Environmental Pollution 286 (2021) 117661

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Iron modification to silicon-rich biochar and alternative water management to decrease arsenic accumulation in rice (*Oryza sativa* L.)☆

Prasanna Kumarathilaka [a], Jochen Bundschuh [a,*], Saman Seneweera [b,c], Alla Marchuk [d], Yong Sik Ok [e]

[a] School of Civil Engineering and Surveying, Faculty of Health, Engineering and Sciences, University of Southern Queensland, West Street, Toowoomba, Queensland, 4350, Australia
[b] Centre for Crop Health, Faculty of Health, Engineering and Sciences, University of Southern Queensland, West Street, Toowoomba, Queensland 4350, Australia
[c] National Institute of Fundamental Studies, Hantana Road, Kandy, 20000, Sri Lanka
[d] Institute for Life Sciences and the Environment, University of Southern Queensland, West Street, Toowoomba, Queensland, 4350, Australia
[e] Korea Biochar Research Center & Division of Environmental Science and Ecological Engineering, Korea University, Seoul, Republic of Korea

ARTICLE INFO

Keywords:
Rice
Inorganic arsenic
Iron-biochar
Microorganisms
Silicon

ABSTRACT

Production of rice grains at non-toxic levels of arsenic (As) to meet the demands of an ever-increasing population is a global challenge. There is currently a lack of investigation into integrated approaches for decreasing As levels in rice agro-ecosystems. By examining the integrated iron-modified rice hull biochar (Fe-RBC) and water management approaches on As dynamics in the paddy agro-ecosystem, this study aims to reduce As accumulation in rice grains. The rice cultivar, *Ishikari*, was grown and irrigated with As-containing water (1 mg L^{-1} of As(V)), under the following treatments: (1) Fe-RBC-flooded water management, (2) Fe-RBC-intermittent water management, (3) conventional flooded water management, and (4) intermittent water management. Compared to the conventional flooded water management, grain weight per pot and Fe and Si concentrations in the paddy pore water under Fe-RBC-intermittent and Fe-RBC-flooded treatments increased by 24%–39%, 100%–142%, and 93%–184%, respectively. The supplementation of Fe-RBC decreased the As/Fe ratio and the abundance of Fe(III) reducing bacteria (i.e. *Bacillus*, *Clostridium*, *Geobacter*, and *Anaeromyxobacter*) by 57%–88% and 24%–64%, respectively, in Fe-RBC-flooded and Fe-RBC-intermittent treatments compared to the conventional flooded treatment. Most importantly, Fe-RBC-intermittent treatment significantly ($p \leq 0.05$) decreased As accumulation in rice roots, shoots, husks, and unpolished rice grains by 62%, 37%, 79%, and 59%, respectively, compared to the conventional flooded treatment. Overall, integrated Fe-RBC-intermittent treatment could be proposed for As endemic areas to produce rice grains with safer As levels, while sustaining rice yields to meet the demands of growing populations.

1. Introduction

Arsenic (As) in rice agro-ecosystems is a global environmental and health concern (Bhattacharya et al., 2012; Zavala and Duxbury, 2008). Throughout the full rice growing cycle, a volume of 500–3000 mm of water needs to be supplied, and this figure depends on the rice genotype, climatic conditions, and soil type (Kumarathilaka et al., 2018a). Particularly in dryer periods, rice cultivation is dependent on both groundwater and surface water (i.e. water from lake, river, and dam) sources. The use of As-contaminated groundwater for irrigating rice is the major source of As to rice agro-ecosystems. Studies have demonstrated that the use of As-contaminated groundwater for irrigating rice has contributed to increased As levels in the paddy soil-water system as well as in rice grains (Brammer and Ravenscroft, 2009; Rahman and Hasegawa, 2011; Vicky-Singh et al., 2010).

Different physico-chemical and biological factors affect As speciation in the paddy soil-water system (Kumarathilaka et al., 2020). Arsenite (As(III)) and arsenate (As(V)) are inorganic As species found in rice agro-ecosystems. Dimethylarsinic acid (DMA(V)) and monomethylarsonic acid (MMA(V)) are the commonly detected organic As

☆ This paper has been recommended for acceptance by Jörg Rinklebe.

* Corresponding author. School of Civil Engineering and Surveying, Faculty of Health, Engineering and Sciences, University of Southern Queensland, West Street, Toowoomba, Queensland, 4350, Australia.
E-mail address: jochen.bundschuh@usq.edu.au (J. Bundschuh).

https://doi.org/10.1016/j.envpol.2021.117661
Received 1 March 2021; Received in revised form 25 May 2021; Accepted 23 June 2021
Available online 26 June 2021

species in paddy agro-ecosystems (Bakhat et al., 2017). Aerobic and anaerobic microorganisms inhibiting in the rice rhizosphere involve in As speciation through different processes such as As oxidation/reduction and As methylation and volatilization (Upadhyay et al., 2018). In terms of toxicity, inorganic As species are more toxic than the organic As species. Arsenic (III) is mainly acquired through nodulin 26-like intrinsic proteins (NIPs) such as OsNIP2; 1 (Lsi1) which is also responsible for Si uptake in rice plants (Zhao et al., 2009). Phosphate transporters share the same pathway for PO_4^{3-} and As(V) in rice. It is suggested that both DMA(V) and MMA(V) are acquired by $Si(OH)_4$ transporters (R. -Y. Li et al., 2009).

The method of rice cultivation (i.e. long-term flooded water management) results in a higher inorganic As concentrations in rice grains than in other cereal crops (Su et al., 2010). The availability of iron (Fe) in the paddy soil-water system plays a major role in sequestrating both As(III) and As(V) through surface complexation (Liu et al., 2006). Even though paddy soils comprise indigenous Fe(III)-hydro(oxides) (i.e. ferrihydrite, hematite, lepidocrocite, and goethite), phase conversion from poorly crystalline ferrihydrite to crystalline Fe oxides such as goethite and hematite diminishes the number of adsorption sites available for the sorption of inorganic As species (Komárek et al., 2013). The external supplementation of Fe(III)-hydro(oxides) to As-contaminated paddy environments may enhance the number of adsorption sites available for As.

Intermittent water management has been recognized as a potential way of decreasing As accumulation in rice agro-ecosystems (Mukherjee et al., 2017). During intermittent water management, the rice field is only flooded to desired levels (~3–5 cm) when the soil is dry and cracks are found (P. Hu et al., 2013). Therefore, intermittent water management practices have been found to cut down the volume of irrigation water required for rice cultivation. Even though intermittent water management practices have reduced As accumulation in rice grains, loss of rice yield is one of the concerns associated with this practice (Basu et al., 2015). The loss of rice yield could adversely affect both farmers' incomes and the demand for rice by ever-increasing populations around the world.

