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An integrated approach of rice hull biochar-alternative water management as a promising tool to decrease inorganic arsenic levels and to sustain essential element contents in rice

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A R T I C L E I N F O

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A B S T R A C T

Arsenic (As) in rice agroecosystems causes a loss of both rice yield and quality of rice grains. In this study, an integrated approach of biochar (BC) and alternative water management is proposed to reduce As content while sustaining essential elemental concentrations in rice. The rice cultivar, *Jayanthi*, was grown, irrigated with 1 mg L^{-1} of As-containing water, under rice hull BC (RBC)-flooded, RBC-intermittent, conventional flooded, and intermittent treatments. The RBC has increased rice yield by 11%−19% in RBC-intermittent and -flooded treatments compared to the flooded treatment. Inorganic As content in rice tissues and abundance of Fe(III) reducing bacteria in the rhizosphere were lowered by 10%−83% and 40–70%, respectively, in RBC-flooded, -intermittent, and intermittent treatments over flooded treatment. Essential elemental concentrations (Fe, Mn, Zn, Mg, and Ca) in unpolished rice grains increased by 45%−329% in RBC-flooded and -intermittent treatments compared to flooded treatment. Overall, the integrated approach of RBC-intermittent practices has lowered inorganic As concentration in unpolished rice grains, while sustaining the levels of essential elements in rice grains, compared to other treatments. An integrated approach of RBC-intermittent practices is suggested for rice grown with As-contaminated water to improve the quality of rice, as well as tackling food-related malnutrition in people.

1. Introduction

The health risks related to ingestion of inorganic arsenic (As) through rice consumption is a worldwide concern. Both natural processes (i.e. weathering of As-bearing rocks and alluvial deposits) and anthropogenic activities (i.e. mining operations, wastewater discharge, and use of arsenical pesticides) lead to the distribution of As in rice agroecosystems (Kumarathilaka et al., 2018a; Wu et al., 2019). Use of groundwater, with high concentrations of mostly geogenic As, for irrigating rice is the major cause of accumulating As in rice tissues. The Food and Agricultural Organization's (FAO) recommended level for total As in irrigation water is <100 μg L^{-1}. However, irrigation water exceeding the FAO's recommended level has been extensively used in major rice producing countries. For instance, Huang et al. (2016) demonstrated that groundwater containing 60–920 μg L^{-1} of As has been used in Vietnam for irrigating rice fields. In Bangladesh, India, and Nepal, As-contaminated groundwater (400–1010 μg L^{-1}) has also been extensively used for rice cultivation (Biswas et al., 2014; Dahal et al., 2008; Dittmar et al., 2007).

Rice accumulates higher levels of inorganic As than other cereal crops. Conventional rice cultivation practices (long-term flooded conditions and short-term non-flooded conditions) cause higher levels of bioavailable inorganic As in paddy soil-water systems and subsequently in rice tissues than other cereal crops (Bakhat et al., 2017). Physico-

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chemical properties such as pH, redox potential (Eh), organic matter, redox sensitive ions (i.e. Fe, Mn, and S), and Si and PO_4^{3-} contents, and microbial diversity influence the mobility and bioavailability of As and As speciation (i.e. arsenite (As(III)), arsenate (As(V)), dimethylarsinic acid (DMA(V)), monomethylarsonic acid (MMA(V))) in paddy agroecosystems (Kumarathilaka et al., 2018b; Li et al., 2019a; Sun et al., 2019).

Physico-chemical and biological methods have been tested to minimize the accumulation of As in rice tissues. However, there are limitations to the practical employability of the above mentioned methods (Kumarathilaka et al., 2020; Li et al., 2019b). For example, supplementation of nutrients such as P, Si, and S to As-contaminated paddy environments is limited, due to the high cost and source scarcity (Desplanques et al., 2006; Mew, 2016). The incorporation of Fe and Mn sources into As-contaminated paddy soils needs to be considered, because of their cost and the potential to the secondary contamination. The main drawback of microbial inoculation to As-contaminated paddy agroecosystems is that inoculated microbes may not adapt to field conditions. Long-term field experiments need to be performed to investigate whether typical rice cultivation conditions are suitable for As hyperaccumulating plant species (Kumarathilaka et al., 2020). Alternative water management practices such as intermittent irrigation have proven to lessen As levels in paddy ecosystems more than conventional flooded water management. However, even though intermittent irrigation practices lead to lessen As accumulation in rice tissues, loss of grain yield has been reported (Chou et al., 2016; Moreno-Jiménez et al., 2014).

The application of biochar (BC) to As-contaminated paddy soils might be a viable option in terms of economic feasibility and practical employability. Biochar is produced through pyrolysis of different biomass types including those from forestry and agricultural crop residues, the organic portion of municipal solid wastes, invasive plant species, animal manures, and wood waste (Ghani et al., 2013; Jayawardhana et al., 2018; Tomczyk et al., 2020). Biochar in soil systems has a wide assortment of benefits such as reducing mobility and bioavailability of heavy metal(loids), improving soil nutrient content, increasing water holding capacity, providing habitat for microbial community, and minimizing greenhouse gas emissions through carbon sequestration (Kumarathilaka et al., 2018c; Xu et al., 2012).

Even though BC has been amended to As contaminated soils, very limited studies have focused on the role and potential of BC in As-contaminated paddy ecosystems. Yin et al. (2017) have applied rice straw BC produced at 450 °C to As-contaminated paddy soils. Khan et al. (2014) have produced sewage sludge biochar to be amended with As-contaminated paddy soils. The typical rice cultivation practices change the redox chemistry of the paddy soil-water system, therefore, in-depth study is needed to understand the interaction between BC and As species in paddy soil-water systems. Moreover, an integrated approach of BC supplementation and intermittent irrigation might sustain the rice yield and improve the rice quality for safer human consumption. There are no reports on the integrated effects of BC and water management practices on As dynamics in paddy agroecosystems. In addition, no study has reported essential elemental levels in rice grains under the integrated approach of BC-water management practices in As-contaminated paddy ecosystems. Taking these facts into account, this study, for the first time, investigated the effect of BC amendments to As contaminated paddy soils under different water management practices on plant growth parameters, As speciation in rice tissues, microbial diversity in the rice rhizosphere, and essential elemental concentration in rice grains. Rice hull was used as the feedstock for producing BC since rice hull could contain high level of Si. Since the supplementation of Si to As-contaminated paddy soils is limited, due to the scarcity of resource and its high cost, the application of Si-rich rice hull BC (RBC) may also be a cost-effective and practically employable option to decrease the accumulation of As in rice tissues. Both flooded and intermittent water managements were practiced in this study.

