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# Do land utilization patterns affect methanotrophic communities in a Chinese upland red soil?

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**Abstract**

Soil samples were collected from three plots under different land utilization patterns including degradation, farming, and restoration. The abundances of methanotrophs were quantified using real-time polymerase chain reaction (PCR) based on the *pmoA* and 16S rRNA genes, and the community fingerprint was analyzed using denaturing gradient gel electrophoresis (DGGE) aiming at *pmoA* gene. Significantly lower 16S rRNA and *pmoA* genes copies were found in the degradation treatment than in farming and restoration. Higher abundances of Type I than those of Type II methanotrophs were detected in all treatments. The treatment of farming was clearly separated from degradation and restoration according to the DGGE profile by cluster analysis. The lowest diversity indices were observed in the F (farming plot), suggesting that the community structure was strongly affected by farming activities. There were significantly positive correlations between the copy numbers of *pmoA* also Type II-related 16S rRNA genes and soil available K content. Strong negative and positive correlations were found between Type I and soil pH, and available P content, respectively. We concluded that the vegetation cover or not, soil characteristics including pH and nutrients of P and K as a result of anthropogenic disturbance may be key factors affecting methanotrophic communities in upland soil.

**Key words**: DGGE; land utilization; methanotrophs; *pmoA*; real-time PCR
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## Introduction

Methane ($CH_4$) is the second important greenhouse gas, following carbon dioxide ($CO_2$), which contributes up to 17% of the global warming (IPCC, 2007). The concentration of atmospheric $CH_4$ is determined by $CH_4$ production and consumption. $CH_4$ is produced mainly from natural wetlands, ruminant animals, landfills, and rice field (Lowe, 2006). In addition to the major sink of tropospheric $CH_4$ oxidation, another important $CH_4$ sink is microbiological oxidation of $CH_4$ in aerobic upland soils. Atmospheric $CH_4$ oxidation in upland soils is mediated by methane-oxidizing bacteria (methanotrophs), which utilize $CH_4$ as the sole source of carbon and energy (Knief et al., 2003; Lau et al., 2007; Menyailo et al., 2008). Methanotrophs are obligately aerobic and are classified into two main groups of Type I and Type II based on differences in phylogeny, physiology, morphology, and biochemistry. Type I methanotrophs (γ-Proteobacteria) assimilate the intermediate formaldehyde via the ribulose monophosphate pathway, whereas Type II methanotrophs (α-Proteobacteria) utilize the serine pathway for assimilating formaldehyde (Hanson and Hanson, 1996). It is important to improve our knowledge about the community structures of methanotroph, a key component for establishing the possible increase strategy of $CH_4$ oxidation in soil environment.

Identification of methanotrophs in soils always faces challenges, because many of them are difficult to culture. Fortunately, the application of culture-independent molecular approaches gives us powerful means to detect soil methanotrophs. Over the past decade, numerous researchers have used molecular techniques to study the microbial ecology of methanotrophs in various natural environments (McDonald et al., 1995; Horz et al., 2001; Lin et al., 2004; Lau et al., 2007; Chen et al., 2008; Rastogi et al., 2009). For discriminating the Type I and Type II methanotrophs, group-specific PCR primers were designed to selectively amplify 16S rRNA gene of methanotrophs at the family or genus level (Henckel et al., 1999; Wise et al., 1999; Gulledge et al., 2001; Chen et al., 2007). Besides the 16S rRNA gene, methanotrophic functional genes had also been used to detect the presence and abundance of $CH_4$ oxidizers (Fjellbirkeland et al., 2001; Horz et al., 2001). These marker genes include *pmoA*, *mmoX*, and *mxaF*, which encode subunits of particulate methane monooxy-

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genase, soluble methane monooxygenase, and methanol dehydrogenase, respectively. Compared with 16S rRNA gene, functional genes have advantages in much higher sensitivity of detection in complex environments and in identifying putative uncultivated members of the functional group (McDonald et al., 2008). Among these functional genes, *pmoA* can be detected in all known methanotrophs, with an exception of *Methylocella* spp. (Dedysh et al., 2005). The *pmoA* gene thus had been widely used to characterize methanotrophic diversity and composition in various soils (Knief et al., 2003; Mohanty et al., 2007; Chen et al., 2008; Zhou et al., 2008; Rastogi et al., 2009).

