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Full Length Research Paper

Methanotrophic abundance and community fingerprint in pine and tea plantation soils as revealed by molecular methods

Yong Zheng[1]*, Xiao-Fang Tian[2], Ju-Pei Shen[3] and Li-Mei Zhang[3]

[1]State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, People's Republic of China.
[2]College of Land Resources and Environment, Jiangxi Agricultural University, Nanchang 330045, People's Republic of China.
[3]State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, People's Republic of China.

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Understanding the community structure of methane-oxidizing bacteria (methanotrophs) is important to assess the microbial oxidation of the greenhouse gas methane (CH_4) in soil under different land uses. Soil samples were collected from two plantation plots of pine and tea in southern China. Methanotrophic abundance was quantified with quantitative real-time polymerase chain reaction (qPCR) based on the 16S rRNA and *pmoA* genes, and the community fingerprint was characterized with denaturing gradient gel electrophoresis (DGGE) targeting the *pmoA* gene. No significant difference in the gene copy numbers of methanotrophs was found between the pine and tea land-use, regardless of 16S rRNA and *pmoA* genes. Higher abundance of type I (1.35 vs 1.66×10^8 copie g^{-1} soil) over type II methanotrophs (8.59 vs 10.9 × 10^7) were found both in pine and tea plantation soils. Apparent differences in methanotrophic community fingerprint were observed between the pine and tea treatments. Correlations analysis between methanotrophic abundance and soil characteristics, combining with canonial correspondence analysis (CCA) regarding community fingerprint and environmental parameters indicated that soil pH and available phosphorus were the most important factors potentially affecting the methanotrophic community diversity in the acidic red soil.

Key words: Denaturing gradient gel electrophoresis (DGGE), land use, methanotrophs, *pmoA* gene, quantitative real-time PCR (qPCR).

INTRODUCTION

Methane (CH_4) with an atmospheric concentration of 1.77 ppmv is the second most important greenhouse gas contributing roughly 17% to the observed global warming (IPCC, 2007). Methane-oxidizing bacteria (methanotrophs) can utilize CH_4 as a sole carbon and energy source and currently remove 30 Tg annually from the atmosphere (IPCC, 2007), which suggests that they play a crucial role in the mitigation of global warming. Methanotrophs are ubiquitous in the environments including paddy field and wetland soils (Bodelier et al., 2000; Yun et al., 2010), upland and forest soils (Kolb, 2009), landfill soils (Chen et al., 2007; Chang et al., 2010), and some other ecological niches (Abell et al., 2009; Zheng et al., 2012).

Methanotrophs are traditionally classified into type I (γ-Proteobacteria) and type II (α-Proteobacteria) groups based on their differences in phylogeny, physiology, morphology, and biochemistry (Trotsenko and Murrell,

*Corresponding author. E-mail: zhengy@im.ac.cn.

Abbreviations: DGGE, Denaturing gradient gel electrophoresis; **qPCR,** quantitative real-time PCR; **OM,** organic matter; **TN,** total nitrogen; **AP,** available phosphorus; **AK,** available potassium; **CCA,** Canonial correspondence analysis.

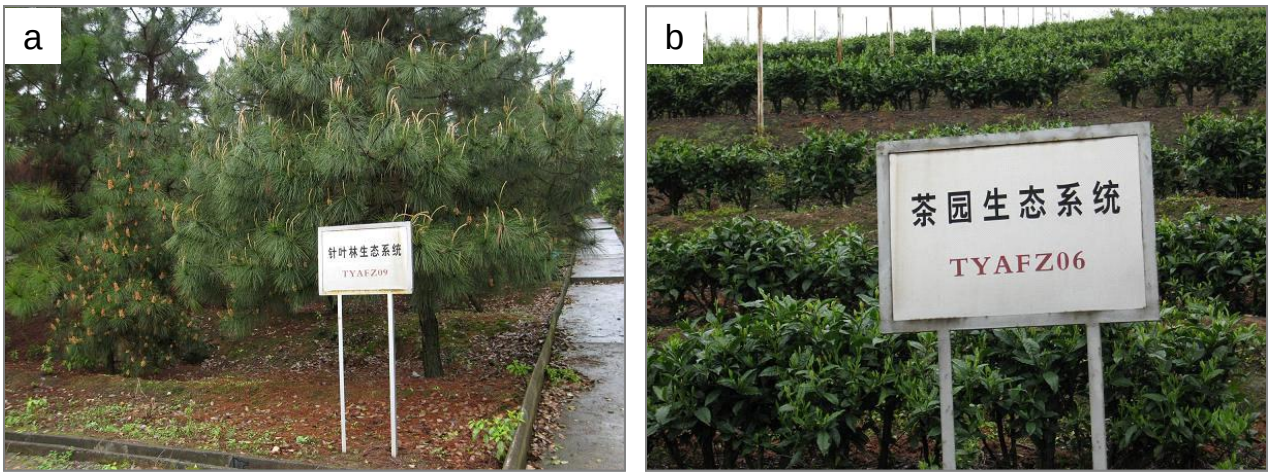

Figure 1. Photographs of the pine (a) and tea (b) land utilization patterns in an acidic red soil sampled in this study.

