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Shifts in the community composition of methane-cycling microorganisms during lake shrinkage

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ABSTRACT

Intensification of anthropogenic activities has resulted in shrinkage of lakes, which are important sources and sinks of the greenhouse gas methane. Given the increasing interest in the potential importance of CH_4 flux, we employed Illumina Miseq pyrosequencing to examine the effect of lake shrinkage (in a lake that had decreased from 3.7 km^2 to 3.0 km^2 in area during the last 20 years) on the community abundance, activity and structure of methane-cycling microorganisms under four types of soils during lake recession: Sediment (the sediment of the lake, at the maximum depth of 6 m), Slope (soil at a lake water depth of 0.6 m), Marsh (the seasonally flooded marsh and wetland formed after recession of the lake) and Meadow (the meadow formed after recession of the lake, about 200 m from the lake). The results showed that lake shrinkage decreased the relative abundance and transcripts of soil methanogens and methanotrophs along the gradient from Sediment to Slope to Marsh. Meadow, having the longest drying time, had both higher soil nutrient concentrations (including dissolved organic carbon, total carbon and total nitrogen), and higher relative abundance of 16S rRNA genes and transcripts of methanogens and methanotrophs. Results of methanogen community structure analysis indicated 'resistance' to change under lake shrinkage, with all communities dominated by Methanosaetaceae except the Slope community, which was dominated by Methanoregulaceae, while the dominant methanotroph changed from *Methylomicrobium* (a Type I methanotroph) (in Sediment, Slope and Marsh) to *Methylocaldum* (Type I) and *Methylosinus* (Type II) (in Meadow) under lake shrinkage. Taken together, the microbial community data indicate that CH_4 production and consumption potentially recedes under lake shrinkage, whereas the formation of Meadow may increase CH_4 emission and oxidization potential.

1. Introduction

Wetlands are important sources and sinks of the greenhouse gas methane, accounting for 62% of the global (natural) CH_4 budget (Conrad, 2009; IPCC, 2001). According to the Intergovernmental Panel on Climate Change (IPCC) (2007), natural wetlands emit 100–231 Tg of methane to the atmosphere annually, which contributes 20–39% of the global emission of methane (Denman et al., 2007; IPCC, 2007). CH_4 constitutes the second most significant greenhouse gas after CO_2, and it accounts for up to 20–30% of the global warming effect (IPCC, 2007).

With the climate change associated with global warming, and with human activities, the total number of large lakes (> 40 ha) decreased from 10,882 to 9172, a decline of 1170 or ~11%, between 1973 and 1997–98 (Smith et al., 2005). Data from a recent study has also shown that, between the 1960s and 1980s, and during 2005–2006, 243 lakes vanished in northern China (Ma et al., 2010). Such dynamic changes in lake area, if representatives of lakes, might strongly affect the global CH_4 budget. Although the recession of lakes has been studied worldwide by using remote sensing techniques (Qiao and Liu, 2006; Quincey et al., 2007), no study has estimated the influence on GHG (greenhouse gas) fluxes.

Microbes in the environment, including methane-producing archaea (methanogens) and methane-oxidizing microbes (methanotrophs), drive both global methane emissions and terrestrial sinks (Nazaries et al., 2013). Present evidence from lakes suggests that the majority of methane production in lakes occurs in anoxic sediment by methanogens (Harriss et al., 1988). The methane thus produced can be exported from the anoxic sediment by ebullition or by diffusion, and then eventually enters the water column. Once the methane reaches oxic sediment or water, a large proportion of it is oxidized by methanotrophs. Other

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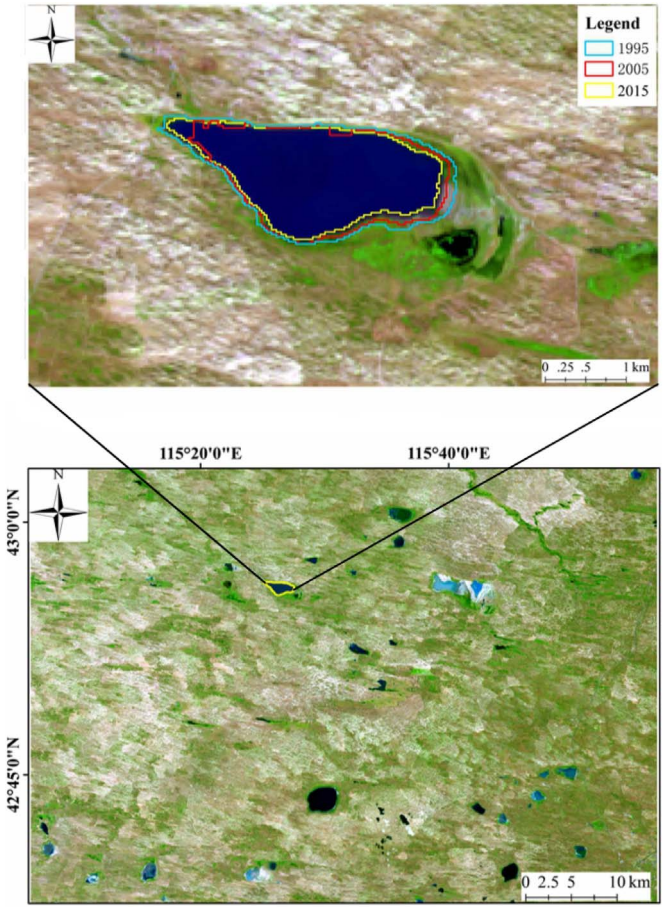

