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# Microbial Community Structure and Activity Linked to Contrasting Biogeochemical Gradients in Bog and Fen Environments of the Glacial Lake Agassiz Peatland

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**The abundances, compositions, and activities of microbial communities were investigated at bog and fen sites in the Glacial Lake Agassiz Peatland of northwestern Minnesota. These sites contrast in the reactivity of dissolved organic matter (DOM) and the presence or absence of groundwater inputs. Microbial community composition was characterized using pyrosequencing and clone library construction of phylogenetic marker genes. Microbial distribution patterns were linked to pH, concentrations of dissolved organic carbon and nitrogen, C/N ratios, optical properties of DOM, and activities of laccase and peroxidase enzymes. Both bacterial and archaeal richness and rRNA gene abundance were >2 times higher on average in the fen than in the bog, in agreement with a higher pH, labile DOM content, and enhanced enzyme activities in the fen. Fungi were equivalent to an average of 1.4% of total prokaryotes in gene abundance assayed by quantitative PCR. Results revealed statistically distinct spatial patterns between bacterial and fungal communities. Fungal distribution did not covary with pH and DOM optical properties and was vertically stratified, with a prevalence of *Ascomycota* and *Basidiomycota* near the surface and much higher representation of *Zygomycota* in the subsurface. In contrast, bacterial community composition largely varied between environments, with the bog dominated by *Acidobacteria* (61% of total sequences), while the *Firmicutes* (52%) dominated in the fen. Acetoclastic *Methanosarcinales* showed a much higher relative abundance in the bog, in contrast to the dominance of diverse hydrogenotrophic methanogens in the fen. This is the first quantitative and compositional analysis of three microbial domains in peatlands and demonstrates that the microbial abundance, diversity, and activity parallel with the pronounced differences in environmental variables between bog and fen sites.**

Peatland ecosystems are generally classified into ombrotrophic (rain-fed) bogs and minerotrophic (groundwater-fed) fens, depending mainly on trophic status and water source (12). These ecosystems accumulate carbon in the form of a partially decomposed peat layer. The important role of peatlands in global carbon sequestration (78) and emission of greenhouse gases (38) is well established, but the response of these ecosystems to changing climate is still uncertain due to inherent spatial and temporal heterogeneity as well as the complex interplay of hydrological and biogeochemical processes (52).

Despite the important role of peatlands in the global carbon cycle, microbial communities in these environments remain understudied. Peats have been gradually recognized as a home to diverse microbial assemblages primarily through cultivation-based studies (29). Modern molecular techniques have begun to be employed to explore microbial community structure in peatlands. However, the focus of this research was restricted largely to specific functional guilds of microorganisms, such as methanogens (7, 65), methanotrophs (32), fermenters (34), sulfate reducers (60), or microbes involved in nitrogen cycling (76). To date, cultivation-independent studies of peatland microbial diversity have provided only a limited phylogenetic resolution and depth of coverage due to practical limitations of the techniques used, e.g., terminal restriction fragment length polymorphism (T-RFLP) and clone library construction (2, 8, 15, 20, 44, 55, 58, 61, 73). Studies of fungi, for example, have suffered from the lack of a high-quality curated database for taxonomic assignment (6).

Walker et al. and others proposed that climate change, partic-

ularly warming, is likely to alter the vegetation balance in northern terrestrial biomes (74). Grasses, such as sedges, and other vascular plants (*Carex*, *Eriophorum*) will be favored and expand in biomass and areal extent, while bryophytes (mosses, such as *Sphagnum*) will likely decrease in areal extent and importance. These changes could have important ramifications for the carbon cycle, since plant species composition is thought to intimately drive microbial carbon turnover. For example, geochemical evidence indicates that the reactivity of organic matter, and therefore its susceptibility to microbial degradation, is dependent upon plant species composition in peatlands (11, 14). Dissolved organic matter (DOM) produced in *Sphagnum* woody plant-dominated bogs is significantly more recalcitrant and aromatic in character than is DOM within vascular plant (sedge)-dominated fens (70). In this study, we sought to compare the microbial ecology of a bog and fen as an analogous scenario to plant species transitions that are projected to occur during climate change. We provide a comprehensive investigation of peatland microbial communities that is closely coupled to geochemical investigations focused on the car-

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bon cycle. Here, we show that the shift of microbial abundance, community composition, and activity parallels with the stark differences in pH and DOM reactivity observed at a bog and fen in the Glacial Lake Agassiz Peatland (GLAP) of northern Minnesota.

## MATERIALS AND METHODS

**Sampling and chemical characterization.** Field sampling was conducted within the Glacial Lake Agassiz region of northwestern Minnesota. Approximately 56% (or nearly 5,000 km<sup>2</sup>) of this region is covered by peatlands, which average 2 to 3 m in depth and were established approximately 5,000 years ago in response to climatic cooling (30). Four peat cores were sampled on 11 August 2009 in the Red Lake II raised bog complex, whose landform patterns are similar to those found in other large peat basins (30). The distance between the bog and fen site was approximately 5 km. The water table was at 0 m. Two cores were from a bog, which is an acidic and nutrient-deficient environment that receives water inputs primarily from precipitation and is covered by *Sphagnum* moss. The remaining two cores were taken from a minerotrophic fen, which is influenced by inputs of more alkaline, nutrient-rich groundwater and is dominated by sedges. Cores from each site were sectioned into surface (0 to 10 cm) and subsurface (40 to 50 cm) samples.

