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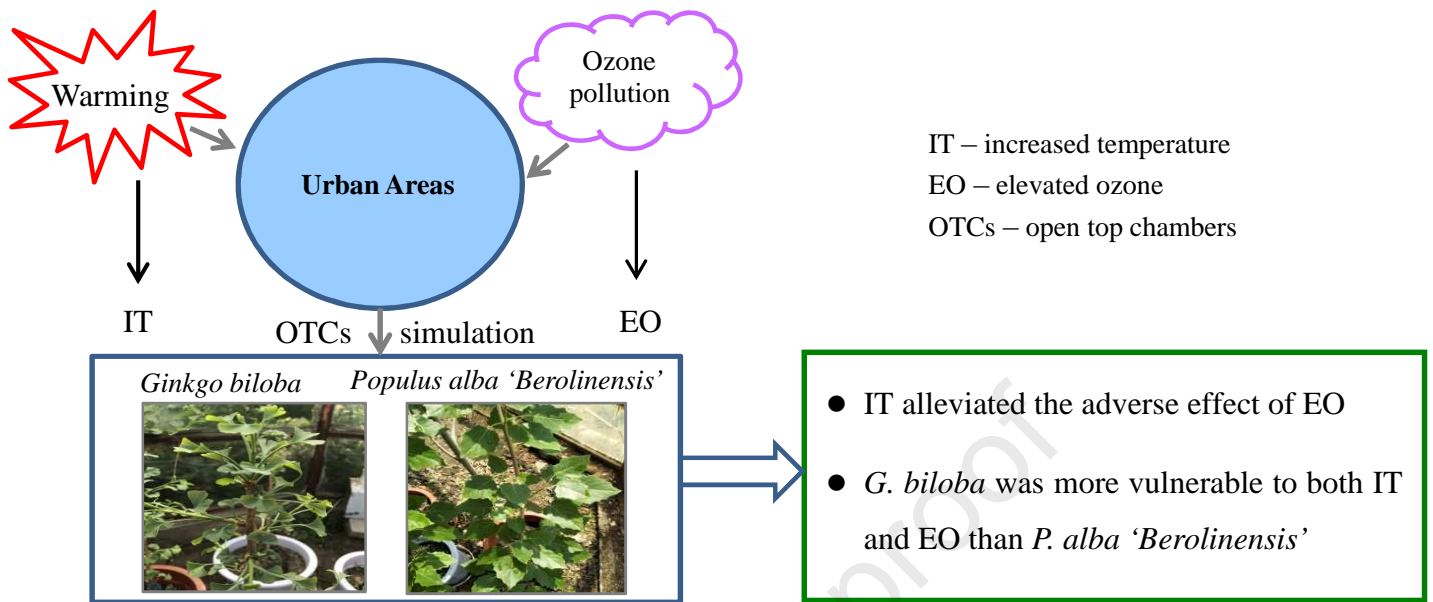
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# Experimental warming alleviates the adverse effects from tropospheric ozone on two urban tree species

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**Running title: The combined effects of air warming and ozone on urban trees**

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**Abstract**

Atmospheric warming and increasing tropospheric ozone (O<sub>3</sub>) concentrations often co-occur in many cities of the world including China, adversely affecting the health status of urban trees. However, little information is known about the combined and interactive effects from increased air temperature (IT) and elevated O<sub>3</sub> (EO) exposures on urban tree species. Here, *Ginkgo biloba* and *Populus alba* 'Berolinensis' seedlings were subjected to IT (+ 2°C of ambient air temperature) and/or EO (+ 2-fold ambient air O<sub>3</sub> concentrations) for one growing season by using open-top chambers. IT alone had no significant effect on physiological metabolisms at the early growing stage, but significantly increased photosynthetic parameters, antioxidative enzyme activities ( $P < 0.05$ ). EO alone decreased physiological parameters except for increased oxidative stress. Compared to EO exposure alone, plants grown under IT and EO combined showed higher antioxidative and photosynthetic activity. There was a significant interactive effect between IT and EO on net photosynthetic rate, stomatal conductance, water use efficiency, the maximum quantum efficiency of PSII photochemistry, the actual quantum efficiency of PSII, enzyme activities, aboveground biomass and root/shoot ratio ( $P < 0.05$ ), respectively. These results suggested that during one growing season, IT mitigated the adverse effect of EO on the tested plants. In addition, we found that *G. biloba* was more sensitive than *P. alba* 'Berolinensis' to both IT and EO, suggesting that *G. biloba* may be a good indicator species for climate warming and air pollution, particularly under environmental conditions as they co-occur in urban areas.

**Key words:** Climate change; *Ginkgo biloba*; Oxidative stress; Photosynthesis; Physiological response; Poplar

**44 Capsule**

45 There was a significant interactive effect between increased air temperature and elevated O<sub>3</sub> on  
46 growth and physiological changes of urban tree species.

47 Increased air temperature alleviated the adverse effect of O<sub>3</sub> stress on urban plants by maintaining  
48 high antioxidative level and protecting photosynthetic apparatus during one growing season.

## Introduction

Global warming and tropospheric ozone (O<sub>3</sub>) pollution are crucial environmental drivers on a regional to global scale (Rotundo et al., 2019; Wang et al., 2019a, 2019b; Xu et al., 2019; Gao et al., 2020). Global warming is progressing at an unprecedented pace, altering plant species composition and biodiversity worldwide (IPCC, 2014; Lawal et al., 2019). It is estimated that global air temperature will enhance by over 1.5 °C by the late 21st century, depending on the selected scenario (IPCC, 2018). The increase in surface air temperature in China has been faster than the mean global rate, and more frequent temperature extremes are expected to occur in future (Piao et al., 2010; Fischer et al., 2012; Li et al., 2018). Shenyang, the biggest city with population in the northeastern China has been one of the regions with the greatest temperature increases in China since the 1980s (Zhao et al., 2009a; He et al., 2013). In general, increased temperatures are expected to stimulate plant growth (Mäenpää et al., 2011; Qi et al., 2015; Shestakova et al., 2016), affect physiological and biochemical metabolisms (Riikonen et al., 2009; Mäenpää et al., 2011, 2013; Fu et al., 2013; Tacarindua et al., 2013; Kumagai and Sameshima, 2014), delay autumn phenophase (Wolkovich et al., 2012; He et al., 2016), alter plant distribution (Kelly and Goulden, 2008), and affect biodiversity of forest ecosystems (Grimm et al., 2013). Urban forests play a crucial role in improving urban air quality, alleviating urban heat island effects, maintaining environmental health and human well-being (Beckett et al., 1998; Mullaney et al., 2015; Chen et al., 2017). However, urban trees are often suffering from the impacts of abiotic factors such as air warming and atmospheric pollution in the urban environment (Dmuchowski et al., 2019). In addition, global warming and regional emission of greenhouse gases can aggravate urban warming during summer months (Fujibe, 2009; Bartholy and Pongracz, 2018). It is therefore important to better understand how urban climate including environmental warming and air pollution will affect the physiological characteristics of urban trees to maintain

sustainability of urban forest ecosystems (Rotzer et al., 2019).

O<sub>3</sub> is considered as one of the main air pollutants and a powerful greenhouse gas, with rising concentrations at global and regional scale (Ziemke et al., 2019). The increasing of ground-level O<sub>3</sub> concentrations in urban areas is one of the most important environmental issues in Asian countries including China despite of sustained efforts by the government to control ozone concentrations (Ueno and Tsunematsu, 2019; Chen et al., 2019). In many Chinese cities, O<sub>3</sub> has taken over PM<sub>2.5</sub> as the major air pollutant, especially on cloudless days in summer (Chen et al., 2019). The highest 8-h average O<sub>3</sub> concentration often reaches 80 ppb during the summer in some cities of northeast China (Aunan et al., 2000; Gao et al., 2020). Due to the strong oxidative toxicity, O<sub>3</sub> generally causes negative impacts on forest plants (Feng et al., 2015; Nowak et al., 2018). Increasing O<sub>3</sub> concentrations can induce oxidative stress, lead to early leaf senescence, and thus strengthen deleterious impacts on physiological metabolisms and inhibits growth of trees (Xu et al., 2015; Moura et al., 2018; Dai et al., 2019; Xu et al., 2020).

Indeed, further air warming and an increase in tropospheric O<sub>3</sub> concentrations may coexist in many large cities in the world including China. Therefore, to make a better understanding of the effects of increased temperature and elevated O<sub>3</sub> in combination on urban trees is vital for the adaptation and management of urban forests to regional and future climate change (Wang et al., 2018; Nowak et al., 2018). So far, far less is known about the combined and interactive impacts of increased air temperature and ozone on physiological metabolisms of urban plants.

