Microbe mediated immobilization of arsenic in the rice rhizosphere after incorporation of silica impregnated biochar composites

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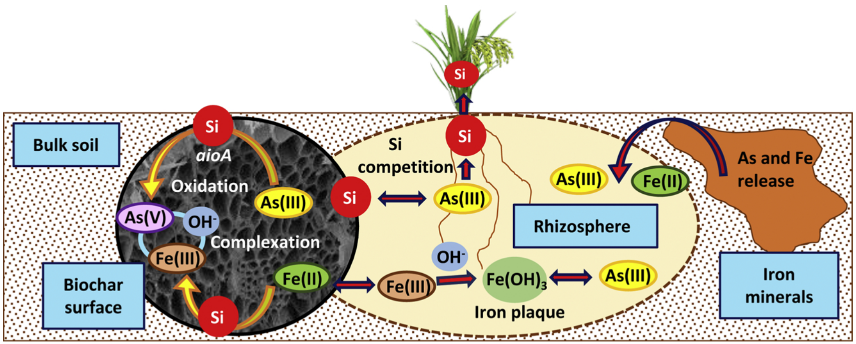
GRAPHICAL ABSTRACT



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ABSTRACT

This study mechanistically addressed for the first time, the contradiction between the application of many biochars to paddy soil and increased arsenic (As) release as employed by most of previous studies. Three types of biochar containing natural and chemical forms of Si: (i) unmodified rice husk biochar (RHBC), (ii) RHBC modified with Si fertilizer (Si-RHBC), and (iii) RHBC modified with nanoparticles of montmorillonite clay (NM-RHBC) were applied in As-contaminated paddy soil to examine their potential to control the mobility of As in the soil-microbe-rice system. Both Si-RHBC and NM-RHBC decreased As concentration in porewater by 40–65 %, while RHBC decreased by 30–44 % compared to biochar unamended soil from tillering to maturing stage. At tillering stage, RHBC, Si-RHBC and NM-RHBC amendments significantly decreased As(III) concentration in the rice rhizosphere by 57, 76 and 73 %, respectively compared to the control soil. The immobilization of As is due to: (i) lowering of microbe mediated As release from iron minerals, (ii) oxidation of As(III) to As(V) by *aioA* gene,

and (iii) adsorption on a Si-ferrihydrite complex. The decrease of more toxic As(III) and its oxidation to less mobile As(V) by Si-rich biochar amendments is a promising As detoxification phenomenon in the rice rhizosphere.

1. Introduction

For the global population, rice (*Oryza sativa* L.) is the staple food for nearly three billion people, predominantly Southeast Asians with an average intake of 500 − 600 g (dry weight) per day (Jia et al., 2014). It is estimated that over 400 million metric tons of milled rice are ingested annually by human beings (Zhu et al., 2008). Paddy ecosystems tend to be extensively polluted by arsenic (As) from As-contaminated irrigation water, As containing pesticides, mining and industrial processes. The flooding nature of rice fields produces an anaerobic condition which promotes the mobility of As in paddy soil, thereby increasing its bioavailability for the uptake by rice. More than 50 % of total As accumulated in the rice grain is inorganic and the health risks of dietary As are dominantly associated with inorganic As species, particularly arsenite (As(III)) (Zhu et al., 2008). Long term exposure to even little dosage of inorganic As through the intake of As-contaminated rice can lead to a serious threat to human health causing cancers (in skin, lungs, liver, etc.), cardiovascular, respiratory and neurological diseases as well as diabetes mellitus and damage to DNA, proteins and lipids (Kumarathilaka et al., 2018). Moreover, the existence of elevated levels of more mobile As species in paddy fields may disrupt the adjacent fresh water reservoirs, aquatic creatures and other crop plants. Therefore, there is an urgent necessity to control the dynamics of speciation, mobility and transformation of As in the rice rhizosphere in order to reduce the accumulation of toxic As species in rice grains as well as to mitigate the environmental risk of As-contaminated paddy fields.

Accumulation of As in rice grains is regulated by the speciation and mobility of As in the rhizosphere depending on physicochemical and microbial properties of paddy soil. Physicochemical factors, such as pH, redox potential (Eh), dissolved organic carbon (DOC), dissolved organic matter (DOM), concentrations of metals (Al, Mn, Fe, etc.) and nutrients (N, P, Si, S, etc.) play a vital role in controlling the speciation and mobility of As in paddy soils (Yang et al., 2018). Various types of microorganisms associated with the rice rhizosphere system can promote the biotransformation of As through oxidation of As(III), reduction of arsenate (As(V)), As(III) methylation and As(V) respiration which strongly affect the mobility, and bioavailability of As to rice (Zhang et al., 2017). Bacteria containing As functional genes, including respiratory arsenate reductase (*arrA*), arsenite oxidase (*aioA*), arsenate reductase (*arsC*) and arsenite methyltransferase (*arsM*) play a key role in regulating the speciation and mobility of As in paddy soil (Rosen, 1999). The *arrA* and *arsC* genes are responsible for reducing As(V) to As (III) which is less adsorbed on soil minerals, thereby resulting in increased release of As in paddy soil. The oxidation and methylation of As (III) are catalysed by *aioA* and *arsM*, respectively, which are recognized as natural detoxification pathways of the As biotransformation cycle in the paddy rice system (Zhang et al., 2017; Gu et al., 2017). The abundance of these As functional genes is generally dependent on the bacterial community structure that can be evaluated based on the diversity of *16S rRNA* genes. Biochar as a soil amendment has been recognized as an excellent hosting substrate for various types of microbes in soil (Yang et al., 2018; Herath et al., 2015; Bandara et al., 2017). It has been recently revealed that the incorporation of rice straw biochar in paddy soil can promote the abundance of *Bacillus* bacteria which is likely to be responsible for As release in paddy soil under anaerobic conditions (Yang et al., 2018). Meanwhile, this paddy soil amended with straw biochar upregulated the abundance of As(V) reducing genes, such as *arrA* and *arsC* in the rice rhizosphere. However, until now, studies regarding the mechanistic interpretations of biochar-microbe mediated immobilization of As in paddy soil have been scarce.

Several studies have reported that the application of biochar derived from bio-waste materials increase the mobility of As in biochar amended soil compared to the unamended soil (Chen et al., 2016; Wang et al., 2017; Qiao et al., 2018a). The addition of biochar produced from orchard prune residues (pyrolyzed at 500 °C) with an application rate of 30 % (w/w) has mobilized As into porewater while reducing the accumulation of As in tomato plants through a phosphorous (P) uptake pathway (Beesley et al., 2013). However, this study has not provided a reasonable mechanism for the increased porewater As levels and decreased As accumulation in the plant parts. Similarly, the addition of a Si-rich biochar derived from rice straw into paddy soil has increased the release of As in rice rhizosphere, whereas the concentration of As(III) in rice grains decreased compared to the control due to a mechanism of root-uptake transporter with Si (Yang et al., 2018). In this study, the addition of rice straw biochar tends to promote reductive dissolution of iron minerals in soil, thereby elevating the release of As into the rice rhizosphere. A field scale study has demonstrated a negligible impact of the addition of biochar produced from wheat straw on the concentration of As in rice grains (Ma et al., 2014). Therefore, it is clear that the application of pristine biochar in As-contaminated soils has shown an inconsistent effect on the mobility of As and its accumulation in the above ground parts of the plants.

Despite that most of the added pristine biochar increased the mobility of As, the application of modified biochar materials as soil amendments has promoted the immobilization of As in biochar amended soils to a considerable extent (Zhu et al., 2019). Biochar mixed with some biomass in certain fractions (Chen et al., 2018) and chemically modified biochar types, such as bismuth impregnated biochar (Zhu et al., 2019), birnessite-loaded biochar (Wang et al., 2019), iron-modified biochar (Wu et al., 2018), zero-valent iron impregnated biochar (Qiao et al., 2018b) and phosphorus-modified biochar (Zhang et al., 2019a) have been successfully applied for the immobilization of As in As-contaminated soils, including paddy soils. The application of a mixture of biochar (derived from rice straw pyrolyzed at 400 °C) and oyster shells reduced the mobile fraction of As(III) and As(V) from 374.9 μg/L to 185.9 μg/L and 119.8 μg/L to 56.4 μg/L, respectively at pH 4–5 in an extremely As-contaminated soil (~ 15,000 mg/kg) (Chen et al., 2018). Paddy soil amended with zero-valent iron impregnated biochar has reduced the accumulation of As in rice grains by 61 % compared to the untreated soil (Qiao et al., 2018b). In a similar study, the incorporation of iron-modified biochar, such as biochar-FeOS, biochar-$FeCl_3$, and biochar-Fe reduced the mobile fraction of As in paddy soil, by 14–30 %, 11–28 %, and 18–35 %, respectively (Wu et al., 2018). One of very recently studies demonstrated that bismuth-impregnated biochar produced from wheat straw can reduce the bioavailability of As in paddy soils through the regulation of ferrolysis under flooding conditions (Zhu et al., 2019). However, the modification of biochar via chemical methods using excessive concentrations of such toxic metal ions such as Al and Mn might also cause in their accumulation in rice grains to a significant extent which is of particular concern in terms of food security and human health. Therefore, the application of silica (Si)-rich amendments has received much attention at present for controlling the mobility of As in the rice rhizosphere as well as the accumulation and speciation of As in rice grains (Limmer et al., 2018; Seyfferth et al., 2016; Wu et al., 2015; Liu et al., 2014).

