

Article

The Effects of Dual Ozone and Drought Stresses on the Photosynthetic Properties of *Acer rubrum* and *A. pictum*

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Abstract: Ozone (O₃) pollution is accompanied by drought stress, especially at high temperatures. Tree species in cities often face dual stresses from O₃ and drought. In this study, *Acer rubrum* ‘Autumn Blaze’ and *A. pictum* were used as test plants in open-top-chambers (OTCs) to investigate the trees most tolerant to increasing O₃ and drought stresses in urban gardens. The results showed that the dual stresses induced a change in *A. rubrum*’s leaf coloration from green to red. The leaf representation in *A. rubrum* was more variable than that of *A. pictum*. The leaf pigment content affected the plant leaf color difference, and the Chl and Car contents of both species were negatively correlated with L*. Under the dual stresses of O₃ and drought, the changes in the net photosynthetic rate (Pn) and transpiration rate (Tr) were less variable in *A. rubrum* than *A. pictum*. The stomatal conductance (Gs) was more sensitive to higher O₃ stress, the effect of which was enhanced by moderate drought (MD) conditions on Gs. The Tr decreased more significantly under drought stress, which mitigated the effect of O₃ stress on the stomatal limit value (Ls). *A. rubrum* displayed differential color changes, resulting in greater structural heterogeneity within the garden landscape. The saplings adjusted their photosynthetic parameters under the dual stresses, whereas the dual stresses played an antagonistic role in protecting *A. rubrum*, suggesting that *A. rubrum* can resist O₃ and drought. Our study suggests that *A. rubrum* is an alternative tree species for inclusion in urban gardens exposed to increasing O₃ and drought stresses.

Keywords: Aceraceae; chlorophyll; photosynthetic parameters; stomata; transpiration; urban trees



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1. Introduction

Tropospheric ozone (O₃) is the primary component of atmospheric photochemical smog and greenhouse gases, which is a specific term for “surface O₃” [1], and has strong oxidizing properties. Rapid development during the industrial age significantly increased the emissions of O₃ precursors, such as nitrogen oxides (NO_x = NO + NO₂) and volatile organic compounds (VOCs), thereby elevating the concentration of near-surface O₃ [2]. Notably, the O₃ concentration in China has been increasing at an annual rate of 0.3%–2.0%, and it is estimated to double by 2100. The north-central regions of North and East China are the most O₃-polluted areas of China [3], with O₃ concentrations considerably above the damage threshold of 40 ppb for sensitive plants [2].

Near-surface O₃ seriously hinders the growth of natural plants [4,5] and reduces the quality and yield of crops [6,7]. Furthermore, O₃ can cause yellow and green color loss, spots, dryness, wilting, and early aging in leaves. Tropospheric O₃ can reduce the photosynthesis rate, cause stomatal closure in plants, hinder photosynthetic electron transport, inhibit plant photosynthesis [6,8,9], cause the stomata on the leaf surface to lag or fail [10,11], and increase water consumption by transpiration [11,12].

Drought stress is the most common challenge for trees. The primary contributors to drought stress are a high-temperature climate and lack of irrigation conditions caused by

limited water resources and the fragility of the ecological environment. Currently, the lack of water resources and the fragility of the ecological environment remains challenging in urban landscape construction [13]. In drought conditions [14,15], plants' response to physiological factors, such as net photosynthetic rate, transpiration rate, stomatal conductance, and hydraulic conductivity, is limited [16]; therefore, drought can affect plant photosynthesis [17]. When plants are subjected to drought stress, the stomata close as stress levels increase; this reduces water loss and increases plant resistance to drought [18]. It also affects the absorption of carbon dioxide, ultimately inhibiting photosynthesis. For the whole plant, the damage caused by drought stress is more severe than that of O₃ stress alone [19]. Water scarcity and drought remain long-standing and challenging problems worldwide [20].

The primary manifestation of the compounding effect of O₃ and drought is the synergistic aggravation of plant damage, while an antagonistic effect reduces plant damage. The response depends on the plant and species, the magnitude and duration of the drought, and high O₃ conditions [21,22]. The impact of multiple stresses on plants is complex, and the interaction between multiple factors at the same time needs to be further investigated. For example, moderate water restriction helps to slow down the harmful damage caused by O₃ to poplars (*Populus deltoides* cv. '55/56' × *P. deltoides* cv. 'Imperial') [23]. However, Hao et al. [24] and Ye et al. [25] found that the seedlings of *Alstonia scholaris* and *Syzygium hainanense* were more affected by the dual stresses of O₃ and drought than by their individual stress effects and that *S. hainanense* seedlings showed stronger anti-O₃ and drought resistant responses than *A. scholaris*. Another study has shown that plants are unaffected by dual stresses [26], suggesting a lack of consensus regarding the effects of these dual stresses on different plants.

Acer rubrum L. (American red maple) and *A. pictum* Thunb. ex Murray (mono maple) are important broad-leaved landscaping tree species in North China [27,28]. The urban landscape provides an important ecosystem for the city's inhabitants; therefore, the woody plants play an essential role in fine-scale monitoring through unmanned aerial systems (UAS) imagery of the urban environment and the effect of O₃ [29]. Research on the effects of O₃ and drought stress on plant photosynthetic physiology is mainly focused on grass crops, fruits, and vegetables. However, the photosynthetic physiology of landscaping tree species has yet to be investigated, especially for *A. rubrum* and *A. pictum*, the two main species of Aceraceae used in urban landscapes in China. So far, previous studies have primarily investigated drought as a single stress and drought resistance when evaluating this tree species [30,31]; hence, data on the dual stresses of this tree species still need to be provided.

In this study, we considered the seedlings of two tree species as research objects and explored their response to variable environmental conditions, such as an O₃ fumigation gradient and drought stress. In addition, changes in the appearance and physiological indicators were explored to provide a reference for the selection and configuration of landscaping tree species. For example, these species could exhibit physiological and ecological changes that reduce the ornamental effect of a garden landscape; alternatively, environmental stress could have little impact on the physiological indicators.

