Soil Biology & Biochemistry 97 (2016) 144–156

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Responses of methanogenic and methanotrophic communities to warming in varying moisture regimes of two boreal fens

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ARTICLE INFO

Article history:
Received 16 November 2015
Received in revised form
14 March 2016
Accepted 15 March 2016
Available online 24 March 2016

Keywords:
Methane oxidation
Methane production
Methanotrophs
Methanogens
Climate change
Drying
T-RFLP
Microarray
qPCR
Boreal peatland
Methane fluxes

ABSTRACT

Peatlands are one of the major sources of the powerful greenhouse gas methane (CH_4). Our aim was to detect responses of methanogenic archaeal and methane-oxidizing bacterial (MOB) communities that control the methane (CH_4) cycle to climatic warming. This study took place in two boreal fens three years after experimental warming in un-manipulated wet and drier regimes, thus simulating future climate scenarios. We determined active methanogen and MOB communities as transcripts of *mcrA* and *pmoA* genes, along with the abundance of these genes, CH_4 production and oxidation potentials, and *in situ* CH_4 fluxes. Methanogenic community remained similar, although methanogen abundance decreased after warming. In the wet regime, this decrease resulted in a small but significant reduction on the potential CH_4 production in such peat layers where the average production potential was high. Drying alone, however, reduced the potential CH_4 production more than warming, and this impact was strong enough to mask the small warming impact in the drier regime. Warming did not affect the MOB community or the potential CH_4 oxidation in the wet regime; however, type Ib MOB abundance decreased and MOB related to genus *Methylocapsa* became typical after warming in the drier regime of the southern fen. The *in situ* measured CH_4 fluxes indicated similar patterns as potential measurements; both warming and drying reduced methane emissions, drying more than warming. These results indicate that methanogens and MOB may have different controlling patterns on CH_4 fluxes when facing global warming. These patterns may further differ not only between moisture regimes, but inside the same habitat type, here boreal fen. Irrespective of this variation, the *in situ* CH_4 fluxes still seem to respond similarly across sites.

1. Introduction

On a mass basis, methane (CH_4) warms atmosphere more in a 100 year perspective than carbon dioxide, and thus, its increasing atmospheric concentration contributes to global warming (IPCC, 2013). The magnitude of CH_4 emissions from unmanaged peatlands varies widely, depending on, e.g., water level, temperature and vegetation (Jaatinen et al., 2005; Saarnio et al., 2007; Turetsky et al., 2014). This means that changes in climate have a great potential for changing peatland CH_4 emissions, the direction and magnitude depending on how variables interact with one another. The climate of northern Eurasia is expected to become warmer and gradually wetter (Arzhanov et al., 2012; Monier et al., 2013). A broad range of summer rainfall among regions is predicted (Screen, 2013; ROSHYDROMET, 2008), meaning some peatlands will suffer from drought more frequently (Gorham, 1991; Roulet et al., 1992). Consequently, responses of northern peatlands to climate warming should be considered under both wet and dry future scenarios.

Experimental studies on the CH_4 cycle with peat soil in

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http://dx.doi.org/10.1016/j.soilbio.2016.03.007
0038-0717/

mesocosms have reported decreased CH_4 emissions as responses to warming and drying (Moore and Dalva, 1993). Complex controls over the CH_4 fluxes due to a set of interactive factors operating at different time frames have been observed, however (Updegraff et al., 2001). The plant community and its productivity are among the controlling factors (Updegraff et al., 2001; White et al., 2008) and any responses of these to warming may have an effect on its own right. In fens, *in situ* CH_4 emissions are known to correlate positively with the coverage of graminoids while sites dominated by shrubs generally show lower effluxes (e.g., Turetsky et al., 2014). Adding to the perplexity, studies conducted *in situ* with open top chambers (OTC) found elevated CH_4 emissions in a rich fen in Alaska (Turetsky et al., 2008), and no changes in a dry boreal bog in Canada (Munir and Strack, 2014) or in three boreal fen sites in Finland (Pearson et al., 2015) after soil warming (due to OTC) and drying (due to water-level drawdown). Put together, previous studies suggest that warming, along with changes in water level, may affect the different factors that control the CH_4 fluxes in different ways, and may thus result in unpredictable alterations in the CH_4 emissions. Clearly, a thorough simultaneous analysis of the different factors affecting the CH_4 fluxes, and their interactions, is needed. Furthermore, the analysis should include the key microbial organisms controlling the processes of CH_4 production and oxidation.

The key microbial organisms responsible for the processes of the CH_4 cycle are methanogenic archaea and methane oxidizing bacteria (MOB). Microbial organisms and their responses to changes in temperature and/or water level could identify the reasons for possible shifts in the balance of CH_4 fluxes and be extremely important for future GHG predictions. Several studies have already shown that water level and temperature are among the key determinants affecting the activity and community of both methanogens and MOB in peatlands (Jaatinen et al., 2005; Turetsky et al., 2008; Larmola et al., 2010; Yrjälä et al., 2011), and have important regulatory roles in CH_4 oxidation and production processes (Börjesson et al., 2004; Whalen, 2005; Juottonen et al., 2008). Yet, the largest changes are expected to be targeted to MOB community, which are reported to be most active in the uppermost peat layers on top or at the water level (Jaatinen et al., 2005; Yrjälä et al., 2011), since oxygen availability is limiting the activity in the deeper layers.