$CH_4$ oxidation in upland soils is strongly affected by anthropogenic factors such as tillage and land use (Hütsch, 1998), which can influence future carbon stock in soil and vegetation (Schulp et al., 2008). Due to the key role that methanotrophs play in the biogeochemical carbon cycle and in global climate change, the effects of land utilization patterns on methanotrophic community have attracted wide attention. A number of works have been performed to evaluate the influence of different land use changes on the $CH_4$ uptake capacity (Dobbie et al., 1996; Singh et al., 1998; Prieme and Christensen, 1999; Tate et al., 2007). However, the contrasting results were often observed. For example, Menyailo et al. (2008) demonstrated that land use change resulted in lower biomass and soil $CH_4$ uptake, but did not affect the diversity of methanotrophs. A similar study recently reported that tree species affected atmospheric $CH_4$ oxidation in grassland soil without altering methanotrophic community (Menyailo et al., 2010). We hypothesize that these discrepancies largely resulted from the highly distinct characteristics of soil tested across a wide variety of environments, and this point has not been fully addressed.

Therefore, in this study, we applied a combination of real-time polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) approaches to compare the methanotrophic abundance and community fingerprint of a subtropical upland red soil under three different land utilization patterns, including a degradation plot, a farming plot, and a restoration plot. The aim was to test whether different land utilization patterns cause changes in methanotrophic abundance and diversity.

## 1 Materials and methods

### 1.1 Site description

Soil samples were collected from the Taoyuan Experimental Station of Agro-ecosystem Observation (28°55′N, 111°26′E) of the Chinese Academy of Sciences, Hunan Province, China. This region has a mean annual precipitation of 1440 mm and a mean annual temperature of 16.5°C. The soil was derived from quaternary red clay and classified as Hapludults. A standard surface runoff observation field was set up since 1995. The field has a sloping gradient of 8–11° and a projected area of 1 ha. Three different land utilization plots were selected in our study: (1) degradation (D), an original utilization of natural vegetation succession plot in which the surface vegetation was harvested and moved out twice yearly in May and November; (2) farming (F), a general plantation plot in which local crops were planted and managed; (3) restoration (R), a natural vegetation succession plot where the surface vegetation was removed in 1995 and left to regenerate without other disturbances, thus very densely vegetation coverage was showed when sampling. The soil samples were taken in surface 0–10 cm in October 2007. The samples from upper, lower and middle positions of the same land utilization plot were used together to represent three replicates of that treatment. Soil samples were sieved (< 2 mm) and stored at –20°C for DNA extraction and at 4°C for soil chemical analyses.

### 1.2 Soil chemical 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 routine methods (Lu, 1999). Selected characteristics of the soil samples are listed in Table 1.

Soil DNA was extracted from 0.5 g (fresh weight) soil samples using MoBio UltraClean™ Soil DNA Isolation Kit (MoBio Laboratories, Solana Beach, USA) according to the manufacturer's instructions. Soil DNA extract was eluted by 80 μL elution solution.

### 1.3 Real-time PCR of methanotrophic *pmoA* and 16S rRNA genes

Real-time PCR was performed on an iCycler iQ5 thermocycler (Bio-Rad, USA). The quantification was based on the fluorescent dye SYBR-Green I, which binds to double-stranded DNA during PCR amplification. The 25-μL reaction mixture consisted of 12.5 μL of SYBR® *Premix Ex Taq*™ (Takara Bio, Otsu, Shiga, Japan), 1 μL of BSA (25 mg/mL), 0.5 μL of each primer (10 μmol/L)

**Table 1** Characteristics of the tested soil samples under three different land utilization patterns

| Treatment | Soil property | | | | |
|---|---|---|---|---|---|
| | pH | OM (g/kg) | TN (g/kg) | AP (mg/kg) | AK (mg/kg) |
| Degradation | 4.20 ± 0.04 ac | 21.4 ± 1.64 b | 2.45 ± 0.20 a | 0.99 ± 0.08 b | 54.1 ± 7.73 b |
| Farming | 3.91 ± 0.06 b | 16.0 ± 1.52 c | 1.94 ± 0.16 b | 28.9 ± 6.35 a | 70.3 ± 8.78 b |
| Restoration | 4.18 ± 0.02 a | 24.6 ± 0.40 a | 2.84 ± 0.34 a | 1.36 ± 0.15 b | 102 ± 17.0 a |

OM: soil organic matter; TN: total nitrogen; AP: available phosphorus; AK: available potassium.
Data are presented as mean ± SD ($n$ = 3). The different letters (a–c) within the same column indicate significant differences between treatments at $P$ < 0.05.