2. Materials and Methods

2.1. Test Area

This study was performed at an experimental field station in Yanqing District, Beijing (40°48' N, 115°60' E). The station experiences a temperate and semi-humid continental climate; the average annual temperature and precipitation are 9.9 °C and 467 mm, respectively; the warmest month is July, with an average temperature of 24.5 °C [32].

2.2. Test Materials and Design

The experimental materials were 1–2-year-old seedlings of *Acer rubrum* 'Autumn Blaze' and *A. pictum*, which are widely used as landscaping plants in northern Chinese

cities. Polyethylene plastic flowerpots with a height of 20 cm and diameter of 25 cm were uniformly used for potting. Each pot was filled with a uniform mixture of local sandy loam and substrate at a 3:1 ratio, with the pH ranging from 5.5 to 6.5.

The fumigation facilities included nine open-top air chambers (OTCs, 2.2 m high, and 11.34 m²), with three O₃ and three H₂O levels, in a 3 × 3 treatment design. The three O₃ levels were non-filtered ambient air (NF), NF with an O₃ addition of 40 nmol/mol (NF40), and NF with an O₃ addition of 60 nmol/mol (NF60), with each O₃ level having three replicates (OTCs). Each chamber had three pots for each of the two varieties of seedlings [33,34]. Three H₂O levels, including well-watered conditions (WW, approximately 75%–80% field capacity), moderate drought stress (MD, approximately 50%–55% field capacity), and severe drought stress (SD, approximately 30%–35% field capacity), were applied to each O₃ treatment. Experimental and control fields were randomly distributed.

2.3. Test Methods

2.3.1. Determination of Plant Leaf Color Parameters

A colorimeter is an instrument suitable for nondestructive testing of plant color. It can accurately measure the leaf color of plants, directly output the leaf color parameters L*, a*, and b*, and quantitatively describe its brightness and color. Leaf color was determined by using a CR-400 (Konica Minolta, Inc., Tokyo, Japan) type fully automatic colorimeter. The measured light source was the built-in D₆₅ standard light source, the window diameter was 8 mm, and CIE L a b color space was selected. Three leaves were selected for each treatment, and each leaf was repeatedly determined three times. The brightness value (L*), color value (a*), and color value (b*) are directly measured by the colorimeter. In this space, L* indicates the brightness of the measured sample color, where 0 indicates black, 100 indicates white, and the greater the L* value, the higher the brightness. The a* value indicates the red and green concentration of the measured sample color, which is positive for red and negative for green; the larger the a* value, the deeper the red, the smaller the a* value, the darker the green. The b* value indicates the yellow-blue concentration of the measured sample color, positive for yellow and negative for blue; the larger the b* value, the darker yellow, and the smaller the b* value, the darker blue. All indicators were determined repeatedly in triplicate.

Photosynthetic pigments were sampled using a 0.7 cm diameter punch, 2 leaf discs per leaf, and extracted with 2 mL of 95% ethanol solution in 4 °C shading until completely faded to white. Subsequently, the absorbance values of the extracts were determined at 664, 649, and 470 nm, and carotenoids (Car), chlorophyll a (Chl a), and chlorophyll b (Chl b) were calculated. The sum of Chl a and Chl b was calculated as the total chlorophyll content. The calculation formula for the photosynthetic pigment concentration is as follows:

$$\text{Chl a} = 13.95 \times \text{OD}_{664} - 6.88 \times \text{OD}_{649} \quad (1)$$

$$\text{Chl b} = 24.96 \times \text{OD}_{649} - 7.32 \times \text{OD}_{664} \quad (2)$$

$$\text{Chl} = \text{Chl a} + \text{Chl b} = 6.63 \times \text{OD}_{664} + 18.08 \times \text{OD}_{649} \quad (3)$$

$$\text{Car} = (1000 \times \text{OD}_{470} - 2.05 \times \text{Chl a} - 114.8 \times \text{Chl b})/245 \quad (4)$$

The unit in the formula is mg·L⁻¹.

Finally, the chlorophyll content in the plant leaves was obtained:

Chlorophyll content = (sample chlorophyll concentration × dilution multiple × extract volume)/leaf area

The unit in the formula is mg·L⁻¹.

2.3.2. Determination of the Photosynthetic Indices in Plants

Sunny and calm weather conditions were selected to sample and measure photosynthetic indicators. From 09:00 to 12:00 every day, healthy, uniformly sized, and uniformly

colored leaves were randomly selected from mature leaves in the middle and upper parts of the plants. A Li-6400 photosynthetic analyzer (Li-cor Portable, Lincoln, OR, USA) was used. The test conditions were as follows: the leaf chamber temperature was 30 °C, the light intensity was 1000 $\mu\text{mol}/\text{m}^2/\text{s}$, the CO_2 concentration was 400 $\mu\text{mol}/\text{mol}$, and the flow rate was 500 $\mu\text{mol}/\text{s}$. Photosynthetic gas parameters were determined under the open gas path. Three leaves were selected for each treatment, and each leaf was repeatedly determined three times. Photosynthetic gas parameters include the net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and transpiration rate (Tr). Water use efficiency (WUE_g) was calculated as Pn/Tr , and the stomatal limit value (Ls) was calculated using $1 - \text{Ci}/\text{Ca}$.

2.3.3. Determination of the O_3 and Soil Moisture Content

O_3 was produced by an O_3 generator (HY003, Jinan Chuangcheng Co., Jinan, China). Pure oxygen was mixed with natural air by a 1.1 kW Fengda air-blower (Shanghai, China), and the outlet cylinder at the inner center of the hood rotated 360° to fill the O_3 gas and maintain the target O_3 concentration of the plant canopy; natural air was fed into the NF treatments. O_3 fumigation (08:00–18:00 every day, except rainy days) lasted from 15 July to 23 September 2021. A Teflon solenoid valve system was used to sample the gas at the canopy height of the OTC plants. Subsequently, the system was measured using an ultraviolet (UV) absorption O_3 analyzer (Model 49i; Thermo Scientific, Franklin, MA, USA) and periodically calibrated using a 49i-PS calibrator (Thermo Scientific). The average concentration range of NF over the entire period was 41.71 ± 0.29 nmol/mol. The soil water content was quantified using a field soil moisture meter (TRIME-HD, Chicago, IL, USA). There were three NF treatments corresponding to the three drought levels.

