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Homoacetogenesis: A potentially underappreciated carbon pathway in peatlands

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ABSTRACT

Due to anaerobic conditions, peatland soils store globally significant amounts of carbon and are an important source of methane, a potent greenhouse gas. One component of anaerobic carbon cycling, homoacetogenesis (i.e., acetate formation from carbon dioxide and dihydrogen via the acetyl-CoA pathway), has rarely been quantified in natural environments because it is commonly viewed as being thermodynamically unfavorable. Here we show that in a laboratory incubation using a tracer method, homoacetogenesis occurred at significant rates in soils from three peatlands (bog, intermediate fen, and cedar swamp) despite thermodynamic conditions that appeared to be unfavorable for this process. Homoacetogens consumed dihydrogen at rates up to 3- to 10-times faster than methanogens, and homoacetogenesis accounted for 16–63% of total acetate production, with the balance likely coming from fermentation processes. Our results show that homoacetogenesis can play an important role in regulating acetate dynamics, methane production, and carbon cycling in peatlands.

1. Introduction

Understanding the mechanisms controlling anaerobic carbon (C) cycling in peatlands is essential because they contain up to one-third of the world's soil carbon, emit globally significant amounts of methane (CH_4), and are likely to represent an important feedback to future climate change (Gorham, 1991; Ise et al., 2008). The basic dynamics of organic C mineralization in wetland soils are in principle well known (Bridgham et al., 2013; Megonigal et al., 2004; Reddy and DeLaune, 2008). During anaerobic carbon mineralization, acetate is a key intermediate metabolite that provides substrates for various groups of microorganisms, such as sulfate and iron-reducing bacteria, and methanogens (Fig. 1). Acetate can be produced by the fermentation of more complex organic polymers or via homoacetogenesis (i.e., acetate formation from carbon dioxide (CO_2) and dihydrogen (H_2)). The production of acetate is an important control over CH_4 dynamics because acetate serves as the substrate for acetoclastic methanogenesis, while homoacetogens potentially compete for H_2 with hydrogenotrophic methanogens (Drake et al., 2009; Megonigal et al., 2004).

Homoacetogenesis is the reduction of CO_2 with H_2 to acetate via the acetyl-CoA pathway ($2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$) (Diekert and Wohlfarth, 1994; Drake et al., 2006). This reaction is generally considered to be thermodynamically unfavorable as a result of low H_2 partial pressure in bulk porewater, which is common in many anaerobic habitats, including peatlands (Goodwin and Zeikus, 1987), because of H_2 consumption by microorganisms such as sulfate and iron reducers (Megonigal et al., 2004). In methanogenic environments (i.e. those with low concentrations of inorganic TEAs), methanogens and homoacetogens are the main H_2 consumers, with methanogens generally thought to dominate (Hoehler et al., 1999; Lovley and Klug, 1983; Jones and Simon, 1985). Nonetheless, it has been suggested that homoacetogens can outcompete methanogens at low temperature (Conrad and Wetter, 1990; Kotsyurbenko et al., 1993; Nozhevnikova et al., 1994; Liu and Conrad, 2011), likely due to higher growth rates at low temperature than methanogens (Conrad et al., 1989; Kotsyurbenko et al., 1996,

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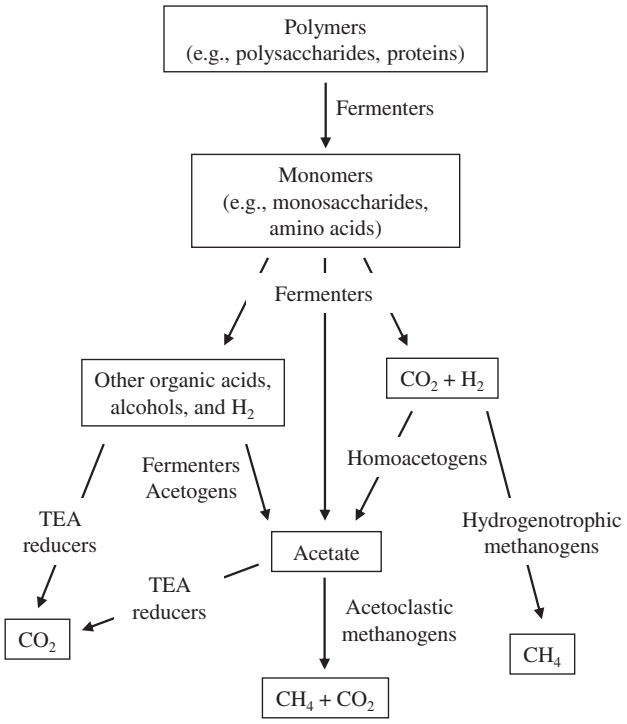

Fig. 1. Pathways for anaerobic decomposition of complex organic carbon. TEA = terminal electron acceptor.
Adapted from Megonigal et al. (2004).

2001). Past studies have also implied that homoacetogenesis can occur under seemingly thermodynamically unfavorable conditions, yet the mechanisms remain elusive (Conrad et al., 1989; Heuer et al., 2009).