**Table 2** Primers and thermal conditions for real-time quantitative PCR

| Target group | Primer name | Sequence (5′–3′) | Thermal profile | Reference |
|---|---|---|---|---|
| MOB | A189 | GGNGACTGGGACTTCTGG | 95°C for 2 min followed by 40 cycles of 1 min at 94°C, 1 min at 56°C, plate read at 83°C. | Holmes et al., 1999 |
| | mb661 | CCGGMGCAACGTCYTTACC | | Kolb et al., 2003 |
| Type I MOB | MB10γ | AAGCGGGGGATCTTCGGACC | 95°C for 2 min followed by 36 cycles of 1 min at 94°C, 1 min at 60°C, plate read at 83°C. | Henckel et al., 1999 |
| | 533r | TTACCGCGGCTGCTGGCAC | | |
| Type II MOB | MB9α | GTTCGGAATAACTCAGGG | 95°C for 2 min followed by 36 cycles of 1 min at 94°C, 1 min at 60°C, plate read at 83°C. | Henckel et al., 1999 |
| | 533r | TTACCGCGGCTGCTGGCAC | | |

MOB: methane-oxidizing bacteria or methanotrophs.

and 1 μL of 10-fold diluted extracted DNA (1–10 ng) as template. The A189, MB10γ and MB9α primer sets (Table 2) were used to determine in triplicates, respectively, the *pmoA* gene copy numbers of total methanotrophs and the 16S rRNA gene copy numbers of the Type I and II methanotrophs in the soil samples.

Real-time PCR assay was carried out with the protocol for each target group as shown in Table 2. For methanotrophic 16S rRNA gene assay, in order to obtain lower background fluorescence signal in the negative controls due to host DNA carryover in the cloned DNA polymerase preparations (Suzuki et al., 2000), 36 cycles were used for this amplification. Assays for total methanotrophs target group were run using 40 cycles. In each real-time PCR amplification, following the three temperature steps, a melting curve analysis was performed to confirm PCR product specificity by measuring fluorescence continuously as the temperature increased from 55 to 95°C. 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.

The primer pair A189/mb661 was used to amplify group-specific *pmoA* gene fragment from the pure culture DNA extract of *Methylocystis parvus* (NCIMB 11129). Meanwhile, the primer pairs MB10γ/533r and MB9α/533r were applied to amplify Type I and Type II methanotroph-specific 16S rRNA gene fragments from the environmental DNA extract and *Methylosinus sporium* (NCIMB 11126), respectively. The PCR products were gel-purified with Agarose Gel DNA Purification Kit (TaKaRa Biotechnology, Dalian, China) and ligated into the pGEM-T Easy Vector (Promega, Madison, USA), then the ligation products were used to transform *Escherichia coli* JM109 competent cells following the instructions of the manufacturer. After re-amplification with the vector-specific primers T7 (5′-TAATACGACTCACTATAGG) and SP6 (5′-ATTTAGGTGACACTATAGAA), the positive clones were selected to extract plasmid DNA with a MiniBEST Plasmid Purification Kit (TaKaRa Biotechnology, Dalian, China). The plasmid DNA concentration was determined using a Nanodrop® ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies, USA) and the copy numbers of *pmoA* and 16S rRNA gene were calculated directly from the concentration of the extracted plasmid DNA. Ten-fold serial dilutions of a known copy number of the plasmid DNA were subjected to real-time PCR assay in triplicate to generate an external standard curve.

### 1.4 PCR and DGGE analysis of *pmoA* fragments

In the PCR amplification, a GC-rich sequence (CCC CCC CCC CCC CGC CCC CCG CCC CCC GCC CCC GCC GCC C) was attached to the 5′ end of primer A189, therefore, the PCR products had a GC clamp to prevent complete melting during separation in the denaturing gradient. The PCR condition and the subsequent DGGE analyses were described previously (Zheng et al., 2008). In brief, the PCR products were loaded onto 6% (*W/V*) polyacrylamide (37.5:1, acrylamide:bisacrylamide) gel with a denaturing gradient from 40% to 60% (100% denaturant contains 40% (*V/V*) formamide and 7 mol/L urea). Electrophoresis was run at 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).

