EFFECTS OF ARBUSCULAR FUNGI ON GROWTH INDEXES AND PHOTOSYNTHETIC APPARATUS OF *SALIX VIMINALIS* IN PB CONTAMINATED SOIL

WEI, N. – HAO, N. A. – ZHU, S. Q. – YIN, J. H.[†] – AN, B.[†*]

College of Horticulture, Jilin Agricultural University, Changchun 130118, China

[†]These authors contributed equally to this work.

[*]Corresponding author
e-mail: jlndbya@126.com

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Abstract. The effects of arbuscular mycorrhizal fungi *Funneliformis mosseae* on the growth, chlorophyll content, photosynthetic parameters and chlorophyll fluorescence characteristics of *Salix viminalis* growing on Pb contaminated soil (0, 50, 200 and 400 $mg \cdot kg^{-1}$) were studied. The results show that: In Pb contaminated soil, the root activity of *Salix viminalis*, and the content of chlorophyll, especially chlorophyll-a, decreased significantly with the increase of Pb content. The mycorrhizal infection rate of *Salix viminalis* roots was 50-70% after inoculation with *F. mosseae*. The root activity of *Salix viminalis* was increased and the chlorophyllin degradation of *Salix viminalis* leaves in Pb contaminated soil was alleviated. The infection of *F. mosseae* could improve the photosynthetic electron transfer ability of PSII in *Salix viminalis* leaves under Pb stress. It could promote the activity of OEC of PSII donor side and the electron transfer ability of Q_A to Q_B of PSII receptor side in *Salix viminalis* leaves, which ensured that the leaves of *Salix viminalis* had relatively high PSII activity in Pb soil.Therefore, the inoculation of *F. mosseae* could improve the tolerance to Pb and reduce the toxicity of Pb to *Salix viminalis*.
Keywords: *Salix viminalis, AMF, Pb, photosystem, photosynthesis*

Introduction

Lead is one of the most harmful elements in heavy metal pollutants. The pollution in soil mainly comes from industrial production and transportation (Soffianian et al., 2014; Sauliutė and Svecevičius, 2015). Pb stress inhibited the growth and development of plants, especially the root. Pb toxicity may cause swelling, bending, thickening and shortening of plant roots (Hall, 2002; Chen and Zhao, 2009; Pallara et al., 2013; Chen et al., 2017a,b,c). The toxic effect of Pb depends on the stress time and heavy metal concentration. In addition, different plants have different tolerance to Pb. For example, hyperaccumulators are more resistant to Pb than sensitive plants (Ogbomida et al., 2018). For example, Jiang and Liu (2010) studied the effect of Pb stress on the cellular defense system of garlic meristems. The results showed that after Pb treatment for 48-72 hours, the mitochondria swelled; the cristae disappeared; the endoplasmic reticulum and Golgi humoral bubbled; the plasmalemma was damaged, and the color of nucleus deepened. The high concentration of Pb can reduce the biomass of rice plant varieties, such as *Najas indica*, guixiang zhang, and nongxiang-18. In addition, Pb stress leads to fewer smaller, and more fragile leaves or leaves with dark purple patches on their surface (Gupta et al., 2010).

Arbuscular mycorrhizal fungi (AMF) exist in soil of most ecosystems, even if it is contaminated soil. AMF can form symbiotic arbuscular consortium with plant roots, which can accelerate the phytoremediation process, improve the remediation efficiency

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and increase the stability of heavy metals (Zhang et al., 2007; Xue and Gao, 2017). The toxicity also has a growth deceleration or damaging impact on the photosynthetic apparatus. The toxic effect of Pb depends on the amount of time the organism is subjected to it and the concentration. In addition, different plants vary in their level of tolerance to Pb. For example, hyperaccumulating plants have a stronger tolerance to Pb than sensitive plants (Arena et al., 2017). When the concentration of heavy metals increased, the host's uptake of heavy metals increased as well, resulting in a negative effect on plant growth. Under heavy metal stress, AMF promotes plant growth, alleviates the toxicity of heavy metals to plants or has no effect on plant growth. Therefore, it has important theoretical value and practical significance to study the mechanism of AMF combined with plants to perform and accelerate the remediation of heavy metal contaminated soils (Qu et al., 2009; Rozpądek et al., 2016; Xue and Gao, 2017; Rizwan et al., 2017).

Salix viminalis grows rapidly, has large biomass, and has strong ability to accumulate heavy metals, *Salix viminalis* has the advantages of rapid growth, high biomass and strong ability to accumulate heavy metals, which is widely used in phytoremediation and biomass energy development in heavy metal contaminated soil areas, and has certain ecological and economic value (Zhai et al., 2016). Therefore, improving the resistance of *Salix viminalis* seedlings to heavy metal stress is the key to ensure the survival and growth of seedlings after transplanting. At present, there are a lot of studies on arbuscular mycorrhizal fungi can improve the resistance of plants to heavy metals, but there are few studies on arbuscular mycorrhizal fungi on the growth index and photosynthetic characteristics of *Salix viminali*s in Pb contaminated soil. In this study, the effects of arbuscular mycorrhizal fungi *F. mosseae* on the growth, photosynthetic gas exchange parameters and chlorophyll fluorescence parameters of *Salix viminalis* under Pb contaminated soil were studied from the perspective of plant photosynthesis mechanism, in order to provide some basic data for suggesting the mechanism of arbuscular mycorrhizal fungi to improve the resistance of *Salix viminalis* to Pb stress.

