Received: 4 February 2024 | Revised: 29 February 2024 | Accepted: 18 March 2024

DOI: 10.1002/ecs2.4955

ECOSPHERE
AN ESA OPEN ACCESS JOURNAL

ARTICLE

Macrosystems Ecology

Biological and physical controls of methane uptake in grassland soils across the US Great Plains

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Funding information
Division of Environmental Biology, Grant/Award Number: 1054956

Handling Editor: Dawn M. Browning

Abstract

The grassland biome is an important sink for atmospheric methane (CH_4), a major greenhouse gas. There is considerable uncertainty in the grassland CH_4 sink capacity due to diverse environmental gradients in which grasslands occur, and many environmental conditions can affect abiotic (e.g., CH_4 diffusivity into soils) and biotic (e.g., methanotrophy) factors that determine spatial and temporal CH_4 dynamics. We investigated the relative importance of a soil's gas diffusivity versus net methanotroph activity in 22 field plots in seven sites distributed across the US Great Plains by making approximately biweekly measures during the growing seasons over 3 years. We quantified net methanotroph activity and diffusivity by using an approach combining a gas tracer, chamber headspace measurements, and a mathematical model. At each plot, we also measured environmental characteristics, including water-filled pore space (WFPS), soil temperature, and inorganic nitrogen contents, and examined the relative importance of these for controlling diffusivity and net methanotroph activity. At most of the plots across the seven sites, CH_4 uptake rates were consistently greatest when WFPS was intermediate at the plot level. Our results show that variation in net methanotroph activity was more important than diffusivity in explaining temporal variations in net CH_4 uptake, but the two factors were equally important for driving spatial variation across the seven sites. WFPS was a significant predictor for diffusivity only in plots with sandy soils. WFPS was the most important control on net methanotroph activity, with net methanotroph activity showing a parabolic response to WFPS (concave down), and the shape of this response differed significantly among sites. Moreover, we found that the WFPS level at peak net methanotroph activity was strongly correlated with the mean annual precipitation of the site. These results suggest that the local precipitation regime determines unique sensitivity of CH_4 uptake rates to soil moisture. Our findings indicate that grassland CH_4 uptake may be predicted using local soil water conditions. More variable soil moisture, potentially induced through predicted future extremes of rainfall and drought, could reduce grassland CH_4 sink capacity in the future.

KEYWORDS
gas diffusivity, grassland CH_4 uptake, mean annual precipitation, mean annual temperature, net methanotroph activity, soil texture, the US Great Plains, water-filled pore space

INTRODUCTION

Grassland soils are an important sink for atmospheric methane (CH_4), a greenhouse gas contributing approximately 20% of the current global warming potential created by the major greenhouse gases (Etminan et al., 2016). Estimated global uptake of CH_4 by grasslands ranges from 1 Tg C·year^{-1} (Potter et al., 1996) to 3.7 Tg C·year^{-1} (Yu et al., 2017), and there is considerable uncertainty in estimated CH_4 sink strength of the grassland biome (Dutaur & Verchot, 2007; Xu et al., 2016). Such varying estimates and uncertainty in grassland CH_4 uptake are in part because grasslands occur in diverse environmental conditions. For instance, grasslands can be found along mean annual precipitation and temperature (MAP and MAT) gradients between 250 and 1000 mm, and between 0 and 26°C, respectively (Blair et al., 2014; Whittaker, 1975). Such large gradients in precipitation and temperature regimes in the grassland biome create diverse abiotic and biotic conditions which regulate soil CH_4 fluxes.

Soil CH_4 fluxes of well-drained, oxic soils in grasslands are controlled by both abiotic and biotic factors. Gas diffusivity is the most prominent abiotic factor, which controls supply rates of CH_4 from the atmosphere into the soil matrix where methanotrophs (CH_4 consuming bacteria) reside, whereas CH_4 consumption by methanotrophs represents the major biotic factor (Brumme & Borken, 1999; Smith et al., 2003). Gas diffusivity is, in principle, controlled by soil texture and water conditions with higher sand and less soil water content leading to higher gas diffusivity (Ball et al., 1994; De Bernardi et al., 2019; Grover et al., 2012; Moldrup et al., 2001). However, the influences of soil texture and water content can be weakened by the presence of soil macropores, which permit greater gas diffusivity (Ball et al., 1994). A soil's capacity for methanotroph activity arises from the methanotrophic bacteria, which use CH_4 as the primary source of energy (Hanson & Hanson, 1996). Like other bacterial activity, CH_4 oxidation by methanotrophs is controlled by environmental conditions in soils, including water availability (Borken et al., 2006; Schnell & King, 1996), temperature (Chen et al., 2011; van den Pol-van Dasselaar et al., 1998, but see King & Adamsen, 1992), and pH (Smith et al., 2000; Xiu-jun et al., 2001, but see MacDonald et al., 1996). In addition, CH_4 oxidation by methanotrophs can be diminished by high levels of ammonium (NH_4^+) (Adamsen & King, 1993; Bronson & Mosier, 1994; Dunfield & Knowles, 1995), likely because methanotrophs can oxidize NH_4^+, when abundant, in addition to CH_4 (Hanson & Hanson, 1996). The net methanotroph activity of soils can be further diminished by the activity of methane-producing microbes, which can occur even in well-drained oxic soils (Angel et al., 2012). In general, methanogenesis is considered negligible in oxic soils as methanogens are anaerobes, thus the presence of oxygen prohibits this process (Fetzer & Conrad, 1993; Yuan et al., 2009). However, environmental conditions, such as high soil water content (Moyano et al., 2013) and oxygen demand (Keiluweit et al., 2018), can create anoxic microsites which trigger methanogenesis (Brewer et al., 2018). Thus, the net CH_4 oxidation via methanotrophy in grassland soils can be offset by methanogenesis, resulting in reduced net CH_4 uptake or even occasional net CH_4 emission (e.g., Kammann et al., 2001). Together, the suite of environmental factors regulating each component of a net CH_4 flux—diffusivity and net methanotroph activity—make it challenging to estimate spatial and temporal variability in grassland CH_4 sink capacity.