2.4. Data Analysis

WPS Office Excel was used for statistical data processing, and SPSS software (version 26.0) was used to conduct the analyses. The effects of O_3 and drought on the dependent variables and the dual stress were analyzed using variance analysis, and the mean and standard deviation were calculated using Duncan's multiple comparison test. Origin 2021 software was used to draw graphs. There were three replicates of each stress and three subsamples. In each replicate, three test plants (3 pots) were collected from the middle leaves of a single plant, and at least 3–4 leaves were collected on a single seedling for determination.

3. Results

3.1. Morphological Changes in the Leaves of *A. rubrum* and *A. pictum*

As seen in Figure 1 and Table 1, the leaves of *A. rubrum* and *A. pictum* showed changes in appearance following exposure to the dual stresses of O_3 and drought. *A. rubrum* had the highest brightness value, L^* , at NF40 + SD, and *A. pictum* had the highest L^* at NF60 + SD. Under drought single stress, *A. rubrum* had the highest saturation, a^* , under WW, and the lowest a^* at NF60. *A. pictum*'s a^* was not significantly different under the compound stress of O_3 and drought. *A. rubrum* had the highest yellow and blue saturation, b^* , under NF40 + SD, and the lowest b^* under NF + MD. Among them, *A. rubrum* appeared to have significant differential changes visually with the dual stress of $\text{O}_3 \times$ drought. The difference in the leaf color of *A. rubrum* was the most pronounced (Figure 1). The leaf color change in NF60 was more affected by increasing drought compared to that of NF. While the leaf color changed from green to bright red in NF+WW, *A. rubrum* remained in the green leaf stage in NF60+SD. At the same time, the leaf size of *A. rubrum* decreased with drought stress. Changes in the appearance and morphology of *A. pictum* leaves were primarily reflected in the yellowing and chlorosis of leaves, from the leaf edge to the interior, and the degree of leaf cracking. Under O_3 single stress, the degree of leaf cracking of *A. pictum* showed three palmate lobes in NF and NF40, but the trilobate trend disappeared at NF60. The number of serrations on the leaf edge of *A. pictum* increased in NF60. The leaf size of *A. pictum*

did not change significantly under single stress yet showed a shrinking trend under the dual stresses, suggesting that they affected the leaf appearance compared to trees without environmental stress exposure.

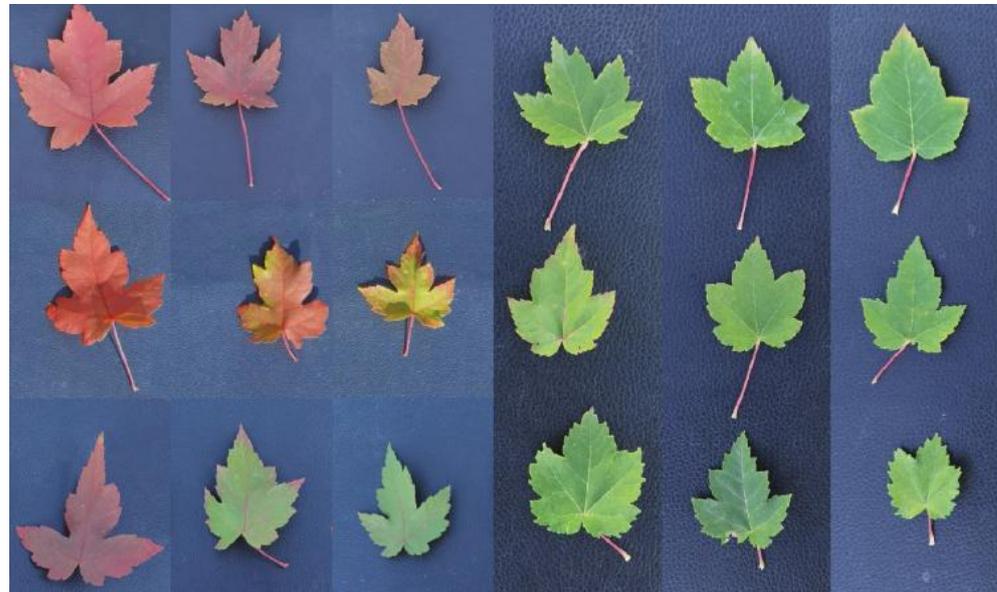


Figure 1. Effects of O_3 and drought stresses on leaf appearance of *Acer rubrum* and *A. pictum*. The left and right sets of nine photographs (3×3) show the processed *A. rubrum* and *A. pictum*, respectively. The treatments of the two sets in the top rows from left to right are NF + WW, NF + MD, and NF + SD, respectively; the two sets in the second row are NF40 + WW, NF40 + MD, and NF40 + SD, respectively; the two sets in the third row are NF60 + WW, NF60 + MD, and NF60 + SD, respectively.

Table 1. Comparison of leaf color parameters between *Acer rubrum* and *A. pictum* under nine different treatments.

