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ORIGINAL RESEARCH ARTICLE

The response system of the growth, physiological and uptake characteristics of *Pinus bungeana* under ozone stress

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ABSTRACT

Objective: To study the changes of growth, physiological and absorption characteristics of *Pinus bungeana* under ozone (O_3) stress, to elucidate the correlations among the indicators, and to determine its degree of response to O_3. **Methods:** The growth, physiological characteristics and O_3 uptake capacity of *Pinus bungeana* seedlings were measured in an open-top O_3 fumigation manual control experiment with three concentration gradients (NF: normal atmospheric O_3 concentration, NF40: normal atmospheric O_3 concentration plus 40 nmlol/mol; NF80: normal atmospheric O_3 concentration plus 80 nmol/mol), and the relationships between the characteristics of *Pinus bungeana* under different O_3 concentrations were investigated with correlation analysis, redundancy analysis and analysis of variance. **Results:** (1) Plant height growth (ΔH), diameter growth at 50 cm (ΔDBH), stomatal size (S), stomatal density (M), stomatal opening (K), stomatal conductance (G_s), net photosynthetic rate (P_n), transpiration rate (E_t), water use efficiency (WUE), maximum photochemical efficiency (F_v/F_m), chlorophyll content (CHL), whole tree water consumption (W), and O_3 uptake rate (F_{O_3}) all decreased with the increase of O_3 concentration; while intercellular CO_2 concentration (C_i) and relative conductivity (L) increased with the increase of O_3 concentration; (2) growth indicators of *Pinus bungeana* under O_3 stress (ΔH, ΔDBH) were the most correlated with O_3 uptake status (F_{O_3}, W), followed by photosynthetic indicators (P_n, WUE, E_t, G_s, C_i) and growth indicators (ΔH, ΔDBH) and stomatal characteristics (K, M, S) under O_3 stress, some physiological indicators (L, F_v/F_m) were relatively weakly correlated with photosynthesis (P_n, WUE, E_t, G_s, C_i) and stomatal (K, M, S); (3) all the indicators of *Pinus bungeana* were significantly different under O_3 treatments of NF and NF80 ($P < 0.05$), ΔH, ΔDBH, M, CHL, P_n, G_s, W and F_{O_3} were most significantly different under NF and NF40 treatments, and K, S, WUE, F_v/F_m, E_t, C_i, L were more significantly different under NF40 and NF80 treatments. **Conclusion:** The experiment proved that the growth of *Pinus bungeana* was slowed, photosynthetic capacity was reduced, and the absorption capacity of O_3 was further reduced by long-term exposure to high concentration of O_3. The growth of *Pinus bungeana* was most correlated with the changes of O_3 absorption characteristics, and the stomatal characteristics were most correlated with photosynthetic physiological characteristics, and the reduction of photosynthetic capacity etc. further led to the curtailment of its growth.

Keywords: Pinus Bungeana; O_3 Stress; Growth; Physiological Characteristics; Ozone Uptake; Correlation

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1. Introduction

Human activities have long influenced atmospheric ozone (O_3) concentrations, and from the last 100 years or so to the present, human activities have led to the increasing emissions of O_3 into the atmosphere[1]; tropospheric O_3 is one of the major air pollutants today, and its precursors are generally nitrogen oxides (NO_x) and volatile organic compounds (VOCs), among others[2]. Due to accelerated urbanization, human activities have led to a large increase of O_3 precursors emitted into the atmosphere, creating conditions for an increase in near-surface O_3 concentrations. O_3-induced pollution is more serious in urban areas, especially in the urban agglomerations of Beijing-Tianjin-Hebei, Yangtze River Delta and Pearl River Delta in China[3,4]. In 2019, $PM_{2.5}$, PM_{10}, SO_2, NO_2, and CO decreased to varying degrees in 338 prefecture-level and above cities across China, only the O_3 concentration was 148 μg/m^3, a year-on-year increase of 6.5%[5], which has attracted academic attention. In recent years, O_3 concentrations have also been increasing year by year in Beijing[6], and monitoring results from the Beijing Municipal Environmental Protection Bureau show that from 2013–2015, the maximum daily (8 h) average O_3 concentration in Beijing increased from 183.4 μg/m^3 to 202.6 μg/m^3[7,8], and O_3 has become the primary pollutant in the region in summer; in 2017 May, the Ministry of Environmental Protection informed the media that according to the latest air quality forecast results, the primary pollutant in Beijing, Tianjin, Hebei and surrounding areas is O_3; the maximum daily 8-h sliding average 90th percentile concentration value of O_3 in Beijing in 2019 was 191 μg/m^3, exceeding the national secondary standard (160 μg/m^3) of 19.4%[9].

In recent years some scholars have applied other methods such as artificially controlled air chamber simulation experiments (closed O_3 fumigation experiment method, large artificial climate chamber method, open-top chambers (OTC), and also free-air gas concentration enrichment (FACE)) to study the damage of O_3 on crops and trees[10]. Several researchers have found from various indicators of individual plants that long-term exposure to O_3 results in a series of physiological changes, such as premature leaf decay and abscission, reduced number of stomata[11], reduced photosynthetic carbon fixation capacity, and reduced carbon assimilates in the return roots[12,13]. Schaub *et al.*[14] demonstrated that O_3 uptake affects photosynthesis and conductance of wild Prunus serotina, especially later in the growing season.

