Experimental sulfate amendment alters peatland bacterial community structure

Strickman, R. J. S.^a, Fulthorpe, R.R.^a, Coleman Wasik, J.K.^{b,1}, Engstrom, D.R.^b, and Mitchell, C.P.J.^{a*}

Department of Physical and Environmental Sciences, University of Toronto Scarborough, Scarborough, Ontario, Canada^a; St. Croix Watershed Research Station, Science Museum of Minnesota, Marine on St. Croix, Minnesota, United States^b

* Address correspondence to Carl Mitchell, carl.mitchell@mail.utoronto.ca.

¹ Present address: Department of Plant and Earth Science, University of Wisconsin-River Falls, River Falls, Wisconsin 54022, United States

Abstract

As part of a long-term, peatland-scale sulfate addition experiment, the impact of varying sulfate deposition on bacterial community responses was assessed using 16S tag encoded pyrosequencing. In three separate areas of the peatland, sulfate manipulations included an eight year quadrupling of atmospheric sulfate deposition (experimental), a 3-year recovery to background deposition following 5 years of elevated deposition (recovery), and a control area. Peat concentrations of methylmercury (MeHg), a bioaccumulative neurotoxin, were measured, the production of which is attributable to a growing list of microorganisms, including many sulfate-reducing *Deltaproteobacteria*. The total bacterial and *Deltaproteobacterial* community structures in the experimental treatment differed significantly from those in the control and recovery treatments that were either indistinguishable or very similar to one another. Notably, the relatively rapid return (within three years) of bacterial community structure in the recovery treatment to a state similar to the control, demonstrates significant resilience of the peatland bacterial community to changes in atmospheric sulfate deposition. Changes in MeHg accumulation between sulfate treatments correlated with changes in the *Deltaproteobacterial* community, suggesting that sulfate may affect MeHg production through changes in the community structure of this phylum.

Keywords: wetland, microbial ecology, sulfate reducing bacteria, mercury, methylmercury

1. Introduction

Atmospheric sulfate deposition may exert a significant impact on bacterial community structure and activity in anoxic, aquatic ecosystems. Sulfate is an electron acceptor for sulfate reducing

bacteria (SRB), whose by-products affect the growth of other metabolic guilds including sulfide oxidizers and, under some conditions, methanogens (Muyzer and Stams, 2008; Pester, 2012). Peatlands, which extend over more than four million square kilometres and store up to 30% of the world's soil carbon (Limpens et al., 2008), host diverse and active communities of SRB, even in the upper, periodically unsaturated layers (Schmalenberger et al., 2007) that are the most exposed to atmospheric inputs. Although changes in sulfate deposition have the potential to alter interactions among peatland bacteria, and thus affect major biogeochemical cycles and ecosystem functioning (Fuhrman 2009), sulfate as a bacterial driver has been little studied in comparison to the effects of other atmospherically deposited nutrients on bacterial communities (Andersen et al., 2013). Previous investigations have focused on very broad taxonomic or metabolic divisions, and indicate an increase in gram-negative bacteria (Pennanen et al., 1998), stimulation of SRB paired with inhibition of methanogens (Gauci and Chapman, 2006; Vile et al., 2003), or similarities in the diversity of a limited number of SRB from sites that received similar levels of nutrients, including sulfate (Steger et al., 2011). Here high-throughput DNA sequencing was used to investigate the influence of sulfate on the peatland bacterial community as a whole..

The response of bacterial communities to disturbance, including changes in atmospheric nutrient supply, is an important and variable aspect of bacterial ecology (Griffiths and Philippot, 2012). Bacterial communities may resist or rapidly recover from some disturbances (Allison and Martiny, 2008), while in other circumstances a disturbance may trigger a long-term change in bacterial community structure (McKew et al., 2011; Nelson et al., 2015) or functioning (Currey et al., 2009), analogous to a shift to an alternative stable state (Scheffer et al., 2001). In a short-term experimental manipulation of sulfate deposition in an ombrotrophic peatland, slight shifts in microbial enzyme activities were observed in an area of the peatland recovering from sulfate

additions (Seifert-Monson et al., 2014), but the effects of sulfate on bacterial community structure remain unknown.

