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SHORT COMMUNICATION

Relationship between archaeal community structure and vegetation type in a fen on the Qinghai–Tibetan Plateau

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Abstract The contribution of different methanogenic precursors probably depends on vegetation in the cold Zoige peatlands. This study was carried out to elucidate the relationship between archaeal community dynamics and vegetation type over growing season. Soil samples were collected monthly during the growing season from two vegetation types (communities dominated by *Carex muliensis* vs. *Eleocharis valleculosa*) on an open fen at the Wetland National Nature Reserve of the Zoige peatlands on the Qinghai–Tibetan Plateau. Archaeal community structure was determined with terminal restriction fragment length polymorphism analysis of the 16S rRNA gene fragment. *Methanosarcinales*, *Methanosaeta*, *Methanomicrobiales*, *Methanobacteriales*, uncultured RC-II, and uncultured *Crenarchaeota* were detected in both vegetation types. The results suggested that seasonal change affects the activity rather than the structure of the archaeal community over the growing season. Ordination analyses indicated that archaeal community composition was related to vegetation type and plant height.

Keywords Qinghai–Tibetan Plateau · Peatland · Methanogenic archaea · Plant growth

Introduction

Natural wetlands contribute 20–30% of the total global emission of the important greenhouse gas, methane (CH_4) (IPCC 2007). In anoxic wetlands, CH_4 is an end product of the anaerobic degradation of organic matter by methanogenic archaea (Conrad 1999). Microbial activity is exclusively responsible for methane production in natural ecosystems (Garcia et al. 2000).

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Methanogenic populations have been studied in different habitats such as fen (Galand et al. 2003), rice soil (Großkopf et al. 1998), marine and lake sediments (Koch et al. 2009), and peatlands (Cadillo-Quiroz et al. 2006). A variation in the composition of methanogenic communities among wetlands has been related to vegetation, pH, temperature, water table, and hydrology (Galand et al. 2003; Juottonen et al. 2005; Cadillo-Quiroz et al. 2006; Rooney-Varga et al. 2007; Kotiaho et al. 2010), but little is known about the spatiotemporal dynamics of methanogenic populations (Kruger et al. 2005; Wagner et al. 2005; Hoj et al. 2006; Rooney-Varga et al. 2007), especially in natural wetlands. Juottonen et al. (2008) found that archaeal community composition and size in a boreal fen varied only slightly with seasonal changes. In an Italian rice field, Kruger et al. (2005) found that the seasonal changes in the methanogenic processes were probably caused by changes in the activity rather than in the size of the methanogenic community. Watanabe et al. (2006) suggested that difference in soil type (sampling region) influenced the community structure of methanogens. Rooney-Varga et al. (2007) indicated a link between vegetation type and archaeal community composition. However, more research is needed to increase our understanding of the dynamics of methanogenic archaeal communities as related to vegetation type in natural wetlands under varying climatic and soil conditions.

The Zoige Plateau (average elevation of 3,400 m asl) on the eastern edge of the Qinghai–Tibetan Plateau is a "hotspot" of CH_4 emission (Jin et al. 1999). It is a complete and orbicular plateau that covers an area of 2.8×10^4 km^2, surrounded by a series of alpine mountains (average elevation of 4,000 m asl). Numerous alpine wetlands and lakes have developed on the plateau, accounting for 17.8% of the plateau area. During the peak growing season on the Zoige Plateau, methane emissions varied among wetland areas that differed in vegetation type (Chen et al. 2009a); this variation was found to be related to key factors including standing water table and plant community height, but its relation with the methanogenic archaea were not characterized.

Whether plant growth affects the composition of the archaeal community on the Zoige Plateau remains unclear. Methanogenic archaea and methanogenic pathway on the plateau have been studied several times (Zhang et al. 2008a, b; Jiang et al. 2010). Jiang et al. (2010) reported that the methanogenic pathway was strongly correlated with vegetation type in Zoige peatlands. Previously, studies have also demonstrated a link between vegetation type and methanogenesis pathways, but only a few have linked terminal decomposition pathways to microbial community analysis (Galand et al. 2005; Rooney-Varga et al. 2007). It is urgent to characterize the microbial communities that are responsible for these processes in order to understand how their dynamics are related to environmental and biogeochemical factors in this area, which is a sensitive area for climate change.