Some studies observed tight correlations between CH_4 uptake and diffusivity in oxic, well-drained soils, leading to a conclusion that CH_4 fluxes were primarily controlled by gas diffusivity (Born et al., 1990; Curry, 2007; Dörr et al., 1993; Keller & Reiners, 1994; Kruse et al., 1996; Striegl, 1993). However, other studies did not show such clear trends (e.g., Ball et al., 1997; Livesley et al., 2013), suggesting substantial roles of the biotic component in soil CH_4 fluxes. Indeed, capability for methanotrophic activity to dominate net CH_4 uptake was demonstrated via an approach that combined measurements of net CH_4 uptake and gas diffusivity in situ in combination with mathematical modeling by von Fischer et al. (2009): Over the course of a summer month with little precipitation in Shortgrass Steppe (SG), soil CH_4 uptake gradually declined, which was associated with a slight but steady increase in gas diffusivity paired with rapid decline in net methanotroph activity. Just after a major precipitation event for the first time in 2 months, soil CH_4 uptake spiked, which was driven by a drop in gas diffusivity in combination with dramatically stimulated net methanotroph activity (von Fischer et al., 2009). That novel approach provided a mechanistic method to quantify how soil CH_4 fluxes are controlled by both diffusivity and net methanotroph activity, and an opportunity to explore better ways to predict CH_4 dynamics and sink capacity of grassland soils. Furthermore, we may be able to predict the two major components if we understand how primary environmental conditions, such as soil water content and temperature, control the two components. This systematic understanding is critical as we need an ability to accurately estimate CH_4 sink capacity of the grassland biome where many ecosystems are exposed to environmental changes such as climate warming which can cause, for instance, extreme precipitation regimes (Knapp et al., 2015; Stocker et al., 2013).

In this study, our overarching goal was to better understand the relative importance of abiotic and biotic factors in determining CH_4 sink capacity of grasslands across the US Great Plains, and how these factors were controlled by other environmental factors such as edaphic characteristics, soil water content, and soil temperature. We measured CH_4 fluxes in diverse grasslands including short-, mixed- and tall-grass prairies and a desert grassland during growing seasons over the course of 3 years. This extensive dataset can provide us insights into spatial and temporal variations of CH_4 dynamics in grasslands which cover up to 40% of the earth's land surface (Blair et al., 2014). Our first objective was to assess how CH_4 fluxes, diffusivity, and net methanotroph activity (defined as CH_4 oxidation via methanotrophy, offset by methanogenesis) were influenced by soil characteristics, including water-filled pore space (WFPS), texture, temperature, inorganic N contents, topographic position (i.e., upland and lowland) which affects soil structure, water, and nutrient biogeochemistry (Hook & Burke, 2000). Our approach evaluated the relative importance of these potential controls. Our second objective was to determine the relative contributions of gas diffusivity and net methanotroph activity for explaining variation in soil surface CH_4 uptake, using the approach developed by von Fischer et al. (2009). Finally, we assessed the characteristics of net methanotroph activity in response to WFPS at the regional scale (Objective 3). To broaden the range of natural environmental conditions in our seven study sites, we took advantage of ongoing field treatments at three of the sites that simulate predicted global changes: increased precipitation (Blankinship et al., 2010), altered fire regimes (Sullivan et al., 2011), and nitrogen deposition (Aronson & Helliker, 2010).

MATERIALS AND METHODS

Study sites

We selected a total of 22 plots in seven sites along the environmental gradient across the US Great Plains (Table 1; Appendix S1: Figure S1). MAP and MAT range from 244 to 835 mm and 6.1 to 13.3°C, respectively (Table 1). At each site, we have established two to six 10 × 10 m plots (modified where experiments had been already in place) to sample natural and experimentally induced heterogeneity. At six sites, including Makoce (MA), Nine Mile (NM), Ordway (OR), SG, Sand Hill (SH), and Sevilleta (SV), paired lowland (toe-slope of hill, but not permanently inundated) and upland (hill summit) plots were selected as the different topographic positions can induce persistent patterns in soil moisture and nutrient levels (Hook & Burke, 2000) which in turn affect CH_4 dynamics and methanotroph and methanogen ecology (Table 1). At Konza (KZ), we included two plots on an irrigation treatment that mimics soil conditions on the wettest edge of the Great Plains (Knapp et al., 2001), and two plots subject to different prescribed fire regimes (Hartnett et al., 1996). At SG, we used a long-term (>15 years) experiment with factorial, supplemental rainfall and nitrogen additions, where 30 kg $ha^{-1}\cdot year^{-1}$ of N in the form of ammonium nitrate, and 210 mm of water had been added between May and September annually (Nicole Kaplan, Colorado State University, personal communication). At SV, we took advantage of field treatments of the summer monsoon, where plots receive ambient rainfall, ambient plus weekly 5 mm additions, or ambient plus 20 mm monthly additions (Thomey et al., 2011). Within each of the 22 plots, we established six ~1 m radius sampling areas for repeated sampling of soils and trace gas fluxes.