Materials and methods

Test materials and treatment

The experiment was conducted in the laboratory of soil science of Jilin Agricultural University (Changchun, Jilin Province, China) from March to June 2019. The substrate was composed of organic fertilizer, perlite and yellow soil (6.0: 3.0 : 0.5 : 0.5). The substrate was sterilized at high temperature (121 °C) for 2 h in an autoclave to kill the indigenous mycorrhiza and other microorganisms in the soil. The mycorrhizal strain used in the experiment was *F. mosseae*, and the inoculum was purchased from the China Arbuscular Mycorrhizal Fungi Germplasm Resource Bank and was numbered "BGCXJ01". The mycorrhizal agent used for inoculation contained spores, hyphae and mycorrhizal segments, and each gram of mycorrhizal agent contained 40-50 spores. The plants that were tested materials were approximately 20 cm tall. The physicochemical properties of sterilization matrix were determined to be pH 7.66 (soil / water = 1:2.5, w v), organic matter 16.85 $g \cdot kg^{-1}$, ammonium nitrogen 7.37 $mg \cdot kg^{-1}$, nitrate nitrogen 25.77 $mg \cdot kg^{-1}$, available phosphorus 11.48 $mg \cdot kg^{-1}$, available potassium 108.96 $mg \cdot kg^{-1}$, and total Pb 3.58 $mg \cdot kg^{-1}$. Before transplanting, the seedlings are irrigated once to make the relative water content of the soil basically reach saturation.

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The seedlings are transplanted into a cultivation bowl with a diameter of 12 cm and a height of 15 cm, and one plant is planted in each pot.

A two factor test AMF×Pb was arranged in a completely randomized block. The appropriate amount of $Pb(NO_3)_2$ solution was added to the substrate and evenly mixed, so that the Pb content in the soil Pb test medium was established at 4 gradients of 0, 50, 200 and 400 $mg \cdot kg^{-1}$. The control group was treated with the same amount of distilled water, and each Pb level was treated with inoculation of *F. mosseae* (+AMF) and no inoculation of arbuscular mycorrhizal fungi (CK). The mycorrhizal fungi were inoculated by adding 50 g of soil containing fungi per kilogram of substrate, and the treatment of non-inoculation of mycorrhizal fungi involved the addition of same amount of sterilized soil. *Salix viminalis* cuttings with the same growth status were selected (the number of leaves, height, number of new roots and length were close to each other), and then the cuttings were fixed by filling holes with substrate. Each treatment was repeated in five pots for a total of 40 pots. The pot was cultured in an incubator with temperature of 25 °C, light intensity of 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, a photoperiod of 12 / 12 h (light/dark) and relative humidity of approximately 75%.

The pots were watered regularly and managed at the seedling stage. Fresh 0.25 × Hoagland's nutrient solution (35 ml pot^{-1}) was cultured for one month, and the relevant data were measured when the seedlings were approximately 25-35 cm high. Hoagland's nutrient solution was used [10 µM H_3BO_3, 6mM KNO_3, 8M $Ca(NO_3)_2$, 1M $NH_4H_2PO_4$, 2.6M $MgSO_4$, 0.5mM $CuSO_4$, 1.0M $ZnSO_4$, 1.6µM $MnSO_4$, $(NH_4)Mo_2PO_4$, 2µM Fe-EDTA, pH 6.0±0.5], Na_2EDTA, DTE, sucrose, Tris HCl (50 mm, pH 7.5).

Determination items and methods

The growth parameters were determined: After measuring the plant height, the plant was taken out from the substrate, and the root length of single plant was measured after washing the substrate on the surface of root system. After the water on the surface of root system was dried with absorbent paper, the aboveground and underground parts were put into aluminum box to kill (105 °C, 30 min) and dried (60 °C, 30 h) to constant weight, and then the biomass of each plant was calculated. Repeat 5 times.

Mycorrhizal infection rate, chlorophyll content and root activity were measured: The infection rate of mycorrhiza was determined by section staining. The root system was rinsed with tap water, put into 1n KOH solution at 80 °C for 1h, then washed with distilled water, put it into 20% H_2O_2 solution for 30min, and then put in 1n HCl for 2 min. Finally, it was stained with 0.5% acid fuchsin and pressed into tablets. The number of cells stained was observed under the microscope. Soil organic matter content was determined by k2cro7-h2so4 oxidation method, soil pH value was determined by electrode method, and soil field water holding capacity was determined by drying method. Mycorrhizal infection rate (%) = root segments forming clumps / number of measured root segments × 100; Root activity was measured by TTC method (Shao et al., 2009).

Determination of Pb Content in the Underground and Aboveground Parts: The collected aboveground and underground samples were rinsed with tap water and deionized water for several times to remove dust and residues on the surface as far as possible. The samples were placed in an oven at 80 °C for at least 48 h until they reached a constant weight, and the dry weight was recorded. The dried plant samples were then placed in a micro grinder to make a uniform powder. 0.5 g powder samples of roots, stems and leaves were weighed, nitric acid and perchloric acid (4:1 in volume

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ratio) were added and placed for 12 h, digested until the solution was transparent, filtered, and the volume was fixed to 50 ml. Lead content was measured by atomic absorption spectrophotometer. Repeat 5 times.

Determination of photosynthetic gas exchange parameters: The net photosynthetic rate (P_n), stomatal conductance (G_S), transpiration rate (T_r) and intercellular CO_2 concentration (C_i) of the second fully expanded functional leaf of *Salix viminalis* from top to bottom were measured by Li-6800 photosynthetic measurement system (licor company, USA) from 9:00 am to 11:00 am, The concentration of CO_2 was fixed at 400 $\mu L \cdot L^{-1}$ in a CO_2 cylinder. The net photosynthetic rate (P_n), stomatal conductance (G_s), transpiration rate (T_r) and intercellular CO_2 concentration (C_i) of *S. viminalis* leaves under different treatments were measured. Repeat 5 times.

The OJIP curve of chlorophyll fluorescence was determined: After 30 min dark adaptation, the OJIP curve of leaves after dark adaptation was measured by handy PEA (PPsystem Company, UK). According to Strasser et al.'s (1995) method, the OJIP curves were standardized by $V_{O\text{-}P}=(F_t-F_o)/(F_m-F_o)$ and $V_{O\text{-}J}=(F_t-F_o)/(F_J-F_o)$, respectively. The relative variable fluorescence (V_J and V_K) of J point at 2 ms and K point at 0.3 ms were obtained, respectively. The differences between the standardized $V_{O\text{-}P}$ and $V_{O\text{-}J}$ curves of different treatments and the control were calculated, expressed as $V_{O\text{-}P}$ and $V_{O\text{-}J}$, respectively. The measured OJIP curve was analyzed by JIP test Strasser et al. (1995).