Species	Treatments		CIE L a b Color Coordinates		
	O_3	Drought	Brightness value L*	Red and Green Saturation a*	Yellow and Blue Saturation b*
<i>A. rubrum</i>	NF	WW	51.22 ± 1.07de	37.64 ± 0.68b	14.71 ± 0.74c
	NF	MD	50.73 ± 0.39de	24.93 ± 1.13c	7.88 ± 0.15d
	NF	SD	56.64 ± 0.4bc	24.72 ± 0.51c	14.55 ± 0.2c
	NF40	WW	52.19 ± 0.34cde	42.56 ± 0.17a	22.77 ± 0.4b
	NF40	MD	54.32 ± 0.12bcd	35.17 ± 1.03b	21 ± 0.07b
	NF40	SD	68.11 ± 2.19a	3.86 ± 1.27d	46.87 ± 1.34a
	NF60	WW	47.21 ± 0.24e	21.87 ± 0.38c	5.81 ± 0.54d
	NF60	MD	56.8 ± 0.95bc	−8.05 ± 1.07e	16.14 ± 0.19c
	NF60	SD	58.65 ± 1.37b	−13.09 ± 0.28f	16.94 ± 1.69c
<i>A. pictum</i>	NF	WW	57.38 ± 0.24e	−16.1 ± 0.44b	20.19 ± 0.4c
	NF	MD	60.81 ± 0.42d	−18.97 ± 0.15cd	20.89 ± 1.31c
	NF	SD	70.26 ± 0.25b	−18.32 ± 0.23bcd	20.77 ± 0.44c
	NF40	WW	64.58 ± 0.05c	−19.53 ± 0.93d	33.39 ± 0.29a
	NF40	MD	59.54 ± 0.46d	−16.75 ± 0.76bc	23.96 ± 0.2b
	NF40	SD	70.05 ± 0.2b	−19.56 ± 0.38d	26.5 ± 0.38b
	NF60	WW	57.34 ± 0.5e	−18.22 ± 0.17bcd	23.95 ± 0.47b
	NF60	MD	56.3 ± 0.22e	−12.04 ± 0.41a	13.39 ± 0.15d
	NF60	SD	76.95 ± 0.7a	−18.39 ± 0.3bcd	20.19 ± 0.4c

NF, non-filtered ambient air; NF40, NF with an O_3 addition of 40 nmol/mol; NF60, NF with an O_3 addition of 60 nmol/mol; WW, well-watered conditions; MD, moderate drought stress; SD, severe drought stress. The data are means ± SE. n = 9. Lowercase letters mean with the different letters are significantly different at $p < 0.05$ in different treatments.

3.2. Effects of O_3 and Drought Stresses on Photosynthetic Pigments in Leaves

The total chlorophyll (Chl a + b) and chlorophyll a (Chl a) contents of *A. rubrum* and *A. pictum* decreased significantly with an increase in stress degree under O_3 single stress,

drought single stress, and dual stress (Figure 2, Table 2), while the carotenoid (Car) and chlorophyll b (Chl b) contents of both species decreased significantly with an increase in stress degree under single and dual stresses ($p < 0.001$). *A. rubrum* had the lowest Car at NF40 + SD, and *A. pictum* had the lowest Car at NF60 + SD. The Chl a/b of *A. rubrum* did not change significantly under each stress, first increasing and then decreasing under O₃ single stress, with the overall Chl a/b ratio of each stress treatment remaining at levels between 2:1–3:1.

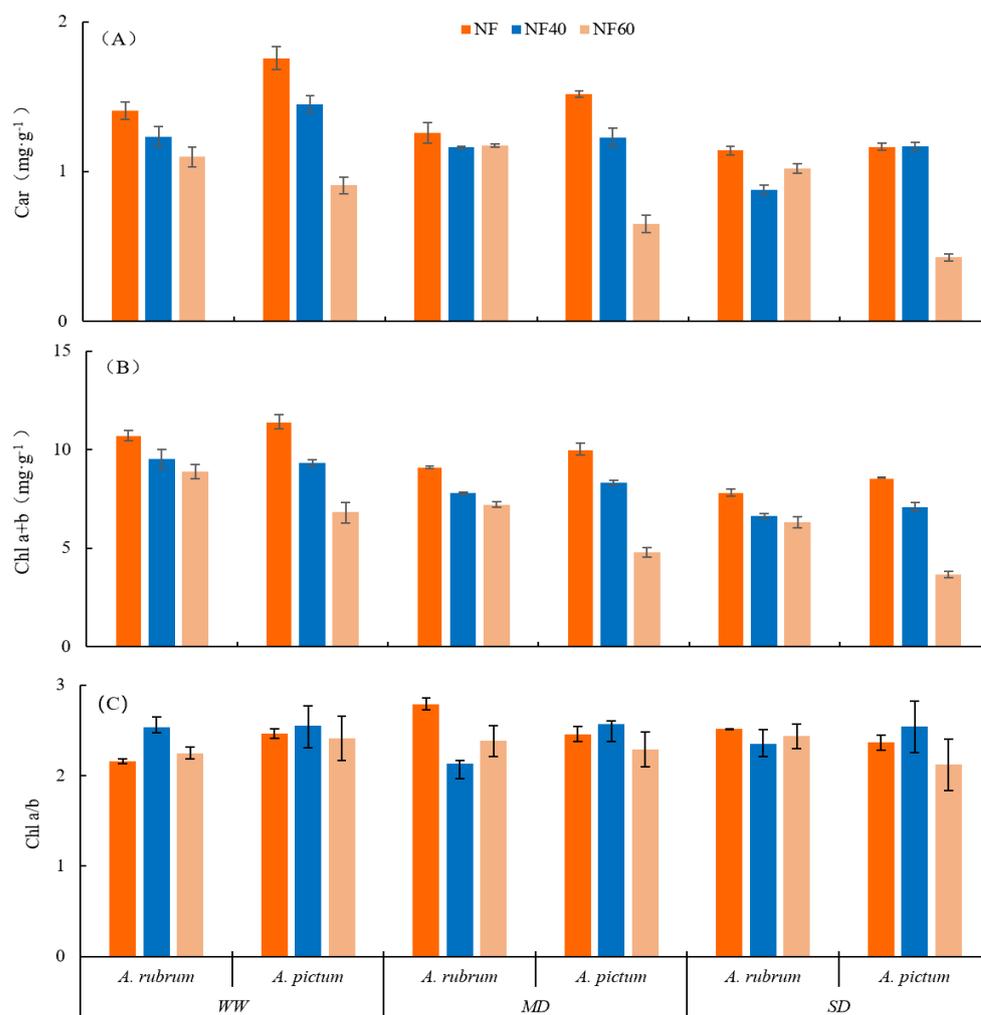


Figure 2. Effects of O₃ and drought stresses on photosynthetic pigments in leaves of *Acer rubrum* and *A. pictum*. Abbreviations: (A) carotenoids (Car), (B) total chlorophyll (Chl a + b), and (C) proportion of chlorophyll a:b (Chl a/b) in leaves of *A. rubrum* and *A. pictum*.