Sample collection

Soil samples were collected in summer 2011 to assess their static characteristics, including texture and soil organic carbon (SOC) and nitrogen (N) contents. Six soil cores (10 cm in depth, 5 cm in diameter) were collected at each of the 22 locations, and analyzed at EcoCore in Natural Resource Ecology Laboratory, Colorado State University. Soil texture was determined using a hydrometer method (Elliott et al., 1999; Gee & Bauder, 1986). To determine SOC and N content, ~5 g of each soil sample was dried at 60°C for 48 h and finely ground in a ball mill. Total C, including SOC and inorganic carbon (C), and N were measured using a LECO CHN-1000 analyzer (LECO Corp., St. Joseph, Minnesota, USA). Inorganic C content, if any, was determined using a pressure-calcimeter method following Sherrod et al. (2002). Soil organic C content was determined based on the difference between total C and inorganic C contents. Stable isotope ratios of soil organic C and total N were determined by a VG Isochrom continuous flow isotope ratio mass

TABLE 1 Characteristics of the seven study sites, and the 22 plots across the sites.

Site	MAP (mm)	MAT (°C)	Elevation (m)	Grass type	US state	Coordinates[a]	Plot ID	Plot description
Konza (KZ)	835	13.1	100	Tall	KS	39.107° N 96.609° W	KZC	Control
							KZ + W	Water[b]
							KZ4	4-year burn[c]
							KZ20	20-year burn[c]
Makoce (MA)	627	7.8	498	Tall	SD	43.553° N 96.968° W	MAL	Lowland
							MAU	Upland
Nine Mile (NM)	721	10.7	356	Tall	NE	40.866° N 96.818° W	NML	Lowland
							NMU	Upland
Ordway (OR)	513	6.1	600	Mixed	SD	45.703° N 99.112° W	ORL	Lowland
							ORU	Upland
Shortgrass Steppe (SG)	320	8.6	1650	Short	CO	40.813° N 104.777° W	SGC	Control
							SG + N	Nitrogen[d]
							SG + W	Water[d]
							SG + NW	Nitrogen and water[d]
							SGL	Lowland
							SGU	Upland
Sand Hill (SH)	576	8.1	881	Mixed	NE	42.246° N 99.654° W	SHL	Lowland
							SHU	Upland
Sevilleta (SV)	244	13.3	1480	Desert	NM	34.355° N 106.884° W	SVC	Control/upland[e]
							SV + WS	Small water[e]
							SV + WL	Large water[e]
							SVB	Bluegrama/lowland

[a] Latitude and longitude in the WGS84.

[b] Water had been added to meet actual evapotranspiration since 1991 (Knapp et al., 2001).

[c] 4- and 20-year burn plots had been burned every 4 and 20 years, respectively, since the mid-1970s (Hartnett et al., 1996).

[d] Nitrogen and water had been added 30 kg N·ha^{-1}·year^{-1} (as ammonium nitrate) and 210 mm between May and September, respectively, since 1996.

[e] Water had been added in the small (5 mm week^{-1}) and large (20 mm month^{-1}) water experiments since 2006.

spectrometer (Isoprime, Manchester, UK) coupled with a Carlo Erba NA 1500 elemental analyzer (Carlo Erba Strumentazione, Milano, Italy). In case soils had inorganic C, the samples were treated with HCl fumigation (Harris et al., 2001) before δ^{13}C analyses, and δ^{15}N analyses were conducted separately using soils without HCl fumigation. Stable isotope ratios of SOC of grasslands in the Great Plains reflect relative production of C_3 and C_4 plants, which is primarily controlled by summer temperature (von Fischer et al., 2008), whereas δ^{15}N of total N could reflect MAP (Amundson et al., 2003). Thus, δ^{13}C of SOC and δ^{15}N of total N could be considered as indices to integrate summer temperature and MAP, respectively.

Soil bulk density was determined at three depths (5, 15, and 25 cm) following Elliott et al. (1999). At each plot, a soil pit (0.7 × 0.4 m and 0.5 m deep) was excavated in the vicinity of the gas sampling area. Three soil corers (5 cm in diameter and 10 cm in height) were horizontally inserted into soils at each depth. The three soil samples collected at each depth were combined in a plastic bag and brought back to the lab. Rocks larger than 2 mm in diameter were removed by sieving with a 2-mm screen and their volume was calculated by the water displacement method. Sieved soils were dried at 105°C for 48 h, and the dry soil masses in combination of rock volumes were used to determine the soil bulk density. Total porosity was calculated using soil bulk density and particle density (2.65 g cm^{-3}, Keller et al., 2011).

Methane and CO_2 fluxes and diffusivity were measured using a chamber-based method following von Fischer et al. (2009). Six cylindrical soil collars (20 cm in diameter, 15 cm in height) were driven into the soil to 10 cm in depth at each of the 22 plots in summer 2010. During the growing seasons between 2011 and 2013, these fixed collars were repeatedly used to measure CH_4 and CO_2 fluxes and diffusivity. A dome-shaped chamber head attached with a 30-cm Tigon tube was used for the measurements. The tube had a Luer Lock adapter at the end which could be attached to a syringe. The chamber head could be attached to a collar tightly with milled grooves on their mating surfaces, and a strip of closed cell foam was placed between them to keep the seam airtight. The headspace made of the collar and chamber head was approximately 6.5 L.

