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Community structure of *Anaeromyxobacter* in Fe(III) reducing enriched cultures of paddy soils

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Abstract

Purpose *Anaeromyxobacter* is a typical representative genus of dissimilatory metal-reducing microbes. However, the community structure and metabolic function of *Anaeromyxobacter* have rarely been reported because of the limited number of *Anaeromyxobacter* isolations. Therefore, this study aimed to investigate the community structure and succession of *Anaeromyxobacter* in a Fe(III)-reducing enriched culture of paddy soils.

Materials and methods A 40-day anaerobic incubation of paddy soils enriched with ferrihydrite and goethite was conducted to investigate the response of the community structure and succession of *Anaeromyxobacter* to iron oxide addition.

Results and discussion The dominant *Anaeromyxobacter* in paddy soils were potentially capable of Fe(III) reduction. Ferrihydrite enrichment increased the absolute abundance of *Anaeromyxobacter* by 0.01×10^8 to 3.2×10^8 copies g^{-1} soil, while goethite enrichment increased the absolute abundance of *Anaeromyxobacter* by 0.004×10^8 to 1.8×10^8 copies g^{-1} soil. Iron oxide enrichment significantly influenced the richness of *Anaeromyxobacter* during the later stages of incubation but had a negligible influence on the evenness. Nonetheless, Fe(II) accumulation was stimulated by ferrihydrite enrichment after paddy soil was incubated for 5 days, whereas goethite had a negligible effect on Fe(II) accumulation. Redundancy analysis revealed that *Anaeromyxobacter* community succession was closely correlated with the processes of Fe(III) reduction.

Conclusions Exogenous ferrihydrite addition showed a greater influence than goethite on the *Anaeromyxobacter* community during anaerobic incubation of paddy soils. The difference in inherent amorphous iron oxide content in paddy soils was also decisive in the distinct community structure and succession of *Anaeromyxobacter* in paddy soils.

Keywords *Anaeromyxobacter* community · Fe(III) reduction · Ferrihydrite · Goethite · Paddy soil

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1 Introduction

The redox process of iron (Fe) oxides in paddy soil is a prominent biogeochemical process when the soil redox potential decreases to the range of − 100–100 mV after submergence (Johnston et al. 2014; Weber et al. 2006). Fe(III) reduction in submerged paddy soil is considered a microbial-mediated biogeochemical process, which is important in the global element cycle and remediation of polluted soils (Burgin et al. 2011; Hori et al. 2010; Li et al. 2012a; He et al. 2018). It is widely accepted that microorganisms are the main drivers mediating dissimilatory Fe(III) reduction (Lovley 2006; Xu et al. 2014).

Anaeromyxobacter, a typical representative genus of dissimilatory Fe(III)-reducing microbes, can use acetate or H_2 as electron donor to reduce Fe(III) to Fe(II) and gain energy to maintain growth in the process (He & Sanford 2003; Treude et al. 2003; Lin et al. 2007). Many studies have indicated that *Anaeromyxobacter* community is strongly related to the

Fe(III) reduction process in various environments (Hori et al. 2007 and 2010; Zhu et al. 2011). Although it has been widely isolated for example, from paddy soils, river sediments, and compost, *Anaeromyxobacter* is less dominant in such habitats, resulting in a lack of knowledge regarding the metabolic function of *Anaeromyxobacter*. Except for the critical role in Fe(III) reduction, *Anaeromyxobacter* could alternatively take halogenated phenols, nitrate, nitrous oxide, fumarate, oxygen, U (VI), As(V), and Se (IV) as electron acceptors competing with Fe(III) (Wu et al. 2006; Sanford et al. 2007; Thomas et al. 2009; Strycharz et al. 2010; He and Yao 2011; Sanford et al. 2012; Tonomura et al. 2015). This suggests the potential role of *Anaeromyxobacter* in the bioremediation of heavy metals, radioactive metals, and organic halide pollution. Therefore, investigating the succession and diversity of *Anaeromyxobacter* could improve understanding of the metabolic versatility of *Anaeromyxobacter*.

Because iron oxides are the terminal electron acceptor of the electron transport chain, the abundance and species of iron oxide greatly affect the process of microbial Fe(III) reduction (Qu et al. 2004; Bonneville et al. 2009). Amorphous iron oxide and lepidocrocite as representatives of weakly crystalline iron oxides are easily reduced by Fe(III)-reducing bacteria, whereas microbial reduction of goethite, hematite, and their aluminum substitutes as representatives of crystalline iron oxides is more difficult (Qu et al. 2004). The different species of iron oxide can influence the bacterial community structure in paddy soil. Li et al. (2012b) enriched bacteria in *Geobacter* and *Clostridium* genus with ferrihydrite and found that *Clostridium* spp. was the dominant population in goethite enrichment. Although Fe(III)-reducing bacterial strains were isolated and identified in the enriching culture with different species of iron oxides, only a few studies had investigated the difference of the *Anaeromyxobacter* community during anaerobic incubation with ferrihydrite and goethite (Hori et al. 2010; Ding et al. 2015; Hori et al. 2015).

In addition, the abundance and species of iron oxides in paddy soil vary greatly due to the differences of parent materials and anthropogenic management of paddy soil (Yuan et al. 2016). The succession and diversity of *Anaeromyxobacter* community over flooding time in different types of paddy soils remain unclear. Moreover, the success of the *Anaeromyxobacter* community in response to the exogenous addition of the different species of iron oxide also requires further research. Therefore, this study investigated the changes of *Anaeromyxobacter* community in the enriched culture of different paddy soils with ferrihydrite and goethite based on the qPCR and high-throughput sequencing technology. Furthermore, the relationship between the *Anaeromyxobacter* community and the process of Fe(III) reduction was also analyzed to better understand the biogeological mechanism of Fe(III) reduction in paddy soil.

