Biogeochemistry (2012) 109:117–131
DOI 10.1007/s10533-011-9644-5

Methanogen diversity and community composition in peatlands of the central to northern Appalachian Mountain region, North America

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Received: 26 March 2011 / Accepted: 31 July 2011 / Published online: 24 September 2011

Abstract Methanogenic archaea are ubiquitous in peat soils; however, their diversity and distributions within and among peatland ecosystems are not well known. We used comprehensive clone libraries of 16S rRNA gene sequences to investigate spatial patterns in diversity (richness, evenness of taxa) and composition (taxonomic, phylogenetic) of the methanogenic community in six peatlands arrayed 775 km from eastern Ontario, Canada to West Virginia, USA. Five sites were *Sphagnum* (moss) and shrub dominated; one site was sedge dominated; and, potential rates of methane (CH_4) production ranged from 15 to 450 nmol/g day. The gradient allowed us to examine influences of site conditions, site history, and climate on community composition. The region had representatives of methanogens from four taxonomic orders. We observed 29 operationally defined units (OTUs) based on >97% sequence identity. One OTU accounted for 43% of all clones, whereas 15 OTUs were rare with <1% of the total number of clones. The number of OTUs per site ranged from 4 to 21, and statistical analysis suggested diversity of 4–43 per site. Eighteen of the OTUs were endemic to one site; albeit, most endemics occurred in the sedge dominated site. One OTU was cosmopolitan, occurring in all six sites. We found a positive relationship between methanogen diversity and rates of CH_4 production per site (Pearson $r = 0.93$). Turnover in community composition between sites was weakly related to geographic distance between sites, whereas variation in soil pH and annual temperature played larger roles. About 50% of the variation in community composition was unexplained by distance, pH, mean climate, and site age. We conclude that methanogen diversity in peatlands of the central Appalachian region is shaped by present-day environmental conditions, suggesting an influence of impending climatic and environmental changes.

Keywords 16S ribosomal RNA · Beta diversity · Environmental gradient · Microbial communities · Peatland · Spatial turnover

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Introduction

High latitude peatlands are a harsh environmental for plants and animals. Winters are long and cold; soils are

waterlogged and often poorly aerated; and, low supplies of nutrients and solutes contribute to persistently acidic conditions. Hence, relatively few plant and animal species thrive in peatlands (Rydin and Jeglum 2006). Soil properties also would suggest that microbial diversity is limited in peatlands. Organic matter decomposes slowly (Moore and Basiliko 2006), and thus peat soils are an accumulation of partially decomposed plant remains, often several meters thick. However, long ago Selman Waksman and others (Begak 1926; Waksman and Purvis 1932) noted that peat soils harbored diverse communities of microorganisms, some with large populations, which recent studies corroborate (Dobrovol'skaya et al. 2009). Thus the origin and maintenance of microbial diversity in peatlands is poorly understood and requires further study.

The distribution and abundance of anaerobic microorganisms in cool temperate peatlands presents an ideal system to test ecological concepts about diversity and community composition. Although peatlands are large and extensive across northern boreal landscapes, they become small and isolated within a matrix of forest and/or agricultural land towards the southern end of their distribution. For anaerobic microorganisms, the matrix of soils should be inhospitable and act as a barrier that limits dispersal among isolated habitats (cf., Boyd et al. 2010). Dispersal limitation is one mechanism that contributes to beta diversity (sensu Whittaker 1972), defined as the difference in community composition or species richness among separated habitats. As a result, similarity in community composition should decrease with increasing geographic distance between sites, i.e., the so-called distance decay of similarity (Soininen et al. 2007; Morlon et al. 2008). There are several reasons for dispersal limitations (Hubbell 2001; Martiny et al. 2011). In addition to physical barriers, there are biological reasons, such as progeny growing close to parents. Furthermore, for species with small population size, the so-called rare biosphere (Sogin et al. 2006), the probability of successful dispersal is less than that for species with large population size (Woodcock et al. 2006).

However, there is a second mechanism for beta diversity. Environmental conditions that determine species habitat, i.e., niche, also tend to vary along gradients. Therefore, niches are likely to differ for sites separated by large distance. In general niche diversity reflects variation in climate, geology, and lag in ecosystem recovery after marked disturbances from the last glacial cycle (Duivenvoorden et al. 2002). For example, isolated peatlands situated along the axis of the Appalachian Mountains in eastern North America occur along a historical and climatic gradient (Wieder and Yavitt 1994). Although Appalachian peatlands are Pleistocene age, peat accumulation began 18,000 years ago in southern counterparts but only 8,000 years ago in central counterparts (Maxwell and Davis 1972; Wieder 1985). Presently, climate conditions between the central region (cooler) and southern region (warmer) encompass the range of predicted scenarios of climate change. Also, local patterns in hydrogeology create variation in soil pH, which is a master variable for microbial community composition (Fierer 2008). Taken together, the physical and biological factors that determine niches should differ with increasing distance apart (Dumbrell et al. 2010).

Here we focus on CH_4-producing microorganisms, i.e., methanogens, which are strict anaerobes in the *Euryarchaeota* that make CH_4 from a limited number of substrates: CO_2 and H_2 (i.e., hydrogenotrophic methanogenesis), formate, methanol, methylamines or acetate (i.e., aceticlastic methanogenesis). Despite relying on few metabolic substrates, methanogens are phylogenetically diverse, including the orders *Methanosarcinales*, *Methanomicrobiales*, *Methanobacteriales*, *Methanocellales* (Rice Cluster I), and other yet to be described examples (Garcia et al. 2000; Cadillo-Quiroz et al. 2008). Studies in individual peatlands suggest the presence of representatives from these diverse clades (Hales et al. 1996; Basiliko et al. 2003; Horn et al. 2003; Kotsyurbenko et al. 2004), although there have been only a few studies that have used extensive clones libraries or in-depth sequencing.

We used 16S rRNA-based molecular methods to construct clone libraries and to examine the composition of methanogenic communities in six peatlands arrayed across 775 km distance in eastern North America. We sampled peatlands dominated by *Sphagnum* mosses and shrubs, and included one site dominated by sedges (*Carex* sp.) as an out-group. Our specific aims were:

1. To determine richness and diversity of methanogens in peatlands of the Appalachian Mountain region.

2. To assess the relative importance of environmental conditions and spatial distances in determining diversity across the region.
3. To consider impact of future climatic change on methanogen diversity.

Methods

Study area

The six peatlands span a geographic distance of ~775 km (Fig. 1; Table 1). The northern site, Mer Bleue bog is a raised bog, i.e., convex in shape, situated in a melt-water channel of the post-glacial Ottawa River in Ontario, Canada. Peat accumulation began between 7000 and 8500 years before present (ybp), and the present-day bog phase has been continuous for the last 6500 years BP. We sampled between high (50 cm tall) hummocks in Mer Bleue bog. Three sites were located in central New York State. Rome bog is situated in a 61-km^2 mosaic of sand dunes and low peat bogs. We sampled between low (20 cm tall) hummocks in Rome bog. Chicago bog occurs in a local landscape called kame-and-kettle topography, characterized by rounding hills of stratified sand and gravel called kames, dipping into depressions of glacial drift called kettles. Chicago is a quaking bog, and we sampled between high hummocks. Michigan Hollow is nutrient-rich fen peatland, dominated by sedges and located in a hummocky, confused topography with poor drainage, called the Valley Heads Moraine. Clay in the glacial till prevents drainage. Peat accumulation began about 13,000 ybp in central New York State. The two southern sites are located south of the Wisconsin-age glacial boundary in North America, and they began peat accumulation about 18,000 years ago. The Glades bog is a raised bog with 5-m of peat accumulation, and we sampled in a wet lawn. Big Run bog has 1.5 m peat depth, and we sampled from a wet carpet.

