Atmospheric nitrogen dioxide at different concentrations levels regulates growth and photosynthesis of tobacco plants

Wang Jiechen, Wang Yue, Zhang Huihui, Guo Dandan and Sun Guangyu

1. Introduction

With the acceleration of urbanization and industrialization, atmospheric nitrogen oxide pollution caused by vehicle exhaust emissions has gradually replaced atmospheric sulfuric acid pollutants (Yin et al. 2005). About one-third of urban atmospheric NO\textsubscript{2} comes from emissions from stationary sources (steel, cement, power production, etc.), and the remaining two-thirds come from motor vehicle exhaust (Wang et al. 2012). The annual average concentration of NO\textsubscript{2} in the air was 53 ppb in the United States, which can reach 20 \mu L\textsuperscript{−1} in urban areas with dense traffic (United States Environmental Protection Agency 2018). After entering the atmosphere, NO\textsubscript{2} reacts chemically with water vapor in the air to form nitric acid, which acidifies rainwater to form acid rain. In recent years, annual average emissions have markedly risen, making the acid rain problem exceptional prominent, and its secondary product, nitrous gas, has increased the accumulation of O\textsubscript{3} in the lower layers of the atmosphere, causing harmful fumes over cities and seriously harming the health of humans, animals, and plants (Liao et al. 2008).

Atmospheric NO\textsubscript{2} mainly enters plant leaves through their stomata. NO\textsubscript{2} enters the leaves to form NO\textsubscript{2}, which can be reduced to ammonia by ferredoxin or nicotinamide adenine dinucleotide phosphate (NADPH) (Paneque 1964). It has been suggested that NO\textsubscript{2} may inhibit the fixation of carbon dioxide in the dark reaction by competing with carbon assimilation for NADPH (Takahashi et al. 2005; Adam et al. 2008). Chen et al. (2010) performed NO\textsubscript{2} fumigation on camphor tree seedlings with 4 \mu L\textsuperscript{−1} NO\textsubscript{2}, and found that the net photosynthetic rate, dark respiration, and maximum photochemical quantum yield of photosystem II (PSII) in leaves significantly decreased.

However, some relevant studies have found that plants exposed to moderate amounts of atmospheric NO\textsubscript{2} over a long time period exhibit increased absorption and metabolism of nutrients used by plants for growth and development, which is known as the plant vitality effect of NO\textsubscript{2} (Takahashi et al. 2005; Adam et al. 2008). Chen et al. (2010) performed NO\textsubscript{2} fumigation on camphor tree seedlings for 60 days and found that 0.1 and 0.5 \mu L\textsuperscript{−1} NO\textsubscript{2} fumigation treatments increased N content and nitrate reductase (NR) activity and promoted seedling growth, while 4 \mu L\textsuperscript{−1} NO\textsubscript{2} fumigation inhibited growth. Wang et al. (2019) fumigated mulberry seedlings with 4 \mu L\textsuperscript{−1} NO\textsubscript{2} and found that the net photosynthetic rate, photorespiration rate, and PSII maximum photochemical quantum yield of the leaves were each significantly improved. Yu et al. (1988) found that 8 \mu L\textsuperscript{−1} NO\textsubscript{2} significantly increased the N content of both spinach and green bean leaves. Thus, responses to NO\textsubscript{2} differed substantially among both species and NO\textsubscript{2} concentrations.

The use of phytoremediation technology to control air pollution is a frontier new subject that has developed rapidly in recent years. Wei et al. (2017) proposed that phytoremediation is an environmentally friendly, cost-effective way of remediation of air pollutants. The key to this technology is plants that can adsorb or absorb pollutants and support microbes in biodegradation or biotransformation of pollutants. So far, a large number of research reports have focused on the response of plants under a specific NO\textsubscript{2} concentration.
the effects of long-term and different concentration gradients of NO$_2$ on plants are rarely reported. More importantly, most of studies have focused on the role of garden woody plants or forest trees under air pollution, the attention to herbaceous plants is low. In the research of Morikawa et al. (1998) on the assimilation ability of 217 green plants to atmosphere NO$_2$, some herbaceous plants also have high assimilation ability to NO$_2$, including Tobacco. Tobacco had NO$_2$-N content > 4.9, it can be considered 'NO$_2$-philic' because of the important effect of nitrogen dioxide on it. In this study, tobacco was selected as a model plant to study the growth and photosynthetic response of herbaceous plants with high assimilation ability to atmospheric NO$_2$, and to explore whether there is a NO$_2$ threshold concentration that can be absorbed and utilized to promote the growth of herbaceous plants? If there is a concentration threshold, how do plant growth physiological characteristics change with concentration within the threshold range? Which concentration is most beneficial to plant growth? This result provides a theoretical basis for the absorption of atmospheric NO$_2$ through herbaceous plants and the restoration of the gas polluted environment.

2. Materials and methods

2.1. Plant material and growth conditions

This research was conducted in the Plant Physiology Laboratory of Northeast Forestry University, Harbin, Heilongjiang Province, China, from May to December 2019. The seeds were provided by the Heilongjiang Mudanjiang Tobacco Science Research Institute. Tobacco seeds were sown into plug trays in May. At the two true leaf stages, the seedlings were transplanted into culture pots with a 15-cm upper diameter, a 12-cm lower diameter, and a 15-cm height. One plant was transplanted into each pot, filled with 500 g of a substrate comprised of a uniform 2:1 mixture (v/v) of turf soil and vermiculite. The seedlings were cultivated in an artificial growth chamber maintained at 25 ± 2°C, 65 ± 5% humidity, and 400 µmol m$^{-2}$ s$^{-1}$ light intensity, with a 14/10 h light/dark cycle. Once each week, plants were watered with 200 mL of Hoagland nutrient solution (Lian 2002). When the seedlings had 5–6 true leaves, tobacco seedlings that appeared to be healthy and growing uniformly were selected for fumigation.

2.2. Experimental setup

The NO$_2$ fumigation device was composed of a customized NO$_2$ artificial growth chamber, NO$_2$ tank, pressure reducing valve, and connecting pipe (Wang et al. 2020). The temperature, humidity, and light intensity in the climate box during fumigation were the same as during the seedling period.

