Soil Biology and Biochemistry 118 (2018) 156–160

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

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

Methylotrophic methanogenesis in *Sphagnum*-dominated peatland soils

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ARTICLE INFO

Keywords:
Bog
Isotopes
Methane
Methanogenesis
Methylotrophic
Wetlands

ABSTRACT

Although it is generally assumed that CH_4 production in peatlands is dominated by the acetoclastic and hydrogenotrophic methanogenic pathways, we found evidence that methylated substrates (including methanol, methylamines, and dimethylsulfide) support methanogenesis in these organic-rich environments. The role of methylotrophic methanogenesis was investigated in three northern Minnesota peatlands by amending surface soils with ^{13}C-labeled substrates. Labeled methanol was converted to enriched δ ^{13}C-CH_4 at all three sites. Additionally, dimethylsulfide and monomethylamine were processed through methanogenic pathways, although this was not evident at every site. The addition of ^{13}C labeled methanol and monomethylamine also resulted in the enrichment of δ ^{13}C-CO_2, further suggesting that either methanogens or non-methanogenic microbes were processing methylated substrates. Results demonstrate the potential for methylotrophic methanogenesis in northern peatlands and suggest that further studies are warranted to quantify the amount of CH_4 produced by this process in comparison to traditional pathways of methanogenesis.

1. Introduction

Peatland ecosystems play an important role in the global carbon cycle, and thus global climate regulation, by storing large quantities of carbon in their soils and releasing a significant fraction of the greenhouse gas methane (CH_4) that enters the atmosphere annually (Bridgham et al., 2006, 2013; Keller and Medvedeff, 2015). A pressing question in global biogeochemistry remains whether the vast stores of carbon in peatland soils will be released to the atmosphere as carbon dioxide (CO_2) and/or CH_4 in the face of ongoing climate change. To address this question, and inform biosphere-climate models, an improved mechanistic understanding of CH_4 cycling is required for peatland ecosystems.

It is generally assumed that CH_4 production (methanogenesis) occurs through two dominant pathways. In the acetoclastic pathway, acetate is split to form CO_2 and CH_4, while in the hydrogenotrophic pathway, CO_2 is reduced to CH_4 using H_2 as an electron donor (Conrad, 1999; Whalen, 2005; Bridgham et al., 2013). This view is so engrained in our thinking about peatland CH_4 cycling that the rates of acetoclastic methanogenesis have often been calculated as the difference between total CH_4 production and measured rates of hydrogenotrophic methanogenesis (e.g., Duddleston et al., 2002; Avery et al., 2003; Keller and Bridgham, 2007; Ye et al., 2012, 2014). However, it has long been known that methanogens can also produce CH_4 using a variety of methylated substrates, including methanol, methylamines and dimethylsulfide (Boone et al., 1993; Zinder, 1993). In some ecosystems use of these substrates makes up only a small proportion of the total CH_4 produced (Lovley and Klug, 1983), but there are indications that these pathways are important in some natural environments, including salt marshes, intertidal zones (Oremland et al., 1982, 1989; King et al., 1983; King, 1984; Franklin et al., 1988) and hypersaline ecosystems (Smith et al., 2008; Kelley et al., 2012, 2015; Tazaz et al., 2013). Despite the importance of peatlands in the global carbon cycle, few studies have investigated the importance of methylotrophic pathways in these ecosystems. Horn et al. (2003) demonstrated that the addition of methanol had no effect on net CH_4 production in a bog soil. In contrast, Zhang et al. (2008) used qPCR to demonstrate an increase in the methanogen population in response to the addition of both methanol and trimethylamine in a wetland soil. Thus, in peatlands, limited and conflicting evidence exists on the role of methylated substrates in CH_4 production and the direct conversion of methylated substrates to CH_4 has rarely been measured.

