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# Arsenic mobility and bioavailability in paddy soil under iron compound amendments at different growth stages of rice<sup>☆</sup>



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## ABSTRACT

Iron (Fe)-based solids can reduce arsenic (As) mobility and bioavailability in soils, which has been well recognized. However, to our knowledge, there are few studies on As uptake at different growth stages of rice under Fe compound amendments. In addition, the formation of Fe plaques at different growth stages of rice has also been rarely reported. Therefore, the present study was undertaken to investigate As mobility and bioavailability in paddy soil under Fe compound amendments throughout the whole growth stage of rice plants. Amendments of poorly crystalline Fe oxides (PC-Fe), FeCl<sub>2</sub>+NaNO<sub>3</sub> and FeCl<sub>2</sub> reduced grain As by 54% ± 3.0%, 52% ± 3.0% and 46% ± 17%, respectively, compared with that of the non-amended control. The filling stage was suggested to be the key stage to take measures to reduce As uptake. At this stage, all soil amendments significantly reduced As accumulation in rice plants. At the maturation stage, PC-Fe amendment significantly reduced mobile pools and increased immobile pools of soil As. Besides, PC-Fe treatment promoted the transformation of Fe fractions from dissolved Fe to adsorbed, poorly crystalline and free Fe oxides. Moreover, significant positive correlations between soil Fe fractions and As fractions were found. Accordingly, we hypothesized that Fe compound amendments might affect the concentration distribution of Fe fractions first and then affect As fractionation in soil and its bioavailability to rice plants indirectly. The formation of Fe plaques varied with growth stages and different treatments. Significantly negative correlations between mobile pools of As and Fe or As in Fe plaques indicated that Fe plaques could immobilize mobile As in soils and thus affect As bioavailability. Overall, the effect of the soil amendments on reduction of As uptake varied with growth stages and different treatments, and further research on the key stage for reducing As uptake is still required.

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## 1. Introduction

Arsenic (As) is the most ubiquitous environmental toxin and carcinogen (Wang et al., 2015). It has been regarded as one of the top item on the US priority list of hazardous substances by the US Environmental Protection Agency (EPA) and the Agency for Toxic Substances and Disease Registry (ATSDR) (The US Environmental Protection Agency and Agency for Toxic Substances and Disease Registry, 2015). Rice consumption is considered as a major route

of As ingestion for people who rely on rice as a staple food, and this As ingestion threatens human health and deserves more attention (Meharg et al., 2009; Ma et al., 2014).

In the soil environment, Fe redox cycling significantly affects the bioavailability and mobility of As, especially in paddy soil, because of its characteristic alternating wetting and drying cycles (Yamaguchi et al., 2011; Liu et al., 2015; Yu et al., 2016a). On the one hand, the reductive dissolution of Fe(III) oxyhydroxides that occurs under flooding conditions leads to the release of adsorbed As into the soil solution (Islam et al., 2004; Liu et al., 2015; Rawson et al., 2016); on the other hand, Fe(III) oxyhydroxides produced by Fe(II) oxidation under drying conditions or secondary Fe minerals formed during Fe(III) reduction can immobilize As (Chen et al., 2008; Hohmann et al., 2009, 2011; Wang et al., 2009a; Muehe

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et al., 2016). Similarly, Fe plaques that formed at the root surface in the microaerophilic rhizosphere environment also have great significance for the mobility of As (Wang et al., 2009b; Yamaguchi et al., 2014; Hu et al., 2015). Fe plaques can sequester As because of the high specific surface area of Fe (oxyhydr)oxides and their coprecipitation with As (Liu et al., 2006). However, As that is sequestered in Fe plaques can be released into soil solution quickly as a result of Fe reductive dissolution that occurs under flooded conditions (Wang et al., 2009b). Overall, the mobility and bioavailability of As in soils depends largely on retention by Fe oxyhydroxides or on release by reductive dissolution of Fe oxyhydroxides (Zheng et al., 2013; Muehe and Kappler, 2014).

The processes of Fe redox cycling coupled to the mobilization of As are affected by many factors such as the types of Fe minerals (Dixit and Hering, 2003), the pH and Eh (Al-Abed et al., 2007; Yamaguchi et al., 2011) and the microbial species (Kocar et al., 2006; Jiang et al., 2013). Of these factors, microbial activities and diversity play important roles in As mobilization coupled to Fe redox cycling such as Fe-reducing bacteria and Fe-oxidizing bacteria, especially nitrate-dependent Fe(II) oxidizing bacteria, which can oxidize Fe(II) coupled to nitrate reduction under flooding conditions in soils. It was reported that nitrate-dependent Fe(II) oxidizers are widespread in different habitats, including paddy soils (Ratering and Schnell, 2001; Li et al., 2016) and the respiratory nitrate-reducing, Fe(III)-reducing, sulfate reducing, and methanogenic microbial communities that could all potentially contribute to the nitrate-dependent Fe(II) oxidation in anaerobic environments (Weber et al., 2006). Previous studies also showed that nitrate-dependent Fe(II)-oxidizing bacteria can effectively immobilize As during Fe(II) oxidation (Hohmann et al., 2009; Sun et al., 2009; Hitchcock et al., 2012). Accordingly, we speculated that simultaneous amendments of nitrate and Fe(II) might promote As immobilization in paddy soil, which requires further investigation.

Previous studies have shown that Fe compounds such as Fe(II), goethite, ferrihydrite, hematite, and ferrous or ferric sulfate all can effectively reduce the bioavailability and mobility of As in soils (Kim et al., 2003; Hartley and Lepp, 2008; Kumpiene et al., 2008; Chen et al., 2014; Garau et al., 2014; Simón et al., 2015). These amendments can reduce mobile As and increase poorly crystalline/crystalline Fe oxide-bound As via adsorption, complexation or coprecipitation with As and also enhance the binding of As to the Fe plaques on the root surfaces. However, to our knowledge, there is little information about As uptake at different growth stages of rice under Fe compound amendments. In addition, there are few reports on the key growth stage to reduce As uptake under Fe compound amendments. Generally, the whole growth period of rice plants lasts for approximately 150 days and comprises seven growth stages, i.e., the seedling stage, tillering stage, jointing stage, booting stage, flowering stage, filling stage and maturation stage (Li et al., 2015b). Besides, the seeding, tillering and jointing stages are considered as vegetative stage and the last four stages are considered as reproductive stage. Rice grows actively from tillering stage to filling stage and the powerful root system of rice has formed before booting stage. At the maximum tiller number stage (tillering stage), the ability to secrete oxygen for the root system of rice reaches a peak and at this stage, the amounts of Fe plaques formed are the largest. As indicated in a previous study, As uptake in rice plants varied with the growth stages (Zheng et al., 2011). Accordingly, it is highly necessary to investigate As uptake at different growth stages of rice under Fe compound amendments. In addition, the formation of Fe plaques also varies with growth stage because the release of O<sub>2</sub> from rice roots into rhizosphere soil depends on the age of the roots (Zhang et al., 2012). However, the formation of Fe plaque at different growth stages of rice has rarely been reported. Therefore, the objective of this study were (i) to compare

the effects of different soil amendments including poorly crystalline Fe oxide (PC-Fe), FeCl<sub>2</sub>, FeCl<sub>2</sub>+NaNO<sub>3</sub> on the mobility and bioavailability of As in paddy soils throughout the whole growth stage; (ii) to compare the effects of different soil amendments on Fe plaque formation and As sequestration by Fe plaques throughout the whole growth stage; and (iii) to evaluate the biomass and As accumulation in different rice tissues at different growth stages under Fe compound amendments.