Fen and bog pore water samples were also collected at the same depths as the peat samples (0 to 10, 40 to 50 cm) using piezometers and a peristaltic pump. Experimental approaches for sample handling and the determination of dissolved organic carbon (DOC) and organic nitrogen were described previously (11, 71). Optical properties of chromophoric DOM (CDOM) were determined using absorption and fluorescence spectroscopy as described in reference 71. Two optical properties ( $E_2/E_3$  ratio and  $SUVA_{254}$ ) were included here to indicate their link with shifts in microbial community composition. The  $E_2/E_3$  ratio of absorbance at 250 to 365 nm is used to estimate DOM molecular size.  $E_2/E_3$  decreases as molecular size increases, because high-molecular-weight DOM absorb at a longer wavelength (21). The  $SUVA_{254}$  (liters mg C<sup>-1</sup> m<sup>-1</sup>) is calculated by normalizing the UV absorbance at 254 nm by the concentration of DOM.  $SUVA_{254}$  correlates with the fraction of aromatic compounds contained in CDOM (77).

Assays for polyphenolic oxidase enzyme activity followed the protocol described in reference 26, with addition of H<sub>2</sub>O<sub>2</sub> to determine total potential activities of both polyphenolic oxidases and peroxidases. Briefly, 1 g of wet peat was suspended in 9 ml of acetate buffer (10 mM, pH 4) at 4°C and blended for 5 min to release adsorbed enzyme in suspension. Enzyme reactions began by mixing the soil suspension with 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS; final concentration of 5 mM) in the buffer (pH 4) and incubating in the dark at 4°C for 10 min. To stop the reaction, the mixture was centrifuged at 10,000 rpm for 10 min. The absorbance of oxidized ABTS was detected with a spectrophotometer at 420 nm.

**DNA extraction, PCR amplification, pyrosequencing, and qPCR.** Total genomic DNA was extracted from 0.5 g peat soil per sample (in triplicate) using a MoBio PowerSoil DNA extraction kit (MoBio, Carlsbad, CA) by following the manufacturer's protocol. The extracted DNA was pooled, amplified, and sequenced at the Emory University Genomics Facility (Atlanta, GA) using the standard pyrosequencing protocol (Roche 454, Branford, CT). PCR amplification of the bacterial small-subunit (SSU) rRNA gene and fungal internal transcribed spacer (ITS) regions of the rRNA gene was performed using the primer pairs of 27F/534R (43) and ITS1F/ITS4 (28), respectively. Targeted gene fragments were amplified in 50- $\mu$ l reaction mixtures containing 5 PRIME Master-Mix and 10 ng genomic DNA. Thermal cycling was performed by following the manufacturer's protocol with annealing temperature at 55°C for 35 cycles. Real-time PCR was performed to quantify the abundance of total bacterial and archaeal SSU rRNA genes according to the procedures described elsewhere (40). The original genomic DNA was diluted to 1:40 to remove inhibition effects. Fungal quantitative real-time PCR (qPCR) was performed following the protocol described in reference 62. Standard

curves were created with PCR amplicons from genomic DNA of an *Ascomycoota* isolate from GLAP using primers EukA and EukB (49).

**Clone library construction.** Construction of clone libraries for archaeal SSU rRNA gene sequences was based on PCR products amplified using primers ARC344F and ARC915R (63). PCR products were purified with a MinElute PCR purification kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions. The amplicons were cloned using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA). Colonies were picked into 0.85% NaCl and sent to Sequetech for plasmid isolation and sequencing. Sequence processing and phylogenetic analysis followed the procedures described elsewhere (47).

**Processing of pyrosequencing data and diversity estimation.** All 454 sequencing data were analyzed in QIIME (9). The sequencing reads were first binned and filtered to remove low-quality sequences, which included those sequences of <200 bp in length with an average quality score of <25 and containing ambiguous characters or a homopolymer run exceeding 6 nt. Sequences without the correct primer sequence were excluded. A workflow script in QIIME was used to pick operational taxonomic units (OTUs) at the 97% sequence identity level through building OTU tables. For bacterial SSU rRNA gene sequences, the PyNAST-aligned representative sequences were used to identify chimeric sequences using ChimeraSlayer in QIIME. Chimera check of fungal ITS gene sequences was done with UCHIME (23) in a *de novo* mode. Taxonomy was assigned to each representative sequence by using BLAST with a maximum E value of 0.001 against the Greengenes core set for bacteria and using the QIIME-compatible UNITE database for fungi ([http://qiime.org/home\\_static/data\\_files.html](http://qiime.org/home_static/data_files.html)). For all OTU-based analyses, the original OTU table was rarefied to depths of 44,576 bacterial sequences and 774 fungal sequences per sample, to minimize the effects of a different sampling effort in comparing alpha- and beta-diversity across the samples.

