

Effect of grazing intensities on the activity and community structure of methane-oxidizing bacteria of grassland soil in Inner Mongolia

Xiao-Qi Zhou · Yan-Fen Wang ·
Xiang-Zhong Huang · Jian-Qing Tian ·
Yan-Bin Hao

Received: 22 January 2007 / Accepted: 31 July 2007 / Published online: 12 October 2007
© Springer Science+Business Media B.V. 2007

Abstract The effects of different grazing intensities on in situ methane flux and the structure and diversity of the methanotrophic community are measured in the typical grassland of Inner Mongolia. Four grazing intensity sites founded in 1989, control (CK), low-intensity grazing (LG), middle-intensity grazing (MG) and heavy-intensity grazing (HG), were selected. Group-specific PCR-DGGE (polymerase chain reaction-denaturing gradient gel electrophoresis) of 16S rRNA genes for the type I and type II methanotrophs was used to characterize the composition of the methanotrophic community. DGGE patterns were further analyzed using the method of the Shannon-wiener index *H* and non-metric multi-dimensional scaling (MDS). The results showed that there were no significant differences in methane flux among different sites, yet methanotrophic communities showed significant differences. MDS analysis showed that type I

methanotroph community composition at the CK site were significantly different from the three other sites. For type II methanotrophic community composition, it was similar between CK and HG site, and between LG and MG site, while that at the former two sites were significantly different from latter two ones. Additionally Shannon indices of type II methanotrophs were higher at the LG and MG sites than two other sites. Though grazing intensities had an impact on the structure of the methanotrophic community, management-induced changes in the structure of methanotrophic community did not reflect methane consumption capacity across sites. These results suggest that methane consumption is a complex process in soil, and we should be cautious when speculating on the change of methane consumption rates based on a change of methanotrophic community structure.

Keywords Grazing intensities · Methane flux · DGGE · Methanotroph · Grassland

Introduction

Atmospheric methane is one of the most important greenhouse gases and contributes approximately 20% of global warming (IPCC 2001). The most important natural source of methane is natural wetlands where methanogenesis occurs (Wuebbles and Hayhoe 2002). Much of this methane (700 Tg year⁻¹) is oxidized by aerobic methane-oxidizing bacteria (methanotrophs) in the oxidic cover layer of these anaerobic soils

X.-Q. Zhou · Y.-F. Wang · X.-Z. Huang ·
J.-Q. Tian · Y.-B. Hao
Department of Biology, Graduate University of Chinese
Academy of Sciences, Beijing 100049, China

X.-Q. Zhou
Institute of Botany, Chinese Academy of Sciences,
Beijing 100093, China

Y.-F. Wang (✉)
College of Resources and Environment, Graduate
University of Chinese Academy of Sciences,
Beijing 100049, China
e-mail: yfwang@gucas.ac.cn

before it is released into the atmosphere. Aerobic soils also oxidize a significant amount (20–60 Tg year⁻¹) of the atmospheric methane (Hütsch 2001).

Methanotrophs are obligately aerobic, gram-negative bacteria that utilize methane as their sole sources of carbon and energy. They can be divided into two distinct physiological groups, type I and type II, based on their phylogenetic affiliation, intracellular membrane arrangement, carbon assimilation pathway and phospholipid fatty acid (PLFA) composition (Hanson and Hanson 1996; Bowman 2000). Type I is a kind of low-affinity methanotroph that can only grow in the high methane concentration and low oxygen concentration. Type II methanotrophs are high-affinity organisms that can grow in the low concentration of atmospheric methane (Bender and Conrad 1992). Notably, cultivated isolates of methanotroph were not able to grow on the low mixing ratio of atmospheric methane (Benstead et al. 1998). A new independent culture technique, however, proved that a kind of methanotroph exists in soil which could be the predominant methanotrophs consuming atmospheric methane (Roslev and Iversen 1999; Bull et al. 2000; Crossman et al. 2005).