The objectives of the current study were to analyze the diversity of methanogenic archaeal communities in two predominant vegetation types: *Carex muliensis* (CM) and *Eleocharis valleculosa* (EV). The composition of archaeal communities was evaluated by terminal restriction fragment length polymorphism (T-RFLP) analysis of archaeal 16S rRNA genes, thus including also other groups of Euryarchaeota and *Crenarchaeota*. The second aim was to quantify the relationships between environmental variables and composition of archaeal community.

Materials and methods

Site description and sample collection

Soil samples were collected from an open fen at the Wetland National Nature Reserve of Zoige (33°56′ N, 102°52′ E; 3,430 m asl). The alpine wetlands of this region cover an area of 6,180 km^2, which represents 31.5% of the whole Zoige Plateau, according to the Zoige wetlands classification of *Mires of the Zoige Plateau*. This region is located in the cold Qinghai–Tibetan climatic zone. The mean annual precipitation is approximately 650 mm, and the mean annual temperature is 1.7°C (Chen et al. 2009b). The warmest temperatures (9.1–11.4°C) occur in July, and the coldest temperatures (−8.2 to −10.9°C) occur in January (Chen et al. 2009a). The pH values are around 6.6–7.0. Soil samples in the depth of 10–15 cm were collected monthly from June to August 2007. Each sample was represented by three soil cores, and there were 18 samples in total (three samples per month and per vegetation). Other details concerning soil sampling are provided by Zhang et al. (2008b). The samples were placed in ice chests and transported to the laboratory for immediate processing.

DNA extraction, PCR amplification, and phylogenetic analysis

Total DNA was extracted with a FastDNA SPIN kit for soil according to the manufacturer's instructions (Qbiogene, Carlsbad, CA). Guanidine thiocyanate (5.5 mM; Sigma) was used to remove PCR-inhibiting compounds (mainly humic acids) from the extraction. The archaeal 16S rRNA gene was amplified with the primer pair A109f/A915r or A915r labeled at the 5′ end with 6-carboxyfluorescein (Stahl and Amann 1991). The PCR was performed as follows: a primary denaturation step of 3 min at 94°C; followed by 32 cycles of 30 s at 94°C, 45 s at 53°C, and 90 s at 72°C; and a final DNA synthesis for 5 min at 72°C.

PCR products were purified with a QIAquick PCR purification kit (QIAGEN, Hilden, Germany).

The purified 16S rRNA fragments of about 800 bp were cloned into the pUCm-T vector and sequenced by Sangon Biological Engineering Technology and Services (Shanghai, China). Chimera sequences of 16S rRNA genes were identified by Chimera Check of Ribosomal Database Project II (release 8.1) (Cole et al. 2005). The 16S rRNA sequences were submitted to GenBank, and a search for similar sequences was conducted using the BLAST algorithm. The best matching sequences were retrieved from the database and aligned, and a similarity analysis was performed by ClustalX (Thompson et al. 1994). The phylogenetic tree was constructed using the neighbor-joining method implemented in MEGA 4.0 (Tamura et al. 2007). The topologies of the resultant tree were evaluated by bootstrap analysis (Felsenstein 1985) based on 1,000 resamplings.

Terminal restriction fragment length polymorphism analysis

The T-RFLP analysis was performed as described previously (Chin et al. 1999). Briefly, labeled purified PCR products were subjected to T-RFLP analysis with the restriction enzyme Taq*I* (Promega, USA) and were analyzed with an ABI Prism 373 DNA sequencer (Applied Biosystems, USA). The electropherograms were analyzed with GeneScan version 2.1 (Applied Biosystems). Relative amplicon frequencies were determined based on relative signal intensities of terminal restriction fragments (T-RFs) as indicated by peak heights (Lueders and Friedrich 2003). Signals with peak heights less than 100 relative fluorescence units were regarded as background noise and were excluded from the analysis. The percentages of fluorescence intensity represented by single T-RFs were calculated relative to the total fluorescence intensity of all T-RFs.

Measurement of physicochemical parameters

Total carbon (C), total nitrogen (N), and dissolved organic C (DOC) were measured according to O'Halloran (1993) by the Chengdu Institute of Biology, Chinese Academy of Science. Air temperature, soil temperature at 5- and 10-cm depths, and plant community height (the average height of vascular plants) were recorded during sampling. Acetate concentration in the pore water was measured by gas chromatography (Zhang et al. 2008b).

