



# PM<sub>2.5</sub>-bound SO<sub>4</sub><sup>2-</sup> absorption and assimilation of poplar and its physiological responses to PM<sub>2.5</sub> pollution

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## ABSTRACT

PM<sub>2.5</sub> has raised serious environmental concerns in recent years. Plants that act as sinks for these particles can effectively mitigate air pollution. The major water-soluble inorganic ion of PM<sub>2.5</sub> is SO<sub>4</sub><sup>2-</sup>, whose absorption and assimilation into plants remain unknown. This study simulated the formation of PM<sub>2.5</sub> particles through a PM<sub>2.5</sub> generation system to investigate the uptake and assimilation of PM<sub>2.5</sub>-bound SO<sub>4</sub><sup>2-</sup> in *Populus × euramericana* cv. 'Neva', and to determine the physiological responses of poplar to PM<sub>2.5</sub> pollution. PM<sub>2.5</sub>-bound SO<sub>4</sub><sup>2-</sup> was absorbed and assimilated into poplar leaves after slight and heavy PM<sub>2.5</sub> treatments (SPT and HPT, respectively) by monitoring the changes in the contents of SO<sub>4</sub><sup>2-</sup>, S-containing products, and the activities of SO<sub>4</sub><sup>2-</sup> assimilation enzymes. However, the absorption and assimilation of PM<sub>2.5</sub>-bound SO<sub>4</sub><sup>2-</sup> exhibited different change patterns in the two treatments. In addition, analyses of total N and non-S-containing amino acids indicated that HPT enhanced N assimilation in poplar leaves due to high S treatment, whereas SPT did not evidently affect N metabolism. In response to PM<sub>2.5</sub> pollution, antioxidant defense was triggered with increasing concentration of reactive oxygen species in poplar seedlings. The reduced ascorbate content decreased, whereas the levels of the oxidized ascorbate and soluble sugars increased. These findings could be related to stress resistance. PM<sub>2.5</sub> pollution treatments also influenced photosynthetic physiology. The values of net photosynthetic rate, stomatal conductance, intercellular CO<sub>2</sub> concentration, and transpiration rate decreased and were higher in HPT than in SPT. These results provide novel insights into improving air quality through plants.

## 1. Introduction

Ambient air pollution, especially fine particles (PM<sub>2.5</sub>), has attracted widespread social attention in recent years because of its adverse impact on air quality and human health (Yadav et al., 2010; Sgrigna et al., 2015). PM<sub>2.5</sub> is emitted directly from natural sources or indirectly from heterogeneous chemical reactions (Song et al., 2015). It is considered a carrier of contaminants and toxic species, including water-soluble inorganic ions, carbonaceous species, and elements (Zhang et al., 2014). Water-soluble inorganic ions are the dominant components of PM<sub>2.5</sub>, comprising approximately half of the total components (Chan and Yao, 2008). SO<sub>4</sub><sup>2-</sup> is the most abundant constituent of water-soluble inorganic ions (Kang et al., 2011); this compound is secondarily formed through chemical reactions involving the precursor gas SO<sub>2</sub> (Tian et al., 2016). In the reaction, SO<sub>2</sub> is oxidized into H<sub>2</sub>SO<sub>4</sub> in gas or aqueous phase on the surface of pre-existing particles; then, H<sub>2</sub>SO<sub>4</sub> is condensed or nucleated into new particles (Yin et al., 2014). The conversion of SO<sub>2</sub> to H<sub>2</sub>SO<sub>4</sub> mainly leads to the formation of sulfate, which increases the size of PM<sub>2.5</sub> (Khan et al., 2016).

Plants can intercept or adhere atmospheric particles, such as PM<sub>2.5</sub>, mainly by their leaves (Terzaghi et al., 2013). In our previous study, a large amount of particles were intercepted by poplar leaves; PM<sub>2.5</sub> was the main component of total particles (> 70%) and was abundant in S from motor vehicle emission (Wang et al., 2016). Some scholars have compared the ability of various tree species to capture PM<sub>2.5</sub> particles and performed component analysis and source identification of PM<sub>2.5</sub> on tree leaves (Saebo et al., 2012; Hu et al., 2014; Liang et al., 2016). However, little is known about whether and how PM<sub>2.5</sub>-bound various components are absorbed and assimilated into plants, such as the metabolism of PM<sub>2.5</sub>-bound SO<sub>4</sub><sup>2-</sup>. Furthermore, the responses of plants to PM<sub>2.5</sub> pollution remain poorly understood.

*Populus × euramericana* cv. 'Neva' is a common pollution-resistant tree species used in northern China due to its high survival rate and ability to resist biotic and abiotic stresses. Thus, *P. × euramericana* cv. 'Neva' seedlings were used as experimental materials in the present study. An aerosol generation system was used to simulate the formation of PM<sub>2.5</sub> particles. In the system, sulfate solution was converted to solid particles. The goal of this study was to examine the absorption and

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assimilation of  $\text{PM}_{2.5}$ -bound  $\text{SO}_4^{2-}$ , including the changes of several  $\text{SO}_4^{2-}$  assimilation enzyme activities and related S-containing product contents, as well as the physiological responses of poplar to  $\text{PM}_{2.5}$  pollution. This study expands our knowledge on the absorption and assimilation of  $\text{PM}_{2.5}$  components and the response mechanism of plants to  $\text{PM}_{2.5}$  pollution. Results provided a theoretical basis for improving air quality through plants.

## 2. Materials and methods

### 2.1. Plant materials and growth condition

In mid-April 2015, *P. × euramericana* cv. ‘Neva’ cuttings were planted in individual 10 L pots containing loam soil, turf soil, and vermiculite (6:3:1, v/v/v) in the experimental nursery of Beijing Forestry University, Beijing (40° 00′ N, 116° 20′ E, 49 m above sea level). Experiments were performed in early August 2015 when the average height and stem base diameter of the seedlings were  $82.8 \pm 12.4$  cm and  $7.1 \pm 1.1$  mm, respectively. Experiments with each treatment were performed simultaneously, and the experiments were repeated thrice.

### 2.2. $\text{PM}_{2.5}$ generator

To simulate  $\text{PM}_{2.5}$  formation, an aerosol generator designed by Mai et al. (2013) was used to generate aerosol particles with controlled concentration and size. Liquid particles were first generated using a  $2 \text{ g L}^{-1}$   $\text{Na}_2\text{SO}_4$  solution. The formed liquid particles were dragged by an air flow in a dry pipe and transformed into solid particles with diameter of about 80 nm. The solid particles were subsequently passed into the plant growth chamber (100 cm × 50 cm × 100 cm).  $\text{PM}_{2.5}$  concentration in the chamber was maintained by regulating the rotary flow meter and was measured using the DM1781 Dustmate apparatus (Turnkey Instruments Ltd., Northwich, England, UK). A Schematic diagram of the experimental setup is shown in Fig. 1.

