

# Nitritriacetic acid enhanced lead accumulation in *Athyrium wardii* (Hook.) Makino by modifying rhizosphere characteristics

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## Research Article

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# Abstract

Chelant-assisted phytoremediation may modify plant rhizosphere, which is closely relevant to heavy metal (HM) accumulation in plants. This work focused on the effects of nitrilotriacetic acid (NTA) on rhizosphere characteristics to investigate the mechanism of lead (Pb) accumulation in *Athyrium wardii* (Hook.) Makino with exposure to 800 mg kg<sup>-1</sup> Pb. After NTA application, Pb accumulation in the underground part of *A. wardii* increased by 14.3%, accompanying with some changes for the rhizosphere soils. Soil pH decreased by 0.37 and the dissolved organic carbon (DOC) content in the rhizosphere soils significantly increased by 7.6%. The urease, acid phosphatase and catalase activities in the rhizosphere soils significantly increased by 104.8%, 19.7% and 27.1%, respectively. However, a slight inhibition on microbial activities was observed in the rhizosphere of *A. wardii* after NTA application. Soil respiration decreased by 8.9% and microbial biomass carbon decreased by 8.9% in the rhizosphere soils, indicating that NTA addition recruited some microorganisms to maintain rhizosphere functions in Pb-contaminated soils, while inhibited others with low tolerance to Pb. Results suggest that lower pH, more DOC exudation and higher soil enzyme activities after NTA application contributed to the increase of Pb accumulation in *A. wardii*. This study gave some preliminary evidence for NTA-assisted Pb remediation by *A. wardii* by modifying rhizosphere characteristics.

## 1 Introduction

Lead (Pb) is one of the abundant elements and most hazardous heavy metal (HM) in the environment. A review published by Li et al. (2014) points out that Pb is the second largest inorganic pollutants in mines across China. Pb can persistently exist in soils for decades or even centuries with a half-life up to 740–5900 years (Saifullah et al., 2015; Attinti et al., 2017). Pb is therefore a great threat to food chain and human health, causing a range of diseases (Tighe et al., 2019). Phytostabilization, with native tolerant plants, has been proven to be an economic and environmental strategy for Pb remediation (Anjum et al. 2014; Khalid et al., 2017), showing great applicability in the vegetation development and restoration of mining areas (Dary et al, 2010; Sarwar et al. 2017).

In order to enhance Pb accumulation in plant, lots of researches have proved the possibility and practicability of using biodegradable chelators, such as nitrilotriacetic acid (NTA), which shows high efficiency in promoting Pb uptake by *Brassica carinata* (Quartacci et al, 2007), water spinach (Hseu et al, 2013), *Siegesbeckia orientalis* L. (Lan et al, 2013), *Daucus carota* (Babaeian et al, 2016) and *Scirpus triqueter* (Hu et al, 2017). This strategy makes use of NTA to desorb HM from soil particles into soil solutions, thus enhancing the availability and mobility of HM in soils and improving its uptake by plants (Shahid et al., 2012).

Rhizosphere, a dynamic hotspot for the interaction of plant roots, soils and microbes, is of vital importance in altering HM availability. Chelating agent application alters the dynamic characteristics of rhizosphere, including soil pH, cation exchange capacity, organic compounds and microbe, thus influencing HM availability (Li et al., 2011; Zhan et al., 2018). Soil pH is always regarded as the most

important factor affecting HM availability (Tian et al., 2018). Soil pH decline could be attributed to the excretion of root exudates, thus increasing the content of dissolved organic carbon (DOC) in the rhizosphere soils (Li et al., 2013). Microorganisms, another rhizosphere processes, is of vital importance in plant absorbing of HM. The microorganism colonized in the rhizosphere promote plant growth and affect the availability of HM in soil, thereby improving HM accumulation in plant (Yang et al., 2013; Ayangbenro and Babalola, 2017; Wang et al., 2019). Some microorganisms are sensitive to HM with low tolerance and thereby a decrease on microbial activities, and some high metal resistant microorganisms can survive to keep function on plant growth and HM uptake by plants under metal stress (Shi and Ma, 2017; Antoniadis et al., 2017; He et al., 2019). As the most active soil components, soil enzymes are involved in metabolic process, soil nutrient cycling and the purification of pollutants (Yang et al., 2013; Wang et al., 2019). Some enzymes, such as urease, catalase and acidic phosphatase are most common enzymes and widely accumulated in soils. These enzymes are extracellular enzyme and derived from plant roots and microorganisms, which are closely related to the transformation, accumulation and decomposition of soil organic nitrogen, carbon, phosphorus (Cang et al., 2009; Huang et al., 2016). They are involved in many biochemical processes, subsequently affecting HM availability in the rhizosphere soils by changing pH, complexation and oxidation-reduction reactions (Antoniadis et al., 2017). These researches do prove that these rhizosphere processes play a key role in HM availability and mobility in rhizosphere soils. So, whether and how NTA application modify the rhizosphere soils and, consequently, HM availability in soils? Some work therefore has been processed. It was found that the addition of NTA leads to soil acidification by the exchange of hydrogen ion (Saifullah et al., 2015; Yu et al., 2020). Chelating agents may have a negative effect on microbial growth and enzyme activities. For example, ethylene diamine tetraacetic acid (EDTA) seriously decreased the bacterial count and inhibited catalase and urease activity in soil of *S. nigrum* (Han et al., 2019). Yet, the processes of how NTA affect rhizosphere characteristics and HM availability in soil associated with HM accumulation in plants still need more investigation.