The typical grassland of Inner Mongolia is one of the representative parts of Eurasia grasslands and is regarded as an important sink for atmospheric methane (1.8–2.3 kg C ha⁻¹ year⁻¹) (Wang et al. 2005). There are about 3.13×10^8 km² of grassland in China. However, these grasslands have undergone considerable change over the last decades because of grassland management. This has led to more intensive grazing regimes that may eventually result in grassland degradation (Chen and Wang 2000). Effects of grazing intensities on the microbial communities have been conventionally studied by techniques based on the cultivation of the soil microorganisms or by measuring their metabolic activities, e.g., by studying the microbial biomass, soil respiration, dehydrogenase, urease, proteinase, and transferase activities (Liu and Liao 1997). In the past decade a new strategy for the PCR-DGGE method has been widely used to detect the temporal and spatial variation of general bacteria and functional groups. The method has successfully described the structure and diversity of microbial community in wastewater sludge (Boon et al. 2002), grassland soil (Clegg 2006), and agricultural soils (Seghers et al. 2003, 2004).

The goal of this study was to evaluate the effects of different grazing intensities on the behavior and structure of methanotrophic community with specific primers of 16S ribosomal RNA (rRNA) and denaturing gel gradient electrophoresis (DGGE). We combined a kinetic description of the methane consumption capacity of these experimental treatments with a molecular analysis of the methanotrophic populations involved. We hypothesize that management may induce a change in the structure and diversity of the methanotrophic community, which may in turn lead to a change in methane consumption capacity of soils.

Materials and methods

Site description and soil sampling

The experimental site, 600 m × 300 m, is located in the Xilin River Basin (43°32' N, 116°40' E, 1200 m a.s.l.) with temperate terrestrial monsoon climate. The site is 300 m north of the bank of the Xilin River, and the soil type is a sandy calcareous chestnut. The plant community is dominated by *Artemisia frigida*, *Cleistogenes squarrosa* and *Carex* spp. This plant population is regarded as one of degradation stages of the *Leymus chinensis* and *Stipa grandis* community due to long-term over-grazing. Soil organic C in the surface layer is 0.5%. According to the long-term records at the Inner Mongolia Grassland Ecosystem Research Station, the temperate semi-arid steppe climate shows a dry spring and a moist summer. Average annual air temperature is -0.4°C, and average air temperature in January and July are -23°C and 17.9°C, respectively. Precipitation is ca. 350 mm/year, which is mainly falling in June, July and August.

Grazing management experimental plots were established in 1989 (Chen and Wang 2000). There are six stocking rates (0.00, 1.33, 2.33, 4.00, 5.33, 6.67 sheep ha⁻¹) with three different replicates per treatment except for the 0.00 sheep ha⁻¹ grazing intensity (that is, control site). Every site area is 100 m × 100 m, and all 16 experimental plots were set up by a randomized block design. Four different stocking rates (0.00, 1.33, 4.00, 6.67 sheep ha⁻¹) were selected, which were designated as control (CK), low-intensity grazing (LG), middle-intensity grazing (MG) and heavy-intensity grazing (HG).

Every year, grazing began on May 20 and terminated on October 5. Each plot was rotationally grazed three times per year, each time for 15 days with a rotation interval of 30 days, yielding a total grazing period of 45 days per plot. This rotational grazing facilitated the restoration succession of the degraded *A. frigida* grassland. Soil characteristics under different grazing intensities are listed in Table 1 (Chen and Wang 2000).

Soil samples were taken from a depth of 0 to 5 cm with a 3-cm diameter auger in August 2004. From each study plot, 20 soil cores were collected to form one bulk sample, and soil physical properties were measured at the same time. Other samples were transported to the laboratory and stored at -20°C for molecular analysis after sieving (mesh size <2 mm) to remove plant material.

CH₄ consumption analyses

Methane consumption rates were measured in situ in 1998 and 1999 as well as in August 2004 and in July 2005 using a closed static chamber method (Wang and Wang 2003). The chambers were equipped with a battery-driven ventilator, and a small vent for pressure equilibration was used for gas flux measurements. Three replicate chambers, $0.5\text{ m} \times 0.5\text{ m} \times 0.5\text{ m}$, were used in every plot. During measurement, the chambers were 10–15 m from each other. Gas samples were taken from the chambers with 10-min intervals in 40 min after closure using 10-ml gas tight syringes. All gas samples were analyzed within 6 h with gas chromatograph (HP 5890 II) in the laboratory. To calculate the CH₄ flux rate, liner regressions were performed on the four gas samples collected from each flux chamber. Finally, the CH₄ flux rate was adjusted

using air pressure and air temperature within the chamber headspace (Wang et al. 2005).