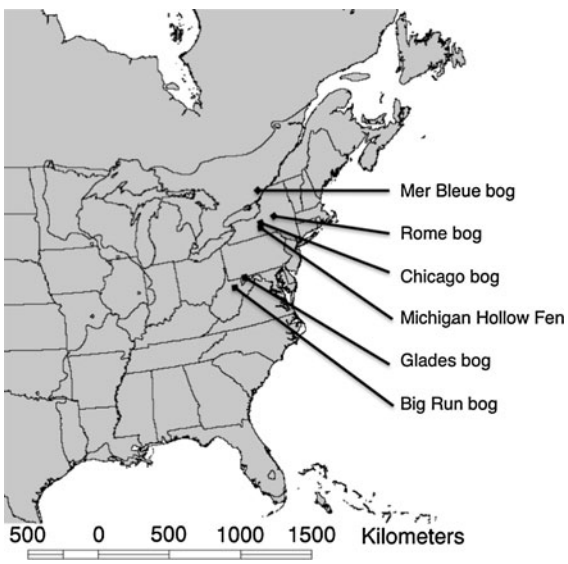


Fig. 1 Geographical distribution of six peatlands of the central to northern Appalachian Mountain region, North America

More details are available for sites in the following references: Mer Bleue (Moore et al. 2002); Rome (Kurczewski 1999); Chicago (Dettling et al. 2006); Michigan Hollow (Bernard and MacDonald 1974); The Glades (Maxwell and Davis 1972); Big Run (Wieder 1985).

Soil samples were taken between July and September in 2004. Within each site we collected samples at five points starting at a random point and located subsequent samples at 3 m intervals along a randomly selected cardinal direction (north, south, east, or west). Samples were collected by removing surface vegetation and peat (10 cm diameter) to reveal the water table level, then cutting a ~75 cm^3 block of peat soil immediately below the water level. A sample of soil water was taken to determine pH. Samples were stored individually in a clean canning jar, with no air spaces, for transport to the laboratory at Cornell University. Upon arriving at the laboratory, peat samples were either immediately processed for genomic DNA purification or stored in smaller aliquots at −80°C.

16s rRNA gene amplification, cloning and screening

Nucleic acids were extracted using 0.5 g of peat soil from each of the five peat samples from each sampling site, using the PowerSoil DNA Extraction Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) as previously described (Cadillo-Quiroz et al. 2006). DNA quality was evaluated by 1% agarose gel electrophoresis, followed by DNA dilution (1:10) before PCR amplification.

PCR amplification of the 16S rRNA gene was done in triplicate for each peat sample using the 1Af

Table 1 Study sites

Local name (size)	Flora	Lat. location	MAT (°C)	MAP	pH	CH_4 rate (nmol/g day)
Mer Bleue (3340 ha)	*S. cappillifolium*	45.24	5.8	940	3.8	30
	S. *fuscum*	Ontario				
	S. *magellanicum*					
	Heath hrubs					
Rome (27 ha)	*S. cappillifolium*	43.24	7.7	1041	3.8	100
	Heath shrubs	NY State				
Chicago (4.5 ha)	*S. fuscum*	42.56	7.9	890	4.2	150
	Heath shrubs	NY State				
Mich. Holl (48 ha)	*Carex* sedge	42.33	8.3	900	6.8	450
		NY State				
Glades (32 ha)	*S. fallax*	39.56	8.7	1231	4.8	20
	Mixed Shrubs	Maryland				
Big Run (15 ha)	*S. fallax*	39.12	8.8	1300	4.8	250
	Sedges	West Virginia				

MAT mean annual temperature, *MAP* mean annual precipitation

(5′-TCY GKT TGA TCC YGS CRG AG-3′) and 1100Ar (5′-TGG GTC TCG CTC GTT G-3′) primers (Hales et al. 1996). PCR reactions and concentration of the reagents were as described in Cadillo-Quiroz et al. (2006), using Hotmaster Taq polymerase (Promega, Madison, WI, USA). The PCR conditions were 2 min at 95°C, followed by 25 cycles of 1 min at 95°C, 1 min at an annealing temperature of 54°C, and 1 min 30 s at 72°C, plus a final elongation step of 8 min 30 s at 72°C.

Clone libraries were constructed for each sample (6 sites × 5 samples per site) using the TA Cloning kit (Invitrogen, Carlsbad, CA, USA) and clones were screened for the correct insert size using the M13 primers. Fifty clones per library were selected and analyzed by restriction digestion (Cadillo-Quiroz et al. 2006) for a total of 250 clones per site. Briefly, 20 µl of M13 amplified PCR product was digested with a mixture of *Hha*I (20 U) and *Hae*III (20 U) enzymes (New England Biolabs, USA). Digested DNA products were resolved by gel electrophoresis and clones were binned according to their restriction fragment patterns.

From each restriction fragment group, 1/3 to 1/5 of the clones were randomly selected and were submitted for forward and reverse sequencing. The complete insert sequence was then assembled. Sequences were aligned using the SILVA aligner in the ARB software (Ludwig et al. 2004). Screening for possible chimeric sequences was made using Mallard (Ashelford et al. 2006). Operational taxonomic units (OTUs) were determined using mothur (Schloss et al. 2009) at 90%, 97%, and 99% sequence identity. The taxonomic identity of OTUs was determined using BLASTn search (Altschul et al. 1990). We constructed a maximum likelihood tree from representative sequences from each OTU at the 97% similarity threshold. Using jModelTest, both Akaike and Bayesian information criteria (AIC, BIC) agreed upon the GTR + I + G model substitution (Posada 2008). We used the above model and the SPR tree improvement to build a tree in Phyml3 (Guindon and Gascuel 2003). Bootstrap tests were performed with 100 replications. Sequences were deposited to Genebank under accession JN649067 to JN649324.

For an estimate of methane production, portions of fresh soil (not frozen) were placed in Mason jars, sealed with a headspace of O_2-free N_2, and incubated for 40 days. The headspace gas was sampled periodically using a gas tight syringe to estimate concentrations of CH_4. An equal volume of N_2 was added to replace the gas sample taken. Concentrations of CH_4 were analyzed by gas chromatography using a 3-m Poropack-Q column (80/100 mesh) to separate CH_4 and a flame ionization detector to quantify concentration. Slopes of linear regression of total gas production

versus time were used to estimate production rates, normalized per gram of dry peat soil. The pH of soil water was determined using an electrode.

Data analysis

Sampling effort in each site was assessed using rarefaction. The rarefaction curves were constructed using EstimateS 8.0 (http://viceroy.eeb.uconn.edu/estimates; Colwell 2005). Coverage of the libraries was determined using the formula presented in Good (Good 1953). Estimates of library richness were made using the nonparametric estimators ACE and Chao1 (Magurran 2004). We calculated the three most common indices of diversity: the Shannon index (H′) of diversity, the reciprocal of Simpson's index (1/D) of evenness, and the reciprocal of the Berger-Parker index (1/d) of dominance (Magurran 2004).

Compositional similarity between all pairwise comparisons of sites, i.e., beta diversity, was quantified using the taxonomic Jaccard index and the Yue and Clayton index in mothur (Schloss et al. 2009). Jaccard reflects the compositional similarity between two sites as the likelihood that an OTU occurs in just one site (Jaccard 1912). The Yue and Clayton index calculates proportions of the community represented by shared and unshared OTUs, placing more emphasis on shared that are similar in abundance than those of dissimilar abundance (Yue and Clayton 2005). We also examined differences in phylogenetic composition among sites using weighted UniFrac (Lozupone and Knight 2005). UniFrac calculates a metric comparing the sum total branch length for all the sequences from each sample with the branch length shared by each sample pair. This metric represents the degree of divergence between sequences without classifying as OTUs.

