

# Gaseous NO<sub>2</sub> effects on stomatal behavior, photosynthesis and respiration of hybrid poplar leaves

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**Abstract** In this study, we used poplar as a model plant and investigated the effects of gaseous nitrogen dioxide (NO<sub>2</sub>, 4 μl l<sup>-1</sup>) on stomatal conductance, photosynthesis, dark- and photorespiration of *Populus alba* × *Populus berolinensis* hybrid leaves using the photosynthesis system and scanning electron microscope technique. The results showed that net photosynthetic rates were significantly reduced in leaves exposed to 4 μl l<sup>-1</sup> NO<sub>2</sub> for 48 h as compared with leaves exposed to ambient carbon dioxide 380 μl l<sup>-1</sup> and ambient NO<sub>2</sub> <0.1 μl l<sup>-1</sup> (the control) and the leaves exposed for 14 h. The decline of net photosynthetic rate was caused mainly by NO<sub>2</sub> treatment. Dark respiration rates were dependent on co-action of the two factors (leaf temperature and NO<sub>2</sub> treatment time). Post-illumination carbon dioxide burst in the exposed leaves occurred at 13–15 s after turning the light off, whereas this phenomenon was absent in the control leaves.

**Keywords** Nitrogen dioxide · Photorespiration · *Populus* trees · Post-illumination CO<sub>2</sub> burst · Stomatal behavior

## Introduction

Foliar uptake of NO<sub>2</sub> gas mainly occurs through stomata, with a small fraction of cuticular deposition (Rennenberg and Gessler 1999). Stomatal characteristics such as stomatal dimension and conductance (Chaparro-Suarez et al. 2011; Breuninger et al. 2013), epidermis physicochemical properties such as cuticular chemical compositions and water films (Lendzian and Kerstiens 1988; Range et al. 1993; Jetter et al. 1996) and related environmental factors (Geßler et al. 2000; Chaparro-Suarez et al. 2006) have influences on foliar NO<sub>2</sub> uptake. On the other hand, gaseous NO<sub>2</sub>, due to its corrosive and highly oxidizing nature, also has impacts on stomatal dynamics and stomata-related physiological and biochemical processes, particularly photosynthesis and dark respiration. The effects of gaseous NO<sub>2</sub> on stomatal aperture seem to be species-specific, so while for *Ilex rotunda*, there was a positive correlation between NO<sub>2</sub> concentration and stomatal aperture (Takagi and Gyokusen 2004); for *Raphanus sativus*, the correlation was negative (Mazarura 2012). Studies on gaseous NO<sub>2</sub> effects on leaf photosynthesis and/or respiration process are scarce and mainly focused on herbaceous plants with only a few studies in woody plants (Pallardy 2007). Inhibitory effects of NO<sub>2</sub> on photosynthesis and dark respiration have been reported for *Phaseolus vulgaris* (Srivastava et al. 1974a, b), *Glycine max.* Merr (Carlson 1983), *Vulpia microstachys* (Vallano et al. 2012), and *Raphanus sativus* L. (Mazarura 2012); whereas stimulatory effects of NO<sub>2</sub> on dark respiration have been found for *Glycine max.* Merr. cv. Williams (Sabaratnam et al. 1988) and *Lolium*

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*multiflorum* (Vallano et al. 2012). Oleksyn (1984) showed that in *Pinus sylvestris* the net photosynthesis, dark respiration, and photorespiration declined after 30 min exposure to gaseous NO<sub>2</sub>; however, these parameters increased or were unaffected by a longer time of fumigation. The results of Van Hove et al. (1992) showed that NO<sub>2</sub> reduced dark respiration rate of Douglas fir, but did not affect net photosynthetic rate as compared with the control. Eller et al. (2011) also reported no significant difference in photosynthetic rates of the sugar maple leaves exposed to 40 nl l<sup>-1</sup> NO<sub>2</sub> and the control (ambient CO<sub>2</sub> 365 μl l<sup>-1</sup> and ambient NO<sub>2</sub> <1 nl l<sup>-1</sup>).