**Statistical analyses.** Microbial diversity expressed as Chao1 richness and equitability was calculated in QIIME based on the OTU tables rarefied to the same sampling depths. Pairwise comparisons of microbial community structure were computed as Bray-Curtis distances and were visualized using principal coordinate analysis in PRIMER version 6. Pearson correlations between the OTU abundance matrix and the environmental matrix shown in Table 1 were calculated with PRIMER Permanova+. Environmental parameters having their Pearson correlation with the first two PCO axes larger than >0.5 were displayed as vectors in the principal coordinate analysis. Mantel tests by RELATE in PRIMER were performed to calculate the significance of fit between the community similarity matrix and distance matrices of each environmental variable with 1,000 permutations. A distance-based multivariate linear model (DistLM) was performed to select the best combination of predictor variables explaining the community variation, with significance tests by permutation.

Comparison of spatial patterns in bacterial and fungal distribution was assessed using a Procrustean analysis at the overall degree of association between bacterial and fungal OTU abundance matrices. Peres-Neto and Jackson demonstrated the advantages of the procrustean approach over the Mantel test for detecting matrix association (59). Procrustes analysis was carried out in QIIME, which outputs the correlation value  $M^2$  ( $1 - r^2$ , where  $r$  is Pearson correlation coefficient) and  $P$  values indicating significance levels. Smaller values of  $M^2$  indicate higher concordance between two data matrices.

**Nucleotide sequence accession numbers.** Archaeal sequences from this study are available in GenBank (JQ807525 to JQ807565).

## RESULTS

**Pore water geochemistry, enzyme activity, and rRNA gene abundance in peat soils.** The bog and fen sites showed largely contrasting geochemical conditions linked to plant species composition (Table 1). Bog pore waters were more acidic (pH of ~4) than the fen (pH of ~6) and accumulated approximately twice as much DOC in comparison to that of the fen. Dissolved organic nitrogen content was about 10% higher in the bog, and it de-

TABLE 1 Biogeochemical comparison between the bog and fen environments<sup>a</sup>

Site	Depth (cm)	pH	Amt			C/N ratio	E <sub>2</sub> /E <sub>3</sub>	SUVA <sub>254</sub>	Enzyme activity (μmol/g/h)	Abundance of bacteria and archaea	% of archaea	Abundance of fungi
			DOC (mM)	DON (μM)	Ammonia (μM)					(×10 <sup>8</sup> )/g	(×10 <sup>6</sup> )/g	
Bog	0–10	4.2	5.6	59.7	14	93.3	4.8 ± 0.1	4.6 ± 0.1	3.0 ± 0.8	2.5 ± 0.4	1.2 ± 0.4	4.8 ± 0.7
Bog	40–50	4.3	6.2	44.1	94.2	139.6	5.3 ± 0.1	4.0 ± 0.0	2.3 ± 0.8	1.9 ± 0.3	4.1 ± 0.8	2.3 ± 0.4
Fen	0–10	5.7	2.9	53.7	11.6	53.9	6.9 ± 0.3	3.0 ± 0.0	22.6 ± 3.4	6.9 ± 0.7	1.3 ± 0.9	14.0 ± 0.8
Fen	40–50	5.9	3.6	39.6	53.9	90.6	7.3 ± 2.5	3.3 ± 0.1	19.1 ± 1.9	6.0 ± 0.4	10.5 ± 1.3	3.4 ± 0.5

<sup>a</sup> Numbers following “±” indicate standard deviations. All assays associated with peats are normalized to the wet soil weight. The last 3 columns are total prokaryotic rRNA gene abundance, percentages of archaeal rRNA gene abundance, and fungal 18S rRNA gene abundance.

creased with depth at both sites. Ammonia concentrations increased by a factor of 5 from the surface to the subsurface at both sites and did not substantially differ between sites.

Several independent parameters indicated that DOM was more recalcitrant in bog pore waters. The C/N ratio was about 1.6 times lower in the fen than in the bog for the same depth, and the bog subsurface showed the highest C/N ratio. The difference in DOM quality between sites was further reflected in its optical properties: E<sub>2</sub>/E<sub>3</sub> ratio and SUVA<sub>254</sub>. Lower E<sub>2</sub>/E<sub>3</sub> ratios in the bog are representative of a higher-molecular-weight DOM relative to the fen. In addition, the SUVA<sub>254</sub> was higher in the bog than in the fen, suggesting that the bog DOM is more aromatic in nature than that in the fen.

