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RESEARCH ARTICLE

Biochar-assisted phytoextraction of arsenic in soil using *Pteris vittata* L

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Abstract

The alkaline nature of biochar provides a potential for soil arsenic (As) mobilization and, hence, enhancing efficiency of As phytoextraction by combining with As hyperaccumulator. To testify the feasibility and potential risk of the above strategy, biochar effect on As transfer in a paddy soil and accumulation in *P. vittata* was investigated in a pot experiment. By leaching soil (total As concentration 141.17 mg/kg) with simulated acid rain (pH 4.2), As the concentration in leaching eluate increased proportionally with increasing biochar ratio. Coincident with elevated soil As mobility, apparent enhancement in As uptake and translocation in *P. vittata* was determined with 1–5% biochar amendment after 40 days of plant growth. Furthermore, diffusive gradients in thin film (DGT) technique were employed to characterize any potential risk in vertical downward migration of As at 2-mm resolution. A significantly increasing profile of DGT-As ranging from on average 20 μg/L in CK to 50–100 μg/L in 1–3% biochar treatments was recorded over 0–60 mm depth, with 25–71% lower labile As in the rhizosphere than non-rhizosphere zone with few exceptions. As compared to Chinese quality standard for groundwater (Class IV 50 μg/L), biochar ratio at ≤ 1% was suggested for local water safety while actual application should take the physicochemical characteristic of tested soil into account. Our results demonstrated the biochar-assisted *P. vittata* phytoremediation can serve as an emerging pathway to enhance efficiency of soil As phytoextraction. The combination of DGT techniques and greenhouse assay provided a powerful tool for evaluating the gradient distribution of heavy metal in rhizosphere and accessing corresponding ecological risk at more precise scale.

Keywords Arsenic · Hyperaccumulator · *Pteris vittata* L. · phytoextraction · Biochar · Diffusive gradients in thin films (DGT)

Introduction

Arsenic (As) is ubiquitous in the earth's crust ranging from < 1 to > 100 mg/kg (Cullen and Reimer 1989). Since the process of industrialization and urbanization, arsenic is widely accumulated in aquatic and terrestrial environments caused by anthropogenic activities including mining, metal processing, and consumption of As-laden agrochemicals (Leist et al. 2000). As an

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unessential element for human beings, arsenic exceedance would trigger damage of the kidney, lung, and liver, even increasing mortality rates through polluted drinking water, rice diet, inhalation, and direct skin contact (Althobiti et al. 2018, Argos et al. 2010, Irem et al. 2019). There are thousands of people suffering from As contamination all around the world, especially in the region of South Asia such as Bangladesh, India, and China (Brammer and Ravenscroft 2009, Moreno-Jimenez et al. 2012, Shah et al. 2019).

Under the pressure of escalating soil As pollution and its consequent threat toward humans, phytoremediation has been increasingly considered as a cost-efficient and environmentally harmonious method (Salt et al. 1998). *Pteris vittata* L. (*P. vittata*) is one of the widely acknowledged As-hyperaccumulators that is resistant to As poisoning, with aboveground As concentration being up to 1442–7526 mg/kg (dry weight) (Ma et al. 2001, Niazi et al. 2011). However, the extraction efficacy of *P. vittata* is limited by growth rate, plant biomass, and soil properties especially As availability in tested sites (Mathews et al. 2010, Singh et al. 2015, Ye et al. 2011). Former researchers have done numerous work to facilitate phytoextraction efficiency (Niazi et al. 2016). For example, the supplement of phosphate rock was identified to promote more As uptake of *P. vittata* than phosphorous fertilizer (Lessl et al. 2014). Chemical additive such as sodium polyacrylate and water-soluble chitosan were investigated to mobilize As and then expedite As extraction from soil (Yan et al. 2012, Yang et al. 2017). Nevertheless, the above materials mainly consisted of phosphorous-containing substances and other chemical complexes, which imposed potential risks of secondary pollution and eutrophication in water body.

Biochar(BC) is a promising material made by biomass such as farm and forestry wastes via oxygen-depleted thermal degradation (pyrolysis) (Ok et al. 2015). Relative to other common organic amendments for soil, BCs embraced myriad surface functional groups, high cation exchange capacity (CEC), proper alkalinity, and sustainable recalcitrance (Jindo et al. 2014, Kuzyakov et al. 2014, Wu et al. 2012). It has been well established that the utility of BC could mitigate climate change and ameliorate soil physicochemical properties and thus plant growth as well as rehabilitating contaminated soil (Atkinson et al. 2010, Lehmann et al. 2011). Nevertheless, due to its alkaline nature, BCs have been increasingly recognized to enhance soil As mobility, posing potential threats to ecological security (Beesley et al. 2014, Liang et al. 2017, Zeng et al. 2018). From the perspective of phytoextraction, however, this property of BCs could be employed to facilitate an elevated As bioavailability and thus enhance As depletion by its hyperaccumulator as exemplified by *Pteris vittata*.