A set of chamber flux measurements took approximately 15 min. Then, 30 s after the chamber head was tightly placed onto a collar, 60 mL of diluted inert gas (sulfur hexafluoride, SF_6) was injected into the headspace via the attached tube using a syringe and mixed. Four 30 mL gas samples were collected with a syringe from the headspace 1, 2, 3, and 4 min after the SF_6 injection. The gas samples were placed in pre-evacuated glass vials with septa and kept in a cooler until they were brought back to the lab. Gas samples were analyzed for CH_4, CO_2, and SF_6 concentrations by gas chromatography on a Shimadzu GC14B (Shimadzu, Kyoto, Japan) in the lab at Colorado State University.

At the end of each gas sampling, we collected soil samples in the vicinity of each soil collar to assess gravimetric soil water content, pH, and inorganic N content (i.e., NH_4^+ and NO_3^-). For each soil collar, three soil cores were collected approximately 1 m away from the collar and three randomly selected directions and pooled. Soil samples were placed in polyethylene bags and transported back to the lab on ice in a cooler. The soils were kept at 4°C until processing. All the soils were sieved with a 2-mm screen before downstream analyses. Gravimetric water contents were determined by drying soils at 105°C for 48 h. Volumetric soil water contents were calculated using gravimetric water contents and bulk soil density. WFPS was calculated by dividing volumetric soil water content by total porosity. Soil pH was measured following Thomas (1996). In a plastic container, 10 g of fresh soil and 10 mL of deionized water were stirred together and pH was measured using an Orion 3 Star Benchtop pH meter (Thermo Fischer Scientific, Waltham, Massachusetts, USA). Inorganic N was extracted from 5 g of each soil in 45 mL of 1 M KCl for 12 h followed by filtering through a filter paper (Grade 454, VWR International, Randnor, Pennsylvania, USA). Concentrations of inorganic N were measured colorimetrically on an Alpkem autoanalyzer (OI Analytical, College Station, Texas, USA) at the Natural Resource Ecology Laboratory of Colorado State University.

Soil temperature was monitored using data loggers (Decagon ech2o, Pullman, Washington, USA) which recorded continuous soil temperature and moisture readings (Decagon 5T soil moisture and temperature probes, Pullman, Washington, USA). Soil temperature was also measured using handheld digital thermometers (Hanna Instruments, Rhode Island, USA) inserted to 10 cm in depth at each chamber when gas samples were collected.

Gas flux analyses

Rates of CH_4 and CO_2 fluxes, and gas diffusivity using SF_6 were calculated based on changes in concentrations of sequentially collected gas samples from each chamber at each sampling event following von Fischer et al. (2009) using JMP (SAS Institute, Cary, North Carolina, USA). To calculate net methanotroph activity (μ, "methanotroph activity" in von Fischer et al., 2009), only CH_4 fluxes of net uptake (i.e., negative values) were chosen. Out of a total of 2978 net CH_4 flux measurements at the chamber level, 2219 measurements were qualified for calculating net methanotroph activity. Using the qualified net CH_4 flux measurements and corresponding diffusivity at the chamber level, μ was estimated via an equation (von Fischer et al., 2009):

$$q(x=0) = C_0\sqrt{Da\mu},$$

where q is the net CH_4 flux (in moles per square centimeter per minute), C_0 is the initial gas concentration (in moles per cubic centimeter), D is diffusivity (in square centimeters per second), a is the air-filled porosity (in cubic centimeters per cubic centimeter), and μ is the net methanotroph activity (per minute).

The net CH_4 flux rates in *Results* are expressed in a commonly used unit (in milligrams of carbon per square meter per day). We also note that a net CH_4 flux rate is a multiplicative product of diffusivity and net methanotroph activity in this equation. This nature was used to assess the relative importance of diffusivity and net methanotroph activity for the observed net CH_4 flux at the plot level by calculating the CV.

Statistical analyses

All statistical computations were carried out in R (version 3.4.3, R Core Team, 2017). A principal components analysis (PCA) was employed to summarize the diversity of climate and edaphic factors of the 22 plots across the seven sites. The factors used in the analysis were MAP and MAT (Table 1) and static edaphic characteristics, including bulk density, soil texture, SOC, total N, C:N ratio, and stable isotope ratios of SOC and total N. In addition, we used 10 dynamic soil characteristics, including WFPS, soil respiration rates, soil temperature, inorganic N concentrations (i.e., N-NH_4^+ and N-NO_3^-) and their ratios, pH, net CH_4 flux rates, diffusivity and net methanotroph activity quantified during the three growing seasons between 2011 and 2013. For the 10 dynamic soil characteristics, mean values at the chamber level ($N = 6$ per plot) were used for the PCA. The analysis was conducted using the built-in *prcomp* function in R (R Core Team, 2017) and results were visualized using SigmaPlot (Systat Software, San Jose, CA, USA).

