Microbial Ecology

Comparison of Bacterial Communities in New England Bogs Using Terminal Restriction Fragment Length Polymorphism (T-RFLP)

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Wetlands are major sources of carbon dioxide, methane, and other greenhouse gases released during microbial degradation. Despite the fact that decomposition is mainly driven by bacteria and fungi, little is known about the taxonomic diversity of bacterial communities in wetlands, particularly Sphagnum bogs. To explore bacterial community composition, 24 bogs in Vermont and Massachusetts were censused for bacterial diversity at the surface (oxic) and 1 m (anoxic) regions. Bacterial diversity was characterized by a terminal restriction fragment length (T-RFLP) fingerprinting technique and a cloning strategy that targeted the 16S rRNA gene. T-RFLP analysis revealed a high level of diversity, and a canonical correspondence analysis demonstrated marked similarity among bogs, but consistent differences between surface and subsurface assemblages. 16S rDNA sequences derived from one of the sites showed high numbers of clones belonging to the Deltaproteobacteria group. Several other phyla were represented, as well as two Candidate Division-level taxonomic groups. These data suggest that bog microbial communities are complex, possibly stratified, and similar among multiple sites.

Sphagnum bogs store one-third of the earth's carbon, and in New England they are responsible for 36% of methane emissions [10]. In spite of the importance of microorganisms to the decomposition of peatland vegetation [13, 21, 39, 40] and thus their effect on nutrient release, there has been little study of the taxonomic diversity of peatland microbial communities.

Bacterial numbers are relatively low in peat compared to aerated soils [30]. Bacterial density in high-latitude peatlands, including fens and bogs, peaks in

sludge. Surface samples tended to be less degraded and lighter in color with intact *Sphagnum* particulate, whereas 1 m samples were generally moderately to completely degraded, with a dark color, and lower water content. Subsamples (20 mL) were decanted into smaller tubes and fixed in the field with glutaraldehyde (final concentration 2.5%) for microscopic counts. A soil sample from outside the wetland, classified as a hemist histosol, was also taken at one site. All samples were kept on ice in the field, and stored at 4 C in the laboratory until DNA extraction, and all extractions were performed within 48 h of collection. Archival material was stored at 20 C.

Bacterial Abundance. We analyzed 59 samples. Four samples were not countable and were therefore omitted from the study, leaving a set of 28 surface and 27 one-meter samples. Prior to staining, we diluted fixed samples 5- to 40-fold for optimum counting The dilution

Table 2. Two-way ANOVA of bacterial counts from surface and 1-m samples taken at 22 New England bogs

Source	df	SS	MS	F ratio	P value
Bog	21	5×10^{15}	2×20^{14}	1.172	0.360
Depth	1	9×10^{14}	9×10^{14}	4.746	0.041
Bog × Depth	21	4×10^{15}	2×10^{14}	29.926	0.001
Error	396	2.543×10^{15}	6.42×10^{12}		
Total	439	1.222×10^{16}			

Bog and depth were random effects in the mode, and there was a significant Bog × Depth interaction.
df: degrees of freedom; SS: sum of squares; MS: mean square.

two of the enzymes fell within the 3-bp gate and a third one fell outside by 2 bp, a wider gate was accepted.

Phylogenetic analyses were performed using a TIGR in-house pipeline (Dongying Wu, unpublished data) that automatically assigns taxonomic tags to environmental small subunit rRNA (SSU rRNA) sequences based on neighbor-joining phylogenetic trees. The 16S rDNA sequences obtained in this study were searched against the Ribosomal Database Project (RDP) rRNA database by BLASTN. Top matches, as well as selected matches within different score ranges, were aligned to the query sequence using CLUSTALW, and the alignments automatically trimmed using a Perl script based on alignment quality score for all the columns. A neighbor-joining phylogenetic tree based on the automatically trimmed alignments was constructed, and the taxonomy of the

