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The Variation of Microbial Communities in a Depth Profile of an Acidic, Nutrient-Poor Boreal Bog in Southwestern Finland

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Abstract

Natural bacterial communities impact the motility of isotopes, such as radionuclides, in the environment. As a result of post glacial crustal rebound radionuclides may escape the deep geological repository for spent nuclear fuel on Olkiluoto Island, Finland, and reach surface environments. Lastensuo Bog, a 5300-year-old raised bog in southwestern Finland, functions as analogue ecotope for bogs formed in Olkiluoto due to the crustal rebound. A core comprising the depth profile (0 - 7 m depth) of the bog including surface *Sphagnum* moss, peat and bottom clay was obtained using a stainless steel corer. High throughput sequencing was used to characterize the bacterial communities throughout the bog's depth profile. A total of 12,680 bacterial Operational Taxonomic Units (OTUs) (97% sequence similarity) were detected comprising altogether 40 different bacterial phyla. Of these, 13 phyla were present at all depths, accounting for 97% - 99% of the whole bacterial community. The bacterial communities differed notably through the bog's depth profile, dividing it into five distinct strata: 1) the *Sphagnum* moss layer; 2) 0.5 - 3.7 m; 3) 3.7 - 4.0 m; 4) 5.5 - 6.0 m deep peat; 5) the former seabed clay at 6.5 - 7.0 m depth. *Acidobacteria, α-* and *γ-Proteobacteria* dominated the surface community, but in the peat *Acidobacteria* contributed with up to 85% of the bacterial community. The estimated bacterial population density ranged between 2×10^9 and 5×10^{10} 16S rRNA gene copies g^{-1} dry-weight peat. This study revealed that Lastensuo Bog had a highly diverse bacterial community. Most of the taxonomic groups belonged to thus far poorly characterized and uncultured bacteria with unknown physiological role. However, new insights into the distribution of bacterial taxa and their putative roles in organic carbon break down with-

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in the bog ecosystem have been obtained and an important baseline for further studies has been established.

Keywords

Ombotrophic Bog, Peat, Sequencing, Bacterial Community

1. Introduction

Pristine wetlands play a significant role in global carbon cycling and function as sinks for atmospheric CO_2 [1] [2]. They harbour one third of the global soil carbon pool [3] [4] and are an important global source of methane [5], which is produced during anaerobic degradation of organic matter. Wetlands are important biotopes in northern areas and cover up to one third of the land surface in Finland [6]. In addition, water-logged peatlands might be considerable reservoirs of freshwater in the Northern Hemisphere. Ombotrophic raised bogs represent unique habitats for microorganisms, being very acidic and receiving water and minerals only from precipitation. These conditions favour the dominance of *Sphagnum* mosses, which form acidic, nutrient-poor, decomposition-resistant *Sphagnum* peat [7] [8].

The Olkiluoto Island, on the west coast of Finland, is selected for deep geological disposal of spent nuclear fuel in Finland. The nuclear waste will be placed in copper-lined cast iron canisters at about 400 m depth in the bedrock, embedded in bentonite clay in order to protect the canisters and prevent leakage of radionuclides into the environment [9]. After the last deglaciation, Olkiluoto Island lay below the surface of the Baltic Sea, and rose above the sea about 2500 - 3000 years ago [10]. The on-going crustal rebound in the region has changed and changes the coastal area, and within 6000 years the Olkiluoto Island will develop into an inland site and new bogs will form in the area [11]. This is the earliest time considered for radioactive releases from the deep spent fuel repository to be able to reach the biosphere if the nuclear waste canisters were to lose their integrity [12]. In the safety assessment for the long-lived radionuclides present in spent nuclear fuel, the Lastensuo Bog is identified as representative of biotopes expected to develop in Olkiluoto in the future due to crustal rebound [13].

Although wetland microbiology has been studied for decades, progress has been hampered by methodological challenges, such as inability to cultivate the majority of the microorganisms under laboratory conditions [14] [15]. Developments in molecular biology technologies (RLFP, cloning of 16S rRNA gene fragments) in recent years have resulted in an increase in our knowledge of acidic peat microbial inhabitants [14]. Most studies have concentrated on particular groups of microorganisms, such as *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, mehanotrophic or methanogenic microorganisms [16]-[21]. Only a few studies employing high throughput sequencing have been conducted to date to elucidate the whole bacterial community structure in acidic peatlands [15] [22]-[24]. Thus, the structure of the microbial communities and functional roles of many microbial inhabitants of northern wetlands remain unknown.

Sufficient understanding of the function of the microbial community in peat bogs in the region is important to support long-term safety assessments for geological disposal of nuclear waste. The aims of this study included the establishment of a baseline describing the bacterial communities at different depths of Lastensuo Bog, a 5300-year-old raised bog in Finland. This information will be used for further studies focusing on the involvement of the bacteria in the mobilization or impediment of radionuclides. In addition, we aimed to describe the bacterial communities throughout the bog profile in detail and to estimate the main metabolic pathways presented in the bacterial community of Lastensuo Bog.

2. Materials and Methods

2.1. Site Description

The sampling area, Lastensuo Bog, is located on the western coast of Finland approximately 30 km north-east of the Olkiluoto repository site. This south-west sloping raised bog is 440 ha in extent and is surrounded by hummocky till soils [11] [25]. The bog is 44 to 48 m above sea level and the maximum thickness of the peat layer is approximately 6 m. The underlying soil consists of clay and sand originating from the former seabed. Gyttja is found on top of the clay layer.

In terms of ecotopes, the central bog area consists of treeless or near-treeless *Sphagnum fuscum* bog, *Sphagnum fuscum* pine bog, ridge hollow pine bog and hollow bog [11] [25]. Towards the edges the ecotope change from low sedge bog and cotton grass pine bog to tall sedge pine fen and forested peatlands. Of the main peat types, 58% is *Sphagnum* peat, 8% sedge-moss peat, 19% sedge peat and 15% few-flowered sedge [25]. According to radiocarbon dating, the peat accumulation in the mire started 5300 years ago, when the area emerged from the Baltic Sea, and the average peat increment has been 1.08 mm/year [25].

The ecotope at the sampling plot, located at the centre of the bog, is almost treeless hummock-hollow pine bog [11]. The vegetation on the hummocks and hollows of the sampling plot were characterized in 2010 [11] and the vegetation on the hummocks included *Eriophorum vaginatum*, *Erica* spp., *Rubus chamaemorus*, *Vaccinium oxycoccus*, *Sphagnum fuscum*, *Andromeda polifolia*, *Vaccinium uliginosum*, *Empetrum nigrum*, *Cladina stellaris, Cladonia* spp., *Cetraria islandica* and *Dicranum* spp. In partly vegetated, partly muddy hollows identified plants included *Rhunchospora alba*, *Scheuzeria palustris* and *Sphagnum* spp.

2.2. Sampling

The peat samples from the Lastensuo Bog were collected in May 2011 and June 2013 from one sampling plot (61˚17'31"N, 21˚50'22"E, WGS84 coordinate system). The samples collected in 2011 were used for the geochemical characterization of the peat and the peat samples used for the isolation of nucleic acid were collected in 2013. The samples were taken from seven different peat layers: 0.5 - 1.0 m, 1.5 - 2.0 m, 2.5 - 3.0 m, 3.5 - 3.7 m, 3.7 - 4.0 m, 4.5 - 5.0 m, 5.5 - 6.0 m and 6.5 - 7.0 m. In addition, surface moss, mainly *Sphagnum* spp. (hereafter referred to as 0 m) was collected. The samples were taken using a Russian peat corer, made of stainless steel with a diameter of 15 cm and a nest length of 50 cm. The samples were placed into 2 L plastic bags and the sub-samples for nucleic acid isolation were transferred aseptically to sterile 50 ml Falcon tubes. All samples were cooled during transportation and samples for DNA extraction were stored frozen at −18˚C until thawed for DNA extraction.

2.3. Sample Characterization

From the peat samples dry weight (105˚C) and the organic matter (OM) contents (550˚C, measured by the loss on ignition method) were analyzed as described elsewhere [26]. Humification degree (von Post scale) was estimated based on [27]. The pH was measured in 0.01 M $CaCl_2$ solution (ISO10390 standard) using a gel-filled electrode. All analyses were performed in triplicate.

