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ORIGINAL ARTICLE

# The Response of Plant Photosynthesis and Stomatal Conductance to Fine Particulate Matter ($PM_{2.5}$) based on Leaf Factors Analyzing

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**Abstract** The effects of particles on the photosynthesis of vegetation, which is a sink for fine particulate matter ($PM_{2.5}$) deposition, are still not well understood. Here, we carried out indoor measurements to evaluate the variation dynamics of net photosynthetic rate and stomatal conductance of four plant species with different leaf characteristics under different $PM_{2.5}$ levels. Then tree leaves were sampled and the groove proportion, leaf trichome density, stomatal density and stomatal size were quantitatively studied by scanning electron microscopy (SEM). The stomatal conductance of the 4 species had a close positive correlation with photosynthetic rate. Net photosynthetic rate and stomatal conductance declined over time at elevated $PM_{2.5}$, and the rate of the decline became more rapid with higher concentration of $PM_{2.5}$. The inhibiting effect might be caused by the closure of the stomata and the decrease of stomatal conductance, which was proved by the reduction of the stomatal size of under the condition of $PM_{2.5}$ pollution. Leaf trichome and groove seemed to show a protective role for plants from $PM_{2.5}$ exposure and be responsible for the difference of photosynthetic rate and stomatal conductance under the condition of $PM_{2.5}$ pollution. The higher groove proportion and the presence of trichomes on the leaf surface in *Neolitsea aurata* and *Lindera kwangtungensis* absorbed some particulate matters and buffered the effect of $PM_{2.5}$ pollution on stomata.

**Keywords:** Leaf morphology, Leaf trichome and groove, Net photosynthetic rate, $PM_{2.5}$, Stomata, Stomatal conductance

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## Introduction

As a common air pollutant, $PM_{2.5}$ (Fine particulate matter) has become one of the most important air pollutants in the world, which has attracted wide attention (Janssen 1997). $PM_{2.5}$ is mainly discharged from power stations, vehicle exhaust, industrial processors and so on, and reacts with secondary particles in the atmosphere to form nitrogen oxides (NOX), sulfur dioxide ($SO_2$), ammonia and volatile organic compounds (VOCs) (Bosco 2005). $PM_{2.5}$ has caused serious public health concerns because of the easy penetration of particles through the alveoli. And the associated pollution problems have become increasingly serious due to global climate change (Gilmour 2004), such as reduction of air visibility, forest damage, crop loss, and decrease of ecosystem diversity (Seigneur et al. 2000; Gallagher et al. 2002; Finer et al. 2008). Phytoremediation has attracted considerable attention for its ability to purify air by reducing particles velocity and capturing particles (Popek et al. 2015). Trees can intercept and adhere to $PM_{2.5}$ by leaf surface roughness and trichomes (Wang et al. 2008), and $PM_{2.5}$ can also be directly absorbed through stomatas and lenticels (Nowak et al. 2013; Schaubroeck et al. 2014), which eventually affecting $PM_{2.5}$ in the air. Rita Baraldi's study also showed that species with hairy, waxy, and rough leaves had higher particle retention potential than those without hairy and smooth leaves (Baraldi 2018). Therefore, forest ecosystem has the function of reduction of pollution and purifying the air (Escobedo et al. 2011). However, $PM_{2.5}$ may also block stomatas in plant leaves, affect plant respiration, and ultimately reduce plant photosynthesis (Hirano et al. 1995).

Although trees contribute to mitigate the effects of air pollution, their responses to air pollution are highly species-specific due to high levels of environmental air pollution (Larcher 2003). At present, some scholars have studied the mechanism of settling particulates by different tree species from the perspective of leaf structural morphology. Amit Pal


**Fig. 1.** Photographs of leaves of four selected species in the study.

**Table 1.** Main characteristics of leaves associated with $PM_{2.5}$ adsorption for selected species in our study

| Species | Roughness of leaf surface | Covered by leaf trichome |
|---|---|---|
| *Neolitsea aurata* | Coriaceous | yes |
| *Gordonia acuminata* | Coriaceous | no |
| *Lindera kwangtungensis* | Papery | yes |
| *Rhamnus esquirolii* | Papery | no |

studied the changes of leaf micromorphology and structure of eight plants in India under different levels of pollution, which showed that the thickness of waxy layer, villi length and stomatal density of leaves changed significantly under heavy pollution (Pal 2002). Takashi Kiyomizu's study show that Rhododendron and duckweed could avoid air pollution by regulating stomatal density, while Ginkgo biloba could tolerate air pollution through its small stomatal density and large mesophyll thickness (Kiyomizu 2018). The plugging effect of particles on stomata is also documented (Hirano et al. 1995; González et al. 2014). The effect of $PM_{2.5}$ pollution on leaf surface morphology may result in stomatal blockage and decreased stomatal conductance, which has a negatively effects on gas exchange and water absorption (Baldocchi and Meyers 1998; Bukaveckas et al. 2011). And eventually photosynthetic characteristics of plants is changed (Pryzybysz et al. 2014). Therefore, a clear understanding of the photosynthetic responses of different tree species to atmospheric $PM_{2.5}$ pollution is essential for improving the benefits of the multiple services of forest ecosystem.