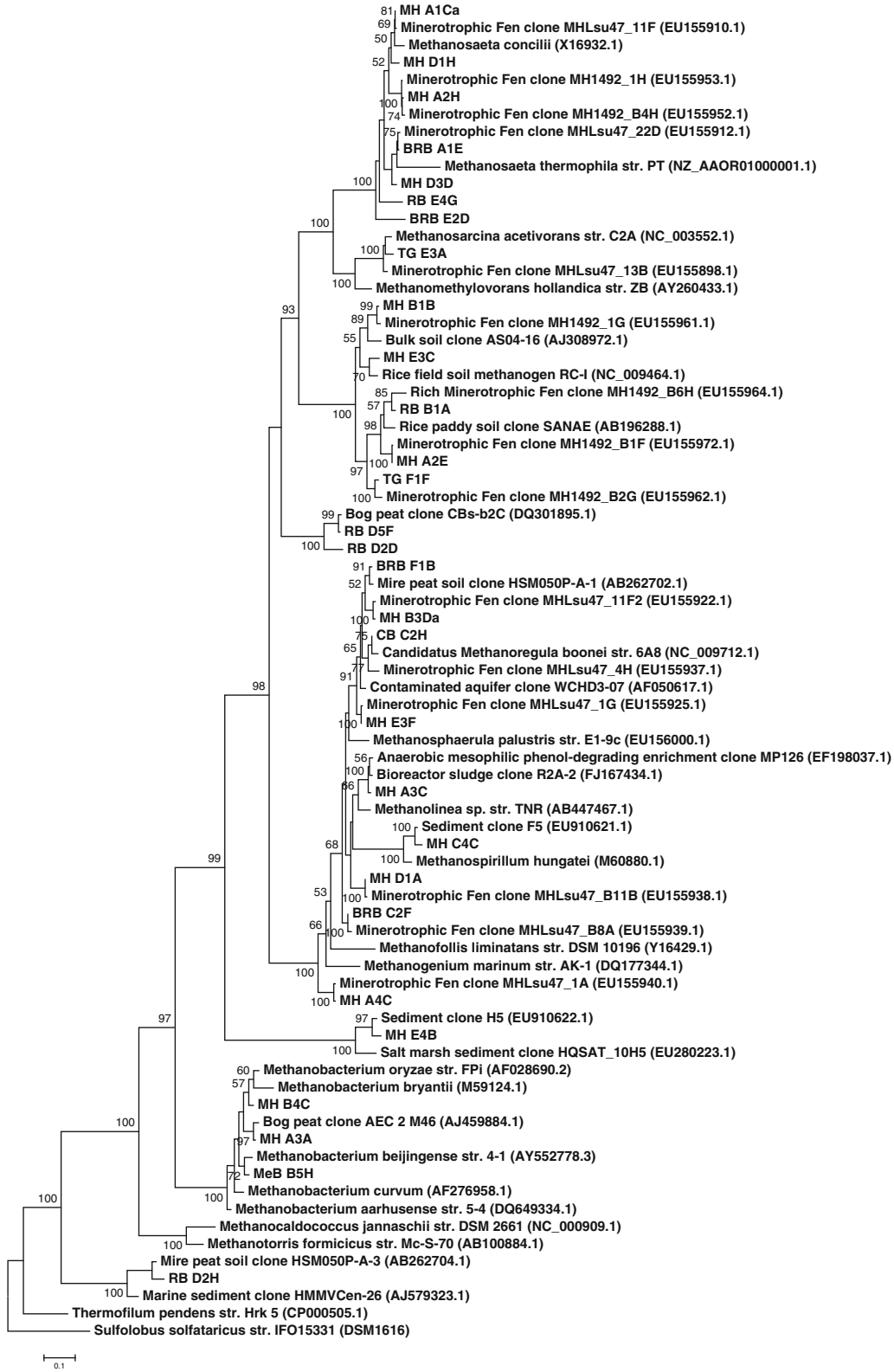
We used distance–decay relationships to assess rates of decline in similarity of community composition and increases in environmental distance as a function of geographic distance (Morlon et al. 2008). For compositional distance we used the Sorensen (Bray-Curtis) index. Environmental variables were soil–water pH, mean annual temperature, mean annual precipitation, and an estimate of peatland age (Table 1). Mantel tests of matrix correspondence were carried out to test for correlations between composition, environmental, and geographical distance matrices. The Mantel test is especially appropriate for determining which environmental variable or geographic distance could potentially explain the observed variation in community composition between plots. We also used multiple regressions on distance matrices to quantify the proportion of variation in compositional distances that could be explained by environmental and geographic distances. Multiple regressions allow a more accurate representation of environmental effects than Mantel tests, as each environmental variable is entered in the analysis independently. Our approach follows that of Duivenvoorden et al. (2002) and Jones et al. (2006).

Results

General diversity observations

We characterized methanogen community composition using the 16S rRNA gene region targeted by 1Af and 1100r primers. The 1Af–1100r combination is optimal for *euryarchaeal* coverage, as other primer sets miss some of the known methanogens (Cadillo-Quiroz et al. 2008). Within the study area surveyed, we examined 1,400 clones, which grouped into 29 OTUs using a 97% sequence similarity cutoff.

Some sequences were associated with methanogen groups that have had longstanding cultured members, such as the *Methanosarcinaceae* (MS) and *Methanosaetaceae* (MT) (Fig. 2; Table 2). In most sites, however, many of the phylotypes resided in recently cultured groups such as the E2 group (Cadillo-Quiroz et al. 2006), also called the fen cluster (Galand et al. 2005) from which *Methanoregula* was cultured (Bräuer et al. 2006), the E1 group from which *Methanosphaerula* was cultured (Cadillo-Quiroz et al. 2008), and Rice Cluster I, now known as the *Methanocellales* (Sakai et al. 2007). The clones associated with E2 were numerically dominant or co-dominant at all sites, except for MT and E1 dominance in the Michigan Hollow fen (Table 2). We also had sequences related to uncultured groups, including Rice Cluster III and a clade we have called the subaqueous cluster (SC) (Cadillo-Quiroz et al. 2006). We have no reason to exclude these groups until members are cultivated to determine physiology. Subordinate groups varied among sites: MS and RC III in Mer Bleue bog; RC II in Rome bog; E1 in Michigan


MH A1Ca
Minerotrophic Fen clone MHLsu47_11F (EU155910.1)
Methanosaeta concilii (X16932.1)
MH D1H
Minerotrophic Fen clone MH1492_1H (EU155953.1)
MH A2H
Minerotrophic Fen clone MH1492_B4H (EU155952.1)
Minerotrophic Fen clone MHLsu47_22D (EU155912.1)
BRB A1E
Methanosaeta thermophila str. PT (NZ_AAOR01000001.1)
MH D3D
RB E4G
BRB E2D
Methanosarcina acetivorans str. C2A (NC_003552.1)
TG E3A
Minerotrophic Fen clone MHLsu47_13B (EU155898.1)
Methanomethylovorans hollandica str. ZB (AY260433.1)
MH B1B
Minerotrophic Fen clone MH1492_1G (EU155961.1)
Bulk soil clone AS04-16 (AJ308972.1)
MH E3C
Rice field soil methanogen RC-I (NC_009464.1)
Rich Minerotrophic Fen clone MH1492_B6H (EU155964.1)
RB B1A
Rice paddy soil clone SANAE (AB196288.1)
Minerotrophic Fen clone MH1492_B1F (EU155972.1)
MH A2E
TG F1F
Minerotrophic Fen clone MH1492_B2G (EU155962.1)
Bog peat clone CBs-b2C (DQ301895.1)
RB D5F
RB D2D
BRB F1B
Mire peat soil clone HSM050P-A-1 (AB262702.1)
Minerotrophic Fen clone MHLsu47_11F2 (EU155922.1)
MH B3Da
CB C2H
Candidatus Methanoregula boonei str. 6A8 (NC_009712.1)
Minerotrophic Fen clone MHLsu47_4H (EU155937.1)
Contaminated aquifer clone WCHD3-07 (AF050617.1)
Minerotrophic Fen clone MHLsu47_1G (EU155925.1)
MH E3F
Methanosphaerula palustris str. E1-9c (EU156000.1)
Anaerobic mesophilic phenol-degrading enrichment clone MP126 (EF198037.1)
Bioreactor sludge clone R2A-2 (FJ167434.1)
MH A3C
Methanolinea sp. str. TNR (AB447467.1)
Sediment clone F5 (EU910621.1)
MH C4C
Methanospirillum hungatei (M60880.1)
MH D1A
Minerotrophic Fen clone MHLsu47_B11B (EU155938.1)
BRB C2F
Minerotrophic Fen clone MHLsu47_B8A (EU155939.1)
Methanofollis liminatans str. DSM 10196 (Y16429.1)
Methanogenium marinum str. AK-1 (DQ177344.1)
Minerotrophic Fen clone MHLsu47_1A (EU155940.1)
MH A4C
Sediment clone H5 (EU910622.1)
MH E4B
Salt marsh sediment clone HQSAT_10H5 (EU280223.1)
Methanobacterium oryzae str. FPi (AF028690.2)
Methanobacterium bryantii (M59124.1)
MH B4C
Bog peat clone AEC 2 M46 (AJ459884.1)
MH A3A
Methanobacterium beijingense str. 4-1 (AY552778.3)
MeB B5H
Methanobacterium curvum (AF276958.1)
Methanobacterium aarhusense str. 5-4 (DQ649334.1)
Methanocaldococcus jannaschii str. DSM 2661 (NC_000909.1)
Methanotorris formicicus str. Mc-S-70 (AB100884.1)
Mire peat soil clone HSM050P-A-3 (AB262704.1)
RB D2H
Marine sediment clone HMMVCen-26 (AJ579323.1)
Thermofilum pendens str. Hrk 5 (CP000505.1)
Sulfolobus solfataricus str. IFO15331 (DSM1616)
0.1

