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

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ABSTRACT

Nitrogen dioxide (NO_2) is recognized as a toxic gaseous pollutant. However, an appropriate concentration of NO_2 can promote the growth of plants. In this study, tobacco was exposed to different concentrations of NO_2. The results that 1, 4, and 8 μL L^{-1} NO_2 fumigation increased the net photosynthetic rate (P_n), maximum photochemical efficiency (F_v/F_m) and light energy utilization capacity parameters (PI_{ABS}) of leaves, and promoted the growth of seedlings. About 16 μL L^{-1} NO_2 caused the leaves to wither, and the plants were about to die after five days of fumigation. About 8 μL L^{-1} NO_2 obviously promoted the growth of plants, but meanwhile, the old leaves began to show signs of damage, such as bruising and yellowing. Therefore, 8 μL L^{-1} may be the threshold concentration of herbaceous plants with high NO_2 assimilation ability. At an intermediate concentration (4 μL L^{-1}) within the threshold range, NO_2 is most beneficial to the growth of herbaceous plants.

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KEYWORDS
Nitrogen dioxide; tobacco; photosynthesis; growth; concentration threshold

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_x 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_2 in the air was 53 ppb in the United States, which can reach 20 μL L^{-1} in urban areas with dense traffic (United States Environmental Protection Agency 2018). After entering the atmosphere, NO_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 exceptionally prominent, and its secondary product, nitrous gas, has increased the accumulation of O_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_2 mainly enters plant leaves through their stomata. NO_2 enters the leaves to form NO_2^-, which can be reduced to ammonia by ferredoxin or nicotinamide adenine dinucleotide phosphate (NADPH) (Paneque 1964). It has been suggested that NO_2 may inhibit the fixation of carbon dioxide in the dark reaction by competing with carbon assimilation for NADPH produced by the photoreaction, thereby reducing the photosynthetic rate. (Hill 1970; Srivastava 1975; Sabaratnam et al. 1988). Chen et al. (2009) found that 18.0, 22.5, and 27.0 μL L^{-1} NO_2 reduced the net photosynthetic rate and chlorophyll content of camphor tree seedlings and inhibited photosynthesis in seedling leaves. Wang et al. (2020) fumigated tobacco seedlings with 16 μL L^{-1} NO_2 and obtained consistent results. Hu et al. (2014, 2015) fumigated hybrid poplar (*Populus alba* × *P. berolinensis*) seedlings with 4 μL L^{-1} NO_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_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_2 (Takahashi et al. 2005; Adam et al. 2008). Chen et al. (2010) performed NO_2 fumigation on camphor tree seedlings for 60 days and found that 0.1 and 0.5 μL L^{-1} NO_2 fumigation treatments increased N content and nitrate reductase (NR) activity and promoted seedling growth, while 4 μL L^{-1} NO_2 fumigation inhibited growth. Wang et al. (2019) fumigated mulberry seedlings with 4 μL L^{-1} NO_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 μL L^{-1} NO_2 significantly increased the N content of both spinach and green bean leaves. Thus, responses to NO_2 differed substantially among both species and NO_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 phylloremediation 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_2 concentration

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(Li 1982; Zhang 2005; Pan 2012; Sheng 2018), 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 concentration threshold 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 $ETR = 0.85 \times 0.5 \times PFD \times \Phi_{PSII}$, where Φ_{PSII} is the actual photochemical efficiency ($\Phi_{PSII} = 1 - F_s/F'_m$), 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, thickener 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 leaf 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 higher 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% ($p < .05$),