Because of the effect of particulate matters deposition on the leaf stomatal conductance and net photosynthetic rate, it is necessary to study the role of leaf surface micro-morphology in this process. Pervious works qualitatively examined on the leaf roughness, leaf hair, stomatal density, and stomata size (Sæbø et al. 2012). However, a detailed quantitative research of the influence of leaf morphology is important. In this paper, the variations of stomatal conductance and net photosynthetic rate of 4 tree species under different concentrations of $PM_{2.5}$ were examined using a chamber device. Then tree leaves were sampled and the groove proportion, leaf trichome density, stomatal density and stomatal size were quantitatively studied by scanning electron microscopy (SEM). In order to explore the effect and mechanism of atmospheric particles on plant photosynthesis, we analyze the interaction between leaf morphology and stomatal conductance, expecting to provide basis for forest management and tree species selection under high pollution conditions in contemporary

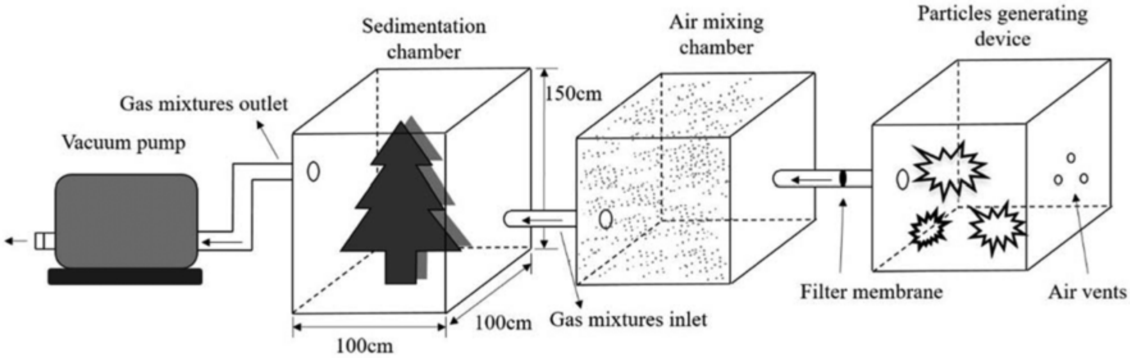


**Fig. 2.** Schematic of the mixture chamber.

developing countries.

## Results and Discussion

### Photosynthetic Physiological Parameters of 4 Selected Species

The variation in net photosynthetic rate and stomatal conduct under the condition of different photosynthetically active radiation without $PM_{2.5}$ was shown in Fig. 3A and Fig. 3B. The trend of net photosynthetic rate and stomatal conduct was similar, presenting parabolic shape with a single peak in each curve. The $P_n$ and $g_s$ values of each species was proportional increased in the range of 0-500 μmol $m^{-2}s^{-1}$, and then the growth rate was weakened in the range of 500-1300 μmol $m^{-2}s^{-1}$. When PAR was over1300 μmol $m^{-2}s^{-1}$, the $P_n$ and $g_s$ of some species began to decrease and the remaining species such as *Gordonia acuminate* continued to rise until reaching the peak. Overall, the $P_n$ *of Gordonia acuminate* was highest comparing with other species, and the $g_s$ *of Lindera kwangtungensis* was the lowest in the 4 species. The Fig. 3C showed the relationship between $P_n$ and $g_s$. $P_n$ showed an exponential increase ($P < 0.01$) with the increase of $g_s$ and $R^2$ values ranged from 0.9323 to 0.9929. This result illustrated that the $g_s$ of 4 species had a close positive correlation with the $P_n$, which was similar to the study of Wong. And a series of models were established base on it, which means that the change of stomatal conductance could maximize carbon capture and minimize water loss, and ultimately affect photosynthetic rate (Cowan, 1978; Wong, 1985a, b, c).

### Effect of Fine Particulate Matters on Photosynthesis

Fig. 4 showed the variation of photosynthetic rate and stomatal conductance of 4 tree species with the time under different concentrations of $PM_{2.5}$. The variation extent of $P_n$ and $g_s$ was different significantly among different species. On the whole, the decrease of $P_n$ and $g_s$ of *Rhamnus esquirolii* and *Lindera kwangtungensis* was small and the values were 9.90% and 28.51% (*Rhamnus esquirolii*) and 13.16% and 22.81% (*Lindera kwangtungensis*). *Neolitsea aurata* decreased most and the values were 20.73% and 32.11%. This difference of $P_n$ and $g_s$ among different species might be caused by the roughness of leaf surface (Terzaghi et al. 2013). The roughness of leaf surface played an important role in the absorption of $PM_{2.5}$ because of the different of water potential of different leaves. The distribution of cuticle wax and moisture absorption