*Athyrium wardii* (Hook.) Makino, a perennial fern in *Athyriaceae* family, was found in a Pb-Zn mining with high tolerance to Pb with extensive roots. It has been proven to be an appropriate plant for the phytostabilization of Pb-contaminated soils (Zou et al. 2011; Zhao et al. 2016). Previous study pointed out that NTA could be useful as a potential chelator to enhance Pb phytostabilization by *A. wardii*. Meanwhile, 2 mmol kg<sup>-1</sup> NTA applied for 1 week was a better alternative for improving Pb accumulation in *A. wardii* (Zhan et al. 2019). Although previous works have elucidated that NTA addition improved Pb accumulation in *A. wardii*, what happened to rhizosphere environment in response to NTA associated with Pb accumulation is still unclear. We hypothesize that NTA application may modify rhizosphere characteristics to enhance Pb availability in soils, resulting in a great increase of Pb accumulation in *A. wardii*. Therefore, a pot experiment was implemented to investigate the changes of soil pH, DOC, microbial activities and enzyme activities in the rhizosphere soils of *A. wardii* after NTA addition, which is beneficial to reveal the mechanism of NTA-enhanced Pb accumulation in *A. wardii*.

## 2 Materials And Methods

## 2.1 Soil and plant preparation

Seedlings of *A. wardii* were gathered from an old Pb-Zn mine area located in Yingjing, Ya'an, Sichuan Province, PR China (102°31'E, 29°47'N). After separating into similar size, the seedlings of *A. wardii* were pre-cultured in vermiculite with 1/4 Hoagland solution for 14 days. Then, uniform and well-growing plants were prepared for transplantation.

Grey fluvo-aquic soils and humic substances used for soil preparation, were collected from Dujiangyan, Sichuan Province, PR China. Grey fluvo-aquic soils were obtained from 0- to 20-cm surface soil of farmland uncontaminated with Pb. Humic substances derived from plant litter. Grey fluvo-aquic soils and humic substances were fully mixed (2:3, w/w). After 1 month of homogenization, the soil properties were as follows, total nitrogen of 1.97 g kg<sup>-1</sup> (TN), available nitrogen of 39 mg kg<sup>-1</sup> (AN), organic matter of 42.4 g kg<sup>-1</sup> (OM), available phosphorus of 28.6 mg kg<sup>-1</sup> (AP), available potassium of 113 mg kg<sup>-1</sup> (AK), and pH of 5.38. The Pb treatment were homogeneity added with 800 mg kg<sup>-1</sup> Pb, added as Pb(NO<sub>3</sub>)<sub>2</sub> solutions, and the available Pb after 1 month of homogenization was 89.3 mg kg<sup>-1</sup>.

## 2.2 Pot experiment

The pot experiment comprised five treatments: (1) CK, (2) NTA2, (3) Pb800, (4) NTA2 + Pb800, (5) NTA2 + Pb800-NP, presented in Table 1. Each treatment is with four replicates, and totally 20 pots were arranged randomly. Each pot (5 L) was filled with 5 kg of soils prepared before. The pot experiment was imposed in the glass room with rainproof facilities and natural light in Sichuan Agricultural University, Chengdu, China.

Table 1  
Experimental design.

Treatment	NTA	Pb	Planting
CK	-	-	+
NTA2	+	-	+
Pb800	-	+	+
Pb800 + NTA2	+	+	+
PB800 + NTA2-NP	+	+	-

The plus (+) means "with". The minus (-) means "without". CK is for the treatment with planting but without NTA and Pb application. NTA2 is for the treatment with planting and 2 mmol kg<sup>-1</sup> of NTA application but without Pb application. Pb800 is for the treatment with planting and 800 mg kg<sup>-1</sup> of Pb application but without NTA application. Pb800 + NTA2 is for the treatment with planting and 2 mmol kg<sup>-1</sup> of NTA and 800 mg kg<sup>-1</sup> of Pb application. Pb800 + NTA2-NP is for the treatment with 2 mmol kg<sup>-1</sup> of NTA and 800 mg kg<sup>-1</sup> of Pb application but without planting.

After 30-day of plant growth, 1 L of deionized water dissolved with 2 mmol kg<sup>-1</sup> NTA was applied into pot at once. In the treatments with no NTA, 1 L of deionized water took place of NTA. Each pot was irrigated

with the same amount of deionized water per 3 days to maintain water holding capacity of 70% approximately in plant growth period. All pots were placed at random and switched in the stochastic position to keep a coincident growth condition.

After adding NTA for 7 days, the plants were harvested and separated into aboveground and underground part. The underground part was soaked in 20 mmol L<sup>-1</sup> Na<sub>2</sub>-EDTA for a quarter to remove Pb sucked on the surface. Then all samples were washed cleanly by deionized water and dried at 75°C to a constant weight. Subsequently, the dried plant samples were ground and stored for Pb analysis. Rhizosphere soils were collected by brushing soils tightly adhering to the roots (approximately 2mm to the root). Some soil samples were stored at 4°C for the analysis of soil respiration, microbial biomass carbon (MBC) and soil enzymes. These indicators were determined within a week. The other soil samples were naturally air-dried, ground, and sieved through 1-mm and 0.15-mm mesh for Pb determination.

## 2.3 Plant analysis

Determination of Pb in plants was according to Zhao et al. (2016). In short, HNO<sub>3</sub> and HClO<sub>4</sub> (5:1, v/v) were used to digest about 0.3 g plant samples. After cooling down, the digested solutions were moved to 50 mL volumetric flask and made up to volume, and then filtered through 0.22 µm membrane filters. Finally, a flame atomic absorption spectrophotometry (AA900T, Perkin Elmer, America) was used to determine Pb concentrations in the filtrates.

## 2.4 Soil analysis

### 2.4.1 Soil chemical properties

The analysis of soil chemical properties, including pH, TN, AN, AP, AK, and OM were followed by the measures of the International Organization for Standardization (Margesin and Schinner, 2005).

For the determination of total Pb in soils, HNO<sub>3</sub>, HClO<sub>4</sub> and HF (5:1:1, v/v/v) were applied to digest about 0.1 g soil samples by a similar method to plant. Determination of soil available Pb was extracted with diethylenetriaminepentaacetic acid (DTPA) solutions, as described by Zhao et al. (2016).