Ordination analyses of community composition data

To visualize the differences in the archaeal community between samples and seasons, we performed correspondence analysis (CA) and canonical correspondence analysis (CCA) with CANOCO version 4.5 for Windows (Microcomputer Power, Ithaca, NY, USA). A total of 18 samples, eight T-RFs, and six environmental variables (including temperature, vegetation type, plant community height, total nitrogen, organic matter, and DOC) were subjected to a CCA with biplot scaling focused on intersample distances.

Sequence accession numbers

The 16S rRNA gene sequences obtained in this study has been deposited in the EMBL, GenBank, and DDBJ nucleotide sequences databases under accession numbers HQ407436 to HQ407463.

Results

The figures EV6, EV7, and EV8 refer to data from *E. valleculosa* stands and CM6, CM7, and CM8 refer to data from *C. muliensis* stands both sampled in June, July, and August of 2007, respectively. One clone library of archaeal 16S rRNA genes which contained 30 clones was constructed from EV6, and one of 30 clones was generated from CM6. The sequencing analysis showed that the archaeal community consisted of *Methanosarcinaceae*, *Methanosaeta*, *Methanomicrobiales*, *Methanobacteriales*, uncultured Rice Cluster II (RC-II), and uncultured Crenarchaeotal (Fig. 1). The spatiotemporal dynamics of the archaeal community in soil was estimated using T-RFLP analyses. Based on silico analyses of clone sequences, different terminal restriction fragments (T-RFs) could be assigned to different archaeal lineages as follows: 78 bp to *Methanobacteriales*, 86 bp to *Methanomicrobiales*, 182 bp to *Methanosarcinaceae*, 281 bp to *Methanosaeta*, 358 bp to *Methanosarcinaceae*, 387 bp to *Methanomicrobiales*, 488 bp to RC-II, and 735 bp to uncultured *Crenarchaeota*. T-RFLP patterns were similar among the soil samples regardless of sampling date and vegetation type (Fig. 2). However, the relative abundance of the T-RFs varied among months within each type of vegetation.

The predominant amplified fragment, which was 281 bp in both plant stands, had a relative abundance of 17–24% at the EV site and 23–33% at the CM site (Fig. 2). At the EV site, the next most abundant fragments were 78 and 86 bp. The relative abundance of the 182-bp fragment was less than 10% at the EV site but was between 15% and 20% at the CM site (Fig. 2).

The community dynamics were further investigated by CA of the T-RFLP data (Fig. 3). The first CA axis, which is correlated with the main compositional variation (64.7%), separated archaeal communities of CM from those of EV,