### 2.3. $\text{PM}_{2.5}$ pollution treatments and sampling

$\text{PM}_{2.5}$  at 100 and  $300 \mu\text{g m}^{-3}$  concentrations were set as the slight pollution treatment (SPT) and heavy pollution treatment (HPT), respectively, according to the Chinese ambient air quality standards (GB3095-2012). When the  $\text{PM}_{2.5}$  concentration in the plant growth chamber reached the required level, the poplar seedlings were placed in the chamber and exposed to  $\text{PM}_{2.5}$  particles between 8:30–11:30 a.m. and 2:30–5:30 p.m. for 1 day. A preservative film was placed covering the surface of the pot under the shoot to prevent the generated  $\text{PM}_{2.5}$  particles from entering the soil. Seedlings exposed to particles generated by distilled water instead of  $\text{Na}_2\text{SO}_4$  solution in a similar chamber were used as the controls.

Fresh mature leaves were collected before treatment (0 day) and at 1, 3, 5, and 7 days after  $\text{PM}_{2.5}$  pollution treatment from the treated and control seedlings. Some leaf samples were used to directly analyze  $\text{SO}_4^{2-}$  and total N contents. The remaining samples were immediately frozen in liquid N and stored at  $-80^\circ\text{C}$  for determination of isozymes, amino acids, and antioxidant index.

### 2.4. Determination of $\text{SO}_4^{2-}$ and total N

Leaf samples were thoroughly washed thrice with deionized water to remove the adherent particles. The samples were inactivated at  $105^\circ\text{C}$  for 30 min and dried at  $80^\circ\text{C}$  until constant weight was achieved. According to the method of Smith et al. (1997), the dried samples were ground into fine powder and sieved through 60- and 200-mesh sieves. The dry powder sieved through a 60-mesh sieve was placed into a glass tube with deionized water, boiled for 10 min, transferred to 25 mL volumetric flask, and passed through a  $0.45 \mu\text{m}$  filter. The obtained filtrate was used to analyze  $\text{SO}_4^{2-}$  content through ion chromatography analysis (ICS-2100, Thermo Fisher Scientific, Inc., USA). The dry powder sieved through a 200-mesh sieve was used to determine the total N by using the Vario MACRO cube elemental analyzer (Elementar Analysensysteme GmbH, Germany).

### 2.5. Determination of key enzymes and amino acids related to $\text{SO}_4^{2-}$ assimilation

Leaf samples stored at  $-80^\circ\text{C}$  were used to determine the activities of key enzymes involved in  $\text{SO}_4^{2-}$  assimilation. The activity of adenosine-5'-triphosphate sulfurylase (ATPS) was assayed by measuring the molybdate-dependent formation of pyrophosphate through the method described by Lappartient and Touraine (1996). Sulfite reductase (SiR) activity was determined using the method proposed by Brychkova et al. (2012). O-Acetylserine (thiol) lyase (OASTL) activity was assessed through cysteine (Cys) formation from O-acetyl-serine and sulfide by using the method described by Warrilow and Hawkesford (2000).

Leaf powder passed through an 80-mesh sieve was used to measure S-containing amino acids, namely, Cys and methionine (Met). The powder samples were subjected to hydrolysis according to the method of Hanafy et al. (2013). The total contents of Cys and Met in the samples were measured by using an amino acid analyzer (Sykam, Germany). Glutathione (GSH) level was determined using the enzymatic cycling assay proposed by Nagalakshmi and Prasad (2001).

### 2.6. Determination of non-S-containing amino acids

Twelve non-S-containing amino acids, namely, aspartic acid (Asp), glutamic acid (Glu), valine (Val), serine (Ser), leucine (Leu), arginine (Arg), glycine (Gly), phenylalanine (Phe), threonine (Thr), isoleucine (Ile), histidine (His), and tyrosine (Tyr), were extracted from frozen leaf

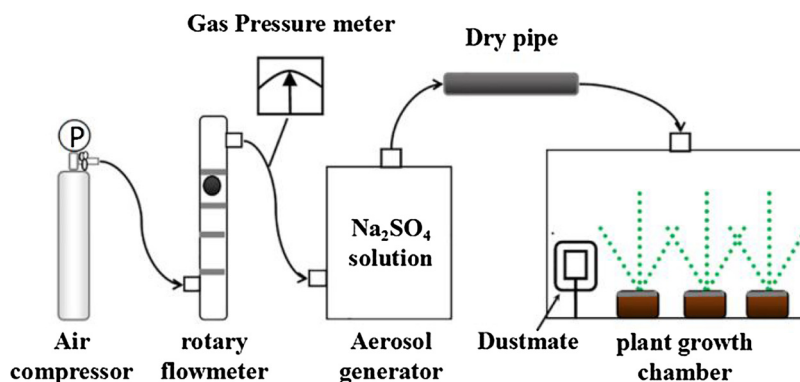


Fig. 1. Schematic diagram of the experimental setup.

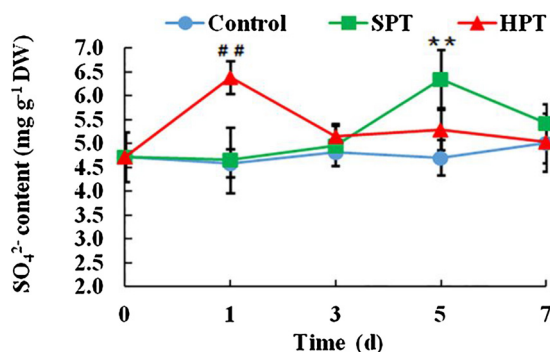


Fig. 2.  $\text{SO}_4^{2-}$  content in *P. × euramericana* cv. 'Neva' leaves after slight and heavy  $\text{PM}_{2.5}$  pollution treatments (SPT and HPT, respectively). All data in the current and following figures or tables represent the mean values of three independent experiments  $\pm$  standard deviation (SD) ( $n = 3$ ). \* indicates significant differences at  $P < 0.05$  and  $P < 0.01$  between the control and SPT. # indicates significant differences at  $P < 0.05$  and  $P < 0.01$  between the control and HPT.

samples by acid hydrolysis through the methods described by Larsen et al. (2009). According to the method of Guo et al. (2013), the 12 amino acids were analyzed by an Agilent 1100 high-performance liquid chromatography system (Agilent Technologies, Inc., Santa Clara, CA, USA) and precolumn derivatization using o-phthalaldehyde and 9-fluorenylmethoxycarbonyl. Amino acids were quantified by comparing the sample peak areas with the standard curves of the external standards (Sigma–Aldrich Corporation, St. Louis, MO, USA). The liquid chromatography software Chemstation Plus Family was used for data acquisition and analysis.

## 2.7. Analyses of antioxidant-related indices

Leaf samples stored at  $-80^\circ\text{C}$  were used to determine the contents of the following: reactive oxygen species (ROS); total ascorbates including reduced ascorbate (ASA) and oxidized ascorbate (DHA); and soluble sugars. ROS was measured using an ELISA kit (Baiminjing Biotech Co., Ltd, Beijing, China). Total ascorbate and ASA contents were assayed at 525 nm in a micro-plate reader through the method of Murshed et al. (2013). DHA content was determined by subtracting the ASA content from the total ascorbate content. Soluble sugars were quantified by phenol–sulfuric acid method described by Dubois et al. (1956).