## 2. Materials and methods

### 2.1. Experimental design and sampling

Five treatments with three replicates for each treatment were set up as follows: (1) untreated soil (control), (2) PC-Fe (5 g/kg dry weight of soil, preparation with 0.1 M Fe(NO<sub>3</sub>)<sub>3</sub> and 1 M NaOH at pH 7.0) + soil (3) FeCl<sub>2</sub> (0.54 mM/kg dry weight of soil) + soil, (4) NaNO<sub>3</sub> (7.5 mM/kg dry weight of soil) + soil, and (5) FeCl<sub>2</sub> (0.54 mM/kg dry weight of soil) + NaNO<sub>3</sub> (7.5 mM/kg dry weight of soil) + soil. These treatments are designated as the control, PC-Fe, FeCl<sub>2</sub>, NaNO<sub>3</sub>, and FeCl<sub>2</sub>+NaNO<sub>3</sub> sequentially hereafter. The soil with a total As concentration of 132 mg/kg used for pot experiments was collected from rice paddy fields in the downstream area of the Lianhuashan tungsten mine, which is located in the (sub) tropical areas within the Guangdong province of China. The analysis methods for soil properties have been reported in previous studies (Chang et al., 2014) and detailed information can be found in Table S1. After being air-dried, the soil samples for cultivation and chemical analysis were passed through 5 mm and 2 mm mesh sieves, respectively. Approximately 6 kg of the soils that were mixed well with amendments were placed in each pot (8 cm in diameter and 15 cm in height) and submerged in water (2–3 cm above the soil surface) for a week before the rice seedlings were transplanted. In addition, chemical fertilizers including N, P and K (N: P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O = 1.5: 1: 1.5) were applied at a rate of 0.15 g/kg dry weight of soil. To minimize the effect of N application, N fertilizer was applied by spraying urea at the rate of 0.3% (rice plant in each pot was sprayed with 1 L 0.3% urea). The rice cultivar (U U 128) was grown, and when there were three leaves, the prepared seedlings were transplanted to pots containing four seedlings per pot. To separate the rhizosphere soil from the bulk soil, a nylon mesh bag (height of 20 cm, diameter of 80 mm, containing 600 g soil) was placed in the central of each pot (Ultra et al., 2009). The pot soil was maintained under flooded conditions (with 2 cm of water above the soil surface) during the whole growth period. Rhizosphere soil, rice plant and Fe plaque samples were collected at the four different growth stages (the tillering stage (April 25, 2013, day 15 after transplantation), jointing stage (May 10, 2013, day 30 after transplantation), filling stage (July 25, 2013, day 106 after transplantation) and maturation stage (August 20, 2013, day 132 after transplantation)).

### 2.2. As/Fe fractionation and the total As/Fe concentration in soil

A sequential As/Fe fractionation scheme was employed to determine the following operationally defined As/Fe fractions: dissolved As/Fe, which was determined by adding 20 ml of ultrapure water (pH = 7.0) to 1 g of soil and shaking for 16 h, followed by centrifugal separation at 4500 r/min for 30 min, filtration, and the collection of the filtrate to determine the concentration of As/Fe. The HCl-extractable As/Fe was determined by adding 25 ml of 0.5 M HCl to 0.5 g of soil and shaking for 4 h, followed by filtration and the collection of the filtrate to determine the As, Fe and Fe(II) concentrations. The dithionite-citrate-bicarbonate (DCB)-extractable As/Fe was determined by adding 1.0 g soil to 50-ml

polyethylene centrifuge tubes and treating with 20 ml of DCB solution in a 75 °C water bath with shaking at 120 rpm for 10 min. Then, 1.0 g of sodium dithionite was added, and after shaking the mixture for 5 min, 1.0 g of sodium dithionite was added again and shaken for another 10 min. The mixture was then centrifuged at 2500 rpm for 5 min, and the supernatant was collected in a 50-ml volumetric flask (if the supernatant was turbid, a saturated solution of potassium chloride or sodium chloride was added and the mixture was centrifuged at 2500 rpm for 5 min). A 20-ml volume of DCB solution was added to the residue and the extraction procedure above was repeated, and then the whole supernatant was collected to determine the As/Fe concentration. To determine the poorly crystalline Fe and poorly crystalline Fe-bound As (oxalate-extractable As), 0.5 g of soil was added to 50-ml polyethylene centrifuge tubes, and the tubes were placed in a shaker and shaken for 4 h at 25 °C with 25 ml of oxalic acid-ammonium oxalate buffer solution and then filtered to determine the As/Fe concentration. For sorbed As ( $\text{NH}_4\text{H}_2\text{PO}_4$ -extractable As), the extraction procedure for this fraction has been reported in previous studies (Khan et al., 2010; Liu et al., 2015). In brief, 1.0 g of soil was extracted with 25 ml of 0.05 M  $\text{NH}_4\text{H}_2\text{PO}_4$  solution in 50-ml centrifugation tubes, which were centrifuged, and all the supernatant was collected to determine the As concentrations. To evaluate the total As content in the soil, approximately 0.5 g of each soil sample was digested in 5 ml of  $\text{HNO}_3$  and  $\text{HClO}_4$  (4:1, v:v) at 140–160 °C and then analyzed with a hydride generation-atomic fluorescence spectrometer (AFS-820, Beijing Titan Instruments Co., China). For the total Fe content, approximately 0.1 g of each soil sample was digested by mixed acids ( $\text{HF}:\text{HNO}_3 = 7:1$ , v:v) until nearly dry. Then, 5 ml HF was added and the mixture was digested continually until nearly dry. Subsequently, 2 ml  $\text{HClO}_4$  was added and the samples were digested continually until they reached complete digestion. Finally, 1 ml  $\text{HNO}_3$  was added to dissolve the residue, which was transferred to a volumetric flask to analyze with an atomic absorption spectrometer.

### 2.3. As concentrations in rice plants

For each treatment and growth stage, there were three pots with four rice plants in each pot and total 60 pots of rice plants were planted. Four rice plants in each pot were collected and mixed as one sample. The rice plants were divided into different rice tissues, e.g., rice plants sampled at maturity were divided into grain, straw and root fractions. All the plant materials were washed with tap water and then rinsed with deionized water more than three times. After being dried, they were ground in a carnelian mortar before further As analysis. The analysis methods for As in rice plants have been described in detail in previous studies (Liu et al., 2010, 2015). In brief, plant samples of approximately 1 g each were digested with 1.0 ml of  $\text{HClO}_4$ , 1.5 ml of  $\text{H}_2\text{SO}_4$  and 4.0 ml of  $\text{HNO}_3$  at 110–130 °C until a clear solution was obtained. The total As concentrations were analyzed with a hydride generation-atomic fluorescence spectrometer (AFS-820, Beijing Titan Instruments Co., China).

### 2.4. Root Fe plaque extraction and As/Fe concentrations in Fe plaque

Fe plaque was extracted from the roots using DCB solution as described previously (Liu et al., 2006; Hu et al., 2015). After DCB extraction, the roots were oven-dried at 70 °C for 3 days and weighed. The As and Fe concentrations in the DCB extracts were measured with a hydride generation-atomic fluorescence spectrometer and an atomic absorption spectrometer, respectively.

### 2.5. Statistical analyses

Statistical analyses of the experimental data were performed with SPSS® 18.0 (SPSS, USA) software. The statistical significance of the differences between different treatments or at different growth stages of rice plants were determined by analysis of variance with  $p < 0.05$ . The correlation analysis was conducted by Pearson correlation with a significance level at  $p < 0.05$  (two-tailed). The amounts of As in the rice plants ( $A_{sT}$ , mg) and the transfer coefficient (TF, %) were estimated with the following equations:

at tillering stage, jointing stage and filling stage:

$$A_{sT} = A_{s\text{straw}} \times W_{\text{straw}} + A_{s\text{root}} \times W_{\text{root}} \quad (1)$$

$$TF = \frac{A_{s\text{straw}} \times W_{\text{straw}}}{A_{s\text{root}} \times W_{\text{root}}} \times 100 \quad (2)$$

at maturation stage:

$$A_{sT} = A_{s\text{straw}} \times W_{\text{straw}} + A_{s\text{root}} \times W_{\text{root}} + A_{s\text{grain}} \times W_{\text{grain}} \quad (3)$$

