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Variations in potential CH_4 flux and CO_2 respiration from freshwater wetland sediments that differ by microsite location, depth and temperature

Michael R. Brooker*, Gil Bohrer, Paula J. Mouser

Department of Civil, Environmental and Geodetic Engineering, The Ohio State University, 470 Hitchcock Hall, 2070 Neil Ave, Columbus, OH, United States

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ABSTRACT

Wetlands are a valued ecosystem because of their ability to improve water quality through pollutant removal, their high biodiversity, their carbon sequestration capabilities, and their ability to dampen storm hydrographs through water storage. However, wetlands also contribute to global warming, most obviously through microbial methane production. Driving the biochemical processes that enable methanogenesis is a diverse community of microorganisms under the influence of multiple environmental factors that are poorly constrained in greenhouse gas flux models. Sediments were collected from several distinct microsites across a fresh-water, constructed temperate wetland in Columbus, Ohio, U.S.A. Depth and temperature were tested for their influence on methane emission rates under two controlled temperatures. Pyrosequencing analysis of the 16S rRNA gene was used to investigate microbial communities associated with microsites and depths. Unvegetated, open-water and vegetated microsite sediments had similar potential methane flux rates when cores were separated into shallow and deeper depths. Deeper sediments lacked the ability to produce detectable methane without substrate addition. A 10 °C increase in temperature accelerated the rate of methane production and carbon dioxide respiration by 2–3 fold across all microsites. Methanogens were initially most prevalent in sediments collected from open-water microsite sediments, although had little effect on potential methane flux rates during incubations. These results suggest that upper limit methane emissions are similar across microsites in the absence of other field scale effects, such as redox boundaries, vegetation, or hydrologic fluctuations.

1. Introduction

The management of wetland ecosystems is complicated by an environmental dilemma whereby they provide ecosystem services to human society by acting as nitrogen and carbon sinks, biodiversity reserves, and flood control; but are also major contributors of the greenhouse gas, methane (Bowden, 1987; Bridgham et al., 2006). Early 20th century areal losses resulting from drainage or dredging of wetlands and the excessive use of fertilizers in modern agricultural practices have collectively increased nitrogen loading to surface waters and subsequent hypoxic conditions in large drainage basins such as the Laurentian Great Lakes and Gulf of Mexico (Conroy et al., 2011; Dahl, 1990; Rabalais et al., 2002; Vitousek et al., 1997). To reduce these water quality impacts, protections for wetlands were mandated by the Clean Water Act (33 USC § 1251 et seq.). As a result, altered or destroyed wetlands in the United States now require mitigation or replacement. Outside these requirements, the creation of new wetland areas has been proposed to purify surface waters and to support other important ecosystem services, such as providing habitat for rare species (*e.g.* Mitsch et al., 2001).

One of the impediments to prescribing the creation of wetlands for nutrient removal is whether the design considers the effect of greenhouse gasses (GHG) generation in its assessment of short- or long-term ecosystem services. Wetlands account for more global methane flux than any other type of land surface coverage (Intergovernmental Panel on Climate Change (IPCC), 2007). Although wetlands are known carbon sinks by sequestering carbon dioxide for long-term storage, they initially promote global warming due to methane having a global warming potential 25 times higher than carbon dioxide over a 100 year period (Intergovernmental Panel on Climate Change (IPCC), 2007; Mitsch et al., 2012).

* Corresponding author. Tel.: +1 5174149409; fax: +1 6142923780.
E-mail address: brooker.26@osu.edu (M.R. Brooker).

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In order to estimate the GHG budgets from wetlands, methane emissions are modeled as fluxes across broad spatial scales. Unfortunately, these models may not account for the mosaic of ecological patches within wetlands, known as microsites, which emit methane at variable rates across small spatial and temporal scales (Altor and Mitsch, 2008; Bridgham et al., 2013). For example, an order of magnitude difference in instantaneous flux has been measured across unique microsites within a 2 ha wetland area (Sha et al., 2011). GHG models incorporate key environmental factors, namely hydrological conditions, substrate composition, vegetation, and temperature (Bridgham et al., 2013; Kettunen, 2003; Walter and Heimann, 2000). Variability is particularly evident between vegetated *versus* open water microsite areas within a wetland (Altor and Mitsch, 2008). A better understanding of the variability in methane emissions across these microsites will allow for more accurate predictions of GHG from wetlands.

Vegetation introduces the majority of carbon substrate to wetlands by assimilating carbon dioxide that is subsequently released to sediments through the production of root exudates or detrital material (Bernal and Mitsch, 2012). The carbon-rich sediments drive microbial activity, depleting oxygen in these hydric systems (Pester et al., 2010). As oxygen is depleted, microorganisms seek alternate electron acceptors for respiration, each with successively reduced energy yields. The presence and concentration of electron acceptors, including nitrates, sulfate, and humic substances diminish methane production through competition for carbon resources (Klupfel et al., 2014; Laanbroek, 2010; Liu et al., 2011). Rates of methanogenesis generally do not peak until sulfate has been depleted. However, despite competition for substrates that can be used in other electron accepting processes, methanogens also dependent upon the production of simple substrates by other microorganisms.

