Soil Biology & Biochemistry 95 (2016) 78–86

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Co-variation in methanotroph community composition and activity in three temperate grassland soils

Craig R. Judd [a, b, 1], Akihiro Koyama [a, *], Mark P. Simmons [a], Paul Brewer [a, b], Joseph C. von Fischer [a, b]

[a] Department of Biology, Colorado State University, Ft. Collins, CO 80523, United States
[b] Graduate Degree Program in Ecology, Colorado State University, Ft. Collins, CO 80523, United States

ARTICLE INFO

Article history:
Received 6 July 2015
Received in revised form
1 December 2015
Accepted 18 December 2015
Available online 5 January 2016

Keywords:
Grassland soils
Methanotroph community composition
Methanotroph phylogeny
Michaelis–Menten kinetics

ABSTRACT

Methane is a potent greenhouse gas, and the uptake of methane by methanotrophic bacteria in oxic, well-drained soils is a key global sink. Field studies at the ecosystem scale have observed significant temporal and spatial variation in methane uptake rates, but there is considerable uncertainty about the roles of abiotic and biotic factors, including methanotroph community composition, in structuring these patterns. Here, we present an analysis of Michaelis–Menten kinetics of methane uptake in soils collected from three North American temperate grassland sites of differing soil moisture regimes and their methanotroph community composition. The three sites were Konza Prairie in Kansas, Shortgrass Steppe in Colorado and Sevilleta in New Mexico with mean annual precipitation of 835, 320 and 244 mm, respectively. Michaelis–Menten kinetics and methanotroph community were assessed via lab incubation and *pmoA*-based phylogeny, respectively. Across the precipitation gradient we observed distinct variation in Michaelis–Menten kinetics and methanotroph community composition. Both K_M and V_{Max} values of the Michaelis–Menten kinetics followed the trend of the mean annual precipitation (Konza Prairie > Shortgrass Steppe > Sevilleta). The observed six methanotroph clades were all within the γ-proteobacteria division, and included two novel clades found in Shortgrass Steppe and Sevilleta. The methanotroph communities were dominated by *Methylococcus* spp, JR2 clade, and USC gamma, in Konza Prairie, Shortgrass Steppe and Sevilleta soils, respectively. The distinct differences in the community composition among the three sites may help explain the functional variation of upland methanotrophy observed in K_M. Taken together, the coincident differences in Michaelis–Menten kinetics we observed suggest that methanotroph community composition can be important for CH_4 uptake in controlled environments, potentially playing a role in the variation in methane uptake in the fields.

1. Introduction

Methane (CH_4) is a potent greenhouse gas that is 25 times more powerful than CO_2 in the 100-year time scale (Lelieveld et al., 1998). Despite its relatively low concentration in the atmosphere (approx. 1.8 ppm) compared to CO_2 (approx. 400 ppm), CH_4 contributes to 32% of the current radiative forcing created by the major greenhouse gases (IPCC, 2013). The uptake of CH_4 by upland (i.e. oxic, well-drained) soils is a key part of the global CH_4 budget, removing an estimated 30 Tg CH_4 yr^{-1} (Ciais et al., 2014). Variation in observed rates of CH_4 uptake can arise from multiple factors including methanotroph abundance (Menyailo et al., 2008), temperature (Del Grosso et al., 2000), ammonium concentrations (Aronson and Helliker, 2010) and soil diffusivity (e.g. von Fischer et al., 2009) which is controlled by soil bulk density (Del Grosso et al., 2000) and soil water content (e.g. Schnell and King, 1996). While soil gas diffusivity is a relatively straightforward function of soil porosity and diffusivity (Hillel, 1982), methanotroph activity, shaped by methanotroph abundance and community composition, and their sensitivity to the environment, is a complex ecophysiological response.

A number of studies have argued that community composition can play a substantial role in controlling process dynamics in

* Corresponding author. Present address: Department of Biology, Algoma University, 1520 Queen Street East, Sault Ste. Marie, Ontario P6A 2G4, Canada. Tel.: +1 705 949 2301x1051.
E-mail address: akihiro.koyama@algomau.ca (A. Koyama).
[1] Present address: Office of Forensic Science, Philadelphia Police Department, 843-849 N 8th Street, Philadelphia, PA 19123, USA.

http://dx.doi.org/10.1016/j.soilbio.2015.12.014
0038-0717/

phylogenetically narrow microbial groups that specialize on a biogeochemical process (e.g. Schimel and Schaeffer, 2012). Methane uptake by methanotrophic bacteria in upland soils can be an ideal model system for linking microbial diversity with ecosystem function because the process is likely among the simplest biogeochemical processes (von Fischer et al., 2009).

One way to characterize ecophysiology of methanotroph community is by the Michaelis–Menten kinetics (Michaelis and Menten, 1913), which have been widely used to describe CH_4 uptake by soils as well as cultured methanotrophs (Gulledge et al., 2004). The Michaelis–Menten kinetics model fits reaction rate over a concentration gradient to two parameters; V_{Max} (the maximum velocity) and K_M (the substrate concentration at half of the maximum velocity). While V_{Max} reflects effective enzyme content (i.e. methanotroph quantity), K_M represents enzyme affinity for substrate and enzyme efficiency (i.e. methanotroph quality) (German et al., 2011). Therefore, K_M of CH_4 uptake in a controlled environment can provide a means to assess the relationship between methanotroph community composition and function.

