Methanogenic Archaea In Peatlands

By: **Suzanna L. Brauer**, Nathan Basiliko, Henri M.P. Siljanen, & Stephen H. Zinder

Abstract

Methane emission feedbacks in wetlands are predicted to influence global climate under climate change and other anthropogenic stressors. Herein, we review the taxonomy and physiological ecology of the microorganisms responsible for methane production in peatlands. Common in peat soils are five of the eight described orders of methanogens spanning three phyla (Euryarchaeota, Halobacterota and Thermoplasmatota). The phylogenetic affiliation of sequences found in peat suggest that members of the thus-far-uncultivated group Candidatus Bathyarchaeota (representing a fourth phylum) may be involved in methane cycling, either anaerobic oxidation of methane and/or methanogenesis, as at least a few organisms within this group contain the essential gene, mcrA, according to metagenomic data. Methanogens in peatlands are notoriously challenging to enrich and isolate; thus, much remains unknown about their physiology and how methanogen communities will respond to environmental changes. Consistent patterns of changes in methanogen communities have been reported across studies in permafrost peatland thaw where the resulting degraded feature is thermokarst. However much remains to be understood regarding methanogen community feedbacks to altered hydrology and warming in other contexts, enhanced atmospheric pollution (N, S and metals) loading and direct anthropogenic disturbances to peatlands like drainage, horticultural peat extraction, forestry and agriculture, as well as post-disturbance reclamation.

Brauer SL, Basiliko N, Siljanen HMP, Zinder SH. Methanogenic archaea in peatlands. FEMS Microbiology Letters. 2020;367(20):1f. doi:10.1093/femsle/fnaa172. Publisher version of record available at: https://academic.oup.com/femsle/article/367/20/fnaa172/5928548

MINIREVIEW – Environmental Microbiology

Methanogenic archaea in peatlands

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One sentence summary: Peat-forming wetlands and peatland microorganisms that produce the greenhouse gas methane.

Editor: Rich Boden

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ABSTRACT

Methane emission feedbacks in wetlands are predicted to influence global climate under climate change and other anthropogenic stressors. Herein, we review the taxonomy and physiological ecology of the microorganisms responsible for methane production in peatlands. Common in peat soils are five of the eight described orders of methanogens spanning three phyla (Euryarchaeota, Halobacterota and Thermoplasmatota). The phylogenetic affiliation of sequences found in peat suggest that members of the thus-far-uncultivated group *Candidatus* Bathyarchaeota (representing a fourth phylum) may be involved in methane cycling, either anaerobic oxidation of methane and/or methanogenesis, as at least a few organisms within this group contain the essential gene, *mcrA*, according to metagenomic data. Methanogens in peatlands are notoriously challenging to enrich and isolate; thus, much remains unknown about their physiology and how methanogen communities will respond to environmental changes. Consistent patterns of changes in methanogen communities have been reported across studies in permafrost peatland thaw where the resulting degraded feature is thermokarst. However much remains to be understood regarding methanogen community feedbacks to altered hydrology and warming in other contexts, enhanced atmospheric pollution (N, S and metals) loading and direct anthropogenic disturbances to peatlands like drainage, horticultural peat extraction, forestry and agriculture, as well as post-disturbance reclamation.

Keywords: methane; fen; bog; climate; sedge; permafrost

INTRODUCTION

Peatlands (a.k.a. bogs and fens, mires and muskeg) are climate change feedback hotspots in the terrestrial biosphere. These wetland ecosystems cover less than 3% of global land area yet hold an estimated 40% of all terrestrial organic carbon (C) as soil organic matter called peat (Gorham 1991; Lehner and Döll 2004; Yu *et al.* 2010; Scharlemann *et al.* 2014). Peatlands are able to store tremendous amounts of carbon owing to their often low pH, saturated conditions, low redox potentials, recalcitrant and inhibitory organic compounds in soil and thus extremely low rates of microbial decomposition relative to organic matter inputs from primary production (Moore and Basiliko 2006). Despite overall net uptake of atmospheric C, these saturated, anoxic, organic-rich conditions are also conducive to methane (CH_4) production, the terminal step in anaerobic decomposition (Conrad 2009; Zinder 1993). Methane is a much more potent greenhouse gas than carbon dioxide (CO_2), and methane emissions feedbacks to climate and other environmental changes in peatlands are challenging to predict. Although methanogens represent prototypical members of the domain Archaea (Woese, Kandler and Wheelis 1990), methanogens in peatlands are notoriously challenging to enrich for and isolate; thus, much remains unknown about their physiology and how methanogen communities will respond to environmental changes. Here we review and synthesize the taxonomy and physiological ecology of methanogens in peatlands, including highlighting relatively recent discoveries of putative methanogens in the phylum Crenarchaeota. We briefly cover what is known about methanogen community responses to critical contemporary environmental changes (e.g. permafrost thaw) and end by recommending strategies that may contribute to improving our understanding of the known-unknown (i.e. detected, but not isolated) methanogens in peatlands.

Methanogen taxonomy and physiological ecology

Anaerobic methanogenesis is carried out exclusively by members of the archaeal domain. Thus far, methanogens include eight orders, each of which contains at least one cultured representative: the Methanococcales (marine and not found in peat), Methanopyrales (hyperthermophiles not found in peat), Methanobacteriales, Methanomicrobiales, Methanocellales, Methanonatronarchaeales (haloalkaliphiles not found in peat), Methanosarcinales and the Methanomassiliicoccales (Lyu *et al.* 2018); note that the GTPD terminology is used throughout. Additionally, metagenomic data have facilitated identification of several other groups of putative methanogens including one in the Euryarchaeota, *Candidatus* Methanofastidiosales, also known as WSA2 (Nobu *et al.* 2016); one in the Halobacterota, *Candidatus* Methanoflorentaceae, also known as RC-II (Mondav *et al.* 2014), as well as two in the Crenarchaeota: *Candidatus* Methanomethylicia, also known as Verstraetearchaeota (Vanwonterghem *et al.* 2016), and candidate phylum Bathyarchaeota also known as MCG (Zhou *et al.* 2018a; Evans *et al.* 2015; Baker *et al.* 2020). A total of five of these orders and two of the candidate taxa are common in peat: Methanomicrobiales, Methanocellales and Methanosarcinales in the phylum Halobacterota; Methanobacteriales in the phylum Euryarchaeota; Methanomassiliicoccales (RC-III) in the phylum Thermoplasmatota, as well as candidate family Methanoflorentaceae (in the Methanomicrobiales) and candidate phylum Bathyarchaeota (Table 1 and Figs 1–3). Isolates for a number of the orders are relatively recent; with the first Methanocellales isolate (formerly recognized as RCI) described in 2008 (Sakai *et al.* 2008) and Methanomassiliicoccales in 2012 (Dridi *et al.* 2012). Similarly, the first assembled metagenome of a member of the candidate family Methanoflorentaceae was reported in 2014 (Mondav *et al.* 2014) and putative methanogenic metabolism in the candidate phylum Bathyarchaeota first described in 2015 (Evans *et al.* 2015). Although the Methanocellales (RCI group) had been recognized via community fingerprinting as key putative peatland methanogens prior to the first isolation, the other recently-described groups generally were not. It is also important to note that the type strain isolate(s) for each order were not always enriched from peat soils, for example, the prototypical *Methanomassiliicoccus sp.* isolate was from human feces (Dridi *et al.* 2012).