2. Materials and methods

2.1. Paddy soil collection and characterization

Paddy soils were collected from the ploughing layer (0–20 cm) in New South Wales, Australia (34° 35′ 53.5″ S, 146° 21′ 38.1″ E). A composite sample was prepared by mixing each subsample. After being air-dried, the soil samples for pot experiments and chemical analysis were passed through 5 and 2 mm sieves, respectively. The pH and electrical conductivity (EC) of the soil were measured in 1:10 suspensions of soil-to-distilled water using a digital pH and conductivity meter (PC2700, EUTECH Instruments). A moisture meter (ISSCO, MB 120, OHAUS Corporation) was used to measure the moisture content of soils. Total organic carbon content of the soil was determined through a loss-on-ignition method (Rayment and Lyons, 2014). Cation exchange capacity of the soil was determined using the 1 M/ammonium extraction (pH adjust to 7.0) method (Rayment and Lyons, 2014). Soil samples were digested in a microwave digestion system (Multiwave 3000, Anton Paar) before being analysed for total concentration of metal(loids). The EDTA (Ethylenediaminetetraacetic acid) extraction solution was used to determine bioavailable concentrations of metal(loids). In brief, 20 mL of 0.05 M / EDTA was added to 1 g of soil. The mixture was then stirred for 3 h, and centrifuged, and the supernatant was filtered through the membrane filter technique. Arsenic concentration in paddy soils was measured using inductively coupled plasma mass spectrometry (ICP-MS) (PerkinElmer NexION™ 300X). The concentration of other elements was determined using an atomic absorption spectrophotometer (AAS) (AAS-7000, Shimadzu).

2.2. Biochar production and characterization

Rice hulls were obtained from Grain and Grape, Victoria, Australia to produce RBC. Rice hulls were subjected to a slow pyrolysis process under continuous N_2 flow in a muffle furnace (CS2, RIO GRANDE) at 600 °C for 2 h to produce RBC. The heating rate of the pyrolysis process was set as 7 °C min^{-1}. The produced RBC was ground and sieved through a 2 mm aperture to gain homogeneous particle size.

The pH and EC of RBC were measured in 1:5 suspensions of RBC-to-distilled water using the digital pH and conductivity meter. The proximate analysis for RBC (moisture content, volatile matter, ash content, and resident matter) were performed according to the modified thermal analysis methods described elsewhere (Ahmad et al., 2012). In brief, moisture content was determined by calculating weight loss when RBC was heated at 105 °C for 24 h. Volatile matter content was measured as the weight loss when RBC was heated at 450 °C for 30 min in a covered porcelain crucible. Ash content was also determined as the weight loss when RBC was heated at 700 °C for 30 min in an open-top porcelain crucible. Resident matter content was calculated using the difference between the portion of RBC which was not ash and moisture, volatile matter, and ash contents. Specific surface area of the RBC was determined through adsorption isotherms using the Brunauer–Emmett–Teller (BET) equation. Total pore volume and average pore size of the RBC were measured from the N_2 adsorption data using the Barret–Joyner–Halender (BJH) method. The morphology of the RBC was characterized by Scanning Electron Microscopy (SEM) (JCM-6000, JEOL).

2.3. Pot experiment

A pot experiment was conducted to examine the influence of water management and RBC amendment on the mobility and phytoavailability of As species in the soil solution and substantial uptake and accumulation in rice tissues. The rice cultivar, *Jayanthi*, was used for this study. Rice seeds were surface sterilized with 10% H_2O_2 for 5 min and germinated in moist compost. After three weeks, plants of uniform size (three seedlings per pot) were transferred into polyvinylchloride pots containing paddy soils (2.5 kg). Before transplanting, each pot was flooded with As-containing water (1 mg L^{-1}) and amended with RBC (1% w/w). There were four different treatments: (1) continuous flooding with RBC (1% w/w); RBC-flooded, (2) intermittent irrigation with RBC (1% w/w); RBC-intermittent, (3) conventional flooding without RBC; Flooded, and (4) intermittent irrigation without RBC; Intermittent. To ensure the wet cultivation of rice, in continuous flooding treatments, pots were flooded (~5 cm) during the entire growing season, whereas in the intermittent irrigation treatments, pots were only flooded (~5 cm) when the soil was dry and cracks were formed. This depth was maintained by adding As-containing water to the appropriate pots, having a gauge marked on the side. The total volume of water consumed during the whole growing season was calculated. All the treatments were in triplicate and regularly randomized to ensure uniform light and temperature.

Table 1
Selected physico-chemical properties and elemental composition in paddy soils.

Parameter	Value	
pH	6.59 ± 0.06	
EC (dS m^{-1})	0.04 ± 0.01	
Moisture content (%)	5.79 ± 0.21	
Organic matter (%)	7.22 ± 0.36	
Cation exchange capacity (cmol/kg)	14.37 ± 1.16	
Metal(loid) concentration (mg kg^{-1})	Total	EDTA-Extractable
As	3.10 ± 0.27	0.04 ± 0.01
Fe	12,720.26 ± 130	1147.74 ± 22.5
Mn	288.15 ± 9.56	197.82 ± 12.3
Si	2190.10 ± 24.3	153.43 ± 14.6
Zn	32.55 ± 2.58	2.23 ± 0.29
Mg	877.05 ± 26.8	37.78 ± 5.31
K	1284.50 ± 33.4	66.65 ± 4.68
Ni	23.99 ± 1.27	2.35 ± 0.62
Co	119.05 ± 7.92	42.5 ± 4.93
Ca	5531.58 ± 29.6	513.64 ± 15.6
Al	43,718.86 ± 147	563.88 ± 13.8

2.4. Soil solution analysis

Soil solution samples were collected at 30-day intervals until harvest. The pH and EC of soil solutions were measured. The redox potential (Eh) was recorded using an oxidation-reduction potential (ORP) meter (YK-23RP, Mother Tool) by inserting the platinum electrode into the paddy soil surface. The Fe, Mn, and Si concentrations in the paddy water were determined using AAS. Ions such as PO_4^{3-}, NO_3^-, NO_2^- and SO_4^{2-} concentrations in the paddy water were measured by an ion chromatography system (ICS-2000, Dionex).