The application of biochar (BC) in agricultural ecosystems has been found to increase crop productivity since BC releases essential nutrients into soil pore water (Jeffery et al., 2011). Biochar is produced under an O_2 free environment at higher temperatures (~400–800 °C) by using organic feedstocks (Cha et al., 2016; Jayawardhana et al., 2018). Biochar production by using waste organic feedstocks is a technically feasible and economically viable option. The BC in agricultural soil-water systems can also adsorb contaminants through physical and chemical adsorption processes (Jayawardhana et al., 2019; Kumarathilaka et al., 2018b). In addition, BC in agricultural soil-water systems can promote C sequestration and enhance the water holding capacity (Ghani et al., 2013). The BC can be modified through physical and chemical activation processes to enhance the properties of BC to improve contaminant removal and nutrient supplementation (Sizmur et al., 2017; Wang and Wang, 2019). The modification of BC with Fe sources and the subsequent application of Fe-modified BC to As-contaminated paddy soils may enhance the available adsorption sites for As species (Wu et al., 2018). Moreover, the selection of Si-rich organic feedstock to produce BC leads to an increased Si concentration in the paddy soil-water system which could decrease the uptake of highly toxic As(III) by rice roots.

There is a lack of studies designed to examine the integrated effects of the BC composites and water management strategies on the phytoavailability of As in paddy rice soils and rice yield. Taking these facts into account, in this study, Fe-modified rice hull BC (Fe-RBC) was applied to As-contaminated paddy soil-water systems under different water management practices (i.e. conventional flooded water management and intermittent water management). We hypothesized that an integrated approach could decrease As accumulation in rice tissues while sustaining the rice yield.

2. Materials and methods

2.1. Collection and characterization of paddy soils

Paddy soils were collected from New South Wales, Australia (34° 35' 53.5" S, 146° 21' 38.1" E), air-dried, sieved, and then thoroughly mixed to obtain a composite sample. Physico-chemical properties of the soil such as pH, electrical conductivity (EC), moisture content, cation exchange capacity, and total and EDTA (ethylene-diamine-tetra-acetic acid)-extractable concentrations of metal(loid)s were determined. Table S1 summarizes methods used for the determination of physico-chemical characteristics of paddy soils and obtained values for each parameter.

2.2. Preparation and characterization of iron modified biochar

Biochar samples were prepared and characterized according to the methods described elsewhere (Ahmad et al., 2012; Kumarathilaka and Vithanage, 2017). The rice hull biomass was pyrolyzed at 600 °C for 2 h by using a muffle furnace (RIO GRANDE) at a heating rate of 7 °C min^{-1} and under continuous N_2 flow to obtain rice hull biochar (RBC). The produced RBC was washed with deionized water several times (3–4 times), oven-dried at 85 °C, and placed in a sealed container before use. Fe-modified RBC (Fe-RBC) was prepared through direct hydrolysis of iron salt. Solutions of Fe salt were prepared by dissolving 7.23 g of $Fe(NO_3)_3.9H_2O$ in 40 mL of deionized water. A weight of 10 g of RBC was added to the Fe solution and the mixture was stirred for 12 h using a magnetic stirrer (BIBBY HB502). The mixture was then dried at 105 °C in an oven (STERIDIUM). The dried composites were washed with deionized water several times for removing excess Fe(III)-hydro(oxides). After the washing step, the composite was again dried at 80 °C and the resulting product was the Fe-RBC.

The physico-chemical properties and characterization of BC such as pH, EC, proximate analysis (moisture content, mobile matter, ash content, and resident matter), Brunauer-Emmett-Teller (BET surface area), total pore volume, and average pore size were determined according to methods reported by Ahmad et al. (2012). The EDTA extraction solution was used to determine bioavailable As, Fe, and Si contents in Fe-RBC. Spectroscopic analyses including Scanning Electron Microscopy (SEM) (JCM-6000, JEOL) and Fourier Transform Infrared Spectroscopy (FTIR) (IRAffinity-1S, Shimadzu) analysis were also performed to examine the morphology and spectral characteristics of Fe-RBC before and after the modification.

2.3. Pot experiment of rice

A pot experiment was designed to assess the effects of flooded/intermittent irrigation and Fe-RBC supplementation on the accumulation of As species in rice tissues as well as on the rice growth parameters. Rice seeds (*Ishikari*) were surface sterilized with 10% H_2O_2 before germinating them in moist compost for three weeks. Subsequently, uniform size and healthy seedlings were transplanted in pots that contained 2.5 kg of paddy soils. There were four different treatments: (1) continuous flooding with Fe-RBC (1% w/w); Fe-RBC-flooded, (2) intermittent irrigation with Fe-RBC (1% w/w); Fe-RBC-intermittent, (3) conventional flooding without Fe-RBC; flooded, and (4) intermittent irrigation without Fe-RBC; intermittent. Two different water management regimes were designed as follows: (1) flooded water management: a ~5 cm water level was maintained, (2) intermittent water management: irrigated intermittently to 5 cm water level, in particular when the soil has dried. The water levels in each treatment were maintained by adding 1 mg L^{-1} of As(V)-containing water. All the treatments consisted of triplicates. The water supplementation throughout the rice growing season under each treatment was calculated. Plant growth and yield parameters including plant height, root length, shoot biomass, and grain weight per pot were also measured.

2.4. Chemical analysis

Soil solution samples were collected until grain maturity at 30-day intervals. The Fe and Si concentrations were determined by atomic absorption spectrophotometer (AA-7000, Shimadzu). The levels of total carbon (TC) and total organic carbon (TOC) were measured by a total organic carbon analyzer (TOC-VCSH, Shimadzu).

Iron plaque on root surfaces was extracted using a Dithionite-Citrate-Bicarbonate (DCB) solution as described elsewhere (Amaral et al., 2017). After harvesting, roots were thoroughly washed with tap water to remove soil attached to the roots. Then roots were rewashed with distilled deionized water several times. A 10 g of fresh roots were agitated with DCB solution for 3 h. The DCB solution comprised 40 mL of 0.3 M sodium citrate ($Na_3C_6H_5O_7.2H_2O$), 5 mL of 1.0 M sodium bicarbonate ($NaHCO_3$), and 3 g of sodium dithionite ($Na_2S_2O_4$). After 3 h agitation, roots were removed from the DCB solution and rinsed with distilled deionized water, with the rinse solution added to the DCB solution. Then, DCB extracted solution was bought to the volume of 100 mL. The concentrations of As and Fe in the extracted solution were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) (PerkinElmer NexION™ 300X) and AAS, respectively.

To determine As species in rice tissues (roots, shoots, husks, and unpolished rice grains), powdered rice tissue samples were digested in a microwave digestion system (Multiwave 3000, Anton Paar) following a method described by Herath et al. (2020a). The concentrations of As species in rice tissues were measured by Ultra-High Performance Liquid Chromatography - Inductively Coupled Plasma Mass Spectrometry (UHPLC-ICP-MS) (Flexar, PerkinElmer - PerkinElmer NexION™ 300X). For accuracy and precision, As speciation analysis was validated using a certified reference material (CRM) for rice. The ERM-BC211 from the European Commission was used as the CRM for As speciation analysis. Reagent blanks and continuing calibration verification (CCV) were included in each batch. The optimized operational conditions for UHPLC-ICP-MS system to analyze As species is described elsewhere (Herath et al., 2020a).