Despite the expansive phylogenetic diversity of methanogens spanning three phyla (Euryarchaeota, Halobacterota and Thermoplasmatota) and potentially a fourth phylum (Crenarchaeota), their metabolic pathways are extremely limited and include only: (1) CO_2-reducing methanogens, using H_2 (hydrogenotrophs), formate or even ethanol or isopropanol as electron donors; (2) aceticlastic methanogenesis (acetate splitting) and (3) methylotrophic methanogenesis, in which methyl compounds like methanol or methylamines serve as methane precursors that are either fermented to CH_4 or CO_2, or are directly reduced to CH_4 by H_2. Most methanogens, including most of those in the orders Methanomicrobiales, Methanocellales, Methanobacteriales, Methanococcales, Methanopyrales and Methanosarcinales can carry out hydrogenotrophic methanogenesis and are able to reduce CO_2 using H_2 and/or formate as an electron donor (Zinder 1993; Liu and Whitman 2008). Aceticlastic methanogenesis is carried out by two genera in the order Methanosarcinales *Methanosarcina* and *Methanosaeta*/*Methanothrix* (there is controversy about the name of this genus). Acetate utilization in the genus *Methanosarcina* has likely recently (475–250 Ma) evolved due to horizontal gene transfer of *ackA* and *pta* from an organism within the class Clostridia (Fournier and Gogarten 2008; Rothman *et al.* 2014). Methanosarcinales can also carry out methylotrophic methanogenesis and are the most metabolically versatile order of methanogens. Similar to at least one member of the Methanobacteriales, *Methanosphaera stadtmanae*, a gastrointestinal isolate that can only grow using methanol and H_2 (Fricke *et al.* 2006), the cultured members of the remaining two orders, Methanonatronarchaeales and Methanomassiliicoccales appear to be obligate methylotrophs that reduce methanol or trimethylamine, using either H_2 or formate as the electron donor (Kröninger, Gottschling and Deppenmeier 2017; Sorokin *et al.* 2018).

Acetate is the dominant CH_4 precursor in most freshwater anaerobic soils, although this is not always the case in acidic peatlands and/or permafrost- affected peatlands. Aceticlastic methanogenesis often accounts for 2/3 or more of total methanogenesis in some peatlands (Schulz and Conrad 1996; Conrad, Klose and Claus 2002; Metje and Frenzel 2007). Correspondingly, aceticlastic methanogens can predominate among methanogenic populations in peat (Kotsyurbenko *et al.* 2004; Zhang *et al.* 2008b). However, in acidic peatlands where the decomposition of organic matter is incomplete and/or turnover rates are much lower compared to other freshwater systems (Conrad 2020), hydrogenotrophic methanogenesis can play a much more important role (Lansdown, Quay and King 1992; Popp *et al.* 1999; Chasar *et al.* 2000; Metje and Frenzel 2005). This trend has been observed in collapsing peat underlain by thawing permafrost described below (McCalley *et al.* 2014). Methylotrophic methanogenesis is generally considered to represent only a small proportion of total methanogenesis in most freshwater systems, including peatlands (Conrad 2020).

Although methanogenic activity is readily detected in acidic peat soils that have pH values as low as 3.5 (Goodwin and Zeikus 1987; Bergman, Svensson and Nilsson 1998; Høj, Olsen and Torsvik 2008; van Winden *et al.* 2012), very few novel genera or species of methanogenic archaea have been isolated from peat. These include two novel genera, *Methanoregula boonei* (Bräuer *et al.* 2006, 2011) and *Methanospherula palustris* (Cadillo-Quiroz *et al.* 2008; Cadillo-Quiroz, Yavitt and Zinder 2009), within what has been called the R10 cluster (Hales *et al.* 1996) or fen cluster (Juottonen, Galand and Yrjälä 2006) of the Methanomicrobiales, several species of *Methanobacterium* (König 1984; Krivushin *et al.* 2010; Shcherbakova *et al.* 2011; Cadillo-Quiroz *et al.* 2014) and one species each of *Methanolobus* (Zhang *et al.* 2008a) and *Methanoculleus* (Tian, Wang and Dong 2010). Note that

Table 1. Archaeal orders containing methanogens or potential methanogens found in peat.

Order	Phylum	Representative genera	Metabolism[a]
Methanosarcinales	Halobacterota	*Methanosarcina*, *Methanosaeta/thrix*	H, M, RM, A
Methancellales (Rice cluster I)	Halobacterota	*Methanocella*	H
Methanomicrobiales	Halobacterota	*Methanoregula* *Methanosphaerula* 'Methanoflorens' (RCII)	H
Methanomassilii-coccales (Rice cluster III)	Thermoplasmatota	*Methanomassiliicoccus*	RM
Methanobacteriales	Euryarchaeota	*Methanobacterium*	H (RM in *Methanosphaera*)
'Bathyarchaeales' (Miscellaneous Crenarchaeotal Group; Rice cluster IV)	Bathyarchaeota	'Bathyarchaeota' clones	M?, AMO?

[a] Metabolisms: H, hydrogenotrophic; M, methylotrophic (disproportionating); RM, reductive methylotrophic; AMO, anaerobic methane oxidation.