Table 2. Multi-way ANOVA of photosynthetic parameters of *Acer rubrum* and *A. pictum* under O₃ and drought stress.

Factor	Chl a		Chl b		Car		Chl a + b		Chl a/b	
	F	p	F	p	F	p	F	p	F	p
O ₃	148.259	0.000 **	73.943	0.000 **	43.910	0.000 **	190.436	0.000 **	1.690	0.150
Drought	88.018	0.000 **	61.817	0.000 **	19.238	0.000 **	124.486	0.000 **	0.193	0.871
Species	4.760	0.090	8.876	0.005 **	0.056	0.382	9.542	0.019 *	0.071	0.754
O ₃ × drought	0.566	0.754	1.573	0.699	0.374	0.842	0.476	0.900	1.307	0.474
O ₃ × species	35.985	0.000 **	22.015	0.000 **	23.139	0.000 **	48.461	0.000 **	1.538	0.747
Water × species	0.187	0.621	1.275	0.174	2.491	0.131	0.188	0.949	1.059	0.119
O ₃ × drought × species	1.302	0.953	0.996	0.560	1.150	0.382	0.655	0.803	1.672	0.958

* $p < 0.05$, significant difference; ** $p < 0.01$, highly significant difference. Chl, Chlorophyll; Car, Carotenoids.

3.3. Effects of O_3 and Drought Stresses on Photosynthetic Parameters

Compared to *A. rubrum*, the difference in the leaf net photosynthetic rate (Pn) of *A. pictum* was more significant under the dual stress of O_3 and water. Compared with WW, water deficiency (MD and SD) significantly decreased Pn by 57.6% and 78.8% under the NF treatment ($p < 0.05$), 41.3% and 51.6% under NF40 treatment, and 3.2% and 42.9% under NF60 treatment, respectively. Under the O_3 single stress, the Pn of *A. rubrum* first decreased and then increased slightly, but the overall change was not significant. Meanwhile, in *A. pictum*, the Pn decreased first and then increased slightly with an increase in O_3 concentration under the WW and MD treatments. Among the *A. pictum* samples, the Pn of the O_3 stress treatments (NF40 and NF60) under the WW condition was significantly lower than that of the NF treatment by 41.1% (NF40 treatment) and 28.4% (NF60 treatment) ($p < 0.05$). However, the Pn of *A. rubrum* increased with O_3 enrichment under SD treatment, and the Pn of the O_3 stress increased by 65.9% (NF40 treatment) and 31.4% (NF60 treatment) compared to NF (Figure 3A).

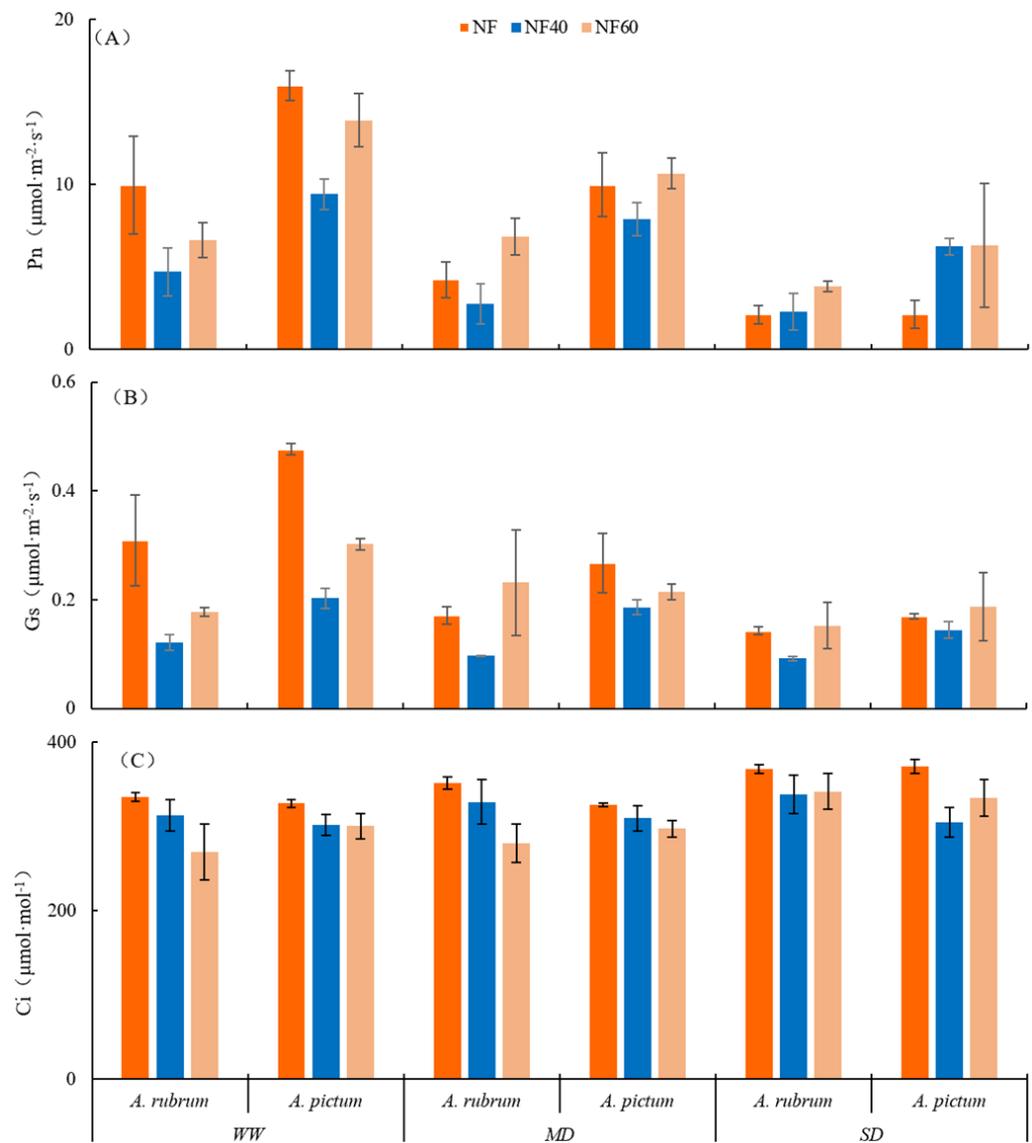


Figure 3. Cont.