2.3. Experimental design

In the experiment, four NO$_2$ concentrations were set up to fumigate tobacco seedlings, 1, 4, 8, and 16 µL L$^{-1}$, respectively. At the same time, the seedlings placed in a standard artificial growth chamber without NO$_2$ fumigation were used as the control treatment (CK). Each treatment included four replicates. Fumigations were conducted twice daily, at 8:00–11:00 am and 2:00–5:00 pm, and the fumigation is continuous for 15 days at each concentration. During the fumigation process, the indoor temperature, humidity, and light intensity are consistent with the original growth environment of the seedlings. Before the start of fumigation (0 d), and on the 5th, 10th, and 15th days of fumigation, the gas exchange parameters and chlorophyll fluorescence parameters of mature leaves of the plant were measured, and samples were taken to determine the growth indicators and chlorophyll content of each treatment.

2.4. Measured variables and methods

2.4.1. Determination of growth indicators

Plant growth parameters including plant height, single leaf area, single leaf number, and specific leaf weight. The distance from the base of the stem to the top of the tobacco plant was recorded as plant height. The fourth to last fully expanded leaf of the tobacco seedling was measured to determine leaf length and width. For each leaf measured, the product of the measured leaf length and width were multiplied by the leaf area index, which is 0.6345 in tobacco (Liu 1996), to determine the leaf area. To determine specific leaf weight, a punch was used to take fresh samples from the same leaf position of a leaf from the same portion of each measured seedling. The samples were dried at 105°C for 30 min until they were no longer green and further dried at 60°C to a constant weight, at which point the dry mass was obtained (Zhang et al. 2018; Zhang et al. 2020a). The dry mass of the leaf per unit area was calculated as the specific leaf weight. Compared with measurements obtained before fumigation, the plant height, percentage of leaf area, and specific leaf weight increase for each treatment on the 5th, 10th, and 15th days of fumigation and the increase in leaf number per plant were calculated.

2.4.2. Determination of chlorophyll content

To assess chlorophyll content, 0.1 g samples of fresh leaves were obtained from the control and treatment groups, respectively, which were extracted with 25 mL of 96% (v/v) ethanol. The chloroplast pigment extract was measured with a spectrophotometer at 663 and 645 nm, allowing the calculation of chlorophyll a ($C_a$), chlorophyll b ($C_b$), and total chlorophyll ($C_a + C_b$) contents according to the method described by Lichtenthaler and Wellburn (1983).

2.4.3. Determination of gas exchange parameters

The gas exchange parameters were measured with a portable photosynthesis instrument (LI-COR6400, LI-COR, Lincoln, NE, USA), with a fixed light intensity of 1000 µmol m$^{-2}$ s$^{-1}$ and a CO$_2$ concentration of 400 cm$^3$ m$^{-3}$. This setup was used to measure the net photosynthetic rate ($P_n$), stomata conductance ($G_s$), intercellular CO$_2$ concentration ($C_i$), and transpiration rate ($T_r$) of the fourth (from the bottom) mature expanded leaf of each plant from 9:00 to 11:00 am.

2.4.4. Determination of fluorescence parameters

The chlorophyll fluorescence parameters were measured on the fourth (from the bottom) mature expanded leaf of the tobacco seedlings by using a FMS-2 pulse modulation fluorometer (Hansatech, King’s Lynn, UK). After 30 min dark adaptation, the initial fluorescence ($F_o$) and maximum fluorescence ($F_m$) were measured to calculate the maximum photochemical efficiency ($F_v/F_m$) of PSII. Then the
maximum fluorescence ($F_m'$) and steady-state fluorescence ($F_s'$) under light adaptation were measured after 3 min of applying 1000 µmol m$^{-2}$ s$^{-1}$ actinic light (PFD) to the leaves with the built-in light source of FMS-2. Non-photochemical quenching NPQ = ($F_m' - F_m'')/F_m'$ and electron transfer rate ETR = 0.85 × 0.5 × PFD × ΦPSII, where ΦPSII is the actual photochemical efficiency ($Φ_{PSII} = 1 - F_o/F_m'$), 0.5 is the distribution ratio of leaves, and PFD is the light intensity (µmol m$^{-2}$ s$^{-1}$) (Zhang et al. 2020b, 2020c).

The chlorophyll fluorescence kinetics curve (OJIP curve) of the third fully developed leaf of the tobacco seedlings that were dark adapted (30 min) were determined by M-PEA multi-function plant efficiency analyzer (Hansatech, UK). During the determination of the OJIP curve, the induced light intensity of the pulsed red light was 3000 µmol m$^{-2}$ s$^{-1}$. Following the method for calculation described by Stasser et al. (1995), the absorption of light energy performance index ($PI_{ABS}$) were determined by JIP-test analysis.

### 2.5. Data processing and statistical methods

Excel (2016, Microsoft Corp, USA) and SPSS (22.0, IBM Corp, USA) software were used for statistical analysis of the data. Growth indicators, chlorophyll content, gas exchange parameters, and chlorophyll fluorescence parameters were determined from four biological replicates and expressed as mean ± standard deviation (SD) values. One-way ANOVA and least significant difference tests (LSD) were used to compare the data from treatments at different concentrations and different times. The statistical significance levels considered were $p < .05$ and $p < .01$.

### 3. Results and analysis

#### 3.1. Plant morphology

As Figure 1 shows, after 15 days of NO$_2$ fumigation, compared with CK plants, leaf morphology changes under 1 µL L$^{-1}$ NO$_2$ fumigation were not substantial; 4 µL L$^{-1}$ NO$_2$ made leaves darker green, thicker and increased in area; 8 µL L$^{-1}$ NO$_2$ further increased the dark green coloration and thickness of leaves, but the edges of leaves were curled, and the older leaves showed burn spots. In contrast, 16 µL L$^{-1}$ NO$_2$ fumigation for five days caused the older leaves to wither and plants to die.