In Beijing, with the increasing O_3 pollution, the effect of O_3 on plants is also increasing. Through field observations, Wan *et al.*[15] found that a large number of trees, shrubs and herbaceous plant leaves in the suburbs of Beijing were damaged by O_3. With the premise that O_3 concentrations will continue to increase in the future, the degree of plant damage will also continue to increase[16]. The relationship between different O_3 concentrations on the indicators of *Pinus bungeana* is less studied and the extent of this effect is less explored. Which indicators of *Pinus bungeana* respond most rapidly at elevated O_3 concentrations, which indicators are most affected, and how these indicators affect the growth and development of *Pinus bungeana* are not clearly defineded and require in-depth analysis. To this end, this study investigates the changes in growth, physiology, photosynthesis, and O_3 uptake indicators of *Pinus bungeana* under elevated O_3 concentrations using an open-top chambers, thus providing data and theoretical support for studying the response of trees to O_3 stress from a physiological perspective.

2. Materials and methods

2.1 Overview of the study site and experimental materials

The study site, Beijing Botanical Garden (39°48′N, 116°28′E), is located at the foot of the Fragrant Hill, with an altitude of 76 m, and belongs to temperate continental climate. The average annual temperature is 11.6 ℃, with an average temperature of -3.7 ℃ in January and 26.7 ℃ in July. The extreme high temperature is 41.3 ℃, the extreme low temperature is -17.5 ℃, the annual precipitation is 634.20 mm, and the relative humidity ranges from 43% to 79%. *Pinus bungeana* is a common landscaping species in North China and is widely planted in the study area. It is well adapted and resistant to adversity, and is an excellent tree species for afforestation in mountainous and semi-arid areas, as well as one of the preferred species for urban greening. In this study, *Pinus bungeana* seedlings were selected as the material for artificially controlled pot planting trials. The *Pinus bungeana* seedlings were all 3 years old, and some studies have shown that 3-year-old trees are in the early stages of growth and development, making the results more significant[17]. The seedlings pot has a diameter of 40 cm and a height of 50 cm. The size, crown width, basal diameter and height of *Pinus bungeana* seedlings in different air chambers were basically the same.

2.2 Experimental methods

Nine 3-year-old *Pinus bungeana* seedlings were placed in each open-top chamber (OTC). A total of 15 OTCs were set up, and the O_3 generator was used to control the O_3 concentration entering the chamber, and the O_3 generator outlet was equipped with an O_3 concentration detector BMT 964. One of the OTCs was fumigated without any O_3-related treatment at NF concentration (normal atmospheric O_3 concentration); the other two were fumigated at NF 40 (normal atmospheric O_3 concentration plus 40 nmol/mol) and NF 80 (normal atmospheric O_3 concentration plus 80 nmol/mol), respectively, and every three OTCs were grouped into five groups.

Table 1. Different processing methods in experiment

Types	Treatment steps
NF	Normal atmospheric O_3 concentration, no treatment
NF 40	Normal atmospheric O_3 concentration plus 40 nmlol/mol, growing season (May to October) Daily O_3 fumigation treatment from 8:00 to 16:00
NF 80	Normal atmospheric O_3 concentration plus 80 nmol/mol, growing season (May to October) daily O_3 fumigation treatment from 8:00 to 16:00

2.3 Determination of functional trait indexes

At the beginning and end of the experiment, the height and diameter at 50 cm of *Pinus bungeana* plants were measured with a ruler, and the difference was calculated to obtain the change in plant height (ΔH) and the change in diameter at 50 cm (ΔDBH), respectively. The number of stomata in the field of view was observed using an optical microscope, and the field of view area was calculated; the field density (M) = the average number of stomata in the field of view/field area in "per/mm^2". The actual size of stomata (S/μm^2) and stomatal opening (K/μm^2) can be obtained by the proportional method. The net photosynthetic rate (P_n/μmol·m^{-2}S^{-1}), transpiration rate (E_t/mnol·m^{-2}S^{-1}), intercellular CO_2 concentration (C_i/μmol·mmol^{-1}), and stomatal conductance (G_s/mnol·m^{-2}S^{-1}) of *Pinus bungeana* under different treatments were measured by CI340 photosynthesizer, and water utilization (WUE/μmol·mmol^{-1}), $WUE = P_n/E_t$ were calculated by equation. The chlorophyll content CHL/mg·g^{-1} was measured by spectrophotometer. The maximum photochemical efficiency F_v/F_m was determined using a Yaxin-1161G chlorophyll fluorometer; the relative conductivity (L) was obtained by measuring the initial and final conductivity values by a conductivity meter (DDS-307) to calculate the ratio. Whole-tree water consumption (W) and O_3 uptake rate (F_{O_3}) were determined and calculated by the trunk sap flow technique[18].

2.4 Data processing

The test data were organized using SPSS 24.0 software and Canoco 5.0 software, and cluster analysis, correlation analysis, redundancy analysis and one-way ANOVA were performed to investigate the correlation between the indicators and whether there were significant differences in the indicators under different O_3 concentration treatments to systematically evaluate the damage of different concentration gradients of O_3 on *Pinus bungeana*.

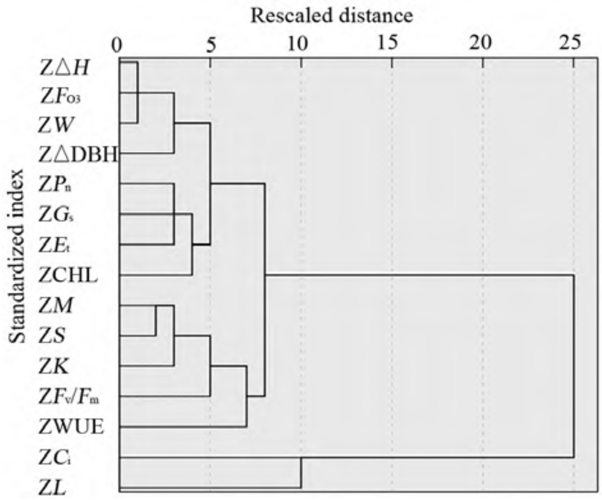

Figure1. Cluster analysis.