The DOC was measured based on Jones and Willett (2006). Firstly, soils were extracted with deionized water with a water-to-soil ratio of 5:1 (v/w), and then spined at 200 rpm for 16 h at 25°C. Following this, the suspension was centrifuged for 25 min at 10,000×g. After filtering through 0.45 µm membrane filter, the filtrates for DOC concentrations were analyzed by the total organic carbon analyzer (MultiN/C2100TOC, Analytik Jena AG, Germany).

### 2.4.2 Soil enzyme activities

Soil enzymes were measured following the method mentioned by Cang et al. (2009). In brief, urease, catalase and acidic phosphatase activity were measured following the method of sodium phenol - sodium hypochlorite colorimetry, permanganimetric method and P - nitrophenyl disodium phosphate colorimetric method, respectively.

## 2.4.3 Soil microbial activities

As described by Vance et al. (1987), procedures were utilized to measure MBC. Fresh soil samples of 10 g were placed in a vacuum desiccator and full of gasified ethanol-free  $\text{CHCl}_3$  at room temperature. After fumigation at 25°C for 24 h in the darkness, removing residual  $\text{CHCl}_3$  firstly, then the soil samples were extracted with 0.5 M  $\text{K}_2\text{SO}_4$ , and shaken for 30 min at 25°C on a thermostatic oscillator. Prior to analysis, the extracts were filtered through 0.45  $\mu\text{m}$  membrane filter and subsequently were assayed by the total organic carbon analyzer (MultiN/C2100TOC, Analytik Jena AG, Germany). MBC was calculated as the difference in withdrawable organic carbon between the fumigated and unfumigated soils, using a conversion factor of  $k_c$  (0.45).

Soil respiration was referred to method of Lu et al. (2013) and determined by measuring the content of carbon dioxide ( $\text{CO}_2$ ) emissions for incubation at 25°C within 24 hours.

## 2.5 Statistical analysis

The mean data originated from four replicates in this study were calculated. Descriptive statistics were performed using SPSS 22.0. Duncan's multiple range test was used to compare the means at a significant level of  $p < 0.05$ . All values were processed using origin 9.1 and Excel 2016.

## 3 Results

### 3.1 Pb accumulation

The biomass of *A. wardii* both in aboveground and underground part treated with NTA2 showed no significant changes compared with CK (Fig. 1). The biomass in aboveground and underground part of *A. wardii* grown in Pb-contaminated soils significantly decreased compared with CK. NTA showed no effect on the biomass of *A. wardii* both in aboveground part and underground part in Pb-contaminated soils.

In general, NTA application didn't affect Pb accumulation in aboveground part of *A. wardii* in Pb-contaminated soils (Fig. 2). Pb accumulation in underground part of *A. wardii* with Pb800 + NTA2 increased by 14.3% compared with Pb800, indicating NTA promoted the Pb accumulation in underground part of *A. wardii*.

### 3.2 Pb availability in the rhizosphere soils

The concentration of available Pb in the rhizosphere soils with Pb800 + NTA2 significantly decreased by 23.6% compared with Pb800 (Fig. 3). The concentration of available Pb with the treatment of Pb800 + NTA2-NP was 1.35 times higher than that of Pb800 + NTA2, indicating that NTA application promoted Pb mobilization and Pb depletion in the rhizosphere soils due to the uptake by *A. wardii*.

### 3.3 pH and DOC in the rhizosphere soils

There was no significant difference for soil pH between the treatments of Pb800 and CK (Fig. 4a). In general, NTA application significantly reduced the pH of rhizosphere soils compared with CK. In Pb-contaminated soils, the pH in rhizosphere soils remarkably decreased by 0.37 units after NTA addition.

There was no difference for DOC in the rhizosphere soils of *A. wardii* between CK and NTA2 (Fig. 4b). With the exposure to 800 mg kg<sup>-1</sup> Pb, the DOC in rhizosphere soils of *A. wardii* significantly increased by 7.6% after NTA application. The DOC in the rhizosphere soil treated with Pb800 + NTA2 was 1.52 times higher than that of Pb800 + NTA2-NP.

### 3.4 Soil enzyme activities in the rhizosphere soils

In general, NTA application significantly increased the activities of urease, catalase and acid phosphatase in the rhizosphere soils of *A. wardii* (Fig. 5). When exposed to 800 mg kg<sup>-1</sup> Pb, urease, catalase and acid phosphatase activities in the rhizosphere soils significantly increased by 104.8%, 27.1% and 19.7% after NTA application, respectively. The urease and acid phosphatase activities in the rhizosphere soils treated with Pb800 + NTA2 significantly increased compared with Pb800 + NTA2-NP.

### 3.5 Microbial activities in the rhizosphere soils

Soil respiration showed no significant difference between the treatments of NTA2 and CK (Fig. 6A). Soil respiration in the rhizosphere soils treated with Pb800 significantly reduced by 19.6% compared with CK. Soil respiration in the rhizosphere soils significantly reduced by 8.9% after adding NTA in Pb-contaminated soil. Soil respiration in the rhizosphere soils in the treatment of Pb800 + NTA2 significantly increased by 21.1% in comparison with Pb800 + NTA2-NP. The same trend was observed for MBC (Fig. 6B). When exposed to 800 mg kg<sup>-1</sup> Pb, MBC significantly decreased by 16.2% after NTA application. MBC in the rhizosphere soils treated with Pb800 + NTA2 significantly increased by 26.7% compared with Pb800 + NTA2-NP.

The qCO<sub>2</sub> is the ratio of soil respiration and MBC (Fig. 6C). There is no significant difference for qCO<sub>2</sub> in the rhizosphere soils of *A. wardii* between the treatments of NTA2 and CK. qCO<sub>2</sub> in the rhizosphere soils of *A. wardii* treated with Pb800 was 1.37 times higher than that of CK. qCO<sub>2</sub> showed no significant difference between the treatments of Pb800 + NTA2 and Pb800.

