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Soil microbial community composition is correlated to soil carbon processing along a boreal wetland formation gradient

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ABSTRACT

Climate change is modifying global biogeochemical cycles. Microbial communities play an integral role in soil biogeochemical cycles; knowledge about microbial composition helps provide a mechanistic understanding of these ecosystem-level phenomena. Next generation sequencing approaches were used to investigate changes in microbial functional groups during ecosystem development, in response to climate change, in northern boreal wetlands. A gradient of wetlands that developed following permafrost degradation was used to characterize changes in the soil microbial communities that mediate C cycling: a bog representing an "undisturbed" system with intact permafrost, and a younger bog and an older bog that formed following the disturbance of permafrost thaw. Reference 16S rRNA databases and several diversity indices were used to assess structural differences among these communities, to assess relationships between soil microbial community composition and various environmental variables including redox potential and pH. Rates of potential CO_2 and CH_4 gas production were quantified to correlate sequence data with gas flux. The abundance of organic C degraders was highest in the youngest bog, suggesting higher rates of microbial processes, including potential CH_4 production. In addition, alpha diversity was also highest in the youngest bog, which seemed to be related to a more neutral pH and a lower redox potential. These results could potentially be driven by increased niche differentiation in anaerobic soils. These results suggest that ecosystem structure, which was largely driven by changes in edaphic and plant community characteristics between the "undisturbed" permafrost bog and the two bogs formed following permafrost thaw, strongly influenced microbial function.

1. Introduction

Climate change is modifying global biogeochemical cycling, often in disparate fashion [1–3]. The modification of global biogeochemical cycles is particularly evident in northern ecosystem carbon cycling [4]. Owing to thousands of years of carbon accumulation and slow rates of decomposition, boreal peatlands have been historical sinks for atmospheric carbon. However, direct and indirect effects of climate change are causing extensive degradation of permafrost in interior Alaska that are reshaping these historical carbon sinks [5]. Because ice occupies more volume than water, when ice-rich permafrost thaws, soil collapses into relic ice space; this collapse leads to inundation in low-lying areas and subsequent wetland formation. This process also leads to changes in plant community composition as part of the ecosystem state change from peat forest to peat wetland [5,6]. Given the anoxic and waterlogged conditions of these ecosystems, boreal wetlands are a significant source of atmospheric CH_4 and CO_2 and to a lesser extent N_2O [7–9]. Despite these ecosystems functioning as a long-term carbon sink, some models predict that Alaska will be a net source of greenhouse gases by the end of this

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century [10]. A unifying framework incorporating physical concepts such as thermodynamics and energy flow may help to highlight emerging patterns in response to climate change [11,12].

Soil microbial communities play an integral role in the C cycle and the metabolism of other nutrients, such as nitrogen and sulfur for energy acquisition [13]. For example, the production of CH_4, a potent greenhouse gas, is mediated by archaea—a domain of microorganisms. A better understanding of the microbial-scale conditions and processes that produce greenhouse gases will better inform models that operate at the ecosystem scale. Despite the large role that microbial communities play in nutrient cycles, less is known about microbial responses to climate change [14,15]. This is of particular concern because of the potential positive feedbacks that may enhance further climate warming. In this study, the soil microbial community was examined in Alaskan boreal wetlands that were at different stages of ecosystem development, and the soil microbes were framed as mediators of carbon (C) cycling and soil energy flow. The study venue was a gradient of developing boreal wetlands and the soil microbial community was used to provide a mechanistic understanding of changes in C cycling following a permafrost thaw disturbance at a site of actively degrading permafrost.

Rates of soil processes, such as biogeochemical cycling, may also be related to the abundance of soil microbial organisms [16,17]. Advances in tools developed specifically for analyzing soil microbial communities have streamlined workflow for analyzing millions of DNA sequences [18]. For example, next generation sequencing approaches have been used to show increases in cellulose processing microbial genes as well as denitrification and ammonification genes following permafrost thaw [19]. Advances in next generation sequencing technologies have also enhanced the ability to correlate microbial-scale mechanisms and processes with ecosystem-scale processes [17]. For example, while recent studies have shown a wide range of bacterial groups capable of degrading complex soil organic carbon (SOC) such as cellulose and hemicellulose, one recent study found that the majority of bacterial genes encoding SOC degradation enzymes are found in the bacterial phyla *Bacteroidetes, Actinobacteria*, and *Verrucomicrobia* [20]. These techniques were used in this study to investigate complex SOC-degrading community change along a permafrost thaw gradient.

This investigation asked: How does the soil microbial community change as a result of permafrost thaw and subsequent wetland formation, and how does this relate to functional processes? Although more is known about microbial response to climate change than a few years ago, these responses have proven to be unpredictable [21]. The objectives of this study were: 1) To investigate how soil microbial structure responded to permafrost thaw and wetland development along a space-for-time gradient, or chronosequence, of sites; 2) To measure the influence of the water table position and redox potential on microbial communities; 3) To assess changes in microbial functional groups and corresponding change in functional processes such as potential CO_2 and CH_4 production and; 4) To quantify soil microbial community diversity to assess how microbial community complexity changed following permafrost degradation. Wetland formation following permafrost thaw represents a post-disturbance ecosystem state change, and increased soil waterlogging and vegetation community change are key metrics of that ecosystem state change. Thus, shifts in microbial community abundance were expected that reflected these changes. In other words, the abundance of different bacterial and archaeal phyla would be tightly coupled to ecosystem state, or ecosystem type.