Linear mixed-effect models were used to assess the relative importance of environmental factors for net CH_4 fluxes, diffusivity, and net methanotroph activity using the *lmer* function in the package *lme4* (Bates et al., 2014). Datasets were selected in ways that common predictive variables could be used in each model. Specifically, a total of four datasets were (1) six sites (MA, NM, OR, SG, SH, and SV) which had paired upland/lowland plots, (2) four KZ plots with precipitation and fire treatments, (3) four SG plots with N and water treatments, and (4) four SV plots with precipitation treatments and vegetation types (Table 1). In each analysis, a full model was first built using all the potential predictive variables which could affect diffusivity or net methanotroph activity based on a priori knowledge. The predictive variables included WFPS^2 because past studies showed nonlinear responses of net CH_4 fluxes and net methanotroph activity to soil water content (e.g., Bowden et al., 1998; Dijkstra et al., 2011, 2013; Saari et al., 1998). Then, a series of reduced models with fewer predictive variables were built, and we used model selection techniques to find a best-fit model using Akaike information criterion (AIC, Akaike, 1974). Using full and the best-fit models, we assessed the relative explanatory power of predictive variables using mean sum of squares (MSS) statistics. Overall fit of regression models was also assessed by calculating conditional R^2 which incorporated both fixed and random effects (Nakagawa & Schielzeth, 2013). A significance α level of 0.05 was used to assess statistical significance.

To assess the relative contributions of diffusivity and net methanotroph activity for temporal and spatial variations of net CH_4 uptake rates, CVs were employed. To assess the temporal contributions, averages and SDs were calculated using individual data points at the plot level for diffusivity and net methanotroph activity, with which CVs were derived. The spatial contributions were assessed at two levels, within-site and across the seven sites. First, chamber-level averages were calculated for upland and lowland plots at each site. At KZ which did not have upland/lowland plots, the control and water-addition plots were employed (Table 1). The resulting 12 average values of diffusivity and net methanotroph activity for each site were used to calculate site-level averages and SDs, which were used to calculate CVs. The site-level CVs should address spatial variabilities of diffusivity and net methanotroph activity within each site. To assess the spatial contributions across the seven sites, site-level averages were first calculated by averaging the 12 chamber-level means at each site. Using the site-level averages, CVs across the seven sites were calculated for diffusivity and net methanotroph activity.

RESULTS

Environmental and edaphic characteristics and biogeochemical dynamics

Climatic (Table 1) and soil characteristics (Table 2) suggest that our 22 plots across the seven sites captured much of the broad environmental diversity that might affect net methanotroph activity and gas diffusivity, and thus CH_4 dynamics in the US Great Plains grasslands. The seven sites ranged in MAP and MAT, from 244 mm (SV) to 835 mm (KZ), and 6.1°C (OR) to 13.3°C (SV), respectively (Table 1). Soil bulk densities varied from 0.8 g cm^{-3} (MAL, MAU, and NMU) to 1.5 g cm^{-3} (SG + N, SG + W, SG + NW, and SHU) (Table 2). Soil textures were diverse, with different mixture ratios of sand, silt, and clay (Table 2; Appendix S1: Figure S2) resulting in a range of soil types: clay loam (MAU, NMU, OR plots, and SGL), loam (NMU and ORL), sandy clay loam (SG and SV plots), sandy loam (SGN, SGU, SGW, SVC, SVL, and SVS), silty clay loam (KZ and MA plots), silty loam (NML), and sand (SH plots). Soil organic C and total N varied over one order of magnitude, ranged from 4.4% (SV + WS) to 60.4% (MAU) and 0.5% (SHU) to 5.2% (MAU), respectively, resulting in C:N ratios ranging from 6.5 (SVC) to 12.9 (KZ20) (Table 2). $\delta^{13}C$ and $\delta^{15}N$ ranged from −21.6‰ (SHL) to −14.9‰ (KZ4), and −1.4‰ (SHU) to 6.2‰ (SV + WL), respectively (Table 2).

Covariation of CH_4 dynamics with environmental and ecological factors

Net CH_4 flux rates, diffusivity, and estimated net methanotroph activity varied across the plots and time during the three growing seasons (Figure 1; Appendix S1: Figure S3). It was also the case for environmental and ecological factors, which could affect net CH_4 fluxes, including WFPS, soil temperature, soil pH, soil respiration (Appendix S1: Figures S3 and S4), soil NH_4^+ ($[NH_4^+]$) and

TABLE 2 Soil characteristics of the 22 plots across the seven sites.

