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The shift of sulfate-reducing bacterial communities from the upland to the paddy stage in a rapeseed-rice rotation system, and the effect from the long-term straw returning

Shipu Lu[b], Shun Han[a], Yifan Du[b], Hong Liu[b], Hailing Nie[a], Xuesong Luo[a,b,*], Qiaoyun Huang[a,b], Wenli Chen[a]

[a] State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, China
[b] College of Resources and Environment, Huazhong Agricultural University, Wuhan 430070, China

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ABSTRACT

Sulfate reduction is an essential process in the biogeochemical sulfur cycle in soils. It is mainly driven by dissimilatory sulfate-reducing bacteria (DSRB). The responses of the DSRB community in a rapeseed-rice rotation system to the environmental variability caused by the shift from the upland to the paddy stages, and the effects of the long-term straw returning on this shift remains unclear. We surveyed the sulfate reducing potential (SRP) and the structure of the DSRB community by high-throughput sequencing that targeted *dsrB* (and the xenologous *dsrB*) in soils. The SRP, ranging from 1.2 to 5.8 μmol d^{-1} d w g^{-1}, was increased in the paddy stage, and it was likely to be enhanced by the long-term straw returning. Highly abundant DSRB optimal taxonomic units (OTUs) were found to be affiliated with the *Nitrospirae* supercluster (including the uncultured DsrAB lineage WX, lineage 10, and an unknown clade with GU372064), the Environmental supercluster 1 (including the uncultured DsrAB lineage 8 and an unknown lineage related to EF065019), and the *Firmicutes* group (including the uncultured DsrAB lineage 6 and an unknown lineage between the uncultured DsrAB lineages 2 and 3). The environmental transformation from the upland to the paddy stages led to a decrease in the α-diversity and the number of detectable OTUs. The abundance of a few dominant DSRB OTUs was changed by either the environmental transformation from the upland to the paddy stages or the effects caused by the long-term straw returning. In this study, the changes in the DSRB community structure correlated with the decrease in soil pH and total phosphorus content, and the increase in available sulfate and moisture contents.

1. Introduction

Dissimilatory sulfate-reducing microorganisms play a key role in the biogeochemical cycles of soils, and sulfate reduction is energetically more favorable than fermentative processes and methanogenesis (Muyzer and Stams, 2008). In paddy soils, the redox reactions involved in sulfate reduction are promoted in the presence of rice plants (Liesack et al., 2000). Sulfate reducers, nitrate reducers, mathanogen and iron reducers compete for electron donors, such as acetate (Achtnich et al., 1995; Chidthaisong and Conrad, 2000). In addition, rice roots were found to stimulate sulfate reduction in situ (Wind and Conrad, 1997). Several dissimilatory sulfate-reducing bacteria (DSRB), belonging to *Desulfobacteraceae*, *Desulfobulbaceae*, *Desulfovibrionaceae*, *Syntrophobacteraceae*, and *Peptococcaceae*, have been isolated by cultivation or identified from paddy fields using molecular biology techniques (Wind and Conrad, 1997; Wind et al., 1999; Scheid and Stubner, 2001; Scheid et al., 2004; Lu et al., 2006; Knief et al., 2012). The ecology of DSRB in paddy soils was investigated in several studies. For example, gypsum was found to stimulate the growth of DSRB phylogenetically related to *Syntrophobacter*, *Desulfovibrio*, *Desulfobulbaceae*, and *Desulfobacteraceae* in the rice rhizosphere of plants cultivated on a paddy soil (Wörner et al., 2016). DSRB abundances in paddy soils from Wanshan Hg mining area were positively correlated with methylmecury emission. Spatial variation of the DSRB communities was related to methylmecury, organic matter content, NH_4^+, and SO_4^{2-} (Liu et al., 2014). Long-term acid mine drainage irrigation also led to an increment of the DSRB abundance in paddy soils (Wang et al., 2016).

In the Wuxue long-term rapeseed-rice rotation system, the microbial community is exposed to different crops associated with the upland and flooding cycles. When the soils are flooded and planted with rice each

Abbreviations: TN, total nitrogen; TP, total phosphorus; TK, total potassium; MOI, moisture; TC, total carbon

* Corresponding author at: State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, China.
E-mail address: xuesongluo@mail.hzau.edu.cn (X. Luo).

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year, the activity of dissimilatory sulfate-reducing bacteria (DSRB) would be stimulated (Liesack et al., 2000). The response of the whole DSRB community to the environmental changes caused by the transformation from the upland to the paddy stages remains unclear. In this system, a long-term straw returning experiment was also designed as described previously (Peng et al., 2016; Han et al., 2017). Assessment of the ecological effect of long-term straw returning on the DSRB community is also interesting to study. This work utilized *dsrB* amplicon sequencing, real-time PCR, and sulfate reducing potential (SRP) measurements, coupled with statistical analyses, to assess the ecological effects of the long-term straw returning and the rice-planting paddy stage conditions on the DSRB community.

2. Materials and methods

2.1. Study site

The long-term straw returning group (three randomized plots) and the untreated control (three randomized plots) were settled in the Wuxue Field Experiment Station (29°59′N, 115°38′E) in Hubei Province, China, for seven years. Three crop (rice-rice-rapeseed) rotations were employed in the system. For the straw returning plots, approximately 5200 kg ha^{-1} y^{-1} straw was tilled into the soil. In June 2014 (just after the rapeseed was harvested, and the rice was not planted; BP) and July 2014 (the rice-growing stage; GR), one kg soil was sampled from each plot. A total of 12 soil samples were obtained. To avoid bias, six random cores from 5 to 25 cm layer were mixed to prepare one sample for a plot. The soil properties were determined as described by Han et al. (2017). Briefly, soil moisture and sulfate content significantly increased to 41–47% and 145–183 mg kg^{-1}, respectively, in the paddy stage. In addition, rice planting resulted in a decrease in soil pH and total phosphorus content. Compared to the data for the control samples, that for the straw-returning plots revealed a decrease in total phosphorus content, especially in the upland stage.