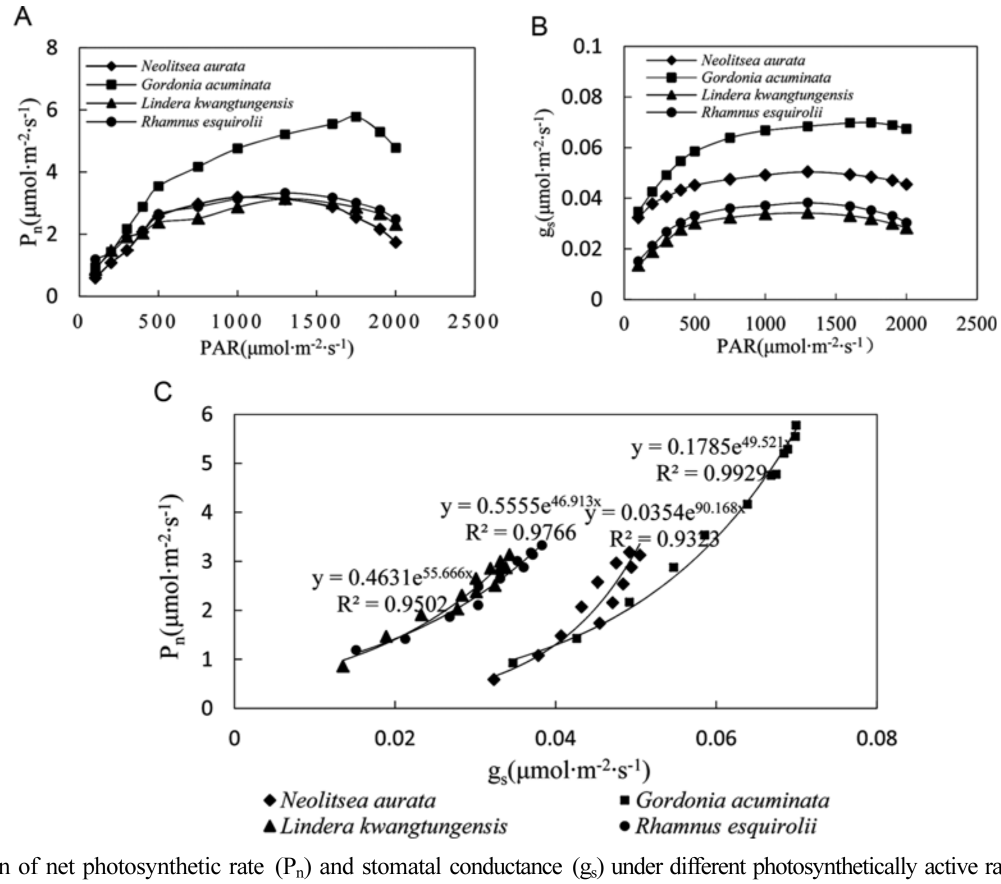


**Fig. 3.** Variation of net photosynthetic rate ($P_n$) and stomatal conductance ($g_s$) under different photosynthetically active radiation gradients without $PM_{2.5}$.

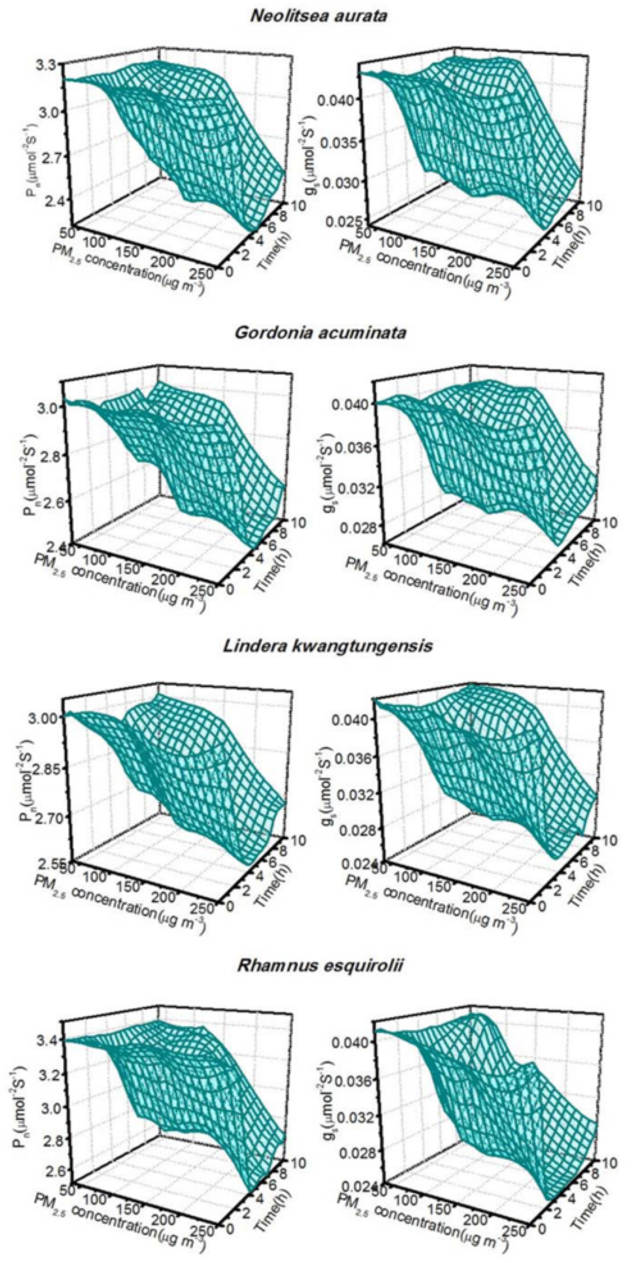


**Fig. 4.** Variation of net photosynthetic rate and stomatal conductance in different $PM_{2.5}$ concentration levels in the mixture chamber of four selected species.