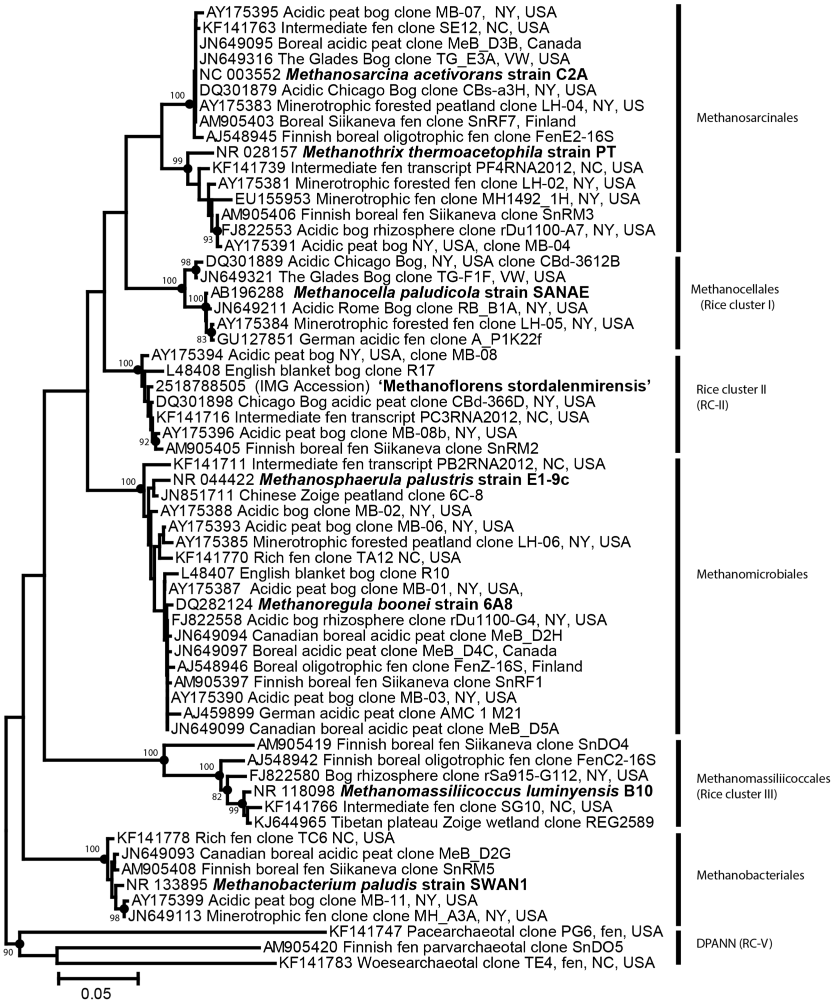

Figure 1. Neighbor-joining tree inferring the phylogenetic relationship between the SSU rRNA sequences retrieved from known cultured methanogens and the related sequences from peat. Bootstrap values ≥ 65 shown for nodes that were also supported by maximum likelihood. Supported nodes are marked with filled circles. Scale bar indicates fractional differences in nucleotide sequences.

no novel species of aceticlastic methanogens have been isolated from acidic peat. In past studies, researchers have made additions of acetate to peat microcosms that were either inhibitory or non-stimulatory (Williams and Crawford 1984; Goodwin and Zeikus 1987; Bridgham and Richardson 1992; Watson and Nedwell 1998; Blodau, Roehm and Moore 2002). Because of the toxicity of even low concentrations (3–5 mM) of acetate at low pH, some strategies for enriching aceticlastic methanogens in low pH environments have included co-culturing with syntrophic partners (Schmidt *et al.* 2016) or successive additions of low concentrations (≤ 1 mM) of acetate (Bräuer, Yavitt and Zinder 2004). However, eventual isolation of these strains have thus far proven unsuccessful (Horn *et al.* 2003; Sizova *et al.* 2003; Bräuer, Yavitt and Zinder 2004).

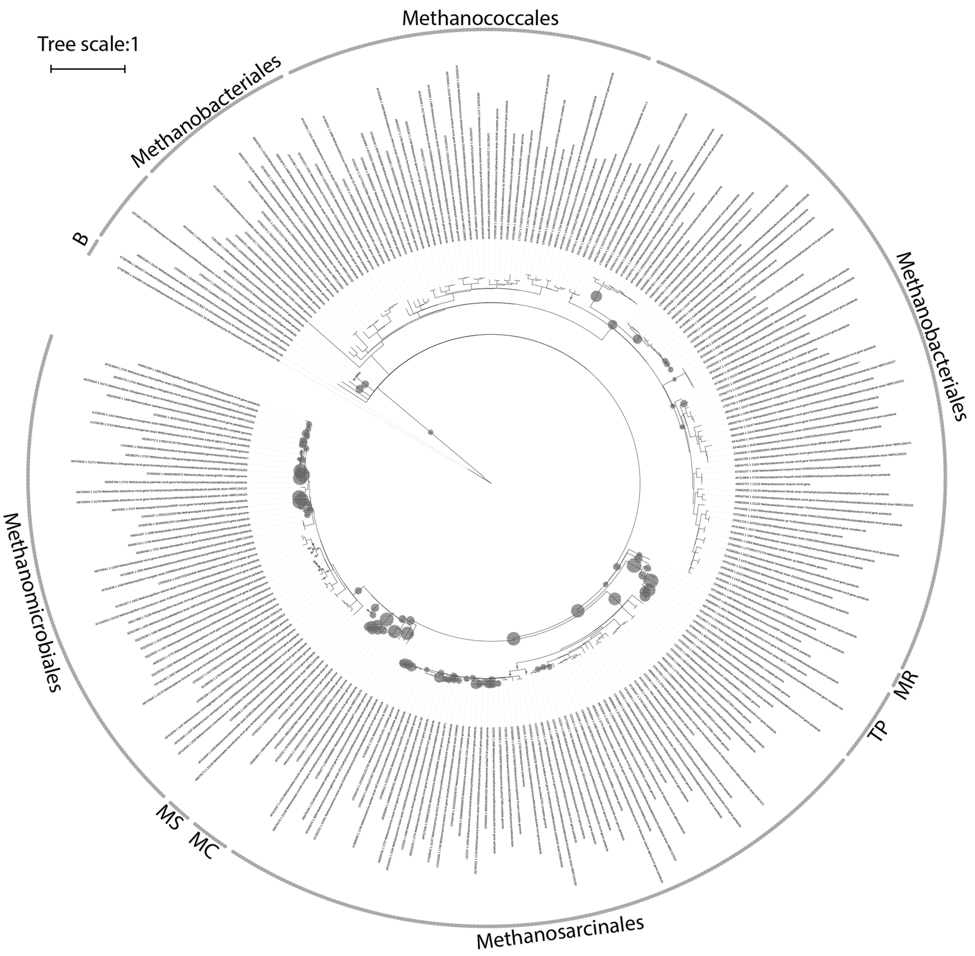

Figure 2. Phylogenetic relationship between short metagenomic methyl-coenzyme M reductase alpha subunit (mcrA) sequences found in bogs and known methanogen and Bathyarchaeotal mcrA sequences inferred using the iTOL-tree (Letunic and Bork 2019) with RaxML (Stamatakis 2014). Metagenomic mcrA genes were obtained by searching with an mcrA gene hmmer-profile (Wheeler and Eddy 2013) against an NCBI-SRA database of all bog ecosystems. The databases of peatland related ecosystems were collected with the SRAdb (Zhu *et al.* 2013) package in R (R Core Team 2019). In total, 203 bog SRA files were screened for mcrA genes.