3. Results and analysis

3.1 Cluster analysis of each characteristic index of *Pinus bungeana* under O_3 stress

Using SPSS software, all the measured indicators were standardized and subjected to systematic cluster analysis (**Figure 1**). It was possible to classify the 15 indicators into categories 15, 13, 8, 7, 5, 4, 3, 2, and 1. To facilitate the integrated analysis and to combine the knowledge of plant physiology, we divided all the indicators tested into 5 major categories and 7 subcategories. The first category includes ΔH, F_{O_3}, W, ΔDBH, P_n, G_s, E_t and CHL, which are subdivided into ΔH, F_{O_3}, W, ΔDBH and P_n, G_s, E_t, and CHL; the second category is M, K, S and Fv/Fm; the third category is WUE; the fourth category is C_i; and the fifth category is L.

The first category of indicators mainly reflects the growth, O_3 absorption and photosynthesis capacity of *Pinus bungeana*, where plant height and diameter at 50 cm respond to the growth of *Pinus bungeana*, and water consumption and O_3 absorption rate respond to the O_3 absorption of *Pinus bungeana*. Typical correlation analysis showed a high positive correlation between the two data sets (P < 0.001) with a correlation of 0.998. This indicates that the variation in plant height (ΔH), O_3 uptake rate (F_{O_3}), water consumption (W) and diameter change at 50 cm (ΔDBH) show very similar changes with the increase of O_3 concentration. P_n, G_s, E_t and CHL responded to the photosynthetic physiological properties of *Pinus bungeana*, which were close to the growth status and O_3 uptake status from the intra-group mean linkage distance, indicating that some of the changes in photosynthetic physiological properties of *Pinus bungeana* may be closely related to its growth changes and O_3 uptake status.

The second group of indicators, M, K, S and F_v/F_m, mainly reflected the stomatal characteristics and maximum photochemical efficiency, which decreased with the increase of O_3 concentration. The correlation between stomatal indicators and F_v/F_m was positive, with a correlation coefficient of 0.946 (P < 0.001).

The third category of indicators was water use efficiency (WUE), which showed a gradual decrease with the increase of O_3 concentration.

The fourth category of indicators was intercellular CO_2 concentration (C_i), which gradually increased with the increase of O_3 concentration in *Pinus bungeana*. The fifth category of indicators is relative conductivity (L), and the closest indicator to its change is intercellular CO_2 concentration (C_i).

3.2 Correlations among the characteristic indicators of *Pinus bungeana* under O_3 stress

C_i and L were positive correlated with a correlation coefficients of 0.775, and negative with the other physiological factors (**Table 2**), indicating that the trend of the changes was the same with the increase of O_3 concentration, while the increase of intercellular CO_2 concentration (C_i) and relative conductivity (L) indicated that the leaf cell structure of *Pinus bungeana* might be damaged to some extent. Both Δ*H* and ΔDBH had the highest correlations with *W*, F_{O_3}, with correlation coefficients above 0.92, indicating that the uptake of O_3 and water depletion of *Pinus bungeana* significantly affected its own growth. *M* and *K* had the highest correlation with *S*, with correlation coefficients above 0.92, indicating that changes in stomatal density and openness were strongly correlated with stomatal size. WUE was highly correlated with G_s with a correlation coefficient of 0.79. The correlations between P_n, E_t and G_s were high with correlation coefficients greater than 0.91. These three indicators were closely related to the photosynthetic characteristics of *Pinus bungeana*, and the changes of the three were consistent with the increase of O_3 concentration. The results of redundancy analysis showed that C_i and *L* were highly positively correlated with O_3 concentration, while the rest of the indices were negatively correlated with the first three, and the photosynthetic physiological characteristics such as WUE, CHL, P_n, E_t were the most correlated with O_3 concentration, and the photosynthetic performance of *Pinus bungeana* became lower and lower with the increase of O_3 concentration.

Table 2. Correlation analysis of indices of *Pinus bungeana*

Index	Δ*H*	ADBH	*M*	*K*	*S*	WUE	P_n	E_t	G_s	C_i	F_v/F_m	CHL	*L*	*W*	F_{O_3}
Δ*H*	1														
ADBH	0.889	1													
M	0.727	0.836	1												
K	0.728	0.758	0.883	1											
S	0.823	0.891	0.963	0.924	1										
WUE	0.649	0.798	0.819	0.735	0.846	1									
P_n	0.83	0.817	0.784	0.802	0.86	0.652	1								
E_t	0.821	0.815	0.743	0.744	0.817	0.71	0.928	1							
G_s	0.888	0.842	0.827	0.857	0.915	0.789	0.932	0.91	1						
C_i	-0.787	-0.882	-0.821	-0.8	-0.876	-0.81	-0.929	-0.953	-0.908	1					
F_v/F_m	0.728	0.76	0.826	0.875	0.919	0.695	0.851	0.769	0.879	-0.797	1				
CHL	0.819	0.78	0.826	0.781	0.859	0.675	0.879	0.844	0.914	-0.814	0.833	1			
L	-0.708	-0.763	-0.859	-0.878	-0.851	-0.733	-0.74	-0.715	-0.791	0.775	-0.787	-0.733	1		
W	0.98	0.921	0.808	0.772	0.872	0.699	0.867	0.863	0.909	-0.842	0.761	0.87	-0.755	1	
F_{O_3}	0.991	0.92	0.744	0.75	0.849	0.697	0.827	0.832	0.894	-0.815	0.755	0.821	-0.707	0.972	1