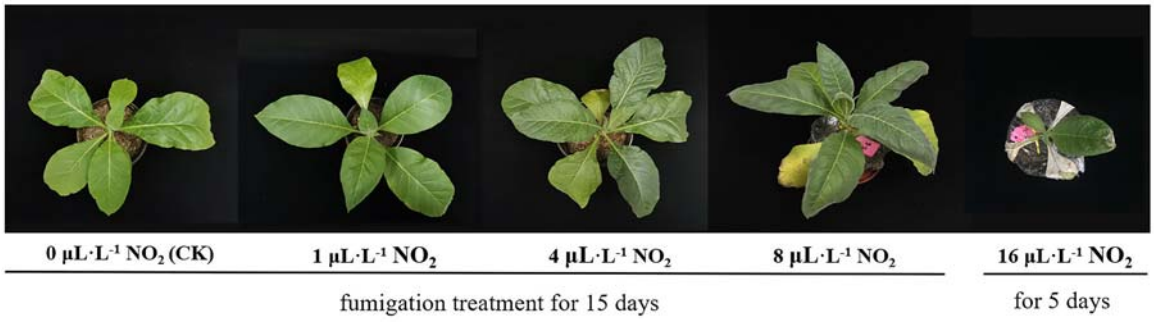

Figure 1. Leaf phenotypes of tobacco seedlings after fumigation with different concentrations NO_2 for 15 days (except 16 μL L^{-1} NO_2, which was only conducted for five days).

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 μL 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 μL L^{-1} NO_2 treatment followed by the 8 μL L^{-1} NO_2 treatment and then the 1 μL L^{-1} NO_2 treatment. While the 1 μL L^{-1} 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 μL L^{-1} NO_2 and 16 μL L^{-1} NO_2 treatments decreased gradually with time (Figure 4(a)). Under the 1 and 4 μL 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 μL 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 μL L^{-1} NO_2 for five days, P_n decreased significantly, by 61.86% compared with CK plants ($p < .05$).

The G_s under the CK, 1 μL L^{-1} NO_2 and 8 μL L^{-1} NO_2 treatments decreased with treatment time. In contrast, G_s under the 4 μL L^{-1} NO_2 treatment increased significantly in the first five days, and then gradually decreased, while that under the 16 μL L^{-1} NO_2 treatment increased significantly after five days of fumigation (Figure 4(b)). Within 15 days of fumigation, G_s of the 1 μL L^{-1} NO_2 treatment was lower than that under the CK treatment, and the difference was not significant. G_s under the 4 μL 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_s under 4 μL 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 μL L^{-1} NO_2 treatment reduced G_s compared with CK plants; the difference in G_s 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 μL L^{-1} NO_2 treatment significantly reduced G_s, by 34.25% ($p < .05$), compared with CK plants.

The C_i of leaves under the CK and 1, 4, and 8 μL L^{-1} NO_2 treatments all increased slowly with time, reached their peak on the 10th day, and then decreased. When treated with 16 μL 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 μL 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 μL L^{-1} NO_2 treatment was significantly reduced by