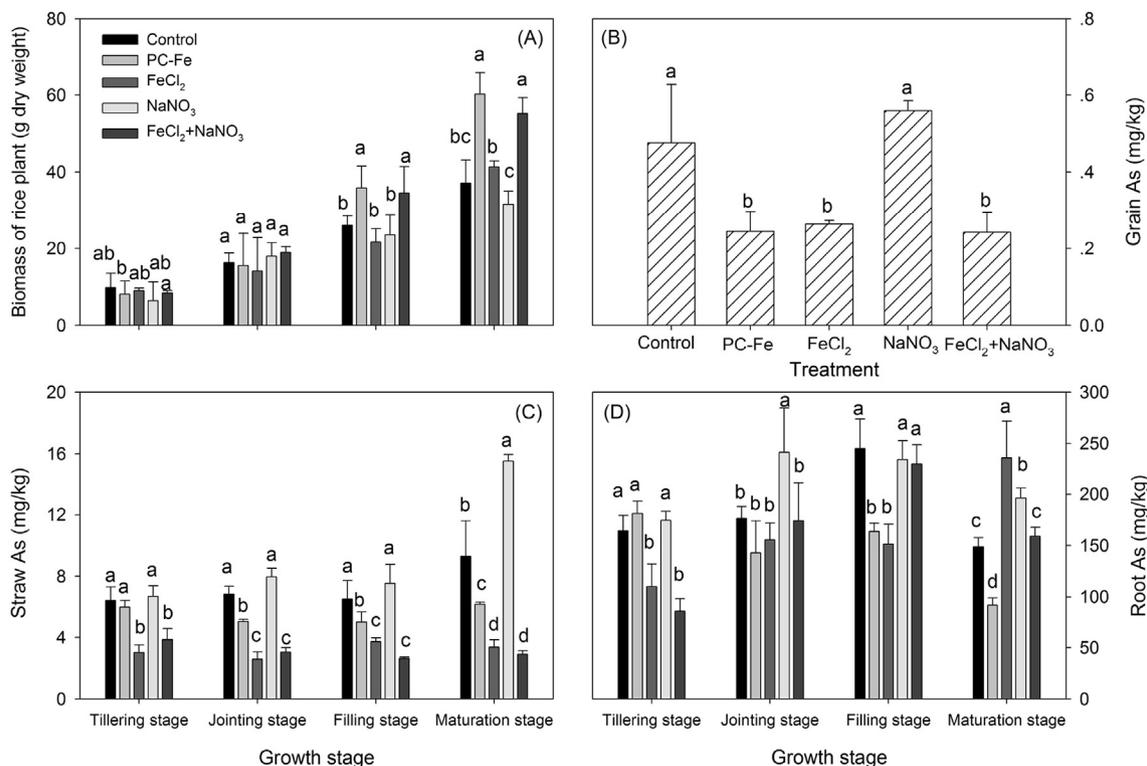
$$TF = \frac{A_{s\text{straw}} \times W_{\text{straw}} + A_{s\text{grain}} \times W_{\text{grain}}}{A_{s\text{root}} \times W_{\text{root}}} \times 100 \quad (4)$$

where  $A_{s\text{straw}}$ ,  $A_{s\text{root}}$  and  $A_{s\text{grain}}$  are the concentrations (mg/kg) of As in rice straws, roots (without Fe plaque) and grains, respectively.  $W_{\text{straw}}$ ,  $W_{\text{root}}$  and  $W_{\text{grain}}$  are the dry weights (kg) of rice straws, roots (without Fe plaque) and grains, respectively.

## 3. Results

### 3.1. Biomass and As accumulation for rice plants under different treatments

The biomass showed an increasing trend with the growth stages of rice plant, and at each growth stage, the soil amendments exerted different impacts on the accumulation of dry matter in the rice plants (Fig. 1A). For example, adding PC-Fe and  $\text{FeCl}_2+\text{NaNO}_3$  significantly increased the dry matter yields of rice plants by 62% and 49%, respectively, at the maturation stage in comparison with that of the control. Similarly, compared with the biomass of the control, 38% and 32% increases were observed for PC-Fe and  $\text{FeCl}_2+\text{NaNO}_3$  amendments, respectively at the filling stage. By contrast, the  $\text{NaNO}_3$  amendment significantly reduced the dry matter yields of rice plants by 15% at the maturation stage, compared with that of the control. The As concentrations in above-ground rice tissues, especially in grains, were much lower than those in the roots (Fig. 1), indicating that As was primarily accumulated in the roots, which was consistent with our previous studies (Liu et al., 2015; Yu et al., 2016b). The As concentrations in straw were relatively steady during the first three growth stages for all the treatments, but at the maturation stage under  $\text{NaNO}_3$  treatment, they increased markedly (Fig. 1C). Comparatively, the As concentrations in rice roots in the control,  $\text{NaNO}_3$  and  $\text{FeCl}_2+\text{NaNO}_3$  treatments increased during the first three growth stages and decreased at the maturation stage (Fig. 1D). By contrast, under PC-Fe treatment, they showed a decreasing trend throughout the growth stage of the rice plant, especially at the maturation stage, and for the maturation stage under  $\text{FeCl}_2$  treatment, the As concentrations increased substantially. In particular, at the maturation stage,  $31\% \pm 22\%$ ,  $68\% \pm 7.8\%$  and  $64\% \pm 6.0\%$  reductions were found for As concentrations in straw, and  $54\% \pm 3.0\%$ ,  $52\% \pm 3.0\%$  and  $46\% \pm 17\%$  reductions were observed for As concentrations in grain, relative to that in the control for the PC-Fe,  $\text{FeCl}_2+\text{NaNO}_3$  and  $\text{FeCl}_2$



**Fig. 1.** Biomasses of rice plants and As concentrations in rice plant tissues under different soil amendments (control, poorly crystalline Fe oxide (PC-Fe), FeCl<sub>2</sub>, NaNO<sub>3</sub>, FeCl<sub>2</sub>+NaNO<sub>3</sub>). The data are the means  $\pm$  SD ( $n = 3$ ). Within each group of five values, any two means with different letters and the same letter indicate a significant difference and no significant difference between the treatments at a  $p < 0.05$  level, respectively.

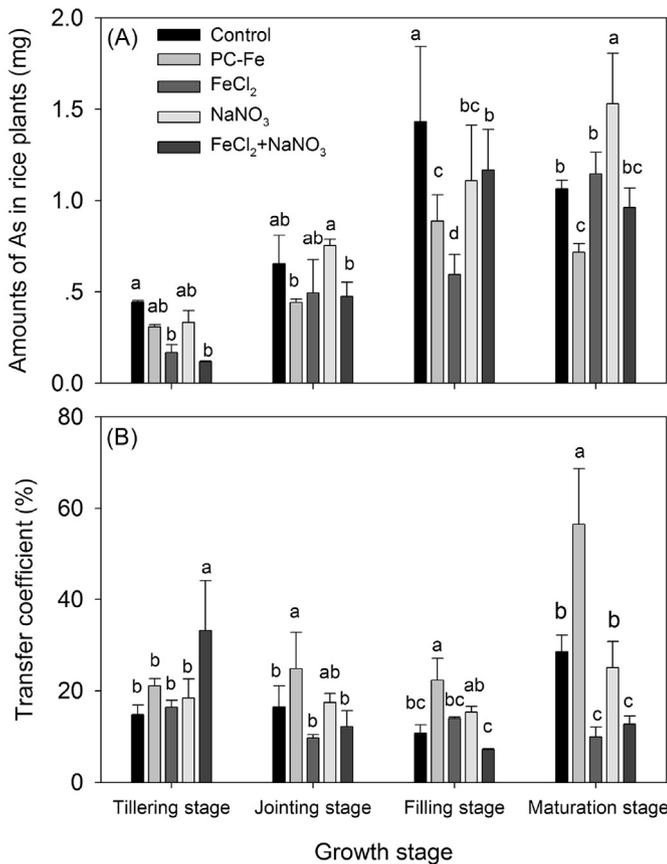
treatments, respectively. However, the As concentrations in roots at the maturation stage for the FeCl<sub>2</sub>, NaNO<sub>3</sub>, and FeCl<sub>2</sub>+NaNO<sub>3</sub> treatments were increased by  $49\% \pm 17\%$ ,  $32\% \pm 2.6\%$  and  $7.2\% \pm 1.4\%$ , respectively, compared with that of the control.

The amounts of As accumulation during the four growth stages of rice plants were calculated on the basis of the As concentrations and dry weights of rice plant tissues. These quantities showed an increasing trend during the first three rice growth stages under all the treatments, whereas a decreasing trend was found for the control, PC-Fe and FeCl<sub>2</sub>+NaNO<sub>3</sub> and an increasing trend was observed for the FeCl<sub>2</sub> and NaNO<sub>3</sub> treatments at the maturation stage (Fig. 2A). It appears incomprehensible that As accumulation decreased at the maturation stage under the control, PC-Fe and FeCl<sub>2</sub>+NaNO<sub>3</sub> compared with those at the filling stage. Comparison of As accumulation in different rice tissues showed that As accumulation in rice root was predominant and concentrations of As in root at the maturation stage under the control, PC-Fe and FeCl<sub>2</sub>+NaNO<sub>3</sub> were significantly lower than those at the filling stage, respectively (Fig. S1). Since the rice plants at the different growth stages were collected in different pots, the significant decreases of root As at the maturation stage may be related to ontogenetic variation in rice plants and As detoxification in physiology. According to the reports concerning As detoxification in rice plant (Duan et al., 2012; Zangi and Filella, 2012; Kumar et al., 2015), extrusion of As is the major As detoxification mechanism. The amounts of As in rice plants under PC-Fe and FeCl<sub>2</sub>+NaNO<sub>3</sub> treatments over the four growth stages were significantly or slightly lower than that of the control, and at the filling stage, all the soil amendments significantly reduced As accumulation in the rice plants (Fig. 2A). The TF was defined as the amount of As in the above-ground rice tissues divided by the amount of As in the rice roots, and the TF values were relatively steady in the FeCl<sub>2</sub> and

NaNO<sub>3</sub> treatments throughout the four growth stages of the rice plants. Conversely, at the jointing stage under FeCl<sub>2</sub>+NaNO<sub>3</sub> treatment, these values showed a rapidly declining trend compared with those at the tillering stage and then became steady. At the maturation stage under PC-Fe treatment, the TF increased dramatically (Fig. 2B). It is worth noting that although the amount of As accumulation in rice plants under PC-Fe treatment was significantly or slightly lower than that of the control, the TF of As under the PC-Fe treatment was significantly or slightly higher than that in the control (Fig. 2B). These results indicated that Fe compound amendments can affect As accumulation from soil to rice plants and As distribution in rice plants simultaneously, and these two effects were not consistent. In our opinion, the high percentage of transfer at maturation in the case of PC-Fe amendment should be mainly related to the significantly lower As concentration in the roots at the maturation stage under the PC-Fe amendment compared with the other treatments (Fig. 1D). Generally speaking, all soil amendments can effectively reduce the As accumulation at the filling stage, whereas at the maturation stage, only the PC-Fe treatment exhibited the reduction effect.

