Microbial driven iron deduction affects arsenic transformation and transportation in soil-rice system

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Microbial driven iron reduction affects arsenic transformation and transportation in soil-rice system.

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Abstract

1. INTRODUCTION

Arsenic (As) is the most widespread toxic element in nature. The average concentration in the Earth's crust and soil is 2.1 and 5 mg/kg respectively, ranking 20th in the crustal abundance of elements (Frost et al., 2003; Zhao et al., 2010). Agricultural and industrial production, including mining, smelting, fertilizer and pesticide applications, wood preservation and feed additions are important sources of environmental As contamination (Bahar et al., 2012; Charlet and Polya, 2006). Anthropogenic emissions of As in global soils is between 2.84×10^5 - 9.4×10^5 t (Zhu et al., 2014). Soil As pollution not only causes secondary pollution of surface waters and groundwater, but for paddy soils, it also reduces crop yield and quality, whilst adversely affecting human health through food chain transfer (Khan et al., 2010). In 2014, the Ministry of Environmental Protection and the Ministry of Land and Resources surveyed China's soil pollution status, revealing that 19.4% of farmland soils were contaminated with As (Zhao et al., 2015). Arsenic contamination has become a global environmental problem, especially in Southeast Asia, where groundwater and soil As contamination have attracted the attention of researchers from around the world (Seyfferth et al., 2014). Rice is the most important food crop in China and the staple food of half of the world's population (Khush, 2013). Studies have shown that per capita, for Chinese residents, As intake is about 42 μg/d, whilst As in rice accounts for 60% of the total daily intake (Zhu et al., 2008). Arsenic contamination seriously threatens the health of populations with a rice-based diet, and

this has become the main exposure route besides contaminated drinking water (Singh et al., 2015).

Microbial conversion of As is an important part of its geochemical cycle (Huang et al., 2011). Sforna et al. (2014) revealed that the microbial metabolism of As began 2.7 billion years ago, including redox and methylation of As in microbial cells (Sforna et al., 2014). Microbial regulation of As determines its morphological transformation and hence its environmental fate and bioavailability in paddy soils (Zhang et al., 2015). Arsenic oxidizing bacteria such as *Paracoccus sp. SY* and *Alkalilimnicola ehrlichii*, from arsenic-contaminated soils and alkaline hypersaline soda lakes, for example, can oxidize trivalent arsenic to pentavalent arsenic, and so reduce its mobility and bioavailability (Yamamura and Amachi, 2014; Zhang et al., 2015). Arsenate-resistant microorganisms and certain dissimilatory arsenate-reducing prokaryotes such as *Chrysiogenes arsenatis* and *Bacillus selenatarsenatis*, can reduce As(V) to As(III) by intracytoplasmic reductase (ArsC) and anaerobic respiration. Arsenate adsorbed on the surface of iron (hydrogen) oxides acts as a terminal electron acceptor, thus leading to the activation of As in paddy soils, consequently promoting absorption and accumulation of As in rice (Yamamura et al., 2005; Zhang et al., 2015). Arsenic methylation is catalyzed by As(III) adenosylmethionine methyltransferase (ArsM), in which the methyl group is transferred from adenosylmethionine to As(III), and is subsequently methylated from As(III) to $As(CH_3)_n$ (Ye et al., 2012). Since the toxicity of monomethylarsine is much less than inorganic arsenic, promoting the methylation of As in soils is an effective way to alleviate arsenic pollution in

77 farmlands. Zhang et al. (2015) found that As transformation genes in paddy soils were
78 widely distributed, highly diverse and abundant, and mainly from rice rhizosphere
79 bacteria, such as Proteobacteria, Gemmatimonadales, and Funicutes (Zhang et al.,
80 2015). Soil physicochemical properties (pH, EC, total carbon, nitrogen, As and iron,
81 C/N ratio, sulfate and nitrate ions) and rice rhizosphere environments (mucigels,
82 polysaccharides, amino acids and organics secreted by the roots) all affect As
83 metabolism by microorganisms, which can increase microbial abundance and change
84 the microbial community structure (Bais et al., 2006).

85 Soil minerals and organic matter greatly affect As mobility, bioavailability, and
86 toxicity in soils (Kim et al., 2015). Iron (hydrogen) oxide is a very common mineral in
87 soil, including ferrohydrite, hematite, goethite, fibrite, magnetite etc (Doušová et al.,
88 2011). Due to its large specific surface area, positive surface charge and sufficient
89 adsorption sites, it has a strong adsorption capacity for anions such as arsenate
90 (Ackermann et al., 2010). Through microbial action, the redox process of iron leads to
91 adsorption, release and coprecipitation processes, and may cause transformation of As
92 species (Shi et al., 2018). Identifying the mechanism of Fe(II, III) redox system on As
93 speciation is an effective way to control As pollution in paddy fields, whilst
94 microbial-mediated processes of dissimilatory iron reduction, play an important role
95 in the As biogeochemical cycle (Borch et al., 2010). The transportation and speciation
96 of heavy metals such as chromium, arsenic and selenium in anaerobic circumstances
97 are closely related to iron reduction processes (Yan et al., 2004). Stroud et al. (2011)
98 reported that the reduction of Fe(III) caused the release and reduction of As(V)

adsorbed on iron (hydrogen) oxide (Stroud et al., 2011). However, recent studies have shown that dissimilatory iron reduction processes may promote the adsorption of As, because of formed secondary iron minerals which promote As fixation, causing a decrease in As mobility (Guo et al., 2013; Tufano and Fendorf, 2008). Furthermore, generated As(III) may be more likely to adsorb on iron minerals than As(V) (Jiang et al., 2013). Jiang et al. (2013) showed that under the action of iron-reducing bacteria such as *S. oneidensis MR-1* and *Shewanella sp. HN-41*, the concentration of As(V) in solution decreased because As(V) and Fe(II) produced in solution formed a ferrous arsenate coprecipitate (Jiang et al., 2013).

Radial oxygen loss (ROL) from rice roots, results in iron (hydrogen) oxide plaque formation, being mainly composed of ferrihydrite, goethite and fibrite, which can strongly adsorb As on root surfaces, consequently reducing As transportation to aboveground rice tissues (Wu et al., 2016). Studies have revealed that the microaerobic status of rhizosphere soils has led to the relative abundance of As oxidizing bacteria being higher than that of As reducing bacteria. This has enhanced oxidation of As(III) and promoted an increase in As adsorption by the iron plaque (Jia et al., 2014). However, understanding how the rhizosphere process affects microbial activity and the subsequent effects on As migration, speciation, and rice As accumulation, requires further investigation.

Although there are studies regarding the effects of radial oxygen loss from roots on As accumulation in rice, as well as As biotransformation related genes in paddy soils, little research has been conducted on the effects of iron reduction genes on As

biotransformation, and As transportation and speciation in soil-rice systems. The objectives of the present work were to 1) to study the effects of iron reduction genes on As biotransformation related genes' abundances in paddy soils; 2) to study the effects of iron reduction genes on rice rhizosphere physicochemical properties, As/Fe concentrations in soil pore water, as well as As transportation and speciation in different rice genotypes.

2. MATERIALS AND METHODS

Experimental setup

The contaminated paddy soils for pot experiments were collected from a paddy filed (1-20 cm depth) around a mining area in Chenzhou City, Hunan Province. Mean soil As and Fe concentrations were 130.20 mg/kg^{-1} and 40.03 g/kg^{-1} respectively; other selected basic soil properties are listed in Table S1. Four rice genotypes were selected for the investigation; two hybrid subspecies, Shenyou 9586 (SY-9586) and Fengyuanyou 299 (FYY-299) and two indica subspecies, Xiangwanxian 17 (XWX-17) and Xiangwanxian 12 (XWX-12). Seeds were surface sterilized by soaking in 30% H_2O_2, and subsequently transferred to a petri dish covered by moist filter paper for germination to seedlings (~2-3 cm). Seedlings were then cultivated in a nutrient solution for 2 weeks prior to the pot investigation (Wu et al., 2017). Four uniform rice seedlings of each genotype (SY-9586, FYY-299, XWX-17 and XWX-12) were selected and transplanted into the central area (rhizosphere) of 1 kg contaminated paddy soil in polyethylene pots (30 cm high, bottom diameter 24 cm, top diameter 28

cm) and covered with a nylon mesh (24 μm) bag (height of 15 cm; diameter of 12 cm). At the same time, the outside area of soil (bulk soil) was kept away from the rice roots, using 9 kg contaminated paddy soil (Jia et al., 2013a). Three rice seedlings were planted in each pot, and each genotype rice seedling was replicated four times, totaling 16 pots. Rice seedlings were grown under flooding conditions (water 2 cm higher than the soil surface) to simulate the actual paddy field environment. Pots were placed randomly in a greenhouse (25 °C during the day and 20 °C at night, with 70% relative humidity) and natural light was supplemented with sodium light (1200 Lux), providing a photoperiod of 12 h light/12 h dark. Plants were cultured until harvest. The rhizosphere and bulk soil solutions of the four rice cultivars were sampled every 15 days as follows: tillering stage (15d), jointing stage (30d), heading stage (45d), early stage of filling (60d), mid-filling stage (75d), late stage of filling (90d) and maturity stage (105d)) using Rhizon Soil Moisture Samplers (Rhizosphere, Netherlands). Soil solutions were analyzed for pH, electrical conductivity (EC), total As, and As speciation (As(Ⅲ), As(Ⅴ), DMA, MMA) and iron (Fe) content. At harvesting, root, straw, husk, and grain were separated, subsequently dried and ground for determination of Fe (as iron plaque), total As concentrations and As speciation.

