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Research Letter

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Beyond the usual suspects: methanogenic communities in eastern North American peatlands are also influenced by nickel and copper concentrations

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One sentence summary: Nickel and copper concentrations, pH, temperature, water table and percentage coverage of Sphagnum mosses strongly influence methanogenic communities in 17 peatlands along a latitudinal gradient in eastern North America.

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ABSTRACT

Peatlands both accumulate carbon and release methane, but their broad range in environmental conditions means that the diversity of microorganisms responsible for carbon cycling is still uncertain. Here, we describe a community analysis of methanogenic archaea responsible for methane production in 17 peatlands from 36 to 53 N latitude across the eastern half of North America, including three metal-contaminated sites. Methanogenic community structure was analysed through Illumina amplicon sequencing of the *mcrA* gene. Whether metal-contaminated sites were included or not, metal concentrations in peat were a primary driver of methanogenic community composition, particularly nickel, a trace element required in the F_{430} cofactor in methyl-coenzyme M reductase that is also toxic at high concentrations. Copper was also a strong predictor, likely due to inhibition at toxic levels and/or to cooccurrence with nickel, since copper enzymes are not known to be present in anaerobic archaea. The methanogenic groups Methanocellales and Methanosarcinales were prevalent in peatlands with low nickel concentrations, while Methanomicrobiales and Methanomassiliicoccales were abundant in peatlands with higher nickel concentrations. Results suggest that peat-associated trace metals are predictors of methanogenic communities in peatlands.

Keywords: methanogens; peat; mcrA; fen; bog; metal

INTRODUCTION

Peatlands, the most prevalent (4.2 million km^2) wetland in the world (Xu *et al.* 2018), play unique roles in both the long-term storage and contemporary fluxes of atmospheric carbon in the form of carbon dioxide and methane (Gorham and Jansens 1992; Lehner and Döll 2004; Petrokofsky *et al.* 2012). In the anoxic layer of peat, methanogenic archaea produce methane, a greenhouse gas from the decomposing organic matter utilizing either methylotrophic, hydrogenotrophic and/or aceticlastic mechanisms (Zinder 1993; Ferry 2011; Bräuer *et al.* 2020). Despite the importance of microbes in regulating carbon cycling and greenhouse gas emissions in peatlands, the factors structuring peatland microbial communities are still uncertain. Among potential controlling factors, the role of trace metals is perhaps the most poorly understood.

Given the critical role of metal enzymes in methanogenesis, methanogens are likely to be highly sensitive to metal concentrations. In methanogenic archaea, the trace metals iron, nickel, zinc, cobalt, molybdenum and tungsten are required in large amounts (Glass and Orphan 2012). Iron and zinc are required in the heterodisulfide reductase essential for the the second-to-last step in methanogenesis (Thauer *et al.* 2010). Nickel is required in the F_{430} cofactor in methyl-coenzyme M reductase in the final step in methanogenesis (Nayak *et al.* 2020). Cobalt is another important enzyme in methanogenesis as it is contained within methyltransferases required for methyl transfer from methyl-tetrahydromethanopterin to coenzyme M (Gottschalk and Thauer 2001). Finally, molybdenum is required for nitrogen fixation in methanogens (Lobo and Zinder 1990; Lobo 1992). Copper is of less importance for methanogenic archaea, but is required in other organisms for enzymes operating at a higher redox potential, such as enzymes involved in aerobic methane oxidation (Messerschmidt 2010; Glass and Orphan 2012). If concentrations of iron, nickel and cobalt are too low, methanogenesis is limited (Glass and Orphan 2012). On the other hand, metal concentrations that are too high can also be toxic to methanogens (Glass 2015), especially copper, zinc and nickel, with copper being the most inhibitory (Chen, Cheng and Creamer 2008). In largely ombrotrophic peatlands, natural abundance of metals can be quite low (Shotyk 1988; Gotelli *et al.* 2008; Warren *et al.* 2017), whereas regional pollution may lead to accumulation of metals (Watmough and Orlovskaya 2015; Fiałkiewicz-Kozieł *et al.* 2018; Pratte *et al.* 2018; Rosca *et al.* 2019), which may also impact the microbial communities (Fiałkiewicz-Kozieł *et al.* 2015; Luke *et al.* 2015). Thus, in addition to other more well-studied factors such as as pH, water table depth, C:N ratios and *Sphagnum* coverage, we hypothesize that both low (limiting) and high (toxic) metal concentrations could structure methanogen communities.