Methanogens living in ombrotrophic bogs must adapt to high concentrations of protons (low pH) and to extremely low concentrations of ions like sodium and potassium; thus, methanogen diversity is generally reduced in bogs and increases with increasing pH and nutrient contents along a bog to poor fen, to intermediate and rich fen gradient. Bräuer *et al.* (2015) examined the genome sequence of *M. boonei*, and described some aspects of its genome that were congruent with adaptation to these conditions. The deduced amino acid sequence of the ion-pumping AtpCK subunit of the A_1A_o ATPase/synthase in *M. boonei* belonged to an AtpCK group able to pump either protons or Na+, whereas the other AtpCK group can only pump Na+. The membrane-bound Mtr methyltransferase complex plays a key role in methanogen energy conservation, and the MtrE subunit has been found to pump Na^+ in methanogens. The MtrE in *M. boonei* lacks the amino acid motif considered essential to pumping Na^+, and may be a proton pump. Finally, most methanoarchaea only have genes encoding the low- and medium-affinity K^+ transporters, Trk and Kup, respectively. *M. boonei* is one of the few methanoarchaea possessing genes encoding the high-affinity ATP-driven Kdp transporter. Other methanoarchaea with predicted Kdp transporter genes include *Methanobacterium* strains SWAN-1 and AL-21, both isolated from acidic bogs, and *Methanosphaerula palustris* isolated from a fen where the porewater K+ concentration was only 3–8 μM. Interestingly, these genes are all related to a family from *Geobacter*, suggesting horizontal gene transfer that allowed these methanoarchaea to adapt to hypokalemic environments. Other genome studies of Methanomicobiales (Browne *et al.* 2017) have similarly revealed that unique transporters for additional scarce nutrients (besides K+) such as Co, Ni, Mg, Fe, No_3^-, HCO_3^- and sulfonate were present in the peat-dwelling strains *M. palustris* and *M. boonei* but not strains from other habitats such as sewage sludge, marine sediments, oilfields, saline swamp mud or a tar pit. Comparison genomes included those for *Methanoregula formicica, Methanolinea tarda, Mehtanoculleus marisnigri, Methanolacinia petrlearia, Methaplanus limicola, Methanospirillum hungatei* and *Methanocorpusculum labreanum*.

One, thus-far-uncultivated, elusive phylum is the Bathyarchaeota. Sequences clustering in this phylum have been detected in a wide variety of peatlands throughout the globe including those in Finland (Galand *et al.* 2002; Putkinen *et al.* 2009), Norway (Høj, Olsen and Torsvik 2008), China (Tian *et al.* 2012; Gu *et al.* 2013; Cao *et al.* 2014; Wei *et al.* 2014; Xiang *et al.* 2017), Slovenia (Stopnišek *et al.* 2010), Japan (Akiyama *et al.* 2011; Narihiro *et al.* 2011), Germany (Hunger *et al.* 2011; Steger *et al.*

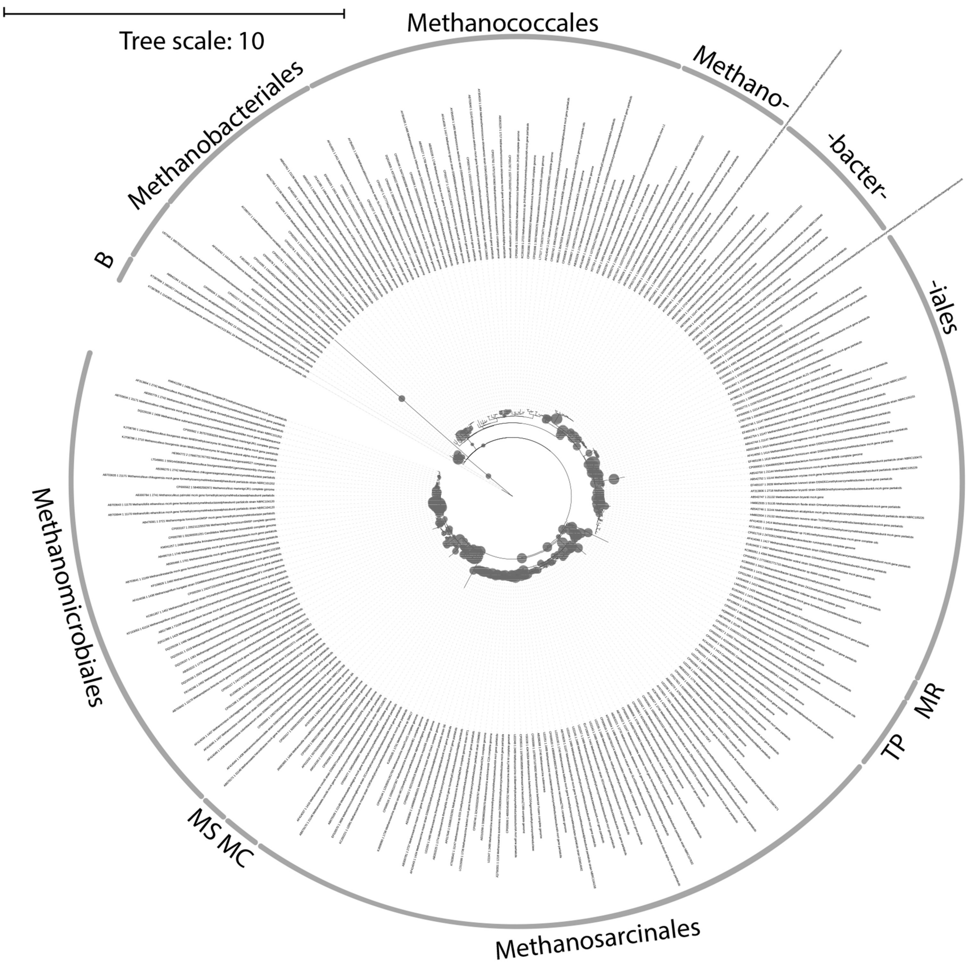

Figure 3. Phylogenetic relationship between short metagenomic methyl-coenzyme M reductase alpha subunit (mcrA) sequences found in fens and known methanogen and Barhyarchaeotal mcrA sequences inferred using the iTOL-tree (Letunic and Bork 2019) with RaxML (Stamatakis 2014). Metagenomic mcrA genes were obtained by searching with an mcrA gene hmmer-profile (Wheeler and Eddy 2013) against an NCBI-SRA database of all fen ecosystems. The databases of peatland related ecosystems were collected with the SRAdb (Zhu *et al.* 2013) package in R (R Core Team 2019). In total, 272 fen SRA files were screened for mcrA genes.

2011), Denmark (Görres, Conrad and Petersen 2013), Brazil (Etto *et al.* 2012), as well as several US peatlands including those in Alaska (Rooney-Varga *et al.* 2007), Minnesota (Lin *et al.* 2012), North Carolina, (Hawkins, Johnson and Bräuer 2014), New York (Cadillo-Quiroz et al. 2006, 2008, 2010) and West Virginia (Yavitt *et al.* 2012). Members of this class have been previously known as RC-IV (Großkopf, Stubner and Liesack 1998), group 1.3 crenarchaeota (Høj, Olsen and Torsvik 2008), the deep peat group (Putkinen *et al.* 2009) or more commonly, the MCG or miscellaneous crenarchaeotal group (Kubo *et al.* 2012). Sequences from this group can represent a large portion (≥ 50%) of the archaeal population in some peatlands, for example in China (Xiang *et al.* 2017), Brazil (Etto *et al.* 2012) the southeastern continental US (Hawkins, Johnson and Bräuer 2014) and Alaska (Rooney-Varga *et al.* 2007). Yet in other studies, the bathyarchaeotal sequences are considerably rarer. This large variation in the proportions and abundance of Bathyarchaota detected may depend on environmental conditions (Xiang *et al.* 2017; Yu *et al.* 2017; Zhou *et al.* 2018a, b; Pan *et al.* 2019), primers used (Cadillo-Quiroz *et al.* 2010), or other unknown factors (Biddle *et al.* 2006).