2.5. Microbial diversity analysis

Rhizosphere soils collected at the flowering stage of the rice were used for microbial diversity analysis. DNeasy® PowerSoil® Pro kit (QIAGEN) was used for extracting DNA, according to the manufacturer's instructions. Polymerase Chain Reaction (PCR) amplicons were generated using the primers and conditions summarized in Table S2. Thermocycling was completed with an applied biosystem (Veriti™ 384) and a Platinum SuperFi II PCR master mix (Invitrogen, Australia) was used for the primary PCR. The first stage PCR was cleaned using magnetic beads, and samples were visualized on 2% Sybr Egel (Thermo Fisher Scientific). A secondary PCR was carried out to index the amplicons with the same polymerase master mix. The resulting amplicons were cleaned again using magnetic beads, quantified by fluorometry (Quanti-Fluor®dsDNA System, Promega), and normalized. The equimolar pool was cleaned for a final time using magnetic beads to concentrate the pool and then measured using a High-Sensitivity D1000 Tape on an Agilent 2200 TapeStation system. The pool was diluted to 5 nM and molarity was confirmed again using a Qubit™ dsDNA high sensitivity assay kit (Thermo Fisher Scientific). This process was followed by sequencing on an Illumina MiSeq (San Diego, CA, USA) with a V3, 600 cycle kit (2 × 300 base pairs paired-end). Paired-end reads were assembled by aligning the forward and reverse reads using PEAR software (version 0.9.5). Primers were identified and trimmed. Trimmed sequences were processed using Quantitative Insights into Microbial Ecology (QIIME 1.8) software.

2.6. Data analysis

Reported values were the means of three replicates. Kruskal-Wallis one-way ANOVA method was used to test the mean ranks of the data. The statistical significance of plant growth parameters, As/Fe in root plaque, and As concentration in rice roots, shoots, husks, and unpolished grains under different combinations of Fe-RBC supplementation and water management regimes were evaluated by using Duncan's multiple range test with $p < 0.05$ by using the SAS 9.1 statistical package. Origin 6.0 software package was used for making graphs.

3. Results and discussion

3.1. Characterization of iron-modified rice hull biochar

Table S3 summarizes physico-chemical properties of Fe-RBC. The Fe-RBC possesses a pH of 5.33. Relatively high percentages of resident matter and mobile matter in Fe-RBC, 41.36 w/w and 23.24 w/w, respectively, indicated that the application of Fe-RBC to paddy soils could enhance the carbon availability in these soils. The resident matter is the organic fraction of BC that could remain stable in the paddy agro-ecosystem for a very long time. The mobile matter is the organic fraction of BC that migrates into the paddy agro-ecosystem and becomes a food source for soil microorganisms (Gorovtsov et al., 2019). A 31.33% of ash content in Fe-RBC demonstrated that inorganic minerals and residues remained in the Fe-RBC structure after Fe modification. The BET results demonstrated a well-developed pore structure in Fe-RBC with a 142.60 $m^2 g^{-1}$ of surface area and 0.1650 mL g^{-1} of pore volume. According to the average pore diameter value (4.6285 nm), it can be revealed that mesopores have been developed in Fe-RBC. As shown in the SEM image (Fig. 1(b)), Fe has been successfully incorporated into the RBC structure. Fig. 1(c) and (d) show FTIR spectra before and after modification to Fe-RBC. The broad and strong band at 3261 cm^{-1} was due to the hydroxyl groups (-OH) of the RBC. The bands at 1049 and 1056 cm^{-1} corresponded to the C-OH stretching of carboxylic acids and alcohols. New peaks have appeared at 1695 and 1321 cm^{-1} after modification of RBC with Fe. These findings were consistent with the results reported by X. Hu et al. (2015). The band at 1695 cm^{-1} in Fe-RBC can be attributed to stretching vibration of the carboxyl groups which could complex with Fe(III) (Pehlivan et al., 2013).

The EDTA-extractable As in Fe-RBC was not detectable whereas EDTA-extractable Fe and Si concentrations in Fe-RBC were 1396.5 and 196.27 mg kg^{-1}, respectively. The presence of Fe in the paddy soil-water system could decrease the bioavailability of inorganic As for rice plant uptake. Similarly, the presence of Si in the paddy soil-water system can also reduce As(III) uptake by rice roots. Since both Si and As(III) are acquired by the same Si transporter in rice roots, high Si concentration in the rice rhizosphere decreases the uptake of As(III) by rice roots (Kumarathilaka et al., 2020). Moreover, uptake of Si by rice plants increases the rice plant's resistance to both biotic (fungal infections) and abiotic stresses (strong wind, heavy rain, and salinity).

3.2. Plant growth parameters and water usage

Plant growth parameters such as plant height, root length, shoot weight, and grain weight per pot under different treatments are summarized in Table 1. The results showed that the Fe-RBC-flooded treatment has significantly ($p \leq 0.05$) increased plant height, root length, and grain weight compared to the conventional flooded treatment. There was a 4% increase in plant height in the Fe-RBC-flooded treatment in comparison to the flooded treatment. In the case of root length and shoot weight, Fe-RBC-intermittent and Fe-RBC-flooded treatments recorded the highest growth parameters (6%–14% increment for root length and 6%–13% increment for shoot weight) over the flooded treatment. Most importantly, grain weight per pot increased in Fe-RBC-intermittent and Fe-RBC-flooded treatments by 24% and 39%, respectively, compared to

