Do water regimes affect iron-plaque formation and microbial communities in the rhizosphere of paddy rice?

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Abstract

Pot experiments were conducted to investigate the effect of soil water regimes on the formation of iron (Fe) plaque on the root surface of rice seedlings (*Oryza sativa* L.) and on the microbial functional diversity in a paddy soil. The rice seedlings were subjected to three moisture regimes (submergence, 100%, and 60% water-holding capacity [WHC]), and were grown for 5 and 11 weeks. Aerobic lithotrophic Fe(II)-oxidizing (FeOB) and acetate-utilizing Fe(III)-reducing bacteria (FeRB) in the rhizosphere and non-rhizosphere soil were determined at 5 weeks using the most probable number (MPN) method. The carbon substrate use patterns of the microbial communities in the rhizosphere and non-rhizosphere soil samples were determined at 11 weeks using Biolog-GN2 plates. The amount of Fe plaque (per unit dry root weight) was much higher under submerged conditions than at lower soil moisture contents and decreased with plant age. There was a positive correlation between the amount of Fe plaque and phosphorus accumulated in the Fe plaque at both sampling times ($r = 0.98$ and 0.92 , respectively, $n = 12$). Numbers of FeOB and FeRB in the submerged soil were lower than in aerobic soil, but by two orders of magnitude higher in the rhizosphere than in the bulk soil. On the other hand, the functional diversity of the rhizosphere microbial communities was much higher than that of the non-rhizosphere soil, irrespective of soil water regimes. We conclude that soil flooding results in a decreased number and diversity of Fe-oxidizing/reducing bacteria, while increasing the Fe-plaque formation.

Key words: Biolog / Fe(II)-oxidizing bacteria (FeOB) / Fe(III)-reducing bacteria (FeRB) / most probable number / Shannon Index

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1 Introduction

Iron (Fe) plaque is a deposit of reddish-brown Fe oxides (hydroxides, oxyhydroxides) on the root surfaces of aquatic plants (Chen et al., 1980). Rice, a major aquatic crop plant, usually forms Fe plaque on its root surfaces. The oxides in Fe plaque are commonly dominated by poorly crystalline forms such as ferrihydrite, but can contain more crystalline Fe forms such as lepidocrocite (γ -FeOOH) and/or goethite (α -FeOOH) (Chen et al., 1980; Hansel et al., 2001). The oxidized Fe compounds, whose chemical and physical properties are similar to those of Fe oxides in soil, have a high capacity for binding phosphorus (P) (Christensen et al., 1997) and may influence the assimilation of P by aquatic plants. Formation of Fe plaque is mainly due to the oxidation of ferrous to ferric iron, which is facilitated by the release of oxygen and oxidants into the rhizosphere (Chen et al., 1980; Ando et al., 1983). The amounts of Fe plaque formed may depend on the availability of ferrous Fe in the vicinity of the root and the oxygenation capacity of the root system. The formation of Fe plaque on roots of wetland plants has been found to be related to soil chemical properties such as the amount of total free Fe(III) oxides and Fe carbonates (Macfie and Crowder, 1987), and close negative correlations have been found

between redox potential and concentrations of Fe and manganese (Mn) plaques (Christensen and Wigand, 1998). Liu et al. (2004a) demonstrated that P starvation might induce the formation of Fe plaque, and Fe plaque in turn may influence arsenate uptake by rice plants. In a recent study, Liang et al. (2006) confirmed that soil moisture regimes efficiently affected the Fe-plaque formation (6-fold higher under flooding condition than under field-capacity condition). In addition, it has been suggested that a rapid microbially mediated cycling of Fe occurs in the rhizosphere of *Juncus* roots under changing redox conditions (Küsel et al., 2003). The discovery of both lithotrophic Fe-oxidizing bacteria (FeOB) (Emerson et al., 1999) and Fe-reducing bacteria (FeRB) (King and Garey, 1999) on the roots of wetland plants indicates that plaque-associated microbes may directly influence plaque formation. Emerson et al. (1999) found that there was a positive correlation between cell numbers (FeOB) and the total amount of Fe present in *Sagittaria australis*, but the same correlation was not found for *Leersia oryzoides*. Johnson-Green and Crowder (1991) also reported that axenic roots accumulated significantly more Fe oxides than did non-axenic roots. Moreover, Weiss et al. (2004) demonstrated that the percentage

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of poorly crystalline Fe(III) was significantly correlated with the percentage of Fe-reducing bacteria. Though the role of the microbial oxidation of Fe(II) in the formation of Fe plaque is still a matter of debate, the microorganisms must mediate the Fe biogeochemistry cycle both in the rhizosphere and the non-rhizosphere soil.