2.4. Nucleic Acid Isolation

Approximately 0.5 g subsamples of moss, peat or clay were used for DNA extraction from 0 m (*Sphagnum* moss), peat from 0.5 - 1.0 m, 1.5 - 2.0 m, 2.5 - 3 m, 3.5 - 3.7 m, 3.7 - 4.0 m, 5.5 - 6.0 m depth and former seabed clay from 6.5 - 7.0 m depth. Community DNA was isolated directly from the thawed samples with the Nucleo Spin Soil DNA extraction kit (Macherey-Nagel). Two parallel extractions were performed for each depth one of which was extracted with buffer SL1 (sample a) and the other with buffer SL2 (sample b) with the addition of the enhancer solution, according to the manufacturer's protocol. An additional extraction was performed using the same buffers without the enhancer solution in order to maximize the amount of DNA extracted. The DNA extraction proceeded as instructed by the manufacturer. The DNA was eluted in 100 µl elution buffer and the DNA concentration of each extract was measured using the NanoDrop-1000 spectrophotometer.

2.5. Amplicon Library Preparation

Libraries for 454 high throughput (HTP) amplicon sequencing were prepared by PCR from each DNA extraction. Bacterial 16S rRNA gene fragments covering the V1 - V3 variable regions were amplified with primers 8F and P2 [28] [29] equipped with adapter and MID sequences (tags) at their 5' end in a single round PCR as described in [30]. PCRs were performed with the KAPA HiFi polymerase (KapaBiosystems, Inc., Boston, MA, USA) in 1 × HF buffer. Each 50 µl reaction contained 0.5 mM dNTP and 1 µM of primers. The PCR conditions consisted of an initial denaturation step of 30 s at 98˚C, followed by 35 cycles of 10 s at 98˚C, 15 s at 55˚C and 15 s at 72˚C, and a final extension step at 72˚C for 5 min. The sequencing was performed at Macrogen Inc., Korea, using the FLX 454 (454 Life Sciences, Branford, CT, USA).

2.6. Real-Time Quantitative PCR

Bacterial 16S rRNA genes were quantified by qPCR with LightCycler® 480 SYBR Green I 2× Master mix (Roche, Finland) using primers P1 and P2 [29]. Reactions were performed in triplicate for each sample. Each reaction contained 1 µl of extracted DNA as template and 5 pmol of both forward and reverse primers. The qPCR was performed on a Roche LightCycler 480 (Roche Applied Science, Germany) on white 96-well plates (Roche Applied Science, Germany). The qPCR conditions consisted of an initial denaturation at 95˚C for 10 minutes followed by 45 amplification cycles of 15 seconds at 95˚C, 30 seconds at 55˚C and 30 seconds at 72˚C with a quantification measurement at the end of each elongation. A final extension step of three minutes at 72˚C was performed prior to a melting curve analysis. This consisted of a denaturation step for 10 seconds at 95˚C followed by an annealing step at 65˚C for one minute prior to a gradual temperature rise to 95˚C at a rate of 0.11˚C s^{-1} during which the fluorescence was continuously measured. The number of bacterial 16S rRNA genes was determined by comparing the amplification result (Cp) to that of a dilution series of *E. coli* 16S rRNA genes in plasmid.

2.7. Sequence Processing and Analysis

Sequence reads were analysed using QIIME [31] to remove adapter, barcode and primer sequences, and to exclude sequences that did not meet the quality criteria (*i.e.*, no barcode and primer mismatches, no ambiguous nucleotides, maximum eight nucleotide long homopolymer stretches and defined minimum length of 200 bp). The bacterial 16S rRNA genes sequences were grouped into Operational Taxonomic Units (OTUs; 97% sequence similarity) and classified using the GreenGenes 13_8 16S rRNA gene sequence reference database [32]. The sequencing coverage was evaluated by rarefaction analysis and the estimated species richness and diversity indices were calculated. For comparable α- and β-diversity analyses the data sets were normalized by random subsampling of 1147 sequences/sample. Samples with lower number of sequences were excluded. Microbial metabolic pathways were estimated based on the 16S rRNA gene data using the PICRUSt software [33] on the web based Galaxy application [34]-[36].

2.8. Accession Numbers

The sequences have been submitted to the European Nucleotide Archive (http://www.ebi.ac.uk/ena) under accession numbers ERS515436 - ERS515450.

2.9. Statistical Analyses

The relationships between the measured peat characteristics, amount of bacteria, community richness (Chao 1) and diversity (Shannon index H') of the bacterial community, and bacterial community structure, were analysed by univariate analysis. Prior to the analysis the normal distribution of the data was evaluated by the Shapiro-Wilk test ($p < 0.05$). Hypotheses about normality were rejected for pH, moisture, organic matter content, Shannon index and the abundance of taxonomic groups. Only richness (Chao 1) was close to normal distribution. Therefore, we used Spearman's correlation analysis, since the Spearman's rank correlation coefficient does not assume that the relationship among the variables is linear. All statistical analyses were performed using OriginPro 8.6 (OriginLab, USA).

3. Results and Discussion

3.1. Characteristics of the Surface, Peat and Clay Samples

The humification degree of the peat samples increased as a function of depth from H3 in the 0.5 - 1.0 m layer to H4 - H5 in the middle layers and finally to H6 in the deepest peat layer at 5.5 - 6.0 m (**Table 1**). The deepest sampling layer (6.5 - 7.0 m) below the peat layers was light grey clay. The organic matter (OM) content of the samples was measured by the loss on ignition (LOI) method, and although the OM content and LOI are not exactly the same they strictly correlate with each other. For simplicity, here we refer to LOI as OM content. The OM content was relatively constant, 99.6% ± 0.2% on average, down to the depth of 4.0 m and decreased to 95.0% ± 0.2% at 5.5 - 6.0 m. The OM content of the deepest (clay) layer was 15.3%. Water content of the peat ranged from 66.2% to 92.7%. Water content was lowest in the 2.5 - 3.0 m peat layer of the bog. The pH of the bog

Table 1. Characteristics of the peat sample obtained from different depths in Lastensuo bog.

Layer	pH*	Humification degree	Water content, %	Organic matter content**, %
0 m (surface)	3.1	H1	92.0	99.2
0.5 - 1.0 m	3.1	H3	88.8	99.5
1.5 - 2.0 m	3.0	H4	90.9	99.6
2.5 - 3.0 m	3.2	H4	66.2	99.8
3.5 –3.7 m	3.2	H4/H5	94.1	99.8
3.7 - 4.0 m	3.3	H5	91.0	99.8
5.5 - 6.0 m	4.0	H6	83.3	95.0
6.5 - 7.0 m	5.3	Clay	73.2	15.3

*pH was measured in 0.01 M $CaCl_2$ solution; **Organic matter was determined by loss on ignition (LOI) method.

was approximately the same, 3.1 - 3.3, to the depth of 3.5 - 4.0 m and increased to 4.0 and 5.3 at depths of 5.5 - 6.0 m and 6.5 - 7.0 m, respectively.

3.2. Microbial Counts—qPCR

The number of bacterial 16S rRNA gene copies determined by qPCR was used as a proxy for estimating the size of the bacterial community at different depths in the bog. The 16S rRNA gene copy number is not a precise indicator of bacterial population size because different bacterial species contain varying numbers of 16S rRNA gene copies in their genomes. However, here we assumed that the bacteria inhabiting this oligotrophic environment are slow growing organisms with only a low number of 16S rRNA genes in their genomes. The obtained gene copy number is, thus, used as a proximation for bacterial number. All peat samples except that from 2.5 - 3.0 m depth had similar numbers of bacterial 16S rRNA genes: 1×10^{10} - 5×10^{10} copies g^{-1} dry weight (DW) peat. The lowest bacterial numbers were detected in the peat sample from the 2.5 - 3.0 m depth (2×10^9 copies g^{-1} DW) and the clay layer (5×10^9 copies g^{-1} DW).

3.3. Bacterial Community Composition and Diversity

HTP sequencing of bacterial 16S rRNA genes with 454 technologies identified a total of 12,680 bacterial Operational Taxonomic Units (OTUs) sharing 97% internal sequence homology. The highest number of OTUs in the normalized data was observed at 0 m with 488 identified and up to 1143 estimated (Chao 1) bacterial OTUs (**Table 2**). This sample also had the highest diversity index (H' = 8.28, normalized to equal number of reads/sample). In general, the lowest number of identified (<200) and estimated OTUs (<600) and diversity index (<6) was shown in samples between 1.5 to 4 m depth, with the exception of one of the parallel samples from 2.5 - 3.0 m, which had a slightly higher number of identified OTUs. The rarefaction analyses showed that bacterial community was quite well characterized (**Figure S1**). However, the non-normalized sequence data showed that >70% of the diversity was in general obtained from the uppermost (0 m) and the deepest (5.5 - 7.0 m) samples, with the exception of one of the parallel samples from 1.5 - 2.0 m and 2.5 - 3.0 m, for which <70% of the diversity was obtained by the HTP sequencing.