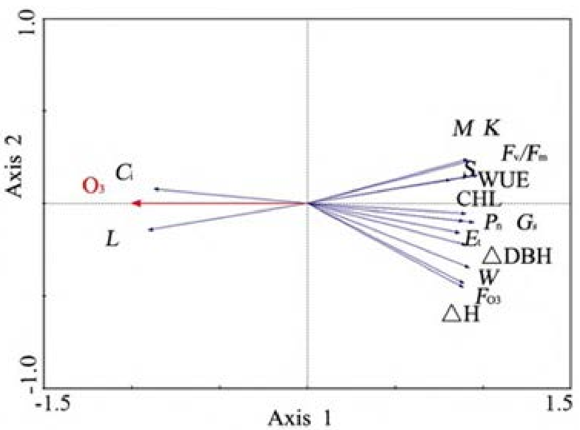

Figure 2. RDA sequence of O_3 and the growth, physiology and uptake characteristics of *Pinus bungeana*.

3.3 The effects of different varied O_3 concentrations on *Pinus bungeana*

The results of chi-square test showed that the variation of plant height (Δ*H*), transpiration rate (E_t) and intercellular CO_2 concentration (C_i) did not meet the chi-square test ($P < 0.05$), while the rest of the indicators passed the chi-square test. These indicators were subjected to ANOVA to obtain the differences in indicator values of *Pinus bungeana* under different treatments.

In the control test, the differences in all indicators of *Pinus bungeana* varied significantly ($P < 0.001$), indicating that the high O_3 concentration had a large effect on all indicators tested in the test (**Table 3**). The *F* values were ranked in order of magnitude: F_{O_3} > *W* > *S* > *M* > G_s > K > ΔDBH > F_v/F_m > *L* > CHL > P_n > WUE, indicating that O_3 uptake characteristics and stomatal changes were the most sensitive and responsive under NF, NF40 and NF80 treatments, followed by the growth condition of *Pinus bungeana* and finally the physiological and photosynthetic characteristics.

Table 3. Single factor variance analysis

Index	Group	Square sum	Freedom	Mean square	Mean square	Significance
ΔDBH	Between groups	0.624	2	0.312	40.133	0.000
	Within group	0.093	12	0.008		
	Total	0.717	14			
M	Intergroup	58,677.271	2	29,338.636	63.085	0.000
	Within group	5,580.785	12	465.065		
	Total	64,258.056	14			
K	Intergroup	14,533.211	2	7,266.606	46.204	0.000
	Within group	1,887.246	12	157.271		
	Total	16,420.457	14			
S	Intergroup	60,601.615	2	30,300.807	204.818	0.000
	Within group	1,775.282	12	147.940		
	Total	62,376.897	14			
WUE	Between groups	0.050	2	0.025	13.455	0.001
	Within group	0.022	12	0.002		
	Total	0.073	14			
P_n	Between groups	3.237	2	1.619	22.819	0.000
	Within group	0.851	12	0.071		
	Total	4.088	14			
G_s	Between groups	388.872	2	194.436	60.243	0.000
	Within group	38.731	12	3.228		
	Total	427.603	14			
F_v/F_m	Between groups	0.017	2	0.009	39.141	0.000
	Within group	0.003	12	0.000		
	Total	0.020	14			
CHL	Between groups	2.231	2	1.115	24.805	0.000
	Within group	0.540	12	0.045		
	Total	2.771	14			
L	Between groups	49.890	2	24.945	30.213	0.000
	Within group	9.908	12	0.826		
	Total	59.798	14			
W	Intergroup	0.209	2	0.105	307.373	0.000
	Within group	0.004	12	0.000		
	Total	0.213	14			
F_{O_3}	Between groups	3,172.262	2	1,586.131	450.051	0.000
	Within group	42.292	12	3.524		
	Total	3,214.554	14			

Since plant height change (Δ*H*), transpiration rate (E_t) and intercellular CO_2 concentration (C_i) did not satisfy chi-square ($P < 0.05$), non-chi-square multiple comparisons were used (**Table 4**). The height changes (Δ*H*) of *Pinus bungeana* seedlings under different O_3 concentration treatments showed significant differences ($P < 0.05$), with highly significant differences ($P < 0.001$) between the height changes (Δ*H*) of *Pinus bungeana* seedlings in the NF gas chamber compared with the NF40 and NF80 gas chambers, and relatively small differences in ΔH between the NF40 and NF80 treatments. In contrast, the differences in transpiration rate (E_t) and intercellular CO_2 (C_i) concentrations were the opposite, with the most significant differences under NF40 and NF80 treatments ($P < 0.001$), followed by NF and NF80 treatments, and non-significant differences under NF and NF40 treatments ($P > 0.05$).