2.5. Plant growth parameters

Plant height, root length, shoot biomass, and grain production per pot were measured to evaluate the plant growth and yield under different combinations of RBC and water management regimes. Plant height was recorded at 30 day intervals by using a meter scale. The root length and grain production per pot were measured after harvesting the plants. Shoot biomass was determined after drying the harvested rice shoots in an oven (STERIDIUM) at 60 °C for 72 h.

2.6. Arsenic speciation analysis

Rice tissue samples (roots, shoots, husks, and unpolished grains) were digested in the microwave digestion system for As speciation analysis. Acid cleaned digestion vessels were used and 0.5 g of rice tissues was added into the vessels with 7 mL of 0.2 M HNO_3. The digestion program consisted of a 20 min ramping time to 95 ºC with a 90 min holding time, and a 10 min holding time before cool down. All digested solutions were diluted to 20 mL by adding Milli-Q water. Each sample was filtered through a glass fiber filter prior to analysis. The concentration of As species in extracted solutions was determined using Ultra-High Performance Liquid Chromatography – Inductively Coupled Plasma Mass Spectrometry (UHPLC-ICP-MS) (Flexar, PerkinElmer – PerkinElmer NexION™ 300X). Arsenic species were separated using an anion exchange column (PRP-X100, 250 × 4.1 mm, 10 μm (Hamilton, USA)). A solution containing 8.5 mM of $NH_4H_2PO_4$ and 8.5 mM of NH_4NO_3 (1:1) at pH 6.0 was used as the mobile phase which was pumped through the column isocratically (Herath et al., 2020). Arsenic species in each sample were identified by comparing their retention times with those of the standards (As(III), DMA(V), MMA(V), and As (V)). A certified reference material (CRM), ERM-BC211, was used to verify digestion efficiency in rice tissue samples.

2.7. Determination of essential elements

Unpolished rice grain samples were digested in the microwave digestion system . A weight 0.5 g of rice tissues (oven-dried at 65 °C for 48 h) was added to the vessels with 6 mL of conc. HNO_3 and 1 mL of 30% H_2O_2. The digestion program comprised a 20 min ramping time to 180 °C with a 45 min holding time and a 10 min holding time before cool down. All digested solutions were diluted to 20 mL by adding Milli-Q water. Each sample was filtered through a glass fiber filter prior to analysis. A method blank and continuing calibration verification (CCV) were run for each analytical batch. In addition, a certified reference material (CRM), ERM-BC211 was used to verify digestion efficiency in rice tissue samples. The concentration of elements such as Fe, Mn, Mg, K, Ca, Zn, Ni, and Co, were determined using AAS.

2.8. Microbial diversity analysis

DNA was extracted from the freshly collected rhizosphere soils at the flowering stage of the rice by using the DNeasy® PowerSoil® Pro kit (QIAGEN), according to the manufacturer's instructions. The fluorometry has been used for quality assurance of samples for Polymerase Chain Reaction (PCR) analysis. The V3-V4 region of the 16S rRNA gene was amplified using a set of primers (forward primer: 341F (CCTAYGGGRBGCASCAG) and reverse primer: 806R (GGACTACNNGGGTATCTAAT). The pooled DNA products were utilized to make an Illumina Pair-End library. Paired-ends reads were assembled by aligning the forward and reverse reads using PEAR (version 0.9.5) on the Miseq platform (San Diego, CA, USA). Trimmed sequences were processed using Quantitative Insights into Microbial Ecology (QIIME 1.8.4) software.

2.9. Statistical analysis

Statistical analysis of the experimental data was performed with SAS 9.1 software. Statistical graphing was done using Origin 6.0 software package. The statistical significance of As concentration in rice tissues under different combinations of RBC amendments and water management practices were determined by using Duncan's multiple range test with $p < 0.05$.

3. Results and discussion

3.1. Paddy soil characterization

Paddy soils were slightly acidic with a moisture content of 5.79% (Table 1). Paddy soils were characterized by a high amount of organic matter (7.22%) and were in the range of cation exchange capacity in typical paddy soils (Dong et al., 2016). Total As concentration in paddy soils (3.10 mg kg^{-1}) was well below the European Union's

Table 2
Physicochemical properties and EDTA extractable elemental concentrations in RBC.

Parameter	Value
pH	9.81 ± 0.09
EC (dS m^{-1})	0.73 ± 0.03
Proximate analysis (%)	
Moisture	1.08 ± 0.10
Volatile matter	22.88 ± 1.37
Ash	30.25 ± 2.68
Resident matter	45.79 ± 3.48
Specific surface area (m^2 g^{-1})	201.39
Total pore volume (mL g^{-1})	0.2026
Average pore diameter (nm)	4.0240
EDTA-extractable metal(loid) concentration (mg kg^{-1})	
As	ND
Fe	112.59 ± 5.67
Mn	170.70 ± 4.38
Si	246.31 ± 9.37
Zn	1.71 ± 0.35
Mg	229.37 ± 10.6
K	53.62 ± 6.58
Ni	1.38 ± 0.21
Co	42.96 ± 4.39
Ca	1771.74 ± 24.6
Al	50.69 ± 3.47

Note: ND: not detectable.

recommended maximum value of 20 mg kg^{-1} of As to be present in agricultural soils (Shrivastava et al., 2017). The EDTA-extractable As concentration was 0.04 mg kg^{-1} which was 1.29% of the total As content in paddy soils. Total Fe and Mn concentrations in the paddy soil were 12,270.26 and 288.15 mg kg^{-1}, respectively. Extractable percentage of Mn (68.65%) was higher than that of Fe (9.02%). Extractable concentrations of Fe and Mn exceeded the critical concentrations (10 and 5 mg kg^{-1}, respectively) in paddy soils (Pirzadeh et al., 2010). Paddy soils also contained other essential elements, including Si, Zn, Mg, K, Ni, Co, and Ca, which are important for the growth of rice plants as well as for protection against biotic and abiotic stresses (Table 1).