Chemical analysis of soil pore water and iron plaque

The pH and redox potential (Eh) of soil pore waters were determined by a pH probe and meter (PHS-3C, Shanghai Precision Instrument Co., P.R. China). EC was measured by a conductivity meter. Iron and As contents were determined by atomic

absorption spectrometer (AAS) and atomic fluorescence spectrometer (AFS) respectively. As speciation was determined by high-performance liquid chromatography-hydride generation atomic fluorescence spectrometry (HLCP-HG-AFS) (Wu et al., 2016).

Iron and As concentrations of rhizosphere iron plaque were determined by DCB (dithionite−citrate−bicarbonate solution) extraction (Wu et al., 2016). Iron and As concentrations of the extraction were determined by AAS (AAS, TAS-990, Beijing Puxi Instruments Co., P.R. China) and HG-AFS (HG-AFS, AFS-8230, Beijing Jitian Instruments Co., P.R. China) respectively.

Plant analysis

Harvested mature rice plants were divided into four parts: root, straw, husk and grain. Material was washed with tap water and then thoroughly rinsed with deionized water. Rice samples were then divided into two parts, half was used to extract Fe plaque, and the other half was placed in a vacuum freeze-drying oven for desiccation. Following desiccation, the material was ground under liquid nitrogen conditions, and arsenic contents and speciation of each part of the rice samples were determined. For determination of total As in rice, 0.5 g of ground sample was digested with 1.0 ml of perchloric acid ($HClO_4$) and 4 ml of nitric acid (HNO_3) (HNO_3: $HClO_4$=4:1) at 110-130℃ in a heating block until the a clear solution was obtained with a certified reference plant material (GSV-2，GWB07603) for quality control purpoes. For determination of different As species in rice, 0.5 g of ground sample was extracted

using 25 ml of 1% nitric acid (HNO_3) at 95℃ for 1.5h, then extractions were centrifuged at 5000 r/min for 10 min and the supernatant filtered (0.22 mm) (Wu et al., 2016). Arsenic speciation was determined by HLCP-HG-AFS (Wu et al., 2016).

Soil DNA extraction

Rhizosphere and non-rhizosphere soils were analyzed by quantitative PCR (qPCR) for arsenic functional genes and iron reduction genes for the four rice genotypes at 15 days (tillering stage), 30 days (jointing stage), 75 days (filling stage) and 105 days (maturity stage). Soil samples were used to extract total microbial DNA using QuantiFast® SYBR® Green PCR Kit (Qiagen， Germany) according to the manufacturer's instructions.

Quantitative real-time PCR analysis of arsenic functional genes and iron reduction genes

To amplify aioA, arsC , arsM and Geo gene abundance in each sample, the primers of qPCR AroAdeg2F/AroAdeg2R(Inskeep et al., 2007), amlt-42-f/amlt-376-r(Sun et al., 2004), arsMF1/arsMR2(Jia et al., 2013a) and Geo564F/Geo840R(Somenahally et al., 2011) were used in a LightCycler® 480 Ⅱ Fluorescence quantitative PCR instrument (Roche，Swiss), respectively. Details of qPCR programs are presented in the Supporting Information.

Statistical Analysis

Correlations between soil pH, EC, As contents, DCB-extractable Fe/As contents in iron plaque, plant total As contents, rice genotypes as well as gene copy number were drawn with Origin 9.0. The relationship between gene abundance for aioA, arsC, arsM and Geo and physicochemical characteristics of soil solution samples were evaluated using redundancy analysis (RDA) in CANOCO 5. Significant differences were determined using one-way analysis of variance (ANOVA), and *P< 0.05 was used as a statistically significant difference.

3. RESULTS

Arsenic/iron in soil solutions

通过图 1 及表 1、S2 分析不同时期水稻土壤溶液 pH、EC、铁砷浓度变化，深入挖掘根际、非根际及不同水稻品种与土壤溶液性质的关系。

Fig. 1 shows the pH, EC and concentrations of total Fe, As and As(Ⅲ) in rhizosphere and non-rhizosphere soil solutions from the four rice genotypes (SY-9586, FYY-299, XWX-17, and XWX-12) at their different growth stages. During the culture period, the differences between pH and the rhizosphere and non-rhizosphere soil solutions were not significant ranging from 7.41 to 8.80, but in general the pH value firstly increased and then decreased, achieving a maximum pH of 8.80 at the middle of the rice filling stage. Electrical conductivity (EC) of soil solutions and non-rhizosphere soil solutions revealed a significant upward trend at 0-60d, then significantly decreased, and then subsequently increasing again after 90d, with a

range between 116.3-820.0 ms/cm. Iron content in rhizosphere and non-rhizosphere soil solutions showed an increase, reaching a maximum at maturity stage, ranging between 0.90-72.1 mg/L. Rhizosphere soil solutions were slightly lower than those of non-rhizosphere solutions. The trend of As concentration in soil solutions was similar to that of Fe, showing an upward trend with a range of 73.54 - 453.00 μg/L; As concentrations in rhizosphere soil solutions were slightly higher than those in non-rhizosphere solutions. Arsenite concentrations in rhizosphere solutions gradually increased before the filling stage, but then decreased slightly during the maturity stage, with a range between 98.5 - 453.0 μg/L (Fig. 1). Similar to total As and Fe, the concentrations of As(Ⅲ) in rhizosphere soil solutions were higher than those in non-rhizosphere solutions. In addition, the concentrations of Fe, total As and As(III) in the rhizosphere soil solutions of hybrid rice genotypes were higher than those of indica rice genotypes after early filling stage.

Table 1 and Table S2 show the correlations of various physicochemical properties in rhizosphere and non-rhizosphere soil solutions respectively, which present similar trends. In rhizosphere solutions, correlations between pH and Fe (*P<0.05)/As(Ⅲ) (***P <0.001) concentrations displayed a significant negative correlation, with correlation coefficients -0.223 and -0.503, respectively. EC significantly affected the concentrations of Fe (***P <0.001) and total As (***P <0.001), with correlation coefficients of -0.455 and 0.375 respectively. Concentrations of Fe in soil solutions were significantly positively correlated with total As (***P <0.001) and As(Ⅲ) (***P <0.001), with correlation coefficients 0.473 and 0.673 respectively. Total As and

As(Ⅲ) concentrations revealed a significant positive correlation (***P<0.001, correlation coefficient=0.447). In non-rhizosphere soil solutions, there were significant negative correlations between pH and EC (*P <0.05)/Fe (***P <0.001)/ As(Ⅲ) (***P <0.001), with correlation coefficients of -0.271, -0.351 and -0.582, respectively. EC significantly affected concentrations of Fe (***P < 0.001) and As(Ⅲ) (***P < 0.001), with correlation coefficients -0.445 and 0.417 respectively. There were significant positive correlations between Fe concentration and total As (*P <0.05) / As(Ⅲ) (***P <0.001) in soil solutions, with correlation coefficients 0.232 and 0.836 respectively. Meanwhile, total As and As(Ⅲ) concentration also showed a significant positive correlation (*P <0.05, correlation coefficient =0.244).

Arsenic/Iron in Rice and Iron Plaque

根据表 3 分析水稻根表铁膜中铁、砷含量与水稻孔隙水性质的关系；根据表 2 数据得出不同水稻品种不同部位砷积累形态的分布关系，结合表 2、3 得出环境因素对水稻砷积累的影响。

Table S3 presents root, straw and grain biomass of the four rice genotypes (SY-9586, FYY-299, XWX-17 and XWX-12). Biomass of root, straw and grain were 43.1-65.1 g/pot, 61.5-119.8 g/pot and 8.2-15.5 g/pot respectively. The concentrations of Fe and As in iron plaque from different rice genotypes are presented in Table S4. The results reveal that Fe concentration in iron plaque of XWX-17 was significantly higher than the other genotypes, with As concentration also being the greatest. There were significant genotypic differences in Fe concentration in the iron plaque (*P <0.05).

Arsenic species in roots, straws, husks, and grains from the four rice genotypes (SY-9586, FYY-299, XWX-17 and XWX-12) are presented in Table 2. Total As contents in roots, straws, husks, and grains of the four rice cultivars were calculated by adding the different As species together, being 259.50 - 283.40 mg/kg, 15.50 -22.80 mg/kg, 6.26 - 10.31 mg/kg and 5.01 - 7.47 mg/kg respectively (Table 2). Arsenic mainly existed in rice plants as inorganic As species (As(Ⅲ) and As(Ⅴ)), whilst organic As species (DMA and MMA) accounted for only a small proportion of total As. The contents of As(Ⅲ) and total As accumulated in the straws, husks, and grains of indica rice (XWX-17 and XWX-12) were lower than that of hybrid rice (SY-9586 and FYY-299). In rice grains, As(Ⅴ) and MMA were not detected, at the

same time, there were significant genotypic differences between As(Ⅲ) and DMA concentrations.

