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

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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 KM and VMax 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 KM. Taken together, the coincident differences in Michaelis–Menten kinetics we observed suggest that methanotroph community composition can be important for CH4 uptake in controlled environments, potentially playing a role in the variation in methane uptake in the fields.

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1. Introduction

Methane (CH4) is a potent greenhouse gas that is 25 times more powerful than CO2 in the 100-year time scale (Lelieveld et al., 1998). Despite its relatively low concentration in the atmosphere (approx. 1.8 ppm) compared to CO2 (approx. 400 ppm), CH4 contributes to 32% of the current radiative forcing created by the major greenhouse gases (IPCC, 2013). The uptake of CH4 by upland (i.e. oxic, well-drained) soils is a key part of the global CH4 budget, removing an estimated 30 Tg CH4 yr–1 (Ciais et al., 2014). Variation in observed rates of CH4 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
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 methanotrophic community is by the Michaelis–Menten kinetics (Michaelis and Menten, 1913), which have been widely used to describe CH4 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; Vmax (the maximum velocity) and Km (the substrate concentration at half of the maximum velocity). While Vmax reflects effective enzyme content (i.e. methanotroph quantity), Km represents enzyme affinity for substrate and enzyme efficiency (i.e. methanotroph quality) (German et al., 2011). Therefore, Km of CH4 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 mono-oxygenase (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 CH4 biogeochemistry and build next-generation CH4 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 CH4 uptake kinetics. We hypothesized that the three study sites along the precipitation gradient had distinct methanotroph community composition and Km values, and that the two results co-varied.

2. Material and methods

2.1. Sampling sites and soil characteristics

We documented community composition, and ecophysologies of methanotrophs in three distinctly different grasslands along precipitation gradient within the LTER network: Konza (eastern Kansas, KZ), Shortgrass Steppe (north central Colorado, SC) 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 grama (Bouteloua gracilis) (toeslope) and a black grama (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 CH4 oxidation enzyme kinetics, methanotroph community composition, and soil characteristics, including SWC, pH, and inorganic N concentrations ([NH4+] and [NO3−]). 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 CH4 oxidation as a function of CH4 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, Ocheleta, Oklahoma, USA) and aluminum crimp tops. Gas concentrations were manipulated by adding CH4 to reach desired concentrations in the headspace. We employed four levels of CH4 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 CH4 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 CH4 uptake rates, which were assessed in previous trial assays. At sampling, we removed 10 mL of air and analyzed the samples for CH4 concentration on a LGR DLT-100 CH4/CO2 analyzer (Los Gatos Research, Mountain View, California, USA) for CH4 concentrations. Gas samples were injected into a stream of N2 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 CH4 concentration. Uptake rates were calculated using up to four headspace CH4 concentrations collected during the
incubation for each sample. Headspace CH₄ concentrations typically showed a linear relationship with incubation time, indicating that our method did not have issues of biphasic kinetics reported by Steenbergh et al. (2010), and that pressure change caused by repeated gas sampling from the headspace did not affect CH₄ uptake during the incubation. Lineweaver-Burk plots were used to calculate V₅₀ and Kₘ values.

2.4. Soil chemistry

Within 48 h of soil collection, soils were processed to measure soil pH, gravimetric water content and exchangeable inorganic N. The N assay used 10 g (dry weight) of soil and 50 mL of 2.0 M KCl, stirred for 1 h. The solution was filtered through Whatman no. 1 filter paper. Concentrations of NH₄⁺ and NO₃⁻ were determined colorimetrically on an Alpkem autoanalyzer (OI Analytical, College Station, Texas, USA).

2.5. Data analysis

All computations were carried out using R (R Development Core Team, 2014). We conducted ANOVA with the sites (i.e. KZ, SG and SV) as a fixed effect. Data were log transformed to restore normality when necessary. Tukey multiple comparisons were conducted to assess significant differences, when detected, among the three sites. A significance level of p ≤ 0.050 was used to assess statistical significance, and all p-values are for two-sided confidence intervals.