The objective of this study was to determine the potential for methylotrophic methanogenesis in three *Sphagnum*-dominated peatlands in northern Minnesota. Our experimental approach was to amend soils with ^{13}C-labeled traditional substrates (acetate and sodium

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https://doi.org/10.1016/j.soilbio.2017.11.025
Received 11 July 2017; Received in revised form 18 November 2017; Accepted 29 November 2017

bicarbonate/H_2) and methylated substrates (methanol, monomethylamine ("MMA"), dimethylsulfide ("DMS")) and to monitor δ^{13}C-CH_4, δ ^{13}C-CO_2, and net CH_4 and CO_2 production in laboratory incubations.

2. Materials and methods

2.1. Study sites

Three *Sphagnum*-dominated peatlands in northern Minnesota, U.S.A were sampled in this study. Bog Lake Fen (N47°30.304′, W93°29.339′) and S1 Bog (N47°30.388′, W93°27.256′) are located within the Marcell Experimental Forest (U.S. Forest Service). Zim Bog (Zim Bog, N47°10.745, W92°42.877') is an ombrotrophic bog located approximately 90 km northwest of Duluth, Minnesota. These sites have been described in detail previously (Medvedeff et al., 2015).

2.2. Sample collection

Soil was collected using a serrated knife from 0 to 25 cm below the water table from Bog Lake Fen and Zim Bog in June 2014 and from S1 Bog in June 2015. Porewater was collected from piezometers (25 cm depth) at all three sites using a peristaltic pump at the date of soil collection. Samples were frozen until analysis at Chapman University. In the laboratory, soils were thawed, manually homogenized, large roots and debris were removed, and 7 g (wet weight) of soil were added to 72-mL serum bottles. Serum bottles were flushed with N_2 for 15 min and pre-incubated at 18 °C until measurable CH_4 production was detected (S1 Bog: 11 days; Bog Lake Fen: 15 days; Zim Bog: 22 days).

Following pre-incubation, 7 mL of filtered (0.2 μm), deoxygenated, site-specific porewater was added to each serum bottle in an anaerobic chamber (Coy Laboratory Products Inc., Grass Lake, MI, U.S.A). Soils were amended with 99 atom % ^{13}C-labeled traditional (sodium acetate, sodium bicarbonate) and methylated (methyl labeled - methanol, MMA, and DMS) substrate (Sigma-Aldrich) in triplicate to a final concentration of 10 μM in each serum bottle. Triplicate unamended controls were also prepared for each site. Following addition of porewater and labeled substrates, pH was measured in all bottles after a 30-min equilibration period and bottles were incubated at 18 °C in the dark. Forty μmols of H_2 gas was added to serum bottles in the sodium bicarbonate treatment to ensure methanogens were not electron limited. Equations for methanogenic pathways are included in Table 1.

Headspace gas samples were analyzed for CH_4 and CO_2 production using a gas chromatograph equipped with a flame ionization detector and methanizer (SRI Instruments, Torrance, CA, U.S.A) until CH_4 concentrations reached 0.5% of the headspace. As a result, sampling dates varied by site (Bog Lake Fen: 1, 3, 6, and 13 days; S1 Bog: 1, 3, 10, 17, and 23 days; Zim Bog: 1, 3, 7, 9, 13, 21, 35, 49, 63, and 77 days). On each sampling date, CH_4 and CO_2 concentrations were determined after accounting for pressure, solubility, and pH (Drever, 1997). On the final gas measurement day, 15 mL of headspace gas were transferred to 12 mL Exetainers (LABCO Ltd.; www.labco.uk.com) and analyzed for ^{13}C-CH_4 and ^{13}C-CO_2 on a Thermo-Finnegan Delta V isotope ratio mass spectrometer coupled to a gas chromatograph by a combustion interface at Florida State University, following the approach of Kelley et al. (2015). After isotope samples were collected, soils were transferred into the anaerobic chamber and final pH measurements were obtained.

Table 1
Processing of methanogenic substrates under anaerobic conditions. Δ G values from Whitman et al., (2006) under standard conditions. A Δ G value for methanol coupled to H_2 oxidation was not included.