2. Methods

2.1. Site description and experimental design

The study sites were located along a gradient of wetland formation in peatlands 35 km southeast of Fairbanks, AK. The sites were in the Tanana River floodplain and contained a large number of wetlands that have developed in the last decades to centuries after permafrost degradation. A space-for-time substitution was used to test microbial community responses to permafrost thaw and state change (Table 1). Three different peatlands were selected along this successional gradient: 1) an "undisturbed" forested bog (FB) of short stature black spruce (*Picea mariana*) with a 50 cm active layer and intact permafrost; 2) a bog dominated by *Carex* sp. that formed following permafrost thaw approximately 50–80 years ago that represents a young collapse scar wetland (YCS); and 3) an older bog that thawed approximately 400–500 years ago and had higher bulk density and abundance of shrub and woody species and that represents an old collapse scar wetland (OCS). The 3 sites were located within 100–200 m from one another and experienced similar climate (Table 1; precipitation and temperature). This permafrost thaw disturbance gradient was used to quantify patterns in microbial community structure and biogeochemical function at the study sites; notably, this experimental design did not represent a generalizable pattern for wetland development and did not attempt to unravel the effects of soil and vegetation variation along the gradient of wetland formation.

2.2. Soil sampling and handling

Soils were sampled at each site along the permafrost thaw gradient by collecting five cores at each site using a sharpened steel tube (i.d. 5.4 cm) in June 2012 (when mean air temperature was 10–20 °C). Soil cores were extruded by carefully pushing the core out of the steel tube with a PVC plunger. The extracted soil cores were ~30 cm length and were subsectioned in the field at the position of the water table, where "surface" and "deep" correspond to above and below water table, respectively. The position of the water table in the forested bog was difficult to measure, so the soil cores were subdivided at a morphological change in peat structure and color. Soil core sections were subdivided and handled separately for potential gas flux experiments and molecular analyses. Soil samples that were collected above the water table were stored in sealed plastic bags and kept in a cooler on ice for transport to the lab. The samples that were collected below the water table were stored in plastic bags with bog water, to keep them anaerobic and waterlogged; they were also stored in a cooler on ice. Soils for the potential gas flux experiments were stored at 4 °C until soil incubations were conducted (within 10 days) and soil samples for DNA sequencing were stored at −20 °C until they were processed. Field redox potential was measured by inserting a redox probe 5 cm into the peat soil, and pH by inserting a pH probe 5 cm into peat soil. Redox potential and pH were quantified to investigate the influence of environmental changes along the wetland gradient on microbial community composition. To quantify total soil C and nitrogen, peat soil subsamples were oven dried 1 week at 65 °C, ground first using a Mini Wiley Mill (Thomas Scientific, Swedesboro, NJ), then ground again using a 8000D Dual Mixer/Mill (SPEX CentiPrep, Metuchen, NJ). This second grinding step was to maximize the homogeneity of the sample prior to analysis. Total C and nitrogen were quantified on a Perkin Elmer 2400 CHN Analyzer (Waltham, MA).

2.3. Potential gas flux experiments

Potential gas flux incubations were conducted by placing fresh

Table 1
pH, redox potential, soil C:N, the depth to the water table, and the dominant plant communities along the wetland formation gradient. Numbers are means with standard errors in parentheses. FB: forested bog; YCS: young collapse scar; OCS: old collapse scar. MAT: mean annual air temperature, MAP: mean annual precipitation.

Site[a]	pH	Redox potential (mV)	Soil C:N	Depth to water table (cm)	MAT (°C)[b]	MAP (mm)[b]	Dominant plant community
FB	4.56 (0.16)	312 (112)	26.38 (1.72)	permafrost	−2.9	269	*Picea mariana* stand
YCS	5.08 (0.04)	−153 (57)	31.58 (0.56)	5.80 (0.08)	−2.9	269	*Carex* spp.
OCS	4.27 (0.09)	−4 (44)	30.43 (0.22)	8.00 (0.71)	−2.9	269	Shrub and woody spp.

[a] Sites were located within the Alaska Peatland Experiment (APEX), just outside of the BNZ Creek LTER.
[b] Sites were located within 300 m of one another, and experience similar climate [62].

peat samples (~3 g) in 100 mL Wheaton vials fitted with rubber septa and sealed with aluminum crimp rings as described by Ref. [12]. Briefly, peat soils were homogenized manually by hand and were incubated with surface and deep subsection samples in separate vials. After being stored at 4 °C, the soils were pre-incubated for three days to allow soil temperatures and microbial activity to stabilize. Potential gas fluxes were measured at standardized conditions and thus were not influenced by the temperature change from 4 °C to 20 °C [22].

The position of the water table in boreal wetlands is dynamic. To experimentally simulate water tables either higher or lower than at the time the soil cores were extracted, surface and deep soil samples were incubated both aerobically and anaerobically. To allow for free exchange of O_2, aerobic incubations were conducted by placing a piece of Parafilm over vials until the headspace gas was sampled. Butyl rubber septa were placed on the aerobic incubation vials 1 h prior to collecting headspace gas. Anaerobic conditions in the appropriate vials were established by flushing the vials with N_2 for 3 min. They were immediately capped with butyl rubber stoppers and sealed with aluminum crimp rings to maintain anaerobicity for the length of the incubation experiment. To ensure anaerobicity during sampling, gas samples were collected with a thin needle syringe. All vials were incubated in the dark at room temperature (20–25 °C). This was not to mimic site conditions, but to understand how organic matter quality under standardized temperature conditions influenced potential gas production rates—although these northern soils typically get this warm, especially at the surface, in the summer. Replicate soil samples were destructively harvested at 1, 24, 48, 72, and 192 h over the course of the 8-day (192 h) incubations to quantify aerobic and anaerobic concentrations of CO_2 and CH_4. Samples were destructively harvested in conjunction with other metrics being assessed in another study [12]. Blank empty vials were used throughout the experiment as controls for gas emissions.

Gas samples were stored in 20 mL vials that were evacuated and flushed with N_2 prior to sampling and that were sealed with rubber butyl stoppers and aluminum crimp rings until the incubation study was complete. Gas samples were analyzed on a Varian CP-3800 gas chromatograph fitted with an autosampler. Potential gas fluxes under anaerobic conditions were calculated as the gas concentration difference between subsequent samplings and daily potential gas fluxes under aerobic conditions were calculated based on the time in which the vials were capped (*e.g.* [23]).

