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Temporal variation of magnetotactic bacterial communities in two freshwater sediment microcosms

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Abstract

Magnetotactic bacteria (MTB), which can mineralize nanosized magnetite or greigite crystals within cells, play important roles in biogeochemical processes, for example iron and sulfur cycling, and depositional remanent magnetization acquisitions. Despite decades of research, the knowledge of MTB distribution and ecology is still limited. In the present study, we investigated the temporal variation of MTB communities in freshwater sediment microcosms based on 16S rRNA genes and UNIFRAC analyses. Two microcosms (MY8 and MY11) collected from two separate sites in Lake Miyun (Beijing, China) were analyzed. The majority of retrieved sequences belonged to alphaproteobacterial magnetotactic cocci in both microcosms (representing 64.29% of clones from MY8 and 100% of clones from MY11), whereas so-called '*Magnetobacterium bavaricum*'-like MTB affiliated within *Nitrospira* phylum were exclusively found in microcosm MY8. Over a 3-month period, the temporal variation of MTB communities was evident in both microcosms. In addition, the phylogenetic discrepancy of MTB communities between two microcosms is more prominent than that of the same microcosm at different times, implying adaptation of MTB phylogenetic lineages to specific microenvironments. Among the physical–chemical parameters measured, a strong correlation was shown between nitrate and the main genetic variability of MTB communities, indicating that nitrate may influence the occurrence of MTB phylogenetic lineages in natural environments.

Introduction

Magnetotactic bacteria (MTB) are ubiquitous in aquatic environments, for example marines and lakes. They can form intracellular nanosized magnetite or greigite crystals, known as magnetosomes, which are membrane bound and are generally organized into one or more chains (Schüler, 2008). The net magnetic moment of magnetosome chains can interact with the Earth's magnetic field and thus navigate MTB along local geomagnetic fields (magnetotaxis) (Faivre & Schüler, 2008). It is widely believed that the magnetotaxis in conjunction with aerotaxis and other chemotaxis can help MTB to efficiently locate and maintain the most optimal position in vertically stratified sediments or water columns (Frankel *et al.*, 1997; Pan *et al.*, 2009b). All currently known MTB belong to the *Proteobacteria* and *Nitrospira* phyla based on the comparison of 16S rRNA genes (Amann *et al.*, 2006).

MTB can play important roles in mediating some geochemical processes, for example iron and sulfur cycling (Simmons & Edwards, 2006). Moreover, fossil magnetosomes preserved in sediments are important natural remanent magnetization carriers (Chang & Kirschvink, 1989; Moskowitz *et al.*, 1993; Pan *et al.*, 2005a, b; Kopp & Kirschvink, 2008), and can serve as a potential proxy for paleoenvironmental reconstruction (Snowball *et al.*, 1999; Snowball *et al.*, 2002; Paasche *et al.*, 2004; Kopp & Kirschvink, 2008). Therefore, understanding the patterns of MTB communities in environments is of great importance. A handful of studies have examined the diversity and vertical distribution of MTB in a single location and have shown that the majority of MTB are usually close to the oxic–anoxic transition zone in chemically stratified aquatic habitats (Spring *et al.*, 1992, 1993; Bazylinski *et al.*, 1995; Bazylinski & Frankel, 2004; Simmons *et al.*, 2004; Flies *et al.*, 2005a; Pan *et al.*, 2008; Lin & Pan, 2009; Lin *et al.*,

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2009). However, due to the lack of detailed studies, the distribution of MTB communities between different locations and their temporal variations remain unclear (Spring *et al.*, 1994; Flies *et al.*, 2005b).

Our previous studies revealed that large amounts of MTB (up to 10^6 cells mL^{-1}) existed in sediments from Lake Miyun near Beijing, China, where the enriched MTB affiliated within both *Proteobacteria* and *Nitrospira* phyla (Lin *et al.*, 2008, 2009). In the present study, we used a combination of a cultivation-independent approach and UNIFRAC analysis to investigate the temporal variations of MTB in two freshwater sediment microcosms, which were collected from two separate sites in Lake Miyun, Beijing. The diversity and variation of MTB communities in two microcosms were also compared.