Substrate:	Equation:	Δ G (kJ mol^{-1} of CH_4)
Methanol	$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$	−104.9
Monomethylamine	$4CH_3\text{-}NH_2 + 2H_2O + 4H^+ \rightarrow 3CH_4 + CO_2 + 4NH_4^+$	−75.0
Dimethylsulfide	$2(CH_3)_2\text{-}S + 2H_2O \rightarrow 3CH_4 + CO_2 + 2H_2S$	−73.8
H_2	$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	−135.6
Acetate	$CH_3COOH \rightarrow CH_4 + CO_2$	−31.0
Methanol reduction coupled to H_2 oxidation	$CH_3OH + 4H_2 + CO_2 + 2H^+ \rightarrow 2CH_4 + 3H_2O$	—

2.3. Statistical analyses

Differences between amended treatments and the control for gas production (CH_4, CO_2) and stable isotope composition (δ^{13}C-CH_4 and δ ^{13}C-CO_2) within sites were determined via a Dunnett's control test ($\alpha < 0.05$) at the end of the incubation period. All statistical tests were completed in JMP v. 8.0 (SAS Institute, Cary, N.C.).

3. Results

Addition of traditional and methylated methanogenic substrates had no stimulatory effect on cumulative CH_4 production regardless of site (Fig. 1a–c). Rates of CH_4 production averaged across all treatments were greatest in Bog Lake Fen (4.05 ± 0.68 μmol g^{-1} dw soil d^{-1}) followed by S1 Bog (2.06 ± 0.49 μmol g^{-1} dw soil d^{-1}), with the lowest production rates from Zim Bog (0.58 ± 0.06 μmol g^{-1} dw soil d^{-1}). The addition of sodium bicarbonate (and H_2) decreased CO_2 production relative to the control in two of the three sites (Bog Lake Fen, $p = 0.0009$; Zim Bog, $p < 0.0001$; Fig. 1d–f). No other effects of substrate addition on CO_2 production were observed. Decreased CO_2 production following sodium bicarbonate (and H_2) amendment resulted in lower CO_2:CH_4 ratios in Bog Lake Fen and Zim Bog, although this decrease was only significant in Bog Lake Fen soil ($p < 0.0001$, Fig. 1g).

To determine if substrates were being converted to CH_4, δ ^{13}C-CH_4 values were quantified at the end of the incubation. At all three sites, the addition of traditional substrates (acetate and sodium bicarbonate/H_2) resulted in enriched δ ^{13}C-CH_4 relative to the controls ($p < 0.05$, Fig. 2a–c), although the degree of enrichment varied widely across the sites. Additionally, methylated substrates were processed though methanogenic pathways in all three sites. The addition of methanol resulted in enriched δ ^{13}C-CH_4 in all three peatland soils ($P < 0.0001$; Fig. 2a–c) relative to control signatures. The addition of MMA resulted in enriched δ ^{13}C-CH_4 in Bog Lake Fen ($P < 0.0001$, Fig. 2a) and DMS addition enriched δ^{13}C-CH_4 signatures in Bog Lake Fen and Zim Bog ($P < 0.5$, Fig. 2a, c) relative to the controls. Neither MMA nor DMS amendments resulted in enriched δ ^{13}C-CH_4 in S1 Bog (Fig. 2b) relative to control signatures.

^{13}C-CO_2 values have the potential to provide further insight into substrate processing (Table 1). Traditional substrates (acetate and sodium bicarbonate/H_2) resulted in enriched δ^{13}C-CO_2 in all sites ($P < 0.01$, Fig. 2d–f); however, the processing of methylated substrates was less consistent. Methanol addition resulted in enriched ^{13}C-CO_2 in Bog Lake Fen and Zim Bog (Fig. 2d, f) relative to the control, whereas MMA resulted in enriched ^{13}C-CO_2 in only S1 Bog soil (Fig. 2e). The addition of DMS has no effect on ^{13}C-CO_2 signatures (Fig. 2d–f).

4. Discussion

At all sites, soil microbial communities were able to convert traditional substrates to CH_4 following amendment. This finding was not surprising as these two pathways are historically known to dominate overall methanogenesis in wetlands (Bridgham et al., 2013). Enriched ^{13}C-CH_4 signatures in unamended soil from Bog Lake Fen suggest a prevalence of acetoclastic methanogenesis. More depleted ^{13}C–CH_4