METHODS

Sample collection

A total of 17 peatlands in eastern North America in collaboration with the Global Peatland Microbiome Project (GPMP; Lamit and Lilleskov *et al.* unpublished) were selected for this study (Table S1 and Figure S1, Supporting Information). The peatlands span from small, local peatlands contained in well defined basins, to some of the largest peatland complexes in the world such as the Hudson Bay Lowlands. They range in latitude from 36.08° to 52.72°N, including sites in North Carolina, USA (Pineola, Sugar Mountain and Tater Hill); Tennessee, USA (Ripshin); West Virginia, USA (Cranberry Glades and Big Run); New York, USA (McLean and Purvis Road/Dryden Bog) and Ontario, Canada (Cartier, Daisy Lake, Long Lake, Mer Bleue, Whitson Lake and Victor Mine). In Ontario, three of the peatlands were historically impacted by local smelter emissions (Long Lake, Daisy Lake and Whitson Lake), and three of the peatlands represent unimpacted control sites (Cartier, Mer Bleue and Victor Mine). Multiple contributors to the GPMP project provided peat and environmental data fore each peatland, sampled in triplicate with cores taken at depths of 10–20 cm, 30–40 cm and 60–70 cm beneath the surface. Some peats were not deep enough to sample at 60–70 cm (Sugar and Tater), and some were only sampled at 10 cm (Long, Whitson, Daisy and Cartier). Three sites were only sampled in duplicate (Ripshin, Mer Bleue and Cranberry). Finally, some of the samples contained insufficient quantities of DNA for sequencing: Pineola (five samples); Tater (one sample); VCIM (one sample) and VMOE fen (five samples.) Samples were frozen at −20°C and sent to the USFS lab in Houghton, MI for DNA analysis (Harbison *et al.* 2016).

Environmental and chemistry data

Environmental data were collected for each sample, including conductivity, core temperature, soil pH, *Sphagnum* and vegetation cover (above each core) and water-table depth: see Seward *et*

al. (2020) for details. The average air temperature of the peatland locations was acquired from the National Oceanic and Atmospheric Administration (NOAA) for sites in the United States, and from the Government of Canada's Environmental and Natural Resources for sites in Canada. Elemental analyses of calcium, nickel, copper, molybdenum, tungsten, potassium, magnesium, cobalt, sodium, vanadium, manganese and iron were performed for each peat sample (Carson 2018). Prior to elemental analysis on a Varian 810 ICP-MS, peat was ashed and fully acid digested (Watkinson et al. 2017). For C and N analysis dried ground peat was weighed to within 0.0001 g on a digital balance. Approximately 75 mg of peat was analyzed using an Elementar VarioMacro CNS Analyzer and precision of results was confirmed using blanks and sulfadiazine for CNS recalibration and QA standard (NIST-1515-SRM apple leaves).

Microbial sequence analysis

DNA was extracted from peat samples using the QIAGEN (Germantown, MD, USA) DNeasy PowerSoil Isolation kit and then cleaned using the PowerClean kit. The manufacturer's protocol was followed including an added heating step after bead beating (65°C for 30 min) during DNA extraction. PCR was performed according to the protocol for *mcrA* targeting in soil samples (Juottonen, Galand and Yrjälä 2006). Initial denaturation at 95°C for 5 min, 35 cycles of 95°C for 45 s, annealing at 46°C for 45 s and extension at 72°C for 7 min. The *mcrA* primers mlasF-mod (5′- GGY GGT GTM GGD TTC ACM CAR TA-3′)—mcrAR (5′-CGT TCA TBG CGT AGT TVG GRT AGT-3′; Luton et al. 2002; Juottonen, Galand and Yrjälä 2006; Angel, Claus and Conrad 2012) were used to amplify the *mcrA* gene. PCR products were confirmed by gel electrophoresis in a 1% agarose gel.

Amplified DNA was sent to Metagenombio Inc. (Waterloo, Canada) for Expression Analysis Illumina Sequencing. Raw sequences were quality filtered and trimmed using the BBMap (Bushnell 2014) package, and chimeras were removed using seqtab.nochim. Forward reads were aligned and processed with MacQIIME Version 1.9.1–2 (Caporaso et al. 2010) and USEARCH (Edgar 2010) using an 86.5% confidence threshold for operational taxonomic units (OTUs) assignments; similar to the 85.7% cutoff recommended for *mcrA* functional genes in other papers (Hunger et al. 2011; Barbier et al. 2012), but adjusted to a slightly higher, more stringent cutoff to fit our particular dataset. Taxonomy was assigned using a publically available *mcrA* database (Yang et al. 2014) through DADA2 (Callahan et al. 2016). Bioedit (Hall 2011) was used to create multiple sequence alignments using the clustalW accessory application. Phylip (Felsenstein 1993) and MEGAX (Kumar et al. 2018) were used to phylogenetically analyze protein and nucleotide sequences using known sequences from the NCBI database for comparison. The relative abundance of methanogenic orders derived from the *mcrA* analysis were also compared to a similar dataset of the 16S rRNA genes in a previously published study of these same sites, for comparative purposes (Seward et al. 2020). NMDS plots were created using the VEGAN Community Ecology Package 2.5–6 (Oksanen et al. 2013) in RStudio, version 1.1.463 (Dixon 2003; R-Core-Team 2013). Biplots were created using the envfit command in VEGAN. Dissimilarity was measured using Jaccard distance method with square root transformation and Wisconsin double standardization and the NMDS was plotted using ggplot. Sequences were deposited with the National Center for Biotechnology Information under submission ID: SUB9421949 and BioProject ID: PRJNA740344. OTUs presented in the phylogenetic trees were deposited in GenBank under accession numbers MW842583-MW842617.