The addition of Si-rich amendments, such as chemical Si-fertilizers, Si-minerals, rice husk and straw biomasses and related chars has been attributed in controlling the mobility and speciation of As in paddy soils as well as the As accumulation in rice grains (Bogdan and Schenk, 2008; Seyfferth and Fendorf, 2012; Li et al., 2009). The incorporation of rice husk char (pyrolyzed at 250 °C) which is a natural source of Si, into paddy soil led to a decrease in the accumulation of As in rice plants by 3-fold and in ripe grain and bran by 18 and 15 %, respectively compared to control plants, whereas char treatments increased the concentration of total As in polished rice grains by 50 % (Limmer et al., 2018). Furthermore, this study found that Si amendments in paddy soil can increase the abundance of *arsM* gene which promotes the methylation of As(III) in the rice-rhizosphere. The addition of Si-rich rice husk biomass into paddy soil (1 % w/w) decreased the amounts of inorganic As in rice grains by 25–50 % compared to the control plants (Seyfferth et al., 2016). In a similar study, paddy soil amended with rice husk biomass has significantly decreased the concentration of total As and inorganic As species in rice grains by 40 and 30 %, respectively (Teasley et al., 2017). Based on the existing knowledge, it is obvious that the addition of Si-rich carbon based amendments to soil has resulted in increasing the dissolved As in soil-porewater while lowering its uptake by rice via a Si transport pathway (Ma et al., 2008). Moreover, some studies observed that elevated As levels in rice rhizosphere result in increased amounts of As in rice grains as well. So that the previous findings are inconsistent, lacking in appropriate mechanistic interpretations to understand how the enrichment of Si in the rice rhizosphere can significantly reduce the amounts of grain As under elevated levels of As in porewater. Furthermore, the specific effects of the incorporation of Si-rich biochar materials to As-contaminated paddy soils on microbial mediated As-biotransformation and the uptake of As by rice is still not fully understood. Hence, there remains a necessity to develop a suitable type of Si-based biochar to immobilize more mobile As(III) in paddy soil as well as to decrease its accumulation in the above ground parts of rice plants. In consequence, the present study aimed to; (1) produce a pristine biochar from rice husk biomass (natural source of Si); (2) modify its surface via a Si-based fertilizer (SiO_2) and nanoparticles of Si-rich montmorillonite clay (natural source of Si); (3) investigate the effects of these pristine and Si-impregnated biochar composites as soil amendments on the mobility/immobility of As and its speciation as well as on the abundance of As-biotransformation functional genes and related bacterial communities in the rice rhizosphere; (4) postulate potential mechanisms involved in soil-biochar-microbe interactions in As-contaminated paddy soil. Such a mechanistic interpretation of the impact of Si-rich biochar amendments on As biogeochemistry in the rice rhizosphere could provide new insights to alleviate As bioavailability and accumulation in rice.

2. Materials and methods

2.1. Biochar production, Si modification and characterization

The pristine biochar was prepared from rice husk obtained from the Ricegrowers Limited Trading Corporation as SunRice, New South Wales, Australia. Rice husk was selected in this study as it is a potential natural source of amorphous reactive Si. Rice husk was then washed with distilled water and dried at room temperature. The dried biomass was pyrolyzed at 700 °C with a heating rate of 7 °C min^{-1} for 2 h under nitrogen (N_2) atmosphere in a kiln (Rio PMC, SC2). The produced biochar was firstly ground and sieved to obtain < 1 mm particle size which was labelled as RHBC. Secondly, the surface of RHBC was modified via the impregnation of two different types of Si sources: (i) Si-based fertilizer containing SiO_2 (> 50 %), and (ii) nanoparticles of montmorillonite (MMT) clay $[(Na,Ca)_{0.33}(Al,Mg)_2(Si_4O_{10})(OH)_2{\cdot}nH_2O]$ which is a natural source of Si. The MMT was obtained from the National Institute of Fundamental Studies (NIFS), Kandy, Sri Lanka. For the preparation of these Si-based composites, $CaSiO_3$ and MMT were mixed with RHBC in the ratio of 1:10 (w/w). Firstly, a suspension was prepared in 1:10 ratio (w/w) Si:RHBC by adding RHBC together with $CaSiO_3$ in 500 mL of deionized water and then the mixture was sonicated for 30 min with an ultrasonicator (600 W, 20 kHz) to bring about a quick particulate diffusion of Si into the pores of biochar. Secondly, the biochar suspension was stirred overnight on a magnetic hotplate stirrer (SCILOGEX MS7-H550-S) at 100 rpm and 50 °C temperature to reach maximum binding of Si particles onto the biochar surface. The slurry was then oven dried at 80 °C, producing a stable Si-impregnated RHBC composite. In order to use a soil amendment, it was ground to 1 mm particle size and labelled as Si-RHBC. The same procedure was followed to produce a composite of MMT-impregnated RHBC which was labelled NM-RHBC. The ratio of Si to biochar was chosen considering the safe levels of Si to be existed in soil (< 1 g kg^{-1}).

Table 1
Summary of biochar characterization data.

Parameter	RHBC	Si-RHBC	NM-RHBC
pH	9.98 ± 0.08	10.41 ± 0.03	9.94 ± 0.01
BET surface area (m^2 g^{-1})	187.7	182.3	189.6
Langmuir surface area (m^2 g^{-1})	291.7	282.9	292.65
Pore volume (mL g^{-1})	0.1968	0.1968	0.1972
Pore diameter (nm)	4.3172	4.3172	4.1599
Element composition (weight %)			
C	55.03 ± 0.40	53.65 ± 0.75	39.86 ± 0.34
O	27.90 ± 0.53	16.87 ± 0.60	32.50 ± 0.43
Si	13.35 ± 0.16	19.42 ± 0.24	22.45 ± 0.18
P	1.06 ± 0.13	3.61 ± 0.25	1.79 ± 0.13
Available Si (mg kg^{-1})	353.0 ± 36.4	617.7 ± 18.2	291.3 ± 24.2
DOC (mg kg^{-1})	477.5 ± 13.2	457.7 ± 5.2	465.1 ± 35.0

Unmodified rice husk biochar = RHBC, RHBC modified with silica fertilizer = Si-RHBC, RHBC modified with nano-montmorillonite clay = NM-RHBC, Dissolved organic carbon = DOC.

Physicochemical properties of the produced RHBC, Si-RHBC and NM-RHBC were determined by several characterization tests to examine their feasibility for the application as soil amendments. Table 1 summarizes the important physical and chemical parameters of these biochar materials. The pH of pristine and Si-modified biochar composites was determined in suspensions of 1:10 (w/v) biochar to deionize water using a digital pH meter (HI 2211). The same suspension was used to measure the DOC of biochar materials after filtering through a 0.45 μm filter by using a TOC analyser (multi N/C 3100, Analytik Jena, Germany). For the analysis of available Si, firstly Si was extracted by using 0.025 M of citric acid and then its concentration was analysed by using a colorimetric method (ammonium molybdite) as discussed elsewhere (Lu, 2010). The surface area of biochar materials was determined by the BET method as discussed elsewhere (Peterson et al., 2012). The natural silicon structure (O—SiO—) of the RHBC was verified by Fourier-transform infrared spectroscopy (FTIR) (IRAffinity-1S, Shimadzu) with a reference granular silicate material (S1). Moreover, changes in the surface morphology of biochar after impregnation of Si were examined using scanning electron microscope integrated with energy-dispersive X-ray spectroscopy (SEM-EDS) (Quanta 250 FEG). The composition of elements present in pristine and modified biochar composites was quantified using the extracted spectra obtained from the SEM-EDS (Table 1). The prominent peak at 1.8 keV in the SEM-EDS spectrum was attributed to Si and the intensity of this peak in Si-RHBC and NM-RHBC composites significantly increased compared to the RHBC which further confirmed the effective impregnation of Si after the modification process. The SEM-EDS images and corresponding elemental spectra are provided in supplementary materials (S2 and S3).

2.2. Soil sampling and characterization

Paddy soil was collected from As-contaminated paddy fields located at Qiyang city in Hunan province, China. The average amount of As present in the selected paddy soil was 85 mg kg^{-1} which is much higher than the permissible limits for As in agricultural soils (20 mg kg^{-1}) as recommended by the European Union (EU). Soil samples were dried under sun light and mechanically sieved to < 2 mm particle size, and then subjected to basic characterization tests, including pH, analysis of tracer elements, total organic matter (TOM), DOC, available Si and mineral composition. Some important physico-chemical properties of this paddy soil are summarized in Table 2.

Soil pH was measured in suspensions of 1:20 (w/v) soil to deionized water using a digital pH meter. The same suspension was used to measure the DOC of biochar materials after they were filtered through a 0.45 μm filter using the TOC analyser. The concentration of trace elements, including total As, Fe, and manganese (Mn) was determined by completely dissolving the soil samples in a closed vessel device using a hot block digestion system (AIM600/SEAL) with a mixture of concentrated HNO_3, HCl and HF and the digested soil samples were analysed using inductively coupled plasma mass spectrometry (ICP-MS) (NexION 300X, PerkinElmer, USA). The amount of available Si was determined by following the same methods given in Section 2.1. Different types of minerals associated with this paddy soil was evaluated by XRD using Cu Kα (k = 1.54 Å) radiation at 45 kV (Rigaku, SmartLab) and major mineral phases identified biochar amended and unamended soils are shown in supplementary materials (S4). The XRD spectra data revealed the existence of hematite ($\alpha-Fe_2O_3$), amorphous silica (SiO_2), and goethite ($\alpha-FeO(OH)$ minerals in paddy soil used in this study.