2.2. SRP measurement

The SRP of the landfill cover soil was determined as described by Xia et al. (2014) with one modification; the pH of the medium was adjusted to a value consistent with the pH of the soil.

2.3. Measurement of sulfate reducing bacteria abundance by qPCR

Soil DNA was extracted using the using the E.Z.N.A.™ soil DNA kit (Axygen Scientific Inc., Union Cit, CA) according to manufacturer's instructions. Quantitative PCR assays were conducted using an ABI7500 FAST Real-time PCR system. *DsrB* F1a-h (mix primers) and *dsrB* 4RSI2a-h (mix primers) were utilized for amplifying *dsrB*. *DsrB* F2a–h and *dsrB* 4RSI2a-h (mix primers) were used to quantify *xdsrB* (xenologous *dsrB*) (Lever et al., 2013). The 20 μl PCR reaction mixtures contained 10 μl SYBR Premix Ex Taq II (2×) (Takara, Bio Inc., Shiga, Japan), 0.9 μl of a 10 mM solution of each primer (final concentration 9 pmol μl^{-1}), 6.2 μl DEPC-treated water, 2.0 μl soil DNA (diluted 10-fold) or 2.0 μl standard plasmid. The original concentration of the standard plasmid is 410 ng μl^{-1}. It was serially diluted for use. Inhibitory effects from soil DNA sample on PCR performance were tested for several samples by running PCRs with templates of (1) a known amount (concentration and volume) of the standard plasmid mixed with a soil DNA sample (1:1, v/v), (2) the same amount of the standard plasmid mixed with water (1:1, v/v), (3) the same soil DNA sample mixed with the same volume of water. The measured cycle threshold (Ct) values for (2) were compared with those measured for (1) and (3). In each PCR assay, negative controls for avoiding contamination from the extraction and the PCR reagents were run (Table 1).

2.4. dsrB fragment sequences and phylogenetic analysis

The composition and diversity of *dsrB* genes amplified from the soil samples were examined using a barcode Illumina sequencing method. A first PCR was performed using the same primers described for the qPCR (see paragraph 2.3). A second round PCR was performed under the same conditions as described above except that different barcodes were added at the 5′ end of the forward and reverse primers, respectively. This approach was needed because direct amplification using the barcode primers was unsuccessful for most soil DNAs. Triplicate PCR reactions (25 μl each) were performed for each sample, and the resulting PCR products were pooled. The PCR products obtained in the second step were subjected to gel extraction. Two point five nanograms of barcoded DNA products from each visually confirmed, purified, and requantified sample was pooled into a single 1.5 ml Eppendorf tube for sequencing. Paired-end multiplex sequencing was performed using an Illumina MiSeq instrument (Personalbio, Shanghai, China). Raw data analysis was performed following the procedures described as Pester et al. (2012b) with some modifications, which were (i) that only illumina reads over 175 nt were analyzed (97% of the obtained high quality reads) and (ii) that taxonomic classification was done using the Bayesian classifier provided by the mothur software package (Schloss et al., 2009). Reads sequenced from both ends were pooled for all analyses because they overlapped by at least 10% (more than overlapping 40 bp out of 340 possible bp). To discriminate against errors introduced by pyrosequencing, high-quality sequences were initially clustered at 97% identity level, and cluster representatives were further translated and screened for insertion/deletion errors using FrameD (Schiex et al., 2003). Correct sequences were further clustered at 90% of the sequences identity for a putative species as suggested by Pelikan et al. (2016). Alpha diversity metrics were calculated in QIIME (Caporaso et al., 2010). The Operational Taxonomic Unit (OTU) table was standardized and subjected to the following analyses.

Sequences from high abundant OTUs (at least > 1% in one type of the samples) were translated and aligned to the pre-aligned core 1292 sequences of the DsrAB ARB database (Müller et al., 2015 and Pelikan et al., 2016). Alignments were manually curated to reduce artificial diversity caused by alignment errors. Neighbor-joining trees were produced, and the reliability of the phylogenetic reconstructions was evaluated by bootstrapping (1000 replicates) using MEGA 6. After phylogenetic analysis, the taxonomic affiliations for these OTUs were listed in Table 2 and Table 3.

2.5. Data analysis

The program Mothur (http://www.mothur.org/wiki/MainPage) was used to calculate the distribution of OTUs in samples. Data regarding soil properties and SRP were processed with Microsoft Excel 2010. The statistical procedures, including analysis of variance or SNK test, were carried out by SPSS 19.0 statistical software (IBM Co., Armonk, New York, USA). Redundancy analysis was carried out by Canocal 4.5. Monte Carlo permutation test (999 random permutations) was used to explore the significant effects of environmental variables on SRB community. SRB communities were compared between sample types (i.e. different sampling stage, different sampling stage in the same treatment, and different treatments in the same stage) by the multi-response permutation procedure (MRPP) analysis using PC-ORD (Version 5.0; Bruce McCune and MJM Software, 1999). To identify which OTUs were stimulated or repressed by the temporal effect and/or the long-term straw returning, we tested for between group differences using the general linear model as implemented in the R package edger 3.6.8 (Robinson et al., 2010).