Materials and methods

Sample collection and microcosm setup

The MTB-bearing sediment samples used in this study were collected from two separate sites (MY8 and MY11) in the southern margin of Lake Miyun near Beijing, China (Fig. 1). At the time of sampling, site MY8 was not connected with site MY11 by water. Approximately 10 L of surface sediments (depth 5–10 cm) from each site were collected in 2008, which were transferred to 20-L aquaria and overlaid with lake water (microcosms) in a laboratory. The microcosms were loosely covered and stored in dim light at room temperature without disturbance.

Magnetic enrichment of MTB

MTB in the sediment were magnetically enriched using a double-ended open magnetic separation apparatus (MTB trap), which could simultaneously collect both north- and south-seeking MTB (Jogler *et al.*, 2009). Specifically, about 200 mL of surface sediments from each microcosm were scratched and directly transferred to the 'MTB trap' (500 mL in volume). A homogeneous magnetic field, about seven times that of the Earth's magnetic field, was applied for cell enrichment for 6 h. The retrieved MTB cells were then washed with sterile-distilled water twice and stored at − 20 °C until further processing. For the microcosm MY8, MTB were collected in 2009 on 26 February (MY8a), 18 March (MY8b) and 23 April (MY8c), respectively; for the microcosm MY11, MTB were collected in 2009 on 25 February (MY11a), 18 March (MY11b) and 24 April (MY11c), respectively.

Physical–chemical analyses

The oxygen concentrations of surface sediments in microcosms were determined using an HQ40d Oxygen Meter (HACH). Pore water was separated from the surface sediments by centrifugation at 1000 ***g*** for 20 min as described previously (Liu *et al.*, 2003). The pH of pore water was measured using a Mettler Toledo Delta 320 pH meter. Physical–chemical analyses of various anions and major cations were conducted at the Analytical Laboratory Beijing Research Institute of Uranium Geology, using a Dionex-500 chromatograph (BioPortfolio) and 785 DMP Titrino (Metrohm AG). The concentrations of total iron of pore water were measured using HR-ICP-MS (Finnigan MAT).

PCR amplification of 16S rRNA genes

PCR amplifications of nearly complete 16S rRNA genes of MTB were carried out using bacterial universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) based on the previous report (Lin *et al.*, 2008). The PCR amplification program consisted of 5 min at 95 °C, 30 cycles of 1.5 min at 92 °C, 1 min at 50 °C and 2 min at 72 °C; the final extension was carried out at 72 °C for 10 min. To avoid potential sample biases, duplicate PCR products for each sample were pooled

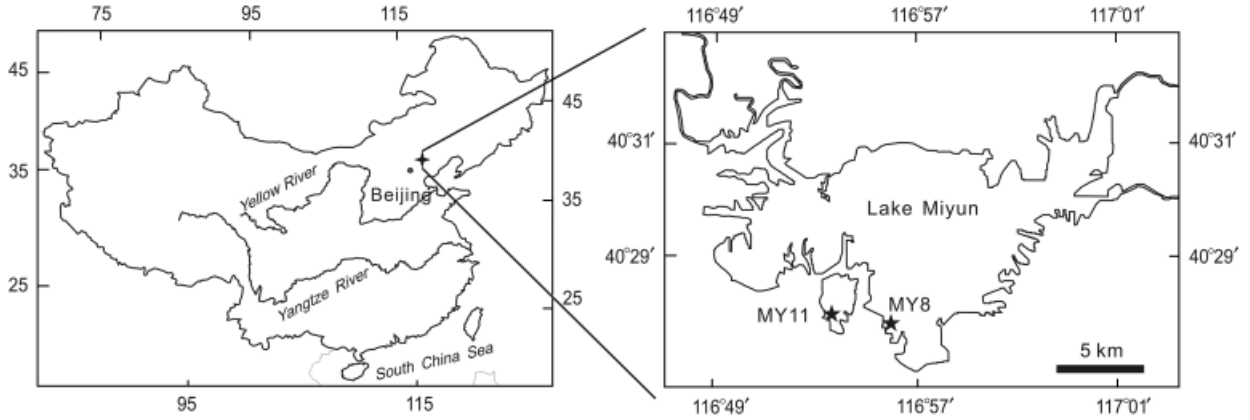

Fig. 1. Schematic diagrams showing the locations of sampling sites (MY8 and MY11) in the Lake Miyun near Beijing, China.

and then purified by 0.8% (w/v) agarose gel electrophoresis. PCR controls with no template were negative.