According to Table 3, pH, EC and the concentration of Fe, As and As(Ⅲ) in the rhizosphere soil solutions had different effects on the formation of iron plaque on the root surface and the total As concentration in different parts of rice. There were significant positive correlations between Fe concentrations in rhizosphere soil solutions and total As concentrations in rice roots (*P <0.05), with a correlation coefficient of 0.588. The concentration of As(Ⅲ) in soil solution was significantly positively correlated with Fe and As concentration in iron plaque, with correlation coefficients of 0.708 and 0.602 respectively. This indicates that the concentration of As(Ⅲ) in rhizosphere soil solutions significantly increased the Fe/As contents in iron plaque, promoting the formation of iron plaque on root surfaces and the subsequent sequestration of arsenic.

Arsenic functional transformation genes and iron reduction gene abundance on rice rhizosphere and non-rhizosphere soil

根据图 2 分析不同水稻品种根际和非根际在生长时期内土壤功能基因拷贝数的变化关系，结合图 3 明确土壤溶液理化性质对土壤功能基因丰度的影响。

According to Fig. 2 (A) and (B), at the tillering stage, copy numbers of the aioA gene in non-rhizosphere and rhizosphere soils were 1.03×10^{11}~7.12×10^{11} and 0.96×10^{11}~9.05×10^{11} mg^{-1} dry soil respectively. For the whole growth period of rice, except for XWX-12, the copy number of aioA in non-rhizosphere soils from the other

three rice genotypes firstly increased but then decreased, reaching a maximum at the jointing stage. There were no significant genotypic differences during the whole growth period. The copy number of aioA gene in rhizosphere soil firstly increased at jointing stage, then decreased during the filling period, but increased again at the maturity period; this was significantly higher in XWX-12 in comparison to the other three rice genotypes at the filling period (*P <0.05). The copy number of aioA gene ranged from 2.35×10^{11} ~ 9.91×10^{11} copies mg^{-1} dry soil of rhizosphere soil at the maturity period, which was significantly higher than that of non-rhizosphere soils. From Fig. 2 (C) and (D), except for the rhizosphere soil of XWX-12, the copy number of As reduction genes (arsC) in rhizosphere and non-rhizosphere soils of the four rice genotype soils showed a consistent trend during the whole growth period, which increased during the joint and filling period, but decreased slightly at maturity. The range of arsC gene copy number in rhizosphere and non-rhizosphere soil during the filling stage was 7.69×10^{10}~9.19×10^{10} copies mg^{-1} dry soil and 6.25×10^{10}~8.69×10^{10} copies mg^{-1} dry soil respectively, which means the rhizosphere environment conditions had little effect on the copy number of arsC genes. At maturity, the copy number of arsC genes in rhizosphere soil of indica rice (XWX-12 and XWX-17) was significantly higher than that of hybrid rice (SY-9586 and FYY-299). Compared with the aioA gene copy number, the arsC copy number decreased by an order of magnitude. In non-rhizosphere soil, the copy number of As methylation genes (arsM) showed a different pattern in different rice genotype soils: the copy number of arsM showed a gradual declining trend that increased

significantly during the maturity period in SY-9586 and XWX-12 rice soils. In comparison, FYY-299 and XWX-17 soils revealed that the copy number of arsM showed a gradual increase throughout the entire growth period. The copy number of arsM ranged from 1.43 to 2.50×10^{13} copies mg^{-1} dry soil at the maturity stage, and there were no genotypic differences throughout the whole growth period. In the rhizosphere soil, except for XWX-12, arsM gene copy numbers in the other three rice genotype soils showed a similar trend during the rice growth period, which firstly increased, but then fell, and then increased reaching the highest value during the maturity period, which ranged from 1.69×10^{13} to 3.77×10^{13} copies mg^{-1} dry soil. At the filling stage, the copy number of arsM in the rhizosphere soil of XWX-12 was significantly higher than that of the other three rice genotypes (*P <0.05). From Fig. 2, the copy number of arsM was one order of magnitude higher than that of aioA. The copy number of iron reduction genes (Geo) gradually increased in rhizosphere and non-rhizosphere soil from the four rice genotypes during the entire growth period. Independent of rhizosphere or non-rhizosphere soil, the relative abundances of iron-reducing bacteria in indica rice genotype XWX-17 and XWX-12 soils were lower than that of those in hybrid rice SY-9586 and FYY-299 soils after the filling stage.

Fig. 3 presents the RDA analysis between the physicochemical properties of soil solutions and the abundance of soil functional genes. Rice growth period (P=0.002), EC (P=0.012), Fe (P=0.002), the total As (P=0.002) and As(Ⅲ) (P=0.004) contents in soil solutions significantly impacted the gene abundances, with explanatory rates

being 70.59%, 19.98%, 53.36%, 61.40%, and 30.0%, respectively. The first and second axes account for 24.25% and 6.79% of the total variance respectively. The cosine value of the included angle between superposed gene vector and environmental factors represents the correlation degree, which is the explanatory rate (Zhang et al., 2015)).

Effects of iron reduction on arsenic functional transformation genes and arsenic speciation transformation in paddy soils

根据图 4 确定铁还原基因与砷还原及甲基化基因的相关关系，根据图 5 分析土壤砷功能转化基因 arsC、arsM 丰度及铁还原基因 Geo 丰度分别与土壤溶液 pH、Fe、As 和 As(Ⅲ)含量相关性，

The abundance of iron reduction gene (Geo) in soils was significantly positively correlated with the abundance of As reduction gene (arsC) (***P <0.001) and As methylation gene (arsM) (**P <0.01) (Fig. 4), which means that increasing Geo gene abundance led to enhanced arsC and arsM gene abundance; iron reduction in soil therefore promoted As reduction and methylation.

Fig. 5 shows the linear fitting results between pH, Fe, total As and As(Ⅲ) contents in soil solutions and As/Fe functional genes respectively. There were negative correlations between pH and the gene abundances of arsC, arsM, and Geo, with significant correlation between pH and arsC gene abundance (**P <0.01). The contents of Fe, As and As(Ⅲ) in soil solutions were significantly positively correlated with Geo and arsM (***P <0.001), and arsC (**P <0.01) abundances. The results

revealed that in flooded paddy soils, iron reduction is gradually enhanced, iron (hydrogen) oxide dissolves and Fe/As is released into the soil solution, resulting in the abundance and activity of As reducing and methylating microorganisms. The enhanced abundance of arsC and arsM genes promoted the reduction and methylation process of As, which led to increasing As(Ⅲ) concentrations in soil solutions. The mobility and speciation of As in soils may be principally driven by microorganisms, especially those involved in the biological reduction process of iron (References).

Effect of iron reduction on arsenic uptake and speciation in rice

Fig. 6 shows the effect of microbial iron reduction processes on As accumulation in different rice tissues and in iron plaque. Geo gene abundance was significantly positively correlated with As concentration in roots and grains (*P < 0.05). The concentrations of As(Ⅲ) and total As in grains were significantly negatively correlated with abundance of arsC genes (*P <0.05). In order to clarify the mechanism for the effect of functional genes on the concentrations of total As and grain As(Ⅲ) in rice, considering that iron plaque was one of the main factors affecting the uptake and speciation of As, we fitted the correlation between functional genes and the concentrations of Fe/As in root surface iron plaque (Fig. 6(G) and (H)). It can be seen from the figure that the concentrations of DCB-extracted Fe/As were positively correlated with arsC (R=0.43, R=0.18, respectively), indicating that As reducing processes may promote formation of iron plaque on roots as well as sequestration of As, consequently reducing As uptake in rice.

Under flooded conditions, the ferrous ion released from iron (hydrogen) oxide by iron-reducing bacteria, formed iron plaque due to the ROL effect of rice. Arsenite may then be removed from the soil solution by iron plaque, which then releases the iron minerals coupled with As. Enhancement of iron reduction gene abundance in soil may increase the abundance of arsC and arsM genes, which promote the release, reduction and methylation of As in soil solution, thereby affecting the accumulation of As(Ⅲ) and DMA in rice grains (Fig. 7).

4. DISCUSSION

The pH of rhizosphere and non-rhizosphere soil solutions increased before the middle stage of grain filling period, and then gradually decreased at maturity (Fig. 3 (A) and (B)), which was similar to previous studies (Honma et al., 2016; Takahashi et al., 2004; Yamaguchi et al., 2011). During the whole growth period of rice, the soil was under continuous flooded conditions, resulting in anaerobic reduction (Wu et al., 2016). Consequently, the decomposition of soil organic matter and the dissolution of minerals such as iron and aluminum (hydrogen) oxides, caused by biological and non-biological processes, led to the deprotonation of soil, which consumed H^+ from the soil solution and increased pH (Zou et al., 2017). Before the filling period, ions in soil were slow-released into the soil solution, resulting in an increase in EC. Subsequently, with an increase in soil pH, alkali metals in the solution precipitate and the concentration of H^+ decrease, causing a rapid decline in EC in solution. Following this, the continuous decrease in soil Eh promotes dissolution of iron (hydroxide)