2 Materials and methods

2.1 Soil sampling

Soil samples were collected from the upper 20 cm of two paddy fields located in the Experimental Station of Hunan Academy of Agriculture Science, Changsha, Hunan province (HN; 28°12′26″N, 113°5′36″E; collected in August 2014) and Huilong town, Qionglai city, Chengdu, Sichuan province (SC; 30°18′8″N, 103°40′53″E, collected in April 2018), respectively. After rice was harvested, five sites were selected in each paddy field along an S-shaped pattern for the collection of a composite sample. Subsequently, the visible plant residues in the samples were removed. The soil samples were then air-dried, filtered through a 1-mm sieve, and stored at room temperature. The basic chemical properties of the two soil samples were determined by standard methods (Table 1) (Page et al. 1982).

2.2 Synthesis of iron oxide

Ferrihydrite and goethite were used in the present study, which represented an amorphous iron oxide and crystalline iron oxide, respectively. Both iron oxides were synthesized according to the methods of Schwertmann and Cornell (1991). The lattice structure of the iron oxides was analyzed by X-ray diffractometer (D8 ADVANCE A25, Bruker, Germany) and is shown in Fig. S1 (Electronic Supplementary Material–ESM).

2.3 Treatments and anaerobic incubation experiments

Two series of enriched cultures of paddy soils were conducted through the addition of ferrihydrite or goethite. Paddy soil without iron oxide supplement was considered as the control treatment (CK). A series sample of exactly 3000 g of soil was mixed with 3 mL ferrihydrite or goethite suspension or 3 mL distilled water in 10-mL sterile serum bottles. The concentration of added iron oxides was 5 mg Fe per gram air-dried soil. Subsequently, serum bottles were covered with rubber stoppers, purged with nitrogen gas (N_2) for 5 min, sealed with aluminum covers, and then incubated in a controlled environment incubator in darkness at 25 °C for 40 days. During the anaerobic incubation, three bottles of slurry samples were randomly selected from each treatment as three biological replicates and stored at − 80 °C for extraction of total soil DNA on days 1, 5, 10, 20, 30, and 40. Meanwhile, three bottles were randomly selected from each treatment to determine the Fe(II) content using the o-phenanthroline colorimetric method as previously described (He and Qu 2008).

Table 1 Basic chemical properties of tested paddy soils ($\bar{x}$ ±s, $n = 3$)

Soils	pH	Amorphous Fe (mg g^{-1})	Free Fe (mg g^{-1})	Acid-soluble Fe (mg g^{-1})	Dissolved organic matter (mg g^{-1})
HN	5.40 ± 0.03a	5.16 ± 0.17a	17.95 ± 0.45a	5.85 ± 0.07a	5.21 ± 0.23a
SC	4.93 ± 0.02b	6.77 ± 0.21b	13.79 ± 0.34b	7.82 ± 0.11b	7.03 ± 0.15b

Note: Means followed by different letter within the same column indicate significant difference at $p < 0.05$. HN, soil collected from the Experimental Station of Hunan Academy of Agriculture Science, Changsha, Hunan Province. SC, soil collected from Huilong town, Qionglai City, Chengdu, Sichuan Province. The same below

2.4 Total microbial DNA extraction

After the slurry samples were thawed and thoroughly mixed, 800 μL of the slurry was pipetted into a weighed 2-mL centrifuge tube containing glass beads. The tube containing the slurry sample was weighed again to determine the weight of the collected slurry, and then centrifuged (D3024R, Scilogex, USA) at 4 °C at 5500 rpm (2870×*g*) for 15 min to remove the liquid. Thereafter, the rest of the soil sample was subjected to DNA extraction with the Soil DNA Kit (D5625-01, Omega Bio-Tek, Norcross, GA, USA), and the extracted total DNA was stored at − 80 °C.

2.5 Quantitative polymerase chain reaction

To determine the absolute abundance of *Anaeromyxobacter*, the extracted soil total DNA in triplicate was used as template for the quantitative polymerase chain reaction (qPCR), and Fac12-66F/Fac12-432R (Treude et al. 2003) was used for the specific primer of *Anaeromyxobacter*. The qPCR was performed in triplicate in a 25-μL mixture containing 1 μL of DNA template, 0.5 μL of each primer, 0.5 μL of ROX Reference Dye (TaKaRa Bio, Otsu, Japan), 12.5 μL of SYBR Premix Ex TaqTM (TaKaRa Bio, Otsu, Japan), and 10 μL of sterile ddH_2O. Subsequently, the qPCR using a StepOnePlusTM Real-Time PCR Instrument (Thermo Fisher Scientific, USA) was started with pre-denaturation at 95 °C for 3 min, and followed by 40 cycles of denaturation at 95 °C for 20 s then annealing at 55 °C for 40 s and extension at 72 °C for 45 s.

2.6 Community analysis

Partial sequence of the bacterial 16S rRNA gene, targeting the V3-V4 hypervariable region, was amplified with primer pairs 341F/806R. Sequencing was performed on an Illumina HiSeq PE250 platform (Novogene Bioinformatics Institute, Beijing, China). All the raw sequence data were filtrated to remove the low-quality sequence reads, such as reads containing ambiguous bases and/or those shorter than 200 bp. The effective sequences were clustered into operational taxonomic units (OTUs) with a threshold of 97% similarity, and then classified taxonomically via the Silva database (http://www.arb-silva.de).