Chlorophyll content were measured: Chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl a+b) and chlorophyll a/b (Chl a/b) were calculated respectively (Shao et al., 2009).

Data processing methods

Excel and SPSS software (Version. 22) were used to conduct statistical analyses on the measured data. The data in the figure was denoted as mean ± standard deviation (SD). Two-way ANOVA was used to detect the significant differences of heavy metal concentration, AMF inoculation and their interaction on all variables.

Results and analysis

Effects of arbuscular mycorrhizal fungi (F. mosseae) on the growth characteristics of Salix viminalis in Pb contaminated soil

With the increase of Pb content in soil, the plant height, root length and biomass of *Salix viminalis* decreased significantly. However, when the Pb content was 50 $mg \cdot kg^{-1}$, the root length of *Salix viminalis* decreased, but the plant height and biomass of different parts did not change significantly, even the ratio of root to shoot increased. The plant height, root length and biomass of *Salix viminalis* inoculated with *F. mosseae* were higher than those without inoculation under different soil Pb contents. When the Pb content in soil was 400 $mg \cdot kg^{-1}$, the inoculation of *F. mosseae* increased the plant height, root length and biomass (*Fig. 1*). The root length, aboveground biomass and underground biomass of *F. mosseae* treatment were 18.07% ($P < 0.05$), 32.32% ($P < 0.05$), 21.33% ($P < 0.05$) and 32.51% ($P < 0.05$), respectively.

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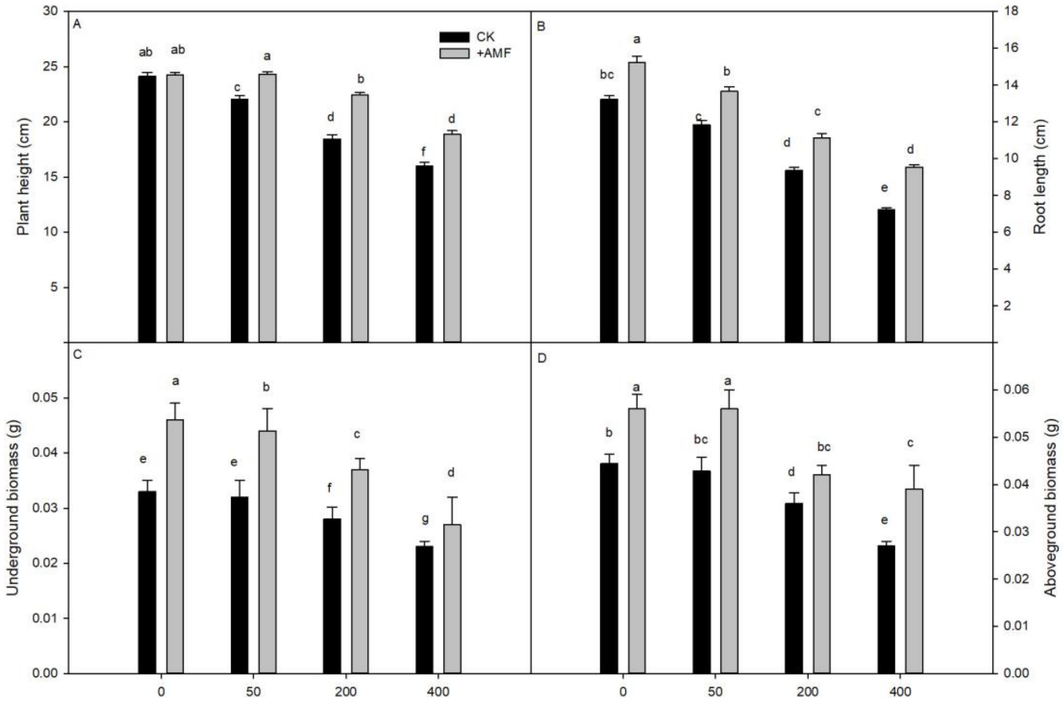

Figure 1. *Growth characteristics of Salix viminalis mycorrhizae in Pb contaminated Soil. The aboveground parts were placed in aluminum boxes and killed by treatment at 105°C for 30 min, dried to a constant weight at 60°C for 30 h), and the biomass was weighed; Underground biomass was treated as the aboveground biomass. CK: CK + 0AMF contents; +AMF: CK + different amounts of arbuscular mycorrhizal fungi (AMF). Note: Data in the figure are the mean ± SD values followed by different lowercase letters indicate a significant difference (p<0.05)*

Effects of F. mosseae inoculation on the Pb content in the underground and aboveground parts

Figure 2A,B show that both the Pb contents in the underground and aboveground parts of *Salix viminalis* represented significant increasing tendencies with the increasing Pbcontent in soil, and the Pb content in the underground part of *Salix viminalis* was higher than in the aboveground plant parts. Inoculation with *F. mosseae* significantly improved the absorption of Pb in *Salix viminalis* roots. The Pb content in the underground plant parts in the +AMF treatment were significantly higher than the plants not inoculated, except in the treatments without Pb, as there was no significant difference found in the absorption of Pb in *Salix viminalis*, the Pb content in the aboveground part of *Salix viminalis* in the +AMF treatment was not significantly different from the Pb content in the aboveground part of the non-inoculated *Salix viminalis.*

Effects of arbuscular mycorrhizal fungi (F. mosseae) on mycorrhizal infection rate and root activity of Salix viminalis in Pb contaminated soil

The mycorrhizal infection rate of *Salix viminalis* roots inoculated with *F. mosseae* decreased significantly with the increase of Pb content in soil. When the Pb content in soil reached 400 mg·kg^{-1}, the infection rate of roots decreased by 45.51 % ($P < 0.05$) (*Fig. 3A*). Although the root activity of *Salix viminalis* also decreased with the increase

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of Pb content in soil, the root activity of *Salix viminalis* was increased in different degrees under different soil Pb contents due to the infection of *F. mosseae* (*Fig. 3B*).