Considering the key limitation of extraction efficiency, As availability was chosen as major index to assess contaminant level and actual risk toward organisms and ecosystem instead of total As concentration. Diffusive gradients in thin films (DGT) is an emerging technique to measure heavy metal availability in site, reflecting interface dynamic procedure, metals bioavailability in soil, and bioaccumulation in plants (Zhang and Davison 2015). Different from traditional test methods, DGT mimics the plants uptake based on Fick's First Law of Diffusion, determining metals availability without element redistribution (Zhang et al. 2001). In recent years, DGT has been implemented to evaluate mobilization, resupply ability, and pertinent kinetic information of soil metals (Egene et al. 2018, Wang et al. 2018). Further, phytoextraction is largely correlated with As activity near rhizosphere while the corresponding researches are still warranted (Hinsinger and Marschner 2006). So this work focuses on study area of rhizosphere and non-rhizosphere to investigate As transportation and availability in the vertical profile.

The objectives of the present work were to (1) elucidate the available As two-dimensional (rhizosphere, non-rhizosphere) distribution in soil with influence of *P. vittata* and biochar and (2) investigate the possibility of new decontamination solution combining *P. vittata* with biochar and its potential environmental risk.

Materials and methods

Soil pretreatment and biochar preparation

The soil used in this work was collected from a historically As mining-impacted cropland (29° 39' 38" N, 111° 02' 16" E) adjacent to Shimen Realgar Mine in Changde, Hunan province of China (Fig. 1a). The studied area belonged to subtropical monsoon climate with mean temperature of 28.6 °C and average annual precipitation of 1540 mm predominated by the acid rain. This site has been suffering from critical As contamination with total As concentration in agriculture land ranging from 475.4 to 1229.3 mg/kg (Tang et al. 2016). Soil samples were air-dried at room temperature, mixed thoroughly, and ground. To remove large debris and gravel fraction and get more homogeneous test portion, the soil was passed through a nylon sieve of 0.154 mm (100 meshes) for subsequent chemical analysis (Alloway 2012).

Rice straw, which was the most abundant biomass resource in most areas of southern China, was chosen as the feedstock for biochar production. In this work, the rice straw was obtained from a farmland free of As contamination in Huailiu village (27°36' 39" N, 111°59' 42" E; Loudi, Hunan province of China) (Fig. 1b). After being washed thoroughly and oven-dried at 60 °C, the feedstock was subjected to slow pyrolysis in a muffle furnace (BF51732 BPMC-1, Thermo Scientific, USA) under N_2 atmosphere. The temperature was raised by 6 °C min^{-1} and kept at 450 °C for 1 h. After cooling, the product was crushed to pass a 2-mm sieve and referred to as RBC (Rice straw derived biochar). The basic properties of studied