3.2. Biochar characterization

Characteristics of the RBC were summarized in the Table 2. The produced RBC possesses an alkaline nature (9.81). The resident matter of RBC is greater than that of mobile matter indicating loss of volatile matter through thermal decomposition. The amount of mobile matter and resident matter in RBC (68.67%) is an important factor in terms of short- and long-term carbon availability in paddy soils. A relatively high percentage of ash (30.25%) in RBC demonstrates that inorganic minerals and residues remain after the pyrolysis of RBC. The SEM image (Fig. S1) and BET data demonstrate the well-developed pore structure and the development of mesopores (4.0240 nm) in RBC.

EDTA extractable elemental concentrations indicated that As is not detectable in RBC. Redox sensitive elements including Fe and Mn (112.59 and 170.70 mg kg^{-1}, respectively) have been incorporated in RBC. Most importantly, RBC contains 246.31 mg kg^{-1} of extractable Si. In addition, RBC comprises essential elements such as Zn, Mg, K, Co, Ca, and Al (Table 2). The presence of redox sensitive elements and other essential elements in RBC may influence the As dynamics in the rice paddy-soil system and subsequent accumulation of As in rice tissues, while improving the quality of rice grains.

3.3. Analysis of soil solution

After 30 days of transplantation, paddy soil surface turned into moderately reduced conditions, as shown in Fig. 1(a). The Eh of each treatment gradually decreased over time for the following 90 days when there was a slight increase in Eh at day 120. Flooded water management practices have indicated a decreased Eh value compared to the Eh values of intermittent water management practices. Highly reduced conditions in flooded water management favor reduction of redox sensitive elements such as Fe and Mn which could directly influence the mobility and bioavailability of As in the paddy soil-water system. The pHs of RBC-

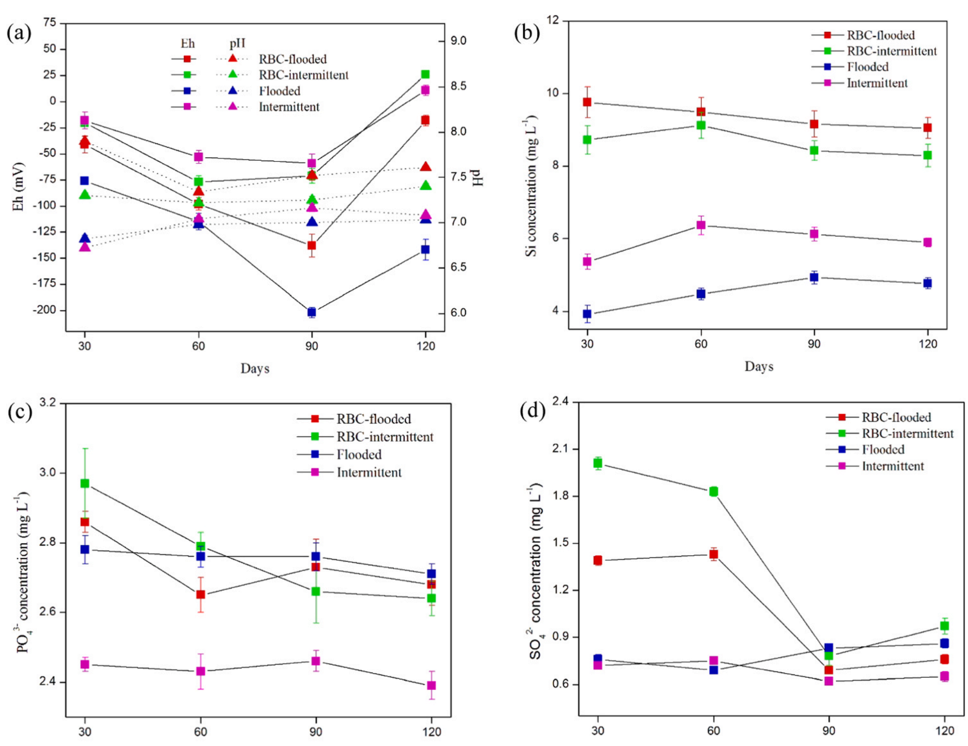

Fig. 1. Temporal variation of pH-Eh (a) and concentrations of Si (b), PO_4^{3-} (c), and SO_4^{2-} (d) in paddy water under different combination of RBC and water management methods.

Table 3
Plant growth parameters and water usage efficiencies per pot under different combination of RBC and water management approaches.

Parameter	RBC-flooded	RBC-intermittent	Flooded	Intermittent
Plant height (cm)				
30 days after transplanting	10.67 ± 0.11	11.39 ± 0.12	10.50 ± 0.09	10.17 ± 0.07
60 days after transplanting	15.61 ± 0.16	15.11 ± 0.08	16.00 ± 0.07	14.17 ± 0.11
90 days after transplanting	20.42 ± 0.14	18.39 ± 0.09	19.06 ± 0.10	18.50 ± 0.12
120 days after transplanting	41.26 ± 0.19	38.93 ± 0.22	38.47 ± 0.16	39.01 ± 0.36
At the harvest (142 days)	52.42 ± 0.32	47.33 ± 0.28	45.72 ± 0.37	47.28 ± 0.40
Grain weight (g)	11.32 ± 0.15	10.54 ± 0.27	9.49 ± 0.19	8.83 ± 0.23
Root length (cm)	27.72 ± 1.57	22.19 ± 1.37	21.17 ± 0.92	20.38 ± 1.12
Shoot dry weight (g)	16.61 ± 0.43	16.06 ± 0.27	15.33 ± 0.15	14.38 ± 0.17
Water usage (cm^3)	11,167	8815	13,300	8994

flooded and -intermittent treatments were higher than that of the flooded and intermittent treatments (Fig. 1(a)). The alkaline nature of RBC may have increased pH in RBC-flooded and -intermittent treatments.