In addition to the formation of Fe plaque, we hypothesized that soil moisture contents also influence rhizosphere microbial communities and the microorganisms (FeOB and FeRB) which may be involved in rhizosphere Fe cycle. The goal of our study was (1) to examine how soil water content affect Fe-plaque formation on roots of rice; (2) to investigate the functional diversity of the microbial communities particularly the abundance of FeOB and FeRB in the rhizosphere and the bulk soil; and (3) to explore the interactions between FeOB and FeRB and Fe-plaque formation.

2 Materials and methods

2.1 Preparation of rice seedlings

Rice seeds (*Oryza sativa* L.) cv. Jiahua-1, which was previously identified to form abundant Fe plaque on its root, were sterilized in 30% H₂O₂ (w:w) solution for 10 min, followed by thorough-washing with de-ionized water. The seeds were germinated in non-sterilized moist perlite, washed with distilled water three times. After 3 weeks, uniform seedlings were transplanted into a rhizobag system, separating the rhizosphere from a bulk-soil compartment. The rhizobags were 37 µm nylon meshbags, 6 cm in diameter, 7 cm in height, filled with 150 g soil, planted with one seedling, and transferred to PVC pots (7.5 cm diameter, 14 cm height). The gap between rhizo-bag and PVC pot was filled with 300 g of paddy soil from Jiaxing, Zhejiang province, China. The soil was a silty clay loam with pH 7.1 (H₂O), a CEC of 7.45 cmol kg⁻¹, 14.6 g kg⁻¹ organic C, and 18.8 g kg⁻¹ Fe. To ensure adequate mineral nutrition, N, P, and K were added at the start of the experiment at the following rates: 0.2 g N kg (urea), 0.03 g P kg (CaHPO₄ · 2H₂O), 0.08 g K kg (K₂SO₄).

2.2 Treatments

The soil water-holding capacity (WHC) was measured according to the standard method recommended by the Soil Science Society of China (Lu, 1999). Plants were grown under three moisture regimes, maintained by weighing and irrigation with distilled water twice per day as follows: (1) submerged, maintaining a flood-water depth of 2–4 cm above the soil surface, (2) 100% WHC, and (3) 60% WHC. Each treatment was replicated four times. After transplanted into the PVC tube, plants were grown for 5 and 11 weeks in a controlled-environment growth chamber with a 14/10 h light/dark cycle (260–350 µmol photons m⁻² s⁻¹), day/night temperature of 25°C/20°C, and 70% relative humidity.

2.3 Soil sampling

At harvest, nylon bags were carefully removed from the PVC pots. The soil adhering to the roots of individual plant was

removed with an aseptic metal spoon and designated as rhizosphere soil (Nicolaisen et al., 2004). Non-rhizosphere soil was defined as soil without rice roots, i.e., outside of nylon bags. Fresh soil samples were kept at 4°C until substrate-utilization analyses and FeOB and FeRB enumeration.

2.4 Extraction and determination of Fe plaque from roots

Roots were washed with tap water to remove soil particles adhering to the root surface and rinsed using distilled water more than three times. The fresh root material was incubated for 60 min at 25°C in 40 mL of dithionite-citrate-bicarbonate (DCB solution) containing 0.03 M sodium citrate (Na₃C₆H₅O₇ · 2H₂O) and 0.125 M sodium bicarbonate (NaHCO₃), with the addition of 0.6 g sodium dithionite (Na₂S₂O₄). Roots were rinsed three times with deionized water, and the washing solution was added to the DCB extracts. The resulting solution was diluted to 100 mL with deionized water (Taylor and Crowder, 1983). After DCB extraction, roots and shoots were oven-dried at 70°C for 3 d and weighed. The concentrations of Fe and P in the DCB extracts were measured by an inductively coupled plasma–optical emission spectrometer (ICP-OES, Optima 2000 DV, Perkin Elmer, USA).