2.7 Data analysis

The OTUs belonging to the *Anaeromyxobacter* genus (named A-OTUs) were selected, and the relative abundance of each A-OTU was calculated as the ratio of its sequence reads to that of the total bacteria. The diversity of the *Anaeromyxobacter* community was assessed by the Marglef index and the evenness index based on the A-OTU. The similarity of the *Anaeromyxobacter* community, based on the A-OTU, was determined by a principal component analysis (PCA) using CANOCO 4.5 (http://www.canoco5.com). The phylogenetic tree analysis was constructed using MEGA 6.0 with the neighbor-joining method (www.megasoftware.net). A redundancy analysis was conducted to analyze the relationship between the *Anaeromyxobacter* and environmental factors using CANOCO 4.5 software.

3 Results

3.1 Absolute abundance of *Anaeromyxobacter*

The variation in the absolute abundance of *Anaeromyxobacter* with incubation time was considerably different between the HN and SC paddy soils (Fig. 1). Iron oxide enrichment resulted in significant increases in the absolute abundance of *Anaeromyxobacter* in both soils, which was ranked in the order of ferrihydrite > goethite > CK. In HN soil, the absolute abundance of *Anaeromyxobacter* increased near linearly from 0.12×10^8 to 0.87×10^8 copies g^{-1} soil along with the incubation time from 1 to 40 days of incubation. Ferrihydrite enrichment enhanced the absolute abundance of *Anaeromyxobacter* to between 0.14×10^8 and 1.68×10^8 copies g^{-1} soil, and goethite enrichment enhanced the absolute abundance of *Anaeromyxobacter* to between 0.13×10^8 and 1.31×10^8 copies g^{-1} soil. The promotion of iron oxides on the growth of *Anaeromyxobacter* was more apparent at 20–40 days of incubation relative to the early stage ($p < 0.05$). In SC soil, abundance of *Anaeromyxobacter* increased rapidly and peak at day 5, then decreased to minimum on day 10, and increased slightly until the end of the incubation. Compared with the CK treatment, the absolute abundance of *Anaeromyxobacter* at days 5–10 of incubation was

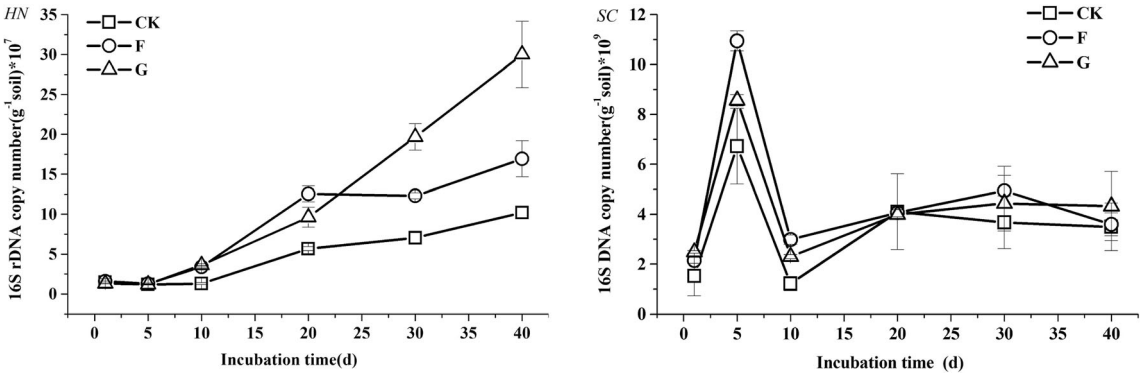

Fig. 1 Absolute abundance of A-OTUs in the paddy soils during anaerobic enrichment culture with different species of iron oxides (CK, the original soil without adding iron oxides; F, soil with added ferrihydrite; G, soil with added goethite; paddy soils were collected from Hunan Province (HN) and Sichuan province (SC), respectively; $n = 3$ for each treatment, the same below)

significantly enhanced by 0.6–1.5 times in the ferrihydrite enrichment and 0.3–0.9 times in the goethite enrichment ($p < 0.05$).

3.2 Community structure and relative abundance of *Anaeromyxobacter*

According to the result of high-throughput sequencing, *Anaeromyxobacter* genus accounted for 0.03–2.31% and 0.86–5.19% of the total bacteria in the HN and SC paddy soil, respectively, which was assigned to 23 and 36 microbial OTUs, respectively (Fig. 2). The variation trend of relative abundance over incubation time was parallel to that of absolute abundance. For the HN paddy soil, the relative abundance of *Anaeromyxobacter* gradually rose to a peak of 2.84% on day 30 of the incubation and then declined slightly. Compared with the CK treatment, iron oxide addition showed evident influence to the growth of *Anaeromyxobacter* during 30–40 days of incubation ($p < 0.05$). The relative abundance of *Anaeromyxobacter* during 30–40 days of ferrihydrite-enriched incubation was 1.29–1.34 times higher than the CK treatment, while that in the goethite-enriched incubation was 1.05–1.12 times higher than that of the CK treatment. A-OTU_29 was the dominant species and occupied 39–73% of *Anaeromyxobacter* genus after 10 days of incubation. It was closely related to uncultured *Anaeromyxobacter*, which was identified in the environment of goethite as the sole electron acceptor (Fig. 3). Coincidently, the growth of A-OTU_29 at 20–40 days was stimulated in the goethite enrichment but inhibited in the ferrihydrite enrichment. Another dominant population, A-OTU_476 was closely related to uncultured *Anaeromyxobacter*, which has been identified in the uranium-contaminated underground water and sediments, indicating the potential of metal-reducing ability.