Peatland sites were grouped into four different standard categories of peatland based on pH and calcium concentrations (Mitch and Gosselink 2000; Fig. 1). All of these standard categorizations matched the classifications provided by the principal investigators that regularly study those sites (based on factors such as vegetation, soil pH, soil chemistry and von Post humification scales) except for McLean Bog which was classified between the criteria for bog and poor fen. While this site is often considered a bog, it also has sedge encroachment along the edges and has experienced some degree of pH increase since 2001 (Bräuer 2006).

RESULTS AND DISCUSSION

Methanogen community phylogenetics

Phylogenetic analysis of the *mcrA* gene sequences revealed OTU representatives from four orders of methanogens known to be common in peat (Bräuer et al. 2020): Methanomassiliicoccales (three OTUs), Methanomicrobiales (13 OTUs), Methanocellales (five OTUs) and Methanosarcinales (14 OTUs). Methanosarcinales were the most abundant (Fig. 2A), corroborating profiles of mesotrophic and oligotrophic fens in Finland using the *mcrA* gene (Juottonen et al. 2005), and in Canada using the SSU rRNA gene(Godin et al. 2012). When compared across peatland type (Fig. 3), the abundance of Methanomicrobiales (fen cluster) increased in rich and intermediate fens, compared to bogs and poor fens; however, the relative proportion of Methanomicrobiales was small (representing less than 30% of sequences). In contrast, SSU rRNA gene (16S) profiling from these same sites demonstrated a predominance of Methanomicrobiales (representing greater than 50% of total sequences; Fig. 2B) supporting most other studies (Basiliko et al. 2003; Galand, Fritze and Yrjälä 2003; Rooney-Varga et al. 2007; Juottonen et al. 2015; Martí et al. 2015). Such differences between the SSU rRNA gene and *mcrA* gene data may be attributed to primer bias inherent in functional gene analyses (Gaby and Buckley 2017). For example, a recent study from one particular bog used in our analyses, McLean Bog, conducted metagenome analyses, unbiased by PCR amplification and demonstrated that nearly all metagenome-assembled genomes related to known methanogens belonged to the Methanoregulaceae within the Methanomicobiales (220 genome equivalents) while other groups, including Methanosarcinaceae, Methanomethyliaceae, Methanomassiliicoccus and Methanobacteriaceae were found in much lower (fewer than 10 genome equivalents total) relative abundance (St. James et al. 2021). To compensate for these PCR biases, unweighted analysis methods (e.g. presence-absence) were used for ordination analyses of methanogenic communities in the samples. The phylogenetic trees for both the protein (Fig. 4) and the nucleotide (Figure S2, Supporting Information) *mcrA* sequences support the presence of four primary methanogenic orders in the 17 peatlands sampled.

Methanogen community composition

A non-metric multidimensional scaling (NMDS) ordination plot using an unweighted Jaccard distance matrix of *mcrA* OTUs (Figure S3, Supporting Information) revealed pH as a major driver of methanogen community assemblages, corroborating work by Seward et al. (2020). Lower pH values (below 5) were associated with the hydrogenotrophic group Methanocellales, consistent with results from ombrotrophic bogs (Lansdown, Quay and King 1992; Popp et al. 1999; Basiliko et al. 2003; Metje and