In much of Europe and North America, atmospheric deposition of sulfate has greatly increased since the onset of the industrial revolution (Mayewski et al., 1990), although stricter emissions regulations introduced in the 1980s have substantially decreased sulfate deposition in these same regions (Kahl et al., 2004). Sulfate deposition to aquatic systems such as lakes and wetlands is of particular significance because it has been repeatedly observed to increase levels of methylmercury (MeHg). MeHg is a bioaccumulative neurotoxin that is produced by bacteria in permanently or transiently anoxic aquatic environments (Branfireun et al., 1990; Coleman Wasik et al., 2012; Munthe et al., 2007). Peatlands are a wetland type wherein particularly high production and accumulation of MeHg is often observed in periodically inundated near-surface layers (Mitchell et al., 2008). Mercury (Hg) methylation ability has been confirmed in many *Deltaproteobacterial* SRB (Gilmour et al., 2013; Ranchou-Peyruse et al., 2009), and experimental sulfate additions have been shown to increase MeHg concentrations via metabolic stimulation of SRB (Gilmour et al., 1992; Yu et al., 2010). However, outside of the direct metabolic stimulation of SRB, it is not known if sulfate-mediated changes in bacterial community structure relate to net MeHg production, which could potentially occur via syntrophic interactions between methylating and nonmethylating taxa (Bae et al., 2014; Pak and Bartha, 1998).

In this study, the *in situ* response of the bacterial community of a sub-boreal peatland in Minnesota to relatively long-term experimental increases and decreases in sulfate deposition, along with the associated changes in MeHg concentrations were assessed. The primary objective here was to assess how changing sulfate loading influences the composition of the peatland bacterial community, with a secondary aim to determine if any relationship exists between MeHg

accumulation within the peat and bacterial community structure. It is hypothesized that increased sulfate loading will lead to significant changes in the peatland bacterial community composition. Given the relatively rapid declines in MeHg concentrations observed by Coleman Wasik et al. (2012) once experimental sulfate additions were ceased in this same study wetland, it is hypothesized that the bacterial community structure will also change relatively rapidly with decreased sulfate loading and that changes in bacterial community structure can be related to changes in MeHg accumulation and loss.

2. Experimental Procedures

2.1. Site Description and Experimental Design

An 8-year manipulation of an experimental sub-boreal peatland in the Marcell Experimental Forest, Minnesota (47°32'N, 93°28'W) simulated realistic increases in sulfate deposition as well as recovery from augmented sulfate deposition, and the impact of these changes on net MeHg accumulation (Coleman Wasik et al., 2012, 2015; Jeremiason et al., 2006). The peatland, referred to as “S6”, covers approximately 2.4 ha and includes an ombrotrophic, elongate, 10-25 m wide center (bog) with *Sphagnum* hummocks and an overstory of tamarack (*Larix laricina*) and black spruce (*Picea mariana*). The bog has a 2-10 m wide margin (lagg) vegetated with alders (*Alnus rugosa*) and willows (*Salix spp.*; Figure 1). In addition to direct precipitation inputs, the lagg intermittently receives water via shallow interflow from a surrounding 6.5 ha upland forest, whereas the bog area is precipitation fed only (Mitchell et al., 2009). Sulfate deposition to a portion of the S6 peatland was experimentally increased by ~4x over ambient rates in order to simulate the deposition received by areas in the northeastern US and eastern Canada before strict emissions regulations came into effect in the 1980s. Source water for the additions was obtained