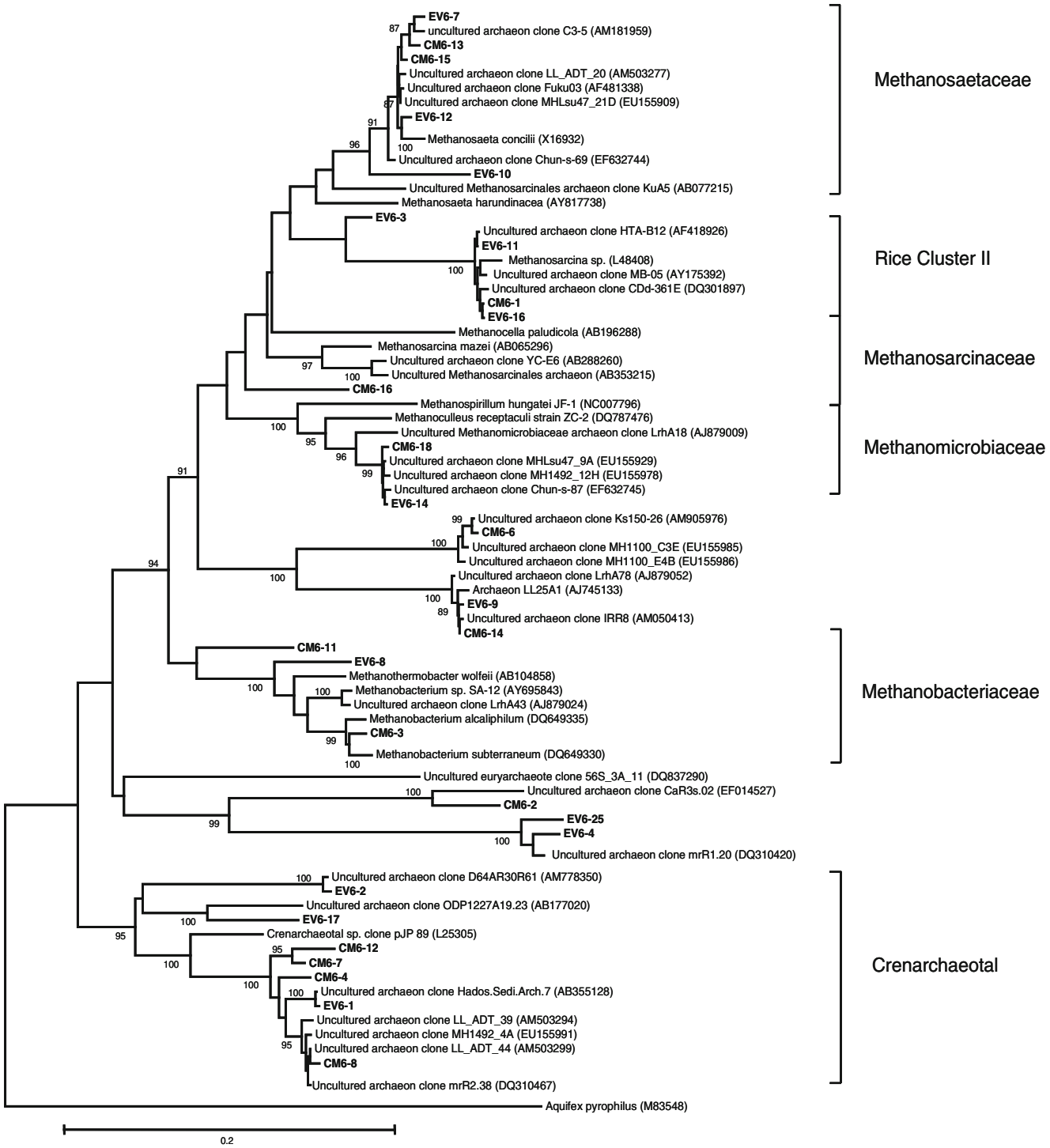

Fig. 1 Phylogenetic relationship of the representative archaeal 16S rRNA gene sequences obtained from EV6 soil (soil collected beneath *E. valleculosa* in June) and CM6 soil (soil collected beneath *C. muliensis* in June). The sequences obtained from this study are indicated by bold characters. The tree, which is based on a consensus length of 780 bp of 16S rRNA gene sequences, was constructed by the neighbor-joining method and was rooted with *Aquifex pyrohilus*. The topology of the tree was estimated by bootstraps based on 1,000 replications. Numbers at branch points are percentages supported by bootstrap evaluation. The GenBank accession number of each reference sequence is shown in parentheses after each strain. The *bar* represents 2% sequence divergence

indicating that the archaeal community composition in CM was distinct from that in EV (Fig. 3a). The second CA axis accounts for 18.9% of the variation which separated CM6 and CM7 data from CM8 data, and separated EV8 and EV6 data from EV7 data. Hence, the difference in T-RFLP data among the sampling dates was much smaller than the

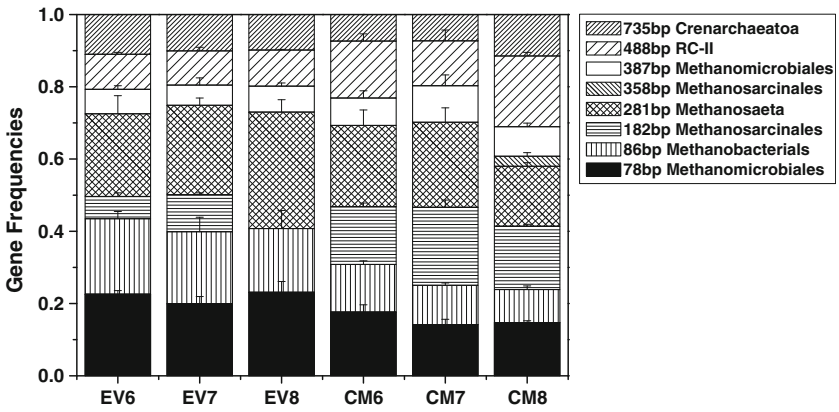

Fig. 2 Relative abundance of individual T-RFs from T-RFLP analysis targeting archaeal 16S rRNA genes amplified from DNA extracted from soil beneath EV or CM in June (6), July (7), or August (8) of 2007.

difference between the two vegetation types. Furthermore, the effect of sampling time was not significant (P=0.08).

The analysis of the CA axis and environmental factors showed that vegetation type and plant height were negatively correlated with the CA axis one (correlation coefficient of −0.78 and −0.40, respectively; P<0.05). Total N was correlated with the CA axis two (correlation coefficient of 0.56; P<0.01). The temperature was not significantly correlated with any axis (P=0.12).