#### 3.2. Growth indicators

Compared with measurements before fumigation, the plant height, leaf area, specific leaf weight, and leaf number for each treatment increased with time over the fumigation period. But the leaves treated with 16 µL L$^{-1}$ NO$_2$ withered after five days of fumigation, so no leaf area or specific leaf weight data were captured (Figure 2). Under fumigation with 1 µL L$^{-1}$ NO$_2$, the growth percentage in plant height and leaf area of tobacco seedlings and the increase in the number of leaves were higher throughout the fumigation period compared with CK plants. However, the differences were not significant, but the percentage increase in leaf-specific weight after 15 days of fumigation increased significantly, by 25.25% ($p < .05$) compared with CK plants. Under 4 µL L$^{-1}$ NO$_2$ fumigation, the growth percentage in plant height of seedlings was higher throughout the fumigation period compared with CK plants, but the difference was not significant. The percentage increase in leaf area and specific weight and the increase in leaf number were significantly higher, by 25.68% ($p < .05$), 33.36% ($p < .05$), and 28.57% ($p < .05$) compared with CK plants after 15 days of fumigation. Under 8 µL L$^{-1}$ NO$_2$ fumigation, the growth percentage of plant height increased significantly at the 10th day compared with CK plants. After 15 days, the growth percentage increased significantly, by 26.58% ($p < .05$), and the growth percentage of leaf area decreased significantly, by 67.85% ($p < .05$). The increase in the number of leaves and the growth percentage of specific leaf weight increased significantly, by 51.43% ($p < .05$) and 73.70% ($p < .05$), compared to CK plants. After five days of fumigation with 16 µL L$^{-1}$ NO$_2$, the growth percentage of plant height was slightly lower than that of CK plants, and the increase in leaf number was slightly lower than that of CK plants. However, the difference was not significant.

#### 3.3. Chlorophyll content

As fumigation durations increased, chlorophyll a, chlorophyll b, and total chlorophyll contents of CK plants did not change significantly. However, the chlorophyll contents of leaves under 1, 4, and 8 µL L$^{-1}$ NO$_2$ fumigation treatments increased with time. The chlorophyll contents of leaves under 16 µL L$^{-1}$ NO$_2$ fumigation decreased significantly with time ($p < .01$) (Figure 3(a–c)). After 15 days of 1, 4, and 8 µL L$^{-1}$ NO$_2$ fumigations, the chlorophyll a, chlorophyll b, and total chlorophyll contents were significantly higher than those of the CK. Chlorophyll a content increased by 21.51% ($p < .05$), 40.10% ($p < .05$), and 92.59% ($p < .05$),

![Figure 1](image-url)  
*Figure 1. Leaf phenotypes of tobacco seedlings after fumigation with different concentrations NO$_2$ for 15 days (except 16 µL L$^{-1}$ NO$_2$, which was only conducted for five days).*
respectively, chlorophyll b increased by 19.81% \((p < .05)\), 30.01% \((p < .05)\), and 78.68% \((p < .05)\), respectively, and the total chlorophyll content increased 21.08% \((p < .05)\), 37.41% \((p < .05)\), and 88.88% \((p < .05)\), respectively. After five days of 16 \(\mu\)L \(-1\) NO\(_2\) fumigation, chlorophyll a, chlorophyll b, and total chlorophyll contents of leaves were significantly lower than that of CK plants, which decreased by 22.92% \((p < .05)\), 23.01% \((p < .05)\), and 22.94% \((p < .05)\), respectively. The chlorophyll \(a/b\) ratio of each treatment was higher than that of the CK treatment, with the ratio highest in the 4 \(\mu\)L \(-1\) NO\(_2\) treatment followed by the 8 \(\mu\)L \(-1\) NO\(_2\) treatment and then the 1 \(\mu\)L \(-1\) NO\(_2\) treatment. While the 1 \(\mu\)L \(-1\) NO\(_2\) treatment was not significantly different from the CK treatment, the other treatments were \((p < .05)\) (Figure 3(d)).

### 3.4. Photosynthetic parameters

The \(P_n\) of tobacco seedling under CK, 8 \(\mu\)L \(-1\) NO\(_2\) and 16 \(\mu\)L \(-1\) NO\(_2\) treatments decreased gradually with time (Figure 4(a)). Under the 1 and 4 \(\mu\)L \(-1\) NO\(_2\) treatments, \(P_n\) increased over the first five days and then gradually decreased. After 15 days of fumigation, \(P_n\) under the 1, 4, and 8 \(\mu\)L \(-1\) NO\(_2\) treatments significantly increased, by 15.97% \((p < .05)\), 30.88% \((p < .05)\), and 16.90% \((p < .05)\), respectively. After fumigation with 16 \(\mu\)L \(-1\) NO\(_2\) for five days, \(P_n\) decreased significantly, by 61.86% compared with CK plants \((p < .05)\).

The \(G_i\) under the CK, 1 \(\mu\)L \(-1\) NO\(_2\) and 8 \(\mu\)L \(-1\) NO\(_2\) treatments decreased with treatment time. In contrast, \(G_i\) under the 4 \(\mu\)L \(-1\) NO\(_2\) treatment increased significantly in the first five days, and then gradually decreased, while that under the 16 \(\mu\)L \(-1\) NO\(_2\) treatment increased significantly after five days of fumigation (Figure 4(b)). Within 15 days of fumigation, \(G_i\) of the 1 \(\mu\)L \(-1\) NO\(_2\) treatment was lower than that under the CK treatment, and the difference was not significant. \(G_i\) under the 4 \(\mu\)L \(-1\) NO\(_2\) treatment increased compared with the CK treatment in the early stage of fumigation and significantly increased, by 13.49%, compared with the CK treatment, on the 10th day \((p < .05)\); after 15 days of fumigation, \(G_i\) under 4 \(\mu\)L \(-1\) NO\(_2\) fumigation was slightly lower than that under the CK treatment, but the difference was not significant. Within 15 days of fumigation, the 8 \(\mu\)L \(-1\) NO\(_2\) treatment reduced \(G_i\) compared with CK plants; the difference in \(G_i\) was the largest on the 10th day, which was 10.83% lower than that under the CK treatment \((p < .05)\). After five days of fumigation, the 16 \(\mu\)L \(-1\) NO\(_2\) treatment significantly reduced \(G_i\) by 34.25% \((p < .05)\), compared with CK plants.