For SC paddy soils, the relative abundance of *Anaeromyxobacter* in the CK treatment increased rapidly to the peak of 4.65% on day 5, but decreased to 4.01% on day 10, and then fluctuated in the range of 4.38–4.46%. Ferrihydrite enrichment resulted in slight increases in relative abundance of *Anaeromyxobacter* to 4.45–5.19% during 5–20 days of incubation. For goethite enrichment, the relative abundance of *Anaeromyxobacter* was decreased to 3.66% at

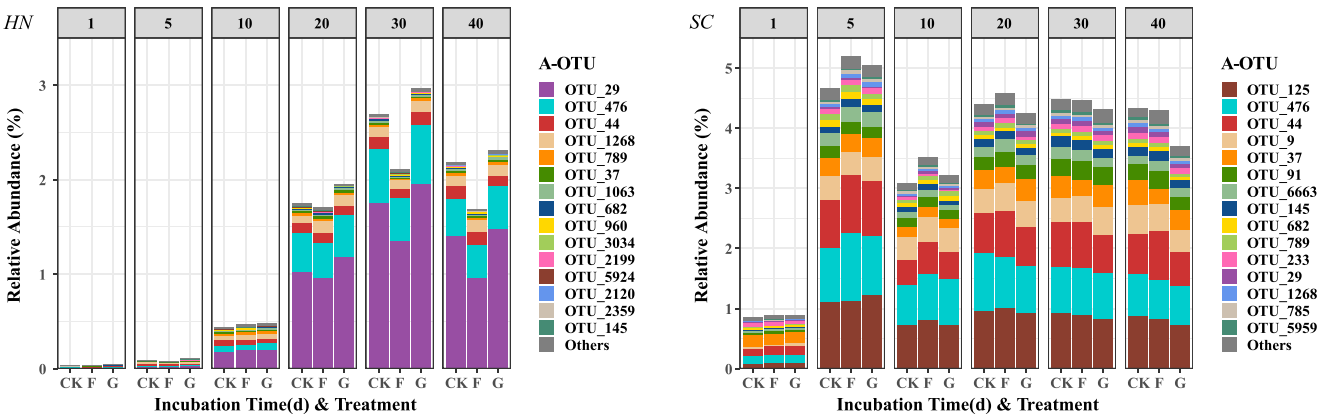

Fig. 2 Relative abundance of A-OTUs in paddy soils during anaerobic enrichment culture incubation with different species of iron oxides

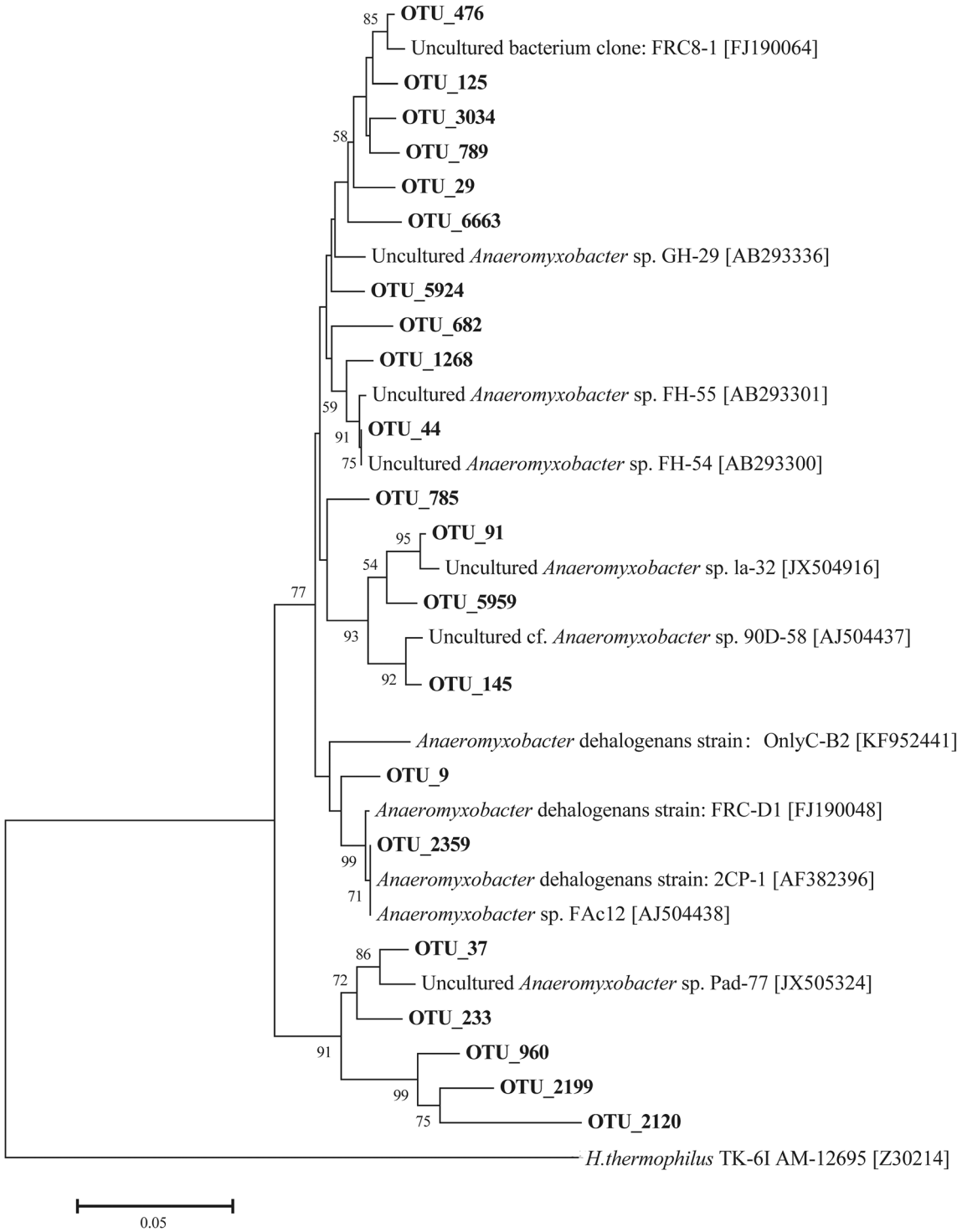

Fig. 3 Phylogenetic tree analysis of *Anaeromyxobacter* on the 16S rDNA sequences. Bold font OTUs represent the A-OTUs based on high-throughput sequencing. Values > 50% (1000 replications) are shown at the branches. GenBank accession numbers are in the parentheses. The scale bar represents 5% sequence divergence. *Hydrogenobacter thermophilus* (*H. thermophilus*) is used as the outgroup