The sum of phenol oxidase and peroxidase activity was about 8 times higher on average in the fen than in the bog, and the activity declined about 19% on average to the subsurface (Table 1). The spatial variation between the enzyme activity and rRNA gene abundance was correlated. The abundance of prokaryotic rRNA genes was 2.5 × 10<sup>8</sup> and 1.9 × 10<sup>8</sup> per gram of wet soil in the bog surface and subsurface, respectively. SSU rRNA gene abundance was about 3 times lower in the bog than in the fen. The proportion of archaea was >3 times higher in the subsurface than at the surface of both sites, with the lowest percentage (1.2%) in the bog surface and the highest (10.5%) in the fen subsurface. Fungal rRNA gene abundance comprised on average 1.4% of the total prokaryotic rRNA gene abundance. At the surface, fungal rRNA gene abundance was 2.9 times higher in the fen than in the bog. Fungal gene abundance declined with depth at both sites.

**Microbial species richness and evenness.** Microbial diversity was determined based on the analysis of 215,928 bacterial SSU rRNA gene sequences, 10,609 fungal ITS sequences, and 181 archaeal SSU rRNA gene sequences (Table 2). Microbial richness and evenness were estimated by using Chao1 and equitability (1 = complete equitability). Bacterial and archaeal species richness was

more than twice as high in the fen as in the bog site for both depths. Fungal richness was highest in the bog surface, followed by the fen site, and was lowest in the bog subsurface. Similar patterns in microbial evenness were observed for these samples, with the equitability ranging from 0.39 (fungi in the bog subsurface) to 0.93 (archaea in the fen subsurface).

**Microbial community composition.** Bacteria were diverse in the peat soils, and their OTUs were affiliated with a total of 53 phyla (Fig. 1; see also Table S1 in the supplemental material), with the most abundant 10 phyla including (in order of decreasing abundance) *Acidobacteria*, *Firmicutes*, *Proteobacteria*, *Verrucomicrobia*, *Chloroflexi*, *Planctomycetes*, *Actinobacteria*, *Bacteroidetes*, *Chlorobi*, and TM7 (Fig. 1). Bacterial community compositions were markedly different between bog and fen sites. The bog and fen environments were dominated by *Acidobacteria* and *Firmicutes*, respectively. The 20 most abundant bacterial OTUs were affiliated with *Acidobacteria* group 1 (dominant in the bog), *Clostridium akagii* (50% at the fen subsurface), *Verrucomicrobia* subdivision 3, *Pelosinus* sp., and uncultured *Oxalobacteraceae* species (see Fig. S1 and Table S2 in the supplemental material).

Distribution patterns of bacterial clades associated with several functional guilds also indicated the environmental differences between the bog and fen sites and across depths (see Table S2 in the supplemental material). For example, levels of both type I (*Methylococcaceae*) and type II (*Methylocystaceae*) methanotrophs were on average 10 times higher at the surface than at the subsurface for both sites. Metal respiring bacteria (*Geobacter* and *Shewanella*) were detectable only at the fen site.

Archaeal community composition was dominated by methanogenic *Euryarchaeota*, including members of *Methanosarcinales*, *Methanomicrobiales*, and *Methanococcales* (Fig. 2; see also Fig. S2 in the supplemental material). The C2 clade was the dominant phylotype of *Crenarchaeota* observed in all samples. In the bog, the archaeal community was dominated by phylotypes affiliated with

TABLE 2 Microbial diversity indicated by Chao1 richness and equitability evenness in the bog and fen sites<sup>a</sup>

Site	Depth (cm)	Bacteria			Archaea			Fungi		
		Sequence read	Chao1	Equitability	Library size	Chao1	Equitability	Sequence read	Chao1	Equitability
Bog	0–10	71,199	7,783	0.57	43	18	0.66	774	243	0.84
Bog	40–50	44,576	8,559	0.55	45	13	0.48	1,189	89	0.37
Fen	0–10	49,361	22,100	0.73	47	90	0.91	6,436	210	0.75
Fen	40–50	50,487	19,873	0.47	46	40	0.93	2,139	220	0.85

<sup>a</sup> Calculation was based on OTU tables rarefied to the same sequencing depth, namely, the smallest one of either total pyrosequencing reads or clone library size. The sequence read is the number of sequences generated per sample. Chao1 richness is estimated from OTU number with 97% sequence identity. Library size is the number of clones picked for sequencing.

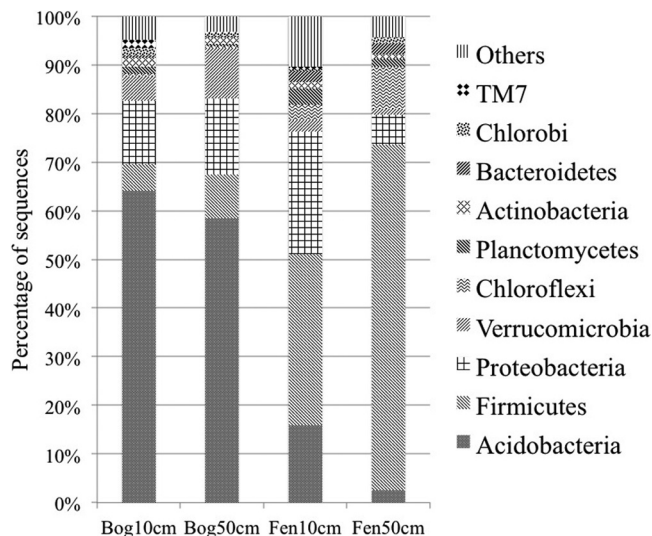


FIG 1 Bacterial community composition in the bog and fen sites. “Others” contains the other 43 phyla relatively rare in these sites (see Table S1 in the supplemental material).

