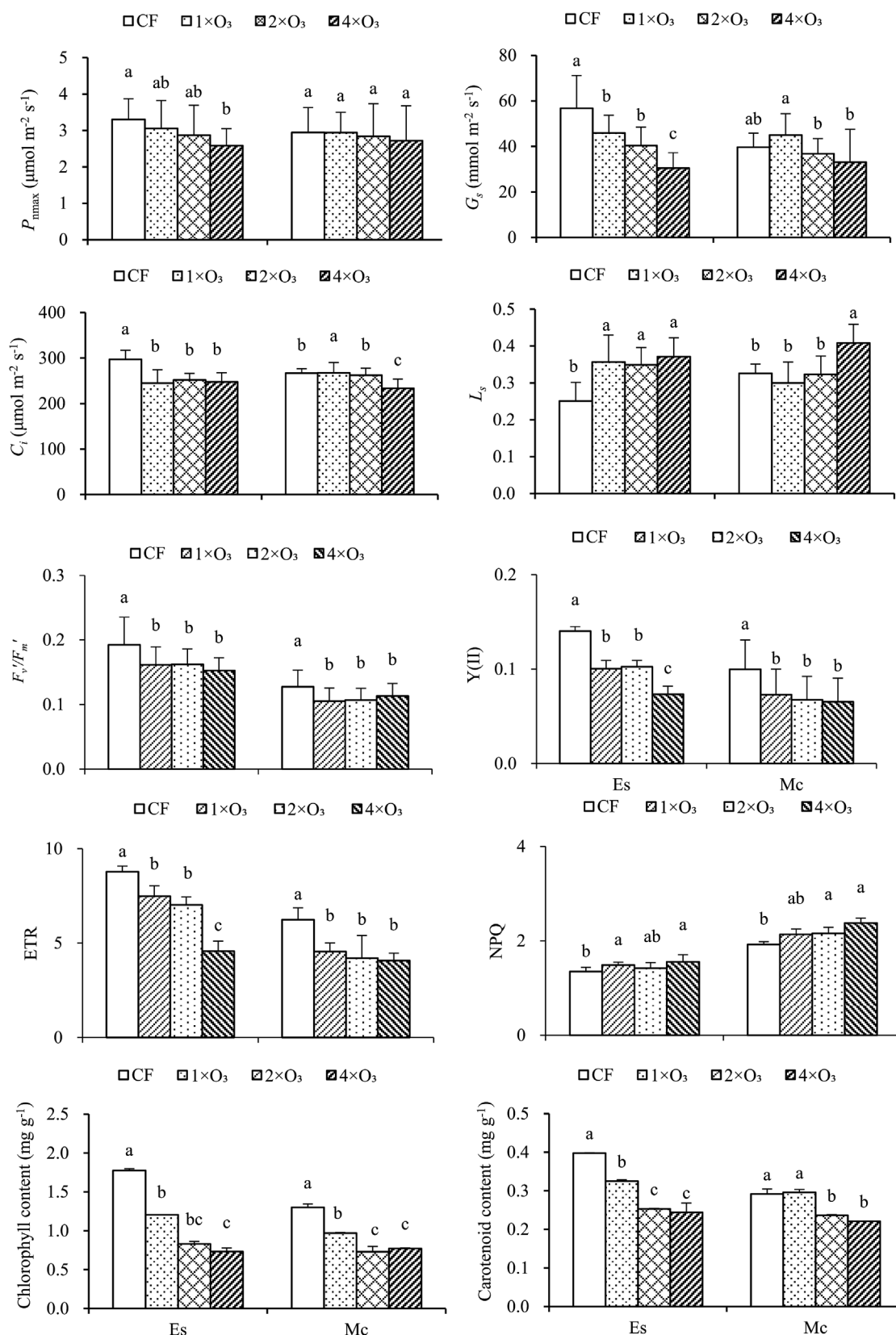


Fig. 3. Mean values of leaf gas exchange, fluorescence parameters and photosynthetic pigments of *E. sylvestris* (Es) and *M. chapensis* (Mc) grown under ozone stress in December 2013. Different letters indicate that the means are significantly different between treatments. Error bars represent standard deviations of means ($n = 15$).