Incorporation of RBC to paddy soils increased the Si concentration in paddy water (Fig. 1(b)). In comparison, RBC-flooded treatment indicated a 46.3% increment of Si concentration compared to the flooded treatment after 120 days of transplantation. Similarly, RBC-intermittent treatment showed a 27.3% increment of Si levels in the paddy water, which was greater than that of intermittent treatment. As summarized in Table 2, RBC contained 246.31 mg kg^{-1} of EDTA-extractable Si which might be continuously released into the paddy soil-water system. The availability of Si in the paddy soil–water system is an important factor for a reduced As(III) uptake by rice roots (Kumarathilaka et al., 2018b). Silicon can also increase the plant resistance to abiotic (i.e. wind and strong rain) and biotic stresses (i.e. insect and fungal infections). Due to the scarcity of the resource, as well as high cost, Si supplementation in As-contaminated paddy ecosystems is limited. Since rice hulls contain 246.31 mg kg^{-1} of EDTA-extractable Si, the incorporation of RBC may be a cost-effective method to introducing Si into As-contaminated paddy soil-water systems for decreasing As uptake by rice roots.

Anions such as PO_4^{3-} and SO_4^{2-} were detected in paddy water in all treatments (Fig. 1(c) and (d)). The PO_4^{3-} concentration in all the treatments slightly varied throughout the rice growing cycle (2.43–2.97 mg L^{-1}). The presence of PO_4^{3-} in paddy water led to a reduced uptake of inorganic As(V) by rice plants. The RBC-flooded and -intermittent treatments have shown approximately two times higher SO_4^{2-} concentrations in paddy water than in flooded and intermittent treatments. The SO_4^{2-} in the paddy water may also decrease the accumulation of As in rice grains by alternating the metabolism in rice plants (Zhao et al., 2009). Concentrations of NO_3^- and NO_2^- in paddy water in all treatments were below the limit of detection (LOD).

3.4. Plant growth parameters

Table 3 summarizes plant growth parameters and water usage in all treatments. Rice plants in the RBC-flooded treatment recorded the highest plant height, followed by the RBC-intermittent, intermittent, and flooded treatments. Similarly, highest grain weight per pot (11.32 g) was recorded in the RBC-flooded treatment, which was an approximately 19% greater increment of rice yield compared to the flooded treatment. Root length and shoot dry weight were also highest

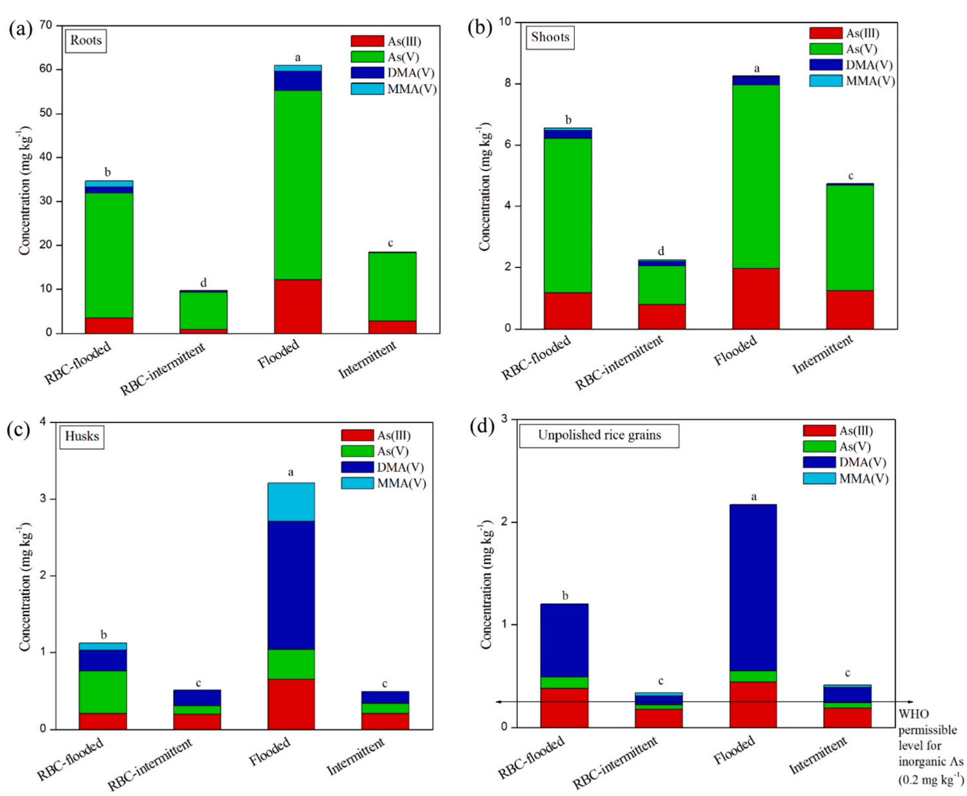

Fig. 2. The concentration of As species in rice roots (a), shoots (b), husks (c), and unpolished rice grains (d) under different combination of RBC and water management regimes. Treatments labelled with same letter are not significantly different from each other for the total As concentration (Duncan's multiple range test; $p < 0.05$).