Construction of clone libraries, restriction fragment length polymorphism (RFLP) analysis and sequencing

Purified PCR products were cloned into the pMD19-T vector and chemically DH5α competent cells (TaKaRa) according to the manufacturer's instruction. A total of six 16S rRNA gene clone libraries (MY8a, MY8b, MY8c, MY11a, MY11b and MY11c) were constructed. Thirty positive clones from each library were randomly selected. The cloned inserts were amplified by PCR with the primers specific for the pMD19-T vector. The PCR products were analyzed by electrophoresis in 0.8% (w/v) agarose. Twenty false-positive clones were removed and in total 160 clones with the correct-size fragment were further screened by RFLP analysis with MspI and RsaI restriction endonucleases (MBI Fermentas) as described previously (Lin *et al.*, 2009). The resulting fragments were checked by gel electrophoresis in 3% (w/v) agarose in 1× Tris-acetate-EDTA buffer. Clones with identical patterns were defined as operational taxonomic units (OTUs). Representatives of each OTU were selected for sequencing of both strands (Beijing Genomics Institute, China).

Phylogenetic analysis

All successful sequences were submitted to the GenBank databases for comparison using the BLASTN algorithm (Benson *et al.*, 2005). They were also submitted to the SEQMATCH program of the ribosomal database project-II (RDP-II) to assess 16S rRNA gene taxonomy (Cole *et al.*, 2009). The sequences, which were not likely to belong to known MTB, might originate from non-MTB contaminations and were therefore excluded for further analysis. The occurrence of chimeric sequences was determined using the CHECK_CHIMERA program of the RDP-II (Cole *et al.*, 2009) and the BELLEROPHON server (Huber *et al.*, 2004). The remaining sequences were then aligned with their close relatives using CLUSTALW (Thompson *et al.*, 1994), and a phylogenetic tree was subsequently constructed with MEGA v4.0 using the neighbor-joining method (Tamura *et al.*, 2007). The robustness of tree topologies was verified by 100 bootstrap resamplings.

Statistical analyses

The unweighted UNIFRAC algorithm (Lozupone *et al.*, 2006, 2007) was used to compare MTB communities across the six clone libraries in this study. UNIFRAC considered the phylogenetic distance between taxa and could reflect the occurrence of distinct microbial lineages among different communities based on phylogenetic information. For the UNIFRAC analysis, a phylogenetic tree of 16S rRNA gene sequences of MTB retrieved in this study was generated by PHYLIP program (http://evolution.genetics.washington.edu/phylip.html) using the neighbor-joining method and exported as NEWICH format, which was submitted to the UNIFRAC web interface (http://bmf2.colorado.edu/unifrac/index.psp) with the environment file. Principal coordinates analyses (PCoA) and Jackknife environment clusters were performed to separate or group MTB communities (Lozupone *et al.*, 2007). A Jackknife environment cluster tree was projected using TREEVIEW software (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html). In order to correlate the physical–chemical factors with the main component of the genetic variability of MTB (PC1 factor of PCoA), Pearson's correlations were computed using SPSS software v13.0 (SPSS Inc., Chicago).

Nucleotide sequence accession numbers

The 16S rRNA gene sequences of MTB acquired in the present study had been deposited in the GenBank/EMBL/DDBJ databases under accession numbers GQ468507–GQ468519.

Results

Characteristics of pore water

The results of pH, temperature, oxygen and the concentrations of anions and cations of pore water of six samples from two microcosms are summarized in Table 1. The pH of each microcosm ranged from 7.35 to 7.64, suggesting that all samples were circumneutral or slightly alkaline. The temperature ranged from 15 to 17 °C. The concentrations of oxygen in surface sediments in which MTB were enriched were 0.29 and 0.10 mg L^{-1}, respectively, for microcosms MY8 and MY11 in April, indicating microaerobic conditions. Overall, the concentrations of most anions and cations of MY8 decreased over time, and yet the corresponding changes of MY11 were rather irregular. MY8a had higher concentrations of Cl^- (18.8 μg mL^{-1}), Na^+ (24.5 μg mL^{-1}), K^+ (4.25 μg mL^{-1}), Mg^{2+} (20.5 μg mL^{-1}) and iron (626 μg L^{-1}) than the other samples, whereas MY11c was highly enriched in SO_4^{2-} (128 μg mL^{-1}) and Ca^{2+} (42.4 μg mL^{-1}). The concentrations of NO^{3-} of MY8 (0.39–0.74 μg mL^{-1}) were higher than that of MY11 ($\leq$ 0.24 μg mL^{-1}). The concentrations of F^- were relatively constant for all samples.