◀ **Fig. 2** Maximum likelihood phylogenetic tree from representative sequences from each OTU at the 97% similarity threshold plus close cultured and uncultured relatives. Bootstrap values of 100 replicates are included in tree nodes

Hollow fen; and MT and E1 in Big Run bog. Only Chicago bog and the Glades bog had fairly similar representation in subordinate groups with MS and E1.

We binned DNA sequences into OTUs based on sequence identities of 90%, 97%, or 99%. We constructed rarefaction curves for the clone library data (Fig. 3) and found clear asymptotes at 90% and at 97% sequence identities for all six sites. Therefore, we sampled methanogen diversity completely at those levels of identity. However, the lack of asymptotes at 99% identity suggests further micro-diversity in the methanogens per site.

The sites had 4–20 OTUs at 97% sequence identity (Table 3). The estimated coverage of the clone libraries was high, ranging from 90 to 98%. One OTU accounted for 42.6% of all clones, whereas 15 OTUs were rare with <1% of the total number of clones. Eighteen of the OTUs were endemic to one site, with 15 of the endemic OTUs residing in Michigan Hollow fen. There were two endemics in Rome bog, and one endemic occurred in Big Run bog. One OTU was cosmopolitan, occurring in all six sites. Two OTUs occurred in five sites; two occurred in four sites; two occurred in three sites; and, three occurred in two sites. The statistical estimators of OTU richness (Chao1, ACE) predicted richness of 4 to 74 OTUs.

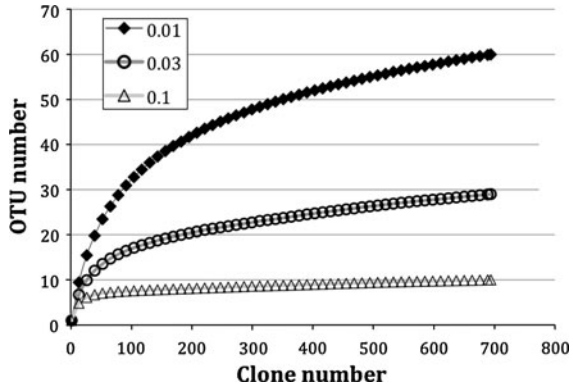


Fig. 3 Rarefaction analysis of OTUs (Operational Taxonomic Units) from composite sequence data including all samples and sites at three sequence similarity values: 90% (0.1), 97% (0.03) and 99% (0.01)

Therefore, for all six sites, sampling achieved >70% of the predicted richness.

We applied diversity indices to complement richness of OTUs (Table 4). The Shannon index, which emphasizes the richness component of diversity, ranked Michigan Hollow fen the most diverse and Mer Bleue bog the least diverse community. The Simpson index and the Berger-Parker index, place more weight on the evenness component of diversity, also concluded that the Michigan Hollow fen has the greatest diversity. However, in terms of evenness Mer Blue bog, Chicago bog, and Big Run bog have the less diverse communities.

Table 2 Phylogenetic compositions of peatland euryarchaeota in six sites

Taxon	% of rDNA clones in site					
	Mer Bleue bog	Rome bog	Chicago bog	Michigan Hollow fen	Big Run bog	Glades bog
MS	21	3	17	1	9	13
MT		4	6	46	11	6
SC		9	2			
RC-I			4	2	1	5
RC-II		36	4	2	3	
E2	55	47	56	1	38	53
E1	1		11	39	38	26
MBD				2		
MB		1		9		
RC-III	23					

Table 3 Richness of methanogen 16S rRNA gene sequence libraries from six peatlands

Site	No. of sequences	No. OTUs[a] observed	Estimates	
			Chao1	ACE
Mer Bleue bog	75	4	4 (4, 4)	4 (4, 12)
Rome bog	124	7	7 (7, 14)	8 (7, 20)
Chicago bog	122	8	8 (8, 14)	9 (8, 20)
Michigan Hollow fen	125	20	27 (28, 66)	43 (30, 74)
Glades bog	125	7	7 (7, 10)	7 (7, 10)
Big Run bog	125	9	9 (9, 15)	11 (9, 24)

[a] OTUs at 97% identity; 95% CI in parentheses

Table 4 Diversity Indices of methanogen 16S rRNA gene sequence libraries from six peatlands

Site	Shannon H′	Simpson's 1/D	Berger-Parker 1/d
Mer Bleue bog	1.06	2.55	1.80
Rome bog	1.50	3.81	2.14
Chicago bog	1.60	3.55	1.75
Michigan Hollow fen	2.42	8.04	3.90
Glades bog	1.68	4.67	2.08
Big Run bog	1.70	4.04	1.83

Similarity in community composition among pairs of sites

Both the Jaccard index (Table 5) and the Yue and Clayton index gave the same relationships for taxonomic similarity among pairs of sites at the 97% sequence identity. For the Jaccard index, values ranged from as low as 0.08, which indicates considerable dissimilarity in composition, to as large as 0.70. In general, sites had low compositional similarity when paired with Michigan Hollow fen, and Rome bog had a relatively similar composition when paired with each of the other bog sites. The largest value occurred between Chicago bog in New York State and Big Run bog in West Virginia with a compositional similarity of 0.70. In contrast, UniFrac, which measures phylogenetic distance rather than taxonomic distance, gave a different picture of similarity in community composition (Mantel, Jaccard matrix versus UniFrac matrix Observation $= -0.55$, $P < 0.01$). The UniFrac distance measure indicated quite similar composition between bog sites located close together; e.g., between Glades bog in Maryland and Big Run bog in West Virginia, as well as between Rome bog and Chicago bog in New York State (Fig. 4). We also noted that taxonomic structure at a finer scale of 99% sequence identity, i.e., Theta-YC values, gave the same associations among pairs of sites as that for the phylogenetic UniFrac index (Table 6).

The plot of community similarity versus geographic distance revealed no significant distance–decay relationship for methanogens across the study area (slope $= 0.02$, $P = 0.25$). Furthermore, at a smaller spatial scale among the five replicate samples per site, the distance–decay relationship also was not significant within each of the six peatlands (all slopes -0.01 to 0.02, Ps > 0.20). Therefore, we found little evidence for distance decay in similarity within each site as well as among the study sites.