Site	Plot	Bulk density ($g \cdot cm^{-3}$)	Soil texture (%)			Soil organic C and N properties				
			Sand	Silt	Clay	SOC ($mg \cdot g^{-1}$)	Total N ($mg \cdot g^{-1}$)	C:N ratio	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
KZ	KZC	1.0[a]	22.5[b]	42.7[b]	34.6[b]	44.6 (3.9)	3.1 (0.1)	11.7 (0.1)	−15.3 (0.4)	2.5 (0.1)
	KZ + W	1.0[a]	19.1[b]	43.4[b]	37.4[b]	40.8 (2.0)	3.2 (0.2)	12.7 (0.2)	−15.4 (0.2)	2.6 (0.2)
	KZ4	1.3 (0.1)	2.9 (0.6)	60.1 (1.1)	37 (1.2)	35.3 (1.1)	3.0 (0.1)	11.8 (0.1)	−14.9 (0.1)	3.6 (0.0)
	KZ20	1.2 (0.2)	9.9 (0.9)	55.7 (1.2)	34.4 (0.7)	36.5 (0.7)	3.8 (0.2)	12.9 (0.2)	−16.8 (0.5)	1.6 (0.3)
MA	MAL	0.8 (0.1)	17 (0.6)	49.3 (0.8)	33.8 (0.3)	56.5 (1.7)	4.5 (0.1)	12.4 (0.2)	−18.2 (0.2)	3.5 (0.2)
	MAU	0.8 (0.1)	20.2 (1.5)	40.8 (1.3)	39.1 (0.7)	60.4 (2.4)	5.2 (0.2)	11.6 (0.2)	−18.8 (0.3)	3.4 (0.1)
NM	NML	0.9 (0.0)	21.7 (1.8)	55 (1.9)	23.3 (1.1)	34.4 (1.3)	2.9 (0.1)	11.7 (0.2)	−18.3 (0.1)	2.4 (0.2)
	NMU	0.8 (0.0)	32.5 (1.0)	40.4 (0.5)	27.1 (0.8)	39.0 (0.6)	3.4 (0.1)	11.6 (0.2)	−18.0 (0.4)	−0.3 (0.1)
OR	ORL	1.1 (0.1)	35.8 (1.7)	36.3 (1.6)	27.9 (0.5)	50.0 (1.7)	4.5 (0.1)	11.1 (0.1)	−21.4 (0.1)	5.1 (0.2)
	ORU	1.2 (0.1)	39 (1.7)	26.9 (1.2)	34.1 (1.2)	31.4 (0.6)	3.1 (0.1)	10.1 (0.1)	−19.9 (0.2)	4.5 (0.3)
SG	SGC	1.3[c]	63 (0.7)	14.1 (0.5)	22.9 (0.9)	12.7 (0.7)	1.3 (0.1)	9.6 (0.2)	−16.3 (0.2)	2.7 (0.5)
	SG + N	1.5[c]	63.8 (3.0)	16.3 (2.3)	19.9 (1.4)	13.1 (0.5)	1.4 (0.0)	9.6 (0.2)	−16.9 (0.4)	4.9 (0.2)
	SG + W	1.5[c]	58.4 (3.4)	18.1 (0.8)	23.5 (3.1)	12.7 (1.2)	1.3 (0.1)	10.1 (0.2)	−17.3 (0.4)	2.8 (0.1)
	SG + NW	1.5[c]	58.8 (3.1)	14.1 (1.0)	27.1 (2.6)	15.5 (0.8)	1.5 (0.1)	10.0 (0.1)	−17.7 (0.3)	3.0 (0.1)
	SGL	1.2 (0.0)	50.8 (2.4)	22.8 (1.5)	26.3 (2.1)	17.0 (2.1)	1.6 (0.2)	10.6 (0.3)	−17.2 (0.2)	3.7 (0.2)
	SGU	1.3 (0.0)	65.3 (1.6)	15.8 (1.2)	18.8 (1.3)	10.7 (0.2)	1.1 (0.0)	9.8 (0.2)	−17.2 (0.2)	2.4 (0.2)
SH	SHL	1.4 (0.0)	93.4 (0.6)	4.4 (0.9)	2.2 (0.8)	6.7 (0.9)	0.7 (0.1)	11.1 (0.1)	−21.6 (0.3)	0.7 (0.1)
	SHU	1.5 (0.0)	94.3 (1.0)	4.3 (1.4)	1.3 (0.6)	4.6 (0.6)	0.5 (0.0)	10.1 (0.1)	−20.7 (0.4)	−1.4 (0.4)
SV	SVC	1.3 (0.1)	61 (1.4)	20.4 (0.8)	18.7 (1.8)	4.5 (0.2)	0.7 (0.0)	6.5 (0.3)	−16.8 (0.3)	5.9 (0.1)
	SV + WS	1.3 (0.1)	64.5 (1.7)	18.4 (0.7)	17.1 (1.3)	4.4 (0.3)	0.7 (0.0)	6.7 (0.3)	−15.5 (0.2)	6.1 (0.2)
	SV + WL	1.3 (0.1)	63.3 (2.3)	20.1 (0.9)	16.6 (1.5)	4.7 (0.4)	0.7 (0.0)	7.1 (0.3)	−16.7 (0.2)	6.2 (0.2)
	SVB	1.4 (0.1)	61.1 (1.5)	19.7 (1.2)	19.2 (0.9)	5.5 (0.7)	0.7 (0.1)	7.9 (0.3)	−16.7 (0.2)	6.0 (0.3)

Note: See Table 1 for an explanation of site abbreviations.

Abbreviation: SOC, soil organic carbon.

[a] Seastedt and Ramundo (1990).

[b] From Konza long-term ecological research archive.

[c] From Shortgrass Steppe long-term ecological research archive.

NO_3^- ($[NO_3^-]$) contents, and ratios of $[NO_3^-]$ to total inorganic N content (i.e., $[NH_4^+]$ and $[NO_3^-]$) (Appendix S1: Figures S3 and S5). The PCA showed overall covariation among the environmental factors (e.g., temperature, precipitation, and edaphic characteristics; Table 2) and the field measurements (e.g., CH_4 and CO_2 fluxes) averaged over the three growing seasons at the chamber level (Appendix S1: Figure S6) where the two primary axes cumulatively explained 62.1% of the variability (Figure 2). The 22 plots were evenly distributed along the two primary axes, suggesting that our study design had extensive coverage along the environmental gradients of interests. This indicates that our study plots can provide an ideal system to obtain insights for grassland CH_4 dynamics across the US Great Plains.