Extraction and PCR amplification of total DNA from soil

Nucleic acids were extracted from 0.5 g of each sample by using the procedures described by Zhou et al. (2007). In brief, soil was placed in a 2-ml screw-cap tube containing a mixture of ceramic and silica particles (Bio101, Carlsbad, Calif.); the mixture was homogenized for 30 s in a FastPrep bead beater cell disrupter (Bio101). After the nucleic acids had been precipitated and washed twice in 75% (vol/vol) ethanol, the final DNA was re-suspended in 100 μl TE buffer. Furthermore, the crude extract was purified with Qiagen gel extraction Kit (Qiagen, Inc.). DNA concentrations were measured by Biospec-mimi (Shimadzu, Kyoto, Japan).

All the primers and PCR conditions are listed in Table 2. For more information about these primers and PCR conditions, we refer readers to the original papers. In order to increase the sensitivity and to facilitate the DGGE by analyzing fragments of the same length, a nested PCR technique was applied. In the first round different group-specific primers and one set of bacterial primers were used, each with their own corresponding PCR protocol. During the second PCR round, the obtained fragments were re-amplified by using the bacterial primers P338F and P518r in the same PCR protocol (Table 2).

The final concentration of different components in the mixture was as follows: 0.4 μM of each primer, 200 μM of each deoxynucleoside triphosphate, 1.5 mM MgCl₂, 1 \times thermophilic DNA polymerase 10 \times reaction buffer (MgCl₂-free), 1.25 U per 50 μl of

Table 1 Soil characteristics in different stocking rates

Site	pH	TOC (g kg ⁻¹)	NH ₄ ⁺ -N (mg kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)	Total P (%)	Soil bulk density (g cm ⁻³)
CK	6.92 \pm 0.02	1.79 \pm 0.07a	4.09 \pm 0.72a	3.06 \pm 0.43a	0.14 \pm 0.02	1.16 \pm 0.03b
LG	7.14 \pm 0.01	1.47 \pm 0.08b	3.00 \pm 0.43a	1.39 \pm 0.37b	0.13 \pm 0.02	1.31 \pm 0.03a
MG	7.07 \pm 0.02	1.47 \pm 0.05b	3.47 \pm 0.71a	1.25 \pm 0.18b	0.13 \pm 0.03	1.31 \pm 0.03a
HG	7.13 \pm 0.02	1.56 \pm 0.07b	3.44 \pm 0.65a	1.00 \pm 0.14b	0.1 \pm 0.03	1.33 \pm 0.03a

CK, control; LG, low-intensity grazing; MG, middle-intensity grazing; HG, heavy-intensity grazing

Different letters showed significant differences ($P < 0.05$)

Table 2 PCR primers used in this study

Target	Primers	PCR conditions						References	
		Numbers of cycles	Denaturation		Annealing		Elongation		
			°C	min	°C	min	°C		min
<i>First PCR round</i>									
Type I methanotrophs	methT1dF, methT1bR	30	95	1	55	1	72	2	(Wise et al. 1999)
Type II methanotrophs	27f, methT2R	30	95	1	55	1	72	2	(Lane 1991; Wise et al. 1999)
<i>Second PCR</i>									
Bacteria	P338F ^a , P518r	30	94	1	56	1	72	1	

Before the run of cycles, the temperature was held at 95°C for 7 min, and after each run the temperature was kept 72°C for 10 min for final template elongation

^a A 5'GC-clamp was added for DGGE analysis (Muyzer et al. 1993)

Taq DNA polymerase (Promega, Madison, WI), and DNase and RNase free filter sterilized water (LiYuan Apparatus Com., Beijing, China). All reactions were performed in a PTC-200 thermal cycler (MJ Research Co., USA). During the first PCR round, ~50 ng of purified DNA was added to 24 µl of PCR master mix, and in the second PCR round, 1 µl of amplified product from the first round was added to 24 µl of PCR mixture. After each PCR, the size of the amplification product was verified on a 1.2% agarose gel.