2.5 Substrate-use patterns

The rhizosphere and bulk soil samples were collected to detect the physiological profiles of the community level (CLPP) by Biolog GN microplates (BIOLOG, Hayward, USA), containing 95 different carbon (C) sources, according to the method firstly described by Garland and Mills (1991). Ten grams dry weight of fresh soil were mixed with 100 mL autoclaved NaCl (0.85%), agitated for 1 min, and then put on ice for 1 min, which was repeated three times. After further dilution in 0.85% NaCl, 150 µL bacterial suspension (1:1000 dilutions) was inoculated into each Biolog GN2 plate wells. This dilution was chosen because pre-tests had shown that it was the lowest dilution that did not cause interference in the assay by co-extracted soil components, causing unspecific turbidity and absorbance. The plates were cultured for up to 7 d at 25°C in the dark, and the color development was measured every 24 h at 590 nm using a microplate reader of Biolog Microstation System (Biolog Inc., Hayward, CA).

2.6 Biolog-data analyses

Final values for the GN2 plates were calculated as the optical-density readings at 590 nm minus the A1 (control) well optical density. Negative well optical-density values were set to zero. The average well-color development (AWCD) value was calculated as the mean of the 95 blanked optical-density values. Visual impression of GN2 plates indicated that bacterial growth occurred in a majority of the plates after 96 h, so we chose the 96 h-incubation period for evaluating bacterial plates. The Shannon index of diversity (*H*) was used to determine the microbial richness (Schutter and Dick, 2001) and calculated as:

$$H = -\left(\sum_{i=1}^s P_i \times \ln P_i\right),$$

whith P_i being the proportion of species i in the sample, and

$$P_i = A_i / \sum_{i=1}^s A_i,$$

whith A_i being the catabolic activity of C source i (negative A_i values were set to zero). Shannon evenness, a measure of how evenly bacterial communities were distributed in soil samples, was calculated as $H' = H / \ln s$, where s is the number of C sources with $A_i > 0$. When significant ($\alpha = 0.05$) treatment differences were found, protected LSD values were calculated to separate the means. Shifts in physiological profiles of community level (CCLP) of different water regimes were analyzed by principal-component analysis (PCA) of normalized 96 h optical-density values. The utilization of substrate groups (carbohydrates, carboxylic acids, amino acids, amines and amides, polymers, and miscellaneous) within a plate were also analyzed (Zak et al., 1994).

2.7 Enumeration of aerobic lithotrophic FeOB and acetate-utilizing FeRB

An additional experiment was conducted under the same conditions as described above to investigate the abundance of aerobic lithotrophic FeOB and acetate-utilizing FeRB, using the most probable number (MPN) method (Weiss et al., 2003). The homogenized soil was diluted (1:100) and incubated for 1 h in a 10 mM sodium pyrophosphate solution to detach the bacteria from the soil particles. The soil suspension was diluted to 10^{-3} to 10^{-8} in modified Wolfe's mineral media (MWMM) and incubated in gradient tubes for the enumeration of FeOB (Emerson et al., 1999). A three-tube MPN series was inoculated from each dilution tube, which was used as a source to inoculate Fe-reducing medium (Widdel and Bak, 1992), containing sulfate-free MWMM, 10 mM sodium acetate, 50 mM sodium bicarbonate, 0.01% yeast extract (w/v), $1 \mu\text{L mL}^{-1}$ Wolfe's trace minerals, and 10% poorly crystalline Fe(III) as $\text{Fe}(\text{NO}_3) \cdot 9\text{H}_2\text{O}$, adjusted to pH 7–8 (Schwertmann and Cornell, 2000). The presence of FeOB was confirmed by the development of a discrete band at the oxic–anoxic interface within the gradient tube. The visual consumption of the Fe(III) oxides and the presence of Fe(II) was shown by color development with ferrozine (Sigma Chemical Co, adjusted to pH 7), indicating the presence of FeRB (Stookey, 1970). A subset of tubes positive for FeOB and FeRB was examined microscopically to confirm cell growth. Abundance of FeOB and FeRB was calculated using the Most Probable Number Calculator version 4.05 (©1996 Albert J. Klee, Risk Reduction Engineering Laboratory, United States Environmental Protection Agency, Cincinnati, Ohio, free software available at <http://www.epa.gov/nrlcwww/other.htm>).