The diversity indices including Shannon ($H$) and Evenness ($E_H$) were calculated based on the analysis of DGGE data using Quantity One software. A description of $H$, $E_H$ and their calculation can be found in the previous report (Zheng et al., 2008).

### 1.5 Data analysis

All statistical analyses and Pearson correlation were carried out using SPSS version 11.5, and one-way analysis of variance (ANOVA) followed by S-N-K-test was used to check quantitative differences between treatments. $P < 0.05$ was considered to be statistically significant.

## 2 Results

### 2.1 Real-time PCR quantification of methanotrophic communities in soils

*pmoA*-Targeted quantitative PCR was performed to detect the abundance changes of total soil methanotrophs in response to different land utilization patterns. Treatment of degradation (D) contained $1.56 \times 10^6$ copies/g dry soil which was significantly lower than treatment of farming (F) ($2.44 \times 10^6$ copies/g dry soil) and treatment of restoration (R) ($2.77 \times 10^6$ copies/g dry soil) ($P < 0.05$) (Fig. 1). No significant difference was observed between the F and the R treatments (Fig. 1).

Group specific assays based on 16S rRNA gene-targeted quantitative PCR indicated apparent changes in the abundance of soil Type I and Type II methanotrophs under different land utilization patterns (Fig. 2). For Type I

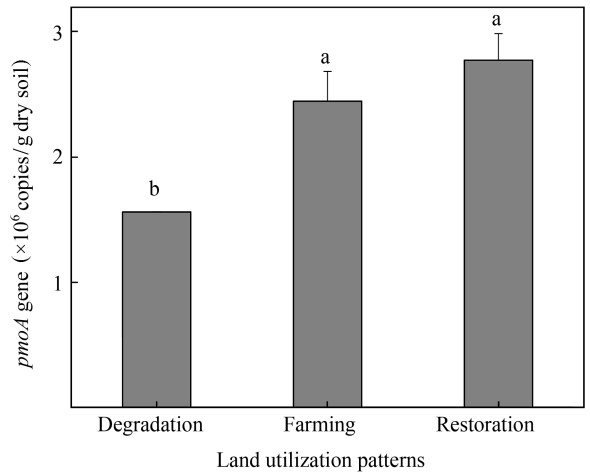


**Fig. 1** Quantification of *pmoA* gene copy numbers of methanotrophs in a Chinese upland red soil with different land utilization patterns. The different letters above bars indicate significant differences between treatments at $P < 0.05$.

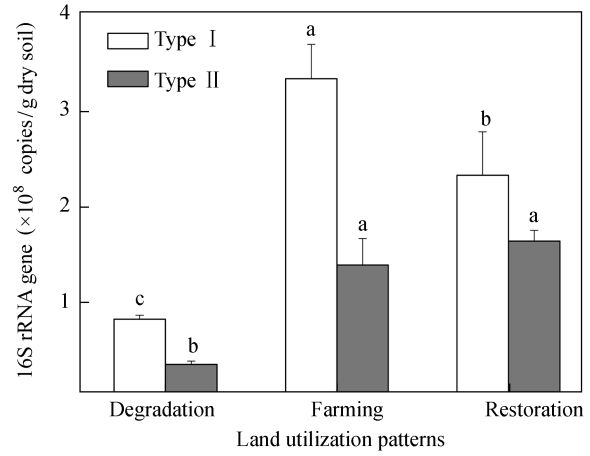


**Fig. 2** Quantification of 16S rRNA gene copy numbers of Type I and Type II methanotrophs in a Chinese upland red soil with different land utilization patterns. The different letters above bars indicate significant differences between treatments at $P < 0.05$.

methanotrophs, significantly different abundance was observed among soil treatments of farming ($3.34 \times 10^8$ copies/g dry soil), restoration ($2.33 \times 10^8$ copies/g dry soil) and degradation ($8.24 \times 10^7$ copies/g dry soil). Similarly, the lowest Type II methanotrophic 16S rRNA gene copies ($3.52 \times 10^7$ copies/g dry soil) was found in the D treatment, which was significantly lower than the treatments of F ($1.39 \times 10^8$ copies/g dry soil) and R ($1.64 \times 10^8$ copies/g dry soil), while no significant difference was observed between the F and R treatments (Fig. 2). Specifically, the ratios of Type I to Type II were 2.34, 2.40 and 1.42 in the treatments of degradation, farming and restoration, respectively. It appeared that Type I accounted for a significant higher percentage (59%–71%) of the total methanotrophs than Type II (29%–41%) did in all the land utilization patterns.