oxides, and the release and transformation of As species, resulting in an increase of ion concentrations in the soil solution, which increases EC (adding references). During the entire rice growth period, the abundances of As/Fe reduction genes (arsC and Geo) were very high and presented a gradual increasing trend. Meanwhile, arsC gene abundance in the rhizosphere was higher than that in the non-rhizosphere, as well as the changing trend of total As and As(Ⅲ) concentrations in soil solutions. However, Geo gene abundance in the rhizosphere was lower than that of non-rhizosphere soil solutions. The abundance of Geo gene in soil was significantly correlated with Fe and total As concentrations in soil solutions (***P <0.001), which indicated that microbial processes were the main driving mechanism for the release of As/Fe in soil. In addition, pH was significantly correlated with Fe and As(Ⅲ) concentration, both in rhizosphere and non-rhizosphere soil solutions, suggesting that pH was also an important factor in promoting As/Fe release. Flooded conditions led to an increase in soil pH and the decrease of Eh. Studies have shown that when Eh falls below +100mV, iron (hydroxide) oxides are reductively dissolved, and at the same time, the relative abundances of dissimilated iron reducing bacteria (FeRB) and As reductive microorganisms increase (Somenahally et al., 2011; Yamaguchi et al., 2014). There was a significant correlation between Fe and total As concentrations in rhizosphere and non-rhizosphere soil solutions (*P <0.05), because FeRB can couple the oxidation process of organic matter and obtain energy by reducing iron (hydrogen) oxides (Somenahally et al., 2011). The reductive dissolution of iron (hydrogen) oxides caused the majority of adsorbed As to be released into the soil solution, as well as

insoluble Fe (III) being reduced to soluble Fe(Ⅱ) ions (Somenahally et al., 2011). Studies have revealed that dissimilatory iron reduction processes are ubiquitous in paddy soils, with iron production accounting for 24 % of the total reduced iron (Hori et al., 2010). On the other hand, it can be seen from Fig. 3 (G), (H), (I) and (J) that under flooded conditions, the majority of As species in rhizosphere and non-rhizosphere soil solutions was As(Ⅲ), accounting for 94% of total As. Under anaerobic conditions, the parameters of soil Eh, DOC, EC, SO_4^{2-} and total As/Fe concentration including As(Ⅲ) in soil solutions, significantly affected the relative abundance of arsC genes and changed the abundance of As reductive microorganisms (Wang et al., 2017; Zhang et al., 2015; Zheng et al., 2017). In this study, during the whole rice growth period, pH, EC, total As/Fe concentration of soil solutions increased, relative abundance of arsC genes increased rapidly, and the microbial activity of As reductive microorganisms was enhanced, which all resulted in the conversion of As(Ⅴ) to As(Ⅲ), so that the majority of As in soil solution was As(Ⅲ). There are two common approaches to the As reduction process in rice soil. Firstly, the reductive dissolution of iron (hydrogen) oxides leads to the release of adsorbed As(Ⅴ) into solution, and then As(Ⅴ) is reduced to As(Ⅲ) by As reducting bacteria; secondly, adsorbed As(Ⅴ) was directly reduced to As(Ⅲ) on the surface of iron (hydrogen) oxides, and then As(Ⅲ), which has a weaker adsorption capacity, is then released into solution (Zhang et al., 2015). Our results show that the relative abundance of Geo genes in indica rice (XWX-17 and XWX-12) soil is lower than that of hybrid rice (SY-9586 and FYY-299), in both rhizosphere and non-rhizosphere soils, after rice

growth to the filling stage. Furthermore, the abundance of Geo genes in non-rhizosphere soils was higher than that of rhizosphere soils, indicating that aerobic conditions in the rhizosphere decreased the abundance and activity of Fe reductive microorganisms.

Under anaerobic conditions, a large amount of iron (hydroxide) oxide in the soil was reduced to Fe(Ⅱ). Due to aerobic conditions in the rhizosphere，Fe(Ⅱ) migrated by diffusion in the non-rhizosphere soil solution and was oxidized to Fe(Ⅲ), which then is deposited on the root as an iron plaque (Pan et al., 2016). The soils around rice roots can therefore be divided into three areas: 1) the iron plaque region attached to the root surface directly, which has the strongest oxidation, 2) the rhizosphere soil region adjacent to the rice root, which is affected by the aerobic conditions in the rice rhizosphere and the reductive condition caused by the flooded environment, and 3) the non-rhizosphere soil region, which was less affected by ROL, and mainly dominated by reductive conditions (Wu et al., 2016). All three regions have their own unique biochemical properties (Somenahally et al., 2011). A lack of aerated tissues in immature rice roots and decreasing Eh in deeper rhizosphere soils, means that iron plaque could not adhere to the root surface and fix As (Fig. 1 (G))(Wang et al., 2015; Yamaguchi et al., 2014). Our results show that the concentration of As(Ⅲ) of hybrid rice in rhizosphere soil solutions at filling and maturity stages was higher than that of indica rice (Fig. 1(I)). Table 3 reveals that there was a significant positive correlation ($P < 0.05$) between As(Ⅲ) concentration in rhizosphere soils and As concentration in iron plaque, with a correlation coefficient of 0.602. Yamaguchi et al. (2014) found that

there was no dissolved As(Ⅴ) in soil solutions under anaerobic conditions, so As mainly existed in trivalent form, however, a small part of As(Ⅲ) was converted into As(Ⅴ) under the action of rhizosphere ROL, thus, the speciation of As in the iron plaque was likely to be determined by the Eh of rice soil (Yamaguchi et al., 2014). In addition, there was a significant positive correlation between the content of As(Ⅲ) in rhizosphere soil solutions and the content of Fe in iron plaque (*P <0.05), with a correlation coefficient of 0.708, indicating that As(Ⅲ) could affect the formation of iron plaque, which was consistent with Lee et al. (Lee et al., 2013).

Arsenic biotransformation mediated by microorganisms significantly affected As environmental behavior and bioavailability in paddy soils. Studies have shown that As metabolic genes are mainly derived from the Proteobacteria, Gemmatimonadales and Firmicutes in rice rhizosphere soils (Das et al., 2017). There are many factors affecting the activities of arsenic-metabolizing microorganisms, including soil pH, EC, total carbon, nitrogen, As, iron, C/N ratio, sulfate ions and nitrate ions (Zhang et al., 2015). In addition, rice rhizosphere conditions can also play an important role in the physicochemical properties of soils, microbial compositions and activities in paddy soils. For example, the mucus, polysaccharides, amino acids and organic acids secreted by roots can increase microbial abundance and change microbial community structure (Zhang et al., 2015). The results showed that the aioA gene abundance in the rhizosphere was higher than that in non-rhizosphere soils (Fig. 2(A)), which was consistent with the research results of Jia et al. (Jia et al., 2014). Compared with non-rhizosphere soils, the micro-aerobic environment produced by ROL from rice

roots, was more suitable to the survival of As-oxidative microorganisms. Therefore, aioA gene abundance in rhizosphere soils was higher than non-rhizosphere soils; in addition, we found the aioA gene rhizosphere abundance with SY-9586, was significantly lower than that of the other three rice genotypes (Fig. 2(A)). This may be due to the lower radial oxygen loss of its root. Total As and As(Ⅲ) content in the rhizosphere soil solution of SY-9586 was also higher than that of the other three rice genotypes (Fig. 1(G), (I)), which indicated that the rhizosphere could affect the abundance of As oxidative microorganisms, and change the bioavailability of As and plant As uptake. Under sterile conditions, As(Ⅲ) is hardly oxidized to As(Ⅴ), relying only on the chemical oxidation ability of O_2 (Rhine et al., 2005). Therefore the enhancement of microbial-mediated As oxidation in the rhizosphere is effective for reducing As availability and plant As uptake. The arsC gene abundance in the rhizosphere was higher than that of non-rhizosphere soils and indica rice with high radial oxygen loss was higher than that of hybrid rice (Fig. 2(C), (D)). Jia et al. (2014) found that arsC gene abundance in the rhizosphere was higher than 50.8% of non-rhizosphere soils (Jia et al., 2014), which was consistent with our results. Furthermore, our research reveals that there was a certain amount of methylated As in rice plants, especially in grains, accounting for up to 39% of the total As. Rice does not have the ability to methylate As, and the methylated As accumulated in the plants was derived from soil (Jia et al., 2013b). Methylated As in soil was derived from human activities, atmospheric deposition and microbial methylation, of which the third is the main source (Huang et al., 2011). Studies have shown that there is no As

methylation process taking place in the soil under sterile conditions, indicating that it is a biological process. Furthermore, As methylation by microorganisms occured quickly, especially when the soil was in an anaerobic state (Huang and Matzner, 2006). Current research shows that arsM gene abundance was higher than that of aioA and arsC by one and two orders of magnitude, respectively. Except for the rice genotype of XWX-17, arsM gene abundance in the rhizosphere was higher than that of non-rhizosphere soils for the other three rice genotypes, and generally showed an increasing trend with the continuation of growth period. However, there was no DMA and MMA detected in pore waters, which may be due to several reasons. Firstly, the activity of As methylated microorganisms, rather than arsM abundance, may have decided the methylation process of As in soil. Studies have shown that arsM gene abundance and organic As concentration in soil were associated with pH into positive and negative correlations, respectively, with the greater activity of microbial As methylation under acid condition, however the pH of the experimental soil ranged from 7.3 ~ 8.5 (Zhao et al., 2013). Jia et al. found that the change of rhizosphere environment and addition of rice straw increased the abundance of arsM genes significantly, possibly because of the improved rhizosphere environment and the increase of DOC, which promoted the activities of As methylating microorganisms (Shimizu et al., 2011). In flooded soils, the reductive dissolution of iron (hydrogen) oxides caused by iron-reducing bacteria, resulted in a massive release of arsenic into the soil solution (Cummings et al., 1999), which promoted the accumulation of organic As in grains (Huang and Matzner, 2006). Studies showed that iron-reducing

552 bacteria existed widely in paddy soil, and were mainly anaerobic bacteria (Hori et al.,
553 2010). Current studies show that the Geo gene abundance of rhizosphere soil is lower
554 than that of the non-rhizosphere, and with continuous flooding of growth periods,
555 both Geo gene rhizosphere and non-rhizospheres showed gradually increasing trends,
556 which was consistent with the research of Somenahally et al. (Somenahally et al.,
557 2011; Somenahally et al., 2011).