## 2.8. Photosynthetic parameter analyses

The 9th to 11th mature and fully expanded leaves of the treated and control seedlings were selected to measure the photosynthetic parameters. Net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular  $\text{CO}_2$  concentration (Ci), and transpiration rate (Tr) were measured using a Li–COR 6400 gas exchange system (Li–COR Inc., Lincoln, NE, USA) before the treatment and at 1, 3, 5, and 7 days after the treatment. Pn, Gs, Ci, and Tr were measured in a  $6\text{ cm}^2$  leaf chamber by applying a saturated photosynthetic photon flux density ( $800\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$ ). Leaf temperature, relative humidity, and external  $\text{CO}_2$  concentration were maintained at  $25^\circ\text{C}$ ,  $50\% \pm 5\%$ , and  $430 \pm 10\text{ }\mu\text{mol mol}^{-1}$ , respectively. All measurements were performed between 9:00 and 11:00 daily.

## 2.9. Observation of stomatal morphology

For each treatment, mature leaves were collected from seedlings before the treatment and at 1, 3, 5, and 7 days after the  $\text{PM}_{2.5}$  pollution treatment. The leaves were cut into small cubical portions (about  $5\text{ mm} \times 5\text{ mm}$ ) and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) at  $4^\circ\text{C}$  for 2 h. The samples were then washed with

phosphate buffer thrice and dehydrated in an ascending series of ethanol (50%–100%, 15 min for each concentration). The samples were immersed in tert-butanol and stored at  $4^\circ\text{C}$  for 30 min. The samples were freeze dried and coated with gold–palladium. Scanning electron microscopy analysis (Quanta200, FEI, USA) was conducted to observe the stoma morphology of the leaves under different treatment conditions.

## 2.10. Statistical analysis

Results were subjected to statistical analysis using SPSS 21.0 statistical package. Significant differences were tested by multiple comparisons of least significant difference (LSD) at  $P < 0.05$  and  $P < 0.01$ .

## 3. Results

### 3.1. $\text{PM}_{2.5}$ -bound $\text{SO}_4^{2-}$ absorption and assimilation in *P. × euramericana* cv. 'Neva' leaves exposed to $\text{PM}_{2.5}$ particles

#### 3.1.1. Changes in the $\text{SO}_4^{2-}$ content in *P. × euramericana* cv. 'Neva' leaves

As shown in Fig. 2, the content of  $\text{SO}_4^{2-}$  in poplar leaves increased to varying degrees after slight and heavy  $\text{PM}_{2.5}$  pollution treatments, but the change trends considerably differed between the two treatments. At day 1 after SPT, the content of  $\text{SO}_4^{2-}$  did not change compared with that in the control group. Thereafter, the  $\text{SO}_4^{2-}$  content gradually increased (LSD test,  $P = 0.008$ ), peaked at day 5, and decreased. Meanwhile, the  $\text{SO}_4^{2-}$  content significantly increased (LSD test,  $P < 0.001$ ), peaked at day 1 after HPT, and decreased to near the control level.

#### 3.1.2. Activities of $\text{SO}_4^{2-}$ assimilation-related enzymes in *P. × euramericana* cv. 'Neva' leaves

ATPS, SiR, and OASTL are key enzymes involved in  $\text{SO}_4^{2-}$  assimilation, and their activities can directly reflect the assimilation efficiency of  $\text{SO}_4^{2-}$  in plants (Reich et al., 2016). Fig. 3 shows the changes in the activities of these three key enzymes in poplar leaves after SPT and HPT. The activities of the three enzymes significantly increased (LSD test,  $P = 0.002$ ;  $P = 0.005$ ;  $P < 0.001$ , respectively) at day 1 after SPT and then decreased gradually to a level similar to that in the control. By contrast, the activities of the three enzymes decreased (LSD test,  $P = 0.018$ ) at day 1 after HPT and then increased gradually to near the control level.

#### 3.1.3. Changes in the contents of S-containing amino acid and S-containing substance in *P. × euramericana* cv. 'Neva' leaves

Cys, Met, and GSH are the products of  $\text{SO}_4^{2-}$  assimilation in plants (Per et al., 2017). As shown in Fig. 4, SPT and HPT changed the amino acid contents in poplar leaves. After SPT, the contents of Cys and Met increased in a fluctuating manner and were higher than those in the control to some extent (Fig. 4A and B). After HPT, the Cys content decreased first and then gradually increased to near the control level. The Met content was slightly higher than in the control but lower than that after SPT (Fig. 4A and B).  $\text{PM}_{2.5}$  pollution treatment also affected GSH level in poplar leaves (Fig. 4C). The content of GSH increased after SPT and was higher than in the control. After HPT, the GSH content minimally changed initially and then evidently decreased to a level lower than in the control.

### 3.2. Physiological responses of *P. × euramericana* cv. 'Neva' to $\text{PM}_{2.5}$ pollution

#### 3.2.1. Changes in the contents of total N and non-S-containing amino acids in poplar leaves

The contents of total N and non-S-containing amino acids in poplar leaves exhibited different change patterns after SPT and HPT (Figs. 5 and 6). After SPT, the contents of total N and 12 non-S-containing

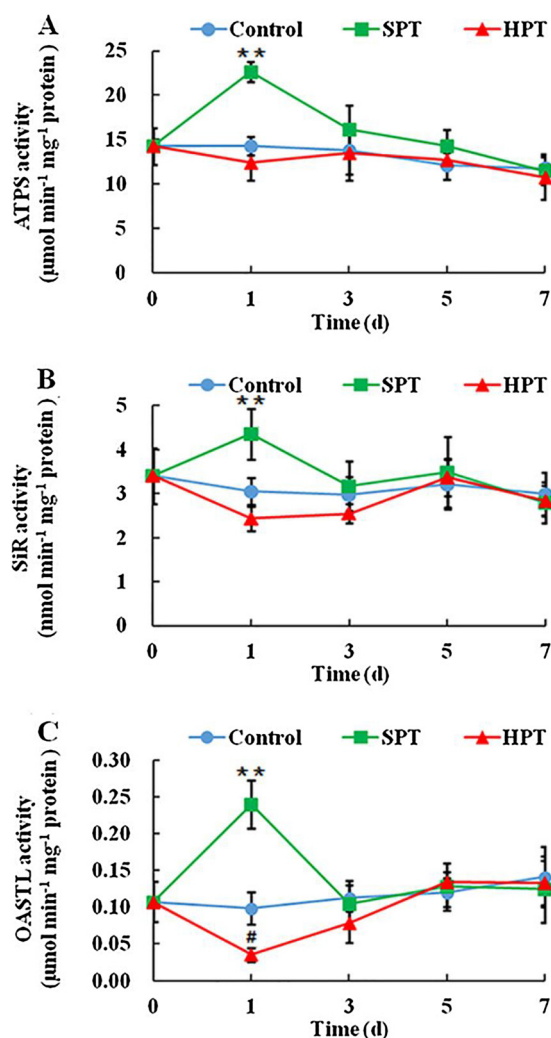


Fig. 3. Activities of ATPS (A), SiR (B), and OASTL (C) enzymes in *P. x euramericana* cv. 'Neva' leaves after slight and heavy  $\text{PM}_{2.5}$  pollution treatments (SPT and HPT, respectively) ( $n = 3$ ). \* indicates significant differences at  $P < 0.05$  and  $P < 0.01$  between the control and SPT. # indicates significant differences at  $P < 0.05$  and  $P < 0.01$  between the control and HPT.

amino acids (Asp, Glu, Val, Ser, Leu, Arg, Gly, Phe, Thr, Ile, His, and Tyr) in poplar leaves did not evidently change. However, the total N and amino acid contents significantly increased after HPT and were higher than in the control.