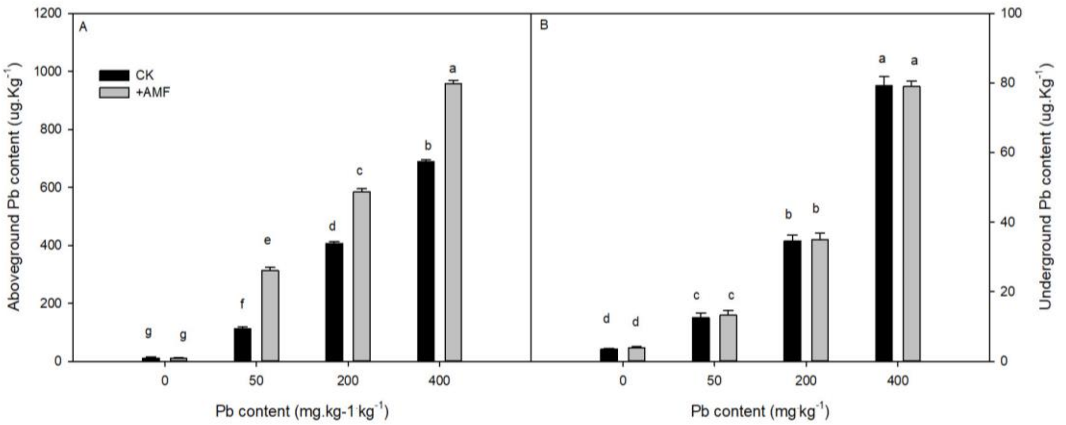

Figure 2. *The Pb Content in the Underground and Aboveground Parts. A: Aboveground Pb Content; B: Underboveground Pb Content. CK: CK + AMF contents; +AMF: CK + different amounts of arbuscular mycorrhizal fungi (AMF). Note: Data in the figure are the mean ± SD; values followed by different lowercase letters indicate a significant difference (p<0.05)*

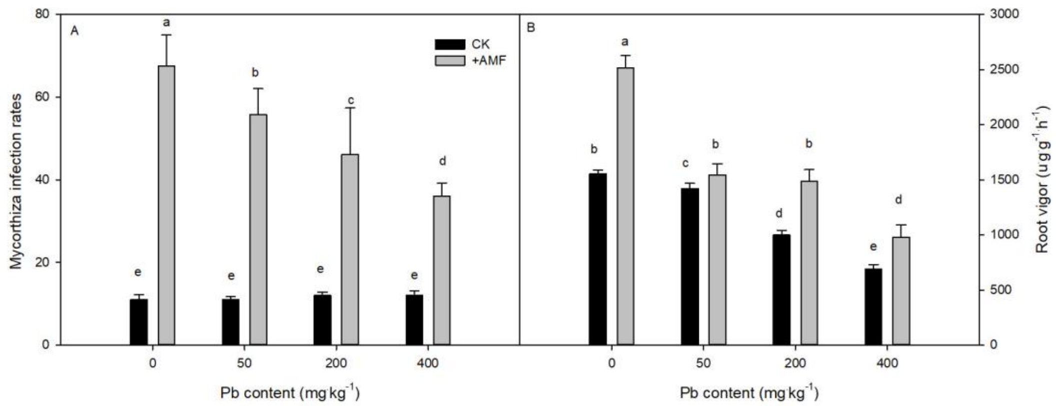

Figure 3. *Infection rate and the Pb Content in the mycorrhizal infection rate androot activity. A: mycorrhizal infection rate; B: Root vigor. CK: CK + AMF contents; +AMF: CK + different amounts of arbuscular mycorrhizal fungi (AMF). Note: Data in the figure are the mean ± SD; values followed by different lowercase letters indicate a significant difference (p<0.05)*

Effects of arbuscular mycorrhizal fungi (F. mosseae) on chlorophyll content of Salix viminalis leaves in Pb contaminated soil

With the increase of Pd content in soil, the contents of chlorophyll a, chlorophyll b and total chlorophyll in *Salix viminalis* leaves decreased significantly, and the decrease degree of chlorophyll a content was greater than that of chlorophyll b, which showed that the chlorophyll a/b value also decreased with the increase of Pb content in soil (*Fig. 4D*). Under different soil Pb content, there was no significant difference in chlorophyll b content between the treatment of *F. mosseae* and the control, but there was significant difference between chlorophyll a and total chlorophyll.

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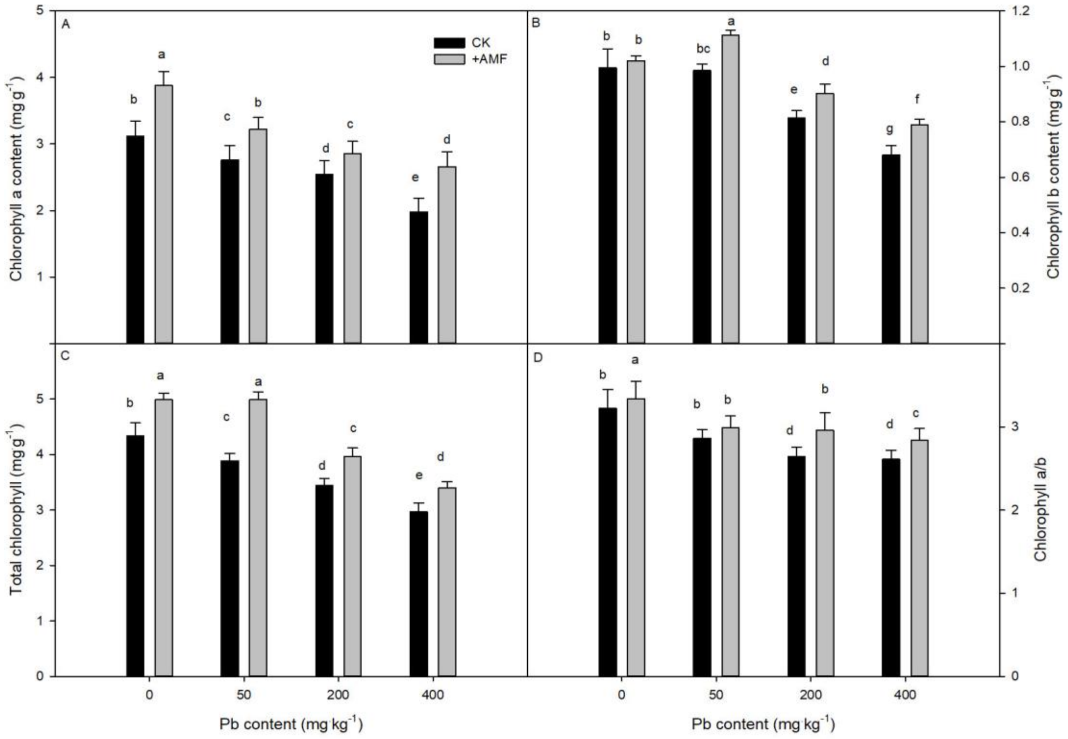