Fig. 1. Satellite image of Zhagsti Lake on 14 September 2015. Blue, red and yellow lines represent the extracted lake boundary for the years 1995, 2005 and 2015, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

methane escapes oxidation and is emitted to the upper layer of the water column and atmosphere (Bastviken et al., 2004). Methanogens are strictly anaerobic microbes, while methanotrophs occur often at the anoxic/oxic interface of various habitats (Peters and Conrad, 1996). Thus, we hypothesized that changes in water habitat resulting from lake recession might lead to changes in the community composition of *in situ* methane-cycling microorganisms as they adapt to the new conditions. Consequently, we also hypothesized that lake shrinkage might have negative effects on CH_4 emission, as anoxic areas decrease and oxic areas increase.

Along with the Arctic region, Antarctica and the Tibetan Plateau, the Mongolian Plateau (MP) is among the world's most sensitive regions to climate change (Sha et al., 2015). Remote sensing data have shown significant lake loss/shrinkage across the MP linked to warmer climate and intense human activities (Lu et al., 2015). In our study, we chose Zagsti Lake, which is located in central Inner Mongolia, as a representative atrophying lake to investigate. Zagsti is one of the lakes that has obviously receded (Fig. 1), and the ecological environment around the lake is less disturbed by human or animals. We investigated the changes in the pattern of methane-cycling microorganisms in the Zagsti lake ecosystem. To do this, we employed pyrosequencing targeting 16S rRNA genes and transcripts of methanogens and methanotrophs, and tested for correlations between the data and a range of environmental variables.

2. Materials and methods

2.1. Soil samples

Zhagsti Lake is located in the southern part of Hunshandake Desert (115°28′E, 42°55′N), with a total area of about 3.0 km^2. The maximum depth is 6 m and the average water depth is 3.5 m. Based on Landsat time series satellite imagery, the spatio-temporal variation of Zhagsti Lake from 1995 to 2015 was extracted using object-based image classification. Fig. 1 shows the shrinking landscape of Zhagsti Lake where our sampling survey was conducted. The soil is dominated by chestnut soil. The experimental design for the sampling of soil included six replicate samples at each of four sites (Fig. S1): (1) Sediment (the sediment of the lake, at the maximum depth of 6 m); (2) Slope (soil at a lake water depth of 0.6 m); (3) Marsh (the marsh and wetland formed after the recession of the lake, seasonally flooded); (4) Meadow (the meadow steppe formed after the recession of the lake, no flooding, about 200 m from the lake), which was the first area to dry up when the lake began to recede. Soil sampling was performed in May 2015. At each of the four sites, sampling was arranged in three randomized blocks with two replicates fully randomized within each block, giving a total of 24 experimental plots. Soil samples from each plot were ground to < 2 mm. To store soil for DNA and RNA analysis, we conserved sub-samples in RNA later. Molecular analysis was by high-throughput pyrosequencing and was undertaken at the Institute of Soil Science, Chinese Academy of Sciences. Sub-samples of soil for analysis of physiochemical properties were air-dried and analyzed for pH, TC (total carbon), TN (total nitrogen) and DOC (dissolved organic carbon).

2.2. Soil properties

Soil properties were determined as previously described (Murphy et al., 2005; Zhou et al., 2015). Total soil C and N (%) were determined simultaneously by the Dumas method using a LECO CN 2000. Soil DOC was extracted with 0.5 M K_2SO_4 and determined with a Shimadzu TOC-TN analyzer (Shimadzu Corp., Kyoto, Japan). Soil pH was determined in 1:2.5 (weight:volume) ratios of soil with distilled water using a Skalar SP10 automated pH system.

2.3. Soil nucleic acid extraction

Soil DNA was extracted from 0.5 g soil using the FastDNA spin kit for soil (Qbiogene, Inc., Irvine, CA) according to the manufacturer's protocol. The quality and abundance of the extracted DNA was measured by gel electrophoresis (0.8% agarose) and a NanoDrop spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE), and stored at − 20 °C.

RNA was extracted using the protocol of Griffiths et al. (2000), with the modification that the step using glass beads was performed twice. The RNA extraction method is detailed in Ding et al. (2015). RNA was purified by the RNeasy mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA contamination was ruled out by PCR amplification using the universal 16S rRNA gene primers (515F, 5′-GTGCCAGCMGCCGCGG-3′; 907R, 5′-CCGTCAATTCMTTTRAGTTT-3′) with purified RNA as a template. The total RNA was converted to complementary DNA (cDNA) using a PrimeScript 1st Strand cDNA Synthesis Kit (TaKaRa) and random hexamers, and was stored at − 80 °C for amplicon sequencing.

2.4. Pyrosequencing analysis of the total 16S rRNA genes and transcripts

The 16S rRNA genes of the V4 regions were analyzed by pyrosequencing on an Illumina MiSeq platform (PE 300). A slight modification from the protocol of Mettel et al., 2010 was made by PCR amplifying the 16S rRNA genes, with the universal primers 515F-GTGCCAGCMGCCGCGG and 907R-CCGTCAATTCMTTTRAGTTT extended