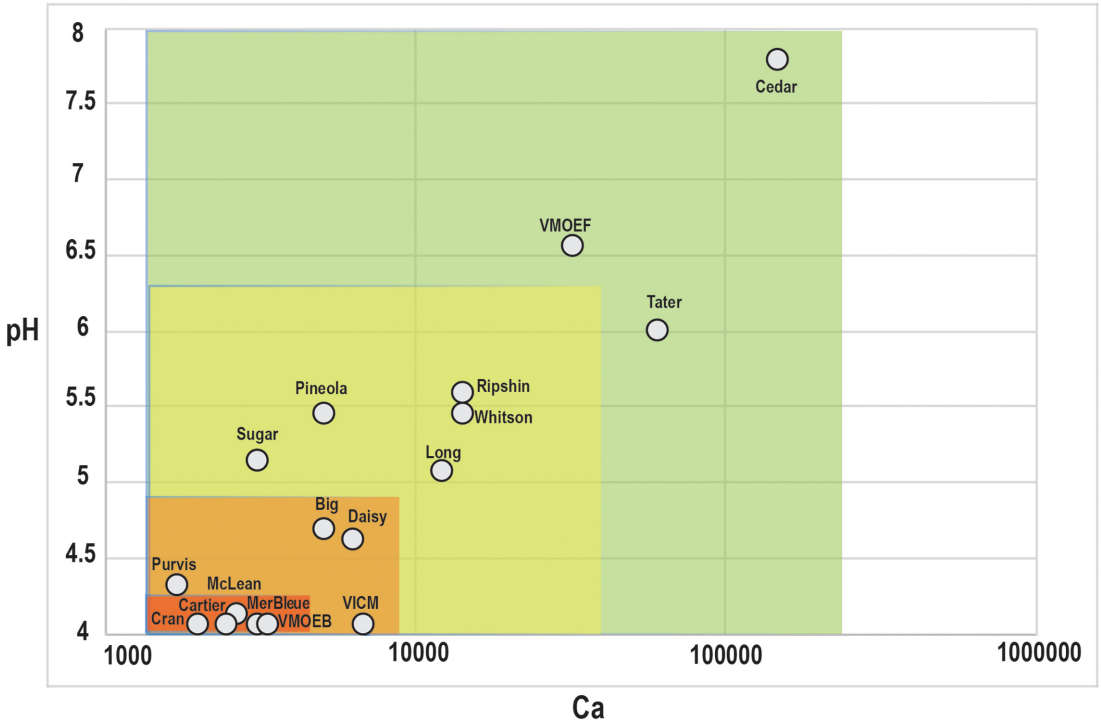

Figure 1. Peatland sites classified by calcium concentration (mg/kg dry peat) and pH. Red = bog, orange = poor fen, yellow = intermediate fen and green = rich fen.

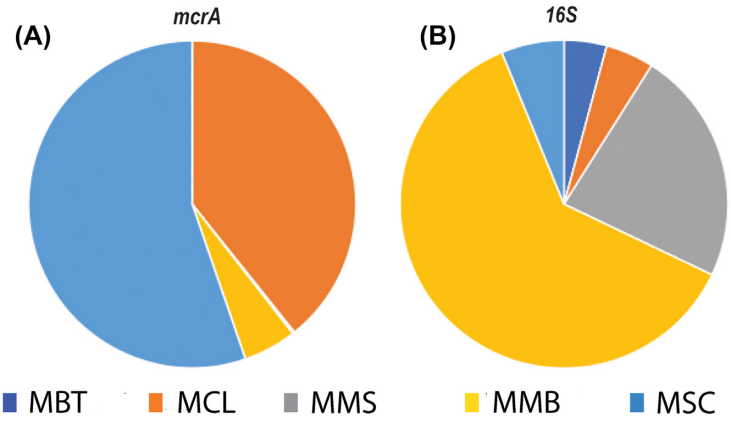

Figure 2. Relative abundance of *mcrA* sequences **(A)** and 16S rRNA gene sequences **(B)** for methanogenic orders representing greater than 1% abundance. Methanogenic groups are abbreviated as follows: MBT, Methanobacteriales; MCL, Methanocellales; MMS, Methanomassiliicoccales; MMB, Methanomicrobiales and MSC, Methanosarcinales.

Frenzel 2005). The peat samples with a higher pH (≥ 5) contained aceticlastic Methanosarcinales and increased proportions of both the 'fen cluster' (Methanomicrobiales) and the reductive methylotrophic group Methanomassiliicoccus (RC-III) supporting results from more pH neutral fens and rice paddies (Großkopf, Stubner and Liesack 1998; Juottonen *et al*. 2005; Godin *et al*. 2012). Methanosarcinales, which are capable of thriving in a wider range of pH environments (Hunger, Gößner and Drake 2015), did not clearly associate with either the low or high pH sites.

Environmental influence on methanogen community composition

An NMDS biplot was created to visualize which environmental factors drive methanogenic community assemblages (Fig. 5). *Sphagnum* cover, temperature, peat pH, water table depth and elemental concentrations were considered for this plot, and compared to the Jaccard distances of the peatland methanogenic assemblages, revealing that nickel concentration was the strongest predictor of the community composition. Other factors shown to drive methanogen community structure included temperature, *Sphagnum* cover, water table depth and pH; although unlike nickel, the effect of these environmental factors was not statistically significant (Table S2, Supporting Information). There is often a strong link between plant types and pH in peatlands where more neutral sites tend to have less *Sphagnum* cover and more *Carex* species present, while acidic sites have greater *Sphagnum* coverage (Verhoeven, Maltby and Schmitz 1990; Strack, Waller and Waddington 2006; Strack *et al*. 2017). These *Sphagnum*-covered bogs contain microbes that are able to thrive in low nutrient environments (Galand *et al*. 2005;