*Ginkgo biloba* and *Populus alba* 'Berolinensis' are two of the most urban tree species in urban forests in many cities of Northeast China (Lu et al., 2011; Xu et al., 2015). In several countries, *G. biloba* is regarded as one of ancient living plant fossils and recommended for urban afforestation and roadside tree species due to its resistance to the adverse conditions in urban environments, for example in the US (Hurley and Emery, 2018; McBride and Lacan, 2018),

Europe (Beus, 2014; Dmuchowski et al., 2019), and Asia (Li et al., 2011, 2014; Xu et al., 2015). *P. alba* 'Berolinensis' is a hybrid of *P. alba* × *P. berolinensis* in Northeast China (Wang et al., 2008). This poplar is commonly planted as street tree species in cities, well known for its excellent ecological properties such as rapid growth and high adaptation capacity to environmental stress (Huang et al., 2017; Jiang et al., 2018). In our previous work, we carried out several studies on the physiological responses of both tree species to O<sub>3</sub> stress; especially He et al. (2006) observed that the short-term O<sub>3</sub> fumigation induced acclimation of the antioxidant defense system in leaves of *G. biloba*. Lu et al. (2009) found that O<sub>3</sub> induced significant oxidative stress, leading to increased hydrogen peroxide accumulation in *G. biloba* leaves. He et al. (2007) and Li et al. (2011) found that *G. biloba* exhibited a significant inhibition in growth under increasing O<sub>3</sub> concentrations. The results from Xu et al. (2015) indicated that decreased net photosynthetic rate in leaves of *G. biloba* by 35.6% after 60 days of elevated O<sub>3</sub> exposure. Fu et al. (2014) found that elevated O<sub>3</sub> (160 ppb) significantly induced the accumulation of membrane lipid peroxidation products and decreased net photosynthetic rates in *P. alba* 'Berolinensis' leaves. Recently, we also found that this hybrid poplar leaves exposed to 120 ppb showed high malondialdehyde content and low net photosynthetic rate, compared to those in ambient O<sub>3</sub> exposures (Xu et al., 2019).

The current work is based on a multi-factorial (elevated O<sub>3</sub> combined with increased air temperature) experimental design to test for combined effects on *G. biloba* and *P. alba* 'Berolinensis' grown in open top chambers (OTCs). This study focused on changes in multi-physiological parameters of two common urban tree species under increased air temperature and elevated O<sub>3</sub> concentrations during one growing season.

The purposes of our present study were: (1) to determine the responses of growth and photosynthetic characteristics to increased air temperatures and elevated O<sub>3</sub>; (2) to compare the



difference in the effects of increased air temperature and O<sub>3</sub> fumigation on oxidative stress, the activities of antioxidant enzymes of *G. biloba* and *P. alba* 'Berolinensis' leaves at different growing stages; (3) to explore the interactive impacts on growth and photosynthesis of the two tree species. In this study, we postulated that increased air temperature can mitigate the negative impacts of O<sub>3</sub> stress on the two urban tree species by antioxidant systems which change the strategy of adapting oxidative stress. The findings obtained will deepen our understanding of the physiological responses and ecological adaptations of urban tree species to environmental pollutants, which will contribute to developing management strategies for urban forests under future climate change and air pollution conditions.

## 1. Materials and Methods

### 1.1. Experimental site and plant materials

This experiment was performed in Shenyang Arboretum (41° 46' N, 123° 26' E) of the Chinese Academy of Science (CAS) and closely located to a commercial center in Shenyang city of Northeast China, Liaoning Province. Elevation of the place is 41 m and the area was 5 ha (Xu et al., 2006). The place is affected by semi-humid continental monsoon climate. More detailed records in annual temperature and precipitation of this area can be found in our previous experiments (Xu et al., 2014b; Xu et al., 2005; Xu et al., 2017).

Three-year-old *G. biloba* seedlings and one-year-old *P. alba* 'Berolinensis' cutting seedlings with consistent size in stem diameter and plant height ( $70 \pm 5$  cm) were selected from a local nursery and planted into plastic pots (30 cm in diameter and 25 cm in depth) on 15 March 2018. Pot soil (2 kg) was mixed of sand: peat: clay = 3:2:1 (v:v:v). All plants were cultivated for 30 days in a glass greenhouse under a designed environmental condition (Xu et al., 2020). The nutritional status of soil in pot is 2.37 mg/g N, 1.78 mg/g P and 6.24 mg/g K, respectively. The

pots were regularly supplied enough water and the soil water content was maintained to the field capacity during the growing period.

## 1.2. Experimental designs

This study was set up in open-top chambers (OTCs) with four treatments: ambient air (AA, control, 40 ppb O<sub>3</sub>), increased temperature (IT, + 2°C to AA), elevated O<sub>3</sub> (EO, 80 ppb O<sub>3</sub>), and increased temperature combined with elevated O<sub>3</sub> (IT + EO). Twelve OTCs with 4 m in diameter and 3 m in height were distributed randomly without mutual shading (Li et al., 2011; Xu et al., 2014a). During the treatments, the plants were watered twice a week and fertilized once at the beginning of the experiment. After 30 d (15 April), the plants were exposed to AA and EO for 8 h d<sup>-1</sup> from 09:00 to 17:00, except during bad weather conditions such as thunderstorm. We applied a fully computer controlled O<sub>3</sub> elevation model, which continuously added generated O<sub>3</sub> to meet the target elevation level (2-fold ambient air O<sub>3</sub> concentration). Air warming was provided with elongated (25.5 cm long and 10.0 cm wide) 1000 Watt ceramic infrared heaters (IRCER12101, 120V, Mor Electric Heating Assoc., Inc. USA). Three ceramic infrared heaters were fixed horizontally at 120 degrees above the canopy inside each IT OTC. According to the growth of seedlings, the heaters were lifted to keep a constant distance of 80 cm above the top of the seedling canopy. The exposure for IT was run 24 hr/day. The similar methods for IT by air warming exposure were referred according to the study by Ghimire et al. (2017).

O<sub>3</sub> was produced from an ozone generator (Xinhang-2010, Shenyang, China). O<sub>3</sub> concentrations in OTC were monitored by an automated time-sharing system connected to an ozone analyzer (S-905, Aeroqual Inc., Auckland, New Zealand) and recorded using a data logger (CR800, Campbell Scientific Inc., Logan, UT, USA). More detailed methods may be referred in our previous experiments (Xu et al. 2015, 2017). All treatments of this experiment were

independently repeated three times under the same conditions. The experiment lasted for six months (15 April to 20 October 2018). Under natural condition, air temperature, air humidity, precipitation (mm) and soil surface temperature at study site during the experiments were automatically recorded by a meteorological observation station (CR-1000, Campbell Scientific Inc., Logan, UT, USA), which is shown in Fig. S1. Fully expanded mature canopy leaves ( $n = 3$ ) were sampled at 09:00 every 45 days from the experiment beginning for physiological measurements including photosynthetic pigment contents, gas exchange and chlorophyll fluorescence parameters, oxidative stress and antioxidative enzyme activities. By the end of the experiment, growth parameters for both tree species were measured under each treatment.

### 1.3. Measurements

#### 1.3.1. Photosynthetic and chlorophyll fluorescence parameters

Leaf photosynthetic pigments were extracted in 95% ethanol (v/v) and quantified spectrophotometrically (UV-1800, Shimadzu, Japan). Chlorophyll a (Chla) and b (Chlb) contents were determined at wavelength of 649 and 665 nm. Carotenoids (Car) were measured at 470, 649 and 665 nm according to the modified methods of Lichtenthaler (1987).

Leaf gas exchange measurements were conducted on two fully developed healthy leaves (3-5<sup>th</sup> leaf from the tip of a shoot) from three randomly selected plants per OTC. Net photosynthetic rate ( $P_n$ ), stomatal conductance ( $g_s$ ), vapor pressure deficit ( $VPD$ ) and transpiration ( $Tr$ ) were recorded by using a portable infra-red gas analyzer (Li-6400, Li-Cor Inc. Lincoln, NE, USA) at a saturated photosynthetic photon flux density (PPFD, 1000  $\mu\text{mol}/\text{m}^2\cdot\text{sec}$ ) under a constant condition of relative humidity (40 to 60%) and leaf temperature (25 $^{\circ}\text{C}$ ) from 9:00 to 11:00. Water use efficiency (WUE) was gained by  $P_n$  divided by  $Tr$  according to Fischer and Turner (1978).

Leaf fluorescence parameters were determined by a chlorophyll fluorometer (FMS-2, Hansatech Instruments, Ltd, Norfolk, UK) to measure the minimum dark fluorescence yield ( $F_o$ ), the maximal fluorescence yield ( $F_m$ ), the maximum quantum efficiency of PSII photochemistry ( $F_v/F_m$ ), the actual quantum efficiency of PSII ( $\Phi$ PSII), the quenching of photochemical efficiency of PSII ( $qP$ ) and the non-photochemical quenching (NPQ) (Maxwell and Johnson, 2000).  $F_v/F_m = (F_m - F_o)/F_m$ .  $\Phi$ PSII,  $qP$ , NPQ were measured from steady-state fluorescence ( $F_s$ ), maximum fluorescence ( $F_m'$ ) and minimum fluorescence ( $F_s'$ ) after the samples were adapted to light.  $\Phi$ PSII =  $(F_m' - F_s)/F_m'$ ,  $qP = (F_m' - F_s)/(F_m' - F_o')$  and NPQ =  $(F_m - F_m')/F_m'$  (Genty et al., 1989).

### 1.3.2. Oxidative stress and antioxidative enzyme activities

Leaf oxidative stress was assessed by malondialdehyde (MDA), the product of membrane lipid peroxidation, and Leaf electrolyte leakage (EL) and superoxide anion radical ( $O_2^{\bullet-}$ ) accumulation in leaves. MDA content was determined according to the method of Dhindsa et al. (1981). EL was measured by a digital conductivity meter (DDS-11A).  $O_2^{\bullet-}$  production rate was determined by hydroxylamine hydrochloride oxidation method according to Elstner and Heupel (1976).

