Environ Sci Pollut Res (2018) 25:91–103
DOI 10.1007/s11356-016-8081-3

RESEARCH IN SOIL POLLUTION AND REMEDIATION IN CHINA

Enhanced iron(III) reduction following amendment of paddy soils with biochar and glucose modified biochar

Rong Jia[1,2] · **Lina Li**[1,2] · **Dong Qu**[1,2] · **Nana Mi**[2]

Received: 13 July 2016 / Accepted: 8 November 2016 / Published online: 17 November 2016

Abstract Although biochar application to paddy fields has been widely studied, its effects on Fe(III) reduction have not yet been investigated. Paddy soil slurry and soil microbial inoculation incubation were conducted with unmodified biochar (UMB) or glucose-modified biochar (GMB) additions at different particle sizes. The Fe(II) concentration and pH value were determined regularly, and Fe(III) reducing capacity (FeRC) was evaluated by modeling. Fe(III) reduction potential (a) was increased by 0–1.96 mg g^{-1} in response to UMBs addition, and a more remarkable increase in a was related to the decrease of particle size. The dissolved organic carbon of UMBs was responsible for the majority of the biochar reducing capacity. UMBs addition increased the contribution of free Fe and nitrate nitrogen to FeRC, while it decreased that of available phosphorus. Moreover, GMBs led to greater promotion of FeRC than the corresponding UMBs, with an increase in a of 2.9–16% in soil slurry and reduction rate of 13–35% in microbial inoculation incubation. The maximum Fe(III) reduction rate (V_{max}) with GMBs addition was faster or invariable than UMBs, while the time to V_{max} (T_{Vmax}) was shorter or stable. The effect of GMBs on Fe(III) reduction was less sensitive as GMB particle size increased. Compared with UMBs addition, pH declined remarkably in response to GMBs. These findings suggest that GMBs can effectively stimulate Fe(III) reduction in paddy fields, while simultaneously alleviating the pH increase usually caused by pristine biochar application.

Keywords Iron(III) reduction · Biochar · Glucose · Adsorption · Dissolved organic matter · Paddy soil

Responsible editor: Roberto Terzano

Electronic supplementary material The online version of this article (doi:10.1007/s11356-016-8081-3) contains supplementary material, which is available to authorized users.

✉ Dong Qu
dongqu@nwafu.edu.cn

Rong Jia
jiarong@nwafu.edu.cn

Lina Li
lizhou51@126.com

Nana Mi
minana@nwafu.edu.cn

[1] State Key Laboratory of Soil Erosion and Dryland Farming on Loess Plateau, Northwest A&F University, Yangling, Shaanxi Province, China

[2] College of Natural Resources and Environment, Northwest A&F University, No. 3 Taicheng Road, Yangling, Shaanxi Province 712100, China

Abbreviations

Fe(III)	Iron(III)
Ox-Fe	Amorphous Fe (oxalate-extractable Fe)
DCB-Fe	Free iron (sodium hydrosulfite-sodium citrate-sodium bicarbonate-extractable Fe)
DOC	Dissolved organic carbon
UMBs	Unmodified biochars
GMBs	Glucose-modified biochars
FeRC	Fe(III) reducing capacity
a	Fe(III) reduction potential
V_{max}	Maximum Fe(III) reduction rate
T_{Vmax}	Time to maximum Fe(III) reduction rate
CCA	Canonical correspondence analysis

Introduction

Paddy fields with repeated redox alternations could provide favorable conditions for redox reactions of nutrients and active metals with variable valency (Kögel-Knabner et al. 2010). Iron [Fe(III)] reduction is a ubiquitous and important geobiochemical process under anoxic conditions such as in submerged paddy fields (Hori et al. 2015; Lovley 2006). Owing to the high relative abundance of Fe(III) as a terminal electron acceptor and its significance in electron transfer (Feng et al. 2013), Fe(III) cycling is considered as important as carbon, nitrogen, phosphorus, and sulfate cycling (Johnston et al. 2014; Kögel-Knabner et al. 2010; Li et al. 2012). Fe(III) reduction is a preferential electron sink for the oxidation of organic pollutants such as pentachlorophenol (Chen et al. 2012; Liu et al. 2013) and dichlorodiphenyltrichloroethane (Li et al. 2010) in submerged paddy soils. Moreover, the toxicity and mobilization of heavy metals are found to be closely related to Fe(III) reduction (Myers et al. 2004; Wang et al. 2009; Whittleston et al. 2013). Furthermore, Fe(III) reduction as a competitor to electrons could suppress the emissions of methane during methanogenesis (Jaeckel and Schnell 2000). This process also exerts strong influences on the bioavailability of nutritive elements (Kumar et al. 2014; Li et al. 2016).

In the last 10 years, biochar has been widely produced because it provides a means of sequestering carbon to offset carbon emissions relative to the burning of plant- and animal-based biomass (Atkinson et al. 2010; Glaser et al. 2002). A growing number of studies have investigated the application of biochar to soil with emphasis on its effects on soil properties, soil fertility, and crop yield (Anderson et al. 2011; Herath et al. 2013; Zhang et al. 2012). Biochar has also been shown to have positive effects on climate change mitigation, (in)organic pollutant sorption and degradation, and microbial activity (Beesley et al. 2011; Kammann et al. 2012; Lehmann et al. 2011; Ren et al. 2016). Moreover, many modified biochar products have been shown to provide benefits, including enhanced soil fertility and removal of soil contaminants. Khan proposed a nutrient-impregnated charcoal by taking biochar as a controlled-release fertilizer carrier and N, P, and K fertilizer as a nutrient source (Khan et al. 2007). Modified biochar prepared with $KMnO_4$, NaOH, and Fe-Mn oxides before pyrolysis or with H_2O_2 and $AlCl_3$ before application was responsible for higher sorption of heavy metal ions (Pb^{2+}, Cu^{2+}, AsO_4^{3-}) than the pristine biochars (Ding et al. 2016; Payne and Abdel-Fattah 2005; Qian et al. 2013; Wang et al. 2015; Wang et al. 2014; Zuo et al. 2016).

However, few studies have investigated the effects of biochar application on Fe(III) reduction in submerged paddy soils. Nevertheless, biochar amendment-induced changes in soil pH, organic matter content, microbial community, and nutritive elemental bioavailability were all indirectly related to Fe(III) reduction (Cui et al. 2011; Koide et al. 2011; Lehmann et al. 2011). More importantly, the redox activity revealed by the aromatic carbon and quinone structures indicates that the biochar could accept and donate electrons, resulting in an electron shuttling function (Graber et al. 2014; Kappler et al. 2014). Thus, biochar amendment into paddy fields may have great influences on Fe(III) reduction.

Organic carbon as an electron donor was found to be one of the dominant factors influencing Fe(III) reduction (Lentini et al. 2012; Peng et al. 2015). Previous studies suggested that microbial dehydrogenation and hydrogen production coupled to organic matter fermentation were of significance to microbial Fe(III) reduction for providing preferential substrates (Jia et al. 2015). Yi et al. (2012) indicated that glucose was the most dominant electron donor for Fe(III)-reducing microorganisms in paddy soils, followed by pyruvate, lactate, and acetate. Microbial community structure analysis showed that the abundance and activity of fermentative Fe(III) reducers like *Clostridium*, *Pseudomonas*, and *Bacillus* are much higher than the obligate ones like *Anaeromyxobacter*, *Geobacter*, and *Shewanella* in flooded paddy fields, especially during the stage of rapid Fe(III) reduction (Bongoua-Devisme et al. 2013; Lentini et al. 2012; Li et al. 2011; Zhu et al. 2011). Moreover, being decomposed into lactate and acetate during anaerobic fermentation, glucose could provide substrates for both fermentative and obligate Fe(III)-reducing bacteria (Feng et al. 2013; Yi et al. 2012). Hence, because of the ability of biochar to sorb low molecular weight dissolved organic carbon thus increasing the input of electron donors, biochar modified with glucose is expected to exert profound effects on Fe(III) reduction in paddy soil.

Therefore, the present study was conducted to (i) investigate the response of Fe(III) reduction to biochar addition and elucidate the potential mechanism and (ii) evaluate the ability of glucose-modified biochar to accelerate Fe(III) reduction in paddy soils. To accomplish this, anaerobic soil slurries incubation and soil microbial inoculation incubation were conducted after amendment with pristine biochar or glucose-modified biochar of different particle sizes. The results of this study demonstrate the response of important biochemical processes to biochar application and suggest an effective method for productive and strategic biochar application to rice paddy fields.

Materials and methods

Soil sampling and characterization

Paddy soils were sampled from eight drained post-harvest paddy fields representative of China's major rice production provinces. The sites were situated in (1) Nanchang County, Jiangxi Province (NC; 28° 33′ N, 115° 56′ E); (2) Fenghua County, Zhejiang Province (FH; 29°45′ N, 121° 26′ E); (3) Hanzhong, Shanxi Province (HZ; 33° 09′ N, 107° 25′ E); (4)

Yongji County, Jilin Province (YJ; 43° 44′ N, 125° 54′ E); (5) Guiyang, Guizhou Province (GY; 26° 22′ N, 106° 42′ E); (6) Qionglai, Sichuan Province (QL; 30° 25′ N, 103° 44′ E); (7) Zhongwei, Ninxia Hui Autonomous Region (ZW; 37° 29′ N, 105° 08′ E); and (8) Songyuan, Jilin Province (SY; 44° 34′ N, 124° 05′ E). In each paddy field, the top 20 cm soil was sampled at five sites along a S-shaped pattern and mixed to give a composite sample. The samples were then air-dried, after which the residual plant parts were removed and samples were passed through a sieve with a mesh size of 1 mm. The sieved samples were then kept in polyvinylchloride bottles in darkness at room temperature until use (Peter Mayer and Conrad 1990). The major nutrient elements and iron oxides in the tested paddy soils were determined by standard methods (Page 1982) and are given in Table 1.