The results of the Mantel tests confirmed that geographic distance had a non-significant effect on methanogen community distances (Observation $= -0.15$, $P = 0.67$). Among the environmental variables examined, the pH matrix had a significant correlation with the community matrix (Obs. $= 0.74$,

Table 5 Abundance based Jaccard similarity among methanogen 16S rRNA gene sequence libraries (at 97% threshold of similarity) from six peatlands

Site	% of rRNA clones in site					
	Mer Bleue	Rome	Chicago	Michigan	Big Run	Glades
Mer Bleue bog						
Rome bog	0.22					
Chicago bog	0.44	0.50				
Michigan Hollow fen	0.09	0.08	0.12			
Big Run bog	0.30	0.33	0.70	0.16		
The Glades bog	0.38	0.27	0.67	0.12	0.60	

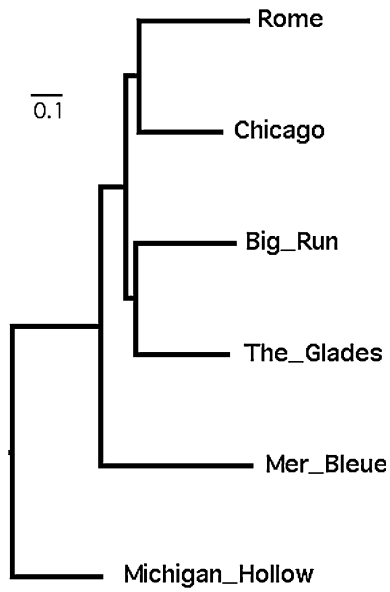


Fig. 4 Neighbor joining tree based on distances of peatland populations computed using UniFrac

$P < 0.003$). Although neither of the climatic matrices showed a significant correlation with the community matrix, there was a slightly stronger correlation with mean annual temperature (Obs. = 0.23, $P = 0.21$) than with mean annual precipitation (Obs. = −0.20, $P = 0.75$). The correlation with site age also was not significant (Obs. = 0.45, $P = 0.55$).

The results of the multiple regressions revealed that geographic distance alone explained only 2% of the variation in community similarity ($R^2 = 0.021$, $P < 0.05$). The environmental variables alone explained 20% of the variation in similarity ($R^2 = 0.20$, $P < 0.001$), presumably driven by the variation in pH, whereas the combined effect of environment and distance explained about the same amount of variation ($R^2 = 0.25$, $P < 0.001$). The significant interaction of environment and distance reflects the strong gradient in temperature across the study area (Table 1). Importantly, 53% of the variation in methanogen community similarity remained unexplained by either distance or environment (pH, mean climate, site age).

Discussion

General discussion

Of the 29 OTUs in the study region, 15 were defined as rare, i.e., they were <1% of the 1,400 clones examined. The so-called rare biosphere (cf., Sogin et al. 2006) for methanogens in peatlands is relatively unexplored. With 50% of the total OTUs being rare, however, the role of rare microbes in peatlands deserves further study (cf., Lyons et al. 2005). The most abundant OTU was 42.6% of the total, whereas another 13 OTUs each accounted for 2 to 9% of the total. Therefore the rank-abundance curve for the entire data set was one dominant, a few subdominants, and several rare phylotypes. This type of curve is typical of communities with moderate diversity as defined by Fuhrman (2009).

The number of rare OTUs also is important because it is used to estimate the expected number of phylotypes had the sampling effort been greater. In our case, sampling coverage was excellent at the 97% similarity threshold for OTUs, and we detected essentially all of the methanogen OTUs in the clone library per site, i.e., the estimated richness was only one or two OTUs greater than the observed richness. In Michigan Hollow fen, however, the estimated richness was considerably greater than the observed richness, in part because this site contained so many rare OTUs.

Table 6 Phylogenetic UniFrac distance among methanogen 16S rRNA gene sequence libraries from six peatlands

Taxon	Fraction of rRNA clones in site					
	Mer Bleue	Rome	Chicago	Michigan	Big Run	Glades
Mer Bleue						
Rome	0.75					
Chicago	0.70	0.48				
Michigan Hollow	0.82	0.77	0.76			
Big Run	0.66	0.60	0.51	0.81		
The Glades	0.73	0.56	0.48	0.75	0.48	

The methanogen community in Michigan Hollow fen also was the most diverse as indicated by the diversity indices. For example, Simpson's diversity index takes into account the species richness as well as abundance of each species. Expressing the index as 1/D, the smallest value is 1.0, i.e., one species is present. Hence greater values indicate greater diversity. The maximum value is the measured species richness. Therefore Michigan Hollow fen and Big Run bog had the same species richness, but the results suggest that a few species were dominant at Big Run bog, whereas there was more evenness among species in the fen at Michigan Hollow. This was borne out by the Simpson index and Berger-Parker index that emphasize evenness, with the greatest value in Michigan Hollow fen. Certainly more sites need to be studied in order to confirm whether methanogen communities are more diverse in fen peatlands than in bogs.

The ecological literature is filled with studies showing that biological diversity is linked to ecosystem processes (plant productivity, organic matter decomposition, nutrient cycling). In our study, for example, there was a positive relationship between diversity of methanogens and potential rates of CH_4 production per site, with a Pearson correlation coefficient of 0.93 ($P < 0.001$). Although only a statistical correlation, the pattern suggests that greater methanogen diversity supports greater rates of CH_4 production. The methanogen data for the Glades bog are particularly informative in light of previous studies conducted there. For instance, Williams and Yavitt (2003) found that peat soils from the Glades bog had low rates of CH_4 production year round; however, reason(s) for consistently low rates were not readily apparent. Now it appears, that the peat provides a poor habitat for a diverse community of methanogens, and the depleted community limits CH_4 production. The same conclusion applies to Mer Bleue with only four OTUs: this can be considered a methanogen-poor community, supporting low rates of CH_4 production (Beer et al. 2008). In contrast, 20 OTUs in Michigan Hollow fen is about typical for methanogen diversity, compared to two other peatland studies that examined diversity using clone libraries (Utsumi et al. 2003; Steinberg and Regan 2008), and Michigan Hollow is a notable source of atmospheric CH_4 (Smemo and Yavitt 2006).

The observed phylotypes in our study region included members of well-known groups as well as members of minor *euryarchaeal* groups. For example, the *Methanomicrobiales* were well represented in our sites. *Methanomicrobiales* are a large, diverse group of hydrogenotrophic methanogens with deep branches. Members occur in acidic bogs as well as in rich fens (Bräuer et al. 2006). *Methanosaeta* also were well represented, especially, in soil from Michigan Hollow fen. *Methanosaeta* are strictly aceticlastic methanogens, suggesting that acetate is a methanogenic substrate in all of the sites, except possibly in Mer Bleue bog. This agrees with results in Beer et al. (2008) that acetate did not fuel much of the CH_4 production in Mer Bleue bog. Rice Cluster I is a novel order of methanogens recently proposed as the *Methanocellales*, and its phylogenic location is between the *Methanosarcinales* and *Methanomicrobiales* (Sakai et al. 2007). Although found originally in rice paddies, they are common in peat soils and elsewhere (Conrad et al. 2006). In addition, RC II was well represented; RC II is a *euryarchaeotal* group that might contain methanogens based on its phylogenic position and evidence of, at least, one methanogenic phenotype (Grosskopf et al. 1998). The SC group is particularly interesting because a sequence associated with this group is the archaeal partner in an enrichment culture showing anaerobic oxidation of CH_4 (Raghoebarsing et al. 2006), which is though to be CH_4 production in reverse. Rice Cluster III also is an interesting group (Kemnitz et al. 2005) with no cultivated members. Given the large number of RC III clones in Mer Bleue, this should be an excellent site for further cultivation studies.

Similarity in community composition among pairs of sites

The second part of our study examined similarity in community composition among sites. We did observe OTU turnover between sites, and thus there was beta diversity in the study region. However, the variation in methanogen community similarity was not related directly to geographic distance between sites; hence, no distance–decay relationship, both within peatlands and across sites. Despite the lack of a distance–decay relationship for methanogens, distance played an indirect role via an interaction with the gradient in

annual temperature across the study region. Furthermore, variation in soil pH played a large role in structuring community composition.