*Populus* species are commonly planted along roadside in urban areas of many countries for amenity purposes, and has been reported to have high capacity of absorption of NO<sub>2</sub> and resistance to its effects (Okano et al. 1989) when compared with the other broad-leaved tree species. Morikawa et al. (1998) investigating 107 woody plants showed that *Populus nigra* had the second highest NO<sub>2</sub>-N assimilation capacity (Morikawa et al. 1998). Takahashi et al. (2005) also found that *P. nigra* had a higher NO<sub>2</sub> assimilation capability when compared to the other roadside trees. The physiological mechanisms of the high tolerance to NO<sub>2</sub> are still not well known. Several earlier publications focused on NO<sub>2</sub> effects on poplar growth, and found that low concentrations (0.1 μl l<sup>-1</sup>) of NO<sub>2</sub> had no significant influence on height, leaf area and dry weight of 1-year-old yellow-poplar seedlings (Dochinger and Jensen 1985), and 0.5 μl l<sup>-1</sup> NO<sub>2</sub> had a significant stimulation on leaf growth of Carolina poplar and Lombardy poplar, but a higher concentration (1 μl l<sup>-1</sup>) significantly decreased the stem growth (Eastham and Ormrod 1986). To date, only limited studies on NO<sub>2</sub> effects on photosynthesis or respiration of *Populus* trees have been conducted. These studies showed that low concentrations (80–135 nl l<sup>-1</sup>) of NO<sub>2</sub> gas had no significant impacts on leaf CO<sub>2</sub> assimilation and stomatal conductance of hybrid *Populus × euramericana* (Siegwolf et al. 2001) and *Populus × euramericana* ‘Dorskamp’ (Schmutz et al. 1995) poplar clone cuttings. Recently we found exogenous sodium sulfide improves morphological and physiological responses of a hybrid *Populus* species to nitrogen dioxide (Hu et al. 2014a). In spite of these studies, the relationship between instantaneous plant performance (dark respiration and photorespiration) and foliar NO<sub>2</sub> uptake (Sparks 2009), and adaptability of ultrastructure of mesophyll tissue adjacent to stomata and chemical elements composition (such as Nitrogen, Phosphorus, Calcium, and Magnesium) of leaf surface to gaseous NO<sub>2</sub> and nitrate transporters of NO<sub>2</sub>-treated leaves (Hu et al. 2014a, b) are still unknown.

In this study, we assumed that high concentration (4 μl l<sup>-1</sup>) of NO<sub>2</sub> fumigation has significant impact on epidermis and stomata-related physiochemical characteristics

of hybrid poplar leaves; photorespiration plays a potential role in the plant response to gaseous NO<sub>2</sub>. So we investigated gaseous NO<sub>2</sub> effects on stomatal dynamics, photosynthetic and respiratory characteristics of the hybrid poplar clone cuttings and discussed the potential mechanisms of the interactions between *populus* trees and gaseous NO<sub>2</sub>.

## Materials and methods

### Plant materials and growth conditions

Twelve two-year-old seedlings of hybrid poplar clonal cuttings (*Populus alba × P. berolinensis*) were grown in pots (20 cm in diameter, 30 cm in height) filled with a soil/sand mixture (3:1 v/v) at outdoor conditions. Environmental conditions were monitored over a time period of 4 h (8:30–12:30 am) when measurements were taken. The mean temperature was 30.8 °C (max/min temp of 38.5 °C/22.95 °C); air relative humidity was 65 %; air CO<sub>2</sub> concentration was approximately 380 μl l<sup>-1</sup> and photosynthetic photon flux density (PPFD) was 1,000–1,900 μmol m<sup>-2</sup> s<sup>-1</sup>. Seedlings were watered using tap water every day.

### NO<sub>2</sub> fumigation

Open-top glass chambers (0.6 cm × 0.40 cm × 0.80 cm) were used for NO<sub>2</sub> fumigation. Stocks of NO<sub>2</sub> source were supplied by the Special Gas Products Co., Ltd. (Dalian city, China). NO<sub>2</sub> concentration in this study was set to 4 μl l<sup>-1</sup>, following Morikawa et al. (1998), Takahashi et al. (2005), and Kondo et al. (2008). 4 μl l<sup>-1</sup> NO<sub>2</sub> was supplied directly from a 40-L cylinder. Mean concentration of NO<sub>2</sub> within the chamber was monitored using an NO<sub>2</sub> analyzer (MIC-500-NO<sub>2</sub>, Shenzhen Yiyuntian Electrical Co., Ltd., China). The control was not fumigated by NO<sub>2</sub> and the treated seedlings (six replicates) were fumigated by 4 μl l<sup>-1</sup> NO<sub>2</sub> for averaged 6 h per day. Leaf stomata and physiology (such as net photosynthetic rate, P<sub>n</sub>) were measured when the leaves were exposed to NO<sub>2</sub> during 48 h.