Antioxidative enzymes were extracted according to Cho and Seo (2005). Superoxide dismutase (SOD) activity was analyzed by the ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) (Dhindsa et al., 1981). Catalase (CAT) activity was assayed by ultraviolet absorption method (Azevedo et al., 1998). Ascorbate peroxidase (APX) activity was measured according to the method reported by Nakano and Asada (1981). Glutathione reductase (GR) activity was determined according to the method described by Krivosheeva et al. (1996).

### 1.3.3. Growth parameters

Before harvesting, relative water content (RWC), plant height, basal diameter and specific leaf weight (SLW) were measured for all plants at the end of the experiment (20 October 2018).  $RWC (\%) = (FW - DW) / FW \times 100\%$ , with FW standing for fresh weight, DW for dry weight. SLW ( $g/cm^2$ ) is the dry weight of unit area leaves. Root/shoot (R/S) ratio was determined by root biomass per plant divided by above-ground biomass.

## 1. 4. Statistical analysis

The data were subjected to the analysis of variance (ANOVA) with SPSS 18.0 (SPSS 18.0, Chicago, IL, USA). Prior to analysis, the assumption of normality and homogeneity was tested with Shapiro-Wilk and Levene tests, respectively. The repeated measures ANOVA was carried out for main effects and interactions of time, tree species, EO and IT on all physiological and growth parameters. One-way ANOVA was used to compare the difference of each parameter between the treatments for each tree species at each sampling date. The least significance differences (LSD) was used to analyze the significant difference between the treatments. The single and interactive impacts of IT and EO, sampling time and tree species on growth, and physiological parameters in leaves were studied by general linear model (GLM). Data shown in all tables and figures are means  $\pm$  SD,  $P < 0.05$  was regarded as statistically significant.

## 2. Results

### 2.1 Effects of increased temperature and/or elevated $O_3$ on photosynthetic characteristics of two urban tree species

During 90 days of exposure, IT alone had no significant effect on the contents of photosynthetic pigments including Chla, Chlb and Car in leaves of *G. biloba* and *P. alba*

235 'Berolinensis' (Fig. 1), but increased significantly their contents after 135 days of exposure  
 236 compared to control. Elevated O<sub>3</sub> alone decreased the contents of these pigments in leaves of the  
 237 two tree species during the whole growing season. Chla contents of *G. biloba* and *P. alba*  
 238 'Berolinensis' leaves exposed to O<sub>3</sub> for 45, 90, 135 and 180 days decreased significantly by 13.2,  
 239 28.1, 72.1, 81.8% and 14.4, 18.0, 62.5, 78.3%, respectively (Fig. 1a & b,  $P < 0.05$ ). Under the  
 240 combination of IT and EO, the content of each photosynthetic pigment in leaves showed the  
 241 similar trend of changing and is higher than that under EO alone, and lower than that under IT  
 242 alone, regardless of tree species. According to GLM analysis, the individual effects of IT, EO,  
 243 time (i.e. different treatment times), and tree species were significant for all the photosynthetic  
 244 pigments (Table S1). However, the interactive effect was significant for the content of Chla under  
 245 the combination of IT, EO, species, and time. No significant interaction of temperature, O<sub>3</sub>, tree  
 246 species and time was observed on Car change in leaves of two urban tree species (Table S1,  $P >$   
 247 0.05).

248 Compared with control, IT alone increased gas exchange parameters including  $P_n$ ,  $g_s$ ,  $VPD$   
 249 and WUE of the two tree species (Fig. 2). IT alone significantly increased  $P_n$  of *G. biloba* and *P.*  
 250 *alba* 'Berolinensis' by 33.2% and 48.0%, 42.3% and 49.5% after 135 and 180 days of exposure,  
 251 respectively ( $P < 0.05$ ). After 90 days,  $P_n$  and  $g_s$  of plants showed no significant change under IT  
 252 ( $P > 0.05$ ). EO alone decreased  $P_n$  and  $g_s$  of the two tree species at each sampling time (Fig. 2a,  
 253 b, c, d). The plants exposed to IT and EO combined showed higher  $P_n$  and  $g_s$  than those being  
 254 exposed to EO alone at each sampling time. Compared with control at each sampling time, IT  
 255 had no significant impact on  $VPD$ . WUE in both *G. biloba* and *P. alba* 'Berolinensis' showed no  
 256 significant change between the different treatments after 45 and 135 days. EO alone increased  
 257  $VPD$  at each sampling time regardless of tree species, decreased significantly WUE in leaves of  
 258 *G. biloba* and *P. alba* 'Berolinensis' by 36.1% and 41.9% after 180 days ( $P < 0.05$ ), respectively

(Fig. 2g & h). GLM revealed significant interactive effect of IT and EO on  $Pn$  ( $P = 0.002$ ),  $gs$  ( $P < 0.001$ ),  $VPD$  ( $P < 0.001$ ) and  $WUE$  ( $P = 0.009$ ), respectively (Table S1).

IT had no significant effect on  $F_v/F_m$ ,  $\Phi PSII$ ,  $qP$  and  $NPQ$  at most of sampling dates except after 180 days with a significant increase of  $\Phi PSII$  and  $qP$ , respectively (Fig. 3c, d, e, f). EO decreased  $F_v/F_m$ ,  $\Phi PSII$  and  $qP$ , but increased  $NPQ$  at each sampling time (Fig. 3g & h). GLM revealed interactive effect of IT and EO on  $F_v/F_m$ ,  $\Phi PSII$  and  $NPQ$  (Table S1).

## 2.2 Effects of increased temperature and/or elevated $O_3$ on oxidative stress of two urban tree species

After 45 days of exposure, IT had no significant effect on MDA content, EL and  $O_2^{\bullet-}$  production rate, but increased the levels of these parameters after 90 days, particularly for MDA content and  $O_2^{\bullet-}$  production rate in leaves of *G. biloba* (Fig. 4a & e,  $P < 0.05$ ). IT decreased significantly MDA content, and  $O_2^{\bullet-}$  production rate in leaves of *G. biloba* and *P. alba* 'Berolinensis' after 135 and 180 days. Regardless of tree species, EO induced a rise in MDA content, EL and  $O_2^{\bullet-}$  production rate at each sampling point ( $P < 0.05$ ), respectively, compared with control. For *G. biloba*, EO increased significantly MDA content after 90, 135 and 180 days by 91.6%, 38.4%, 63.8%, EL by 94.4%, 97.6%, 83.8% and  $O_2^{\bullet-}$  production rate by 26.1%, 29.4% and 60.7% (Fig. 4a, c, e,  $P < 0.05$ ), respectively. For *P. alba* 'Berolinensis', EO increased significantly MDA content after 90, 135 and 180 days of exposure by 18.6%, 31.9%, 52.1% (Fig. 4b,  $P < 0.05$ ). In addition, MDA content, EL and  $O_2^{\bullet-}$  production rate showed a lower level in plants combined with IT and EO than those of  $O_3$  alone regardless of tree species. GLM analysis showed a significant interactive effect of IT and EO on MDA content ( $P = 0.006$ ), EL ( $P < 0.001$ ) and  $O_2^{\bullet-}$  production rate ( $P < 0.001$ ), respectively (Table S1).

## 2.3 Effects of increased temperature and/or elevated $O_3$ on the activities of antioxidative

## enzymes in leaves of two urban tree species

No significant effect was observed in SOD, CAT and APX activities in leaves of *G. biloba* and *P. alba* 'Berolinensis' under IT condition after 45 days of exposure (Fig. 5). EO increased the activities of these enzymes regardless of tree species, particularly for SOD activity with a significant rise by 13.7% and 18.2% in leaves of *G. biloba* and *P. alba* 'Berolinensis' after 45 days (Fig. 5a & b,  $P < 0.05$ ), respectively. IT increased all the enzyme activities including GR activity in leaves of the two tree species after 135 and 180 days of exposure (Fig. 5g & h). EO decreased the activities of SOD, CAT, APX and GR in leaves of the two tree species after 90, 135 and 180 days, respectively. SOD activity decreased significantly by 41.6%, 31.3% and 39.1% in *G. biloba* and by 27.8%, 31.2% and 33.6% in *P. alba* 'Berolinensis' after 90, 135 and 180 days ( $P < 0.05$ ), respectively. Compared to EO alone, the combination of IT and EO induced higher enzyme activities at these three sampling points regardless of tree species. By GLM analysis, we observed that no significant interaction between IT and EO on change in SOD activity ( $P = 0.366$ ), CAT ( $P = 0.451$ ), APX ( $P = 0.079$ ) and GR ( $P = 0.558$ ). However, significant interaction between IT and  $O_3$  on changes in SOD ( $P < 0.001$ ), CAT ( $P = 0.007$ ) and APX ( $P = 0.028$ ) activities was found when combined with time treatment (Table S1).

## 2.4 Effects of increased temperature and/or elevated $O_3$ on growth of two urban tree species

IT and EO significantly affected all the growth parameters except for plant height (Table 1). Compared to AA, IT alone increased significantly shoot, root and total weight by 37.4%, 34.8% and 36.0% in *G. biloba*, and 32.9%, 35.5% and 34.2% in *P. alba* 'Berolinensis', respectively ( $P < 0.05$ ). EO decreased significantly RWC, specific leaf weight, shoot weight, root weight, total biomass and R/S ratio by 38.6%, 42.3%, 35.2%, 61.5%, 49.2% and 36.4% in *G. biloba*, and 20.0%, 29.4%, 17.9%, 35.1%, 26.4%, and 20.0% in *P. alba* 'Berolinensis', respectively ( $P <$



0.05). Each growth parameter of the two tree species showed a higher value under the combination of IT and EO compared to the single effect of IT and EO alone. GLM revealed significant interactive effect of IT and EO on RWC ( $P = 0.002$ ), basal diameter ( $P = 0.032$ ), shoot weight ( $P = 0.014$ ) and R/S ratio ( $P < 0.001$ ).