### 2.2 DGGE fingerprinting of methanotrophic communities in soils

The DGGE profiles of methanotrophic community of the tested soil samples are shown in Fig. 3a. The F treatment (lanes 4–6), with a minor complexity of the banding pattern, showed a clearly lower diversity in the methanotrophic community. More bands were detected in the upper part of the DGGE gel of the treatments of degradation and restoration (lanes 1–3 and 7–9) than treatment farming. Cluster analysis of the DGGE patterns revealed more significant differences among the treatments than among the replicates within each treatment (Fig. 3b). The degradation and restoration treatments had a relatively similar banding pattern (41%) and clustered together initially, and distinguished themselves from the farming treatment (36%). In addition, DGGE fingerprints revealed significantly lower Shannon's diversity index $H$ and Evenness ($E_H$) values for the farming treatment than for another two treatments (data not shown). These results suggest that farming (plot F) could alter the methanotrophic community more considerably.

### 2.3 Statistical characterization of environmental factors affecting methanotrophic communities in soils

In the present study, the correlation coefficients between methanotrophic abundances and selected soil characteristics were calculated and shown in Table 3. There was a significantly positive correlation between the copy numbers of *pmoA* gene and soil AK. No significant correlation was found between *pmoA* and soil pH or AP. In contrast, the quantifications of the copy numbers of Type I methanotrophic 16S rRNA gene were significantly negative and positive correlations, respectively, with soil pH and AP, but not with soil AK (Table 3). Similar to *pmoA* gene, a strong positive correlation ($P < 0.05$) exist between the copy numbers of Type II methanotrophic 16S rRNA gene and soil AK, no significant correlation was found between Type II and soil pH or AP. In addition, the sum of 16S rRNA gene copy numbers (Type I + Type II) was found correlated negatively with soil pH (Table 3).

**Table 3** Pearson correlation coefficients between methanotrophic abundance and soil characteristics

| Target | Selected soil basic characteristics | | |
|---|---|---|---|
| | pH | AP | AK |
| *pmoA* | –0.333 (0.381) | 0.297 (0.437) | 0.819** (0.007) |
| Type I | –0.817** (0.007) | 0.799** (0.010) | 0.326 (0.392) |
| Type II | –0.310 (0.416) | 0.304 (0.427) | 0.788* (0.012) |
| Type I + Type II | –0.680* (0.044) | 0.666 (0.050) | 0.518 (0.153) |

* and ** mean the correlation reached the 5% and 1% significant level, respectively. The $P$ values are listed in the parentheses.

## 3 Discussion

### 3.1 Soil methanotrophic abundance under different land utilization patterns

As a culture-independent approach for quantifying methanotrophic abundance in soils, real-time PCR has more advantages than FISH, MPN, and PLFA methods (Kolb et al., 2003). Furthermore, comparing with the competitive or MPN-PCR, real-time PCR can avoid the bias of endpoint analysis where different amounts of