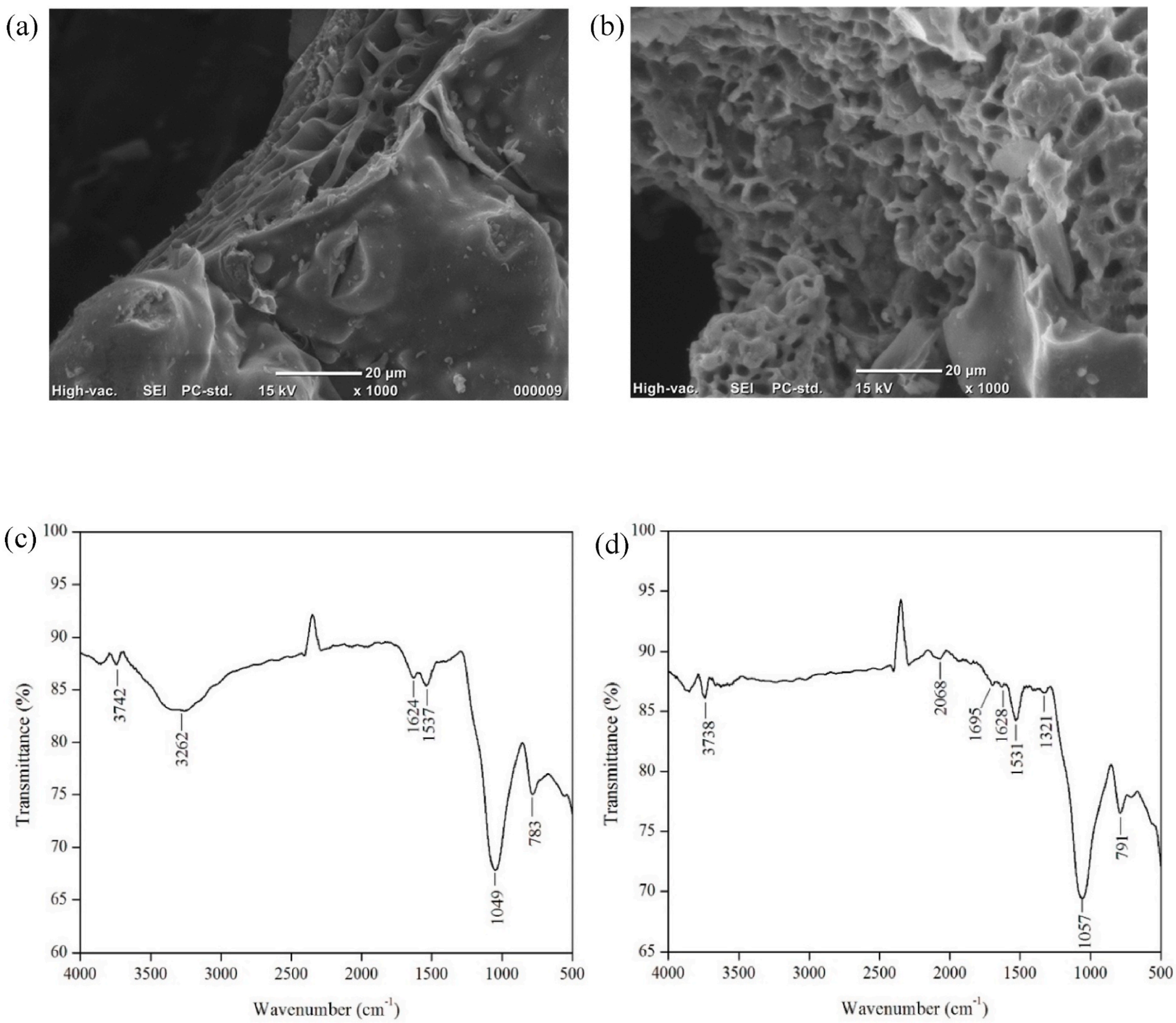

Fig. 1. Scanning electron microscopy images and FTIR spectrums; before (a and c) and after modification to iron-modified rice hull biochar (Fe-RBC) (b and d).

Table 1
Plant growth parameters and water usage efficiencies per pot under different combinations of iron-modified rice hull biochar (Fe-RBC) and water management practices. Treatments labelled with the same letter are not significantly different from each other for a plant growth parameter in the respective row.

Parameter	Fe-RBC-Flooded	Fe-RBC-Intermittent	Flooded	Intermittent
Plant height (cm)	71.11 ± 0.83a	69.44 ± 0.76b	68.67 ± 0.72b	66.25 ± 0.66c
Root length (cm)	19.26 ± 0.93a	17.94 ± 0.86a	16.92 ± 1.10b	16.75 ± 0.78b
Shoot dry weight (g)	17.37 ± 0.69a	16.36 ± 0.57a	15.39 ± 0.85a	14.98 ± 0.72a
Grain weight (g)	14.39 ± 0.24a	12.83 ± 0.37b	10.38 ± 0.28c	10.23 ± 0.17c
Water usage (cm^3)	7411	6065	8925	6327

the conventional flooded treatment. Recent studies by Irshad et al. (2020) and Yu et al. (2017) have also demonstrated that the incorporation of modified BCs in As-contaminated paddy environments has significantly increased root, shoot, and grain biomass in rice plants.

The application of Fe-RBC increased water usage efficiency in the paddy soil-water system. For instance, water usage in RBC-flooded treatment decreased by approximately 17% in comparison to the conventional flooded treatment. This figure for Fe-RBC-intermittent treatment was 32%. Since BC could enhance the water holding capacity in agricultural soils, supplementation of Fe-RBC increased water retention in paddy soils while minimizing the water requirement for irrigating rice (Karhu et al., 2011). Therefore, application of Fe-RBC in rice agro-ecosystems could reduce the cost for irrigation water supplementation. Moreover, Fe-RBC reduced the introduction of As-contaminated water in rice agro-ecosystems.

3.3. Analysis of paddy pore water and root plaque

Fig. 2 shows the temporal variation of Fe, Si, TC and TOC concentrations in paddy pore water under different treatments. The incorporation of Fe-RBC to paddy soils has increased Fe concentrations in the paddy pore water. For instance, Fe concentrations in Fe-RBC-flooded and Fe-RBC-intermittent treatments increased by 100%–142% in comparison to the conventional flooded treatment after 120 days of the transplantation. The higher Fe concentration in paddy pore water under Fe-RBC-flooded and Fe-RBC-intermittent treatments corresponded to the release of Fe into paddy pore water from Fe-RBC. The availability of Fe in the paddy soil-water system is an important factor to a decrease bioavailability of As. Since both As(III) and As(V) are complexed with Fe (III)-hydro(oxides), the bioavailability of inorganic As species for rice plant uptake is reduced (Xu et al., 2017). In addition, Fe could retain in rice roots, forming Fe plaque, which also decreases As uptake by rice