The \(C_i\) of leaves under the CK and 1, 4, and 8 \(\mu\)L \(-1\) NO\(_2\) treatments all increased slowly with time, reached their peak on the 10th day, and then decreased. When treated with 16 \(\mu\)L \(-1\) NO\(_2\) for five days, \(C_i\) increased significantly, by 24.36% \((p < .01)\) compared with the measurement from before fumigation (Figure 4(c)). The \(C_i\) values of the 1 and 4 \(\mu\)L \(-1\) NO\(_2\) treatments were lower than that of the CK treatment within 15 days of fumigation, and the difference was significant at the 10th day. By the end of fumigation, the differences were significantly reduced, by 5.56% \((p < .05)\) and 3.74% \((p < .05)\), respectively. The \(C_i\) value under the 8 \(\mu\)L \(-1\) NO\(_2\) treatment was significantly reduced by

![Figure 2. Percentage increases in plant height (a), leaf area (b), and specific leaf weight (c) and increase in the number of leaves (d) in tobacco seedlings after fumigation with different concentrations of NO\(_2\) for 15 days (NS indicates plant died, specifically under 16 \(\mu\)L \(-1\) NO\(_2\) fumigation for 5 days). Note: Different lowercase letters indicate significant differences among different NO\(_2\) treatments at the \(p < .05\) level, while different capital letters indicate significant differences within the same NO\(_2\) treatment but among different treatment durations at the \(p < .01\) level.](image-url)
7.30% \((p < .05)\) compared with the CK treatment on the 10th day, and there were no corresponding significant differences on the 5th and 15th days compared with the CK treatment. Lastly, the 16 \(\mu\text{L}\cdot\text{L}^{-1}\) NO\(_2\) treatment significantly increased \(C_i\) by 12.57% \((p < .05)\) compared with CK plants when fumigated for five days.

The \(T_r\) values of the CK treatment and the 1, 4, 8, and 16 \(\mu\text{L}\cdot\text{L}^{-1}\) NO\(_2\) treatments decreased over time. At the end of fumigation, the \(T_r\) values of the CK treatment and the 1, 4, 8, and 16 \(\mu\text{L}\cdot\text{L}^{-1}\) treatments decreased by 12.12% \((p < .01)\), 37.43% \((p < .01)\), 23.83% \((p < .01)\), 39.18% \((p < .01)\), and 6.32% \((p > .01)\), respectively (Figure 4(d)). Each of the
four NO$_2$ fumigation treatments had lower $T_i$ values relative to the CK treatment throughout the entire fumigation period. After 15 days of fumigation, the 1, 4, and 8 μL L$^{-1}$ treatments were significantly reduced, by 31.82% ($p < .05$), 10.65% ($p < .05$), and 30.43% ($p < .05$), respectively. After five days, $T_i$ under the 16 μL L$^{-1}$ NO$_2$ treatment was slightly lower than that under the CK treatment, but the difference was not significant.

### 3.5. Chlorophyll fluorescence parameters

As shown in Figure 5(a), the CK treatment and the 1, 4, and 8 μL L$^{-1}$ NO$_2$ treatments all showed a non-significant decreases in $F/F_m$ over 15 days, while the 16 μL L$^{-1}$ treatment significantly reduced $F/F_m$ by 20.54% ($p < .01$). Compared with CK plants, $F/F_m$ was increased by 1.38% ($p > .05$), 3.67% ($p < .05$), and 3.42% ($p < .05$) under the 1, 4, and 8 μL L$^{-1}$ NO$_2$ treatments after 15 days of NO$_2$ fumigation. After 16 days of fumigation treatment with 16 μL L$^{-1}$ NO$_2$, $F/F_m$ decreased significantly, by 19.73% ($p < .05$).

$PI_{ABS}$ under the CK and 1 μL L$^{-1}$ NO$_2$ treatments did not change significantly over 15 days of NO$_2$ fumigation. $PI_{ABS}$ under the 4 μL L$^{-1}$ NO$_2$ treatment increased significantly by the 5th day, by 29.93% ($p < .01$), while $PI_{ABS}$ under the 8 μL L$^{-1}$ treatment significantly increased, by 82.61% ($p < .01$), by the 15th day (Figure 5(b)). In contrast, $PI_{ABS}$ under the 16 μL L$^{-1}$ treatment significantly decreased, by 86.38% ($p < .01$), by the fifth day of fumigation. Compared with the CK treatment, the 1 and 4 μL L$^{-1}$ NO$_2$ treatments had significantly increased $PI_{ABS}$ values, by 36.53% ($p < .05$) and 58.13% ($p < .05$), after 15 days of NO$_2$ fumigation, and the 8 μL L$^{-1}$ NO$_2$ treatment was significantly increased in $PI_{ABS}$, by 92.78% ($p < .05$). The 16 μL L$^{-1}$ NO$_2$ treatment caused significant reductions in $PI_{ABS}$, by 93.66% ($p < .05$), compared with the CK treatment after five days of fumigation.

After 15 days, the ETR under the CK and 16 μL L$^{-1}$ NO$_2$ treatments decreased significantly, by 18.21% ($p < .01$) and 33.38% ($p < .01$), respectively, while those of the 1 and 8 μL L$^{-1}$ NO$_2$ treatment did not change significantly. In contrast, the ETR under the 4 μL L$^{-1}$ NO$_2$ treatment increased significantly, by 39.22% ($p < .01$), over the 15 days (Figure 5(c)). Compared with the CK treatment, the ETR under the 1 μL L$^{-1}$ NO$_2$ treatment exhibited a non-significant increase after 15 days of fumigation, while the 4 and 8 μL L$^{-1}$ NO$_2$ treatments had increased significantly, by 29.56% ($p < .05$) and 59.75% ($p < .05$), respectively. After five days of fumigation with 16 μL L$^{-1}$ NO$_2$, the ETR was significantly reduced, by 25.62% ($p < .05$).