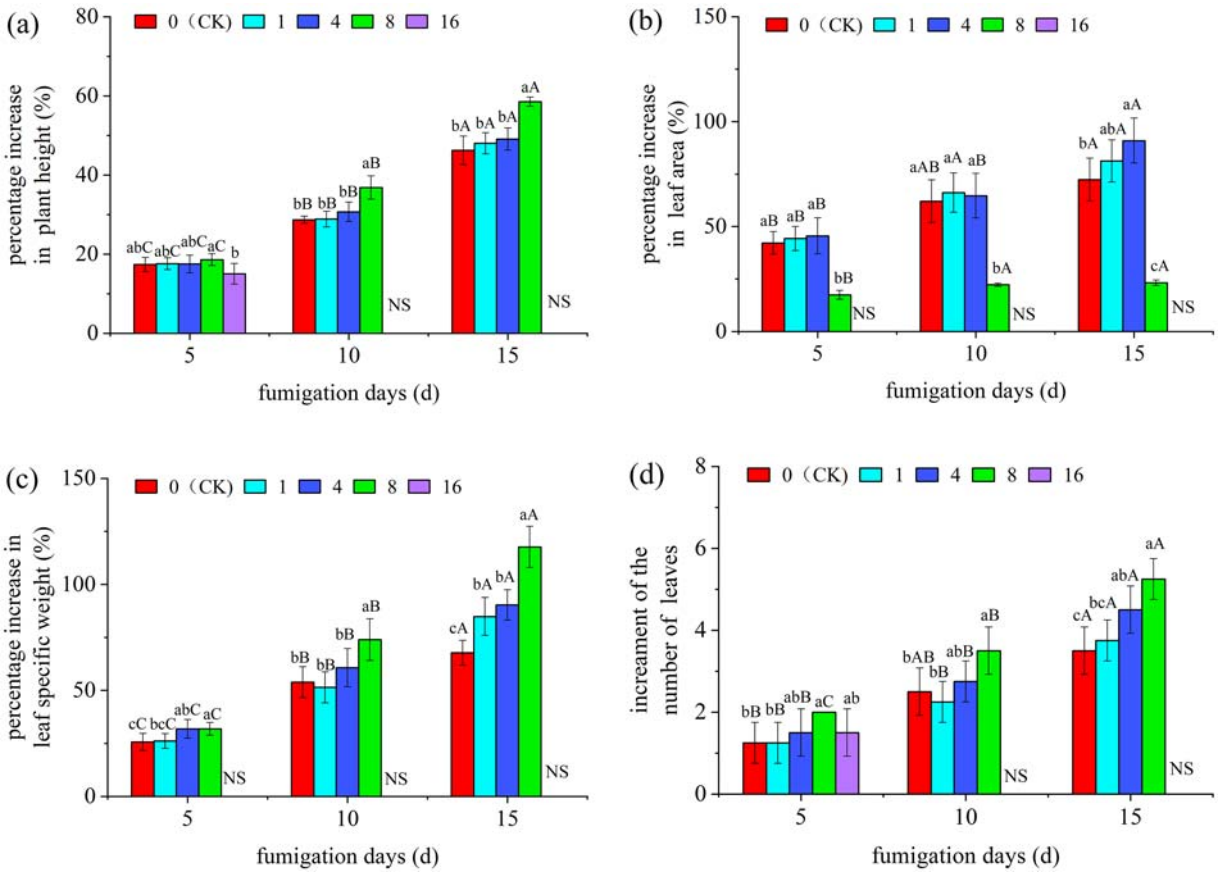

Figure 2. Percentage increases in plant height (a), leaf area (b), and specific leaf weight (c) and increase in the number of leaves (d) in tobacco seedlings after fumigation with different concentrations of NO_2 for 15 days (NS indicates plant died, specifically under 16 μL L^{-1} NO_2 fumigation for 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.