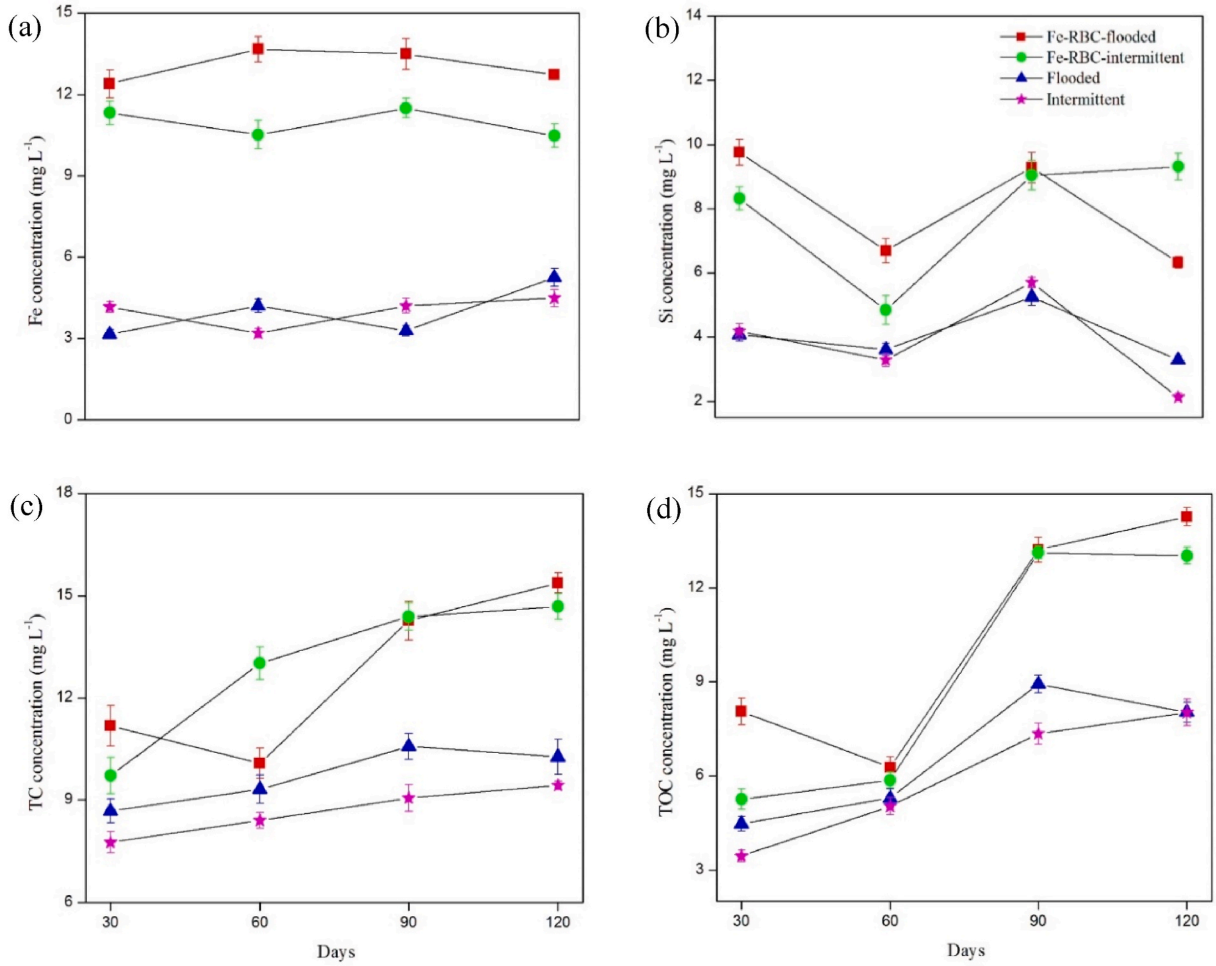

Fig. 2. Variation of Fe (a), Si (b), TC (c), and TOC (d) concentrations in paddy water under different combination of iron-modified rice hull biochar (Fe-RBC) and water management approaches.

roots. The supplementation of Fe-RBC has also increased Si concentration in paddy pore water in Fe-RBC-flooded and Fe-RBC-intermittent treatments by 93% and 184% (after 120 days of the transplantation), respectively, compared to the conventional flooded treatment. The availability of Si in the paddy soil-water system plays a major role in decreasing As(III) uptake by rice roots. As discussed in section 3.1, both $Si(OH)_4$ and As(III) are acquired by Si transporters in rice roots. The higher Si concentration in the paddy pore water decreases As(III) uptake by rice roots (Zhang et al., 2020).

The TC and TOC concentrations in the paddy pore water increased following the application of Fe-RBC in paddy soils (Fig. 2(c) and (d)). In Fe-RBC-flooded and Fe-RBC-intermittent treatments, TC increased by 43%–50% compared to the conventional flooded treatment. In addition, TOC concentration in Fe-RBC-flooded and Fe-RBC-intermittent treatments increased by 72% and 62%, respectively, in comparison to the flooded treatment. As discussed in section 3.1, Fe-RBC contained 74.6 w/w of mobile matter and resident matter which has increased the C availability in the paddy soil-water system. The availability of C in the paddy soil-water system could promote the activity of the microbial community which is responsible for the transformation of As in direct and indirect ways (Ma et al., 2014).

Fig. 3 shows the As/Fe ratio in root plaque under different treatments. The As/Fe ratio in Fe-RBC-flooded, Fe-RBC-intermittent, and intermittent treatments significantly ($p \leq 0.05$) decreased by 57%, 88%, and 69%, respectively, in comparison to the conventional flooded treatment. The decreased As/Fe in rice roots indicated the retention of Fe in the root plaque, as well as sequestration of As in Fe(III)-hydro

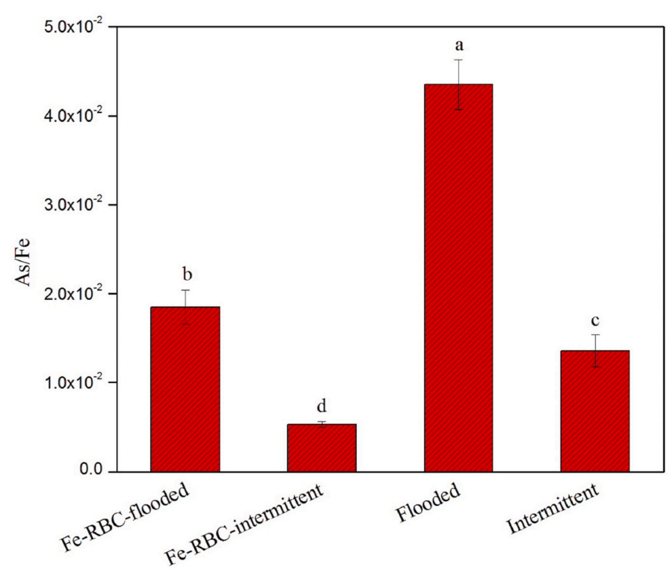

Fig. 3. The ratio of As/Fe in root plaque under different combinations of iron-modified rice hull biochar (Fe-RBC) and water management techniques. Treatments labelled with different letters are significantly different from each other for the As/Fe in root plaque (Duncan's multiple range test; $p < 0.05$).

(oxides) in the root plaque. Lin et al. (2020) also reported that the incorporation of Fe–Mn–La-impregnated BC composites in As-contaminated paddy soils has increased the Fe plaque on the surface of rice roots. The increased Fe plaque on rice roots leads to an enhancement of As retention, minimizing As uptake by rice roots (Qiao et al., 2018).

3.4. Diversity and abundance of microorganisms

Microbial diversity and abundance in paddy agro-ecosystems are important factors since microorganisms directly and indirectly affect the mobility and bioavailability of As in paddy soil-water systems. In this study, 21 different phyla, 67 classes, 110 orders, 170 families, and 221 genera were recorded. The phyla Actinobacteria was prominent in all treatments, containing 36.1%, 37.9%, 27.0%, and 36.5%, respectively, in Fe-RBC-flooded, Fe-RBC-intermittent, flooded, and intermittent treatments. Following the Actinobacteria, Proteobacteria (15.9%–17.0%), Chloroflexi (14.6%–18.7%), Acidobacteria (9.6%–10.8%), Firmicutes (7.8%–9.5%), and Gemmatimonadetes (6.0%–7.3%) dominated in all treatments (Fig. 4(a)). Das et al. (2016) reported that both