The relationships between environmental variables and archaeal community composition are shown in Fig. 3b. Like CA, CCA analysis indicated that vegetation type accounted for most of the variability in archaeal community composition (P=0.006 by Monte Carlo permutation test; Fig. 3b). After inclusion of vegetation class in the model, total N was determined as the second most important environmental variable (P=0.02).

Discussion

In Zoige wetland, two predominant plant populations, *C. muliensis* (CM) and *E. valleculosa* (EV) covering about 95% of the whole site grow in the water. Acetoclastic and hydrogenotrophic methanogensis are two major pathways of methane production in most environments. In CM and EV, fragments of 281, 182, and 358 bp accounted for 30–60% of the total fragments, and fragments of these lengths correspond to acetoclastic methanogens (*Methanosaeta* and *Methanosarcinaceae*). Abundant *Methanosaeta* and *Methanosarcinaceae* in our samples supported Jiang et al. (2010) findings that acetoclastic methanogenesis pathway dominated in both plant stands. A similar relationship between methanogenic community structure and methanogenesis was also found in peat (Rooney-Varga et al. 2007) and fen (Galand et al. 2003). In Alaska peat, hydrogenotrophic methanogens, *Methanomicrobiaceae* and *Methanobacteriaceae*, were predominant and hydrogenotrophic methanogenesis was the dominant methane production pathway (Rooney-Varga et al. 2007).

With the exception of *Methanosaeta* and *Methanosarcinaceae*, RC-II, *Methanomicrobiaceae*, and *Methanobacteriaceae* existed in Zoige wetlands, and many of the 16S rRNA gene sequences of these groups were clustered with sequences from fen, bog, and paddy soil (Hales et al. 1996; Lu and Conrad 2005; Cadillo-Quiroz et al. 2006). *Methanomicrobiaceae* and *Methanobacteriaceae* are considered as hydrogenotrophic methanogens. Therefore both hydrogenotrophic and acetoclastic methanogenesis occurred in this site. Crenarchaea was less prevalent in Zoige wetland than the other abovementioned archaea and grouped with sequences from paddy soil and sediments (Inagaki et al. 2006; Conrad et al. 2008; Peng et al. 2008). However, the function of these groups is unknown.

Our result agreed with previous reports that seasonal changes in archaeal community structure were small in rice fields and mires (Kruger et al. 2005; Juottonen et al. 2008), whereas it disagreed with Chen et al. (2008), who reported significant seasonal variation of CH_4 flux in both *C. muliensis* and *E. valleculosa* stands in the same region where we collected our samples. This inconsistency could be explained by several reasons. Firstly, changes in the activity rather than composition of the methanogenic community may be due to changes in sampling time. Similar results were also reported by Kruger et al. (2005). However, in Alaska peatland, Juottonen et al. (2008) reported that archaeal size and structure were similar in winter and summer. Secondly, the CH_4 flux does not only depend on methanogenesis but also on CH_4 oxidation at the ecosystem scale (Metje and Frenzel 2007). In Zoige wetland, probably CH_4 oxidation control CH_4 flux during growing season because methane oxidizer abundance and activity was controlled by soil water content (Yun et al. 2010), and Chen et al (2009a) reported that the key influence factor