2008). Identification of methanotrophs always faces challenges, because most of them are difficult to be cultured. Fortunately, the application of culture-independent molecular approaches, such as denaturing gradient gel electrophoresis (DGGE), clone library and quantitative real-time PCR (qPCR) provide powerful means to detect methanotrophs in various environments (Lau et al., 2007; Chen et al., 2008; Shrestha et al., 2010).

Methanotrophs have unique functional genes, *pmoA* and *mmoX*, which encode subunits of particulate methane monooxygenase and soluble methane monooxygenase, respectively. Particularly, *pmoA* is a preferred gene and extensively used to detect methanotrophs in soils (Mohanty et al., 2007; Yrjälä et al., 2011). In addition, group-specific PCR primer sets can be used to selectively amplify 16S rRNA gene of type I and type II methanotrophs at the family or genus level (Henckel et al., 1999; Wise et al., 1999; Chen et al., 2007; Martineau et al., 2010).

Land use change is widely recognized as one of the most important driving forces for global carbon cycles (Yang et al., 2009). Lower CH_4 sink activity was found in the disturbed forest ecosystem than those in the subtropical, tropical and pine forest ecosystems (Singh, 2011). The conversion from forest soil to agricultural soil resulted in a reduction in CH_4 uptake by 60% (Dobbie et al., 1996). The highest CH_4 oxidation was shown in the forest soil, followed by the natural grassland and arable soils (Boeckx et al., 1998). Generally, the response of soil methanotrophic community and abundance to different land utilization patterns is important to mirror the CH_4 uptake capacity. Moreover, land-use change was regarded as the main factor in structuring soil microbial community, irrespective of the environment (Yao et al., 2006; Xue et al., 2010). To date, however, there is no consistent point of view concerning methanotrophic community under different land uses. In a previous study, three different land-use upland soils of a natural forest, a 16-year-old reforested site, and an agricultural field also differed in the methanotrophic community composition (Knief et al., 2005). However, Menyailo et al. (2008) demonstrated that land use change did not affect the diversity of methanotrophs. A similar study recently reported that tree species affected atmospheric CH_4 oxidation without altering methanotrophic community (Menyailo et al., 2010). Therefore, whether land use would have an effect on ecological traits (that is, abundance and community diversity) of methanotrophs is not very clear, especially in the acidic soil.

In the current study, a combined application of qPCR and DGGE was used to determine: (1) Whether there is any difference in methanotrophic abundance and community fingerprint in an acidic red soil under the pine and tea land utilizations (plantations) and (2) the key factors affecting methanotrophic abundance and community fingerprint in this acidic soil ecosystem.

MATERIALS AND METHODS

Site description

This study was carried out in Taoyuan Experimental Station of Agro-ecosystem Observation (28° 55' N, 111° 26' E) of the Chinese Academy of Sciences, Hunan Province in southern China. This site has a mean altitude of 95 to 125 m a.s.l., a mean annual temperature of 16.5°C, and a mean annual precipitation of 1440 mm. The soil was developed from quaternary red clay and classified as Hapludults. The field has a sloping gradient of 8 to 11° and a projected area of 1 ha. The vegetations were removed in 1995 and the field was divided into different plots for establishing different land utilization patterns. Two different land utilization patterns were selected in the present study: Pine treatment (P) and tea treatment (T), in which pine and tea were planted since 1995, respectively. The breed of the pine and tea in this study were *Pinus elliottii* and oil-tea *Camellia*, respectively. Each plant occupied an area of ca 11 m^2, and both of the pine and tea plots were divided into 15 terrace. (Figure 1). The pine plot was scarcely disturbed after plantation since 1995 (Ying et al., 2010). However, for the tea plantation plot,

one kg of compound fertilizer (that is, NPK) was applied into each terrace during the early stage of tea's growth annually. In addition, no herbicide was used to both plantation areas, whereas the weeds were removed artificially only in the tea plantation plot. Soil samples were collected in the surface of 0 to 10 cm on the 18th of October, 2007 from three positions designated as upper (U), middle (M) and lower (L) places, which were parallel to the third, eighth and thirteenth terrace in the slope, respectively, to avoid the effect of landform and to give reliable assessment of land utilization patterns (Ying et al., 2010). These samples were named by combining their land utilization patterns (P and T) and positions (U, M, and L). For instance, samples from upper, middle and lower positions of the pine plot were named PU, PM, and PL, respectively. Since there was no replicate of plot, three parallels were done as replicates for DNA extraction and subsequent qPCR and DGGE analysis.

Soil characteristics analysis and DNA extraction

Soil pH was determined with a soil to water ratio of 2:5 (*W*/*V*). Soil organic matter (OM) was measured using the $K_2Cr_2O_7$ oxidation method, and total nitrogen (TN) was determined using the Kjeldahl method (Bremner, 1996). Soil available phosphorus (AP) and available potassium (AK) contents were determined by methods described by Lu (1999). Soil DNAs were extracted from 0.5 g soil samples using MoBio UltraClean™ Soil DNA Isolation Kit (MoBio Laboratories, Solana Beach, USA) according to the manufacturer's instructions. The quality and quantity of the extracted DNA were analyzed by electrophoresis on a 1.0% agarose gel and by spectroscopic analysis (NanoDrop Technologies, USA).