Table 4. Non-homogeneous multiple comparison

Dependent variable				(I-J) Mean value difference	Standard error	Significance	95% confidence interval	
							Lower limit	Upper limit
Δ*H*	Tamheni	NF	NF40	4.85393*	0.17787	0.000	4.1706	5.5373
			NF80	5.01060*	0.17990	0.000	4.3355	5.6857
		NF40	NF80	0.15667*	0.04629	0.033	0.0135	0.2998
E_t tamheni	Tamheni	NF	NF40	0.28700	0.08376	0.059	-0.0131	0.5871
			NF80	0.42500*	0.08224	0.014	0.1207	0.7293
		NF40	NF80	0.13800*	0.03225	0.009	0.0397	0.2363
C_i tamheni	Tamheni	NF	NF40	-28.91200	9.02670	0.087	-63.1628	5.3388
			NF80	-52.02600*	9.42784	0.008	-85.1489	-18.9031
		NF40	NF80	-23.11400*	3.59442	0.002	-34.8660	-11.3620

Table 5. Multiple comparisons

Dependent variable				Mean value significance	Standard error	Significance	95% confidence interval	
							Lower limit	Upper limit
ΔDBH	LSD	NF	NF40	0.35667*	0.05577	0.000	0.2352	0.4782
			NF80	0.48133*	0.05577	0.000	0.3598	0.6028
		NF40	NF80	0.12467*	0.05577	0.045	0.0032	0.2462
M	LSD	NF	NF40	48.19933*	13.63914	0.004	18.4822	77.9165
			NF80	150.03933*	13.63914	0.000	120.3222	179.7565
		NF40	NF80	101.84000*	13.63914	0.000	72.1229	131.5571
K	LSD	NF	NF40	25.09933*	7.93147	0.008	7.8181	42.3805
			NF80	74.89933*	7.93147	0.000	57.6181	92.1805
		NF40	NF80	49.80000*	7.93147	0.000	32.5188	67.0812
S	LSD	NF	NF40	66.13333*	7.69260	0.000	49.3726	82.8941
			NF80	155.13333*	7.69260	0.000	138.3726	171.8941
		NF40	NF80	89.00000*	7.69260	0.000	72.2393	105.7607
WUE	LSD	NF	NF40	0.05098	0.02735	0.087	-0.0086	0.1106
			NF80	0.14016*	0.02735	0.000	0.0806	0.1997
		NF40	NF80	0.08918*	0.02735	0.007	0.0296	0.1488
P_n	LSD	NF	NF40	0.69400*	0.16844	0.001	0.3270	1.0610
			NF80	1.12800*	0.16844	0.000	0.7610	1.4950
		NF40	NF80	0.43400*	0.16844	0.024	0.0670	0.8010
G_s	LSD	NF	NF40	7.47900*	1.13623	0.000	5.0034	9.9546
			NF80	12.38300*	1.13623	0.000	9.9074	14.8586
		NF40	NF80	4.90400*	1.13623	0.001	2.4284	7.3796
F_v/F_m	LSD	NF	NF40	0.02813*	0.00935	0.011	0.0078	0.0485
			NF80	0.08147*	0.00935	0.000	0.0611	0.1018
		NF40	NF80	0.05333*	0.00935	0.000	0.0330	0.0737
CHL	LSD	NF	NF40	0.56133*	0.13412	0.001	0.2691	0.8536
			NF80	0.93867*	0.13412	0.000	0.6464	1.2309
		NF40	NF80	0.37733*	0.13412	0.016	0.0851	0.6696
L	LSD	NF	NF40	-1.53133*	0.57468	0.021	-2.7835	-0.2792
			NF80	-4.40000*	0.57468	0.000	-5.6521	-3.1479
		NF40	NF80	-2.86867*	0.57468	0.000	-4.1208	-1.6165
W	LSD	NF	NF40	0.22800*	0.01166	0.000	0.2026	0.2534
			NF80	0.26800*	0.01166	0.000	0.2426	0.2934
		NF40	NF80	0.04000*	0.01166	0.005	0.0146	0.0654
F_{O_3}	LSD	NF	NF40	29.72600*	1.18732	0.000	27.1390	32.3130
			NF80	31.86160*	1.18732	0.000	29.2746	34.4486
		NF40	NF80	2.13560	1.18732	0.097	-0.4514	4.7226

The differences of the remaining indicators under different treatments were compared by chi-square analysis of variance (**Table 5**), and the differences of ΔDBH under NF and NF40, NF and NF80 treatments were highly significant (P < 0.001), and the differences of ΔDBH under NF40 and NF80 treatments were smaller, indicating that the growth of *Pinus bungeana* had been more significantly inhibited under NF40 treatment; K and S were significantly different in NF, NF40 and K and S were significantly different (P < 0.001) under NF, NF40 and NF80 treatments, indicating that stomatal characteristics were most actively changed under different ozone concentrations, with the most significant differences in K and S under the O_3 concentration treatments of NF and NF80, followed by NF40 and NF80; M was more significantly different (P < 0.05) under NF and NF40 treatments; WUE was the most different under NF and NF80 treatments, followed by NF40 and NF80. The difference between NF and NF40 was not significant (P > 0.05), indicating that water utilization of *Pinus bungeana* was significantly reduced only under higher concentrations of O_3 stress; P_n and G_s were the most different under NF and NF80 treatments (P < 0.001), more significant under NF and NF40 treatments (P < 0.05), and relatively small differences under NF40 and NF80 treatments; F_v/F_m had the least significant differences under NF and NF40 treatments, significant differences under NF40 compared to NF80 F_v/F_m, and the most significant differences between NF and NF80 (P < 0.001); CHL had the most significant differences under NF and NF80 treatments, followed by NF and NF40, and the smallest difference between NF40 and NF80; L was the least significant difference under NF and NF40 treatment, while the other two groups were significantly different in comparison (P < 0.001); W and F_{O_3} were less different under NF40 and NF80 treatment, where W was more significant (P < 0.05) in both groups, F_{O_3} was not significant (P > 0.05), and W and F_{O_3} showed larger difference under the treatment of NF and the other two groups.