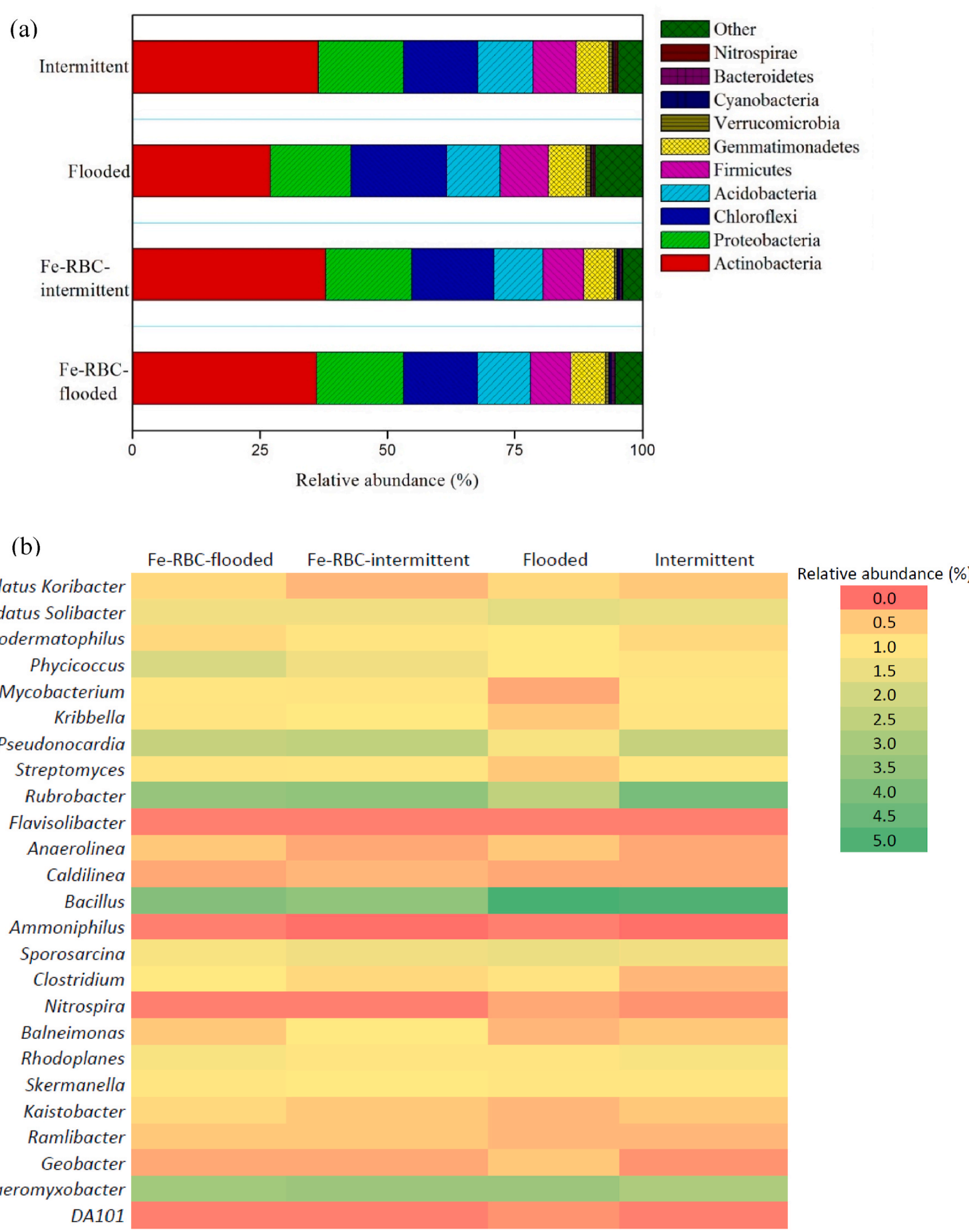

Fig. 4. Relative abundance of phylogenetic groups (a) and prominent genera at genus level (b) in the rice rhizosphere under different combinations of iron-modified rice hull biochar (Fe-RBC) and water management regimes.

Proteobacteria and Firmicutes involve the transformation of As in the rice rhizosphere.

Fig. 4(b) shows the relative abundance of different genera in the rice rhizosphere under different treatments. More importantly, the relative abundance of Fe(III) reducing genera such as *Bacillus* and *Clostridium* (belong to Firmicutes) and *Geobacter* and *Anaeromyxobacter* (belong to Proteobacteria) was higher in the conventional flooded treatment in comparison to other treatments. Iron (III) reduction to Fe(II) by Fe(III) reducing bacteria could release inorganic As species complexed with Fe (III) into the paddy soil-water system (Kumarathilaka et al., 2020). The abundance of *Bacillus* decreased by 24%, 29%, and 2%, respectively, under Fe-RBC-flooded, Fe-RBC-intermittent, and intermittent treatments compared to the conventional flooded treatment. In addition, the abundance of *Clostridium*, *Geobacter*, and *Anaeromyxobacter* in Fe-RBC-flooded, Fe-RBC-intermittent, and intermittent treatments, respectively, decreased as follows in comparison to the conventional flooded treatment: *Clostridium*: 36%, 64%, and 45%, *Geobacter*: 40%, 60%, and 40%, and *Anaeromyxobacter*: 6%, 15%, and 3%. The decreased abundance of Fe(III) reducing genera in Fe-RBC-flooded, Fe-RBC-intermittent, and intermittent treatments compared to the conventional flooded treatment may reduce the mobility and bioavailability of As in the paddy soil-water system. Herath et al. (2020b) also reported that the application of Si impregnated biochar composites in As-contaminated paddy soils has decreased the relative abundance of Fe(III) reducing genera. The incorporation of Fe-RBC (i.e. provides nutrients and substrates) and the selection of water management strategies (i.e. alter the redox chemistry) could create new habitats for microorganisms and consequently, the abundance and the composition of microbes may be shifted among different treatments. Therefore, it is suggested to study the long-term effects of Fe-RBC-water management approaches on the abundance and composition of microorganisms in As-contaminated paddy soils.

3.5. *Arsenic speciation in rice plant tissues with respect to potential mechanisms*

Total As concentrations (sum of As(III), DMA(V), MMA(V), and As (V)) in rice roots, shoots, husks, and unpolished rice grains decreased following the addition of Fe-RBC to paddy soils (Fig. 5). In comparison to the conventional flooded treatment, total As content in rice tissues in Fe-RBC-flooded, Fe-RBC-intermittent, and intermittent treatments, respectively, significantly ($p \leq 0.05$) decreased as follows: rice roots (37%, 62%, and 55%), shoots (21%, 37%, and 11%), husks (40%, 71%, and 48%), and unpolished rice grains (10%, 44%, and 17%). Most importantly, integrated Fe-RBC-intermittent treatment has decreased most toxic inorganic As concentrations in rice tissues in comparison to