### CO<sub>2</sub> exchange rate–time curve

Net photosynthetic rate ( $P_n$ ), dark respiration rate ( $R_{\text{dark}}$ ), transpiration rate ( $T_r$ ), and stomatal conductance ( $G_s$ ) were measured using LI-6400XT Portable Photosynthesis System (LI-COR Biosciences, United States). Concentration of CO<sub>2</sub>, relative humidity, and photosynthetic photon flux density (PPFD) were controlled via an automatic control device of LI-6400XT. The parameters  $P_n$ ,  $R_{\text{dark}}$ ,  $T_r$ , and  $G_s$

were measured as follows. Leaves were pre-lighted at  $1,200 \mu\text{mol m}^{-2} \text{s}^{-1}$  [under  $\text{CO}_2$  concentration of  $370\text{--}380 \mu\text{l l}^{-1}$ , air temperature ( $32\text{--}39^\circ\text{C}$ ), and 65 % relative humidity conditions] for 8 min to obtain a stable photosynthesis rate. Then, the light was turned off for about 180 s to automatically record air temperature ( $T_a$ ), leaf temperature ( $T_l$ ),  $P_n$ ,  $R_{\text{dark}}$ ,  $T_r$ , and  $G_s$  at an interval of 2 s. Post-illumination  $\text{CO}_2$  burst (PIB) was determined according to the method of Laisk and Sumberg (1994). Decker (1955) concluded that this phenomenon “Post-illumination  $\text{CO}_2$  burst” was a product of light respiration. Six to eight fully expanded leaves from different seedlings were measured repeatedly for each fumigation time.

### Measurement of stomatal dynamics

Scanning Electron Microscope was used to estimate stomatal dynamics of the leaves. Mature leaves exposed to gaseous  $\text{NO}_2$  for different times were collected, then washed with tap water and cut into  $5 \text{ mm}^2$  fragments. The fragments were fixed by 2.5 % glutaraldehyde for 6 h, and air was exhaled to immerse the fragments below the fluid level. After dehydration through a grades alcohol series, the samples were critical point dried, mounted on stubs, and coated with gold in a high-vacuum evaporation unit (Lin et al. 2001). Samples were examined at 5 kV acceleration voltages under a Hitachi S-4800 scanning electron microscope.

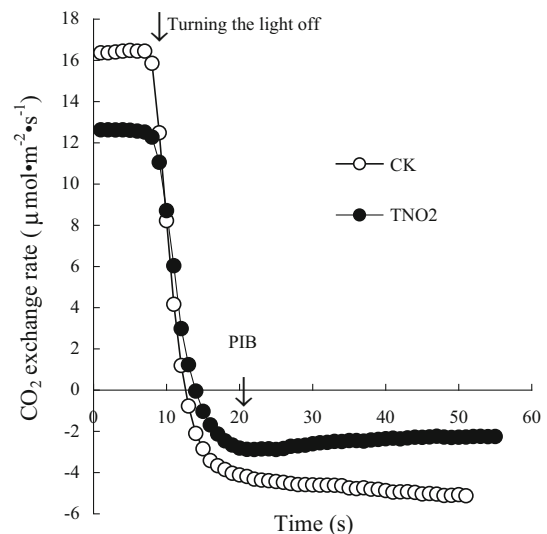
### Statistic analysis

Standard deviation (SD) in graphs was conducted using Microsoft Office Excel Statistical Package (Microsoft Office Excel, 2013). The comparison of averages and correlation coefficients was based on  $t$  test at a significance level of 5 % ( $p < 0.05$ ) or 1 % ( $p < 0.01$ ).