2.6. DNA extraction and PCR amplification

DNA was extracted from 0.4 to 0.5 g subsamples of soil using PowerSoil DNA Isolation Kits (MoBio Laboratories, CA) following the manufacturer’s protocol; extracted DNA was stored at −20 ºC. PCR amplification reactions were performed in 25.5 microliter (µL) reaction mixtures in a Veriti 96 well thermal cycler (Applied Biosystems, CA). The PCR reaction mix contained 22.5 µL of Platinum PCR SuperMix (Invitrogen Corp.), 1 µL of 10 mM A189F forward primer (GGNGACTGGGACTTCTGG, Holmes et al., 1995), 1 µL of 10 mM mb661R reverse primer (CCGGMGCAACGTCYTTACC, Costello and Lidstrom, 1999) and 1 µL of extracted DNA. The thermal profile consisted of an initial denaturing and enzyme activation step of 94 ºC for 5 min, followed by 40 cycles of 94 ºC for 45 s, 52.5 ºC for 45 s and 72 ºC for 45 s. A final extension was carried out at 72 ºC for 10 min.

Annealing temperature was experimentally optimized to increase PCR yield and limit amplification of undesired products, using a temperature gradient from 49 ºC to 60 ºC. The size and purity of each PCR product was analyzed in a 1% agarose gel with a 100 base pair ladder. If multiple bands were present, correct sized products were gel extracted and cleaned using the Wizard SV Gel and PCR Clean-up System (Promega Corp., Wisconsin, USA).

2.7. Cloning and sequencing of pmoA genes

PCR products were then ligated into the pJET1.2/blunt cloning vector using the CloneJET PCR Cloning kit (Fermentas, Thermo Scientific, Waltham, Massachusetts, USA) according to the manufacturer’s instructions. One Shot TOP10 Chemically Competent E. coli cells (Invitrogen Corp.) were then transformed with the ligated pJET1.2 plasmid using the manufacturer’s protocol. Clones were screened for pmoA gene inserts using vector-specific primers (pJET1.2F and pJET1.2R). DNA sequencing was carried out by the Colorado State University Proteomics and Metabolomics DNA sequencing facility using an ABI 3130 Genetic Analyzer and by the University of Chicago Cancer Research Center DNA sequencing facility using a 3730XL 96-capillary automated DNA sequencer.

Despite repeated efforts, we were only successful at amplifying pmoA from a fraction of the soils sampled. Thus, our community composition dataset includes results from four KZ summit soils (30 sequences), one KZ toeslope soil (18 sequences), two SG summit soils (20 sequences), one SG toeslope soil (22 sequences), one SV summit soil (33 sequences) and two SV toeslope soils (11 sequences). Because the community composition results were not balanced with respect to field sampling, we were not able to quantitatively assess how methanotroph community composition controlled enzyme kinetics of CH₄ oxidation in the lab. However, our recent study showed that differences in community composition between the topographic positions were relatively small compared to those among the three sites (unpublished results). Thus, our results presented in this study most likely represented community composition of the three sites.

We note that examination of extracted DNA (as is done in this study) is not necessarily an examination of the active pool of organisms present in a sample (Molin and Givskov, 1999; Nannipieri et al., 2003). However, because DNA degrades relatively quickly in soil (Widmer et al., 1996; Dale et al., 2002), the composition of the soil DNA pool must result from a dynamic equilibrium where the losses from the soil DNA pool are balanced by inputs, presumably from growth of the resident methanotroph populations. We assert that a parsimonious interpretation of our molecular results is that the observed community composition reflects the genetic potential of the sites, with the relative abundances of different clades reflecting temporally integrated population sizes.