40 days of incubation. Like A-OTU_476, A-OTU_125 was closely related to uncultured *Anaeromyxobacter* with potential capacity of metal reduction. A-OTU_44 was another dominant species of *Anaeromyxobacter* that occupied 39–73% of the *Anaeromyxobacter* genus after 1 day of incubation in SC paddy soil. It was closely related to uncultured *Anaeromyxobacter*, which was identified in the environment of ferrihydrite as the sole electron acceptor. The growth of A-OTU_44 was stimulated with ferrihydrite enrichment but suppressed with goethite enrichment. A-OTU_9 was also related

to *Anaeromyxobacter* that was capable of ferrihydrite reduction.

3.3 α-Diversity index of *Anaeromyxobacter*

The Marglef and evenness diversity of *Anaeromyxobacter* could characterize the richness and uniformity of *Anaeromyxobacter* communities, respectively (Fig. 4). For the HN paddy soil, the Marglef index of *Anaeromyxobacter* gradually increased within the first 10 days of anaerobic incubation and was then maintained at approximately 3.0 during the subsequent incubation time. Compared with the CK treatment, goethite enrichment led to a remarkable increase in the Marglef index by 24.50% on day 30 of incubation ($p < 0.05$), whereas ferrihydrite enrichment caused no significant difference on the Marglef index during the entire incubation. For SC paddy soil, the Marglef index of *Anaeromyxobacter* fluctuated in the range of 3.8–4.5 during the anaerobic incubation. Ferrihydrite enrichment showed significant stimulation on the richness of *Anaeromyxobacter*, and the Marglef index on day 1 and day 20 of incubation in the ferrihydrite enrichment was 0.58 and 0.55 higher than that of the CK treatment ($p < 0.05$). There was no evident influence of goethite enrichment on the richness of *Anaeromyxobacter*.

The evenness index of *Anaeromyxobacter* in HN paddy soil decreased rapidly within the first 10 days of anaerobic incubation, and then remained at a stable level. For SC paddy soil, the evenness index of *Anaeromyxobacter* remained at approximately 0.3 after 5 days of anaerobic incubation. Nonetheless, iron oxide enrichment led to few differences in the evenness index of *Anaeromyxobacter* at an identical time of incubation.

3.4 PCA analysis for the community structure of *Anaeromyxobacter*

PCA was performed to illustrate the differences in the community structure of *Anaeromyxobacter* between all samples (Fig. 5). Axis 1 explained 96.4% and 67.2% of variation in the HN and SC soil, respectively, and axis 2 explained 1.4% and 9.6%, respectively. For the HN soil, samples incubated for 1, 5, and 10 days in the enrichment and CK treatment were clustered into three distinct groups according to the incubation time, implying that the form of iron oxide had a limited influence on the diversity during the first 10 days of incubation.