Figure 4. *Effect of F. mosseae on the chlorophyll content of Salix viminalis leaves in Pb-contaminated soil. A: Chlorophyll a content; B: Chlorophyll b content C: Total chlorophyll content: chlorophyll a content+ chlorophyll b content; D: Chlorophyll a/b content: chlorophyll a content/chlorophyll b content; AMF, arbuscular mycorrhizal fungi. CK: CK +0 AMF contents; +AMF: CK + different AMF contents. Note: Data in the figure are mean ± SD; values followed by different lowercase letters indicate a significant difference (p<0.05)*

Effects of arbuscular mycorrhizal fungi (F. mosseae) on photosynthetic characteristics of Salix viminalis leaves in Pb contaminated soil

With the increase of Pb content in soil, P_n, G_s and T_r of *Salix viminalis* leaves decreased. When the Pb content in soil increased to 400 mg·kg^{-1}, P_n, and tr increased by 31.33% ($P < 0.05$), and 13.55% ($P > 0.05$), respectively (*Fig. 5*).

Effects of inoculation of arbuscular mycorrhizal fungi (F. mosseae) on OJIP curve of Salix viminalis leaves in Pb contaminated soil

With the increase of Pd content in soil, the relative fluorescence intensity of o point increased significantly, while the relative fluorescence intensity of P point decreased significantly, especially F_m (*Fig. 6B*). The change range of OJIP curve of *Salix viminalis* leaves inoculated with *F. mosseae* was significantly less than that without inoculation. The results of quantitative analysis of the relative fluorescence intensity (F_o and F_m) of O and P points showed that when the soil Pb content was 0 and 50 mg·kg^{-1}, there was no significant difference between the treatments inoculated with *F. mosseae* and those without inoculation. However, when the soil Pb content was 200 and 400 mg·kg^{-1}, the fo content of *Salix viminalis* leaves inoculated with *F. mosseae* was significantly lower than that without inoculation.

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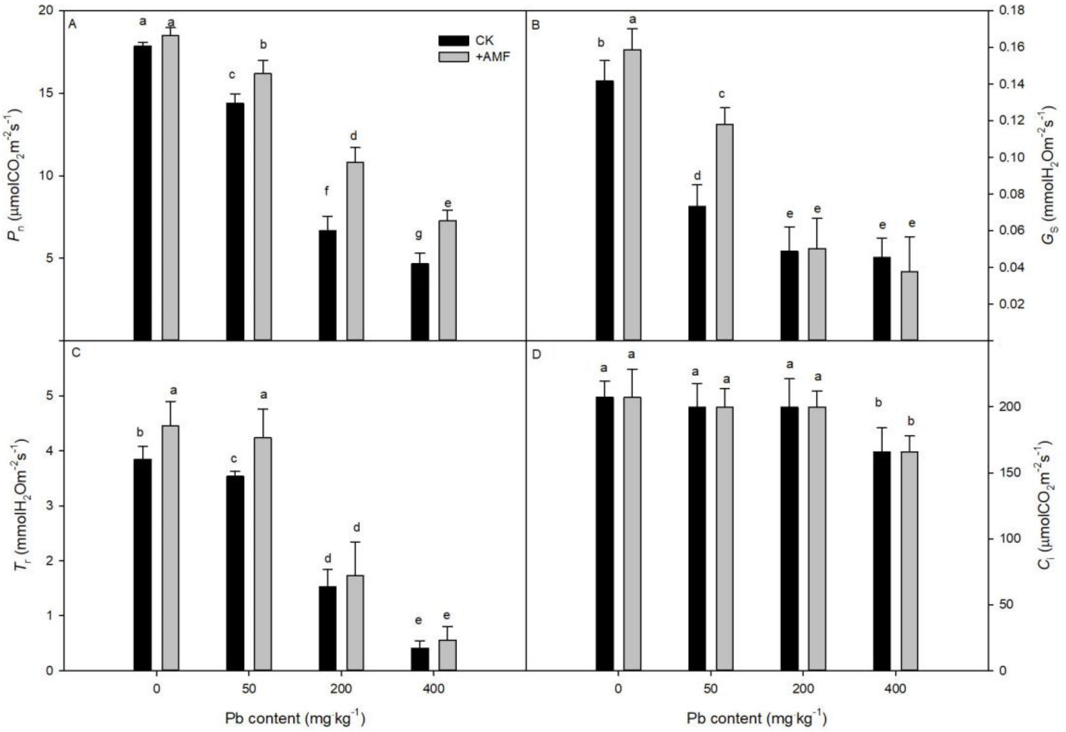

Figure 5. *The photosynthetic characteristics of Salix viminalis leaves in Pb contaminated soil. CK: CK + 0 AMF contents; +AMF: CK + different AMF contents. A: P_{N}:- Net photosynthetic rate; B: G_s-Stomatal conductance; C: Tr-Transpiration rate; D: C_i- Intercellular CO_2; AMF, arbuscular mycorrhizal fungi. Note: Data in the figure are mean ± SD; values followed by different lowercase letters indicate a significant difference (p<0.05)*