After 15 days of fumigation, only the 16 μL L$^{-1}$ NO$_2$ treatment exhibited a significant reduction in NPQ, by 16.83% ($p < .01$), compared with the measurement before fumigation. Compared with the CK treatment after 15 days of treatment, the NPQ under the 1 and 8 μL L$^{-1}$ NO$_2$ treatments increased by 7.57% ($p > .05$) and 8.33% ($p < .05$), respectively, while NPQ under the 4 μL L$^{-1}$ treatment decreased by 9.98% ($p > .05$). After five days of fumigation with 16 μL L$^{-1}$ NO$_2$, NPQ decreased significantly, by 10.07% ($p < .05$), compared with CK plants (Figure 5(d)).

### 4. Discussion

The present study confirms that low and high concentrations of NO$_2$ can respectively promote and inhibit the growth of...
tobacco seedlings, with the latter even leading to death. The NO$_2$ enters the apoptotic body cavity of leaves and undergoes a disproportionation reaction to produce nitrate (NO$_3^-$) and nitrite (NO$_2^-$), which are reduced to ammonium (NH$_4^+$) by NO$_3^-$ reductase (NRA) and NO$_2^-$ reductase (NiR), and then enters GS-GOGAT (Glutamine synthetase/glutamate synthetase) cycle, finally produce amino acids, proteins, chlorophyll, etc. (Maeck 1995; Sakakibara et al. 1996; Tischner 2000). In this experiment, NO$_2$ at a concentration of 8 μL L$^{-1}$ and below promoted the increase in plant height, leaf number, and specific leaf weight of tobacco seedlings. Therefore, NO$_2$ at these concentrations may be used as a gaseous nitrogen fertilizer, which is converted into organic nitrogen through nitrate assimilation for plant growth and development. However, it has been suggested that NO$_2$ has less contribution as a nitrogen source and is more likely to be used as a multifunctional signal to stimulate plant growth, nutrient absorption, and metabolism (Takahashi et al. 2005). Takahashi et al. (2014) analyzed the $^{15}$N/$^{14}$N ratio by mass spectrometry and found that NO$_2$-derived N (NO$_2$-N) comprises $<$3% of total plant N in some species, but Faller (1972) held the opposite view. He fumigated the nitrogen-deficient sunflower (Helianthus annuus) with NO$_2$ (1.5–6 μL L$^{-1}$ for three weeks) and found that the symptoms of N deficiency were reduced, and the leaf nitrogen increased by 70–116%, and proposed a certain concentration of NO$_2$ can be used as the sole source of nitrogen for plants. This difference may be related to plant species or NO$_2$ concentration. Morikawa et al. (1998) investigated the absorption and resistance of 217 species of green plants to NO$_2$ in the atmosphere and found that the largest difference in NO$_2$ absorption of leaves among different plants was more than 600 times. Ma et al. (2007) performed NO$_2$ fumigation of cabbage plants for 24 h and found that 0.25 and 0.5 μL L$^{-1}$ NO$_2$ treatments promoted seedling growth, while 1 and 2 μL L$^{-1}$ NO$_2$ caused visible damage to leaves. In this study, 8 μL L$^{-1}$ is the critical concentration for plants to absorb NO$_2$ to promote their own growth. About 16 μL L$^{-1}$ NO$_2$ severely inhibited plant growth. When the concentration of NO$_2$ is too high, reactive oxygen species will continue to accumulate in plants, leading to peroxidation of membrane lipids and destruction of cell membranes, thus causing damage to plants and even death (Wellburn et al. 1990; Yuan et al. 2005; Jiao et al. 2006). The promotion of NO$_2$ on plant growth is mainly attributed to whether it is a source of N or a signal; there is no clear report yet. However, within a certain concentration threshold, NO$_2$ can promote plant growth, has been confirmed by a large number of studies. Takahashi et al. (2014) obtained a similar conclusion when they fumigated Arabidopsis thaliana with 50 ppb NO$_2$ and also found that NO$_2$ promotes plant growth by controlling cell proliferation and expansion. Takahashi et al. (2011) also conducted an interesting experiment, fumigating tomato seedlings with NO$_2$. The results showed that the fruit yield increased by 40% and the number of flowers per plant increased by 60%, which indicated that NO$_2$ can increase fruit yields by stimulating tomato flowering.

Chlorophyll is one of the most important pigments related to photosynthesis, and chlorophyll content is often used as an indicator of damage caused by air pollution. In this experiment, 1, 4, and 8 μL L$^{-1}$ NO$_2$ fumigation increased tobacco chlorophyll $a$, chlorophyll $b$, and total chlorophyll contents, and the higher the concentration, the more obvious the increase until reaching a concentration of 8 μL L$^{-1}$ NO$_2$. When the NO$_2$ concentration reached 16 μL L$^{-1}$, the chlorophyll content of tobacco leaves decreased significantly. This result is similar to previous studies on the response of chlorophyll to the concentration of NO$_2$ (Chen et al. 2009; Hou et al. 2012). The leaves absorb NO$_2$-N, and NO$_2$-N is assimilated into nitrogenous amino acids, which are used to synthesize pigment proteins. These results indicate that low concentrations of NO$_2$ (8 μL L$^{-1}$ and below) may use NO$_2$-N to promote the synthesis of chlorophyll, especially the synthesis of chlorophyll $a$, which shows a significant increase in chlorophyll $a/b$. The chlorophyll $a$ of plant photosynthesis exists in the PSI and PSII reaction center complexes. It can not only capture light energy but also effectively convert light energy into active chemical energy. Therefore, the increase of chlorophyll $a$ improves the absorption and conversion of light energy of tobacco leaves. However, the tolerance of plants to NO$_2$-N is limited. The high concentration of NO$_2$ (16 μL L$^{-1}$) exceeded the absorptive capacity and scavenging capacity of plants, and produced reactive oxygen species (ROS), which causes oxidative stress on tobacco leaves, thereby destroying the plant cytochrome system and decolorizing chlorophyll, resulting in a significant decrease in chlorophyll content. Similar phenomena have been observed in plants such as Avena sativa L. (Pleijel et al. 1994), and Hedera helix (Della Torre et al. 1998) and may be caused by the disintegration of pigments by ROS (Sakaki et al. 1983).