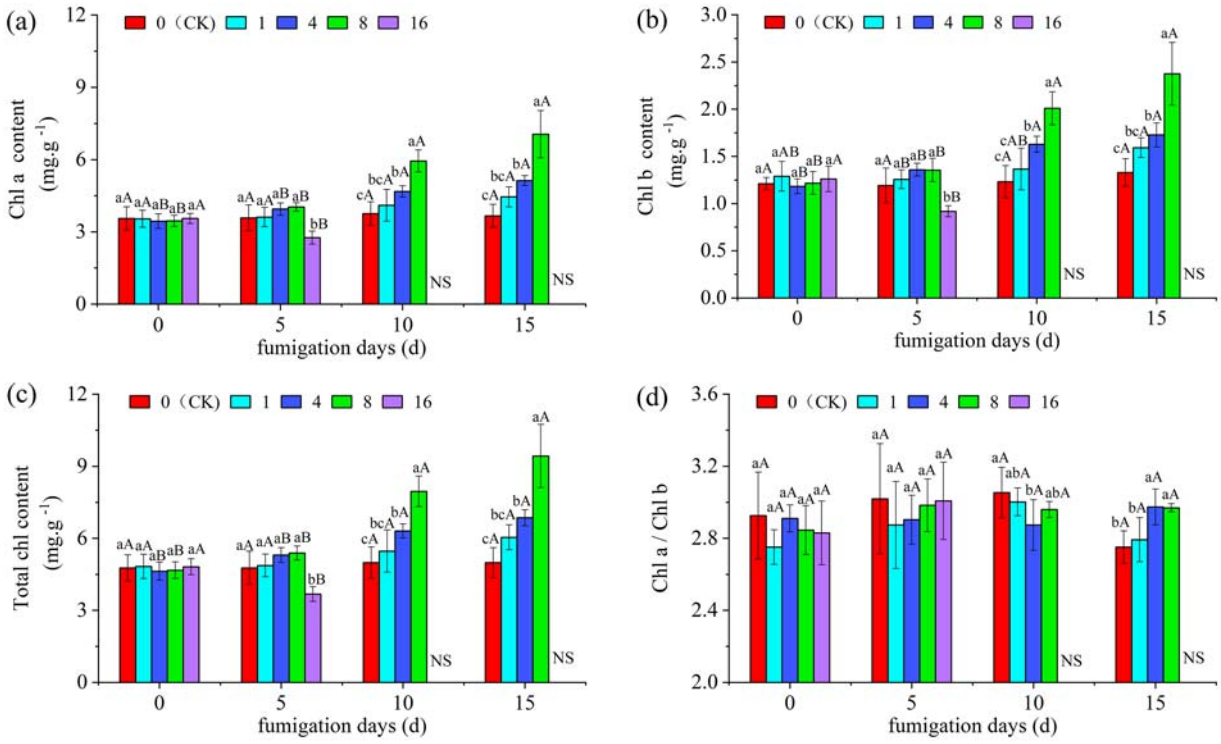

Figure 3. Effects of different concentrations of NO_2 on chlorophyll a (Chl a) (a), chlorophyll b (Chl b) (b), total chlorophyll contents (c), and Chl a/Chl b (d) in leaves of tobacco seedlings.

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 μL 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 μL 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 μL 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