### 3.2.2. Antioxidant response of poplar leaves to $\text{PM}_{2.5}$ pollution

$\text{PM}_{2.5}$  pollution treatments activated the defense response in poplar leaves (Fig. 7). The ROS content in poplar leaves increased rapidly at day 1 after the two treatments (all LSD test,  $P < 0.001$ ). The ROS content was highest in samples treated with HPT, followed by SPT and the control (Fig. 7A). The ASA content initially decreased (all LSD test,  $P < 0.001$ ) and then increased, whereas the DHA content initially increased (LSD test,  $P < 0.001$ ;  $P = 0.002$ , respectively) and then decreased after SPT or HPT (Fig. 7B and C). The ASA/DHA ratio was lower after the two treatments than in the control (Fig. 7D). In addition, the soluble sugar content increased after SPT and HPT and was not significantly different between the two treatments (Fig. 7E).

### 3.2.3. Photosynthetic physiology responses of poplar leaves to $\text{PM}_{2.5}$ pollution

The SEM image showed that  $\text{PM}_{2.5}$  pollution led to different degrees

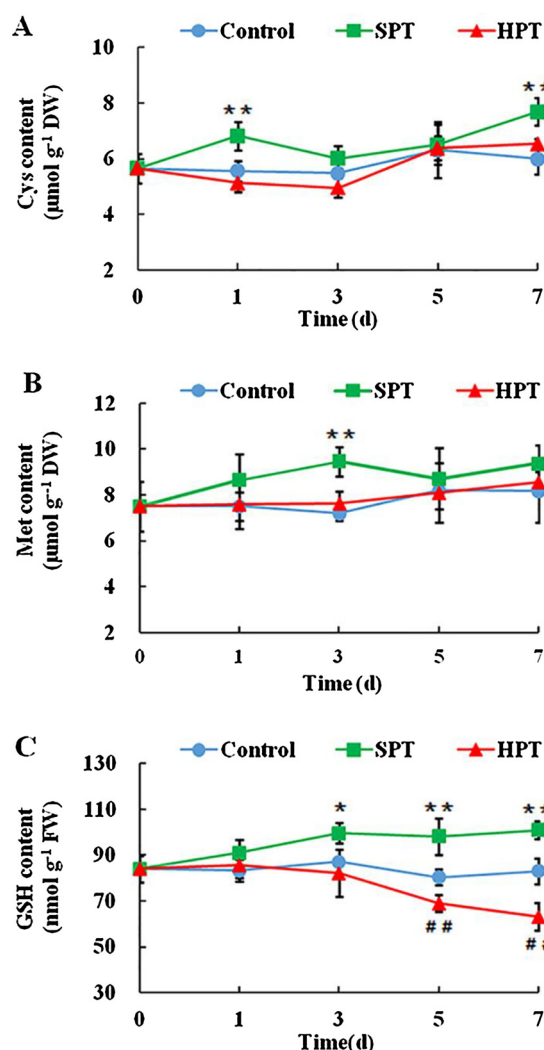


Fig. 4. Contents of Cys (A), Met (B), and GSH (C) in *P. x euramericana* cv. 'Neva' leaves after slight and heavy  $\text{PM}_{2.5}$  pollution treatments (SPT and HPT, respectively) ( $n = 3$ ). \* indicates significant differences at  $P < 0.05$  and  $P < 0.01$  between the control and SPT. # indicates significant differences at  $P < 0.05$  and  $P < 0.01$  between the control and HPT.

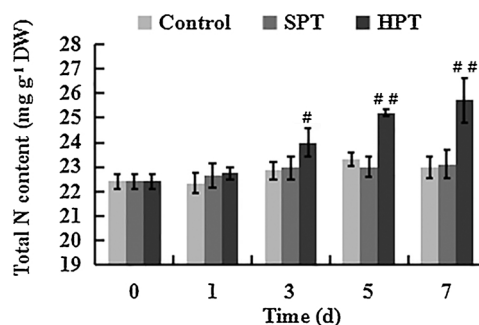


Fig. 5. Total N content in *P. x euramericana* cv. 'Neva' leaves after slight and heavy  $\text{PM}_{2.5}$  pollution treatments (SPT and HPT, respectively) ( $n = 3$ ). \* indicates significant differences at  $P < 0.05$  and  $P < 0.01$  between the control and SPT. # indicates significant differences at  $P < 0.05$  and  $P < 0.01$  between the control and HPT.

of stomatal closure (Fig. 8), which was confirmed by the analysis of stoma length/width ratio (Table 1). The stomatal closure reached the maximum level at day 1 and then gradually decreased after the two treatments. However, HPT resulted in a more severe stomatal closure