of membrane structure on leaf surface of papery-leaved species was more difficult to be affected by external environment (Sæbø et al. 2012). Our results showed that the variation of $P_n$ and $g_s$ of 4 species had a high consistency, which might be due to the synergistic effect of plant physiological function (Xu and Baldocchi 2003). This result was consistent with our research in the 3.1 section, which meant $g_s$ affected the variation of $P_n$. Fig. 4 also showed that the effects of different concentrations of $PM_{2.5}$ on photosynthesis were significantly different under the controlled conditions of environmental factors in the chamber. When the concentration of $PM_{2.5}$ belowed 50 μg m$^{-3}$, the $P_n$ and $g_s$ of 4 species changed little. But with the increase of $PM_{2.5}$ concentration, net photosynthetic rate and stomatal conductance decreased rapidly. When the input concentration of $PM_{2.5}$ was μg m$^{-3}$, $P_n$ and $g_s$ decreased to the bottom 3-5 hours after the start of the gas mixture supply and then returned to the position 10-34% lower than original value. In addition, the stomatal conductance of *Gordonia acuminate* and *Rhamnus esquirolii* decreased faster than that of *Neolitsea aurata* and *Lindera kwangtungensis* under the influence of $PM_{2.5}$. The reason might be the delayed effect of $PM_{2.5}$ on stomatal conductance due to the difference of grooves and trichomes on leaf surface of 4 species. When fine particles deposited on leaves, grooves and trichomes contacted first and absorbed some particulate matters, which providing a buffer time for plant leaves.to close the stomata

## The Variation of Stomatal Morphology and Number Under Different $PM_{2.5}$ Concentrations

The stomatal morphology of 4 species studied were illustrated in Fig. 5. The leaves of *Neolitsea aurata* had stomata on both the obverse and the reverse, and their shape was elliptical. Stomatal density and size of *Neolitsea aurata* were small, while the surrounding cells were bulged and the outer area was smooth. The stomata of *Gordonia acuminata* were on the back of the leaves and appeared circular in shape. The stomatal density and size were larger. And due to the uneven arrangement of cells around the stomata, it was advantageous to the retention of dust particles. The stomata of *Lindera kwangtungensis* were also on the back of the leaves and appeared elliptical in shape. Stomatal size was smaller than that of *Gordonia acuminata*, and the stomata were surrounded by tuberculate or verrucous protuberance. The stomata of *Rhamnus esquirolii* appeared elliptical and the surrounding area was smooth, which were on the back of the leaves. In addition, it could be seen from Fig. 5 that the stomatal morphology of plant leaves changed under the condition of $PM_{2.5}$ pollution. Some stomata of plant leaves were in closed and semi closed state due to stomatal blockage. In previous studies, more qualitative analysis of stomatal morphology was carried out, but few quantitative studies were conducted (Chai et al. 2002). In our study, the stomatal density was counted on the fixed leaf area, and the stomatal size was quantified. Fig. 6 showed that there was no significantly change in the stomatal density of the leaves of four different plants under different levels of $PM_{2.5}$ concentrations. Meanwhile, stomatal size did not change significantly at 35, 75 and 115 g m$^{3}$ $PM_{2.5}$ concentrations, but decreased significantly at 150 and 250 g m$^{3}$ $PM_{2.5}$ concentrations (Fig. 7). *Neolitsea aurata*, *Gordonia acuminata*, *Lindera kwangtungensis* and *Rhamnus esquirolii* were reduced by 6.31-31.24% respectively, and the