558 Our results indicated that the flooded soil conditions increased the abundance of
559 Geo genes. With the enhancement of iron reduction processes, the abundance of arsC
560 and arsM genes increased, which promoted the reduction and release of As in soil
561 solution and the accumulation of DMA in rice grains (Fig. 5 and 6). Studies have
562 revealed that there are a large number of bacteria and fungi, which contain the aioA,
563 arsC, arsM and Geo functional genes, such as Proteobacteria (α-, β-, γ- and
564 δ-Proteobacteria, etc.) (Zhang et al., 2015). As a result of iron reduction, the increase
565 in As may trigger microbial detoxification and promote methylation of As through the
566 Challenger pathway, As(V) → As(III) → MMA(V) → MMA(III) → DMA(V) →
567 DMA(III)→TMAO(V)→TMA(III), resulting in an increase of arsC and arsM gene
568 abundance (Somenahally et al., 2011; Ye et al., 2012). Our results also indicated iron
569 plaque formation and As fixation were found to have an extremely significant positive
570 correlation (**$p<0.01$) with arsC gene abundance (Fig. 6), and have a significant
571 positive correlation (*$p<0.05$) with As(III) in the soil solution (Table 3). This may
572 have enhanced arsC gene abundance leading to a large increase in As(III) content in
573 soil solution, consequently promoting the formation of iron plaque and As fixation.

Yamaguchi et al. found that iron plaque mainly adsorbed As(III) under anaerobic conditions, reducing the absorption of As(III) in rice. However, the inhibitory effect of iron plaque on As(III) absorption in rice is much stronger than the increase of As(III) concentration in soil solution, resulting in arsC gene abundance being significantly negatively correlated with total As in rice and As(III) in grain. Iron plaque had a weak DMA adsorption capacity, and rice could not adsorb methyl As, so the increase of arsM gene abundance may promote the accumulation of DMA in rice grains.

土壤淹水条件下，铁还原菌还原土壤中的铁（氢）氧化物释放的二价亚铁离子，在水稻根际渗氧作用下形成铁膜，吸收土壤溶液中的三价砷离子，同时释放出铁矿物上耦合的砷。淹水条件下土壤中铁还原基因丰度的提高，导致 arsC 和 arsM 基因丰度升高，从而促进 As 在土壤溶液中的释放、还原和甲基化，进而影响水稻植株籽粒中三价砷及 DMA 的累计。

Under flooded conditions, iron-reducing bacteria caused ferrous ion release from iron (hydrogen) oxides, but ferrous ions could form iron plaque under the ROL effect of rice roots, and iron plaque further absorbed As(Ⅲ) in the soil solution (Fig. 7). Meanwhile, iron-reducing bacteria also caused iron minerals to release As (Fig. 7). The iron reduction gene abundance in soils was significantly correlated with the abundance of arsenic reduction gene arsC and arsenic methylation gene arsM (Fig. 4). This demonstrated that the iron reduction process causing the release, reduction and methylation of As in soil solution, was thereby affecting the accumulation of As(Ⅲ) and DMA in rice grains.

5. CONCLUSION

1) During the rice growth period, the pH range of rhizosphere and non-rhizosphere soil solutions ranged from 7.41 to 8.80, both of which increased and then subsequently decreased. The EC range was 116.3 - 820.0 mS/cm, during the tillering stage and early stage of filling, showing a significant upward trend, which then decreased significantly. The contents of Fe, As and As(III) in the rhizosphere and non-rhizosphere soil solution of rice showed similar trends, all of which showed a gradual increase; Fe increased from 0.78 to 72.21 mg/L, As ranged from 73.5 to 453.0 μg/L and As(III) ranged from 62.1 to 340.2 μg/L. Iron content was significantly positively correlated with As and As(III) contents (***$p<0.001$), As content and As(III) content also showed a significant positive correlation (*$p<0.05$).

2) With the growth of rice, aioA gene abundance decreased gradually, and the abundance of arsC, arsM and Geo genes increased gradually. The abundance of aioA, arsC and arsM genes in rhizosphere soils was higher than that in non-rhizosphere soils. The abundance of Geo genes in the rhizosphere was lower than that in non-rhizosphere soils. Correlation analysis showed that Geo gene abundance was significantly positively correlated with arsC (***$p<0.001$) and arsM gene abundance (**$p<0.01$), respectively. The contents of Fe, As and As(III) in soil solutions were significantly positively correlated with Geo, arsC and arsM (**$p<0.01$). This indicated that the cascade of Fe/As in soil solution reduced and dissolved by reduction processes, may trigger the detoxification mechanism of As, increase the abundance of arsC and arsM genes, firstly reducing As(Ⅴ) as As(III), and then by methylation

processes to become DMA and MMA. The increase of As(III) concentration in the soil solution significantly promoted the formation of iron plaque. The inhibitory effect of iron plaque on As(III) absorption was much stronger than the promoting effect of arsC genes on As(III) concentration in the soil solution. Therefore, arsC gene abundance was significantly negatively correlated with total arsenic and As(III) in rice grains (*P<0.05). The adsorption effect of iron plaque on DMA was very weak, so the abundance of arsM genes may have significantly promoted the accumulation of DMA in rice grains. RDA analysis revealed that rice growth period (**p<0.01), EC (*P<0.05), Fe (**p<0.01) / As(**p<0.01) concentrations and As(III) (**p<0.01) are the main factors affecting soil arsenic metabolism and iron reduction gene abundance, whereas, the effects of the rhizosphere environment and rice genotypes were not significant.

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Table 1 Correlation matrix of physicochemical variables of soil solution samples in the rhizosphere of rice (n=84)

	pH	EC	Fe	As	As(Ⅲ)
pH					
EC	-0.101				
Fe	-0.223*	-0.455**			
As	-0.138	0.375**	0.473**		
As(Ⅲ)	-0.503**	0.203	0.673**	0.447**	

*indicated that there is a significant difference（*P <0.05）

indicated that there is a fearfully significant difference（P <0.01）

Table 2 Proportions of As species in root, straw, husk and grain of different rice genotypes（mean ±SD，n=3）

varieties	part	As(Ⅲ) concentration (mg/kg)	As(Ⅴ) concentration (mg/kg)	DMA (mg/kg)	MMA (mg/kg)	As[a]
SY-9586	root	123.3±3.6ab	117.5±4.3a’	16.7±0.7A	22.7±3.8A’	280.3
FYY-299		128.9±19.3ab	114.3±4.7a’	23.4±5.2AB	16.7±2.9B’	283.4
XWX-17		162.2±71.9a	120.6±5.9a’	30.7±7.1B	22.7±5.5A’	280.3
XWX-12		95.2±14.6b	131.1±10.0b’	17.6±8.8A	15.8±2.9B’	259.5
SY-9586	straw	12.1±9a	5.1±1. 3a’	2.9±1.1A	1.8±0.9A’	21.93
FYY-299		11.4±1.7a	8.7±1.1b’	2.3±0.1AB	0.3±0.1B’	22.80
XWX-17		7.6±2.1a	5.9±2.3a’	1.7±0.2BC	0.3±007B’	16.46
XWX-12		9.2±0.9a	4.7±1.5a’	1.3±0.3C	0.3±0.04B’	15.50
SY-9586	husk	4.77±0.64a	3.29±0.45a’	1.33±0.23A	0.93±0.44A’	10.31