from a dilute (conductivity $\sim 10 \mu\text{S cm}^{-1}$), low mercury ($\text{Hg} < 1 \text{ ng l}^{-1}$) ombrotrophic pond in an adjacent watershed and amended with concentrated sodium sulfate to a final sulfate concentration of $\sim 200 \text{ mg l}^{-1}$, as described previously in Jeremiason et al. (2006) and Coleman Wasik et al. (2012). From spring of 2001 to fall of 2008, this system was used to deliver $32 \text{ kg ha}^{-1} \text{ yr}^{-1}$ of sulfate to both the bog and lagg areas of an experimental section of the wetland via a PVC pipe sprinkler system during 3-4 simulated rainfall events (6-10 mm) per year. An unamended area of the peatland, hydrologically up-gradient of the experimental area, was maintained as a control (Figure 1). In 2006, sulfate additions to the up-gradient one-third of the experimental section ended, creating a recovery treatment subject only to an average background deposition rate of 4.6 kg ha^{-1} (NADP, 2006). Additions to the downstream two-thirds of the experimental section continued through the end of 2008 with a final sulfate amendment in October. In addition to this three-level sulfate amendment factor (experimental, recovery, and control), the natural topography of the peatland created another two-level factor (ombrotrophic bog area versus the slightly more minerotrophic lagg area), for a total of six treatments (Figure 1) that could be sampled to compare the effects of sulfate deposition on the bacterial community as well as the concentration of MeHg in the peat.

In late-May 2009, a period when the water table was above the peat surface, three peat cores were collected in each of the six treatment areas described above, with each core serving as a replicate. From each of these eighteen cores, peat was collected from the 2-4 cm depth interval and kept frozen until analysis. Peat samples from each core were split into two subsamples for analyses of 1) bacterial community composition and 2) total Hg (THg) and MeHg concentrations, according to methods described below.

2.2. Bacterial analyses

2.2.1. DNA extraction and sequencing

Total community genomic DNA was extracted from each of the three replicate samples from all six treatment areas (18 samples total). Using the MoBio PowerSoil™ DNA Extraction Kit (MO BIO Laboratories, Solana Beach, CA, USA), DNA was extracted from 0.25 g of thawed peat according to the manufacturer's instructions. An Eppendorf Nanodrop 1000 (Thermo Scientific) was used to estimate DNA concentration ($\text{ng } \mu\text{l}^{-1}$) and purity (260/280 and 260/230 ratios). These eighteen samples were subjected to pyrosequencing of bacterial 16S ribosomal gene amplicon. The hypervariable V1-V3 region was targeted using 28f (5'-GAGTTGATCNTGGTCAG—3') and 519r (5'—GTNTTACNGCGGCKGCTG—3') (Amann et al., 1995) by bTEFAP (bacterial tag encoded FLX amplicon pyrosequencing), carried out by Research and Testing Inc. (Lubbock, Texas) on a Roche 454 FLX Titanium genome sequencer. Sequences were processed using Qiime 1.4.0. (Caporaso et al., 2010). Samples were demultiplexed, forward and (if present) reverse primers were trimmed. Low quality samples were identified and filtered out using the default parameters. Sequences were chimera filtered and denoised using OTUpipeline with USEARCH (Edgar, 2010) and both de novo and database (GOLD) based chimera filtering with intersection; MINSIZE was 2. Sequences were assigned to operational taxonomic units (OTUs) at an identity threshold of 97% (Kunin et al., 2010) using the RDP method trained on the Feb 4, 2011 release of Greengenes with default settings. Sequences were aligned using PYNAST trained against the 2011 release of the Greengenes core set, and a phylogenetic tree constructed with FASTTREE algorithm (Price et al., 2010) and default settings. To allow comparison of community compositions between samples that returned different numbers of reads, all community analyses were carried out on weighted abundances, calculated as the number of reads per OTU in a given sample divided by the total number of reads for that sample.

2.2.2 Sequence accession numbers

All sequences that passed quality control steps and were included in the analysis were deposited to the EMBL-EBI database under accession numbers ERS452350-ERS452367.