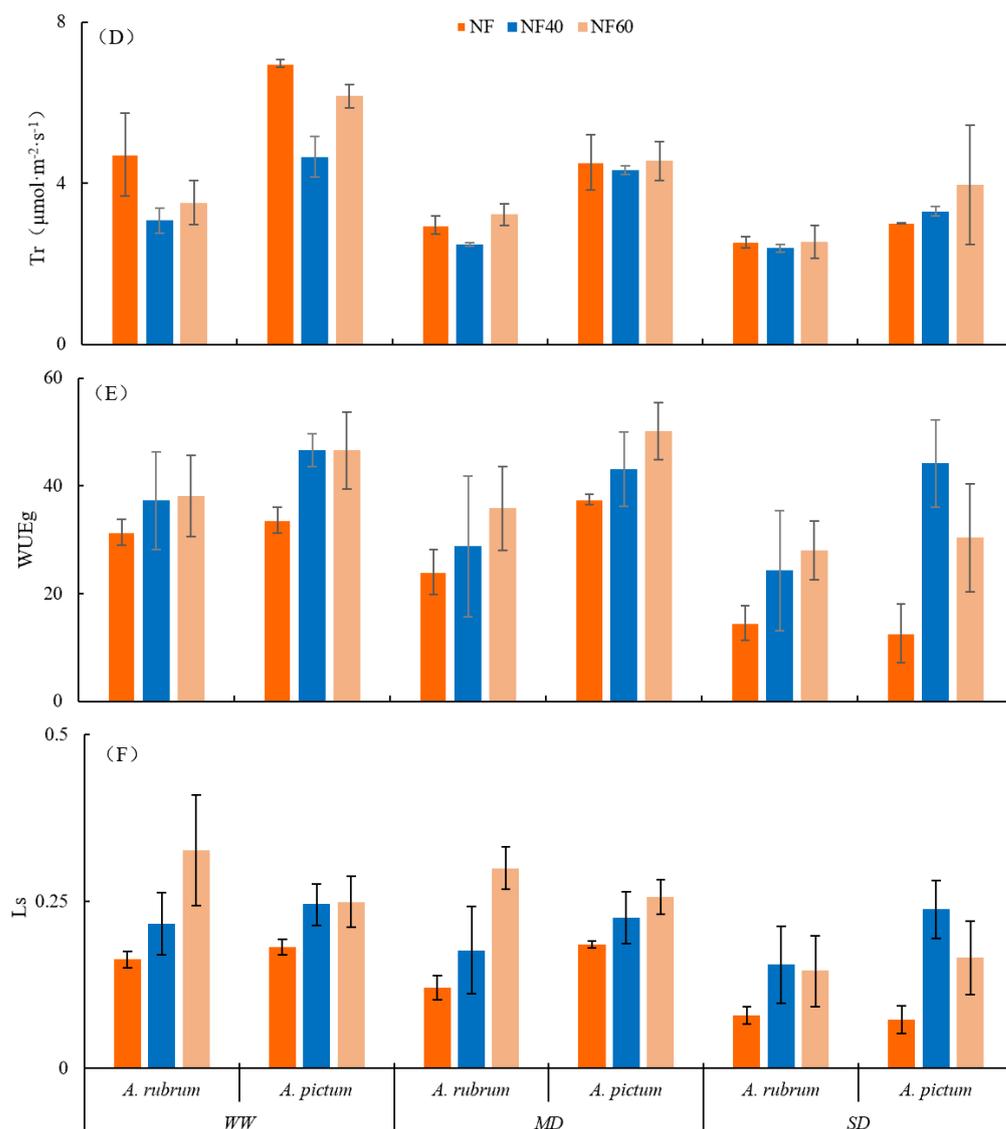


Figure 3. Effects of O₃ and drought stresses on leaf photosynthesis gas exchange parameters in *Acer rubrum* and *A. pictum*. Abbreviations: (A) net photosynthetic rate (Pn), (B) stomatal conductance (Gs), (C) intercellular CO₂ concentration (Ci), (D) transpiration rate (Tr), (E) water use efficiency (WUE_g), and (F) stomatal limit values (L_s) in leaves of *A. rubrum* and *A. pictum*.

Under the dual stresses, the stomatal conductance (Gs) of *A. rubrum* decreased significantly ($p < 0.01$), and the stress treatment groups showed a declining range of more than 50% with NF + MD treatment (60.5%) < NF60 + SD treatment (63.5%) < NF40 + MD treatment (68.7%) < NF60 + MD treatment (70.3%). Furthermore, the Gs of *A. pictum* leaves also decreased under each stress treatment, which showed that NF + MD treatment (57.4%) < NF60 + SD treatment (63.5%) < NF40 + MD treatment (60.9%) < NF60 + WW treatment (64.3%) < NF60 + MD treatment (69.7%) (Figure 3B).

Compared to NF, the intercellular CO₂ concentration (Ci) of *A. rubrum* decreased slightly, by 2.9% and 2.4%, under NF40 and NF60, respectively. *Acer pictum* also showed a downward trend, while both *A. rubrum* and *A. pictum* increased slightly with the degree of drought stress, but the changes were not significant under drought single stress (Figure 3C). This shows that, on the whole, the Ci of *A. rubrum* and *A. pictum* is more affected by O₃ than by drought as a single stress.

Under the dual stresses, the transpiration rate (Tr) of the leaves of *A. rubrum* and *A. pictum* decreased ($p < 0.01$), with the most significant changes in *A. rubrum* observed

for NF60 + SD (43.8%) < NF + SD (45.9%) < NF40 + MD (47.5%) < NF40 + SD (49.3%); the most significant change in Tr of *A. pictum* was NF60 + SD (43.2%) < NF40 + SD (52.7%) < NF + SD (56.6%) (Figure 3D). The change in the Tr range for Tr in *A. rubrum* was smaller than that of *A. pictum* under stress, i.e., the change in Tr was more stable in the former than the latter. The Tr of *A. rubrum* and *A. pictum* showed different degrees of significant decline under SD and, hence, were more sensitive to drought stress.