A total of 40 different bacterial phyla of which 13 phyla were present at all depths were detected (**Figure 1**). These phyla covered 97% - 99% of all sequence reads of each sample. A detailed heat map showing the relative abundance of the detected genera is presented in the supplementary material (**Table S1**). On the surface, the microbial community was dominated by acidophilic *Acidobacteria* (Subdivisions 1 and 3), *α*- and *γ*-*Proteobacteria* (*Acetobacteraceae* and *Sinobacteraceae*). *Plantomycetes*, candidate division WPS-2 and *Verrucomicrobia* were also detected in the 0 m sample. *Acidobacteria* clearly dominated the bacterial community in the peat layers between 0.5 m and 3.7 m and remained the most abundant phylum between 3.7 m and 6.0 m, although their relative abundance decreased below 50% at this depth. The relative amount of proteobacterial sequences in the peat declined significantly with depth, but increased again in the clay layer. In subsurface peat, the *Proteobacteria* accounted for only 0.9% - 11.7% of the bacterial community showing the lowest abundance at 2.5 - 3.7 m

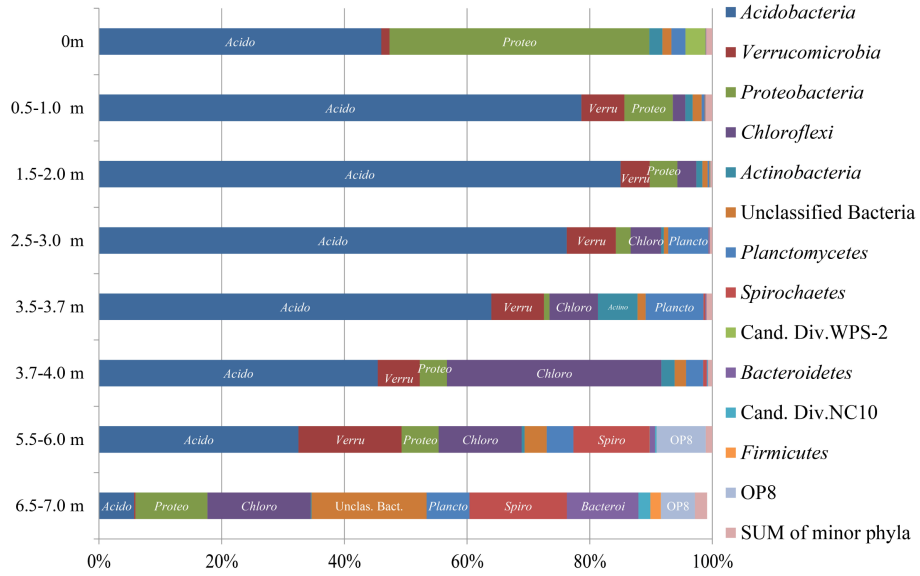

Figure 1. Relative distribution of dominant phyla in samples collected from eight different depths. Operational Taxonomic Units (OTUs) are defined at 97% sequence identity threshold. "Sum of minor phyla" designates the combined relative sequence abundance of those phyla and candidate taxa which present <1.0% in any sample. Minor groups: phyla—*Armati- monadetes*, *Chlorobium*, *Lentisphaerae*, *Nitrospirae*, *Fibrobacteres*, *Elusimicrobia*, *Cyanobacteria*, *Gemmatimonadetes*, and *Caldiserica*; candidate divisions—AC1, AD3, BRC1, FCPU426, GN04, GOUTA4, NKB19, TM7, OD1, OP1, OP3, OP11, SC4, TM6, WS1, WS2, WS3 and WS4.

Table 2. Observed species richness and diversity estimates from high throughput sequencing analysis (97% Operational Taxonomic Unit, OTU, similarity).

Depth	Sample	Number of sequence reads	Observed number of OTUs*	Estimated number of OTUs* Chao 1	Diversity estimators		OTUs consisting of single sequences*	OTUs consisting of double sequences*	Observed number of OTUs, total	Estimated number of OTUs, total Chao 1	Diversity obtained from all sequences, %
					Shannon*	Simpson*					
0 m	a	3799	485	980	8.28	0.99	289	83	877	1161	75.6
	b	10,822	488	1143	8.20	0.99	314	74	1275	1369	93.2
0.5 - 1 m	a	3621	351	924	6.95	0.98	235	47	686	1103	62.2
	b	12,545	299	628	6.52	0.96	180	48	1149	1677	68.5
1.5 - 2 m	a	45,647	190	541	5.17	0.92	122	20	1830	2346	78.0
	b	11,861	157	420	4.84	0.91	95	16	629	1109	56.7
2.5 - 3 m	a	10,177	185	465	5.66	0.96	109	20	671	1084	61.9
	b	21,238	215	514	5.98	0.96	130	27	1089	1460	74.6
3.5 - 3.7 m	a	30							19	32	59.0
	b	6442	183	523	5.53	0.95	108	16	469	795	59.0
3.7 - 4 m	a	1147	172	327	5.04	0.90	92	26	172	327	52.6
	b	7768	177	372	4.76	0.86	103	26	5080	780	65.1
5.5 - 6 m	a	7698	281	568	6.50	0.97	168	48	814	1158	70.3
	b	13,426	272	739	6.39	0.97	176	32	1073	1391	77.1
6.5 - 7 m	a	4359	355	682	7.26	0.98	202	61	655	801	81.7
	b	7455	340	633	7.04	0.98	194	63	764	851	89.8

*normalized to 1147 sequences: a—DNA sample obtained by extraction with the buffer SL1; b—DNA sample obtained by extraction with the buffer SL2.

depth. The phylum *Chloroflexi* was not detected in the surface sample, but was abundant in the deep peat and bottom clay layers, with highest representation at 3.7 - 4.0 m (34.9%). The phylum *Planctomycetes* was present throughout the depth profile, although its relative amount in the peat between 0.5 and 2.0 m was less than 1.0%. The highest relative abundance of *Planctomycetes* was detected at 2.5 - 3.7 m depth (6.6% - 9.4%). *Actinobacteria* were also detected in all samples with a maximum relative abundance from 3.5 - 3.7 m depth (6.5%).

The highest bacterial diversity was obtained from the peat at 5.5 - 6.0 m and the former seabed clay at 6.5 - 7.0 m. In the peat *Acidobacteria* (32.5%), *Verrucomicrobia* (16.8%), *Chloroflexi* (13.5%), *Spirochaetes* (12.4%), OP8 (8.0%), *Proteobacteria* (6.0%), and *Planctomycetes* (4.4%) were the dominating phyla. The bacterial community in the clay was represented by *Chloroflexi* (16.8%), *Spirochaetes* (15.9%), *Proteobacteria* (11.8%), *Bacteroidetes* (11.7%), *Planctomycetes* (7.0%) and *Acidobacteria* (5.72%). In addition, the bacterial sequences that could not be assigned to any known bacterial phyla were present at high numbers (18.8%).

3.4. Correlation of Bacterial Community and Bog Core Characteristics

Correlation analysis (**Table S2**) showed that the copy number of 16S rRNA genes had a significant positive correlation with OM ($r = 0.86$; $p < 0.01$) content, and negative with pH ($r = -0.5$; $p < 0.05$). Bacterial richness (based on Chao 1 estimator) and diversity (Shannon index) correlated only with OM content.

Only phylum *Actinobacteria* showed positive relationship with water content of the samples. Nevertheless, ten phyla or classes correlated with pH and four phyla or classes with OM content (**Table S3**). The peat samples had very low pH ranging from 3.1 in the surface sample to pH 4.0 in the deepest (5.5 - 6.0 m below the surface) peat sample, while the bottom clay layer had pH 5.3. Low pH most probably served as an ecological filter and the absence of the correlation between the pH and many taxonomic groups was due to the small variations in pH between the samples. Acidobacterial TM1, the largest group in all subsurface peat samples, except in layer 3.7 - 4.0 m, did not correlate with pH, water or OM content. The class *Dehalococcoidetes* (GIF9), dominating the whole bacterial community in the sample collected from 3.7 - 4.0 m showed only positive relationship with pH. The distribution of phylum *Verrucomicrobia* did not correlate with any measured parameters.

