Soil Biology & Biochemistry 81 (2015) 204–211

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Nitrogen and methanogen community composition within and among three *Sphagnum* dominated peatlands in Scandinavia

Magalí Martí [a, d, *], Heli Juottonen [b, 1], Bjorn J.M. Robroek [c, 2], Kim Yrjälä [b, 4], Åsa Danielsson [a, 3], Per-Eric Lindgren [d, 5], Bo H. Svensson [a, 6]

[a] Department of Thematic Studies – Environmental Change, Linköping University, SE-581 83 Linköping, Sweden
[b] MEM-group, Department of Biosciences, University of Helsinki, FIN-00014 Helsinki, Finland
[c] Ecology and Biodiversity Group, Institute of Environmental Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands
[d] Division of Medical Microbiology, Department of Clinical and Experimental Medicine, Linköping University, SE-581 85 Linköping, Sweden

ARTICLE INFO

Article history:
Received 10 July 2014
Received in revised form
29 October 2014
Accepted 18 November 2014
Available online 29 November 2014

Keywords:
Methanogenic archaea
mcrA gene
Peatland
Microtopography
T-RFLP
qPCR

ABSTRACT

Ombrotrophic raised bogs are nutrient poor acidic peatlands accumulating organic matter. They are widely spread on northern latitudes and are substantial sources of methane emissions to the atmosphere being of great concern from a climate change perspective. We investigated the methanogen community composition along microtopographic gradients within three bogs in Scandinavia, receiving different amounts of nitrogen precipitation. Methanogenic community analyses by terminal restriction fragment length polymorphism of the *mcrA* gene showed different profiles among the three sites, while no influence of the microtopographic gradients was observed. Peat temperature and dissolved organic carbon were the major edaphic variables explaining 38% of the variation of the methanogenic community diversity among the bogs. The family *Methanoregulaceae* (hydrogenotrophic methanogens) showed the largest relative proportion and highest activity in all three sites. Quantitative PCR of the *mcrA* gene and transcripts showed that the most northern site, receiving the lowest atmospheric nitrogen load, had significantly lower abundance and activity of methanogens (4.7×10^6 and 2.4×10^4 *mcrA* copies per gram of soil, respectively), compared to the most southern site (8.2×10^7 and 4.6×10^5 *mcrA* copies per gram of soil, respectively), receiving the highest nitrogen load. No patterns of the *mcrA* gene and transcript abundances were observed along the microtopography. The results indicated that the difference in occurrence of methanogens is mainly due to geoclimatological conditions rather than site intrinsic microtopographic variation. The study further suggests that environmental changes on the site intrinsic topography will not affect the methanogenic activity, while increasing average temperatures in Scandinavian ombrotrophic raised bogs might contribute to an increase of the methanogenic archaeal activity resulting in an increase of methane production.

1. Introduction

Peatlands cover approximately 3% of the Earth's land area, but hold about 30% of the total soil carbon. This is due to a comparatively low degradation of organic matter in relation to net primary production leading to sequestration of atmospheric CO_2 (Gorham, 1991). However, peatlands are also an important source

* Corresponding author. Department of Thematic Studies – Environmental Change, Linköping University, SE-581 83 Linköping, Sweden. Tel.: +46 70 089 56 21.
E-mail addresses: magali.marti.genero@liu.se (M. Martí), heli.juottonen@ebc.uu.se (H. Juottonen), b.j.m.robroek@uu.nl, bjorn.robroek@epfl.ch (B.J.M. Robroek), kim.yrjala@helsinki.fi (K. Yrjälä), asa.danielsson@liu.se (Å. Danielsson), per-eric.lindgren@liu.se (P.-E. Lindgren), bo.svensson@liu.se (B.H. Svensson).
[1] Present address: Department of Ecology and Genetics, Limnology, Uppsala University, SE-752 36 Uppsala, Sweden.
[2] Permanent address: École Polytechnique Fédérale de Lausanne (EPFL), School of Architecture, Civil and Environmental Engineering (ENAC), Laboratory of Ecological Systems (ECOS), Batiment GR, Station 2, CH-1015 Lausanne, Switzerland. Tel.: +41 21 69 30895.
[3] Tel.: +46 13 282922.
[4] Tel.: +358 9 19159220.
[5] Tel.: +46 10 1038586.
[6] Tel.: +46 13 282280.

http://dx.doi.org/10.1016/j.soilbio.2014.11.016
0038-0717/

of atmospheric CH_4 due to production by methanogenic archaea (Eriksson et al., 2010).

Hydrology, plant functional types, and water chemistry are used to divide boreal peatlands into two types: ombrotrophic bogs and fens. The ombrotrophic bogs are mainly *Sphagnum*-dominated creating an acidic environment (pH $\leq$ 4) and nutrient limitation, since nutrients are only provided by precipitation (Gore, 1983). Due to their location and impact by anthropogenic air pollution, peatlands are subjected to differences in nutrient supply, which then may affect the plant community and microbial community composition.

The main factors controlling the rates of methane production in peatlands are the water table fluctuations, plant functional type composition, and peat temperature and chemical characteristics (Svensson and Sundh, 1992; Granberg et al., 1999; Ward et al., 2013). The abundance of methanogens differs between areas with high and low water table, however, the variation in the methanogenic community composition among peatlands is mainly explained by peat pH and temperature, where the hydrogenotrophic pathway has been found to be dominant at acidic peat pH and low temperature (Kotsyurbenko et al., 2007; Yavitt et al., 2011). There is no consensus on the effects of peat edaphic variables on the methanogenic community composition, which rather seems to depend on the peatland type investigated and the scope of the study. Thus, it is important to include different peatland types, of which ombrotrophic bogs are the least investigated, to be able to predict how a changing climate may impact the methanogenic community responsible for the production of the greenhouse gas methane.