acetoclastic *Methanosarcinales*, which accounted for 65% and 91% of the total archaea in the bog surface and subsurface, respectively (Fig. 2). Samples from the fen contained more diverse groups of archaea, including both acetoclastic and hydrogenotrophic methanogens. Clone GLAP87 accounted for 55% and 75% of total archaeal sequences at the bog surface and subsurface, respectively. Interestingly, this OTU shares 98% sequence similarity with a *Methanosarcina* sp. isolated from a peat bog of northern England (33), but it is phylogenetically distant from well-characterized members of acetoclastic *Methanosarcinales* (see Fig. S2 in the supplemental material), suggesting diverse ecological adaptation strategies of different members of the *Methanosarcinales*.

Fungal community structure was distinct in the bog subsurface and dominated by sequences affiliated with the *Zygomycota* (90% of total sequences) (Fig. 3). A high relative abundance (23% of

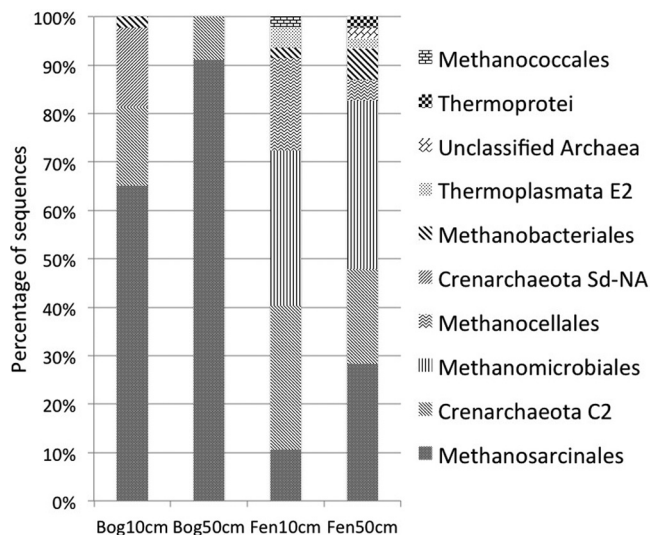


FIG 2 Archaeal community composition in the bog and fen sites.

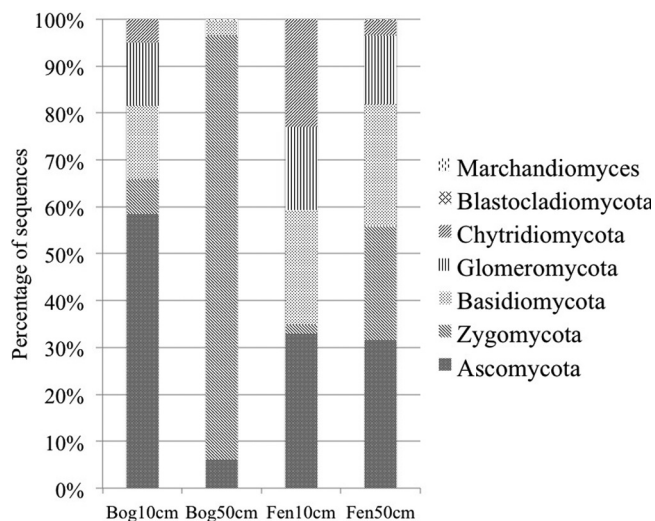


FIG 3 Fungal community composition in the bog and fen sites.

total) of *Zygomycota* sequences was also observed in the fen subsurface, in contrast to less than 5% of *Zygomycota* sequences observed in both surface samples. The bog surface was dominated by phylotypes affiliated with the *Ascomycota*. The surface bog and the two fen samples showed a similar relative abundance of *Basidiomycota* and *Glomeromycota* sequences, while sequences from the *Chytridiomycota* in the fen surface were about one order of magnitude higher in relative abundance than those in the other 3 samples on average. Among the 20 most abundant fungal OTUs detected in this study, only four were classified to the genus level, including *Mortierella* spp., *Stilbella* sp., *Cryptococcus* sp., and *Rhizopus* sp. (see Table S2 in the supplemental material).

**Community comparison across the depth and sites.** Principal coordinate analysis based on Bray-Curtis similarity separated the bog bacterial communities from those of the fen along the first principal coordinate axis (PCO1) (Fig. 4A). PCO1 explains 57.3% of total variation, and this component negatively correlates with environmental parameters, such as pH, total SSU rRNA gene abundance, and  $E_2/E_3$  ratio. PCO1 positively correlates with C/N ratio, DOC concentration, and aromatic fraction (SUVA) (significance level of  $<5\%$ ). PCO2 explained 30.7% of total variation and separates the fen surface from the fen subsurface. Both enzyme activity and DON concentration negatively correlated with the PCO2 component, and the two vectors point to the higher values in the fen surface.