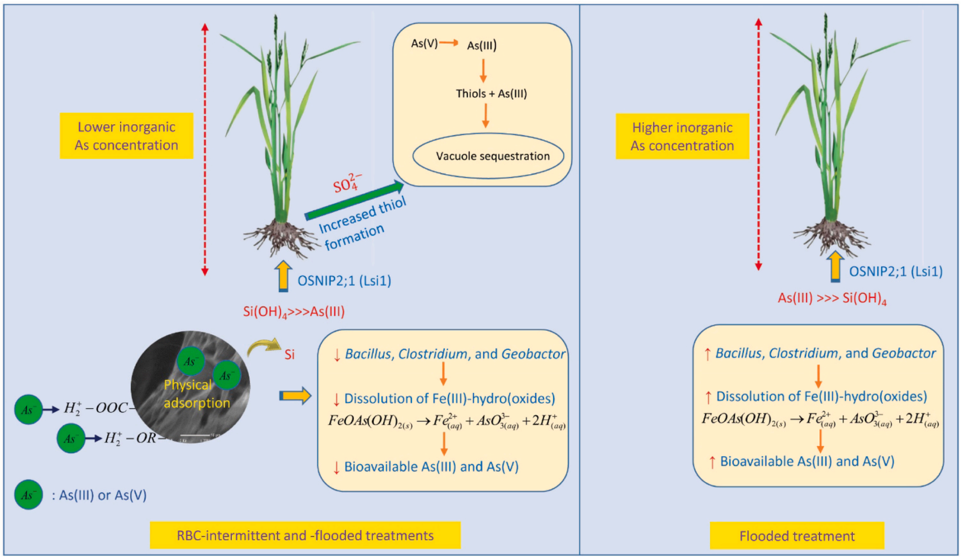

Fig. 3. Possible mechanisms for lowered As accumulation in rice tissues under RBC-intermittent and -flooded treatments compared to flooded treatment.

in the RBC-flooded treatment. The reductive conditions associated with flooded water management may prevent aerobic microbe-related disease damage (Minamikawa et al., 2015). The addition of RBC to paddy soils may also steadily release essential elements into paddy soil solution for plant uptake. As a result, RBC-flooded treatments have reported highest yield and other plant growth parameters such as plant height, root length, and shoot weight.

Water usage efficiency was higher in RBC-flooded and -intermittent treatments than in flooded and intermittent treatments. For instance, RBC-flooded treatment indicated an approximately 16% lower water consumption than that of flooded treatment. The supplementation of RBC to paddy soils may have improved the water holding capacity of paddy soils. However, RBC-intermittent treatment has shown only a 2% less water consumption over the intermittent treatment. During the intermittent irrigation practices, paddy soil is only flooded when the soil becomes dry. This may be a reason for only 2% difference between RBC-intermittent treatment and intermittent treatment. The RBC supplementation to paddy soils has decreased the volume of As-contaminated water required for irrigating rice plants. Consequently, the lower As levels in paddy soil-water systems cause decreased As concentrations in rice tissues. Therefore, an integrated approach of RBC amendment and intermittent irrigation practices would be a promising method to ensure water usage efficiency and to reduce the volume of As-contaminated water required for irrigating rice in As endemic areas worldwide.

3.5. Arsenic speciation in rice tissues

The supplementation of RBC to paddy soils has decreased the accumulation of As (sum of As(III), DMA(V), MMA(V), and As(V)) in rice tissues (i.e. roots, shoots, husks, and unpolished grains) (Fig. 2). In comparison, RBC-intermittent treatment achieved the least As content in rice tissues, followed by RBC-flooded, intermittent, and flooded treatments (Fig. 2). Total As concentration (sum of As(III), DMA(V), MMA (V), and As(V)) in rice roots decreased by approximately 84% in RBC-intermittent treatment when compared with conventional flooded treatment. This figure was 43% and 70%, respectively, in RBC-flooded and intermittent treatments. RBC-intermittent treatment reduced total As content in rice shoots by approximately 73% than that of the flooded treatment, whereas RBC-flooded and intermittent treatments have reduced total As content in rice shoots by 21% and 43%, respectively, compared to the flooded treatment. In rice husks, total As concentration was lowered by 89% in both RBC-intermittent and intermittent treatments in comparison to flooded treatment while this figure was 69% in the RBC-flooded treatment. Total As concentration in unpolished rice grains decreased by 81%, 45%, and 76% in RBC-intermittent, RBC-flooded, and intermittent treatments, respectively, in comparison to conventional flooded treatment. Total As concentrations in rice roots and shoots were significantly different among all different treatments (Fig. 2). In rice husks and grains, total As concentrations were also significantly different among different treatments except between RBC-intermittent and intermittent treatments.

Most importantly, RBC-intermittent treatment decreased the level of most toxic inorganic As species (i.e. As(III) and As(V)) in rice roots, shoots, husks, and unpolished grains (Fig. 2). The respective figures for the decrement of inorganic As levels in RBC-intermittent, RBC-flooded, and intermittent treatments compared to flooded treatment were as follows: roots (83%, 42%, and 67%), shoots (74%, 22%, and 41%), husks (74%, 27%, and 67%), and unpolished rice grains (46%, 10%, and 36%). In all the treatments, the percentage of inorganic As species in rice roots and shoots was higher (~90%) than the percentage of organic As species (i.e. MMA(V) and DMA(V)) in rice roots and shoots (~10%). However, in rice husks and unpolished grains, the percentages of organic As species increased to 30–75% of total As species.

Different metabolic activities in rice plants could involve varied As levels in rice tissues under different treatments (Fig. 3). The supplementation of RBC to paddy soils has increased the Si concentration in paddy water, as shown in Fig. 1(b). Both $Si(OH)_4$ and As(III) acquire nodulin 26-like intrinsic proteins (NIPs) such as OsNIP2;1 (Lsi1) by rice roots. The increased Si/As(III) ratio, due to the RBC supplementation in the paddy soil-water system could decrease the uptake of As(III) by rice roots. Therefore, RBC-intermittent and -flooded treatments have lower As concentrations in rice roots compared to those under the conventional flooded treatment. The concentration of inorganic As species in rice shoots, husks, and unpolished grains are lower compared to the concentration of inorganic As species in rice roots in this study. Following the uptake, both inorganic and organic As species undergo different metabolic activities. The reduction of As(V) to As(III) by As reductase enzymes such as OsHAC1;1, OsHAC1;2, and OsHAC4 occurs in rice roots (Shi et al., 2016). The efflux of As(III) back into the external environment by As(III) efflux transporters and the complexation of As (III) with thiol-rich peptides such as phytochelatins and glutathione may reduce the translocation of inorganic As species from rice roots to shoots (Tripathi et al., 2013). In addition, as shown in Fig. 1(d), relatively high concentrations of SO_4^{2-} in RBC-intermittent and -flooded treatments may enhance the formation of thiol-rich peptides which have a high