Photosynthesis is an important metabolic process in plants. Because of its consistent presence throughout plant evolution, it has always been an important focus of plant physiology and ecology research, and it can be used as an indicator to judge plant growth (Xu 2002). Among photosynthesis-related measures, net photosynthetic rate ($P_n$), stomatal conductance ($G_s$), intercellular carbon dioxide concentration ($C_i$), and transpiration rate ($T_r$) are important indicators that reflect the photosynthetic efficiency of leaves. In previous studies, Okano et al. (1986) found that after a two-week NO$_2$ fumigation of sunflowers, 0.2 μL L$^{-1}$ NO$_2$ increased $P_n$, while 0.5 and 1 μL L$^{-1}$ NO$_2$ decreased $P_n$. However, Sabratnam et al. (1988) significantly increased $P_n$ of soybean by 0.2 μL L$^{-1}$ NO$_2$, and significantly decreased $P_n$ by 0.4 μL L$^{-1}$ NO$_2$ when fumigating soybean. In this study, the appropriate concentration of NO$_2$ (8 μL L$^{-1}$ and below) can enhance the photosynthesis of tobacco leaves, and the enhancement effect is greatest at the intermediate concentration (4 μL L$^{-1}$). When the concentration of NO$_2$ increased to 16 μL L$^{-1}$, the photosynthesis of tobacco leaves was significantly inhibited. Stomata are the channels for gas exchange between plant leaves and the atmosphere, and the degree of closure directly affects photosynthesis and transpiration rates. $G_s$ of tobacco seedlings under 1, 4 and 8 μL L$^{-1}$ NO$_2$ fumigation is not significantly different compared with CK at the late stage of fumigation, indicating that stomatal conductance is not the main factor that promotes the photosynthetic capacity of tobacco plants, and is more likely due to the chlorophyll content increased significantly under these treatments, which promoted the assimilation of CO$_2$, thereby enhancing the photosynthetic activity of the seedlings and increasing the photosynthetic rate. The 16 μL L$^{-1}$ NO$_2$ treatment significantly reduced $P_n$ in tobacco seedlings, while $G_s$ and $T_r$ decreased, and $C_i$ increased, indicating that the 16 μL L$^{-1}$ NO$_2$ severely damaged the photosynthetic machinery of tobacco leaves, reduced the utilization of CO$_2$, and forced the concentration of intercellular CO$_2$ to increase. The
decrease in photosynthetic rate is mainly caused by two aspects. On the one hand, when the volume fraction of harmful gases in the atmosphere is too high, plants will close some stomata to reduce their entry in order to reduce the damage (Xu 1999). The closure of stomata inhibits the plant’s absorption and utilization of CO₂. On the other hand, the stress caused by gaseous pollutants often directly acts on the photosynthetic machinery, the chloroplasts, and the destruction of chloroplasts causes a decrease in photosynthetic activity (Mediavilla et al. 2002). When stomatal conductance and intercellular CO₂ concentration are both reduced, stomatal factors dominate (Eegineer et al. 2016). When the stomatal conductance decreases and the intercellular CO₂ concentration increases, non-stomatal factors dominate (Cakmak et al. 1992). Therefore, the reduction of photosynthetic rate under NO₂ fumigation is mainly affected by a non-stomatal adjustment mechanism, that is, it appears to be caused by the degradation of chlorophyll, which is consistent with the aforementioned results of the reduction of chlorophyll contents observed under this concentration.

Chlorophyll fluorescence parameters are closely related to photosynthesis and can reflect relevant information such as the photosynthesis mechanism and photosynthetic physiological status of plants in different environments. In this study, we analyzed the key chlorophyll fluorescence parameters Fv/Fm, PIABS, ETR, and NPQ. Compared with the CK treatment, Low concentration (8 μL L⁻¹ and below) NO₂ increased the Fv/Fm, PIABS, and ETR values of tobacco seedling leaves, especially the increase of PIABS was more obvious, indicating that the changes of PIABS were more sensitive to environmental stress. The increase of each index was the smallest under the 1 μL L⁻¹ NO₂ treatment. The Fv/Fm and ETR values under 8 μL L⁻¹ NO₂ fumigation were less than those under the 4 μL L⁻¹ NO₂ treatment, but PIABS and NPQ was significantly higher, indicating that the reaction center under the 1 μL L⁻¹ NO₂ fumigation had stronger activity, meaning it could absorb more light energy than under the 4 μL L⁻¹ NO₂ treatment, but the efficiency of light energy utilization was lower. In comparison, 4 μL L⁻¹ NO₂ treatment can better improve the PSII’s original light energy conversion efficiency and electron transfer rate, use more absorbed light energy to produce photochemical power (ATP and NADPH), and promote the process of carbon assimilation. Under unfavorable conditions, NPQ plays an important role in reducing the excessive excitation energy in the plant photosystem (Xu et al. 2018). There was no significant difference in NPQ between the CK treatment and the 1, 4, and 8 μL L⁻¹ NO₂ fumigation after 15 days, indicating that the low concentration of NO₂ is not enough to initiate a photoprotective mechanism that consumes excess excitation energy by increasing heat dissipation, and the energy absorbed by plants can be more used for photochemical reactions. The Fv/Fm, PIABS, ETR, and NPQ were significantly reduced under 16 μL L⁻¹ NO₂ fumigation after five days. This shows that the high concentration of NO₂ destroys the photosynthetic mechanism of tobacco seedlings, reduces the original light energy conversion efficiency and electron transfer rate of PSII. At the same time, the photoprotective mechanism has been severely damaged, unable to relieve the accumulation of excess light energy, thereby inducing the production of ROS, leading to the degradation of photosynthetic pigments and the oxidative damage of photosynthetic organs, causing irreversible damage to plants.