In addition to being implicated in methylotrophic methanogenesis (Evans *et al.* 2015), members of the Bathyarchaeota have been proposed to carry out the anaerobic oxidation of methane (Harris *et al.* 2018), acetogenesis (He *et al.* 2016), photosynthesis (Meng *et al.* 2009), or to utilize carbohydrates, aromatics, proteins, acetate or other organics (Biddle *et al.* 2006; Lloyd *et al.* 2013; Meng *et al.* 2014; Na *et al.* 2015; Lazar *et al.* 2016). However, in the absence of isolated representatives, it is difficult to infer the physiology of Bathyarchaeota in enrichment cultures, or by genomic data alone. For example, in an earlier case, before a representative of the Methanomassiliicoccales (RCIII group) was isolated, it was proposed that members were broader-spectrum soil heterotrophs rather than methanogenic based on the substrates that favored enrichment (Kemnitz, Kolb and Conrad 2005). In hindsight, responses to organic substrate amendments were likely due to indirect effects including stimulation of syntrophic bacteria. Additionally, members of the Bathyarchaeota are not only phylogenetically diverse, but also show a high level of genomic diversity based on metagenomics analyses; thus, there may be high metabolic diversity among species (Meng *et al.* 2014) or among subgroups (Xiang *et al.* 2017; Zhou *et al.* 2018b). About half of the rRNA gene sequences collected in this review (using the NCBI Nucleotide Blast tool) appear to share the highest identity (95–97% ID) with both fosmid clone 37F10 (shown in Figure S1, Supporting Information) and metagenome BE326-BA-RLH, an organism predicted to be involved in anaerobic methane oxidation (Harris *et al.* 2018). These sequences (in the top 2 clusters in Figure S1, Supporting Information) share

91–94% ID with sequences affiliated with MCG cluster 6 in the literature (Kubo *et al.* 2012). The other half (shown in the bottom half of the tree in Figure S1 (Supporting Information), approximately 4 clusters) appear to be most closely (85–90% ID) affiliated with sequences in MCG cluster 8, including *Candidatus* Bathyarchaeota archaeon BA2, an organism predicted to carry out methylotrophic methane production (Evans *et al.* 2015; Berghuis *et al.* 2019; Evans *et al.* 2019). Thus, there is a potential for these Bathyarchaeota may be involved with methane cycling in global peatland ecosystems.

To assess the relative abundance of various methanogenic groups in bogs and fens using a method independent of PCR bias, an extensive search was carried out using a hmmer-profile (Wheeler and Eddy 2013) of the methyl-coenzyme M reductase alpha subunit (mcrA) gene to query the NCBI-SRA database of all peatland ecosystems, either categorized as fens or bogs. Short metagenomic mcrA sequences were aligned to the mcrA reference database with the mafft-aligner-algorithm (Katoh *et al.* 2019). These fragments where carefully selected, evaluated as mcrA with nblast (nt-database) searches and after the global alignment (as nucleic acid and amino acid sequences) with mafft these fragments were closely related to the reference mcrA sequences after analysis with the evolutionary model finder, iQ-tree and UF-boot (Trifinopoulos *et al.* 2016, Kalyaanamoorthy *et al.* 2017, Nguyen *et al.* 2015, Hoang *et al.* 2018). Finally, the sequences were phylogenetically placed in the iTOL-tree (Letunic and Bork 2019) with RaxML (Stamatakis 2014). Results indicate that bogs had a few clusters of Methanomicrobiales, Methanocellales and Methanomassilicoccales, as well as a limited number of Methanobacteriales related mcrA gene sequences (Fig. 2). Fen datasets revealed a dramatic increase in diversity across those four main orders, and notably a significant increase in Methanobacteriales-type mcrA gene sequences (Fig. 3). Additionally, a small number of mcrA sequences related to Methanothermococcus and Methanocaldococcus were found. Finally, results of the RaxML analyses showed Bathyarchaeaota distantly related mcrA sequences. These distantly related sequences were also analyzed after mafft-alignment with iQ-tree, which also tests the best evolutionary model for the data. Results indicate that these groups may be performing methanogenesis, anaerobic methane oxidation or another function especially in fens (Figure S2A, Supporting Information). If additional Bathyarchaeotal mcrA gene sequences are present, they may be too divergent (compared to the two sequences currently known) to be detected; thus, targeted/capture metagenomics should be used to detect Bathyarchaota in future studies (Kushwaha *et al.* 2015; Manoharan *et al.* 2015).

Methanogen community responses to environmental change

Methane feedbacks to environmental changes in wetlands represent an important knowledge gap in the role those wetlands will play in the future global climate system, particularly through 2100 (Dean *et al.* 2018). Because methanogenesis is a strictly anaerobic process with most known methanogens lacking metabolic strategies for protection from reactive oxygen species, CH_4 emissions from peatlands are governed by soil moisture as an overarching control (Blodau 2002). Anaerobic oxidation of some CH_4 produced is common in peatlands (Gupta *et al.* 2013, and see Smemo and Yavitt 2011 for perspectives on AOM in peatlands), yet rates are generally slow, and therefore the position of CH_4 production in peat soil profiles (i.e. in fresher surface organic substrate v recalcitrant deeper substrates) and oxidation by aerobic CH_4-oxidizng bacteria above the water table control net emissions (Blodau 2002). Indeed, average summer water-table position is a strong universal predictor of relative rates of emissions within sites, however emissions can vary by more than two orders of between even botanically-similar sites at the same water-table position (e.g. see Fig. 1B in Moore *et al.* 2011). Vegetation controls on CH_4 emissions are multi-faceted, with both stimulation and suppression of methanogenesis beneath the water table from substrate or O_2 supply respectively, and stimulation and suppression of aerobic oxidation through the supply of O_2 or by aerenchyma serving as chimney allowing CH_4 to evade CH_4-oxidizing bacteria above the water table, respectively (Lai 2009).