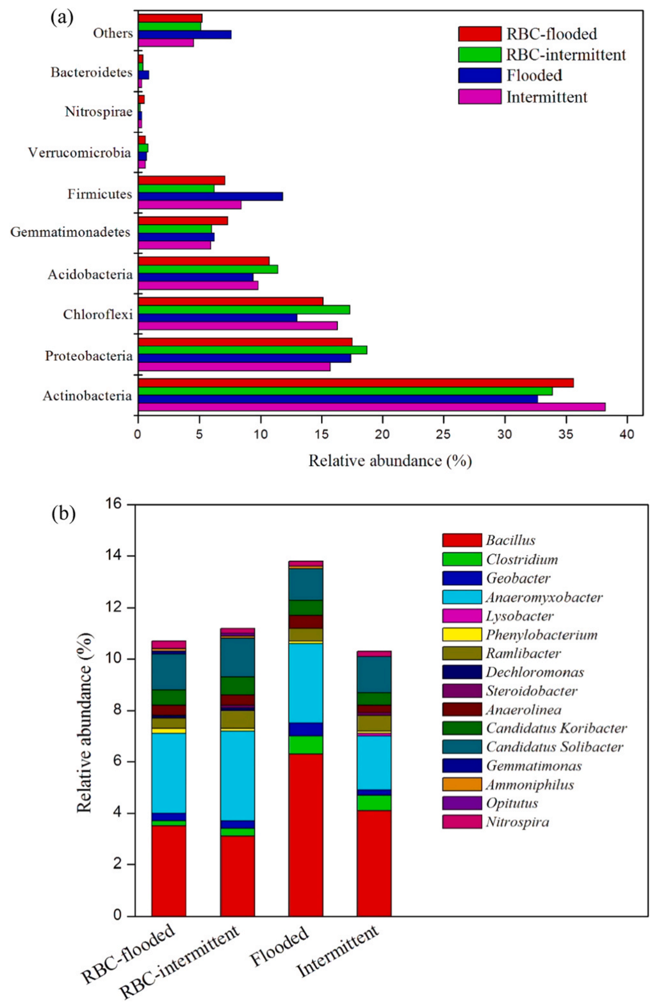

Fig. 4. Relative abundance of phylogenetic groups (a) and the dominated genera at genus level in the rhizosphere (b) under different combination of RBC and water management approaches.

affinity to As(III) in rice roots. Zhang et al. (2011) have also found that the formation of phytochelatins and glutathione increases due to the S in paddy soils. Therefore, the concentration of inorganic As species in rice shoots, husks, and unpolished grains are lower compared to the concentration of inorganic As species in rice roots. In this study, percentages of organic As species in rice husks and unpolished grains are higher than the percentages of organic As species in rice roots and shoots. The translocation efficiency of organic As species in rice plants has been found to be higher than the translocation efficiency of inorganic As species (Raab et al., 2007).

Different mechanisms in RBC lead to varied As accumulation in rice tissues (Fig. 3). The well-developed pore structure in RBC as shown in Fig. S1 may facilitate the diffusion of As species through the process of physical adsorption. Oxygenated functional groups in BC have also been found to minimize the availability of As in aqueous medium through the surface complexation process (Beiyuan et al., 2017). Moreover, supplementation of RBC might promote the abundance of microbial community which involves the oxidation, methylation and volatilization of As in the paddy soil-water system (discussed in detail in Section 3.6). Due to different metabolic activities in rice plants and different mechanisms in RBC, As concentrations in rice tissues in RBC-intermittent and -flooded treatments were lower than the As concentrations in rice tissues in conventional flooded treatment. Rice tissues in intermittent treatment have been shown to have a lower As content compared to rice tissues in RBC-flooded treatment. Comparatively less usage of As-contaminated water for irrigating rice would be the reason for less As content in rice tissues under intermittent treatment over RBC-flooded treatment.

3.6. Microbial diversity

Microorganisms in paddy soil-water systems are important factors in mobilization/immobilization and speciation of As. In this study, 20 different phyla, 66 classes, 116 orders, 180 families, and 228 genera have been identified. The phyla Actinobacteria dominated in the rhizosphere soil, comprising 35.6%, 33.9%, 32.7%, and 38.2% in RBC-flooded, RBC-intermittent, flooded, and intermittent treatments. Proteobacteria (15.7%−18.7%), Chloroflexi (13.0%−17.3%), Acidobacteria (9.4%−11.4%), Gemmatimonadetes (5.9%−7.3%), and Firmicutes (6.2%−11.8%) were other dominant phyla in this study (Fig. 4(a)), suggesting that members of those phyla may actively behave in the rhizosphere. Both Proteobacteria and Firmicutes have been found to comprise diverse genera which involve As-cycling in the paddy agroecosystem (Das et al., 2016).

The relative abundance of different genera in rhizosphere soils were influenced by different treatments, as shown in Fig. 4(b). The abundance of Fe(III) reducing bacteria such as *Bacillus*, *Clostridium*, and *Geobactor* was higher in the conventional flooded treatment than in other treatments. The abundance of *Bacillus* was lowered by 44%, 51%, and 35% in RBC-flooded, RBC-intermittent, and intermittent treatments when compared to conventional flooded treatment. The abundance of *Clostridium* decreased by 71%, 57%, and 14% in RBC-flooded, RBC-intermittent, and intermittent treatments in comparison to flooded treatment. In the case of *Geobactor*, this figure was 40% each for RBC-flooded and -intermittent treatments, and 60% for intermittent treatment. The genera *Anaeromyxobacter* was also found to reduce Fe(III) to Fe(II); however, the abundance of the *Anaeromyxobacter* has not shown a significant pattern in this study. The Fe(III)-(hydro)oxides in the paddy soil-water systems are important factors in governing the mobility of As. Both As(III) and As(V) can be complexed with Fe(III)-(hydro)oxides in the paddy soil-water system; therefore, the mobility and bioavailability of both As(III) and As(V) can be minimized (Kumarathilaka et al., 2018a). The reduction of Fe(III) to Fe(II) by *Bacillus*, *Clostridium*, and *Geobactor* in the conventional flooded treatment may lead to mobilization of more As(III) and As(V) into the soil solution and consequently to a higher As content in rice tissues than other treatments. In contrast, the genera *Lysobacter* was detected in the intermittent treatment. The *Lysobacter* was found to oxidize Fe(II) to Fe(III), therefore, minimizing the bioavailability of As(III) and As(V) for plant uptake. That may be one of the reasons for less As content in rice tissues in intermittent treatment than in the conventional flooded treatment. Overall, RBC amendment into paddy soils and intermittent irrigation practices have decreased the abundance of Fe(III) reducing bacteria and subsequently, lower As content in rice tissues is found compared to conventional flooded treatment. The supplementation of RBC and changes in water management can form new habitats for microbes by adsorbing nutrients and organic substrates on RBC surface and changing the redox chemistry of the rice rhizosphere, leading to a shift in the abundance of microorganisms and community composition. Therefore, long-term effect of the RBC and water management approaches on As-contaminated paddy soil-water systems should be further systematically examined.