### 3.2. Fe fractions in rhizosphere soils throughout the whole growth stage for different treatments

The soil Fe fractions significantly affected As bioavailability and mobility, which has been supported by our previous study, which was also conducted in the same sampling region as the present study (Liu et al., 2015). The concentrations of dissolved Fe in the rhizosphere soils varied with growth stages of rice plants and different treatments ranging from 1.1 to 24 mg/kg. At the jointing and filling stages, the concentrations of dissolved Fe showed an increasing trend under all the treatments (Fig. 3A). At the



**Fig. 2.** Amounts of As accumulation in rice plants and the transfer coefficient, which is defined as the amount of As in the rice plant tissues above the ground divided by the amount of As in the rice roots under different treatments (control, poorly crystalline Fe oxide (PC-Fe), FeCl<sub>2</sub>, NaNO<sub>3</sub>, and FeCl<sub>2</sub>+NaNO<sub>3</sub>) during the rice growth stages. The data are the means  $\pm$  SD ( $n = 3$ ). Within each group of five values, any two means with different letters and the same letter indicate a significant difference and no significant difference between the treatments at a  $p < 0.05$  level, respectively.

maturation stage, they decreased slightly in the PC-Fe, FeCl<sub>2</sub> and FeCl<sub>2</sub>+NaNO<sub>3</sub> treatments compared with the dissolved Fe at the filling stage in the PC-Fe, FeCl<sub>2</sub> and FeCl<sub>2</sub>+NaNO<sub>3</sub> treatments, respectively (Fig. 3A). HCl-extractable Fe(II) is thought to be primarily derived from the microbial reduction of poorly crystalline Fe(III) oxyhydroxide or the poorly ordered Fe(III) oxyhydroxide, ferrihydrite (Lovley and Phillips, 1986). Thus, the amount of HCl-extractable Fe(II) can be used to assess the ability of microorganisms to reduce poorly crystalline Fe(III) oxyhydroxide. The concentrations of HCl-extractable Fe(II) in the PC-Fe treatment at the jointing and filling stages were significantly higher than those in the other treatments, and no significant difference was found between different treatments at the maturation stage (Fig. 3B). Similarly, concentrations of HCl-extractable Fe(III) in the PC-Fe treatment throughout the whole growth stage were also significantly higher than those in the other treatments (Fig. S2). Oxalate can extract the poorly crystalline Fe forms that are available for microbial reduction in soils, and the concentrations of oxalate-extractable Fe under the PC-Fe treatment at the first three growth stages were significantly higher than those in the control; no significant difference was found between different treatments at the maturation stage (Fig. 3C). As for the DCB-extractable Fe, it is considered as free Fe oxides, including crystalline and poorly crystalline Fe oxides (Munch and Ottow, 1980). Similar to oxalate-extractable Fe, the concentrations of DCB-extractable Fe in the

PC-Fe treatment throughout the whole growth stage were significantly higher than those in the other treatments (Fig. 3D).

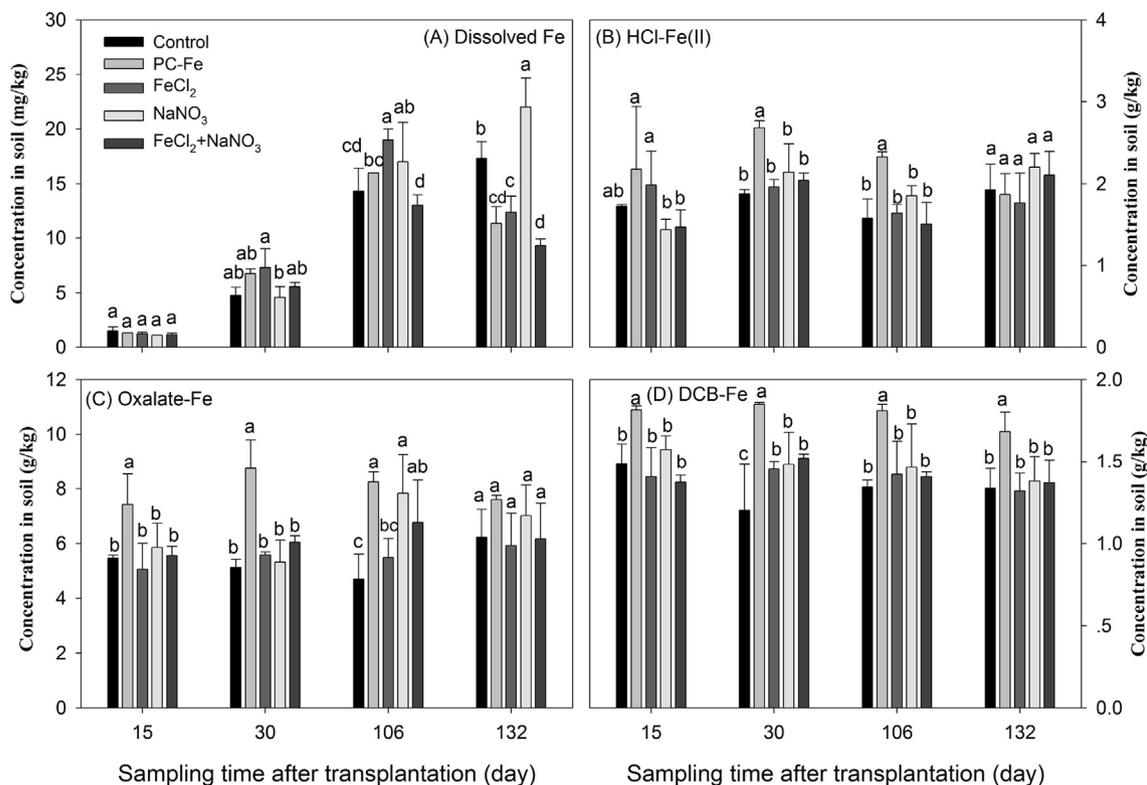
### 3.3. As fractionation in rhizosphere soils over all growth stages under different treatments

The concentrations of dissolved As in the rhizosphere soils of the four growth stages of rice plants varied from 0.047 to 2.0 mg/kg. At the filling and maturation stages for the PC-Fe, FeCl<sub>2</sub> and FeCl<sub>2</sub>+NaNO<sub>3</sub> treatments, the concentrations of dissolved As were significantly lower than those in the control, whereas at the tillering and jointing stages, there were no significant differences between different treatments (Fig. 4A). The As fraction extracted with NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>, which is identified as a suitable predictor of As availability in soils (Huang et al., 2006; Liu et al., 2015), also showed significant variation under different treatments at the whole growth stage. Concentration of NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>-extractable As(III) under the PC-Fe treatment at the jointing stage was significantly higher than those in the other sampling times (Fig. S4). By contrast, under FeCl<sub>2</sub> and FeCl<sub>2</sub>+NaNO<sub>3</sub> treatments, NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>-extractable As(III) reached their significantly higher concentrations at the filling stage (Fig. S4). Notably, they were reduced markedly at the maturation stage in the PC-Fe, FeCl<sub>2</sub> and FeCl<sub>2</sub>+NaNO<sub>3</sub> treatments, and as a result, the NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>-extractable As(III) concentrations under these treatments were significantly lower than that of the control (Fig. 4B). The concentration of HCl-extractable As, which is considered as the portion of As that is adsorbed by Fe oxides and is likely to be released under reducing conditions, varied from 14 to 42 mg/kg. For the PC-Fe treatment, HCl-extractable As exhibited a significantly decreasing trend at the filling stage, and it also decreased sharply at the maturation stage under FeCl<sub>2</sub> treatment and at the filling stage under FeCl<sub>2</sub>+NaNO<sub>3</sub> treatment compared with those at the filling stage under FeCl<sub>2</sub> treatment and the jointing stage under FeCl<sub>2</sub>+NaNO<sub>3</sub> treatment, respectively (Fig. S4). At the maturation stage, concentrations of HCl-extractable As in the PC-Fe and NaNO<sub>3</sub> treatments were significantly higher than that of the control (Fig. 4C).