Composition of MTB clone libraries

Thirteen OTUs were identified from a total of 132 clones after eliminating the putative non-MTB contaminations (23 clones) and putative chimeras (five clones). 16S rRNA genes from microcosm MY8 (libraries MY8a, MY8b and MY8c)

could be divided into five OTUs, as follows: OTU 8 (58.57% of the total clones), OTU 1 (35.71%), OTU 2 (2.86%), OTU 29 (1.43%) and OTU 50 (1.43%) (Fig. 2a). The average distance between these OTUs was 15%, and all sequences were $\leq$ 94% identical. All OTUs except OTU 1 were within the *Alphaproteobacteria* and most related to magnetotactic coccus strains (Fig. 3). OTU 2 was the closest relative to *Magnetococcus* clone CF22 recovered from a freshwater habitat in Northern Germany (Flies *et al.*, 2005b) with 97.25% similarity. OTU 8 and 50 were 96.64% and 97.38%, respectively, similar to *Magnetococcus* clone CF2, which was detected in lake 'Waller See' in Bremen (Flies *et al.*, 2005a).

Table 1. Chemistry of pore water collected from microcosms MY8 and MY11 at different times

	pH	Temperature (°C)	Oxygen ($mg\,L^{-1}$)	F^- ($\mu g\,mL^{-1}$)	Cl^- ($\mu g\,mL^{-1}$)	NO_3^- ($\mu g\,mL^{-1}$)	SO_4^{2-} ($\mu g\,mL^{-1}$)	Na^+ ($\mu g\,mL^{-1}$)	K^+ ($\mu g\,mL^{-1}$)	Mg^{2+} ($\mu g\,mL^{-1}$)	Ca^{2+} ($\mu g\,mL^{-1}$)	Total iron ($\mu g\,L^{-1}$)
MY8a (February)	7.35	15	NM	0.33	18.8	0.74	91.4	24.5	4.52	20.5	39	626
MY8b (March)	7.64	17	NM	0.29	6.67	0.56	41.1	12.3	2.14	11.7	26.2	42.1
MY8c (April)	7.59	17	0.29	0.26	4.95	0.39	72.9	5.8	1.53	7	17.5	BD
MY11a (February)	7.44	15	NM	0.35	6.27	0	97.3	8.3	3.18	15.9	34.4	9.39
MY11b (March)	7.61	17	NM	0.35	7.25	0	63.4	4.7	2.48	10.9	25.9	BD
MY11c (April)	7.43	17	0.1	0.33	8.45	0.24	128	8.3	3.32	18.1	42.4	BD

NM, not measured; BD, below detection.