3.5. Characteristics of the Major Phyla

Acidobacteria The phylum *Acidobacteria* was represented by several subdivisions (**Figure 2(A)**). On the surface (0 m), the most prominent lineage was the Subdivision 1 (class *Acidobacteria*) followed by Subdivision 3 (class *Solibacteres*). The phylum *Acidobacteria* is a large diverse group consisting of at least 26 subdivisions [37], but few isolated and characterized species exist. Their ecological role in elemental cycles, thus, is poorly understood. The isolated members of this group are aerobic or facultative anaerobic organisms. The prevalence of *Acidobacteria*, especially Subdivisions 1, 2 and 3, in acidic low-nutrient *Sphagnum* peat has been documented [23] [24] [38]. The majority of the acidobacterial Subdivision 1 sequences from the Lastensuo Bog could not be assigned to any known genus. The genus *Granulicella* was detected in all peat layers with relative abundance ranging from 2% of total bacterial community in the surface samples to <0.5% at 3.7 - 6.0 m. Members of this genus have been isolated from tundra soil and *Sphagnum* peat of bogs [39] [40]. Acidobacterial Subdivision 1 has an important role in degradation of cellulose in acidic *Sphagnum* peat [41]. This is supported by a complete genomic analysis of two cultured strains of Subdivision 1 [42]. Cellulolytic bacteria have recently been isolated from *Sphagnum* peat samples and characterized [43]. Genomic analyses also suggested that the *Acidobacteria* play a role in nitrogen cycling in soils and sediments with ability for nitrate and nitrite reduction [42]. As this microbial group was dominant in the Lastensuo Bog peat, it may, in addition to contributing to the carbon cycle and the degradation of dead plant biomass, also be the major nitrogen cycling bacterial group in this community.

Sequences assigned to Subdivision 3 (*Candidatus* Solibacter) accounted for almost 34% of all acidobacterial sequences in the moss layer. Their relative amount declined with depth, except for the samples from 2.5 m to 3.7 m, where they accounted for about 20% of all acidobacterial sequences.

In this study we found that an uncultured group represented by a cloned 16S rRNA gene sequence obtained from grass roots, clone TM1 [44], was the most abundant acidobacterial group in all subsurface peat samples as well as in the clay layer accounting for up to 59.8% of the total sequence number retrieved from 1.5 - 2.0 m depth. To the best of our knowledge, there are no reports on such high abundance of this group in acidic peat bogs. Acidobacterial Subdivision 2 was mostly detected in the middle of the peat core collected from 3.5 m to 4.0 m depth. Sequences assigned to class *Holophagae* (Subdivision 8) as well as those represented by several uncultivated clones MVS-40 and OS-K were found only in small amounts.

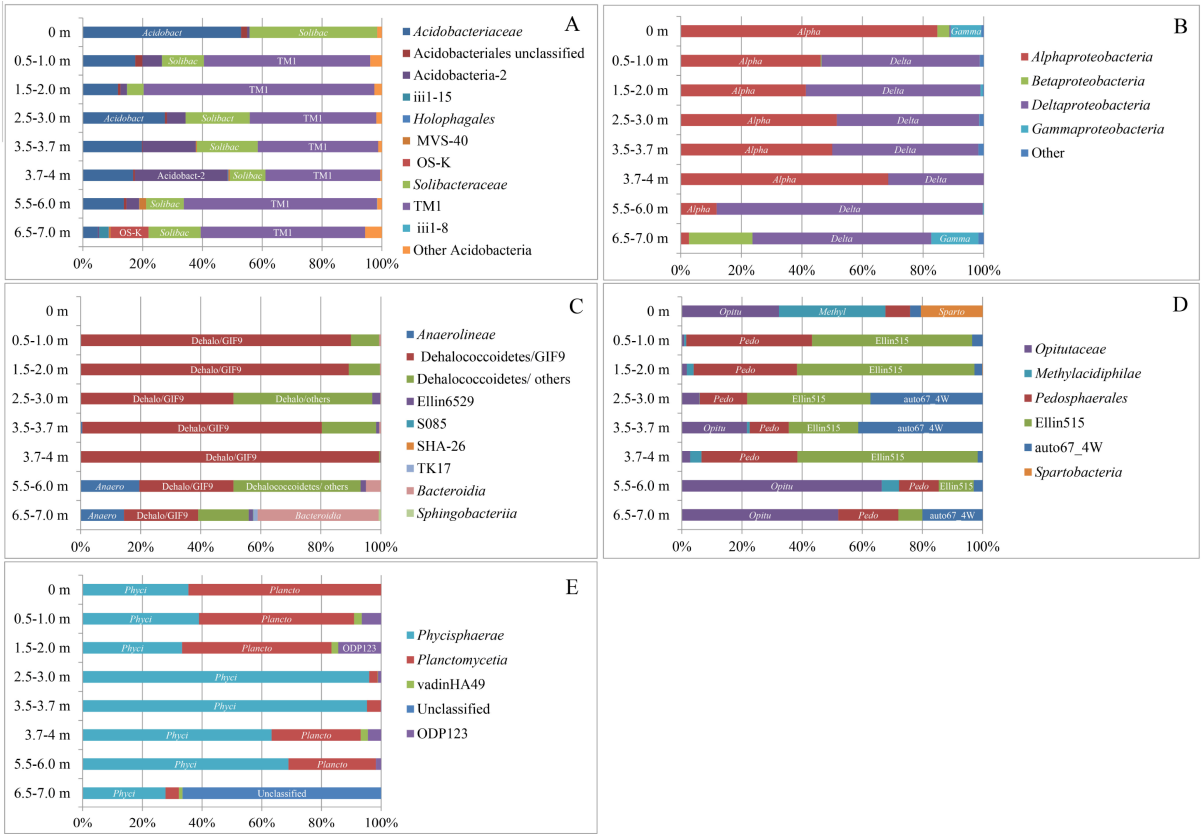

Figure 2. Diversity of the phyla in peat samples in relation to depth: A—*Acidocabteria*; B—*Proteobacter*; C—*Chloroflexi-Bacteroides* lineage; D—*Verrucomicrobia*; E—*Planctomycetes*.

Proteobacteria The *Proteobacteria* were primarily represented by *α*-proteobacterial lineages at depths from the surface to 4.0 m (**Figure 2(B)**). The diversity of *α-Proteobacteria* was high. In the moss layer, the major group was unclassified *Acetobacteraceae* (about 20% of the total community). Representatives of this group inhabit various acidic environments and have often been found in acidic peat [24] [38] [45]-[47]. The family *Methylocystaceae* was present at notable relative abundance in the moss layer (more than 3% of all bacterial sequences) but was also detected in all peat layers and the bottom clay. These bacteria are generally aerobic methanotrophic organisms oxidizing methane emitted from deeper peat layers before it reaches the atmosphere [48]. Nevertheless, as shown for *Methylocystis parvus*, they are able to succeed under anoxic conditions by fermenting a storage compound, poly-*β*-hydroxybutyrate as an alternative to aerobic methane oxidation [49].

β-Proteobacteria were only detected in the moss layer and in the clay from 6.5 - 7.0 m depth, where they were the second largest proteobacterial group. All *β*-proteobacterial sequences found in the clay were grouped to the family *Comamonadaceae,* which was mostly represented by the genus *Hydrogenophaga*, harbouring mostly hydrogen-oxidizing chemoautotrophic microorganisms (1.8% of the total sequence reads).

δ-Proteobacteria were present in the moss layer at only very low abundance. However, their abundance in regard to the other proteobacterial classes increased with depth. In all subsurface samples this lineage was the main proteobacterial group, accounting for more than 80% of all *Proteobacteria* at 5.5 - 6.0 m. The majority of *δ-Proteobacteria* at 0.5 - 1.0 m depth was affiliated with the anaerobic acetate-degrading sulphate reducing genus *Desulfobacca* [50]. In the clay layer the *δ-Proteobacteria* were mostly represented by genus *Syntrophus*, members of which are strict anaerobes living in syntrophic relationship with hydrogen- and formate-utilizing microorganisms [51] such as *Hydrogenophaga*. 16S rRNA gene sequences belonging to *Syntrophus* sp. have recently been retrieved from acidic bog peat [38] [52] [53]. Sequences of uncultured *δ-Proteobacteria* similar to those represented by the environmental clone BPC076 (Accession Number AF154096) originating from a hydrocarbon seep were detected only in peat from 5.5 - 6.0 m depth and clay where they represented 62% and 37%, respectively, of all *δ*-proteobacterial sequences found in these layers.

γ-Proteobacteria were detected in the moss layer and clay and were not found in the peat between 3.5 m and 4.0 m depth. In the surface sample more than 90% of the γ-proteobacterial sequences were affiliated with the order *Xanthomonadales* (4.1% of the total community) and in the clay to methanotrophic *Methylococcales* (1.8%), almost entirely represented by the genus *Crenothrix* (1.8% of the total bacterial community). Surprisingly, the latter was retrieved only from the sample from 6.5 - 7.0 m. The isolated members of this genus are aerobic methane oxidizing bacteria [54]. Recent discovery of a new form of fermentation-based methanotrophy in *Methylomicrobium alcaliphilum*, however, opened the possibility that under anoxic conditions methanotrophs are capable of methane consumption with production of hydrogen [55]. Whether such metabolic activity exists in other *Methylococcales* species is yet to be shown.