In the present study, transcripts of methanogens and MOB, addressing functional active members of the community, are for the first time explored from boreal peatlands, representing *Carex*-dominated fens, with respect to climate change. We studied the impact of moderate warming that was applied for three years on organisms responsible for the CH_4 cycle in two alternative moisture regimes: an un-manipulated wet regime and a drier regime (due to moderate water-level drawdown), both reflecting the future climate scenarios. We explored the simultaneous abundance (qPCR) and community changes of methanogens and MOB by analyzing transcriptional products of *mcrA* and *pmoA* genes from the most active peat layers with T-RFLP (*mcrA*) followed by cloning and sequencing, and microarray (*pmoA*) data using multivariate analysis combined with multilevel mixed models. We measured both the potential CH_4 production and oxidation under controlled conditions from the peat profile down to 50 cm depth and the *in situ* CH_4 flux mediated by methanogens and MOB. Sporadic measurements of CH_4 fluxes in the sites were already done during the first two years following the experimental setup (Pearson et al., 2015). Instead, our new measurements reflect a situation when both microbial and plant communities have had three years to adapt to the changed temperature and water-level conditions. Earlier, Updegraff et al. (2001) observed that treatment effects on CH_4 fluxes became progressively stronger over three growing season.

Earlier we observed that within the boreal zone, different peatland types may show differing microbial responses to global change factors (Jaatinen et al., 2005, 2007; Peltoniemi et al., 2009). Now we wanted to see, whether the responses are similar within a peatland type and may thus be generalized over a climatic region. Thus, we set up the experiment in two boreal fen sites which differ in latitude (mid boreal vs north boreal). Fens were chosen for study sites since they are more vulnerable to disturbance than bogs, especially with respect to drying (Laine et al., 1995; Komulainen et al., 1999; Jaatinen et al., 2007).

The following hypotheses were addressed: 1) warming in wet, un-manipulated control leaves the methanogenic community unaffected, while the MOB community changes. 2) The changed MOB community increases CH_4 oxidation potential. 3) Increased oxidation potential together with non-affected production potential results in decreased *in situ* CH_4 fluxes. 4) Drying further enhances the effect of warming and induces changes in both methanogen and MOB communities, and thus 5) decreases potential CH_4 production and increases potential CH_4 oxidation since activity may move into deeper peat layers.

2. Materials and methods

2.1. Study sites, experimental design and soil sampling

Our study sites were in Orivesi (the southern fen, Lakkasuo, 61°48′ N 24°19′ E) and Kittilä (the northern fen, Lompolojänkkä, 67°60′ N 24°12′ E). A detailed description of the study sites, experimental design, vegetation patterns, peat characteristics and sampling procedure is found in Pearson et al. (2015) and Peltoniemi et al. (2015). Element concentrations prior to experiment start are shown in Table S1 in Supplementary Material. Briefly, both experimental sites consisted of two moisture regime plots; one with an undisturbed wet moisture regime and the other, drier regime, with lowered water level achieved with a shallow drainage ditch 30 cm deep. Both moisture regime plots were divided into six subplots; three controls with no temperature manipulation and the other three with artificial warming with small open-top chambers (OTC), widely used to simulate climate warming (Marion et al., 1997; Hollister and Webber, 2000). We shall call the un-manipulated moisture regime "wet", and suggest that it also covers the conditions under increased precipitation. Since the surface position of these sites changes according to changes in water storage and gas content in the peat, the water levels are not likely to become clearly higher, especially if the surface is floating (as is often the case in wet fens) (Roulet, 1991; Strack et al., 2006; Reeve et al., 2013). For the purpose of this study, the conditions under lowered waters levels are called the "drier" regime, and the treatment applied to achieve this is called drying. We choose "drier" rather than "dry", since the latter might give a wrong impression as these sites remain rather wet even after moderate water-level drawdown. These sites also adjust to water-level drawdown with a lowering surface position, which makes the measurable water-level drawdown smaller than the change in water storage might imply.

The shallow ditch lowered water levels by, on average, 6 and 3 cm in the southern and northern fen, respectively (Table 1). OTCs increased the average daily air temperature during the growing season by 1.5 °C at 15 cm above the peat surface. With respect to controls, the effective temperature sum in the OTCs increased by 320 and 200 degree days in the southern and northern fens, respectively. In 2011, the average daily temperature immediately below the moss layer was approximately 0.8 °C higher and at 5 cm depth 0.3 °C higher under the OTCs compared to control subplots. Notable differences were not observed in soil layers 5 cm below the surface.

Sample cores were taken with a 6 × 8 cm box-corer from each subplot in September 2011. Sampling was conducted to the depth of 20–50 cm; some sample cores were so wet that only first 20 cm of peat could be obtained. Cores were divided into sub-samples at 10 cm intervals from the peat surface (L1: 0–10, L2: 10–20, L3: 20–30, L4: 30–40 and L5: 40–50 cm) (Table 1). In total, 100 sub-