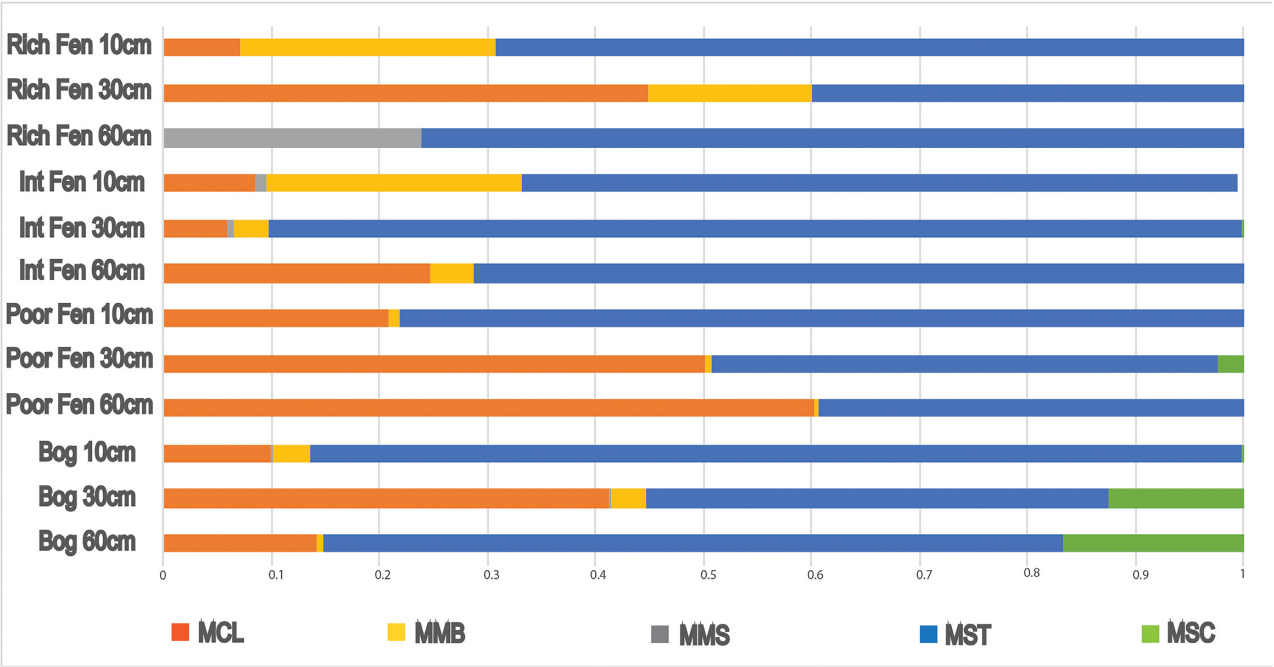

Figure 3. Average methanogen community composition (based on the *mcrA* gene) of peatland sites organized by peatland classification and depth. Methanogenic groups are abbreviated as follows: MST, *Methanosaeta*; MSC, *Methanosarcina*; MCL, Methanocellales; MMB, Methanomicrobiales and MMS, Methanomassiliicoccales.

Keller and Bridgham 2007). Low soil pH also inhibits complete degradation, which is favorable for hydrogenotrophic methanogenesis (Conrad 2020). Aceticlastic methanogens tend to thrive in minerotrophic peatlands that have *Carex* sedges and are nutrient rich (Kelly, Dise and Martens 1992), and also tend to increase with increasing rates of decomposition along permafrost thaw gradients (Hodgkins *et al.* 2014; McCalley *et al.* 2014). The temperature effect on peatland diversity could be due to the geographical location itself. Warmer peatlands tend to have faster rates of decomposition and peatland microbiomes are impacted by both temperature and latitude as well as pH (Seward *et al.* 2020). The community composition of methanogens can be altered by the soil temperature (Kotsyurbenko *et al.* 2019) and/or regional climate factors. Methanogen diversity appears to increase with increasing temperature (Utsumi *et al.* 2003), however certain methanogenic groups such as *Methanobacteria*, *Methanosarcina* and Methanomicrobiales are abundant in colder peatlands (Kwon *et al.* 2017).

Nickel was the strongest predictor of the methanogen community structure, whereas it had no relationship with overall microbial community (primarily bacteria, identified using 16S primers) of the same peatland samples (available at https://link.springer.com/article/10.1007/s00248-020-01510-z/figures/6; Seward *et al.* 2020). Similarly, a study of plant diversity in 18 total sites in the Sudbury, Ontario area in Canada, including both impacted and control sites found that pH, not nickel or other metals, was the primary driver of plant communities (Barrett and Watmough 2015), mirroring the bacterial data. Further, studies of salt marsh sediments found that total microbial biomass was unaffected by nickel addition (Capone, Reese and Kiene 1983). Thus, the effect of nickel is likely specific to the methanogenic communities since nickel is required for nickel–iron hydrogenases and the methyl-coenzyme reductase cofactor F_{430} (DiMarco, Bobik and Wolfe 1990). Low bioavailability of nickel has also been shown to limit methanogenesis in bioreactors (Gonzalez-Gil, Kleerebezem and Lettinga 1999), and indeed potentially in peatlands (Basiliko and Yavitt 2001). However, very high concentrations of nickel have also been associated with detrimental effects on methanogens and methane productions (Chen, Cheng and Creamer 2008). Peatlands with low concentrations of nickel have communities dominated by Methanocellales and *Methanosarcina*, suggesting that these methanogenic groups may be inhibited by higher concentrations of nickel in soil (Figure S4, Supporting Information; Paulo *et al.* 2017; Wang *et al.* 2019). In contrast, the Methanomicrobiales appear to thrive with elevated concentrations of nickel, possibly due to the diversity of Methanomicrobiales, effective mechanisms for regulating or tolerating metal uptake, or other factors in the environment that covary with the high metal concentrations, such as *Sphagnum* loss in the three impacted sites, Long, Daisy and Whitson (Carson 2018).