### 3. Discussion

The results presented here extend our insights into the combined and interactive effects of air warming and O<sub>3</sub> pollution on plants. So far, the combined effects of air IT and EO have been investigated on a few crops (Wang et al., 2019a) and several field tree species (Riikonen et al., 2013; Hartikainen et al., 2020). However, knowledge of the combined and interactive effects of the two abiotic factors is still limited on the widely planted urban tree species such as ginkgo and poplar tested in this study, both of them being used as bio-indicating species to climate change and air pollution in Chinese cities.

#### 3.1. Photosynthetic response to increased temperature and/or elevated O<sub>3</sub>

Photosynthetic responses to IT and air pollution of terrestrial plants can potentially alter ecosystem C cycling (Jassey et al., 2015). The contents of photosynthetic pigments, leaf gas exchange and chlorophyll fluorescence are the most commonly used parameters in the assessment of photosynthetic response to various abiotic stresses including warming and ozone (Albert et al., 2011; Guidi and Calatayud, 2014; Zhang et al., 2018). In this study, warming by air IT increased the contents of photosynthetic pigments including chlorophyll and carotenoids in leaves of *G. biloba* and *P. alba* 'Berolinensis'. The increase of contents in photosynthetic pigments under warming may be associated with higher photosynthetic performance indicating plant seedlings adaptability in the new condition for survival to deal temperature stress during acclimatization (Jeon et al., 2006). Similar studies were found that total chlorophyll

concentrations increased in needles of *Abies faxoniana* (Wang et al., 2012) and *Picea asperata* (Zhao and Liu, 2009), and in maize leaves under 2°C warming (Tan et al., 2017). Contrary to warming, EO usually induced the reduction of photosynthetic pigments in plants. Our results in this experiment supported the study of Leitao et al. (2007) where high O<sub>3</sub> concentration decreased total chlorophyll and Car contents in maize leaves. Higher level of Car in plants might have played a protective role (Singh et al., 2014). In this study, plants under the combination of IT and EO showed higher Car content than that under EO alone, which might indicate a mitigation of adverse O<sub>3</sub> effects on the photosynthetic apparatus by preventing photo-oxidative destruction of chloroplasts.

Moderate warming can enhance plant photosynthesis (Martinez et al., 2014). In this study, the warming by + 2°C increased *P<sub>n</sub>* of the two tree species, particularly for *G. biloba* at earlier growing stage, indicating that regional warming can promote carbon gain and increase carbon sink function of urban vegetation by the means of more nutrient and biomass accumulation from photosynthesis. Our findings are in accordance with the study by Fan et al. (2015), who confirmed that the experimental warming significantly enhanced *P<sub>n</sub>* and consequently the carbon gain in winter wheat from the Yangtze River Basin of China. Similarly, Zheng et al. (2018) found that the experimental warming significantly increased the maximum *P<sub>n</sub>* of maize leaves in the North China Plain. Warming tended to increase photosynthesis of trees such as *Erica multiflora*, *Globularia alypum*, and *Pinus halepensis* (Prieto et al., 2009) and grass species (Valencia et al., 2016).

However, some studies showed that warming decreased photosynthesis in leaves of perennial grasses (Zhong et al., 2014), soybean (Zhang et al., 2016a) and boreal tree species (Reich et al., 2018). These conflicting findings indicated that photosynthetic characteristics of plants under warming might be related to plant species or cultivars, the intensity and duration of

warming and/or other environmental conditions (Valencia et al., 2016) or even to warming techniques (Boeck et al., 2007). Under warming,  $g_s$  is suggested to increase in order to avoid overheating of the leaves (Urban et al., 2017; Drake et al., 2018). In current study, we found that IT increased  $g_s$  in leaves of the two urban tree species. However, the evidence of  $g_s$  adaptation to IT in trees over the long-term has been observed in both directions, i.e. increase and decrease  $g_s$  (Way et al., 2015). In this experiment, IT increased  $VPD$  and  $WUE$  in *G. biloba* leaves but increased  $VPD$  in *P. alba* 'Berolinensis' leaves at early growing stage. Similar results showed that IT enhanced  $VPD$  of soybean leaves at different growing stages, which may indicate that IT could exacerbate the drying effects on soybean and lead to reductions in photosynthesis (Zhang et al., 2016a).

As expected,  $P_n$  and  $g_s$  declined in plants that were exposed to high  $O_3$  concentrations (Feng et al., 2011). In this study, we noticed that EO decreased  $P_n$  and  $g_s$  and concurrently increased  $VPD$  in *G. biloba* and *P. alba* 'Berolinensis' leaves. Similar results were found in urban tree species in our studies (Xu et al., 2015, 2019). Actually, the reduction of  $g_s$  by stomatal closure with increasing  $VPD$  under EO could prevent excessive water loss under high evaporative demand for adaptation to the adverse environments (Medina and Gilbert, 2016). Under the combination of IT and EO,  $P_n$  and  $g_s$  showed higher values than those under IT or EO alone at each sampling time, indicating that increasing temperature increased the adaptation of photosynthesis to environment stresses (Ashraf and Harris, 2013; Xu et al., 2015). EO effects on  $P_n$  were partly compensated by increased air temperature, showing an interactive effect of the treatments (Table S1,  $P < 0.01$ ). The reason for the alleviation of ozone stress under warmer environments could be due to the increasing of VOCs emissions (Hartikainen et al. 2009), which exerted a protection from  $O_3$  stress (Loreto and Velikova 2001). In this study,  $WUE$  decreased significantly under elevated  $O_3$ , which showed that  $O_3$  stress might adversely affect

photosynthesis and increase water demand for plant growth (Masutomi et al. 2019). Mäenpää, et al. (2011) found that elevated ozone concentrations decreased the ratio of photosynthesis to stomatal conductance, and thus indirectly decreased WUE in leaves of silver birch and European aspen.

Chlorophyll fluorescence parameters were used to study plant photosynthetic performance and as indicator for abiotic stresses (Dai et al., 2009; Kalaji et al., 2018).  $F_v/F_m$  was often applied to detect injury of plant photosynthetic apparatus by elevated temperature (Zhou et al., 2018; Cao et al., 2019). Warming may increase plant photosynthesis by increasing the efficiency of PSII by means of increased  $F_v/F_m$  and  $\Phi_{PSII}$  (Yin et al., 2009). In this study, IT and its combination with EO exposure resulted in higher  $F_v/F_m$ ,  $\Phi_{PSII}$  and  $qP$  for *G. biloba* and *P. alba* 'Berolinensis' leaves than those under  $O_3$  exposure alone, indicating that moderate warming enhanced the efficiency of PSII that can improve  $P_n$  by accelerating the energy transport from PSII to PSI of plants (Hussain et al., 2019). Our result is consistent with earlier findings where  $F_v/F_m$  decreased in  $O_3$ -exposed leaves of bean indicating photoinhibition in PSII reaction centers (Guidi et al., 2002).

NPQ is one of the most key photoprotective mechanisms during plant growth (Moustaka et al. 2015). The increased NPQ appeared to serve as a photosynthetic protective mechanism to avoid injury accumulation of excitation energy with lower partitioning of photochemical activity in no-carbon-assimilative processes under high  $O_3$  concentration (Pellegrini, 2014). In this study,  $O_3$ -treated leaves showed higher NPQ levels than those of leaves exposed to ambient air and warming conditions, indicating that non-radiative dissipative mechanisms are involved in the dissipation of excess excitation energy (Lorenzini et al., 1999) and greater photo-inactivation of PSII in  $O_3$ -exposed plants (Pellegrini et al., 2011). The lower NPQ under IT associated with the increased PAR fraction absorbed by PS-II-antenna may have triggered

reactive oxygen species (ROS) production in the chloroplast, which was properly controlled by the maintenance of high antioxidative enzyme activities (Neves et al., 2019).

### 3.2. Oxidative and antioxidative responses to increased temperature and/or elevated O<sub>3</sub>

Oxidative stress induced by IT causes the membrane peroxidation and decreases the cell membrane stability of plants (Mittler et al., 2012). In current study, MDA content, EL and O<sub>2</sub><sup>•-</sup> accumulation showed slightly higher levels under IT than those under AA, indicating that oxidative stress temporarily occurred under IT probably due to young and weak leaves at early growing stage. Similar results showed that enhanced temperature (+ 1~3°C) increased MDA content in leaves of poplar clones (Yang et al., 2018) and herb species (Qin et al., 2020). However, it seemed that antioxidative enzyme activities such as SOD, CAT, APX and GR increased with further growth and development of plants in order to alleviate the oxidative stress by IT in this experiment. Similar results showed that a slight increase in air temperature of about 2°C was able to increase the antioxidant ability in *Stylosanthes capitata* (Martinez et al., 2014) and a significant increase of the total antioxidant capacity was found in *Betula pendula* saplings exposed to elevated temperature (+ 1.3°C) (Riikonen et al., 2009). Zhang et al. (2016b) found that soil warming (+ 5°C) increased significantly the activities of CAT and APX, but decreased SOD and peroxidase (POD) activities in leaves of *Cunninghamia lanceolata*. Some studies have also shown that a prolonged exposure to IT decreased antioxidant enzyme activities in leaves of plants such as grass species (Wang et al., 2003, Du et al., 2009). In this study, increased air temperature maintained higher antioxidative enzyme activities during the entire growing season than those under elevated O<sub>3</sub>, which could contribute to optimal warming adaptation for plant growth, as manifested by the lower MDA content and higher  $P_n$  and  $F_v/F_m$ .