DGGE

DGGE analysis was performed using a Dcode system (Bio-Rad, Hercules, CA) as described previously (Muyzer et al. 1993). For the generation of bacterial community profiles, polyacrylamide gels (8% acrylamide, 0.5× TAE (20 mM Tris, 20 mM acetate, 1 mM EDTA [pH 8.0]), 37:1 acrylamide:bisacrylamide) were cast using 40–60% denaturant [100% denaturant was defined as 7 M urea with 40% (vol/vol) formamide]. Electrophoresis was run overnight for 12 h at a constant temperature 60°C at 110 V. After electrophoresis, the gels were soaked for 30 min in SYBR green II nucleic acid gel stain (1:10,000 dilution; FMC Bioproducts, Rockland, ME). The stained gels were immediately photographed on UV transillumination table with a Hewlett Packard Scanjet 5370C. The DGGE patterns were

determined using the Labworks software (Labworks TM software version 4.0. UVP, UK).

Cloning and sequencing analysis

The specific methanotrophic gene fragments of the first PCR round were cloned into the pGEM-T Easy vector (Promega) according to the manufacturer's instructions. Plasmid DNAs were isolated from randomly selected clones and screened for inserts of the expected sizes. In addition, 1 band from the type II methanotrophs pattern was excised from the gel and re-amplified with the same reaction procedure. The PCR products were purified and then were cloned into plasmids with a pGEM-T easy vector kit (Promega) as well. The plasmid DNA was sequenced with primers (T7 reverse/forward) on an ABI PRISM 3700 DNA analyzer (ABI Biosystems, Inc.). The nucleotide sequence obtained in this study was sent to the NCBI database. The accession numbers is DQ420621.

Statistics

The means and standard errors of the mean (SEM) of the methanotroph analytical results are presented per dry matter weight (dry wt) of soil. Analysis of variance (ANOVA) followed by Tukey's test was performed to test for within-treatment differences in methane consumption rates in different grazing intensities. Significant differences are reported at the 0.05 level.

The DGGE gel photographs were screened for their presence (1) or absence (0) of bands in the DGGE patterns. A similarity matrix was generated with the binary data using the Jaccard coefficient and analyzed with non-metric multidimensional scaling (MDS) as described by Jaatinen et al. (2004).

The DGGE patterns were analyzed with the labworks software (Labworks TM software version 4.0. UVP, UK). Band intensity of each lane in the densitometric curve was read. The diversity of the microbial community was examined by the Shannon index of general diversity H (Sun et al. 1993). H was calculated on the basis of the band intensity on the gel tracks, which was reflected as peak heights in the densitometric curve. The equation for the Shannon index is:

$$H = - \sum (n_i/N) \log(n_i/N)$$

where n_i is the height of the peak and N the sum of all peak heights of the densitometric curve.

Results

Methane consumption rates

Methane fluxes at different stocking rates are shown in Fig. 1. The methane consumption rates at all sites were negative, which showed Inner Mongolia grassland as a sink for atmospheric methane, consistent with previous studies in this area (Wang et al. 2005). The large variations of methane uptake flux observed at one site at the different sampling times confirm flux rates observed by Wang et al. (2005). In total, methane uptake rates were slightly higher on 9/8/98, 7/1/99, 8/1/99 and 9/1/99 than at the other sampling times (Fig. 1). The mean annual precipitation in 1998, 1999, 2004 and 2005 was 357, 214, 364 and 162 mm, respectively. Methane uptake flux at one site at one sampling time also showed large variations (error bars, Fig. 1). When the data of different sampling time at the same site were averaged, however, no significant differences were observed among different grazing sites. Statistical analysis of all methane fluxes at the different sampling times showed that the averages of methane flux in CK, LG, MG and HG sites were 75, 83, 85 and 75 $\mu\text{g C m}^{-2} \text{h}^{-1}$, respectively.