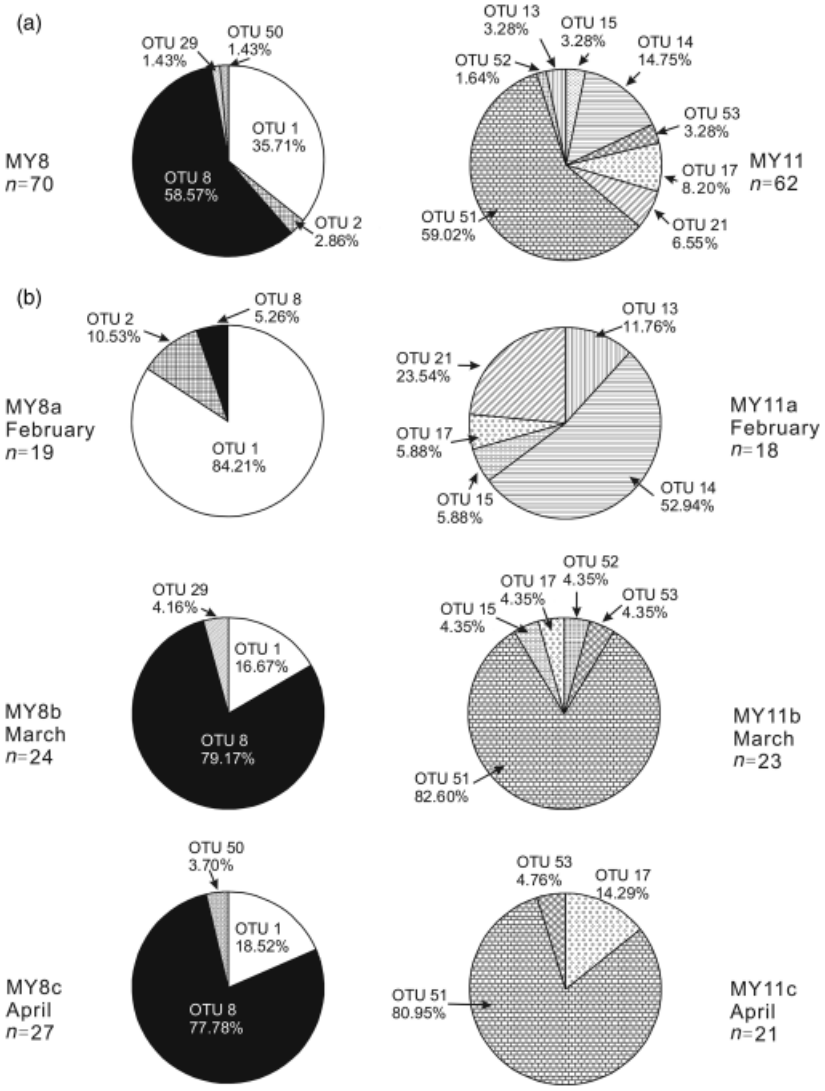

Fig. 2. Distributions of 16S rRNA gene sequences of MTB in clone libraries. (a) The overall distributions of sequences for combined libraries from microcosms MY8 and MY11, separately; (b) the distributions of sequences in each clone library collected at different times.

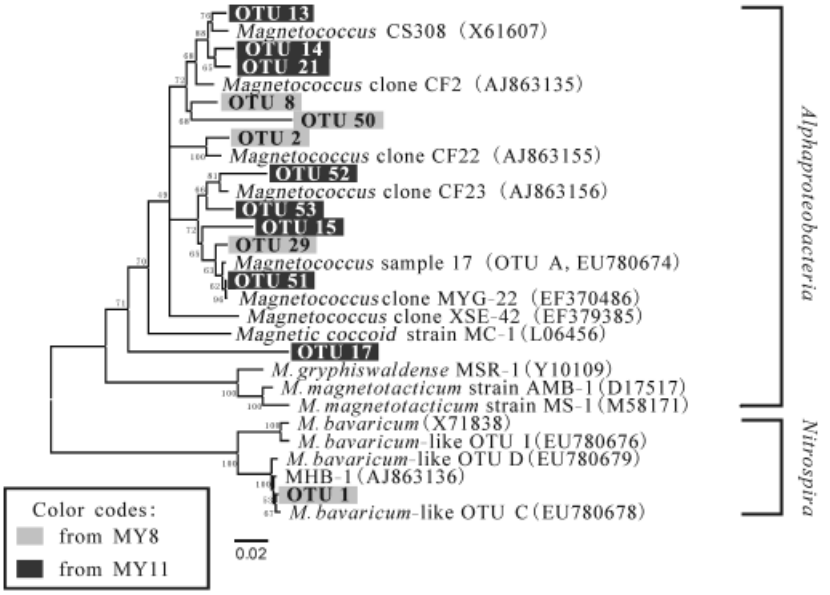

Fig. 3. Neighbor-joining phylogenetic tree of MTB 16S rRNA genes retrieved in this study and the related sequences collected from the GenBank database. The percentages of bootstrap replicates are indicated at the nodes.

OTU 29 was found to share high similarity (98.36%) to *Magnetococcus* clone MYG-22, which was previously recovered from the same place (Lin *et al.*, 2008). Phylogenetic analysis of OTU 1 had shown that it clustered within the *Nitrospira* phylum and was 99.53% similar to the '*Magnetobacterium bavaricum*'-like clone OTU C (Lin *et al.*, 2009).

Eight OTUs were identified from microcosm MY11 (libraries MY11a, MY11b and MY11c). OTU 51 was encountered most frequently and represented 59.02% of the total clones (Fig. 2a). The other OTUs included OTU 13 (3.28%), OTU 14 (14.75%), OTU 15 (3.28%), OTU 17 (8.20%), OTU 21 (6.55%), OTU 52 (1.64%) and OTU 53 (3.28%, Fig. 2a). All OTUs from microcosm MY11 were affiliated with *Alphaproteobacteria* and showed $\leq$ 98% similar (Fig. 3). OTUs 13 and 14 had 97.47% and 96.92% sequence identities, respectively, with magnetotactic coccus CS308 (Spring *et al.*, 1992). OTUs 52 and 53 were closely related to *Magnetococcus* clone CF23 (98.76% and 97.74%, respectively) (Flies *et al.*, 2005b). OTUs 15, 17 and 21 were 96.85%, 89.04% and 97.06% identical to *Magnetococcus* clones MYG-22, XSE-42 and CF2, respectively. Furthermore, OTU 51 was found to share high identity (99.66%) to *Magnetococcus* clone OTU A, which was recovered from the same site previously (Lin *et al.*, 2009).