## 4 Discussion

In chelating agent-assisted phytoremediation process, it's the critical point that chelating agents promote the accumulation of HM in plants (Saifullah et al., 2015; Sarwar et al., 2017; Yu et al., 2020). NTA shows great practicability for assisting Pb accumulation in plants due to its high chelating ability, which has been applied successfully to a variety of plants, such as *Festuca arundinacea* (Zhao et al., 2013), *Daucus carota* (Babaeian et al., 2016) and *Scirpus triqueter* (Hu et al. 2017). In this study, NTA application enhanced Pb accumulation in the roots of *A. wardii* grown in Pb-contaminated soils, keeping consistent

with the previous studies of Zhao et al. (2016) and Zhan et al. (2019), indicating that it's an effective measure to add NTA for improving Pb uptake in *A. wardii*.

HM accumulation in plants mainly depends on the availability of HM in soils (Khalid et al., 2017; Zhang et al., 2019). According to our results, Pb availability in the rhizosphere soils of *A. wardii* dramatically changed among different treatments. Interestingly, Pb availability in the rhizosphere soils significantly decreased with NTA addition under Pb exposure, whereas Pb accumulation in roots significantly increased and Pb availability with Pb800 + NTA2-NP significantly increased compared with Pb800 + NTA2. It indicates a Pb mobilization and therefore enhanced Pb availability in the rhizosphere soils and also Pb uptake by roots of *A. wardii* after NTA application. NTA application improved Pb availability in the rhizosphere soils. NTA reduced the adsorption of Pb and other HM on pure minerals and soils, resulting in an increase for exchangeable Pb and Pb bound to carbonates along with a decrease for Pb bound to organic matters and Fe-Mn oxides (Yu et al., 2020). Thus, after NTA application, more Pb existed in a rapidly available form in soils, thereby enhancing Pb accumulation in *A. wardii*.

It is well known that rhizosphere characteristics are closely related to HM mobilization in soils. Lower pH and more exudation of DOC have contributed to mobilizing HM (Li et al., 2013; Han et al., 2019). Soil pH is often emphasized as an important factor on solubility and speciation of HM in soils, particularly in soil solutions (Tian et al., 2018; Yu et al., 2020). The result showed that pH in the rhizosphere soils of *A. wardii* after NTA application significantly decreased. The addition of NTA leads to soil acidification by the exchange of hydrogen ion, thus increasing Pb availability (Begum et al., 2012; Zhan et al., 2019). The plantation of *A. wardii* further reduced soil pH. This may be attributed to root exudates, which could be proved by the increase of DOC in the rhizosphere with the plantation of *A. wardii*. Meanwhile, an interesting phenomenon is that NTA application stimulated the production of DOC. Similar DOC increase was also found in the study of Usman et al. (2013) after NTA and EDTA application. The increase of DOC in the presence of chelating agents is due to its high affinity for organic substances. Meanwhile, DOM is a mixture composed of aromatic and aliphatic hydrocarbon structures with many functional groups (e.g. carboxyl, amidogen, hydroxyl and so on), which show high affinity with HM, ultimately improving HM solubility and availability and thus facilitating HM uptake by plant (Li et al., 2013; Borggaard et al., 2019).

In addition, the increased organic compounds in the rhizosphere soils also increase enzyme activities (Bárta et al., 2014; Huang et al., 2016). The activities of urease, acid phosphatase and catalase in the rhizosphere soils of *A. wardii* significantly increased after NTA addition in Pb-contaminated soils, possibly due to the increase of DOC. The increase of enzyme activities was prone to increase Pb availability, resulting in improving Pb accumulation in plant. They are involved in nutrient cycling, which related to plant growth and thus resulting in an increase of HM accumulation (Xian et al., 2015; Hou et al., 2017). What's more, they can combine with HM ions by complexation and thereby improving HM availability directly (Antoniadis et al., 2017).

NTA-enhanced Pb availability in the rhizosphere soils was closely related to NTA-metal complexes, with which microorganisms have to endure and cope (Usman et al., 2013; Huang et al. 2015). MBC can be

regarded to reflect soil microbial biomass and soil quality. As the key process of CO<sub>2</sub> transport from terrestrial ecosystems to the atmosphere, soil respiration is evaluated to assess HM effects on soil microbe (Zhou et al. 2017; Zhan et al., 2018). qCO<sub>2</sub> represents the bioenergetic status of microbial biomass. Generally, qCO<sub>2</sub> shows higher value with more serious HM pollution (Parelho et al., 2016; Zhou et al., 2017). In this study, soil respiration, MBC and qCO<sub>2</sub> in the rhizosphere soils of *A. wardii* demonstrated no apparent difference after NTA application in non-contaminated soils, indicating that NTA itself had no effect on microbial activities. However, a slight inhibition on microbial activities was observed after NTA application in Pb-contaminated soils. This was mainly attributed to the mobilization of Pb by NTA application. Some soil microorganisms are sensitive to heavy metals with low tolerance, which are now known to select only a small subset of the actual soil population. Along with Pb mobilization in the rhizosphere, the mobile HM could combine with the active protein groups and suppresses the growth of microorganisms (Yang et al., 2013; Xian et al., 2015; Ayangbenro and Babalola, 2017). Therefore, increased release of Pb ions after NTA application showed toxicity to some soil microorganisms, thus inhibiting microbial growth and activity. Functional microorganisms retained as the enzyme activities were stimulated, indicating that rhizosphere-competent microorganisms can tolerate high concentrations of HMs and also have potential to adapt to the selective rhizosphere environment. The NTA-induced Pb mobilization selected some keystone taxa representing stable groups to maintain rhizosphere functions. Rhizosphere processes are complex and a further study on rhizosphere microbial community is needed to get a deeper understanding on the role of rhizosphere microbes in the processes of Pb remediation and restoration by *A. wardii* with NTA application.