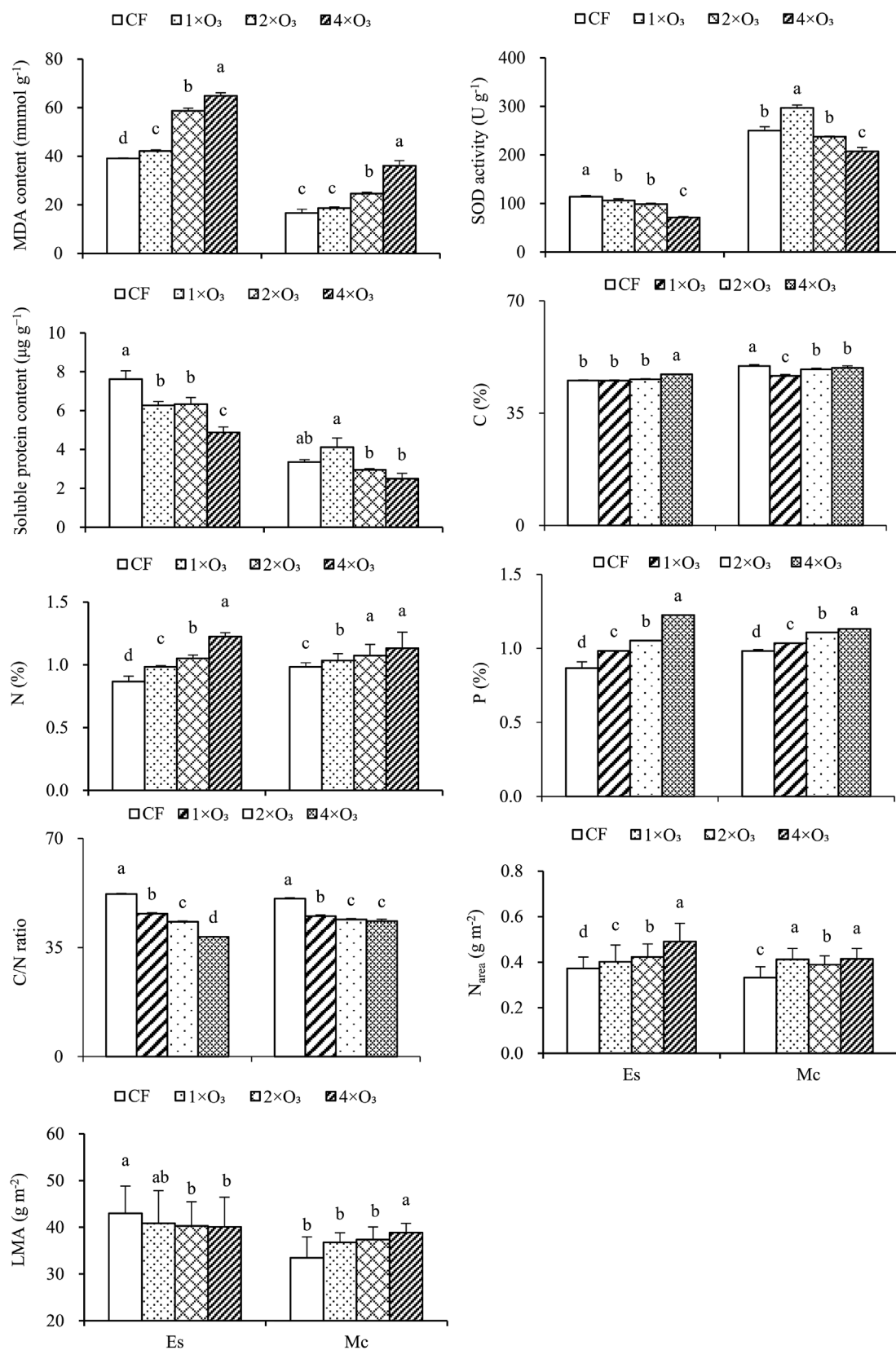


Fig. 4. Mean values of physiological and biochemical traits of *E. sylvestris* (Es) and *M. chapensis* (Mc) grown under ozone stress in December 2013. Error bars represent standard deviations of means (n = 15).