Three of the peatland sites in this study (Long Lake, Daisy Lake and Whitson) have enriched copper and nickel concentrations in the peat from the Copper Cliff smelter in Sudbury, Ontario (Pennington and Watmough 2015), which was found to negatively impact the *Sphagnum* coverage of these peatlands and may play a role in shaping methanogenic communities. For example vegetation has been shown to influence methanogenic community composition in at least one other study (Galand, Fritze and Yrjälä 2003). Similarly, another study found that peatlands impacted by the Copper Cliff smelter had both reduced methanogen abundances and shifted methanogen order prevalence compared to control peatlands (Carson 2018). However, the change in community composition revealed here is not solely due to these three impacted sites since nickel was still a strong predictor of methanogenic community composition when these impacted sites were removed from analysis (Figure S5, Supporting Information). A total of two of the sites (Sugar and Big Run) had average nickel concentrations close to 50 mg/kg (Table S1, Supporting Information), a concentration shown to stimulate methanogens in rice paddy soil (Wang *et al.* 2019). Whether this concentration is stimulatory *in situ*, or simply shapes the

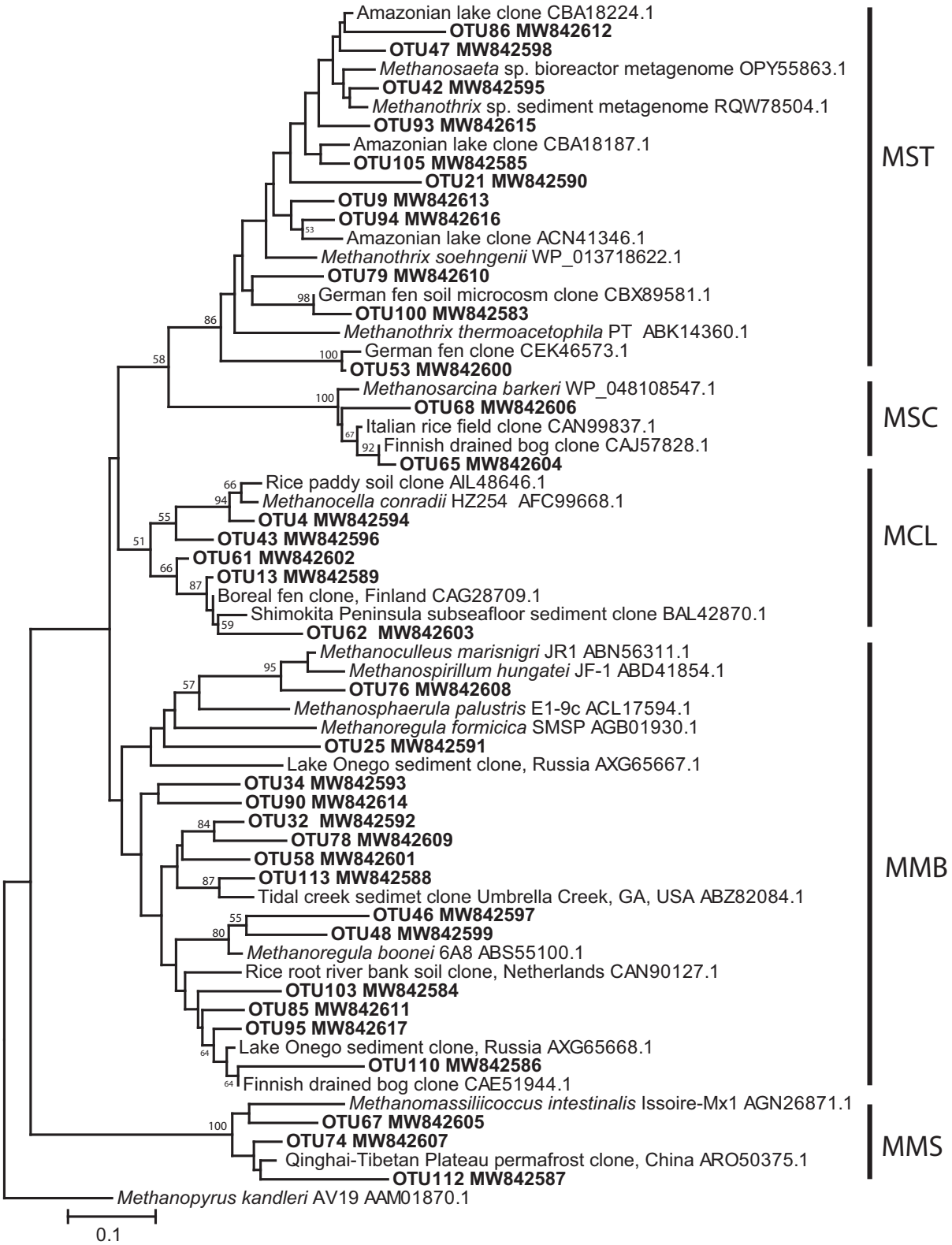

Figure 4. Neighbor-joining tree inferring the phylogenetic relationship between the translated *mcrA* gene sequences (McrA protein sequences) retrieved from peatlands sampled in this study to select sequences retrieved from publicly available databases. Bootstrap values ≥ 50 are shown for nodes that were also supported by maximum likelihood. Scale bar indicates fractional differences in nucleotide sequences. Methanogenic groups are abbreviated as follows: MST, *Methanosaeta*; MSC, *Methanosarcina*; MCL, Methanocellales; MMB, Methanomicrobiales and MMS, Methanomassiliicoccales.