Hou et al. (2012) found that 0.83 μL L⁻¹ NO₂ increased the chlorophyll content of Arabidopsis leaves, while 1.95 μL L⁻¹ NO₂ decreased the chlorophyll content. Sabrannam et al. (1988) found that NO₂ at a concentration of 0.2 μL L⁻¹ and below is beneficial to soybean growth. Ma et al. (2007) found that the concentration of 0.5 μL L⁻¹ and below is beneficial to the growth of cabbage, and the concentration of 1 μL L⁻¹ and above NO₂ will inhibit the growth of leaves. Through previous research results, we found that the favorable concentration of NO₂ in general herbaceous plants was below 2 μL L⁻¹. Chen (2009) found that 0.1 and 0.5 μL L⁻¹ NO₂ increased the net photosynthetic rate of camphor tree leaves, 4 μL L⁻¹ NO₂ reduced the net photosynthetic rate, and proposed that the concentration threshold of woody plants is about 4 μL L⁻¹. In our study, 8 μL L⁻¹ NO₂ significantly promoted the chlorophyll content of tobacco, enhanced the photosynthetic rate and photochemical efficiency, and at the same time, the old leaves showed signs of injury such as scars. When the concentration exceeds the damage threshold of the plant, the plant leaves first show the symptoms of damage (Chen 2009), indicating that tobacco is on the verge of maximum tolerance to 8 μL L⁻¹ NO₂. Therefore, 8 μL L⁻¹ is the threshold concentration for the absorption and utilization of NO₂ by herbaceous plants with high NO₂ assimilation ability. This threshold is significantly higher than that of ordinary herbs and even higher than the NO₂ threshold of some woody plants. When the concentration exceeds the threshold and rises to 16 μL L⁻¹, all indicators indicate that the plant is seriously injured. Chen (2009) fumigated camphor seedlings with high concentrations of NO₂ for three days, and found that 18–22.5 μL L⁻¹ NO₂ was the threshold for short-term damage to woody plants. Therefore, compared with woody plants, herbaceous plants appear to be less resistant to high concentrations of NO₂. However, as far as the level of NO₂ pollution in the atmosphere is concerned, herbaceous plants still have a high research value for alleviating air pollution.

5. Conclusions

The proper concentration of NO₂ can promote the synthesis of chlorophyll, increase the conversion efficiency of plant PSII light energy, and enhance the absorption, transformation, and utilization of light energy, thereby promoting the growth of plants. About 8 μL L⁻¹ is the NO₂ threshold concentration for herbaceous plants with a high assimilation ability to absorb NO₂ to promote growth, higher than general herbs and even some woody plants. Within the threshold range, the intermediate concentration (4 μL L⁻¹) is most beneficial to plant growth and photosynthesis. When this threshold concentration is exceeded, NO₂ will inhibit the growth of plants, causing plants to wither and even die within a short time. Therefore, when the concentration of atmospheric NO₂ is lower than the absorption threshold concentration of herbaceous plants, NO₂ can be absorbed by plants to reduce air pollution. Our research provides a reference for the application of herbaceous plants in garden plants under air pollution.

Disclosure statement

No potential conflict of interest was reported by the author(s).
Funding
This work was supported by the National Natural Science Foundation of China (31870373).

Authors’ contributions
J.W.: writing-original draft; Y.W.: writing-review and editing; H. Z.: methodology; D.G.: data curation; and G.S.: supervision.

References
United States Environmental Protection Agency Home Page. 2018. [Accessed 2018 Dec 8]. Available from: http://www3.epa.gov/tnn/naaqs/criteria.html.

Adam SEH, Shigeto J, Sakamoto A, Takahashi M, Morikawa H. 2008. Erratum: atmospheric nitrogen dioxide at ambient levels stimulates growth and development of horticultural plants. Botany. 86 (4):442-447.

Cakmak I, Marschner H. 1992. Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. Plant Physiol. 98 (4):1222-1227.

Chen ZM, Chen YX, Du GJ, Wu XL, Li F. 2014. Effects of 60-day NO2 fumigation on growth, oxidative stress and antioxidant response in Cinnamomum camphora seedlings. J Zhejiang Univ Sci B. 11:190-199. (in Chinese).

Chen ZM, Chen YX, Du GJ. 2009. Effects of NO2 on Cinnamomum camphora seedlings growth and photosynthesis. Chin J Appl Ecol. 20:1650-1656. (in Chinese).

Della Torre G, Ferranti L, Lupattelli M, Pocceschi N, Figoli A, Nali C, Hu YB, Bellaloui N, Tigabu M, Wang JH, Diao J, Wang K, Yang R, Sun J, Jiao J, Li CZ, Huang JB. 2006. Protective effects of NO2 on Cinnamomum camphora seedlings growth and photosynthesis. Chin J Appl Ecol. 20:1591-1597. (in Chinese).

Lichtenthaler HK, Wellburn AR. 1984. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem Soc Trans. 11:591-592.

Liu GS. 1996. A comparative study on various measuring methods of tobacco leaf area. J Anhui Agric Sci. 24:139-141. (in Chinese).

Ma CY, Xu X, Hao L, Cao J. 2007. Responses of brassica campestris seedlings to nitrogen dioxide stress and modulation of hydrogen peroxide. Sci Agric Sin. 40:2556-2562. (in Chinese).

Maek G. 1995. Organ-specific changes in the activity and subunit composition of glutamine-synthetase isoforms of barley (Hordeum vulgare L.) after growth on different levels of NH4+. Planta. 196 (2):231-238.

Meditavilla S, Santiago H, Escudero A. 2002. Stomatal and mesophyll limitations to photosynthesis in one evergreen and one deciduous Mediterranean oak species. Photosynthetica. 40(4): 553-559.

Morikawa H, Hijiki A, Nohno M, Takahashi M, Kamada M, Nakata M, Toyoohara G, Okumura Y, Matsui K, Kitani S, etal. 1998. More than a 600-fold variation in nitrogen dioxide assimilation among 217 plant taxa. Plant Cell Environ. 21(2):180-190.

Okano KY, Totsha T, Fukuwaza TT. 1986. Absorption of nitrogen dioxide by sunflower plants grow at varies levels of nitrate. New Phytol. 102:551-562.

Pan W, Zhang WQ, Zhang FQ, Liu JP, Duan JP, Chen J. 2012. Decontamination ability of sulfur dioxide and nitrogen dioxide for 38 young landscaping plants in Guangzhou city. Ecology and Environmental Sciences. 21:606-612. (in Chinese).

Paneque A, Ramirez JM, Del Campo FF, Losada M. 1964. Light and dark reduction of nitrite in a reconstituted enzymic system. J Biol Chem. 239:1737.

