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RESEARCH ARTICLE

Winter cover crops alter methanotrophs community structure in a double-rice paddy soil

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Abstract

Methanotrophs play a vital role in the mitigation of methane emission from soils. However, the influences of cover crops incorporation on paddy soil methanotrophic community structure have not been fully understood. In this study, the impacts of two winter cover crops (Chinese milk vetch (*Astragalus sinicus* L.) and ryegrass (*Lolium multiflorum* Lam.), representing leguminous and non-leguminous cover crops, respectively) on community structure and abundance of methanotrophs were evaluated by using PCR-DGGE (polymerase chain reaction-denaturing gradient gel electrophoresis) and real-time PCR technology in a double-rice cropping system from South China. Four treatments were established in a completely randomized block design: 1) double-rice cropping without nitrogen fertilizer application, CK; 2) double-rice cropping with chemical nitrogen fertilizer application (200 kg ha^{-1} urea for entire double-rice season), CF; 3) Chinese milk vetch cropping followed by double-rice cultivation with Chinese milk vetch incorporation, MV; 4) ryegrass cropping followed by double-rice cultivation with ryegrass incorporation, RG. Results showed that cultivating Chinese milk vetch and ryegrass in fallow season decreased soil bulk density and increased rice yield in different extents by comparison with CK. Additionally, methanotrophic bacterial abundance and community structure changed significantly with rice growth. Methanotrophic bacterial *pmoA* gene copies in four treatments were higher during late-rice season (3.18×10^7 to 10.28×10^7 copies g^{-1} dry soil) by comparison with early-rice season (2.1×10^7 to 9.62×10^7 copies g^{-1} dry soil). Type I methanotrophs absolutely predominated during early-rice season. However, the advantage of type I methanotrophs kept narrowing during entire double-rice season and both types I and II methanotrophs dominated at later stage of late-rice.

Keywords: Chinese milk vetch, ryegrass, methanotrophic bacteria, community structure, double-rice

1. Introduction

Atmospheric methane (CH_4) has received an extensive attention as a greenhouse gas, which perturbs the radiation balance of climate and contributes 25 times more toward warming than carbon dioxide over a hundred year time horizon (IPCC 2007). Concerning about potential environmental impacts of global warming, intensifying study on major sources and sinks of CH_4 is extremely urgent. Rice paddy is one of main sources of anthropogenic CH_4,

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contributing 20–40 Tg CH_4 emission per year (IPCC 2007). Rice cultivation area in China occupy 19% of the global rice planting area and CH_4 emission from rice paddies notably increased from 4.13 Tg yr^{-1} in 1970 to 6.85 Tg yr^{-1} in 2009. Moreover, relative higher methane emissions were observed in southern China, where double-rice cropping was widely adopted (Zhang *et al*. 2014). However, paddy soil is also a sink of atmospheric CH_4 on account of the existence of methanotrophs.

Methanotrophic bacteria play an influential role in global methane budget by attenuating the methane flux from the soil into the atmosphere. About 30–90% of CH_4 produced in paddy soil is oxidized by methanotrophs in aerobic regions of the soil (i.e., the surface soil layer and the rice rhizosphere) before emitting to the atmosphere (Groot *et al*. 2003) and CH_4 emission was significantly correlated with transcripts of methanotrophic *pmoA* genes (Lee *et al*. 2014). The attenuation process is greatly influenced by environmental factors including concentration of methane, oxygen and soil nitrogen availability etc. Additionally, methanotrophic bacterial community structure on rhizosphere is a further crucial factor critically affecting attenuation process and it varies with seasons, soil properties, cultivars, and managements (Horz *et al*. 2001).