The water use efficiency (WUE_g) of *A. rubrum* increased with single-factor O_3 stress, whereas that of *A. pictum* increased and then decreased significantly ($p < 0.05$). In the case of drought stress, the WUE_g of *A. rubrum* decreased with a decreasing water gradient, but the difference among the treatments was not significant. However, the SD treatment of *A. pictum* under NF and NF60 was significantly lower than that of WW by 62.4% and 53.9%, respectively, and the difference between MD + NF40, MD + NF, and MD + NF60 treatments under MD was the smallest (Figure 3E).

In the case of O_3 single stress, the stomatal limit values (Ls) of *A. rubrum* and *A. pictum* showed an overall increasing trend with increasing O_3 concentration. The Ls of *A. rubrum* under the NF40 and NF60 treatments increased by 24.7% and 50.1% (WW treatment), 31.7% and 48.9% (MD treatment), and 48.7% and 45.5% (SD treatment) compared to NF. Under the NF40 and NF60 treatments, the Ls of *A. pictum* increased by 25.9% and 27.0% (WW treatment), 17.8% and 27.7% (MD treatment), and 69.5% and 56.1% (SD treatment), respectively, compared to NF ($p < 0.05$). Under drought stress, the Ls of *A. rubrum* decreased under MD and SD treatments by 25.9% and 51.2% (NF treatment) and 18.3% and 28.4% (NF40 treatment), respectively, and 55.3% under SD treatment compared to WW (NF60 treatment). Under the NF and NF60 treatments, the Ls of *A. pictum* showed a small fluctuation and then decreased significantly with water deficiency and fluctuated only slightly with the water gradient under the NF40 treatment. In contrast, Ls under SD treatment decreased by 60.1% (NF treatment) and 33.7% (NF60 treatment), and Ls under MD and SD treatments decreased by 8.1% and 3.1% (NF40 treatment), respectively. There was no significant difference between the treatments of *A. rubrum* under dual stress, but the difference between those of *A. pictum* was significant ($p < 0.05$) (Figure 3F; Table 3).

Table 3. Multi-way ANOVA of photosynthetic parameters of *Acer rubrum* and *A. pictum* under O_3 and drought stress.

Factor	Pn		Gs		Ci		Tr		WUE _g		Ls	
	F	p	F	p	F	p	F	p	F	p	F	p
O_3	4.637	0.904	12.843	0.014 *	5.169	0.002 **	4.162	0.252	5.872	0.002 **	5.169	0.002 **
Drought	26.680	0.000 **	12.927	0.000 **	2.990	0.046 *	23.012	0.000 **	5.579	0.001 **	2.990	0.046 *
Species	39.816	0.000 **	15.036	0.002 **	0.009	0.924	46.676	0.000 **	7.514	0.005 **	0.009	0.924
$O_3 \times$ drought	4.129	0.008 **	4.178	0.007 **	0.990	0.877	2.689	0.027 *	0.687	0.506	0.990	0.877
$O_3 \times$ species	0.096	0.762	0.581	0.375	1.627	0.245	0.269	0.607	0.744	0.520	1.627	0.245
Water \times species	2.590	0.054	1.992	0.112	0.283	0.594	2.349	0.041 *	0.557	0.866	0.283	0.594
$O_3 \times$ drought \times species	0.706	0.647	0.694	0.594	0.568	0.570	0.499	0.617	0.341	0.977	0.568	0.570

* $p < 0.05$, significant difference; ** $p < 0.01$, highly significant difference. Pn, net photosynthetic rate; Gs, stomatal conductance; Ci, intercellular CO_2 concentration; Tr, transpiration rate; WUE_g , water use efficiency; Ls, stomatal limit values.

The impact of *A. rubrum* on Ls under O_3 stress was greater than that of *A. pictum*. During SD, the antagonism between drought and O_3 slowed down the damage to the stomata of *A. rubrum*, whereas *A. pictum* did not show self-preservation mechanisms under SD.

4. Discussion

4.1. Stress Changes in Leaf Color and Appearance

As a color-changing leaf tree species, *A. rubrum* is one of the most important landscaping tree species globally. Following the reproductive and vegetative growth of flowers and leaves in the growing season, its leaf color gradually changes from emerald green to

fiery red in late summer and early autumn as temperatures drop [35]. This study showed that high concentrations of O₃ and drought conditions interfered with the discoloration of the leaves of *A. rubrum*, delaying or even changing the color of the leaves [36,37]. At the same time, the leaf area of *A. rubrum* decreased to different degrees with stress levels, whereas the leaf area and leaf crack degree of *A. pictum* were most affected. These results are similar to recent studies on *Acer truncatum* Bunge [38,39]. These results show that O₃ and drought stresses have a particular impact on the appearance of *A. rubrum* and *A. pictum* leaves, affecting the garden landscape. Furthermore, our results show that under O₃ stress, the degree of leaf cracking in *A. pictum* tends to slow down. The dual stresses of O₃ and drought tended to cause the leaf area of *A. pictum* to shrink compared to the control leaves.