PC1 (42.3%) captured site and ecosystem properties controlling water availability, including MAP, WFPS, and soil texture (i.e., sand, silt, and clay contents) (Figure 2; Appendix S1: Figure S7). PC1 also encompassed soil properties resulting from local water availability, such as SOC and total N (Figure 2; Appendix S1: Figure S7). Along PC1, all the plots in SG and SV with the lowest MAP had positive scores, and the plots in wetter sites (i.e., KZ, NM, MA, OR) had negative scores (Figure 2). The two SH plots had positive scores in PC1 despite relatively high MAP (Table 1) because the SH soils had poor water-holding capacity due to the high sand contents (Table 2; Appendix S1: Figure S2).

PC2 (19.8%) represented MAT, and some soil properties, such as pH, $\delta^{13}C$, and inorganic C contents (Figure 2; Appendix S1: Figure S7). Along with PC2, the plots in SV and KZ had the most positive scores due to their highest MAT (Table 2). While MAP and WFPS loadings are very similar, MAT and soil temperature at the chambers (Temp)

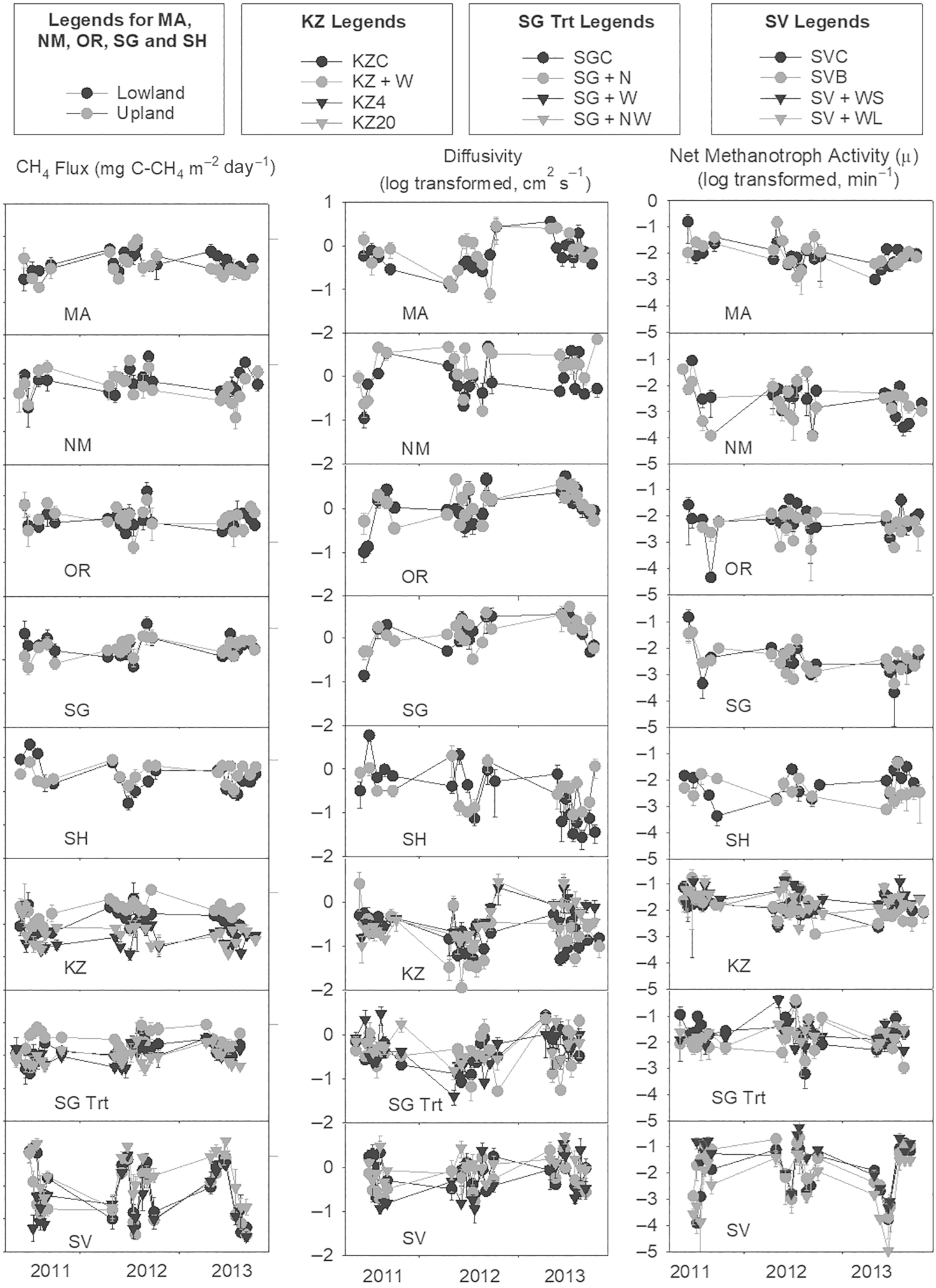


FIGURE 1 CH_4 flux rates, diffusivity, and net methanotroph activity (μ) during growing seasons between 2011 and 2013 (mean ± SE; $N = 6$). See Table 1 for an explanation of site abbreviations.