2.8. Phylogenetic analysis

To conduct phylogenetic analysis on our pmoA sequences, we used pmoA and amoA sequences available at GenBank (Benson et al., 2000). These included 141 sequences found in grasslands by Horz et al. (2005), those of cultured type I methanotrophs (e.g. Methylobacter albus, Methylohalobius crimeensis, Methylocaldum szegediense), Upland Soil Cluster (USC) gamma (e.g. WB5FH-A), cultured type II methanotrophs (e.g. Methanocapsa acidophila, Methanosinus trichosporium, Methylocystis echinoides), and amoA (e.g. Nitrosooccus oceatus). Nucleotide-based alignments were obtained using the default alignment parameters in MUSCLE ver. 3.6 (Edgar, 2004). Manual adjustments to the MUSCLE alignments were performed in MacClade ver. 4.03 using the procedure outlined by Simmons (2004), following Zurawski and Clegg (1987). Five out-of-frame insertions were inferred for four different sequences: a 2-bp insertion for KNZ27_12, two 1-bp insertions for AY786014 γ-proteobacteria GCS245, a 1-bp insertion for FJ686567 Methylococcus, and a 1-bp insertion for AM698044 clone 49. All of the insertions were autapomorphic and hence no gap characters (Simmons et al., 2007) were scored. There were no ambiguously aligned nucleotide positions excluded from the phylogenetic analyses, but 31 nucleotides in regions of individual sequences that could not be unambiguously aligned with the remaining sequences were scored as ambiguous. The nucleotide alignment consisted of 477 positions, with the relative abundances of different clades reflecting temporally integrated population sizes.

Phylogenetic analyses were performed using three different character-sampling strategies: all nucleotide characters, nucleotide characters from first and second codon positions only, and amino acid characters. The matrix of all nucleotide characters was found to exhibit significant nucleotide-frequency heterogeneity for the parsimony informative characters, as determined by the chi-square test implemented in PAUP* ver. 4.0 b10 (which ignores
phylogenetic correlations; Swofford, 2001). Shifts in nucleotide composition are typically concentrated at third codon positions (e.g. Prager and Wilson, 1988; Hasegawa et al., 1993) and convergent changes in nucleotide composition can exacerbate long-branch attraction (Woese et al., 1991; Lockhart et al., 1992). Therefore, the phylogenetic analyses based on the amino acid characters and first and second codon positions only were expected to be more robust to this potential problem than the analyses of all nucleotide characters. No significant differences in nucleotide-frequency heterogeneity for the parsimony informative nucleotide characters from the first and second codon positions only were inferred.

Equally weighted parsimony jackknife analyses (JK; Farris et al., 1996) were conducted for each data matrix using PAUP* with the removal probability set to approximately e−1 (36.7879%), and “jac” resampling emulated. Two-thousand jackknife replicates were performed with 100 random addition TBR searches (each with a maximum of ten trees held) per replicate.

We used jModeltest ver. 0.1.1 (Posada, 2008) and ProtTest ver. 2.2 (Abascal et al., 2005) to select the best-fit likelihood model for each data matrix using the Akaike Information Criterion (Akaike, 1974). Following Yang (2006) and Stamatakis (2008), invariant-site models (Reeves, 1992) were not considered because models that incorporated the gamma distribution (Yang, 1993) were considered. The models selected were GTR and GI for all nucleotide characters, TVMef + G for first and second codon positions, and JIT + F for amino acid characters.

Maximum likelihood (Felsenstein, 1973) analyses of nucleotide characters from each of the molecular data matrices were performed as (fallible; Gaut and Lewis, 1995; Siddall, 1998) tests for long-branch attraction. Likelihood analyses were conducted using RAxML ver. 7.03 (Stamatakis, 2006). Given that RAxML only implements the GTR Q-matrix for nucleotide characters, this Q-matrix were applied to both nucleotide-based character-sampling strategies. Optimal likelihood trees were searched for using 1000 independent searches starting from randomized parsimony trees with the GTRGAMMA and PROTCATJTTF models for nucleotide and amino acid characters, respectively. Likelihood bootstrap (BS) analyses (Felsenstein, 1985) were conducted with 2000 replicates and ten searches per replicate using the “–f I” option, which “refine[s] the final BS tree under GAMMA and a more exhaustive algorithm” (Stamatakis, 2008).

All phylogenetic analyses were repeated after excluding seven terminals with the greatest amount (16–41%) of missing/inapplicable data to test for increased resolution and branch support after elimination of these potential “wildcards” (Nixon and Wheeler, 1992). The nucleotide and amino acid-based data matrices have been deposited in GenBank (JN374690 – JN374809). Phylogenetic trees were created using TreeGraph 2 (Stover and Müller, 2010).

All phylogenetic analyses performed assumed hierarchical relationships among the sampled terminals. This assumption could be violated by recombination among the sampled lineages. The pairwise homoplasy index (PHI) test of recombination (Bruen et al., 2006) was performed in SplitsTree 4 (Huson and Bryant, 2006) to the matrix of all nucleotide characters using the default window size of 100 bp and 1000 permutations to test for significance. No significant evidence for recombination was inferred (p = 0.67).