Pelej H, Skarby L, Ojapera K, Sellédén G. 1994. Exposure of oats, Avena sativa L., to filtered and unfiltered air in open-top chambers: effects on grain yield and quality. Environ Pollut. 86 (2):129-134.

Sakakiribara H, Shimizu H, Hase T, Yamazaki Y, Takao T, Shimonishi Y, Sugiyama T. 1996. Molecular identification and characterization of cystolic isoforms of glutamine synthetase in maize roots. J Biol Chem. 271:29561-29568.

Sheng QQ, Zhu ZL. 2018. The NO2 resistance study and garden application of seven colored plants in nanjing. J Centr South Univ Forest Technol. 38:81-87. (in Chinese).

Srivastava HS, Jolliffe PA, Runeckeles VC. 1975. Inhibition of gas exchange in bean leaves by NO2, Can J Bot. 53(5):466-474.

Strasser RJ, Srivastava A, Goyvindje G. 1995. Polyphasic chlorophyll a fluorescence transient in plants and cyanobacteria. Photochecm Photobiol. 61(1):32-42.

Takahashi M, Furuhashi T, Ishikawa N, Horiguchi G, Sakamoto A, Tsukaya H, Morikawa H. 2014. Nitrogen dioxide regulates organ growth by controlling cell proliferation and enlargement in Arabidopsis. New Phytol. 201:1304-1315.

Takahashi M, Morikawa H. 2014. Nitrogen dioxide is a positive regulator of plant growth. Plant Signal Behav. 9(2):e28033-15.

Takahashi M, Nakagawa M, Sakamoto A, Ohsumi C, Matabara T, Morikawa H. 2005. Atmospheric nitrogen dioxide gas is a plant vitalization signal to increase plant size and the contents of cell constituents. New Phytol. 168:149-154.

Takahashi M, Sakamoto A, Ezura H, Morikawa H. 2011. Prolonged exposure to atmospheric nitrogen dioxide increases fruit yield of tomato plants. Plant Biotechnol. 28:485–487.

Tischner R. 2000. Nitrate uptake and reduction in higher and lower power plants grow at varies levels of nitrate. New Phytol. 102:551-562.

Wang Y, Zhang WQ, Zhang FQ, Liu JP, Duan JP, Chen J. 2012. Decontamination ability of sulfur dioxide and nitrogen dioxide for 38 young landscaping plants in Guangzhou city. Ecology and Environmental Sciences. 21:606-612. (in Chinese).

Wang JH, Wang Y, Li J, Liu Y. 2012. Characteristics of atmospheric NO2 in the Beijing-Tianjin-Hebei region and the Yangtze river delta analyzed by satellite and ground observations. Environ Sci. 33(11):3686–3692. (in Chinese).

Wang Y, Zhang HH, Wang JC, Zhao HC, He GQ, Huang D, Yang FW, Zhao MC, Che YH, Teng ZY, etal. 2020. Elevated NO2 damages the photosynthetic apparatus by inducing the accumulation of superoxide anions and peroxynitrite in tobacco seedling leaves. Ecotoxicol Environ Safe. 196:110534.
Wei X, Lyu S, Yu Y, Wang Z, Liu H, Pan D, Chen J. 2017. Phylloremediation of air pollutants: exploiting the potential of plant leaves and leaf-associated microbes. Front Plant Sci. 8:1318.

Wellburn AR. 1990. Tansley review No. 24: Why are atmospheric oxides of nitrogen usually phytotoxic and not alternative fertilizers? New Phytol 115:395–429.

Xu DQ. 1999. Photosynthetic rate, photosynthetic efficiency and crop yield. Bull Biol. 34:8–10. (in Chinese).

Xu DQ. 2002. Photosynthesis efficiency. Shanghai: Shanghai Science and Technology Press. 33 (in Chinese).

Xu N, Zhang HH, Zhong HX, Wu YN, Li JB, Li X, Yin ZP, Zhu WX, Q Y, Sun GY. 2018. The response of photosynthetic functions of F1 cutting seedlings from Physocarpus amurensis maxim (♀) × Physocarpus opulifolius “diabolo” (♂) and the parental seedlings to salt stress. Front Plant Sci. 9:714.

Yin YL, Luo YM, Zhang TL, He JF, Xu JH. 2005. Concentrations of metallic elements, N and S in Sabina chinensis cv. pyramidalis leaves along urban-rural roadways of Nanjing city. Chin J Appl Ecol. 5:929–932. (in Chinese).

Yu SW, Shimazaki K, Li L. 1988. Response of spinach and kidney bean plants to nitrogen dioxide. Environ Pollut. 55(1):1–13.

Yuan L, Keremu VL, Zhang LQ. 2005. Effects of NaCl stress on active oxygen metabolism and membrane stability in Pistacia vera seedlings. Chin J Plant Ecol. 29:985–991.

Zhang HH, Li X, Che YH, Wang Y, Li MB, Yang RY, Xu N, Sun GY. 2020a. A study on the effects of salinity and pH on PSII function in mulberry seedling leaves under saline-alkali mixed stress. Trees, 34, 3, 693–706.

Zhang HH, Li X, Guan YP, Li MB, Wang Y, An M, Zhang YH, Liu GJ, Xu N, Sun GY. 2020b. Physiological and proteomic responses of reactive oxygen species metabolism and antioxidant machinery in mulberry (Morus alba L.) seedling leaves to NaCl and NaHCO₃ stress. Ecotoxicol Environ Saf. 193:110259.

Zhang HH, Li X, Xu ZS, Wang Y, Teng ZY, An MJ, Zhang YH, Zhu WX, Xu N, Sun GY. 2020c. Toxic effects of heavy metals Pb and Cd on mulberry (Morus alba L.) seedling leaves: photosynthetic function and reactive oxygen species (ROS) metabolism responses. Ecotoxicol Environ Saf. 195:110469.

Zhang XX, He R, Shi Y, Shao JP, Zhou Z. 2005. Study on the reaction of 13 indigenous tree species for greening in Yunnan to SO₂ and NO₂. J West China Forest Sci. 34:41–46. (in Chinese).