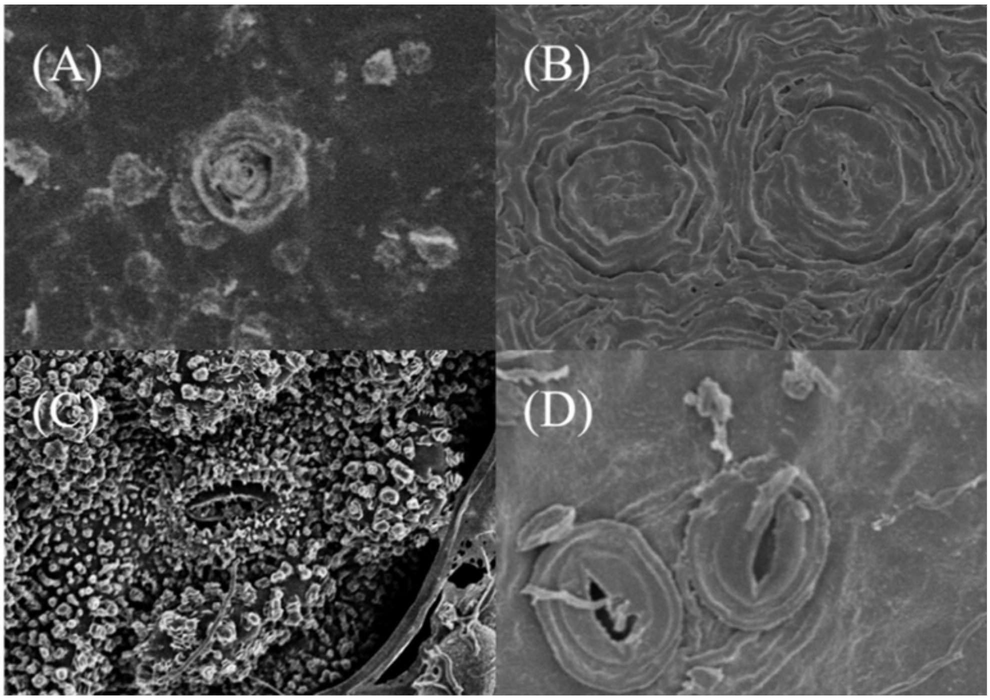


**Fig. 5.** The stomatal morphology of the tested trees under the condition of $PM_{2.5}$ pollution [(A)-(D) represents *Neolitsea aurata*, *Gordonia acuminata*, *Lindera kwangtungensis*, and *Rhamnus esquirolii*, respectively].

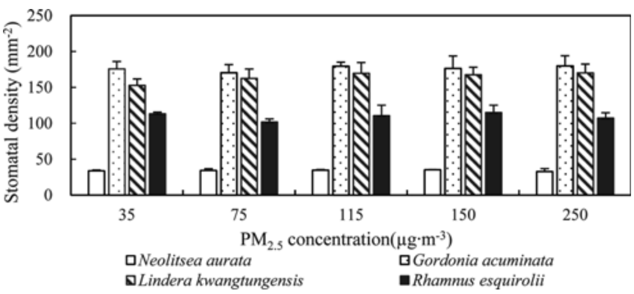


**Fig. 6.** Variation of stomatal density in different $PM_{2.5}$ concentration levels of 4 selected species.

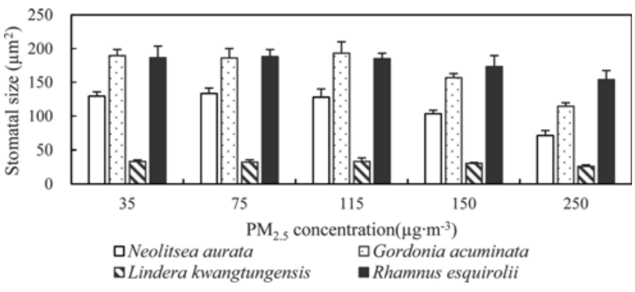


**Fig. 7.** Variation of stomatal size in different $PM_{2.5}$ concentration levels of 4 selected species.

degree of reduction increased with $PM_{2.5}$ concentration. This result was similar to the finding of Zhang (Zhang 2015), suggesting that plant leaves could adapt to environmental changes by closing stomata and reducing stomatal size under the condition of $PM_{2.5}$ pollution. The reduction percentage of stomatal size of the four species was in the order of *Neolitsea aurata*, *Gordonia acuminata*, *Lindera kwangtungensis* and *Rhamnus esquirolii*, which meant that the change of stomatal morphology of *Neolitsea aurata* and *Gordonia acuminata* was the largest under different levels of $PM_{2.5}$ pollution. Previous studies (Lehndorff et al. 2006; Song et al. 2015) had shown that particulate matter could enter leaf through the stomata and accumulate around it, which resulted in blockage and closure of the stomata, the decrease of stomatal size, and ultimately leading to a reduction of stomatal conductance.

## Characteristics of Leaf Trenches and Trichomes Among 4 Tree Species

Because of the differences in leaf morphology, leaf texture and the distribution of leaf vein, the vascular bundles, folds and trichomes on leaves surface of different tree species were also significantly different in size, quantity and density. Fig. 8 showed several groove patterns on leaf surface: reticulate, nodular or verrucous, smooth or rarely wrinkled, and corrugated grooves. Different types of folds could cause significant differences in the ratio of leaf grooves (Fig. 8). The leaf surface grooves of *Neolitsea aurata* and *Lindera kwangtungensis* were mostly reticulated or scaly and the groove proportions of these grooves were mostly in the 15%-20% level, which could reach more than 20% on some finer reticulated grooves (Fig. 10). The leaf surface grooves of