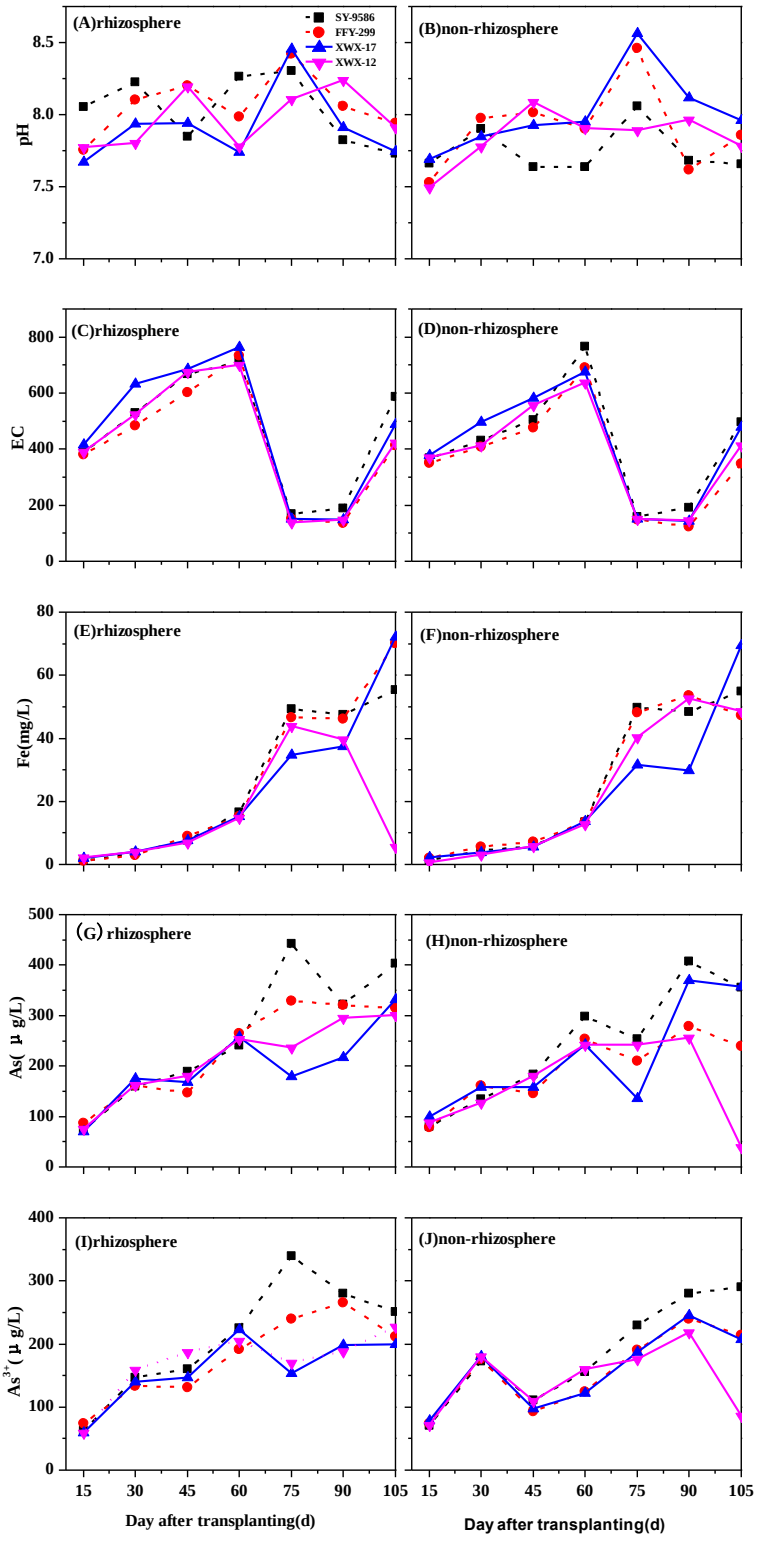
FYY-299		2.88±0.86b	3.27±0.49a’	1.83±0.26B	0.42±0.04B’	8.38
XWX-17		2.90±0.55b	1.31±0.25b’	1.74±0.15B	0.32±0.03B’	6.26
XWX-12		2.53±0.63b	3.17±2.53a’	1.64±0.11B	0.46±0.21B’	7.79
SY-9586	grain	5.73±0.73a	ND	1.75±0.42A	ND	7.47
FYY-299		4.44±0.23b	ND	1.70±0.02A	ND	6.14
XWX-17		3.39±1.12c	ND	1.61±0.30A	ND	5.01
XWX-12		2.71±0.69c	ND	2.32±0.39B	ND	5.03

Note: a indicated that the total arsenic content added by the four arsenic speciation contents

ND indicated that no relevant content was detected

Table 3 Correlation of Fe/As concentrations, total As concentrations in rice roots, straw, husks, as well as grains, with physicochemical variables of soil solution samples in the non-rhizosphere of rice (n=12)

	rhizosphere				
	pH	EC	Fe	As	As(Ⅲ)
Fe in iron plaque	0.391	0.491	0.539	0.231	0.708*
As in iron plaque	0.532	0.392	0.215	0.287	0.602*
As in root	0.053	0.310	0.588*	0.397	0.135
As in straw	0.230	0.246	0.020	0.277	-0.010
As in husk	0.205	-0.115	0.239	0.165	0.244
As in grain	-0.363	-0.242	0.215	0.224	0.439



821

822 Fig. 1. Temporal changes of pH, EC, and concentration of iron (Fe), total As and
823 As(Ⅲ) in soil solutions in rhizosphere and non- rhizospheres of four genotypes
824 rice after 15 d, 30 d, 45 d, 60 d, 75 d, 90 d, and 105 d growth

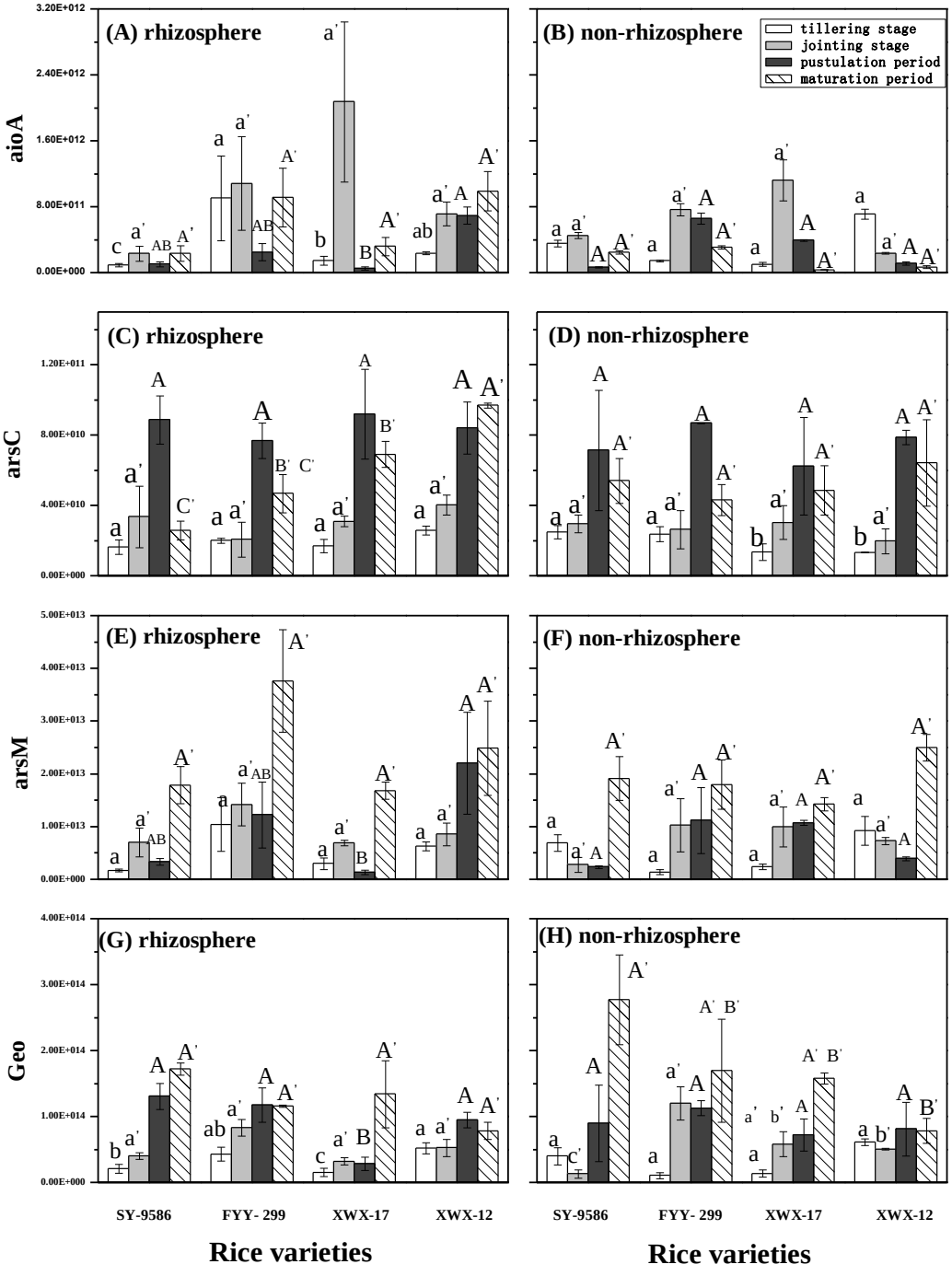


Fig. 2. Copy numbers of aioA, arsC, arsM and Geo genes present in rhizosphere and nonrhizosphere soil samples in four growth stages of tillering, jointing, filling and maturation of rice with SY-9586、FYY-299、XWX-17 and XWX-12 genotypes