Table 3. Taxonomic assignment for the most represented ribotypes at 1 m and surfaceatph55nqbased onr16r5nq9fT-334.747Sr16

location, averaging to approximately three unique ribo types per site. In contrast, a single ribotype classified as a Planctomycetes was present in 46 of 49 (94%) samples (Table 3). Ribotypes present in more than 75% of the samples were classified based on their RDP library match (Table 3). In 1 m samples, four out of the eight most common ribotypes were assigned as Deltaproteobacteria. In surface profiles, the two major groups were Proteobacteria, with half belonging to the Delta and the other to the Epsilon subdivision. When data for both depths were analyzed together, 10 ribotypes were present in at least 75% of samples. Firmicutes and Deltaproteobacteria assignments were most frequently represented with three ribotypes each.

Statistical analysis of the ribotypes profiles showed

(Fig. 2B). Although the first two canonical dimensions accounted for only 13.7% of the data set variance, vertical zones could be almost completely separated based on their bacterial ribotype profiles. The majority of this variance is accounted for by the separation of two surface samples in CC1 (QP and RP); and two other 1 m sites in CC2 [Chickering Pond (CHP) and Chickering (CHI)] (Fig. 2A). Even without these outliers, a detailed view of the plot (Fig. 2B) clearly shows the segregation of the microbial communities into two separate groups (1 m and surface).

Individual samples taken from a different area in the same bog were typically very similar (PON and SPR) at 1 m depth. Surface samples replicated in this manner exhibited more unique communities from within the same bog (CHO and PON), although they still clustered with their respective depth. The single soil sample, obtained near one of the bogs, clustered with bog surface samples.

Correlations between the bacterial communities and environmental data including water chemistry (pH, DOC, ammonium, DON, NO_3-N, SO_4, Cl, P-SR, Al, Ca, Cu, Fe, K, Mg, Mn, Na, Si, and Zn) and plant chemistry (C, H, N, Ca, Mg, Fe, Cr, Cu, K, Al, Co, B, Cd, Mo, Mn, Na, Ni, P, Pb, S, and Zn) for three different species (pitcher plant, leather leaf, and Sphagnum) were conducted. Vegetation data, from the bog and the surrounding forest, and geographical data including latitude, longitude, bog area, and elevation were also used in correlation analyses [18]. None of the tested parameters showed any significant correlation to specific ribotypes or general community structure. The only link between sample segregation and chemistry was Ca^{2+} levels. Two sites, Chickering (8.59 mg/L) and Colchester (8.24 mg/L), showed approximately 8-fold higher Ca^{2+} levels when compared to the average of all other bogs [0.98 mg/L, SD = 7 (excluding Chickering and Colchester)].

Based on the CCA plots generated (Fig. 2), ribotypes contributing to the majority of the variance among the data were identified (Table 4). Canonical dimensions showing the greatest variance shift depending on the data included. Limiting the set of ribotypes to those explain-
in(inc2e)]TJ0.160.0784314/2
in(inc2e)]TJ0.160.e

clones were identified as possible members of separate candidate divisions (TM7 and WS3). Other sequences were identified as being part of deep-rooted lineages such as Actinobacteria [12

surface and subsurface samples; we expected to find distinct communities in these two microhabitats because of anoxic conditions in the subsurface samples.

Although bogs are oligotrophic and acidic, overall diversity was high, with 817 ribotypes detected. There were more unique ribotypes in surface vs subsurface samples (199 vs 64), but total bacterial counts were higher for the subsurface samples (Fig. 1 and Table 2). Although no data are available to explain this increase in abundance with depth, an increase in bacterial numbers in anoxic zones could correlate with a decreased amount of competition or predation by organisms present in the aerated zones. Alternatively, as counts were normalized based on cells/mL of sample, higher counts might have been an effect of solids content.

Canonical correspondence analysis of the ribotype data showed a clear separation of samples based on sampling depth but relatively poor separation of bogs sampled at the same depth (Fig. 2). At this level of discriminating power, all of the truly ombrotrophic bogs sampled were fairly similar in their bacterial community composition. Chickering bog, which has been classified elsewhere as a poor fen, was identified as an outlier based on T-RFLP profiles. This was possibly correlated to increased

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