The poorly crystalline Fe oxide-bound As was extracted with oxalic acid-ammonium oxalate buffer solution. The availability of this As depends on soil properties and environmental factors (Hsu et al., 2012), and it also fluctuated throughout the whole growth stage of rice plants. At the jointing stage, all the soil amendments increased the concentrations of oxalate-extractable As, whereas at the filling stage, no significant difference was found between the soil amendments (except for NaNO<sub>3</sub>) and the control (Fig. 4D). At the maturation stage, the PC-Fe treatment significantly increased concentrations of oxalate-extractable As compared with the control. The concentrations of Fe oxide-bound As that were extracted by DCB solution in the PC-Fe, FeCl<sub>2</sub> and FeCl<sub>2</sub>+NaNO<sub>3</sub> treatments all showed a decreasing trend at the filling stage, with the value for the PC-Fe treatment being significantly higher than those of the other treatments (except for NaNO<sub>3</sub>) (Fig. 4E). At the maturation stage, the PC-Fe amendment also significantly increased the concentrations of DCB-extractable As in comparison with the other treatments.

### 3.4. As and Fe variations in Fe plaques at the whole growth stage under different treatments

Fe plaques have been demonstrated to have substantial implications for the environmental fate of As according to greenhouse and field experiments (Yamaguchi et al., 2014; Yu et al., 2016a). The amount and composition of Fe plaques vary with growth stage of rice plants and thus affect As uptake (Zhang et al., 2012). The Fe concentrations in Fe plaques decreased sharply at the jointing stage



**Fig. 3.** Concentrations of Fe fractions in soils, including dissolved Fe, HCl-extractable Fe(II) (HCl-Fe(II)), dithionite-citrate-bicarbonate (DCB)-extractable Fe (DCB-Fe) and oxalate-extractable Fe (oxalate-Fe) at the tillering stage (15 days after transplantation), jointing stage (30 days after transplantation), filling stage (106 days after transplantation) and maturation stage (132 days after transplantation) under different treatments (control, poorly crystalline Fe oxide (PC-Fe), FeCl<sub>2</sub>, NaNO<sub>3</sub>, and FeCl<sub>2</sub>+NaNO<sub>3</sub>). The data are the means  $\pm$  SD ( $n = 3$ ). Within each group of five values, any two means with different letters and the same letter indicate a significant difference and no significant difference between the treatments at a  $p < 0.05$  level, respectively.

compared with those at the tillering stage and then became relatively steady at the filling and maturation stages (Fig. 5A). At the tillering stage for the PC-Fe and FeCl<sub>2</sub> treatments, the Fe concentrations were significantly higher than that of the control, whereas no significant difference was found between different treatments over the last three growth stages (Fig. 5A). The significantly higher Fe in Fe plaques under the PC-Fe and FeCl<sub>2</sub> treatments may be related to two aspects: (1) PC-Fe and FeCl<sub>2</sub> amendments provided more Fe(III) and Fe(II) sources to Fe plaque formation; (2) PC-Fe and FeCl<sub>2</sub> amendments could activate some microbial community such as iron reducing and iron oxidizing bacteria which further promote Fe plaque formation.

The As concentrations that were adsorbed to Fe plaques also showed clear variations throughout the whole growth stage under different treatments. Concentrations of As in Fe plaques increased rapidly at the jointing stage under PC-Fe and FeCl<sub>2</sub>+NaNO<sub>3</sub> treatments, and at the filling stage, they increased slightly and decreased rapidly, respectively. The As sequestered in Fe plaques under FeCl<sub>2</sub> treatment at the tillering and jointing stages were significantly higher than those in the control, while no significant difference was found between FeCl<sub>2</sub> treatment and the control at the filling stage (Fig. 5B). Notably, As concentrations in Fe plaques decreased markedly at the maturation stage for all the treatments (Fig. 5B). At the jointing and filling stages under PC-Fe treatment, they were significantly higher than those in the other treatments (Fig. 5B), and no significant difference between different treatments was found at the maturation stage. In addition, significantly positive correlations were found between the As and Fe concentrations in Fe plaques under the different treatments in the four growth stages of rice plants (Fig. S5), indicating an important role

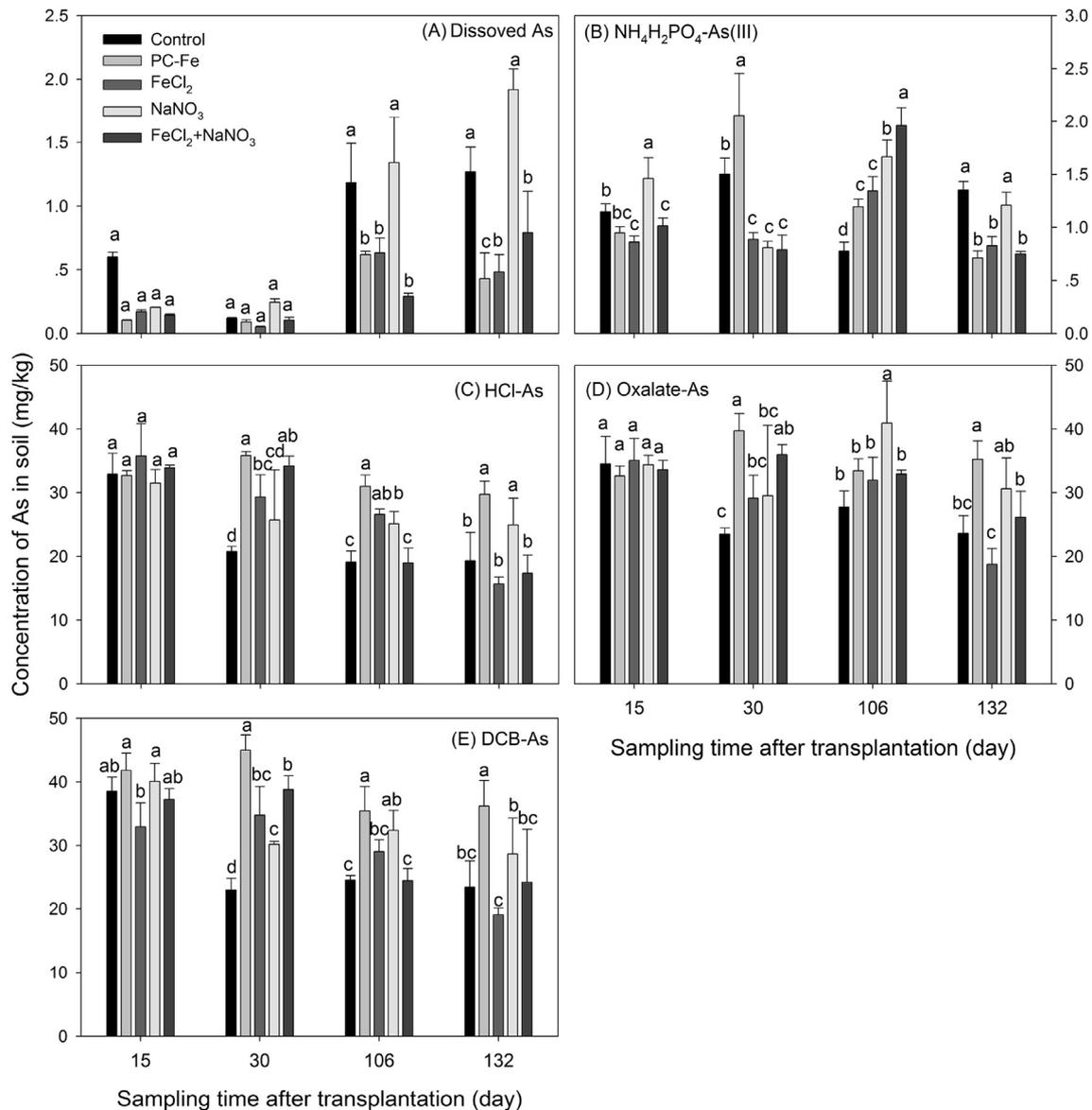
for Fe plaques in As sequestration.