2.4. DNA extractions

Soil samples were thawed from −20 °C and DNA samples were extracted in duplicate from soil subsamples taken from each of the 5 cores and 2 soil sections from each site using a PowerSoil DNA extraction kit (MoBio). Concentrations of extracted DNA were quantified the using a QuBit 2.0 Fluorometer (Invitrogen); the higher concentration of DNA duplicate was then diluted to ~ 5 ng uL^{-1} for use in downstream analyses with DI H_2O. All DNA extractions were conducted in the Wetland Ecosystem Ecology Laboratory at Arizona State University in Tempe, AZ.

2.5. Targeted genes and primer design

To investigate the influence of ecosystem state change on the bacterial and archaeal communities, PCR and next-generation DNA sequencing technologies were used. Existing published primer sets were modified for the 16S rRNA gene (515F and 909R primers [24]) and an Illumina MiSeq 2000 was used to sequence these genes. PCR products generated from the extracted DNA samples were all pooled to multiplex them into a single lane on the Illumina MiSeq 2000, using unique barcodes for each sample. To ensure that there was a unique barcode for each extracted DNA sample, 7 forward primers and 5 reverse primers were used in different permutations for each sample. This multiplexing approach made it possible to simultaneously combine the DNA samples for sequencing into a single sample and identify each unique sample from each site along the gradient of wetland development for downstream analyses. The 16S rRNA gene from bacteria and archaea was amplified by modifying the following universal primers with the barcode sequences: primer 515F, 5′ ATCACGGTGYCAGCMGCCGCGGTA 3′ for the forward primer and primer 909R, 5′ TCGAAGTGTGCCCCGYCAATTCMTTTRAGT 3′ for the reverse primer [24]. We used the following thermocycler conditions for 16S rRNA PCR: 98° for 30 s, then 25 cycles of 98° C for 10 s, 63 °C for 15 s, and 72 °C for 15 s, and finished with a final 72 °C 2-min annealing step. All amplified PCR products were verified by fragment size using 1% agarose gel electrophoresis.

2.5.1. 16S rRNA PCR amplicon normalization

To account for variations of PCR yield across samples, a normalization step for all of the targeted gene products was conducted. This step was used to ensure that each sample was properly represented on the Illumina MiSeq sequencing. A SequalPrep Normalization Plate Kit (Invitrogen) was used and manufacturer's instructions were followed. Each well of the plate eluted roughly the same amount of DNA and the more labor-intensive process to quantify and re-aliquot each sample prior to pooling for next-generation sequencing was avoided.

2.5.2. 16S rRNA illumina amplicon sequencing and analysis

The normalized pooled amplicons were sequenced using a 300 base pair paired-end read on an Illumina MiSeq 2000. Sequence data were downloaded from Illumina BaseSpace and the raw forward and reverse sequence reads were merged using Paired-End reAd mergeR (PEAR, http://sco.h-its.org/exelixis/web/software/pear/index.html). The output from PEAR was a single merged fastq file that was directly used with Quantitative Insights Into Microbial Ecology [18]. A number of data processing steps were performed using the default QIIME workflow, including assigning multiplexed reads to soil samples based on their unique barcode sequence in the mapping file, determining operational taxonomic units based on 97% sequence similarity using uclust, and assigning taxonomy based on reference databases using the May 2013 version of greengenes. In addition to using the QIIME workflow for

16S rRNA analysis, phyloseq, an R package was used to perform further data analyses, data visualization, and presentation [25].

2.6. mcrA amplification and quantitative PCR

To quantify the number of methanogens in the peat samples using quantitative PCR, the *mcrA* gene was amplified using the following primers: 5′ GGTGGTGTMGGDTTCACMCARTA 3' [26] for the forward primer and 5′ CGTTCATBGCGTAGTTVGGRTAGT 3′ for the reverse primer [26]. PCR for *mcrA* was optimized with the following conditions: 98° for 30 s, then 25 cycles at 98° C for 10 s, 58 °C for 15 s, and 72 °C for 15 s and completed the final annealing reaction at 72 °C for 2 min. To create standards for qPCR, established protocols were followed [26].

Using the linearized plasmids containing the *mcrA* gene as a standard, qPCR was conducted using a 384 well format ABI7900HT thermocycler and Maxima SYBR Green qPCR Master Mix Kit (Thermo Scientific) in 20 μL reactions. For *mcrA* qPCR reactions, 1 ng of template DNA and 0.3 μM forward and reverse primers were used. A three-step cycling protocol with the following conditions was used: Initial denaturation at 95 °C for 10 min, then 40 cycles of denaturing at 95 °C for 15 s, annealing at 58 °C for the *mcrA* gene for 30 s, and extension at 72 °C for 30 s. Samples for qPCR were diluted to test for sample inhibition. A melting-curve analysis was performed to verify that there were no primer dimers. All samples and standards were run in duplicate.

2.7. Calculating alpha diversity

The QIIME workflow (qiime.org) was used to compute a metric of alpha diversity to assess shifts in the soil microbial community at various stages of ecosystem development. Alpha diversity compares OTU-level diversity within a site. With this approach, alpha diversity variation among sites and different edaphic factors was assessed. Faith's phylogenetic diversity––a measure of alpha diversity computed as the sum of branch lengths of a phylogenetic tree––was used to measure alpha diversity.

2.8. Statistical analysis

Analysis of Variance (ANOVA) was performed on the rates of potential gas production (CO_2, CH_4) across site, soil section, and oxygen status of the incubation (aerobic or anaerobic). To examine the effects of environmental parameters on alpha diversity, linear regressions were performed in R. In all of the ANOVA analyses, multiple *post hoc* analyses using Tukey's Honest Significant Difference (HSD) test were used to compare means that were significantly different. Results with $p < 0.05$ were considered to be significant and $p < 0.01$ to be highly significant. Assumptions of equal variance and normality were tested in R for the ANOVA models by using residuals *vs.* fitted and normal quantile-quantile plots, respectively. Data that did not meet the assumptions of the ANOVA were log transformed.