***Chloroflexi-Bacteroidetes* lineage** *Chloroflexi* were absent from the moss layer but present in all subsurface samples (**Figure 2(C)**). They were the second largest bacterial group (34.9%) in the peat from 3.7 - 4.0 m depth. In all subsurface samples to the depth of 4.0 m, *Chloroflexi* were represented almost entirely by the class *Dehalococcoidetes*, from 97% to 100% of all *chloroflexi* sequences. The majority of the *Dehalococcoides* sequences were assigned to candidate group GIF9 originally isolated from a chlorobenzene contaminated groundwater-treated reactor [56]. Members of the class *Dehalococcoidetes* so far isolated and physiologically characterized are anaerobic obligate organohalide-respiring microorganisms that use hydrogen as an electron acceptor [57] [58]. The appearance of these bacteria might be connected to the accumulation of halogenated organic compounds as a result of humification of plant material [59]. Currently, however, there is no evidence to conclude that the metabolism of group GIF9 would be as unusual as it is for the isolated *Dehalococcoides* species. The lineage *Anaerolineae* appeared only in the deepest peat collected from 5.5 - 6.0 m and the clay. Members of the *Anaerolineae* are filamentous anaerobic bacteria, which have been identified from diverse environments, including arctic permafrost, sediments, and anaerobic methanogenic sludge bioreactors [60]-[62]. Characterized *Anaerolineae* strains are able to ferment carbohydrates [63]. In addition, some *Anaerolineae* species have been found to benefit from syntrophic relationships with hydrogenotrophic methanogens.

The phylum *Bacteroidetes* was almost entirely represented by a taxonomically unclassified group of the order *Bacteroidales* (**Figure 2(C)**). The isolated and characterized members of this group are mostly anaerobic or facultative anaerobic microorganisms found in a large variety of environments, from sediment to biogas reactors [64]. A nitrogen-fixing facultative anaerobe, which belongs to this order, was also recently characterized [65].

Verrucomicrobia *Verrucomicrobia*l sequences were assigned to four classes, namely, *Opitutae*, *Pedosphaerae*, *Methylacidiphilae* and *Spartobacteria*, of which the two last groups are present only in a few of the tested samples and only at very low abundance levels (**Figure 2(D)**). The majority of the verrucomicrobial sequences belonged to the class *Pedosphaerae*, which were most abundant in subsurface peat layers. Sequences assigned to the subdivision 4 (class *Opitutae*) were retrieved from all depths, with the exception of the surface sample. The majority of the sequences formed an unknown group of the family *Opitutaceae*. This group was abundant in the peat layer from 5.5 to 6.0 m depth where it accounted for more than 11% of the total bacterial community. *Verrucomicrobia*l Subdivision 4 was reported from an acidic peat bog [24] [38]. However, unlike in our study it was abundant only in surface samples. Bacteria from this family are known to be fermentative anaerobes [66] and to have the ability to degrade lignocellulose and fix nitrogen [67]. The abundance of the class *Pedosphaerae* was uniform in the layers from 0.5 m to 6.0 m depth. In the moss layer and deepest clay layer this group was minor. The majority of pedosphaerael sequences remained unassigned to any known *Pedosphaerae* group, but was closely similar to a group of uncultured *Pedosphaerae* represented by clone Ellin515 obtained from soil [68]. Sequences assigned to the sub-phylum 6, *i.e.*, class *Methylacidiphilae*, which is currently known to contain only methanotrophs [16] [69], were detected in all samples, but were present at low abundance.

Planctomycetes *Planctomycetes* bacteria were present at all depths, but in the peat at 0.5 - 2.0 m depth, they were present at very low abundance (**Figure 2(E)**). In the peat layer from 2.5 m to 6.0 m depth the *Planctomycetes* were mostly represented by the *Phycisphaerae* lineage, which accounted for almost 9% of the total community in peat from 3.5 - 3.7 m. The *Phycisphaerae* have recently been isolated from marine algae [70]. The pure cultured species of this group are facultative anaerobes able to reduce nitrate to nitrite. In addition, the *Phycisphaerae* species heterotrophic, with the ability to hydrolyse different sugars and organic acids. In the clay layer at 6.5 - 7.0 m sequences affiliated with unclassified *Planctomycetes* were predominant (4.6%). *Planctomycetes* isolated from acidic peat available in pure culture are aerobic or facultative anaerobic chemoorganoheterotrophic bacteria [71]-[74]. However, certain members of the *Planctomycetes* are able to perform the ANAMMOX process, *i.e.*, the anaerobic oxidation of ammonia to N_2 gas [75].

Spirochaeta The role of *Spirochaeta* in acidic peatland environment is unclear. Some members of this genus are sulphur- and thiosulphate-reducers. However, these bacteria are more often associated with high pH. Genes encoding nitrogenase enzymes (used in nitrogen fixation), closely related to those from *Spirochaeta* have been detected in a bacterial community associated with *Sphagnum* moss [76].

Candidate Divisions Among the detected candidate divisions the three most abundant ones were NC10, OP8, and WPS-2. Sequences assigned to the candidate division OP8 (current name *Aminicenantes*) were not found in the samples above 4.0 m, but they were abundant in the peat samples collected from 5.5 - 6.0 m and the clay from 6.5 - 7.0 m below the surface (8.0% and 5.6% of the total, respectively). This division was established based on sequences found from hot spring [77]. *In silico* database mining has shown that this phylogenetically diverse group is ubiquitous, found in different environmental niches, although more often in anoxic low salinity environments [78]. To our knowledge this is the first report of this group recovered from an acidic bog.

Candidate division NC10 was also absent in the surface samples, appearing only at relative low abundance level (less than 0.05%) from 1.5 - 3.0 m, 3.7 - 4.0 m and 5.5 - 6.0 m, but accounted for 1.9% of the total community in the clay at 6.5 - 7.0 m. This group harbours the denitrifying methanotrophic organism "*Candidatus Methylomirabilis oxyfera*" [79], which performs nitrite-dependent anaerobic methane oxidation in anaerobic environments [80]. Sequences affiliated with candidate division WPS-2 were present in the surface sample with relative abundance 3.3%, but were also recovered at very small amounts from all subsurface samples, less than 0.05% of the total community. This division has also previously been seen in acidic peat of northern wetland [24].

3.6. Predicted Metabolic Pathways of the Bacterial Community

The metabolic pathways presented in the bacterial community at different depths of the sample core were predicted based on the 16S rRNA gene data using the recently developed software PICRUSt [33]. The most abundant metabolic pathways contributing >1% of the predicted genes in the bacterial community are presented in **Figure 3**. The accuracy of the metabolic estimator depends on quantity and quality of annotated genomes. Because of the large proportion of poorly described taxa in the Lastensuo Bog samples, the metabolic profiling can be biased and needs to be updated when new genome sequences are annotated. Nevertheless, this approach gave us additional information on the bacterial community. Little variation in the predominant metabolic pathways was observed between the different depths. However, the clay layer at the bottom showed a slightly different pattern than the moss and peat samples. The carbon fixation, nitrogen metabolism, oxidative phosphorylation, sulphur metabolism, amino sugar and nucleotide sugar metabolism, starch and sucrose metabolism, peptidases and specific secretion systems showed the lowest representation in the clay compared to the moss and peat samples. The clay layer had the highest representation of purine metabolism, pyrimidine metabolism, glycolysis/gluconeogenesis, pyruvate metabolism, glycine and serine and threonine metabolism compared to the moss and peat samples from the various depths. Starch and sucrose metabolism were most abundantly repre- sented in the moss and peat samples, where *Acidobacteria* was the most common bacterial group. In addition, the relative abundance of methane-metabolizing and nitrogen-cycling bacterial groups identified throughout the peat profile is mirrored by the high predicted number of genes involved in these processes throughout the vertical profile.

4. Conclusions

The high throughput sequence analyses revealed a very high diversity within bacterial communities in the sampled acidic peat profile from Lastensuo raised bog. The community was dominated by *Acidobacteria*, especially the TM1 lineage, which has not been reported for acidic *Sphagnum* peat before. In addition, many known aerobic methane oxidizing bacterial groups were identified, which might employ alternative metabolic routes for oxidizing methane anaerobically or use completely different life styles under anaerobic conditions. Many nitrogen- and carbon dioxide-fixing bacteria were identified, which could represent primary producers in the anaerobic peat where the majority of the microbial community consisted of OM degraders. In addition to fermentation, nitrate-respiring microorganisms were also frequently detected. The statistical analyses showed that the sample characteristics, such as pH value, water or OM contents did not influence the bacterial richness or diversity. Additionally, dominant taxonomic groups, such as classes *Dehalococcoidetes* (GIF9) and acidobacterial TM1 as well as phylum *Verrucomicrobia*, did not correlate with the above mentioned parameters. The prediction of bacterial metabolic pathways using the PICRUSt software showed that the clay layer differed only slightly from peat or moss samples.