## 5 Conclusion

NTA application altered rhizosphere characteristics of *A. wardii* grown in Pb-contaminated soils. Reduced pH, increased exudation of DOC and higher enzyme activities were observed after NTA application, making a great contribution to the enhancement of Pb accumulation in *A. wardii*. However, a slight inhibition on microbial activities was observed in the rhizosphere of *A. wardii* after NTA application. This is possibly because that NTA addition recruited some microorganisms to maintain rhizosphere functions in Pb-contaminated soil, while inhibited others with low tolerance to Pb. NTA is an effective chelating agent for improving Pb accumulation in *A. wardii*. Further experiments are necessary to explore the changes of rhizosphere microbial community to get an insight on the role of microbes in the rhizosphere processes of NTA-assisted phytoremediation by *A. wardii* in Pb-contaminated soils.

## Declarations

## Ethical approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Availability of data and materials

The data and materials will be available on request.

## Competing interests

The authors declare no conflict of interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Authors contributions

Tingxuan Li contributed to the experiment design and paper revisions. Yunhong Zhang contributed to the pot experiment, chemical and data analysis and writing. Huagang Huang contributed to the collection of plant seedlings and chemical analysis. Haiying Yu contributed to chemical and data analysis. Juan Zhan contributed to chemical analysis and paper revisions. Daihua Ye contributed to paper revisions. Zicheng Zheng, Xizhou Zhang and Yongdong Wang contributed to the collection of plant seedlings and data analysis.

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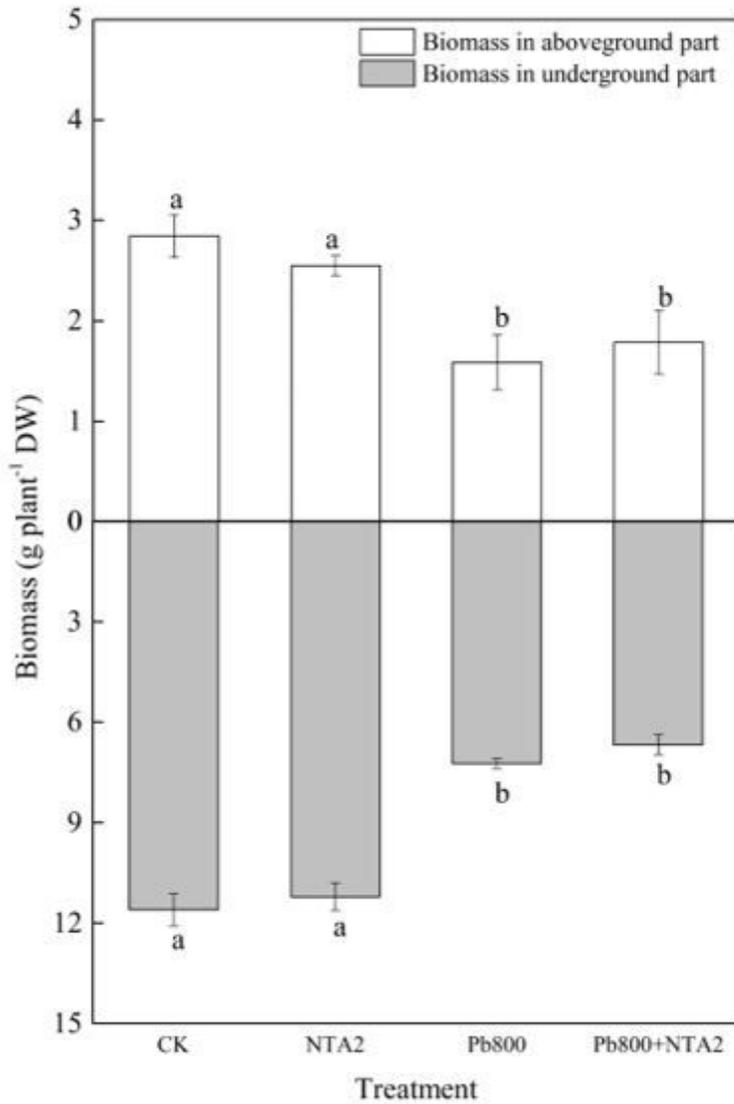
## Table

Table 1 Experimental design.

Treatment	NTA	Pb	Planting
CK	-	-	+
NTA2	+	-	+
Pb800	-	+	+
Pb800+NTA2	+	+	+
PB800+NTA2-NP	+	+	-