3. Results

In this study next generation sequencing was used to characterize microbial community composition along a gradient of wetland development. The study also aimed at relating microbial community composition to carbon cycling via methanogenesis. The sequencing data revealed 3990 operational taxonomic units (OTUs) across the 30 soil samples along the gradient of wetland development when the analysis was limited to OTUs with an abundance of greater than 100. There were a total 6.91×10^6 sequence reads and sequencing depth ranged from 54,801 reads to 343,063 reads across all of the samples. The mean sequencing depth was 230,219 with a standard error of 16,989. The median sequence depth was 253,174. Next, taxonomic results of microbial community composition along the gradient of wetland development are presented.

3.1. Microbial community structure across wetland gradient and soil depth

Following permafrost thaw at the forested bog, shifts in microbial community composition were observed at the young and old wetlands. Along the gradient of sites following permafrost thaw, a marked change in relative proportion of several bacterial phyla was observed. For example, an increase in *Bacteroidetes* at YCS relative to FB, a decrease in *Actinobacteria*, and an increase in *Chloroflexi* was observed. The soil microbes that were dominant across the permafrost thaw gradient included 11 phyla (Fig. 1) that accounted for 75–90% of the total microbial community. Across the gradient, the surface soil microbial community was not different from the subsurface soil microbial community at the phylum level (Fig. 1). The remaining fraction was either unclassified—~9% at FB, ~14% at YCS, and ~9% at OCS—or was composed of phyla that occurred at low frequencies. The most common bacterial phyla were the *Proteobacteria*, *Actinobacteria*, and *Acidobacteria*; these three phyla accounted for 65–70% of the total community at all sites (Fig. 1). Within the *Proteobacteria*, *Alphaproteobacteria,* followed by *Gammaproteobacteria* and *Deltaproteobacteria,* were the most common bacterial classes accounting for ~16%, 5%, and 4% of the total bacterial sequences, respectively. Less common, but still relatively abundant phyla included: *Bacteroidetes*, *Plantomycetes*, *Chloroflexi*, and *Cyanobacteria*.

Archaeal phyla were most prominent at the young bog site, making up 5% of the total microbial community. However, along the disturbance gradient, dramatic changes in archaeal phyla abundance were observed; for example, almost no *Euryarchaeota*—an archaeal phylum containing methanogens— were observed at the forested bog underlain by permafrost (Figs. 1 and 2). An intermediate abundance compared to the forested bog and young bog of *Euryarchaeota* phyla was observed at the old bog site. Within the *Euryarchaeota* phylum *Methanobacteriales* were the most prominent order observed. Along the gradient of wetland formation, shifts in relative proportion of several functionally important bacterial phyla were observed.

3.2. Microbial community and carbon cycling

3.2.1. Complex soil organic carbon degrading microbial community

Shifts in the soil microbial community along the gradient of wetland development have implications for the functioning of these systems. The majority of soil organic carbon degradation in northern peatlands has been attributed to three microbial groups: *Bacteroidetes, Actinobacteria*, and *Verrucomicrobia* [20]. Across the gradient of wetland development, the sequencing data revealed a high proportion of these soil organic carbon degraders; *Bacteroidetes*, *Actinobacteria*, and *Verrucomicrobia* were present across all sites and soil depths and represented roughly 20% of the overall proportion of the microbial community (Fig. 1). *Bacteroidetes* and *Actinobacteria* were a larger proportion of the SOC degraders compared to *Verrucomicrobia*; the latter represented ~2% of the overall microbial community.

3.2.2. Methanogen communities

The presence of three different orders of methanogens from the *Euryarchaeota* phylum was observed: the *Methanobacteriales*, the *Methanomicrobiales*, and the *Methanosarcinales* (Fig. 2). *Methanobacteriales* were the most abundant of the three orders,

followed by *Methanosarcinales*, then *Methanomicrobiales* (Fig. 2). The abundance of methanogens varied by site along the gradient of wetland development (Fig. 2). Methanogens were almost entirely absent at the FB site and the most abundant at the youngest wetland site, YCS. An intermediate abundance of methanogens was observed at the older bog site, OCS, but not above the water table. Additionally, all three orders of methanogens were found at YCS, but only two orders of methanogens at OCS.

3.2.3. CO_2 and CH_4 potential production

Across the gradient of wetland development, similar rates of aerobic CO_2 production were observed from surface peats across all sites (Fig. 3a). Similar rates of CO_2 production were also found among all sites in anaerobic incubations of the deep peat (Fig. 3b). Not surprisingly, much higher rates of potential aerobic CO_2 production were observed from surface peats relative to anaerobic CO_2 production from deep peats (Fig. 3). Almost no potential production of CH_4 was observed from the forested bog (FB) underlain by permafrost. The highest rates of potential CH_4 production were found from the newly formed wetland, YCS, and the rate of potential CH_4 production from the older wetland site, OCS, was between the forested bog site and newly formed wetland (Fig. 4a). The highest numbers of *mcrA* gene copies—a gene responsible for CH_4 production—were found in YCS soils, followed by OCS, and finally FB (Fig. 4b). Finally, the number of these gene copies was highly correlated with the magnitude of potential CH_4 production (Fig. 4c). Notably, this relationship was not a function of microbial community size as all sites had similar absolute numbers of 16S rRNA genes (Fig. 5a and b).