## 4. Discussion

### 4.1. Effects of the growth stage on As accumulation in rice plants under different treatments

The As accumulation amounts in rice plants at the filling stage were significantly higher than those at the other growth stages in the control, PC-Fe, and FeCl<sub>2</sub>+NaNO<sub>3</sub> treatments, indicating the importance of this stage and its contribution to total As accumulation (Fig. S6). The net accumulation amounts of As at different growth stages were also calculated (average accumulation amount of As at one growth stage minus that at the previous growth stage). For the control, PC-Fe, and FeCl<sub>2</sub>+NaNO<sub>3</sub> treatments, the highest levels of net accumulation amount of As occurred at the filling stage (Fig. S6). Significantly positive correlations between the concentration of dissolved As at the filling stage and grain As or straw As at the maturation stage (Fig. S7) also indicated the important contribution of bioavailable As at the filling stage to As uptake in rice plants at the final stage. Noteworthy, the 76-day period from the end of the tillering stage to grain filling designated as the filling stage in our study also consists of the booting and flowering stages, and during this period, the rice plants grew vigorously, as characterized by a very rapid increase in biomass at the filling stage (Fig. 1A).

More As accumulation at the filling stage (106 days after transplantation) under the control, PC-Fe, and FeCl<sub>2</sub>+NaNO<sub>3</sub> treatments (Fig. S6) may be related to the following two aspects: first, the rice grew actively at this stage, and the uptake capacities of



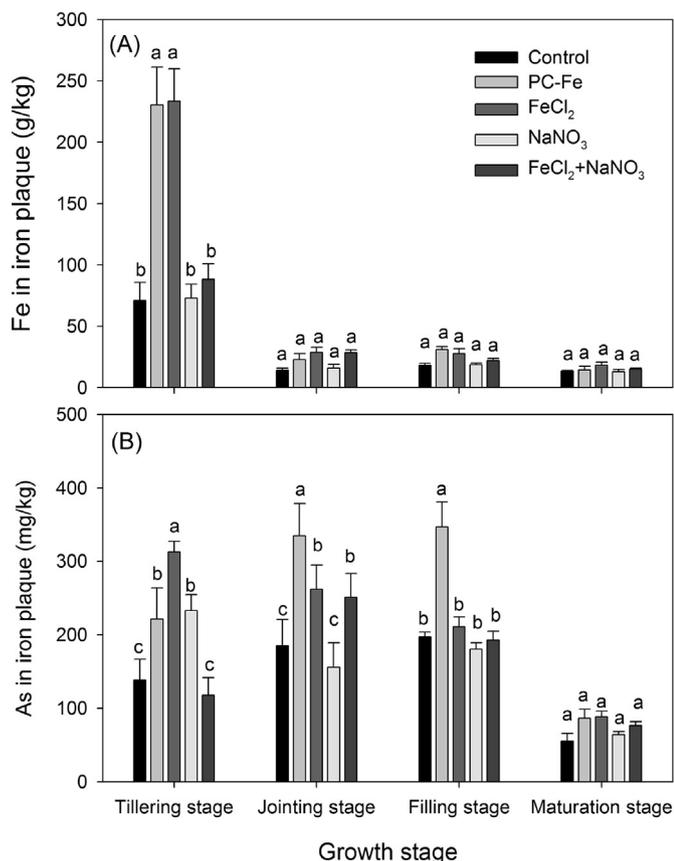
**Fig. 4.** Concentrations of As fractions in soils, including dissolved As,  $\text{NH}_4\text{H}_2\text{PO}_4$ -extractable As(III) ( $\text{NH}_4\text{H}_2\text{PO}_4$ -As(III)), HCl-extractable As (HCl-As), oxalate-extractable As (oxalate-As) and dithionite-citrate-bicarbonate (DCB)-extractable As (DCB-As) at the tillering stage (15 days after transplantation), jointing stage (30 days after transplantation), filling stage (106 days after transplantation) and maturation stage (132 days after transplantation) under different treatments (control, poorly crystalline Fe oxide (PC-Fe),  $\text{FeCl}_2$ ,  $\text{NaNO}_3$ , and  $\text{FeCl}_2+\text{NaNO}_3$ ). The data are the means  $\pm$  SD ( $n = 3$ ). Within each group of five values, any two means with different letters and the same letter indicate a significant difference and no significant difference between the treatments at a  $p < 0.05$  level, respectively.

nutritional elements as well as hazardous elements were increased (Guo et al., 2002). Second, the powerful root system at this stage increased the contact area of roots with soil and thus promoted As uptake (Li et al., 2015b). In addition, all soil amendments significantly decreased As uptake at the filling stage (Fig. 2A). Furthermore, the TF values showed a decreasing trend at the filling stage, indicating a lower translocation of As from roots to shoots at this stage (Fig. 2B). All these results suggested that the filling stage may be the key stage to take measures to reduce As uptake. Nevertheless, this is a preliminary result because no sample was collected in our study at the booting and flowering stages, which are the two stages between the tillering and filling stages. A previous study (Li et al., 2015b) reported that the jointing and booting stages were the key stages for As uptake. By contrast (Hu, 2014), found that As accumulations in rice grains, roots and husks primarily occurred at the filling and maturation stages. Therefore, further research is

required to examine the key stage for reducing As uptake.

#### 4.2. pH effect on As accumulation in rice plants under soil amendments

The amounts of As uptake in the whole rice plants or the As concentrations in the rice plant tissues under  $\text{NaNO}_3$  treatment were significantly higher than or comparable to those in the other treatments at most of the growth stages (Figs. 1 and 2). However, the As uptake by rice plants under  $\text{FeCl}_2$  treatment was significantly lower than or comparable to those in the other treatments at most of the growth stages (Figs. 1 and 2). This difference may be related to pH changes that occurred in response to soil amendments (Fig. S8). The pH in the rhizosphere soils decreased at the jointing stage under all the treatments, and then it rose markedly at the filling stage under  $\text{NaNO}_3$  treatment and rose slightly under the PC-



**Fig. 5.** Concentrations of As and Fe in Fe plaques under different treatments (control, poorly crystalline Fe oxide (PC-Fe), FeCl<sub>2</sub>, NaNO<sub>3</sub>, and FeCl<sub>2</sub>+NaNO<sub>3</sub>) at the four rice growth stages. The data are the means  $\pm$  SD (n = 3). Within each group of five values, any two means with different letters and the same letter indicate a significant difference and no significant difference between the treatments at a  $p < 0.05$  level, respectively.

Fe and FeCl<sub>2</sub> treatments, respectively. The pH values reached their highest levels at the filling stage for the control, NaNO<sub>3</sub> and PC-Fe treatments, and they were lowest at the filling stage under FeCl<sub>2</sub>+NaNO<sub>3</sub> treatment, respectively. At the filling stage, the NaNO<sub>3</sub> amendment significantly increased the pH in the rhizosphere soils, and the FeCl<sub>2</sub> and FeCl<sub>2</sub>+NaNO<sub>3</sub> treatments significantly reduced the pH (Fig. S8).

The significant increase in the pH under NaNO<sub>3</sub> treatment could be attributable to denitrification. The process of denitrification consumes H<sup>+</sup> and thus leads to an increase in the pH (Trudell et al., 1986). By comparison, adding FeCl<sub>2</sub> reduced the pH in the rhizosphere soils, which was consistent with a previous study (Hu, 2014). It is widely accepted that the pH can significantly affect the bioavailability of As (Huang et al., 2006; Beesley et al., 2014; Yin et al., 2015). On the one hand, the quantity of negative charges on the surface of the soil colloid (clay, Fe/Mn/Al oxides/hydroxides, etc.) increases as the soil pH increases, and then, more As is desorbed (Yin et al., 2015). Accordingly, an increase in the pH can enhance the desorption of As in soils and thus increases its mobility and bioavailability. On the other hand, the reductive dissolution of Fe oxides is also highly pH-dependent (Gotoh and Patrick, 1974). A decrease in pH favors the dissolution of Fe oxides, and as a result, the adsorbed As is released into the soil solution (Al-Abed et al., 2007). Therefore, the As released from soils with respect to pH is indicated as a V-shaped profile with a decreasing trend under acidic conditions and an increasing trend under alkaline conditions (Al-

Abed et al., 2007). Moreover, a significantly positive correlation was obtained between bioavailable fractions of As and pH values ranging from 6.75 to 7.5 (Das et al., 2013). The pH in the rhizosphere soils of our present study ranged from 7.0 (FeCl<sub>2</sub> treatment) to 8.0 (NaNO<sub>3</sub> treatment). Accordingly, the increased As concentrations in the plant tissues under NaNO<sub>3</sub> treatment could be related to the pH increase relative to the control, and the decreased As concentrations in the plant tissues under FeCl<sub>2</sub> treatment could be related to the pH decrease compared with that of the control, which can be supported by the significantly positive correlation between the pH in the rhizosphere soil and straw As at the filling stage (Fig. S9).