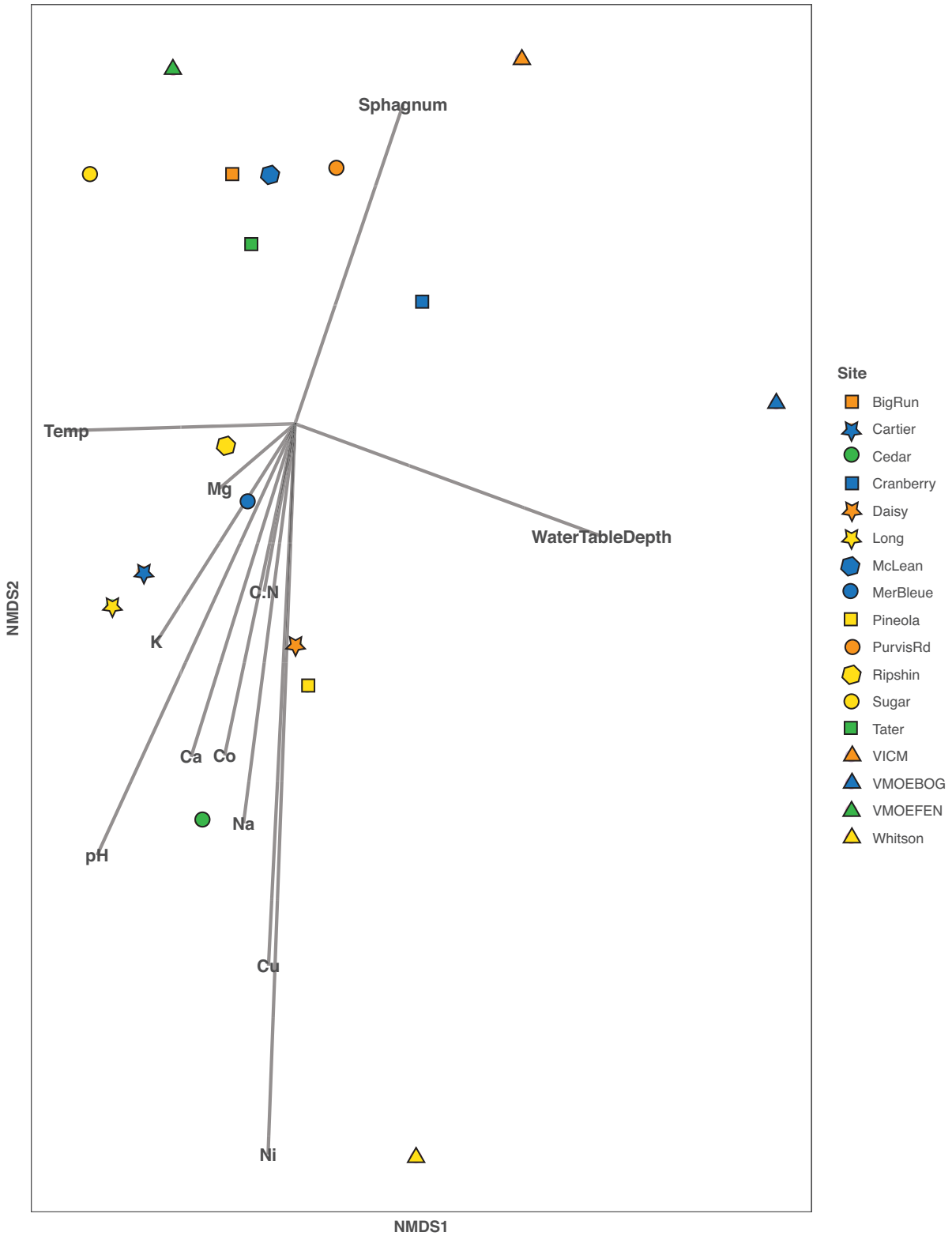

Figure 5. Non-metric multidimensional scaling (NMDS) biplot based on Jaccard distances for *mcrA* OTUs for methanogenic communities across the peatlands sampled in this study overlaid with vectors for environmental factors.

community composition is unclear. Another study has demonstrated that that certain methanogens can tolerate nickel concentrations, up to 200–1200 mg/L, and that they may be able to adapt to even higher concentrations (Sanchez *et al.* 1996).

All six of the peatlands with elevated nickel, including the three impacted sites (Long Lake, Daisy Lake and Whitson) and three of the sites in the southern Appalachian mountains (Pineola, Ripshin and Tater Hill) also had elevated copper above levels reported to be inhibitory to aceticlastic methanogens in anaerobic digesters, >20.7 mg/L (Karri, Sierra-Alvarez and Field 2006) and an additional five peatlands (Mer Bleue, Sugar, Cedar, Big Run and Cartier) had average copper levels exceeding that reported to inhibit hydrogenotrophic methanogens (8.9–10 mg/L; Karri, Sierra-Alvarez and Field 2006). The source of high nickel and copper concentrations in sites not impacted by smelter activity is unclear. However, *Sphagnum* species are can effectively relocate nutrients such as N and P from the older, dying portions of the plant to the younger and growing portions in the capitulum and have been shown to concentrate copper (Shotyk 1988). Further, the long polymers of uronic acids associated with *Sphagnum* have a high cation exchange capacity and can absorb and retain metals that are deposited by precipitation or other water sources (Rydin and Clymo 1989; Ringqvist and Öborn 2002).

