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## Soil-specific responses of methanotrophic communities in the rice rhizosphere to genotype and nitrogen fertilisation

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## Abstract

One of the most significant agricultural sources of the greenhouse gas methane is lowland rice production. An approach to reduce methane flux from the rice field is to develop rice varieties that support methane oxidation in the roots and surrounding rhizosphere by methanotrophs. However, the effect of different genotypes on methanotrophic communities in comparison to other influence factors such as soil or nitrogen fertilisation management is still poorly understood. We conducted pmoA amplicon sequencing of root and rhizosphere samples collected from planted (Kasalath, Nipponbare, IR64, and O. rufipogon) and unplanted (as a control) paddy soil microcosms. The microcosms contained either Italian or Philippine paddy soil and were either fertilised with additional N  $(50 \text{ kg ha}^{-1})$  or not. 2,865 amplicon sequence variants were identified across 171 samples. Compositional analysis showed that *Methylocystis* sp. dominated in the Italian paddy soil, while *Methylococcus* sp. and uncultured rice paddy clusters were more commonly detected in the Philippine soil. Beta diversity analysis based on Aitchison distance and PERMANOVA showed that the two soils had significantly different ( $R^2=0.332$ , P=.001) methanotrophic communities. On a per soil basis, the effect of genotype was significant in Philippine soil ( $R^2=0.082$ , P = .001) where Kasalath had a similar community with Nipponbare, but different to O. rufipogon. In the Italian soil, the fertilisation schemes ( $R^2=0.025$ , P=.004) and genotypes  $(R^2=0.064, P=.004)$  resulted in significantly different methanotrophic communities, with Kasalath and Nipponbare hosting similar communities, especially in the root compartment. Our findings indicate that community assembly of methanotrophs in the rice root and rhizosphere depends on the native population present in the soil and that plant-genotype effects and nitrogen fertilisation-responses are soil specific. Despite being phylogenetically distant, i.e. Nipponbare to Kasalath, they established similar methanotrophic communities, which indicates that the relatedness of the genotypes is not necessarily reflected in the preferential recruitment of dis-/similar methanotrophic communities.

**Keywords:** Greenhouse gas, methane, methanotrophs, microcosm, PmoA, rhizosphere, rice, rice variety

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