O₃ exposure, were considered as compensatory responses in the seedlings to reduce the negative effects of O₃ exposure on nutrient absorption.

For *E. sylvestris*, exposure to O₃ induces a reduction in the leaf soluble protein and the of total N use efficiency for soluble protein content and causes a decrease in photosynthetic parameters, which are in line with the findings in some previous studies (Yamaguchi et al., 2010; Watanabe et al., 2013). Some studies suggested that O₃-induced reduction in Rubisco content without reduction in N content (Häikiö et al., 2009; Watanabe et al., 2018). Given the increase in structural protein nitrogen, the N content allocated to plant photosynthetic mechanisms decreased under elevated O₃, which reduced the PNUE (Guan and Wen, 2011; Watanabe et al., 2013). Therefore, the decrease in the soluble protein and the increased foliar N content and N_{area} of both species under elevated O₃ concentration exposure in this study (Fig. 4) indicated that more N allocation to structural protein was utilized to build structural defenses instead of altering photosynthesis capacity.

4.3. O₃ adaptability strategy and compensatory mechanisms

A significant interaction between O₃ and tree species was showed in all physiological and biochemical traits (Table 1). The P_{nmax} of *E. sylvestris* seedlings under exposure to the 4 × O₃ concentration (160 ppb) significantly decreased. In contrast, there was no significant difference in G_s and P_{nmax} for the *M. chapensis* seedlings treated with different O₃ levels, suggesting that the adaptability strategy for O₃ was strongly dependent on tree species. It should be noted that as O₃ concentration increased, the LMA of *E. sylvestris* decreased but that of *M. chapensis* increased, which was likely due to a compensatory increase in the mesophyll tissues density and/or leaf thickness (Dai et al., 2017). Plant sensitivity to O₃ was correlated with O₃ uptake, leaf physiological properties, internal resource allocation for defense, repairs, and the compensatory strategies for reducing O₃ damage (Wieser and Matyssek, 2007; Agathokleous, 2018). The increase in leaf thickness enhanced the foliage assimilation capacity per unit area and sustained a high level of photosynthesis under elevated O₃ (Niinemets, 1999). Furthermore, the increased LMA indicated a higher apoplastic leaf fraction, suggesting that a decreased O₃ uptake and oxidative products reached the plasmalemma (Dizengremel et al., 2013). The needle-leaf evergreen species and broadleaf deciduous species with high LMA are less sensitive to O₃ (Feng et al., 2018). A lower O₃ load and internal O₃ exposure per unit leaf mass (Li et al., 2016; Feng et al., 2018) due to the increased LMA can account for the high O₃ tolerance and low sensitivity of *M. chapensis* to O₃ fumigation in terms of leaf biomass, leaf gas exchange and fluorescence parameters, which was consistent with our first hypothesis. *E. sylvestris* seedlings used stomatal closure as a defense barrier against O₃ uptake, while *M. chapensis* seedlings increased LMA, probably leading to a dilution of O₃ and ROS within the leaf cells. In addition, for *M. chapensis*, the larger increase in MDA for lipid peroxidation and the smaller decreases in antioxidants (carotenoid content and SOD) led to smaller decreases in leaf biomass, total biomass, P_{nmax} and chlorophyll α fluorescence parameters (F_v/F_m , F_v'/F_m' , Y(II) and ETR) than *E. sylvestris*. These findings indicated that the higher antioxidant activities improved the tolerance of seedlings to O₃, which supports our second hypothesis.

Some studies reported that the susceptibility of plants to O₃ effects varied among species with different growth patterns probably due to their distinct compensatory mechanisms (Manninen et al., 2009). *E. sylvestris* exhibited a determinate-like growth pattern with the rapid height growth of the seedlings ceasing in early June (Dong et al., 2016). Compared with *E. sylvestris*, *M. chapensis* had a longer vegetative period, which was from May to November every year (Yu et al., 2012). Compared with *M. chapensis*, the rapid phenological development and determinate growth pattern of *E. sylvestris* suppressed the compensatory growth in its shoots and leaves in response to O₃ stress. Furthermore, *M. chapensis* has high cold tolerance and is naturally distributed in valleys

at elevations from 500 to 1500 m, while *E. sylvestris* is naturally distributed in the hilly area at elevations below 1000 m (Editorial Committee of Flora of China, 1996). The high O₃ tolerance of *M. chapensis* corresponded with the average O₃ levels in the species' natural environments because of the higher average O₃ levels in the higher elevation of the mountainous area (Jin et al., 2008).