2.3. Pot experiments

A pot experiment was conducted in a greenhouse from July to December in 2018 in Nanjing (Jiangsu province, China). Biochar unamended soil (control) and paddy soil amended with RHBC, Si-RHBC and NM-RHBC at 0.25 % (w/w) of application rate were used in this pot experiment. Firstly, 3 kg of soil was thoroughly homogenized with each biochar material and then plastic pots (20 cm diameter x30 cm height) were filled with soil mixed with different biochar materials. Secondly, the pots with biochar amended soil were continuously flooded with tap water for one week. The rice cultivar Shenyou 957 was used for the potted plants. Three rice seedlings were placed in each pot and there were four treatments (including control soil) and triplicates for each treatment. The biochar treatments were labelled as: (1) Control soil (biochar unamended soil), (2) 0.25 % RHBC, (3) 0.25 % Si-RHBC and (4) 0.25 % NM-RHBC. The temperature of the greenhouse was controlled at 28 °C during the day and 20 °C at night. The pH and Eh of soil were measured in situ using portable pH and Eh meters at major rice growing stages, including tillering, heading and maturing. There was approximately 14 h of light and 10 h of darkness per day. The pots were randomized inside the greenhouse and rearranged once every week. A representative image showing the differences in the production of biomass and rice grains at the maturing stage is provided in the supplementary materials (S5).

2.3.1. Porewater analysis

Porewater was collected by immersing porewater collectors into 10 cm depth of the rhizosphere soil during the main rice growing stages, including tillering, heading and maturing. Approximately 15 mL of porewater was collected from each pot, filtered through 0.45 μm filter paper, and diluted with 1 % nitric acid while vigorously shaking. Species of As, including As(III), As(V), monomethylarsonate (MMA(V)) and dimethylarsinate (DMA(V)) were immediately analysed by HPLC-ICP-MS (Series 200, NexION 300X, PerkinElmer, USA). The concentration of total As and other trace elements such as Fe, and Mn present in diluted porewater samples was analysed by ICP-MS. The concentration of DOC and available Si was determined by following the methods given in Section 2.1. For the analysis of Fe(II), 5 mL of porewater was separately collected and immediately diluted just after the collection with 0.5 M HCl acid to prevent its oxidation to Fe(III) and precipitation. The concentration of Fe(II) was then determined by a spectrophotometer (Shimadzu UV-1800) following the phenanthroline method explained elsewhere (Xue et al., 2020).

2.3.2. Analysis of plant materials

Rice plants were harvested after the maturation of grains. The harvested plants were thoroughly washed with tap water and then dried in an oven at 60 °C for 48 h. The dried plant materials were separated into straw and grains and then ground to powder form. For the analysis of total As concentration, approximately 0.2 g of powdered materials were treated with 5.0 mL of concentrated HNO_3 acid (analytical grade) in 50 mL of polypropylene tubes and digested at 180 °C for 45 min in a microwave digestion system (CEM, Mars, USA). After the digestion was completed, the solutions were diluted with deionized water to a final volume of 10 mL and filtered through 0.45 μm filter paper. Finally, the total concentration of As in rice straw and grains was determined by ICP-MS. The concentration of As species present in rice grains was determined by following the method discussed elsewhere (Herath et al., 2019). A certified rice reference material (GBW 10045) was used for the quality control of the digestion procedure and quantification of As in rice grains.

2.3.3. Quantification of 16S rRNA and As functional genes

Soil samples were collected from the pots at the maturing rice growing stage and transported to the laboratory in liquid nitrogen (N_2). The maturing stage was selected because at this stage biochar is well-equilibrated with the bulk soil and rhizosphere which allows it to reach the climax of most of the microbe mediated As biotransformation processes in the rice rhizosphere. The soil samples were then frozen dried and stored at −80 °C until the extraction was carried out. Total microbial DNA was extracted by using a DNeasy Power-Soil DNA Kit (QIAGEN, Germany) according to the manufacturer's protocol. The concentration of DNA and RNA was determined using a NanoDrop 2000C spectrophotometer (Thermo Scientific, Wilmington, USA). The abundance of bacterial *16S rRNA* genes and four different types of As functional genes, including respiratory arsenate reductase (*arrA*), arsenite oxidase (*aioA*), arsenate reductase (*arsC*) and arsenite methyltransferase (*arsM*) were quantified by real-time PCR (CFX96 Thermocycler, Bio-Rad, USA) (Wang et al., 2014).

2.3.4. High-throughput sequencing of bacterial 16S rRNA gene

The high-throughput sequencing of bacterial 16S rRNA gene was carried out following the procedure given elsewhere (Xu et al., 2019). The DNA fragments of the V4-V5 regions of the bacterial 16S rRNA genes were amplified using the primer pair 515 F (5′-GTGCCAGCMG CCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′). Amplicons were purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA). The pooled DNA products were used to construct Illumina Pair-End library before the amplicon library, being paired-end sequenced (2 × 250) on an Illumina MiSeq platform (Shanghai BIOZERON Co., Ltd). The raw reads were demultiplexed and quality-filtered before the bioinformatics of the resultant high-quality sequences being processed using the Quantitative Insights Into Microbial Ecology (QIIME) with a method discussed in a previous study and the online instruction for QIIME (Zhang et al., 2017).

Table 2
Summary of some important physicochemical properties of soil.

Parameter	Amount
pH	5.83 ± 0.05
Total As (mg kg^{-1})	85.30 ± 3.50
Total Fe (g kg^{-1})	32.30 ± 0.50
Total Mn (mg kg^{-1})	279.34 ± 8.60
TOM	21.92 ± 5.91
DOC	104.00 ± 17.30
Available Si (mg kg^{-1})	151.60 ± 37.56

Total organic matter = TOM, Dissolved organic carbon = DOC.

2.4. Statistical analysis

Statistical analyses were carried out to test whether there is significant difference between the control and biochar treatments in the analysis of As in porewater, rice straw and grains as well as the abundance of As functional genes and bacterial diversity. The test was performed using one-way analysis of variance (ANOVA) followed by post hoc multiple comparisons with the Tukey test ($p < 0.05$) (Ojo et al., 2020). All the statistical analyses were carried out using IBM® SPSS Statistics 25.0. In this study, data presented were the mean ± standard error (SE) of three replicates ($n = 3$). In figures, asterisk (*) and double asterisks (**) indicate the significant difference between biochar treatments and the control at $P < 0.05$ and $P < 0.01$ level, respectively.

3. Results

3.1. Dynamics of porewater chemistry after addition of biochar to paddy soil

Physico-chemical factors of soil-porewater, such as pH, Eh, DOC, available Si and Fe play a vital role in regulating the mobility of As in paddy soil. Fig. 1 depicts the effects of modified and unmodified rice husk biochar on the variation of these physico-chemical properties in the rice rhizosphere.

3.1.1. Variation of soil pH and Eh

The pH and Eh of soil were measured in situ using portable pH and Eh meters and corresponding values measured at different rice growing stages (transplanting day, tillering, heading and maturing stage) are given in Table 3. Interestingly, the addition of Si modified and unmodified biochar treatments to paddy soil did not result in increasing soil pH to a significant extent between the biochar treated and control soil throughout the rice growing period, probably due to the addition of a little dose of biochar (0.25 %) as a soil amendment in this experiment. The average pH of biochar amended soil dropped slightly (6–13 %) from tillering to maturating stages, compared to the pH of control soil on the transplanting day because of the dissolution of silicate present in biochar into a weakly acidic silicic acid (H_4SiO_4) in soil-porewater. To a considerable extent, the incorporation of biochar to paddy soil has been attributed to alteration of the Eh of rice rhizosphere (Table 3). The addition of both modified (Si-RHBC and NM-RHBC) and unmodified (RHBC) biochar significantly increased the Eh of the rice rhizosphere compared to the control soil, particularly at heading and maturating stages. The Si-RHBC and NM-RHBC treatments increased the Eh of rhizosphere from -141.9 (control) to -111.4 and 35.3, respectively at the heading stage and it was further increased to −124.0 and −97.4, respectively at maturing stage. Soils having increased Eh values (positive or less negative) can promote naturally occurring microbe mediated oxidation processes.