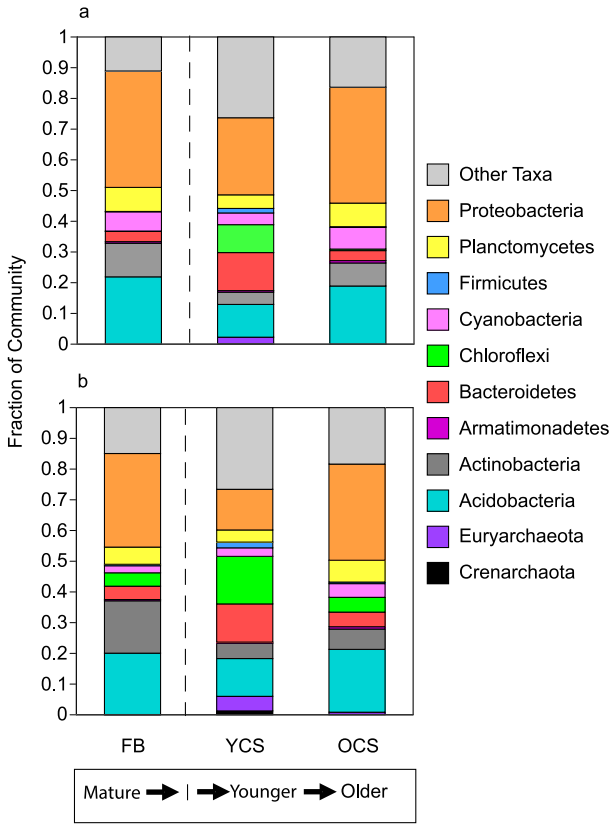

Fig. 1. Phyla level microbial community structure at each site along the gradient of wetland formation on the x-axis. The top panel represents the microbial community structure in the surface peat above the water table, and the bottom panel represents deep peat found below the water table. Bars depict mean of 5 replicates at each site along the wetland development gradient. Legend colors are listed in order that correspond to figure. The vertical dashed line represents the permafrost thaw disturbance that results in the formation of the young collapse scar (YCS) wetland. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

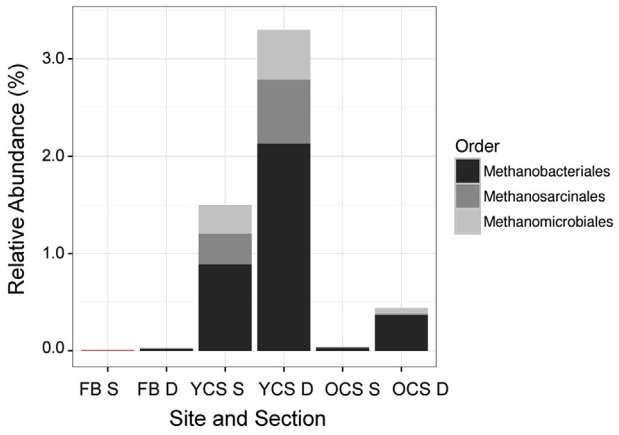

Fig. 2. Relative abundance of different orders of *Euryarchaeota* (methanogens) across the gradient of wetland formation. Bars represent the number of reads that were sequenced in the 5 replicates at each site along the wetland gradient. YCS had the largest and most diverse methanogen community. FB: forested bog, YCS: young collapse scar wetland, OCS: old collapse scar wetland. S: surface soils above water table, D: deep soils below water table.

3.3. Microbial diversity and environmental constraints

Microbial community characteristics can be influenced by environmental parameters. As these ecosystems thaw and become inundated, there are dramatic changes in important environmental parameters. The pH in soils at the intact-permafrost forested bog varied from less than 4.0 to 5.5, while pH at the two Sphagnum wetland sites was less variable; the young wetland had the highest pH and OCS was the most acidic (Table 1, Fig. 6a). Microbial diversity was positively correlated with ecosystem pH ($R^2 = 0.525$, $p < 0.01$; Fig. 6a). Soil C:N did not vary along the gradient and was not correlated with diversity. Considerable variation in redox potential was seen at 5 cm depth along the gradient of permafrost thaw, particularly at the forested bog site (Fig. 6b). Generally, the most reduced soils were found at the YCS site, while OCS had intermediate redox potentials and FB soils were the least reduced at 5 cm depth, with means of −150 mv, −4 mv, and 312 mv, respectively (Table 1). Given that the position of the water table relative to the peat soil surface was different at each site along the gradient, the variation in redox potential was not surprising. Redox potential explained less of the variation in diversity than did pH, although the relationship was still significant ($R^2 = 0.151$, $p < 0.01$; Fig. 6). Higher microbial diversity was found in more reduced environments compared to those environments with higher redox potential values.

4. Discussion

4.1. Community structure across permafrost thaw gradient

The prominent soil microbial phyla that were observed in this study have been also documented by other studies of boreal peatland microbial communities [19,27,28]. A previous study observed that *Proteobacteria* and *Actinobacteria* accounted for ~55% of the microbial phyla in their assessment of northern peatlands bacterial phyla [20]. Within the *Proteobacteria*, however, it has been observed that *Deltaproteobacteria* accounted for ~20% of the

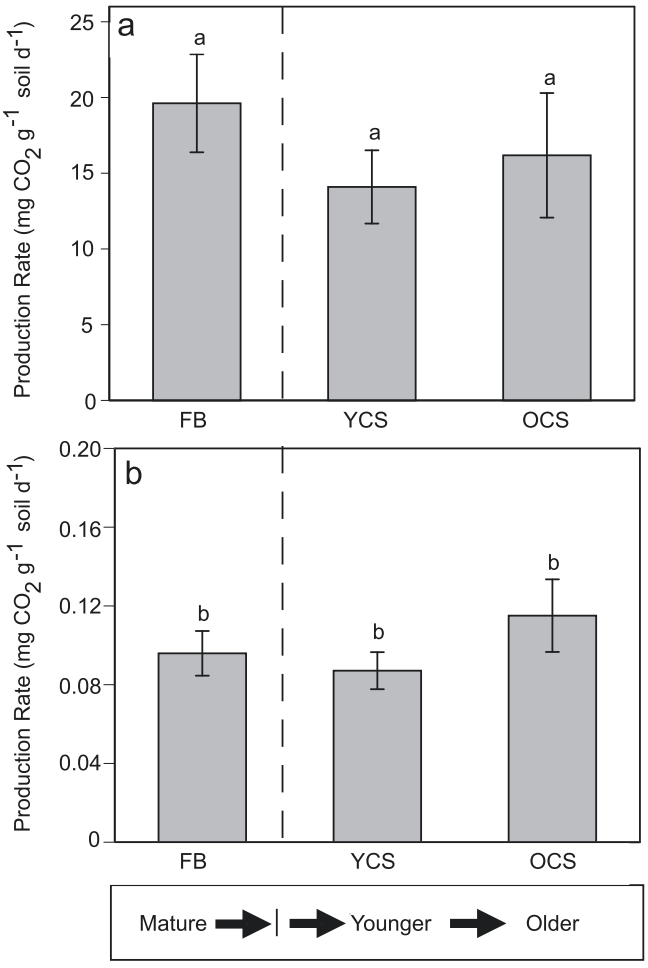

Fig. 3. Potential CO_2 production from aerobic incubations of surface peat (a), and CO_2 production from anaerobic incubations of deep peat (b). Letters above whiskers on box plots represent statistically different relationships. The vertical dashed line represents the permafrost thaw disturbance that results in the formation of the young collapse scar (YCS) wetland.