The distribution pattern of fungal communities (Fig. 4B) was significantly different from that seen in the bacterial community (Fig. 4A) based on a matrix comparison using Procrustes analysis ( $M^2 = 0.59$ ,  $P = 0.11$ ), highlighting a unique fungal community composition in the bog subsurface. PCO1 explained 52.8% of total variation and separated the bog subsurface sample from all others (Fig. 4B). The fungal PCO1 negatively correlates with enzyme activities, DON, and SSU gene abundance and positively correlated with C/N ratio and concentrations of DOC and ammonia. In contrast, several factors (pH, molecular size, and SUVA) that influenced bacterial distribution were less significant (significance level of  $>8\%$ ) for the fungal PCO1, suggesting that bacterial and fungal communities respond differently to environmental stressors (e.g., pH) and DOM properties. PCO2 explained 28.3%

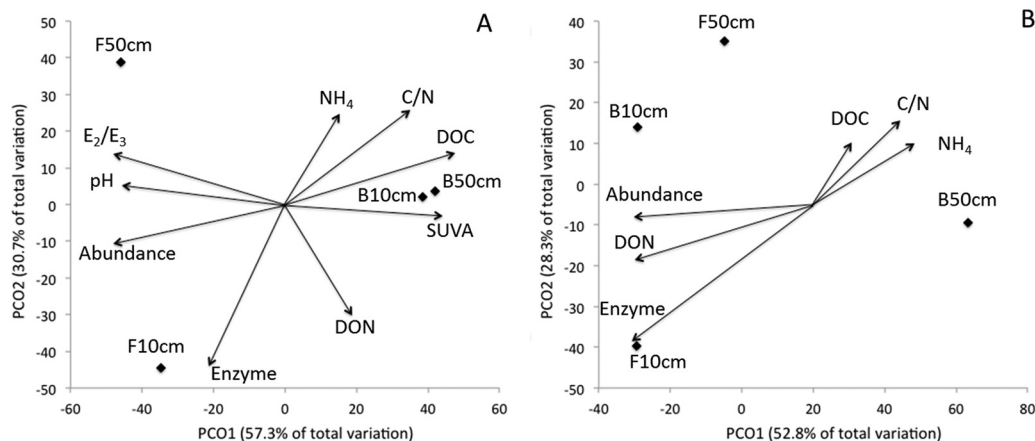


FIG 4 Principal coordinates analyses for bacterial (A) and fungal (B) community composition. Environmental vectors with Pearson correlation of  $R$  of  $>0.5$  are shown. The initials “B” and “F” followed with depth (10 cm or 50 cm) indicate bog and fen, respectively. Abundance represents the total SSU rRNA gene abundance.

of total variation, and enzyme activity is still an important factor correlating with this axis.

To further explore the determinants of community structure, a distance-based multivariate linear model was used to partition community variation into environmental components. Table 3 shows that pH explained the greatest proportion (53.4%) of bacterial community variation. A combination of pH, DOC concentration, and biomass (rRNA gene abundance) predicts most (93.3%) of the bacterial community variation. In contrast, the concentrations of  $\text{NH}_4$ , DON, and DOC are combined to explain 86.0% of total fungal community variation.

## DISCUSSION

**Bacterial and archaeal community composition and their functional significance in peatlands.** Prokaryotes are major players in the mineralization of organic matter via fermentation and respiration in peats (10, 79), especially in the catotelm, where anoxia may limit the metabolism of fungi. We observed a significant correlation between bacterial community composition and pH, concentrations of DOC and DON, C/N ratios, fractions of aromatic compounds, and molecular size of DOM. In corroboration of previous work in peatlands, we observed that low pH in the moss-dominated bog selects for acid-tolerant phylotypes (e.g., *Acidobacteria* group 1) (20, 68), while the fen is dominated by

phylotypes (e.g., *Clostridia* of the *Firmicutes*) favoring a more neutral pH condition. Bacterial community composition in peat soils of the bog was dominated by *Acidobacteria*, whose relative abundance was at the high end of the range reported for soil environments (45), while the relative abundance of *Firmicutes* in the fen was high in comparison to other soil environments and comparable to that observed in human intestinal environments with high fermentation activity (22).

Although more information is available for peatland bacterial communities than for fungi, the respective role of specific bacterial groups in the degradation of *Sphagnum*-derived litter is largely unknown. Acidobacteria are well adapted to acidic, nutrient-poor conditions (24, 68). Recent studies showed that 1/3 of laccase (phenol oxidase) genes in peat soils are affiliated with *Acidobacteria* (3), suggesting their important role in degrading aromatic compounds in peatlands. A potential role for the *Acidobacteria* in degrading cellulose has also been suggested based on the analysis of genome sequences (75) and laboratory tests with novel acidobacterial isolates (56, 57). In contrast, the *Clostridia* appear to thrive at relatively neutral pH in fen soils inhabited by more flowering plants that release labile DOM from their roots and are known to be key players in the degradation of cellulose and labile organic compounds (20, 31, 67). Interestingly, the fen subsurface contained about 50% of bacterial sequences affiliated with *Clostridium akagii*, an  $\text{N}_2$ -fixing fermenter isolated from acidic soils (42), suggesting their adaptation to this environment. Further work is needed to link dominant bacterial groups to the degradation of specific polyphenolic and carbohydrate compounds which are abundant in plant DOM in peatlands.