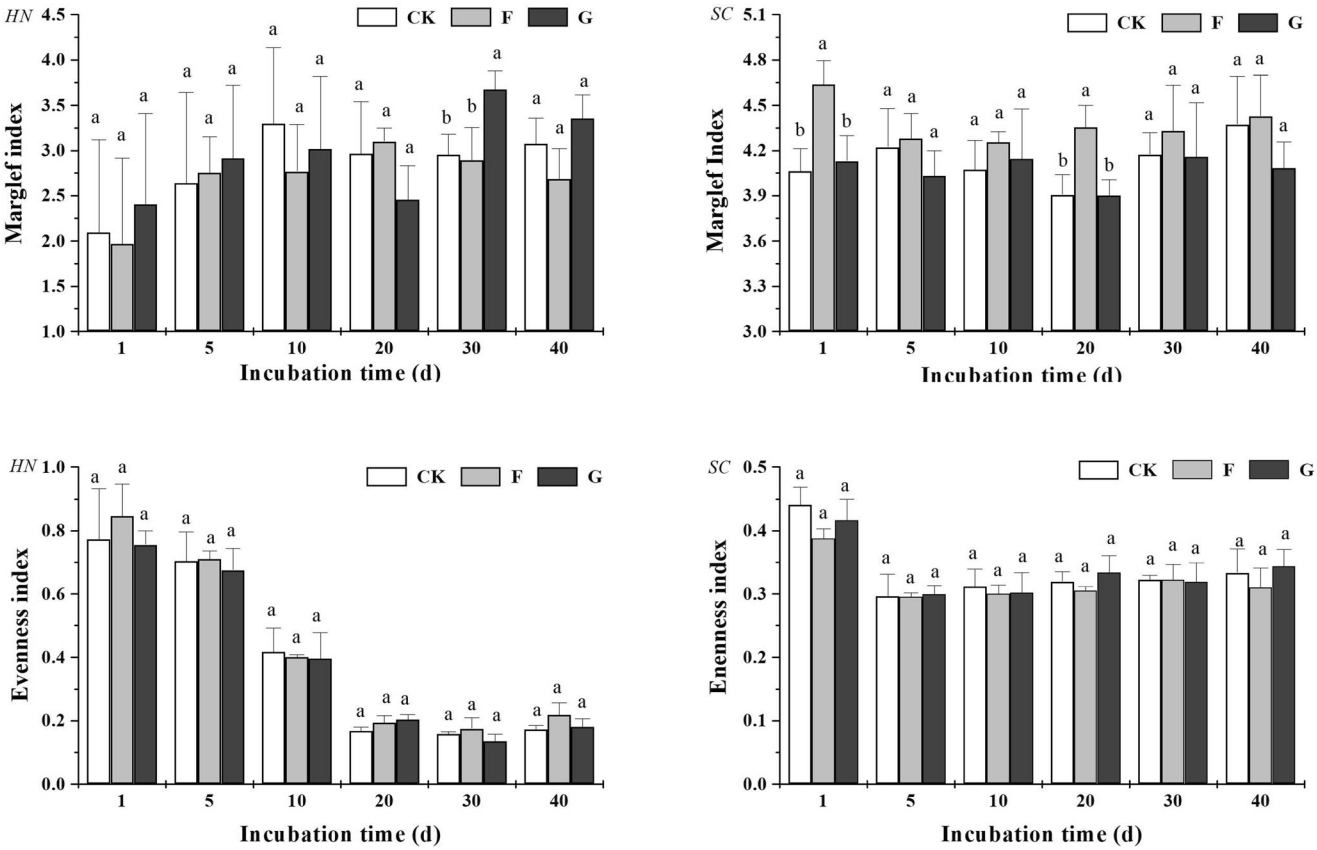

Fig. 4 The Marglef and evenness index of *Anaeromyxobacter* in paddy soils during anaerobic enrichment culture with different species of iron oxides (vertical bars represent standard errors of the means ($n = 3$), different small letters above the column indicate a significant difference between the three treatments at the $p < 0.05$ level, the same below)

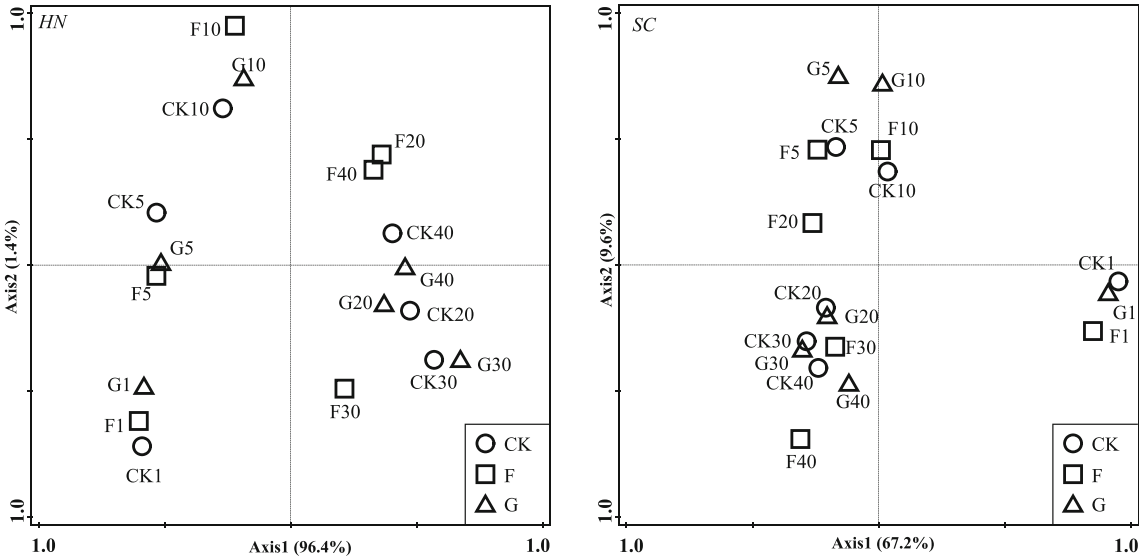

Fig. 5 Principal component analysis (PCA) plot based on the relative abundances of A-OTUs in paddy soils during anaerobic enrichment culture with different species of iron oxides (the number followed by the abbreviations of the treatments represents the anaerobic incubation time. One sample point represents the average of biological replicates ($n = 3$))

During the subsequent part of the incubation (20–40 days), samples in the ferrihydrite enrichment were clustered far from samples in the CK and goethite enrichment. For SC paddy soil, samples were clustered in the ordination plot in three distinct groups: 1 day, 5/10 days, and 20/30/40 days, which suggest a clear succession of *Anaeromyxobacter* with incubation time. During 20–40 days of incubation, samples in CK and the goethite enrichment were similar, whereas samples in ferrihydrite enrichment were still scattered.

3.5 Dynamics of Fe(II) concentration

After the HN and SC paddy soils were submerged, Fe(II) both in the enrichment and CK treatment was accumulated rapidly during the 1–20 days of anaerobic incubation and then gradually increased to the maximum concentration of 8.64 and 7.45 mg g^{-1} on day 40 (Fig. 6). Compared with the CK treatment, ferrihydrite addition in both HN and SC soil increased the Fe(II) accumulation significantly by 8.3–27.6% and 15.4–27.5% during the middle and later stages of anaerobic incubation ($p < 0.05$). Goethite enrichment stimulated Fe(II) accumulation to a minor extent during the late stages of anaerobic incubation and no significant differences were found between the CK and goethite enrichment in both HN and SC soils.