2.8 Statistical analysis

Principal-component analysis on the Biolog data, and analysis of variance (ANOVA) on plant biomass, Fe and P concentrations, utilization of substrate groups, and Shannon diversity were performed using SPSS 11.5. at 5%-error probability.

3 Results

3.1 Effect of water regimes on Fe-plaque formation and P sequestration

Rice roots in the submerged treatment developed reddish-brown coatings, the typical color of Fe oxides, while no visible Fe coatings were observed at 60% WHC and 100% WHC. The amounts of Fe plaque differed significantly between soil-moisture treatments, ranging from 0.6 at 60% WHC to 27.8 g Fe kg^{-1} at soil submergence. Roots in the submerged treatment had the highest amounts of Fe plaque, and those in the other treatments had similar amounts of Fe plaque (Tab. 1). The amounts of Fe plaque harvested at 11 weeks were generally lower than those at 5 weeks.

The amount of P adsorbed to the plaque increased significantly with the amount of plaque formed, and significant positive correlations were found (data not shown, Pearson coefficient > 0.9 ; $p < 0.05$).

3.2 Biolog: Substrate-use patterns

For the three different water regimes, the average well-color development (AWCD) generally followed a sigmoid pattern with incubation time (Fig. 1). The AWCD of plates inoculated with rhizosphere soil increased rapidly after 24 h and reached significantly higher values within 144 h than plates inoculated with bulk soil. For the rhizosphere soil, the AWCD values were not affected by soil moisture content. However, for the non-rhizosphere soil, AWCD values with 60% WHC were significantly higher than in the other two treatments.

The PCA clearly indicated the degree to which profiles for each community were unique and reproducible and was used to identify contribution rate of different carbon substrates in different soil samples. Principal-component analysis scores were obtained after analysis of the covariance matrix of 96 h OD590 values. The first covariance PC axis accounted for 34%, the second accounted for 7% (Fig. 2). Principal-component axis 1 had the highest loadings on substrates for which there were consistent differences in the degree of substrate oxidation (*i.e.*, OD590 values) between microorganism communities. Principal-component analysis of Biolog GN meta-

Table 1: Iron and P concentrations in DCB extracts of rice seedling s with different soil moisture contents at 5 and 11 weeks.

Water regime	Fe concentration (g kg ⁻¹)		P concentration (g kg ⁻¹)	
	5 weeks	11 weeks	5 weeks	11 weeks
Submerged	27.8 ± 0.8	12.5 ± 2.3	0.73 ± 0.08	0.81 ± 0.09
100% WHC	0.8 ± 0.1	0.6 ± 0.1	0.26 ± 0.01	0.18 ± 0.03
60% WHC	2.3 ± 0.5	0.6 ± 0.1	0.32 ± 0.03	0.28 ± 0.05

Figure 1: Average well-color development profiles (A590) (AWCD) for samples from rhizosphere soil (circles) and bulk soil (squares) in the water treatments submerged, 100% WHC, and 60% WHC.

bolic fingerprints indicated differences in substrate utilization between rhizosphere and non-rhizosphere. The second axis showed a clear separation of the submergence from 100% WHC and 60% WHC.

Figure 2: Principal-component analysis (PCA) performed on the Biolog data after 96 h incubation of soil extracts. Symbols: circles, rhizosphere soil; squares, bulk soil.

Table 2: Shannon richness and evenness of AWCD after 96 h incubation of soil extracts from 60% WHC (water-holding capacity), 100% WHC, and submerged paddy soil. Means (mean $\pm$ SE, $n = 4$) followed by the same letter are not significantly different ($p < 0.05$).