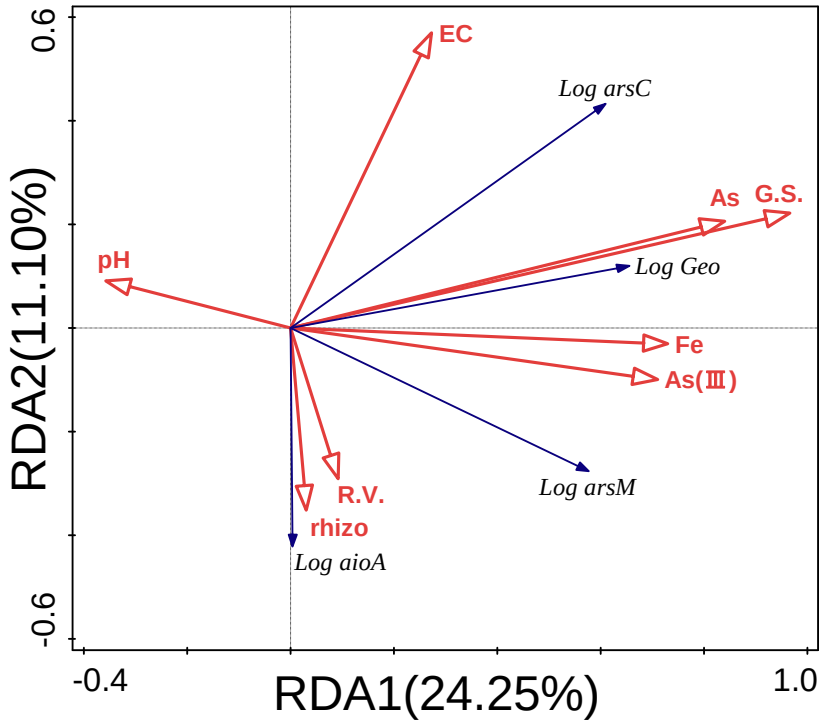
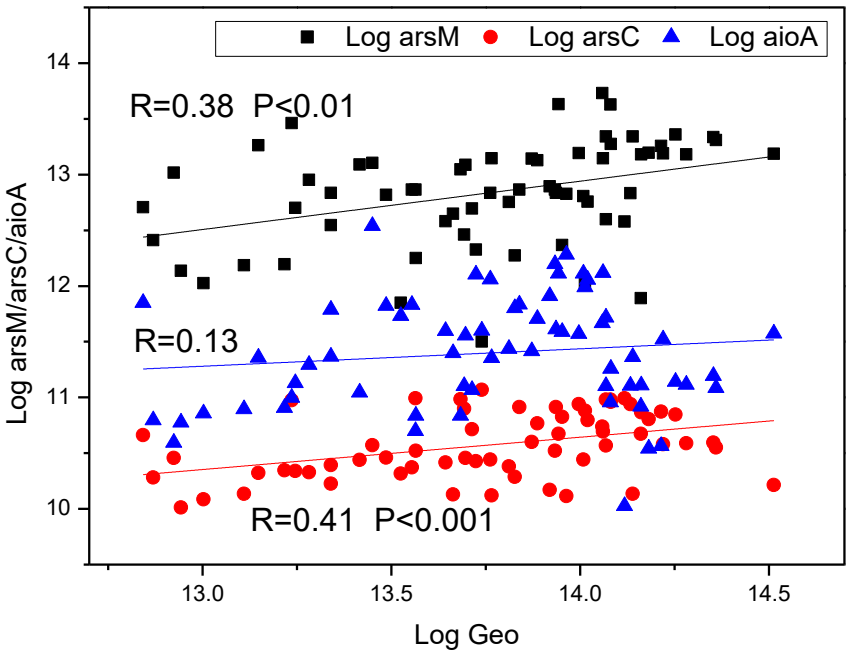
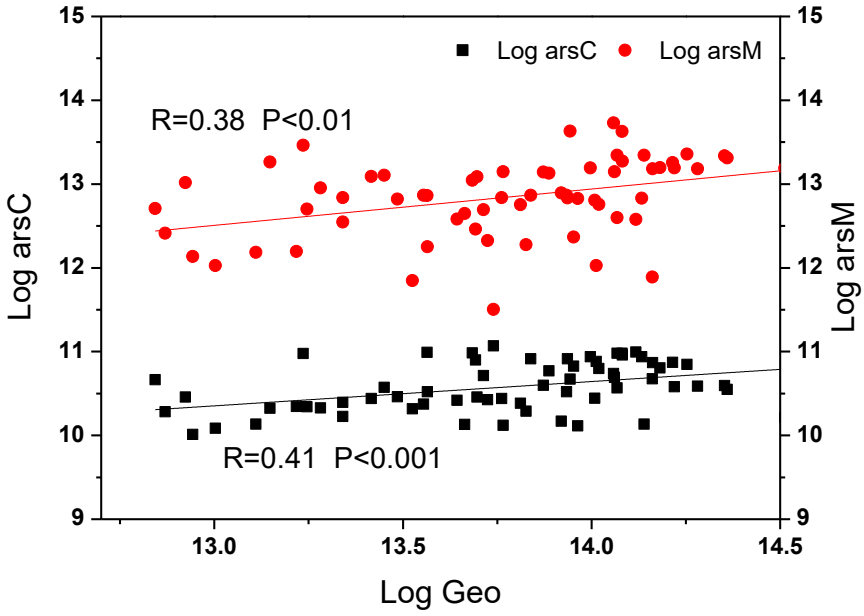


Fig. 3. RDA correlation of gene abundances of aioA, arsC, arsM and Geo with physicochemical variables of soil solution samples.

Note: G.S.: growth stage; As(Ⅲ) means As(Ⅲ) concentration in pore water; R.V.: rice variety; Solid black arrows indicate the functional genes; Faint arrows indicate environmental factors.