4 Discussion

4.1 Differences in the *Anaeromyxobacter* community between paddy soils

The succession of microbial community structure could be significantly different according to the physicochemical properties

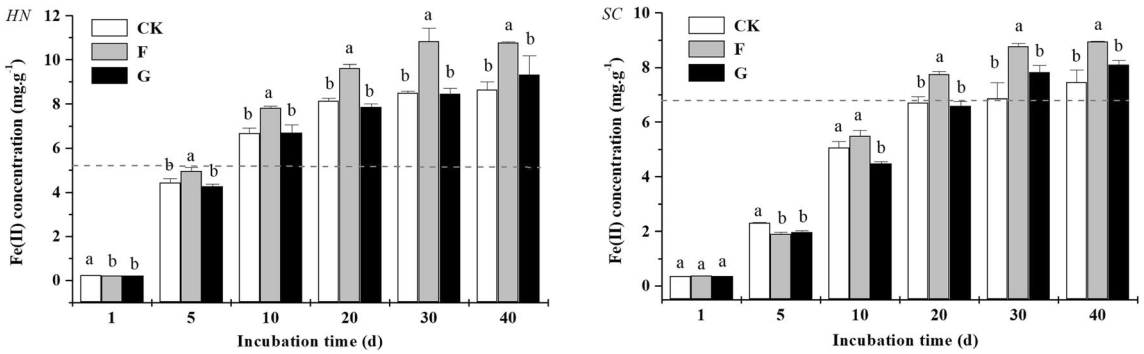

Fig. 6 Dynamics of Fe(II) concentration in the flooded paddy soils during anaerobic enrichment culture with different species of iron oxides. The dotted line indicates the amount of amorphous iron of paddy soils

of paddy soils (Unger et al. 2009). In our study, the abundance and diversity index of *Anaeromyxobacter* in the SC paddy soil was significantly higher than that in the HN paddy soil. This could be because the inherent amorphous iron oxide content in the SC paddy soil was higher than that in the HN paddy soil (Table 1). Amorphous iron oxide has a large specific surface area and high solubility and is thus easily reducible by microorganisms (Bonneville et al. 2004; Bosch et al. 2010). Most iron-reducing bacteria are preferentially enriched in anaerobic enrichment culture with amorphous iron oxide (Li et al. 2012b; Zhang et al. 2013) (Fig. S2-ESM). Thus, a higher content of amorphous iron oxide might stimulate the growth of *Anaeromyxobacter* capable of Fe(III) reduction. Another possible explanation could be attributed to the lower initial pH value of the SC paddy soil (Table 1). Lower pH value can enhance the stability of iron oxide (Bonneville et al. 2004), which can supplement the easily reducible iron oxide for *Anaeromyxobacter*. In addition, Qu et al. (2004) found that the crystalline iron oxide in paddy soil could be reduced by iron-reducing bacteria during the late stages of anaerobic incubation. Studies have also suggested that some *Anaeromyxobacter* can couple the respiratory metabolism of goethite with acetate oxidation (Hori et al. 2010). In the present study, we found that A-OTU_29 was potentially capable of goethite reduction, and A-OTU_44 and A-OTU_9 were potentially capable of ferrihydrite reduction (Thomas et al. 2009; Hori et al. 2010). Thus, A-OTU_29 was awakened after day 5 when the inherent amorphous iron was reduced in the HN soil (Figs. 2 and 6). However, A-OTU_29 was less prominent in the SC soil because the inherent amorphous iron was finally reduced until day 30 of incubation, whereas A-OTU_44 and A-OTU_9 were identified throughout the incubation of SC soil.

4.2 Effect of iron oxide enrichment on *Anaeromyxobacter* community

The ferrihydrite addition can supply sufficient easily reducible electron acceptor to Fe(III)-reducing bacteria (Li et al. 2012b; Qu et al. 2004). In the present study, the *Anaeromyxobacter* community in the SC soil preferred to take ferrihydrite as an electron acceptor. Thus, it is not surprising that ferrihydrite enrichment stimulated the growth of *Anaeromyxobacter* during 5–10 days of incubation in SC soil. The relative abundance of A-OTU_44 and A-OTU_9 in SC soil was also increased to a minor extent. With the gradual reduction of amorphous iron oxide in the later stages of incubation, the competition of Fe(III)-reducing bacteria for amorphous iron oxide in paddy soil would be intensified (You et al. 2011). Therefore, ferrihydrite enrichment enhanced the absolute abundance of *Anaeromyxobacter* after 20 days of incubation in the HN soil. Accordingly, the diversity of *Anaeromyxobacter* increased when the inherent amorphous iron reduced. The relative abundance of A-OTU_29 clearly declined in the ferrihydrite-enriched culture in the HN soil because it was less competitive with ferrihydrite as the electron acceptor.

Goethite cannot easily be used by Fe(III)-reducing bacteria because of its high mineral crystal size, small specific surface area, and low solubility (Cornell and Schwertmann 2003; Bosch et al. 2010). However, the relative abundance of A-OTU_29 in the HN soil was increased under goethite enrichment. The community structure of *Anaeromyxobacter* in goethite-enriched cultures was close to that of the CK treatment, whereas the community structure of *Anaeromyxobacter* at the later stage of incubation in ferrihydrite-enriched cultures was significantly different to the CK and goethite treatments. This suggests that ferrihydrite had a greater influence on the community structure of *Anaeromyxobacter* than goethite during the anaerobic incubation of paddy soils.