Methane-oxidizing bacteria (methanotrophs) are diverse and consume a significant fraction of greenhouse gas produced in peatlands. In the GLAP bog and fen, type II (*Methylocystaceae* of *Alphaproteobacteria*) methanotrophs were the dominant methane-oxidizing bacteria and accounted for 0.8 to 1.6% (or  $\sim 10^6$  sequences/g) of the total community (see Table S2 in the supplemental material), comparable to that in other acidic soils (19, 39). In contrast, type I (*Methylococcaceae* of *Gammaproteobacteria*) methanotrophs were barely detectable, especially in the fen. Relative to the type I methanotrophs, type II methanotrophs generally have the ability to fix  $\text{N}_2$  and grow better at lower  $\text{O}_2$  concentra-

TABLE 3 Sequential tests of predictor variables for bacterial and fungal community variation using the distance-based multivariate linear model

Variables	Adjusted $R^{2a}$	$P$ value	Proportion of variation explained	Cumulated variation explained
<b>Bacterial community</b>				
pH	0.457	0.005	0.534	0.534
DOC	0.699	0.013	0.251	0.785
Gene abundance	0.882	0.023	0.148	0.933
<b>Fungal community</b>				
$\text{NH}_4$	0.314	0.002	0.412	0.412
DON	0.454	0.049	0.198	0.610
DOC	0.755	0.015	0.250	0.860

<sup>a</sup> Correlation coefficient adjusted for number of parameters.

tions (1). This is consistent with our observation of the dominance of type II methanotrophs in this high C/N ratio peat environment with low O<sub>2</sub> concentrations, especially in the subsurface, although we do not exclude other environmental factors affecting the abundance and diversity of methanotrophs (46).

Surprisingly, sequences from members of the *Methanosarcinales*, known as acetoclastic methanogens, predominated in the bog and in the subsurface of both sites, in contrast to the previous observations that hydrogenotrophic methanogenesis is the primary pathway in the ombrotrophic bog and oligotrophic fen of GLAP (13) or in other northern peatlands in general (36, 37). The isotopic fractionation of mineralization products, CO<sub>2</sub> and CH<sub>4</sub>, also indicates that hydrogenotrophic methanogenesis should dominate and is energetically more favorable in the bog than in the fen at GLAP (14). Although methanogens are perhaps the best studied of microbial groups in peatlands, linkages between the phylogenetic structure of archaeal communities and pathways of methanogenesis are not fully resolved (41, 65). For example, Kotsyurbenko et al. (41) reported that *Methanosarcina* spp. accounted for approximately half of the archaeal community in acidic peat, while members of *Methanosarcinaceae* were undetectable in acidic environments of Alaskan peatlands (65).

It should be recognized that members of the *Methanosarcinales* (e.g., *Methanosarcina*) exhibit the highest physiological diversity among methanogens and are capable of most major metabolic pathways for methane production (27). In addition, the most abundant *Methanosarcina* clone in our samples, GLAP87, is phylogenetically distant from well-characterized cultures of the *Methanosarcinales* (see Fig. S2 in the supplemental material) but closely related (>98% identity) to an isolate from a peat bog of northern England (33), suggesting their adaptation and potentially novel metabolism in bog environments. *Methanosarcina* species are believed to succeed in nature by following a generalist strategy (81). Thus, the ecological function and controls of methanogenesis in the acidic bog may need to be reevaluated.

**Fungal abundance, community composition, and distribution in peatlands.** Assessing the relative contributions of fungi and bacteria to the microbial biomass and activity is of substantial interest for soil ecology, as fungi and bacteria govern most of C transformation and favor different degradation pathways (69). Our qPCR results showed that fungal SSU rRNA gene abundance was 1 to 2 orders of magnitude lower than that observed in grassland, cropland, and forest soils compared using the same technique (62). This is consistent with the observation that microbial biomass relative to soil organic carbon was much lower in peatlands than in agricultural forest soils (5.6 versus 24 mg microbial C g<sup>-1</sup> soil organic C) (51). Moreover, aromatic and phenolic compounds released from *Sphagnum* litter exhibit antimicrobial properties and may impede the decomposition of carbohydrate-rich DOM (66). These properties of peatland DOM to some extent explain the lower biomass observed in the bog.

More interestingly, we found very low fungus/prokaryote ratios (<0.02), close to the lower end of the range (0.01 to 1 in soils) reported globally (25). This finding is contrary to prior cultivation-dependent studies, which suggested that surface peatlands with extensive hyphal growth are usually dominated by fungal biomass (29, 72). Our qPCR values simply represent ratios of fungal/bacterial SSU gene abundance, instead of biomass. However, our results are consistent with a more recent activity-based study demonstrating a strong bacterial dominance in a bog and fen of

northern peatlands (79). Thus, our culture-independent study, together with activity-based studies, suggests a less important role for fungi in carbon cycling of peatlands relative to that in other soil environments.