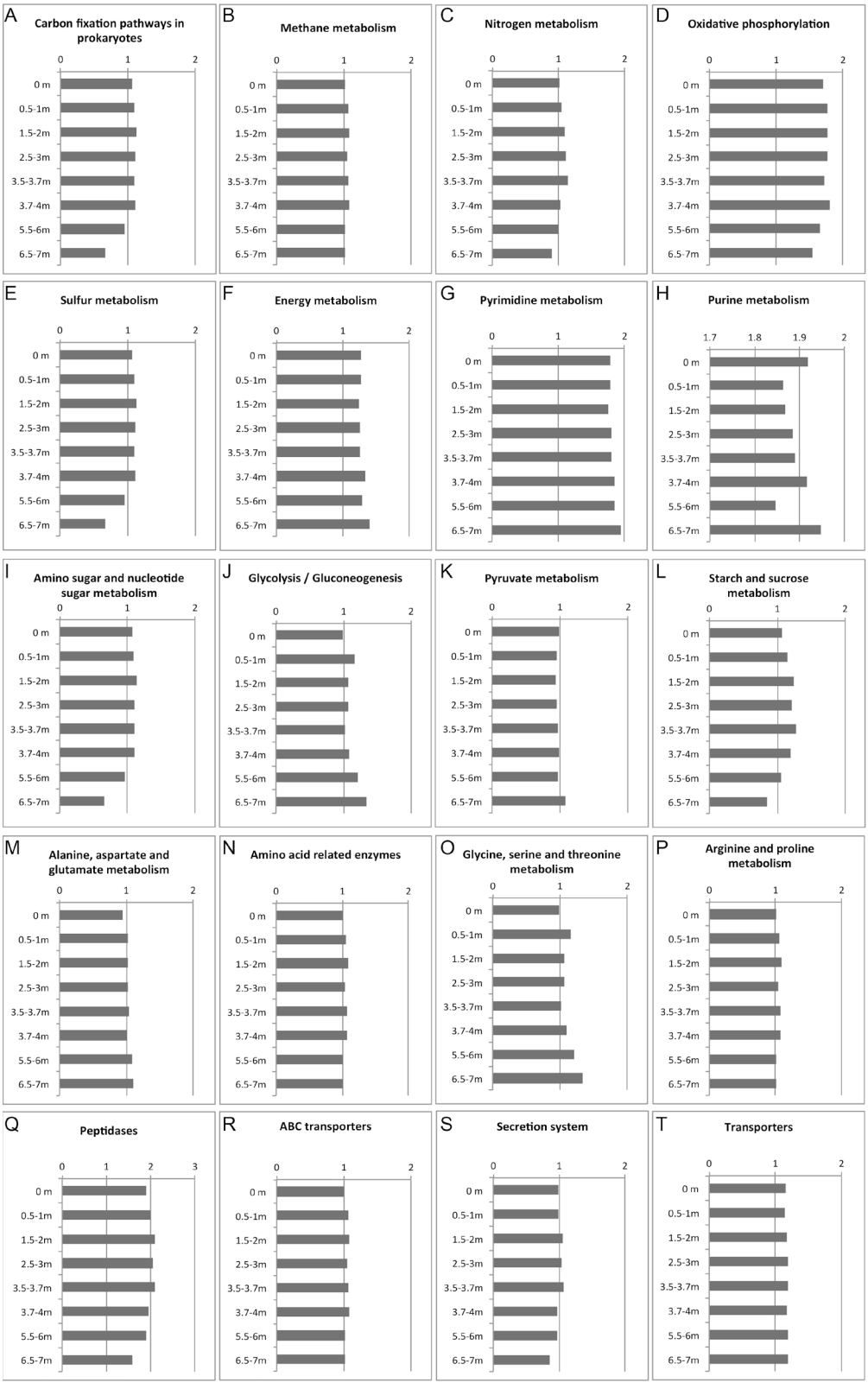

Figure 3. Relative abundance of genes associated with different metabolic activities. From upper left to right: (A)-(F) energy metabolism; (G)-(H) nucleotide metabolism; (I)-(L) carbohydrate metabolism; (M)-(P) amino acid metabolism; (Q) enzyme families; (R)-(T) membrane transport.

The results reported here highlight the necessity for more detailed studies on the Lastensuo Bog characteristics and their effect on bacterial inhabitants. The revealed bacterial community diversity in the acidic ombotrophic Lastensuo Bog can serve as a solid background for further research on bacterial involvement in the fate of radionuclides in case of their release in similar biotopes.

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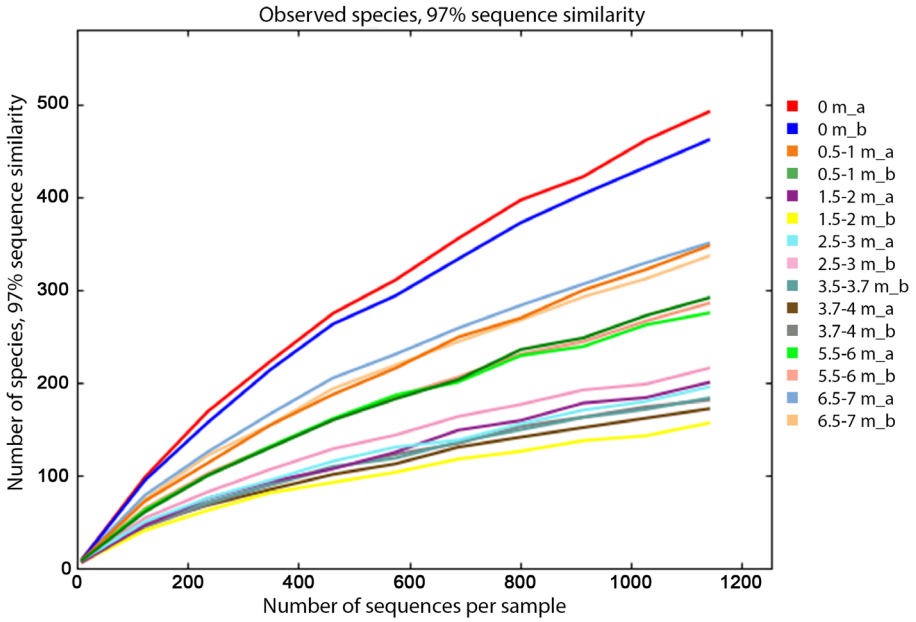

Figure S1. Rarefaction analysis showing the number of different bacterial 16S rRNA gene sequences detected per number of randomly picked sequences. The sequence reads were grouped into Operational Taxonomic Units (OTUs) sharing > 97% sequence similarity between sequence reads. Samples are named as in **Table 2**.

Table S1. Heat map showing the abundance as percentage (%) of total number of sequence reads designated to different taxonomic groups atthe different depths of the bog profile. The colouring describes low (green), medium (yellow) and high (red) relative abundance of sequence reads).

Phylum	Class	Order	Family	Genus	Relative amount at different depths*							
					0 m	0.5 -1 m	1.5-2.0 m	2.5-3.0 m	3.5-3.7 m	3.7-4.0 m	5.5-6.0 m	6.5-7.0 m
Unclassified					0.04	0.75	0.06	0.02	0.34		0.05	0.15
Bacteria					1.48	1.47	0.85	0.70	1.30	1.86	3.62	18.77
AC1	HDBW-WB69											0.08
	SHA-114										0.07	0.20
AD3	JG37-AG-4					0.09	0.10	0.03	0.05	0.08	0.04	
Acidobacteria	Other				0.56	2.70	1.94	1.36	0.76	0.22	0.47	0.30
	Acidobacteria	Acidobacteriales	Other		0.73	1.66	0.63	0.55	0.14	0.22	0.27	
			Unclassified				0.00					
			Acidobacteriaceae	Other	2.20	2.70	2.67	11.74	7.79	0.44	0.98	0.08
				Unclassified	14.92	8.23	5.24	6.19	2.78	6.52	2.86	0.19
				Acidobacterium			0.01	0.01				
				Edaphobacter	0.01	0.02	0.00	0.04			0.00	
				Granulicella	2.16	1.18	1.17	2.35	1.75	0.03	0.33	
			Koribactera-ceae	Other	0.61	0.96	0.45	0.15	0.02	0.21	0.16	0.03
				Unclassified	8.47	7.70	6.74	1.40	0.54	2.57	1.73	0.38
				Candidatus Koribacter	0.48	0.61	0.38	0.53	0.93	1.15	0.17	0.03