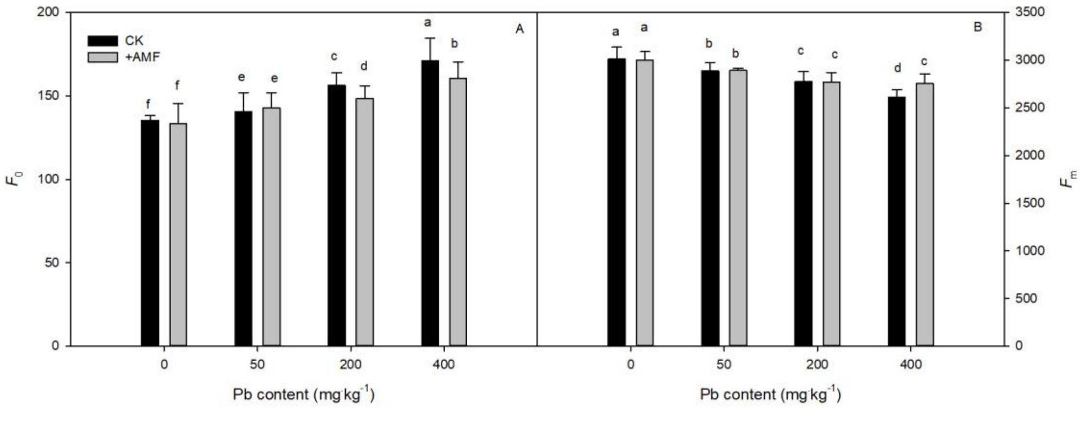

Figure 6. *The F_o and F_m of Salix viminalis mycorrhiza in soil contaminated with Pb. CK: CK +0 AMF contents; +AMF: CK+different AMF contents. A: F_o:-Quantitative analysis of the changes of the relative fluorescence intensity of o-point; B: F_m- Quantitative analysis of the changes of the relative fluorescence intensity P-point; AMF, arbuscular mycorrhizal fungi. Note: Data in the figure are represented as mean ± SD; values followed by different lowercase letters indicate a significant difference (p<0.05)*

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Effects of inoculation with F. mosseae on electron transport of PSII donor and acceptor side in Pb contaminated soil

The relative variable fluorescence V_J of J point at 2 ms on the standardized OJIP curve of *Salix viminalis* leaves increased most significantly, but the increase range of V_J in the treatment of inoculating *F. mosseae* was significantly lower than that of non-inoculation treatment (*Fig. 7*). The results of quantitative analysis of V_J and V_K showed that with the increase of soil Pb content, V_J and V_K increased significantly, and the increase range of V_J was significantly greater than that of V_K. Under different soil Pb contents, V_J and V_K of *Salix viminalis* leaves inoculated with *F. mosseae* were significantly lower than those without inoculation, and the V_J of *Salix viminalis* leaves inoculated with *F. mosseae* was significantly lower than that of non-inoculation treatment under different soil Pb contents, while V_K reached significant difference level only when soil Pb content was 200 mg·kg^{-1} and 400 mg·kg^{-1}.

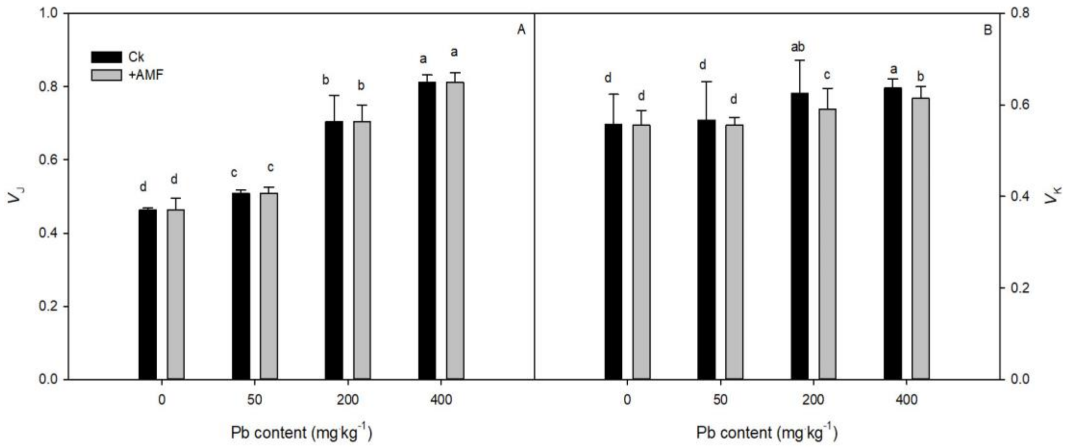

Figure 7. *The PSII and PSI photochemical activity of Salix viminalis leaves in Pb contaminated soil. CK: CK + 0 AMF contents; +AMF: CK+ different AMF contents. A: V_J, the relative variable fluorescence transient at point J (2 ms); B: V_K: the relative variable fluorescence transient at point K (0.3 ms); AMF, arbuscular mycorrhizal fungi. Note: Data in the figure are represented as mean ±SD; values followed by different lowercase letters indicate a significant difference ($p<0.05$)*

Effects of arbuscular mycorrhizal fungi (F. mosseae) on PSII photochemical activity of Salix viminalis leaves in Pb contaminated soil

With the increase of Pb concentration, the Φ_{PSII} of *Salix viminalis* leaves showed a decreasing trend, and the decreasing range was greater than 200 mg·kg^{-1} at 400 mg·kg^{-1}. The increase of ETR in *Salix viminalis* leaves under Pb stress was significantly lower than that of CK, especially at the concentration of 400 mg·kg^{-1} Pb (*Fig. 8B*). Under 400 mg·kg^{-1} Pb stress, NPQ of *Salix viminalis* leaves increased significantly compared with CK (*Fig. 8D*). In the soil without Pb pollution, the PI_{ABS} of *Salix viminalis* leaves inoculated with *F. mosseae* was 17.28 % higher than that without inoculation ($P < 0.05$). With the increase of Pb content in soil, the PI_{ABS} of *Salix viminalis* leaves decreased significantly.

