



## Phylogenetically distinct methanotrophs modulate methane oxidation in rice paddies across Taiwan



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### ABSTRACT

Rice paddies are considerable sources of methane because of the highly reduced soil oxidation–reduction conditions during rice cultivation. In this ecosystem, active methane-oxidizing bacteria are important, because they consume methane while reducing the overall emissions from rice paddy soils. However, the biogeographic distribution of active methanotrophs in paddy soils across Taiwan remains poorly understood. We used DNA-based stable isotope probing (DNA-SIP) to show that phylogenetically distinct type I and type II methanotrophs dominated methane oxidation in geographically different paddy soils across Taiwan. High-throughput sequencing of soil 16S rRNA and *pmoA* genes under field conditions revealed that a type II methanotroph, *Methylocystis*, was predominant in rice paddy soils. In addition, an uncultured novel type I methanotroph cluster (Rice Paddy Clusters) was detected with the closest relatedness to *Methylocaldum* 16S rRNA genes in all rice field soils tested. SIP microcosm incubation, however, suggested that the type I methanotrophs *Methylosarcina* and *Methylobacter* were significantly stimulated during the consumption of high-concentration methane in five paddy soils with pH > 6.07, whereas the *Methylosinus*-like microorganisms of type II methanotrophs dominated aerobic methane-oxidizing communities in acidic soil with pH = 5.17. Furthermore, high-throughput sequencing of <sup>13</sup>C-*pmoA* genes indicated the presence of novel methanotrophs that are phylogenetically distantly related to the type I methanotrophs *Methylosarcina* in four out of five non-acidic paddy soils studied, and the high proportions in the <sup>13</sup>C-DNA suggested that these uncultured methanotrophs play an important role in methane oxidation. These results provide strong evidence for the environmental selection of phylogenetically distinct methanotrophs under field conditions. Moreover, community shifts in active methanotrophs likely occurred in response to environmental variations with fluctuating methane concentrations.

### 1. Introduction

Methane (CH<sub>4</sub>) is an important greenhouse gas and has a 25 times higher potential for retrieving heat than carbon dioxide (CO<sub>2</sub>) (IPCC, 2013), and it is released from ecosystems with long inundation periods such as rice paddies, landfills, and wetlands (Yang and Chang, 1998; Weart, 2004). Among all the different anaerobic ecosystems, it is estimated that rice paddies contribute approximately 5–19% of the CH<sub>4</sub> emission worldwide (Ma et al., 2010). Paddy cultivation is an important agricultural practice that represents a major source of food in Asia. However, the long period of soil inundation during the paddy growing season results in rice paddies being a considerable source of CH<sub>4</sub>. Previous studies have shown that CH<sub>4</sub> emissions from rice paddies ranged from 0.01 to 0.03 μmol m<sup>-2</sup> s<sup>-1</sup> in Taiwan (Tseng et al., 2010),

0.10–0.11 μmol m<sup>-2</sup> s<sup>-1</sup> in Southern China (Liu et al., 2017), 0.06–0.07 μmol m<sup>-2</sup> s<sup>-1</sup> in Japan (Miyata et al., 2000), and 0.38 μmol m<sup>-2</sup> s<sup>-1</sup> in the Philippines (Wagner-Riddle et al., 1996). It is, however, speculated that the methane emissions would otherwise be at least double if the anaerobically generated methane escaped to the atmosphere without microbial consumption by methanotrophs (Le Mer and Roger, 2001).

In paddy fields, oxygen diffusion through rice plants and the water table leads to niche differentiation and fuels aerobic methane oxidation by methanotrophs. In such an ecosystem with a high potential for CH<sub>4</sub> production, it is estimated that 40–90% of methanogenic CH<sub>4</sub> could be converted to CO<sub>2</sub> by methane-oxidizing bacteria before it is emitted to the atmosphere (Roslev and King, 1996; Kruger et al., 2001; Le Mer and Roger, 2001; Megonigal and Schlesinger, 2002). Therefore,

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methanotrophs are considered as the key biofilter in wetland ecosystems for their ability to reduce CH<sub>4</sub> flux. However, because of technical limitations, the taxonomic identities of the active methanotrophs responsible for the vast consumption of methane produced in complex wetland ecosystems are poorly understood.