Water regime	Bulk soil		Rhizosphere soil	
	Shannon richness	Shannon evenness	Shannon richness	Shannon evenness
Submerged	2.94 $\pm$ 0.20b	0.69 $\pm$ 0.04b	3.88 $\pm$ 0.05b	0.89 $\pm$ 0.01c
100% WHC	3.32 $\pm$ 0.23ab	0.83 $\pm$ 0.03a	4.13 $\pm$ 0.04a	0.92 $\pm$ 0.01b
60% WHC	3.73 $\pm$ 0.06a	0.86 $\pm$ 0.02a	4.24 $\pm$ 0.04a	0.94 $\pm$ 0.01a

Microbial substrate-utilization potentials for six types of Biolog substrates (amino acid, carbohydrate, carboxylic acid, polymer, amine, miscellaneous) were determined using AWCD after 96 h of incubation (Fig. 3). Utilization of Biolog substrates was generally greater in the rhizosphere than in the non-rhizosphere, especially the utilization of carbohydrates. Utilization of amino acids, carbohydrates, and carboxylic acids was higher than that of the remaining substrates. Compared to other substrates, amine utilization was the lowest, irrespective of soil moisture content.

A t-test was performed to test the significant differences in Shannon richness and evenness between the water treatments (Tab. 2). Soil water treatments had a significant effect on Biolog Shannon richness and evenness; both parameters increased with decreasing soil moisture contents. The Shannon richness and evenness of the rhizosphere soil were much higher than those of the non-rhizosphere soil irrespective of soil water treatment.

Figure 3: Six types of Biolog substrate-utilization potentials of microbial communities following different water regimes. Within each substrate, means ($n = 4$) followed by the same letter are not significantly different ($p < 0.05$).

3.3 Abundance of FeOB and FeRB

Besides the submergence treatment, the densities both of FeOB and FeRB in the rhizosphere were one to two orders of magnitude higher than those in the non-rhizosphere (Tab. 3). The highest FeOB abundance was observed in the rhizosphere in the treatment of 100% WHC. The lowest FeOB abundance was 6.6×10^4 cells g⁻¹ in the non-rhizosphere submerged soil, which also had the lowest FeRB density of 3.4×10^4 cells g⁻¹. The FeOB and FeRB in the submerged paddy soil were lesser than in the other two treatments.

4 Discussion

4.1 Effect of soil moisture on the formation of Fe plaque and adsorbed P

The submerged rice roots formed more Fe plaque than roots in aerobic soil as reported before (Liang et al., 2006), as possibly larger amounts of dissolved Fe moved to rhizosphere and as the Fe(II) concentration increased both in the rhizosphere and bulk-soil solution after flooding. A subsequent decrease of the Fe(II) concentration in the soil solution could be the result of Fe(II) precipitation as siderite, vivianite, or Fe sulfide. Compared with the submerged soil, the treatments with 100% and 60% WHC underwent several drying–wetting cycles, resulting in changes of physical and chemical soil properties (e.g., redox potential, mobility of ferrous Fe). The mobility of ferrous Fe may have decreased because of its precipitation and adsorption to the soil matrix (Kirk, 2004). Similarly, Zhao et al. (2003) reported that DTPA-extractable Fe and exchangeable Fe in the soil significantly increased during flooding. A low Fe-plaque formation in the treatments with 100% and 60% WHC may indicate a low availability of mobile ferrous Fe in the soil. This study also conformed that the amount of Fe plaque decreased with the growth stage of the rice plants. The roots sampled at 11 weeks were significantly larger than those sampled at 5 weeks, visual appearance of reddish coating was not equal throughout the root system, and the root growth might outpace Fe-plaque formation. As a result, the Fe concentration, calculated on the basis of dry root weight, decreased with advancing growth stages. Increasing Fe(II) in the growth medium could effectively increase the formation of Fe plaque on rice roots (Liang et

al., 2006; Liu et al., 2004b). It may be concluded that the formation of Fe plaque is closely associated with the concentration and the mobility of Fe in the soil.