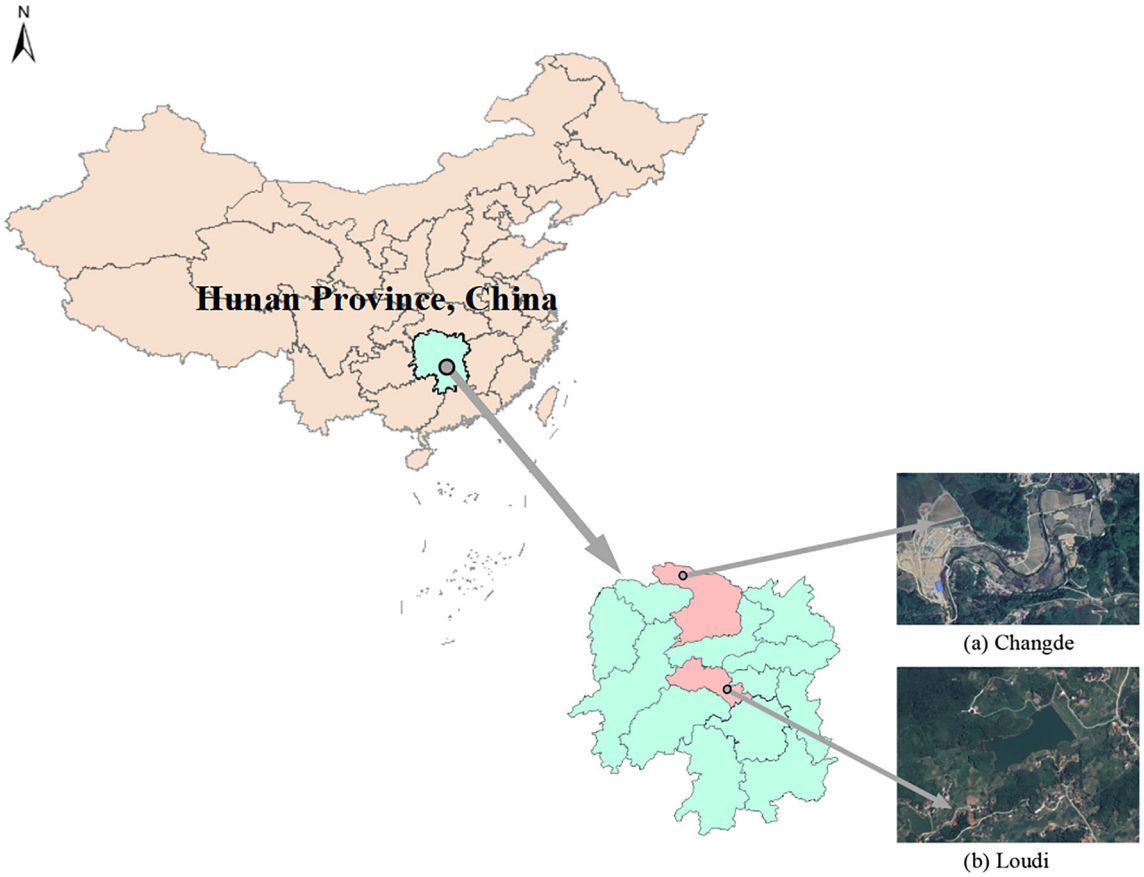

Fig. 1 The location of soil (a) and rice straw (b) sampling sites in Hunan province, south China. Image was from the Google Earth software

soil, RBC, and their mixtures were tested directly prior to planting (Table 1). Relative to soil, the As content in RBC was fewer and RBC-spiked soils did not show obvious As increase, suggesting that the presence of RBC itself would not introduce much arsenic load.

Reagents

All chemicals used in this work were of analytical grade purity and were dissolved in deionized (DI) water (18.2 MO) (Nanopurewater, Barn-stead). All plastic and glassware were soaked in 10% (v/v) HNO_3 for 24 h and then washed by DI water. Sodium arsenate ($Na_2HAsO_4{\cdot}7H_2O$) was purchased from Fisher Scientific.

Leaching column experiment

The columns with internal diameter of 5 cm were filled to a height of 18 cm with soil, RBC, and soil amended with RBC (2% and 5%, w/w) respectively, according to the optimal addition ratio in agriculture soil (Matovic 2011). Each treatment was repeated with three identical columns. The columns were leached upwards from their base, with the bottom of columns being packed with filter paper and quartz sand. Based on the average amount of rainfall in monsoon season in Hunan province, 800 ml of acid solution (HNO_3:H_2SO_4 = 1:4, pH = 4.2) simulating local precipitation of acid rain was introduced into the columns by peristaltic pump at a 27.26 ml/min flow rate (1 rpm). The leachate was collected from the outlet and analyzed for As concentration and pH.

Table 1 Basic properties of studied soil, biochar, and their mixtures

	pH	available P (mg/kg)	total arsenic (mg/kg)	CEC (cmol kg^{-1})
Biochar	9.70 (± 0.05)	1191.93 (± 19.41)	35.68 (± 10.23)	33.4 (± 1.80)
Soil	5.69 (± 0.10)	53.29 (± 4.30)	141.17 (± 0.68)	10.7 (± 1.47)
Soil-2%biochar	6.77 (± 0.06)	70.31 (± 4.17)	138.32 (± 11.78)	22.3 (± 1.14)
Soil-5%biochar	8.01 (± 0.02)	97.64 (± 1.68)	154.22 (± 1.03)	26.9 (± 1.70)

Greenhouse assay

Untreated control soil and RBC-amended soil were used for pot experiments. RBC treatments were prepared by mixing the soil with corresponding RBC additional rate in large plastic containers. Plastic pots (18 × 25 cm; d × h) were then filled with 3500 g of substrate and equilibrated at field capacity for four weeks. Each treatment had three replicate pots.

P. vittata plants were collected from Shimen realgar mining area. After 2-week acclimation in its original soil at greenhouse, every two randomly picked plants of similar size were transferred into each pot and kept in an opened glasshouse to make the *P. vittata* plants grow under almost the same ambient temperature as nature. To provide more detailed information about influence of RBC addition on As contaminated soil, the plant were incubated in soil under two level of RBC (2%, 5% and 1%, 2%, 3%, respectively) (w%) and then harvested at two period (from 20 April to 30 May with mean monthly temperature of 25 °C, from 20 October to 30 November with mean monthly temperature of 14 °C, respectively), which was designated as HA and LA-expt. Soil moisture was maintained around the field capacity with deionized water being added periodically as required during the growing period.

DGT measurement

In this study, the Zr-oxide gel was prepared according to Ding et al. (2011). For assembling the flat DGT probe, the Zr-oxide gel was covered in sequence by diffusive gel (APA gel, 0.80-mm thick) (Scally et al. 2006), and a cellulose nitrate filter membrane (0.13-mm thick, Whatman, 0.45-μm pore size) with an open window of 20 mm × 150 mm (width × length) (Ding et al. 2016).