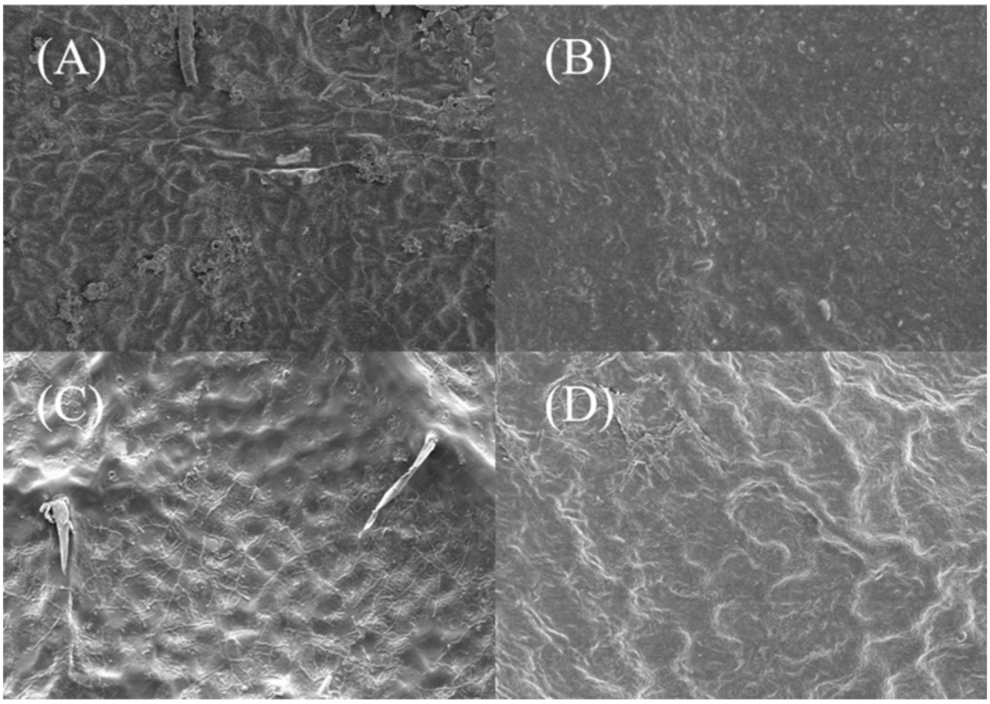


**Fig. 8.** The leaf groove morphology of 4 selected species [(A)-(D) represents *Neolitsea aurata*, *Gordonia acuminata*, *Lindera kwangtungensis*, and *Rhamnus esquirolii*, respectively].

