Research Letter

High abundance and diversity of nitrite-dependent anaerobic methane-oxidizing bacteria in a paddy field profile

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Abstract

The discovery of nitrite-dependent anaerobic methane oxidation (n-damo) mediated by ‘Candidatus Methylomirabilis oxyfera’ with nitrite and methane as substrates has connected biogeochemical carbon and nitrogen cycles in a new way. The paddy fields often carry substantial methane and nitrate, thus may be a favorable habitat for n-damo bacteria. In this paper, the vertical-temporal molecular fingerprints of M. oxyfera-like bacteria, including abundance and community composition, were investigated in a paddy soil core in Jiangyin, near the Yangtze River. Through qPCR investigation, high abundance of M. oxyfera-like bacteria up to 1.0 × 10^8 copies (g d.w.s.)^{-1} in summer and 8.5 × 10^7 copies (g d.w.s.)^{-1} in winter was observed in the ecotone of soil and groundwater in the paddy soil core, which was the highest in natural environments to our knowledge. In the ecotone, the ratio of M. oxyfera-like bacteria to total bacteria reached peak values of 2.80% in summer and 4.41% in winter. Phylogenetic analysis showed n-damo bacteria in the paddy soil were closely related to M. oxyfera and had high diversity in the soil/groundwater ecotone. All of the results indicated the soil/groundwater ecotone of the Jiangyin paddy field was a favorable environment for the growth of n-damo bacteria.

Introduction

Methane (CH4), whose warming potential is 25 times higher than that of carbon dioxide (CO2) on a 100-year timescale, is the second most important anthropogenic greenhouse gas after CO2 (IPCC, 2007). CH4 is mainly produced by methanogenic archaea under strictly anaerobic conditions (Mah et al., 1977) and has been thought to be only consumed by methane-oxidizing bacteria under oxic conditions or oxidized with sulfate anaerobically (Barnes & Goldberg, 1976). However, the conceptual framework of the methane cycle has been changed with the identification of a novel pathway named nitrite-dependent anaerobic methane oxidation (n-damo) which couples the methane oxidation to nitrite reduction (Raghoebarsing et al., 2006).

The n-damo research was mainly performed on enrichment cultures (Raghoebarsing et al., 2006; Ettwig et al., 2008, 2009; Hu et al., 2009), and the responsible n-damo bacteria could be identified as ‘Candidatus Methylomirabilis oxyfera’ affiliated to the ‘NC10’ phylum (Ettwig et al., 2009). Some physiological and biochemical properties of n-damo bacteria, such as the unusual intra aerobic metabolism have been identified (Ettwig et al., 2008, 2010; Zhu et al., 2010; Luesken et al., 2012; Wu et al., 2012). In recent years, with the suitable primers developed, the M. oxyfera-like bacteria have been detected in various natural ecosystems, such as Lake Constance in Germany (Deutzmann & Schink, 2011), Lake Biwa in Japan (Kojima et al., 2012), Qinghai-Tibetan saline lakes in China (Yang et al., 2012), Brunssummerheide peatland in the Netherlands (Zhu et al., 2012), Jiaxing paddy soil in China (Wang et al., 2012a), and Qiantang River in China (Shen et al., 2013). However, because of the limited research cases, the distribution, community composition, and the ecological patterns of n-damo bacteria in natural environments still remain unclear.

The boundary or ecotone between two features in a landscape, for example the land/freshwater interface, is often the biogeochemical hot spot (McClain et al., 2003;...
Wang et al., 2012b,c; Zhu et al., 2013). Rice paddy fields are dry-wet alternation ecosystems, resulting in the soil/water ecotones which are ideal environments for soil microorganisms (Ishii et al., 2011). Paddy field is the major natural CH$_4$ source (Mikaloff Fletcher et al., 2004) and nitrate (NO$_3^-$) sink (Kögel-Knabner et al., 2010), and the fluctuation of groundwater level in a paddy field will also generate an ecotone on a vertical scale. So we hypothesized that the soil/groundwater ecotone of the paddy field may be an ideal habitat for n-damo bacteria.

The aim of this study was to test the temporal-spatial distribution patterns of M. oxyfera-like bacteria in the paddy soil and to ascertain whether the soil/groundwater ecotone contains M. oxyfera-like bacteria with high abundance and diversity.