3. Results

3.1. Soil properties

The three study sites differed in all of the physical and chemical properties that we measured (Fig. 1). Soil water contents were significantly different among the three sites (p < 0.001, Fig. 1), and the trend followed the mean annual precipitation of each site, with KZ having the wettest soils at the time of sampling, the SG was intermediate and SV the driest (Fig. 1). Soil pH levels were significantly different among the sites (p < 0.001, with circumneutral values at the SG and KZ, but significantly more basic at SV (Fig. 1). Soil inorganic N contents were significantly different among the three sites (p < 0.001, Fig. 1). Ammonium contents were highest in KZ and lowest in SV (Fig. 1). KZ and SG soils had similar [NO3 ] with levels at both sites higher than SV (Fig. 1).

3.2. Enzyme kinetics

Both KM and Vmax were significantly different among the three sites (p = 0.007 and < 0.001, respectively, Fig. 1). For KM, KZ was significantly greater than SV, but SG was not significantly different from either KZ or SV (Fig. 1). For Vmax, KZ was significantly greater than SG and SV, and SG and SV were not significantly different each other (Fig. 1).

3.3. Phylogeny of methanotroph community

Our pmoA clone libraries consisted of 48, 42 and 44 sequences from KZ, SG and SV soils, respectively. A simplified consensus phylogram is presented in Fig. 2.

All sequences found in this study are within the γ-proteobacteria (Type I) division (Fig. 2). Thirty-eight sequences amplified from KZ soils were in a well-supported clade with Methylococcus capsulatus. The USC gamma sequences (Knief et al., 2003; Kolb et al., 2005) grouped together with 26 of our sequences, mostly from SV soils (Fig. 2). All DNA-based trees showed a common evolutionary origin for what we label as the “Grassland Methanotroph Clade.” The remaining novel sequences are members of this grassland methanotroph clade, which includes previously described clades (JR2 and JR3, Horz et al., 2005; Zhou et al., 2008) and two novel clades. The first novel clade (SGS) consists of two sequences amplified from SG soils and a pmoA sequence found in Inner Mongolian Steppe soil (YC6617, Fig. 1, Supporting material 1, Zhou et al., 2008). The second novel clade (SEV) is composed entirely of sequences amplified from SV soils. The closest pmoA sequences to the SEV were found in saline alkaline soils by Lake Texcoco in Mexico (Supporting material 1, Serrano-Silva et al., 2014). Both clades are closely related to, but distinct from, JR2 and JR3 clades, which include 45 and 21 sequences, respectively from our study.

We assessed amino acid sequences of the two novel clades found in SG and SV (Fig. 2) by comparing to those reported in Horz et al. (2005, Table 1). Horz et al. (2005) contend that JR2 and JR3 clades represent methanotrophs, in part due to their conserved amino acid residues shared between pmoA and amoA sequences (Holmes et al., 1999; Ricke et al., 2004) and shared residues considered diagnostic of pmoA (Holmes et al., 1999). SGS sequences shared all 16 conserved amino acid residues shared between pmoA and amoA sequences, while SEV sequences shared all but one. SGS and SEV sequences also shared all diagnostic amino acid residues of pmoA as reported in Horz et al. (2005). Notably, a sequence that was resolved as sister to all other members of the SEV clade (SEV3 14), has 17 unambiguously optimized autapomorphies, as mapped onto the DNA parsimony jackknife tree, with several of these being in conserved and diagnostic amino acids (Table 1).

3.4. Community composition

The distribution of methanotroph clades found in each site is presented in Fig. 3. KZ, the wettest site, exhibited the lowest methanotroph diversity and was dominated (79%) by sequences in
the Methylococcus capsulatus clade, with JR2-like sequences present as well (21%). At the intermediate SG site, methanotroph communities were dominated by sequences from the JR2 clade (67%) with JR3 also abundant (25%), while SGS (4%) and USC gamma (4%) sequences made up a minor portion of the overall SG community. At the dry end, SV methanotrophs were dominated by USC gamma sequences (55%), while SEV (18%) and JR3 (20%) sequences also made a substantial contribution to the communities, and JR2 (7%) sequences made the smallest contribution.