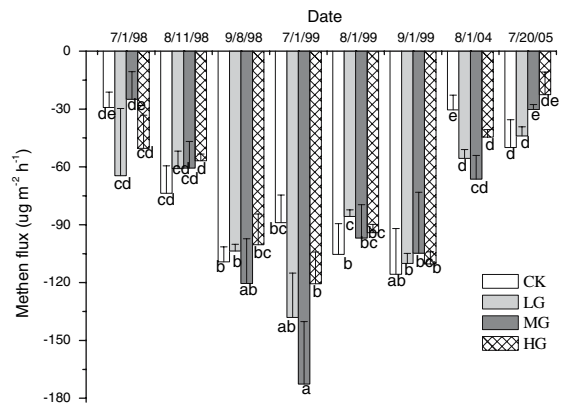


Fig. 1 The methane consumption rates in the different grazing intensity plots. CK, control; LG, low-intensity grazing; MG, middle-intensity grazing; HG, heavy-intensity grazing, different letters showed significant differences ($P < 0.05$)

DGGE analysis

Before PCR-DGGE analysis, DNA yields after extraction were examined, and no significant differences were found between the different samples. Figure 2 shows DGGE patterns of the type I methanotrophs and type II methanotrophs. After further MDS analysis of these patterns, the structure of the type I methanotroph community in the grazing treatments was significantly different from site CK (Fig. 3a). No significant differences were observed between the other grazing intensity treatments. Grazing management greatly affected the structure of the type II methanotrophs community as well. Type II methanotroph communities at the LG and MG sites were significantly different from the other two sites (Fig. 3b). Additionally, the Shannon indices (Table 3) indicated that type I and type II methanotrophs communities at the LG and MG sites were higher than at the CK and HG sites.

Each band in the DGGE pattern was assumed to represent one unique ribotype. In the pattern of type II methanotrophs, a phylotype (band) was only detected in LG and MG soil. To determine more information, the band was further cloned and sequenced. After blasting the sequence in the GenBank, its sequence exhibited 100% similarity to an *uncultured methylobacteriaceae* bacterium clone GASP-WA2W2_D12 (EF072931), which was recently detected in America (unpublished).

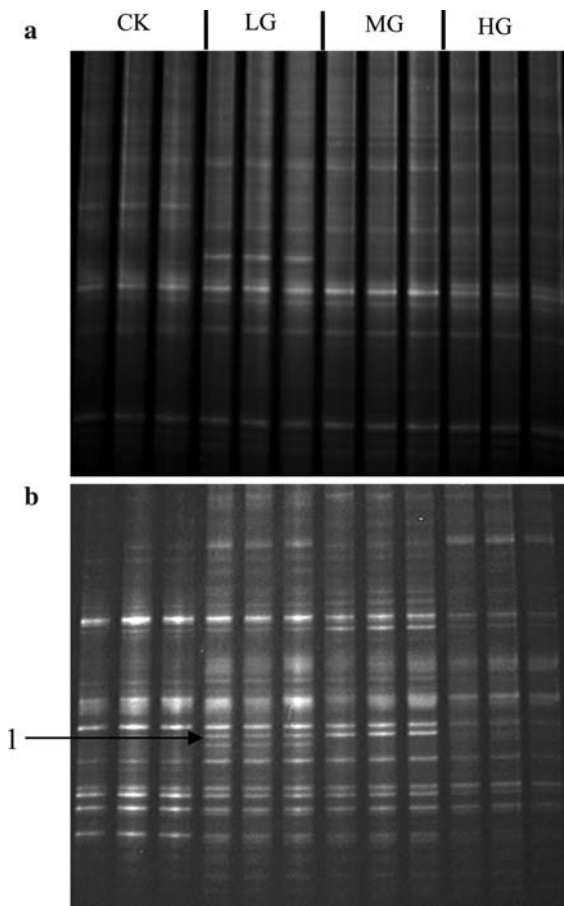


Fig. 2 DGGE patterns of type I methanotrophs (a) and type II methanotrophs (b) in the different grazing intensity treatments in three parallel replicates. CK, control; LG, low-intensity grazing; MG, middle-intensity grazing; HG, heavy-intensity grazing

Discussion

In this study, we examined the effects of different grazing intensities on methane consumption capacity and methanotrophic community structure. The soil-atmosphere exchange of methane comprises two parts: atmospheric methane oxidation by soil and methane produced by the soil methanogenesis (Wager et al. 1996). Methane production in the Inner Mongolia steppe can be negligible for the amount of methane produced by methanogenesis is very little in the aerated soil in semi-arid grassland. The mean methane uptake flux observed agrees with previous studies in this area (Wang et al. 2005). These uptake rates are slightly higher than those reported in the Colorado shortgrass steppe by Mosier et al.