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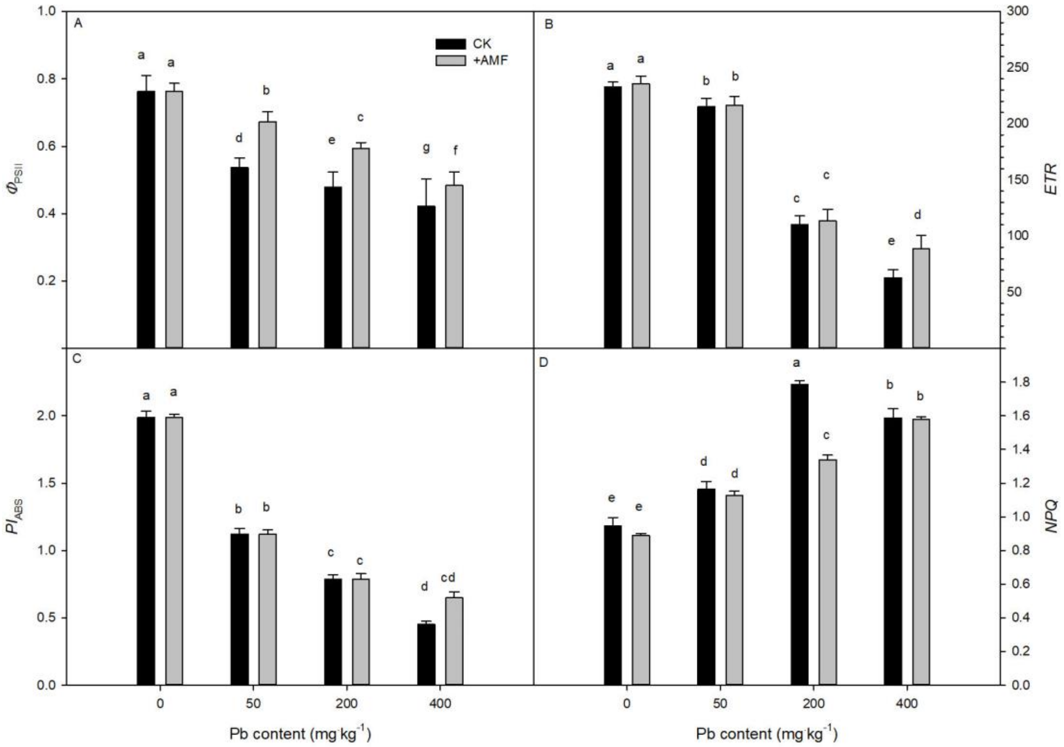

Figure 8. *The PSII photochemical activity of Salix viminalis leaves in Pb contaminated soil. CK: CK + 0 AMF contents; +AMF: CK+ different AMF contents. A: Φ_{PSII}: actual photochemical efficiency; B: ETR: electron transfer rate; C: PI_{ABS}: absorption-based photosynthetic performance index; D: NPQ:non-photochemical quenching. Note: Data in the figure are represented as mean ±SD; values followed by different lowercase letters indicate a significant difference (p<0.05)*

Discussion

Pb can significantly inhibit the growth of plants. In order to maintain normal growth and dry matter accumulation, plants must adapt to the stress environment in terms of external morphological characteristics and physiological functions, so as to ensure that the roots can normally absorb water and Photosynthesis and other physiological processes under adverse conditions (Del et al., 1999a,b; Dong et al., 2008). In this experiment, the growth of *Salix viminalis* in Pb contaminated soil was obviously inhibited, and its plant height, root length, biomass and accumulation decreased significantly with the increase of Pb content in soil, indicating that the growth and development of *Salix viminalis* roots would be hindered by Pb in soil, which may be due to the accumulation of Pb in *Salix viminalis* roots The effect on the growth of aboveground was also studied. The infection level of arbuscular mycorrhizal fungi (*F. mosseae*) on the roots of *Salix viminalis* was relatively high. Under the influence of its infection, the root activity of *Salix viminalis* L. inoculated with *F. mosseae* under different Pb pollution levels was significantly higher than that without inoculation, which increased the root length and root biomass of *Salix viminalis* under Pb pollution.

Pb can also cause disordered grana stacking in chloroplasts, matrix lamellae disappearing and chloroplast function decreasing. Pb stress can also limit the photosynthetic carbon assimilation capacity of plants (Romanowska et al., 2006; Tukaj et al., 2007; Marmiroli et al., 2013). Some studies have found that heavy metal stress

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can increase the activities of carbon assimilation related enzymes such as Rubisco (Kalaji et al., 2016; Liu et al., 2017). However, a large number of studies have shown that heavy metal ions such as Pb^{2+} can combine with functional groups of some enzymes (such as sulfhydryl, - SH) to replace the essential elements in metalloproteins, which will change the conformation of biological macromolecules and inhibit their activities, resulting in the decrease of photosynthetic capacity (Li et al., 2000; Malecka et al., 2001; Qureshi et al., 2007; Skórzyńska-Polit et al., 2010). In this experiment, with the increase of soil Pb content, P_n, G_s and T_r decreased significantly. Inoculation with *F. mosseae* significantly improved stomatal limitation of *Salix viminalis* leaves, and then increased T_r and P_n, which was conducive to the accumulation of assimilates. When Pb content in soil increased to 200 mg·kg^{-1}, C_i of *Artemisia argyi* leaves inoculated with *F. mosseae* continued to decrease, while C_i of leaves without inoculation increased compared with that of 200 mg·kg^{-1} The results showed that Pb content of mg·kg^{-1} destroyed the photosynthetic mechanism of *Salix viminalis* leaves, which was related to the decrease of CO_2 utilization capacity. Even with the decrease of stomatal conductance, C_i was forced to increase. However, C_i in leaves of *Salix viminalis* inoculated with *F. mosseae* was lower than that without inoculation when Pb content reached 200 mg·kg^{-1}, indicating that inoculation with *F. mosseae* could protect the physiological function of photosynthetic apparatus and increase the ability of CO_2 assimilation. In conclusion, inoculation with *F. mosseae* could improve photosynthetic capacity by increasing stomatal opening and the tolerance of photosynthetic apparatus to Pb. According to Farquhar et al. (1982), it can be concluded that the decrease of photosynthetic capacity of *Salix viminalis* leaves caused by Pb stress is the result of both stomatal and non-stomatal factors.