bacterial community and were more abundant than *Alphaproteobacteria* and *Betaproteobacteria* [20]. *Deltaproteobacteria* include syntrophic bacteria, which are capable of providing H_2 gas to methanogens as a substrate for methanogenesis, though ~half of the *Deltaproteobacteria* in this study were the *Myxobacteria*, a bacterial group that does not produce H_2 [29].

Many of the observed phyla across the gradient of wetland development in this study are capable of producing substrates for methanogenesis. A recent 16S rRNA next-generation sequencing study in boreal peatlands showed high numbers of *Acidobacteria*, *Proteobacteria*, *Actinobacteria*, *Plantomycetes*, and *Verrucomicrobia* [28]. Several other studies including a clone library sequencing study of mineral permafrost soils from Northern Norway and pyrosequencing of the 16S rRNA gene from various boreal and arctic locations showed a relatively high abundance of *Actinobacteria*, *Proteobacteria*, and *Bacteroidetes*, bacteria phyla commonly associated with SOC degrading [30,31]. The *Proteobacteria* results are thus supported by other peatland microbial community analyses and suggest that there are an abundance of bacterial groups capable of providing substrate to methanogens for methane production across our sites [20,30].

Methanogens play a large role in anaerobic C cycling in boreal wetlands and peatlands [32]. To assess differences among methanogens along the gradient of wetland formation, the methanogens associated with methanogenesis were analyzed (Fig. 3c). Along the gradient of wetland formation, the highest abundance of methanogens was found in the young bog (YCS) and the old bog (OCS) soils, and this was positively correlated with rates of CH_4 production from these soils (Fig. 4c). Methanogens were found to be in low abundances, relative to the proportion of the whole microbial community, by phyla (Fig. 1). Despite being a large source of atmospheric CH_4, low abundances of methanogenic Archaea have been previously observed in boreal wetlands [19,33]. This relatively low abundance, based on total sequencing data of methanogens, highlights the importance of a low-frequency microbial phylum in the global carbon cycle with methanogenesis, given the magnitude of methane produced at the young bog site (Fig. 4a,c).

Actinobacteria are important degraders of complex organic matter in peatlands [34]. Several previous studies have documented higher abundances of *Actinobacteria* in permafrost soils compared to active layer soils [34–36]. In addition to finding a higher abundance of *Chloroflexi* in active layer soils in this study, especially at depth, higher proportions of *Chloroflexi* were observed in these peatland soils compared to other 16S rRNA gene-sequencing studies from peatlands. For example, while *Chloroflexi* were in the top 11 most abundant phyla in the soils in this study, another study reported that *Chloroflexi* were a rare phylum in their northern peatlands in European North Russia [28]. The high proportion of *Chloroflexi* at YCS may be a result of the accumulation of halogenated organic compounds generated during decomposition [37]. Additionally, the proportion of the microbial community at the YCS site that was *Chloroflexi* was considerably higher than has been previously documented in other soil and clone-sequencing studies, though *Chloroflexi* have been previously observed in active layer soils in other peatland soils [19,27,28]. These results suggest that microbial community changes along the gradient of wetland development could have important implications for how carbon decomposition in peatlands may be altered following permafrost thaw.

4.2. Soil organic carbon degrading microbial community and soil function

Because 16S rRNA gene databases are taxonomically robust and integrate functional information about bacterial isolates from soil metagenomes, the functions of many bacterial groups have been described. For example, many studies have described the bacterial groups of *Bacteroidetes*, *Actinobacteria*, *Verrucomicrobia*, *Alphaproteobacteria*, and *Planctomycetes* in terms of their roles in the carbon cycle and their ability to degrade polysaccharides, including cellulose and hemicellulose [38–40]. These results generally agree with a previously published study on microbial communities and SOC degradation in peatlands [20]. However, a proportion of *Verrucomicrobia* that was five times smaller relative to the whole microbial community was observed in this study, suggesting that *Verrucomicrobia* play a smaller SOC-degrading role in this system. High abundances of *Actinobacteria* were also observed; along with fungi, *Actinobacteria* are one of the main decomposers in peatlands [41,42]. This similarity in abundances of SOC degraders corresponded to similar rates of potential CO_2 production under both aerobic and anaerobic conditions along the gradient of wetland development, suggesting that different individual microbial groups produce CO_2 at similar rates along the gradient, as observed in another study along the gradient [43]. The similar rates of potential aerobic CO_2 production along the gradient (Fig. 3), despite higher bacterial abundances in surface soils at YCS (Fig. 4), suggest that fungi could play an important role in FB and may compensate for