Methanogens utilize simple carbon compounds derived from fermentation processes and are classified based on their mode of respiration: methylotrophs utilize single carbon compounds (*e.g.* acetate) while chemolithotrophs use CO_2 and H_2 (Gottschalk, 1985). Acetate and hydrogen are largely derived from the activity of acetogens or fermentative organisms (Liu and Conrad, 2011). Because chemolithotrophs may be limited for hydrogen from fermentative organisms, they are known to form synergistic bonds with bacteria (termed syntrophs) so that energy is gained for both partners (Schink, 1997). These relationships can be ambiguous, as syntrophic interactions may proceed in either direction of methane production or oxidation (Bridgham et al., 2013; Muyzer and Stams, 2008). Furthermore, the factors limiting methanogenesis may be related to compound availability or the absence of certain microbial members.

The application of new genomic tools now allows for higher resolution investigations on microbial communities and their functions in wetlands as they relate to system geochemistry (Wang et al., 2012). For example, broad surveys of the 16S rRNA gene such as those examined from peatlands and coastal mangroves has revealed the presence of novel phyla (Cleary et al., 2012; Serkebaeva et al., 2013). Analysis of microbial carbon functional potential in freshwater wetlands indicated that higher temperatures increased the relative abundance of lignin-degrading genes (Wang et al., 2012). Furthermore, by tracking genes influencing methane emission, Freitag et al. (2010) related methane generation and oxidation. These studies were primarily based on field samples, where experimental factors are difficult to control. They also focused on peatlands, rice paddies, sub-tropical or saline wetlands, while temperate, freshwater wetlands are more poorly represented (Wang et al., 2012). Thus, experimental studies examining how microsite and temperature influence methane emissions in the presence of certain microbial members will better elucidate the factors controlling its flux at the field scale.

We conducted a bench scale experiment to better explain field-scale variability in methane and carbon dioxide emissions across different wetland microsites. Anaerobic microcosms were prepared from sediment cores and water samples from a constructed, urban temperate wetland. Sediments from three different microsites and two depths were incubated at different temperatures to test the influence of these factors on anaerobic, microbial respiration and specifically the maximum potential methane production. Pyrosequencing analysis of the 16S rRNA gene was used to evaluate differences in starting microbial communities. We hypothesized that methane emissions in microcosms would fall near upper limit methane flux potentials measured at the field scale, and that certain microsites would produce significantly more methane than others.

2. Materials and methods

2.1. Site description and sample collection

Samples used in this study were obtained from the Wilma H. Schiermeier Olentangy River Wetland Research Park (ORWRP) on the campus of The Ohio State University, Columbus, OH, USA (40°0′N and 83°1′E) (Fig. 1). The site is composed of two 1-hectare, urban wetlands constructed for experimental purposes in 1992. Water is pumped from the adjacent Olentangy River into the wetlands at a rate proportional to the river stage. Hydrological regimes and temperatures have been well-described at the site (Mitsch et al., 2012). From November through April, Ohio experiences wetter and cooler (mean air temperature 4 °C) conditions, which results in higher water levels, more frequent flooding, and periodical freezing at the wetland. Water levels are lower between May through October when there is less precipitation and warmer temperatures (mean of 21 °C) (Nahlik and Mitsch, 2010). Like many temperate wetlands, the ORWRP is characterized by several different microsites, including upland areas, open water areas, and areas dominated by a particular vegetation. The depth of standing water in deeper basins is generally 50 to 80 cm, while shallower marsh areas have 20 to 40 cm standing water. Emergent macrophytes flourish at the edges of the standing water margins, while the deeper basins are either unvegetated or host submerged plants. The OM content of these sediment ranges from 5% in the vegetated microsite and 8% in open water sediments. Humic acid represents the majority (80%) of organic matter measured within wetland sediments (Hernandez and Mitsch, 2007).

Soil core and water samples were collected from the ORWRP for two separate bench-scale experiments performed during the dormant-season (December 2011) and growing-season (June 2013). For dormant-season experiments, replicate cores were extracted from a one square meter area to a depth of 30 cm using a 7-cm diameter corer. Cores were collected from three microsites in experimental wetland 1: an open-water, unvegetated area in the deeper basin nearest to the river inflow (OW), an edge microsite dominated by emergent *Typha* sp., lateral to the OW microsite (VEG), and an upland site (UP). Cores for the growing-season experiment were again collected from the OW and VEG microsite areas, separated into shallow (0–15 cm, SH) and deep (15–30 cm, DE) sections during sampling. The *Typha* sp. that dominated the VEG microsite during dormant season sampling had been replaced by another emergent plant, *Scirpus* sp. Cores were transported to the Environmental Biotechnology Laboratory at OSU and stored at 20 °C until use. Water samples were collected from the intake pipe of experimental wetland 1 and stored at 4 °C until use. Soil