Fungi, in particular, are understudied in peat soils, although these microorganisms are thought to play an important role in the carbon cycle (72). In this study, fungal communities appear to respond differently to environmental variables compared to bacteria. Statistical analysis indicates that pH is less important as a selective force in structuring fungal community composition, which can be attributed to the ability of fungi to tolerate a wider pH range for optimal growth as well as their optimal extracellular enzyme activity at low pH (5). Furthermore, our results suggest that concentrations of ammonia, DOC, and DON are more important than the molecular size and aromaticity of DOM in structuring fungal distribution patterns (Fig. 4). The gradient of ammonia with depth may be indicative of a redox condition, which is known to affect the distribution and activity of fungi in peatlands (72). Unpublished data from our group in another peatland site show a sharp decline of fungal 18S gene abundance below 50 cm, further supporting the potential importance of oxygen governing the abundance and activity of fungi. Several studies have demonstrated the relationship between fungal community distribution pattern and plant species composition in mineral soils (50, 53). However, to our knowledge, this is the first study to report the feature that fungal community pattern differentially responds to quantity, quality (e.g., C/N ratio), and reactivity of DOM in peatland ecosystems.

The spatial distribution pattern of the fungal community exhibited a vertical stratification of major phyla, reflecting their response to different environmental conditions (e.g., plant community composition, DOM quantity). Fungal taxa found in this study belong mainly to 3 phyla: *Ascomycota*, *Basidiomycota*, and *Zygomycota*, consistent with previous work in peat soils (2, 29, 72). The predominance of *Ascomycota* and *Basidiomycota* at the surface is consistent with their ability to degrade DOM (e.g., cellulose and polyphenolic compounds) under oxic conditions, which has been observed primarily in laboratory studies (48, 72). In contrast to previous work, members of the *Zygomycota* were detected in abundance in the subsurface of both the bog and fen sites, suggesting that their physiology is distinct from that of the *Ascomycota* and *Basidiomycota*. The *Zygomycota* produce thick-walled, resistant spores that allow the fungi to survive over long periods of dormancy (4). In this aspect, it will be important to determine if members of the *Zygomycota* in the subsurface are active or dormant. Additionally, members of the *Zygomycota* do not respond as well as the *Dikarya* (*Ascomycota* and *Basidiomycota*) to soluble plant degradation products (e.g., cellulose, sucrose) and instead appear to be associated with carbon substrates of animal and fungal origin, such as fungal hyphae (16, 18, 35). For example, *Mortierella* spp., dominant among the zygomycete phylotypes in our samples, are strong digesters of chitin (80), which is the fundamental component of fungal hyphae. Experiments show that zygomycetes degrade chitin nearly as efficiently as chitinolytic actinomycetes (18). Although the vertically stratified distribution of *Zygomycota* as well as *Dikarya* appears consistent with the gradient of carbon quantity and quality in the GLAP peatland, we can only speculate on the functional role of these fungal taxa in degrading organic carbon

substrates. Our findings suggest that the *Zygomycota* may play a more important role in the carbon cycle than was previously perceived (72).

Our findings of both microbial community analysis and geochemical measurements were generally reproducible. Previous studies have reported high reproducibility of 454-based DNA sequencing of the same sample (54) or habitat (64). Reproducibility of microbial community analysis was also verified by a fingerprinting method, which indicated that similarity in community membership (Sorensen similarity) and structure (Bray-Curtis similarity) among triplicated DNA extractions per sample was >85% and >65%, respectively (see Table S3 in the supplemental material). In addition, the spatial variation in our geochemical measurements is also consistent with previous studies of multiple-year measurements at GLAP (17, 70). Therefore, the results reported here are robust and likely representative of peatland microbial communities and chemical environments.

In summary, this study revealed prokaryotic dominance in peatland microbial communities and demonstrated a distinct response of fungal and bacterial communities to environmental variables changing along the depth and vegetation gradients in the bog and fen environments. Ammonia (presumably correlating negatively with oxygen concentration vertically) and pH are determined to be primary environmental parameters structuring fungal and bacterial community composition, respectively. Additionally, DOM molecular size and aromaticity showed a more significant correlation, with the shift in bacterial community composition relative to that in fungi. Our study identified unique distribution patterns of specific microbial groups in the heterogeneous peatland environment, which may select for microbes with poorly recognized metabolic capability. *Zygomycota* prevalent at the subsurface are targets for further functional understanding in their role in peatland carbon cycling. A novel clade of *Methanosarcinales* dominant in the bog indicates their potential capability of both acetoclastic and hydrogenotrophic methanogenesis. A mechanistic understanding of the role of important clades in peatland carbon cycling requires both additional field experiments and ecophysiological studies in the laboratory.

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