3.1.2. Variation of DOC

The DOC plays a significant role in controlling the mobility of As in soil (Fig. 1(a)). The results of DOC analysis in porewater samples demonstrated that the addition of Si-RHBC and NM-RHBC treatments did not result in a significant change of DOC levels in the rice rhizosphere, although it showed a slight upward trend at the heading stage compared to the control soil. In contrast, the application of unmodified RHBC increased the DOC levels from 98.0 mg L^{-1} (control) to 138.3 and 154.9 mg L^{-1} at heading and maturing stage, respectively, whereas it remained steady (98.3 mg L^{-1}) at the tillering stage. When considering the overall trend, it is clear that all the biochar treatments at 0.25 % of application rate have had no significantly change the DOC levels in the rice rhizosphere compared to the control soil throughout the rice growing stages. That being the case, a little dose of biochar (0.25 %) produced at higher pyrolytic temperature (at 700 °C) was applied in this experiment. When a biomass is pyrolized at higher

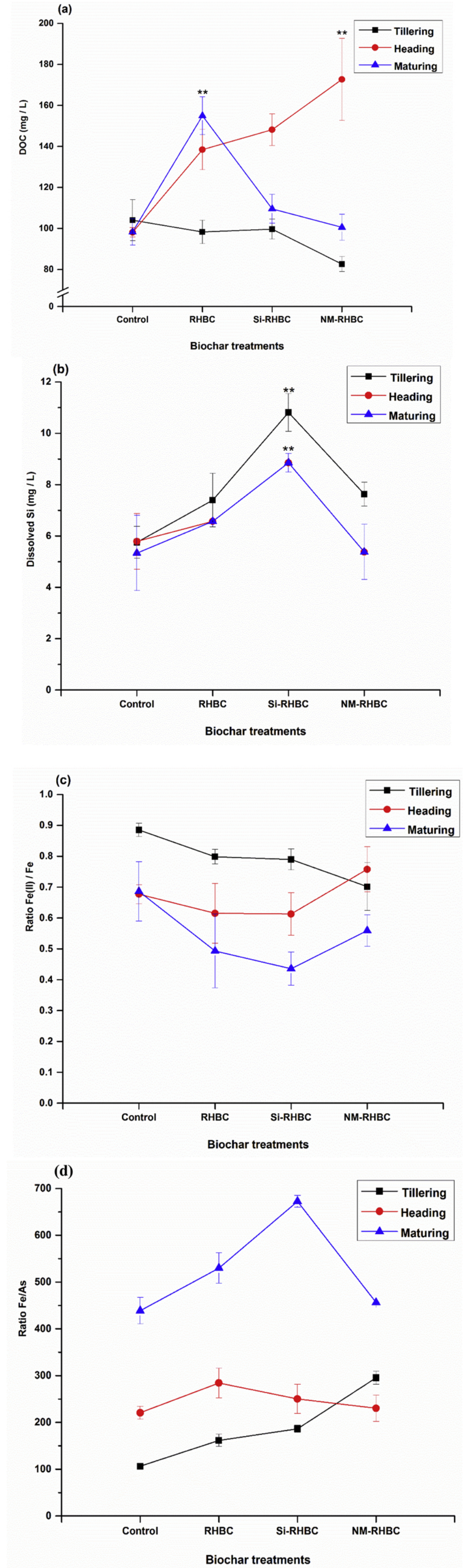

Fig. 1. Variation of: (a) DOC, (b) dissolved Si, (c) dissolved Fe(II), and (d) ratio Fe/As in porewater in biochar amended and unamended paddy soil among different rice growing stages.

Table 3
pH and Eh of soil-porewater in biochar amended and unamended paddy soil among different rice growing stage.

Parameter	Biochar applications	Rice growing stage			
		Transplanting day	Tillering	Heading	Maturing
Soil-porewater pH	Control soil	7.48 ± 0.06	6.80 ± 0.24	6.95 ± 0.02	6.31 ± 0.04
	RHBC	7.29 ± 0.03	6.64 ± 0.11	7.03 ± 0.04	6.28 ± 0.02
	Si-RHBC	7.68 ± 0.17	6.77 ± 0.21	7.00 ± 0.01	6.52 ± 0.08
	NM-RHBC	7.66 ± 0.17	6.68 ± 0.09	7.16 ± 0.11	6.63 ± 0.06
Soil-Eh (mV)	Control soil	−95.1 ± 9.9	−25.5 ± 10.5	−141.9 ± 44.6	−159.0 ± 16.3
	RHBC	−123.0 ± 32.6	−127.9 ± 10.6	21.0 ± 3.3	−70.0 ± 52.3
	Si-RHBC	−103.3 ± 48.3	−119.0 ± 32.1	−111.4 ± 27.8	−124.0 ± 33.5
	NM-RHBC	−139.5 ± 12.7	21.1 ± 8.3	35.3 ± 14.6	−97.4 ± 25.9

Unmodified rice husk biochar = RHBC, RHBC modified with silica fertilizer = Si-RHBC, RHBC modified with nano-montmorillonite clay = NM-RHBC, Dissolved organic carbon = DOC.

temperatures (> 600 °C), a number of labile organic compounds present in the biomass tends to get lost while producing a biochar which then possesses more inorganic carbon rather than organic. Therefore, the addition of a low rate of such biochar to soil cannot increase the concentration of DOC at a significant extent as employed by this study. These observations are in contrast with previous studies where low temperature derived biochar materials (< 600 °C) were applied at higher doses (> 1%) as soil amendments. The elevation of DOC levels has been often attributed to increase the mobility of As in biochar amended soils (Yang et al., 2018; Beesley et al., 2013).

3.1.3. Variation of dissolved Si and Fe

Since this study is mainly based on naturally occurring and synthetic Si-rich biochar materials, the analysis of dissolved Si levels in the rice rhizosphere is of particular concern (Fig. 1 (b). In general, amounts of Si in porewater of soil amended with Si-RHBC composite were higher than that of in RHBC and nano-RHBC treated soils. The average concentration of soluble Si in the control soil was 5.6 mg/L and it was increased by 53–88 % in the rhizosphere of Si-RHBC treatment, whereas during the entire rice growing period, RHBC and NM-RHBC treatments increased by only up to 13–33 % of Si in the rice rhizosphere compared to the control. Hence, it is obvious that Si-RHBC composite provides more available Si into soil-porewater compared to NM-RHBC and RHBC. This is mainly due to the different physical and chemical nature of Si impregnated in biochar. In NM-RHBC, Si is in the form of nanoparticles derived from a natural silicate clay mineral and these nano-Si particles can strongly be retained in the pores of biochar without dissolving much in soil. Further, nano-Si particles can be easily adsorbed on other mineral surfaces available in paddy soil. As a result, relatively a small amount of Si tends to be dissolved in the soil-porewater system. On the other hand, RHBC has a characteristic natural silicate structure which is highly stable, so that it has less capability of releasing a large amount of soluble Si in soil.

The determination of total Fe and its aqueous form of Fe (II) in the rhizosphere is an important indication to investigate the speciation and transformation of As over a paddy rice system. Hence, the concentration of soluble total Fe and Fe(II) in porewater was quantified to assess the influence of iron oxide minerals associated with paddy soil on the control of As mobility in rice rhizosphere. Fig. 1(c) shows the variation of Fe(II) concentration in relative to the total Fe in the rice rhizosphere from tillering to maturing stage. Results indicated that Fe(II) is the dominant species, accounting for 67–89 % of total Fe concentration in porewater of both biochar amended and unamended soils. The ratio between the concentration of dissolved As and total Fe in the rhizosphere was determined at different rice growing stages (Fig. 1(d)). Results demonstrated that RHBC, Si-RHBC and NM-RHBC treatments significantly elevated the average Fe/As ratio in porewater from 255 (control soil) to 325, 370 and 327, respectively, during the entire rice growing stages. However, there was no significant difference among the different biochar treatments. It has been previously reported that As(III) is strongly oxidized to As(V) and adsorbed on iron oxide minerals such as ferrihydrite, when the Fe/As molar ratio becomes higher than 200 (Fe/As > 200) in the system (Zhao et al., 2011).

3.2. Effects of Si-rich biochar on controlling the mobility of As in paddy soil

Total concentrations of As in porewater collected at different rice growing stages were determined to evaluate the effects of unmodified and Si impregnated biochar composites on controlling the mobility of As in the rice rhizosphere. Fig. 2 illustrates the variation of dissolved As and its speciation in porewater of biochar amended and unamended paddy soil over different rice growing stages.

The concentration of total As in soil porewater has gradually decreased from the tillering stage to the maturating stage (Fig. 2(a)). In the tillering stage, RHBC, Si-RHBC and NM-RHBC amendments significantly decreased ($P < 0.01$) the concentration of As in porewater by 44.3, 62.5, 64.9 %, respectively compared to biochar unamended paddy soil, thereby noticeably diminishing the mobility of As in rice rhizosphere. Similarly, the application of all the RHBC, Si-RHBC and NM-RHBC amendments to paddy soil decreased the levels of As in porewater at the heading and maturing stages, however, the trend fluctuated among treatments at the heading stage. During the maturing stage, RHBC, Si-RHBC and NM-RHBC amendments reduced the concentration of As in soil porewater by 31, 45, and 40 %, respectively relative to the biochar unamended paddy soil. The impregnation of Si into rice husk biochar in the form of fertilizer and nano-particles resulted in decreasing the concentration of As in porewater by 33–37 % at the tillering stage, followed by 13–21 % at the maturing stage compared to RHBC. Hence, the trend of As immobilization capabilities of different biochar materials varied in the order of Si-RHBC > NM-RHBC > RHBC. It is obvious that the modification of the biochar surface via different forms of Si has led to a reduction in the mobility of soluble As in paddy soil to a significant extent compared to the unmodified biochar.