At end of experiment before plant harvest, DGT devices were inserted into both the rhizosphere and non-rhizosphere soils at water content of 70% and kept for 24 h. The DGT devices were then retrieved from soils and rinsed by deionized water to remove soil residue. The ZnO binding gels were peeled from devices, sliced at 2-mm intervals, and extracted with 0.8 mL of 1 M NaOH for 24 h at room temperature. Arsenic concentration in the extraction was analyzed with an atomic fluorescence spectrometer (AFS6500, Haiguang, Beijing). Detailed procedure for deployment of this flat type of DGT with subsequent processing and calculation can be found elsewhere (Ding et al. 2015, Sun et al. 2014).

Soil and plant analyses

After 40 days growth, *P. vittata* plants were harvested and washed thoroughly. The plants were then separated into roots, stems, and fronds. Oven-dried fern tissues were grounded and passed through a 1-mm sieve. The total arsenic concentration of plant and soil was analyzed with AFS following microwave-digestion (CEM MARS 6, Matthews, NC, USA) with HNO_3/HCl as requirement of EPA Method 3051a (USEPA, 1986). Soil pH was determined in water extract at soil-to-solution ratio of 1:2.5 (1:20 for biochar) by a redox potential depolarization automatic analyzer (FJA-6, Nanjing, China) (China Ministry of Agriculture, NY/T 1377-2007, determination of pH in Soil). The soil available phosphorous was tested by Mehlich 3 method (Mehlich 1984). The cation exchange capacity (CEC) of all soil batch was analyzed by the method described by Liang et al. (2006). To attain the soil buffering capacity, the titration curve was also investigated through description of Xu et al. (2012). Briefly, 2g air-dried samples from each treatment were shaken for 1 h to mix up with the 5 mL solution containing 0, 0.3, 0.5, 1.0, and 1.5 mL of 0.1 M HCl (NaOH), respectively. Then, the suspensions were equilibrated in calorstat for 7 days at 25 °C before pH analysis, followed by daily 2min mixture.

Statistical analysis

All results presented were the average of triplicates ± SD. Differences between treatments were analyzed by one-way analysis of variance (ANOVA) followed by Fisher's test ($p < 0.05$) for multiple comparison with SPSS v.21.

Results and discussion

Effect of RBC on As leachability in column test

Frequent acid precipitation in southern China has been contributing to soil acidification, which may consequently induce mobilization of toxic metals in soil substrates (Zhang et al. 2007). In the initial period of acid leaching (the first 20 collections) with a total of 3.15×10^{-3} cmol H^+ input (Fig. 2a) or titration with a total of 3×10^{-3} cmol H+ input (Fig. 3), leachate pH of the soils with 2% and 5% RBC (w/w) declined slowly, both of which were consistently higher than that of the unamended control. Afterwards, leachate pH from the soil-2% RBC decreased more steeply with continuous acid leaching while soil-5% RBC still maintained a stable pH higher than 6 (Fig. 2a), indicating a more persistent acid buffering capacity of the soil with higher BC incorporation. Leaching pH of soil was lower than mixture by 0.46–1.74 unit in previous period and went parallel with soil-5% RBC mixture since 17th sampling, which may attribute to the exhausted alkaline component in biochar such as carbonates and the function of soil buffer capacity for acids (Yuan et al. 2011). The pH buffering capacity (pHBC) of media was further calculated through the linear simulation of relationship between acid/base introduction versus corresponding pH with great fitting degrees (Table S1). As a result, the buffering capacity of soil, soil-2% RBC, soil-5% RBC, and RBC were 3.20,