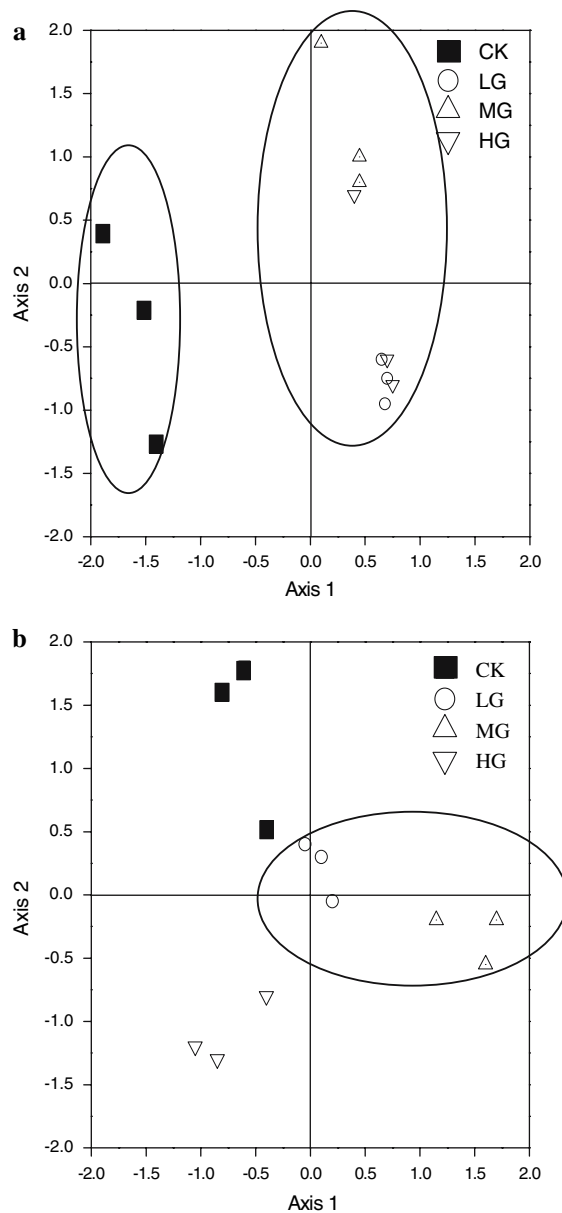


Fig. 3 Non-metric multi-dimensional scaling (MDS) analysis DGGE patterns of type I methanotrophs (a) and type II methanotrophs (b). CK, control; LG, low-intensity grazing; MG, middle-intensity grazing; HG, heavy-intensity grazing

(25–45 $\mu\text{g C m}^{-2} \text{ h}^{-1}$) (1991, 1996) and in an English grassland (10–19 $\mu\text{g C m}^{-2} \text{ h}^{-1}$) (Kammann et al. 2001).

Compared with type I methanotrophs, the type II methanotrophs are likely to be the main group in the grassland soil in Inner Mongolia. These organisms oxidize atmospheric methane at low concentration.

Table 3 Shannon diversity (H) and evenness (E) of different treatments

	Treatment CK	Treatment LG	Treatment MG	Treatment HG
Type I methanotroph	0.98 ± 0.05b	1.03 ± 0.02a	1.06 ± 0.09a	0.97 ± 0.03b
Type II methanotroph	1.13 ± 0.04b	1.26 ± 0.02a	1.21 ± 0.03a	1.09 ± 0.02b

CK, control; LG, low-intensity grazing; MG, middle-intensity grazing; HG, heavy-intensity grazing

Different letters showed significant differences ($P < 0.05$)

Compared with the CK and HG sites, methane uptake at the LG and MG sites was slightly higher. The type II methanotrophic phylotype was obvious at the LG and MG sites and disappeared at the CK and HG sites. This implies that the phylotype seems to play an important role in oxidizing atmospheric methane in this area. The further sequence analysis indicated that it is a kind of *uncultured Methylobacteriaceae* bacterium.