Generally, EO exposures can generate oxidative stress in plant tissues by inducing an

over-production of ROS such as  $O_2^{\bullet-}$  and  $H_2O_2$  (He et al., 2006; Caregnato et al., 2013). MDA accumulation in plants can reflect the status, which they are suffering from oxidative stress induced by increasing  $O_3$  concentrations (Dai et al., 2017). In this study, we found that  $O_3$  induced a significant increase in MDA content and  $O_2^{\bullet-}$  accumulation in leaves of tree species, indicating that peroxidation and denaturation of membrane lipids occurred under  $O_3$  stress and became more significant as growing period was progressing. Similar results were found when high  $O_3$  increased oxidative stress and accumulation of ROS and MDA in maize leaves (Singh et al., 2014), wheat cultivars (Wang et al., 2014; Fatima et al., 2018) and poplar clones (Podda et al., 2019; Shang et al., 2020).

Plant protection against oxidative stress under additional abiotic stressors was properly achieved through enzymatic and non-enzymatic antioxidant systems (Hasanuzzaman et al., 2012; Voss et al., 2013; Soares et al., 2019). The antioxidant enzymes such as SOD, CAT, APX and GR are responsible for ROS detoxification. The variations of their activities are a common trait in plants under abiotic stresses such as ozone (Fatima et al., 2018; Soares et al., 2019). SOD activity in this study was found to increase at early growing stage, and decrease at the following growing stages under EO. The increase of SOD activity could provide early protection to scavenge ROS induced by  $O_3$  stress during early foliar development and the decrease at late stages could result from an increased oxidative stress. Similar changes were observed in CAT and APX activities in leaves of the two tree species (Fig. 5), consistent with the results confirmed by Singh et al. (2014) where SOD, CAT and APX activities in maize leaves were stimulated by elevated  $O_3$  at two developmental stages. In this study, GR activity however showed significant decrease at different growing stages regardless of tree species during  $O_3$  exposure. Similar results were found where EO decreased GR activity at different sampling dates in *G. biloba* leaves after 30-day fumigation during growing season (He et al., 2006) and in palm leaves during 8-h exposure (Du et al., 2018).

Under the combination of EO and IT, oxidative stress induced by O<sub>3</sub> in plants was mitigated by IT, compared to that under EO alone. This finding was confirmed by the reductions in MDA contents, EL and O<sub>2</sub><sup>•-</sup> production rate found in leaves of the two tree species under the combination of the two abiotic factors. The interactive effects of O<sub>3</sub> and temperature on these parameters of oxidative stress were statistically significant (Table S1,  $P < 0.01$ ) and antagonistic, resulting in ameliorative effects during the combined treatments due to the high activities of antioxidant enzymes under IT than EO. This implied that increased air temperature was beneficial and decreased the O<sub>3</sub> sensitivity, as confirmed by non-significant differences in oxidative stress, enzyme activities, photosynthetic characteristics and growth, particularly the total biomass between the combined treatment of IT and EO and ambient air at the end of this experiment.

### 3.3. Growth response to increased temperature and/or elevated O<sub>3</sub>

Air warming generally increases plant growth and consequently biomass accumulation. In this study, IT (ambient + 2°C) significantly increased growth of the two broadleaf tree species. Similar findings were reported for other deciduous tree species such as European beech (Overdieck et al. 2007), silver birch and European aspen (Mäenpää et al., 2011) and for coniferous species such as *A. faxoniana* (Wang et al., 2012). Mäenpää et al. (2013) found that rising nocturnal temperature increased growth and shoot biomass of silver birch, which is recognized as a specific response of trees to warmer temperature in nights.

However, warming by means of increased air temperature also resulted in negative or neutral impacts on growth (Chen et al., 2017; Iturrate-Garcia et al., 2017), which is in contrast to our current results. IT can usually enhance plant growth under modest temperature or dramatic precipitation (Gustafson et al., 2017). In addition, moderate warming (+ 3°C) increased photosynthetic rates, but decreased biomass production due to the increased maintenance costs

through o respiration f plants (Gustafson et al., 2017). In our current study, air warming did not alter root/shoot ratio, indicating no change in the functional balance and distribution between carbon gain and nutrient supply under IT (Wang et al., 2012). Contrary to our present results, Tacarindua et al. (2013) found that the above-ground biomass of soybean significantly reduced by warming of 2~3°C. Dry biomass decrease was associated with a decline in net photosynthesis and stomatal conductance. The indicated contradictory results suggested that plant growth in response to warming might depend on plant species or cultivars, the intensity, frequency and duration of increasing temperature and the season (Silveira and Thiebaut, 2017), or environmental factors including O<sub>3</sub> pollution.

Generally, increased O<sub>3</sub> concentrations can inhibit plant growth in many species. In the current experiment, elevated O<sub>3</sub> induced a significant reduction of growth in the two urban tree species, especially in biomass. Similar previous studies found that elevated O<sub>3</sub> concentrations decreased growth and biomass accumulation in crops (Mills et al., 2011; Feng et al., 2015) and trees including *G. biloba* and poplar clones (Xu et al., 2015, 2019; Zhang et al., 2018; Shang et al., 2020). In addition, this study showed that the percentage of reduction in total biomass was larger in *G. biloba* (49.2%) than in *P. alba* 'Berolinensis' (26.4%) exposed to EO alone. Basal diameter in O<sub>3</sub>-exposed *G. biloba* showed a significant decrease but not in O<sub>3</sub>-exposed *P. alba* 'Berolinensis'. These results might imply that the former was more sensitive to O<sub>3</sub> than the latter, as manifested by higher levels of MDA content, EL and O<sub>2</sub><sup>•-</sup> accumulation in *G. biloba* leaves exposed to O<sub>3</sub> during the whole growing season (Fig. 4). It is well known that the reduction of the root/shoot (R/S) ratio is considered a typical plant response to O<sub>3</sub> exposure (Matyssek et al., 2008). In this study, EO decreased significantly R/S ratios of the two tree species, which could indicate that shoot was prioritized over root system for resource allocation (Keutgen et al., 2005).

Under the current combination of abiotic factors, air warming throughout the experimental



period were perhaps more beneficial for plants to adapt the adverse environment by adjusting the physiological and biochemical processes. In other words, our findings suggest that air warming may be suitable for growth of urban tree species, which could alleviate the adverse effect of high  $O_3$  concentration on plants, as supported by the similar values for all growth parameters under the combination of increased air temperature and elevated  $O_3$  concentration, compared to those under ambient air and  $O_3$  alone. This was in accordance with a recent study that increased temperature attenuated the negative impact of  $O_3$  on the biomass of wheat cultivars (Changey et al., 2018). In fact, increased temperature can reduce the uptake of  $O_3$  through decreasing stomatal conductance, and improve the capacity of plants to defend against oxidative stress through increased photosynthetic efficiency (Mäenpää et al., 2011). Furthermore, global warming may alter the adaptive ability of plants or change their phenology and distribution range adapt to the adverse environmental conditions including  $O_3$  pollution in a changing world (Riikonen et al., 2009; Hartikainen et al., 2012).

#### 4. Conclusions

The current results highlighted the importance of a better understanding how trees responded and adapted to regional air warming and air pollution. The two urban tree species tested in this study showed similar physiological responses to adapt increased air warming and elevated  $O_3$  exposure. We found that increased temperature lead to enhanced above-ground biomass of urban trees. *G. biloba* was more sensitive to both increased temperature and elevated tropospheric  $O_3$  concentrations than *P. alba* 'Berolinensis'. Elevated  $O_3$  concentrations induced oxidative stress, inhibited growth and photosynthesis, which being greatly mitigated by increasing temperature. Significant interactive and antagonistic effects between increased temperature and  $O_3$  pollution were found on photosynthetic performance, above-ground biomass

and root/shoot ratio. The results presented in this study based on one growing-season experiment using young plants. In addition, the physiological changes of the two tree species at different growing stages in this study cannot be generalized as the response to regional warming is largely species-specific and can depend on the leaf age and the development stage of plants. In particular, our results suggest that the effects of air warming and ozone pollution on growth and physiological metabolisms are likely to be limited in northern temperate and boreal tree species of continental climate. However, elucidating how rising temperatures interact with elevated O<sub>3</sub> may have significant implications for predicting the phenological characteristics and the responses of forest function and structure to projected future climate change, particularly under the regional warming and the ground-level ozone pollution frequently co-occurring during the growing season for plants in urban areas.

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## Figure captions

**Figure 1.** Changes in chlorophyll a (Chla; a & b), chlorophyll b (Chlb; c & d) and carotenoids (Car; e & f) contents in leaves of *Ginkgo biloba* and *Populus alba* 'Berolinensis' exposed to increased temperature (IT, ambient air + 2 °C) and/or elevated O<sub>3</sub> (EO, ambient air + 40 ppb O<sub>3</sub>) during the experimental treatments. AA-ambient air (40 ppb O<sub>3</sub>).