4. Discussion

Throughout the experimental period, it could be measured that the growth indicators of *Pinus bungeana* showed differences with different concentrations of O_3 fumigation treatments, with the height and diameter of *Pinus bungeana* plants under NF80 treatment being lower than NF40, and those under NF40 treatment being lower than NF, indicating that O_3 inhibited the growth of *Pinus bungeana* seedlings. This is consistant with the results of hybrid poplar *Populus tremula* × *P. Tremuloides* conducted by Niu *et al*.[19], in which the plant height, diameter, and biomass of hybrid poplar under the influence of O_3 were lower than the values in normal environment. Cluster analysis, correlation analysis, and redundancy analysis revealed that *Pinus bungeana* growth indicators (ΔH, ΔDBH) had the highest correlation with O_3 uptake status (F_{O_3}, W), which is due to the fact that O_3 entering the plant affects the function of various parts, thus leading to a reduction in water uptake by the plant and a certain effect on the supply of plant organs, which can further lead to the slow growth of plant.

Stomata are the main channels through which gases enter the plant. Generally speaking, the larger the stomatal opening and size, the more O_3 will enter the plant; when the tolerance limit of the plant body is exceeded, the plant will narrow the stomata. This was confirmed by the experimental results, where the stomatal density, opening and size of *Pinus bungeana* were reduced under the treatment of NF, NF40 and NF80 concentrations of O_3. It has been pointed out that stomatal density tends to increase with the increase of pollution in urban environments, while stomatal area and stomatal opening decrease[20], probably because the nature of O_3 involved in this study is quite different from that of $PM_{2.5}$ and PM_{10} in the environment, and in urban polluted atmospheres, some stomata are blocked by suspended particulates affecting the water vapor exchange process of plants, and the adoption of increasing the number of stomata to compensate is also possible . It has also been shown that elevated O_3 concentrations reduce stomatal flexibility, making stomata less responsive to the external environment[21-23]. The response rate of stomatal closure and the amount of O_3 entry are related to the sensitivity of different tree species to

O_3, and the more sensitive species have smaller tolerance values at the same O_3 concentration, which may cause a failure of cellular defense of the plant, not only increasing stomatal opening and size, but also making the plant unable to close stomata quickly. This does not occur for *Pinus bungeana* in the test which may be due to the fact that the stomatal size and opening of *Pinus bungeana* are lower than those of trees with wider leaves, and they inhale less in the same concentration of O_3 and have higher tolerance values. Stomatal characteristics (K, M, S) and photosynthetic physiological indicators (P_n, WUE, E_t, G_s, C_i, CHL, F_v/F_m, L) correlated the most, because plant leaves are the main site of photosynthesis, and stomata are the main channel for O_3 to enter the leaves, and as O_3 concentration increases, the stomatal size, density and opening of plants gradually decrease, which will lead to a large reduction of CO_2 entering the leaves, thus reducing the photosynthetic rate, transpiration rate, stomatal conductance and water use efficiency of plants.

Photosynthesis is the most basic physiological process of plants, and the effect of long-term O_3 environment on plants is also expressed in photosynthetic properties. The decrease in stomatal conductance (G_s) leads to an increase in stomatal resistance, making it difficult for CO_2 and H_2O to enter the plant, which further affects changes in transpiration rate (E_t) and water consumption (WUE). Chlorophyll content (CHL) is an important indicator of the photosynthetic capacity of trees and has an impact on plant photosynthetic rate and primary productivity[24], and it was found that the CHL of *Pinus bungeana* decreased with the increase of O_3 concentration because O_3 can damage the structure and components of chloroplasts[25-27]. The experimental results showed that increasing O_3 concentration led to a significant decrease in G_s, E_t and WUE of *Pinus bungeana*. Photosynthetic rate reflects the photosynthesis ability of the plant under certain environment, and the study showed that the net photosynthetic rate (P_n) of *Pinus bungeana* decreased by 11.71% and 18.81% under O_3 treatment with NF40 and NF80 concentrations, respectively. In contrast, the intercellular CO_2 concentration (C_i) of *Pinus bungeana* increased with the increase of O_3 concentration in the experiment, indicating that the factors causing the decrease in photosynthetic capacity of *Pinus bungeana* were mainly non-stomatal factors. The Photosynthetic physiological indicators (P_n, WUE, E_t, G_s, C_i, CHL, F_v/F_m, L) of *Pinus bungeana* were higher in correlation with growth indicators (ΔH, ΔDBH), stomatal characteristics (K, M, S). In contrast, the intercellular CO_2 concentration increased significantly with the increase of O_3 concentration in this experiment, indicating that the factors causing the reduction of photosynthetic indexes such as photosynthetic rate in *Pinus bungeana* are not only stomatal factors, but may be caused by non-stomatal factors such as reduced assimilation capacity of leaf pulp cells[28]. In addition, it has been shown that O_3 elevation blocks the photosynthetic electron transport chain[29] and reduces the chlorophyll fluorescence parameter F_v/F_m (maximum photochemical quantum yield), which is consistent with the findings in this experiment on *Pinus bungeana*, where chlorophyll decomposition under O_3, a strong oxidant, leads to an impairment of plant photosynthesis, which also further contributes to a decrease in maximum photochemical rates (F_v/F_m) etc. The magnitude of relative conductivity (L) reflects the degree of plant cell membrane damage, and the degree of injury to the membrane system of plants can be understood by measuring the value of the relative conductivity change in plants[30]. This study showed that the L value of *Pinus bungeana* gradually increased with the increase of O_3 concentration, which is because O_3 is a strong oxidant that can change membrane permeability, and O_3 entered the leaves through the stomata, the cell membrane was damaged, and the relative conductivity (L) then increased, indicating that the cell membrane of *Pinus bungeana* leaves had been significantly damaged. Compared with the NF concentration, the water use efficiency (WUE) decreased by 2.62% and 6.70% under NF40 and NF80, respectively, indicating that the increased O_3 concentration inhibited the water consumption of *Pinus bungeana*. The reduced photosynthetic capacity then made it difficult to maintain the nutrients required for normal plant growth, further leading to slower plant growth.