## Results

### Effects of $\text{NO}_2$ on photosynthesis and respiration processes

At the steady status of photosynthesis, the net photosynthetic rate of leaves exposed to  $\text{NO}_2$  was significantly lower than that of the control leaves (CK) (Fig. 1). After turning the light off,  $\text{CO}_2$  exchange rates of CK and  $\text{NO}_2$ -exposed leaves sharply decreased for about 10 s, and then slowly reached a relatively steady state in subsequent 60 s (Fig. 1). For CK leaves, post-illumination  $\text{CO}_2$  burst (PIB) was absent and it was lower than dark respiration rate ( $R_{\text{dark}}$ ). In contrast,  $\text{NO}_2$ -exposed leaves showed a typical characteristic of PIB where the maximum PIB occurred at



**Fig. 1** Post-illumination transients in  $\text{CO}_2$  exchange rates. *Populus alba*  $\times$  *P. berolinensis* leaves were exposed to  $380 \mu\text{l l}^{-1} \text{CO}_2$ , 21 %  $\text{O}_2$ , and PPFD of  $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$  in a steady photosynthesis state. At time = 10 s, light was switched off. Values of the post-illumination  $\text{CO}_2$  burst of the leaves exposed to gaseous  $\text{NO}_2$  for 48 h (TNO2) (black circle) and CK leaves (white circle) were read at arrow

13–15 s after turning the light off (Fig. 1); PIB value was higher than  $R_{\text{dark}}$ . Both  $R_{\text{dark}}$  and PIB (absolute values) of  $\text{NO}_2$ -exposed leaves were lower than that in CK leaves. These results indicated an inhibitory effect of gaseous  $\text{NO}_2$  on photosynthesis and dark respiration processes.

We statistically analyzed the results of  $P_n$  and  $R_d$  in different leaf temperatures/the same  $\text{NO}_2$  treatment time and in different treatment times at similar leaf temperatures. The results showed that in a given PPFD of  $1,200 \mu\text{mol m}^{-2} \text{s}^{-1}$ , net photosynthetic rates ( $P_n$ ) of the  $\text{NO}_2$ -treated leaves were dependent on the treatment time ( $R^2 = 0.5966$ );  $P_n$  values declined with the increase in treatment time. In contrast, leaf temperature had a weak correlation with  $P_n$  values ( $R^2 = 0.0151$ ). The  $t$  test analysis showed a significant difference in the effect of  $\text{NO}_2$  treatment time and leaf temperature on  $P_n$  values ( $P = 0.048$ , at the significance level of 0.05). The  $t$  test analysis also showed that there was no significant difference in the effect of  $\text{NO}_2$  treatment time ( $R^2 = 0.4022$ ) and leaf temperature ( $R^2 = 0.5093$ ) on  $R_d$  values ( $P = 0.504$ , at the significance level of 0.05). Dark respiration rates of the treated leaves were determined by the co-action of leaf temperature and  $\text{NO}_2$  treatment time. However, the two factors had an opposite effect on  $R_d$  values:  $R_d$  was correlated positively with leaf temperature, but negatively with  $\text{NO}_2$  treatment time.

### Effects of gaseous $\text{NO}_2$ on stomata morphology

Figure 3a showed a typical SEM image of *Populus alba*  $\times$  *P. berolinensis* leaf, including upper and lower