2.2.3 Sequence analysis

To assess the impact of the varying sulfate treatments on the bacterial community, weighted UNIFRAC distance matrices, a phylogenetically aware beta diversity metric designed for analysis of large genomic datasets (Lozupone and Knight, 2005), were used to compare the relatedness of bacterial community composition from different treatments. Based on differences in hydrology and nutrient status (Mitchell et al., 2008), the bacterial communities of lagg and bog sites were analysed separately. Hierarchical clustering was applied to UNIFRAC matrices to visualize patterns of relatedness between bacterial communities (Hamady et al., 2009), and PERMANOVA (permutational analysis of variance) to assess the significance of the revealed patterns (Anderson, 2001; McArdle and Anderson, 2001). Alpha diversity was assessed using the Chao1 diversity index, which estimates the true diversity of a population by extrapolating from the abundance distribution of the subsample (Chao, 1984). Mantel tests were used to test correlations between bacterial community structures and peat MeHg content. These tests were conducted in Qiime with 999 permutations at $\alpha < 0.05$. In addition, the *Deltaproteobacteria*, which host many SRB and confirmed mercury methylators, were separately assessed for the relationship of their community structure to sulfate amendment and %MeHg (percent of THg as MeHg) via the same methods used for the whole bacterial community. Two-tailed tests of Pearson's correlations at $\alpha \leq 0.01$ were used to relate specific OTUs with %MeHg in peat.

2.3. Chemical Analyses

As for the bacterial community analyses, one peat sample was analysed from each of three cores collected from each of the six treatment areas, yielding 18 samples. THg analysis in peat samples was carried out via microwave digestion in concentrated HNO₃, cold-vapor generation (using SnCl₂), dual gold-trap amalgamation, and detection by cold vapor atomic fluorescence spectroscopy (CVAFS) on a Tekran 2600 total-Hg analyser. MeHg analysis was carried out via isotope dilution-gas chromatography-inductively coupled plasma mass spectrometry (ID-GC-ICPMS) after the method of Hintelmann et al. (1997). Samples were enriched with a known spike of Me¹⁹⁹Hg and distilled in 8M H₂SO₄ and 20 % KCl in acid-cleaned Teflon vessels (Horvat et al., 1993). MeHg in distillates was liberated from solution through aqueous phase ethylation with sodium tetraethylborate, concentrated on a Tenax trap, thermally desorbed in a stream of argon gas, and passed through a heated chromatography column hyphenated to an Agilent Technologies 7700x ICP-MS machine. Blanks, duplicates, and certified reference materials (MESS-3 for THg; IAEA-405 for MeHg) were used for ongoing quality control (Supplementary Table 1). Porewater for sulfate analysis was collected using a mini Teflon piezometer and peristaltic pump, and analysed using a Dionex DX-500 ion chromatograph with standard methods (Coleman Wasik et al., 2012). Porewater sulfate concentrations were assessed once per treatment in 2009 at the time of core collection and interpretations were supplemented with historical data taken from the treatment areas by Coleman Wasik et al. (2012 and 2015).

3. Results and Discussion

3.1 Sulfate Deposition Manipulation Effects

In this same peatland, experimental increases in sulfate deposition resulted in peak pore water sulfate concentrations ($3.8 \pm 2.2 \text{ mg l}^{-1}$ in 2008) immediately following experimental additions,

declining significantly over a period of approximately two weeks, but with concentrations remaining elevated above those observed in the control (Coleman Wasik et al., 2012, 2015). Sulfate concentrations in the recovery treatment porewaters declined to levels similar to those in the control ($< 1 \text{ mg l}^{-1}$) by early 2006, the first year without experimental sulfate addition to the recovery treatment area, although sulfate release after drought events continued to be elevated in the recovery treatment area for the remainder of the study, suggesting that strong internal sulfur cycling persisted in the recovery treatment area and likely through the time that samples from this study were collected (Coleman Wasik et al., 2012, 2015). Sulfate reduction is clearly sulfate-limited in this peatland, with relatively rapid conversion of sulfate to sulfide and subsequent accumulation into the reduced and organic sulfur pools within the peat (Coleman Wasik et al., 2012). During porewater sampling in spring 2009, concomitant with peat collection for this study, sulfate concentrations were highest in the experimental treatment in both bog (2.99 mg l^{-1}) and lagg (1.57 mg l^{-1}) areas and lowest in the control treatment area (bog: 1.09 mg l^{-1} , lagg: 0.98 mg l^{-1}) with recovery area levels nearly identical to concentrations in the control area (Figure 2). Due to difficulty in collecting porewaters, only a single measurement was collected in each treatment, but the overall pattern of sulfate levels closely mirrors the long-running patterns observed in this experimental system (Coleman Wasik et al., 2012, 2015). These results were extensively explored by Coleman Wasik et al. (2012, 2015) and suggest that the sulfur cycle in the experimental area was significantly altered by sulfate additions, whereas sulfur cycling in the recovery area had more closely returned to pre-disturbance conditions.