Although the majority of clones in microcosm MY8 (64.29%) and all clones in microcosm MY11 belonged to alphaproteobacterial magnetotactic cocci, no identical OTU was found between them. The most related OTUs from MY8 and MY11 were OTU 29 and OTU 51 with 98.89% similarity. Other OTUs from MY8 showed $\leq$ 97% similar to that from MY11 (Fig. 3).

Temporal variation of MTB communities

The communities of MTB within each microcosm did vary from February to April (Fig. 2b). For microcosm MY8, although '*M. bavaricum*'-like OTU 1 was most dominant in MY8a (84.21%), it dramatically decreased in March and April, and only left 16.67% and 18.52% in the libraries MY8b and MY8c, respectively. OTU 8 comprised 5.26% of MY8a; however, it significantly increased to 79.17% and 77.78% in MY8b and MY8c, respectively, and became the most dominant group. OTUs 2, 29 and 50, on the other hand, were time specific. For microcosm MY11, OTU 14 was the dominant group in MY11a (52.94%), but it was not observed in MY11b and MY11c (Fig. 2b). In contrast, OTU 51, not detected in MY11a, became the most dominant OTU in MY11b (82.60%) and MY11c (80.95%). OTU 17 was relatively evenly distributed over time (4.35–14.29%). OTU 15 was detected only in MY11a (5.88%) and MY11b (4.35%), while OTU 53 was only found in MY11b (4.35%) and MY11c (4.76%). Other OTUs were time specific, for example OTUs 13 and 21 were solely observed in MY11a and OTU 52 was specifically detected in MY11b.

UNIFRAC and statistical analyses

The MTB communities in six clone libraries were compared using unweighted UNIFRAC analysis. The PCoA plot showed that MTB clustered by microcosms rather than collection time (Fig. 4a). Samples from microcosm MY11 clustered together to the left along PC1, which accounted for 66.7% of the variation, while samples from microcosm MY8 grouped to the right. This result was supported by Jackknife environment clusters with high Jackknife values (Fig. 4b). Pearson's

correlation analysis between the unweighted PC1 factors and the physical–chemical variables demonstrated that the former significantly correlated with the concentrations of NO_3^- (Table 2, $P < 0.05$).

Discussion

Because few efforts have been made to explore the distribution and ecology of MTB, so far, knowledge on spatiotemporal variations of MTB communities is scarce. In the present study, a combination of a molecular approach, UNIFRAC analysis of phylogenetic data and Pearson's correlation analysis of two freshwater sediment microcosms provides an insight into the dynamics of MTB communities in nature.

16S rRNA gene analysis shows that the majority clones of both microcosms MY8 and MY11 belong to magnetotactic cocci within *Alphaproteobacteria* (64.29% of clones from MY8 and all clones from MY11), which is normally the dominant type of MTB found in most freshwater and marine environments (Amann *et al.*, 2006; Lin & Pan, 2009; Pan *et al.*, 2009a). The presence of '*M. bavaricum*'-like MTB, confirmed by our previous observation in Lake Miyun (Lin *et al.*, 2009), is only detected in microcosm MY8 (Fig. 3). We find that the diversity of MTB in each microcosm is not stable within 3 months, and it shows rapid short-term variations (Fig. 2b). The changes are especially prominent between February and March for both microcosms. Considering their incubation in the laboratory without disturbance, these results suggest that the MTB population is very sensitive to the imperceptible changes in microenvironments. Our results are consistent with the previous report that the MTB community was found to be dynamic during long-term incubation in one microcosm (Flies *et al.*, 2005b). Together, MTB communities are microenviroment-sensitive and thus potential proxies for changes of ecology and climate. However, because of only three individual samples from each microcosm, we lack the statistical power to determine correlations between measured physical–chemical factors and the dynamics of MTB communities over time. Therefore, at this stage, we cannot determine the specific factors that influence the observed temporal variation in MTB communities.