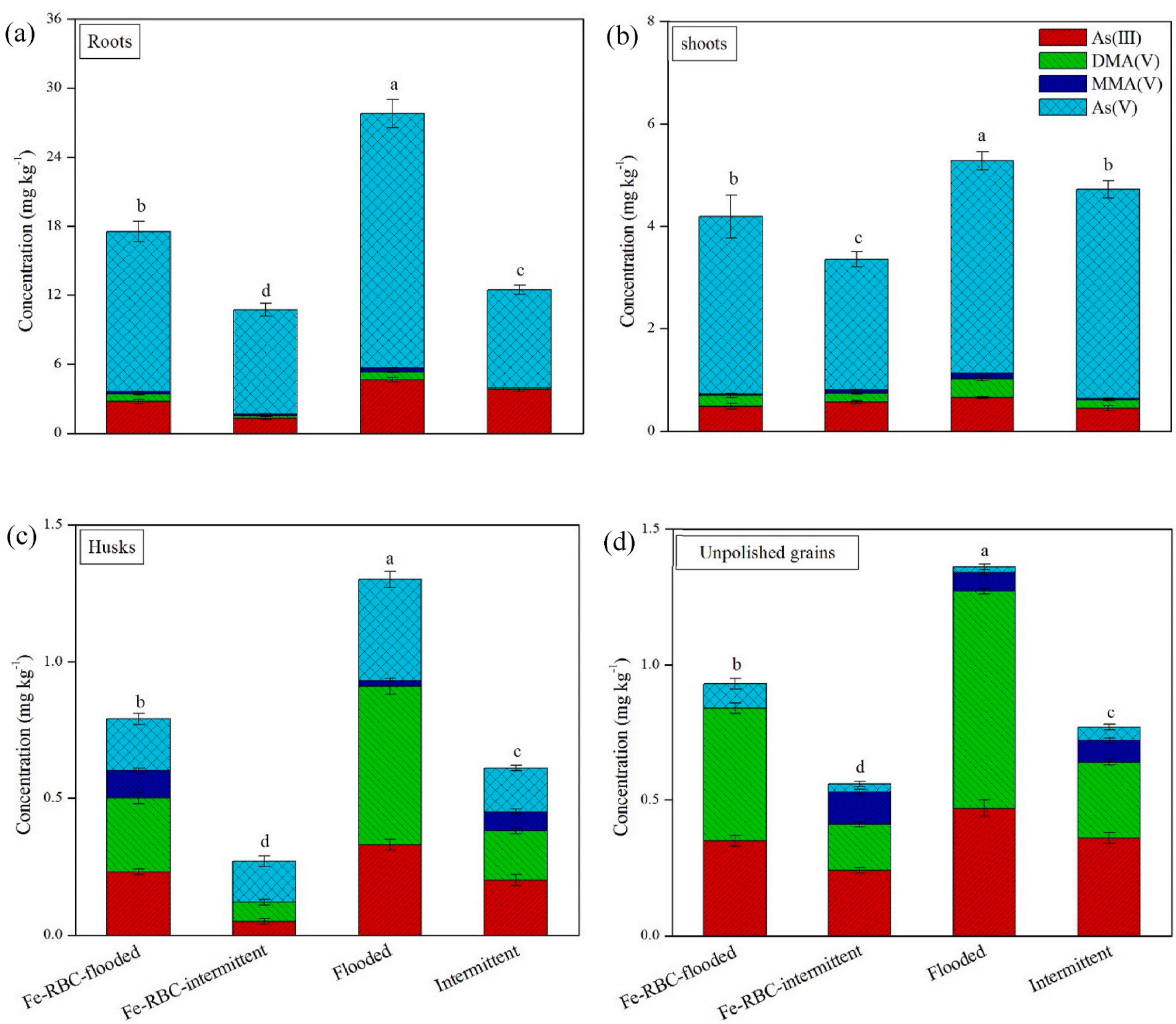

Fig. 5. The concentration of As(III), DMA(V), MMA(V) and As(V) in rice roots (a), shoots (b), husks (c), and unpolished rice grains (d) under different combinations of iron-modified rice hull biochar (Fe-RBC) and water management approaches. Treatments labelled with the same letter are not significantly different from each other for the total As concentration (Duncan's multiple range test; $p < 0.05$).

conventional flooded treatment. For instance, inorganic As concentration in rice roots, shoots, husks, and unpolished rice grains under Fe-RBC-intermittent treatment was lowered by 61%, 36%, 71%, and 44%, respectively, compared to flooded treatment. This figure under Fe-RBC-flooded treatment was 38%, 18%, 40%, and 10%. Moreover, inorganic As concentration in rice roots, shoots, husks, and unpolished rice grains under intermittent treatment decreased by 55%, 11%, 53%, and 43%, respectively, in comparison to conventional flooded treatment.

The translocation efficiency of inorganic As species from root to shoot in rice plants under each treatment has been decreased in this study. The percentages of inorganic As species in rice roots (95%–99%) and shoots (91%–96%) were higher than the percentages of organic As species in rice roots (1%–5%) and shoots (4%–9%). However, the percentage of inorganic As species in rice husks and unpolished grains decreased to 53%–74% and 36%–53%, respectively. In contrast, percentage of organic As species in rice husks (26%–47%) and unpolished rice grains (47%–64%) increased compared to the percentage of organic As species in rice roots and shoots.

Different mechanisms could involve decreasing As accumulation in rice tissues under Fe-RBC and alternative water management approaches (Fig. 6). As shown in Fig. 1(b), Fe-RBC possessed a well-developed pore structure. Arsenic species could diffuse into the pores of Fe-RBC through physical adsorption processes. Zhu et al. (2019) also found that bismuth impregnated BC in As-contaminated paddy soils has adsorbed As through the physical adsorption. As indicated in Fig. 1(d), oxygenated functional groups (i.e. alcoholic and carboxylic) could complex with inorganic As species through the surface complexation. H. Li et al. (2017) demonstrated that the complexation and electrostatic interactions are major mechanisms for As adsorption in BC. In addition, As(III) could be precipitated on the Fe-RBC surface (Wang et al., 2021). The modification of RBC with Fe could also minimize the bioavailability of inorganic As species in the rice rhizosphere since inorganic As species could complex with Fe(III)-hydro(oxides) on the RBC surface as shown in Fig. 6. Supplementation of Fe-RBC to paddy soils had increased the Fe concentration in the paddy soil-water system (Fig. 2(a)). The presence of Fe in the rice rhizosphere is an important factor to sequestrate inorganic As species (Xu et al., 2017). Even during the flooded water management conditions, rice roots diffuse O_2 into the rhizosphere through aerenchyma cells in a process called redial oxygen loss (ROL). As a result, more oxidative conditions develop in the rhizosphere zone compared to the bulk soils (Wu et al., 2012). Oxidative conditions in the rice rhizosphere promote the oxidation of Fe(II) to Fe(III) which has a high affinity with both As(III) and As(V) (Xu et al., 2017). In addition, oxidative conditions in the rice rhizosphere enhance the formation of Fe(III) plaque on rice roots (Lee et al., 2013). As shown in Fig. 3, Fe plaque has sequestrated As in the rhizosphere, limiting the bioavailability of As for rice root uptake. Moreover, Fig. 4(b) shows decreased abundance of Fe (III) reducing bacteria in the rice rhizosphere, following the supplementation of Fe-RBC in paddy soils. Therefore, the tendency to release As(III) and As(V), which are complexed to Fe(III) hydro(oxides), into the paddy soil-water system decreases, due to the supplementation of Fe-RBC in paddy soils.