3.2. Bacterial Community Description

A total of 241700 raw sequences were obtained from 18 core samples, 44038 of which were removed due to low read quality or during sample denoising and chimera filtering, leaving 197662 high quality sequences (8662—13110 per sample). Sequences clustered into 7050 operational taxonomic units (OTUs). Between 1480 and 2270 OTUs were obtained from each sample. The bacterial communities of the S6 peatland were diverse and harbored many rare taxa. Of the final 7050 OTUs, 29 were not of bacterial origin and 916 could not be assigned past the rank of Bacteria. Thirty phyla, 63 identified classes, 86 identified orders, and 109 identified families were represented in the sequences. Of the 5497 OTUs that could be assigned to the order level, 978 were placed into undescribed novel orders. Undescribed families made up 40% (1936) of the 4889 OTUs that could be assigned to this level. Sixteen phyla were rare, representing less than 1% of any sample and 12 were completely absent from at least one treatment. The most abundant phylum was *Proteobacteria*, representing on average 30—52 % of the weighted total community in each treatment, which included the classes *Alphaproteobacteria* (21—36%), *Betaproteobacteria* (0.10—3.0%), *Deltaproteobacteria* (2.5—6.6%), *Gammaproteobacteria* (3.6—7.6%) and unassigned *Proteobacteria* (0.31—1.1%). Thirteen other phyla represented on average more than 1% of the total community in any treatment (Figure 3a), with the most commonly detected families being *Acidobacteraceae*, *Bradyrhizobiaceae*, *Hyphomicrobiaceae*, two other undefined families in *Rhizobiales*, and *Acetobacteraceae* in *Rhodospirales* (Supplementary Tables 2-5). Overall, both bog and lagg bacterial communities were similar in terms of taxonomic composition and the relative abundances of phyla to other communities retrieved from *Sphagnum* peatlands around the northern hemisphere (Dedysh et al., 2006; Juottonen et al., 2005; Lin et al., 2012; Pankratov et al., 2005; Serkebaeva et al., 2013). Species richness, estimated using the Chao1 index, was consistently and significantly (ANOVA; $F=14.76$, $p = 0.002$) higher in the lagg sites (2704 ± 197 OTUs) than the bog sites (2338 ± 341

OTUs). The control treatment also had significantly greater species diversity (2747 ± 221 OTUs) than the experimental (2392 ± 217 OTUs, $p = 0.026$) or recovery (2425 ± 426 OTUs, $p = 0.043$) treatments, which did not significantly differ from one another. Notably, the *Desulfovibrionaceae* and *Syntrophobacteraceae* that contain sulfate-reducing taxa, made up only a small fraction (0.00-0.0025%; Supplementary Table 4) of the whole community, and did not differ significantly among treatments in either bog or lagg areas.

3.3. Bacterial Community Responses to Sulfate Amendment

The experimental manipulation of sulfate deposition had a significant impact on the composition of the whole bacterial community within the peatland. PERMANOVA analysis revealed that the lagg zone communities differed significantly among treatments ($F_{(2,6)} = 3.65$, $p = 0.003$). Hierarchical clustering analysis showed the recovery and control communities to be most similar, while the experimental communities were distinct (Figure 4a). In the bog areas, although the community clustering was visually less distinct than in the lagg (4b), bacterial communities did differ significantly among sulfate treatments ($F_{(2,6)} = 1.99$, $p = 0.019$). The significantly greater taxonomic diversity of the lagg community suggests that it has a greater degree of ecological redundancy within functional groups, leading to greater resilience to sulfate perturbation than the bog community (Luck et al., 2003). In both lagg and bog areas, recovery and control *Deltaproteobacterial* communities were highly similar to each other, but distinct from *Deltaproteobacterial* communities in the experimental treatments. There was a composite cluster of communities from control and recovery treatments and a distant cluster composed solely of experimental communities (Figure 4c, d). These patterns were similar and significant in both lagg ($F_{(2,6)} = 3.600$, $p = 0.003$) and bog ($F_{(2,6)} = 3.138$, $p = 0.006$) areas.