Previous studies indicated that long-term agricultural practices may shift the structure of the methanotrophic community, which results in the change of methane oxidation (Adamsen and King 1993; Seghers et al. 2003). Are the changes in methane oxidation related with the shifting of structure and diversity of methanotrophic community induced by management practices? The research of Jaatinen et al. (2004) indicated that fire significantly increased the methane oxidation rate, but the community structure did not change through comparing *pmoA* recovery products. It is possible that shifts occurred on a fine phylogenetic scale not distinguishable by *pmoA* products. However, Seghers et al. (2003) found long-term use of herbicides atrazine and metolachlor resulted in shifting of methanotrophic community structure, but no change in abundance and activity of methanotrophs. In this study, comparing the change of methane uptake flux and the change of methanotroph communities structure at different stocking rates sites, we found that although grazing intensity had an impact on the methanotrophic community structure, it did not greatly impact the methanotrophic activity. As one of the primary land uses in Inner Mongolia, the practice of grazing sheep is a complex event. A range of specific factors, such as fecal and urine deposition, shifts in plant rhizosphere exudation, shifts in plant composition and the change in soil structure and aerobicity, changes soil characteristics (Clegg 2006). Methanotroph activity in soil is affected by many factors, one of which is ammonium concentration in soil (Bodelier and Laanbroek

2004). The $\text{NH}_4^+\text{-N}$ concentrations at the different sites were not significantly different, which may have helped to account for no difference in methane consumption rates across sites (Table 1).

Recently more and more functional groups that participate in consuming atmospheric CH_4 have been found in terrestrial ecosystems (Bull et al. 2000; Henckel et al. 2000; Knief et al. 2003). Recent studies have indicated that atmospheric methane oxidization can also be mediated by a kind of unknown methanotroph, which is similar to type II methanotrophs in the phylogenetic tree (Bull et al. 2000; Henckel et al. 2000; Crossman et al. 2005). The type II methanotroph primers used in this study, which targeted all of the *methylosinus/methylocystis* groups, are degenerate primers and detect some unknown methanotrophs (Wise et al. 1999). We detected a kind of unknown type II methanotroph in the Inner Mongolian grassland soil and its role in consuming atmospheric methane needs to be further studied.

Acknowledgments This work is financially supported by the National Science Foundation of China (no. 90411001) and President Foundation of the Chinese Academy of Sciences. We greatly thank Cui Xiao-Yong and two anonymous reviewers for going through this manuscript with great care and giving valuable comments on it.

References

- Adamsen APS, King GM (1993) Methane consumption in temperate and subarctic forest soils: rates, vertical zonation, and responses to water and nitrogen. *Appl Environ Microbiol* 59:485–490
- Bender M, Conrad R (1992) Kinetics of CH_4 oxidation in oxic soils exposed to ambient air or high CH_4 mixing ratios. *FEMS Microbiol Lett* 101:261–269
- Benstead J, King GM, Williams HG (1998) Methanol promotes atmospheric methane oxidation by methanotrophic cultures and soils. *Appl Environ Microbiol* 64:1091–1098
- Bodelier PLE, Laanbroek HJ (2004) Nitrogen as a regulatory factor of methane oxidation in soils and sediments. *FEMS Microbiol Ecol* 47:265–277