5. Conclusions

In this study, O₃-induced effects on two representative evergreen broad-leaved tree species native to South China, *E. sylvestris* and *M. chapensis*, were studied for the first time. The significant decrease in the R/S ratios of both species indicated that O₃ stress shifted the allocation of biomass to the aerial part rather than below-ground part. The reductions in F_v/F_m , F_v'/F_m' , Y(II) and ETR and an increase in NPQ suggested that fluorescence impairment occurred in both species, which ruled out the photoinhibitory damage of O₃ to the PSII reaction centers and noncyclic electron transport. A significant interaction between O₃ and tree species was showed in all physiological and biochemical parameters, indicating that negative effect of O₃ stress was strongly dependent on tree species, as demonstrated by greater effects of O₃ on *E. sylvestris* than *M. chapensis*. *M. chapensis* showed high O₃ tolerance during the ten-month experimental period as indicated by the leaf biomass, photosynthetic, physiological and fluorescence parameters (F_v/F_m , Y(II) and ETR). Compared to *E. sylvestris*, the higher SOD activity as a hormetic-compensatory mechanism, the increase in the LMA and its longer vegetative period of *M. chapensis* contributed to the high tolerance of this species to O₃ stress.

Author contributions

Lan Pan: Lan Pan analyzed and interpreted of the data, drafted of the article, and added important intellectual content in the critical revision of the article. **Xiao-jun Zou:** Xiao-jun Zou analyzed and interpreted of the data. **Gan-wen Lie:** Gan-wen Lie designed the setup of experiment, collected and assembled the data, analyzed and interpreted of the data. **Li Xue:** Li Xue designed the setup of experiment, obtained the funding, provided the experimental materials, and reviewed the manuscript. **Hong-yue Chen:** Hong-yue Chen design the setup of experiment, obtained the funding and provided the experimental materials.

Declaration of competing interest

We declare that we have no conflicts of interest to this work.

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References

- Agathokleous, E., 2018. Environmental hormesis, a fundamental non-monotonic biological phenomenon with implications in ecotoxicology and environmental safety. *Ecotox. Environ. Safe.* 148, 1042–1053.
- Agathokleous, E., Saitanis, C.J., Wang, X., Watanabe, M., Koike, T., 2015. A review study on past 40 Years of research on effects of tropospheric O₃ on belowground structure, functioning, and processes of trees: a linkage with potential ecological implications. *Water Air Soil Pollut.* 227, 33.
- Alonso, R., Elvira, S., Castillo, F.J., Gimeno, B.S., 2001. Interactive effects of ozone and drought stress on pigments and activities of antioxidative enzymes in *Pinus halepensis*. *Plant Cell Environ.* 24, 905–916.
- Biswas, D.K., Xu, H., Li, Y.G., Sun, J.Z., Wang, X.Z., Han, X.G., Jiang, G.M., 2010. Genotypic differences in leaf biochemical, physiological and growth responses to ozone in 20 winter wheat cultivars released over the past 60 years. *Global Change Biol.* 14 (1), 46–59.