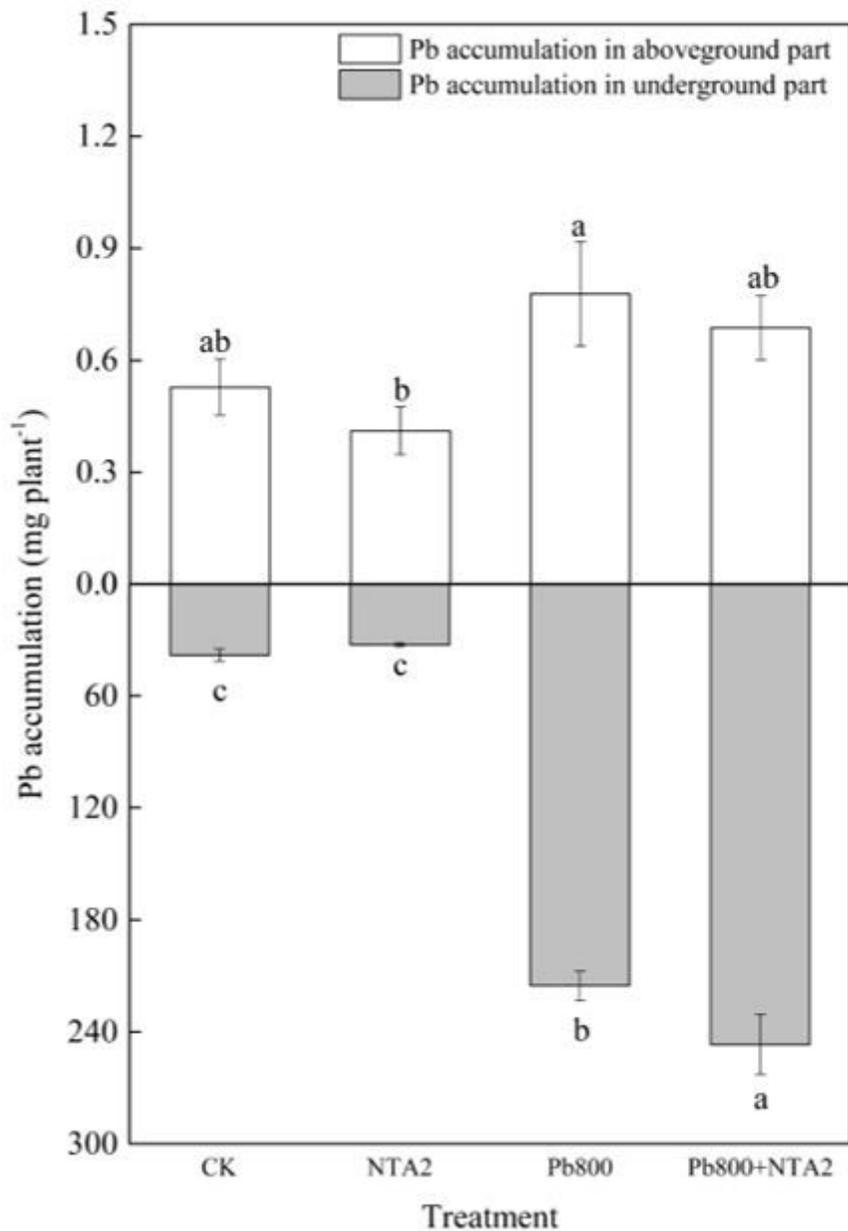
The plus (+) means “with”. The minus (-) means “without”. CK is for the treatment with planting but without NTA and Pb application. NTA2 is for the treatment with planting and 2 mmol kg<sup>-1</sup> of NTA application but without Pb application. Pb800 is for the treatment with planting and 800 mg kg<sup>-1</sup> of Pb application but without NTA application. Pb800+NTA2 is for the treatment with planting and 2 mmol kg<sup>-1</sup> of NTA and 800 mg kg<sup>-1</sup> of Pb application. Pb800+NTA2-NP is for the treatment with 2 mmol kg<sup>-1</sup> of NTA and 800 mg kg<sup>-1</sup> of Pb application but without planting.

## Figures



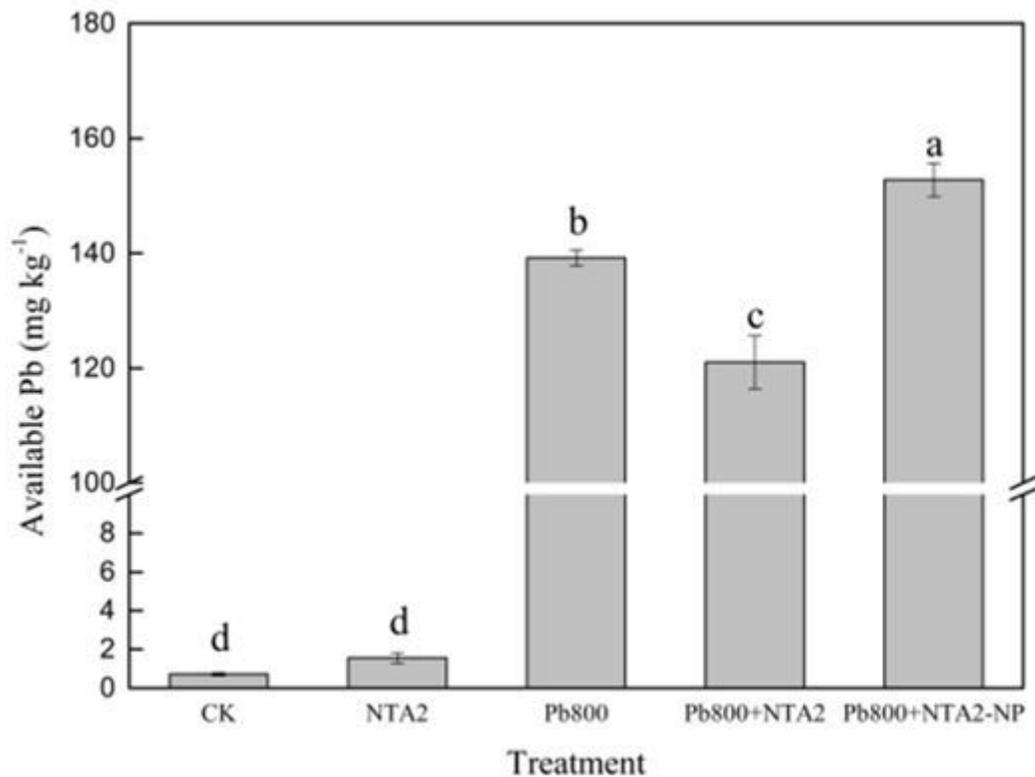
**Figure 1**

Effect of NTA application on biomass in aboveground and underground part of *A. wardii*. CK is for the treatment with planting but without NTA and Pb application. NTA2 is for the treatment with planting and 2 mmol kg<sup>-1</sup> of NTA application but without Pb application. Pb800 is for the treatment with planting and 800 mg kg<sup>-1</sup> of Pb application but without NTA application. Pb800+NTA2 is for the treatment with planting and 2 mmol kg<sup>-1</sup> of NTA and 800 mg kg<sup>-1</sup> of Pb application. Data are means  $\pm$  SE (n=4). The lowercase letters refer to significant difference ( $P < 0.05$ ) Duncan's multiple range test) among different treatments.