The only previous work to investigate the effect of sulfate on an entire peatland bacterial community, rather than a metabolic or taxonomic subset, inferred changes in bacterial community structure by assessing changes in populations of bacterivorous testate amoebae (Payne et al., 2009). This indirect approach suggested that sulfate changed the bacterial community structure, a finding confirmed here using a replicated experimental manipulation of sulfate, paired with direct characterization of the bacterial community. The mechanism by which sulfate alters the peatland bacterial community appears to be relatively subtle, and further investigations are needed to link changes in the deposition of sulfate with specific taxonomic and functional changes. It is beyond the scope of this study to explain conclusively how sulfate changes bacterial communities; however, despite the apparent low abundance and responsiveness of confirmed SRB to sulfate treatments, results indicate that sulfate may directly alter the bacterial community structure through its effect on organisms that use it in their metabolism. Experimental sulfate additions may have stimulated unknown sulfate reducers, the full diversity of which is not described (Pester, 2012; Steger et al., 2011), or conferred a competitive advantage upon rare but disproportionately dominant sulfate reducers that have been estimated to carry out up to 50% of the anaerobic carbon mineralization in peatlands (Blodau et al., 2007; Keller and Bridgham, 2007), even when their abundance is very low (Andersen et al., 2013). Alternatively, it has been previously observed that changes in atmospheric sulfate inputs can restructure the bacterial community indirectly via mechanisms such as redirection of carbon flow away from methanogens (Gauci and Chapman, 2006; Lovley and Klug, 1983) with accompanying negative effects on methanotrophs, as well as stimulation of sulfide oxidizers as they consume the H_2S produced by sulfate reduction. Such a redirection of carbon flow with associated restructuring may be occurring in this system.

The effects of altered sulfate deposition regime on the bacterial community appear to be

relatively short-lived and reversible. The recovery of bacterial communities in the lagg and near recovery in the bog within three years of the cessation of sulfate amendments suggests that the bacterial community structure is resilient to changes in the supply of labile sulfur. Similar findings were obtained by Seifert-Monson et al. (2014), who compared microbial enzyme activities in the former experimental area of the S6 peatland with newer microcosm sulfate amendments in the recovery and control areas. These authors observed no differences in enzyme activity between the former experimental area, which was in the process of recovering from sulfate amendment, and unamended control microcosms. At the time these authors sampled the S6 peatland, the experimental area had been unamended for approximately three years and was better considered a recovery treatment, suggesting that its microbial community was now functionally similar to that of the recovery treatment at the time it was sampled for this current study. In this experimental environment, added sulfate becomes increasingly recalcitrant as it is repeatedly recycled and progressively converted to less bioavailable organic forms (Coleman Wasik et al., 2012, 2015). Thus, most alterations to ecosystem function as a result of sulfate-mediated changes in the bacterial community are hypothesized to be reversible on the timescale of a few years, although it should be noted that when sulfate deposition has been elevated for a longer time, the recovery period is also likely to be longer. This study specifically simulated the effects of historical reductions in sulfate deposition and suggests that emission reductions in North America and Europe over the last few decades have likely been sufficient for the peatland microflora to return to a structure similar to that found in areas with lower deposition.