Quantification of 16S rRNA and *pmoA* genes by qPCR

Methanotrophic abundances were analyzed based on the 16S rRNA and *pmoA* genes copy numbers using qPCR, which were performed on an iCycler iQ5 thermocycler (Bio-Rad, USA). The 25-µL reaction mixture consisted of 12.5 µL of SYBR® *Premix Ex Taq*™ (TaKaRa Biotechnology, Dalian, China), 1 µL of BSA (25 mg/mL), 0.5 µL of each primer (10 µmol/L) and 1 µL of 10-fold diluted extracted DNA (1 to 10 ng) as template. The primer pairs of MB10γ/533r, MB9α/533r, and A189/mb661 were used to determine in triplicate, respectively, the type I-, type II 16S rRNA and *pmoA* genes copy numbers of methanotrophs in the soil samples.

The qPCR assays were carried out with the protocol described previously (Zheng et al., 2008, 2012). Briefly, the amplification of 16S rRNA gene was programmed as follows: Initial denaturation for 2 min at 95°C, followed by 40 cycles of 1 min at 94°C, 1 min at 60°C, plate read at 83°C. For the PCR of *pmoA* gene, we used the same thermo-cycling conditions, except for the annealing temperature at 56°C. For confirming specificity of the PCR product, a melting curve analysis was performed by measuring fluorescence continuously as the temperature increased from 55 to 95°C after the above three temperature steps. The external standard curves for qPCR were set up by amplifying type I and type II-related 16S rRNA and *pmoA* genes fragments from environmental DNA extract, *Methylosinus sporium* (NCIMB 11126), and *Methylocystis parvus* (NCIMB 11129), respectively. All assays were performed at least in triplicate DNA samples from each soil sample.

Data analysis was carried out with iCycler software (version 1.0.1384.0 CR). The parameter C_t (threshold cycle) was determined as the cycle number at which a statistically significant increase in the reporter fluorescence was detected.

Methanotrophic community structure analyzed by PCR-DGGE

The extracted DNAs were used as template to produce PCR products for subsequent DGGE. The primers A189-gc and mb661 were selected to amplify the methanotrophic *pmoA* gene fragment. The composition of PCR mixtures and a touchdown thermocycling condition were described previously (Zheng et al., 2008). The obtained PCR products were loaded onto 6% (*W*/*V*) polyacrylamide (37.5:1, acrylamide: bisacrylamide) gel with a denaturing gradient from 40 to 60%, where 100% denaturant contains 7 M urea and 40% (*V*/*V*) formamide. Electrophoresis was run at the temperature of 60°C, started at 150 V for 10 min and then at 120 V for 6 hr. The gel was then stained for 30 min in SYBR green gold nucleic acid gel stain (1:10000), photographed by a GBOX/HR-E-M (Gene Company Limited, Syngene, UK).

Data analysis

Canonical correspondence analyses (CCA) of methanotrophic community structure were performed using CANOCO for Windows 4.5 software (ter Braak and Šmilauer, 2002). A Monte-Carlo permutation test based on 499 random permutations was used to analyse the relationship between methanotrophic community and soil properties. The resulting ordination biplot approximated the weighted average of each species (DGGE bands) with respect to environmental variables, which were represented as arrows. The length of the individual arrow indicated the relative importance of soil characteristics in explaining variation in methanotrophic community structure.

All statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) software (version 13.0). The significance or nonsignificance of a given variance was determined by calculating the respective '*t*' and S.D. ± values, considering the land utilization as source of variation. Correlations of methanotrophic abundance with soil physicochemical characteristics were done by Pearson correlation method.

RESULTS

Soil physicochemical properties

Some selected soil properties are listed in Table 1. The results showed that soil pH, available phosphorus (AP), and extracted DNAs were significantly lower ($P < 0.05$) in the pine than in tea treatment. However, significantly higher available potassium (AK) was found in the pine than those in tea treatment. There was no significant difference of soil organic matter (OM) and total nitrogen (TN) contents between these two treatments.

Methanotrophic abundance

The abundances of type I and type II methanotrophs were separately measured through the quantification of group-specific 16S rRNA gene copies. Consistently higher copies of type I (1.35×10^8 and 1.66×10^8 g^{-1} soil in pine and tea, respectively) than type II (8.59×10^7 and 10.9×10^7) methanotrophs were detected (Figure 2). However, no significant difference both in the 16S rRNA gene copies of type I and type II methanotrophs was found between pine and tea plots. Similarly, there was no significant difference ($P > 0.05$) in the quantification of *pmoA* gene between the treatments of pine and tea, in

Table 1. Selected characteristics of the tested soil samples under two land utilization patterns.

Soil characteristic	pine plot	tea plot	*P* value
pH	3.92 ± 0.09[bB]	4.24 ± 0.11[a]	0.017
OM (g kg^{-1})[A]	19.1 ± 0.8[a]	22.5 ± 2.1[a]	0.057
TN (g kg^{-1})	2.06 ± 0.17[a]	2.51 ± 0.28[a]	0.077
AP (mg kg^{-1})	1.43 ± 0.28[b]	15.2 ± 3.7[a]	0.007
AK (mg kg^{-1})	89.4 ± 3.7[a]	71.8 ± 5.8[b]	0.012
DNAs (μg g^{-1})	0.473 ± 0.007[b]	0.530 ± 0.009[a]	0.001

[A]OM, TN, AP, and AK are the abbreviations of soil organic matter, total nitrogen, available phosphorus, and available potassium, respectively. [B]Mean ± S.D. (n = 3). The different letters (a and b) within the same row indicate significant differences between treatments at $P < 0.05$.