Besides the dissolved Fe(II) in soil solution, the formation of Fe plaque on the rice root can be affected by factors such as oxygen released from rice roots, P starvation, and the rice genotypes (Armstrong, 1967; Liu et al., 2004a, b). The Fe-plaque formation on the root surface of wetland plants has been attributed to the gas exchange between the atmosphere and the anaerobic soils (Van der Nat and Middelburg, 1998). As a result, the change in oxygen transported from the conduit could influence the formation of Fe plaque. However, the morphology of rice roots under different water regimes was not observed in this study.

Similar to this study, Zhang et al. (1999) found that Fe plaque might act as a P pool. In the present study, the amount of P adsorbed on the Fe plaque was correlated with rice-plant biomass. This suggests that P trapped in Fe plaque may be available to the plant, which is supported by Wigand and Severson (1997).

4.2 Effect of soil moisture on the microorganisms

The bacterial communities of rhizosphere and non-rhizosphere soil in the different treatments were also evaluated using Biolog. All data (AWCD, PCA, and six types of Biolog substrate utilization) indicated that the rhizosphere community differed from that in the non-rhizosphere. This agrees with numerous earlier reports (Kent and Triplett, 2002). A different utilization of C sources between rhizosphere and non-rhizosphere microorganisms suggests a different availability of these C sources in the rice rhizosphere and non-rhizosphere, which may influence the proliferation of particular communities of microorganisms. This microbial diversity may have arisen from the variation in C compounds exuded by the rice roots. In the present experiment, the microbial community utilized carbohydrates the most, but utilized amines and polymers poorly (Fig. 3), suggesting that the microbes are adapted to simple rather than to complex C resources in rice rhizosphere.

Table 3: Most-probable-number (MPN) enumerations of Fe(II)-oxidizing bacteria (FeOB) and Fe(III)-reducing bacteria (FeRB) in rhizosphere and bulk paddy soil with different water regimes. Overflow of the MPN calculator version 4.05 with the series of dilution is denoted by a “NA”.

Water regime	FeOB		FeRB	
	MPN (cell g ⁻¹)	95% confidence interval	MPN (cell g ⁻¹)	95% confidence interval
Bulk soil				
Submerged	6.6×10^4	1.6×10^4 – 2.7×10^5	3.4×10^4	9.8×10^3 – 1.2×10^5
100% WHC	7.1×10^4	1.7×10^4 – 3.0×10^5	6.0×10^5	1.5×10^5 – 2.5×10^6
60% WHC	7.7×10^4	1.8×10^4 – 3.3×10^5	8.2×10^4	1.9×10^4 – 3.6×10^5
Rhizosphere soil				
Submerged	5.4×10^4	1.3×10^4 – 2.2×10^5	5.4×10^4	1.3×10^4 – 2.2×10^5
100% WHC	NA	NA	3.5×10^6	9.4×10^5 – 1.3×10^7
60% WHC	9.0×10^5	2.1×10^5 – 3.9×10^6	2.9×10^6	8.1×10^5 – 1.0×10^7

The changes in microbial-community diversity in response to different moisture contents were detected in the paddy soil. Similarly, effects of moisture content have been shown to influence the diversity and cultivable activity of bacterial communities in the rhizosphere of an established upland grassland soil (Griffiths et al., 2003). It was concluded that controlled soil drying decreased the substrate-utilization response, and subsequent re-wetting of dried soil led to the recovery of the microbial population. In the present study, abundances of FeOB and FeRB in the submerged soil were less than in the other two treatments. It is well known that in non-rhizosphere soil, anoxic conditions are established under flooded conditions and fermentation and methanogenesis are the major microbial pathways after depletion of other electron acceptors. Decreasing the water content changes the redox conditions towards oxic condition, and therefore the community will adapt to more aerobic microorganisms. It is known that under oxic condition, the species richness and evenness increases, and the oxic incubation condition may favor the aerobic bacteria. However, Biolog plate used for environmental probes has different drawbacks (Preston-Mafham et al., 2002). Although Biolog analysis has the limitation of selecting for fast growing bacteria, thereby promoting minimal fungal metabolism, and some fungi cannot reduce the tetrazolium dye (Dobranic and Zak, 1999), the growth-based techniques (Biolog) could partly indicate the relative diversity between different treatments, and it can be concluded that the submerged paddy soil contained fewer aerobic bacteria despite the microaerophilic environment of the rhizosphere. Soil moisture directly affects the osmotic status of bacterial cells and can indirectly regulate substrate availability, diffusion of gases, soil pH, and temperature (Griffiths et al., 2003). As a consequence, these factors will induce changes of structure and function of soil bacterial communities.