- Boon N, De Windt W, Verstraete W, Top EM (2002) Evaluation of nested PCR-DGGE (denaturing gradient gel electrophoresis) with group-specific 16S rRNA primers for the analysis of bacterial communities from different wastewater treatment plants. *FEMS Microbiol Ecol* 39:101–112
- Bowman J (2000) The methanotrophs—the families *Methylococcaceae* and *Methylocystaceae*. In: Dworkin M (ed) *The prokaryotes*. Springer, New York, USA
- Bull ID, Parekh NR, Hall GH, Ineson P, Evershed RP (2000) Detection and classification of atmospheric methane oxidizing bacteria in soil. *Nature* 405:175–178
- Chen ZZ, Wang SP (eds) (2000) *Chinese typical grassland ecosystem*. Science Press, Beijing, China
- Clegg CD (2006) Impact of cattle grazing and inorganic fertilizer additions to managed grasslands on the microbial community composition of soils. *Appl Soil Ecol* 31:73–82
- Crossman ZM, Ineson P, Evershed RP (2005) The use of ^{13}C labelling of bacterial lipids in the characterisation of ambient methane-oxidizing bacteria in soils. *Org Geochem* 36:769–778
- Hanson R, Hanson T (1996) Methanotrophic bacteria. *Microbiol Rev* 60:439–471
- Henckel T, Jackel U, Schnell S, Conrad R (2000) Molecular analyses of novel methanotrophic communities in forest soil that oxidize atmospheric methane. *Appl Environ Microbiol* 66:1801–1808
- Hütsch BW (2001) Methane oxidation in non-flooded soils as affected by crop production—invited paper. *Eur J Agron* 14:237–260
- IPCC (2001) *Climate change: the scientific basis. Contribution of the working group I to the third assessment report of the Intergovernmental panel on climate change*. In: Houghton JT et al (eds) Cambridge University Press, New York, USA, p 881
- Jaatinen K, Knief C, Dunfield PF, Yrjala K, Fritze H (2004) Methanotrophic bacteria in boreal forest soil after fire. *FEMS Microbiol Ecol* 50:195–202
- Kammann C, Grünhage L, Jäger HJ, Wachinger G (2001) Methane fluxes from differentially managed grassland study plots: the important role of CH_4 oxidation in grassland with a high potential for CH_4 production. *Environ Pollut* 115:261–273
- Knief C, Lipski A, Dunfield PF (2003) Diversity and activity of methanotrophic bacteria in different upland soils. *Appl Environ Microbiol* 69:6703–6714
- Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) *Nucleic acid techniques in bacterial systematics*. John Wiley and Sons Ltd, Chichester, UK, pp 115–175
- Liu LP, Liao YN (1997) Biological characteristics and biodiversity of the soil microorganisms in *Leymus Chinensis* steppe and *Stipa Grandis* steppe under different grazing intensities. In: Chen ZZ (ed) *Inner Mongolia grassland ecosystem research station research on grassland ecosystem*, vol 5. China Scientific Press, Beijing, China, pp 13–22
- Mosier AR, Schimel DS, Valentine DW, Bronson KF, Parton WJ (1991) Methane and nitrous oxide fluxes in native, fertilized and cultivated grasslands. *Nature* 350:330–332
- Mosier AR, Parton WJ, Valentine DW, Ojima DS, Schimel DS, Delgado JA (1996) CH_4 and N_2O fluxes in the Colorado shortgrass steppe, 1, Impact of landscape and nitrogen addition. *Global Biogeochem Cy* 10:387–399
- Muyzer G, De Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol* 59:695–700
- Roslev P, Iversen N (1999) Radioactive fingerprinting of microorganisms that oxidize atmospheric methane in different soils. *Appl Environ Microbiol* 65:4064–4070
- Seghers D, Verthe K, Reheul D, Bulcke R, Siciliano SD, Verstraete W, Top EM (2003) Effect of long-term herbicide applications on the bacterial community structure and function in an agricultural soil. *FEMS Microbiol Ecol* 46:139–146
- Seghers D, Wittebolle L, Top EM, Verstraete W, Siciliano SD (2004) Impact of agricultural practices on the *Zea mays* L. endophytic community. *Appl Environ Microbiol* 70:1475–1482
- Sun RY, Li B, Zhu GY, Sang YC (eds) (1993) *General ecology*. High Education Press, Beijing, China
- Wager C, Griesshammer A, Drake HL (1996) Acetogenic capacities and the anaerobic turnover of carbon in Kansas prairie soil. *Appl Environ Microbiol* 62:494–500
- Wang YS, Wang YF (2003) Quick measurement of CH_4 , CO_2 and N_2O emission from a short-plant ecosystem. *Adv Atmos Sci* 20:842–844
- Wang YS, Xue MB, Zheng XH, Ji BM, Du R, Wang YF (2005) Effects of environmental factors on N_2O emission from and CH_4 uptake by the typical grasslands in the Inner Mongolia. *Chemosphere* 58:205–215
- 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
- Wuebbles DJ, Hayhoe K (2002) Atmospheric methane and global change. *Earth-Sci Rev* 57:177–210
- Zhou XQ, Wang YF, Huang XZ, Hao YB, Tian JQ, Wang JZ (2007) Effects of grazing by sheep on the structure of methane-oxidizing bacterial community of steppe soil. *Soil Biol Biochem* doi: 10.1016/j.soilbio.2007.05.020