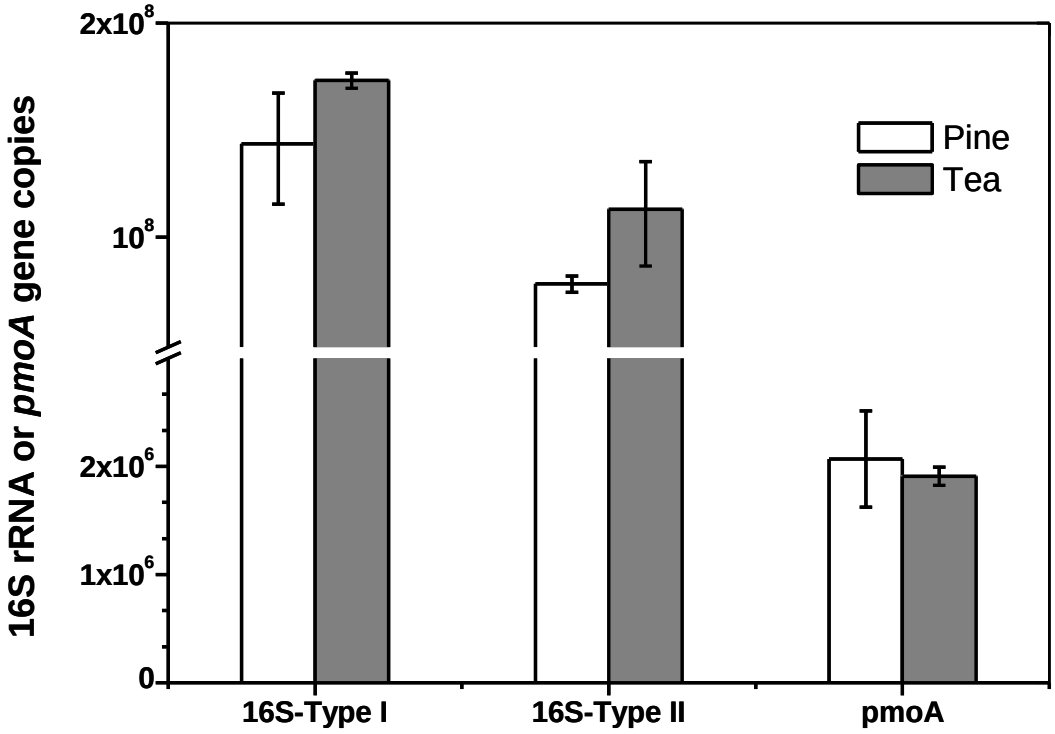

Figure 2. Quantification of 16S rRNA and *pmoA* genes copy numbers of methanotrophs in an acidic red soil with two different land utilization patterns of pine and tea plantation.

which 2.07 × 10^6 and 1.91 × 10^6 g^{-1} soil were obtained, respectively (Figure 2).

There were significantly positive correlations between the 16S rRNA gene copy numbers of type I methanotrophs and soil pH, OM, AP, and soil DNAs contents (Table 2). The 16S rRNA gene copy numbers of type II methanotrophs were significantly positively correlated with soil pH and TN ($P < 0.05$), and marginally positively correlated with soil OM, AP, and extracted soil DNAs (Table 2). However, no significant correlation was observed between methanotrophic *pmoA* gene copies and any soil characteristics ($P > 0.05$, Table 2).

DGGE profiling of methanotrophic *pmoA* gene

The DGGE profile of methanotrophic community was shown in Figure 3a. A total of 32 bands numbering from 01 to 32 were detected in these two treatments via the software of Quantity one. Of these bands, 22 (69%) and 19 (59%) bands were detected in the pine and tea plots, respectively. Nine (28%) bands (no. 5, 8, 15, 16, 21, 22, 25, 29, and 32) were common in pine and tea treatments. A cluster analysis was conducted based on the DGGE fingerprint. Two clusters represent the treatments of pine and tea, respectively. Both of treatments were clearly

Table 2. Pearson correlation coefficients between methanotrophic abundance and soil characteristics.

Soil characteristic	Methanotrophic abundance		
	16S rRNA gene copies		*pmoA* gene copies
	type I	type II	
pH	0.918** (0.010)[B]	0.866* (0.026)	0.059 (0.911)
OM['A']	0.978** (0.001)	0.788 (0.062)	0.077 (0.885)
TN	0.943 (0.050)	0.838* (0.037)	0.190 (0.719)
AP	0.898* (0.015)	0.753 (0.084)	−0.213 (0.686)
AK	−0.425 (0.401)	−0.494 (0.319)	0.535 (0.274)
DNAs	0.844* (0.034)	0.804 (0.054)	−0.140 (0.792)

[A]OM, TN, AP, and AK are the abbreviations of soil organic matter, total nitrogen, available phosphorus, and available potassium, respectively. [B]The symbols of * and ** mean the correlation reached the 0.05 and 0.01 significant level, respectively. The *P* values were listed in the parentheses.