Although ecophysiological differences among the different methanotroph clades have been studied with cultured methanotrophs (Calhoun and King, 1998; Dunfield et al., 1999), soil metagenomic studies indicate that the dominant methanotrophs from many environments remain uncultured (Dumont et al., 2006; Chen et al., 2008). This metagenomic information is primarily based on sequence information from the functional gene methane monooxygenase (MMO). The *pmoA* gene, which codes the first subunit of the particulate MMO enzyme, is widely used as a nearly universal functional gene marker for methanotrophs. Uncultivated methanotrophs with unique *pmoA* sequences have been discovered in a number of soils (e.g. Horz et al., 2005; van Teeseling et al., 2014).

If we are to improve our understanding of the role of methanotrophs in CH_4 biogeochemistry and build next-generation CH_4 cycle models (Schimel, 2000; Green et al., 2008), there is a need to document patterns in methanotroph community composition coincidently with ecophysiological differences among the communities and local environmental variables. To address this goal, we conducted a study on three Long Term Ecological Research (LTER) grasslands along the precipitation gradient in the US Great Plains to explore biotic controls on methanotroph ecophysiology by assessing Michaelis–Menten kinetics. The three grasslands fall along a precipitation gradient, but also vary in other soil properties that may be important for methanotroph ecology. We documented methanotroph ecophysiology via Michaelis–Menten kinetics in the lab, and patterns of methanotroph community. We also documented methanotroph clades found in the grassland soils and their composition via *pmoA*-based sequences and phylogeny. We quantified environmental variables, including soil water content (SWC), pH and inorganic nitrogen (N) concentrations, to assess potential controls over CH_4 uptake kinetics. We hypothesized that the three study sites along the precipitation gradient had distinct methanotroph community composition and K_M values, and that the two results co-varied.

2. Material and methods

2.1. Sampling sites and soil characteristics

We documented community composition, and ecophysiologies of methanotrophs in three distinctly different grasslands along precipitation gradient within the LTER network: Konza (eastern Kansas, KZ), Shortgrass Steppe (north central Colorado, SG) and Sevilleta (central New Mexico, SV). At each site we sampled from summit and toeslope in the landscape to assess local variations. However, the local topographic variations were relatively small compared to those among the three sites, thus, we pooled data collected from summit and toeslope within each site. At KZ (39° 05′N; 96° 35′W) that has 835 mm and 13.1 °C of mean annual precipitation (MAP) and mean annual temperature (MAT), respectively, we collected soil samples at both topographic positions within plots subject to annual prescribed burn. On the SG (40° 49′N; 104° 46′W, 320 mm and 8.6 °C for MAP and MAT, respectively) we sampled at both topographic positions at two sites, one on sandy-loam and one on clay-loam. At SV, (34° 20′N; 106° 43′W, 244 mm and 13.3 °C for MAP and MAT, respectively), measurements and soil samples compared two vegetation types: a local topographic depression dominated by blue gramma (*Bouteloua gracilis*) (toeslope) and a black gramma (*Bouteloua eriopoda*) dominated site (summit).

2.2. Soil collection

We collected soil samples in May 2008; May 28 to 30 for KZ, May 19 and 20 for SG, and May 13 to 15 for SV. Soil core samples (0–10 cm depth, 5.5 cm diameter) were taken from each topographic position of each site (8–12 cores). These cores were used to assess CH_4 oxidation enzyme kinetics, methanotroph community composition, and soil characteristics, including SWC, pH, and inorganic N concentrations ($[NH_4^+]$ and $[NO_3^-]$). Upon collection, the soil cores were placed into collection bags, and stored in a cooler on ice until they were transported to the lab at Colorado State University. Soils were then homogenized and sieved through 2 mm screen where large roots and rocks were removed. To minimize physiological effects of temperature cycling, we held these sieved samples at room temperature for <48 h until they were subsampled for soil chemistry and enzyme kinetic assays. Following the subsampling, the remaining material was frozen to −20 °C for later extraction of DNA.

2.3. Enzyme kinetics

We determined Michaelis–Menten kinetics of CH_4 oxidation as a function of CH_4 concentration using slurried soil samples. Within 48 h of soil collection, 10 g dry weight homogenized soil was placed in 120 mL glass vials and 10 mL of deionized water was added. Vials were sealed with rubber septa (GeoMicrobial Technologies, Ochelata, Oklahoma, USA) and aluminum crimp tops. Gas concentrations were manipulated by adding CH_4 to reach desired concentrations in the headspace. We employed four levels of CH_4 concentrations for the Michaelis–Menten assay; 5, 50, 500 and 5000 μL L^{-1}. To quantify any headspace gas loss during incubation sampling, 40 mL of lab air was injected into triplicate "negative control" vials before incubations began. Soils from three replicate samples from each site were incubated at five different CH_4 concentrations. Following the method modified from Megraw and Knowles (1987), the incubation was carried out in bench top water bath shaker tables at 30 °C and 140 oscillations per minute.

We sampled headspace gases every 8–12 h for up to 48 h, depending on CH_4 uptake rates, which were assessed in previous trial assays. At sampling, we removed 10 mL of air and analyzed the samples for CH_4 concentration on a LGR DLT-100 CH_4/CO_2 analyzer (Los Gatos Research, Mountain View, California, USA) for CH_4 concentrations. Gas samples were injected into a stream of N_2 gas that flowed into the instrument, and sample peaks were integrated using an SRI data acquisition system model 202 and PeakSimple 2000 software (SRI Instruments, Torrence, CA, USA). Calibration was made against commercially prepared and certified standard gases of known CH_4 concentration. Uptake rates were calculated using up to four headspace CH_4 concentrations collected during the