**Figure 2.** Changes in net photosynthetic rate ( $P_n$ ; a & b), stomatal conductance ( $g_s$ ; c & d), vapor pressure deficit (VPD; e & f) and water use efficiency (WUE; g & h) in leaves of *Ginkgo biloba* and *Populus alba* 'Berolinensis' exposed to increased temperature (IT, ambient air + 2 °C) and/or elevated O<sub>3</sub> (EO, ambient air + 40 ppb O<sub>3</sub>) during the experimental treatments. AA-ambient air (40 ppb O<sub>3</sub>).

**Figure 3.** Changes in the maximum quantum efficiency of PSII photochemistry ( $F_v/F_m$ ; a & b), the actual quantum efficiency of PSII ( $\Phi_{PSII}$ ; c & d), the quenching of photochemical efficiency of PSII ( $qP$ ; e & f) and the non-photochemical quenching (NPQ; g & h) in leaves of *Ginkgo biloba* and *Populus alba* 'Berolinensis' exposed to increased temperature (IT, ambient air + 2 °C) and/or elevated O<sub>3</sub> (EO, ambient air + 40 ppb O<sub>3</sub>) during the experimental treatments. AA-ambient air (40 ppb O<sub>3</sub>).

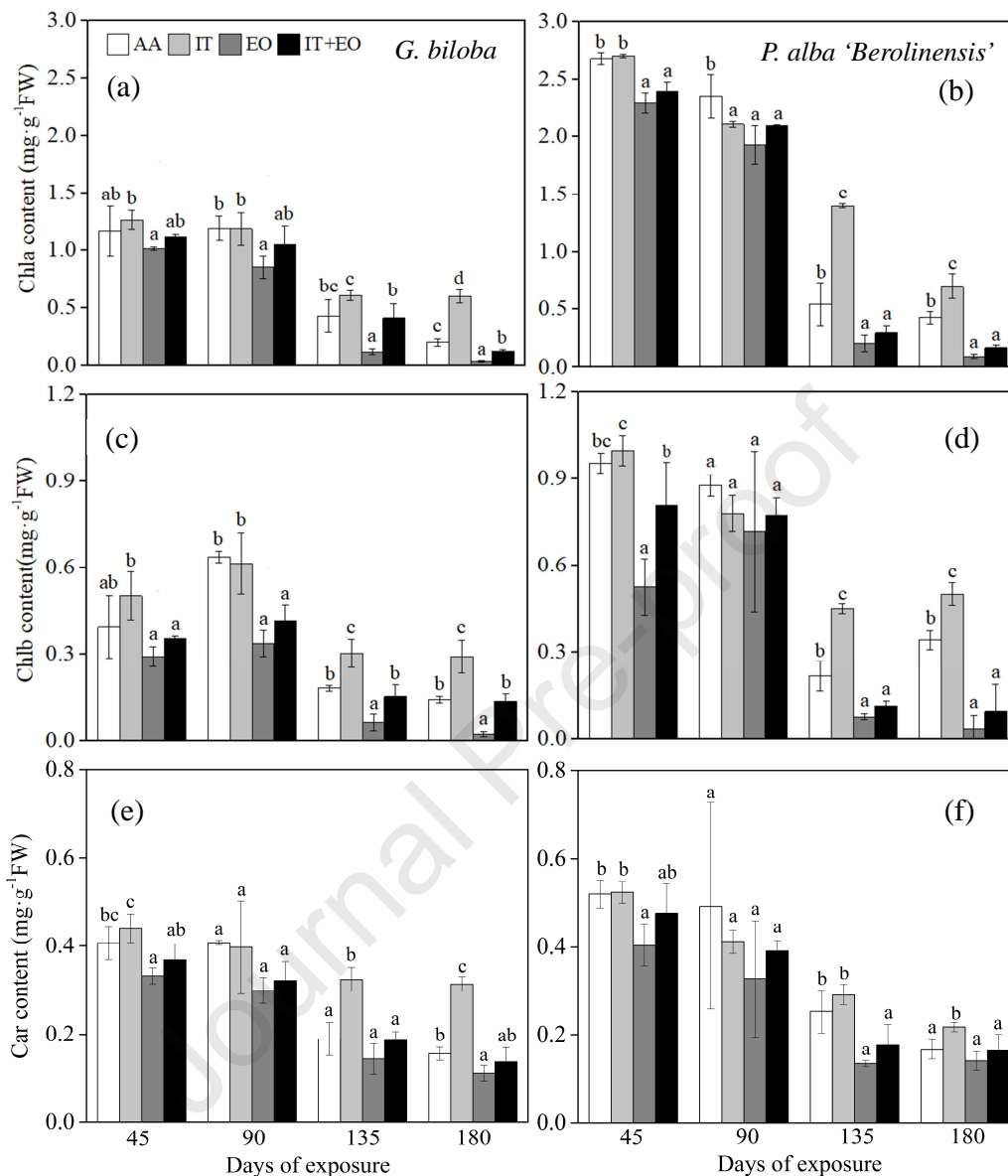
**Figure 4.** Changes in malondialdehyde (MDA; a & b) content, electrolyte leakage (EL; c & d) and O<sub>2</sub>•<sup>-</sup> production rate (e & f) in leaves of *Ginkgo biloba* and *Populus alba* 'Berolinensis' exposed to increased temperature (IT, ambient + 2°C) and/or elevated O<sub>3</sub> (EO, ambient air + 40 ppb O<sub>3</sub>) during the experimental treatments. AA-ambient air (40 ppb O<sub>3</sub>).

**Figure 5.** Changes in the activities of superoxide dismutase (SOD; a & b), catalase (CAT; c & d), ascorbate peroxidase (APX; e & f) and glutathione reductase (GR; g & h) in leaves of *Ginkgo biloba* and *Populus alba* 'Berolinensis' exposed to increased temperature (IT, ambient + 2°C) and/or elevated O<sub>3</sub> (EO, ambient air + 40 ppb O<sub>3</sub>) during the experimental treatments. AA-ambient air (40 ppb O<sub>3</sub>).

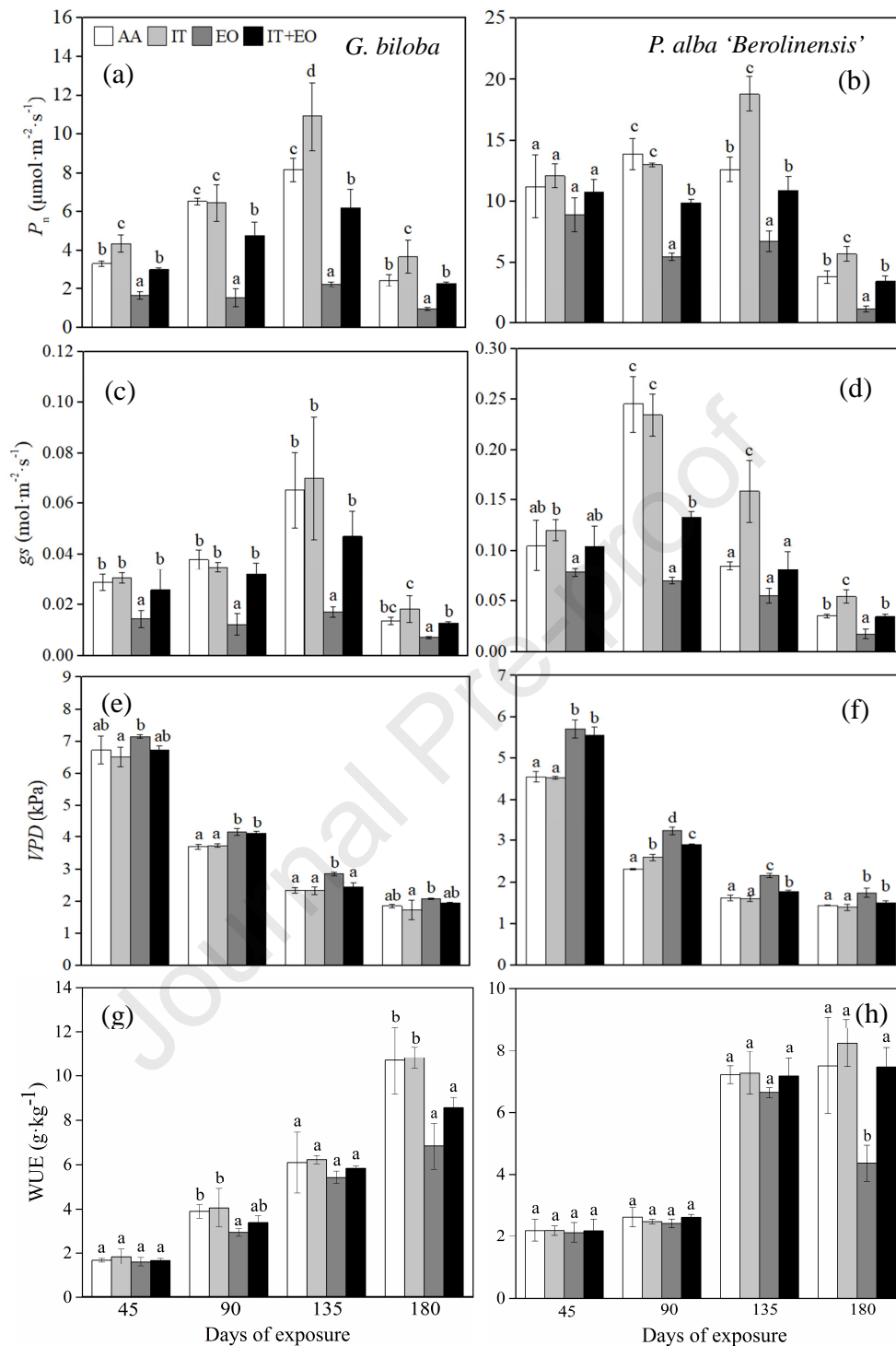
977 **Table 1** ANOVA (*P* values) of growth parameters for the main effects and interactions of EO (ambient air-AA + 40 ppb O<sub>3</sub>) and IT (ambient + 2°C) (n=3).