Results of species analyses demonstrated that As(III) is the dominant species of As (55–76 %) in porewater of both biochar amended and unamended soils that is mainly due to the reductive dissolution of iron oxide minerals under anaerobic conditions in flooded paddy soil (Fig. 2(b)). Interestingly, the addition of modified and unmodified rice husk biochar to paddy soil decreased the concentration of As(III) in porewater during the entire rice growing stages. At the tillering stage, RHBC, Si-RHBC and NM-RHBC amendments significantly ($P < 0.01$) decreased the concentration of As(III) in soil porewater by 57, 76 and 73 %, respectively, whereas in the heading and maturing stages, there was only a slight decrease of As(III) concentrations (20–31 %) in porewater of biochar amended soils. With regard to As(V) levels in porewater at the tillering stage, the RHBC treatment decreased the concentration of As(V) by only 18 %, while Si-RHBC and NM-RHBC treatments reduced it by 41–60 % compared to the biochar unamended soil. From heading to maturing stage, all the biochar amendments

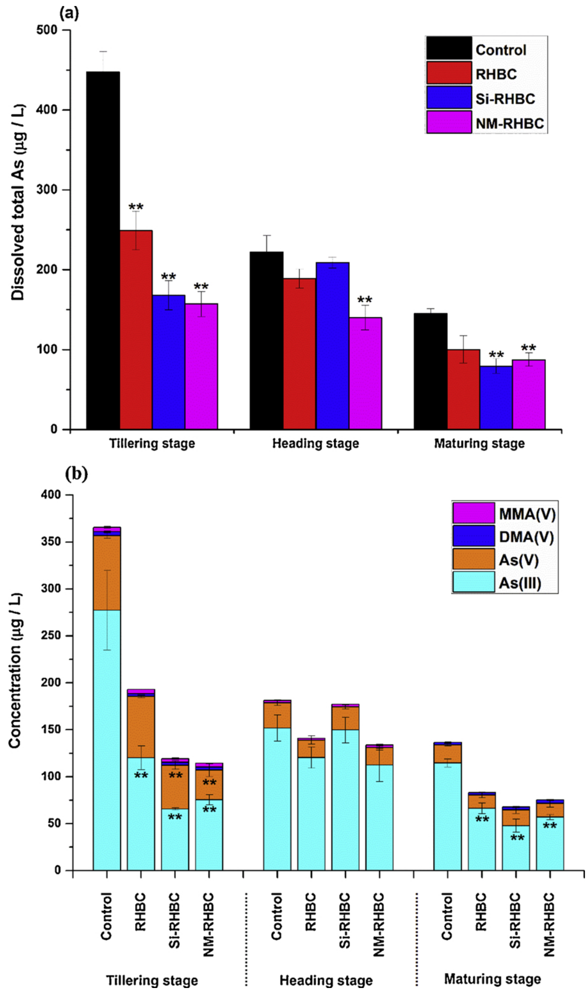

Fig. 2. Effects of modified and unmodified rice husk biochar on the: (a) concentration of total As, and (b) speciation of As in porewater among different rice growing stages.

decreased the concentration of As(V) ranging 13–26 % compared to the biochar unamended paddy soil. The amount of methylated As in porewater of both control and biochar amended soils was negligible (< 1%) compared to the total As throughout the rice growing stages, signifying that the incorporation of these Si-rich biochar amendments do not promote the methylation of As(III). Overall results of As speciation in porewater suggested that paddy soils amended with Si composites applied in this study can encourage the oxidation of highly toxic and mobile As(III) to less moveable As(V) while lowering the levels of soluble As(III) in the rice rhizosphere. This was an interesting phenomenon to understand the mechanisms involved in the immobilization of As(III) under anaerobic conditions after the incorporation of these Si-rich biochar composites in paddy soil.

3.3. *Accumulation of As in plant parts*

Bioaccumulation and toxic effects of As were assessed by the determination of total As and its inorganic and organic species in above ground parts of rice, specifically in the rice straw and unpolished rice grains. Results of total As analysis demonstrated that the average concentration of As in rice straw is almost 23-fold higher than that of the rice grains which is a naturally captivating phenomena in terms of food security and human health risks (Fig. 3). The application of Si-RHBC to paddy soil decreased the amount of As in rice straw and grains by 16 and 8 %, respectively compared to the control plants. Although the NM-RHBC treatment slightly reduced the accumulation of As (14 %) in rice straw, there was not a noticeable difference in the amount of total As accumulated in rice grains compared to the plants grown in biochar unamended soil. The addition of RHBC to paddy soil slightly increased the total As in both rice straw and grains compared to the biochar unamended paddy soil. However, it was not a significant increase ($P > 0.05$) in relative to the plants grown in biochar unamended soil. With compared to the RHBC, the incorporation of Si-RHBC and NM-RHBC amendments was attributed to a decrease in the accumulation of As in rice straw and unpolished grains by 20–22 % and 25–31 %, respectively. Therefore, the results suggested that the addition of biochar treatments had little or insignificant effect on As accumulation in rice grains.

3.4. *Abundance of 16S rRNA and As functional genes in rice rhizosphere*

In order to understand the effects of Si-rich biochar composites on the microbe mediated As biotransformation in the soil-rice system, the abundance of *16S rRNA* genes and As functional genes were quantified in biochar amended and unamended soil (Fig. 4). Results of 16S rRNA analysis indicated that RHBC and Si-RHBC applications elevated the bacterial abundance (*16S rRNA* gene) while possessing a slight decrease in the soil amended with NM-RHBC (Fig. 4(a)). With regard to the *aioA* gene which is responsible for As(III) oxidation, the Si-RHBC treatment significantly ($P < 0.05$) increased the abundance of *aioA* gene which was twice as much as that of the biochar unamended soil (Fig. 4(b)). The RHBC application exhibited an increase of only 25 % in the abundance of *aioA* compared to the control. However, the NM-RHBC treatment did not significantly affect the copy number of aioA gene

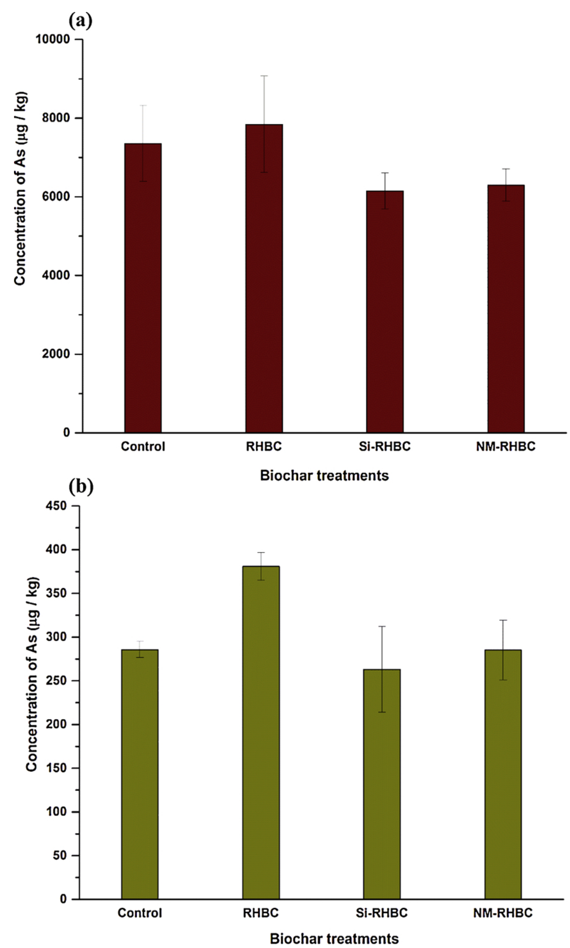

Fig. 3. Effects of Si-rich biochar amendments on the accumulation of As in: (a) rice straw, (b) rice grains.