Responses of peatland methanogen communities to environmental changes are influenced by site characteristics. Peatland classification systems vary to some extent, but in general, bogs are regarded as being primarily rain-fed, while fens are hydrologically connected to groundwater (e.g. National Wetlands Working Group 1997). Although in part dependent on the local climate, fens are typically wetter with more stable water-table positions, have higher soil and pore water pH due to higher base cation concentrations transported in from surrounding mineral soils, and are more likely to be dominated by graminoid (e.g. sedge) vegetation. Bogs are acidic with pH values often ca. 4.0 due to atmospheric inputs of inorganic acids, internal acidity generation from vegetation and low buffering capacity from groundwater-supplied base cations, and are often dominated by *Sphagnum* (a genus) mosses, with evergreen and deciduous woody shrubs and stunted spruce (*Picea sp.)* and tamarack/larch (*Larix sp.)* trees. The chemistry and plant communities across fens can vary, with for example low pH and bog-like vegetation in sites situated in catchments with shallow, coarse-textured soils low in calcium and other base cation elements (i.e. where the groundwater is more like rainwater chemically), to well-buffered pH circumneutral systems with very high calcium concentrations (e.g. see Godin *et al.* 2012 for descriptions of poor fens that resemble bogs to calcareous rich fens). As peat soil profiles grow over time (i.e. because primary productivity by plants exceeds mineralization losses by microbes), conditions often become more bog like, with hydraulic conductivity slowing over time as peat becomes more decomposed and amorphic. This soil profile growth also leads to peatland surfaces and local water table becoming perched higher than the surrounding groundwater and cutting off the supply of base cation elements from nearby mineral soils (Table 2 footnote). Peatlands can display varying degrees of microtopography, often consisting of raised hummocks, low hollows and flat 'lawn' areas that are relatively drier and wetter and have vegetation that is adapted to microsite physicochemical characteristics; and typically bogs display more pronounced undulating hummock-hollow topography (varying every few m) than fens. Permafrost-affected peatlands are often characterized by having raised palsas (e.g. as in Yavitt *et al.* 2006) or larger more continuous peat plateaus that are elevated and isolated from surrounding mineral-influenced groundwater by the permafrost (i.e. perennially frozen soil water within the surface 1m).

Key anthropogenic disturbances facing peatlands include direct and indirect effects of climate change such as higher temperatures (and associated permafrost thaw in permafrost-affected sties), altered precipitation and hydrologic dynamics, as well as altered disturbance regimes such as fire, invasive species, atmospheric pollution (notably enhanced reactive N, S and

Table 2. Methanogen community characteristics in peatlands across natural gradients (A) and under key anthropogenic stressors (B).

A. Natural gradient studies	Location	Notes on study and study sites	Methanogen community profiling method	Key findings (influence/changes/controls on methanogen community structure)	Reference
Fen-bog[a] or other natural trophic gradients	USA	Bog v temperate marsh peatland	Archaeal 16S rRNA gene profiling	High phylogenetic diversity at both sites, including aceticlastic methanogens in the bog (pH 4.1)	Basiliko *et al.* (2003)
	Canada	Rich to poor fen gradient	Archaeal 16S rRNA gene profiling	Methanosarcinaceae were dominant in most sites; Interestingly Methanomicrobiales was absent and Methanosaetaceae abundant in a poor fen (pH 4.7), contrasting a rich fen (pH 6.3) without *Sphagnum* moss	Godin *et al.* (2012)
	Finland	Reciprocal soil transplant between bog and fen in microbe-excluding membrane	*mcrA* gene and transcript profiling	Fen peat placed in an acidic bog led to lower rates of CH_4 production, but acetate utilization (by members of Methanosaetaceae) persisted	Juottonen *et al.* (2020)
	Finland	Bog fen comparison	*mcrA* gene profiling	Aceticlastic Methanosaetaceae dominated meso- and oligotrophic fen communities (and community structures were similar, while members of CO_2-reducing Methanomicrobiales dominated a bog	Juottonen *et al.* (2005)
	China	Adjacent riverine fen (marsh) sites with different *Carex* species	^{13}C-DNA-probing of *mcrA* genes to ID active methanogens	Both sites (pH 5.0 and 5.5) supported CO_2 reducing methanogens; *Carex angustifolia* also supported active aceticlastic *Methanosarcina sp* (but interestingly not *Methanosaeta sp*), while the *Carex lasiocarpa* site did not support aceticlastic methanogenesis	Lin *et al.* (2015)
	USA	Sub-arctic and temperate sties with pH and vegetation differences	Archaeal 16S rRNA gene profiling	Methanomicrobiaceae and Methanobacteriaceae predominated colder sites; Methanosaetaceae only found in sites dominated by *Carex* sp. sedges and with little to no *Sphagnum* moss	Rooney-Varga *et al.* (2007)
Vegetation and/or topographic microforms within sites	Czech Republic	Three plant functional type microforms (*Sphagnum*, *Eriophorum* and *Vaccinium*) sampled each in three acidic (pH peatlands	Prokaryote 16S rRNA gene profiling	Few differences between plant species and sites (only one taxon of *Methanoregula* was more abundant under sedge); Methanomicrobia, Methanobacteria and Thermoplasmatales predominated while Methanosarcinaceae and Methanosaetaceae were detected, but were very rare	Chronakova *et al.* (2019)

Table 2. Continued

A. Natural gradient studies	Location	Notes on study and study sites	Methanogen community profiling method	Key findings (influence/changes/controls on methanogen community structure)	Reference
	Finland	Hummock- lawn microforms in an oligotrophic (pH > 4.4) fen	Archaeal 16S rRNA gene profiling	In surface soils, Methanomicrobiales (CO_2 reducers) dominated hummocks, while more versatile *Methanosarcina sp.* dominated lawns; no difference in deeper soils	Galand *et al.* (2003)
	Finland	Hummock-hollow-lawn microforms in four bogs	*mcrA* gene profiling	Between site differences in methanogen community structure were greater than between forms within sites; CO_2-reducing Methanoregulaceae and RCII/Methanoflorentaceae predominated in all sites; Methanosaetaceae were not detected	Juottonen *et al.* (2015)
	Sweden and Denmark	Hummock to lawn gradients in three bogs (also see below for between-site comparison)	*mcrA* gene and transcript profiling	CO_2-reducing Methanoregulaceae predominated in all bog ($pH < 4.0$) sites and microforms, followed by Methanosarcinaceae in two sites Methanocellales in one site; Methanosaetaceae were not detected	Marti *et al.* (2015)
Soil profile (depth) gradients	Canada	Depth profile in a *Sphagnum* (pH 3.8) bog	^{13}C signatures to predict methanogenic pathways	Acetate utilization predominated in surface soils, while CO_2 reduction predominated at lower depths	Hornibrook *et al.* (1997)

B. Anthropogenic stressors	location	Notes on study and study sites	Methanogen community profiling method	Key findings (influence/changes/controls on methanogen community structure)	Reference
Drought and warming	USA	Controlled microcosm experiment exploring warming (0–40°C) in a *Sphagnum* bog peat	Archaeal 16S rRNA gene profiling	Distinct psychrophilic and mesophilic methanogen communities and associated bacterial syntrophs peak at 4 and 20°C; both methanogen communities are predominantly Methanobacteriaceae taxa, however putative fermentative bacteria shift with *Clostridia sp.* more important at warmer temperatures	Kolton *et al.* (2019)