We expected a greater role for geographic distance, given isolation of the peatland study sites and stringently anaerobic and acidiphilic methanogens in those sites. The surrounding matrix of forest and agricultural soils should have limited dispersal to a greater extent than that for more prosaic microorganisms. Although not a distance–decay relationship per se, looking at similarity in community composition using phylogenetic composition (UniFrac) rather than taxonomic composition revealed an interesting clustering among sites located closer together (Fig. 4). For example, the clustering of the communities in Chicago bog with Rome bog across 120 km of geographic distance suggests evolutionary relationships among the predominant E2 phylotypes in both sites. Likewise, clustering of communities in Big Run bog and the Glades bog located near each other indicates evolutionary dealings among the E1 and E2 co-dominants. These patterns suggest that dispersal of genetic information for anaerobic methanogens across 10 s to 100 km of inhospitable geographic distance is greater than expected. Bryant et al. (2008) also found a stronger clustering among nearby sites for phylogenetic composition than for taxonomic composition for bacteria along an elevation gradient. Phylogenetic clustering for sites located closer together, and possibly taxonomic clustering at the fine, i.e., ecotype, scale (Cohan and Perry 2007), highlights the importance of using as much phylogenic, taxonomic, and ecological information available to understand structuring of microbial communities.

Heterogeneity in pH appeared to play an especially prominent role in our study. Soil pH has been shown to influence microbial diversity in soils at large scales (Lauber et al. 2009). In general, acidity is a stress on physiology, especially for methanogens (Bräuer et al. 2006), that appears to filter the community composition. Our data provide support for this idea at a regional scale, albeit driven in part by Michigan Hollow fen with near neutral pH and the most OTUs. Notably, pH did not have strong spatial gradient across our study area. This is not surprising, as peatland pH is driven by local surface water and ground water hydrology and by local geology. For example, in the study area, acidic bog peatlands occur on soils derived from granite or acidic sandstone parent material, whereas fens can occur in close proximity when the parent material has inclusions of carbonate-rich dolomite. We do note, however, that variation in soil pH correlates with availability of many other chemical compounds, including nutrients and toxins (McBride et al. 1997), and thus much of the unexplained environmental variation in community composition might be pH dependent, rather than a result of pH per se.

Climate also accounted for variation in community composition. O'Brien et al. (2000) have noted that variation in length and quantity of rainfall is an important control on plant species diversity. For plants, water stress during a drought is a constraint that can be a cause of mortality for some species, but not others. Hence annual precipitation often accounts for beta diversity in forests (Davidar et al. 2007). Our findings, however, pointed to a largely role for variation in annual temperature. This makes sense for peatlands, since peat soils have strong water-holding capacity, and peatlands occur in places with restricted drainage (bogs) or local ground water discharge (fens) (Rydin and Jeglum 2006), making soil water content somewhat independent of annual precipitation (Verry et al. 2011). How temperature filters community composition for methanogens, and microorganisms in general, is not clear and likely complicated. One dilemma is that the optimal temperature for a microorganism's physiological activity might not be the same as the regional temperature (Wallenstein and Hall 2011). In other words, microbes often reside outside their thermal optimum, and their adaptation to temperature change depends on temperature regime (Bennett and Lenski 1993). Clearly we need more controlled physiological studies with microbial isolates to better understand how variation in temperature controls species turnover.

The array of sites in this study represented a gradient of glacial history. For example, the last glacial maximum did not affect the southern sites, with spruce woodland—much like that in the present-day boreal forest—occurring south of the glacial boundary in West Virginia and in Maryland (Maxwell and Davis 1972). Peat accumulation in the region began 18,000 ybp (Wieder 1985). In contrast, Mer Bleue was covered with ice until about 14,000 years ago, with peat accumulation in the past 8,000 years. Therefore, Big Run bog and the Glades bog could have served as refuges for methanogens that seeded

the more northern sites as the glaciers retreated. The lowest diversity of methanogens in Mer Bleue bog provides some support for the hypothesis that diversity is less in the youngest site. However, Mer Bleue also had a relatively large contribution from RC III, which were not present in the southern counterparts. Likewise, Big Run bog does not appear to be a refuge, because this site favors E1 and less E2 than that in northern counterparts. Therefore, we conclude tentatively that contemporary environmental factors play a larger role in site diversity than glacial history.

Possible implications of climate and methanogenic communities

The potential consequences of global environmental changes, e.g., climate, land use, and pollution, on biota in ecosystems are still unclear and need to be resolved. The role of atmospheric CH_4 in climate and environmental change is undisputable (Dlugokencky et al. 2011). Concentrations of atmospheric CH_4 have more than doubled since the preindustrial; molecule-for-molecule, CH_4 is much more effective as a greenhouse gas than CO_2; and, CH_4 provides an important link between climate and air quality issues as it provides a critical control on background ozone levels in the troposphere. Thus there is a critical need to understand how major sources of atmospheric CH_4, including peatlands (Rydin and Jeglum 2006), will react to future environmental change, and to forecast feedbacks on the Earth's CH_4 budget. Although methanogens control the production of CH_4 in soils (Conrad 1996), and they can consume CH_4 in some peatlands (Smemo and Yavitt 2011), models that project CH_4 emissions from peatlands are notably devoid of biological information (cf., Meng et al. 2011). In present-day models, the dynamics of CH_4 in soils are projected via the perceived competition between generalized microorganisms for dissolved organic carbon (DOC), i.e., the electrons fueling microbial activity, and electron acceptors, e.g., O_2, NO_3, SO_4, Fe^{3+}, for anaerobic respiration in lieu of CH_4 production; temperature, pH, and moisture help determine reaction rates among microbial mediated reactions. Therefore a role for microorganisms is implied via chemical reactions, but microbes themselves are deemed tangential (Schimel and Gulledge 1998). Perhaps different types of models that incorporate biological knowledge are needed (Todd-Brown et al. 2011).

Because the predictions of global scale models are crucial for climate policy (Prinn et al. 1999), there have been many calls to integrate microbial communities into broad-scale models (Allison and Martiny 2008; Todd-Brown et al. 2011). Methane and peatland methanogens are central to the call. For example, our data would argue that a one-size-fits-all notation for methanogens in models is too simplistic. The diversity of methanogens in peat soils is too great to reduce to one variable. Indeed, approaches that recognize two methanogenic populations, aceticlastic versus hydrogenotrophic (Beer et al. 2008), might not be robust enough to understand and predict CH_4 dynamics. One reason we argue for a larger community perspective for methanogens is that all of our sites had rare OTUs. It is been suggested that rare species will increase in dominance with environmental change in some communities (Hillebrand et al. 2008; Le Roux and McGeoch 2008), driving changes in community dominance patterns (Walther 2010). Our knowledge about the physiology of individual methanogen isolates is too rudimentary to discount the value of diversity. In other words, it is unclear why some methanogen communities maintain 20 OTUs, presumably all with the functional trait of CH_4 production. Is this an extreme example of functional redundancy, i.e., several species with the same functional role in the ecosystem (Rosenfeld 2002)? Or, do different phylotypes have different functional niches in peat soils, with subtle but significant differences in resource use, activity, and/or tolerance to environmental change? Until we know more about the physiology of microbial phylotypes we ought to remind ourselves constantly that diversity and complexity are linked too often in natural and managed systems to ignore (Page 2010).

Clearly our results show that peat soil pH is an important control of methanogen community composition. It is important to keep-in-mind that peat soil pH might be more variable than expected. It is well known, for example, that fossil fuel burning produces chemical compounds that, upon deposition to peatlands, can acidify peat soils (Shotyk 1996). And, recovery from human-induced acidification can take years to decades (Moldan et al. 2001). However, it is also important to recognize there is a complex link

between climate and peat pH (Clark et al. 2005), i.e., drought can induce peat acidification.