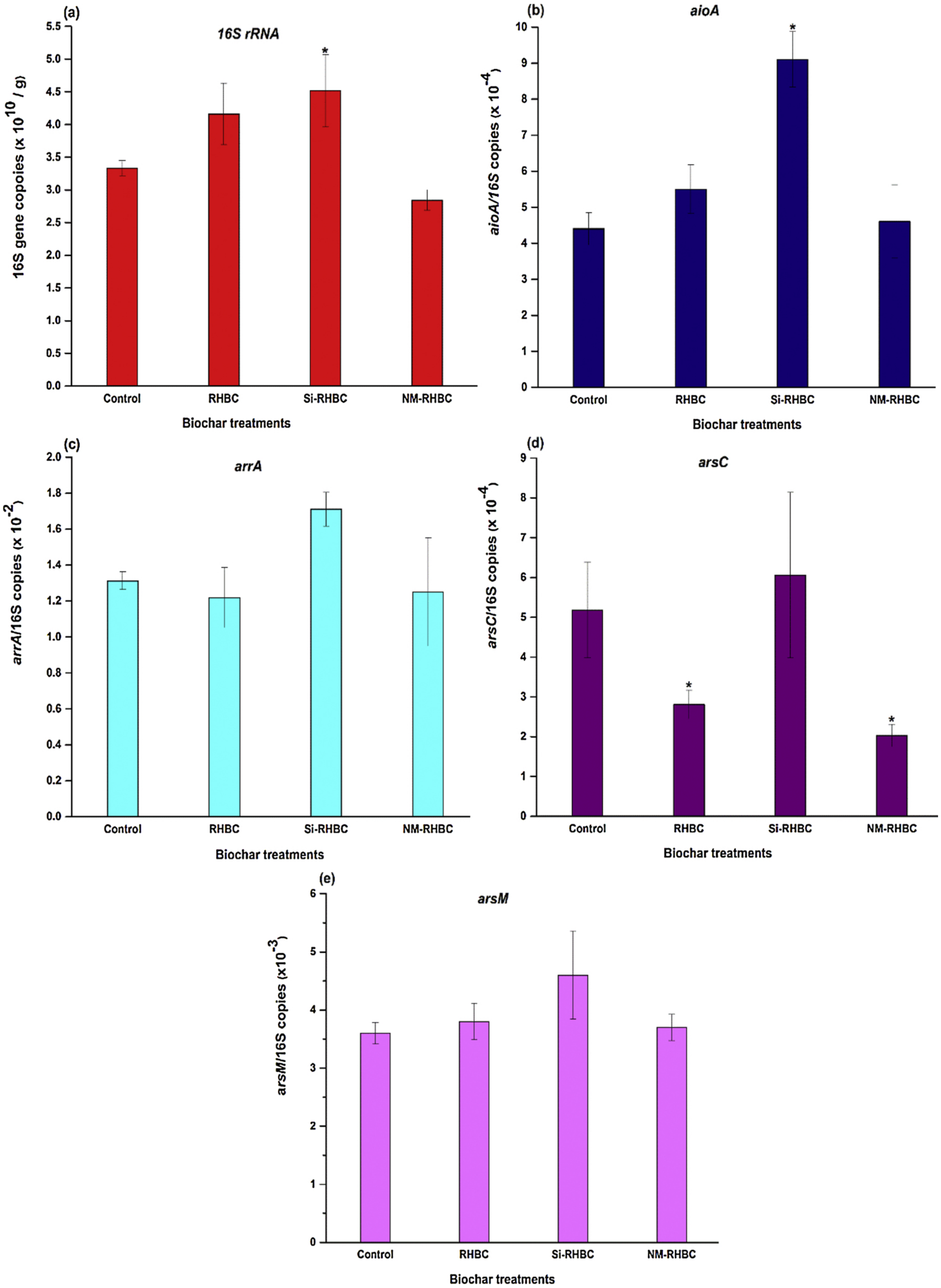

Fig. 4. Effects of Si-rich amendments on the abundance of: (a) *16S rRNA*, (b) *aioA*, (c) *arrA*, (d) *arsC*, and (e) *arsM* in rice rhizosphere amended with and without biochar composites.

while producing a slight increase (only 5% compared to the control) in rice rhizosphere. This is likely to be due to the existence of nano-sized Si mineral particles which may not provide a favourable substrate for *aioA* gene related bacteria to increase their density in the soil treated with NM-RHBC.

Fig. 4(c) and (d) show the effects of Si-rich biochar treatments on the abundance of the respiratory reducing gene (*arrA*) and the As(V) reductase gene (*arsC*) in the rice rhizosphere. Results demonstrated that the application of both RHBC and NM-RHBC amendments caused a slight decrease of the abundance of the *arrA* gene in the rhizosphere (only 7 %). However, the abundance of *arsC* was decreased, ranging 46–61 % in the rhizosphere of both RHBC and NM-RHBC amended soils. In contrast to this, soils amended with Si-RHBC composite slightly increased the copy number of both *arrA* and *ars C* genes, but it was not a significant elevation compared to the biochar unamended soil. With regard to the As(III) methylation gene (*arsM*) in the rhizosphere, there was not a significant impact on its abundance in soils treated with RHBC and nano-RHBC composites compared to the control, while showing a minor increase (1.3-fold) with Si-RHBC treatment in relative to the biochar unamended soil (Fig. 4(e)). Therefore, results of gene quantification are in agreement with the conversion of As(III) to As(V) in biochar amended soils as employed by speciation analyses suggesting that the elevated abundance of *aioA* gene related bacteria can catalyse the anaerobic oxidation of As(III) in the rice rhizosphere. This oxidation is partly encouraged by the dissolved oxygen in the rhizosphere which enters through the root system.

3.5. The diversity of microbial community in rice rhizosphere

Effects of the addition of Si-modified and unmodified biochar on the diversity of microbial communities in the rice rhizosphere were examined based on the high-throughput sequencing of bacterial *16S rRNA* genes. The sequencing bacterial data was characteristic with a total of 38 phyla and *Acidobacteria*, *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, *Tenericutes* and *Proteobacteria* are the dominant phyla in rice rhizosphere (Fig. 5). *Proteobacteria* was the dominant phylum, accounting for 23.3 % of the total abundance and the addition of RHBC and Si-RHBC treatments increased its abundance up to 30 and 26 %, respectively, while slightly decreasing to 20 % in the NM-RHBC treatment. Some sulfur reducing bacteria such as *Desulfuromonas,Pseudomonas* and *Salmonella* as well as iron reducing bacteria, including *Geobacter* and *Deltaproteobacteria (*genus *Anaeromyxobacter)* belong to the phylum *Proteobacteria* and these bacteria have ability to enhance the dissolution of As-bearing sulfur and iron minerals (arsenopyrites, pyrites, goethite, etc.) associated with paddy soil, thereby leading to elevated levels of more mobile As(III) in the rhizosphere (Herath et al., 2018; Voegelin et al., 2019). Moreover, these sulfur reducing bacteria can promote the formation of various sulfur-containing As complexes such as thioarsenic compounds in the paddy-rice system (Herath et al., 2018).

At the genus level, nearly 720 different types of genera were detected in this experiment and *Clostridium sensu stricto 10*, *Candidatus Hepatoplasma*, *Lentimicrobium*, *HOC36_norank* and *Anaerolinea* were the major genera, accounting for 2–21 % of the total abundance. Fig. 6 (a) depicts the variation in the diversity of different microbial communities at genera level in biochar amended and unamended paddy soil. At the phylum level, the relative abundance of *Tenericutes* was 17 % in the biochar unamended soil and it was significantly ($P < 0.01$) reduced to $0.4-0.5$ % in soils amended with all the biochar treatments. Several genus types, including *Candidatus Hepatoplasma*, *Mycoplasmataceae*, and HOC36*_norank* belong to the phylum *Tenericutes* and there was a remarkable decrease of the relative abundance of *Candidatus Hepatoplasma* in biochar amended soils (by 100 %), compared to the control soil (Fig. 6 (a)). For phylum *Bacteroidete*, all the biochar treatments slightly increased its relative abundance from 21 % (control) to 25–34 % in the rice rhizosphere. It has been found that *Bacteroidete* species tend to colonize together with the species of phyla *Proteobacteria*, *Firmicutes* and *Actinobacteria* in rhizosphere (Zhang et al., 2019b). However, the contribution of different species of *Tenericutes* and *Bacteroidete* with regard to the dynamics of As biotransformation in paddy soil is unknown to date.

Phylum *Firmicutes* includes *Geobacter*, *Bacillus*, and *Clostridium (Clostridium_sensu_stricto_1*, *Clostridium_sensu_stricto_8*, *Clostridium_sensu_stricto_10*, and *Clostridium_sensu_stricto_12)* which may be involved in the release of As via the reductive dissolution of iron oxide minerals in paddy soils. The relative abundance of *Bacillus* in soils treated with RHBC, Si-RHBC and NM-MMT was decreased by 34, 33, and 40 %, respectively compared to the biochar unamended soil. Moreover, the application of Si-RHBC and NM-RHBC treatments decreased the abundance of *Clostridium* and *Geobacter* by 9–20 % and 12–16 %, respectively compared to the control soil (Fig. 6(b)). These results suggested that the lowering of the abundance of iron reducing bacteria in the presence of such Si-rich biochar composites may control the dissolution of iron oxide minerals associated with paddy soil, thereby decreasing the mobility of As in paddy soil.

4. Discussion

Application of Si-rich rice straw and husk as soil amendments has become an alternative practice in As contaminated paddy fields on these days. However, some recent studies have revealed that the incorporation of carbon rich amendments, such as biochar, ash, biomass of rice husk and straw into paddy soil results in increasing the mobility of As into the rice rhizosphere, thereby leading to elevated levels of As in porewater (Yang et al., 2018; Wang et al., 2017; Limmer et al., 2018). This is mainly due to the elevation of soil DOC and pH after the application of such carbon-rich char amendments at a higher dose ($> 1\%$) which promotes the release of As from paddy soil. In the meantime, the addition of biochar to paddy soil has caused an increase in the abundance of iron reducing bacteria in paddy soil catalysing the reductive dissolution of iron minerals, thereby releasing As into the rice rhizosphere to a significant extent. This divergent behaviour between the addition of biochar and increased As release in paddy soils was mechanistically addressed by our study because the elevated levels of As in porewater can disturb entire paddy ecosystems as well as adjacent surface water bodies, aquatic creatures, crop productivity and food security. In order to achieve this, we comprehensively studied the dynamics of porewater chemistry, impact of soil microbes and accumulation of As in the above ground parts of rice after the incorporation of three different types of Si-rich biochar materials (RHBC, Si-RHBC, NM-MMT) as demonstrated by the results section.

4.1. Mechanisms of biochar-microbe mediated As immobilization in paddy soil

Results of porewater analysis indicated that the incorporation of all the Si based biochar amendments decreased the concentration of As in porewater compared to biochar unamended soil. However, the capability of As immobilization in Si-RHBC and NM-RHBC composites was greater than that of RHBC indicating the optimistic impact of the modification of RHBC via different forms of Si. Mechanisms of As immobilization after the incorporation of biochar in paddy soil are triggered via several key steps: (i) release of Fe(II) through reductive dissolution of Fe(III)(oxyhydr)oxides; (ii) adsorption of Fe(II) and its oxidation to Fe(III) by silicate (SiO_4^{4-} and SiO_3^{3-}) on the biochar surface; (iii) complexation of Fe(III) with hydroxyl ion and formation of ferrihydrite layer on the biochar surface (BC≡Si−Fe(III)OOH) at near neutral pH; and (iv) micrtobe mediated As(III) oxidation and adsorption of As(V) on BC≡Si−Fe(III)OOH.