3.7. Essential elements in rice grains

The nutritional value of rice grains is of particular importance to sustain rice quality for human consumption (Mwale et al., 2018). There are no reports on the concentration of essential elements in unpolished rice grains under different mitigation techniques used for reducing As content in rice grains. This study revealed that the supplementation of RBC into paddy soils has increased the elemental concentrations of Fe, Mn, Zn, Mg, and Ca in unpolished rice grains (Fig. 5). The supplementation of RBC to flooded and intermittent treatments has increased

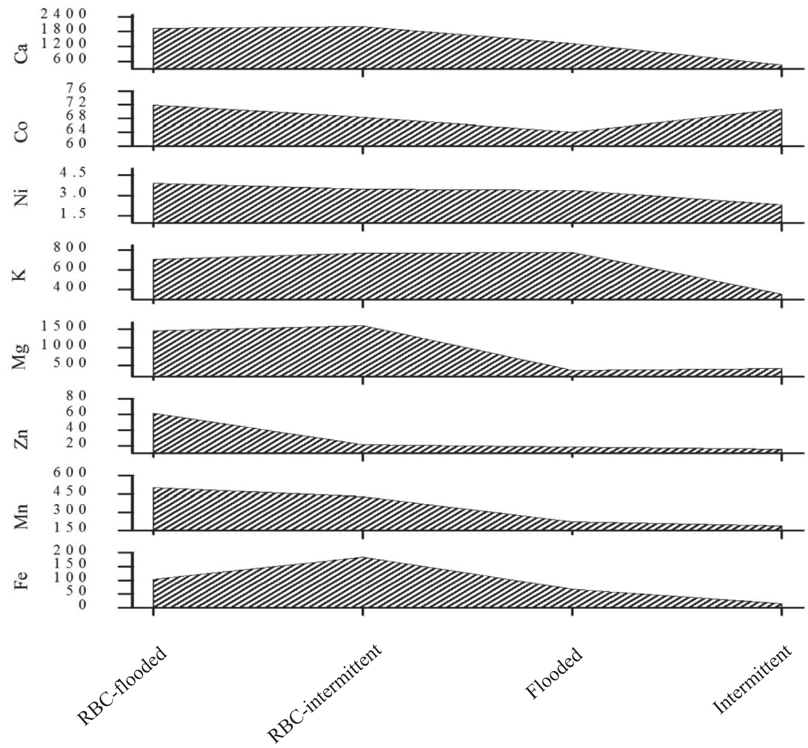

Fig. 5. The variation of essential elements in mg kg^{-1} in unpolished rice grains under different combinations of RBC and water management techniques.

elemental concentrations in unpolished rice grains in comparison to conventional flooded treatment as follows: (1) Fe: 57% and 178%, (2) Mn: 124% and 92%, (3) Zn: 239% and 15%, (4) Mg: 291% and 329%, and (5) Ca: 45% and 52%. However, there was no significant pattern for K, Ni, and Co in unpolished rice grains with the supplementation of RBC to paddy soils. Since RBC steadily releases essential elements into paddy soil solution, unpolished rice grains in RBC-flooded and -intermittent treatments have accumulated higher essential elements than in unpolished rice grains under flooded and intermittent treatments. Therefore, the addition of RBC to paddy soils leads to the enhancement of essential elemental concentrations in unpolished rice grains. Interestingly, the majority of the elemental concentration (i.e. Fe, Mn, Zn, K, Ni, and Ca) in unpolished rice grains was lowered in intermittent treatment compared to the unpolished rice grains in the flooded treatment. Wetting and drying cycles in the intermittent treatment may have lowered the bioavailability of essential elements in the rhizosphere zone compared to the flooded treatment. Even though intermittent treatment has decreased the As content in unpolished rice grains compared to that of RBC-flooded and flooded treatments, lower levels of essential elements in unpolished rice grains are required to be concerned. Overall, incorporation of RBC to paddy soils could sustain the level of essential elements in rice grains and could lead to minimizing food-related malnutrition in people worldwide.

4. Conclusions

The integrated approach of RBC amendment and intermittent water management practices has reduced inorganic As content in rice tissues and improved essential elemental content in unpolished rice grains, ensuring global food safety. Even though the integrated approach of RBC amendment, together with flooded water management practices, has increased rice yield and essential elemental content in unpolished rice grains, the inorganic As content in unpolished rice grains is higher compared to RBC-intermittent and intermittent treatments. The RBC in paddy soil-water system contributes to a reduced As accumulation and to an increased essential elemental content in unpolished rice grains through releasing Si and SO_4^{2-}, and essential nutrients, respectively, into the paddy water. In addition, RBC could adsorb As species through chemi- and physisorption processes to decrease the bioavailable As for plant uptake. The RBC in paddy soil has also reduced the abundance of Fe(III) reducing bacteria, therefore, the mobility and bioavailability of As(III) and As(V) in paddy soil-water systems has decreased. Further studies are required at glasshouse and field scales to optimize the effect of an integrated approach of BC amendment and alternative water management practices under different BC amendment rates, BC types (i.e. pristine BC and modified-BC), and range of As concentrations in irrigation water/paddy soils on decreased inorganic As content and to sustain the essential elemental levels in rice grains for safer human consumption.

CRediT authorship contribution 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. **Yong Sik Ok**: Resources, Supervision.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2020.124188.

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