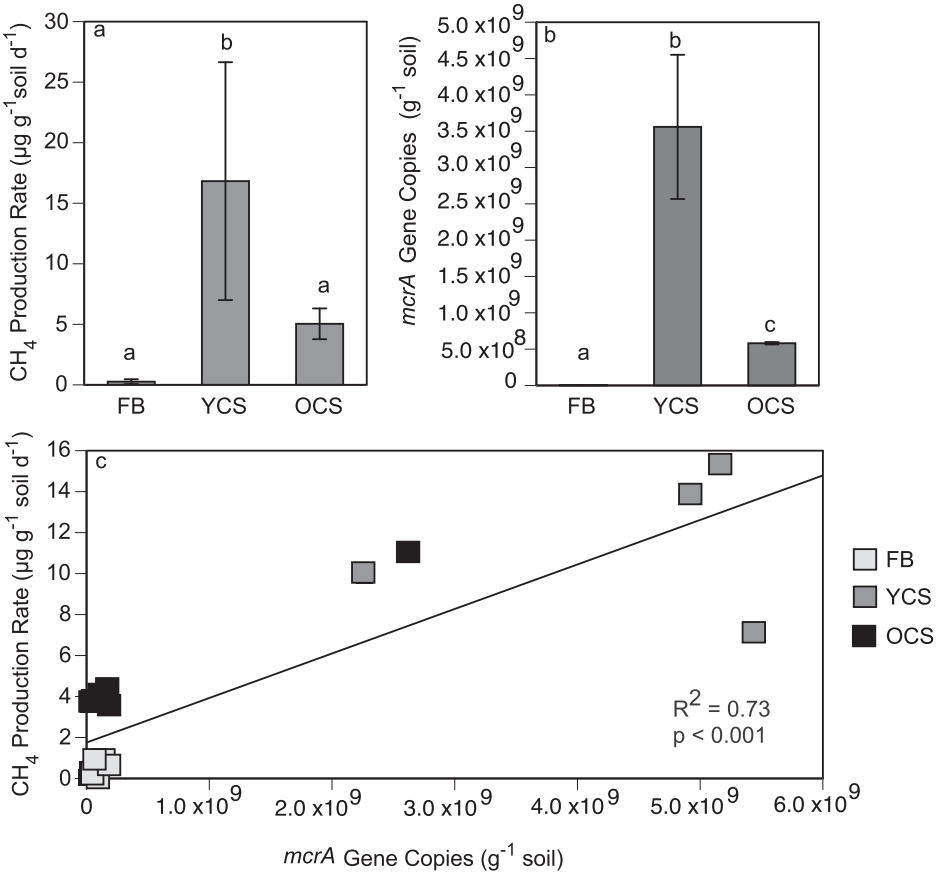

Fig. 4. Potential CH_4 production rate from anaerobic incubations of deep peat (a), mcrA gene abundance across gradient of wetland development (b), linear regression of CH_4 production rate and abundance of mcrA gene (c), $R^2 = 0.73$, $p < 0.001$. Letters above whiskers on box plots represent statistically different relationships.

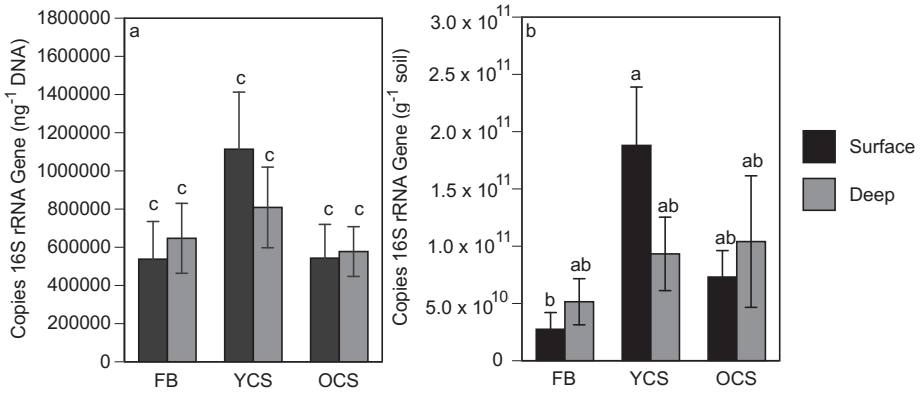

Fig. 5. Copies of 16S rRNA per ng of DNA (a), and per gram of soil (b). FB: forested bog, YCS: young collapse scar wetland, OCS: old collapse scar wetland. Surface: surface soils above water table, Deep: deep soils below water table.

fewer bacteria compared to YCS.

4.3. Anaerobic respiration and fermentative microbial community

Given the complex relationship between microbial community composition and anaerobic respiration, it is difficult to attribute particular taxa to a certain metabolic pathway. However, several recent gene sequencing studies have attributed the majority of anaerobic respiration in peatlands to the *Proteobacteria*, *Actinobacteria*, and [to a lesser extent] *Acidobacteria* phyla, prominent phyla that were observed in this study [20,44,45]. While there are metagenomic studies that suggest a relatively high abundance of denitrification genes in peatlands [19], other studies have found low abundances, suggesting that there may be relatively small pools of mRNA to produce enzymes for denitrification [20].