Table 2. Continued

B. Anthropogenic stressors	location	Notes on study and study sites	Methanogen community profiling method	Key findings (influence/changes/controls on methanogen community structure)	Reference
	Russia	pH (known to decrease with drought) and temperature were controlled in a microcosm study with bog peat (4, 15 and 25°C and pH 3.8, 4.8 and 6)	tracers to partition CO_2 reduction vs. aceticlastic activity; Archaeal 16S rRNA gene profiling	At low temperature and pH, only CO_2 reduction occurs driven by Methanobacteriaceae taxa; while at high temperatures, regardless of pH, aceticlastic activity predominated (60–70%)	(Kotsyurbenko *et al.* 2007)
	Japan	Aridified and sasa-invaded former *Sphagnum* bog (pH 4.2) peatland	*mcrA* and archaeal 16S rRNA gene profiling	Methanomicrobiales taxa predominated in *Sphagnum* sites (90%), but invasion with the broad-leaf bamboo after drought led to no detectable *mcrA* sequences and appearance of other distinct Euryarchaeota taxa	Narihiro *et al.* (2011)
	Finland	Two fen sites at different latitudes with experimental water-table lowering and passive soil warming	*mcrA* gene and transcript profiling	Warming generally did not enhance drying effects (that were larger); aceticlastic Methanosaetaceae taxa and CO_2-reducing Methanobacteriaceae were prominent in the drying regime in both sites (one more oligotrophic); interestingly this corresponded to reduced sedge and increased shrub biomass	Peltoniemi *et al.* (2016)

Table 2. Continued

B. Anthropogenic stressors	location	Notes on study and study sites	Methanogen community profiling method	Key findings (influence/changes/controls on methanogen community structure)	Reference
	China	Mesocosm experiment with temperature and water table	Archaeal 16S rRNA gene profiling	Warming effects were negligible in the 1–2 year experiment; CO_2 reducing Methanobacteriales and Methanomicrobiales taxa were more important in the drier experimental regimes, while RCII (CO_2-reducing, can. 'Methanoflorentaceae') taxa predominated under wetter conditions	Tian *et al.* (2015)
	Finland	Water-table drawdown experiment across a sedge fen	*mcrA* gene profiling	Methanocellales taxa dominated the reference wet areas; the community shifted to the 'Fen cluster' (Methanomicrobiales) with drying	Yrjala *et al.* (2011)
Permafrost thaw and landform changes	Sweden	Palsa degradation to thermokarst gradient	^{13}C signatures to predict methanogenic pathways; Archaeal 16S rRNA gene profiling	Enhanced C lability following thermokarst formation associated with sedges led to enhanced aceticlastic methanogenesis; a single CO_2 reducing taxon (formerly RCII, here isolated as can. '*Methanoflorens stordalenmirensis*') also became dominant and is an important predictor of disturbance and CH_4 cycling feedbacks to thaw	Hodgkins *et al.* (2014); McCalley *et al.* (2014); Mondav *et al.* (2014)
	Canada	Compared palsa, recently collapsed palsa, and surrounding bog that had not been permafrost-affected for longer	Archaeal 16S rRNA gene profiling	Methanogen DNA was not detected in permafrost sites; recently degraded permafrost soils were characterized by Methanosarcinaceae (authors suggested were aceticlastic) and Methanobacteriales (CO_2-reducing) taxa, while the bog was characterized by Methanobacteriales and Methanocellales (RCI)	Yavitt *et al.* (2006)
	Norway	Compared degrading palsa to thermokarst to stabilized depression of a former collapsed palsa	*mcrA* gene profiling	Thermokarst formation led to a mixed CO_2-reducing (Methanobacterium and Methanocellales) and potentially aceticlastic (Methanosarcina) community. Longer-term bog succession had a similar-structured, but much less active community	Liebner *et al.* (2015)

Table 2. Continued

B. Anthropogenic stressors	location	Notes on study and study sites	Methanogen community profiling method	Key findings (influence/changes/controls on methanogen community structure)	Reference
Atmospheric N pollution deposition	Sweden and Denmark	Three bogs of similar pH (3.9) receiving 1.5, 7 and 25 kg N per ha per y also represented different climatic environments	*mcrA* gene and transcript profiling	Substantially higher methanogen numbers in the high N deposition (but also warmer) site; there were subtle community changes, but Methanoregulaceae predominated in all sites and microforms, followed by Methanosarcinaceae in two sites and Methanocellales in one site; Methanosaetaceae were not detected	Marti *et al.* (2015)
Drainage for forestry and wood ash fertilization	Finland	Compared ash-fertilized and non-fertilized within a drained peatland	*mcrA* gene profiling	A drained bog supported only CO_2-reducing methanogens; RCI (now Methanocellales) predominated in both sites; though ash addition selected for Fen Cluster taxa (Methanomicrobiales) that were not in the control site	Galand *et al.* (2005)
Horticultural peat extraction and reclamation	Canada	Eight sites representing pristine, mined and reclaimed acidic bogs	Archaeal 16S rRNA and *mcrA* gene profiling	Methanogens were not detectable in some actively mined (drained sites), but CO_2-reducing methanogens (RCII, now *Candidatus* Methanoflorentaceae) and Methanomicrobiaceae predominated, and in one trial the methanogen community of the restored site resembled the nearby pristine site	Basiliko *et al.* (2013)
Impacts of agriculture and reclamation	Finland and Germany	Rewetted bog sites with dung applied to simulate post-animal grazing reclamation contexts	*mcrA* gene profiling	Dung impacted sites facing rewetting (i.e. again becoming methanogenic) were dominated by enteric *Methanobrevibactor sp.* and had high rates of CH_4 production in stark contrast to reference sites	Hahn *et al.* (2018)

[a] Fens are connected to groundwater sources and although they range in nutrient concentration and pH (rich, intermediate and poor), are higher in base cations and alkalinity than bogs, which are isolated from groundwater sources. A prototypical peatland succession model proceeds from shallow aquatic ecosystem, to rich fen, to poor fen, to bog as the peat soil profile aggrades and hydraulic conductivity decreases- eventually isolating the majority of the system from all water inputs except precipitation. Bogs tend to have a continuous *Sphagnum* moss cover and few sedges, while mesotrophic (intermediate) and eutrophic (rich) fens tend to be dominated by graminoid vascular species (e.g. sedges in the genera *Carex* and *Eriophorum*).

metal loading), and direct drainage exploitation for mining, silviculture and agriculture (e.g. IUCN 2017; Grzybowski and Glińska-Lewczuk 2020). A growing body of literature has explored how these anthropogenic changes affect methane cycling rates, but there are still numerous gaps in understanding how changes influence methanogen communities. Many anthropogenic disturbances lead to shifts in broader ecosystem properties like plant functional types (e.g. with loss of keystone sphagnum mosses from bogs and acidic fens and the encroachment of vascular plants, or sedge and aquatic *Sphagnum* proliferation in thermokarst formation through permafrost degradation), and this means that studies exploring methanogen communities across natural gradients (e.g. fen to bog, different vegetation and hydrological micro-features within sites, etc.) can be useful to make predictions of the effects of anthropogenic disturbances.