#### 4.3. Distributions of As fractions and Fe fractions in rhizosphere soils and their effects on As accumulation in rice plants under different treatments

Generally, fractions of dissolved As and NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>-extractable As(III) are considered as the portions of As that are available to plants in soils, and HCl-extractable As, DCB-extractable As and oxalate-extractable As are not readily available to plants (Khan et al., 2010; Sun et al., 2015), which can be shown by the correlations between As fractions in soils and As concentrations in rice plant tissues. Significantly positive correlations were found between dissolved As or NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>-extractable As(III) in soil and As in straw or grain (Fig. 6 and Fig. S10). However, root As showed a significantly negative correlation with DCB-extractable As and oxalate-extractable As (Fig. S10). In terms of the results at the maturation stage, the PC-Fe amendment can significantly reduce mobile pools and increase immobile pools of soil As and thus reduce As bioavailability. The percentages of As fractions accounting for the total As concentration in the soils at the maturation stage also showed a similar pattern under different treatments (Fig. S11). The percentages of dissolved As in the PC-Fe, FeCl<sub>2</sub> and FeCl<sub>2</sub>+NaNO<sub>3</sub> treatments were significantly lower than that of the control. The soil amendments also significantly reduced the percentages of NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>-extractable As(III) compared with the control. For the immobile pools of As, the PC-Fe treatment slightly increased the percentages of HCl-extractable and DCB-extractable As, and the FeCl<sub>2</sub> and NaNO<sub>3</sub> treatments significantly reduced the percentage of oxalate-extractable As (Fig. S11). Apparently, PC-Fe was the most effective amendment to reduce As mobility in comparison with the other treatments, which was consistent with previous reports (Kim et al., 2003; Simón et al., 2015; Sun et al., 2015). In order to elucidate the variation of As fractionation in soils as only affected by growth stages of rice, the percentages of dissolved As, NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>-extractable As(III) and DCB and oxalate-extractable As accounting for total soil As under the control treatment were calculated. The percentages of dissolved As, DCB and oxalate-extractable As decreased slightly from the tillering stage to the jointing stage and then increased markedly until the maturation stage (Fig. S12). Comparatively, the percentage of NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>-extractable As(III) increased slightly from the tillering stage to the jointing stage and decreased slightly from the jointing stage to filling stage and then increased markedly until the maturation stage (Fig. S12). Generally, the available and not readily available portions of As all showed an increasing trend with the growth stages, similar to the previous studies (Xu et al., 2010; Yang et al., 2015). These variations may be related to pH, Eh and microbial activity variations with different stages of rice growth (Zeng et al., 2005; Tao et al., 2007; Yang et al., 2015). The pH in the control treatment of our present study fluctuated with the growth stages (Fig. S8). All the results mentioned above indicated that soil amendments could affect As fractionation in soils, and these effects varied with the growth stages of rice plants and different treatments.

Similar to As fractionation as affected by soil amendments, the

PC-Fe treatment had a significant impact on the Fe fractions distribution. Accordingly to the concentration variations of Fe fractions under the PC-Fe treatment (Fig. 3), we speculated that PC-Fe treatment may promote the transformation of Fe forms from dissolved Fe to adsorbed, poorly crystalline and free Fe oxides. On the other hand, significantly positive correlations were observed between dissolved Fe and straw As or grain As and significantly negative correlations were found between root As and HCl-extractable Fe(III) or DCB-extractable Fe (Fig. 6 and FigS. 13). Furthermore, significantly positive correlations between soil Fe fractions and As fractions were found, such as dissolved Fe and dissolved As, HCl-extractable Fe and HCl-extractable As or DCB-extractable As, oxalate-extractable Fe and oxalate-extractable As, DCB-extractable Fe and DCB-extractable As or oxalate-extractable As (Fig. 7). Accordingly, the transformation of Fe fractions changed the distribution of As fractions in soils. Therefore, we speculated that Fe compound amendments might affect soil Fe fractions first and then affect the As fractionation in the soil and As bioavailability in rice plants indirectly.

#### 4.4. Fe plaque formation and their effects on As accumulation in rice plants throughout the whole growth stage under different treatments

As reported, the amount of Fe plaques formed was affected by many factors, such as the rice cultivar, growth stage, soil type and soil environmental conditions (Chen et al., 1980; Hu et al., 2013; Wu et al., 2016). At the tillering stage, the Fe concentrations in Fe plaques were significantly higher than those at the other stages (Fig. 5A) because the rice thrived during this stage and its ability to secrete oxygen for the root system was enhanced, and thus more Fe plaques were formed. By comparison, at the maturation stage, the rice roots had lower activity and a lower ability to secrete oxygen, and thus the amounts of Fe plaques formed at this stage were relatively fewer. A previous study also obtained a similar result showing that the As in combined roots and Fe plaque declined

during the last few weeks of the growth season, which may be related to a decline in the rate of fresh Fe plaque formation (Garnier et al., 2010). In addition, the dissolved organic matter in the rhizosphere soil also affects the amount of Fe plaques formed because the decomposition of dissolved organic matter releases many organic ligands, providing protons and electrons, which then promote the dissolution of Fe plaques (Zhang et al., 2012). Dissolved organic matter reportedly increased gradually from the tillering stage and reached a maximum level in the booting and flowering stages, and then it decreased gradually from the filling stage to the maturation stage (Wang et al., 2004). Accordingly, lower contents of dissolved organic matter at the tillering stage may be helpful for Fe plaque formation.

The Fe concentrations in Fe plaques were significantly negatively correlated with  $\text{NH}_4\text{H}_2\text{PO}_4$ -extractable As(III) and As(V) in soils at the tillering stage (Fig. S14). Moreover, significantly negative correlations between As in Fe plaques and  $\text{NH}_4\text{H}_2\text{PO}_4$ -extractable As(III) in soils at the maturation stage and  $\text{NH}_4\text{H}_2\text{PO}_4$ -extractable As(V) in soils at the tillering stage were found. These results indicated that Fe plaques could immobilize  $\text{NH}_4\text{H}_2\text{PO}_4$ -extractable As(III) and As(V) in soils and thus affected As bioavailability (Fig. S13). However, the concentrations of HCl-extractable As in soils were significantly positively correlated with Fe and As in Fe plaques at the filling stage. Similarly, significantly positive correlations were found between As in Fe plaques and DCB-extractable As at the jointing and filling stages (Fig. S15). This trend was consistent with the fact that HCl-extractable As and DCB-extractable As were the portions of As that were adsorbed and bound by Fe oxides, respectively. Fe plaques are a mixture of crystalline and poorly crystalline ferric hydroxides, goethite and lepidocrocite (Huang et al., 2015); therefore, As pools that were adsorbed or bound by Fe oxides in soils exhibited a similar pattern to those of the As portions that were sequestered by Fe plaques. In addition, significantly negative correlations between Fe in Fe plaques and straw As at the jointing stage and root As at the filling stage and grain As at the maturation stage were observed,