The response to increasing temperature is particularly uncertain and unpredictable at the present time. Although temperature had a significant impact on beta diversity across our study area, it was not a simple, straightforward relationship. The interaction of temperature and geographic distance indicates that dispersal and environmental conditions are intertwined in determining community composition. This likely reflects community assemble following the retreat of the last glacial episode in the region, a thermal maximum in temperature approximately 6,000 years ago, followed by cooling until the recent episode of global warming. Therefore, it is reasonable to assume that methanogens are still acclimating and possibly adapting to climate change (Wallenstein and Hall 2011). Understanding microbial acclimation to climate requires experiments with multiple treatments several years of analysis (cf., Brown et al. 2011). For microbial adaptation, we need to utilize the lessons from ideal experiments with common gardens and reciprocal transplants of soils (Yavitt et al. 2005; Bell 2010), in order to learn how environments and new temperature regimes determine community composition.

Finally, we reiterate our plea for more studies with microbial isolates. We simply need to know more about the identity and physiology of different taxa. Since microbial metabolism is key to ecosystem process (Docherty et al. 2011), more emphasis must be placed on elucidating the ecological value of diversity, and discerning how diversity and relatedness in community composition interact.

References

Allison SD, Martiny JBH (2008) Resistance, resilience, and redundancy in microbial communities. Proc Natl Acad Sci USA 105:11512–11519

Altschul SF, Gish W, Miller W et al (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Ashelford KE, Chuzhanova NA, Fry JC, Jones AJ, Weightman AJ (2006) New screening software shows that most recent large 16S rRNA gene clone libraries contain chimeras. Appl Environ Microbiol 72:5734–5741

Basiliko N, Dees PM, Merkel SM, Yavitt JB (2003) Methane biogeochemistry and methanogen communities in two northern peatland ecosystems, New York State. Geomicrobiol J 20:563–577

Beer J, Lee K, Whiticar M, Blodau C (2008) Geochemical control on organic matter decomposition in a northern peatland. Limnol Oceanogr 53:1393–1407

Begak DA (1926) Microbiological study of the high-moor peatland: quantitative assessment of bacteria in the high-moor peat. Pochvovedenie 2:64–75

Bell T (2010) Experimental tests of the bacterial distance–decay relationship. ISME J 4:1357–1365

Bennett AF, Lenski RE (1993) Evolutionary adaptation to temperature II. Thermal niches of experimental lines of *E. coli*. Evolution 47:1–12

Bernard JM, MacDonald JG Jr (1974) Primary production and life history of *Carex lacustris*. Can J Bot 52:117–123

Boyd ES, Hamilton TL, Spear JR, Lavin M, Peters JW (2010) [FeFe]-hydrogenase in Yellowstone National Park: evidence for dispersal limitation and phylogenetic niche conservatism. ISME J 4:1485–1495

Bräuer SL, Yashiro E, Ueno NG, Yavitt JB, Zinder SH (2006) Characterization of acid-tolerant H_2/CO_2-utilizing methanogenic enrichment cultures from an acidic peat bog in New York State. FEMS Microbiol Ecol 57:206–216

Brown JR, Blankinship JC, Niboyet A, van Groenigen CJ, Dijkstra P, Leadley PW, Hungate BA (2011) Effects of multiple global change treatments on soil N_2O fluxes. Biogeochemistry. doi:10.1007/s10533-011-9655-2

Bryant JA, Lamanna C, Morlon H, Kerkhoff AJ, Enquist BJ, Green JL (2008) Microbes on mountainsides: contrasting elevational patterns of bacteria and plant diversity. Proc Natl Acad Sci USA 105:11505–11511

Cadillo-Quiroz H, Bräuer S, Yashiro E, Sun C, Yavitt J, Zinder S (2006) Vertical profiles of methanogenesis and methanogens in two contrasting acidic peatlands in central New York State, USA. Environ Microbiol 8:1428–1440

Cadillo-Quiroz H, Yashiro E, Yavitt JB, Zinder SH (2008) Characterization of the archaeal community in a minerotrophic fen and terminal restriction fragment length polymorphism-directed isolation of a novel hydrogenotrophic methanogen. Appl Environ Microbiol 74:2059–2068

Clark JM, Chapman PJ, Adamson JK, Lane SN (2005) Influence of drought-induced acidification on the mobility of dissolved organic carbon in peat soils. Glob Change Biol 11:791–809

Cohan F, Perry L (2007) A systematics for discovering the fundamental units of bacterial diversity. Curr Biol 17:R373–R386

Colwell R (2005) EstimateS: statistical estimation of species richness and shard species from samples. Version 7.5. User's guide and application. http://viceroy.eeb.uconn.edu/EstimateS

Conrad R (1996) Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2O, and NO). Microbiol Rev 60:609–640

Conrad R, Erkel C, Liesack W (2006) Rice Cluster I methanogens, an important group of *Archaea* producing greenhouse gas in soil. Curr Opin Biotechnol 17:262–267

Davidar P, Rajagopal B, Mohandass D, Puyravaud J-P, Condit R, Wright SJ, Leigh EG (2007) The effect of climatic gradients, topographic variation and species traits on the beta diversity of rain forest trees. Global Ecol Biogeogr 16:510–518

Dettling MD, Yavitt JB, Zinder SH (2006) Control of carbon mineralization by alternative electron acceptors in four peatlands, central New York State, USA. Wetlands 26:917–927

Dlugokencky EJ, Nisbet EG, Fisher R, Lowry D (2011) Global atmospheric methane: budget, changes and dangers. Philos Trans R Soc A 369:2058–2072

Dobrovol'skaya TG, Golovchenko AV, Pankratov TA, Lysak LV, Zvyagintsev DG (2009) Assessment of the bacterial diversity in soils: evolution of approaches and methods. Eurasian Soil Sci 42:1138

Docherty KM, Balser TC, Bohannan BJM, Gutknecht JLM (2011) Soil microbial responses to fire and interacting global change factors in a California annual grassland. Biogeochemistry. doi:10.1007/s10533-011-9654-3

Duivenvoorden JF, Svenning J-C, Wright SJ (2002) Beta diversity in tropical forests. Science 295:636–637

Dumbrell AJ, Nelson M, Helgason T, Dytham C, Fitter AH (2010) Relative roles of niche and neutral processes in structuring a soil microbial community. ISME J 4:337–345

Fierer N (2008) Microbial biogeography: patterns in microbial diversity across space and time. In: Zengler K (ed) Accessing uncultivated microorganisms: from the environment to organisms and genomes and back. ASM Press, Washington, DC, pp 95–115

Fuhrman JA (2009) Microbial community structure and its functional implications. Nature 459:193–199

Galand PE, Fritxe H, Conrad R, Yrjala K (2005) Pathways for methanogenesis and diversity of methanogenic archaea in three boreal peatland ecosystems. Appl Environ Microbiol 71:2195–2198

Garcia Jl, Patel BKC, Ollivier B (2000) Taxonomic, phylogenetic and ecological diversity on methanogenic Archaea. Anaerobe 6:205–226

Good I (1953) The population frequencies of species and the estimation of population parameters. Biometrika 40:237–264

Grosskopf R, Janssen PH, Liesack W (1998) Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultivation and direct 16S rRNA gene sequence retrieval. Appl Environ Microbiol 64:960–969

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52:696–704

Hales BA, Edwards C, Ritchie DA, Hall G, Pickup RW, Saunders JR (1996) Isolation and identification of methanogen-specific DNA from blanket bog peat by PCR amplification and sequence analysis. Appl Environ Microbiol 62:668–675

Hillebrand H, Bennett DM, Cadotte MC (2008) The consequences of dominance: review of evenness effects on local and regional ecosystem processes. Ecology 89:1510–1520

Horn MA, Matthies C, Kusel K, Schramm A, Drake HL (2003) Hydrogenotrophic methanogenesis by moderately acid-tolerant methanogens of a methane-emitting acidic peat. Appl Environ Microbiol 69:74–83

Hubbell SP (2001) The unified neutral theory of biodiversity and biogeography. Princeton University Press, Princeton, NJ