Since soils of most northern peatlands have low concentrations of TEAs (Blodau et al., 2002; Janssens, 2005), current anaerobic carbon cycling theory suggests that methanogenesis should dominate anaerobic decomposition (Fig. 1). However, studies have shown that anaerobic peatland soils frequently produce little CH_4 and accumulate acetate (Hines et al., 2001; Duddleston et al., 2002; Hines et al., 2008; Keller and Bridgham, 2007; Bridgham et al., 2013). The mechanisms underlying these dynamics are very poorly understood, but one possible explanation is that homoacetogenesis may prevail in these soils at typical growing season temperatures (Duddleston et al., 2002). In support of this hypothesis, we recently observed substantial acetate accumulation at 17 °C during an anaerobic incubation of peat soils (Ye et al., 2012). To understand the source of this acetate, we anaerobically incubated soil samples from a bog, an intermediate fen, and a cedar swamp from the Upper Peninsula of Michigan, USA in the laboratory with a ^{14}C-CO_2 tracer and measured rates of CO_2 respiration, homoacetogenesis, hydrogenotrophic methanogenesis, and acetoclastic methanogenesis in these soils.

2. Materials and methods

2.1. Sample preparation

The current study was run concurrently with a large pH-controlled experiment (Ye et al., 2012), which suggested significant rates of homoacetogenesis in peatland soils. To minimize potential pH artifacts, we used peat samples at a pH equal to or closest to their native pHs. Thus, the bog peat was incubated at pH 3.5 (0.2 pH unit lower than the native), fen peat at pH 4.5 (equal to the native), and swamp peat at pH 6.5 (0.5 pH unit higher than the native). pH within a site can easily vary by half of a pH unit seasonally, so these incubation pHs are reasonable.

Peat samples were collected from three peatlands in the Upper Peninsula of Michigan, USA in August 2009 (Table 1). Four cores were extracted with PVC tubes (10-cm diameter, 15-cm length) from below the water table in hollows from each site and transported on ice to our laboratory at the University of Oregon and frozen at −20 °C until use. After thawing at room temperature in an anaerobic glove box filled with 2% H_2 and 98% N_2 gas (Coy Laboratory Products Inc., Grass Lake, MI, USA), each peat core was homogenized by hand after the removal of woody material, large roots, and green vegetation. A subsample was dried at 60 °C for 3 days to determine the moisture content. Approximately 120 g of peat from each core was transferred to a 440 mL Mason jar and mixed well with 240 mL of degassed and deionized water, followed by the adjustment of pH to the targeted value with either 10 N HCl or 10 N NaOH. The pH was adjusted daily in the glove box during the first week of incubation and once every 2 or 3 days afterward when pH changes in most of the slurries were <0.2 pH units. After pH adjustment, the slurries were capped and bubbled with oxygen-free N_2 gas for 10–15 min and then incubated in the dark at 17 °C, which was the average field temperature when the peat cores were collected.

2.2. CO_2 and CH_4 production, CH_4 pathway, acetogenic CO_2 reduction

On Days 2, 7, 15, and 43 of the incubation, 10 g of slurried peat from each sample was transferred into a 40 mL vial (I-Chem, VWR International LLC, CA, USA) in the glove box following pH adjustment. The peat slurries were capped and bubbled with oxygen-free N_2 gas for 5–10 min. Following the addition of 0.1 mL of 3.5 μCi mL^{-1} $NaH^{14}CO_3$, the slurries were gently shaken and incubated at 17 °C in the dark. After incubation for 48 h, slurries were shaken to release trapped gas bubbles and headspace CO_2 and CH_4 were quantified by gas chromatography using a flame ionization detector equipped with a methanizer (SRI Instruments, Torrance, CA, USA), while $^{14}CO_2$ and $^{14}CH_4$ production were measured concurrently with an in-line radioactive gas detector (LabLogic Systems Inc., Brandon, FL, USA). Production of CH_4 and CO_2 were calculated as the sum of production in both gas and liquid phases (Stumm and Morgan, 1995). Porewater was then collected from the same subsamples and filtered with a Whatman GF/F glass fiber filter (Sigma–Aldrich, MO, USA), followed by a second filtration with a 0.22 μm syringe filter (Tisch Scientific, OH, USA). Acetate concentrations of the water samples were determined with a Dionex DX500 ion chromatograph system equipped with an HC-75 column (Hamilton Company, Reno, ND, USA), with the effluent of

Table 1
Physical and biological characterizations of the sampling sites.

	Location	Dominant vegetation	Peat depth	pH	Water-table depth[a]
Bog	Michigan (N46°6′6″, W88°16′25″)	*Sphagnum* spp.	380 cm	3.7	−34 cm
Intermediate fen	Wisconsin (N46°12′48″, W89°30′2″	*Sphagnum* spp.	340 cm	4.5	−23 cm
Cedar swamp	Michigan (N46°14′41″, W89°32′38″)	*Thuja occidentalis* L.	290 cm	6.0	−16 cm

[a] Measured from hollow surface.