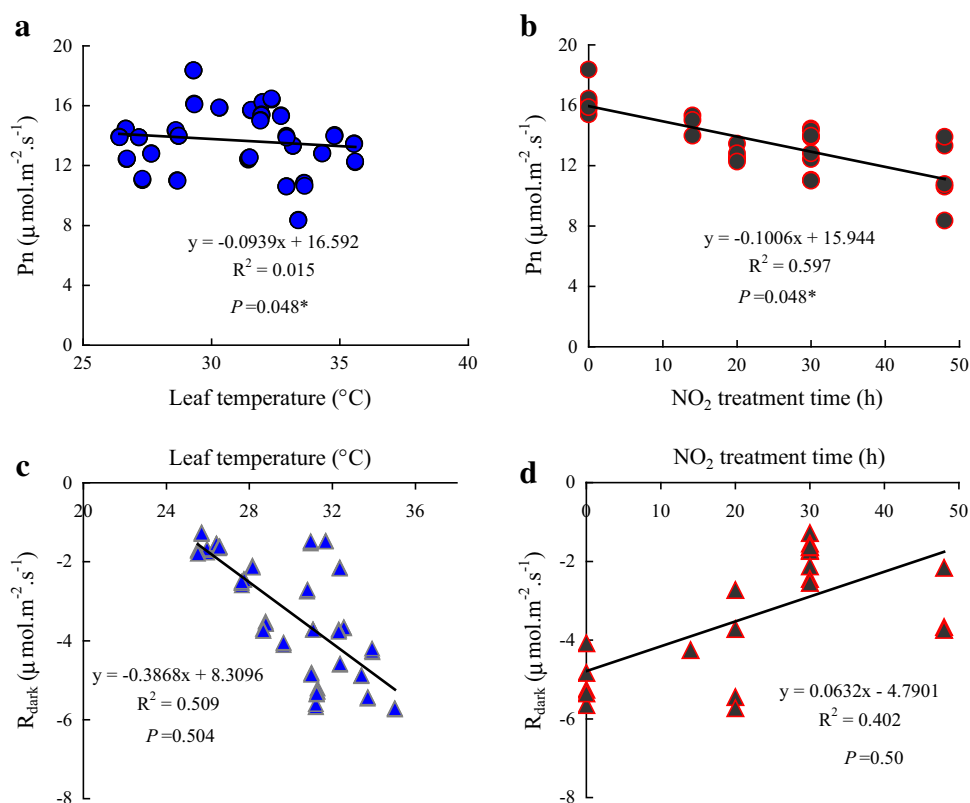
epidermis, palisade/spongy tissues, and epidermal hairs. Dense white trichomes in abaxial leaf surface are visible without any additional equipment. Stomatal distribution was observed in both abaxial and adaxial leaves (amphistomatic) (data as shown in Hu et al. 2014a). Significant differences in stomatal size (length  $\times$  width  $\mu\text{m}$ ) were found in the leaves exposed to  $4 \mu\text{l l}^{-1}$   $\text{NO}_2$  for 0 (Fig. 3b), 14 (Fig. 3c) and 48 h (Fig. 3d). Stomatal opening in the leaves exposed to  $\text{NO}_2$  for 48 h was lower than that of 0-h and 14-h exposed leaves. Exposure to the gaseous  $\text{NO}_2$  for 48 h resulted in a decline in  $T_r$  ( $P = 0.054$ , at the significant level of 0.05) and  $G_s$  ( $P = 0.028$ , at the significant level of 0.05) as compared with the control leaves (Fig. 4). 14 h exposure to  $\text{NO}_2$  led a slight decline in  $G_s$  and increase in  $T_r$  of the treated leaves compared with the control leaves.