Jaccard P (1912) The distribution of the flora in the alpine zone. New Phytol 11:37–50

Jones MM, Tuomisto H, Clark DB, Olivas P (2006) Effects of mesoscale environmental heterogeneity and dispersal limitation on floristic variation in rain forest ferns. J Ecol 94:181–195

Kemnitz D, Kolb S, Conrad R (2005) Phenotypic characterization of Rice Cluster III archaea without prior isolation by applying quantitative polymerase chain reaction to an enrichment culture. Environ Microbiol 7:553–565

Kotsyurbenko OR, Chin KJ, Glagolev MV, Stubner S, Simankova MV, Nozhevnikova AN, Conrad R (2004) Acetoclastic and hydrogenotrophic methane production and methanogenic populations in an acidic West-Siberian peat bog. Environ Microbiol 6:1159–1173

Kurczewski FE (1999) Historic and prehistoric changes in the Rome, New York pine barrens. Northeast Nat 6:327–340

Lauber CL, Knight R, Hamady M, Fierer N (2009) Soil pH as a predictor of soil bacterial community structure at the continental scale: a pyrosequencing-based assessment. Appl Environ Microbiol 75:5111–5120

Le Roux PC, McGeoch MA (2008) Rapid range expansion and community reorganization in response to warming. Glob Change Biol 14:2950–2962

Lozupone C, Knight R (2005) UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol 71:8228–8235

Ludwig W, Strunk O, Westram R et al (2004) ARB: a software environment for sequence data. Nucl Acids Res 32:1363–1371

Lyons KG, Brigham CA, Traut B, Schwartz MW (2005) Rare species and ecosystem functioning. Conserv Biol 19:1019–1024

Magurran AE (2004) Measuring biological diversity. Blackwell, Oxford

Martiny JBH, Eisen JA, Penn K, Allison SD, Horner-Devine MC (2011) Drivers of bacterial beta-diversity depend on spatial scale. Proc Natl Acad Sci USA 108:7850–7854

Maxwell JA, Davis MB (1972) Pollen evidence of Pleistocene and Holocene vegetation on the Allegheny Plateau, Maryland. Quatern Res 2:506–530

McBride MB, Richards BK, Steenhuis T, Russo J, Sauve S (1997) Mobility and solubility of toxic metals and nutrients in soil fifteen years after sludge application. Soil Sci 162:487–500

Meng L, Hess PGM, Mahowald NM et al (2011) Sensitivity of wetland methane emissions to model assumptions: application and model testing against site observations. Biogeosci Discuss 8:6095–6160

Moldan F, Wright RF, Lofgren S et al (2001) Long-term changes in acidification and recovery at nine calibrated catchments in Norway, Sweden and Finland. Hydrol Earth Syst Sci 5:339–350

Moore TR, Basiliko N (2006) Decomposition. Boreal peatland ecosystems. In: Wieder RK, Vitt DH (eds) Ecological studies, vol 188. Springer-Verlag, Berlin

Moore TR, Bubier JL, Frolking SE, Lafleur PM, Roulet NT (2002) Plant biomass and production and CO_2 exchange in an ombrotrophic bog. J Ecol 90:25–36

Morlon H, Chuyong G, Condit R, Hubbell S, Kenfack D, Thomas D, Valencia R, Green JL (2008) A general framework for the distance–decay of similarity in ecological communities. Ecol Lett 11:94–917

O'Brien EM, Field R, Whittaker RJ (2000) Climatic gradients in woody plant (tree and shrub) diversity: water–energy dynamics, residual variation, and topography. Oikos 89:588–600

Page SE (2010) Diversity and complexity. Princeton University Press, Princeton, NJ

Posada D (2008) jModelTest: phylogenetic model averaging. Mol Biol Evol 25:1253–1256

Prinn R, Jacoby H, Sokolov A et al (1999) Integrated global system model for climate policy assessment: feedbacks and sensitivity studies. Clim Change 41:469–546

Raghoebarsing AA, Pol A, van de Pas-Schoonen KT et al (2006) A microbial consortium couples anaerobic methane oxidation to denitrification. Nature 440:918–921

Rosenfeld JS (2002) Functional redundancy in ecology and conservation. Oikos 98:156–162

Rydin H, Jeglum JK (2006) The biology of peatlands. Oxford University Press, Oxford

Sakai S, Imachi H, Sekiguchi Y, Ohashi A, Harada H, Kamagata Y (2007) Isolation of key methanogens for global methane emission from rice paddy fields: a novel isolate affiliated with the clone cluster Rice Cluster I. Appl Environ Microbiol 73:4326–4331

Schimel JP, Gulledge J (1998) Microbial community structure and global trace gases. Glob Change Biol 4:745–758

Schloss PD, Westcott SL, Ryabin T et al (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541

Shotyk W (1996) Peat bog archives of atmospheric metal deposition: geochemical evaluation of peat profiles, natural variations in metal concentrations, and metal enrichment factors. Environ Rev 4:149–183

Smemo KA, Yavitt JB (2006) A multi-year perspective on CH_4 cycling in a shallow peat fen in Central New York State, USA. Wetlands 26:20–29

Smemo KA, Yavitt JB (2011) Anaerobic oxidation of methane: an underappreciated aspect of methane cycling in peatland ecosystems? Biogeosciences 8:779–793

Sogin ML, Morrison HG, Huber JA, Mark Welch D, Huse SM, Neal PR et al (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere". Proc Natl Acad Sci USA 103:12115–12120

Soininen J, McDonald R, Hillebrand H (2007) The distance decay of similarity in ecological communities. Ecography 30:3–12

Steinberg LM, Regan JM (2008) Phylogenetic comparison of the methanogenic communities from an acidic, oligotrophic fen and an anaerobic digester treating municipal wastewater sludge. Appl Environ Microbiol 74:6663–6671

Todd-Brown KEO, Hopkins FM, Kivlin SM, Talbot JM, Allison SD (2011) A framework for representing microbial decomposition in coupled climate models. Biogeochemistry. doi:10.1007/s10533-011-9635-6

Utsumi M, Belova SE, King GM, Uchiyama H (2003) Phylogenetic comparison of methanogen diversity in different wetland soils. J Gen Appl Microbiol 49:75–83

Verry ES, Brooks KN, Nichols DS, Ferris DR, Sebestyen SD (2011) Watershed hydrology. In: Kolka RK, Sebestyen SD, Verry ES, Brooks KN (eds) Peatland biogeochemistry and watershed hydrology at the Marcell Experimental Forest. CRC Press, Boca Raton, FL, pp 193–212

Waksman SA, Purvis ER (1932) The microbiological population of peat. Soil Sci 34:95–109

Wallenstein MD, Hall EK (2011) A trait-based framework for predicting when and where microbial adaptation to climate change will affect ecosystem functioning. Biogeochemistry. doi:10.1007/s10533-011-9641-8

Walther G-R (2010) Community and ecosystem responses to recent climate change. Philos Trans R Soc B 365:2019–2024

Whittaker RH (1972) Evolution and measurement of species diversity. Taxon 21:213–251

Wieder RK (1985) Peat and water chemistry at Big Run Bog, a peatland in the Appalachian Mountains of West Virginia. Biogeochemistry 1:277–302

Wieder RK, Yavitt JB (1994) Peatlands and global climate change: insights from comparative studies situated along a latitudinal gradient. Wetlands 14:233–242

Williams CJ, Yavitt JB (2003) Botanical composition of peat and degree of peat decomposition in three temperate peatlands. Ecoscience 10:85–95

Woodcock S, Curtis TP, Head IM, Lunn M, Sloan WT (2006) Taxa–area relationships for microbes: the undersampled and the unseen. Ecol Lett 9:805–812

Yavitt JB, Williams CJ, Wieder RK (2005) Soil chemistry versus environmental controls on production of CH_4 and CO_2 in northern peatlands. Eur J Soil Sci 56:169–178

Yue JC, Clayton MK (2005) A similarity measure based on species proportions. Commun Stat Theory Methods 34:2123–2131