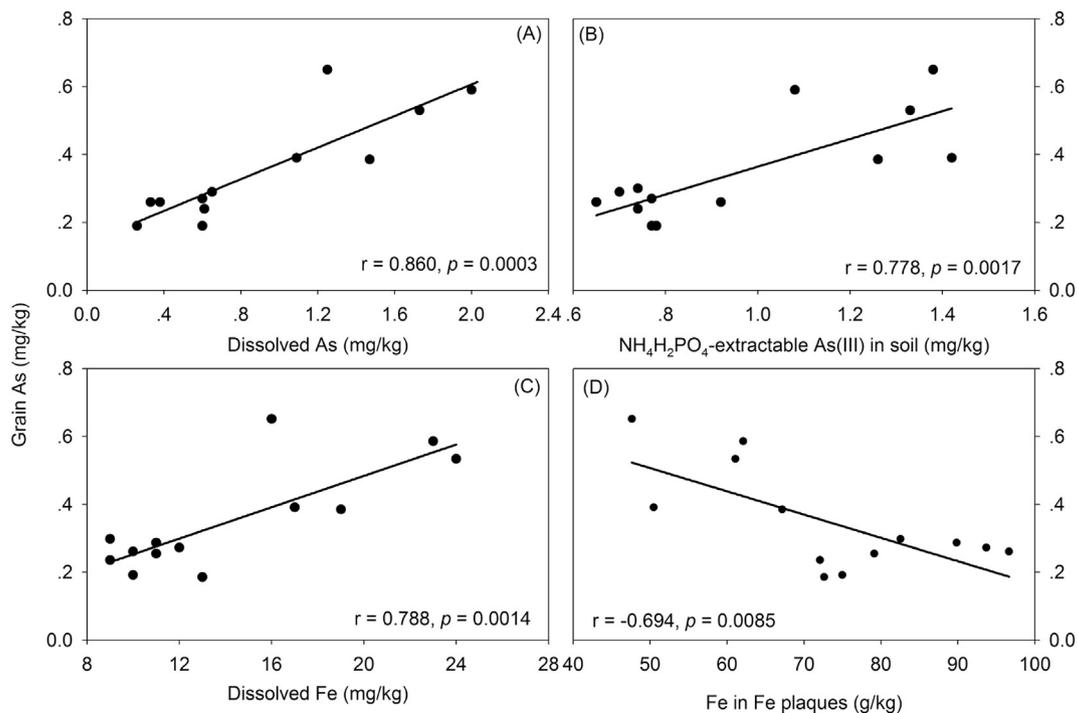


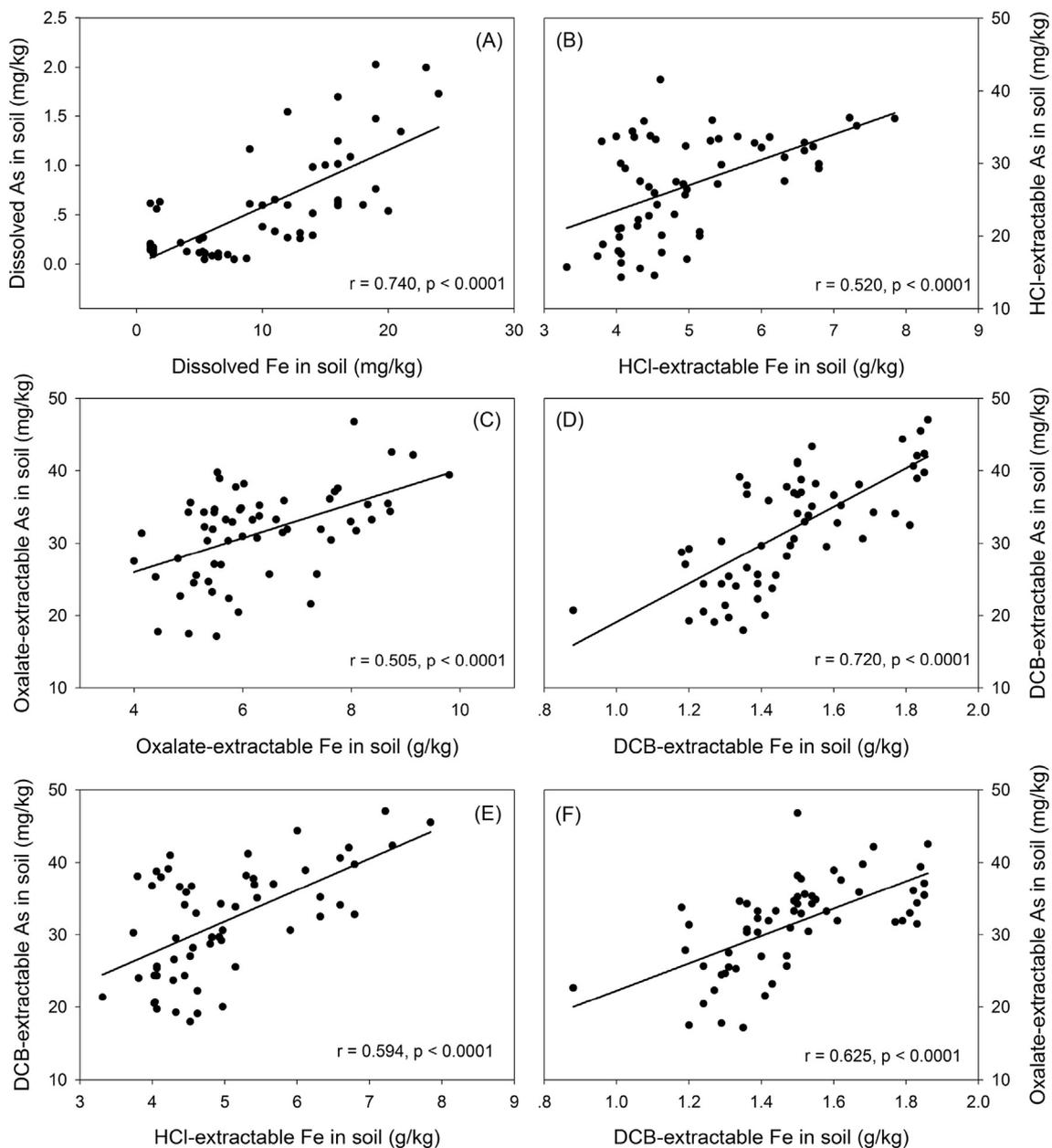
Fig. 6. Correlations between concentrations of As in rice grain and concentrations of dissolved As/Fe,  $\text{NH}_4\text{H}_2\text{PO}_4$ -extractable As(III) in soils and Fe in Fe plaques.

confirming the important role of Fe plaques in reducing As uptake (Fig. 6D and FigS. 16).

**5. Environmental implications**

Based on all the results obtained above, the soil Fe compound amendments exhibited different effects on As mobility and bioavailability over the rice growth stages and with different treatments. First, Fe compound amendments affected the soil pH and thus affected As mobility. Second, Fe compound amendments affected the distribution of Fe fractions and As fractionations in soils and thus affected As mobility and bioavailability. Third, Fe compound amendments affected the amounts of Fe plaques formed and thus affected As sequestration by Fe plaques. Taking grain As an example, the PC-Fe, FeCl<sub>2</sub> and FeCl<sub>2</sub>+NaNO<sub>3</sub> treatments can all significantly reduce the As concentration in rice grain (Fig. 1B).

Significantly positive correlations between grain As and dissolved As and NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>-extractable As(III) indicated that the decrease in the mobile pools of As reduced As accumulation in rice grains (Fig. 6A and B). In addition, significantly positive correlations between grain As and dissolved Fe indicated that the decreased dissolution of Fe oxides reduced As uptake in rice grain (Fig. 6C). Corresponding to these correlations, PC-Fe, FeCl<sub>2</sub> and FeCl<sub>2</sub>+NaNO<sub>3</sub> amendments significantly reduced the percentages of dissolved As and NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>-extractable As(III) and the concentration of dissolved Fe in soils at the maturation stage (Fig. S11). Accordingly, the soil Fe compound amendments reduced the mobile pools of As and Fe and thus reduced As accumulation in rice grain. In addition, the significantly negative correlations between grain As and Fe in Fe plaques suggested that the formation of Fe plaques sequestered soil As and thus reduced As uptake in rice grain (Fig. 6D), and we speculated that this sequestration primarily occurred from the



**Fig. 7.** Correlations between As fractions (dissolved As, HCl-extractable As, dithionite-citrate-bicarbonate (DCB)-extractable As, and oxalate-extractable As) and Fe fractions (dissolved Fe, HCl-extractable Fe, dithionite-citrate-bicarbonate (DCB)-extractable Fe, and oxalate-extractable Fe) in soils.

tillering stage to the filling stage because As concentrations in Fe plaques at these stages were significantly higher than those at the maturation stage (Fig. 5B).

Unexpectedly, the reduction effect on As uptake under  $\text{FeCl}_2 + \text{NaNO}_3$  amendment did not appear to be more effective in comparison with the other amendments. Fe redox cycling and nitrogen cycling are known to be highly intercorrelated, and microbial nitrate-dependent Fe(II) oxidation can directly immobilize As(III) (Li et al., 2015a). In comparison with a laboratory study, the role of the nitrate-dependent Fe(II) oxidation process in As mobility and bioavailability according to field and pot experiments may require further investigation. Currently, the As contamination of groundwater in South and Southeast Asia has caused widespread concern (Rodriguez-Lado et al., 2013), and this contamination can also result in elevated concentrations of As in rice paddy soil and rice plants (Dittmar et al., 2007). Accordingly, exploring effective strategies to reduce As mobility and bioavailability in paddy soils is very urgent. The importance of Fe-based products to As mobility and bioavailability has been well recognized, in materials such as red mud, hematite, Fe grit, ferrihydrite, Fe phosphate, and Fe-rich water treatment residual (Garau et al., 2014; Sun et al., 2015). However, the stage at which the reduction effect on As uptake by Fe-based products exhibited more effectively in paddy soils throughout the whole growth stage has rarely been reported. Our results showed that PC-Fe was the most effective amendment to reduce As accumulation in rice plants, and the filling stage was the key stage for devising measures to mitigate rice As uptake, which will provide more guidance for reducing As mobility and bioavailability in paddy soils by using Fe-based products. These findings have enhanced our insight into the control of As pollution in paddy soil with soil amendments.

#### Author contributions

Huan-Yun Yu and Xiangqin Wang contributed equally to this work.

#### Notes

The authors declare no competing financial interest.

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#### Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.envpol.2017.01.072>.

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