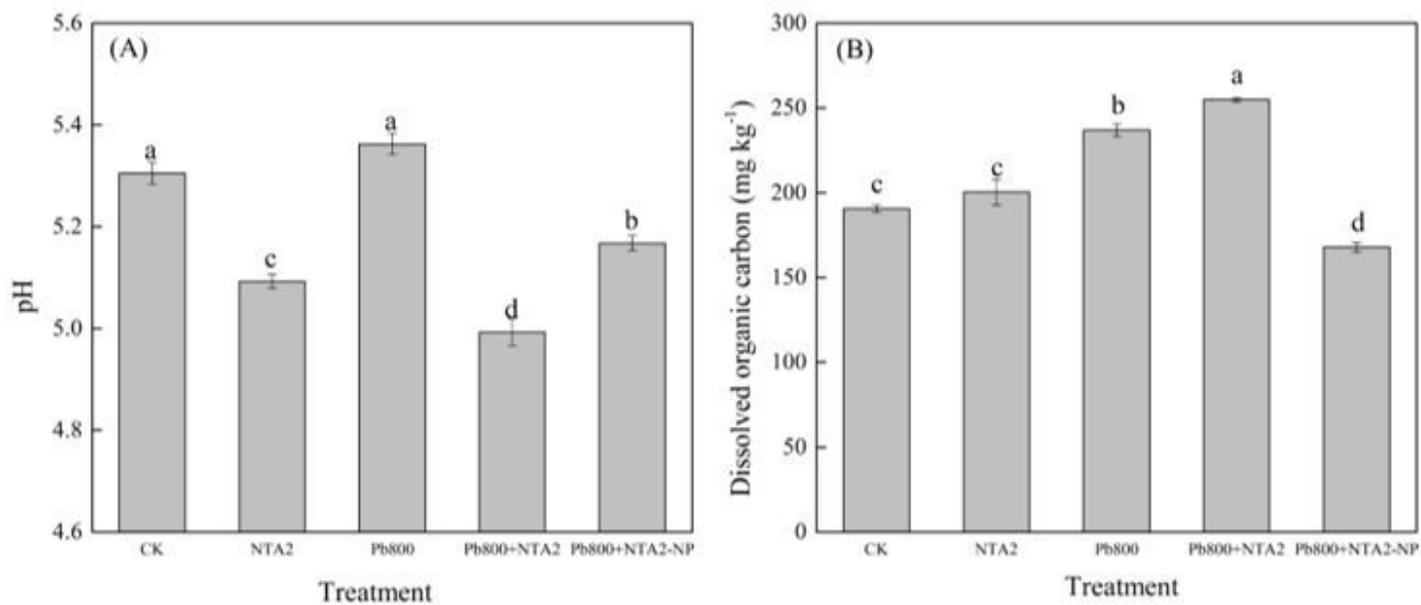
**Figure 2**

Effect of NTA application on Pb accumulation in aboveground and underground part of *A. wardii*. CK is for the treatment with planting but without NTA and Pb application. NTA2 is for the treatment with planting and 2 mmol kg<sup>-1</sup> of NTA application but without Pb application. Pb800 is for the treatment with planting and 800 mg kg<sup>-1</sup> of Pb application but without NTA application. Pb800+NTA2 is for the treatment with planting and 2 mmol kg<sup>-1</sup> of NTA and 800 mg kg<sup>-1</sup> of Pb application. Data are means  $\pm$  SE (n=4). The lowercase letters refer to significant difference ( $P < 0.05$  Duncan's multiple range test) among different treatments.