4.3 A rhizosphere Fe cycle

In the present study, large numbers of FeOB and FeRB were found in both rhizosphere and non-rhizosphere. In general, FeOB and FeRB were more abundant in the rhizosphere than in non-rhizosphere indicating that a more rapid active Fe cycle may exist in the rhizosphere. It has been described that anaerobic Fe(II) oxidation is mediated by members of both *Archaea* and bacteria domains (Straub et al., 1996; Hafenbradl, 1996) in anoxic environments at circumneutral pH. Furthermore, the high abundance of poorly crystalline Fe(III) minerals, which were the main component of Fe plaque, and high levels of labile carbon contributed to the high densities of acetate-utilizing FeRB in the rhizosphere. Weiss et al. (2004) found that the percentage of poorly crystalline Fe (III) was significantly correlated with the percentage of FeRB ($r = 0.76$), reflecting the fact that poorly crystalline Fe(III) minerals are labile with respect to microbial reduction. The FeRB consumed a large amount of poorly crystalline Fe(III) oxides of the Fe plaque with the incubation time. Qu et al. (2004) found that *Geobacter metallireducens* GS-15 could only reduce poorly crystalline Fe(III) minerals in a 4-day incubation. The presence of Fe(III)-dominated Fe plaque readily functions as electron acceptors for FeRB. On the other hand, the rhizosphere Fe(II) oxidation in paddy soils may be due to other biotic processes. For example, nitrate-reduction

coupled to Fe(II) oxidation was reported in the rice paddy soil (Ratering and Schnell, 2001). The ubiquity and diversity of these anaerobic FeOB suggests that nitrate-dependent Fe(II) oxidation, light-independent reactions, have the potential to contribute to anoxic Fe oxidation in the rhizosphere, provided adequate concentrations of a suitable electron acceptor.

It is likely that the microbial metabolism affects the physical and chemical characteristics of the rhizosphere, and subsequently influences Fe-plaque formation. For example, microbial oxidation of root exudates (e.g., glucose, glycine, citrate, and malate) to CO₂ might affect solution pH (Küsel et al., 2003). Similarly, it was reported that the pH of Fe solutions after exposure to axenic and non-axenic seedlings was significantly different (Johnson-Green and Crowder, 1991). The ability of Fe-oxidizing bacteria to compete with the chemical oxidation of Fe(II) may depend on the rhizosphere pH. The kinetics of Fe oxidation are relatively slow at low pH (<4). At low pH, Fe-oxidizing bacteria, such as *Thiobacillus ferrooxidans*, may increase Fe oxidation kinetics (Emerson et al., 1999) and thereby contribute to Fe-plaque formation. Therefore, there may be interactions between plaque formation and microbial processes. However, it is still unknown which species of bacteria play the key role in this process. Further studies are therefore needed to investigate some functional bacteria (e.g., Fe-oxidizing or Fe-reducing bacteria), which may be important in Fe-plaque formation.

5 Conclusion

To conclude, our data implicate marked differences in Fe-plaque formation and soil microbial-community composition in the different soil water treatments. High soil moisture content increased the formation of Fe plaque and in consequence the P adsorption on the root surface. Moreover, based on substrate-utilization analyses, significant changes were observed for the total microbial community in the different moisture treatments. With low moisture content, CCLP of the paddy soil increased, and that of the rhizosphere was significantly higher than that of the bulk soil. Further studies are warranted on some functional bacteria (e.g., Fe-oxidizing or Fe-reducing bacteria), which may be important in Fe-plaque formation and in providing insights into the mechanism of Fe-plaque formation.

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