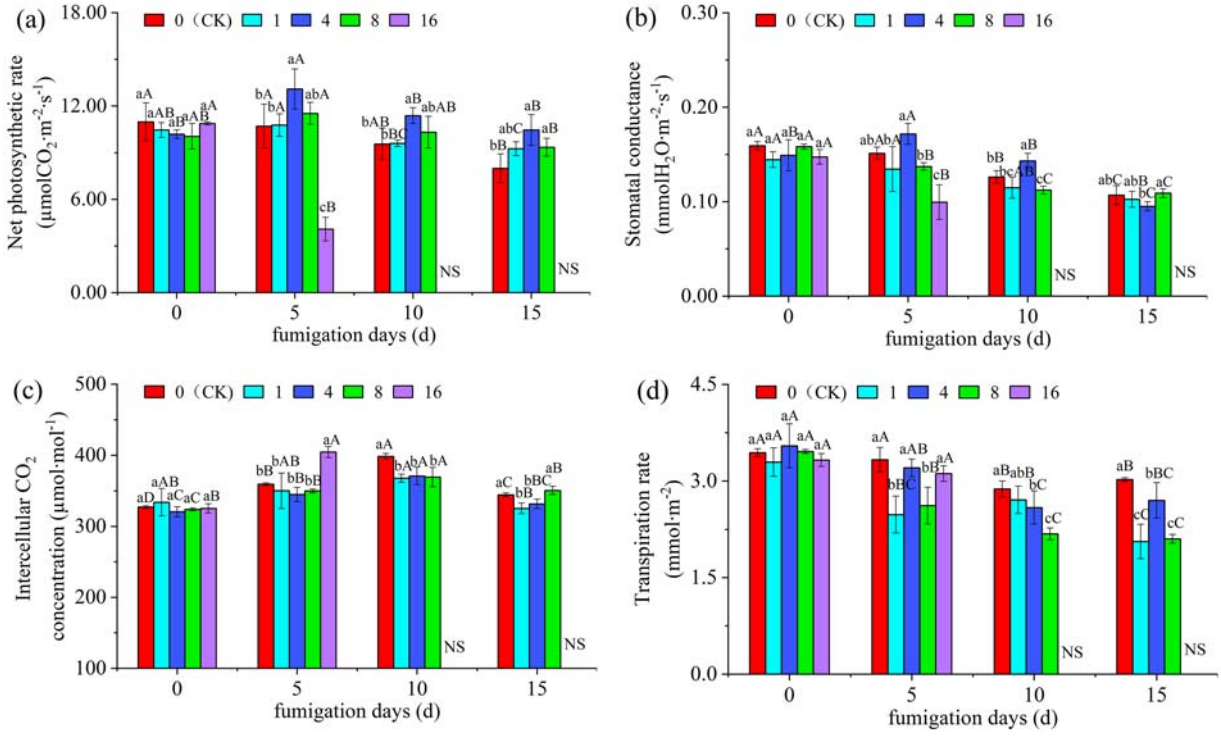

Figure 4. Net photosynthetic rate (P_n) (a), stomatal conductance rate (G_s) (b), intercellular carbon dioxide concentration (C_i) (c), and transpiration rate (T_r) (d) in tobacco seedlings after fumigation with different concentrations of NO_2 for 15 days (NS means plant died, specifically after five days under of 16 μL L^{-1} NO_2).

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 treatments among different treatment durations at the $p < .01$ level.

four NO_2 fumigation treatments had lower T_r 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_r 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_v/F_m over 15 days, while the 16 μL L^{-1} treatment significantly reduced F_v/F_m, by 20.54% ($p < .01$). Compared with CK plants, F_v/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_v/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