4.3 Potential correlation of *Anaeromyxobacter* community with Fe(III) reduction in the Fe(III)-enriched culture

Redundancy analysis (Fig. 7) and Pearson correlation analysis (Table 2) were used to reflect the correlation between the community structure of *Anaeromyxobacter* and the process of Fe(III) reduction. For the two paddy soils, the relative abundance of *Anaeromyxobacter* showed a significant positive correlation with the Fe(II) accumulation ($p < 0.05$), whereas the evenness index of *Anaeromyxobacter* showed a significant negative correlation with the Fe(II) accumulation, suggesting that *Anaeromyxobacter* was closely related to the Fe(III) reduction process. Previous studies also confirmed that *Anaeromyxobacter* played an important role in the process of Fe(III) reduction in the flooded paddy soils (Treude et al. 2003; Wu et al. 2006). The enhanced Fe(II) accumulation under the ferrihydrite enrichment was attributed to the enhanced absolute abundance of *Anaeromyxobacter* in both the HN and SC soils. Furthermore, although the dominant *Anaeromyxobacter* strains in the HN paddy soil were competitive with goethite as the electron acceptor and its abundance and Marglef index increased to a minor extent, the reduced Fe(II) might cover the surface of crystalline goethite (Roden and Urrutia 2002; Roden 2003). This prevented the reduction of goethite by the *Anaeromyxobacter* during the later flooding period and might explain why there was little difference of Fe(II) accumulation between the goethite enrichment and CK treatments. Moreover, *Anaeromyxobacter* is representative of respiratory Fe(III) reducers and can consume low-weight organic acids in paddy soil to reduce Fe(III) to Fe(II). This could explain why the diversity index and relative abundance of *Anaeromyxobacter* showed a significant correlation with the pH value of soil slurry. Additionally, even though there were limited isolations of *Anaeromyxobacter* in paddy soils, the less dominant *Anaeromyxobacter* were still metabolically important. Although this study provided fundamental information for

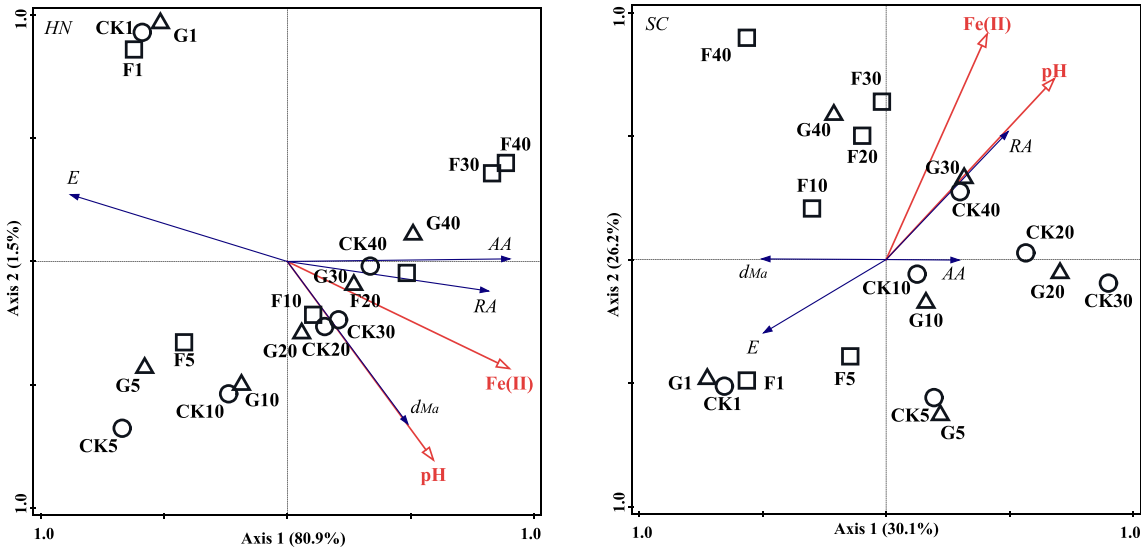

Fig. 7 Redundancy analysis (RDA) for the relationship between the community of *Anaeromyxobacter* and the process of Fe(III) reduction in HN and SC paddy soils. Three symbol types were used: circle for the CK treatment, square for the ferrihydrite treatment, and triangle for the goethite treatment. Species parameters are represented by the arrows with blue line that include the absolute abundance (AA), relative abundance (RA), Marglef index (d_{Ma}), and evenness index (E) of *Anaeromyxobacter*, respectively. Environmental parameters are represented by the arrows with a red line, including Fe(II) concentration [Fe(II)] and soil pH

understanding the role of *Anaeromyxobacter* in Fe(III)-enriched culture, further efforts should be focused on the isolation and characterization of *Anaeromyxobacter* strains that are contributors to the process of element cycling.

5 Conclusions

This study investigated the succession of the *Anaeromyxobacter* community during anaerobic incubation of paddy soils enriched with two species of iron oxides. We revealed that ferrihydrite and goethite could enhance the absolute abundance of *Anaeromyxobacter* in paddy soils. The indigenous iron oxide in the soils could affect the succession of the *Anaeromyxobacter* community. Ferrihydrite had a greater influence than goethite on the *Anaeromyxobacter* community during the later stages of anaerobic incubation. The succession of *Anaeromyxobacter* community was closely related to the process of Fe(III) reduction. These results could provide a theoretical basis for understanding the ecological distribution and metabolic function of *Anaeromyxobacter*, which plays a vital role in the electron transport chain in paddy soils.

Table 2 The Pearson correlation between the community of *Anaeromyxobacter* and the process of Fe(III) reduction in HN and SC paddy soils

Siols		AA	RA	d_{Ma}	E	Fe(II)	pH
HN	CN	1.000	0.766**	0.367	− 0.702**	0.658**	0.396
	RA		1.000	0.469*	− 0.927**	0.787**	0.580*
	d_{Ma}			1.000	− .663**	0.706**	0.797**
	E				1.000	− 0.921**	− 0.762**
	Fe(II)					1.000	0.884**
	pH						1.000
SC	CN	1.000	0.570*	− 0.122	− 0.496*	− 0.448*	− 0.510*
	RA		1.000	− 0.288	− 0.859**	0.673**	0.713**
	d_{Ma}			1.000	0.171	− 0.205	− 0.344
	E				1.000	− 0.481*	− 0.563*
	Fe(II)					1.000	0.947**
	pH						1.000

AA the absolute abundance of *Anaeromyxobacter*, *RA* the relative abundance of *Anaeromyxobacter*, d_{Ma} Marglef index, *E* Evenness index, *Fe(II)* Fe(II) concentration, *pH* soil pH

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