Continued

	Acidobacteria-2				0.30	4.52	1.71	4.59	11.16	12.87	1.27	0.03
	Acidobacteria-6	clone iii1-15										0.16
	Holophagae	Holophagales	Other		0.01		0.01	0.00		0.02	0.02	0.01
			Unclassified			0.01						
			Holophagaceae			0.03	0.01			0.09	0.00	
		MVS-40						0.01	0.20	0.21	0.71	0.03
	OS-K											0.67
	Solibacteres	Solibacterales	Solibacteraceae	Candidatus Solibacter	15.56	9.72	4.30	15.91	12.75	4.94	3.83	0.92
	TM1				0.01	38.60	59.79	31.42	25.17	15.93	19.68	2.90
	iii1-8										0.02	
Actinobacteria	Other				0.02	0.09	0.03	0.02	0.15	0.02	0.02	0.01
	Acidimicrobiia	Acidimicrobiales	Other		0.05		0.00		0.02	0.01		
			Unclassified		1.23	1.09	0.93	0.42	6.29	2.13	0.40	0.08
	Actinobacteria	Actinomycetales	Other		0.31	0.01	0.02				0.01	
			Actinospicaceae				0.01					
			Propionibacteriaceae	Propioni-bacterium			0.00			0.01		
		WCHB1-81	At425_EubF1									0.02
	OPB41											0.02
	Thermoleophilia	Gaiellales	Unclassified								0.01	0.02
			Gaiellaceae								0.02	
		Solirubrobacterales	Other		0.40						0.01	
			Conexibacteraceae	Other	0.02							
				Unclassified	0.08		0.00					

Continued

Armatimonadetes	Other							0.00	0.02			
	Armatimonadia	Armatimonadales	Other		0.29	0.01					0.02	
			Armatimonadaceae	Other	0.02							
				Unclassified	0.01							
			WD294		0.14	0.01	0.02	0.02		0.06	0.01	
	Chthonomonadetes	Chthonomonadales	Chthonomonadaceae	Chthonomonas	0.01		0.00					
	SJA-176						0.00	0.02		0.04	0.04	0.23
	Fimbriimonadetes	Fimbriimonadales	Fimbriimonadaceae	Other					0.05			
				Unclassified		0.01		0.00		0.01		
				Fimbriimonas	0.09		0.00					
BRC1	PRR-11											0.05
Bacteroidetes	Other					0.03	0.04	0.11	0.15	0.08	0.19	0.10
	Bacteroidia	Other										0.02
		Bacteroidales	Other									0.15
			Unclassified			0.01	0.01	0.01	0.05	0.02	0.65	11.17
	Sphingobacteriia	Sphingobacteriales	Unclassified					0.00			0.01	
			Chitinophagaceae		0.06							
			Ekhidnaceae									0.21
			Sphingobacteriaceae		0.04							
Caldiserica	Caldisericia	Caldisericales	Other									0.03
			Unclassified									0.55
	OP5	WCHB1-02	SHBZ1169									0.03
	WCHB1-03											0.26

Continued

Chlorobi	BSV26	A89							0.06	
		BSN164		0.01	0.00					
		C20							0.15	0.28
		PK329							0.15	
		VC38				0.01		0.03	0.00	
	Ignavibacteria	Other				0.00	0.02			
		Ignavibacteriales	Ignavibacteriaceae	0.01	0.01	0.05	0.06			
		SJA-28					0.03		0.04	0.36
Chloroflexi		Other					0.02	0.04	0.90	0.21
	Anaerolineae	Other							0.15	1.85
		A31							0.01	0.08
		Anaerolineales	Other							0.02
			Anaerolinaceae Other						0.02	0.11
			Anaerolinaceae Unclassified						0.03	0.67
			Anaerolinaceae C1_B004							0.03
		GCA004								0.11
		H39							0.01	0.06
		SB-34								0.06
		SJA-15				0.00	0.02	0.01		
		envOPS12					0.03		2.38	0.13
		pLW-97								0.96
	Dehalococcoidetes	Other			0.01	0.04	0.14	0.07	1.31	1.83
		Unclassified		0.19	0.31	2.26	1.30	0.08	4.20	2.19
		Dehalococcoidales	Dehalococcoidaceae Other						0.00	0.05
			Dehalococcoidaceae Unclassified						0.09	0.52
		FS117-23B-02							0.03	0.14
		GIF9		1.84	2.78	2.53	6.33	34.69	4.17	6.96
	Ellin6529					0.13	0.08	0.04	0.23	0.39
	S085									0.05
	SHA-26									0.02
	TK17	S085								0.39

Continued

Cyanobacteria	4C0d-2	MLE1-12		0.16							
		SM1D11		0.05							
	Chloroplast	Other		0.01							
		Chlorophyta		0.05							
		Streptophyta		0.05							
Elusimicrobia	Elusimicrobia	Other								0.01	
		Elusimicrobiales			0.01	0.00	0.02	0.03		0.02	0.01
	Endomicrobia									0.01	
	FCPU426					0.01	0.04	0.11	0.03	0.04	
Fibrobacteres	Fibrobacteria	258ds10									0.05
	TG3	TG3-1	Other			0.01	0.04	0.15	0.28		
			Unclassified				0.01			0.03	0.02
Firmicutes	Other										0.03
	Bacill	Bacillales	Alicyclobacillaceae	Alicyclobacillus				0.02		0.00	0.01
			Thermoactino-mycetaceae	Unclassified			0.01	0.00			
				Shimazuella							0.03
	Clostridia	Clostridiales	Other				0.00				
			Clostridiaceae	Other							0.02
				Clostridium			0.01				0.05
			Ruminococcaceae	Ethanoligenens		0.01		0.00			
			Veillonellaceae	Other							0.02

Continued

		SHA-98	D2								1.57
GN04	MSB-5A5										0.03
GOUTA4											0.02
Gemmatimonadetes	Gemm-1									0.08	
Lentisphaerae					0.04	0.02	0.01		0.04		
NC10	Unclassified										0.08
	12-24	Other								0.01	
		JH-WHS47				0.03			0.13	0.25	1.86
NKB19	Other										0.06
	Unclassified										0.05
Nitrospirae	Nitrospira	Nitrospirales	Thermodesulfo-vibrionaceae			0.01				0.02	
OD1	Other					0.00	0.05	0.08	0.01	0.03	
	Unclassified									0.01	
	ABY1									0.01	
	SM2F11									0.01	
	ZB2								0.03	0.02	
OP1	MSBL6										0.02
OP11	Other									0.01	
OP3	BD4-9						0.00			0.05	
	koll11	GIF10	Unclassified						0.06	0.02	0.02
			kpj58rc	0.01	0.02	0.01	0.03		0.01		
OP8	OP8_1	Other								7.89	0.32
		Unclassified									3.95
		SHA-124								0.13	1.21
	OP8_2										0.07

Continued

Planctomycetes	Other					0.01	0.01	0.01		0.02	0.00	0.12
	Unclassified											4.56
	ODP123	T8-B82				0.03	0.03	0.07	0.02	0.12	0.08	
	Phycisphaerae	Unclassified			0.80	0.19	0.08	6.32	8.98	1.74	3.00	1.90
		Phycisphaerales			0.01			0.01	0.02	0.02		
	Planctomycetia	Gemmatales	Gemmataceae	Unclassified	0.18	0.01						
				Gemmata	0.88	0.01						
			Isosphaeraceae	Other	0.13							
				Unclassified	0.23	0.01	0.01				0.01	
		Pirellulales	Pirellulaceae	Other			0.00					
				Unclassified	0.01	0.23	0.10	0.18	0.43	0.83	1.26	0.30
		Planctomycetales	Planctomycetaceae	Planctomyces	0.05							
	vadinHA49	p04_C01				0.01	0.01	0.01		0.07		0.08
Proteobacteria	Other				0.32	0.09	0.01	0.03	0.02		0.00	0.20
	Alphaproteobacteria	Other			1.54	0.08	0.05	0.01	0.05	0.08	0.01	
		Unclassified			0.32							
		Caulobacterales	Unclassified			0.02						
			Caulobacteraceae	Other	1.40		0.01			0.01		
				Unclassified	0.89		0.01					
				Asticcacaulis	0.04							
				Caulobacter	0.03							
		Ellin329			0.94	0.02	0.02	0.02	0.05	0.08	0.09	0.02
		Rhizobiales	Other		0.79	1.90	0.48	0.22	0.09	1.26	0.09	0.03
			Beijerinckiaceae	Other	0.03	0.02	0.03					
				Methylocella	0.01	0.09	0.04					