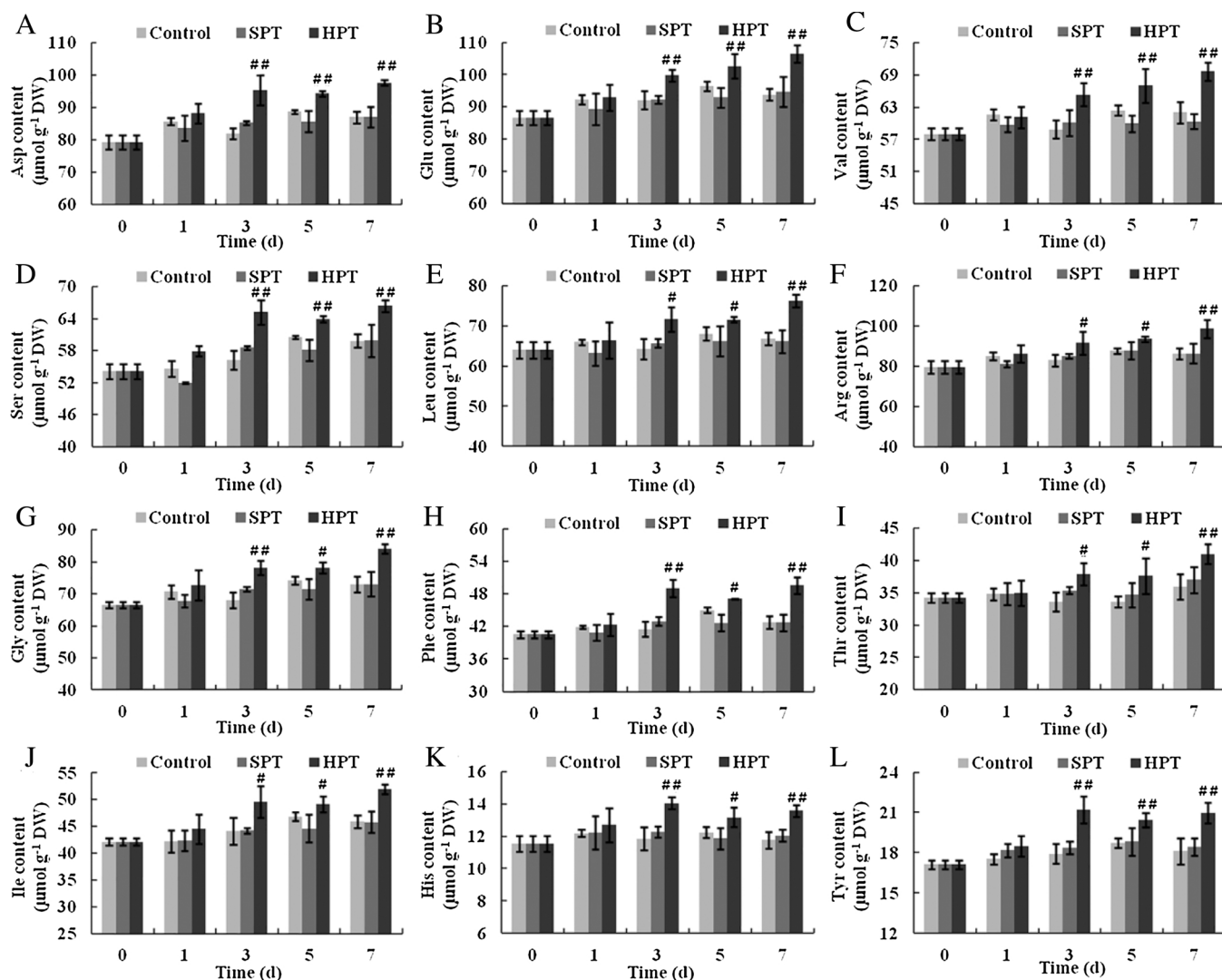


Fig. 6. Non-S-containing amino acids contents in *P. x euramericana* cv. 'Neva' leaves after slight and heavy  $\text{PM}_{2.5}$  pollution treatments (SPT and HPT, respectively) ( $n = 3$ ). \* indicates significant differences at  $P < 0.05$  and  $P < 0.01$  between the control and SPT. # indicates significant differences at  $P < 0.05$  and  $P < 0.01$  between the control and HPT.

than SPT.

The Pn in polar leaves decreased to the lowest point at day 1 after SPT (LSD test,  $P = 0.008$ ) and HPT (LSD test,  $P = 0.001$ ) and then gradually recovered to the level similar to that in the control. The Pn was always lower in HPT than in SPT (Fig. 9A). The Gs decreased to some extent after SPT and HPT, and the decrease was higher in the latter (Fig. 9B). The Ci and Tr in the polar leaves did not evidently change after SPT but were markedly decreased after HPT compared with the control (Fig. 9C and D). In general, HPT exhibited greater effects on Pn, Gs, Ci, and Tr than SPT.

#### 4. Discussion

##### 4.1. Absorption and assimilation of $\text{PM}_{2.5}$ -bound $\text{SO}_4^{2-}$ by *P. x euramericana* cv. 'Neva'

Accumulated evidence showed that  $\text{SO}_4^{2-}$  can be assimilated into S-containing products and stored in cell vacuoles or transported in different tissues and organs after being absorbed by plants (Fig. 10). In plants, Cys is the first formed S-containing amino acid in the  $\text{SO}_4^{2-}$  assimilation pathway; meanwhile, ATPS, SiR, and OASTL are key enzymes involved in Cys biosynthesis. The activities of these three enzymes can directly reflect the assimilation efficiency of  $\text{SO}_4^{2-}$  (Reich

et al., 2016). Cys can be further transformed into S-containing amino acid (Met) and S-containing substance (GSH) (Per et al., 2017). Cys, Met, and GSH are also involved in the long-distance transportation of sulfur (Zalm et al., 2005).

In this study, the  $\text{SO}_4^{2-}$  content increased in poplar leaves after SPT and HPT (Fig. 2), thereby indicating that  $\text{PM}_{2.5}$ -bound  $\text{SO}_4^{2-}$  could be absorbed by poplar. However, the absorption and assimilation of  $\text{SO}_4^{2-}$  exhibited different change patterns under the two treatments. At the initial stage after SPT (day 1), the  $\text{SO}_4^{2-}$  content did not significantly change in poplar leaves (Fig. 2), whereas the activities of  $\text{SO}_4^{2-}$  assimilation enzymes and the contents of S-containing products (Cys, Met, and GSH) increased (Figs. 3 and 4). These results suggest that the absorbed  $\text{SO}_4^{2-}$  could be rapidly assimilated into S-containing products through the catalysis of  $\text{SO}_4^{2-}$  assimilation enzymes at the early stage after SPT. At day 1 after HPT, the contents of  $\text{SO}_4^{2-}$  increased, whereas the activities of  $\text{SO}_4^{2-}$  assimilation enzymes decreased to some extent. The content of S-containing products showed no distinct changes (Figs. 2–4). This phenomenon suggested that the HPT initially caused a stress on poplar, thereby decreasing the activities of  $\text{SO}_4^{2-}$  assimilation enzymes. Thus, the absorbed  $\text{SO}_4^{2-}$  could not be rapidly assimilated into S-containing products, leading to  $\text{SO}_4^{2-}$  accumulation in poplar leaves.

At the late stages after SPT (days 3–7), the contents of  $\text{SO}_4^{2-}$ , Cys,