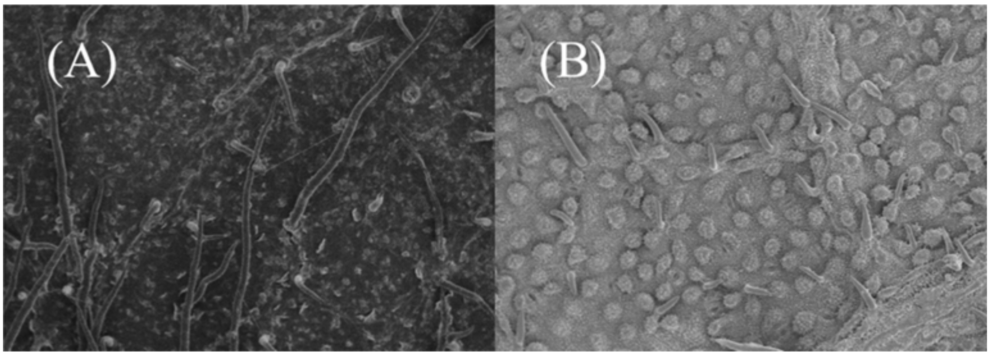


**Fig. 9.** The leaf trichome morphology of 4 selected species. (A) *Neolitsea aurata* (B) *Lindera kwangtungensi*

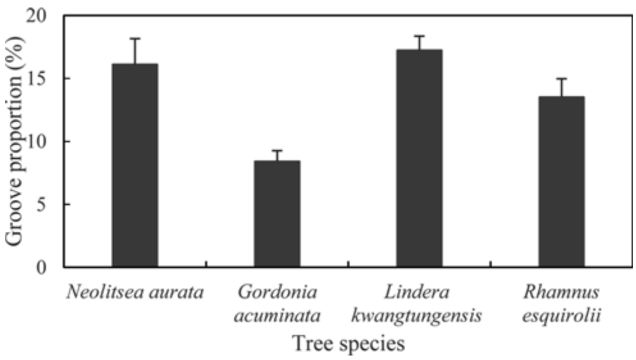


**Fig. 10.** The groove proportion of 4 selected species.

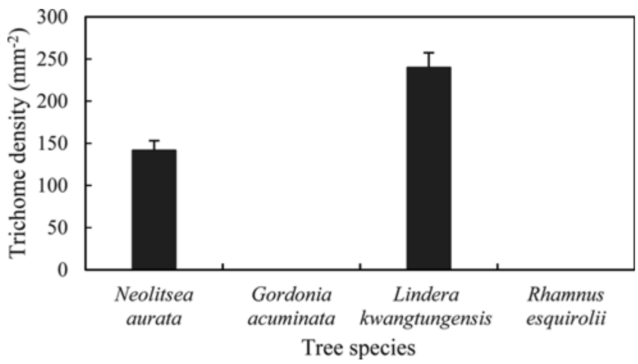


**Fig. 11.** The trichome density of 4 selected species.

*Rhamnus esquirolii* were mostly corrugated and the groove proportion was between 10%-15%. The leaf surface of *Gordonia acuminata* was smooth and the surface groove was lower than 10% (Fig. 10). Leaf surface roughness was the accelerator of $PM_{2.5}$ capture, and grooves were the main part of $PM_{2.5}$ settlement and accumulation. Studies had shown that there was a strong correlation between $PM_{2.5}$ accumulation and groove proportion, while trees species with high groove proportion and low stomatal size had a strong ability to capture $PM_{2.5}$ (Liang 2016). The higher groove proportion and lower stomatal size of *Neolitsea aurata* and *Lindera kwangtungensis* indicated that they had stronger $PM_{2.5}$ capture ability. In addition, Fig. 9 showed that the back of the leaves of *Neolitsea aurata* and *Lindera kwangtungensis* had trichomes. The *Neolitsea aurata* leaves were densely villous, and *Lindera kwangtungensis* leaves were mainly pubescent located between tuberculate protuberances. The density and effect of trichomes in *Lindera kwangtungensis* were lower than *Neolitsea aurata* (Fig. 11). The different types of trichomes had different retention effects to $PM_{2.5}$. The trichome density of *Neolitsea aurata* and *Lindera kwangtungensis* were 141.67 and 240 ($mm^{-2}$), respectively. The leaf surfaces of *Gordonia acuminate* and *Rhamnus esquirolii* had no trichome. Zhao's study (Zhao 2015) showed that when the total leaf trichome number per unit area was less than 50, the retention of $PM_{2.5}$ increased to a certain extent compared with no leaf trichome. And when the total leaf trichome number per unit area was more than 50, the overall retention level was the highest, which meant the increase of leaf trichome was helpful to retain $PM_{2.5}$. The presence of leaf trichome significantly increased leaf surface area and roughness, made fine particles settled and no longer suspended, produced a buffering effect on $PM_{2.5}$ settlement, and increased the time interval of $PM_{2.5}$ acting on stomata. The higher groove proportion and the presence of trichomes on the leaf surface in *Neolitsea aurata* and *Lindera kwangtungensis* buffered the effect of $PM_{2.5}$ on stomata and slowed down the speed of changes in stomatal conductance.

Response of Net Photosynthetic Rate and Stomatal Conductanceto to $PM_{2.5}$ Concentration

Fig. 12 and Fig. 13 showed that the net photosynthetic rate and the stomatal conductance of 4 species had a significant

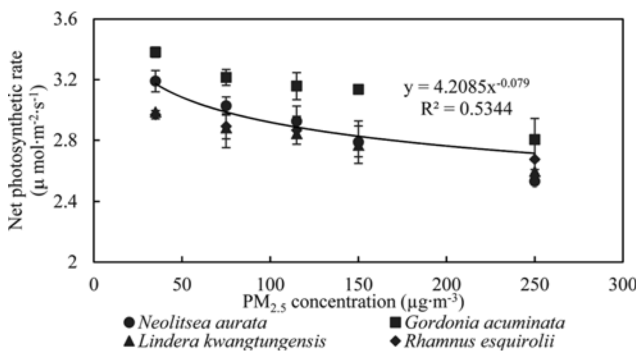


**Fig. 12.** Relationship between net photosynthetic rate and $PM_{2.5}$ concentration.

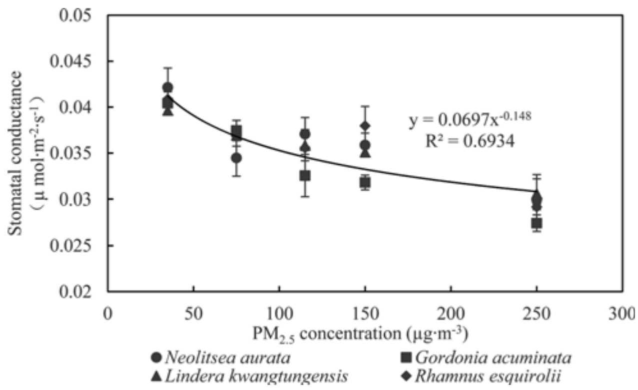


**Fig. 13.** Relationship between stomatal conductance and $PM_{2.5}$ concentration.

power function relationship with the $PM_{2.5}$ concentration ($P < 0.05$) and $R^2$ were 0.53 and 0.69, respectively, indicating that the net photosynthetic rate and the stomatal conductance decreased with the $PM_{2.5}$ concentration in power function. This was consistent with our research shown above. Due to stomatal blockage and closure, the changes of stomatal conductance in leaves might affect the leaf water potential in unstable state and had a negative impact on photosynthetic rate (Bukaveckas et al. 2011; Popek et al. 2013; Pryzybysz et al. 2014). In addition, the net photosynthetic rate of *Gordonia acuminate* was the highest comparing with other species in the four species and *Lindera kwangtungensis* was the lowest. The difference in stomatal conductance values between the four species was small. In forest management and tree species selection, the balance of environmental factors such as $PM_{2.5}$ concentration on different plant species may be a key to improve the ecological environment. Therefore, the relationship between biological cycle and ecological balance should be emphasized, and the impact of $PM_{2.5}$ on human living environment should be concerned. For example, the reduction of plant photosynthetic rate caused by $PM_{2.5}$ mentioned in this paper may result in climate change and eventually global consequences due to the impact of carbon absorption.