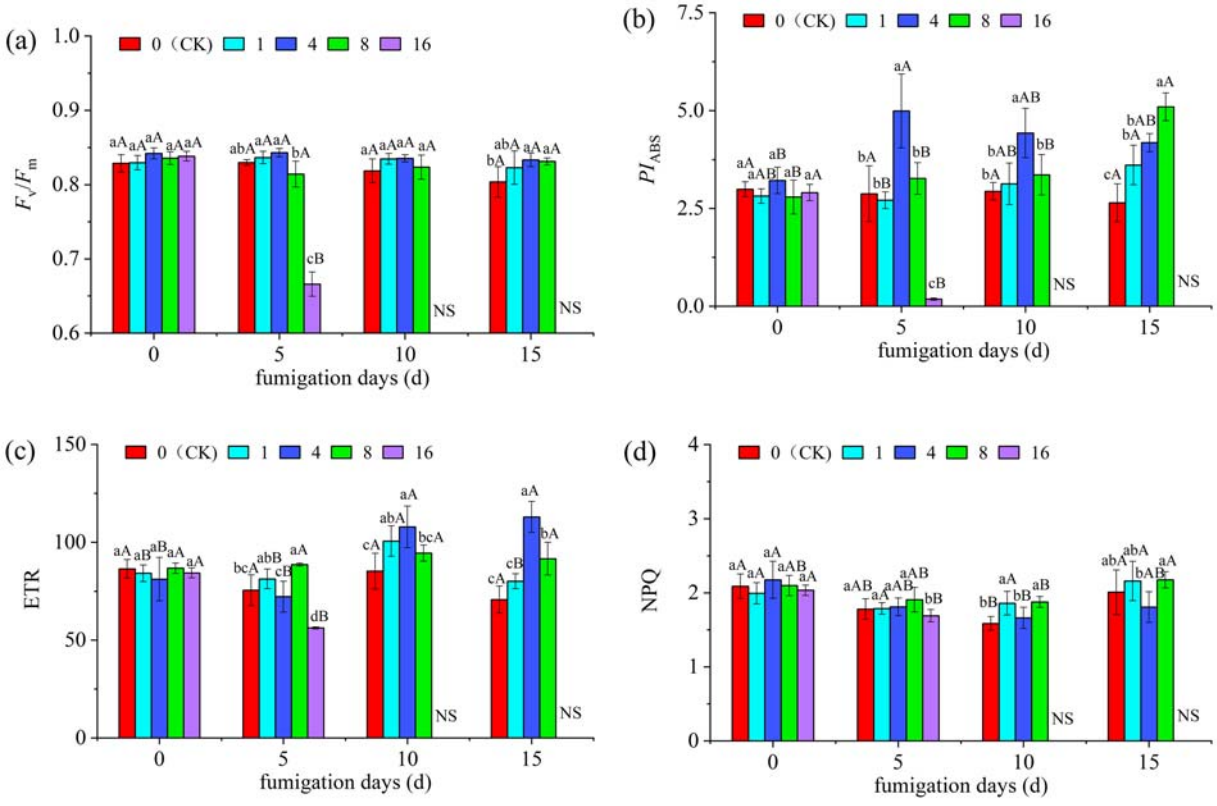

Figure 5. Maximum quantum yield of photosystem II (F_v/F_m) (a), light energy utilization capacity parameters (PI_{ABS}) (b), electron transfer rate (ETR) (c) and non-photochemical quenching (NPQ) (d) of tobacco seedlings after fumigation with different concentrations of NO_2 over 15 days (NS indicates plants died, specifically under 16 μL 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 among time points at the $p < .01$ level.

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 seedings 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 the16 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 CO_2. 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 CO_2 concentration are both reduced, stomatal factors dominate (Eegineer et al. 2016). When the stomatal conductance decreases and the intercellular CO_2 concentration increases, non-stomatal factors dominate (Cakmak et al. 1992). Therefore, the reduction of photosynthetic rate under NO_2 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 F_v/F_m, PI_{ABS}, ETR, and NPQ. Compared with the CK treatment, Low concentration (8 μL L^{-1} and below) NO_2 increased the F_v/F_m, PI_{ABS}, and ETR values of tobacco seedling leaves, especially the increase of PI_{ABS} was more obvious, indicating that the changes of PI_{ABS} were more sensitive to environmental stress. The increase of each index was the smallest under the 1 μL L^{-1} NO_2 treatment. The F_v/F_m and ETR values under 8 μL L^{-1} NO_2 fumigation were less than those under the 4 μL L^{-1} NO_2 treatment, but PI_{ABS} and NPQ was significantly higher, indicating that the reaction center under the 8 μL L^{-1} NO_2 fumigation had stronger activity, meaning it could absorb more light energy than under the 4 μL L^{-1} NO_2 treatment, but the efficiency of light energy utilization was lower. In comparison, 4 μL L^{-1} NO_2 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} NO_2 fumigation after 15 days, indicating that the low concentration of NO_2 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 F_v/F_m, PI_{ABS}, ETR, and NPQ were significantly reduced under 16 μL L^{-1} NO_2 fumigation after five days. This shows that the high concentration of NO_2 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} NO_2 increased the chlorophyll content of Arabidopsis leaves, while 1.95 μL L^{-1} NO_2 decreased the chlorophyll content. Sabratnam et al. (1988) found that NO_2 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 NO_2 will inhibit the growth of leaves. Through previous research results, we found that the favorable concentration of NO_2 in general herbaceous plants was below 2 μL L^{-1}. Chen (2009) found that 0.1 and 0.5 μL L^{-1} NO_2 increased the net photosynthetic rate of camphor tree leaves, 4 μL L^{-1} NO_2 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} NO_2 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} NO_2. Therefore, 8 μL L^{-1} is the threshold concentration for the absorption and utilization of NO_2 by herbaceous plants with high NO_2 assimilation ability. This threshold is significantly higher than that of ordinary herbs and even higher than the NO_2 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 NO_2 for three days, and found that 18–22.5 μL L^{-1} NO_2 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 NO_2. However, as far as the level of NO_2 pollution in the atmosphere is concerned, herbaceous plants still have a high research value for alleviating air pollution.

5. Conclusions

The proper concentration of NO_2 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 NO_2 threshold concentration for herbaceous plants with a high assimilation ability to absorb NO_2 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, NO_2 will inhibit the growth of plants, causing plants to wither and even die within a short time. Therefore, when the concentration of atmospheric NO_2 is lower than the absorption threshold concentration of herbaceous plants, NO_2 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.

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Authors' contributions

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

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