In a study of organic carbon transformations in boreal peatlands,

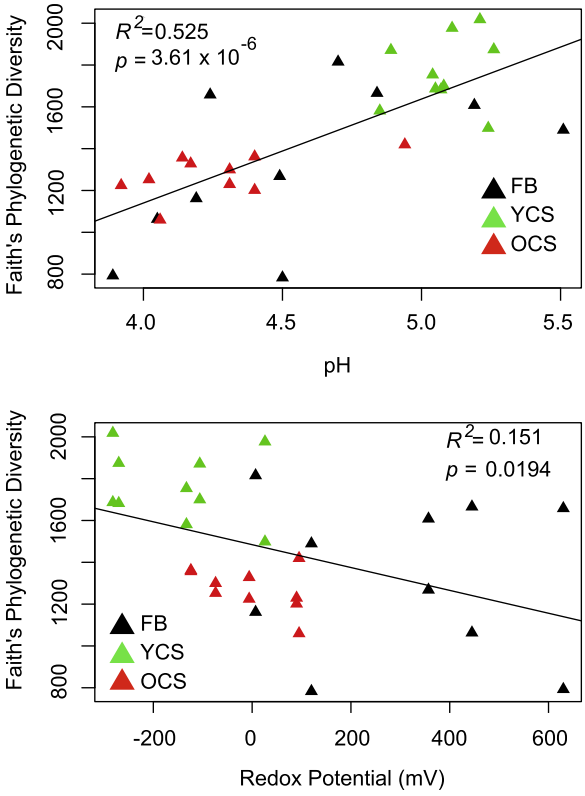

Fig. 6. Alpha diversity across soil development gradient (a) pH as measured with Faith's Phylogenetic diversity. Linear regression explained 52% of the variation of the data points and was significant (P < 0.0001) (b) Alpha diversity across soil redox potential as measured with Faith's Phylogenetic diversity. Linear regression explained 15% of the variation of the data points and was significant (P = 0.0194). Sites were color-coded: YCS was green, OCS was red, and FB was black. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the majority of fermentation and fermentative genes were attributed to the *Firmicutes* and *Actinobacteria* bacterial phyla [20]. These genes include a hydrogenase gene (*hydA*) involved with H_2-producing fermentations and a formyltetrahydrofolate synthetase gene (*fhs*) involved with encoding a key enzyme in homoacetogenesis. Low abundances of the *Firmicutes* were found, but *Actinobacteria* was one of the most abundant phyla and comprised of ~10% of the total microbial population. By creating H_2 and acetate from fermentative pathways while producing CO_2 from SOC decomposition, northern peatland microbial communities provide the substrates needed for methanogenesis, which has important implications for greenhouse gas production.

4.4. Methanogen community and methane production

Methanobacteriales and *Methanomicrobiales* produce CH_4 from CO_2 and H_2, whereas *Methanosarcinales* are more metabolically diverse and can produce CH_4 acetoclastically, hydrogenotrophically, or from methylated compounds [46]. Thus, the composition of different *Euryarchaeota* orders may reveal important environmental constraints among the sites, such as the types of substrates present for methanogens and methanotrophs in the ecosystem. The metabolically versatile *Methanosarcinales*, an order of archaeal methanogens, were found only at YCS, suggesting that YCS contained a diverse pool of substrates for methanogens and methanogenesis (Fig. 2). Additionally, methanogen abundances were greater in deep peats, below the water table, an expected finding from obligate anaerobes that agrees with several studies [20,47,48]. The highest abundances of methanogenic microbes were found at the post-disturbance herbaceous bog sites (YCS and OCS), where the water table was higher than in the forested bog (FB), which likely led to lower oxygen availability. The relative abundance of methanogens strongly correlated with potential CH_4 production along the same gradient of wetland development, because of higher concentrations of methane precursors from fermentation products and a [presumed] lack of oxygen at depth [12,20,49]. These observed rates of potential CH_4 production were similar to other published studies of anaerobic laboratory incubations of northern peat soils [50].

4.5. Alpha diversity across chronosequence and environmental constraints

Considering species diversity has been long thought to increase with ecosystem maturity [51,52], it was surprising that the mature ecosystem (the forested bog) and the old collapse scar bog had lower measures of microbial diversity. One explanation is that soil microbial diversity is not equivalent to whole-ecosystem species diversity. Because Faith's phylogenetic diversity metric is calculated based on branch lengths of a phylogenetic tree, the results suggest that the recently formed wetland, YCS, had more distantly-related OTUs compared to FB and OCS. This was an indication that FB and OCS are more similar to one another than to YCS. However, microbial diversity was strongly and positively correlated with pH [53,54], and small increases in pH were observed from surface to deep peat depths in the laboratory, potentially leading to higher levels of microbial diversity deeper in the soil profile. The correlation of microbial diversity with pH highlights the potential importance of plant-microbe interactions, as *Sphagnum* can control bog pH and microbial activity can control soil pH and redox potential [55,56].

Bog plant communities regulate soil pH and also affect soil Eh, and this feeds back to influence the composition of the microbial community [57]. For example, researchers used T-RFLP to quantify soil microbial diversity across a half-dozen biomes across the Americas [53]. They found a curvilinear relationship between microbial diversity and soil pH; diversity was highest at a pH of roughly 7, and decreased as soils became more acidic or alkaline. Across the ~ 4–5.5 pH range in this study, a linear, increasing relationship between microbial diversity and pH was found (Fig. 6), which corresponds to the more acidic portion of curvilinear relationship in the previous study [53]. This relationship between pH and microbial diversity has been demonstrated in numerous studies across a range of ecosystem types [58–60]. Changes in ecosystem pH associated with wetland development thus have important influence over microbial community composition and the corresponding ecosystem function.

The observed pattern of redox potential along the gradient of wetland sites—lowest redox potential at the newly formed wetland and highest redox potential at the forested bog—was expected based on the position of the water table relative to the soil surface and the abundance of methanogens (Table 1). Few studies have investigated the role of redox potential on microbial community structure and diversity. One study found that when dominant bacterial OTUs were incubated anaerobically for extended periods of time, microbial diversity decreased relative to field conditions [61]. In soils that were incubated with fluctuating redox potentials, microbial diversity also decreased relative to field conditions. This finding contradicts with the results of this study that the most reducing environment tended to be the most diverse. In a study investigating microbial diversity at various depths in a California

lake, it was found that microbial communities were more diverse in more reduced environments—a finding that the authors attributed to a greater potential for niche differentiation in the reduced environments [63]. Along the gradient of developing wetlands that were studied here, higher microbial diversity was found in the more reduced soils, providing evidence of niche differentiation through diverse anaerobic metabolic pathways. Though several environmental factors influence microbial community structure, pH is widely documented as the strongest predictor of microbial community structure on small and large scales across ecosystem types.

5. Conclusion

The gradient of ecosystem development studied here consisted of a bog with intact permafrost that was an "undisturbed" system, and a younger bog and an older bog that formed following a permafrost thaw disturbance. Along the permafrost thaw disturbance chronosequence we found: 1) a greater abundance of methanogens in a young bog formed following permafrost thaw; 2) higher rates of methane production in this young bog; and 3) greater microbial diversity in soils from this young bog. These results suggest that soil microbial processes are tightly linked to ecosystem structure, and that this linkage may be driven by changes in edaphic and plant community characteristics between the "undisturbed" permafrost bog and the two bogs formed following the permafrost thaw disturbance.

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