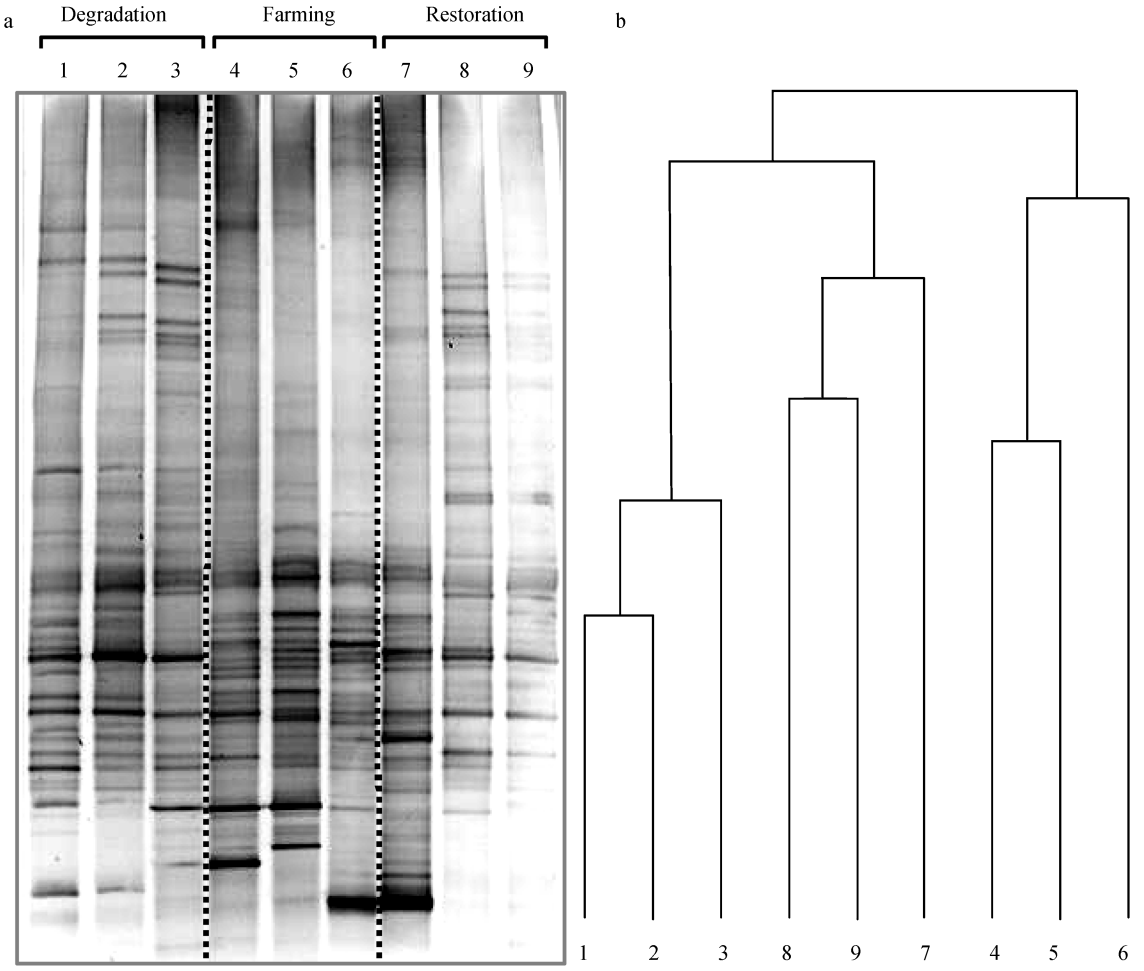


**Fig. 3** (a) DGGE profile of methanotrophic *pmoA* gene fragments amplified from a Chinese upland red soil with different land utilization patterns and (b) cluster analysis of DGGE banding patterns. Lanes 1 to 3, 4 to 6, and 7 to 9 denote soil samples collected from the upper, middle and lower slopes in each plot of degradation, farming, and restoration treatments, respectively.

PCR amplicons are generated from the same initial target DNA concentration (Raeymaekers, 2000). In this study, the quantitative results of methanotrophic *pmoA* gene coincided well with those of 16S rRNA gene. Significant correlations ($P = 0.004$) were found between the quantification of *pmoA* gene and that of methanotrophic (Type I + Type II) 16S rRNA gene (data not shown). However, about two orders (75–140) of magnitude higher copies were quantified based on 16S rRNA gene than *pmoA* gene. It was interpreted that one methanotrophic cell contains 2 *pmoA* gene copies, and about 8 copies of 16S rRNA gene (Fogel et al., 1999; Stolyar et al., 2001). Furthermore, the *pmoA*-targeted primers can not detect the *Methylocella* (Dedysh et al., 2005; Theisen et al., 2005) would be the reason for underestimating the copy numbers of *pmoA* gene. Nevertheless, real-time PCR could be regarded as an effective technique to quantify methanotrophic genes in upland soil tested in the present study.

Significant differences in both of *pmoA* and 16S rRNA genes copy numbers were found among soils exposed to distinct land utilization patterns. The treatment D seemed to have some negative effects on methanotrophs, indicated by the lowest gene copies. Given the fact that the surface vegetation was moved out from this experimental plot every year, the plants could be a critical factor for the growth of soil methanotrophs. Similarly, a recent study demonstrated that the size of methanotroph populations was decreased as a result of removal of the above-ground vegetation cover (Chen et al., 2008). Although the Type I methanotrophic 16S rRNA gene copy numbers in the F treatment was significantly higher ($P < 0.05$) than in the R treatment, no significant differences was found for *pmoA* and Type II methanotrophic 16S rRNA gene copies. This might imply that Type I instead of Type II methanotrophs was selectively influenced by treatments of F and R, and only a subset of Type I methanotrophs was severely affected by different land utilization patterns. A further study will focus on which members of Type I methanotrophs are sensitive to the land use change. Interestingly, there was a higher abundance of Type I than that of Type II methanotrophs in all treatments (ratios ranged from 1.42 to 2.34, Fig. 2). This was inconsistent with a previous study that showed the population size of Type II predominated in the paddy soil (Zheng et al., 2008). The predominance of physiologically distinct methanotrophs in upland versus paddy soils clearly indicated that land use change could