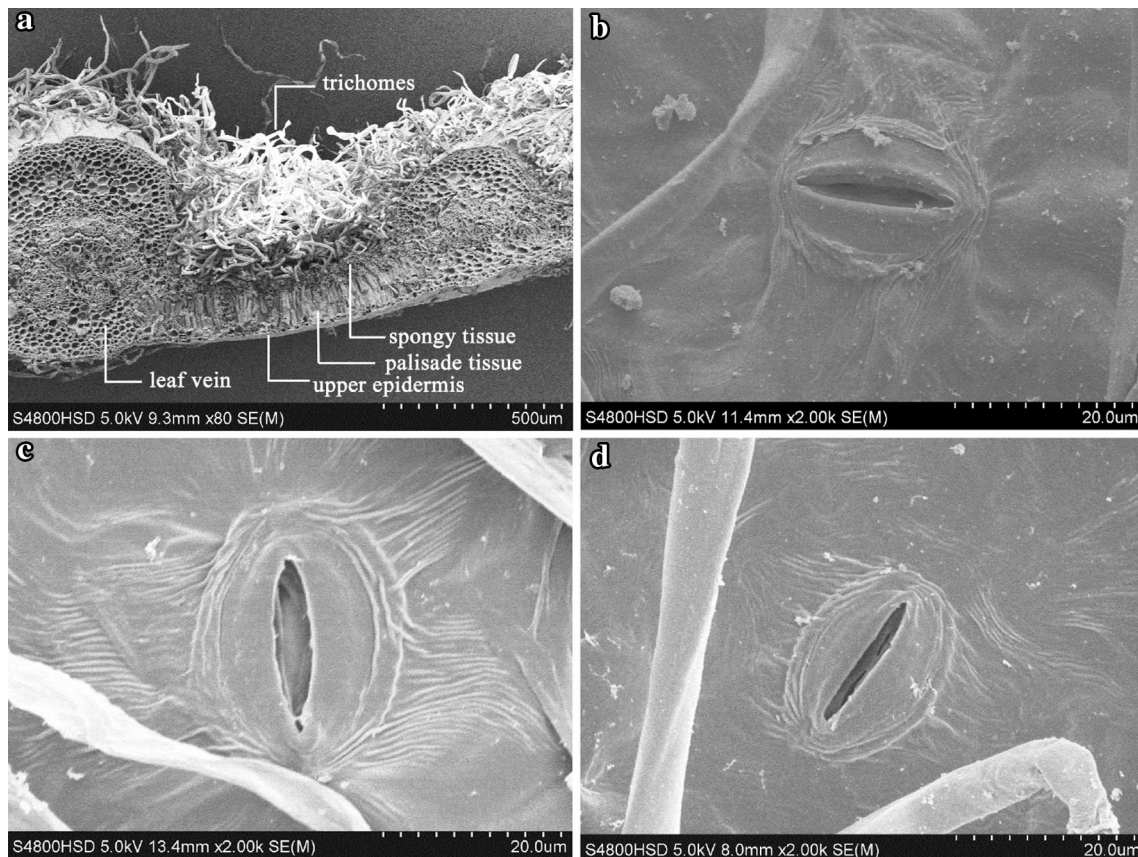
## Discussion

In this study, we found that  $4 \mu\text{l l}^{-1}$   $\text{NO}_2$  had negative influence on net photosynthetic rate ( $P_n$ ) and dark respiration rate ( $R_{\text{dark}}$ ) of leaves of hybrid poplar clone cuttings (*Populus alba*  $\times$  *P. berolinensis*) (Fig. 1). Significant declines in  $P_n$  and  $R_{\text{dark}}$  occurred in the leaves exposed to  $4 \mu\text{l l}^{-1}$   $\text{NO}_2$  for 48 h. Our results are in agreement with

previous research that showed inhibitory effect of gaseous  $\text{NO}_2$  ( $7.39 \mu\text{l l}^{-1}$ , exposed for 2 h) on net photosynthetic rate of the leaves of *Populus euramericana* (Furukawa 1991), but in disagreement with other researchers that hybrid poplar clone cuttings showed stimulatory effects of gaseous  $\text{NO}_2$  on  $\text{CO}_2$  assimilation rates of the leaves of *Populus*  $\times$  *euramericana* 'Dorskamp' (Schmutz et al. 1995) and *Populus*  $\times$  *euramericana* (Siegwolf et al. 2001). Studies with other species such as *Phaseolus vulgaris* (Srivastava et al. 1974a, b) and *Glycine max.* Merr (Carlson 1983; Sabaratnam et al. 1988) also showed inconsistent results of gaseous  $\text{NO}_2$  effects on  $\text{CO}_2$  assimilation rates. The inconsistent results may be caused mainly by differences in gaseous  $\text{NO}_2$  concentration and environmental conditions under which the plants were grown. For example, in the research indicated above, where the stimulatory effects of  $\text{NO}_2$  occurred, the plants were exposed to low concentrations (80–135  $\text{nl l}^{-1}$ ) of gaseous  $\text{NO}_2$ . Besides, it did not evaluate the effects of gaseous  $\text{NO}_2$  on respiration process. Oleksyn (1984) have investigated the effects of gaseous  $\text{NO}_2$  on photosynthesis and respiration processes of *Pinus sylvestris* leaves, and found that 30 min of  $\text{NO}_2$  fumigation led to the decline of net photosynthesis rate, dark respiration rate, and photorespiration rate, but long-term  $\text{NO}_2$  fumigation either increased or unchanged these parameters. In the present study, we only tested the gaseous

**Fig. 2** Correlation between  $P_n$  and leaf temperature (a) and  $\text{NO}_2$  fumigation time (b)/ between  $R_{\text{dark}}$  and leaf temperature (c) and  $\text{NO}_2$  fumigation time (d) in the poplar leaves exposed to  $4 \mu\text{l l}^{-1}$   $\text{NO}_2$ .  $P_n$ , photosynthesis;  $R_{\text{dark}}$ , dark respiration. Statistics analysis was according to the results of  $t$  test at a significance level of 5 % ( $P = 0.05$ ). Asterisk means significant difference at  $P = 0.05$