In paddy soil, the release of As(III) and Fe(II) predominantly occurs through the reductive dissolution of hematite (α-Fe_2O_3) and goethite (α-FeOOH), so that dissolved As(III) and Fe(II) are the prevailing species in soil-porewater under anaerobic conditions (Eq. (1) and (2)). The XRD spectra obtained for biochar amended and unamended paddy soils verified the presence of hematite and goethite, giving prominent peaks at 20.9 and 36.5°, respectively (S3). The reductive dissolution of these iron oxide minerals is catalysed by Fe reducing bacteria, including *Clostridia*, *Bacilli*,and*Actinobacteria* and their availability in biochar amended and unamended soils is confirmed by the *16S rRNA* sequencing analysis detecting genera types, such as *Clostridium sensuu stricto 1*,*Bacillus*,*Geobacter*, *Anaeromyxobacter* and *Pedobacter* to a noticeable extent. The relative abundance of *Bacillus*and*Geobacter* was gradually decreased in soil amended with Si-RHBC and NM-RHBC (Fig. 6 (b)).

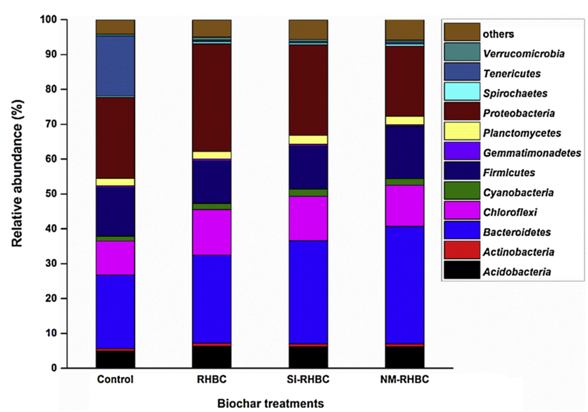

Fig. 5. Relative abundance of bacteria in the rice rhizosphere at phylum level. The relative abundance is expressed as the average relative abundance of the three replicates in each treatment by the average % of the targeted sequences to the total high-quality bacterial sequences of each sample.

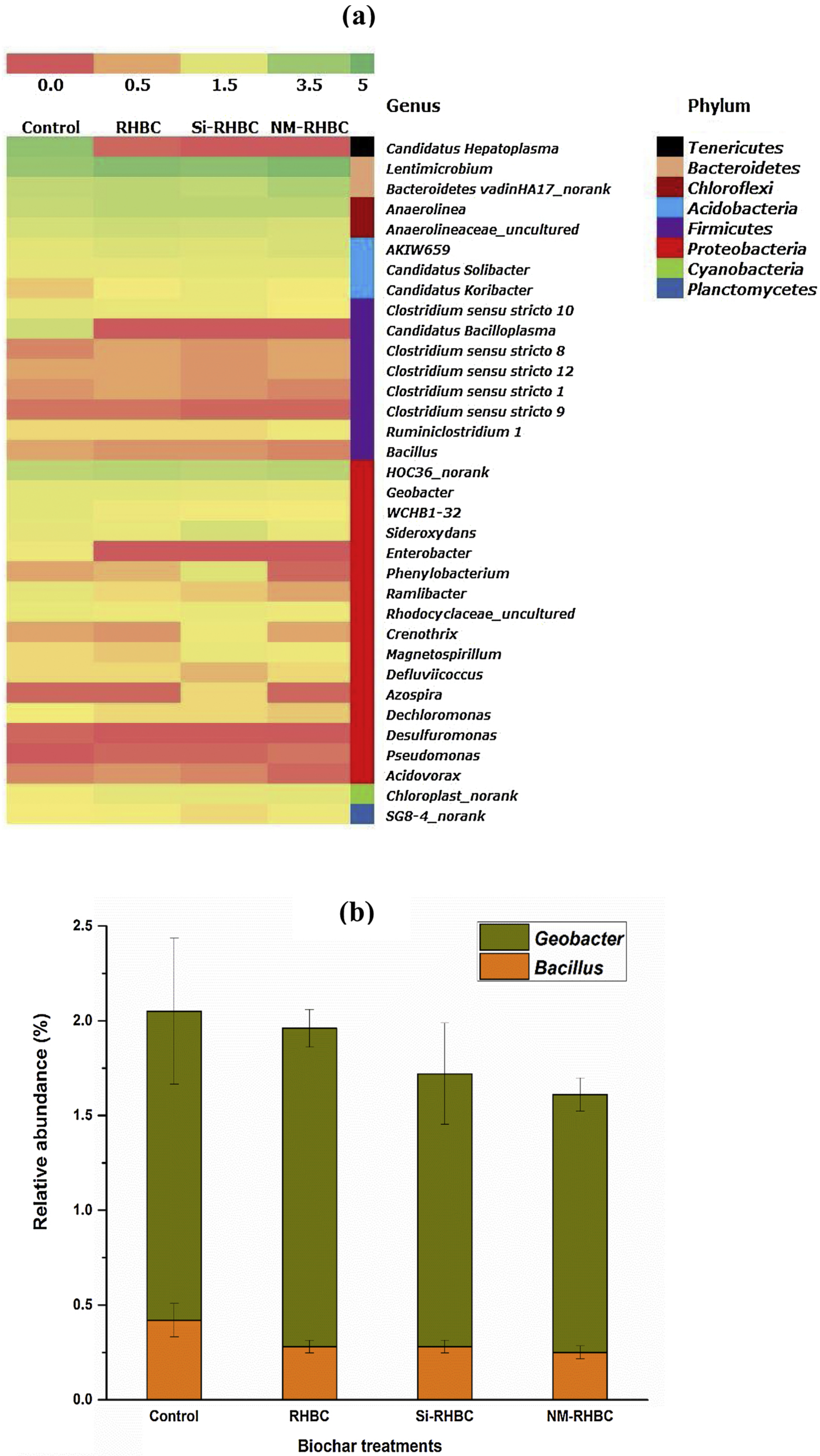

Fig. 6. Impact of biochar amendments on the microbial diversity showing the: (a) heat map of the top 30 abundant genera, and (b) relative abundance of iron reducing bacteria in the rice rhizosphere. The heat map was drawn based on the conversion of relative abundance value of the corresponding genus by [$\log_2$(x 100 + 1)].

This suggests that the application of these Si impregnated biochar composites can limit the dissolution of Fe minerals in the soil, thereby diminishing the release of high As levels in the rice rhizosphere.

$$\alpha - FeOOH_{(s)} + 3H^{+}_{(aq)} + e \overset{Iron\ reducing\ bacteria}{\leftrightarrow} Fe^{2+}_{(aq)} + 2H_2O_{(l)} \quad (1)$$

$$\alpha - Fe_2O_{3(s)} + 6H^{+}_{(aq)} + 2e \overset{Iron\ reducing\ bacteria}{\leftrightarrow} 2Fe^{2+}_{(aq)} + 3H_2O_{(l)} \quad (2)$$

The solid surface of rice husk biochar types applied in this pot experiment can possess naturally occurring silicate as well as impregnated –SiO_2 and nanoparticles of Si_4O_{10} which may also exist as orthosilicate (SiO_4^{4-}) and metasilicate SiO_3^{2-}. The FTIR spectrum obtained for the RHBC is clearly evident with the silicate nature of the biochar surface (S1). The O−SiO− groups attached to the biochar surface are able to form a strong negative charge on the surface of biochar leading to electrostatic interactions with positively charged Fe(II) ions in the rice rhizosphere. Because of this enhanced negative charge on the biochar surface, negatively charged As species cannot be directly adsorbed onto it. Secondly, at near neutral pH, Fe(II) ions bound on the biochar surface are readily oxidized to Fe(III) by silicate groups present in biochar as well as dissolved oxygen (O_2) in soil-porewater as O groups can accept the electrons of this oxidation process. Thirdly, Fe(III) is readily complexed with hydroxyl ions present in the soil-porewater, thereby forming a Si-based ferrihydrite complex on the biochar surface. Eventually, As(III) is adsorbed on this ferrihydrite layer forming a complex of [≡BC(Si)−Fe(O)$_2$As^{III}(OH)$^-$]. Subsequently, this As(III) is oxidized to As(V) which is capable of interacting with ferrihydrite complex while forming a complex of [≡BC(Si)−Fe(O)$_2$As^{V}(OH)$^-$] as expressed by Eq. 3, where BC(Si) ≡−represents the Si-based biochar surface. The feasibility of Fe(II) oxidation and its complexation on the Si-based biochar surface at near neutral pH is corroborated with the findings of recent studies (Zhao et al., 2011; Voegelin et al., 2019; Yang et al., 2015; Kwon et al., 2014). The adsorption of As(III) on ferrihydrite can take place through both inner-sphere and outer-sphere complexation depending on the pH of the medium. The formation of inner-sphere complexation is thermodynamically feasible at near to neutral pH and the inner-sphere complexes of As(III) on ferrihydrite are typically formed by bidentate-binuclear interactions (Zhao et al., 2011). The pH of soil amended with biochar in this study in the range of 6–7 during the entire rice growing stages which suggests that the inner-sphere complexation is the prevailing mechanism responsible for the adsorption of As(III) and its immobilization in biochar amended paddy soil.