particularly select for a certain group of microorganisms, possibly by altering ecological niches such as nutrient concentration and redox status of targeted microorganisms.

### 3.2 Soil methanotrophic composition and diversity under different land utilization patterns

The effects of land use change on $CH_4$ uptake were reported frequently (Prieme and Christensen, 1999; Tate et al., 2007). However, the possible mechanisms mediating $CH_4$ oxidation by soil physicochemical properties or by soil biological parameters were still unclear (Menyailo et al., 2008). In this study, we focused on the biological characterizations of methanotrophs by using molecular approaches. Clear differences in banding patterns were observed in the DGGE profile, suggesting that different land utilization patterns altered the soil methanotrophic community composition. The cluster analysis showed that both treatments of D and R were distinctly different from the F (farming plot), in which the lowest diversity index was obtained from the DGGE data. It could be interpreted that the soil physicochemical properties, especially pH and P, were mainly responsible for the observed differences in community structure. Fierer and Jackson (2006) represented that soil pH was the best predictor of soil microbial community composition. Furthermore, comparing with the restoration plot (R), the farming plot (F) seemed to receive more frequent and intensive disturbance, such as tillage and planting. It was previously testified that the disturbances reduced the capacity to sink atmospheric $CH_4$ by 60% to 90% (Whalen and Reeburgh, 1990; Steinkamp et al., 2001). Here we concluded that the anthropogenic disturbances maybe one of the key factors determining the community structure of methanotrophs and thus controlling the capacity of upland soil as the sink of greenhouse gas $CH_4$.

### 3.3 Linking environmental factors with soil methanotrophic abundance under different land utilization patterns

A correlation analysis was conducted to detect which environmental factor affecting the abundance of methanotrophs. A strong negative correlation ($P = 0.007$) between Type I and soil pH values was observed (Table 3). Ammonia-oxidizers are evolutionarily related to Type I methanotrophs (Holmes et al., 1995), and intensive researches have been conducted regarding soil pH effect on ammonia-oxidizers. For instance, He et al. (2007) demonstrate that there was a significant positive correlation between the population size of ammonia-oxidizers and soil pH. Therefore, we were not certain about the exact mechanisms why Type I methanotrophs responded to soil acidification in a different manner, despite the physiological and metabolic similarity between γ-ammonia-oxidizers and Type I methanotrophs. A positive correlation exists between the abundance of Type I and soil available P content (Table 3). However, no significant correlation was found between Type II or *pmoA* and these two soil characteristics. This may indicate that Type I responded much more rapidly to, and were influenced to a greater extent by changes in soil pH and available P than Type II methanotrophs. In previous studies on Chinese upland red soil, the pH and P were also found to play important roles in affecting soil microbial colony forming units (CFUs) (Zhong and Cai, 2007; He et al., 2008). On the other hand, higher available K appeared to result in higher quantification of Type II methanotrophic 16S rRNA and *pmoA* gene copies. Similarly, the applications of potassium (K) fertilizer was considered as one of the most important environmental factors in controlling the abundance of methanotrophs in paddy soil, in which Type II accounted for a significantly higher percentage than Type I methanotrophs (Zheng et al., 2008).

In conclusion, our results demonstrated that different land utilization patterns had great effects on the abundance and community diversity of methanotrophs in the upland soil. The land use of degradation (D) induced the lowest methanotrophic *pmoA* and 16S rRNA gene copy numbers, while the lowest diversity were observed in the farming plot (F), indicating that the vegetation cover or not, anthropogenic disturbance as well as soil pH and the nutrients of P and K might be key factors influencing methanotrophic community structure. Both *pmoA* and 16S rRNA-targeted assays of methanotroph communities showed similar results. Therefore, the combined use of the functional gene and the 16S rRNA genes is recommended in further study of soil methanotroph ecophysiologys.

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