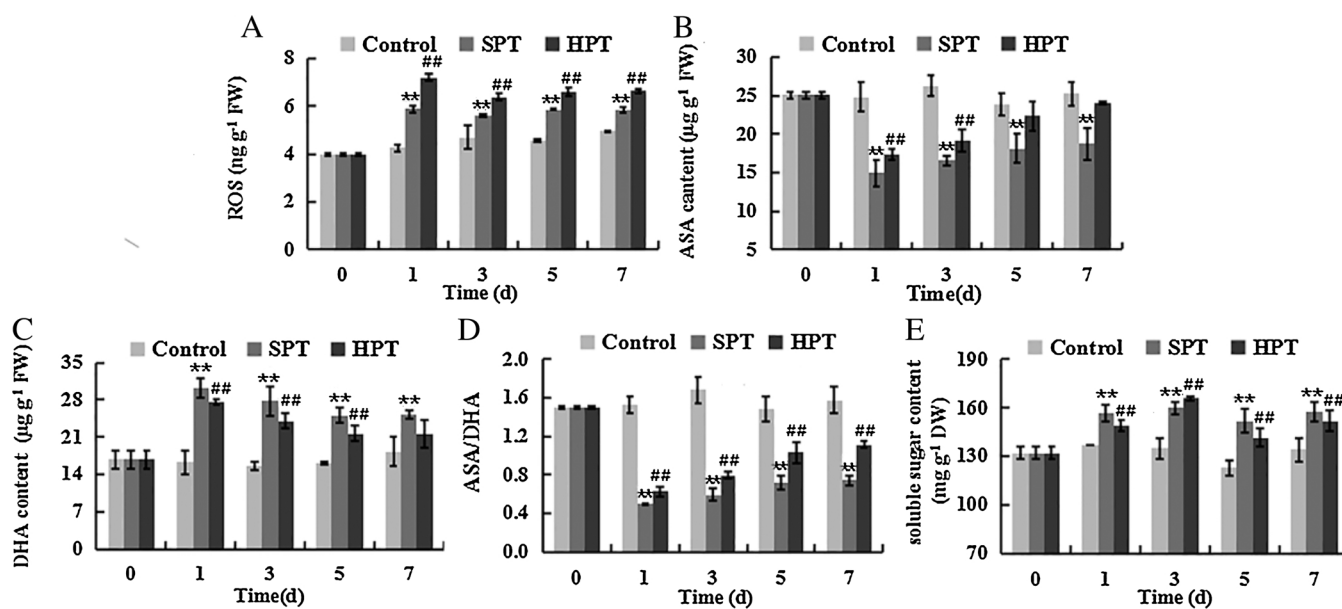


Fig. 7. Contents of ROS (A), ASA (B), DHA (C), ASA/DHA (D), and soluble sugar (E) in *P. × euramericana* cv. 'Neva' leaves after slight and heavy PM<sub>2.5</sub> pollution treatments (SPT and HPT, respectively) ( $n = 3$ ). \* indicates significant differences at  $P < 0.05$  and  $P < 0.01$  between the control and SPT. # indicates significant differences at  $P < 0.05$  and  $P < 0.01$  between the control and HPT.

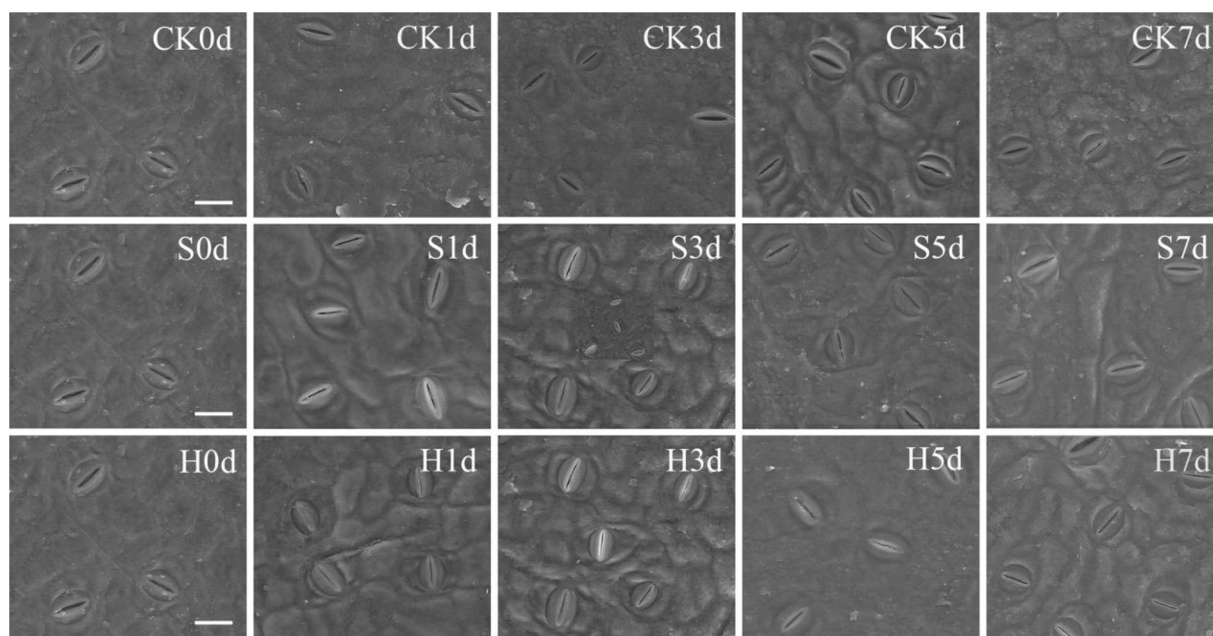


Fig. 8. Scanning electron microscopy micrographs of stomata in *P. × euramericana* cv. 'Neva' leaves after PM<sub>2.5</sub> pollution treatments (2000×). Control check (CK), slight PM<sub>2.5</sub> pollution treatment (S), and heavy PM<sub>2.5</sub> pollution treatment (H). The numbers and lowercase letters following the uppercase letters represent the days of after treatments. Bar = 20 μm.

Met, and GSH increased to some extent, whereas the activities of  $\text{SO}_4^{2-}$  assimilation enzymes gradually decreased to the levels similar to those in the control (Figs. 2–4). The absorbed  $\text{SO}_4^{2-}$  could remain partly assimilated into S-containing products, but the decreased enzyme activities reduced the  $\text{SO}_4^{2-}$  assimilation, thereby causing the significant accumulation of  $\text{SO}_4^{2-}$  in poplar leaves at day 5. At days 3–7 after HPT, the  $\text{SO}_4^{2-}$  content gradually decreased, whereas the activities of  $\text{SO}_4^{2-}$  assimilation enzymes gradually increased to near the control level (Figs. 2 and 3). The recovery of the activities of  $\text{SO}_4^{2-}$  assimilation enzymes enhanced the  $\text{SO}_4^{2-}$  assimilation, thereby reducing the  $\text{SO}_4^{2-}$  accumulation to some extent in poplar leaves. Previous studies showed that GSH is an important form of organic sulfur that can be transported

to various parts of plants, where it is decomposed into amino acids to produce proteins; such proteins sustain growth under stress (Blake-Kalff et al., 1998; Jung et al., 2016). Therefore, the reduced GSH content in poplar leaves at later stages after HPT could be related to the transport of sulfur.