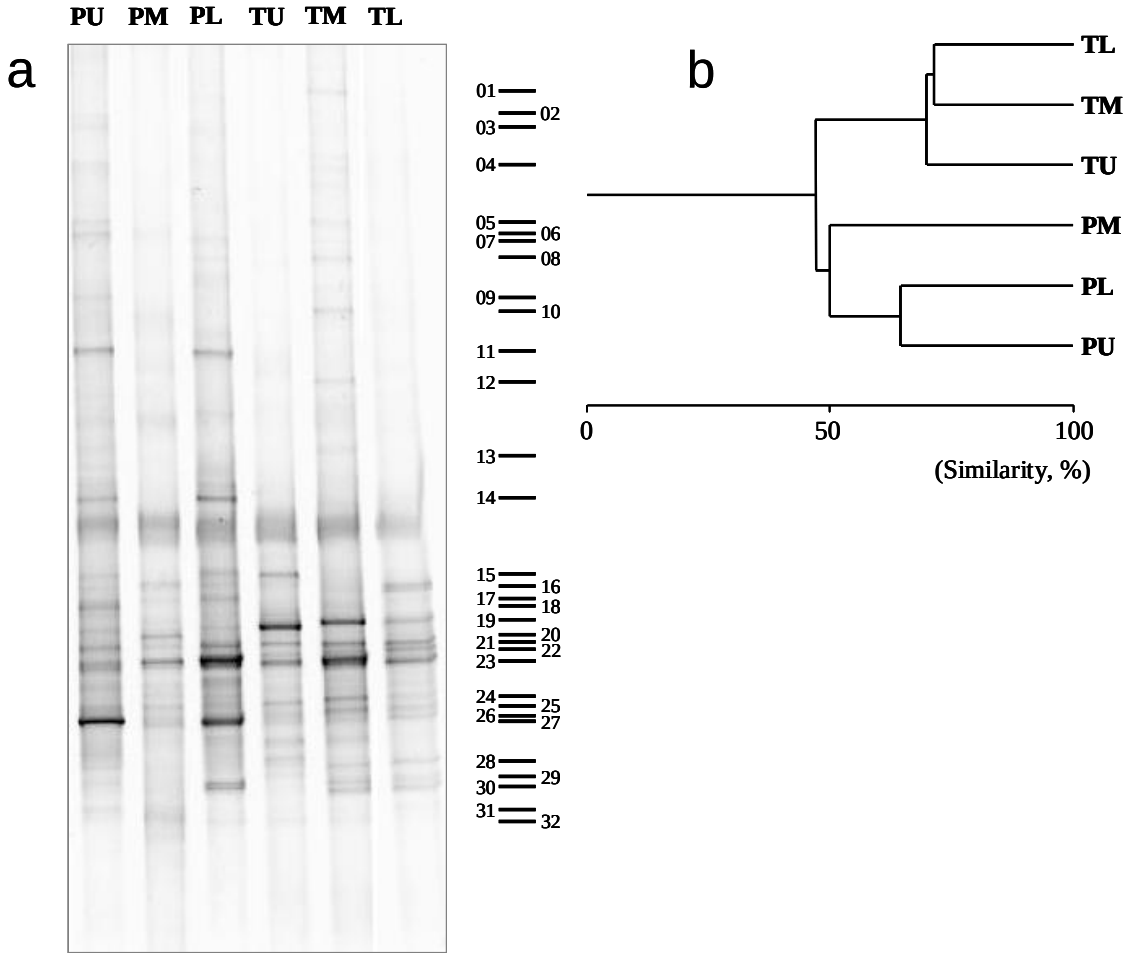

Figure 3. (a) DGGE profiles of methanotrophic *pmoA* gene fragments of soil samples with two different land utilization patterns and (b) Cluster analysis of left DGGE banding patterns. Lanes PU, PM, PL, TU, TM, and TL denoted the upper, middle and lower samples from pine and tea treatments, respectively.

distinguished themselves based on the similarity of DGGE band patterns (Figure 3b). More apparent differences were found between the two treatments than among the replicates within each treatment.