The content of chlorophyll directly affects the photosynthetic capacity and growth of plants. Under Pb stress, the number of chloroplast decreased or the structure of chloroplast was destroyed, which accelerated the degradation of chlorophyll and affected the photosynthetic capacity (Al-aghabary et al., 2005; Mani et al., 2015). In this experiment, Chla and Chlb decreased significantly with the increase of Pb concentration, especially Chla was more sensitive to Pb, which led to the decrease of Chl a/b with the increase of Pb concentration, which was consistent with the results of sheet, Vajpayee and Paiva who found that Chla was more sensitive to heavy metals than Chlb Pb stress not only reduced the light energy capture ability of *Salix viminalis* leaves, but also reduced the light energy utilization capacity. The results showed that the chlorophyll a/b ratio of *Salix viminalis* leaves inoculated with *F. mosseae* was higher than that without inoculation. Therefore, inoculation with *F. mosseae* had a higher chlorophyll a/b ratio than that without inoculation *F. mosseae* not only ensured the light energy capture ability of *Salix viminalis* leaves in Pb contaminated soil, but also increased the activity of reaction center to ensure the normal progress of photosynthesis.

Chlorophyll fluorescence technology is one of the important means to study the photosynthetic mechanism, especially the function of PSII (Zhang et al., 2019a,b). In this experiment, with the increase of Pb content in soil, *F*o on OJIP curve of *Salix viminalis* leaves increased significantly, while F_m showed a decreasing trend. F_v/F_m and piabs parameters reflecting PSII photochemical activity of *Salix viminalis* leaves were significantly decreased. However, the decrease range of F_v/F_m and piabs of *Salix viminalis* leaves inoculated with *F. mosseae* were significantly lower than those without inoculation, indicating that inoculation with *F. mosseae* could significantly reduce the decrease of F_v/F_m and piabs *F. mosseae* treatment could alleviate the decrease of PSII

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photochemical activity in *Salix viminalis* leaves.In order to further analyze the reason why the inoculation of *F. mosseae* could relatively improve the photochemical activity of PSII in *Salix viminalis* leaves under Pb stress, we standardized the OJIP curves of *Salix viminalis*leaves under different treatments. The results showed that compared with the soil Pb content of 0 (CK), J point at 2 ms and 0.3 m on the OJIP curve of *Salix viminalis* leaves under different Pb stress were compared. The relative variable fluorescence V_J and V_K of K point at MS were significantly increased. Many studies have found that under stress conditions, the blocking sites of photosynthetic electron transfer often occur on the electron acceptor side and electron donor side of PSII reaction center (Xu et al., 2018; Zhang et al., 2018). The transfer of Q_A (primary electron receptor of photosystem II electron transport chain) to Q_B (secondary electron receptor of photosystem II electron transport chain) is the main inhibition site, and the activity of OEC on electron donor side is also observed is one of the sensitive parts to adversity. The relative variable fluorescence V_J at 2 ms on the OJIP curve can reflect the accumulation of Q_A^- that is, the enhancement of V_J indicates that the electron transfer from Q_A to Q_B on the PSII receptor side is blocked. The increase of V_K is considered as a specific marker for the damage of OEC activity on the side of PSII electron donor. Rashid et al. Showed that Pb^{2+} competitively inhibited the binding site of Ca^{2+} and Cl^- on 23kd protein in the exoxygenation complex, and affected the activity of 23K protein, thus inhibiting the activity of exoxygenation complex. Yao et al. (2009) also found that the photoinhibition of PSII in *Maize* Leaves under Pb stress was mainly related to the damage of OEC. Therefore, the decrease of photochemical activity of PSII in leaves of *Salix viminalis* was mainly due to the inhibition of electron transfer from Q_A to Q_B and the decrease of OEC activity of PSII donor side. However, the increase of V_J and V_K in *Salix viminalis*leaves inoculated with *F. mosseae* were significantly lower than those without inoculation, which indicated that the PSII activity of *Salix viminalis* leaves could be improved by stabilizing the electron transport of PSII receptor side and donor side.

Conclusion

Both 200 $mg \cdot kg^{-1}$ and 400 $mg \cdot kg^{-1}$ Pb stress resulted in the decrease of chlorophyll (especially Chla) degradation and photosynthetic capacity of *Salix viminalis* leaves. The reduction of photosynthetic carbon assimilation capacity of *Salix viminalis* leaves was limited by both stomatal and non-stomatal factors. The decrease of PSII activity and carboxylation efficiency and the oxidative damage of ROS were important non stomatal factors. The results showed that PSII was more sensitive to Pb stress than PSI, and the damage of PSII donor side was greater than that of PSII receptor side.

Soil Pb pollution first affected the root system of *Salix viminalis*, resulting in the decrease of root activity and underground biomass accumulation, as well as the decrease of chlorophyll content and photosynthetic capacity of leaves. *F. mosseae* could infect the roots of *Salix viminalis*. By inoculating *F. mosseae*, the root activity and chlorophyll content of *Salix viminalis* in Pb contaminated soil were significantly increased. In addition, the photosynthetic carbon assimilation capacity and PSII photochemical activity of *Salix viminalis* leaves were also improved in different degrees. Therefore, inoculation with *F. mosseae* could improve the Pb tolerance of *Salix viminalis* from morphological characteristics and photosynthetic function. Our future study aims to reveal the toxicological effect of Pb on the mechanisms underlying the anti-Pb response in tobacco.

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