#### 4.2. Effect of PM<sub>2.5</sub> pollution treatments on the contents of total N and non-S-containing amino acids in *P. × euramericana* cv. 'Neva'

N assimilation is related to S metabolism, and the requirement of one depends on the supply of the other (Habtegebrail and Singh, 2006; Anjum et al., 2012). Varin et al. (2009) investigated the effect of three

**Table 1**

Changes in stomatal closure (length/width ratio) of *P. × euramericana* cv. 'Neva' leaves after slight and heavy PM<sub>2.5</sub> pollution treatments.

| Time after treatment (days) | Control       | Slight pollution | Heavy pollution |
|-----------------------------|---------------|------------------|-----------------|
| 0                           | 8.06 ± 0.71Aa | 8.06 ± 0.71Ab    | 8.06 ± 0.71Ad   |
| 1                           | 8.20 ± 1.17Ca | 11.66 ± 0.56Ba   | 31.31 ± 1.32Aa  |
| 3                           | 8.29 ± 0.55Ca | 10.55 ± 0.33Ba   | 16.55 ± 1.51Ab  |
| 5                           | 8.29 ± 1.04Ba | 9.05 ± 1.01Bb    | 10.92 ± 0.99 Ac |
| 7                           | 8.15 ± 0.88Ba | 8.39 ± 0.81Abb   | 9.73 ± 0.54 Acd |

Data represent the mean values from three independent experiments ± SD. The different letters indicate significant differences ( $P < 0.05$ ). Different lowercase letters represent significant differences among different sampling times in the same treatment. Different uppercase letters represent significant differences among different treatments at the same time.

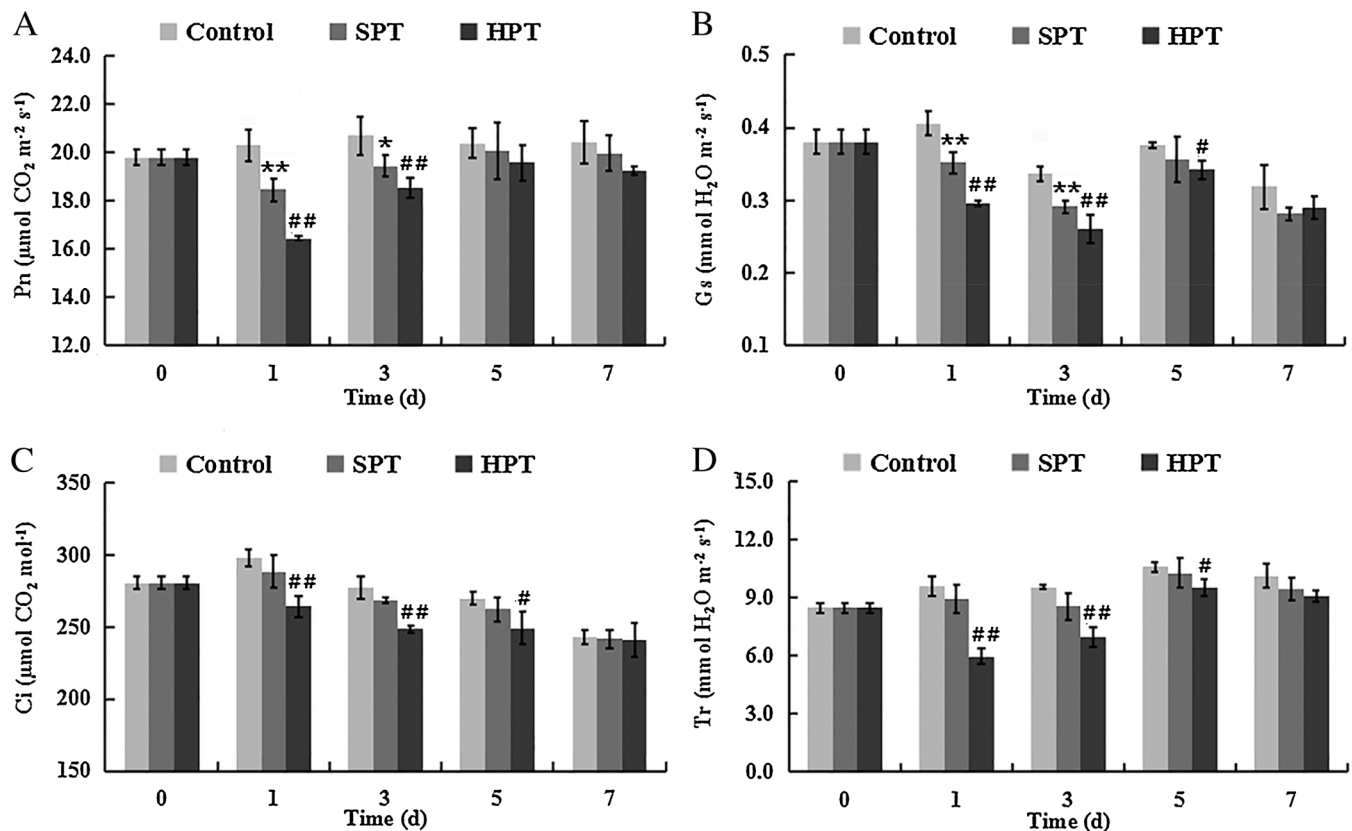
concentrations of  $\text{SO}_4^{2-}$ : “Low S,” “Medium S,” and “High S,” on the growth of *Trifolium repens* in a hydroponic system. Appropriate S availability (Medium S) increased the N content. Ahmad et al. (2010) conducted a field experiment to determine the interactive effect of S and N on *Ammi majus* and reported that sulfur application significantly enhanced N assimilation. In the present study, the total N content in poplar leaves changed minimally after SPT, but it evidently increased after HPT (Fig. 5). Given that the poplar seedlings were exposed to PM<sub>2.5</sub> particles generated from the sulfate solution, HPT led to a higher  $\text{SO}_4^{2-}$  level than SPT. The results suggest that the N assimilation pathway could be enhanced by higher  $\text{SO}_4^{2-}$  level in poplar leaves and the S supply exerted a concentration-dependent effect on N assimilation. The concentration effect of S supply on N assimilation varies among different plant species. Increased N content is usually accompanied by enhanced amino acid synthesis (Anjum et al., 2012). Angell

et al. (2014) revealed that enhanced N assimilation increased amino acid synthesis in a selective culture of seaweeds. The contents of amino acids did not considerably change after SPT but increased after HPT (Fig. 6). Hence, the increased total N content could be attributed to the enhanced synthesis of non-S-containing amino acids.

#### 4.3. Antioxidant response of *P. × euramericana* cv. 'Neva' to PM<sub>2.5</sub> pollution

Exposure of plants to various stresses may disturb the equilibrium between the production and scavenging of ROS (Liu et al., 2011). Adverse conditions can enhance the generation of ROS and lead to oxidative damage in plant cells (Deng and Jin, 2013; Mostofa et al., 2015). In this study, the content of the generated ROS was highest in samples after HPT, followed by SPT and the control (Fig. 7A). Hence, PM<sub>2.5</sub> treatment caused oxidative stress on poplars, and HPT led to more severe stress than SPT.