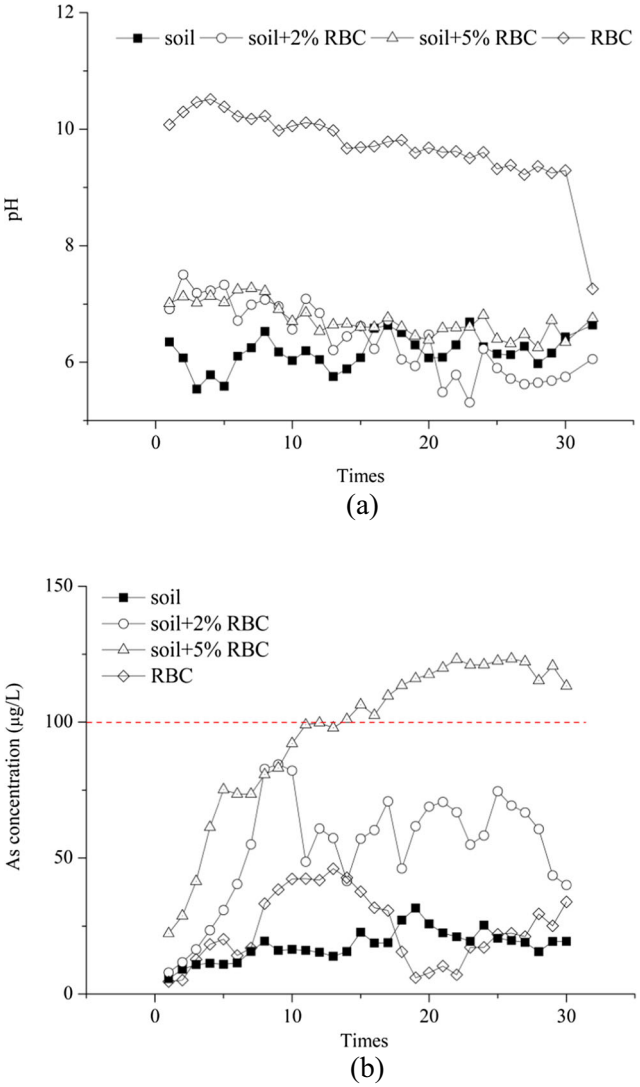

Fig. 2 Dynamics of pH (**a**) and As concentration (**b**) of column eluate in leaching column experiment

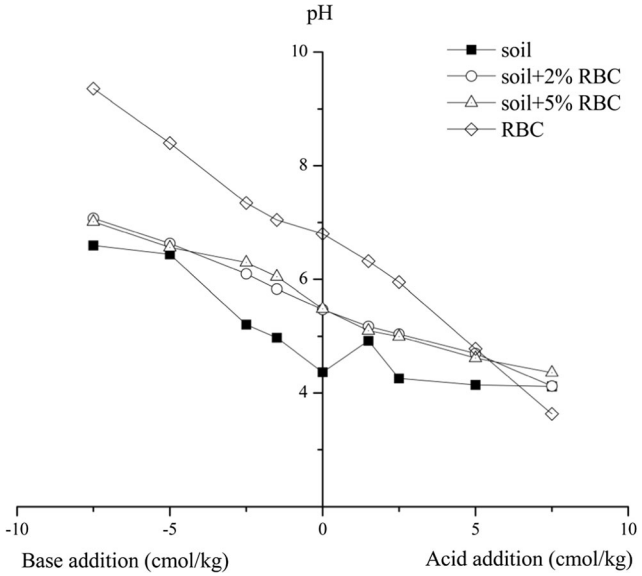

Fig. 3 Titration curve of soil, RBC, and soil-RBC treatments

2.94, 3.00, and 1.61 cmol/kg, respectively. Different from previous study (Shi et al. 2018), there was no promotion of soil pHBC under the effect of RBC, which may be credited to the short equilibration time (40 days) in this study compared to other researches (several years).

The eluate As increased proportionally as a function of RBC ratio, with the steep increase occurring within the initial 10 fractions followed by a stable plateau throughout the rest of leaching period (Fig. 2b). The As content in soil-2% RBC mixture ascended rapidly until ninth sample, descending with fluctuation but still keeping a high level beyond 40 μg/L while As of soil-5% RBC mixed media kept rising (up to 123.14 μg/L) and held 5 times arsenic content as much as the control soil column. With few exceptions, leachate As from the RBC-only control was consistently lower than those from the soil control, suggesting the higher labile As in the RBC-amended soil was not derived from RBC itself. Most likely, As fraction of differential lability in the tested soil had been largely changed with RBC application. According to the results by Yin et al. (2017), soil As concentrations associated with water-soluble, surface-adsorbed, and carbonate-bound fractions exhibited consistent increase with RBC amendment, while As complexed by Fe/Al hydrous oxides was markedly reduced. The increased labile pool of As was most probably resulted from the increased soil pH upon RBC application (Fig. 2a), with OH^- outcompeting $HAsO_4^{2-}/H_2AsO_4^-$ for the anion binding sites on Fe/Al hydrous oxides and other soil constituents. This result highlights, on the one hand, the potential risk of soil As mobilization with RBC amendment, especially in areas suffered from persistent acid precipitation and/or soil acidification. While on the other hand, it provides a possibility to improve soil As bioavailability and hence, phytoextractability upon biochar application with As hyperaccumulating plants growth.