Biochar and biochar modification

Biochar was derived from abandoned branch of apple tree and pyrolyzed at 500 °C for 6–8 h in the absence of air. The biochar consisted of 72% carbon, 24% oxygen, 2.6% hydrogen, and 1.2% nitrogen. The surface area of biochar was 87 m^2 g^{-1}, the pH was 10.43, and the ash content was 14%. To obtain different particle sizes, the biochar was ground to pass through 5.0-, 3.0-, 2.0-, 1.0-, 0.5-, and 0.25-mm sieves. The unmodified biochar was divided into six fractions according to particle size: B1 (0–0.25 mm), B2 (0.25–0.5 mm), B3 (0.5–1.0 mm), B4 (1.0–2.0 mm), B5 (2.0–3.0 mm), and B6 (3.0–5.0 mm); they were collectively named UMBs. The water-dissolved organic carbon (DOC) content, acid-dissolved total iron, and Fe(II) content of the different particle size fractions of biochar are given in Fig. 1.

To modify the biochar with glucose, 5 g of B3, B4, and B5 fractions were added into the same volume (25 mL) of 9 g L^{-1} glucose solution (then named GMB3, GMB4, and GMB5, respectively), and each particle size fraction of biochar was shaken for 6, 12, and 48 h to obtain different levels of biochar modification with glucose. Next, the solids were separated from solution by quantitative filtration and dried for 12 h at 60 °C. Biochars modified with glucose were collectively named GMBs. The adsorption rate of glucose to GMBs under different modification times was determined by the potassium permanganate oxidation method and listed in Table 2 (Blair et al. 1995). Briefly, 0.02 g UMBs or GMBs was added to 20 mL distilled water and 5 mL concentrated sulfuric acid (H_2SO_4) and then bathed for 30 min in boiling water to dissolve the glucose adsorbed on the biochar. After filtration through a 0.45-μm pore size membrane, 5 mL filtered solution was transferred to a 100-mL Erlenmeyer flask with 10 mL of 0.01 mol L^{-1} potassium permanganate ($KMnO_4$) and 5 mL H_2SO_4. The oxidation was conducted in a boiling water bath for 30 min, after which 10 mL of 0.01 mol L^{-1} sodium oxalate ($Na_2C_2O_4$) was added to the hot Erlenmeyer flask, and the excess of $Na_2C_2O_4$ was titrated with 0.01 mol L^{-1} $KMnO_4$. The adsorption rate of glucose to GMBs was calculated as

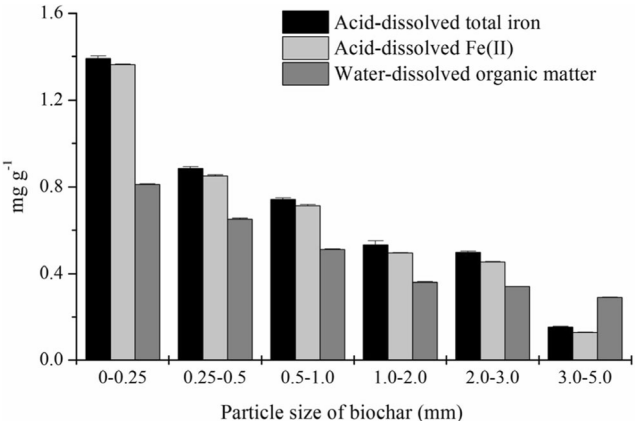

Fig. 1 Content of water-dissolved organic matter, acid-dissolved total iron, and Fe(II) of the different particle size fractions of biochar

Table 1 Soil basic properties of the texted paddy soils (means ± SD, $n = 3$)

Soil	pH	Ox-Fe (mg g^{-1})	DCB-Fe (mg g^{-1})	Organic matter (mg g^{-1})	Available phosphorus (mg kg^{-1})	Available potassium (mg kg^{-1})	Nitrate nitrogen (mg kg^{-1})	Ammoniacal nitrogen (mg kg^{-1})
NC	5.17 ± 0.02	3.62 ± 0.04	13.9 ± 0.2	48 ± 5.	5.7 ± 0.2	114 ± 3	5.26 ± 0.04	37.7 ± 0.5
FH	5.42 ± 0.02	6.15 ± 0.08	10.5 ± 0.2	65 ± 2	11 ± 1	180 ± 4	18.7 ± 0.2	29.7 ± 0.5
HZ	5.82 ± 0.02	6.0 ± 0.2	12.4 ± 0.2	44 ± 2	2.9 ± 0.3	135 ± 4	12.5 ± 0.3	26.1 ± 0.2
YJ	6.21 ± 0.01	4.1 ± 0.1	9.9 ± 0.3	38 ± 3	6.8 ± 0.4	149 ± 3	0.8 ± 0.8	13.37 ± 0.08
GY	7.45 ± 0.01	5.7 ± 0.3	14. 4 ± 0.2	95 ± 7	14.9 ± 0.8	78 ± 5	16.1 ± 0.5	10.0 ± 0.2
QL	7.83 ± 0.02	2.97 ± 0.09	7.8 ± 0.1	35 ± 1	13.0 ± 0.9	70 ± 2	34.8 ± 0.1	12.0 ± 0.4
ZW	8.24 ± 0.02	1.9 ± 0.2	9.7 ± 0.1	22 ± 1	17 ± 3	74 ± 5	7.8 ± 0.4	6.095 ± 0.007
SY	10.15 ± 0.01	0.56 ± 0.01	2.3 ± 0.2	17 ± 1	8.24 ± 0.02	352 ± 5	8.9 ± 0.2	0.68 ± 0.06

NC Nanchang county, Jiangxi Province; *FH* Fenghua county, Zhejiang Province; *HZ* Hanzhong, Shanxi Province; *YJ* Yongji county, Jilin Province; *GY* Guiyang, Guizhou Province; *QL* Qionglai, Sichuan Province; *ZW* Zhongwei, Ninxia Hui Autonomous Region; *SY* Songyuan, Jilin Province; *Ox-Fe* oxalate-extractable Fe (amorphous Fe); *DCB-Fe* sodium hydrosulfite-sodium citrate-sodium bicarbonate-extractable Fe (free Fe)

Table 2 Adsorption rate of glucose to modified biochars (GMBs) after different modification times

Modification time	GMB3 (%)	GMB4 (%)	GMB5 (%)
6 h	6.3 ± 0.4c	3.3 ± 0.7b	3.3 ± 0.3b
12 h	12.5 ± 0.3b	11.9 ± 0.1a	8.7 ± 0.2a
48 h	16 ± 1a	12.2 ± 0.1a	9.0 ± 0.2a

Different letters indicate significant differences among modified biochars with the same particle size but different modification times at $p < 0.05$

GMB3, *GMB4*, *and GMB5*: biochars particle size fractions B3 (0.5–1.0 mm), B4 (1.0–2.0 mm), and B5 (2.0–3.0 mm) modified with glucose, respectively; *6 h*, *12 h*, *and 48 h*: modification times of glucose to biochar for 6, 12, and 48 h, respectively

follows: Adsorption rate = $(G_{GMBs} - G_{UMBs}) / G_{add}$, where G_{GMBs} or G_{UMBs} was equivalent to glucose in GMBs or UMBs according to the titrated volume of $KMnO_4$; G_{add} was the added glucose when modifying biochar.

Preparation of soil microbial inoculation

Ten grams of soils from GY and SY was flooded with 10 mL sterile distilled water in two 25-mL sterile serum bottles to give a 1:1 soil to liquid ratio. After being covered with rubber stoppers, purged with nitrogen gas for 10 min, and sealed with aluminum covers, the serum bottles were incubated in a controlled-environment incubator in darkness at 30 °C for 1 week to recover the microbial community. The soil slurries were then quantitatively transferred to sterile centrifuge tubes with 80 mL sterile distilled water. Next, microbial community extraction was performed at 30 °C for 1 h in a shaking incubator (FUMA QYC 211, Shanghai, China) at 7×*g*, after which the tube was centrifuged (Zongkia KDC-40 Low Speed Centrifuge, Anhui, China) at 164×*g* for 25 min. The supernatant fluid was collected as soil microbial inoculant.

Experiment design

Experiment I To investigate the response of Fe(III) reduction to biochar addition in different paddy soils, soils from eight drained post-harvest paddy fields were amended with biochar at different particle sizes. This was achieved by adding 0.06 g UMBs with particle sizes of 0–0.25, 0.25–0.5, 0.5–1.0, 1.0–2.0, 2.0–3.0, and 3.0–5.0 mm to a series of 10-mL serum bottles containing 3 g paddy soils (B1, B2, B3, B4, B5, and B6, respectively). Serum bottles were then incubated anaerobically by capping with rubber stoppers, flushing with filtered-sterilized nitrogen gas for 5 min, sealing with aluminum covers, and incubating in darkness at 30 °C for 40 days. A control (CK) without added biochar was also prepared for each soil.

Experiment II To provide abundant electron donors and further strengthen the benefits of biochar, GMBs (0.06 g) prepared in 2.2 (GMB3, GMB4, and GMB5) were mixed with 3.000 g soil (GY and SY) in a series of 10-mL serum bottles and then submerged with 3 mL sterilized distilled water at a ratio of 1:1 (*w*/*v*). The serum bottles were subsequently anaerobically incubated as described in the "Experiment design" section.

Experiment III Soil microbial inoculation essay with synthetic ferrihydrite FeOOH as sole electron acceptor was conducted to make Fe(III) reduction more intuitive, as well as to elucidate the potential mechanism of UMBs for accelerating Fe(III) reduction and to evaluate the ability of GMBs. To accomplish this, aliquots (1 mL) of the microbial inoculation and 0.06 g modified biochar (GMB3, GMB4, and GMB5) were incubated anaerobically in series of 10-mL sterile serum bottles with inorganic medium. The inorganic medium contained 1 mL of 5 g L^{-1} NH_4Cl solution as a nitrogen source, 1 mL of 0.025 mol L^{-1} phosphate buffer solution, 1 mL of 1.7 g L^{-1} synthetic ferrihydrite FeOOH, and 1 mL distilled and sterilized water. The corresponding UMBs (B3, B4, and B5) were also conducted to substitute for GMBs as a control group. The (un)modified biochars were considered the sole carbon source in the microbial inoculation essay.

Sample analysis

During anaerobic incubation, for each experiment, three serum bottles for each treatment were taken randomly on days 0, 1, 3, 5, 7, 10, 13, 16, 20, 25, 30, 35, and 40 and analyzed for Fe(II) concentration and pH. The Fe(II) concentration was quantified using ferrozine assay (He and Qu 2008; Lovley and Phillips 1986). Briefly, 0.4 mL of well-shaken subsample was transferred to a polyethylene plastic tube with 4.6 mL HCl solution and allowed to react at 30 °C for 24 h. Next, the tube was centrifuged (Zongkia KDC-40 Low Speed Centrifuge, Anhui, China) at 2009×*g* for 10 min, and 1 mL of supernatant was mixed with a 5 mL aliquot of 1 mol L^{-1} NaAC buffer and a 5 mL aliquot of 1 g L^{-1} phenanthroline to develop color. The concentration of Fe(II) was subsequently determined based on the absorbance at 510 nm. The pH was determined using a calibrated Delta320 pH meter with a compound glass electrode (Mettler-Toledo Instruments, Shanghai, China).

Statistical analyses

Data are expressed as the arithmetic means ± the standard deviation (three replicates). Analysis of variance (ANOVA) was conducted using SAS 8.0 and differences between treatments were identified by *t* tests. A $p < 0.05$ was considered to indicate significance. Changes in Fe(II) concentration with incubation time were simulated with the logistic model:

$C_t = a/(1 + be^{-ct})$ (He and Qu 2008). The Fe(III) reducing process was characterized using kinetic parameters, where a was the Fe(II) reduction potential, b referred to the model parameter, and c was the reaction rate constant. The maximum Fe(III) reduction rate (V_{max}) was calculated as 0.25 ac, and the time to V_{max} (T_{Vmax}) was ln$b$/c. The Fe reduction rate in the microbial inoculation experiment was calculated as the ratio of Fe(II) concentration at the end (the 40th day) of incubation to Fe(III) concentration at the beginning of anaerobic incubation. The contribution of soil properties to the Fe(III) reduction process with UMBs was summarized by canonical correspondence analysis (CCA) using Canoco 4.5 (http://www.canoco5.com) (Hoy et al. 2008). The independent variables included soil organic matter (OM), ammoniacal nitrogen (AN), nitrate nitrogen (NN), available phosphorus (AP), available potassium (AK), free iron (sodium hydrosulfite-sodium citrate-sodium bicarbonate-extractable Fe; DCB-Fe), amorphous iron (oxalate-extractable Fe; Ox-Fe), and soil pH (pH). The dependent variables contained the Fe(III) reducing capacity (FeRC) kinetic parameters a, V_{max}, and T_{Vmax}. The results of CCA were visualized in the form of a biplot using Canodraw 4.5 (http://www.canodraw.com).

Results

Variations in Fe(III) reducing capacity with UMBs addition in paddy soil slurries

Changes in Fe(II) concentration with incubation time were fitted with a logistic model to provide insight into the potential for Fe(III) reduction. As shown in Table 3, the Fe(III) reduction potential (a) varied from 1.27 to 10.4 mg g^{-1} in control soils (CK treatment) from different rice production provinces without biochar addition (Table 3). The highest a was found in soil collected from HZ with the highest maximum Fe(III) reducing rate (V_{max}), 1.9 mg (g day)$^{-1}$, while the lowest a was detected in soil from SY, which was coincident with the lowest V_{max} 0.22 mg (g day)$^{-1}$ (Table 3).

UMB addition at different particle sizes led to an increase in a of 0–1.96 mg g^{-1}, which was 0–20% higher than the control soils (Table 3). A more remarkable increase in a was detected as particle size decreased ($p < 0.01$). a in the B1 and B2 treatments was increased significantly by 4.5–20 and 4.6–19%, respectively, while the addition of B5 and B6 did not seem to cause a significant increase in a. The effect of particle size on V_{max} in NC, HZ, and YJ soils was different from that in FH, GY, QL, ZW, and SY soils. V_{max} declined or remained stable in the former soils, while it increased in the latter soils. T_{Vmax} was significantly shortened or remained stable with UMBs addition at different particle size (except for NC soil amended with B6 fraction of biochar and YJ soil amended with B4 fraction of biochar; Table 3). Generally speaking, the variation in Fe(III) reducing kinetics revealed that biochar addition promoted Fe(III) reduction in paddy soils.

Moreover, the addition of UMBs resulted in an input of 0.009–0.028 mg acid-dissolved total iron per gram of soil (Fig. 1), which was extremely recalcitrant and unavailable to microorganisms in paddy soils. When compared with the acid-dissolved Fe(II) content in paddy soils (1.27 to 10.4 mg g^{-1} soil; Table 3), the low iron input via biochar addition could be neglected in this study.

Variation in Fe(III) reducing capacity with GMBs addition in paddy soil slurries

When compared with CK, GMBs showed a significant promotion effect on Fe(III) reduction (Tables 3 and 4). At the end of the 40-day incubation, Fe(II) accumulation increased by 0.37–1.08 mg g^{-1} in GY paddy soil and 0.11–0.64 mg g^{-1} in SY paddy soil with the addition of GMBs, respectively (Online Resource 1). Furthermore, the extent of the promotion effect was more obvious as biochar particle size decreased. For GMB3, there was a remarkable increase in a of 6.7–10% in GY soil and 15–16% in SY soil (Table 4). For GMB4 and GMB5, a increased by 5.6–8.1% (GMB4) and 2.9–6.4% (GMB5) in GY soil and 15–16% (GMB4) and 14–15% (GMB5) in SY soil, respectively (Table 4). In addition, GMBs had a better effect than the corresponding UMBs, resulting in a significant difference in both soils. These findings indicated a successful effect of modified biochar addition on Fe(III) reduction. Nonetheless, the addition of GMBs had a negligible effect on V_{max} and T_{Vmax} in GY paddy soil. GMBs modified for 12 and 48 h had similar a, V_{max}, and T_{Vmax} values, which demonstrated a higher promotion effect than that for 6 h in GY soil. In SY soil, V_{max} in each GMB treatment varied with modification time, while modification time did not influence a and T_{Vmax} in SY soil (Table 4).

Variations in pH with the addition of UMBs and GMBs in paddy soil slurries

The addition of UMBs led to an increase in soil initial pH that was negatively related to biochar particle size and followed the order B1 > B2 > B3 > B4 > B5 ≈ B6 ≈ CK (Online Resource 2). Following soil flooding, a slow decrease in pH occurred during the first 7 days of incubation, after which it stabilized and there was no significant difference among UMBs treatments. These results suggested that the increase caused by UMBs addition gradually disappeared after anaerobic incubation for 40 days.

With the addition of GMBs, a rapid decrease of pH occurred during the first 7 days of soil slurry incubation (Fig. 2). The pH value then reached a level equal to that of the CK treatment in the GY soil, while it decreased from an initial value of 10.51 to 9.48 in the SY soil,

Table 3 Kinetic parameters of Fe(III) reduction in submerged paddy soils after the addition of unmodified biochar (UMBs) at different particle sizes

Soils	Particle size	Logistic model parameters			Soils	Particle size	Logistic model parameters		
		a/mg g^{-1}	V_{max}/mg (g day)$^{-1}$	T_{Vmax}/day			a/mg g^{-1}	V_{max}/mg (g day)$^{-1}$	T_{Vmax}/day
NC	CK	5.789 ± 0.06c	0.82 ± 0.02a	3.26 ± 0.07b	GY	CK	7.9 ± 0.1d	1.3 ± 0.1d	1.10 ± 0.09ab
	B1	6.712 ± 0.07a	0.62 ± 0.04bc	3.4 ± 0.2b		B1	8.74 ± 0.02a	1.6 ± 0.1a	0.9 ± 0.1b
	B2	6.61 ± 0.06a	0.59 ± 0.02c	3.4 ± 0.2b		B2	8.34 ± 0.06b	1.45 ± 0.01b	0.98 ± 0.04b
	B3	6.36 ± 0.09b	0.65 ± 0.08bc	3.5 ± 0.2ab		B3	8.11 ± 0.06c	1.41 ± 0.05bc	1.07 ± 0.09ab
	B4	5.9 ± 0.1c	0.70 ± 0.02b	3.5 ± 0.2ab		B4	7.96 ± 0.06cd	1.31 ± 0.03cd	1.15 ± 0.06a
	B5	5.9 ± 0.1c	0.67 ± 0.02bc	3.6 ± 0.1ab		B5	8.0 ± 0.2cd	1.28 ± 0.04d	1.16 ± 0.08a
	B6	5.9 ± 0.1c	0.63 ± 0.09bc	3.9 ± 0.4a		B6	7.89 ± 0.04d	1.268 ± 0.001d	1.2 ± 0.1a
FH	CK	9.33 ± 0.04c	1.75 ± 0.06e	2.85 ± 0.08a	QL	CK	5.9 ± 0.1d	0.82 ± 0.04b	3.1 ± 0.2a
	B1	10.16 ± 0.08a	2.5 ± 0.1a	2 ± 0d		B1	7.11 ± 0.08a	1.008 ± 0.002a	3.08 ± 0.08a
	B2	10.15 ± 0.05a	2.27 ± 0.06ab	2.53 ± 0.03cd		B2	7.06 ± 0.04a	0.90 ± 0.09ab	3.3 ± 0.1a
	B3	9.9 ± 0.2b	2.20 ± 0.07bc	2.5 ± 0.1cd		B3	6.522 ± 0.04b	0.87 ± 0.06ab	3.1 ± 0.2a
	B4	9.8 ± 0.1b	2.0 ± 0.2cd	2.6 ± 0.2bcd		B4	6.4 ± 0.1c	1.0 ± 0.2ab	2.8 ± 0.6a
	B5	9.37 ± 0.05c	1.9 ± 0.2de	2.7 ± 0.2abc		B5	6.0 ± 0.06d	0.95 ± 0.03ab	2.98 ± 0.06a
	B6	9.33 ± 0.06c	1.8 ± 0.1e	2.80 ± 0.09ab		B6	5.97 ± 0.04d	0.95 ± 0.09ab	2.8 ± 0.1a
HZ	CK	10.4 ± 0.5c	1.9 ± 0.3a	3.8 ± 0.3a	ZW	CK	5.9 ± 0.1c	0.339 ± 0.009b	4.3 ± 0.4a
	B1	12.3 ± 0.2a	1.7 ± 0.2a	3.9 ± 0.2a		B1	6.8 ± 0.2a	0.41 ± 0.03a	5.2 ± 0.6a
	B2	12.4 ± 0.2a	1.66 ± 0.06a	4.0 ± 0.2a		B2	6.8 ± 0.2a	0.43 ± 0.04a	5.0 ± 0.7a
	B3	12.2 ± 0.2a	1.65 ± 0.02a	4.1 ± 0.1a		B3	6.44 ± 0.1b	0.41 ± 0.02a	4.4 ± 0.6a
	B4	11.50 ± 0.06b	1.9 ± 0.1a	3.4 ± 0.3a		B4	6.2 ± 0.2b	0.39 ± 0.02a	4.7 ± 1.2a
	B5	11.2 ± 0.4b	1.6 ± 0.2a	4.0 ± 0.3a		B5	5.9 ± 0.08c	0.44 ± 0.02a	4.6 ± 0.4a
	B6	10.5 ± 0.2c	1.7 ± 0.4a	3.9 ± 0.4a		B6	5.1 ± 0.1c	0.44 ± 0.04a	4.9 ± 0.2a
YJ	CK	7.3 ± 0.1c	0.72 ± 0.05a	4.34 ± 0.06bcd	SY	CK	1.27 ± 0.02d	0.22 ± 0.02a	1.68 ± 0.06a
	B1	8.0 ± 0.1a	0.73 ± 0.1a	3.65 ± 0.04e		B1	1.46 ± 0.02a	0.36 ± 0.16a	1.0 ± 0.1c
	B2	7.95 ± 0.03a	0.70 ± 0.07a	4.0 ± 0.3de		B2	1.38 ± 0.02b	0.32 ± 0.01a	1.13 ± 0.02c
	B3	7.88 ± 0.09a	0.63 ± 0.06a	4.5 ± 0.3abc		B3	1.343 ± 0.006c	0.27 ± 0.02a	1.21 ± 0.02bc
	B4	7.56 ± 0.09b	0.67 ± 0.05a	4.8 ± 0.1a		B4	1.31 ± 0.02c	0.27 ± 0.06a	1.2 ± 0.2c
	B5	7.13 ± 0.06c	0.7 ± 0.1a	4.3 ± 0.2cd		B5	1.26 ± 0.02d	0.25 ± 0.03a	1.4 ± 0.1bc
	B6	7.16 ± 0.01c	0.62 ± 0.04a	4.7 ± 0.3ab		B6	1.26 ± 0.03d	0.21 ± 0.05a	1.59 ± 0.03ab

Different letters indicate significant differences among treatments with different particle sizes at $p < 0.05$. The coefficient of determination values that ranged from 0.930 to 0.997 indicated that it was appropriate to use logistic model to characterize Fe(III) reducing kinetics

B1, *B2*, *B3*, *B4*, *B5*, *and B6*: biochars with particle size of 0–0.25, 0.25–0.5, 0.5–1.0, 1.0–2.0, 2.0–3.0, and 3.0–5.0 mm, respectively

which lasted until the end of the incubation period (Fig. 2). This decrease of pH after GMBs treatments was higher than that in the CK and UMBs treatments in SY soil, illustrating that the addition of GMBs alleviated the increase of soil pH that occurred in response to the input of UMBs. Moreover, GMBs modified for 12 and 48 h produced a greater decrease in pH than those incubated for 6 h in SY soil (Fig. 2).

Variation in Fe(III) reducing capacity with GMBs during soil microbial inoculation incubation

Table 5 presents the logistic kinetic parameters of Fe(III) reduction with biochar as an electron donor and soil inoculation as a microbial source. With the addition of UMBs, Fe(II) accumulation was 160–261 mg L^{-1} in the GY treatments and 154–215 mg L^{-1} in the SY treatments after 40 days of incubation (Online Resource 3), coincident with the reduction rate at 50–76 and 46–65%, respectively (Table 5). These results revealed that UMBs could provide an electron for Fe(III) reduction. Furthermore, microbial inoculation from GY with UMBs resulted in a much higher Fe(III) reduction rate than that from SY, likely due to differences in the microbial community collected from GY and SY soil.

The addition of GMBs had a remarkable promotion effect on Fe(III) reduction. For a, the increase was 45–76 mg L^{-1} in GY and 81–113 mg L^{-1} in SY with GMBs, respectively (Table 5). The V_{max} with GMBs addition was 1.6–2.5 and 1–3.6 times faster in GY and SY, respectively,

Table 4 Kinetic parameters of Fe (III) reduction in paddy soils after the addition of biochar modified with glucose (GMBs) at different particle sizes

Soils	Treatments	Logistic model parameters			Soils	Treatments	Logistic model parameters		
		a/mg g^{-1}	V_{max}/mg (g day)$^{-1}$	T_{Vmax}/day			a/mg g^{-1}	V_{max}/mg (g day)$^{-1}$	T_{Vmax}/day
GY	B3	8.11 ± 0.06d	1.41 ± 0.05a	1.07 ± 0.09a	SY	B3	1.343 ± 0.006b	0.27 ± 0.02c	1.21 ± 0.02a
	GMB3 (6 h)	8.48 ± 0.06c	1.3 ± 0.2a	1.09 ± 0.08a		GMB3 (6 h)	1.47 ± 0.02a	0.31 ± 0.05bc	0.8 ± 0.1b
	GMB3 (12 h)	8. 60 ± 0.04b	1.2 ± 0.2a	1.0 ± 0.1a		GMB3 (12 h)	1.455 ± 0.004a	0.38 ± 0.06ab	0.8 ± 0.1b
	GMB3 (48 h)	8.75 ± 0.01a	1.25 ± 0.08a	1.2 ± 0.2a		GMB3 (48 h)	1.47 ± 0.01a	0.43 ± 0.06a	0.70 ± 0.03b
	B4	7.96 ± 0.06c	1.31 ± 0.03a	1.15 ± 0.06a		B4	1.31 ± 0.02b	0.27 ± 0.06ab	1.2 ± 0.2b
	GMB4 (6 h)	8.37 ± 0.08b	1.20 ± 0.08a	1.10 ± 0.09a		GMB4 (6 h)	1.46 ± 0.01a	0.31 ± 0.02a	1.40 ± 0.09ab
	GMB4 (12 h)	8.54 ± 0.02a	1.2 ± 0.5a	0.94 ± 0.05a		GMB4 (12 h)	1.47 ± 0.02a	0.278 ± 0.003ab	1.5 ± 0.2ab
	GMB4 (48 h)	8.6 ± 0.1a	1.09 ± 0.13a	1.0 ± 0.3a		GMB4 (48 h)	1.453 ± 0.006a	0.226 ± 0.002b	1.9 ± 0.4a
	B5	8.0 ± 0.2b	1.28 ± 0.04a	1.16 ± 0.08a		B5	1.26 ± 0.02b	0.25 ± 0.03a	1.4 ± 0.1a
	GMB5 (6 h)	8.16 ± 0.09b	1.26 ± 0.06a	1.1 ± 0.4a		GMB5 (6 h)	1.45 ± 0.02a	0.31 ± 0.03a	1.4 ± 0.1a
	GMB5 (12 h)	8.4 ± 0.2a	1.21 ± 0.06a	1.1 ± 0.1a		GMB5 (12 h)	1.46 ± 0.01a	0.34 ± 0.05a	1.5 ± 0.2a
	GMB5 (48 h)	8.43 ± 0.02a	1.2 ± 0.2a	1.1 ± 0.2a		GMB5 (48 h)	1.44 ± 0.03a	0.3 ± 0.1a	1.8 ± 0.4a

Different letters indicate significant differences among treatments with the same particle size but different modification times at $p < 0.05$. The coefficient of determination values ranged from 0.948 to 0.992, revealing that it was well fitted with the logistic equation to simulate the kinetics of variations in Fe(II) concentration

GMB3, *GMB4*, *and GMB5*: modified biochars with particle size of B3 (0.5–1.0 mm), B4 (1.0–2.0 mm), and B5 (2.0–3.0 mm), respectively; *6 h*, *12 h*, *and 48 h*: modification times of glucose to biochar for 6, 12, and 48 h, respectively

than with UMBs. T_{Vmax} was significantly reduced or remained stable in response to the addition of UMBs (Table 5). The response of Fe(III) reduction to GMBs addition was less sensitive as GMBs particle size increased, occurring in the order GMB3 (95–98%) > GMB4 (78–91%) > GMB5 (63–78%) (Table 5). There were no significant differences in a, V_{max}, and T_{Vmax} between 12-h GMB and 48-h GMB at the same particle size (Table 5), which was related to the similar absorption rate. From the viewpoint of microbial inoculation source, the increase in a and V_{max} between UMBs and GMBs addition was higher in the SY treatments than in the GY treatments, indicating that microbial inoculation from SY soil was more sensitive to glucose modification.

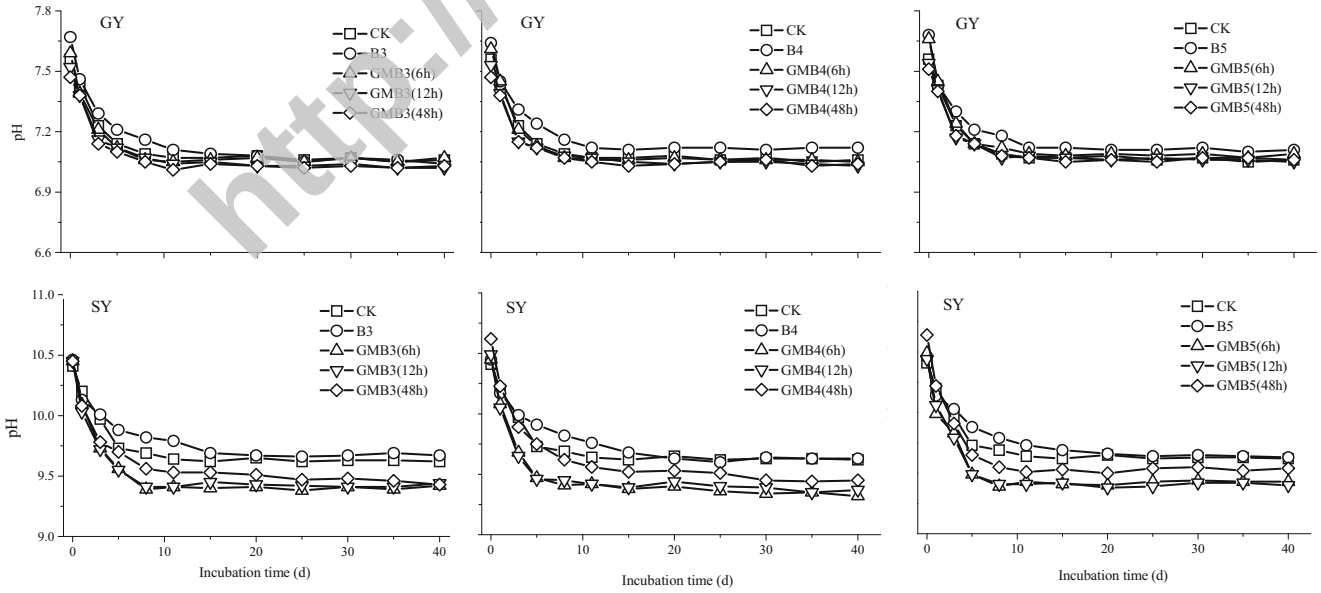

Fig. 2 Changes of pH during Fe(III) reduction process with different particle size fractions of biochar modified with glucose (*GMBs*) in paddy soils. Soil samples were collected from Guiyang municipality, Guizhou Province (*GY*) and Songyuan municipality, Jilin Province (*SY*). *GMB3*, *GMB4*, and *GMB5*: modified biochars with particle size *B3* (0.5–1.0 mm), *B4* (1.0–2.0 mm), and *B5* (2.0–3.0 mm). Six, 12, and 48 h are modification times of glucose to biochar for 6, 12, and 48 h, respectively

Table 5 Kinetic parameters of Fe(III) reduction in microbial inoculation incubations after addition of biochar modified with glucose (GMBs) at different particle sizes

Soils	Treatments	Logistic model parameters			Reduction rate/%	Soils	Treatments	Logistic model parameters			Reduction rate/%
		a/mg g^{-1}	V_{max}/mg (L day)$^{-1}$	T_{Vmax}/day				a/mg g^{-1}	V_{max}/mg (L day)$^{-1}$	T_{Vmax}/day	
GY	B3	258 ± 12b	17 ± 1bc	5.3 ± 0.4a	76 ± 3b	SY	B3	222 ± 7b	10.4 ± 0.4d	8.6 ± 0.5a	65 ± 2c
	GMB3 (6 h)	329 ± 78a	16 ± 2c	5.7 ± 0.5a	96 ± 2a		GMB3 (6 h)	323 ± 3a	19 ± 2c	8.7 ± 0.2a	94.7 ± 0.9b
	GMB3 (12 h)	332.5 ± 4a	23 ± 4ab	4.2 ± 0.2b	97 ± 1a		GMB3 (12 h)	332 ± 3a	22 ± 1b	7.1 ± 0.2b	97.1 ± 0.9ab
	GMB3 (48 h)	326 ± 8a	27 ± 6a	3.6 ± 0.5b	96 ± 2a		GMB3 (48 h)	335 ± 4a	25 ± 1a	7.1 ± 0.2b	98 ± 1a
	B4	222 ± 10c	8 ± 2c	8.2 ± 1.7a	65 ± 3c		B4	190 ± 5b	6.7 ± 0.3c	9 ± 1b	56 ± 1b
	GMB4 (6 h)	264 ± 10b	17 ± 2b	5.1 ± 0.2b	78 ± 3b		GMB4 (6 h)	295 ± 12a	11.4 ± 0.7b	11.3 ± 0.8a	86 ± 3a
	GMB4 (12 h)	295 ± 13a	23 ± 2a	5.2 ± 0.4b	86 ± 4a		GMB4 (12 h)	302 ± 10a	14.1 ± 0.5a	9.8 ± 0.6ab	88 ± 3a
	GMB4 (48 h)	298 ± 6a	21 ± 3a	5.4 ± 0.1b	87 ± 2a		GMB4 (48 h)	310 ± 7a	14 ± 1a	9.5 ± 0.8b	91 ± 2a
	B5	171 ± 5b	5.6 ± 0.3b	9.3 ± 0.6a	50 ± 1b		B5	156 ± 7c	5.7 ± 0.5c	10.11 ± 1.3a	46 ± 2c
	GMB5 (6 h)	221 ± 9a	20.4 ± 0.8a	4.5 ± 0.5b	65 ± 3a		GMB5 (6 h)	237 ± 7b	8.96 ± 0.09b	10.4 ± 0.4a	70 ± 2b
	GMB5 (12 h)	220 ± 3a	21 ± 1a	4.1 ± 0.3b	65 ± 1a		GMB5 (12 h)	267 ± 5a	11.2 ± 0.9a	9.5 ± 0.8a	78 ± 1a
	GMB5 (48 h)	214 ± 7a	20 ± 5a	3.7 ± 0.3b	63 ± 2a		GMB5 (48 h)	2599 ± 2a	10.6 ± 0.9a	9.2 ± 0.3a	75.8 ± 0.4a

Different letters indicate significant differences among treatments with the same particle size but different modification times at $p < 0.05$. The coefficient of determination values ranged from 0.951 to 0.996

GMB3, *GMB4*, *and GMB5*: modified biochars with particle size of B3 (0.5–1.0 mm), B4 (1.0–2.0 mm), and B5 (2.0–3.0 mm), respectively; *6 h*, *12 h*, *and 48 h*: modification times of glucose to biochar for 6, 12, and 48 h, respectively

Variation in pH with GMBs during soil microbial inoculation incubation

As shown in Fig. 3, with the addition of UMBs, a slow increase in pH occurred during the first few days of microbial inoculation incubation, which was followed by a stable level of 7.7 in the GY treatment and 8.0 in the SY treatment. However, after the addition of GMBs, pH markedly decreased at the beginning of the incubation period and reached its minimum on day 5 because of the fermentation of glucose adsorbed onto the GMBs. The pH then increased to a stable value until the end of the experiment (Fig. 3). On the other hand, because of differences in the fermentative ability of the microbial community between GY and SY paddy soil, the pH of the GY treatment decreased of 0.3 units, while that of the SY treatment decreased of 0.6 units.

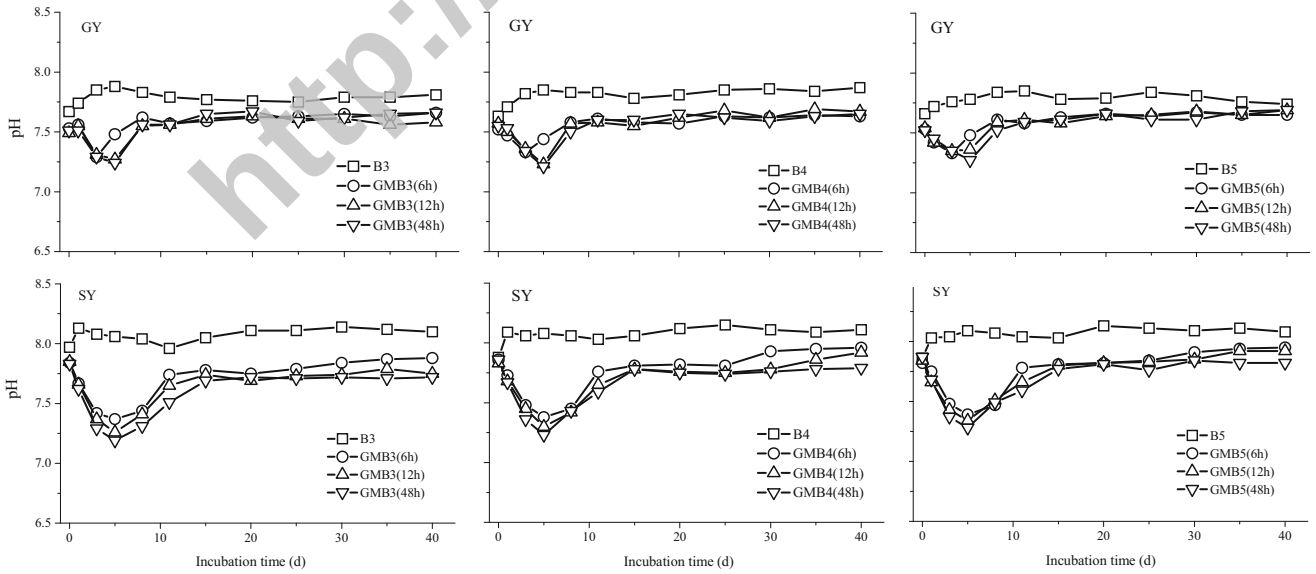

Fig. 3 Changes of pH during Fe(III) reduction process with different particle size fractions of biochar modified with glucose (*GMBs*) in microbial inoculation incubation. Soil microbial inoculations were extracted from soils which sampled from Guiyang municipality, Guizhou Province (*GY*) and Songyuan municipality, Jilin Province (*SY*). *GMB3*, *GMB4*, and *GMB5*: modified biochars with particle size *B3* (0.5–1.0 mm), *B4* (1.0–2.0 mm), and *B5* (2.0–3.0 mm). Six, 12 and 48 h are modification times of glucose to biochar for 6, 12, and 48 h, respectively

Discussion

Mechanism of stimulated Fe(III) reduction with UMBs addition

Biochar is abundant in highly aromatic compounds, among which quinones account for 15–21% (Heymann et al. 2011). Quinones have been reported to act as electron shuttles and are of significance to biochemistry cycles in soils and sediments (Chen et al. 2012; Klupfel et al. 2014; Myers and Myers 1993; Newman and Kolter 2000). Kappler et al. (2014) confirmed that 5 and 10 g L^{-1} biochar could accelerate the rate and extent of Fe(III) reduction by *Shewanella oneidensis MR-1* as quinones in biochar transferred more electrons to Fe(III). In the present study, 154–261 mg L^{-1} Fe(II) accumulated in the microbial inoculation assay when there was no electron donor other than the UMBs (equal to 14–23 μmol Fe(III); Table 5 and Online Resource 3). In addition, the electrons donated by DOC of UMBs were 7.2–20 μmol (based on 1 mol of carbon thoroughly oxidized to CO_2 being able to transfer 4 mol of electron; Fig. 1). Namely, the DOC of UMBs was considered as electron donor and shuttle and responsible for the majority of the reducing capacity and presumably 20% increase of Fe(II) accumulation in paddy soils (Fe(II) accumulation during the 40-day incubation period increased by 0–105 μmol with UMBs addition in paddy soils. These results are in agreement with those of Graber et al. (2014).

CCA revealed that organic matter (OM) and amorphous Fe content (Ox-Fe) (the longest arrows closely related to axis 1 in Fig. 4) were the dominant factors contributing to Fe(III) reduction in paddy soils. However, UMB addition enhanced the contribution of free Fe content (DCB-Fe) to FeRC to as an important parameter together with OM and Ox-Fe (Fig. 4). While arrow of AP was shortened dramatically following the addition of UMBs, this indicated a reduced contribution of soil original available phosphorus content to Fe(III) reduction (Fig. 4). It has been reported that rice straw-derived biochar application to paddy soil could increase the phosphorus availability by decreasing phosphate adsorbed on ferrihydrite (Cui et al. 2011). As a result, more Fe(III) was available to be readily reduced. Furthermore, biochar and amorphous iron oxide could mitigate the emission of nitrous oxide, which competed intensely with Fe(III) for electron donors in denitrifying environments (Easton et al. 2015), resulting in more electron transport to Fe(III). These findings were in agreement with the enhanced contribution of nitrate nitrogen (NN) to FeRC in CCA analysis (Fig. 3).

Most studies conducted to date have demonstrated that microbial activity in paddy soils increased as a result of biochar addition (Lehmann et al. 2011), which might also explain the enhanced microbial- mediated Fe(III) reduction. Microbial community abundance and structures related to Fe(III) reduction should be further explored to clarify the variations in soil microorganisms induced by biochar addition.

Effect of biochar particle size on Fe(III) reduction

The difference in biochar particle size can influence its function in soil. For instance, larger biochar particles with less ash are less efficient in increasing soil pH, while smaller biochar particles are better for glucose adsorption and transport (Online resource 2; Table 2). In particular, the content of DOC was related to biochar particle size (Fig. 1). Namely, the number of electrons donated and shuttled by DOC of biochar was increased with the decrease of biochar particle size. That is why, the increase in Fe(III) reduction potential was significantly correlated with the decrease of biochar particle size. Obviously, in the present study, the rate at which glucose adsorbed to biochar varied with biochar particle size (Table 2). Small particle size is effective in the sorption of organic carbon and attachment of microorganisms (Ameloot et al. 2013; Lehmann et al. 2011). Liang et al. (2016) confirmed that the use of amendments with finer biochar particles resulted in an increase in soil enzyme activity as compared to coarser particles. In the present study, the promotion effect of biochar on Fe(II) accumulation was greater, occurring in the order GMB3 > GMB4 > GMB5 in both the soil slurry assay and microbial inoculation assay. Additionally, a larger particle was associated with a shorter time needed for adsorption saturation. Thus, the effect of GMBs modification time on Fe(III) reduction was less sensitive as GMB particle size increased.

Advantage of GMBs addition over UMBs to Fe(III) reduction and soil pH

Following the addition of UMBs to paddy soils, despite DOC being responsible for Fe(III) reduction, part of the DOC may participate in the reduction of other electron acceptors more readily reduced than Fe(III). Thus, the promotion of Fe(III) reduction by UMBs might be less significant than the theoretical one as estimated in the "Mechanism of stimulated Fe(III) reduction with UMBs addition" section. Based on the pore structure, surface area, and adsorption capacity of biochar, we modified biochar with glucose to increase the DOC content of biochar. Quilliam et al. (2013) calculated that ^{14}C-labeled glucose on biochar could diffuse into soil at 0.48 cm day^{-1} and that it further enhanced the microbial activity in soil surrounding the biochar. Our results demonstrated that, with the addition of GMBs, the Fe(III) reducing potential and maximum Fe(III) reduction rate were remarkably increased or remained stable relative to UMBs in both the soil slurry assay and the microbial inoculation assay. These findings revealed that glucose modification of biochar was important to simulate Fe(III) reduction in paddy soil. Moreover, it is widely believed that biochar addition could lead to positive priming effects on soil organic

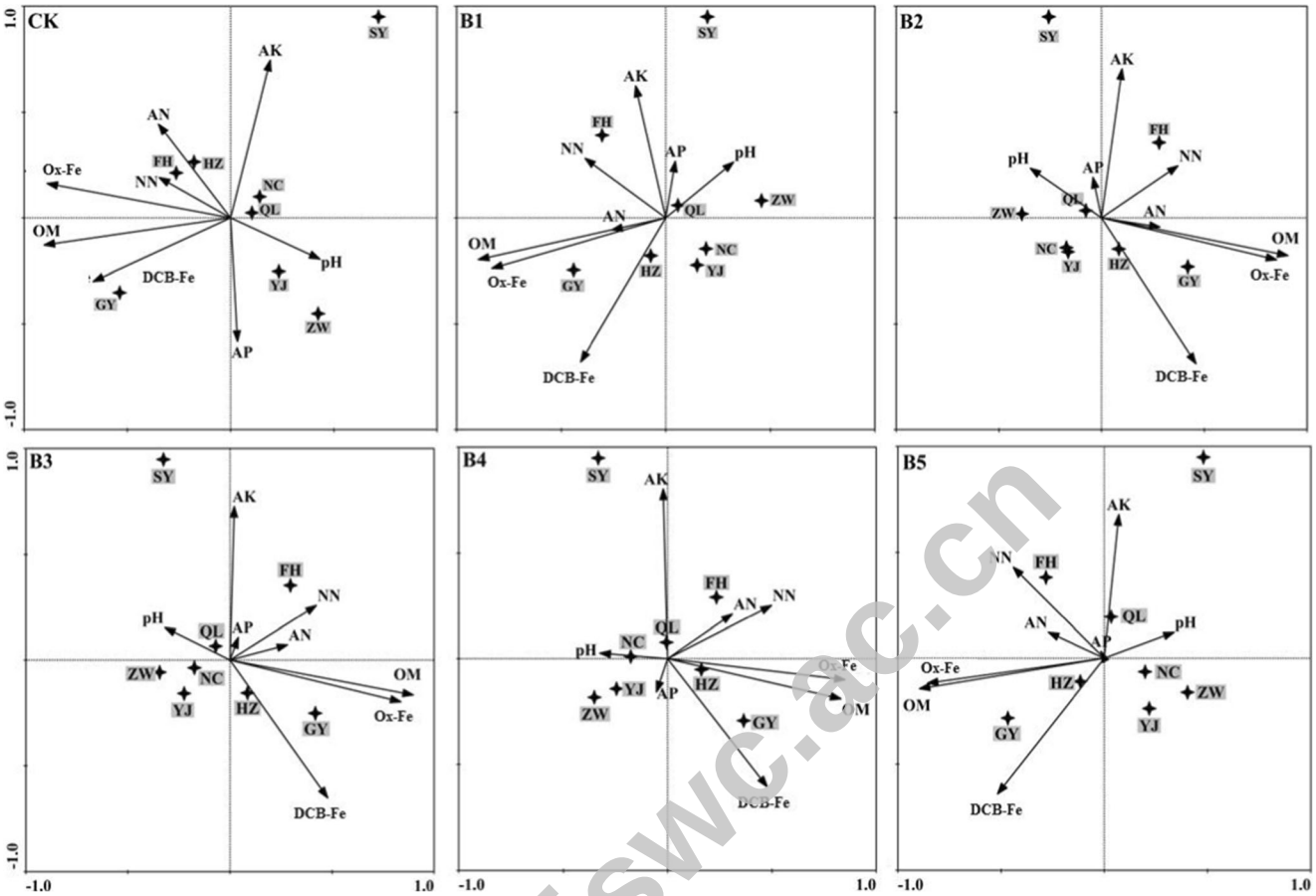

Fig. 4 Biplots based on a canonical correspondence analysis (CCA) of Fe(III) reducing capacity (FeRC) of different paddy soils (*stars*) in relation to soil environmental factors (*arrows*) after the addition of biochar at different particle sizes (*B1–B5*). *B1* to *B5* is the biochar particle size at 0–0.25, 0.25–0.5, 0.5–1.0, 1.0–2.0, 2.0–3.0, and 3.0–5.0 mm, respectively. *OM* soil organic matter, *AN* ammoniacal nitrogen, *NN* nitrate nitrogen, *AP* available phosphorus, *AK* available potassium, *DCB-Fe* sodium hydrosulfite-sodium citrate-sodium bicarbonate-extractable Fe (Free Fe), *Ox-Fe* oxalate-extractable Fe (amorphous Fe), *pH* soil intial pH. Variance of data, explained by axes 1, ranged from 92.4 to 95.8% in the paddy soils with or without unmodified biochar (UMBs) addition

matter mineralization by increasing soil microbial biomass and activities. Quilliam et al. (2013) indicated that the mineralization rate of glucose by microorganisms on the biochar surface was significantly enhanced compared to that in soil without biochar addition. Chen et al. (2012) found that the Fe(III) reducing and dechlorinating bacteria were enriched by the additional presence of electron donors of lactate and electron shuttles of 2,6-anthraquinone disulfonate. Hence, changes in microbial biomass and organic matter turnover could be additional causes for the enhanced Fe(III) reduction after GMBs addition.

For pH, similarly to previous studies (Joseph et al. 2010; Xu et al. 2012), the addition of UMBs resulted in an increase in pH during the paddy soil slurry incubation and the microbial inoculation incubation. This can be explained by the basic groups of biochar, such as –COO– and –O-functional groups and carbonate, which contributed greatly to the alkalinity (Yuan et al. 2011). However, the pH was lower in response to GMBs throughout the anaerobic incubation period in comparison to the UMBs and CK treatments, which was attributed to the production of organic acids by microbial fermentation of the glucose adsorbed on biochar (Lovley 1987; Takai et al. 1963).

Recent studies have demonstrated that fermentative bacteria play a supporting and important role during the Fe(III) reduction in paddy soils with intermediate metabolites (such as low-molecular-weight organic acids and hydrogen) serving as electron donors (Lehours et al. 2010; Lehours et al. 2009; Lentini et al. 2012). Trchounian et al. (2012) indicated that H_2 production or uptake by microorganisms was correlated with the activity and function of hydrogenase, as well as the environmental pH. Jia et al. (2015) further reported that fermentative dehydrogenation and hydrogen production were responsible for microbial Fe(III) reduction. In the soil slurry assay with GMBs addition, the decrease in pH in GY soil was lower, which was coincident with remarkable Fe(II) accumulation, revealing a considerable H_2 uptake of Fe(III)-reducing bacteria relying on *Hyd-2*. Nevertheless, the continuous

decrease in pH in SY soil was higher than that in GY soil, corresponding to a slight increase of Fe(II) accumulation. This result may be attributed to the suppression of hydrogenase activity under strongly alkaline conditions. Moreover, 12-h GMB and 48-h GMB, which had a similar glucose adsorption, presented similar FeRC kinetic parameters in both the soil slurry and the microbial inoculation assay. This is a further indirect evidence that the decrease of pH was caused by microbial fermentation.

Studies have also confirmed that Fe(III) reduction could compete with methanogenesis for electrons in paddy fields. Our finding that biochar addition-simulated Fe(III) reduction provides new insight that may help to explain the mechanism by which methane emissions are mitigated in response to biochar application. Specifically, the application of glucose-modified biochar to paddy fields may enable alleviation of the increase in pH and salinity that is caused by basic groups of biochar in alkaline soil (Bongoua-Devisme et al. 2012). Future studies should be conducted to improve our understanding of the mechanisms involved in biochar-microbe-biochemical process in paddy soils and providing new valuable information for the assessment of productive and strategic biochar application.

Conclusion

Biochar (unmodified biochars; UMBs) of different particle sizes and glucose-modified biochar (GMBs) were added to paddy soils. Soil original organic matter and amorphous Fe content were responsible for the dominant contribution to Fe(III) reducing capacity (FeRC). The addition of UMBs promoted Fe(III) reduction potential, which was negatively correlated to the UMBs particle size. UMBs addition enhanced the contribution of soil original free Fe content and nitrite nitrogen to FeRC, while it reduced that of available phosphorus. Dissolved organic carbon of biochar was redox active and responsible for a 20% increase in Fe(II) formation. Although UMBs enhanced or had no effect on Fe(III) reduction, GMBs with the same particle size had a much greater effect in comparison to pristine biochar. The decrease in pH during anaerobic incubation following GMBs addition was attributed to the organic carbon fermentation by microorganisms. The pretreatment of biochar with glucose can be used to prepare high-efficiency biochars for the stimulation of Fe(III) reduction and alleviation of soil pH increase caused by the basic groups in the biochar.

Acknowledgements Support for this paper was provided by the Foundation of State Key Laboratory of Soil Erosion and Dryland Farming on Loess Plateau of China (Grant No. A318009902-1509) and the National Natural Science Foundation of China (Grant No. and 41571239). The authors were grateful for the anonymous reviewers for their quality comments.

References

Ameloot N, Graber ER, Verheijen FGA, De-Neve S (2013) Interactions between biochar stability and soil organisms: review and research needs. Eur J Soil Sci 64:379–390. doi:10.1111/ejss.12064

Anderson CR, Condron LM, Clough TJ, Fiers M, Stewart A, Hill RA, Sherlock RR (2011) Biochar induced soil microbial community change: implications for biogeochemical cycling of carbon, nitrogen and phosphorus. Pedobiologia 54:309–320. doi:10.1016/j.pedobi.2011.07.005

Atkinson CJ, Fitzgerald JD, Hipps NA (2010) Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. Plant Soil 337:1–18. doi:10.1007/s11104-010-0464-5

Beesley L, Moreno-Jimenez E, Gomez-Eyles JL, Harris E, Robinson B, Sizmur T (2011) A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. Environ Pollut 159:3269–3282. doi:10.1016/j.envpol.2011.07.023

Blair GJ, Lefroy RDB, Lisle L (1995) Soil carbon fractions based on their degree of oxidation, and the development of a carbon management index for agricultural systems. Aust J Agric Res 46:1459–1466. doi:10.1071/AR9951459

Bongoua-Devisme AJ, Cebron A, Kassin KE, Yoro GR, Mustin C, Berthelin J (2013) Microbial communities involved in Fe reduction and mobility during soil organic matter (SOM) mineralization in two contrasted paddy soils. Geomicrobiol J 30:347–361. doi:10.1080/01490451.2012.688928

Bongoua-Devisme AJ, Mustin C, Berthelin J (2012) Responses of iron-reducing bacteria to salinity and organic matter amendment in paddy soils of Thailand. Pedosphere 22:375–393. doi:10.1016/s1002-0160(12)60024-1

Chen M, Shih K, Hu M, Li F, Liu C, Wu W, Tong H (2012) Biostimulation of indigenous microbial communities for anaerobic transformation of pentachlorophenol in paddy soils of southern China. J Agric Food Chem 60:2967–2975. doi:10.1021/jf204134w

Cui HJ, Wang MK, Fu ML, Ci E (2011) Enhancing phosphorus availability in phosphorus-fertilized zones by reducing phosphate adsorbed on ferrihydrite using rice straw-derived biochar. J Soils Sediments 11:1135–1141. doi:10.1007/s11368-011-0405-9

Ding Z, Hu X, Wan Y, Wang S, Gao B (2016) Removal of lead, copper, cadmium, zinc, and nickel from aqueous solutions by alkali-modified biochar: batch and column tests. J Ind Eng Chem 33:239–245. doi:10.1016/j.jiec.2015.10.007

Easton ZM, Rogers M, Davis M, Wade J, Eick M, Bock E (2015) Mitigation of sulfate reduction and nitrous oxide emission in denitrifying environments with amorphous iron oxide and biochar. Ecol Eng 82:605–613. doi:10.1016/j.ecoleng.2015.05.008

Feng C, Yue X, Li F, Wei C (2013) Bio-current as an indicator for biogenic Fe(II) generation driven by dissimilatory iron reducing bacteria. Biosensors & Bioelectronics 39:51–56. doi:10.1016/j.bios.2012.06.037

Glaser B, Lehmann J, Zech W (2002) Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal—a review. Biol Fertil Soils 35:219–230. doi:10.1007/s00374-002-0466-4

Graber ER, Tsechansky L, Lew B, Cohen E (2014) Reducing capacity of water extracts of biochars and their solubilization of soil Mn and Fe. Eur J Soil Sci 65:162–172. doi:10.1111/ejss.12071

He JZ, Qu D (2008) Dissimilatory Fe(III) reduction characteristics of paddy soil extract cultures treated with glucose or fatty acids. J Environ Sci 20:1103–1108. doi:10.1016/S1001-0742(08)62156-7

Herath HMSK, Camps-Arbestain M, Hedley M (2013) Effect of biochar on soil physical properties in two contrasting soils: an alfisol and an andisol. Geoderma 209:188–197. doi:10.1016/j.geoderma.2013.06.016

Heymann K, Lehmann J, Solomon D, Schmidt MWI, Regier T (2011) C 1s K-edge near edge X-ray absorption fine structure (NEXAFS) spectroscopy for characterizing functional group chemistry of black

carbon. Org Geochem 42:1055–1064. doi:10.1016/j.orggeochem.2011.06.021

Hori T, Aoyagi T, Itoh H, Narihiro T, Oikawa A, Suzuki K, Ogata A, Friedrich MW, Conrad R, Kamagata Y (2015) Isolation of microorganisms involved in reduction of crystalline iron(III) oxides in natural environments. Front Microbiol 6:386–401. doi:10.3389/fmicb.2015.00386

Hoy CW, Grewal PS, Lawrence JL, Jagdale G, Acosta N (2008) Canonical correspondence analysis demonstrates unique soil conditions for entomopathogenic nematode species compared with other free-living nematode species. Biol Control 46:371–379. doi:10.1016/j.biocontrol.2008.06.001

Jaeckel U, Schnell S (2000) Suppression of methane emission from rice paddies by ferric iron fertilization. Soil Biol Biochem 32:1811–1814. doi:10.1016/S0038-0717(00)00094-8

Jia R, Li LN, Qu D (2015) pH shift-mediated dehydrogenation and hydrogen production are responsible for microbial iron(III) reduction in submerged paddy soils. J Soils Sediments 15:1178–1190. doi:10.1007/s11368-015-1084-8

Johnston SG, Burton ED, Aaso T, Tuckerman G (2014) Sulfur, iron and carbon cycling following hydrological restoration of acidic freshwater wetlands. Chem Geol 371:9–26. doi:10.1016/j.chemgeo.2014.02.001

Joseph SD, Camps-Arbestain M, Lin Y, Munroe P, Chia CH, Hook J, van Zwieten L, Kimber S, Cowie A, Singh BP, Lehmann J, Foidl N, Smernik RJ, Amonette JE (2010) An investigation into the reactions of biochar in soil. Soil Research 48:501–515. doi:10.1071/SR10009

Kögel-Knabner I, Amelung W, Cao Z, Fiedler S, Frenzel P, Jahn R, Kalbitz K, Kölbl A, Schloter M (2010) Biogeochemistry of paddy soils. Geoderma 157:1–14. doi:10.1016/j.geoderma.2010.03.009

Kammann C, Ratering S, Eckhard C, Muller C (2012) Biochar and hydrochar effects on greenhouse gas (carbon dioxide, nitrous oxide, and methane) fluxes from soils. J Environ Qual 41:1052–1066. doi:10.2134/jeq2011.0132

Kappler A, Wuestner ML, Ruecker A, Harter J, Halama M, Behrens S (2014) Biochar as an electron shuttle between bacteria and Fe(III) minerals. Environmental Science & Technology Letters 1:339–344. doi:10.1021/ez5002209

Khan MA, Kim KW, Mingzhi W, Lim BK, Lee WH, Lee JY (2007) Nutrient-impregnated charcoal: an environmentally friendly slow-release fertilizer. Environmentalist 28:231–235. doi:10.1007/s10669-007-9133-5

Klupfel L, Keiluweit M, Kleber M, Sander M (2014) Redox properties of plant biomass-derived black carbon (biochar). Environmental Science & Technology 48:5601–5611. doi:10.1021/es500906d

Koide RT, Petprakob K, Peoples M (2011) Quantitative analysis of biochar in field soil. Soil Biol Biochem 43:1563–1568. doi:10.1016/j.soilbio.2011.04.006

Kumar N, Omoregie EO, Rose J, Masion A, Lloyd JR, Diels L, Bastiaens L (2014) Inhibition of sulfate reducing bacteria in aquifer sediment by iron nanoparticles. Water Res 51:64–72. doi:10.1016/j.watres.2013.09.042

Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC, Crowley D (2011) Biochar effects on soil biota—a review. Soil Biol Biochem 43:1812–1836. doi:10.1016/j.soilbio.2011.04.022

Lehours AC, Rabiet M, Morel-Desrosiers N, Morel JP, Jouve L, Arbeille B, Mailhot G, Fonty G (2010) Ferric iron reduction by fermentative strain BS2 isolated from an iron-rich anoxic environment (Lake Pavin, France). Geomicrobiol J 27:714–722. doi:10.1080/01490451003597663

Lehours AC, Batisson I, Guedon A, Mailhot G, Fonty G (2009) Diversity of culturable bacteria, from the anaerobic zone of the meromictic Lake Pavin, able to perform dissimilatory-iron reduction in different in vitro conditions. Geomicrobiol J 26:212–223. doi:10.1080/01490450902744012

Lentini CJ, Wankel SD, Hansel CM (2012) Enriched iron(III)-reducing bacterial communities are shaped by carbon substrate and iron oxide mineralogy. Front Microbiol 3:1–19. doi:10.3389/fmicb.2012.00404

Liang C, Gascó G, Fu S, Méndez A, Paz-Ferreiro J (2016) Biochar from pruning residues as a soil amendment: effects of pyrolysis temperature and particle size. Soil Tillage Res 164:3–10. doi:10.1016/j.still.2015.10.002

Li HJ, Peng JJ, Weber KA, Zhu YG (2011) Phylogenetic diversity of Fe(III)-reducing microorganisms in rice paddy soil: enrichment cultures with different short-chain fatty acids as electron donors. J Soils Sediments 11:1234–1242. doi:10.1007/s11368-011-0371-2

Li FB, Li XM, Zhou SG, Zhuang L, Cao F, Huang DY, Xu W, Liu TX, Feng CH (2010) Enhanced reductive dechlorination of DDT in an anaerobic system of dissimilatory iron-reducing bacteria and iron oxide. Environ Pollut 158:1733–1740. doi:10.1016/j.envpol.2009.11.020

Li X, Zhang W, Liu T, Chen L, Chen P, Li F (2016) Changes in the composition and diversity of microbial communities during anaerobic nitrate reduction and Fe(II) oxidation at circumneutral pH in paddy soil. Soil Biol Biochem 94:70–79. doi:10.1016/j.soilbio.2015.11.013

Li Y, Yu S, Strong J, Wang H (2012) Are the biogeochemical cycles of carbon, nitrogen, sulfur, and phosphorus driven by the "FeIII–FeII redox wheel" in dynamic redox environments? J Soils Sediments 12:683–693. doi:10.1007/s11368-012-0507-z

Liu Y, Li FB, Xia W, Xu JM, Yu XS (2013) Association between ferrous iron accumulation and pentachlorophenol degradation at the paddy soil-water interface in the presence of exogenous low-molecular-weight dissolved organic carbon. Chemosphere 91:1547–1555. doi:10.1016/j.chemosphere.2012.12.040

Lovley DR (1987) Organic matter mineralization with the reduction of ferric iron: a review. Geomirobiology Journal 5:375–399. doi:10.1080/01490458709385975

Lovley DR (2006) Dissimilatory Fe(III)- and Mn(IV)-reducing prokaryotes. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) The prokaryotes. Springer, New York, pp. 635–658. doi:10.1007/0-387-30742-7-21

Lovley DR, Phillips EJP (1986) Organic matter mineralization with reduction of ferric iron in anaerobic sediments. Appl Environ Microbiol 51:683–689

Myers CR, Myers JM (1993) Role of menaquinone in the reduction of fumarate, nitrate, iron(III) and manganese(IV) by *Shewanella putrefaciens* MR-1. FEMS Microbiol Lett 114:215–222. doi:10.1111/j.1574-6968.1993.tb06576.x 215-222

Myers JM, Antholine WE, Myers CR (2004) Vanadium(V) reduction by *Shewanella oneidensis* MR-1 requires menaquinone and cytochromes from the cytoplasmic and outer membranes. Appl Environ Microbiol 70:1405–1412. doi:10.1128/aem.70.3.1405-1412.2004

Newman DK, Kolter R (2000) A role for excreted quinones in extracellular electron transfer. Nature 405:94–97. doi:10.1038/35011098

Page AL (1982) Methods of soil analysis, part 2: chemical and microbiological properties. American Society of Agronomy Press, Madison

Payne K, Abdel-Fattah T (2005) Adsorption of arsenate and arsenite by iron-treated activated carbon and zeolites: effects of pH, temperature, and ionic strength. J Environ Sci Health A 40:723–749. doi:10.1081/ese-200048254

Peng QA, Shaaban M, Hu R, Mo Y, Wu Y, Ullah B (2015) Effects of soluble organic carbon addition on CH_4 and CO_2 emissions from paddy soils regulated by iron reduction processes. Soil Research 53:316–324. doi:10.1071/sr14287

Peter Mayer H, Conrad R (1990) Factors influencing the population of methanogenic bacteria and the initiation of methane production upon flooding of paddy soil. FEMS Microbiol Lett 73:103–111. doi:10.1016/0378-1097(90)90656-B

Qian W, Zhao AZ, Xu RK (2013) Sorption of As(V) by aluminum-modified crop straw-derived biochars. Water Air Soil Pollution 224:1610–1615. doi:10.1007/s11270-013-1610-5

Quilliam RS, Glanville HC, Wade SC, Jones DL (2013) Life in the 'charosphere'—does biochar in agricultural soil provide a significant habitat for microorganisms? Soil Biol Biochem 65:287–293. doi:10.1016/j.soilbio.2013.06.004

Ren X, Zhang P, Zhao L, Sun H (2016) Sorption and degradation of carbaryl in soils amended with biochars: influence of biochar type and content. Environ Sci Pollut Res 23:2724–2734. doi:10.1007/s11356-015-5518-z

Takai Y, Koyama T, Kamura T (1963) Microbial metabolism in reduction process of paddy soils (part 2). Soil Science and Plant Nutrition 9: 10–14. doi:10.1080/00380768.1963.10431049

Trchounian K, Pinske C, Sawers RG, Trchounian A (2012) Characterization of *Escherichia coli* [NiFe]-hydrogenase distribution during fermentative growth at different pHs. Cell Biochemistry Biophysics 62:433–440. doi:10.1007/s12013-011-9325-y

Wang H, Gao B, Wang S, Fang J, Xue Y, Yang K (2015) Removal of Pb(II), Cu(II), and Cd(II) from aqueous solutions by biochar derived from $KMnO_4$ treated hickory wood. Bioresour Technol 197:356–362. doi:10.1016/j.biortech.2015.08.132

Wang S, Gao B, Zimmerman AR, Li Y, Ma L, Harris WG, Migliaccio KW (2014) Removal of arsenic by magnetic biochar prepared from pinewood and natural hematite. Bioresour Technol 175C:391–395. doi:10.1016/j.biortech.2014.10.104

Wang X, Chen X, Yang J, Wang Z, Sun G (2009) Effect of microbial mediated iron plaque reduction on arsenic mobility in paddy soil. J Environ Sci 21:1562–1568. doi:10.1016/S1001-0742(08)62456-0

Whittleston RA, Stewart DI, Mortimer RJG, Burke IT (2013) Enhancing microbial iron reduction in hyperalkaline, chromium contaminated sediments by pH amendment. Appl Geochem 28:135–144. doi:10.1016/j.apgeochem.2012.10.003

Xu G, Lv YC, Sun JN, Shao HB, Wei LL (2012) Recent advances in biochar applications in agricultural soils: benefits and environmental implications. Clean-Soil Air Water 40:1093–1098. doi:10.1002/clen.201100738

Yi WJ, Wang BL, Qu D (2012) Diversity of isolates performing Fe(III) reduction from paddy soil fed by different organic carbon sources. Afr J Biotechnol 11: 4407–4417. doi:10.5897/ajb11.1216

Yuan JH, Xu RK, Zhang H (2011) The forms of alkalis in the biochar produced from crop residues at different temperatures. Bioresour Technol 102:3488–3497. doi:10.1016/j.biortech.2010.11.018

Zhang AF, Bian RJ, Pan GX, Cui LQ, Hussain Q, Li LQ, Zheng JW, Zheng JF, Zhang XH, Han XJ, Yu XY (2012) Effects of biochar amendment on soil quality, crop yield and greenhouse gas emission in a Chinese rice paddy: a field study of 2 consecutive rice growing cycles. Field Crop Res 127:153–160. doi:10.1016/j.fcr.2011.11.020

Zhu C, Xia SH, Wang BL, Qu D (2011) Variation of Anaeromyxobacter community structure and abundance in paddy soil slurry over flooding time. African Journal of Agricultural Reseearch 6:6107–6118. doi:10.5897/ajar11.1159

Zuo X, Liu Z, Chen M (2016) Effect of H_2O_2 concentrations on copper removal using the modified hydrothermal biochar. Bioresour Technol 207:262–267. doi:10.1016/j.biortech.2016.02.032