Continued

			Bradyrhizobiaceae	Other	0.87	0.01	0.03					
				Bosea						0.01		0.01
				Bradyrhizobium	0.18		0.00					
			Hyphomicrobiaceae	Unclassified		0.10	0.01	0.00			0.03	0.01
				Rhodoplanes	0.83	0.05	0.16	0.01			0.03	0.05
			Methylocystaceae	Other	0.28	0.68	0.53	0.55	0.17	1.48	0.11	0.01
				Unclassified	3.24	0.07	0.07	0.01			0.03	0.03
				Methylosinus	0.18	0.15	0.16	0.01		0.01	0.07	0.01
			Phyllobacteriaceae							0.01	0.00	0.03
			Rhizobiaceae	Agrobacterium						0.01		0.02
		Rhodospirillales	Other		0.01							
			Acetobacteraceae	Other	7.09	0.20	0.06	0.18	0.09	0.06	0.09	0.03
				Unclassified	12.13	0.17	0.13	0.20		0.01	0.10	0.04
				Acidiphilium	0.18		0.00					
				Acidisoma	0.53							
				Acidocella	2.52	0.01	0.00					0.01
				Tanticharoenia	0.01			0.01				
			Rhodospirillaceae	Other	0.74	0.04	0.02	0.00			0.04	0.02
				Unclassified	0.79		0.03				0.01	0.02
				Magnetospirillum	0.01							
				Telmatospirillum	0.14	0.02	0.02					
		Rickettsiales	Other		0.02							
			Unclassified		0.05		0.00					
			mitochondria		0.04							
		Sphingomonadales	Sphingomonadaceae	Other	0.01			0.02		0.02		
				Novosphingobium	0.03	0.01						

Continued

	Betaproteobacteria	Other			1.54							0.14
		Burkholderiales	Comamonadaceae	Other								0.45
				Curvibacter								0.03
				Delftia								0.05
				Hydrogenophaga								1.78
				Variovorax								0.02
			Oxalobacteraceae		0.09							
		Neisseriales	Neisseriaceae	Chromobacterium	0.01							
		Procabacteriales	Procabacteriaceae		0.04							
		SBla14				0.02						
	Deltaproteobacteria	Other				0.05	0.01	0.01			0.01	0.32
		BPC076									3.31	2.60
		Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio	0.01							
		Desulfuromonadales	Geobacteraceae	Geobacter		0.07	0.02				0.00	
		FAC87			0.01							
		MIZ46			0.03							
		Myxococcales	Other		0.01							
			Unclassified		0.09	0.01		0.01	0.05		0.21	0.04
			Haliangiaceae		0.01							
			Myxococcaceae		0.01							
		Syntrophobacterales	Other			0.01	0.01	0.01	0.02	0.04	0.06	0.15
			Desulfobacteraceae	Other								0.20
				Desulfobacterium								0.06

Continued

			Syntrophaceae	Other		0.22	0.00	0.02	0.03	0.27	0.13	0.31
				Unclassified				0.01		0.01	0.03	1.04
				Desulfobacca		2.99	2.10	0.34	0.02	0.04	0.52	0.03
				Syntrophus		0.29	0.02	0.04	0.06	0.18	0.09	1.26
			Syntrophobacteraceae	Unclassified		0.01					0.01	
				Syntrophobacter		0.09	0.18	0.28	0.03	0.08	0.11	0.03
				Unclassified		0.38	0.25	0.43	0.23	0.77	0.82	0.87
	Gammaproteobacteria	Other			0.08							0.03
		Legionellales	Other		0.03							
			Unclassified		0.05		0.00				0.01	
			Coxiellaceae	Unclassified	0.04							
				Aquicella	0.01							
		Methylococcales	Other									0.02
			Crenotrichaceae	Crenothrix								1.78
		Xanthomonadales	Sinobacteraceae		4.07	0.01	0.03					
			Xanthomonadaceae	Stenotrophomonas				0.00				0.01
SC4											0.02	0.11
Spirochaetes	Spirochaetes	M2PT2-76						0.01				
		Spirochaetales	Spirochaetaceae	Other		0.01	0.00		0.02	0.02	0.03	0.16
				Spirochaeta		0.06	0.07	0.06	0.28	0.51	12.39	15.74
				Treponema			0.01	0.01		0.02		
TM6	Other							0.01				
	SBRH58					0.03						
TM7	TM7-1				0.03	0.13	0.04	0.03		0.01	0.04	0.01

Continued

Verrucomicrobia	Other					0.03	0.01	0.01	0.02	0.01	0.01	
	Opitutae	Opitutales	Other								0.01	
			Opitutaceae	Other		0.01		0.03	1.45	0.08	10.61	0.08
				Unclassified							0.01	
				Opitutus	0.43	0.04	0.08	0.43	0.40	0.11	0.55	0.03
	Methylacidiphilae	Methylacidiphilales	Other		0.01							
			Unclassified		0.46	0.01	0.01	0.01				
		S-BQ2-57				0.04	0.10	0.01	0.08	0.26	0.96	
	Pedosphaerae	Pedosphaerales	Other		0.10	2.57	1.43	0.95	0.88	2.13	1.94	0.03
			Unclassified		0.01	0.34	0.20	0.32	0.23	0.06	0.28	0.01
			Ellin515			3.71	2.80	3.28	1.98	4.10	1.96	0.02
			Pedosphaeraceae	Pedosphaera	0.02		0.00					
			auto67_4W		0.05	0.24	0.12	2.99	3.54	0.11	0.49	0.04
	Spartobacteria	Chthoniobacterales	Chthoniobacteraceae	Other	0.13							
				Unclassified	0.14		0.01					
WPS-2					3.30	0.04	0.08		0.02		0.04	
WS1								0.02			0.01	0.08
WS2	Kazan-3B-09											0.04
	SHA-109											0.03
WS3	PRR-12	PBS-III-9										0.08
WS4							0.00	0.01				

Table S2. Spearman Rank correlationbetween peat characteristics andrichness (Chao 1 values), diversity (Shannon index H') and abundance of 16S RNA gene.

Peat parameters	Richness	Diversity	Abundance of 16S rRNA gene (copy $_{log10}$ g^{-1} DW)
Depth	−0.25	−0.17	−0.57*
pH value	−0.06	0.08	−0.50*
Moisture content	−0.01	−0.21	0.86**
Organic matter content	−0.76**	−0.74**	0.33

*Significance at p = 0.05 level; **Significance at p = 0.01 level.

Table S3. Spearman Rank correlation between the relative abundances of the major bacterial phyla and classes and the peat characteristics.

Phylum/Class	depth	pH	Moisture	organics
Acidobacteria				
Acidobacteria-1	−0.98**	−0.86*	0.26	0.27
Acidobacteria-2	0.05	−0.07	0.31	0.93**
Holophagae	0.12	0.12	−0.10	−0.17
Solibacteres	−0.67	−0.52	0.24	0.61
TM1	−0.29	−0.48	−0.17	0.41
Proteobacteria				
Alphaproteobacteria	−0.81*	−0.69	0.33	0.15
Betaproteobacteria	−0.11	0.08	−0.11	−0.70
Deltaproteobacteria	0.60	0.52	−0.62	−0.61
Gammaproteobacteria	−0.20	−0.20	−0.17	−0.81*
Chloroflexi				
Anaerolineae	0.95**	0.95**	−0.27	−0.28
Dehalococcoidetes	0.93**	0.86**	−0.19	0.05
Other *Chloroflexi*	0.85**	0.85**	−0.54	−0.25
Verrucomicrobia				
Opitutaceae	0.456	0.201	−0.024	0.087
Methylacidiphilae	0.185	0.035	0.199	0.213
Pedosphaerales	−0.151	−0.411	0.241	0.533
Ellin515	−0.224	−0.554	0.082	0.590
auto67_4W	0.047	−0.250	−0.217	0.274
Actinobacteria	−0.36	−0.29	0.93**	0.54
Unclassified Bacteria	0.60	0.71*	−0.07	−0.71*
Spirochaetes	0.98**	0.86**	−0.26	−0.27
Candidate division NC10	0.81*	0.68	−0.36	−0.53
Bacteroidetes	0.74**	0.76**	−0.29	−0.39
Firmicutes	0.35	0.19	−0.25	−0.07
Aminicenantes (Group OP8)	0.73*	0.73*	−0.48	−0.75*
Planctomycetes	0.64	0.71*	−0.12	0.07
Candidate Division WPS-2	−0.73*	−0.73*	0.44	−0.28

*Significance at p = 0.05 level; **Significance at p = 0.01 level.

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