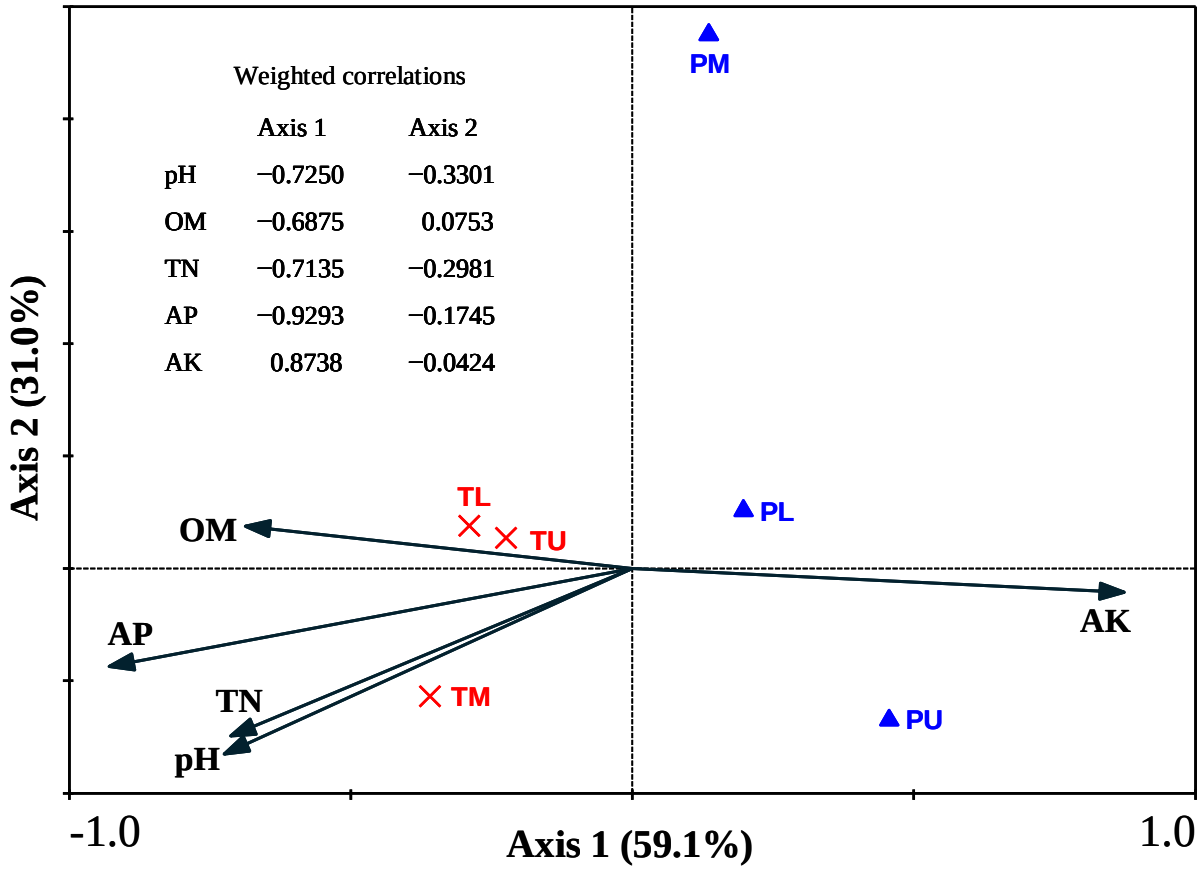

Figure 4. Canonial correspondence analysis ordination diagram of DGGE data, with soil characteristics (pH, OM, TN, AP, and AK) as arrows. The symbols ▲and ✕represent the samples collected from upper, middle and lower samples from pine plot (PU, PM and PL) and tea plot (TU, TM and TL), respectively. Values on the axes indicate the percentage of total variation explanation by the corresponding axis. OM, organic matter; TN, total nitrogen; AP, available phosphorus; AK, available potassium.

Canonial correspondence analysis (CCA) of the DGGE band patterns showed that tea treatment (that is, TU, TM, and TL) clustered more closely, and differed from the pine treatment (Figure 4). The variation within methanotrophic community fingerprint between the two treatments explained 59.1 and 31.0% of the variation by the first and second axes, respectively (Figure 4). A significantly negative correlation was found between methanotrophic community composition (species) and soil AP content (that is, the weighted correlation between AP and axis is −0.9293, $P < 0.05$). However, both of the first and second CCA axes were not correlated with soil pH, OM, TN, and AK ($P > 0.05$).

DISCUSSION

Comparison of methanotrophic abundance

No significant difference in methanotrophic abundance was found between the pine and tea, regardless of the quantification of 16S rRNA and *pmoA* genes. However, there were higher abundances of type I than that of type II methanotrophs both in two treatments (ratios are 1.5 to 1.6). This was consistent with previous studies in agricultural and upland soil (Seghers et al., 2003), whereas was inconsistent with the studies in paddy field soil (Bodelier et al., 2000; Zheng et al., 2008). More recently, overwhelming majority of type I methanotrophs also have been found in an alpine meadow soil in the Tibetan Plateau (Zheng et al., 2012). A possible explanation for these results could be that, comparing with rice field and wetland soils, upland and/or grassland soil have better gas diffusion condition and thus better oxygen supply, which type I methantrophs preferred, because type I methanotrophs seemed to grow preferentially at high O_2 and low CH_4 concentrations (Amaral and Knowles, 1995).

In the present study, significantly positive correlation was found between methanotrophic 16S rRNA gene copy numbers and soil pH, even though no significant difference in 16S rRNA gene copies was observed

between two treatments of pine and tea plantation. This result implied that pH seemed to be an important environmental factor to potentially influence the methanotrophic abundance, probably because the soil samples studied were strongly acidic (3.9 to 4.2). Furthermore, low nutrient is another character of such acidic red soils in southern China (Ying et al., 2010). Similarly, our results also showed that soil organic matter (OM), available phosphorus (AP), and total nitrogen (TN) were significantly correlated with the abundances of type I and type II methanotrophs, respectively. In a previous study of a Chinese upland red soil, we demonstrated that phosphorus (P) could be a key factor to control the microbial colony forming units (He et al., 2008). Considering the high P fixation capacity and low P availability in the red soil, it was not surprising that P was a key fertilizer factor in influencing the methanotrophic abundance in the soil. In turn, for paddy field soil, in which P was not the limited factor, the applications of nitrogen and potassium fertilizers were considered as the important factors determining the abundance of the methanotrophs (Zheng et al., 2008). Our results suggested that the change of soil properties (for example, pH and available phosphorus) resulted from alerted vegetation and/or fertilization management, could potentially influenced the methanotrophic populations size in the future.

