

# METHANE OXIDATION AND STABLE ISOTOPE PROBING OF ACTIVE METHANOTROPHS IN COLD-TEMPERATE RICE FIELDS

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## **Abstract**

Paddy rice fields represent a unique anthropogenic wetland ecosystem which contributes considerably emission to the atmosphere greenhouse gas methane. Yet, the ecology and diversity of methanotrophs that regulate active methane oxidation and attenuate the methane emission potentials remain largely unclear, especially in the less studied cold-temperate regions. Here we used stable isotope probing technique to investigate the methanotrophic potentials, and further identify the active methane oxidizers in 3 different paddy soils from Jian-San-Jiang (one Baijiang-derived, JB and one Meadow-derived, JM) and Qing-An (Meadow-derived, QA) of Northeast China. After microcosm incubation under 1% v/v <sup>13</sup>C-labeled methane condition, all soil <sup>13</sup>C abundances significantly increased from background 1.08% to 1.21% in average, representing an approximately 36.9% methane-derived carbon assimilation induced by methanotrophy. High enrichment of methanotrophic biomarker pmoA genes in <sup>13</sup>C-labeled DNA by quantitative PCR demonstrated great propagation of methanotrophs supported by methane oxidation. High-throughput sequencing of 16SrRNA and pmoA genes from <sup>13</sup>C-labeled DNA further revealed a diverse guild of both type I and II methanotrophs in all three soils. Specifically, Methylobacter-affiliated type I methanotrophs were highest stimulated in JB and JM soils, whereas Methylocystis-affiliated type II dominated the methanotrophic activity in QA soil. Collectively, our results suggest high potentials of methanotrophy by phylogenetically divergent microorganisms in soils from cold-temperate region, implying great physiological diversification of soil methanotrophs that might be due to constant environmental fluctuations in paddies.

**Keywords:** Methanotrophs; Cold-temperate rice paddy; DNA-SIP; High-throughput sequencing