The development of different microhabitats within peatlands, such as hummocks, lawns, and hollows, gives rise to spatial patterns of the environmental conditions (Robroek et al., 2014) which in turn affect methane emissions (Svensson and Roswall, 1984). In addition, these microhabitats are characterized by a different set of peat mosses and accompanying vascular plants (Saarnio et al., 1997; Rydin and Jeglum, 2006; Robroek et al., 2009) which ultimately control the quantity and quality of the organic matter available for microbial decomposition (Kettunen and Kaitala, 1996; Galand et al., 2003; Andersen et al., 2013; Jassey et al., 2013). In short, the composition of the methanogenic archaeal communities in peatlands shows a tight link with the microtopography in the peatland (Galand et al., 2003; Yavitt et al., 2011). Hence, understanding the spatial distribution of the methanogenic population along the lawn-hummock topography may reveal further insight into the regulation of the methane formation capacity linked to the carbon balance of peatlands. The predicted climate change scenario, e.g. increased variability in precipitation and increased temperature (IPCC, 2007), together with increasing N deposition loading (Phoenix et al., 2012) are believed to, directly or indirectly, affect the microtopography and the variables controlling the potential methane production. This may affect the methane emissions and the carbon allocation (Limpens et al., 2008; Luo and Weng, 2011; Jassey et al., 2013).

Our understanding of effects of edaphic variables on the distribution of peatland methanogens is growing, yet consensus is lacking. Hitherto, research on multisite or multifactor effects is still scarce. Therefore, the present study was conducted to elucidate how the methanogenic community is affected by (1) site intrinsic microclimate conditions, (2) differences in N deposition and (3) variation of a suite of edaphic variables. To establish a set of contrasts, where a variation of the variables can be explored, three peatland sites were chosen: 1) Lille Vildmose (LV) in Northern Jutland, Denmark, which is an area heavily affected by farming and, thus, exposed to high deposition rates of nitrogen; 2) Store Mosse (SM) on about the same latitude in Sweden, but with a N deposition at about half of that for the Jutland site; and, 3) Degerö Stormyr (DS), located in Mid Sweden, experiences much lower N deposition and a colder season than the southern sites. The climatic conditions are about the same for the two southern sites. Within these sites similar microtopographic microhabitats as for the plant study (Robroek et al., 2014) were selected for peat sampling, from which the *mcrA* gene and transcript were used to determine the abundance and diversity of methanogens.

2. Materials and methods

2.1. Sites and peat samples collection

In July 2009, peat samples (5 cm^3) were collected from three *Sphagnum*-dominated Scandinavian ombrotrophic raised bogs (Table 1). The atmospheric N deposition data corresponding to 2008 was retrieved from The European Monitoring and Evaluation Programme (EMEP). In each site, five hummock-lawn transects (microtopographic gradients) were sampled at two depths. Using a Holmen auger (Holmen, 1964) two samples were extracted from five microhabitats – north lawn (Lawn N), north hummock slope (Slope N), hummock top (Hummock), south hummock slope (Slope S) and south lawn (Lawn S) – one sample from above the water table level (Above WTL) and another from just below the water table level (Below WTL) (Fig. 1). Samples were kept cold during transport to the laboratory, where they were frozen at −20 °C until further analysis. Water table level and pH were measured in the field at the sampling time. From June 12th to September 7th 2009, temperature was recorded at 5 cm below the *Sphagnum* cover (Robroek et al., 2014). Pore water was collected in July, from which nitrate (NO_3^-), ammonium (NH_4^+), phosphate (PO_4^{3-}) and dissolved organic carbon (DOC) were analyzed. For more details see (Robroek et al., 2014).

2.2. Nucleic acid extraction and reverse transcription

Before nucleic acid extraction, peat samples were thawed overnight at 4 °C. DNA was isolated from 0.25 g wet peat (average water content = 1500 ± 540 (% dry weight)) using the FastDNA®SPIN Kit for Soil and the FastPrep® Instrument (MP Biomedicals, Santa Ana, CA) according to manufacturer's instructions. The extracted DNA was further purified with the OneStep™ PCR Inhibitor Removal Kit (Nordic Biolabs AB, Täby, Sweden) and stored at −20 °C. Total RNA was isolated from 0.75 g wet peat using the

Table 1
Study sites description. Variables values are given by mean ± standard deviation (Robroek et al., 2014).

Site	Degerö Stormyr (DS)	Store Mosse National Park (SM)	Lille Vildmose (LV)
Location	Northern Sweden	Southern Sweden	Denmark
Coordinates (UPS)	64°10′N, 19°33′E	57°16′N, 13°55′E	56°50′N, 10°11′E
Atmospheric nitrogen deposition (kg N ha^{-1} yr^{-1})	1.5	7.0	25
pH	3.96 ± 0.19	3.98 ± 0.03	3.96 ± 0.06
DOC (mg l^{-1})	61.4 ± 6.5	57.4 ± 4.7	64.9 ± 2.8
NO_3^- (mg l^{-1})	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.02
NH_4^+ (mg l^{-1})	0.28 ± 0.23	0.33 ± 0.08	0.18 ± 0.14
PO_4^{3-} (μg l^{-1})	2.80 ± 1.39	2.88 ± 0.40	4.60 ± 2.26
Peat temperature (°C)	13.4 ± 0.2	15.4 ± 0.6	16.0 ± 0.8
Mean annual temperature (°C)	1.0	6.2	7.6