Species	Treatments	RWC (%)	Plant height (cm)	Basal diameter (mm)	SLW (g/cm <sup>2</sup> )	Shoot weight (g)	Root weight (g)	Total weight (g)	R/S ratio
<i>Ginkgo biloba</i>	AA	97.3 ± 1.9b	80.0 ± 14.7a	16.8 ± 2.3b	2.6 ± 0.5b	45.7 ± 2.5c	52.0 ± 3.1c	97.7 ± 4.7b	1.1 ± 0.1b
	IT	97.2 ± 0.8b	76.7 ± 9.9a	16.9 ± 2.2b	3.2 ± 0.7b	62.8 ± 7.1d	70.1 ± 3.1d	132.9 ± 9.3c	1.1 ± 0.1b
	EO	59.7 ± 3.1a	64.7 ± 7.6a	10.3 ± 1.2a	1.5 ± 0.2a	29.6 ± 3.9a	20.0 ± 2.8a	49.6 ± 5.6a	0.7 ± 0.1a
	IT+EO	86.9 ± 19.6b	73.0 ± 6.2a	15.6 ± 0.7b	2.4 ± 0.2b	40.2 ± 1.2b	46.1 ± 2.6b	86.3 ± 3.7b	1.1 ± 0.0b
<i>Populus alba</i> 'Berolinensis'	AA	86.8 ± 1.5b	212.0 ± 2.7a	16.7 ± 0.2a	1.7 ± 0.4b	117.1 ± 6.8b	114.6 ± 5.1b	231.7 ± 1.7b	1.0 ± 0.1b
	IT	86.6 ± 5.2b	201.7 ± 10.4a	17.2 ± 1.5a	1.8 ± 0.1b	155.6 ± 10.7c	155.3 ± 12.0c	310.9 ± 14.7c	1.0 ± 0.1b
	EO	69.4 ± 2.4a	195.7 ± 22.9a	15.6 ± 1.2a	1.2 ± 0.1a	96.1 ± 3.7a	74.4 ± 6.7a	170.5 ± 9.7a	0.8 ± 0.1a
	IT+EO	87.0 ± 2.0b	205.0 ± 10.5a	16.7 ± 1.4a	1.4 ± 0.1ab	115.6 ± 3.1b	112.1 ± 5.9b	227.7 ± 7.5b	1.0 ± 0.0b
Species		0.366	< 0.001***	0.014*	< 0.001***	< 0.001***	< 0.001***	< 0.001***	0.018*
IT		0.002**	0.841	0.011*	0.005**	< 0.001***	< 0.001***	< 0.001***	< 0.001***
EO		< 0.001***	0.123	0.001**	< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001***
Species × IT		0.429	0.764	0.141	0.047*	0.003**	0.003**	< 0.001***	0.110
Species × EO		0.021**	0.764	0.022*	0.050*	0.029*	0.012*	0.002**	0.174
IT × EO		0.002**	0.131	0.032*	0.562	0.014*	0.623	0.139	< 0.001***
Species × IT × EO		0.443	0.690	0.080	0.648	0.195	0.271	0.093	0.043*

978 RWC-relative water content, SLW-specific leaf weight, R/S-root/shoot. \*: *P* < 0.05; \*\*: *P* < 0.01; \*\*\*: *P* < 0.001.

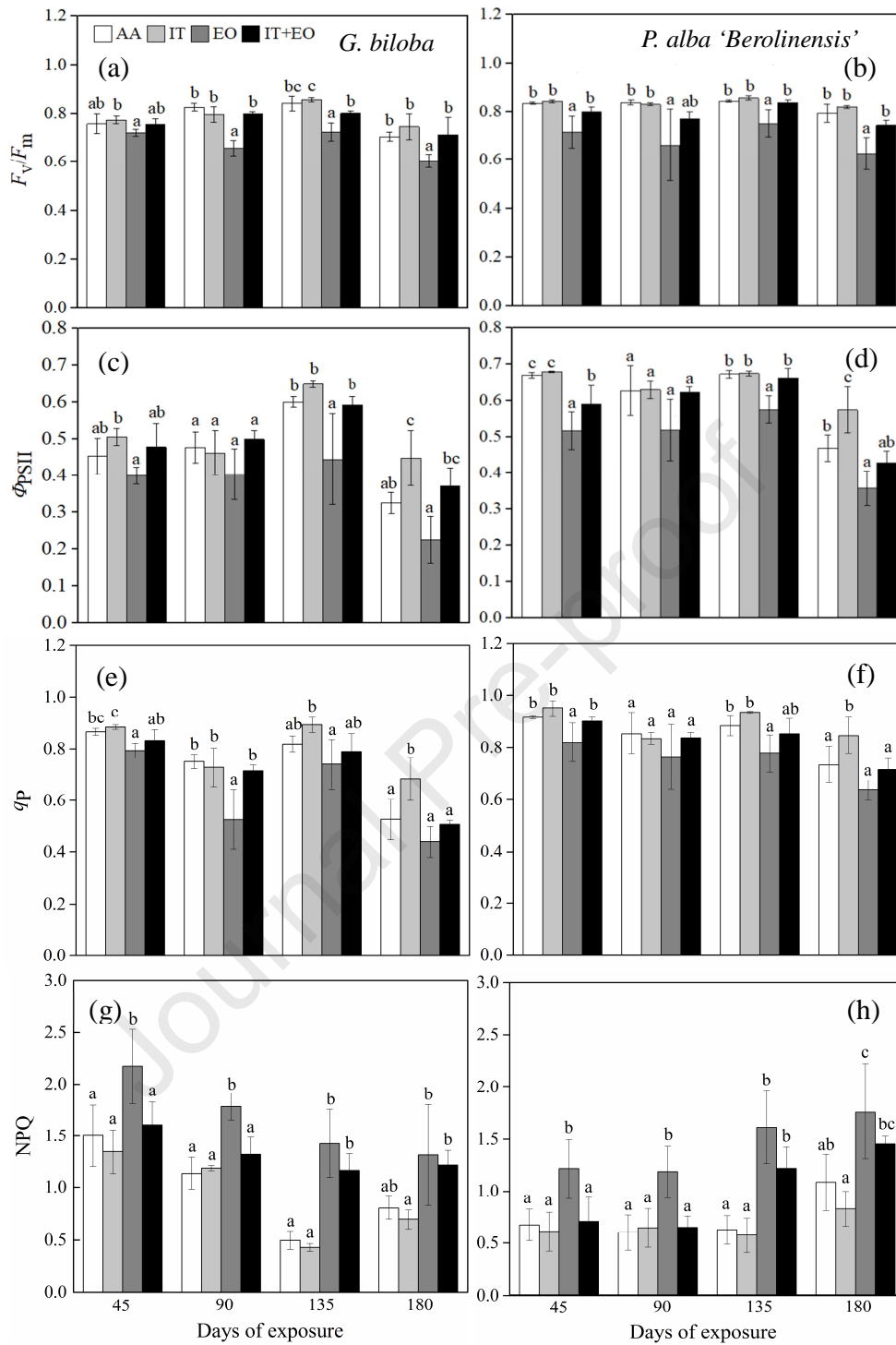


**Fig.1.** Changes in chlorophyll a (Chla; a & b), chlorophyll b (Chlb; c & d) and carotenoids (Car; e & f) contents in leaves of *Ginkgo biloba* and *Populus alba* 'Berolinensis' exposed to increased temperature (IT, ambient air + 2 °C) and/or elevated O<sub>3</sub> (EO, ambient air + 40 ppb O<sub>3</sub>) during the experimental treatments. AA-ambient air (40 ppb O<sub>3</sub>).

