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Water management impacts on arsenic behavior and rhizosphere bacterial communities and activities in a rice agro-ecosystem

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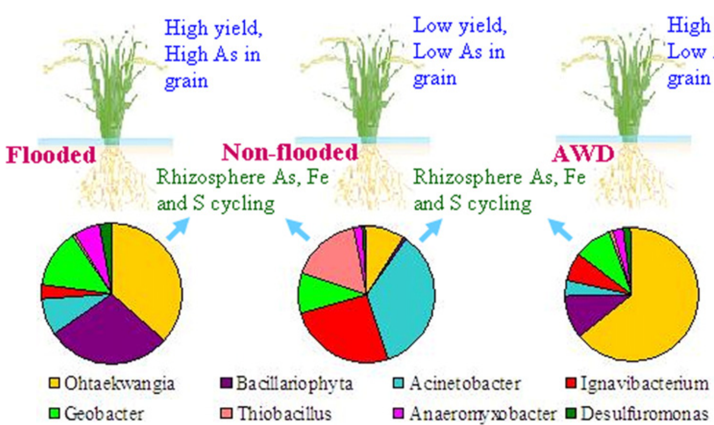
HIGHLIGHTS

- Water management significantly influenced As behavior and rhizosphere microbiome.
- Rhizosphere mocrobiome was studied with high throughput sequence analysis.
- As, Fe and S cycling in rice rhizosphere influenced As contamination in rice paddies.
- Water management significantly influenced As fractions in rhizosphere soil of rice.
- AWD is promising for reducing As level in grain while maintaining grain yield.

GRAPHICAL ABSTRACT



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ABSTRACT

Although rice cultivated under water-saturated conditions as opposed to submerged conditions has received considerable attention with regard to reducing As levels in rice grain, the rhizosphere microbiome potentially influencing As-biotransformation and bioavailability in a rice ecosystem has rarely been studied. In this study, the impacts of flooded, non-flooded and alternate wetting and drying (AWD) practices on rhizosphere bacterial composition and activities that could potentially impact As speciation and accumulation in rhizosphere soil and pore water, As fractions in rhizosphere soil and As speciation and distribution in plant parts were assessed. The results revealed that in addition to pore water As concentration, non-specifically sorbed As fraction, specifically sorbed As fraction and amorphous iron oxide bound As fraction in soil were bio-available to rice plants. In the flooded treatment, As(III) in the pore water was the predominant As species, accounting for 87.3–93.6% of the total As, whereas in the non-flooded and AWD treatments, As(V) was the dominant As species, accounting for 89.6–96.2% and 73.0–83.0%, respectively. The genera *Ohtaekwangia*, *Geobacter*, *Anaeromyxobacter*, *Desulfuromonas*, *Desulfocapsa*, *Desulfobulbus*, and *Lacibacter* were found in relatively high abundance in the flooded soil, whereas the genera *Acinetobacter*, *Ignavibacterium*, *Thiobacillus*, and *Lysobacter* were detected in relatively high abundance in the non-flooded soil. Admittedly, the decrease in As level in rice cultivated under the non-flooded and AWD conditions was mostly linked to a relatively high soil redox potential, low As(III) concentration in the soil pore water, a decrease in the relative abundance of As-, Fe- and sulfur-reducing bacteria and an increase in the relative abundance of As-, Fe- and sulfur-oxidizing bacteria in the rhizosphere soil of the rice. This

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study demonstrated that with substantial reduction in grain As levels and higher water productivity, AWD practice in rice cultivation should be favored over the non-flooded and continuously flooded rice cultivations in As-contaminated sites.

1. Introduction

The health risk associated with ingestion of arsenic (As)-contaminated rice has been of great concern in Southeast Asian countries, where rice is a daily staple food (Zhu et al., 2008). Compared to the other cereals, rice accumulates a larger amount of As because it is commonly grown under flooded conditions where As mobility is high (Takahashi et al., 2004; Xu et al., 2008). Growing rice is mainly dependent on groundwater used for irrigation. It has been suggested that the use of As-contaminated groundwater for irrigation of rice paddies has resulted in elevated As levels in rice grain and a reduced crop yield due to the accumulation of this toxic metalloid in the soil (Bhattacharya et al., 2007; Zhao et al., 2010). In recent years, growing rice under aerobic conditions and alternate wetting and drying (AWD) water management practices in rice cultivation have received considerable attention with regard to reducing As levels in rice while maintaining grain yields (Somenahally et al., 2011; Linquist et al., 2015).

Water management in rice cultivation greatly influences the oxidation–reduction (redox) reactions of paddy soils, which eventually control the fate and behavior of As in rice paddies, since the mobility of As is redox-sensitive (Roberts et al., 2010; Yamaguchi et al., 2011). In aerobically cultivated rice soil, As is less mobile because As(V), which is the dominant form in aerobic soil, is strongly adsorbed on mineral soil components (e.g., iron (hydr)oxides) (Takahashi et al., 2004). By contrast, in anaerobic soil under submerged paddy cultivation, As(V) is reduced to As(III) in soil solid phase followed by the desorption of the latter from soil minerals due to its lower sorption ability as compared to As(V) (Yamaguchi et al., 2014). Prolonged submergence in rice cultivation increases dissolved As(III) concentrations in the soil solution, which further promotes the incorporation of As(III) into rice since the dominant species taken up by rice roots is As(III) (Chen et al., 2005). Numerous studies have recognized that cultivating rice in aerobic conditions as opposed to continuously flooded conditions decreases As concentrations in soil, pore water and in rice grain (Takahashi et al., 2004; Xu et al., 2008; Somenahally et al., 2011; Linquist et al., 2015); however, the impacts of different water management practices on As bioavailability and rhizosphere microorganisms that may play an important role in As-cycling in rice paddies have remained elusive.