Ascorbates, including their reduced and oxidized forms (ASA and DHA), play a critical role in stress resistance by either directly or indirectly scavenging ROS and its products (Anjum et al., 2014). In antioxidative processes, ASA is oxidized into DHA to scavenge ROS (Nahar et al., 2017). Xu et al. (2010) indicated that with the generation of  $\text{H}_2\text{O}_2$ , the ASA content decreased, whereas the DHA content increased, thereby improving the capability of cucumber seedlings to withstand high-temperature stresses. Under Al stress, the ratio of ASA to DHA decreased due to the conversion of ASA to DHA during ROS scavenging in mungbean seedlings (Nahar et al., 2017). The DHA content increased, whereas the ASA and ASA/DHA ratios decreased in the treated poplar seedlings (Fig. 7B–D), thereby indicating that poplars could enhance resistance to PM<sub>2.5</sub> pollution stress by oxidizing ASA into DHA. Several studies showed that the ASA/DHA ratio gradually decreased



**Fig. 9.** Pn (A), Gs (B), Ci (C), and Tr (D) in *P. × euramericana* cv. 'Neva' leaves after slight and heavy PM<sub>2.5</sub> pollution treatments (SPT and HPT, respectively) ( $n = 3$ ). \* indicates significant differences at  $P < 0.05$  and  $P < 0.01$  between the control and SPT. # indicates significant differences at  $P < 0.05$  and  $P < 0.01$  between the control and HPT.

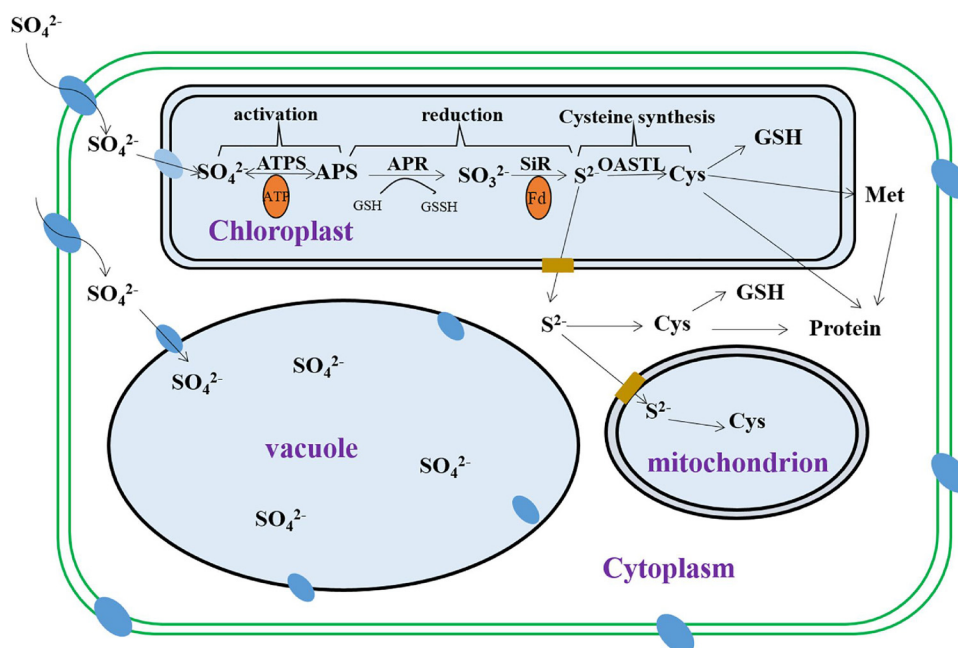


Fig. 10. Schematic of primary  $\text{SO}_4^{2-}$  assimilation in plants (self-drawing).

with increasing stress within a certain range (Deng and Jin, 2013; Homaei and Ehsanpour, 2016). In the present study, the ratio was higher in HPT than in SPT. This result could be explained by the following statement: if we assume that the stability of antioxidant system decreased to some extent when the stress intensity reached a certain threshold, the oxidation of ASA into DHA could not be enhanced continually but slightly decreased in HPT.

Sugars play critical roles in plant growth and development, crop yield and quality, and response to abiotic stresses (Ma et al., 2017). In a previous study, the soluble sugar content increased when sweet potato was exposed to drought conditions, thereby leading to controlled osmotic potential in the leaf and stomatal closure for water loss prevention (Yooyongwech et al., 2017). The soluble sugar content increased after the two treatments compared with the control (Fig. 7E), thereby suggesting that poplar leaves could increase the soluble sugar content to resist  $\text{PM}_{2.5}$  pollution stress.

#### 4.4. Photosynthetic physiology response of *P. × euramericana* cv. 'Neva' to $\text{PM}_{2.5}$ pollution

Stomatal closure is an early physiological response of plants to stress; this phenomenon restricts  $\text{CO}_2$  diffusion into the cells and chloroplasts and ultimately affects photosynthetic physiology (Arias-Moreno et al., 2017).  $\text{PM}_{2.5}$  pollution led to stomatal closure in poplar leaves, and HPT induced more severe stomatal closure than SPT (Fig. 8, Table 1). Stomatal closure may be one of the underlying reasons for Pn reduction (Fig. 9A). In addition to stomatal limitation, particles deposited on the leaves of plants affect the light available for photosynthesis to a certain extent, thereby resulting in non-stomatal limitation for the decline in Pn (Nowak et al., 2014). After SPT, the Pn and Gs decreased, whereas the Ci remained unchanged (Fig. 9A–C). This finding suggests that non-stomatal limitation could be the main reason for Pn reduction in poplar leaves. After HPT, the notable Pn reduction was accompanied by decreased Gs and Ci (Fig. 9 A–C), suggesting that the combination of stomatal and non-stomatal limitations is responsible for the suppression of photosynthesis. In addition, stomata control water loss in plants, and stomatal closure affects Tr (Yang et al., 2014). Tr and Gs markedly decreased after HPT (Fig. 9B and D), thereby indicating that the declined Tr could be due to the decreased Gs in poplar leaves. The decreases in Pn, Gs, Ci, and Tr were greater in HPT than

those in SPT (Fig. 9), revealing that HPT caused more severe stress on poplars than SPT.

## 5. Conclusion

The  $\text{PM}_{2.5}$ -bound  $\text{SO}_4^{2-}$  could be absorbed and assimilated into poplar leaves after SPT and HPT by simulating the formation of  $\text{PM}_{2.5}$  particles. However, the absorption and assimilation of  $\text{SO}_4^{2-}$  displayed different changing trends between the two treatments. HPT promoted N assimilation in poplar leaves due to high S treatment, whereas SPT showed little effect on N metabolism. Both SPT and HPT triggered the antioxidant defense in poplar. These treatments decreased ASA/DHA ratio and increased soluble sugars, thereby leading to enhanced resistance to  $\text{PM}_{2.5}$  pollution stress. Photosynthetic physiology was also influenced by  $\text{PM}_{2.5}$  pollution. The levels of Pn, Gs, Ci, and Tr decreased to varying degrees in the two treatments, and HPT had greater effect than SPT. This work gives novel insight into the different mechanisms underlying the response of poplar to different levels of  $\text{PM}_{2.5}$  pollution.

## 6. Author's contribution

HG, corresponding author of the manuscript, designed experiments and analyzed data. QY and HW performed experiments and wrote the paper. JW and ML participated in the experiments. CL, XX, and WY supervised the experimental work

## 7. Conflict of interest

The authors declare that they have no conflict of interest.

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