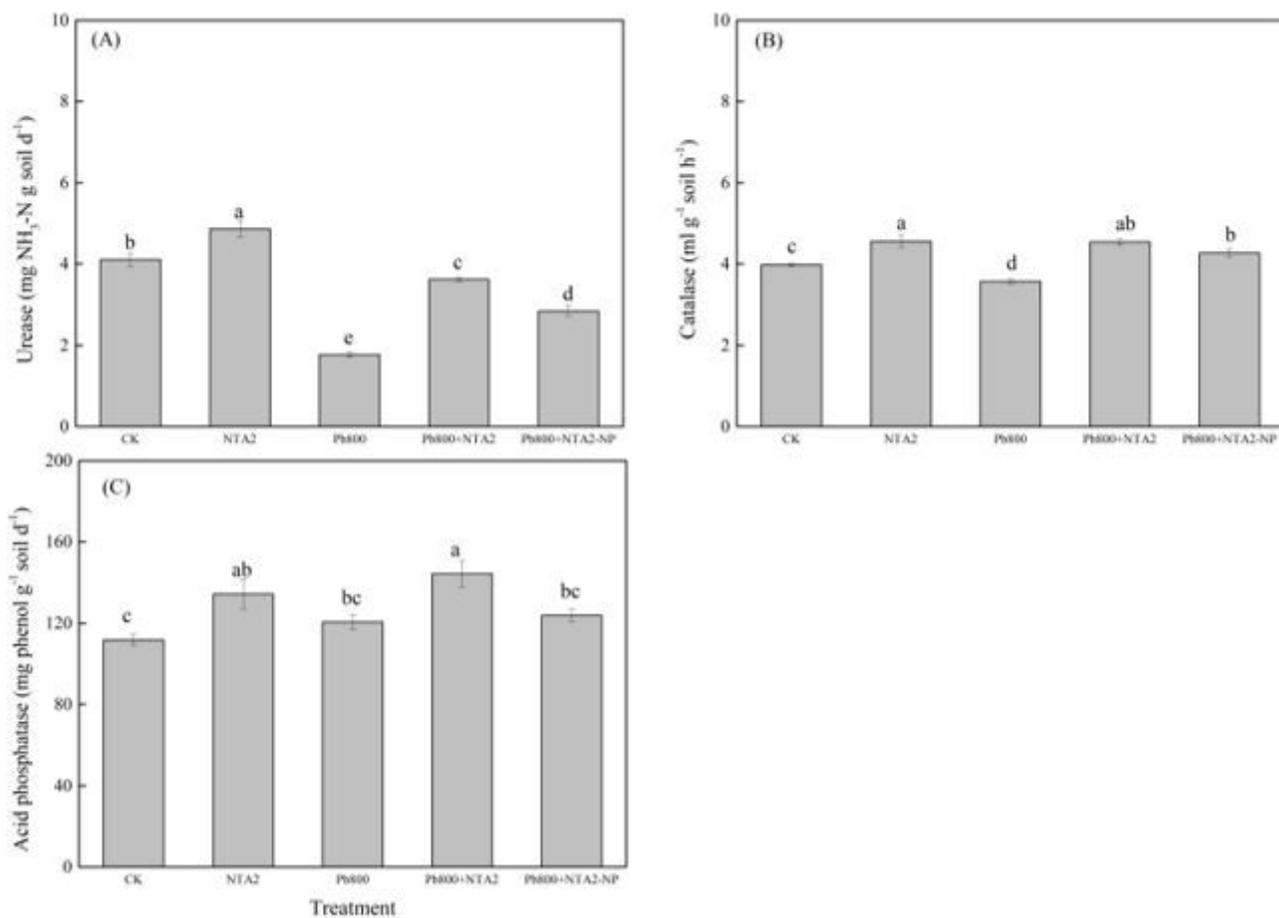
**Figure 3**

Effect of NTA application on available Pb in rhizosphere soils of *A. wardii*. CK is for the treatment with planting but without NTA and Pb application. NTA2 is for the treatment with planting and 2 mmol kg<sup>-1</sup> of NTA application but without Pb application. Pb800 is for the treatment with planting and 800 mg kg<sup>-1</sup> of Pb application but without NTA application. Pb800+NTA2 is for the treatment with planting and 2 mmol kg<sup>-1</sup> of NTA and 800 mg kg<sup>-1</sup> of Pb application. Pb800+NTA2-NP is for the treatment with 2 mmol kg<sup>-1</sup> of NTA and 800 mg kg<sup>-1</sup> of Pb application but without planting. Data are means  $\pm$  SE (n=4). The lowercase letters refer to significant difference ( $P < 0.05$ ) Duncan's multiple range test) among different treatments, the same below.



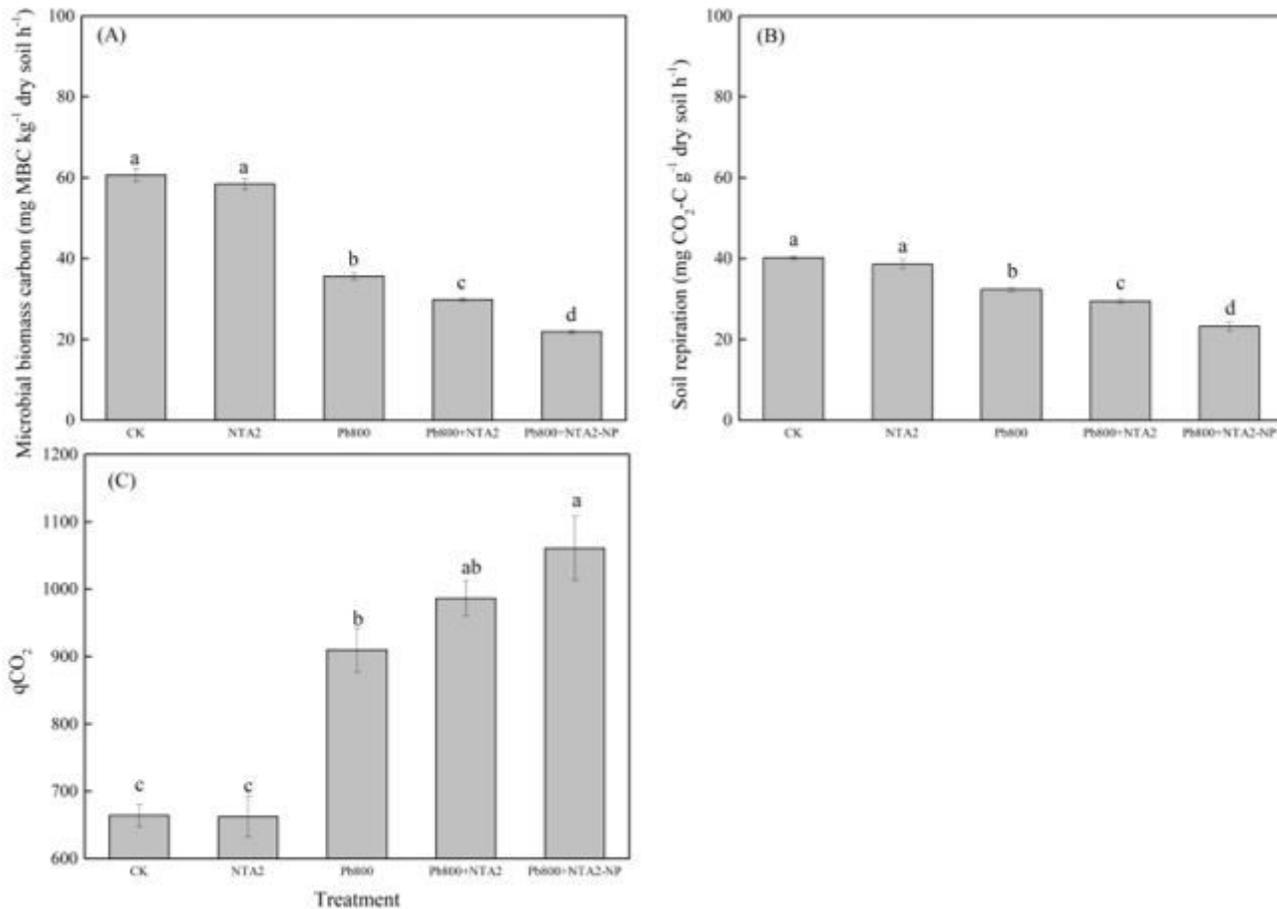
**Figure 4**

Effect of NTA application on pH (A) and DOC (B) in rhizosphere soils of *A. wardii*.



**Figure 5**

Effect of NTA application on soil enzyme activities in rhizosphere soils of *A. wardii*. A-C are for the three soil enzymes of urease, catalase and acid phosphatase.



**Figure 6**

Effect of NTA application on soil respiration (A), microbial biomass carbon (B) and qCO<sub>2</sub> (C) in rhizosphere soils of *A. wardii*.