$$\equiv BC(Si) - Fe(O)_2As^{III}(OH)^{-}_{(s)} \overset{SiO_y^{x-}}{\leftrightarrow} \equiv BC(Si) - Fe(O)_2As^{V}(O)(OH)^{-}_{(s)} + 2OH^{-}_{(aq)} \quad (3)$$

The XRD data showed a slight increase in the intensity of the goethite peak obtained for biochar amended soil, which is likely be due to this newly formed ferrihydrite in a heterogeneous biochar-soil system (S5). However, the ferrihydrite which is newly formed on the biochar surface cannot be clearly distinguished with the aid of only XRD patterns as the newly formed ferrihydrite is almost structurally identical with goethite (α-FeOOH). Therefore, evidence based on Fe-extended X-ray absorption fine structure (EXAFS) is essential to further confirm the formation of this ferrihydrite complex on the biochar surface. It has been revealed that the stability of ferrihydrite formed on an adsorbent via strong electrostatic interactions is more stable than naturally occurring FeOOH minerals, so that the dissolution rate of adsorbed As(V) on the ferrihydrite complex is extremely slow (Zhao et al., 2011).

The oxidation of As(III) to As(V) is catalysed by the ferrihydrite complex as well as the As oxidizing microbes present in soil. Quantification of As functional genes is corroborated with the involvement of the As oxidizing gene (*aioA*) for the oxidation of As(III) in biochar amended soil. The relative abundance of the *aioA* gene was increased in biochar treated soil, suggesting that the biochar surface provides an excellent substrate for *aioA* related microbes to govern the oxidation process. Micro-pores on the biochar surface form a biofilm which can act as an intermediate electron shuttle between microbes and ferrihydrite, promoting the oxidation of As(III) on the [≡BC(Si)-Fe (O)$_2$As^{III}(OH)$^-$]. The pH of biochar amended soil is near neutral pH (6.0–7.0) which is highly favourable for this adsorption pathway. Moreover, such an oxidation nature of biochar amended soils is evident with positive Eh values in biochar amended soils over the rice growing stages. The capacity of As immobilization by Si-RHBC and NM-RHBC is greater than that of unmodified RHBC, suggesting that the formation of BC≡Si−Fe(III)OOH and As(III) adsorption are affected by the biochar modification due to acquiring different chemical and structural properties depending on the type of modification. However, there was no significant difference in the efficiency of As immobilization between Si-RHBC and NM-RHBC treatments, suggesting that the impregnation of nano-size Si particles on the biochar surface does not bring any extra benefit to the As immobilization process.

4.2. Impact of Si impregnation in biochar on As uptake and accumulation in rice

Results obtained from the analysis of plant materials demonstrated that the application of the Si-RHBC composite decreased the concentration of As in both rice husk and grains to a certain degree, whereas the unmodified RHBC treatment slightly increased the accumulation of As in both rice husk and grains compared to the control. Several steps are involved in the mechanisms of As uptake by rice in the presence of Si modified and unmodified rice husk biochar. The amount of As being taken up by rice roots is predominantly controlled by a Si-uptake pathway in the root systems of rice which includes: (i) releasing labile Si from biochar to the rhizosphere; (ii) under high Si levels, downregulation of Si-transporters in roots, thereby limiting the transporters available for As(III) uptake; (ii) creating an increased competition between Si and As(III) for uptake; as a result (iii) decreasing the uptake of As(III) by rice (Seyfferth et al., 2016).

Speciation results revealed that As(III) is the dominant species in porewater while slightly reducing its concentration in the presence of biochar amendments. This is due to the immobilization of As in paddy soil amended with Si-rich biochar composites via the oxidation and adsorption of As(III) on the biochar-ferrihydrite complex. However, more soluble As(III) still prevails in porewater because of a limit in the available active sites for the adsorption of As(III) on the ferrihydrite layer. In the aqueous phase, Si can exist as plant-available H_4SiO_4 (pK_a = 9.8) which is chemically analogous to H_3AsO_3 (pK_a = 9.2) and hence, As(III) can share a root-uptake transport with H_4SiO_4 in the root system (Seyfferth et al., 2016; Teasley et al., 2017). There are two major Si transporter proteins: Lsi1 and Lsi2 in rice, which are responsible for taking H_4SiO_4 into root cells (Li et al., 2009). High Si levels in the rhizosphere can downregulate the Lsi1 and Lsi2 proteins in rice roots As a result, a limited number of Si transporters may available to share with As(III), which creates a strong competition between Si and As(III) for uptake by rice, thereby attenuating the amount of As being taken up by rice and the accumulation in the above ground parts of the rice.

This Si uptake pathway in rice is highly dependent on the amount of plant-available Si in the rice rhizosphere. Biochar amendments which can provide relatively low amounts of plant-available Si into porewater tend to increase the uptake of As(III) while leading to higher levels of As in rice grains. In this study, the unmodified RHBC elevated the accumulation of As in both rice grains and husks. Moreover, the NM-RHBC composite did not significantly change the concentration of As in rice grains compared to the control. The RHBC comprises a stable carbonized Si structure as employed by FTIR data (S1). So that the amount of available Si in RHBC becomes relatively lower than the other two biochar composites (Table 1) and, hence it cannot provide more labile Si in the rhizosphere. In the NM-RHBC composite, Si is associated in the form of nanoparticles which can easily penetrate into the pores, retaining strongly over the inner walls of biochar, which may hinder the release of Si into the rice rhizosphere to a large extent. On the other hand, a certain amount of the soluble Si fraction in porewater is capable of readily binding with some other mineral phases associated with paddy soil. Therefore, lower levels of plant-available Si in the rhizosphere can up-regulate the Si transporters available for As(III) uptake by rice, thereby resulting in an enhanced accumulation of As in rice husk and grains. Although the Si-RHBC composite provided relatively higher amounts of available Si in the rhizosphere, the reduction of grain As was not significant compared to the control. This is likely to be due to the addition of a low application rate of biochar which can release only limited Si levels to the rhizosphere. Therefore, it is noteworthy to mention that the findings of this study reveal that both the application rate of biochar and the form of Si present in biochar amendments are crucial in controlling both the mobility of As in paddy soil and the amount of As being taken up by rice. Therefore, the optimization of the biochar application rate as well as the amount of Si impregnated in biochar are of critical importance to achieve the immobilization of As in paddy soil together with a significant reduction of the As accumulation in rice grains.

5. Conclusions

The present study investigated the potential of Si-rich biochar amendments to control microbe mediated As geochemical processes and reduce the mobility of As in the rice rhizosphere. Results revealed that the incorporation of Si-RHBC and NM-RHBC treatments can effectively immobilize more mobile As(III) in the rhizosphere compared to the unmodified RHBC. This implies that the modification of the RHBC surface via different forms of Si can directly affect the efficiency of As immobilization in biochar amended soil. Mechanisms of As immobilization in the presence of Si-rich biochar composites involved in: (i) the release of Fe(II) via reductive dissolution of Fe(III)(oxyhydr) oxides; (ii) adsorption of Fe(II) and its oxidation to Fe(III) by silicates on the biochar surface; (iii) complexation of Fe(III) with hydroxyl ions and formation of a Si-intermediate ferrihydrite layer on the biochar surface; and eventually, (iv) adsorption of As(III) on this ferrihydrite complex while converting it to a As(V) complex. The abundance of the *aioA* gene in soil amended with Si-RHBC treatment was twice as much as that of the biochar unamended soil. This suggests that the anaerobic oxidation of As(III) to As(V) may be catalysed by the *aioA* gene related bacteria in paddy soil. The relative abundance of iron reducing bacteria; *Bacillus* and *Geobacter* decreased in soil amended with Si-RHBC treatment to a significant extent which implies that the lowering of the abundance of iron reducing bacteria results in decreasing the dissolution of As(III) from iron oxide minerals in paddy soil. Hence, the Si-RHBC composite could be a promising alternative material as a soil amendment for the remediation of As-contaminated paddy soil because of its great ability to microbe mediate immobilization of more mobile As(III). However, the accumulated amounts of As in rice husk and grains showed no significant difference between biochar treatments and the control. This is likely due to insufficient amounts of plant-available Si at 0.25 % of biochar dosage applied in this study. Lower levels of plant-available Si in the rice rhizosphere tend to upregulate the Si transporters available for As(III), thereby increasing its uptake by rice and accumulation in grains. Therefore, findings of this research could direct future research towards the optimization of both the biochar application rate and the amount of Si impregnated in biochar to simultaneously achieve As immobilization in the rhizosphere and significant reduction of grain As.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Indika Herath: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Fang-Jie Zhao:** Conceptualization, Supervision, Validation, Resources, Funding acquisition, Writing - review & editing. **Jochen Bundschuh:** Supervision, Validation, Data curation, Writing - review & editing. **Peng Wang:** Supervision, Project administration. **Jing Wang:** Methodology, Formal analysis. **Yong Sik Ok:** Resources. **Kumuduni Niroshika Palansooriya:** Resources. **Meththika Vithanage:** Resources.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2020.123096.

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