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Seasonal methanotrophy across a hydrological gradient in a freshwater wetland

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ABSTRACT

Wetlands provide significant ecosystem services but are also the largest natural source of methane (CH_4), a critical greenhouse gas. Oxidation of CH_4 in soils/sediments, driven by methanotrophs, offsets CH_4 losses to the atmosphere. Manipulation of flooding regimes to optimize methanotrophy is a potential management strategy to reduce CH_4 emissions from wetlands and offset global climate change. Therefore, the objectives of this study were to determine rates of potential CH_4 oxidation (PMO) and shifts in methanotrophs over hydrological and seasonal gradients. Surface and subsurface soil samples (0–8 or 8–16 cm depths) were analyzed for PMO and profiled for methanotroph community structure using phospholipid fatty acid (PLFA) analysis over four seasons (winter, spring, summer and fall) and 3 landscape positions (upland, intermittently flooded, and permanently flooded sites). PMO rates were highest in the winter. The permanently flooded sites had higher PMO rates than the intermittently flooded sites ($p < 0.05$). Significantly higher PMO rates were observed in the 0–8 cm compared to the 8–16 cm soil depths ($p < 0.05$). PLFA profiling of methanotrophs showed that both Type I and Type II methanotrophs were dominant in winter. Concentrations of the Type II methanotroph PLFA (18:ω9c) was significantly higher ($p < 0.05$) than those for Type I in all seasons and landscape positions. PMO and methanotroph biomass were highest in the winter and in the PFS which suggested substrate (CH_4) concentration was more important in regulating methanotrophy than redox potential or seasonal shifts in temperature under flooded conditions. PFS had the lowest redox potential (which would not favor aerobic CH_4 oxidation), yet it had the highest PMO rates, suggesting anaerobic methane oxidizers may be important in flooded soils.

1. Introduction

Wetlands provide significant ecosystem services like flood control, remediation of wastewater and agricultural runoff, and carbon sequestration. These benefits are, however, offset by the fact that wetlands are the largest natural source of methane. Methane (CH_4) is a critical greenhouse gas and management strategies are necessary not only to limit CH_4 emission, but also to facilitate its biological sequestration into the soil. Manipulation of the flooding regimes can be a potential management strategy to mitigate CH_4 emission especially from constructed wetlands. There is an interest in particularly promoting wetlands with fluctuating or "pulsing" hydrology because of the additional benefits they provide over static wetlands (Mitsch et al., 2005; Roslev and King, 1996). The fringe or pulsing zones of these wetlands provide favorable conditions like higher supply of oxygen for CH_4 consumption through oxidation by CH_4 oxidizing bacteria or methanotrophs in soil. However, most studies in wetlands have been limited to only quantify the surface CH_4 fluxes, and not explored the environmental or hydrological controls on bacterial CH_4 consumption in the soil. The ecophysiology of soil microbial communities and their feedback effects on global C fluxes under changing climate is yet unclear (Bardgett et al., 2008), and particularly the metabolic potential of methanotrophs is under-investigated.

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The rate of CH_4 emission from an ecosystem is controlled by the rates of two opposing processes: (i) CH_4 production in the soil/sediments by methanogenic archaea; and (ii) oxidation of CH_4 in the soil/sediments and the water column by methanotrophic bacteria. But the relationship of potential methane oxidation (PMO) to the methanotroph biomass and community structure is not fully explored in wetlands (Siljanen et al., 2011). Methanotrophs are ubiquitous in soil (Liu et al., 2013; Roy Chowdhury and Dick, 2013; Sundh et al., 2000). The biological oxidation of CH_4 by methanotrophs, which belong to the α-proteobacteria (Type II methanotrophs) and γ-proteobacteria (Type I methanotrophs), represent the major terrestrial sinks for CH_4. The methanotrophs contain specific phospholipid fatty acids (PLFAs) of 16 (Type I methanotrophs) or 18 (Type II) carbon (C) atoms in chain length (Bowman et al., 1991). A number of such methanotrophic biomarker PLFA have been identified using stable isotope probing in numerous studies in a diversity of study sites and previously listed (Roy Chowdhury and Dick, 2013). These PLFAs have also been used to estimate the relative abundance of Type I and II methanotrophs in environments well supplied with CH_4 (Borjesson et al., 1998; Nichols et al., 1987; Sundh et al., 1995b). In this study, we studied the dynamics of the soil methanotroph community structure using the biomarker PLFAs and their relationship to CH_4 oxidation potential from wetlands in response to "pulsing" hydrology. Our objectives were (i) to estimate the rates of CH_4 oxidation in response to seasonal fluctuations in temperature and water-levels in two freshwater wetlands; (ii) study the relative shifts in the methanotrophic community structure along seasonally induced hydrologic gradients; and (iii) understand the relative response within the methanotrophic communities.