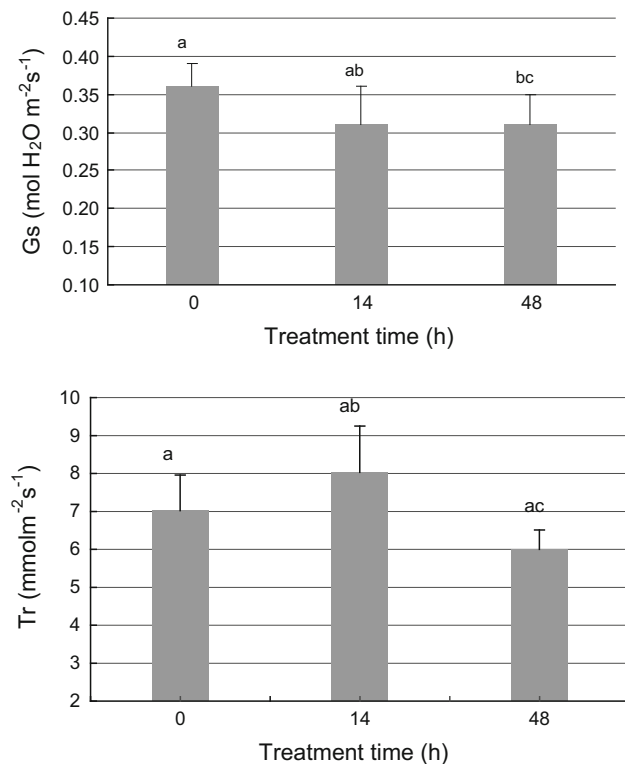


**Fig. 3** Scanning electronic microscope (SEM) images of the leaves of hybrid poplar clone cuttings (*Populus alba* × *P. berolinensis*) when exposed to  $4 \mu\text{l l}^{-1}$   $\text{NO}_2$  for 0 h (a, b), 14 h (c) and 48 h (d) based on two scale bars (20 and 500  $\mu\text{m}$ )

$\text{NO}_2$  effects on photosynthesis and respiration processes for 48 h, and our results showed a declining trend with the increasing time of fumigation (Fig. 2).

Temperature is an important factor affecting plant photosynthesis and dark respiration (Layne et al. 1991). So we analyzed the effects of leaf temperature on photosynthesis and dark respiration of the  $\text{NO}_2$ -treated leaves. There were low correlation coefficients between leaf temperature and  $P_n$  in the same  $\text{NO}_2$  fumigation time (Fig. 2a). Leaf temperature had a significantly positive impact on  $R_d$  of the treated leaves. It agrees with the results of Silim et al. (2010), who found the maximum net photosynthesis rate of *Populus balsamifera* are insensitive to growth temperature relatively to dark respiration rate. The results of Carlson (1983) also showed an inhibitory effect of gaseous  $\text{NO}_2$  on dark respiration and apparent photorespiration. In fact, in the present study, there was a high correlation coefficient ( $R^2 > 0.91$ ) between leaf temperature and  $R_d$  in 0–14 h  $\text{NO}_2$  treatment, but it declined to a low value ( $R^2 = 0.32$ ) in 48-h  $\text{NO}_2$  treatment. So the decline in  $P_n$  and  $R_d$  of 48-h treated leaves was mainly caused by  $\text{NO}_2$  fumigation. The phenomenon of post-illumination  $\text{CO}_2$  burst (PIB) was significantly present in the leaves exposed to gaseous  $\text{NO}_2$

for 48 h. Although we observed PIB in a few leaves exposed to gaseous  $\text{NO}_2$  for 0 and 14 h, this phenomenon was absent in most of the tested leaves (data not shown). The absence of PIB in control leaves might be caused by the masked effects of variable amount of  $\text{CO}_2$  assimilation (Sharkey 1988) and strong mitochondrial respiration during the post-illumination period (Azcon-Bieto and Osmond 1983). Absence of PIB was found at low temperature (13–15 °C) (Kaše and Čatský 1983) and 1 %  $\text{O}_2$ , with the temperature in the range of 15–35 °C (Parys and Romanowska 2000). In our present study, the maximum rate of  $\text{CO}_2$  evolution occurred after 13–15 s of turning the light off. This result was in agreement with the findings of Kaše and Čatský (1983), who reported that at atmospheric  $\text{O}_2$  concentration, the maximum rate of  $\text{CO}_2$  evolution (PIB) of *Phaseolus vulgaris* leaves in each temperature generally occurred at 12–30 s after darkening the leaf. A potential role of photorespiration in foliar uptake of gaseous  $\text{NO}_2$  has been proposed by Hu and Sun (2010). The present study can only demonstrate the inhibitory effects of gaseous  $\text{NO}_2$  on  $\text{CO}_2$  evolution in the light and/or dark, but fails to explain the relationship between photorespiration and foliar  $\text{NO}_2$  uptake. Further research is needed to compare