4.2. Effect of Stress on Photosynthetic Pigments

The contents of Chl and Car in the leaves of both species decreased with a single stress increase, and the variation in the leaf pigment of *A. rubrum* in the stress group under O₃ and drought conditions was smaller than that of *A. pictum*; this shows that *A. rubrum* can cope with O₃ and drought stress better than *A. pictum*. Furthermore, Car changed in response to O₃, consistent with previous findings [40]. The net photosynthetic rate of plants is an important indicator of plant productivity. Previous studies have shown that Chl and Car are important photosynthetic pigments in energy transfer in the photosynthetic system, and changes in their content directly affect the intensity of plant photosynthesis [41,42]. In *A. rubrum*, the Chl and Car were the lowest at NF40 + SD, and the leaves were yellow-green, while b* and L* were the highest. In *A. pictum*, they were the lowest at NF60 + SD, the leaves were green, and L* was the highest. We concluded that the leaf pigment content influenced the leaf color difference, and the Chl and Car contents of both species were negatively correlated with L*. This corresponds to the overall change being more stable in the Pn of *A. rubrum* under the dual stress, compared to the relative results of *A. pictum*. The Chl of *A. rubrum* and *A. pictum* showed significant differences under the single stresses of O₃ and drought, with no significant differences under the dual stresses, showing that the dual stresses could help to alleviate the differential effect of a single stress on the leaf pigment content. Significant differences between the different tree species indicated that the two *Acer* species are different. Significant differences were also observed in the O₃-stress × tree species, while the three-factor stress of the O₃ × drought × tree species was not significant, indicating that drought stress affected the leaf pigment content's differential changes in breaking the dual stress of the O₃ × tree species. There was no significant difference in the value of Chl a/b for each treatment factor before or after O₃ and drought stress, and the two species maintained the biological characteristics of photosynthesis in heliophytic plants in terms of leaf pigment.

4.3. Effect of Stress on Photosynthetic Physiological Indicators

In this study, the Pn of *A. pictum* under O₃ single stress first decreased, then increased slightly, Ci decreased sequentially, and Ls increased sequentially. Studies have shown that O₃ can cause plant stomatal closure, hinder photosynthetic electron transfer, and inhibit plant photosynthesis. Changes in plant photosynthetic efficiency are generally divided into two categories: stomatal limitation caused by stomatal closure and non-stomatal limitation caused by impaired photosynthetic cells and decreased photosynthetic activity [39,43,44]. Therefore, the decrease in Pn is caused by the limitation of the stomata of *A. pictum*. Stomata are the gateway for gas exchange between plants and the environment and regulate important physiological processes, such as transpiration, photosynthesis, and respiration, by controlling CO₂ and water, the main channels through which gaseous pollutants, such as O₃, enter the plant. There was no such change during a severe drought.

The Ls of *A. rubrum* changed more under NF60 than under NF40; that is, the correlation of stomatal restriction was greater in NF60. Under these conditions, the degree of stomatal restriction of *A. rubrum* was positively correlated with the degree of O₃ stress, indicating that *A. rubrum* can cope with this O₃ stress.

The L_s of *A. rubrum* under the NF, NF40, and NF60 treatments decreased with increased drought stress, and changes in L_s were the largest in the SD treatment. *A. rubrum* could cope with O_3 and drought stress during the entire stress period through stomatal self-regulation. The dual stress forms an antagonism to protect the plants. With increasing drought stress, the increase in O_3 concentration was negatively correlated with the change range of P_n , i.e., the dual stress of O_3 and drought played an antagonistic role in protecting the P_n of *A. rubrum*. The ability of this plant to purify polluted air highlights its application value for inclusion in urban landscaping, ecological environment planning, and construction.

The MD condition led to a smaller effect of O_3 stress on the P_n and G_s of *A. pictum*. Therefore, MD can reduce the effects of O_3 stress on the P_n decline [45]. Under SD conditions, the P_n of *A. rubrum* and *A. pictum* increased under O_3 stress, and G_s increased slightly or remained stable, indicating that drought can alleviate the damage caused by O_3 to the P_n of plant leaves, which is consistent with previous studies [22]. Drought induces the closure of stomata, limits the O_3 dose entering the leaves, and plays an antagonistic role in protecting plants, thus stimulating the adaptation and protection mechanisms of plants against adversity and reducing the damage caused by O_3 to leaves [26].

Among the stress treatment groups with a declining range of >50%, the change rate of NF60+MD treatment on the G_s of *A. rubrum* and *A. pictum* was the largest, more prone to a significantly high decline rate in the NF60 treatment group; therefore, we speculate that the impact of O_3 stress on the G_s of *A. rubrum* and *A. pictum* was greater than that of drought stress. In addition, this suggests that G_s is more sensitive to a higher O_3 concentration stress and that the decrease in G_s under MD was positively correlated with O_3 stress, indicating that the MD condition can enhance the effect of O_3 stress on stomatal conductance. At this time, the dual stresses played an antagonistic role in protecting stomatal conductance, making the maximum G_s decline rate of the two tree species appear in MD but not SD at NF60.

Under the dual stresses, the T_r of the stress treatment group decreased in both *A. rubrum* and *A. pictum*, indicating that these two tree species can reduce their leaf transpiration rate in a stressful environment to improve their response to adverse conditions. The WUE_g of *A. rubrum* tended to increase with an increasing O_3 concentration and was highest under the NF60 treatment. We speculate that *A. rubrum* can adapt to environmental pollution caused by increasing atmospheric O_3 concentrations by adjusting its water use efficiency. In contrast, the change in the WUE_g of *A. pictum* indicates a lack of stability or regularity under high O_3 concentrations and drought stress. As a result, O_3 entering the plant through the stomata and cuticle harms plants, and the landscaping and beautification effects are negatively impacted [46]. At the same time, to a certain extent, the O_3 concentration in the air is reduced.

5. Conclusions

According to a comprehensive analysis of leaf appearance, photosynthetic pigment content, and photosynthetic gas exchange parameters, the difference in leaf color of *A. rubrum* under stress is more prevalent. Leaf pigment content affected plant leaf color difference, and the Chl and Car contents of both species were negatively correlated with the L^* . Although stressful environmental conditions have adverse effects on the photosynthetic physiology of *A. rubrum*, these plants can still adapt to environmental changes through self-regulation and resist them. In addition, the dual stresses resulted in an antagonistic response by the plants. Therefore, *A. rubrum* is recommended as a candidate tree species in urban environments exposed to high levels of O_3 and drought and can be used as an indicator plant for O_3 and drought in urban greening construction. However, in this study, the leaf area was not scanned in situ with a leaf area meter, nor was the leaf color compared with a color card or measured using spectrophotometry. Therefore, future studies should record the leaf area and color in the field.

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