By comparing the growth of *Pinus bungeana* in different air chambers, it was found that the differences in all indicators of *Pinus bungeana* under O_3 treatment of NF and NF80 were significant (P < 0.05), indicating that *Pinus bungeana* would respond more significantly to a significant increase in O_3 concentration. Some indicators (ΔH, ΔDBH, M, CHL, P_n, G_s, W and F_{O_3}) showed a more pronounced response when the O_3 concentration was increased to NF40, and showed less significant differences in the higher concentration treatments, indicating that the NF40 concentration gradient affected more the growth and O_3 uptake of *Pinus bungeana* and stomatal density and part of the photosynthetic capacity. While some indicators (K, S, WUE, F_v/F_m, E_t, C_i, L) showed more significant differences under NF40 and NF80 treatments, and relatively less significant differences under NF and NF40 treatments, on the one hand, suggesting that *Pinus bungeana* has a certain resistance to O_3, because NF40 concentration is already close to the O_3-induced; on the one hand, it indicates that *Pinus bungeana* has some resistance to O_3, because NF40 concentration is already close to the threshold of O_3-induced plant injury, and even NF80 already exceeds the threshold of causing plant injury[31]; on the other hand, it indicates that the increase of O_3 concentration to NF80 will have a greater effect on the physiological characteristics, stomatal characteristics and some photosynthesis ability of *Pinus bungeana*.

In this study, only two O_3 concentration gradients were selected for control tests with normal ambient atmospheric O_3 concentrations, and the thresholds were floating values, influenced by the atmospheric O_3 concentrations in the normal environment; only the correlations between growth, photosynthesis, some physiological indicators and absorption characteristics under O_3 stress were studied, and no tests and measurements were performed for their resistance indicators. Pre-treatment of normal ambient O_3 in open-topchambers to achieve relatively consistent and stable concentrations; refinement of the O_3 concentration gradient to study and compare the thresholds of changes in each index, and incorporation of quantitative analysis of resistance (antioxidant system) of *Pinus bungeana* under O_3 stress will be the next research direction.

5. Conclusion

In this study, the functional traits of *Pinus bungeana* were analyzed in response to different concentrations of O_3 stress, and the following conclusions were drawn.

(1) The plant height growth (ΔH), diameter growth at 50 cm (ΔDBH), stomatal size (S), stomatal density (M) stomatal opening (K), stomatal conductance (G_s), net photosynthetic rate (P_n), transpiration rate (E_t), water use efficiency (WUE), maximum photochemical efficiency (F_v/F_m)), chlorophyll content (CHL), whole-tree water consumption (W), and O_3 uptake rate (F_{O_3}) all decreased with the increase of O_3 concentration, while intercellular CO_2 concentration (C_i) and relative conductivity increased with the increase of O_3 concentration.

(2) The correlation between growth indicators (ΔH, ΔDBH) and O_3 uptake (F_{O_3}, W) was highest in *Pinus bungeana* under O_3 stress, followed by photosynthetic indicators (P_n, WUE, E_t, G_s, C_i) and growth indicators (ΔH, ΔDBH) and stomatal characteristics (K, M, S), and some physiological indicators (L, F_v/F_m) were relatively weakly correlated with photosynthesis (P_n, WUE, E_t, G_s, C_i) and stomata (K, M, S).

(3) There was a close relationship between O_3 and functional traits of *Pinus bungeana* plants. Compared to NF concentration, *Pinus bungeana* growth (ΔH, ΔDBH) and O_3 uptake (W and F_{O_3}), as well as stomatal density (M) and some photosynthetic capacity (P_n ,G_s) indicators were significantly reduced at NF40 concentration compared to NF concentration; the physiological properties (F_v/F_m, L), stomatal properties (K, S) and some photosynthetic properties (WUE, E_t, C_i) of *Pinus bungeana* produced more significant differences at NF80 concentration compared to NF40 concentration.