**Materials and methods**

**Sampling site background**

The Yangtze River Plain is the most important rice-planting area in China, which covers 60% of the rice-planting area and 70% of the rice yield in the whole nation (Xu & Ma, 2009). A paddy field located in lower reaches of the Yangtze River was selected for this study (120°3′31″E, 31°56′40″N, Jiangyin, Jiangsu, China). The sampling site was c. 500 m away from the Yangtze River, where the groundwater level was 60 cm below soil surface in summer and 40 cm in winter. Rice-wheat rotation has been carried out for nearly 20 years in this paddy field. Five soil cores each for summer and winter (140 cm, September 30, 2012; 200 cm, February 15, 2013, respectively) were collected using a soil auger. The soil cores were placed in sterile plastic bags, sealed and stored on ice until transported to the laboratory and then sliced every 20 cm and mixed at every depth to form one composite sample. Subsample was used to analyze the physicochemical characteristics immediately after arrival, and the rest was stored at −80 °C for later DNA extraction and molecular analysis.

**Analytical procedures of environmental variables**

Ammonium (NH$_4^+$), nitrate plus nitrite (NO$_3^-$) were extracted from the soil samples with 2 M KCl solution and measured using a Continuous Flow Analyzer (SAN plus, Skalar Analytical, The Netherlands). Soil pH was determined after mixing with water at a ratio (soil/water) of 1:5, and LOI$_{550}$ (Loss on ignition at 550 °C) determined after mixing with water at a ratio (soil/water) plus, Skalar Analytical, The Netherlands). Soil pH was measured using a Continuous Flow Analyzer (SAN

DNA extraction, PCR amplification, cloning, sequencing, and phylogenetic analysis

For each sample of different depths, c. 0.3 g freeze-dried soil was used to extract DNA using the FastDNA Spin Kit for Soil (MP Biomedicals). Concentrations of the extracted DNA were determined by spectrophotometric analysis on a NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific), and the quality was checked by electrophoresis on a 1% (weight/volume) agarose gel. The pmoA genes of n-damo bacteria (encoding the particulate methane monoxygenase (pMMO), an enzyme that catalyzes the first step of n-damo pathway) were amplified using a nested approach with first step primer set of A189F and cmo682, followed by second step primer set of cmo182 and cmo568 according to Luesken et al. (2011) in a C1000™ Thermal Cycler (BIO-RAD). All primers and thermal profiles used in this study are shown in Supporting Information, Table S1.

A total of eight clone libraries were constructed for the representative soil depths with positive PCR results in summer and winter (60–80 cm, 80–100 cm, 100–120 cm, and 120–140 cm of summer samples; 0–20 cm, 60–80 cm, 120–140 cm, and 180–200 cm of winter samples). PCR products were purified and ligated into the pGEM-T Easy cloning vector (Promega) and then transformed to Escherichia coli JM109 competent cells. One hundred clones were randomly picked up from each library and screened by restriction endonucleases HhaI. All representative clones were sequenced by an ABI 3730XL automated sequencer (Biomed, China). Sequences of M. oxyfera-like bacterial pmoA genes obtained in this study were deposited in the GenBank under the accession numbers KF546848 - KF547007, except KF546859, KF546873-KF546874, KF546881, KF546883-KF546884, KF546895-KF546896, KF546906, KF546909-KF546910, KF546915, KF546923, KF546930, KF546937-KF546938, KF546940-KF546943, KF546948, which were found to be chimeric sequences tested through FunGene (Fish et al., 2013). Phylogenetic analysis of pmoA genes was performed using MEGA 5.0 software (Tamura et al., 2011). The phylogenetic tree was constructed by the neighbor-joining method (NJ), and the robustness of tree topology was tested by bootstrap analysis with 1000 replicates. The operational taxonomic units (OTUs) and diversity indices, including Chaol richness estimate and Shannon diversity index, were calculated by DOTUR software for each clone library based on 98% cutoff, incorporating the preselection with restriction endonucleases into the calculation (Schloss & Handelsman, 2005).
Distribution of n-damo bacteria in a paddy field profile

Quantitative PCR analysis
To quantify M. oxyfera-like bacteria and total bacteria at different depths of the soil core, SYBR Green I based quantitative PCR (qPCR) was performed. As qPCR compatible functional gene primers for the M. oxyfera-like bacteria were not available to us, the 16S rRNA genes were quantified in this study. The primers (p1F – p1R and p2F – p2R, Table S1) were designed based on several 16S rRNA gene sequences obtained from the enrichment cultures (Ettwig et al., 2009) and have been used with several environment samples. Two different primer pairs were used for each sample so as to guarantee the accuracy of the observed abundance, as adopted by Ettwig et al. (2009) and Zhu et al. (2012). For total bacteria, primer pair 341F-534R was used targeting the 16S rRNA gene as described by Koike et al. (2007).

Positive clones of 16S rRNA gene of M. oxyfera-like bacteria and total bacteria were selected to isolate plasmids containing corresponding inserts using a GeneJet Plasmid Miniprep Kit (Fermentas MBI, Lithuania), and 10-fold serial dilutions of plasmids were used to construct standard curves. The concentrations of plasmids were determined by a NanoDrop 2000 UV-Vis spectrophotometer (Thermo Fisher Scientific) for the calculation of gene copy numbers. The qPCR was performed on an ABI 7300 real-time PCR instrument (Applied Biosystems) with a SYBR Green qPCR kit (Takara Dalian, China) in triplicate for each sample with 10-fold diluted DNA template. The sequences of primers and thermal profiles are listed in Table S1. The qPCR was performed. As qPCR compatible functional gene primers for the M. oxyfera-like bacteria were not available to us, the 16S rRNA genes were quantified in this study.

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Data analysis
The statistical analyses were conducted by PASW STATISTICS 18.0 (Predictive Analytics Software Statistics). Paired samples t-test was used for the comparison of M. oxyfera-like bacterial abundance in summer and winter. The relations between M. oxyfera-like bacterial diversity/abundance and various environmental variables were computed by Spearman’s bivariate correlation analysis. Multiple linear regression (stepwise regression) was used to identify the most determining variable on M. oxyfera-like bacterial abundance. The level of significance in this study was $\alpha = 0.05$. Graphs were generated using ORIGIN 8.0 software.

Results

Chemical profiles in the paddy soil core
The vertical profiles of the main physicochemical characteristics of the Jiangyin paddy soil core in summer and winter were analyzed as shown in Fig. 1. The content of NO$_3^-$ in this paddy soil core ranged from 0.27 to 3.10 mg kg$^{-1}$ in summer and 0.31 to 1.11 mg kg$^{-1}$ in winter, with the highest values appearing at the soil depth of 20–40 cm and 100–120 cm, respectively, which showed that NO$_3^-$ may leach to deeper soil layers in winter either by influx when the water table was rising or by rainfall combined with fertilization. The content of NH$_4^+$ was 0.85–1.75 mg kg$^{-1}$ in summer and 0.63–7.24 mg kg$^{-1}$ in winter and was quite low in the upper 160 cm. In addition, because of the unsatisfactory condition for sampling, in situ CH$_4$ concentration data could not be determined. However, the observed CH$_4$ fluxes in some paddy fields in the Yangtze River Delta with similar conditions to the Jiangyin paddy field (Dong et al., 2011; Hou et al., 2013; Liu et al., 2014) prove CH$_4$ is available in our sampling site.

Abundance of M. oxyfera-like bacteria in the paddy soil core
To investigate the presence of M. oxyfera-like bacteria in the paddy field core, a PCR survey targeting pmoA genes was conducted using a nested PCR approach resulting in amplification of about 390-bp fragments. Results showed the M. oxyfera-like bacteria were absent at 0–60 cm depth and present at 60–140 cm depth of summer samples and were present in all soil core samples except for 20–40 cm depth of winter samples, while the groundwater level was at a depth of 60 and 40 cm in summer and winter, respectively. The distribution of M. oxyfera-like bacteria indicated that they were not ubiquitous in the whole paddy soil core and their occurrence was not continuous within different seasons, especially in soil above the groundwater level.

To analyze the distribution of M. oxyfera-like bacteria, in detail, their abundance was estimated by quantifying the 16S rRNA gene. The averages of results obtained through two different primer sets were used in the following analysis because there was little difference between them (Fig. 2). The population size of M. oxyfera-like bacteria was up to 0.63 to 1.11 $10^8$ copies (g d.w.s.$^{-1}$) and present at 60–40 cm depth of winter samples, while the groundwater level was at a depth of 60 and 40 cm in summer and winter, respectively. The distribution of M. oxyfera-like bacteria in the paddy field profile seemed to increase from the groundwater level (60 cm in summer and 40 cm in winter), and as the groundwater level fluctuated, the upper edge of high-abundance zone also changed, indicating that where there was groundwater in the paddy soil, there was high-abundance M. oxyfera-like bacteria. No matter in summer or winter, M. oxyfera-like bacteria in soil above the groundwater level were hard to be detected or had low abundance (<3 $10^5$ copies (g d.w.s.$^{-1}$), d.w.s.: dry weight soil), while in soil of the soil/groundwater ecotone, they were detected with quite high abundance (8 $10^6$ copies (g d.w.s.$^{-1}$)). The highest value was observed at 100–120 cm, which was up to 1.0 $10^8$ copies (g d.w.s.$^{-1}$).
in summer and 8.5 × 10⁷ copies (g d.w.s.)⁻¹ in winter. Compared with the *M. oxyfera*-like bacterial abundances in other natural environments including Lake Biwa [2.0 × 10⁵–1.0 × 10⁶ copies (mL sediment)]⁻¹ (Kojima et al., 2012), Brunssummerheide peatland (1.3 × 10⁷–3.2 × 10⁷ copies (g wet soil)]⁻¹ (Zhu et al., 2012), Jiaxing paddy soil (≤1.0 × 10⁵ copies (g d.w.s.)⁻¹) (Wang et al., 2012a), and Qiantang River [1.32 ± 0.16 × 10⁶–1.03 ± 0.12 × 10⁷ copies (g d.w.s.)⁻¹] (Shen et al., 2013), the values in the soil/groundwater ecotone of the Jiangyin paddy field were the highest to our knowledge.

The total bacteria were also quantified to demonstrate the ratio of *M. oxyfera*-like bacteria to total bacteria in the paddy soil core. Targeting 16S rRNA genes, the abundance of total bacteria decreased gradually with the increase of the depth from the surface soil. As a result, a peak value of the ratio of *M. oxyfera*-like bacteria to total bacteria was observed at 100–120 cm of the soil/groundwater ecotone (2.80% and 4.41% in summer and winter, respectively). The results indicated that the soil/groundwater ecotone in the paddy soil core was a preferred habitat for *M. oxyfera*-like bacteria.

To elucidate the potential influencing factors of the distribution of *M. oxyfera*-like bacteria, statistical analyses were performed between the abundance and environmental variables in the paddy soil core. Among the various environmental variables, total carbon (TC) was observed to be the most determining factor (Multiple linear regression (stepwise regression) identified TC to explain 53.3% of the variation of *M. oxyfera*-like bacterial abundance; Table S2) with strongly negative correlation (Spearman correlation coefficient −0.670 for TC vs. *Methylomirabilis oxyfera*-like bacterial abundance, n = 13, P < 0.05; Table 1). As a substrate, the NO₃⁻ content did not show any effect on the abundance of *M. oxyfera*-like bacteria.

**Community structure of *M. oxyfera*-like bacteria**

As high *M. oxyfera*-like bacterial abundance has been found in the soil/groundwater ecotone of the paddy soil core, the community structure and diversity of *M. oxyfera*-like bacteria in the soil profile was investigated. Eight clone libraries of *pmoA* genes with each 100 positive clones were constructed for representative soil depths to analyze the community structure of *M. oxyfera*-like bacteria. After the selection with restriction endonuclease, a total of 139 clones were obtained, 56 for summer (24 for 60–80 cm, 4 for 80–100 cm, 6 for 100–120 cm, 22 for 120–140 cm) and 83 for winter (23 for 0–20 cm, 25 for 60–80 cm, 16 for 120–140 cm, 19 for 180–200 cm).

The *pmoA* gene sequences in the paddy soil had identities to *M. oxyfera* of 85.9–96.9% on the nucleotide level. After the diversity analysis of 139 *pmoA* gene sequences using DOTUR software, a total of 35 unique OTUs (based on a 98% cutoff) were recovered.

A phylogenetic tree of the 35 representative *pmoA* gene sequences for each OTU and some selected GenBank database sequences was calculated and is shown in Fig. 3. Results indicated that the *pmoA* gene sequences...
of M. oxyfera-like bacteria showed a distant relationship with aerobic methanotrophs and were closely related to M. oxyfera. Moreover, the M. oxyfera-like bacteria had high spatial-temporal heterogeneity in the paddy field. On one hand, seasonal population shift of M. oxyfera-like bacteria was observed with few pmoA gene sequences from different seasons in a same soil depth clustering into the same OTUs. On the other hand, the community structures of M. oxyfera-like bacteria in different soil depths were different from each other with few sequences from different soil layers sharing the same OTUs.

To investigate the diversity of M. oxyfera-like bacteria in different soil depths and seasons, diversity indices for every representative soil layer were calculated. The OTU number, Chao1 richness estimate, and Shannon diversity index ranged from 1 to 13, 1 to 24 and 0 to 2.07, respectively (based on a 98% cutoff, Fig. 4). Interestingly, high diversity of M. oxyfera-like bacteria was also detected in the soil/groundwater ecotone. The M. oxyfera-like bacterial diversity showed little temporal but strong spatial heterogeneity which obviously increased with soil depth (as the representative diversity index, Shannon index had strongly positive correlation with depth (r = 0.892,

Table 1. Correlation analysis between abundance of M. oxyfera-like bacterial 16S rRNA genes, Shannon diversity index of pmoA genes and environmental variables in the Jiangyin paddy field

<table>
<thead>
<tr>
<th>NO₃⁻</th>
<th>NH₄⁺</th>
<th>TC</th>
<th>TN</th>
<th>L0550</th>
<th>TP</th>
<th>TS</th>
<th>pH</th>
<th>Fe²⁺</th>
<th>Fe³⁺</th>
<th>Fe</th>
<th>Mn</th>
<th>Moisture content</th>
<th>Depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abundance (n = 13)</td>
<td>*</td>
<td>-0.767*</td>
<td>-0.670*</td>
<td>-0.736**</td>
<td>-0.412</td>
<td>-0.077</td>
<td>-0.601*</td>
<td>0.504</td>
<td>-0.726**</td>
<td>0.054</td>
<td>0.217</td>
<td>0.402</td>
<td>-0.259</td>
</tr>
<tr>
<td>Sig.</td>
<td>0.529</td>
<td>0.011</td>
<td>0.012</td>
<td>0.004</td>
<td>0.162</td>
<td>0.803</td>
<td>0.030</td>
<td>0.079</td>
<td>0.005</td>
<td>0.860</td>
<td>0.477</td>
<td>0.174</td>
<td>0.393</td>
</tr>
</tbody>
</table>

| Shannon (n = 8) | * | 0.238 | 0.190 | -0.024 | 0.025 | -0.262 | -0.310 | -0.252 | -0.195 | 0.228 | -0.524 | -0.476 | -0.310 | -0.479 | 0.892** |
| Sig. | 0.570 | 0.651 | 0.955 | 0.954 | 0.531 | 0.456 | 0.548 | 0.643 | 0.588 | 0.183 | 0.233 | 0.456 | 0.230 | 0.003 |

Signif. Codes (two-tailed): 0.01***; 0.05**. TN: Total Nitrogen; LOIS50: Loss on ignition at 550 °C; TP: Total Phosphorus; TC: Total Carbon; TS: Total Sulfur.

Fig. 2. The abundance of 16S rRNA gene for M. oxyfera-like bacteria and total bacteria of the soil profile in the Jiangyin paddy field in summer and winter. Two sets of primers (p1F – p1R, p2F – p2R) were used for the quantity of M. oxyfera-like bacteria. Error bars indicate standard deviation (n = 3).
The gradually increased diversity implied that the M. oxyfera-like bacterial community structure tended to be stable in this soil/groundwater ecotone, which further showed that the soil/groundwater ecotone was a suitable habitat for M. oxyfera-like bacteria. Moreover, no environmental variables showed any correlation with diversity (Table 1).

Discussion

In this paper, the vertical-temporal distribution of M. oxyfera-like bacterial abundance and diversity in a paddy soil were investigated and some interesting results were obtained. The M. oxyfera-like bacteria were absent or had low abundance in soil above the groundwater level, then increased sharply in the ecotone of soil and groundwater. As the groundwater level changed within different seasons (60 cm in summer and 40 cm in winter), the soil depth where M. oxyfera-like bacterial abundance began to rise also changed. Compared with M. oxyfera-like bacterial abundances reported in other literature, the values at the soil/groundwater ecotone of the Jiangyin paddy field in this study (up to 1.0 \times 10^8 copies (g d.w.s.)\(^{-1}\)) accounted for 2.80% of total bacteria in
summer and 8.5 × 10⁷ copies (g d.w.s.)⁻¹ accounting for 4.41% of total bacteria in winter) were the highest in natural environments to our knowledge. This study demonstrated the high abundance of M. oxyfera-like bacteria in the soil/groundwater ecotone of the Jiangyin paddy soil profile and provided evidence for the hypothesis that the soil/groundwater ecotone is a favorable habitat for M. oxyfera-like bacteria.

In addition to high abundance, high diversity of M. oxyfera-like bacteria also appeared at the soil/groundwater ecotone. The diversity indices in this ecotone reached the highest values with OTU number up to 9 (summer) and 13 (winter), Chao1 richness estimate up to 24 (summer) and 23.5 (winter), and Shannon diversity index up to 1.49 (summer) and 2.07 (winter) based on a 98% cutoff. In contrast, low diversities were observed in Lake Constance (Deutzmann & Schink, 2011), a Jiaxing paddy field (Wang et al., 2012a), and Lake Biwa (Kojima et al., 2012) (Table S3). This result showed further evidence that the soil/groundwater ecotone in the Jiangyin paddy field was a suitable environment for the growth of M. oxyfera-like bacteria.

The high abundance and diversity of M. oxyfera-like bacteria in soil/groundwater ecotone of the paddy soil should be attributed to the substantial availability of substrates and appropriate environmental conditions. As the major resource of CH₄ in natural environments, paddy field contributes c. 18% of global natural CH₄ production (Mikaloff Fletcher et al., 2004), thus is rich in available methane. Moreover, the application of nitrogen fertilizers brings inorganic nitrogen to paddy soil, including ammonium and nitrate. Both the partial ammonium oxidation and reduction of nitrate will provide nitrite for n-damo bacteria (Wang et al., 2011, 2013, 2014; Kojima et al., 2012). Besides, the soil/groundwater ecotone provided suitable conditions for the growth of n-damo bacteria. On the one hand, the groundwater created an anaerobic environment needed by n-damo bacteria because of the low solubility of oxygen in water. On the other hand, the water in soil/groundwater ecotone promoted the substrate supply for the n-damo process. Previous studies reported that low water availability in soil would reduce the rate of substrate diffusion to microbial cells and thus may lead to resource limitation (Stark & Firestone, 1995). With all of the above advantages, the soil/groundwater ecotone of the paddy soil would be a more desirable habitat for n-damo bacteria than the soil above this ecotone, which has also been proved in this study.

The importance of groundwater in the paddy soil for the growth of M. oxyfera-like bacteria was also proved by the study of Wang et al. (2012a). The study on M. oxyfera-like bacteria conducted in a paddy field core in Jiaxing (Zhejiang, China) drew different results that the abundance of M. oxyfera-like bacteria was at most 1.0 × 10⁵ copies (g d.w.s.)⁻¹ in surface soil and decreased with the soil depth to undetectable level below 70 cm depth in the 100-cm sampling scope (Wang et al., 2012a). The values were far lower than those in the soil/groundwater ecotone of the Jiangyin paddy soil in this study, although there was higher nitrate/nitrite loading (up to 53.8 mg kg⁻¹ dry soil) in Jiaxing paddy soil than in Jiangyin. Because there was no groundwater, which could play an important role on constructing an anaerobic environment and enhancing the substrate diffusion rate, within the sampling scope in Jiaxing paddy soil core, the growth of the n-damo bacteria was restricted.
Although $\text{NO}_x$ was a substrate of damo process, there seemed to be no correlation between the $\text{NO}_x$ content and the abundance of $\text{M. oxyfera}$-like bacteria. The reason may be that instead of nitrate, nitrite was reported to be the preferred electron acceptor of the n-damo process (Raghoobarsing et al., 2006; Ettwig et al., 2009; Zhu et al., 2012). In this case, the nitrite needed by $\text{M. oxyfera}$-like bacteria may depend on the partial nitrate reduction or ammonium oxidation (Kojima et al., 2012). So the nitrification or denitrification rate may have an effect on the n-damo process.

In summary, this study showed the distribution patterns of $\text{M. oxyfera}$-like bacteria in the paddy soil and firstly demonstrated the high abundance of $\text{M. oxyfera}$-like bacteria in the soil/groundwater ecotone of this particular paddy soil, which is the highest one in nature to our knowledge. Moreover, high diversity of $\text{M. oxyfera}$-like bacterial community was also observed in this soil/groundwater ecotone. All these results verified that the soil/groundwater ecotone in the Jiangyin paddy field was a favorable habitat for n-damo bacteria.

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References


### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** The primers and thermal profiles used in this study.

**Table S2.** Result of multiple linear stepwise regression on PNR.

**Table S3.** Diversities of *M. oxyfera*-like bacterial *pmoA* gene sequences in other studies (based on a 98 % cut-off).