- Cailleret, M., Ferretti, M., Gessler, A., Rigling, A., Schaub, M., 2018. Ozone effects on European forest growth-Towards an integrative approach. *J. Ecol.* 106 (4), 1377–1389.
- Calatayud, A., Iglesias, D.J., Talón, M., Barreno, E., 2003. Effects of 2-month ozone exposure in spinach leaves on photosynthesis, antioxidant systems and lipid peroxidation. *Plant Physiol. Biochem. (Paris)* 41 (9), 839–845.
- Calatayud, V., Cerveró, J., Sanz, M.J., 2007. Foliar, physiological and growth responses of four maple species exposed to ozone. *Water Air Soil Pollut.* 185 (1–4), 239–254.
- Chaudhary, N., Agrawal, S.B., 2015. The role of elevated ozone on growth, yield and seed quality amongst six cultivars of mung bean. *Ecotoxicol. Environ. Saf.* 111, 286–294.
- Chen, J.C., Wang, X.F., 2002. *Plant Physiology Experiment*. South China University of Technology press, Guangzhou.
- Conklin, P.L., Barth, C., 2004. Ascorbic acid, a familiar small molecule intertwined in the response of plants to ozone, pathogens, and the onset of senescence. *Plant Cell Environ.* 27, 959–970.
- Dai, L., Li, P., Shang, B., Liu, S., Yang, A., Wang, Y., Feng, Z., 2017. Differential responses of peach (*Prunus persica*) seedlings to elevated ozone are related with leaf mass per area, antioxidant enzymes activity rather than stomatal conductance. *Environ. Pollut.* 227, 380–388.
- Derwent, R.G., Simmonds, P.G., Manning, A.J., Spain, T.G., 2007. Trends over a 20-year period from 1987 to 2007 in surface ozone at the atmospheric research station, Mace Head, Ireland. *Atmos. Environ.* 41 (39), 9091–9098.
- Díaz-de-Quijano, M., Schaub, M., Bassin, S., Volk, M., Peñuelas, J., 2012. Ozone visible symptoms and reduced root biomass in the subalpine species *Pinus uncinata* after two years of free-air ozone fumigation. *Environ. Pollut.* 169, 250–257.
- Dizengremel, P., Jolivet, Y., Tuzet, A., Ranieri, A., Le Thiec, D., 2013. Integrative leaf-level ozone phytotoxic dose assessment for forest risk modeling. In: Matyssek, R., Clarke, N., Cudlin, P., Mikkelsen, T.N., Tuovinen, J.P., Wieser, G., Paoletti, E. (Eds.), *Climate Change, Air Pollution and Global Challenges*. Developments in Environmental Sciences 13. Elsevier, Amsterdam, The Netherlands, pp. 267–288.
- Dong, X., Zhang, Y., Xue, L., Hu, T., Hu, J., Lu, G., 2016. Growth analysis on *Elaeocarpus sylvestris* seedlings with different densities. *Ecological Science* 35 (4), 86–90.
- Editorial Committee of Flora of China, 1996. *The Chinese Academy of Sciences. Flora of China*. Science Press, Beijing.
- Feng, Z., Büker, P., Pleijel, H., Emberson, L., Karlsson, P.E., Uddling, J., 2018. A unifying explanation for variation in ozone sensitivity among woody plants. *Global Change Biol.* 24 (1), 78–84.
- Feng, Z., De Marco, A., Anav, A., Gualtieri, M., Sicard, P., Tian, H., Fornasier, F., Tao, F., Guo, A., Paoletti, E., 2019. Economic losses due to ozone impacts on human health, forest productivity and crop yield across China. *Environ. Int.* 131, 104966.
- Gao, F., Catalayud, V., Paoletti, E., Hoshika, Y., Feng, Z., 2017. Water stress mitigates the negative effects of ozone on photosynthesis and biomass in poplar plants. *Environ. Pollut.* 230, 268–279.
- Gimeno, B.S., Bermejo, V., Sanz, J., de la Torre, D., Elvira, S., 2004. Growth response to ozone of annual species from Mediterranean pastures. *Environ. Pollut.* 132, 297–306.
- Guan, L.L., Wen, D.Z., 2011. More nitrogen partition in structural proteins and decreased photosynthetic nitrogen-use efficiency of *Pinus massoniana* under in situ polluted stress. *J. Plant Res.* 124 (6), 663–673.
- Guangzhou Central Meteorological Observatory, 2015. Available at: <http://www.tqyb.com.cn/gz/climaticprediction/bulletin/2015-12-31/466.html>, Accessed date: 17 August 2019.
