

Summary

Methane is a major greenhouse gas and has a high global warming effect, which is about 25 times stronger than that of CO₂. Rice paddy fields under flooded conditions, as an important source, contribute to 5-19% of the global budget of the greenhouse gas methane. Methanogenic archaea, obligate anaerobes, generate methane under flooded soil conditions in rice paddy fields. Therefore, studies on methanogenic archaea in rice paddy fields benefit to understand the mechanism of methane generation in the soil and also are crucial to gain general knowledge for controlling or reducing the methane emission from the fields. Methanogenic archaeal communities exhibit stable structures in the paddy fields under conventional managements. On the other hand, the methanogenesis is influenced by organic matter application and water managements. Extension of flooding period of paddy fields promote anoxic soil conditions and increase methane emission from the fields. In contrast, extended period of drainage for longer than one year in paddy fields induce dry-oxic soil conditions and decrease methane emission from the flooded fields after the drained period. Elevated CO₂ concentration in paddy fields enhances photosynthesis and growth of rice plants and rice root exudation, and results in increase in methane emission from the fields. However, until now no study has been reported on methanogenic archaeal community structure under managements of extended flooding or drainage period and treatments of elevated atmospheric CO₂ concentration under paddy field conditions. In this study, I investigated the responses and behaviors of methanogenic archaeal community composition and abundance in paddy field soil to different water managements and an elevated CO₂ treatment under field conditions by molecular ecological methods.

1. Effect of winter-flooding on methanogenic archaeal community structure in a paddy field under organic farming

The effect of winter flooding on methanogenic archaeal community structure was investigated in Andosols paddy field under organic farming by PCR-DGGE and real-time qPCR methods targeting 16S rRNA gene and *mcrA* gene, encoding α subunit of methyl-coenzyme M reductase. Soil samples were collected from the experimental paddy fields for two years under flooding conditions during winter with organic farming, under non-flooding conditions during winter with organic farming and under non-flooding conditions during winter with conventional farming (non-organic farming). Methanogenic archaeal community compositions were nearly the same irrespective of the treatment and sampling times. Twenty-three DGGE bands were observed from each treatment and 4, 13 and 6 sequences were closely related to *Methanomicrobiales*, *Methanosarcinales* and *Methanocellales*, respectively. Real-time qPCR analysis indicated that the abundance of methanogenic archaeal 16S rRNA gene and *mcrA* gene was not significantly different among the paddy fields. These results showed that the methanogenic archaeal community was not affected by winter flooding under organic farming.

2. Effect of the free-air CO₂ enrichment (FACE) on methanogenic archaeal community structure in a paddy field

Effect of free-air carbon dioxide enrichment (FACE) on methanogenic archaeal community structures in an experimental paddy field with elevated CO₂ (+200 ppm) treatment was investigated by PCR-DGGE and real-time qPCR methods targeting 16S rRNA and *mcrA* genes, respectively. Soil samples were collected from the upper (0-1

cm) and lower (1-10 cm) parts of plow layer soil at two rice-growing stages, 41 and 92 days after transplanting of rice. Methanogenic archaeal community composition showed no conspicuous change under the elevated CO₂ condition, but the community had a slight change in the abundance between the sampling stages in the lower soil. In contrast, abundance of methane-oxidizing bacteria was decreased by the elevated CO₂ estimated by real-time qPCR of *pmoA* (encoding α subunit of particulate methane monooxygenase) gene. The results indicated that the elevated CO₂ in the paddy field under rice growing stages did not influence methanogenic archaeal community structure, but methane-oxidizing bacterial community structure, which may have related to the increase in the methane emission under the elevated CO₂ condition in the FACE paddy field.

3. Effect of paddy-upland rotation on methanogenic archaeal community structure in paddy field soil

Methanogenic archaeal community was investigated in fields where paddy rice under flooded conditions was rotated with soybean under upland conditions at different rotation histories, by PCR-DGGE and real-time qPCR methods targeting 16S rRNA and *mcrA* genes. Soil samples collected from the fields before flooding or seeding, during crop cultivation and after harvest of crops were analyzed. The abundance of the methanogenic archaeal populations decreased to about one-tenth in the rotational plots than in the consecutive paddy (control) plots. The composition of the methanogenic archaeal community was also changed. Most members of the methanogenic archaea consisting of the orders *Methanosarcinales*, *Methanocellales*, *Methanomicrobiales* and *Methanobacteriales* existed autochthonously in both the control and rotational plots, while some were strongly affected in the rotational plots, with fatal effect to some

members belonging to the *Methanosarcinales*. The results indicated that the upland conversion for one or longer than one year in the rotational system affected the methanogenic archaeal community structure and was fatal to some members of methanogenic archaea in paddy field soil.

4. Dynamics of active members of methanogenic archaeal community in soil under paddy-upland rotational conditions

Dynamics of active members of methanogenic archaeal community was investigated under the rotation of paddy rice and soybean for each two or three years, by PCR-DGGE, T-RFLP and real-time qPCR methods targeting 16S rRNA gene, *mcrA* gene and their transcripts, respectively. Soil samples collected from the fields before flooding or seeding, during crop cultivation and after harvest of crops were analyzed through three years. The composition and transcriptional activity of methanogenic archaea were affected by the paddy-upland rotation and relative abundance of active members of methanogenic archaea was changed under different soil conditions in the rotational field. The abundance of *mcrA* transcripts was greatly decreased in the rotational plots after upland conversion longer than one year and autochthonous groups of methanogenic archaea seemed to survive and kept very low transcriptional activities under the upland conditions in the rotational field. Ratio of numbers of the *mcrA* transcripts to those of *mcrA* gene of methanogenic archaea showed positive correlation with the methane production potential of soil in the fields. The results showed that composition and abundance of active members of methanogenic archaea were greatly affected by the upland conversion longer than one year in the rotational field, but the inherent members in soil survived with very low transcriptional activities under the conditions.

In conclusion, this study revealed composition and abundance of methanogenic archaeal communities in the paddy fields under various water managements and the elevated CO₂ treatment and the dynamics and stability of communities in the fields. The findings in this study can be useful for understanding of the mechanisms of methane production in paddy field soil, and controlling and mitigating methane emission from rice paddy fields.