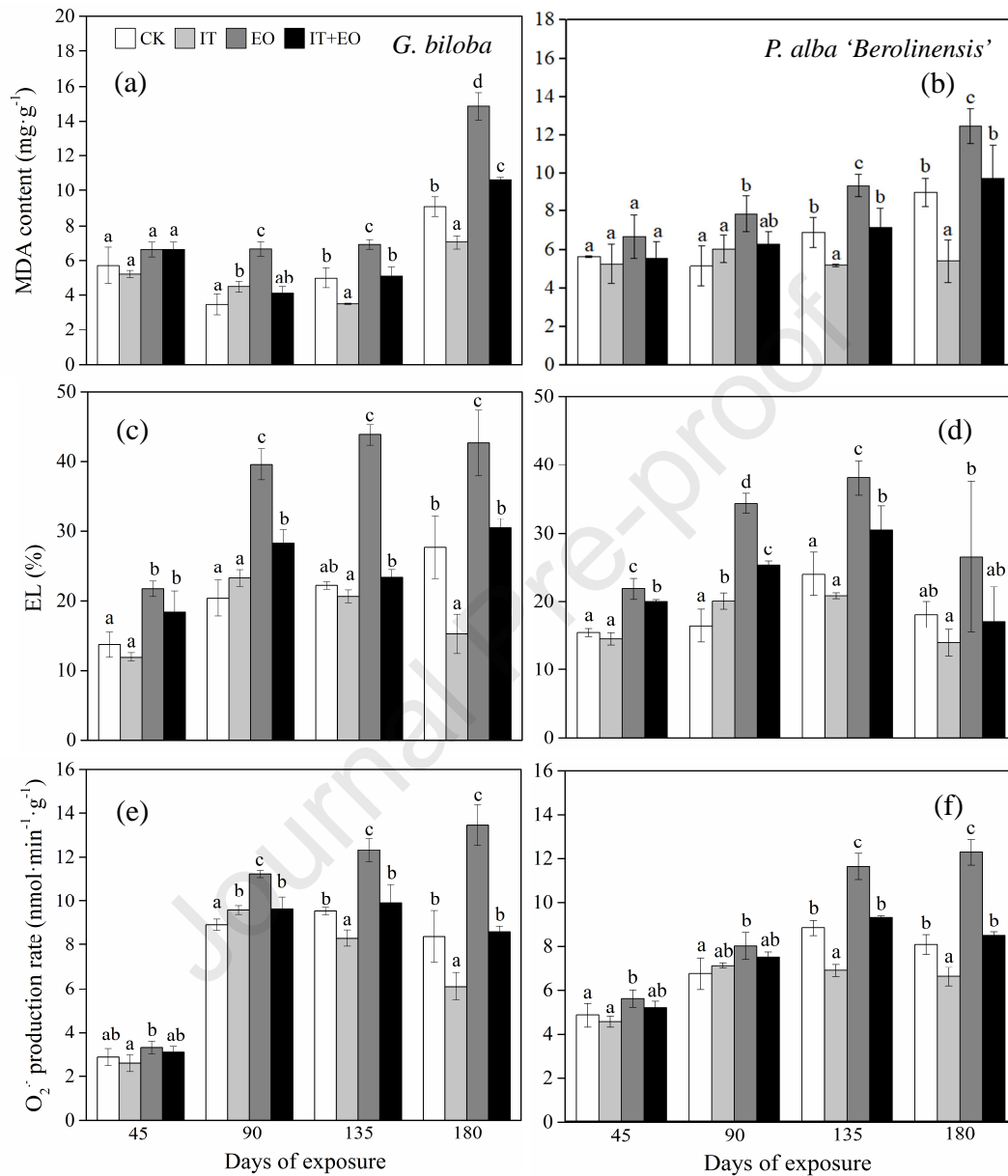


**Fig. 2.** Changes in net photosynthetic rate ( $P_n$ ; a & b), stomatal conductance ( $g_s$ ; c & d), vapor pressure deficit (VPD; e & f) and water use efficiency (WUE; g & h) in leaves of *Ginkgo biloba* and *Populus alba* 'Berolinensis' exposed to increased temperature (IT, ambient air + 2 °C) and/or elevated  $\text{O}_3$  (EO, ambient air + 40 ppb  $\text{O}_3$ ) during the experimental treatments. AA-ambient air (40 ppb  $\text{O}_3$ ).

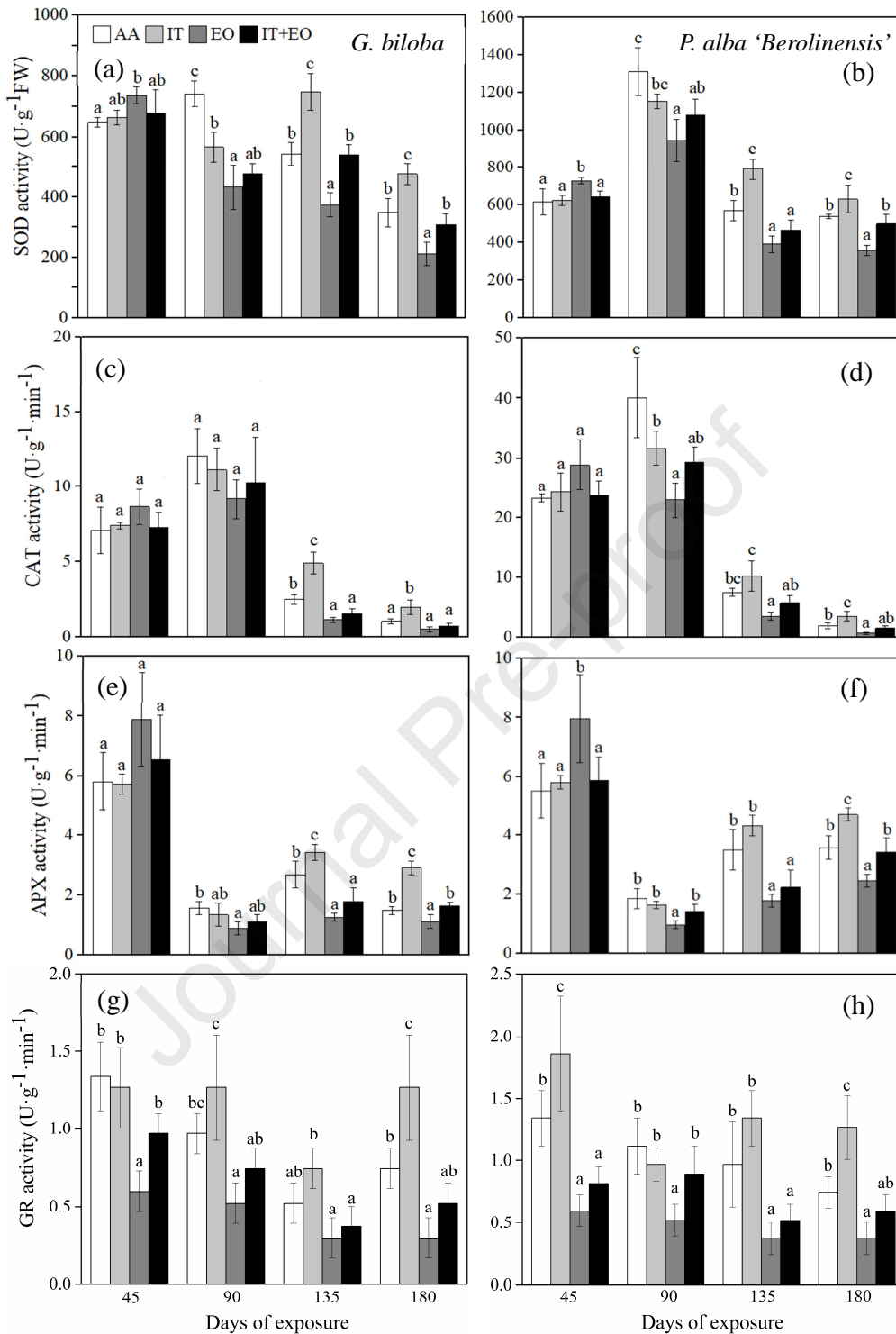




**Fig. 3.** Changes in the maximum quantum efficiency of PSII photochemistry ( $F_v/F_m$ ; a & b), the actual quantum efficiency of PSII ( $\Phi_{PSII}$ ; c & d), the quenching of photochemical efficiency of PSII ( $qP$ ; e & f) and the non-photochemical quenching (NPQ; g & h) in leaves of *Ginkgo biloba* and *Populus alba* 'Berolinensis' exposed to increased temperature (IT, ambient air + 2 °C) and/or elevated  $O_3$  (EO, ambient air + 40 ppb  $O_3$ ) during the experimental treatments. AA-ambient air (40 ppb  $O_3$ ).



**Fig.4.** Changes in malondialdehyde (MDA; a & b) content, electrolyte leakage (EL; c & d) and O<sub>2</sub>•<sup>-</sup> production rate (e & f) in leaves of *Ginkgo biloba* and *Populus alba* 'Berolinensis' exposed to increased temperature (IT, ambient + 2°C) and/or elevated O<sub>3</sub> (EO, ambient air + 40 ppb O<sub>3</sub>) during the experimental treatments. AA-ambient air (40 ppb O<sub>3</sub>).



**Fig.5.** Changes in the activities of superoxide dismutase (SOD; a & b), catalase (CAT; c & d), ascorbate peroxidase (APX; e & f) and glutathione reductase (GR; g & h) in leaves of *Ginkgo biloba* and *Populus alba* 'Berolinensis' exposed to increased temperature (IT, ambient + 2°C) and/or elevated O<sub>3</sub> (EO, ambient air + 40 ppb O<sub>3</sub>) during the experimental treatments. AA-ambient air (40 ppb O<sub>3</sub>).

## Highlights

- Increased temperature led to enhanced above-ground biomass of urban tree species.
- Elevated O<sub>3</sub> inhibited growth and photosynthesis of *Ginkgo biloba* and poplar.
- Increased air temperature mitigated the negative impact from elevated O<sub>3</sub> on growth.
- Significant interactive effects between increased temperature and O<sub>3</sub> pollution were found.
- *G. biloba* was more sensitive than poplar to increased temperature and elevated O<sub>3</sub>.

**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: