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RESEARCH ARTICLE

Comparative effects on arsenic uptake between iron (hydro)oxides on root surface and rhizosphere of rice in an alkaline paddy soil

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Abstract

The iron (Fe) (hydro)oxides deposited around rice roots play an important role in arsenic (As) sequestration in paddy soils, but there is no systematic study on the relative importance of Fe (hydro)oxides on root surface and in rhizosphere soil in limiting As bioavailability. Twenty-seven rice genotypes were selected to investigate effects of Fe (hydro)oxides on As uptake by rice in an alkaline paddy soil. Results indicated that the As content was positively correlated with the Fe content on root surface, and most of As (88–97%) was sequestered by poorly crystalline and crystalline Fe (hydro)oxides in the alkaline paddy soil. The As sequestration by Fe (hydro)oxides on root surface (IAS_{root} 16.8–25.0 mg As/(g Fe)) was much higher than that in rhizosphere (IAS_{rhizo} 1.4–2.0 mg As/(g Fe)); therefore, in terms of As immobilization, the Fe (hydro)oxides on root surface were more important than that in rhizosphere. However, the As content in brown rice did not have significant correlation with the As content on root surface but was significantly correlated ($R^2 = 0.43$, $P < 0.05$) with the partition ratio ($PR_{As} = IAS_{root}/IAS_{rhizo}$) of As sequestration on root surface and in rhizosphere, which suggested that Fe (hydro)oxides on root surface did not play the controlling role in lowering As uptake, and the partition ratio PR_{As} would be a better indicator to evaluate effects of Fe (hydro)oxides around roots on As uptake by rice.

Keywords Rice · Arsenic · Iron (hydro)oxides · Root surface · Rhizosphere soil · Alkaline paddy soil

Introduction

Soil arsenic (As) contamination is becoming more and more serious, causing a worldwide attention because of its adverse effects on the growth of plants, and it may even harm human health through foods produced from the contaminated lands (Shi et al. 2014; Zhu et al. 2016). Arsenic can be easily accumulated in rice, because the waterlogging conditions during rice growth can increase the As mobility (Somenahally et al. 2011; Jia et al. 2014); therefore, rice is considered as the primary source of dietary As for human exposure, especially in some Asian countries (Saifullah et al. 2018).

Inorganic As forms, existing as arsenate (As(V)) and arsenite (As(III)), are the most encountered species in soils and much more toxic than organic forms. Both As(V) and As(III) may change valence states depending on soil pH and redox condition and thus change their relative toxicity and bioavailability. Under waterlogged conditions during rice growth, As(V) bonded with Fe (hydro)oxides can be reduced to As(III) which has higher toxicity and mobility (Stroud et al. 2011; Honma et al. 2016). The As(III)/As(V) ratio is high in the soil solution during flooding (Yamaguchi et al. 2014). On the contrary, oxygen release by rice roots creates an oxidizing microenvironment around rice roots, so the reduced Fe and As surrounding roots are re-oxidized and precipitated around roots (Mei et al. 2009; Jia et al. 2014; Tripathi et al. 2014). The reformed Fe (hydro)oxides around roots are crystalline or poorly crystalline Fe(III), including ferrihydrite, goethite, and lepidocrocite (Liu et al. 2006), and have high affinity for sequestering both As(V) and As(III) (Liu et al. 2004). The ratio of As/Fe in Fe (hydro)oxides around rice roots is much higher than that in Fe minerals in the soil matrix, indicating that As is concentrated in Fe (hydro)oxides in rhizosphere soil (Yamaguchi et al. 2014). Previous research indicated that Fe

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(hydro)oxides deposited in rhizosphere soil are more efficient on immobilizing Pb than that on root surface in paddy soils (Lai et al. 2018). Arsenic, an anion, has different geochemical behavior with Pb^{2+}; do Fe (hydro)oxides in rhizosphere soil play an important role on limiting As uptake into rice? Previous studies normally focused on the Fe (hydro)oxides coated onto root surface (Liu et al. 2004; Seyfferth et al. 2010; Wu et al. 2012), but there are scarce studies on effects of Fe (hydro)oxides in rhizosphere soil.

In addition, Fe (hydro)oxides can be easily formed at low pH and high oxidizing conditions; thus, previous studies on Fe (hydro)oxides effects on As sequestration were mainly done in acidic soils (Syu et al. 2013, 2014; Wu et al. 2016) or culture solution (Liu et al. 2004; Wu et al. 2012), and less such information is available about alkaline soils (Saifullah et al. 2018). However, arsenic in alkaline soils is more mobile and toxic (Saifullah et al. 2018); will the Fe (hydro)oxides easily form around rice roots and play an important role on limiting As uptake by rice? In this study, twenty-seven rice genotypes were selected to plant in an As-contaminated alkaline paddy soil to find the answer. The content of Fe (hydro)oxides and associated As on rice root surface and in rhizosphere soil was measured; the fractions and spatial distribution of Fe (hydro)oxides and associated As on root surface were investigated as well. The objective was to compare effects of As sequestration by Fe (hydro)oxides on root surface and in rhizosphere on limiting the As uptake by rice.

Materials and methods

Soils and rice genotypes

Soils were collected from a paddy field in Yangxin, Hubei Province, China (N 29°48′40 ″; E 115°25′53″). The soil was a sandy loam with pH of 7.7 (1:2.5 H_2O). The total As content was 72.7 mg kg^{-1} measured by nitric acid–hydrogen peroxide (HNO_3–H_2O_2). The soil organic matter was 19.7 g/kg (Nelson and Sommers 1996), total Fe was 28.3 g/kg (Meharg and Rahman 2003), and DCB (sodium citrate–sodium bicarbonate–sodium dithionite) extractable Fe was 7.6 g/kg (Mehra and Jackson 2013), respectively. After being air-dried at room temperature, soils were ground and kept in PVC pots (10 kg soil per pot) for pot experiments.

In this experiment, twenty-seven commonly planted rice genotypes in China were used, viz., Zhenzhunuo (ZZU), Huanghuazhan (HHZ), Ezhong5hao (EZ5), Nongjingsimiao (NJSM), Enuo9hao (EN9), Texianzhan25 (TXZ25), Fengxianzhan (FXZ), Guangliangyouxiang66 (GLYX66), Xinliangyou6hao (XLY6), Liangyou0367 (LY0367), Guangliangyou15 (GLY15), Guangliangyou272 (GLY272), Changyou195 (CY195), Quanliangyou655 (QLY655), Chuanxiangyou6hao (CXY6), Yuefeng202 (YF202), Zhongzheyou634 (ZZY634), IIyouhang2hao (IIYH2), Shenyou9519 (SY9519), IIyou3301 (IIY3301), Tianyou8hao (TY8), Hongxiangyou68 (HXY68), Huayouxiangzhan (HYXZ), Yueyou997 (YY997), Shenyou957 (SY957), Wuyou308 (WY308), and Nei2you111 (N2Y111).

Pot experiments

Pot experiments were conducted in a greenhouse in Huazhong Agricultural University, Hubei Province, China. Before rice transplanting, fertilizers were added into soils thoroughly, and then the soils (10 kg soil per pot) were flooded. The application rates of nitrogen, phosphorus, and potassium fertilizers were 0.14 g N/kg soil by $CO(NH_2)_2$, 0.09 g P_2O_5/kg soil by CaH_2PO_4, and 0.11 g K_2O/kg soil by K_2SO_4, respectively. Then three seedlings were transplanted to each pot at May 15, 2017 when rice plants had three leaves, and three pot replicates were done for each rice genotype. The water level was maintained throughout the rice growing season at 2 cm above the soil surface to simulate the water condition in most paddy fields.

Rice plants were harvested on September 25, 2017 when after all genotypes were ripe, and the rice grains, shoots, roots with Fe (hydro)oxides, and rhizosphere soils were collected. The whole plants were pulled out from pots, and plant shoots were then cut off at ground level. Roots were carefully separated from bulk soil, and tightly adhered soils on roots were shaken off and collected as "rhizosphere" soils. A part of rhizosphere soils were kept at −20 °C as soon as possible, and a small part of rhizosphere soils was air-dried and milled for chemical analyses. Roots with Fe (hydro)oxides were washed immediately by deionized water until no visible soil particles adhered to the roots, which were then kept at −20 °C as soon as possible to avoid the Fe oxidation during the sampling process. Rice grains were subsequently separated into brown rice and husks, and plant materials (e.g., brown rice, husks, and shoots) were oven dried at 70 °C. The total As content of plant materials were digested with HNO_3/$HClO_4$ (4:1, *v*/v) solution and measured by hydride generation atomic fluorescence spectrometry (HG-AFS, AFS-9700, Beijing Jitian Instruments Co., China).

Fe and As contents of Fe (hydro)oxides on root surface and in rhizosphere soils

The Fe (hydro)oxides on root surface were extracted by DCB solution (0.03 mol/L sodium citrate; 0.125 mol/L sodium bicarbonate; and 0.015 g/ml sodium dithionite) in accordance with previous studies (Liu et al. 2004; Lee et al. 2013; Lai et al. 2018). Briefly, 0.5 g root samples were extracted by 30 ml DCB solution for 30 min at room temperature

(25 °C). The roots were then rinsed 3 times with deionized water, and the washings were added to the DCB extracts. The Fe (hydro)oxides deposited in rhizosphere soils were also extracted by DCB solution (Lai et al. 2018). The contents of Fe and associated As in DCB extraction were measured by atomic absorption spectrophotometer (AAS, TAS-990, Beijing Puxi Instruments Co., P.R. China) and hydride generation atomic fluorescence spectrometry, respectively. After DCB extraction, roots were oven dried at 70 °C, and the total As content of roots (with Fe (hydro)oxides removed) were digested with $HNO_3/HClO_4$ (4:1, *v*/v) solution and measured by hydride generation atomic fluorescence spectrometry.

Fractions of Fe and As in Fe (hydro)oxides on root surface

According to the Fe and As contents (low, medium, and high levels) on the root surface, we had selected 6 genotypes which were then cultivated to measure Fe and As fractions on the root surface. To investigate Fe and As fractions on root surface, the proposed sequential extraction procedures were taken following the study of Yu et al. (2018). The process included: step I, extracting with 1 mol/L $MgCl_2$ at 180 rpm for 1 h (for exchangeable Fe and As); step II, extracting with 1 mol/L sodium acetate (pH 4.5) for 1 h (for carbonate Fe and As); step III, extracting with 0.2 mol/L ammonium oxalate (pH 3.2) at 180 rpm for 1 h (for poorly crystalline Fe and As); and step IV, extracting with 0.2 mol/L $Na_3C_6H_5O_7$ and 50 g/L of $Na_2S_2O_4$ powder at 180 rpm for 1 h (for crystalline Fe and As) (Yu et al. 2018). The contents of Fe and As were measured by atomic absorption spectrophotometer and hydride generation atomic fluorescence spectrometry, respectively.

The spatial distribution of Fe and As on rice root

The spatial distribution of Fe and As on rice root (transverse) was evaluated by scanning electron microscopy and energy dispersive X-ray spectrometry (SEM-EDS). Small pieces of roots (about 1–2 mm) were fixed by 2% glutaraldehyde in phosphate buffer (0.05 mol/L, pH 7.2) for 48 h at room temperature. The specimens were dehydrated in an ethanol series (25, 50, 75, 95, and 100%) and then dried using liquid carbon dioxide. The dried specimens were transferred onto sticky, conductive carbon tabs on aluminum stubs and then sputter-coated with gold. The acceleration voltage at the cathode was 15 kV, and representative photomicrographs were obtained at a working distance of 200 um. The relative content of As in Fe plaques on root surface for SY9519 was higher than other rice genotypes, and thus we only performed SEM-EDS on SY9519.

Data analysis

The concentration of As sequestered by Fe (hydro)oxides on root surface and in rhizosphere soil cannot compare directly because of their different denominator of unit (mg/kg soil or mg/kg root). Therefore, an index of As sequestration (IAS) by Fe (hydro)oxides was proposed to eliminate differences in DCB-extraction procedures and sample matrixes (roots and soils). The index was produced as a function of DCB-extracted As concentration (As_{DCB}) and DCB-extracted Fe concentration (Fe_{DCB}), and the equation is described as follows (Lai et al. 2018):

$$IAS = As_{DCB}/Fe_{DCB} \quad (1)$$

where As_{DCB} (mg As/kg) is the concentration of As in the Fe (hydro)oxides extracted by DCB, Fe_{DCB} (g Fe/kg) is the Fe concentration extracted by DCB, and IAS (mg As/g Fe) is the ratio of As_{DCB} to Fe_{DCB}, which is used to express As sequestration by Fe (hydro)oxides on root surface (IAS_{root}) and in rhizosphere soil (IAS_{rhizo}), respectively.

A partition ratio (PR_{As}) was used to indicate the relative tendency of As sequestration by Fe (hydro)oxides on root surface (IAS_{root}) to that in rhizosphere soil (IAS_{rhizo}):

$$PR_{As} = IAS_{root}/IAS_{rhizo}. \quad (2)$$

If $PR_{As} < 1$, it indicates that the sequestration of As by Fe (hydro)oxides on root surface is less than that in the rhizosphere soil; if $PR_{As} > 1$, it means that higher As is sequestered on the root surface than in rhizosphere soil (Lai et al. 2018).

Data presented in this study are means ($n = 3$) ± standard error (SE). Analysis of variance (ANOVA) was used to test As concentrations in rice plant materials (e.g., in roots (with Fe (hydro)oxides removed), shoots, husks, and brown rice) and contents of Fe and As on root surface and in rhizosphere soil. For the comparison of differences between treatments (genotypes), we used the least significant difference (LSD Duncan) test at the level of $P < 0.05$. ANOVA and LSD tests were performed using the SPSS 20 software package.

Results

Arsenic content in different rice tissues

Although different rice genotypes have different abilities of As uptake, As was predominantly distributed in the following order: roots (with Fe (hydro)oxides removed) > shoots > husks > brown rice for all tested genotypes (Fig. 1). The As content in roots (with Fe (hydro)oxides removed) was 117.4–307.7 mg/kg, and there was a significant difference ($p < 0.05$) among different genotypes (Fig. 1A). The content of As in shoots was between 4.6 and 20.6 mg/kg, which was 2.2–

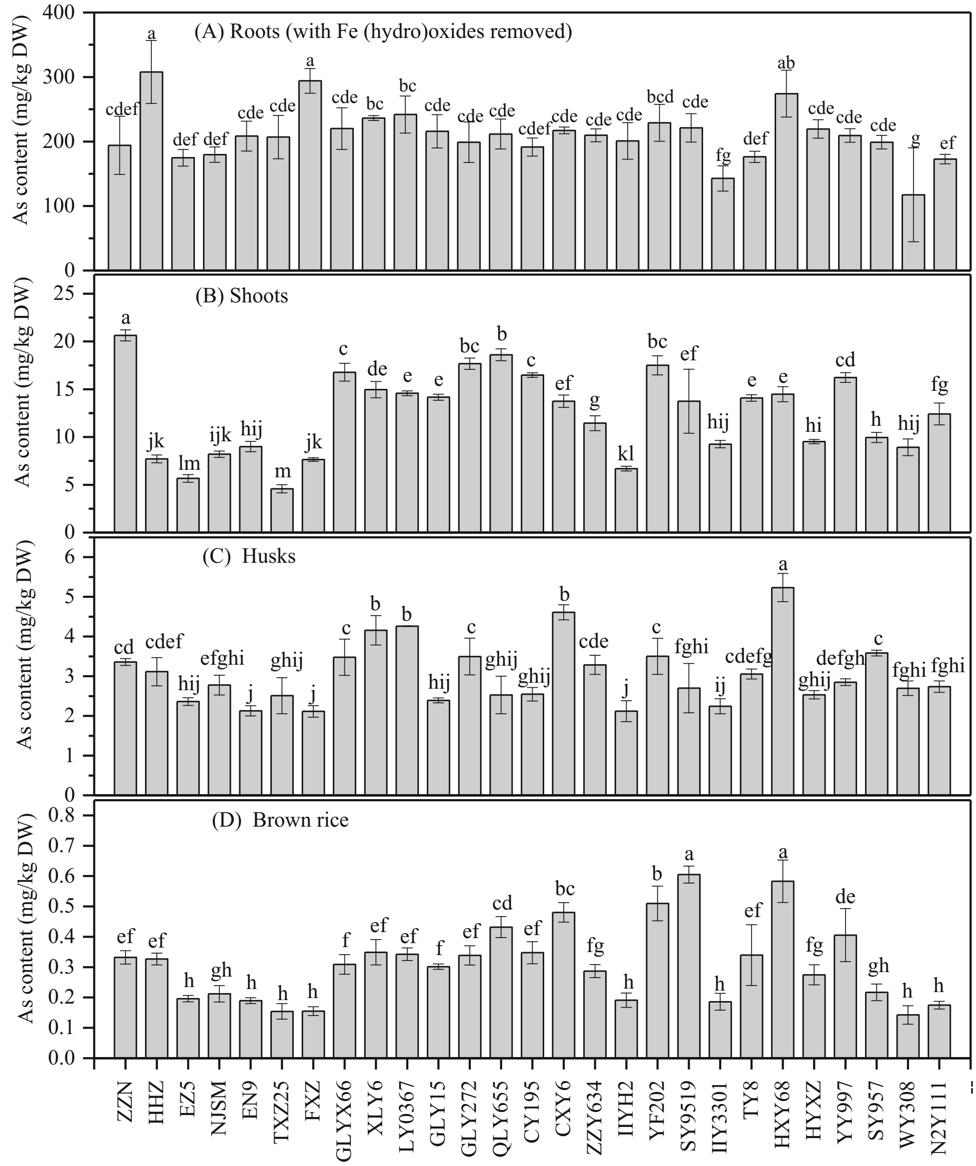

Fig. 1 Total As content in rice roots (with Fe (hydro)oxides removed) (A), shoots (B), husks (C), and brown rice (D) (mg/kg DW; mean ± SE). Different letters on bars indicate significant differences ($p < 0.05$)

12.0% of that in roots (Fig. 1B). The As accumulated in brown rice was significantly different among different genotypes ($p < 0.05$) (Fig. 1D), and the total As content in brown rice of some genotypes (such as TXZ25, IIY3301, and FXZ) was less than the inorganic As limit standard (0.2 mg/kg) set by the (National Health and Family Planning Commission & State Food and Drug Administration of China, GB2762– 2017). Therefore, choosing As low-uptake genotypes to plant in As-contaminated soils is a feasible and cost-effective way to reduce the risk of human As exposure.

Arsenic sequestration by Fe (hydro)oxides on root surface and in rhizosphere soil

The DCB-extracted As from root surface was significantly higher than that in roots (with Fe (hydro)oxides removed) (Table 1, Fig. 1) and even reached 3 times of the latter. The significantly higher amount of As on root surface than that in roots after DCB extraction suggested that As was concentrated on root surface and blocked to enter into root cells. Meanwhile, the As content was significantly related to the Fe content in DCB extraction from root surface ($R^2 = 0.83$, $P < 0.05$) (Fig. 2A), indicating that the As was sequestered by the Fe (hydro)oxides on root surface. The Fe (hydro)oxides deposited on root surface were significantly different among different genotypes ($p < 0.05$, Table 1), which might be due to their different oxygen release capacity by rice roots (Wu et al. 2012).

In addition, the contents of DCB-extracted Fe from rhizosphere soils were 10.2–12.3 g/kg, which were much higher than that from bulk soil (7.6 g/kg), indicating that Fe was also concentrated in rhizosphere soil. The contents of DCB-extracted As had no significant relationship with DCB-

Table 1 Contents of Fe_{DCB} and As_{DCB} indexes of As sequestration (IAS) and partition rates of As (PR_{As})

Rice genotypes	Root surface			Rhizosphere soil			PR_{As}
	Fe_{DCB} (g/kg root)	As_{DCB} (mg/kg root)	IAS_{root} (mg As/(g Fe))	Fe_{DCB} (g/kg soil)	As_{DCB} (mg/kg soil)	IAS $_{rhizo}$ (mg As/(g Fe))	
ZZN	31.6 ± 1.5 de	531.4 ± 40.5 hi	16.8	11.1 ± 0.2 efghi	18.7 ± 2.1 bc	1.7	10.0
HHZ	35.9 ± 0.4 bc	666.3 ± 39.2 cd	18.6	11.6 ± 0.1 abcdef	20.1 ± 1.7 ab	2.0	10.7
EZ5	33.0 ± 2.9 cd	624.3 ± 26.9 de	18.9	11.8 ± 0.3 abcde	17.8 ± 0.6 bc	1.9	12.5
NJSM	37.5 ± 1.9 ab	686.2 ± 27.5 c	18.3	12.3 ± 0.4 a	18.4 ± 0.9 bc	1.7	12.2
EN9	38.2 ± 1.6 ab	776.0 ± 18.5 b	20.3	11.9 ± 0.3 abcd	19.8 ± 1.6 abc	1.8	12.2
TXZ25	30.6 ± 1.9 def	666.5 ± 32.1 cd	21.8	11.9 ± 0.3 abcd	19.2 ± 0.3 abc	2.0	13.5
FXZ	39.8 ± 4.0 a	864.6 ± 30.9 a	21.7	11.8 ± 0.1 abcde	19.5 ± 1.3 abc	1.7	13.1
GLYX66	27.2 ± 2.2 fghi	483.6 ± 18.0 jk	17.8	11.0 ± 0.5 efghi	17.9 ± 1.3 bc	1.5	10.9
XLY6	28.7 ± 2.4 efgh	571.0 ± 23.2 fgh	19.9	10.5 ± 0.3 ij	20.4 ± 0.6 ab	1.5	10.2
LY0367	31.0 ± 2.0 de	608.3 ± 33.0 ef	19.6	10.7 ± 0.5 hij	20.4 ± 2.2 ab	1.6	10.3
GLY15	25.4 ± 2.0 hij	444.5 ± 9.5 kl	17.5	11.5 ± 0.7 bcdefg	18.5 ± 0.8 bc	1.5	10.9
GLY272	26.7 ± 1.2 ghij	537.0 ± 28.0 hi	20.1	10.8 ± 0.1 ghij	17.3 ± 2.5 bc	1.7	12.5
QLY655	30.6 ± 2.2 def	569.8 ± 42.5 fgh	18.6	11.0 ± 0.2 fghi	18.5 ± 1.9 bc	1.6	11.1
CY195	21.7 ± 1.0 kl	379.0 ± 21.6 m	17.4	11.2 ± 0.6 defghi	18.5 ± 0.9 bc	1.5	10.5
CXY6	23.1 ± 2.2 jk	446.0 ± 24.4 kl	19.3	11.1 ± 0.4 efghi	19.2 ± 1.3 abc	1.5	11.2
ZZY634	26.6 ± 2.0 ghij	484.0 ± 19.4 jk	18.2	11.1 ± 0.1 efghi	18.2 ± 3.0 bc	1.6	11.1
IIYH2	23.5 ± 2.2 jk	505.0 ± 21.2 ij	21.5	12.0 ± 0.2 abc	19.8 ± 0.6 abc	1.8	13.0
YF202	23.9 ± 1.7 ijk	431.1 ± 16.7 l	18.1	10.7 ± 0.6 hij	18.3 ± 1.7 bc	1.6	10.6
SY9519	35.6 ± 1.3 bc	681.0 ± 26.0 c	19.1	10.2 ± 0.7 j	18.0 ± 0.9 bc	1.5	10.8
IIY3301	19.2 ± 1.0 lm	478.6 ± 14.7 jkl	25.0	12.2 ± 0.4 ab	21.9 ± 0.4 a	1.8	13.9
TY8	28.7 ± 0.6 efgh	551.3 ± 17.2 gh	19.2	11.3 ± 0.2 cdefgh	18.7 ± 1.4 bc	1.7	11.6
HXY68	26.6 ± 1.3 ghij	467.8 ± 21.4 jkl	17.6	11.0 ± 0.1 fghi	19.6 ± 3.2 abc	1.5	9.9
HYXZ	33.1 ± 1.4 cd	587.8 ± 24.8 efg	17.8	11.0 ± 0.6 fghi	16.9 ± 1.9 c	1.6	11.6
YY997	30.0 ± 3.1 defg	590.8 ± 25.8 efg	19.7	11.2 ± 0.4 defghi	18.1 ± 1.3 bc	1.9	12.2
SY957	16.4 ± 1.18 m	302.8 ± 9.36 n	18.4	10.5 ± 0.4 ij	18.4 ± 0.6 bc	1.4	10.5
WY308	28.6 ± 0.75 efgh	661.0 ± 29.56 cd	23.1	12.0 ± 0.3 abc	20.0 ± 1.3 abc	1.9	13.9
N2Y111	25.3 ± 1.03 hij	454.3 ± 22.24 kl	17.9	12.3 ± 0.3 a	18.2 ± 1.1 bc	1.7	12.1

Fe_{DCB} and As_{DCB} are contents of Fe and As extracted by sodium dithionite–citrate–bicarbonate (DCB). IAS_{root} and IAS_{rhizo} are indexes of As sequestration by Fe (hydro)oxides on root surface and in rhizosphere soil, respectively. PR_{As} is the partition ratio ($PR_{As} = IAS_{root} / IAS_{rhizo}$) of As sequestration by Fe (hydro)oxides on root surface and in rhizosphere. Data are means (n = 3) ± standard error; different letters behind means indicate significant differences among genotypes ($p < 0.05$)

extracted Fe in rhizosphere soil ($R^2 = 0.096$, $P > 0.05$) (Fig. 2B), indicating that Fe (hydro)oxides may not be major factors affecting As accumulation in rhizosphere soil.

The IAS_{root} was varied from 16.8 to 25.0 mg As/(g Fe) and significantly higher than IAS_{rhizo} (1.4–2.0 mg As/(g Fe)) (Table 1), and PR_{As} was much greater than 1 (Table 1). However, the As content in brown rice had neither significant correlation ($R^2 = 0.03$, $P = 0.07$) with the content of As immobilized by Fe (hydro)oxides on root surface nor with IAS_{root}. These results indicated that the Fe (hydro)oxides played a more important role on As immobilization on root surface than in rhizosphere, but they were not the sole factor of controlling As uptake by rice. Even though the Fe (hydro)oxides in rhizosphere soil showed a lower effectiveness than those on root surface, the former also played an important role for sequestering As. Therefore, the partition ratio (PR_{As}) of the relative tendency of As sequestration by Fe (hydro)oxides on root surface and in rhizosphere soil might be a better indicator to evaluate the effects of Fe (hydro)oxides on As uptake by rice, and our results confirmed that the As content in brown rice had a significant negative correlation with PR_{As} ($R^2 = 0.43$, $P < 0.05$) (Fig. 3).

Fractions and spatial distribution of Fe and As on root surface

As shown by above results, the Fe (hydro)oxides on root surface played an important role on the As immobilization; therefore, fractions of Fe and As on root surface were measured. The contents of crystalline and poorly crystalline Fe were

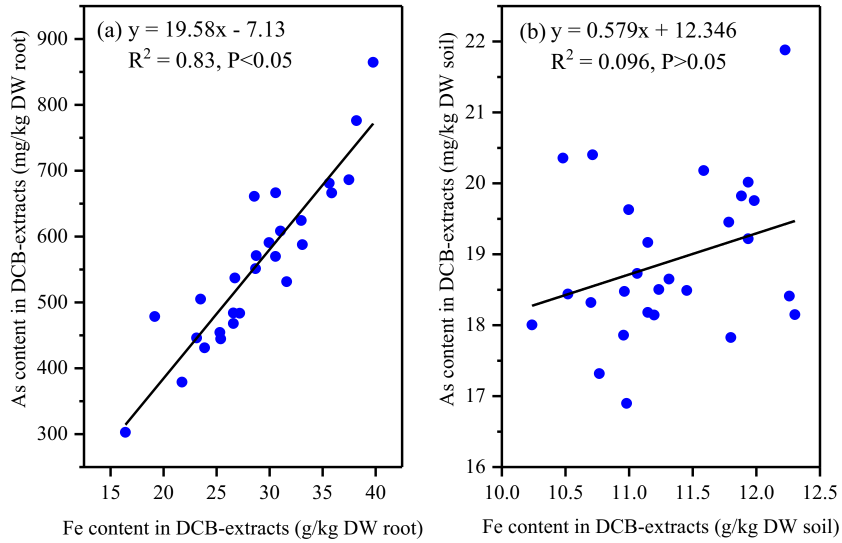

Fig. 2 Relationship between As and Fe contents in Fe (hydro)oxides on root surface (A) and in rhizosphere soil (B)

more than 98% of total Fe on root surface, and contents of crystalline Fe were a little bit higher than that of poorly crystalline Fe (Fig. 4). Meanwhile, the contents of As associated with crystalline Fe were more than 200 mg/kg (DW root) (Fig. 4) and very close to that with poorly crystalline Fe, indicating that As (88–97%) was mainly sequestrated by crystalline and poorly crystalline Fe on root surface. The fractions of Fe and As on root surface were significantly different among rice genotypes (Fig. 4).

SEM-EDS analysis showed that only a small amount of Fe entered root cells; thus, Fe was mainly deposited on root surface (Fig. 5), suggesting that the DCB-extractable Fe was mainly from root surface. SEM-EDS also showed some As entered into root cells, and more As gathered on root surface,

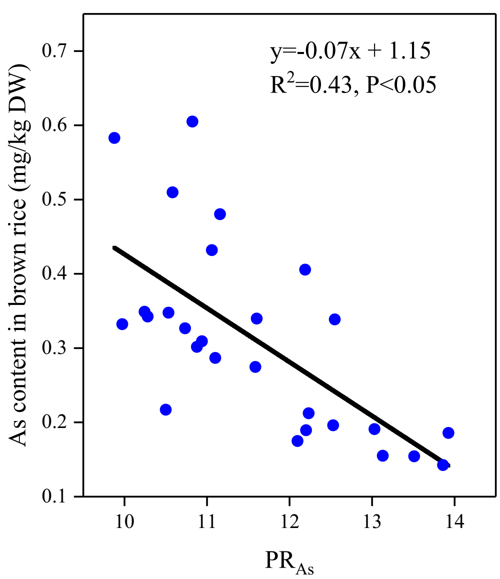

Fig. 3 Relationship between partition rates of PR_{As} and As contents in brown rice. The partition rates of PR_{As} indicate the relative tendency of As sequestration by Fe (hydro)oxides on root surface to that in rhizosphere soil

showing almost the same pattern with Fe (Fig. 5). The results directly confirmed that Fe (hydro)oxides on root surface blocked the entry of As into roots, but it was not the only determining factor.

Discussion

The As uptake by rice is affected by many factors, such as rice genotype, soil moisture, soil microbial community, pH, etc. (Pan et al. 2014; Hu et al. 2015a; Arnamwong et al. 2016; Huang et al. 2016; Liao et al. 2016; Hu et al. 2019). Our results showed that there were significantly different As concentration in brown rice among different genotypes ($p < 0.05$, $n = 27$) (Fig. 1). There had been a report about the differences in Fe (hydro)oxides on the root surface among rice genotypes (Liu et al. 2011). Our results indicated that the amount and composition of Fe and As deposited around roots were significantly different among different genotypes because of their difference in root exudates and oxygen-releasing capacities (Wu et al. 2012). Fe (hydro)oxides sequestered As and decreased the uptake of As by rice plants (Liu et al. 2004; Wu et al. 2012; Syu et al. 2013; Tripathi et al. 2014), and our results proved that the Fe amount had a significant positive correlation with As acquisition on root surface.

Previous studies on effects of Fe (hydro)oxides on As uptake by rice were mainly done in culture solution (Liu et al. 2004; Wu et al. 2012) and acid soils (Syu et al. 2013, 2014; Wu et al. 2016). However, in the case of alkaline soils where the effectiveness of As is greatly increased (Marin et al. 1993), whether can Fe (hydro)oxides be easily deposited on root surface? Our results confirmed that a large amount of Fe precipitated on the root surface and captured a lot of As in the alkaline paddy soil, but Fe (hydro)oxides were not the core factor of controlling As uptake by rice. Pervious researches

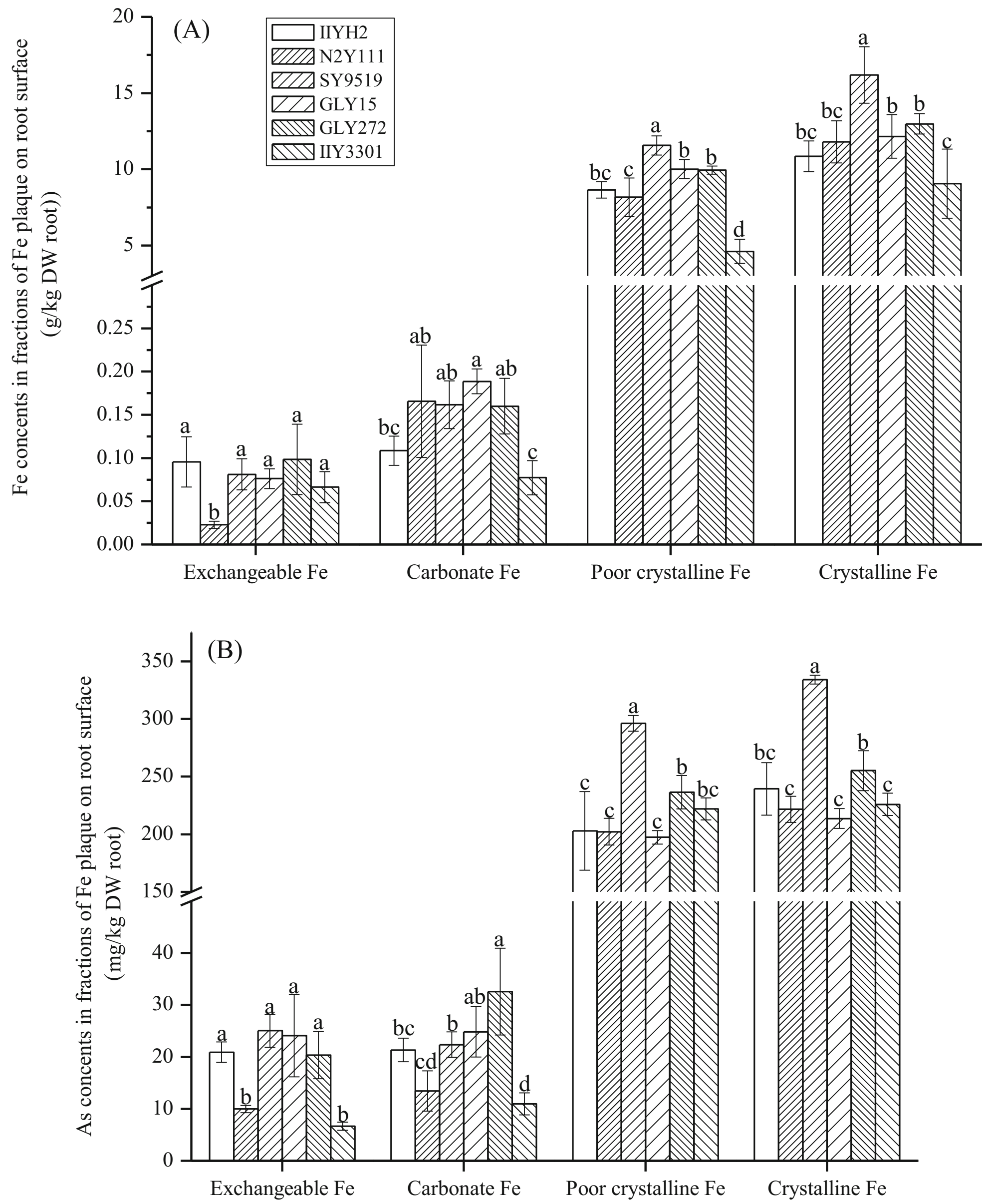

Fig. 4 Fe (A) and As (B) contents in fractions of Fe (hydro)oxides on root surface. Different letters on bars indicate significant differences ($p < 0.05$)

indicated that the diversity and abundance of microorganisms on root Fe (hydro)oxides are critical for As bioavailability (Hu et al. 2015b; Hu et al. 2019); therefore, effects of microbial community are suggested to take into consideration in future research. In addition, different forms of Fe (hydro)oxides have different affinities for immobilizing As. Liu et al. (2015) indicated that amorphous Fe oxide-bound As acts a sink of As. But our results showed that the As was mainly bound to poorly crystalline and crystalline Fe (hydro)oxides in this alkaline paddy soil.

However, there are conflicting reports on the importance of Fe (hydro)oxides to reduce the As uptake by rice plant (Liu et al. 2004; Syu et al. 2013, 2014). Syu et al. (2013) pointed out that Fe (hydro)oxides were the main controlling factor in limiting the As uptake by rice, and 73.8–90.4% of the total As released from soils was sequestered by Fe (hydro)oxides on root surface. But Syu et al. (2014) pointed out that the genotypes with higher Fe plaque formation neither retained more As nor reduced the accumulation and uptake of As by rice plants and Fe (hydro)oxides alone did not control the extent of As accumulation in rice plants. Our experiments were consistent with Syu et al. (2014). For example, contents of Fe (hydro)oxides and associated As of genotype SY9159 were significantly higher than that of genotype IIY3301, but As accumulated in brown rice of genotype SY9519 was much higher than that of genotype IIY3301 (Fig. 1). In addition, neither significant correlation was found between the As content in brown rice and the content of Fe or As on root surface nor between the As content and IAS_{root} (Table 1). Therefore, the accumulation of Fe (hydro)oxides on root surface was not

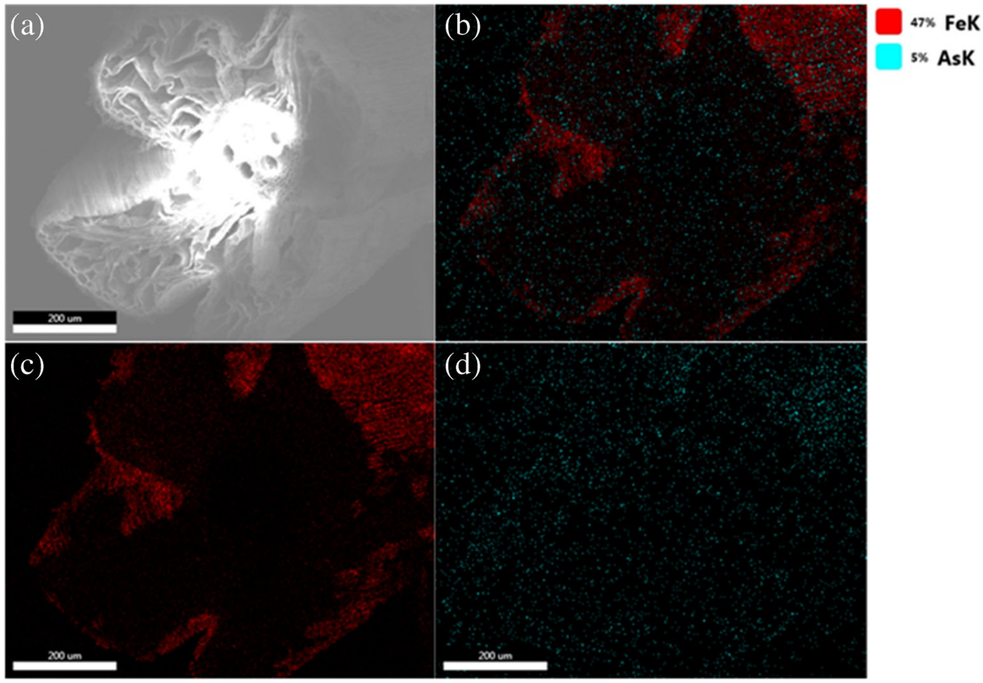

Fig. 5 Transverse distributions of Fe and As on rice root according to SEM-EDS analysis. (A) images of rice root; (B) distribution of Fe (red) and As (green); (C) distribution of Fe (red); (D) distribution of As (green)

the controlling factor in inhibiting As uptake by rice plant. The conflicting results might be due to the different formation of Fe (hydro)oxides (amount and fractions) on root surface at varieties of soil environment (pH, Eh, soil microorganisms, etc.), root anatomy, and other experimental conditions (Syu et al. 2013, 2014; Li et al. 2017). Previous research reported that as the soil pH rose, the bioavailability of As increased in soil, leading to the increase of As content in brown rice (Honma et al. 2016). Conversely, as the soil Eh increased, the bioavailability of As decreased in soil, leading to the decrease of As content in brown rice (Honma et al. 2016). The abundance and activity of As-reducing and As-oxidizing bacteria regulated the speciation and accumulation of As in rice paddies (Zhang et al. 2015). Arsenite (As(III)) could be oxidized to As(V) by heterotrophic and chemoautotrophic microorganisms (Inskeep et al. 2007). However, the abundance of arrA gene (As(III) oxidase) was found to be lower than that of arsC gene (As(V) reductase) in paddy fields (Zhang et al. 2015). As(III) was more weakly bound to soil minerals than As(V); thus, As(V) reduction resulted in the release of As into soil solutions, especially under anaerobic conditions such as in paddy soil (Dixit and Hering 2003).

In this study, we also compared the relative importance of Fe (hydro)oxides on root surface and in rhizosphere soil on As sequestration. Although Fe (hydro)oxides on root surface sequestered much more As than that in rhizosphere soil did ($IAS_{root} > IAS_{rhizo}$), the partition ratio (PR_{As}) of As sequestration by Fe (hydro)oxides on root surface and in rhizosphere soil was more related with the As in brown rice. This results confirmed the above conclusion that Fe (hydro)oxides on root surface were not the controlling factor but Fe (hydro)oxides in rhizosphere soil also played an important role. The application of Fe (hydro)oxides to increase the Fe content in rhizosphere could reduce As accumulation in rice grain (Yu et al. 2017; Xu et al. 2017). Therefore, in practice, except for choosing rice genotypes with high radial oxygen loss, we recommend changing soil conditions, such as Fe content, pH, Eh, and soil moisture, to increase the formation of Fe (hydro)oxides on root surface and in rhizosphere, so as to decrease the As uptake by rice.

Conclusions

In alkaline soils, the Fe (hydro)oxides also could easily deposit on root surface and sequester As, but Fe (hydro)oxides on root surface did not play the controlling role in lowering As uptake; meanwhile, the importance of Fe (hydro)oxides in rhizosphere should not be ignored. The partition ratio (PR_{As}) of As sequestration by Fe (hydro)oxides on root surface to that in rhizosphere soil was a better indicator to evaluate the effects of Fe (hydro)oxides on As accumulation by rice plant.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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