The supplementation of Fe-RBC has increased the TOC concentration in the paddy soil-water system (Fig. 2(d)). Inorganic As species could complex with the dissolved organic compounds in the paddy soil-water system, limiting the bioavailability of As (Kumarathilaka et al., 2018a). The increased TOC following the application of Fe-RBC could also increase the abundance of microorganisms which are responsible for Fe(II) oxidation. As shown in Fig. 2(b), supplementation of Fe-RBC has increased the Si concentration in paddy pore water. Both $Si(OH)_4$ and As (III) are acquired by the same Si transporter, OsNIP2; 1 (Lsi1) (Fleck et al., 2013). This could be attributed to relatively similar dissociation constants of $Si(OH)_4$ and $As(OH)_3$, (pK_a of 9.2 and 9.3, respectively), and their similar sizes with tetrahedral orientation (Zhao et al., 2009). Therefore, higher availability of Si in the paddy soil-water system could increase Si uptake while decreasing the uptake of As(III) through competitive uptake.

An intermittent water management approach rather than the conventional flooded water management could increase the redox potential

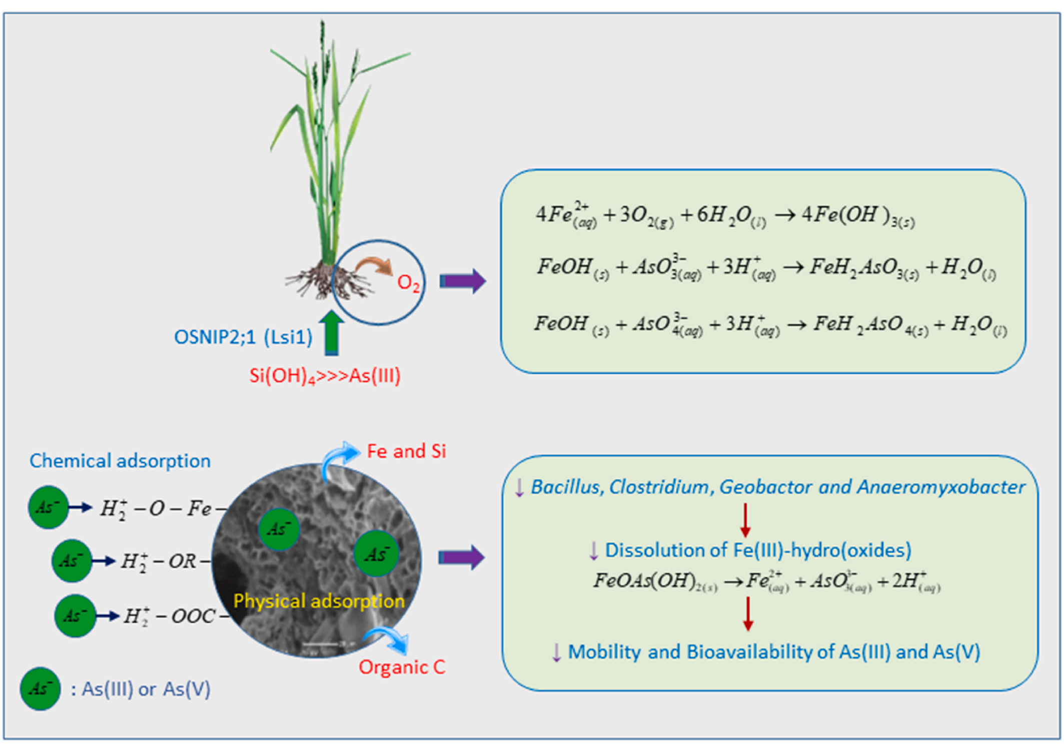

Fig. 6. Potential mechanisms for decreased As mobility and bioavailability in the paddy agro-ecosystem under the supplementation of iron-modified rice hull biochar (Fe-RBC) in paddy soils.

of the paddy soil-water system (P. Hu et al., 2013; Shrivastava et al., 2020). As a result, mobility and bioavailability of inorganic As species are decreased under intermittent water management. Therefore, Fe-RBC-intermittent treatment has significantly ($p \leq 0.05$) decreased As accumulation in rice tissues in comparison to other treatments. Relatively less As accumulation in rice tissues under intermittent treatment compared to the Fe-RBC-flooded treatment could also correspond to less As introduction to the paddy soil-water system through intermittent water management.

Once inorganic As species are acquired by rice roots, there are different As metabolic activities taking place in the rice roots such as reduction of As(V) to As(III), complexation of As(III) with thiol-rich peptides, sequestration of As(III)-thiol-rich peptides in vacuoles, and As(III) efflux back into the external environment (Kumarathilaka et al., 2018c). These different processes are mediated by various enzymes and transporters present in the rice root (Shri et al., 2014). As a result, translocation of inorganic As species from rice roots to above-ground tissues such as shoots, husks, and unpolished rice grains can be gradually decreased. On the contrary, translocation of organic As species from rice roots to above-ground tissues has increased. This could correspond to the dissociation of both MMA(V) and DMA(V) at the cytoplasmic pH (Kumarathilaka et al., 2018c). Moreover, unlike inorganic As species, lack of complexation of organic As species with thiol-rich peptides promoted organic As species to be readily translocated from rice roots to above-ground tissues.

4. Conclusions

Supplementation of Fe-RBC to paddy soils has increased grain weight per pot. Even though Fe-RBC-flooded treatment recorded the highest yield increment per pot compared to the conventional flooded treatment, As concentration in rice tissues under Fe-RBC-flooded treatment was higher in comparison to the Fe-RBC-intermittent and intermittent treatments. The Fe-RBC in the paddy soil-water system has increased Fe, Si, and TOC levels in the paddy pore water and Fe concentrations in the root plaque. The integrated Fe-RBC-intermittent treatment recorded the lowest level of As in rice roots, shoots, husks, unpolished rice grains in comparison to other treatments. Different processes and mechanisms including chemi- and physisorption of As species into Fe-RBC, sequestration of As on Fe plaque, decreased As(III) uptake by rice roots due to the competitive uptake with $Si(OH)_4$ could involve less As accumulation in rice tissues following the supplementation of Fe-RBC in paddy soils. The less abundance of Fe(III) reducing bacteria, following the Fe-RBC supplementation, could also minimize the reduction of Fe(III) which decreases mobility and bioavailability of inorganic As species in the rice rhizosphere. Further research could be focused on examining the As dynamics in paddy agro-ecosystems under different Fe-RBC supplementation rates, Fe and RBC ratios, and As levels. Moreover, this research was conducted on a glasshouse scale. Therefore, the implementation of field-scale experiments to investigate the long-term effects of the integrated Fe-RBC-water management approaches on rice yield and to examine the practical feasibility of applying Fe-RBC in As-contaminated paddy agro-ecosystems should be done in future research.

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.

Acknowledgements

This research was performed as a part of PhD research which was supported by the Australian Research Training Programme-International Stipend and Tuition Fees Scholarship. We thank Dr. Barbara Harmes for proof reading the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.117661.

Credit author statement

Prasanna Kumarathilaka: Conceptualization, Data curation, Investigation, Writing – original draft, Writing – review & editing, Formal analysis, Methodology, Resources, Jochen Bundschuh: Conceptualization, Writing – review & editing, Methodology, Resources, Supervision, Saman Seneweera: Resources, Supervision. Alla Marchuk: Resources, Supervision. Yong Sik Ok: Resources, Supervision

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