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ORIGINAL ARTICLE

Microaerobic iron oxidation and carbon assimilation and associated microbial community in paddy soil

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Abstract Iron oxidation is a prevalent and important biogeochemical process in paddy soil, but little is known about whether and how microbially mediated iron oxidation is coupled with carbon assimilation, particularly under microaerobic conditions. Here, we investigated kinetics of CO_2 assimilation and Fe(II) oxidation in an incubation experiment with paddy soil under suboxic conditions, and profiled the associated microbial community using DNA-stable isotope probing and 16S rRNA gene-based sequencing. The results showed that CO_2 assimilation and Fe(II) oxidation in the gradient tubes were predominantly mediated by the microbes enriched in the paddy soil, primarily *Azospirillum* and *Magnetospirillum*, as their relative abundances were higher in the ^{13}C heavy fractions compared to ^{12}C heavy fractions. This study provided direct evidence of chemoautotrophic microaerophiles linking iron oxidation and carbon assimilation at the oxic–anoxic interface in the paddy soil ecosystem.

Keywords Paddy soil · Microaerobic Fe(II)-oxidation · CO_2 assimilation · SIP

1 Introduction

The carbon (C) cycle plays a fundamental role in sustaining life on Earth, and the global C budget is strongly influenced by human activities, such as those increasing CO_2 emissions (Le Quéré et al. 2009). Although the role of plants in C fixation is extensively recognized, little is known about the microbiota involved in the soil carbon cycle, especially at the oxic–anoxic interface. Chemoautotrophs can use inorganic molecules (e.g. NO_3^- and $S_2O_3^{2-}$) as electron donors to convert CO_2 to organic compounds in soils and sediments (Li et al. 2016). Microaerophilic iron-oxidizing bacteria (FeOB) are chemoautotrophs that can couple Fe and C cycles, using Fe(II) as an energy source and converting CO_2 into biomass under low oxygen (50 μM) conditions (Field et al. 2015; Kato et al. 2015). Numerous microaerophilic FeOB have been found mainly by culture-dependent methods (Emerson et al. 2010). However, studies on the composition of the chemoautotrophic FeOB community in mixed cultures or environmental soils have been rather limited because of the unavailability of universal functional gene markers. Stable isotope probing (SIP) analysis is considered to be a powerful technique to link the taxonomic identity of microorganisms to a specific function in complex environments (Manefield et al. 2002). Paddy soil represents an important ecosystem for Fe- and C-cycling. Owing to the periodic redox reactions and elemental abundance of Fe, microaerobic Fe(II) oxidation around the rice rhizosphere and at the soil–water interface is considered a prevalent biogeochemical process in water-logged paddy soils (Emerson et al. 2010). The present study was designed to

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investigate the active CO_2-assimilating microaerophilic microbial community during carbon assimilation and Fe(II) oxidation in a typical paddy soil from South China via an incubation experiment using DNA-SIP.

2 Results and discussion

2.1 Enrichment of microaerophilic FeOB

Gradient tubes with opposing gradients (Kato et al. 2015) of O_2 and FeS were used for the enrichment of microaerophilic FeOB. Rhizosphere-associated soils were mixed with ultrapure water at a ratio of 1:1 and used as inocula for enrichment of FeOB. Triplicate culture tubes were incubated at 30 °C in the dark. To prevent soil microbes from using alternative electron acceptors, the enriched cultures were subcultured three times and then used as inocula for SIP incubations. Two days after inoculation with the subcultured inocula, a reddish-brown cell band appeared in the inoculated tubes, which was not observed in non-inoculated tubes (Fig. 1a).

2.2 Carbon assimilation and Fe(II) oxidation by microaerophilic FeOB

Autotrophic carbon assimilation was measured by incorporation of ^{13}C-labelled $NaHCO_3$ into cellular biomass, and the microbial biomass carbon (MBC) of cells grown in gradient tubes was measured with the chloroform fumigation–extraction technique (Wu et al. 1990). Throughout the 15 days of culture with inocula, the carbon assimilation amount increased gradually from Day 0 to Day 10, yielding a maximum assimilation amount of 0.60% ^{13}C-$NaHCO_3$ on Day 10 (Fig. 1b). The carbon assimilation amount decreased from Day 10 through 15, implying that carbon assimilation might be slower than carbon consumption.