Methanotrophic community fingerprint

In this study, remarkable differences in banding patterns of DGGE profile were observed, indicating that different methanotrophic community diversity was caused by the land utilization patterns of pine and tea. Land use was regarded as the primary factor in influencing the microbial community. For instance, land use was found to have a greater effect on soil microbial community structure, compared to lime and urea applications in acidic tea orchard soils (Ge et al., 2010; Xue et al., 2010). Specifically, different land use resulted in different methanotrophic community structure characterized by DGGE method (Knief et al., 2005). However, another study showed tree species influenced atmospheric CH_4 oxidation, but not methanotrophic community in grassland soil (Menyailo et al., 2010). A possible explanation for this discrepancy could be that soil properties, rather than tree species were the important factor in determining the methanotrophic community.

Canonial correspondence analysis (CCA) was conducted to detect the relationship between methanotrophic community fingerprint and environmental parameters based on DGGE data; similar reports with respect to bacterial community are available (Zeng et al., 2009; Shen et al., 2010). Our results from CCA showed that soil AP was the most important environmental variable in affecting the community of methanotrophs. This was consistent with a previous study that showed microbial diversity was mainly affected by soil P (He et al., 2008). Therefore, we suggest that some of the soil characteristics might result in more direct and profound effects on the methanotrophic community fingerprint than that the land-use changes.

Conclusion

Here we compared the abundance and community fingerprint of methanotrophs in an acidic red soil under two land utilizations of pine and tea. Our results showed that type I was the dominant methanotrophs and there was no significant difference in methanotrophic abundance between pine and tea plantations. However, clear differences in methanotrophic community profile were detected between these two treatments. Collectively, the results in this study highlight that soil properties (for example, pH and phosphorus) were crucial factors in influencing the methanotrophic community diversity in this acidic red soil.

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REFERENCES

Abell GCJ, Stralis-Pavese N, Sessitsch A, Bodrossy L (2009). Grazing affects methanotroph activity and diversity in an alpine meadow soil. Environ. Microbiol. Rep., 1: 457-465.

Amaral JA, Knowles R (1995). Growth of methanotrophs in methane and oxygen counter gradients. FEMS Microbiol. Lett. 126:215-220.

Bodelier PLE, Roslev P, Henckel T, Frenzel P (2000). Stimulation by ammonium-based fertilizers of methane oxidation in soil around rice roots. Nature, 403:421-424.

Boeckx P, Van Cleemput O, Meyer T (1998). The influence of land use and pesticides on methane oxidation in some Belgian soils. Biol. Fertil. Soils, 27: 293-298.

Bremner JM (1996). Nitrogen–total. Methods of Soil Analysis. In: Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN, Tabatabai MA, Johnston CT, Summer ME (ed.), Part 3 -Chemical Methods. Soil Science Society of America, American Society of Agronomy, Madison, WI. pp.1085-1121.

Chang CY, Tung HH, Tseng IC, Wu JH, Liu YF, Lin HM (2010). Dynamics of methanotroph communities in tropical alkaline landfill upland soil. Appl. Soil Ecol. 46:192-199.

Chen Y, Dumont MG, Cebron A, Murrell JC (2007). Identification of active methanotrophs in a landfill cover soil through detection of expression of 16S rRNA and functional genes. Environ. Microbiol. 9:2855-2869.

Chen Y, McNamara NP, Dumont MG, Bodrossy L, Stralis-Pauese N, Murrell JC (2008). The impact of burning and Calluna removal on below-ground methanotroph diversity and activity in a peatland soil. Appl. Soil Ecol. 40:291-298.

Dobbie KE, Smith KA, Prieme A, Christensen S, Degorska A, Orlanski P (1996). Effect of land use on the rate of methane uptake by surface soils in northern Europe. Atmos. Environ. 30:1005-1011.

Ge CR, Xue D, Yao HY (2010). Microbial biomass, community diversity, and enzyme activities in response to urea application in tea orchard