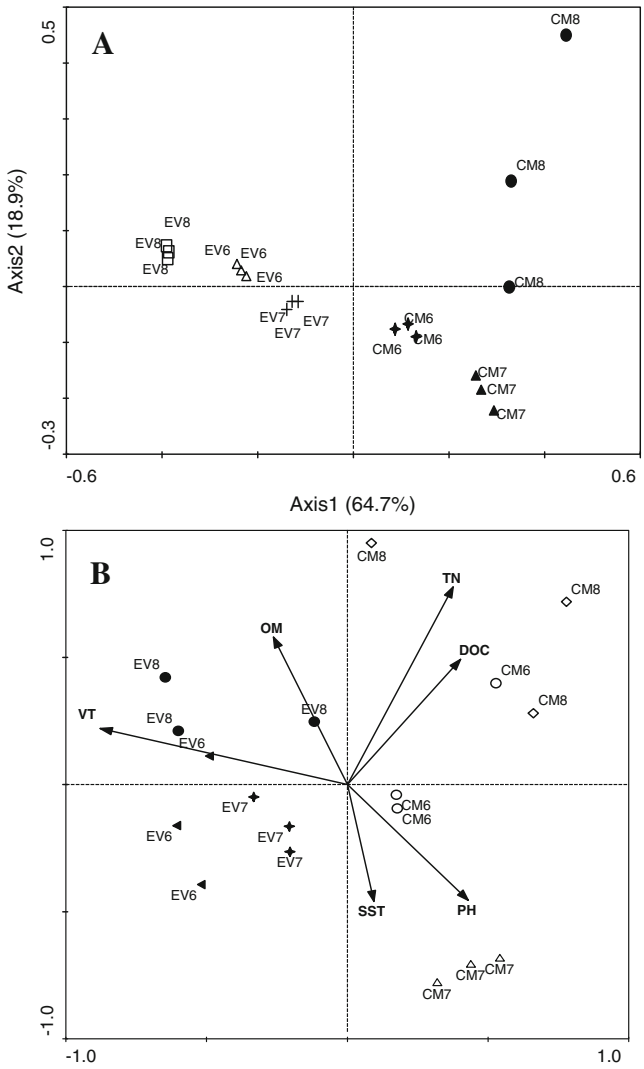

Fig. 3 **a** The ordination plot of CA of T-RFLP fingerprints of the archaeal community detected in soil beneath CM and EV. The *numbers* following CM and EV indicate the month of sampling (6= June, 7=July, and 8=August). **b** CCA ordination diagrams of the archaeal communities are determined by 16S rRNA gene T-RFLP. *Arrows* point to variables associated with community composition, and the *length* of the *arrows* indicates the percentage of data accounted for by that variable. The following abbreviations indicate environmental factors: *VT* vegetation type, *OM* organic matter content, *TN* total N, *DOC* dissolve organic C, *PH* plant height, *SST* surface soil temperature

of CH_4 flux was standing water table in the growing season of Zoige wetland.

Vegetation characteristics are likely to play an important role in biogeochemical activity (Rooney-Varga et al. 2007; Jiang et al. 2010). Our results suggested that composition of methanogenic communities was linked to vegetation type in Zoige wetlands. The composition of methanogenic communities of CM differed from that of EV probably because methanogenic community structure depends on nutrient availability (Galand et al. 2002, 2003). Nutrient availability differs in the rhizosphere of different vascular plants because different plant species produce root exudates that differ in quality and quantity (Bergman et al. 2000; Conrad et al. 2008). Root exudates are C, N, and energy sources for microorganisms. Root exudate-utilizing microbes thrive in summer but utilize recalcitrant dead plant material when plant activity ceases in winter (Lipson et al. 2002; Juottonen et al. 2008). Bergman et al. (2000) reported that different plant communities provided different quantities of substrates to methanogens and that substrate availability was associated with different seasonal changes in CH_4 production. Juottonen et al. (2008) also suggested that substrate quality or quantity may induce small fluctuations in activity and composition of archaeal communities.

The increase in plant biomass can enhance CH_4 production because the increased plant biomass means more C inputs to soil microorganisms including methanogens (Lindau et al. 1991; Banik et al. 1996). We have found that archaeal community composition was correlated with plant community height, which can be considered an indicator of plant biomass (Ding et al. 1999). To the best of our knowledge, this is the first report showing that plant height influences composition of methanogenic communities. Chen et al. (2008), at the same sampling site, reported that CH_4 flux was related to plant height. Over the plant growing season, increase in the organic matter decomposition can promote CH_4 production (Qin et al. 2010). Composition of microbial communities, including methanogenic archaea can be affected by the plant growth (Watanabe et al. 2010). Increase in plant is associated with increase in DOC (Lu et al. 2000a, b), and root-derived DOC is a major C source for methanogenic archaea (Wassmann and Aulakh 2000). Our data show that temperature was not related to methanogenic community structure, confirming what was reported in cold peatlands (Metje and Frenzel 2007).

In conclusion, our results revealed the prevalence of acetoclastic methanogen family *Methanosarcinaceae* and *Methanosaeta*, and showed a relationship between vegetation type (plant communities dominated by *C. muliensis* vs. *E. valleculosa*) and archaeal community composition, suggesting that plants (and/or environmental conditions controlling plant distribution) influence both archaeal community activity and dynamics, thus confirming results by Rooney-Varga et al. (2007). The height of the plant community was also related to archaeal community structure in Zoige wetland. Therefore, it may be possible to use vegetation type and plant height as predictors of methanogenic dynamics in wetland (Rooney-Varga et al. 2007).

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