A search of peer-reviewed literature on the Web of Science Core Collection database (Clarivate Analytics, Boston, MA) at the time of writing yielded 112 works associated with topic words 'methanogen' and 'peatland or fen or bog or mire'. Of these, ca. 16 focused explicitly on how methanogen community structure changed with anthropogenic-type disturbances described above, while ca. 15 contrasted methanogen community structure across sites or microforms (e.g. see descriptions of microtopographic features above) without an explicit link to anthropogenic disturbance; most of these studies are summarized in Table 2. Many anthropogenic disturbances led to changes that are somewhat analogous to changes seen in the prototypical fen to bog succession, only in reverse (Table 2 footnote) with regards to vegetation and hydrological changes. In two studies, one for warming by (Dieleman *et al.* 2015) and one for atmospheric nitrogen pollution deposition by Larmola *et al.* (2013), results demonstrated that *Sphagnum*-dominated poor fen and bog plant communities shifted rather quickly towards those seen in richer, sedge-dominated peatlands. Shifting plant communities commonly mediate microbial feedbacks to environmental changes. Regarding specific impacts on methanogen communities, there are general trends that fens with sedges and higher pH support aceticlastic along with CO_2 reduction pathways (Table 2A), however underlying controlling factors are complex. For example (Kotsyurbenko *et al.* 2007) showed that aceticlastic pathways are absent below pH 4 in bog soils at low temperatures, but can be substantial at low pH when soil temperatures are high. Also, Rooney-Varga *et al.* (2007) demonstrated a clear link between sedges (and little to no *Sphagnum*) and the aceticlastic pathway across extensive study sites (further corroborated by isotope-based methanogenesis pathway analyses by Hines *et al.* 2008), yet Basiliko *et al.* (2003) and Godin *et al.* (2012) illustrated that Methanosaetaceae taxa might be important even in acidic bog and poor fen sites with 100% *Sphagnum* cover. Though it should be noted that the latter two studies were carried out with surface peat soils in relatively lower latitude locations in sub-boreal Canada and the northern continental US, and Kotsyurbenko *et al.* (2007) reported that aceticlastic methanogenesis can occur in low-pH peat soils if the temperatures are high.

A number of climate change impact studies have examined *in situ* warming and drought on methanogen communities in bogs and fens in Europe and Asia (Table 2). Community structure changes appear to be context specific, with cases of shifts from one CO_2-reducing group to another (e.g. Tian *et al.* 2015), increasing importance of aceticlastic methanogens (e.g. Peltoniemi *et al.* 2016), or in one case when drought was coupled with a major plant invasion, loss of all known methanogen taxa (Narihiro *et al.* 2011). Reported impacts in studies of climate change degrading permafrost peatland landforms have been more consistent, at least when the degradation results in thermokarst and sedge expansion after thaw (Table 2). For example, where initial archaeal populations are often dominated by hydrogenotrophic methanogens, as the degree of thaw and rates of decomposition increased, the proportion of aceticlastic methanogens increased (e.g. McCalley *et al.* 2014). It is important to note that there are still relatively few studies, and permafrost degradation can lead to outcomes other than thermokarst formation (and associated shifts in vegetation, methane communities and increased CH_4 emissions) outside of lowlands. For example, the effects of slow active-layer deepening or rapid drainage as gullies form from loss of ice wedges on methanogen communities could be quite different from what has been observed for thermokarst formation following thaw.

A number of case studies have explored direct impacts associated with horticultural peat extraction and reclamation, drainage and forestry and post-agricultural reclamation of peatlands on methanogen communities (Table 2), but given the extent and diversity of context-specific land uses, more work is needed. There are also critical gaps in the literature regarding atmospheric deposition impacts on methanogen communities. Important studies have shown that chronic S loading can suppress methane emissions at large scales (e.g. Gauci *et al.* 2004), while chronic N loading has been shown to enhance emissions from a bog (Juutinen *et al.* 2018), but little is known about the impacts and feedback roles of methanogen communities. Similarly, despite the known role of certain trace metals in CH_4 cycling metalloenzymes (Glass and Orphan 2012), that continental gradients of metals might limit methanogenesis in peatlands (Basiliko and Yavitt 2001), and that metal pollution disrupts vegetation and bacterial and fungal communities in peatlands (Luke *et al.* 2015), work is still needed on methanogen community-metal loading interactions in terms of trace nutrients and potential toxicant effects.

We have attempted to summarize and synthesize what we believe are most of the peer-reviewed studies on methanogen community responses to environmental changes (Table 2). However, given the global extent (ca. 4×10^6 km^2; Parish *et al.* 2008) and variability within and between peatlands, and the types and variability of environmental change pressures, much more work is needed before strong conclusions about feedbacks can be drawn. These studies will ideally go hand in hand with work enriching for, isolating and exploring the physiological ecology of the vast numbers of 'known unknown' peatland methanogens to provide a comprehensive picture of both how and why methanogen communities respond to increasing environmental change pressures.

CONCLUSIONS AND OUTLOOK

Recent progress has contributed substantially to our understanding of the taxonomy and physiological ecology of methanogens in peatlands, yet substantial gaps remain. Environmental omics approaches continue to be refined and play a key role in identifying the 'known-unknown' methane cycling archaeal taxa that are most important in peatland feedbacks to anthropogenic change, but alone they still fail to elucidate fundaments of metabolism and ecology. The isolation of *Methanoflorens stordalenmirensis* in the context of palsa degradation was an excellent example of a paired community biomarker and isolation approach (Mondav *et al.* 2014), and similar work should continue across permafrost peatland thaw, and other environmental change contexts. For example, to date no novel species of aceticlastic methanogens have been isolated from acidic peat, the role of the *mcrA*-containing Bathyarchaeota within the phylum Crenarchaeota is not known, and the potential role of archaea in the omnipresent process of anaerobic CH_4-oxidation in peatlands (Gupta *et al*. 2013) is not clear. Future efforts should clearly focus on novel methods of isolation to acquire a greater diversity of representative organisms in pure culture that are guided by *in situ* studies of methanogen community responses to environmental stressors. In cases where isolation is elusive, long-term enrichment cultures (e.g. with a dominant methanogen and syntrophic bacterium) coupled with physiological measurements and genomic sequencing can yield important information on the physiological ecology and taxonomy of novel methanogens (Carson *et al*. 2019). To guide enrichment and isolation so that efforts are placed on the most interesting and important taxa, meaningful climate change simulation, pollutant (N, S and metals loading) and land-use change studies on methanogen communities are still needed.

ACKNOWLEGEMENTS

HMPS was supported by the Academy of Finland [290315]. SLB, NB, and SHZ did not receive external funds for this work.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSLE* online.

Conflicts of interest. None declared.

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