Molecular surveys have revealed dynamic changes in methanotrophic community structures in paddy fields under various conditions (Jia et al., 2001; He et al., 2012; Daebeler et al., 2014; Sharp et al., 2014; Zheng et al., 2014; Ho et al., 2016). Macalady et al. (2002) found that methanotrophs were mostly present within 0–2 cm of rice paddy soils, and their population increased during rice growing periods. A mid-season drainage is widely employed for rice cultivation in Asia, and it has significant impacts on the abundance of methanotrophs and stimulates methane oxidation activity in rice soils (Ma and Lu, 2010). Therefore, methanotrophic communities under *in situ* conditions are the net result of ecological adaptation to agricultural management practices and evolution under a constantly changing environment. Notably, analyses of the methanotrophic community in rice paddies using high-throughput sequencing of 16S ribosomal DNA genes have only revealed the overall composition of the methanotrophic community, and methanotrophs were found to only represent 0.8–1.8% of the total microbial community in a rice paddy in South Korea (Lee et al., 2014). However, the driving force of niche differentiation among phylogenetically distinct methanotrophs of type I and type II communities remains largely unclear. Understanding the driving force for niche differentiation of methanotrophic communities may help optimize ecological management for reduction of net CH<sub>4</sub> efflux from ecosystems, as Type I and Type II methanotrophs responded differently under changing environments such as CH<sub>4</sub> concentration fluctuations (Graham et al., 1993; Hanson and Hanson, 1996; Shrestha et al., 2010; Ho et al., 2013). For example, the community of type II methanotrophs remains largely constant throughout cultivation periods, whereas the type I methanotrophic community increases during rice cultivation. Nevertheless, whether specific phylotypes of distinct methanotrophs are selected in response to environmental variations during the rice growing season is poorly understood, and the taxonomic identities of active methanotrophs are not well documented.

DNA-based stable isotope probing (DNA-SIP) in combination with isopycnic centrifugation have been demonstrated to be a powerful method for establishing direct links between ecological processes and the taxonomic identities of active microorganisms (Radajewski et al., 2000), particularly for methanotrophs (Cai et al., 2016). Zheng and Jia (2016) used the DNA-SIP technique to demonstrate that the composition and activity of active methanotrophs varied spatially in nine different rice paddies in China. In this study, we hypothesized that methanotrophic communities could vary based on local environment and soil parent material, and they might also serve as seed banks of active communities for methane oxidation under fluctuating environments. To determine the environmental drivers of soil methanotrophic communities, we conducted a high-throughput sequencing survey of methane oxidizers in six geographically different rice soils that largely represent paddy ecosystems of different soil origins across Taiwan. Furthermore, DNA-SIP was employed to elucidate the taxonomic identities of active methanotrophs when soils were exposed to high-concentration methane.

## 2. Materials and methods

### 2.1. Sampling sites

Soil samples were collected from six agricultural research and extension stations: Taoyuan (Site 1; S1), Miaoli (Site 2; S2), Taichung (Site 3; S3), Kaohsiung (Site 4; S4), Hualien (Site 5; S5), and Taitung (Site 6; S6) (Fig. 1). The sampling sites represent the most important regions for rice production, spanning from northern to southern and from eastern to western Taiwan (Table 1). The rice variety used in these regions was



Fig. 1. The six rice paddies sites of the study in Taiwan.

### *Oryza sativa* L.

The S1 site is located in Xinwu Township in Taoyuan City, north western Taiwan. The sampling site has an elevation of about 26 m above sea level, a mean annual precipitation of about 1680 mm, and an annual mean temperature of 21.6 °C. The site contains colluvial soils derived from diluvium red soils and parent materials derived from diluvium materials, which are classified as Oxisols or Ultisols.

The S2 site is located in Gongguan Township in Miaoli County, western Taiwan. The sampling site has an elevation of about 100 m above sea level, a mean annual precipitation of about 1710 mm, and an annual mean temperature of 22.7 °C. The site contains alluvial soils derived from northern sandstones-shales and parent materials derived from sandstone or shale or both materials, which are classified as Inceptisols.

The S3 site is located in Dacun Township in Changhua County, central Taiwan. The sampling site has an elevation of about 19 m above sea level, a mean annual precipitation of about 1380 mm, and an annual mean temperature of 22.4 °C. The site contains order alluvial soils derived from slate calcareous and parent materials derived from slate material, which are classified as Inceptisols.

The S4 site is located in Changzhi Township in Pingtung County, south western Taiwan. The sampling site has an elevation of about 24 m above sea level, a mean annual precipitation of about 2200 mm, and an annual mean temperature of 24.0 °C. The site contains order alluvial soils derived from slate noncalcareous and parent materials derived from slate material, which are classified as Entisols or Inceptisols.

The S5 site is located in Sanxing Township in Yilan County, north eastern Taiwan. The sampling site has an elevation of about 27 m above sea level, a mean annual precipitation of about 2840 mm, and an annual mean temperature of 22.4 °C. The site contains younger alluvial soils derived from northeastern slate noncalcareous and parent

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