- Guidi, L., Nali, C., Lorenzini, G., Filippi, F., Soldatini, G.F., 2001. Effect of chronic ozone fumigation on the photosynthetic process of poplar clones showing different sensitivity. *Environ. Pollut.* 113 (3), 245–254.
- Häikiö, E., Freiwald, V., Julkuntti, R., Beuker, E., Holopainen, T., Oksanen, E., 2009. Differences in leaf characteristics between ozone-sensitive and ozone-tolerant hybrid aspen (*Populus tremula* × *Populus tremuloides*) clones. *Tree Physiol.* 29 (1), 53–66.
- 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 (4), 555–561.
- Hoshika, Y., Pecori, F., Conese, I., Bardelli, T., Marchi, E., Manning, W.J., Badea, O., Paoletti, E., 2013. Effects of a three-year exposure to ambient ozone on biomass allocation in poplar using ethylenediurea. *Environ. Pollut.* 180, 299–303.
- Huntingford, C., Oliver, R.J., Mercado, L.M., Sitch, S., 2018. Technical note: a simple theoretical model framework to describe plant stomatal “sluggishness” in response to elevated ozone concentrations. *Biogeosciences* 15, 5415–5422.
- 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 Soil Pollut.* 214 (1–4), 587–598.
- Institute of Soil Sciences, Chinese Academy of Sciences (ISSCAS), 1978. *Physical and Chemical Analysis Methods of Soils (In Chinese)*. Shanghai Science Technology Press, Shanghai.
- Jin, S., Fan, S., Wang, Z., Nie, H., Yang, G., 2008. The variation characteristics of surface ozone concentration at Waliguan in Qinghai. *China Environ. Sci.* 28 (3), 198–202.
- Jolivet, Y., Bagard, M., Cabané, M., Vaultier, M.N., Gandin, A., Aff, D., Dizengremel, P., Le Thiec, D., 2016. Deciphering the ozone-induced changes in cellular processes: a prerequisite for ozone risk assessment at the tree and forest levels. *Ann. For. Sci.* 73 (4), 923–943.
- Kangasjärvi, J., Jaspers, P., Kollist, H., 2005. Signalling and cell death in ozone-exposed plants. *Plant Cell Environ.* 28, 1021–1036.
- King, J.S., Kubiske, M.E., Pregitzer, K.S., Hendrey, G.R., McDonald, E.P., Giardina, C.P., Quinn, V.S., Karnosky, D.F., 2005. Tropospheric O₃ compromises net primary production in young stands of trembling aspen, paper birch and sugar maple in response to elevated atmospheric CO₂. *New Phytol.* 168 (3), 623–636.
- Li, H.S., 2000. *Experimental Principle and Technology of Plant Physiology and Biochemistry*. Higher Education Press, Beijing.
- Li, P., Calatayud, V., Gao, F., Uddling, J., Feng, Z.Z., 2016. Differences in ozone sensitivity among woody species are related to leaf morphology and antioxidant levels. *Tree Physiol.* 36 (9), 1105–1116.
- Li, P., Feng, Z., Catalayud, V., Yuan, X., Xu, Y., Paoletti, E., 2017. A meta-analysis on growth, physiological and biochemical responses of woody species to ground-level ozone highlights the role of plant functional types. *Plant Cell Environ.* 40, 2369–2380.
- Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods Enzymol.* 148, 350–382.
- Manderscheid, R., Jäger, H.J., Kress, L.W., 1992. Effects of ozone on foliar nitrogen metabolism of *Pinus taeda* L. and implications for carbohydrate metabolism. *New Phytol.* 121 (4), 623–633.
- Manninen, S., Huttunen, S., Vanhatalo, M., Pakonen, T., Hämäläinen, A., 2009. Interand intra-specific response to elevated ozone and chamber climate in northern birches. *Environ. Pollut.* 157 (5), 1679–1688.
- 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. *Environ. Pollut.* 146 (3), 587–607.
- Matyssek, R., Karnosky, D.F., Wieser, G., Percy, K., Oksanen, E., Grams, T.E.E., Kubiske, M., Hanke, D., Pretzsch, H., 2010. Advances in understanding ozone impact on forest trees: messages from novel phytotron and free-air fumigation studies. *Environ. Pollut.* 158, 1990–2006.