Effect of RBC on soil As phytoextraction by *P. vittata*

The introduction of RBC contributed to a distinct phytoextraction potential of *P. vittata* compared to phylotype in native and control soil in HA-expt (Fig. 4a). The As content in frond, stem, and root significantly increased as elevated RBC addition level ($p < 0.05$) except that As concentrated in stem of soil-5% RBC treatment was lower compared to soil-2% RBC treatment but still higher than control. Specifically, the frond As exhibited significant increase by 22.01–82.78% ($p < 0.01$) in the RBC treatments than unamended control of HA-expt. This significant promotion caused by RBC at the ratio of 2% and 5% manifested a possibility of advanced phytoextraction using *P. vittata* combined with RBC. However based on Chinese environmental quality standards for surface water (GB3838-2002), the Class V limit for As in agricultural irrigation water (0.1 mg/L) was partly exceeded

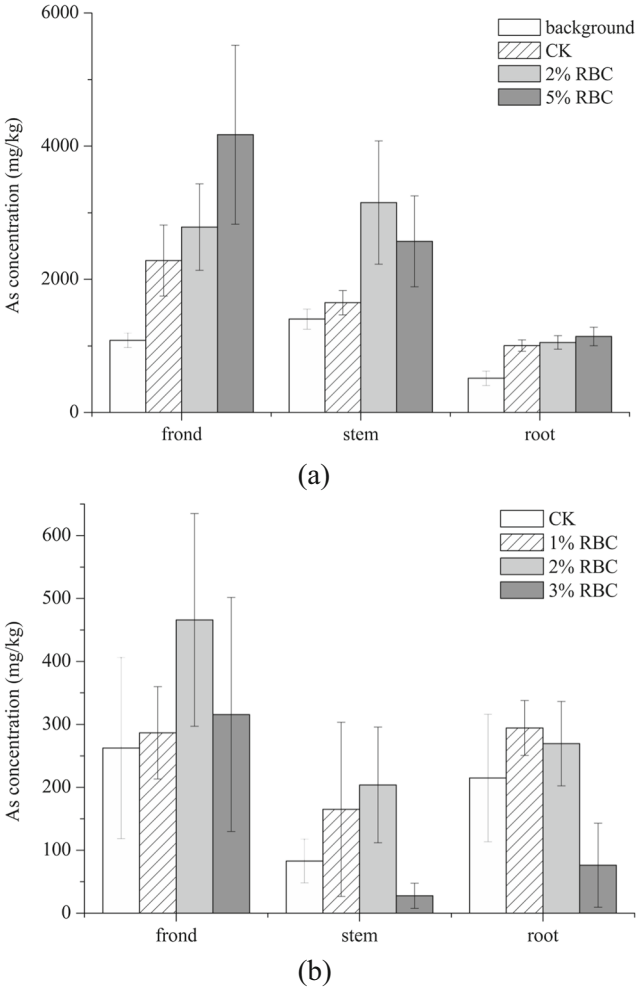

Fig. 4 As concentration of *P. vittata* tissues in greenhouse experiment combined with (**a**) RBC rate of 2% and 5% (HA-expt) and (**b**) RBC rate of 1%, 2%, and 3% (LA-expt)

by leachate As in the 5% RBC treatment (Fig. 2b), indicating the RBC application ratio in the tested soil should be < 5% for environmental safety. Therefore, less biochar proportion, i.e.,1–3%, was used in the following experiments.

Under distinct RBC ratio, the As accumulation patterns among tissues of *P. vittata* were altered in LA-expt. With 1–2% RBC amendment, As level in *P. vittata* root was increased by 3.3–59.85%, in parallel with 1.29–2.10-fold higher As concentration in the aboveground part comparative with control (Fig. 4b). By contrast, both the root and stem As level in *P. vittata* plants were decreased significantly at higher RBC application ratio (3%) compared to other treatments ($p < 0.05$), which was accompanied by a 1.27-time higher As concentration in the frond. In comparison, more efficient As transport from root to stem and frond was determined with increasing RBC ratio, with As translocation factor (ratio of As concentration between aboveground tissue and root) increasing from the value of 1.15–3.26 in 1–2% RBC treatment to 3.67 at the presence of 3% RBC. This result elucidated that elevated RBC application can not only increase As uptake by *P. vittata* but also favor in vivo upward translocation, which was consistent with the higher soil As mobility with increasing RBC ratio (Fig. 2). However, excessive As mobilization due to high rate RBC (3% in this study) deteriorated the uptake capability of *P. vittata* and consequently the proper incorporation rate of RBC should be taken into consideration in the phytoremediation implementation. Previous studies have shown that *P. vittata* was featured by its high root exudation composed mainly of phytic acid and oxalic acid (Lessl and Ma 2013, Tu et al. 2004), which could thus cause much enhanced As bioavailability in the highly acidified rhizosphere upon RBC amendment. In view of district translocation rate between As(III) and As(V) in soil-hyperaccumulator system (Wang et al. 2010), whether the RBC treatment has influenced As redox transformation in the rhizosphere of *P. vittata* is yet to be identified and warrants further investigation.