4. Discussion

4.1. Michaelis–Menten kinetics of CH₄ uptake

Comparing the Michaelis–Menten kinetics of CH₄ oxidation and communities of methanotrophs offers one approach for evaluating ecophysiological differences because the assay measures enzyme function under uniform, controlled laboratory conditions. Since many upland methanotrophs are believed to rely solely on CH₄ oxidation for both C and energy (Dedysh and Dunfield, 2010), it is likely that MMO enzyme structure reflects local conditions to allow the efficacious function in that habitat.

The Michaelis–Menten kinetics of KZ soils were strongly distinct from the other sites in both KM and VMax parameters. While SG and SV were not significantly different from each other, SV tended to have lower KM and VMax values. In general, our observed CH₄ oxidation kinetics were similar to those reported previously in similar soils. High-affinity CH₄ oxidation (KM 4–740 nM) has been consistently measured in upland soils (Knief et al., 2003; Saari et al., 2004), while low-affinity oxidation (KM 1.1–66 μM) is typically found in soils with a larger CH₄ supply (i.e. landfill cover soil or wetland soil; Whalen et al., 1990; Whalen and Reeburgh, 1996). Although the measured kinetics showed variability, the SV and SG soils showed CH₄ affinities typical of atmospheric CH₄ oxidation at 68 and 357 nM, respectively (Fig. 2). Interestingly, KZ soils had average kinetics similar to bogs and other CH₄ emitting soils (1133 nM).

Maximum uptake rates (VMax) demonstrated a similar pattern to KM. SV and SG soils exhibited VMax Values comparable to those in several hardwood, mixed, and pine forest soils (Gulledge and Schimel, 1998; Bull et al., 2000; Knief et al., 2003; Gulledge et al., 2004). However, KZ showed similar VMax Values to other high CH₄ soils, such as bogs and landfill soils (Whalen et al., 1990; Whalen and Reeburgh, 1996).

We hypothesize that the among-site variation in enzyme kinetics reflect differences in soil CH₄ supply. The Michaelis–Menten model predicts that greater substrate affinity (i.e. lower rates of substrate dissociation and thus lower KM) also slows the maximum catalytic rate, thus causing a lower VMax. Such a KM–VMax tradeoff suggests that the optimal KM should rise with soil CH₄ concentration, and this prediction is consistent with studies showing a positive correlation between KM and VMax.
for CH₄ oxidation (in Gulledge et al., 2004). We hypothesize that the highest K_M values at KZ are caused by high soil CH₄ concentrations (>10 ppm) due to endogenous CH₄ production in those soils. Previous studies have found that some well-drained, oxic soils exhibit “occult” or hidden CH₄ production despite net CH₄ uptake (von Fischer and Hedin, 2002). The wetter soil conditions at KZ (Fig. 1) would favor more occult methanogenesis, thus elevating soil CH₄ concentrations. Similarly, the drier SV and SG soils are expected to have less occult methanogenesis, so the greater CH₄ affinity in those methanotroph communities allows them to live off of atmospheric sources of CH₄ (~1.8 ppm).

Fig. 2. Simplified parsimony tree showing phylogenetic relationships between pmoA alleles sequenced in three LTER grasslands and pmoA and amoA alleles available in public-domain databases. Values above branches represent bootstrap support values from maximum likelihood analyses, while values below branches represent jackknife support values from parsimony analyses. Leftmost values represent support values using all DNA nucleotides, middle values represent support values using the first and second codon positions, and rightmost values represent support values using amino acid translations. amoA sequences from public-domain databases were used as outgroups (Ammonia-oxidizing bacteria, A.O.B.). Support values in brackets indicate that the clade was contradicted in the given analysis with bootstrap or jackknife support for the highest contradictory clade shown. Descriptions of YC6617 are in Supporting material 1.
4.2. Methanotroph community composition

Our assessments of the pmoA gene pools indicate that each site has a distinct set of bacteria dominating the local methanotroph community (Fig. 3). Strains related to Methylococcus dominate at KZ, while at SG and SV clades JR2 and USC gamma dominate, respectively (Fig. 3). If these community differences translate to changes in CH4 uptake kinetics as shown in this study, or methanotroph response to external stressors, then community composition has a strong potential to affect ecosystem functions. A review of the literature shows that some methanotroph groups appear to have a restricted biogeographic distribution. For example, the JR2 and JR3 clades have only been found in arid grasslands (Horz et al., 2005; Zhou et al., 2008; Angel and Conrad, 2009; this study), while USC gamma has, thus far, only been found in soils with a pH > 6 (Knief et al., 2003; Zhou et al., 2008; Kolb, 2009; this study).