In addition to abiotic factors (i.e., Eh, pH, organic carbon, metal hydroxides etc.), it has been suggested that microorganisms play a vital role in As bio-transformations (i.e., oxidation, reduction and methylation) and mobilization (Jia et al., 2014). It is noteworthy that compared to the non-rhizosphere soil (bulk soil), rhizosphere is the dynamic region where plant root exudates sustain high microbial activities (Yergeau et al., 2014). Arsenic biotransformation in the rice rhizosphere is likely to be influenced by the stimulating effect of plants on rhizosphere microbes and the natural competitiveness of rhizosphere microbes to resist or to mobilize As and favor their association with plant roots (Yergeau et al., 2014). Arsenic reducing and oxidizing bacteria often coexist in rice rhizospheres, and their abundance and activities regulate As speciation and accumulation in rice paddies (Jia et al., 2014). Apart from As-transforming microorganisms, iron- and sulfur-reducing bacteria, which are dominant in rice soils, (Liesack et al., 2000) can also impact As mobility and bioavailability through the reductive dissolution of As-bearing minerals (Newman et al., 1997; Islam et al., 2004; Oremland and Stolz, 2005). Understanding the role of rhizosphere microbes in As transformation and bioavailability is necessary for the mitigation of As contamination in rice paddies. Therefore, it is imperative to determine the water management impacts on rhizosphere bacterial composition and activities that could potentially impact As transformation and bioavailability in rice paddies.

In this study, we investigated rhizosphere bacterial diversity and activities, speciation and distribution of As in the rhizosphere soil and in the soil pore water and uptake of As by rice when rice was cultivated under (1) flooded (the field was kept waterlogged until 2 weeks before crop harvesting), (2) non-flooded (~60% of saturated volumetric water) conditions, and (3) AWD conditions (alternate wetting and drying, where the field was re-flooded when saturated soil volumetric water reached 40%).

2. Materials and methods

2.1. Site description and experimental design

The field experiment was conducted at Hsuechia experimental site (23°12′4.02″ N and 120°10′50.7″ E) belonging to the Chianan Irrigation Association, Taiwan during the dry season (February–June) in 2014. Mean maximum and minimum temperatures from February to June were 33.0 °C and 19.8 °C, respectively. The mean evaporation rate, sunshine, solar radiation and total rainfall from February to June were 4.03 mm day^{-1}, 2.6 h day^{-1}, 131.0 cal cm^{-2} day^{-1} and 78.5 mm $month^{-1}$, respectively (data collected from Meteorological Workstation of the Chianan Irrigation Association, Hsuechia, Taiwan). The soil was sandy loam in texture with 13.0% clay, 15.0% silt and 71.0% sand, pH (H_2O) 6.4, a cation exchange capacity of 11 cmol kg^{-1}, and total organic carbon at 1.2% (methodological details are presented in the supporting information). Total As content of the soil was 16.0 ± 0.8 mg kg^{-1}, whereas the total As concentration of the groundwater used to irrigate the paddy field was 110 ± 3.0 μg L^{-1}, which was much higher than that of the permissible limit for the irrigation of crop lands (50 μg L^{-1}) in Taiwan (Chou et al., 2014). Even though the total As content of the paddy soil from the study sites is slightly higher than the background level (5–10 mg kg^{-1} is considered as the background level worldwide, Das et al., 2014), irrigation of paddy fields with As-rich groundwater has been reported to enhance the incorporation of this carcinogenic element into plants (Das et al., 2014; Chou et al., 2014).

The field was plowed thoroughly and flooded for 3 days before transplanting for the purpose of puddling and leveling. Rice plants (25-d seedlings of *Oryza sativa* japonica L. *cv.* Tainong 84) were transplanted using a rice transplanter (LY-6300, LEROY, China) at a spacing of 30 cm (row) × 14 cm (plant) with one seedling per hill in the field plots (9 m^2 area). Taifei No. 1 (N:P_2O_5:K_2O = 20:5:10 wt.%, Taiwan Fertilizer Corporation, Taiwan) at a rate of 400 kg ha^{-1} was applied as basal fertilizer before rice transplanting. $(NH_4)_2SO_4$ at a rate of 100 kg ha^{-1} and Taifei no. 5 (N:P_2O_5:K_2O = 16:8:12 wt.%, Taiwan Fertilizer Corporation, Taiwan) at a rate of 100 kg ha^{-1} were applied as the first and second top dressing fertilizers at the tillering (35 days after transplanting (DAT)) and at the panicle initiation (48 DAT) stage, respectively.

Three water management treatments were laid out in a randomized complete block design and were replicated three times. The treatments were: (i) Flooded (the field was kept waterlogged until 2 weeks before crop harvesting), (ii) Non-flooded (flooding was initiated 3 days before rice transplanting and lasted for 3 weeks, and thereafter, the field was maintained at ~60% of saturated volumetric water), (iii) AWD (flooding was initiated 3 days before rice transplanting and lasted for 3 weeks, and thereafter, it was followed by sequences of alternate wetting and drying. The field was re-flooded when saturated soil volumetric water