**Fig. 4** Stomatal conductance ( $G_s$ ) and transpiration rate ( $T_r$ ) of the leaves of hybrid poplar clone cuttings (*Populus alba* × *P. berolinensis*) exposed to  $4 \mu\text{l l}^{-1}$   $\text{NO}_2$  for 0, 14 and 48 h. Values were presented as the mean and standard deviation. Significant differences between exposure times were marked with different letters at the  $P$  values of 0.05 (a and bc) or the  $P$  values of 0.01 (ab and ac)

the differences in  $\text{NO}_2$ -N metabolisms among species having different photorespiration capacities or to evaluate  $\text{NO}_2$ -N metabolisms in species treated with substances that stimulates or inhibits photorespiration.

In this study, we used the SEM/EDS technique to analyze gaseous  $\text{NO}_2$  effects on stomatal dynamics of the hybrid poplar clone cuttings. Our results showed that  $4 \mu\text{l l}^{-1}$   $\text{NO}_2$  resulted in stomatal dysfunction (partial closure of stomata and the decline of stomatal conductance, Figs. 3, 4). Several previous studies have ever reported the impacts of air pollutants (such as  $\text{NO}_2$  and/or  $\text{SO}_2$ ) on the ultrastructure of mesophyll tissues adjacent to stomata. For example, Holopainen et al. (1992) found that air pollutants ( $\text{SO}_2$  and  $\text{NO}_2$ ) have significant impact on the ultrastructure of conifer needles, especially the chloroplasts of mesophyll tissue adjacent to stomata. Gaseous  $\text{SO}_2$  or  $\text{NO}_2$  led to swollen thylakoids and a reduction in the number of grana stacks as compared with the control (Schiffgens-Gruber and Lutz 1992). The results of Rantanen et al. (1994) also demonstrated the significant effects of mixed gaseous  $\text{SO}_2$  +  $\text{NO}_2$  on ultrastructure of mesophyll cells: swelling and slight reduction of thylakoids, enhanced translucence of the plastoglobuli, reduced length of chloroplasts and

starch grains, and increased number of plastoglobuli. Moreover, the deleterious effects of  $\text{SO}_2$ ,  $\text{NO}$ ,  $\text{NO}_2$ , and  $\text{O}_3$  on spruce needles was related to membrane rupture and higher amounts of vacuolar tannins (Tjoelker et al. 2007). In the present study, we only observed the responses of stomata to  $\text{NO}_2$  gas;  $\text{NO}_2$  effects on ultrastructure of guard cells and mesophyll tissue adjacent to stomata was not involved. Toxic effects of gaseous  $\text{NO}_2$  may be mainly caused by generation and accumulation of  $\text{NO}_2$ -derived  $\text{NO}_2^-$  in apoplastic and symplastic space (Yoneyama and Sasakawa 1979; Wellburn 1990; Hu and Sun 2010). However, we did not investigate  $\text{NO}_2^-$  accumulation in the exposed leaves, so the relationship between stomatal behavior and  $\text{NO}_2^-$  accumulation is uncertain.

## Conclusions

This study demonstrated that  $4 \mu\text{l l}^{-1}$  gaseous  $\text{NO}_2$  has significant negative influence on stomata-related physiological processes of *Populus alba* × *P. berolinensis* leaves, particularly photosynthesis and dark- and photo-respiration processes. Photorespiration has a close relation with carbon/nitrogen metabolism. Thus, further research is needed to investigate the relationship between photorespiration, foliar uptake of gaseous  $\text{NO}_2$ , and  $\text{NO}_2$ -N assimilation and compare the differences in  $\text{NO}_2$ -N metabolisms between the species with different photorespiration capacities using photorespiration-stimulatory or -inhibitory substances. Further, estimation of photorespiration rate of leaf exposed to gaseous  $\text{NO}_2$  with more accurate techniques should also be considered in future research.

**Author contribution statement** Dr. Yanbo Hu, corresponding author for experiment design, project conduction, and paper writing; Dr. Muluaem Tigabu and Dr. Bellaloui Nacer, co-authors for project discussion and paper writing; Dr. Jinghong Wang, Jian Diao, Ke Wang, Rui Yang, co-authors for project conduction; Dr. Guangyu Sun, corresponding author for project discussion.

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