## Conclusions

The photosynthetic rate of 4 species showed an significant exponential increase ($P < 0.01$) with the increase of stomatal conductance. $PM_{2.5}$ had a negative effect on plant photosynthesis, and there were differences in the effects among species. The negative effect might be caused by the closure of the stomata and the decrease of stomatal conductance. The reduction of the stomatal size of 4 species under the concentrations of 150 and 250 g $m^{-3}$ $PM_{2.5}$ proved this point. In addition, the difference of leaf surface morphology among 4 species was the reason for the difference of photosynthetic rate and stomatal conductance under the condition of $PM_{2.5}$ pollution. The higher groove proportion and the presence of trichomes on the leaf surface in *Neolitsea aurata* and *Lindera kwangtungensis* absorbed some particulate matters and buffered the effect of $PM_{2.5}$ pollution on stomata.

## Methods

### Experimental Measurements

The experiment (from Juneto November in 2014) are conducted in forest ecological station of Chongqing Jinyun Shan National Nature Reserve (29º49'81"N, 106º22'74"E). We selected four local species of trees (*Neolitsea aurata*, *Gordonia acuminate*, *Lindera kwangtungensis*, and *Rhamuns esquirolii*) (Fig. 1; Table 1) by transplanting fifteen young plants with similar morphology to square stainless steel containers (40 cm × 40 cm × 40 cm), which divided into 5 levels of $PM_{2.5}$ concentration gradient. Three replicates were measured for each species and taken the average.

For indoor test, a series of indicators were observed to examine the correlation between $PM_{2.5}$ and leaf characteristics. Net photosynthetic rate ($P_n$), stomatal conductance ($g_S$), dark respiration rate (Rd), transpiration rate (E), intercellular $CO_2$ concentration ($C_i$), and leaf temperature ($T_L$) were measured by LI-6400XT Portable Photosynthesis System with 6400-02B LED Light Source (LI-COR Inc., Lincoln, Nebraska, USA).

To simulate the natural condition of the $PM_{2.5}$ pollution, indoor experiments used a mixing chamber for omni-directional mixture of plant and particulate matter (Fig. 2). The chamber (100 cm × 100 cm × 150 cm) including tubes of both air inlet and air outlet (diameter of 3 cm) was made of inorganic glass. The inlet tube and the outlet tube were installed respectively at the bottom and the top of the chamber with a diagonal patternto make the mixed air evenly distributed in the whole gas chamber. As Hwang et al. (2011), the particles in the submicron (diameter < 1 μm) and ultrafine (diameter < 0.1 μm) size range were generated using acetylene as the fuel and fully mixed (Ahn et al. 2001; Hwang et al. 2011).

The determination of indoor photosynthetic physiological indices was carried out under the controlled condition of environmental factors mentioned below. Leaf chamber temperature and leaf chamber $CO_2$ concentration were set as 27°C and 750 μmol $mol^{-1}$. These values, which were used to simulate the atmospheric temperature and $CO_2$ concentration in the natural condition, were based on conditions of the local subtropical climate and the surrounding areas of the industrial zone. The photosynthetic characteristics were measured on 3 leaves of a plant at 80 cm under controllable environmental factor conditions. The measurements were carried out under 12 photosynthetically active radiation gradients (100, 300, 400, 500, 750, 1000, 1300, 1600, 1750, 1900 and 2000 mol $m^{-2}$, $s^{-1}$) without $PM_{2.5}$. 5 levels of $PM_{2.5}$ concentration gradient were set as 35, 75, 115, 150, and 250 μg $m^{-3}$ and continuously determinated for 10h in a time interval of 30 minutes in the mixture chamber, as Silva and Mendesp's study of $PM_{10}$ (2011).

After the completion of the experiment under controlled conditions of the chamber, we collected the leaves of four species (10 leaves per species). 5 leaves were used to observe the stomata and the other leaves were used to observe grooves and trichomes after washed by distilled water. The collected leaves were dried at air temperature of 80ºC in a oven. Two small square samples with a length of 5 mm and 1-2 cm to the central vein were cut from both sides of the leaves. The samples were then stick to the observation platform and plated with gold by ion-plating apparatus of Scan Electron Microscopy (SEM, HitachiS-3400 N, Japan). Afterward, the treated samples were magnified to 150~2000 times to observe the leaf morphology. The collection, treatment and observation of leaves were completed within one day.

Groove proportion (G) is expressed as follows:

$$G = M_g / M_t \times 100\% \tag{1}$$

where $M_g$ represents the projection area of groove ($\mu m^2$), $M_t$ represents the area of the leaf sample ($\mu m^2$).

Leaf trichomes per unit area (1 $mm^2$) was counted to calculate trichome density. Stomal number per unit leaf area (1 $mm^2$) was counted to calculate the stomatal density and stomatal size was measured and calculated at the same time.

The experiments carried out in the forest ecological station was under the permission of Chongqing Jin yun Shan National Nature Reserve, and not involved any protected or endangered species.

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## Author's Contributions

YL conceived and designed the study; YW and BW revised and perfected the design of experiments; YL and YW performed the experiments; YL wrote the paper; BW and YW reviewed and edited the manuscript; YL, YW and WY revised the manuscript according to the reviewer's request. All authors read and approved the manuscript.

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