3.4. Relationship Between Sulfate-Mediated Changes in Bacterial Community Structure and MeHg

The major purpose of this study was to investigate the impact of sulfate on the peatland bacterial

community. However, the controlled field manipulation was originally conceived as an experimental investigation into the long-term effects of sulfate on MeHg production (Jeremiason et al., 2006), presenting an opportunity to explore whether bacterial community structure was related to %MeHg in the peat. The percentage of mercury present as MeHg (%MeHg) in peat integrates the long-term methylation and demethylation capacity of a soil, representing net MeHg accumulation (Drott et al., 2008). %MeHg values correlated significantly with MeHg ($r = 0.900$, $p < 0.001$), but not THg ($r = -0.071$, $p = 0.781$) concentrations in the peat, indicating that differences in %MeHg are related to MeHg production and/or accumulation in peat and not a function of inorganic Hg content. As described more fully in previous work (Coleman Wasik et al., 2012, 2015; Jeremiason et al., 2006), sulfate deposition history did alter MeHg concentrations and %MeHg in both the bog and the lagg areas (bog: $F_{(2,6)} = 20.16$, $p = 0.002$, lagg: $F_{(2,6)} = 9.477$, $p = 0.014$). %MeHg was elevated in peat samples from both the bog and lagg areas of the experimental treatment (8 — 16%), intermediate in the bog and lagg areas of the recovery treatments (3 — 7%), and lowest in the control treatment in the bog area (1 — 3%) (Figure 5). In the bog area, %MeHg in the experimental treatment differed significantly from that in both the recovery and control, while the control and recovery were statistically similar to one another. In the lagg area, the experimental treatment had significantly greater %MeHg than the recovery treatment, but the control site had unexpectedly high %MeHg (9—10%; Figure 5). This discrepancy was likely due to sampling a natural MeHg “hot spot” within the control area, as previously described by Mitchell et al. (2008) and Coleman-Wasik et al. (2012).

No correlation was found between the entire bacterial community structure and %MeHg for either the lagg ($r = 0.27$, $p = 0.10$) or bog ($r = -0.13$, $p = 0.53$) areas. Although both mercury methylation and sulfate reduction capacity are found in non-*Deltaproteobacterial* groups, approximately 78% of presently confirmed methylators are sulfate-reducing *Deltaproteobacteria*

(Gilmour et al., 2013). Thus, *Deltaproteobacteria* were chosen as an additional focus for investigating how changes in %MeHg, which had been altered by experimental sulfate additions, may also be related to changes in the bacterial community structure of the S6 peatland. The *Deltaproteobacteria* were represented by 546 OTUs, which together comprised 2.5—7.8 % of the weighted abundance per sample (Figure 3b). The most common of the eight *Deltaproteobacterial* orders present was *Myxococcales*, represented by 305 OTUs. Ten identified families, of which *Syntrophobacteraceae* and *Cystobacteraceae* were most abundant, were also found (Supplementary Table 5).

Unlike the whole bacterial community, *Deltaproteobacterial* communities did exhibit significant or nearly significant correlations with %MeHg for both the bog and lagg areas (bog: Mantel $r = 0.56$, $p = 0.008$, lagg: Mantel $r = 0.36$, $p = 0.057$). This relationship between *Deltaproteobacterial* community structure and %MeHg suggests that the frequently-observed changes in MeHg production in response to sulfate (Branfireun et al., 1999; Coleman Wasik et al., 2012; Jeremiason et al., 2006; Mitchell et al., 2008) are likely related to changes in a subsection of the total bacterial community structure, but the moderate coefficients of variation indicate that this mechanism most likely occurs in addition to sulfate-mediated stimulation of SRB. Macaladay et al. (2000) found a strong relationship between Hg methylation rates and community structure of 37 SRB taxa identified using polar lipid fatty acid (PLFA) analysis in sediment from a California lake. Results from this current study extend this work through a more comprehensive accounting of the bacterial community and suggest that sulfate deposition is an important driver for mercury methylation, not simply because of augmented bacterial activity, but also due to controls on community structure.