The rate of iron oxidation in the culture medium with inocula, as indicated by total iron accumulation, was clearly higher than that in the abiotic control (Fig. 2c). The maximum concentration of Fe was 2.6 mM on Day 15 in inoculated tubes, accounting for 81.1% of total Fe oxidation. The above results indicate that Fe(II) oxidation coupling with carbon assimilation at the oxic–anoxic boundaries was predominantly mediated by the active microaerophilic FeOB enriched in the cultures.

2.3 FeOB community dynamics in density gradient fractions of DNA

The DNA extracts from both ^{13}C-labeled and ^{12}C-unlabeled $NaHCO_3$-supplemented cultures were analyzed using SIP and 16S rRNA gene-based high-throughput sequencing to profile the microbial communities at two times (Days 2 and 15). The sequencing results show that at both times, the genera *Bdellovibrio*, *Magnetospirillum*, *Dechloromonas*,

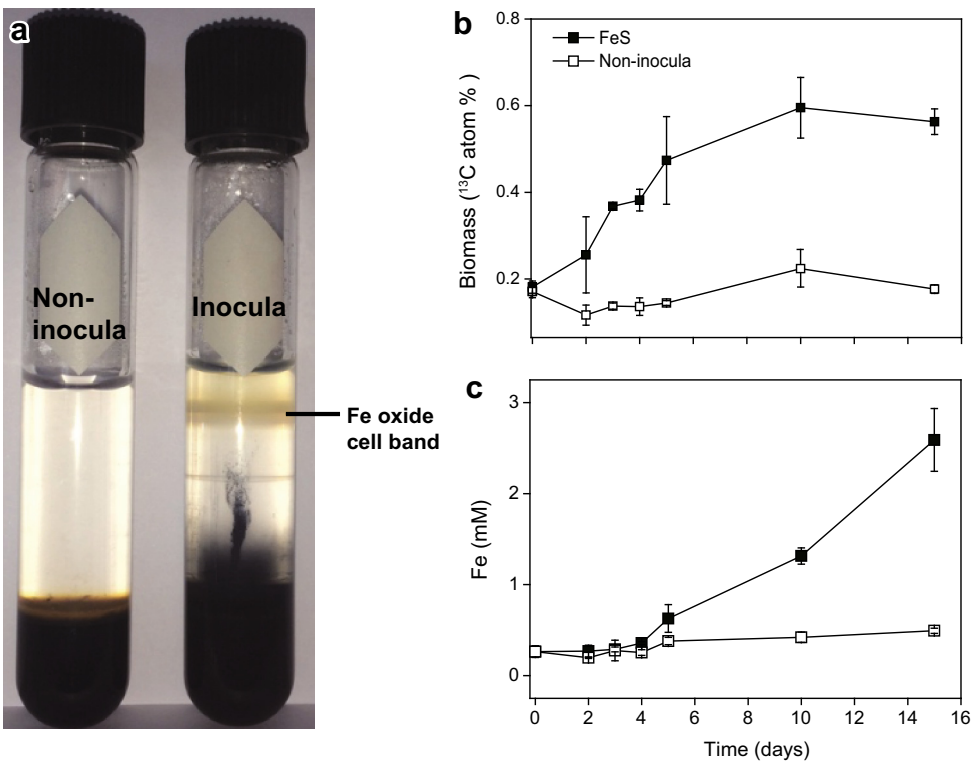

Fig. 1 **a** Gradient growth tubes. The tube on the *left* is a control tube that was not inoculated with cells; chemical iron oxidation occurred in the semisolid layer in this tube. The tube on the *right* was inoculated with subcultured inocula, and a distinct, horizontal cell band of bacterial iron oxidation formed. **b** Time course of microbial assimilation of $H^{13}CO_3^-$ and **c** time course of the concentration of total Fe

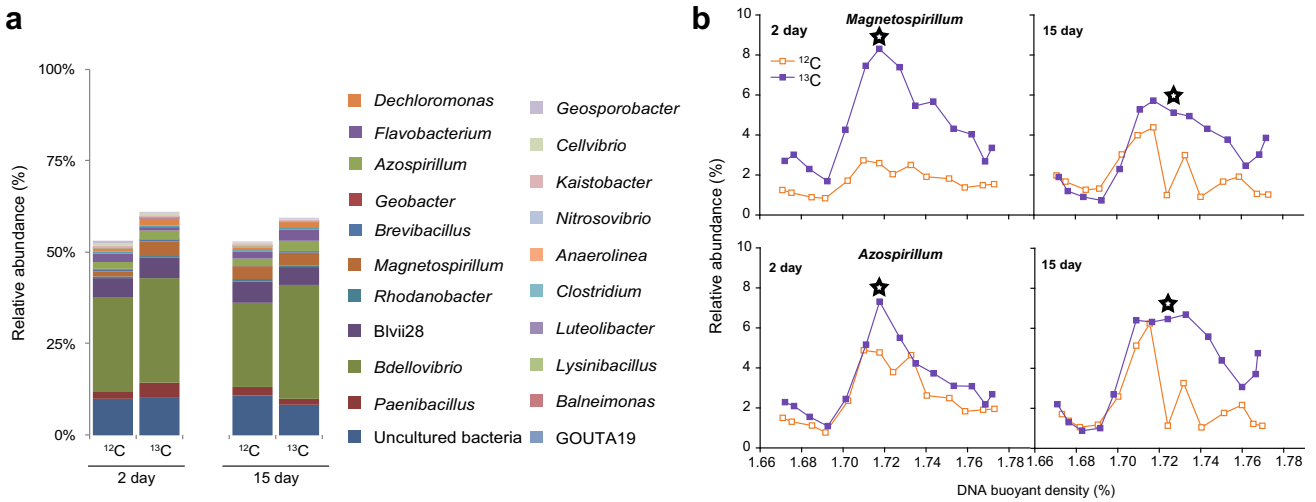

Fig. 2 Relative abundances (%) of **a** dominant genera in cell band of ^{13}C- and ^{12}C-$NaHCO_3$ treatments and **b** two functional populations within the buoyant density gradients of DNA during incubation

and *Azospirillum* (all belonging to Proteobacteria), had conspicuously higher abundances in the microbial communities of labeled treatments than of the unlabeled treatments (Fig. 2a). These genera were thought to be potential CO_2-assimilating microorganisms. Among these potential ^{13}C-assimilating populations, *Magnetospirillum* and *Azospirillum* were apparently enriched in the ^{13}C heavy fractions (Buoyant density >1.720 mg·L^{-1}) but not in the unlabeled heavy fractions (Fig. 2b), indicating these populations possibly play an important role in microaerobic Fe(II) oxidation coupling with CO_2 assimilation in paddy soil. It should be noted that the relative abundance of *Magnetospirillum* and *Azospirillum* decreased in the heavy fractions at the later time point (Day 15), which might be due to the consumption of carbon source in the medium and to the energy deficiency resulting from microbial iron oxidation products entombed in the cell (Emerson et al. 2010)

Two independent techniques (kinetics of iron oxidation/carbon assimilation and SIP analysis) provided evidence that the microaerophilic FeOB from paddy soil were capable of assimilating labeled inorganic-^{13}C at circumneutral pH. Among the active populations, *Magnetospirillum* spp. are magnetotactic bacteria ubiquitously present at the oxic–anoxic transition zone of freshwater sediments and marine waters, that can produce magnetite inside the cell. They were observed for lithoautotrophic growth in gradient tubes with freshwater (Geelhoed et al. 2009), indicating a possible iron-oxidizing capability of this population. Additionally, *Azospirillum*, a heterotrophic nitrate-reducing FeOB, was apparently enriched in the ^{13}C heavy fractions, highlighting that aerobic iron oxidation and inorganic carbon assimilation might also be common abilities of denitrifying bacteria, considering the widespread ability of these bacteria to reduce O_2 and oxidize Fe(II) (Chen et al. 2016).

Our results highlight the complex biogeochemical interactions of Fe and C in paddy soil. The microaerophilic FeOB successfully outcompete abiotic iron oxidation by living at the redox boundary, where O_2 concentrations are low—conditions conducive to the formation of Fe plaque (Kögel-Knabner et al. 2010). Furthermore, CO_2 efflux plays a key role in carbon exchange among the atmosphere, soil, and roots, resulting in active microaerobic iron oxidation coupling with CO_2 fixation at the root–soil and soil–water interfaces in paddy soil. This process is important for maintaining soil fertility, which impacts the sustainability of rice farming (Kögel-Knabner et al. 2010).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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