- Nali, C., Paoletti, E., Marabottini, R., Della Rocca, G., Lorenzini, G., Paolacci, A.R., Ciuffi, M., Badiani, M., 2004. Ecophysiological and biochemical, strategies of response to ozone in Mediterranean evergreen broadleaf species. *Atmos. Environ.* 38, 2247–2257.
- Niinemets, Ü., 1999. Components of leaf dry mass per area - thickness and density - alter leaf photosynthetic capacity in reverse directions in woody plants. *New Phytol.* 144 (1), 35–47.
- Pleijel, H., Eriksen, A.B., Danielsson, H., Bondesson, N., Sellden, G., 2006. Differential ozone sensitivity in an old and a modern Swedish wheat cultivar—grain yield and quality, leaf chlorophyll and stomatal conductance. *Environ. Exp. Bot.* 56 (1), 63–71.
- Polle, A., Pfirrmann, T., Chakrabarti, S., Rennenberg, H., 1993. The effects of enhanced ozone and enhanced carbon dioxide concentrations on biomass, pigments and anti-oxidative enzymes in spruce needles (*Picea abies* L.). *Plant Cell Environ.* 16, 311–316.
- PRDAIR, 2016. Guangdong-Hong Kong-Macao Pearl River Delta regional air quality monitoring network: a report of monitoring results in 2015. Report Number: PRDAIR-2015-5. Available at: http://www.epd.gov.hk/epd/sites/default/files/epd/english/resources/pub/publications/files/PRD_2015_report-en-1.pdf.
- Temple, P.J., Riechers, G.H., 1995. Nitrogen allocation in ponderosa pine seedlings exposed to interacting ozone and drought stresses. *New Phytol.* 130 (1), 97–104.
- Then, Ch., Herbinger, K., Luis, V.C., Heerd, C., Matyssek, R., Wieser, G., 2009. Photosynthesis, chloroplast pigments, and antioxidants in *Pinus canariensis* under free-air ozone fumigation. *Environ. Pollut.* 157, 392–395.
- Vainonen, J.P., Kangasjärvi, J., 2015. Plant signaling in acute ozone exposure. *Plant Cell Environ.* 38, 240–252.
- Vingarzan, R., 2004. A review of surface ozone background levels and trends. *Atmos. Environ.* 38 (21), 3431–3442.
- Vollns, A.V., Kruse, O.M.O., Eriksen, A.B., Oxaal, U., Futsaether, C.M., 2010. In vivo root growth dynamics of ozone exposed *Trifolium subterraneum*. *Environ. Exp. Bot.* 69 (2), 183–188.
- Watanabe, M., Hoshika, Y., Inada, N., Koike, T., 2018. Photosynthetic activity in relation to a gradient of leaf nitrogen content within a canopy of Siebold's beech and Japanese oak saplings under elevated ozone. *Sci. Total Environ.* 636, 1455–1462.
- Watanabe, M., Hoshika, Y., Inada, N., Wang, X., Mao, Q., Koike, T., 2013. Photosynthetic traits of Siebold's beech and oak saplings grown under free air ozone exposure in northern Japan. *Environ. Pollut.* 174, 50–56.
- Wieser, G., Matyssek, R., 2007. Linking ozone uptake and defense towards a mechanistic risk assessment for forest trees. *New Phytol.* 174 (1), 7–9.
- Yamaguchi, M., Watanabe, M., Matsumura, H., Kohno, Y., Izuta, T., 2010. Effects of ozone on nitrogen metabolism in the leaves of *Fagus crenata* seedlings under different soil nitrogen loads. *Trees Struct. Funct.* 24 (1), 175–184.
- Yan, K., Chen, W., He, X., Zhang, G., Xu, S., 2010. Responses of photosynthesis, lipid peroxidation and antioxidant system in leaves of *Quercus mongolica* to elevated O₃. *Environ. Exp. Bot.* 69 (2), 198–204.
- Yu, H., Chen, Z., Shang, H., Cao, J., 2017. Physiological and biochemical responses of *Michelia ichangensis* Rehd. et Wils and *Taxus chinensis* (Pilger) Rehd. to elevated O₃ in subtropical China. *Environ. Sci. Pollut. Res.* 24 (21), 17418–17427.
- Yu, H., Wang, J., Liu, J., Wang, F., Liu, J., 2012. Study on the growth rhythm of *Michelia chapensis*. *J. Anhui Agr. Sci.* 40 (6), 3399–3400.
- Zhang, W.W., Feng, Z.Z., Wang, X.K., Niu, J.F., 2012. Responses of native broadleaved woody species to elevated ozone in subtropical China. *Environ. Pollut.* 163, 149–157.