Coinciding with enhanced As uptake and accumulation by *P. vittata* at the presence of RBC, As removal from RBC-amended soil was increased 1.12–2.28-fold compared to the control. In particular, up to 33.25% of soil As was removed in 1% RBC with *P. vittata* after 40 days of growth, compared to 14.55% in control, suggesting markedly enhanced phytoextraction efficiency by RBC amendment (Fig. 5). However, the As removal rate of soil-2% RBC showed insignificant variation relative to control. This may be explained by the fact that the phytoremediation was a long-term process (Ali et al. 2013) and the alleviating potential of *P. vittata* may partly represent as phytostabilization in site instead of phytoextraction (Lessl et al. 2014, Yan et al. 2012), which needed further research with longer culture period and more proper assessment method. The removal rate under the fortification of 1% RBC was 2 times higher than previous study (Niazi et al. 2012). This result highlighted that the mobilization of soil As by alkaline biochar, as identified in the present (Fig. 2) and other works (Beesley

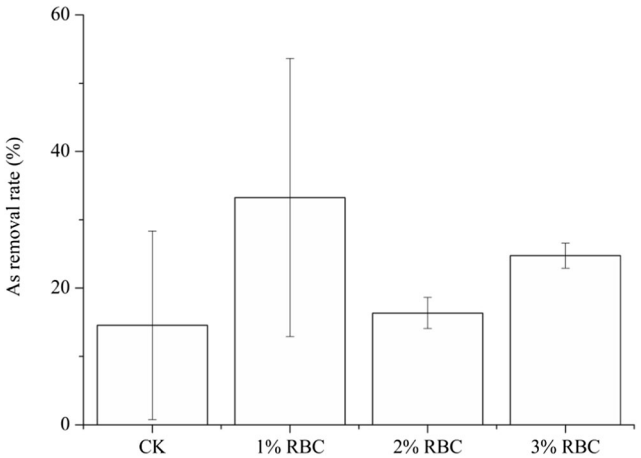

Fig. 5 Soil As removal rate by *P. vittata* in soil and soil-RBC treatments of greenhouse experiment with LA soil.

et al. 2014, Hartley et al. 2009), can transform into positive promotion on soil As removal by phytoextraction with As hyperaccumulator.

Effect of RBC on As leachability with *P. vittata* growth based on DGT analysis

The vertical profile of DGT-labile As of both rhizosphere and non-rhizosphere of *P. vittata* was obtained at end of experiment before harvest (Fig. 6). Without exception, DGT-labile As increased consistently as a function of RBC application ratio, indicating higher As leachability with increasing RBC amount. In particular, apparently higher concentrations of labile As were almost exclusively determined in the non-rhizosphere than rhizosphere, which was in expectation considering the highly efficient As uptake by *P. vittata* as identified by a number of studies (Caille et al. 2005, McGrath and Zhao 2003, Rascio and Navari-Izzo 2011). Very interestingly, DGT-labile As of non-rhizosphere increased tardily from soil interface, reached an ultimate value around − 20 mm and − 30 mm, respectively, in the treatment below and above 1% RBC ratio, followed by gradual decline. It was demonstrated that As infiltration depth was constrained within the top 20 mm of soil in CK and 1% RBC treatment, while much extended and homogeneous percolation of As throughout the 30mm depth range was recorded at the presence of higher RBC (2–3%). This could be largely resulted from the elevated porosity and coarser soil texture with higher RBC input (Lim et al. 2016), which could facilitate water infiltration and As migration to deeper soil layers with limited root length (Ajayi et al. 2016). The DGT-labile As of rhizosphere and non-rhizosphere soil was significantly increased with increasing RBC ratios ($p < 0.01$). The maximum values of rhizosphere and non-rhizosphere (minimum values in rhizosphere) in a serial of treatments were 231.77 (114.04), 101.17 (29.39), 92.38 (22.79), and 34.44 (6.95) μg/L, respectively, also diminished with the decreasing amount of RBC amendment. This result signified that *P. vittata* could partly uptake the extra As but did