soils. Commun. Soil Sci. Plant Anal. 41: 797-810.
He JZ, Zheng Y, Chen CR, He YQ, Zhang LM (2008). Microbial composition and diversity of an upland red soil under long-term fertilization treatments as revealed by culture-dependent and culture-independent approaches. J. Soils Sediments 8:349-358.
Henckel T, Friedrich M, Conrad R (1999). Molecular analyses of the methane-oxidizing microbial community in rice field soil by targeting the genes of the 16S rRNA, particulate methane monooxygenase, and methanol dehydrogenase. Appl. Environ. Microbiol. 65:1980-1990.
IPCC (2007). In Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (ed.), Climate Change 2007: The Physical Science Basis. Working Group I Contribution to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.
Knief C, Vanitchung S, Harvey NW, Conrad R, Dunfield PF, Chidthaisong A (2005). Diversity of methanotroph bacteria in tropical upland soils under different land uses. Appl. Environ. Microbiol. 71:3826-3831.
Kolb S (2009). The quest for atmospheric methane oxidizers in forest soils. Environ. Microbiol. Rep. 1:336-346.
Lau E, Ahmad A, Steudler PA, Cavanaugh CM (2007). Molecular characterization of methanotroph communities in forest soils that consume atmospheric methane. FEMS Microbiol. Ecol. 60:490-500.
Lu RK (1999). Soil and Agro-chemical Analytical Methods (*in Chinese*). China Agricultural Science and Technology Press, Beijing pp.180-194.
Martineau C, Whyte LG, Greer CW (2010). Stable isotope probing analysis of the diversity and activity of methanotroph bacteria in soils from the Canadian high Arctic. Appl. Environ. Microbiol. 76:5773-5784.
Menyailo OV, Abraham WR, Conrad R (2010). Tree species affect atmospheric CH_4 oxidation without altering community composition of soil methanotrophs. Soil Biol. Biochem. 42:101-107.
Menyailo OV, Hungate BA, Abraham WR, Conrad R (2008). Changing land use reduces soil CH_4 uptake by altering biomass and activity but not composition of high-affinity methanotrophs. Glob. Change Biol. 14:2405-2419.
Mohanty SR, Bodelier PLE, Conrad R (2007). Effect of temperature on composition of the methanotroph community in rice field and forest soil. FEMS Microbiol. Ecol. 62:24-31.
Seghers D, Top EM, Reheul D, Bulcke R, Boeckx P, Verstraete W, Siciliano SD (2003). Long-term effects of mineral versus organic fertilizers on activity and structure of the methanotroph community in agricultural soils. Environ. Microbiol. 5:867-877.
Shen JP, Zhang LM, Guo JF, Ray JL, He JZ (2010). Impact of long-term fertilization practices on the abundance and composition of soil bacterial communities in Northeast China. Appl. Soil Ecol. 46:119-124.
Shrestha M, Shrestha PM, Frenzel P, Conrad R (2010). Effect of nitrogen fertilization on methane oxidation, abundance, community structure, and gene expression of methanotrophs in the rice rhizosphere. ISME J. 4:1545-1556.
Singh JS (2011). Methanotrophs: the potential biological sink to mitigate the global methane load. Curr. Sci. 100:29-30.
ter Braak CJF, Šmilauer P (2002). CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination (Version 4.5). Microcomputer Power, New York.
Trotsenko YA, Murrell JC (2008). Metabolic aspects of aerobic obligate methanotrophy. Adv. Appl. Microbiol. 63:183-229.
Wise MG, McArthur JV, Shimkets LJ (1999). Methanotroph diversity in landfill soil: Isolation of novel type I and type II methanotrophs whose presence was suggested by culture-independent 16S ribosomal DNA analysis. Appl. Environ. Microbiol. 65:4887-4897.
Xue D, Huang XD, Yao HY, Huang CY (2010). Effect of lime application on microbial community in acidic tea orchard soils in comparison with those in wasteland and forest soils. J. Environ. Sci. 22:1253-1260.
Yang YS, Xie JS, Sheng H, Chen GS, Li X, Yang ZJ (2009). The impact of land use/cover change on storage and quality of soil organic carbon in midsubtropical mountainous area of southern China. J. Geogr. Sci. 19:49-57.
Yao HY, Bowman D, Shi W (2006). Soil microbial community structure and diversity in a turfgrass chronosequence: land-use change versus turfgrass management. Appl. Soil Ecol. 34:209-218.
Ying JY, Zhang LM, He JZ (2010). Putative ammonia-oxidizing bacteria and archaea in an acidic red soil with different land utilization patterns. Environ. Microbiol. Rep. 2:304-312.
Yrjälä K, Tuomivirta T, Juottonen H, Putkinen A, Lappi K, Tuittila E, Penttilä T, Minkkinen K, Laine J, Peltoniemi K, Fritze H (2011). CH_4 production and oxidation processes in a boreal fen ecosystem after long-term water table drawdown. Glob. Change Biol. 17:1311-1320.
Yun JL, Ma AZ, Li YM, Zhuang GQ, Wang YF, Zhang HX (2010). Diversity of methanotrophs in Zoige wetland soils under both anaerobic and aerobic conditions. J. Environ. Sci. 22:1232-1238.
Zeng J, Yang LY, Li JY, Liang Y, Xiao L, Jiang LJ, Zhao DY (2009). Vertical distribution of bacterial community structure in the sediments of two eutrophic lakes revealed by denaturing gradient gel electrophoresis (DGGE) and multivariate analysis techniques. World J. Microbiol. Biotechnol. 25:225-233.
Zheng Y, Yang W, Sun X, Wang SP, Rui YC, Luo CY, Guo LD (2012). Methanotroph community structure and activity under warming and grazing of alpine meadow on the Tibetan Plateau. Appl. Microbiol. Biotechnol. 93:2193-2203.
Zheng Y, Zhang LM, Zheng YM, Di HJ, He JZ (2008). Abundance and community composition of methanotrophs in a Chinese paddy soil under long-term fertilization practices. J. Soils Sediments 8:406-414.