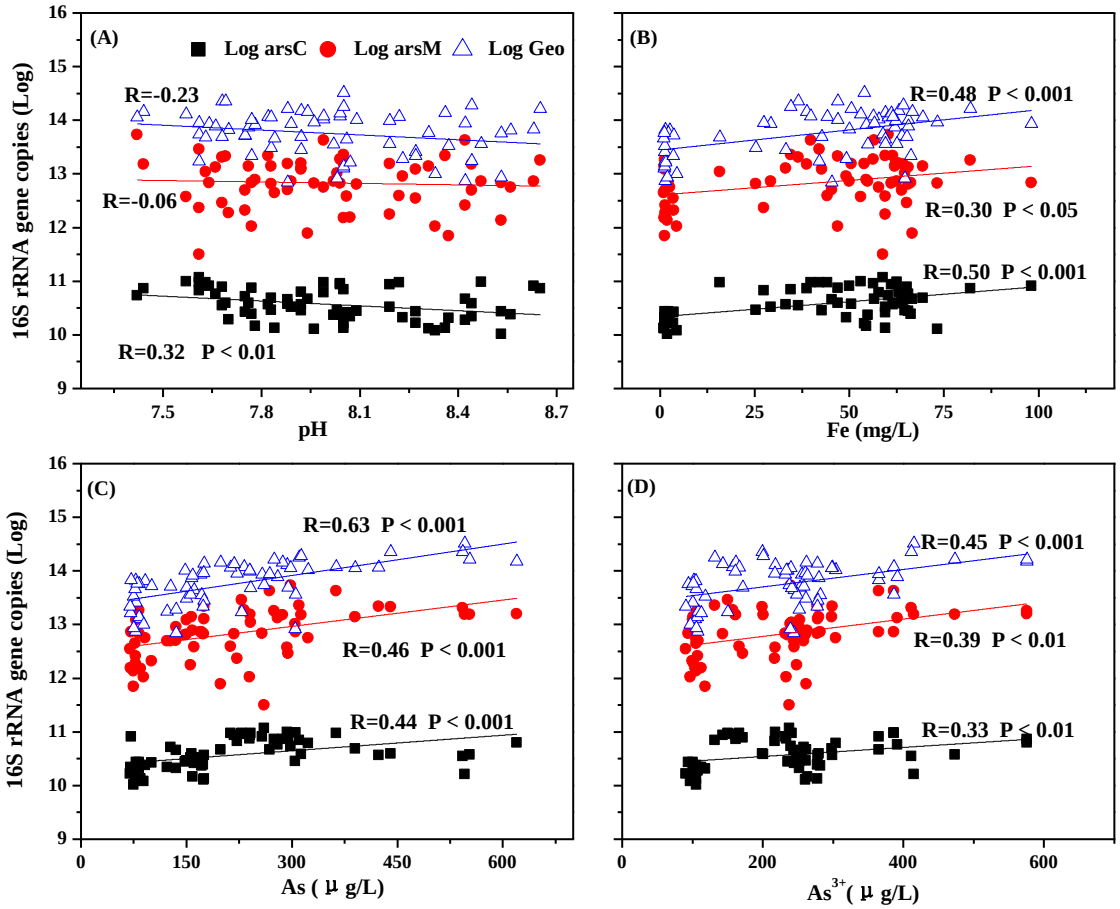




847

848 Fig. 4. Relationship among Geo gene abundance with arsC and arsM gene

849 abundance respectively

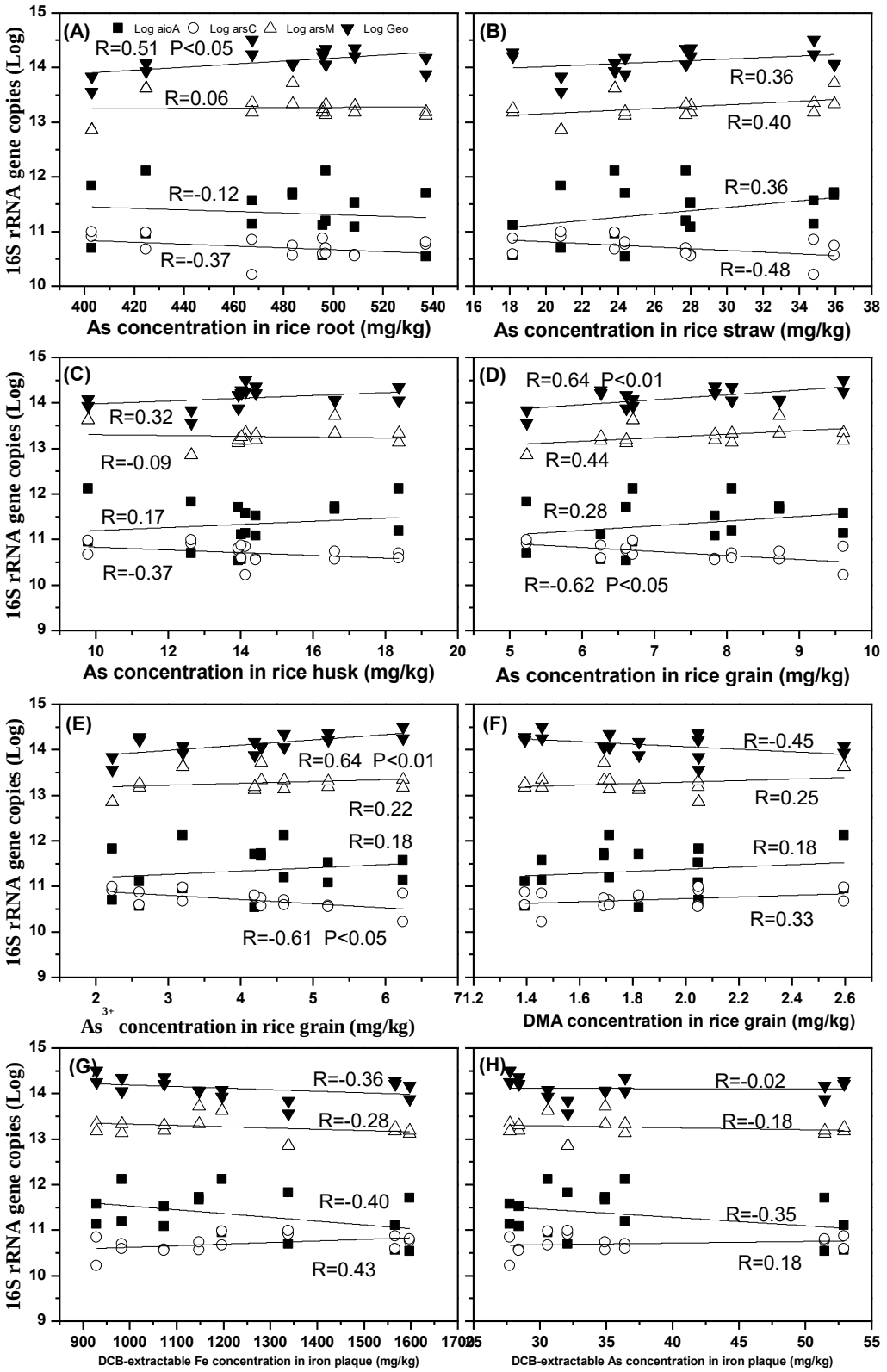


850

851 Fig. 5. Relationship among aioA, arsC, arsM and Geo gene abundance with pH,
852 Fe, As and As(Ⅲ) concentration in soil solution respectively

853

854



855

856 Fig. 6. Relationship among aioA, arsC, arsM and Geo gene abundance with As
857 concentration in rice roots, straw, husk, grain, As(Ⅲ)/DMA concentration in rice grain
858 and DCB-extractable Fe/As concentration in iron plaque respectively.

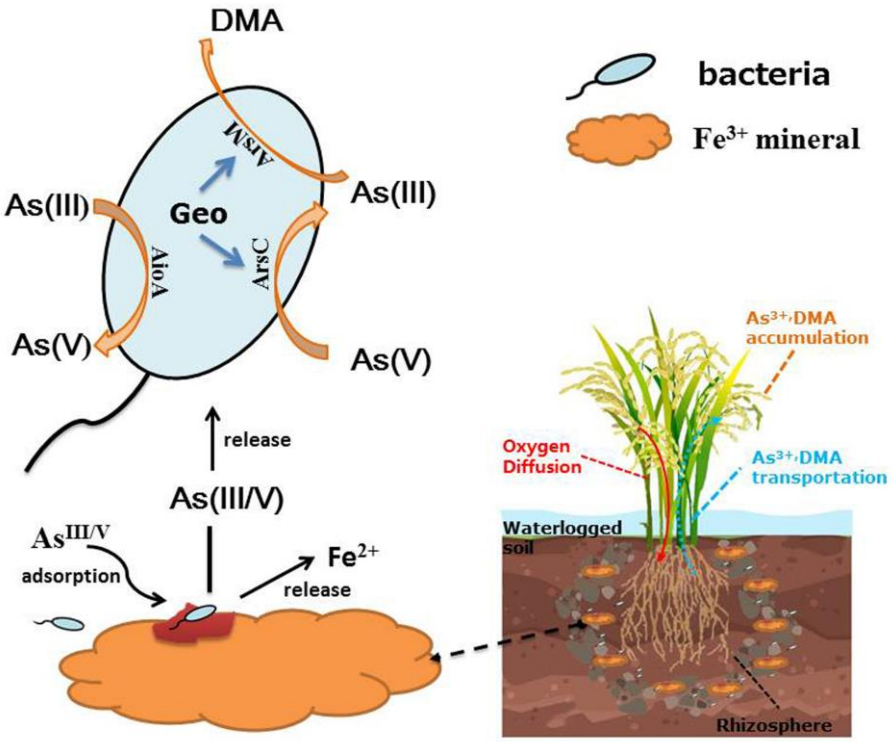


Fig. 7. The influencing mechanism of iron reduction on arsenic uptake in rice.

Supporting Information

Quantitative real-time PCR analysis of arsenic functional genes and iron reduction genes. Soil samples were used to extract total microbial DNA using QuantiFast® SYBR® Green PCR Kit (Qiagen, Germany) according to the manufacturer's instructions. For the quantification of arsenic functional genes and iron reduction genes, each reaction was performed in a total volume of 16 μL, containing 10μL QuantiFast® SYBR® Green PCR Master Mix (Qiagen，Germany) , 0.8 μL DNA template, 0.4 μL of each 10 μM primer, 0.2 μL 10 μM reverse primer, 1μL cDNA and 3.6μL nuclease-free water. The qPCR mixtures were firstly incubated at 95ºC, denatured for 5 min, followed by 40 cycles at 95ºC for 10 s and at 60ºC for 30s. The sequence of primers were as follows: AroAdeg2F(GTCGGYTGYGGMTAYCAYGYYTA)/AroAdeg2(RYTCDGARTTGT AGGCYGGBCG)

amlt-42-f(TCACGCAATACCCTTGAAATGATC)/amlt-376-r(ACCTTTTCACCGTC CTCTTTCGT)

arsMF1(TCYCTCGGCTGCGGCAAYCCVAC)/arsMR2(CGWCCGCCWGGCTTW AGYACCCG) and Geo564F(AAGCGTTGTTCGGAWTTAT)/Geo840R(GGCACT GCAGGGGTCAATA).

Table S1 basic properties of paddy soil used in this study

	pH	EC (ms/cm)	OM[a] (g/kg)	Avail K[b] (mg/kg)	Avail N[c] (g/kg)	Fe (g/kg)	Al (g/kg)	Mn (g/kg)	As (mg/kg)
soil	7.54	1.61	4.1	42.62	0.69	40.03	65.16	2.10	130.2

894

895 Table S2 Correlation matrix of physicochemical variables of soil solution samples in

896 non-rhizosphere soil (n=84)

	pH	EC	Fe	As	As(Ⅲ)
pH					
EC	-0.271*				
Fe	-0.351**	-0.445**			
As	-0.081	-0.023	0.232*		
As(Ⅲ)	-0.582**	0.417**	0.836**	0.244*	

897 *indicated that there is a significant difference（*P <0.05）

898 **indicated that there is a fearfully significant difference（**P <0.01）

899

900 Table S3 Biomass (g/plant, wet weight) of rice roots, straws and grain of four

901 genotypes

Rice varieties	root	straw	grain
SY-9586	41.9±10.3	62.9±9.6	12.7±4.4
FYY-299	65.1±10.4	119.8±48.4	15.5±6.7
XWX-17	43.1±10.2	61.5±8.6	8.2±1.9
XWX-12	63.8±7.2	81.9±5.6	10.1±1.8
Analysis of variance Genotype(G)	NS	*P <0.05	*P <0.05

902 NS indicates that there is no significant difference

903

904 Table S4 Fe and As concentration on Fe plaque of rice roots with different genotypes

varieties	Fe(mg/kg)	As(mg/kg)
SY-9586	1059±91.8b	35.2±8.47a
FYY-299	925±255.8b	34.1±10.1a
XWX-17	1486±298.4a	44.5±10.9a

XWX-12	1156±160.2b	37.3±11.7a
Analysis of variance Genotype(G)	*P <0.05	NS

Table S5 Total As concentrations of rice roots, straws and grain of the four genotypes （mg/kg）（mean ±SD，n=4）

Rice varieties	root	straw	husk	grain
SY-9586	478±26.2	31.4±4.81	14.8±0.84	8.05±1.41
FYY-299	475±27.9	28.3±7.22	17.5±1.26	8.62±0.78
XWX-17	528±31.2	22.3±2.61	13.9±0.06	6.26±1.22
XWX-12	413±16.5	24.5±5.77	7.78±0.29	5.90±1.12
Analysis of variance Genotype (G)	*P <0.05	NS	NS	NS