(4) During the experimental period, *Pi-*

nus bungeana did not stop growing even under NF80 concentration, nor did it show delayed or dysregulated stomatal response, indicating that it could still maintain the balance between its own growth and internal material cycle by adjusting leaf functional traits under O_3 stress conditions, which also tentatively proved its certain resistance to O_3.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Ainsworth EA, Lemonnier P, Wedow JM. The influence of rising tropospheric carbon dioxide and ozone on plant productivity. Plant Biology 2020; 22(Suppl.1): 5–11.
2. Wang T, Xue L, Brimblecombe P, *et al*. Ozone pollution in China: A review of concentrations, meteorological influences, chemical precursors, and effects. Science of the Total Environment 2017; 575: 1582–1596.
3. Ma J, Chu B, Liu J, *et al*. NOx promotion of SO_2 conversion to sulfate: An important mechanism for the occurrence of heavy haze during winter in Beijing. Environmental Pollution 2018; 233: 662–669.
4. Yang Y, Liu X, Qu Y, *et al*. Formation mechanism of continuous extreme haze episodes in the megacity Beijing, China, in January 2013. Atmospheric Research 2015; 155: 192–203.
5. Ministry of Ecology and Environment of the People's Republic of China. Report on the state of the ecology and environment in China 2019. Beijing: Ministry of Ecology and Environment of the People's Republic of China; 2020.
6. Li K, Jacob DJ, Liao H, *et Al*. Anthropogenic drivers of 2013–2017 trends in summer surface ozone in China. Proceedings of the National Academy of Sciences 2019; 116(2): 422–427.
7. Beijing Municipal Ecological and Environment Bureau. Beijing Environmental Statement 2013. Beijing: Beijing Municipal Ecological and Environment Bureau; 2014.
8. Beijing Municipal Ecological and Environment Bureau. Beijing Environmental Statement 2015. Beijing: Beijing Municipal Ecological and Environment Bureau; 2016.
9. Beijing Municipal Ecological and Environment Bureau. Beijing Ecology and Environment Statement 2019. Beijing: Beijing Municipal Ecological and Environment Bureau; 2020.
10. Galant A, Koester RP, Ainsworth EA, *et al*. From climate change to molecular response: Redox proteomics of ozone-induced responses in soybean. New Phytologist 2012; 194(1): 220–229.
11. Wilkinson S, Mills G, Illidge R, *et al*. How is ozone pollution reducing our food supply. Journal of Experimental Botany 2012; 63(2): 527–536.
12. Yamaguchi M, Watanabe M, Matsumura H, *et al*. Experimental studies on the effects of ozone on growth and photosynthetic activity of Japanese forest tree species. Asian Journal of Atmospheric Environment 2011; 5(2): 65–78.
13. Agathokleous E, Saitanis CJ, Wang XN, *et al*. A review study on past 40 years of research on effects of tropospheric O_3 on belowground structure, functioning, and processes of trees: A linkage with potential ecological implications. Water, Air & Soil Pollution 2016; 227(1): 1–28.
14. Schaub M, Skelly JM, Zhang JW, *et al*. Physiological and foliar symptom response in the crowns of Prunus serotina, Fraxinus americana and Acer rubrum canopy trees to ambient ozone under forest conditions. Environmental Pollution 2005; 133(3): 553–567.
15. Wan W, Xia Y, Zhang H, *et al*. The ambient ozone pollution and foliar injury of the sensitive woody plants in Beijing exurban region. Acta Ecologica Sinica 2013; 33(4): 1098–1105.
16. Galloway JN, Townsend AR, Erisman JW, *et al*. Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. Science 2008; 320(5878): 889–892.
17. Li P, Feng Z, Shang B, *et al*. Stomatal characteristics and ozone dose-response relationships for six greening tree species. Acta Ecologica Sinica 2018; 38(8): 2710–2721.
18. Chen B, Li S, Lu S. Ozone uptake characteristics in different dominance hierarchies of poplar plantation. Journal of Beijing Forestry University 2015; 37(7): 29–36.
19. Niu J. Effects of elevated ozone and nitrogen deposition on the growth and physiology of *Ci nnamomum camphora* seedlings [PhD thesis]. Beijing: Chinese Academy of Sciences; 2012.
20. Zhu J, Xu C, Qin G, *et al*. Responses of leaf functional characters of three typical greening plants to air pollution and leaf economic spectrum analysis: A Beijing city as the study case. Journal of Central South University of Forestry & Technology 2019; 39(3): 91–98.
21. Hoshika Y, Carriero G, Feng ZZ, *et al*. Determinants of stomatal sluggishness in ozone-exposed deciduous tree species. Science of the Total Environment 2014; 481: 453–458.
22. Hoshika Y, Omasa K, Paoletti E. Both ozone expo-

sure and soil water stress are able to induce stomatal sluggishness. Environmental and Experimental Botany 2013; 88 (Suppl.1): 19–23.
23. Paoletti E, Grulke NE. Ozone exposure and stomatal sluggishness in different plant physiognomic classes. Environmental Pollution 2010; 158(8): 2664–2671.
24. Cao X, Run L. The exploration of the purification of automobile exhausts contamination by plants. Journal of Central South University of Forestry & Technology 2007; 27(2): 133–136.
25. Pang J, Kobayashi K, Zhu JG. Yield and photosynthetic characteristics of flag leaves in Chinese rice (Oryza sativa) varieties subjected to free-air release of ozone. Agriculture, Ecosystems and Environment 2009; 132(3): 203–211.
26. Feng ZZ, Kobayashi K, Ainsworth E. Impact of elevated ozone concentration on growth, physiology, and yield of wheat (Triticum aestivum). Global Change Biology 2008; 14: 2696–2708.
27. Calatayud A, Barreno E. Response to ozone in two lettuce varieties on chlorophyll a fluorescence, photosynthetic pigments and lipid peroxidation. Plant Physiology and Biochemistry 2004; 42(6): 549–555.
28. Xin Y, Shang B, Chen X, *et al*. Effects of elevated ozone and nitrogen deposition on photosynthetic characteristics and biomass of *Populus cathayana*. Environmental Science 2016; 37(9): 3642–3649.
29. Feng Z, Li P, Yuan X, *et al*. Progress in ecological and environmental effects of ground-level O_3 in China. Acta Ecologica Sinica 2018; 38(5): 1530–1541.
30. Liu D, Zhao S, Wang X, *et al*. The effect of ozone on the leaf damages symptom and physiological characteristics of landscape plants. Zhang Q (editor). China Ornamental Horticulture Symposium 2015; 2015 Aug 18–20; Fujian, Xiamen. Beijing: China Forestry Publishing House; 2015. p. 507–512.
31. Zhang W. Effects of elevated O_3 level on the native tree species in subtropical China [PhD thesis]. Beijing: Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences; 2011.