Winter cover crop cultivation and incorporation is widely regarded as an important management practice on account of the potential to prevent nitrogen loss through nitrate leaching and resulted non-point source pollution in double-rice planting region (Olson *et al*. 2010; Kim *et al*. 2012). These crops are roughly divided into two groups: non-leguminous plants with high-yielding biomass such as ryegrass, barley, wheat, etc., and leguminous plants which perform nitrogen fixation, for instance, sunnhemp, vetch or spiny sesbania (Cho *et al*. 2003). Those two groups both can improve soil physical properties, sustain content of soil organic matter, extend the nutrient persistence, enhance long-term soil fertility levels (Lee *et al*. 2010; Talgre *et al*. 2012), and also contribute to higher rice yield (Kim *et al*. 2013). Soil properties and nitrogen content are positively correlated with the development of methanotrophic community (Conrad 2007). Massive input of organic matters supply sufficient substrate for methanogens and hence increase the methane emission from paddies (Lee *et al*. 2010; Zhu *et al*. 2012; Kim *et al*. 2013). Increased methane concentration in soil can simulate the reproduction of methanotrophs and also affect the community composition (Conrad 2007). However, degration of organic matter intensifies reduced condition in paddy soil and may have negative effects on methanotrophic community (Liu *et al*. 2014).

Due to the uncertainty about the impact of cultivation and incorporation of winter cover crops on methanotroph community structure in previous studies, the present study investigate the effects of leguminous Chinese milk vetch (*Astragalus sinicus* L.) and non-leguminous ryegrass (*Lolium multiflorum* Lam.) as representative winter cover crops on methanotrophic community in double-rice paddy soil by using PCR-DGGE (polymerase chain reaction-denaturing gradient gel electrophoresis) and real-time PCR techniques (targeting *pmoA* genes). PCR-DGGE has been the most widely used technique to estimate microbial ecology on account of its low-cost, easy accessibility and possibility of processing multi-sample analysis (Cahyani *et al*. 2003; Ikenaga *et al*. 2003; Murase *et al*. 2005). As for Q-PCR (real-time quantitative polymerase chain reaction detecting system), due to its high sensitivity and accuracy, it is a powerful tool for nucleic acid quantification (Kruger *et al*. 2005). Additionally, more detailed view of microbial community composition, structure and abundance will be obtained when combining PCR-DGGE with Q-PCR.

2. Results

2.1. Changes of soil ammonium nitrogen content and bulk density after the cultivation of Chinese milk vetch and ryegrass

Cultivating Chinese milk vetch and ryegrass in fallow season had distinct influences on soil ammonium nitrogen content (Table 1). The variation trends of NH_4^+ content were consistent between three soil layers (0–10, 10–20, 20–30 cm). Compared with CK (double-rice cropping without nitrogen fertilizer application), NH_4^+ content in three soil layers respectively increased by 73.3% (P<0.05), 8.5 and 25.0% (P<0.05) after cultivation of Chinese milk vetch. On contrast, NH_4^+ content decreased 28.3% (P<0.05), 37.3% (P<0.05) and 19.4% (P<0.05) after cultivation of ryegrass in three soil layers (0–10, 10–20, 20–30 cm), respectively, by comparison with CK. Additionally, cultivation of Chinese milk vetch or ryegrass significantly decreased soil bulk density in different

Table 1 Soil ammonium nitrogen concentrations (mg kg^{-1}) and bulk density (g cm^{-3}) after harvesting Chinese milk vetch and ryegrass

Treatment[1)]	NH_4^+-N (mg kg^{-1})			Bulk density (g cm^{-3})	
	0–10 cm	10–20 cm	20–30 cm	0–15 cm	15–30 cm
CK	6.0 b	5.9 a	3.6 b	0.86 a	1.09 a
RG	4.3 c	3.7 b	2.9 c	0.71 c	0.88 c
MV	10.4 a	6.4 a	4.5 a	0.74 b	1.03 b

[1)] CK, double-rice cropping without nitrogen fertilizer application; RG, ryegrass cropping followed by double-rice cultivation with ryegrass incorporation; MV, Chinese milk vetch cropping followed by double-rice cultivation with Chinese milk vetch incorporation.

Different letters in each column indicate significant differences (P<0.05).

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