Although copper is needed in trace amounts (Scherer, Lippert and Wolff 1983; Glass and Orphan 2012), it is most commonly reported as an inhibitor to methanogens (Sanchez *et al.* 1996; Gonzalez-Estrella *et al.* 2015); thus the effect of elevated copper is most likely adverse. It is also possible that the effect of copper is the result of cooccurrence with nickel, since these metals are strongly correlated in both 'impacted' and 'unimpacted' sites with $R^2 = 0.754$ for ($n = 24$) and $R^2 = 0.568$ for ($n = 108$) respectively (Figure S6, Supporting Information). Unlike nickel enzymes (such as methyl coenzyme M reductase in the final step of methanogenesis), copper enzymes have not been found in and/or are rare in anaerobic archaea as they function at a higher redox potential. Additionally, the variation in nickel concentration is much greater, spanning four orders of magnitude [Ni] (from 0.17 to 1930 mg/kg), similar to the magnitude of pH/[H+], which also has a large impact on microbial community composition. However, nickel concentration appears to be independent of pH in unimpacted sites (Figure S7, Supporting Information), and thus is not a confounding factor. Interestingly, impacted samples show a significant negative relationship between [Ni] and pH. This may be because atmospheric Ni deposition was accompanied by acid deposition (Hutchinson and Whitby 1977); however the range of pH within the impacted samples is well within the range of the non-impacted samples. Therefore, the combined evidence from our study suggests that nickel is likely the significant player in shaping methanogenic population structure in these peatlands.

Results of this study suggest that other metals beyond nickel and copper such as chromium, molybdenum and tungsten, are strong predictors of methanogen community structure (Figure S8, Supporting Information). These trace metals that, compared with nickel and copper, have a weaker and largely orthogonal relationship to community structure, can interchangeably bind with iron to help transfer electrons from H_2 to other enzymes of methanogenesis (Daas *et al.* 1994), and are needed in relatively high concentrations (Glass and Orphan 2012). Chromium is known to have high to moderate importance to microbial metabolic function and can be toxic in high concentrations, although less toxic than copper (Daas *et al.* 1994). Additionally, molybdenum is found in nitrogenase enzymes in methanogenic archaea (Lobo and Zinder 1990; Lobo 1992).

The impact of peat-associated metals on methanogen diversity in these peatlands indicates how important trace metal concentrations in soils are to methanogens and methanogenesis, and corroborates studies demonstrating high metal requirements for cobalt, molybdenum and nickel by *Methanosarcina barkeri* in culture (Scherer and Sahm 1981), or requirements for continuous input of nickel and cobalt for conversion of methanol to methane by *Methanosarcina* spp. in bioreactors (Gonzalez-Gil, Kleerebezem and Lettinga 1999). Many studies have demonstrated that methanogens are often limited by low metal concentrations (Schönheit, Moll and Thauer 1979; Diekert, Weber and Thauer 1980; Whitman, Ankwanda and Wolfe 1982; Sowers and Ferry 1985; Fathepure 1987; Hausrath *et al.* 2007; Glass and Dupont 2017). Indeed, hydrogenases containing nickel and iron are important for some methylotrophic methanogens (Glass 2015), perhaps explaining the increase in Methanomassiliicoccales with higher nickel concentrations. While trace metal limitation may be important in shaping methanogenic assemblages in oligotrophic sites such as Victor Mine (1–3 nickel and 2–5 copper, mg/kg), Mer Bleue (3 nickel and 10 copper, mg/kg) and McLean Bog (7 nickel and 7 copper, mg/kg), metal toxicity (Glass 2015) may also play a role, especially for three of the sites near Sudbury, Ontario that have experienced historic deposition of metal pollution from a nearby smelter (Whitson, Long Lake and Daisy Lake).

CONCLUSIONS

Overall, these results demonstrate that environmental factors such as pH, *Sphagnum* cover, temperature and nickel concentrations are strong predictors for the methanogenic community assemblages of peatland soils along a latitudinal gradient in eastern North America. The phylogenetic results revealed the presence of four orders of methanogens known to be present in peatlands. The impact of pH on the community structure of the peatland sites can be seen when looking at the trend in peatland sites shifting from Methanocellales at lower pH values (below 5), toward greater relative abundance of Methanomicrobiales and Methanomascillicoccales at pH values of 5 or greater. Nickel concentration however was also revealed as a very significant factor driving community assemblages, with other metals such as copper, molybdenum, chromium and tungsten also likely having an effect. The impact of peat-associated metals on methanogen diversity in these peatlands indicates how important trace metal concentrations in soils are to methanogens and methanogenesis, and are likely shaping changes in methanogen community composition along the gradient.

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SUPPLEMENTARY DATA

Supplementary data are available at *FEMSLE* online.

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