108 OTU's from the lagg and 80 OTU's from the bog were identified that correlated significantly to %MeHg levels in the peat (Supplementary Tables 6-7). Significant, positive

correlations were found for several known methylating taxa. One OTU in *Desulfovibrionaceae*, a family hosting the best-known Hg-methylating genera (Compeau and Bartha, 1985; Gilmour et al., 2011) correlated significantly ($p = 0.007$, $r = 0.817$) with %MeHg, an interesting finding given the extremely low abundance of this family in the bacterial community (0 – 0.25%). In addition, two OTUs in *Syntrophobacteraceae*, a *Deltaproteobacterial* family ($p = 0.001$ - 0.005 , $r = 0.888$ - 0.833), and six OTUs in the *Veillonellaceae* family of the *Firmicutes* ($p = 0.007$ - 0.001 , $r = 0.883$ - 0.927), correlated with %MeHg. Both of these groups contain taxa (*Syntrophus aciditrophicus* SB in *Syntrophobacterales*, and *Desulfitobacterium metallireducens*, *Desulfosporosinus acidiphilus* SJ4, *Desulfitobacterium dehalogenans*, *Desulfosporosinus youngiae*, *Ethanoligenens harbinense* YUAN-3, *Dethiobacter alkaliphilus* AHT 1 in *Firmicutes*) that have been recently identified *in vitro* as Hg methylators (Gilmour et al., 2013). Correlations observed are consistent with the hypothesis that these groups may methylate Hg in peat.

4. Conclusion

This work consistently supports the hypothesis that experimental increases and decreases in sulfate deposition lead to significant, but reversible, changes in the taxonomic composition of a peatland bacterial community. Furthermore, sulfate altered the community composition of the *Deltaproteobacteria*, and the structure of this community was correlated significantly with net MeHg accumulation. These results suggest a sulfate-mediated alteration of the *Deltaproteobacteria* community as a mechanism driving MeHg concentration changes in this peatland. Finally, it is notable that the experimental rate of sulfate deposition in this experiment simulated historical deposition in, and downwind of, industrial areas of eastern Canada and the US prior to aggressive regulation of sulfate emissions. Findings suggest that previous levels of pollution likely altered both the structure and at least one function (MeHg production) of

peatland bacterial communities, and that legislated emissions controls likely led to a significant lessening of these effects with a lag-time of well under a decade.

Author Information

Corresponding Author

Phone: +001 416 208 2744; e-mail carl.mitchell@utoronto.ca

Notes

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Figure Captions

Figure 1 Field Site: Diagram of the sulfate amendment experiment in the S6 peatland of the Marcell Experimental Forest. Black arrows indicate the direction of water flow. Inset map indicates location of the Marcell Experimental Forest in north-central Minnesota, U.S.A. Sulfate amendment schedule is summarized in the lower left corner for reference.

Figure 2 Sulfate Concentrations: Sulfate levels (mg l^{-1}) in porewater from the experimental (Exp.), recovery (Rec.) and control (Cont.) treatments of the lagg and bog areas of the S6 peatland five months after the final sulfate amendment in 2008.

Figure 3 Bacterial Communities: Taxonomy and average relative abundance in each treatment from the lagg (left) and bog (right) areas for the total bacterial community (a) and the *Deltaproteobacteria* (b). Phyla level results are presented for the overall bacterial community, with low abundance phyla (less than 0.5 % of the weighted abundance in any treatment) subsumed into the Bacteria classification for clarity. *Deltaproteobacterial* taxa are weighted by the relative abundance within the *Deltaproteobacteria* rather than the whole bacterial community and are presented at the lowest taxonomic level (phylum, class, or order) to which they could be assigned.

Figure 4 Clustering of Bacterial Communities: Weighted-UNIFRAC based hierarchical clustering trees of bacterial communities sequenced from peat cores obtained from different sulfate amendment treatments (E=experimental; R=recovery; C=control). From top left: overall bacterial communities from the lagg (a) and bog (b) areas, and *Deltaproteobacterial* communities of the lagg (c) and bog (d).

Figure 5 Methylmercury Accumulation: The mean percentage of total Hg as MeHg (%MeHg) $\pm$ standard deviation in peat cores from the three sulfate treatments (Exp. = experimental; Rec. = recovery; Cont. = control). The probabilities ($p \leq 0.05$) associated with each pairwise comparison are indicated above the relevant pair of columns.

Figure 1 (Single Column)

Figure 2 (Single Column)

Figure 3 (Double Column)

a) Total community

b) Deltaproteobacteria

Figure 4 (Single Column)

LAGG

BOG

Figure 5 (Single Column)