2. Materials and methods

2.1. Description of field site

Two experimental wetlands located at the Wilma H. Schiermier Olentangy River Wetland Research Park (ORW) at The Ohio State University, Columbus, Ohio (Mitsch, 2005) were chosen as the site for this study. Details about the ORW can be found in several studies (Mitsch et al., 1998, 2005, 2012). The two wetlands are field-scale replicates with the same basin morphology; the western basin (W1) was planted with 13 native species of macrophytes in May 1994 while the eastern basin (W2) was allowed to naturally colonize. Water from the Olentangy River, a fourth-order river that drains the central part of an agricultural/urban watershed in Ohio, is controlled by pumps into both the experimental wetlands (Mitsch et al., 1998; Nahlik and Mitsch, 2010). During this study period of 2008–2010, the average annual inflow rates ranged from 975 to 1550 $m^3\ d^{-1}$ (36–57 $m\ yr^{-1}$), total nitrogen inflow concentrations averaged 2.85 mg-N L^{-1} and total phosphorus concentrations averaged 160 μg-P L^{-1} (Mitsch et al., 2012; Nahlik and Mitsch, 2010, 2011).

2.2. Experimental design and soil sampling

The experimental design was a randomized block design with the following treatments: three hydrologically distinct landscape positions viz., Upland Site (UPS), Intermittently Flooded Site (IFS) and the Permanently Flooded Site (PFS) in two replicate wetlands W1 and W2, four seasons (winter, spring, summer and fall) and two soil depths viz., 0–8 and 8–16 cm. The IFS were located at 0–15 cm closer to the edge of the wetlands and within the wetland boundaries. This landscape position was subjected to pulsed flooding with water levels ranging from 10 to 20 cm during spring and fall, and <1-cm during summer. The soil surface in the UPS and IFS was nearly frozen to an average depth of ~5-cm during winter sampling. The PFS had water levels >20 cm throughout the year and were located near the middle of the wetlands.

Soil samples were collected over a two-year period, from October 2008 to October 2010. During the entire study period, pumping rates were adjusted daily according to a predetermined calculation based on the flow of the Olentangy River. This was done to ensure that the wetlands experienced conditions similar to naturally occurring riverine wetlands and the same amount of water was introduced to each wetland (Nahlik and Mitsch, 2010). From November to April, during typically cold (mean air temperature of 4 °C) and wet conditions in Ohio, the wetlands generally experience more frequent flooding and higher water levels. During the warm (mean air temperature of 21 °C) and dry seasons from May to October, low water levels prevail in the wetlands due to reduced river flow. The seasonal sampling times were: winter (December–January–February), spring (March–April–May), summer (June–July–August) and fall (September–October–November). Soil samples were collected twice every season to capture any effects of short-term flooding within a season (Table 1). The within-season samplings were separated by 3–4 weeks contingent on flooding events and water levels.

Soil samples were collected from the inflow and the outflow of both wetlands W1 and W2. A minimum of 20 soil cores were collected to obtain a representative soil sample for each landscape position and depth. Soil cores were collected using a stainless-steel soil probe and placed in zip-lock bags. Soil samples were immediately sieved and homogenized by passing through a 2-mm mesh screen and stored at appropriate temperatures until analysis. Samples for analysis of PMO rates were stored at 4 °C, and those for PLFA analysis were stored at −20 °C. Potential methane oxidation rates were measured within a day of soil sampling. Phospholipid fatty acids were extracted within a week after soil collection.

2.3. Potential methane oxidation (PMO) rates measurement

Potential methane oxidation rates were measured according to modified methods after Crossman et al. (2004) and Sundh et al. (1995a). 10 mL of standard 99.99% CH_4 gas was injected into a polytetrafluoroethylene (PTFE) gas sampling bag to prepare standard calibration curves. Appropriate volumes of the standard CH_4 gas were taken to prepare four calibration standards of concentrations 0.2, 0.25, 0.3 and 0.35% prepared in 125 mL Wheaton serum bottles with butyl rubber stoppers equipped with crimps and purged with N_2 gas. The standards were run in a staggered manner at the beginning and end of the run of each set of samples by gas chromatography and concentrations calculation based on linear regression of five-point standard curves.

Potential methane oxidation rates were measured in triplicates for each soil sample. Fifteen grams of homogenized soil was transferred to 125 mL Wheaton serum bottles and 15 mL of deionized water was added and shaken vigorously; the bottles were evacuated and refilled with air in three cycles. 0.32 mL of the standard CH_4 was added to create a final concentration of 0.35% (v/v) CH_4. The flasks were incubated at 25 °C, shaken horizontally at 150 rpm, and the CH_4 concentration in the gas phase was monitored every hour for 8 h. Gas samples were removed (0.75 mL to optimize detection and sensitivity) from the incubation bottles using a syringe and analyzed by gas chromatography as described below. PMO rate was subsequently obtained from a linear regression fitted to all measurements above 0.1%. The PMO rates are reported as nmol $CH_4\ g^{-1}$ dry weight soil h^{-1}.

A gas chromatograph (Shimadzu GC-2010, Kyoto, Tokyo) with a RT-QPLOT column was used to measure CH_4. The column was