These emerging patterns along the precipitation gradient may be a result of methanotroph taxa specializing on key environmental properties, but there is much current debate, both in microbial ecology and in the broader field of ecology, about the relative importance of neutral processes (Hubbell, 2001) vs. competitive interactions (Tilman, 1994) for structuring the composition of communities. Under the competition framework, methanotrophs with the optimal suite of traits for the local environment will become dominant in the local community. Under this paradigm, our results suggest that the dominant methanotrophs at each site are abundant because they are best suited to local conditions. We hypothesize that Methylococcus capsulatus are better adapted to wet soils, JR2 and JR3 are best suited for arid grasslands, and USC gamma have traits that allow it to outcompete other methanotrophs in more alkaline soils. The composition of the SG community appears to fall between the KZ and SV communities (Fig. 3), matching the gradient of precipitation across these sites. However, that observation can only be considered a trend at this time since this experiment was not designed to fully address spatial and temporal community dynamics.

The conservation of functional and diagnostic amino acid residues (Table 1) suggests the novel sequences that make up SGS and SEV clades code for methane monooxygenase enzymes as opposed to ammonia monooxygenases (Holmes et al., 1999; Horz et al., 2005). Our phylogenetic analyses provide only weak to moderate support for the grassland clades being sister to a Nitrosococcus clade (59% likelihood bootstrap and 75% parsimony jackknife support, Fig. 2), the only known ammonia-oxidizing bacteria among the γ-proteobacteria. Based on key amino acid differences, Horz et al. (2005) argued that there is strong support for JR2 and JR3 clades (clades that are closely related to our novel SGS and SEV clades) being methane-oxidizing bacteria. With the exception of a single sequence (SEV3 14), our novel SGS and SEV clades share all key amino acids with the JR2 and JR3 sequences as shown in Horz et al. (2005), also suggesting these novel clades to be methane-oxidizing bacteria (Table 1).

5. Conclusion

There is considerable interest in understanding abiotic and biotic factors that control microbial CH4 uptake rates in terrestrial ecosystems. We found that the three grassland sites along the
precipitation gradient have distinct methanotroph communities and CH4 oxidation ecophysiology. We suggest a framework to elucidate CH4 dynamics in grassland ecosystems and the impacts of the local methanotroph communities on them. First, assess environmental regime in the field along environmental gradients (i.e., temperature and precipitation) with fine temporal resolution (e.g., every other week). The environmental regime includes CH4 uptake rates, pH, soil moisture, diffusivity and temperature. Second, identify sizes and composition of methanotroph community in soils collected in the first process. Third, assess ecophysiology of methanotroph activity including their sensitivity to moisture and temperature. Finally, determine controlling factors for CH4 uptake as an ecosystem function using abiotic and biotic components assessed in the previous steps.

This study improves the empirical linkage between methanotroph ecophysiology and community composition. It may be easier to develop this linkage between community composition and ecosystem function because CH4 uptake is, arguably, one of the simplest biogeochemical processes; the substrate supply is easy to quantify and the catalytic organisms are phylogenetically cohesive. We find that the different ecophysiologival patterns parallel differences in methanotroph community composition, though it appears conflicting these to field uptake rates will require a large comprehensive study measuring many factors over an extended time period. Together, our work contributes to understanding mechanisms behind CH4 dynamics in grassland soils, and provides a framework to improve the understanding in the future.

Acknowledgments

This research was supported by NSF DEB 1054956 to JVF and by NSF DEB 0217631 to the Shortgrass Steppes Long-Term Ecological Research project. Anita Kear and Greg Ames contributed invaluable efforts in the field and laboratory. We acknowledge laboratory help and discussion with Noah Fierer, Greg Butters, Charlotte J. Alster and Colleen Webb.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jsolbio.2015.12.014.

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