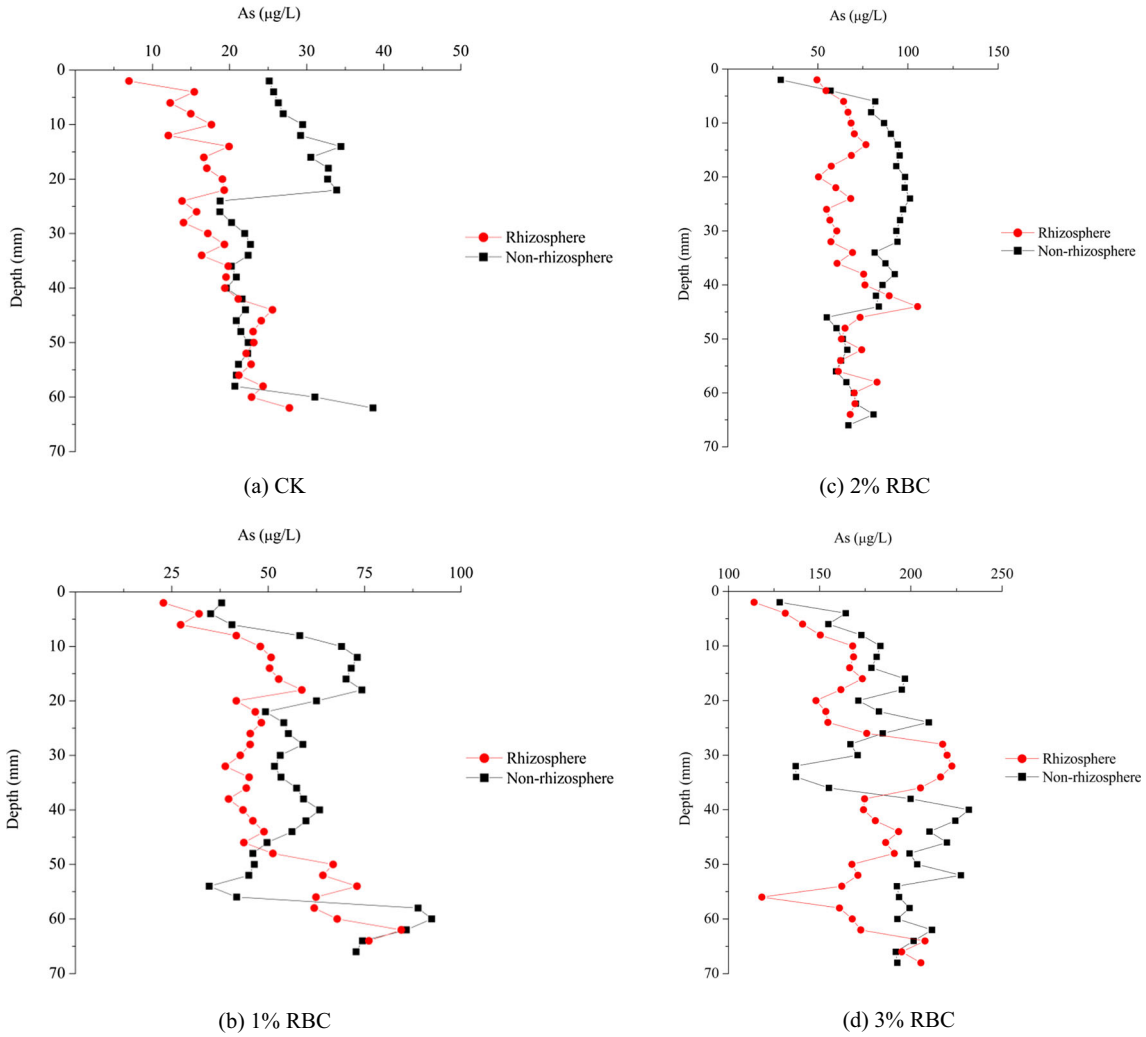

Fig. 6 Profile of DGT-labile As in rhizosphere and non-rhizosphere of *P. vittata*. Treatments included soil control (**a**), soil-1% RBC (**b**), soil-2% RBC (**c**), and soil-3% RBC (**d**). To make sure the clean presentation, the error bars of data were not shown in the figures.

not surpass the entire As load brought by RBC introduction when relative to rhizosphere in control. Nevertheless, the corresponding minimum values in non-rhizosphere were 128.13, 29.39, 34.69, and 18.74 μg/L, respectively, which was a little variant from the sequence of total soil concentration. This incompatibility was probably consequent upon the opposite impact of biochar. On the one hand, the biochar promoted porosity (Guo 2016), nutrient cycle (Tan et al. 2017), and PGP (plant growth promoting) microbe colonization as well as intercepting pathogens in soil (Zhou et al. 2017) thereby intensifying plant metabolism. On the other hand, biochar immobilized arsenic leading to pernicious influence on plant. To protect groundwater for irrigation, the threshold value for As in groundwater was set at 50 μg/L based on Chinese quality standard for groundwater (Class IV limit) (GB/T14848-2017). As phytoextraction by *P. vittata* in this work was suggested to be aided with RBC at ratios ≤ 1%, as shown in Fig. 6.

Therefore, for any target field site with specific geological structure and groundwater level, pre-optimization of biochar ratio is much essential before scaled-up application of biochar-aided phytoextraction to secure groundwater quality.

Conclusion

The present study has demonstrated a promising effect of biochar on increasing As uptake by *P. vittata*, which provides a possible pathway attenuating soil As contamination. Whereas with varying soil property, geological condition as well as root length, pre-optimization of biochar application ratio in field trials is needed to maximize As phytoextraction while avoiding As mobilization into groundwater. Another issue warranting further exploration is how long the promoting effect with biochar can last and the screening of final stabilization method for residual As, which are relating closely to the remediation time frame and reuse type of recovered land soil.

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