As evident in Fig. 4, the UNIFRAC analysis clearly shows that the six MTB communities cluster by the microcosm rather than by the collection time, indicating that the phylogenetic discrepancy of MTB communities collected from distinct microcosms exceeds the temporal variation in each microcosm. Because the microcosms were collected from two separate sites in Lake Miyun (Fig. 1), the above results suggest a potential adaption of different MTB lineages to their respective microenvironments. This is also supported by the distributions of MTB OTUs in clone libraries, as shown in Fig. 2, that no identical OTU is observed between the two microcosms and '*M. bavaricum*'-like MTB exclusively exist in microcosm MY8.

A significant correlation between the phylogenetic distance of MTB communities from the six clone libraries and nitrate concentrations of corresponding pore water is noted here (Table 2). Petermann & Bleil (1993) reported that nitrate or other nitrous oxides could be reduced by most MTB in deep marine environments and might contribute to their vertical distribution, which was supported by observations that the majority of cultivated MTB could utilize nitrous compounds as terminal electron acceptors for respiration (Flies *et al.*, 2005a). A similar situation is expected for uncultivated '*M. bavaricum*'-like MTB as well, because the phylogenetic nonmagnetic relatives of these MTB in *Nitrospira* phylum are nitrite-oxidizing bacteria that can oxidize nitrite to nitrate in environments (Daims *et al.*, 2001). Together, these results suggest that nitrate may play an important role in the occurrence and distribution of MTB lineages in distinct microenvironments.

Because the measurements of oxygen and iron are rudimentary in this study, we are not able to run statistical analyses for these factors; therefore, their contributions are unknown. In addition, biotic factors, such as competition, antagonistic interaction and predation that can influence the shifts in bacterial communities (Janse *et al.*, 2000; Czárán *et al.*, 2002; Kirkup & Riley, 2004; Sestanovic *et al.*,

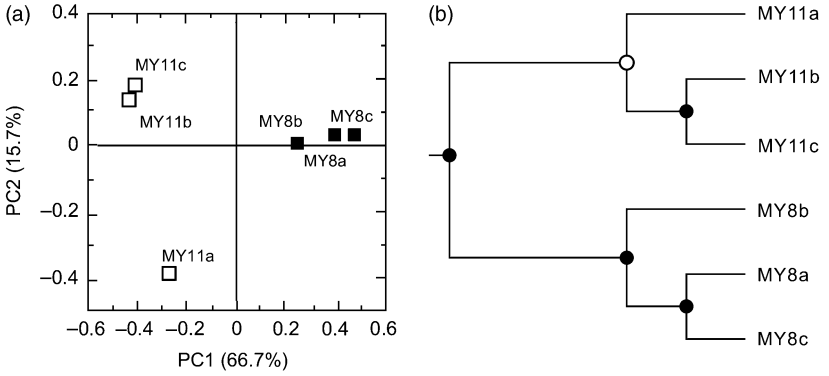

Fig. 4. PCoA (a) and Jackknife environment clusters (b) of MTB sequences among six clone libraries as calculated using unweighted UNIFRAC analysis. Circles represent Jackknife support at the nodes in (b). ●, > 99.9%; ○, > 70.0%.

Table 2. Pearson's correlations showing relationships between physical–chemical parameters and PC1 factors that resulted from PCoA in Fig. 4a

	Pearson's correlation (Significant *P*)									
	pH	Temperature	F^-	Cl^-	NO_3^-	SO_4^{2-}	Na^+	K^+	Mg^{2+}	Ca^{2+}
PC1 factors	0.060 (0.909)	−0.115 (0.829)	−0.761 (0.079)	0.293 (0.573)	0.824 (0.044)*	−0.406 (0.425)	0.510 (0.301)	−0.120 (0.820)	−0.207 (0.694)	−0.400 (0.432)

*Correlation is significant at the 0.05 level.

2004; Brussaard *et al.*, 2005), are not considered either. Based on the present study, the variations of particular microorganisms, such as ammonia-oxidizing microorganisms and nitrite-oxidizing bacteria that can influence the concentrations of nitrate in sediments (Daims *et al.*, 2001), may be responsible for the distribution and variation of MTB communities over location and time. Therefore, further studies are necessary to better understand the mechanisms of variation of MTB communities by more extensive sampling efforts and monitoring more abiotic and biotic factors, not only in microcosms but also in field studies.

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