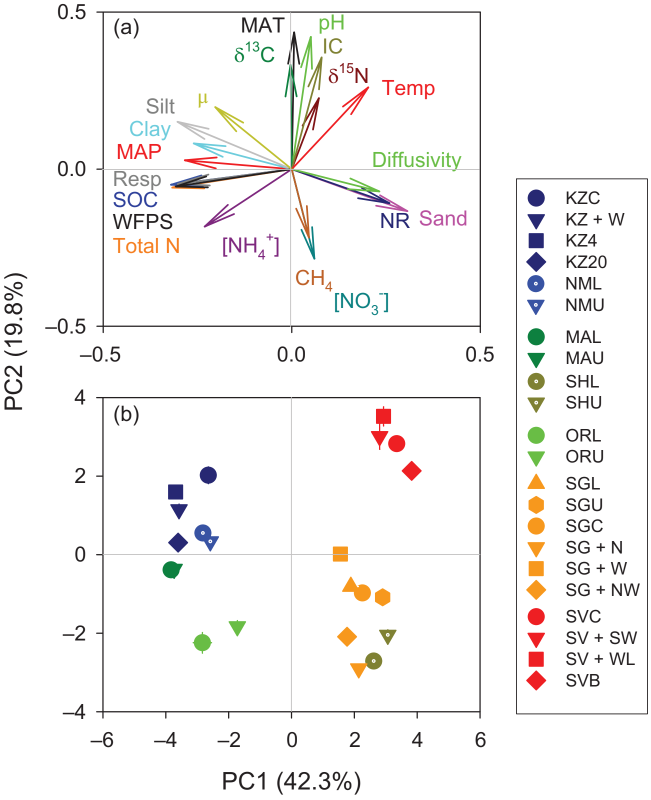


FIGURE 2 The result of a principal components analysis using environmental and soil characteristics of all the 22 plots. (a) Loadings of environmental and soil characteristics and (b) scores of the plots for PC 1 and 2 in the X and Y axes, respectively. The plot symbols in the legend are vertically arranged with the MAP from the wettest on top (KZ) to the driest on the bottom (SV). Each symbol in (b) represents a mean of six scores for a plot (Appendix S1: Figure S3). Error bars show standard errors of means ($N = 6$). IC, inorganic C content; NR, $[NO_3^-]$ to [total inorganic N] ratio; Resp, soil respiration; Temp, soil temperature; WFPS, water-filled pore space; μ, net methanotroph activity. See Table 1 for an explanation of site abbreviations.

were not aligned in a similar magnitude (Figure 2; Appendix S1: Figure S7), most likely because the soil temperature was measured only during the growing seasons. Net CH_4 flux rates and net methanotroph activity (μ) loaded in opposing directions as expected (Figure 2) because negative and positive net CH_4 flux rates meant net CH_4 sink and source, respectively (Figures 1 and 2).

Factors controlling net CH_4 fluxes, diffusivity, and net methanotroph activity (Objective 1)

Environmental factors controlling net CH_4 flux rates were assessed using MSS statistics (Figure 3). WFPS had the highest MSS value in both the full ($R^2 = 0.467$) and best-fit models ($R^2 = 0.466$) for the sites with the lowland/upland plots, followed by $WFPS^2$ (Figure 3). This was also the case for the KZ, SG field treatment, and SV sites (Figure 3). This suggests that net CH_4 uptake was consistently highest when WFPS was intermediate at a given plot (Figure 4; Appendix S1: Table S1) despite the substantial differences in environmental factors controlling soil moisture conditions, including MAP (ranging from 244 to 835 mm), mean WFPS (ranging from 9.7% to 52.9%; Appendix S1: Figure S5) and soil texture (Appendix S1: Figure S2) across the seven sites. The best-fit model also retained three-way interactions among location (i.e., upland/lowland), site and $WFPS/WFPS^2$, even though their MSS values were relatively small (Figure 3). This indicates that there were some variations in the relationship of the overall hump-shaped net CH_4 uptake to observed WFPS variations between upland and lowland and among the five sites (Figure 4; Appendix S1: Table S1). In KZ, $WFPS^2$ and WFPS had the highest MSS values, followed by ratio of $[NO_3^-]$ to [total inorganic N] (NR), the field treatments (Trt) (water addition and prescribed burning every 4 and 20 years, Table 1), $[NO_3^-]$, interactions between the field treatments and $WFPS^2/WFPS$, and pH in both full ($R^2 = 0.266$) and best-fit models ($R^2 = 0.264$) (Figure 3). In the field treatment plots at SG (SG Trt), both full ($R^2 = 0.081$) and best-fit models ($R^2 = 0.081$) had relatively low predictability, where only WFPS and $WFPS^2$ ratio were retained in the best-fit model ($R^2 = 0.081$) (Figure 3; Appendix S1: Table S1). In SV, WFPS and $WFPS^2$ had the highest MSS values in the full ($R^2 = 0.440$) and best-fit models ($R^2 = 0.434$). The SV best-fit model retained interactions between the field treatments (Trt) and $WFPS/WFPS^2$, indicating that net CH_4 uptake in response to soil water conditions depended on the field treatments with the precipitation regimes (Table 1, Figure 3; Appendix S1: Table S1). In addition, the SV best-fit model suggested soil temperature was an important predictor for net CH_4 uptake (Figure 3). We note that, overall, 6.6% of CH_4 flux data were positive, meaning CH_4 sources (Figures 1 and 4). Positive CH_4 fluxes were observed across the seven sites, including the driest SV when WFPS was low (Figure 4; Appendix S1: Figure S8).

Environmental factors controlling diffusivity were also assessed using MSS statistics (Figure 5a). At the lowland/upland sites, WFPS had the highest MSS value in both full ($R^2 = 0.418$) and best-fit models ($R^2 = 0.422$) followed by study site × WFPS interaction, study sites and sand contents (Figure 5a). The high MSS value of study site × WFPS interaction indicates inconsistent

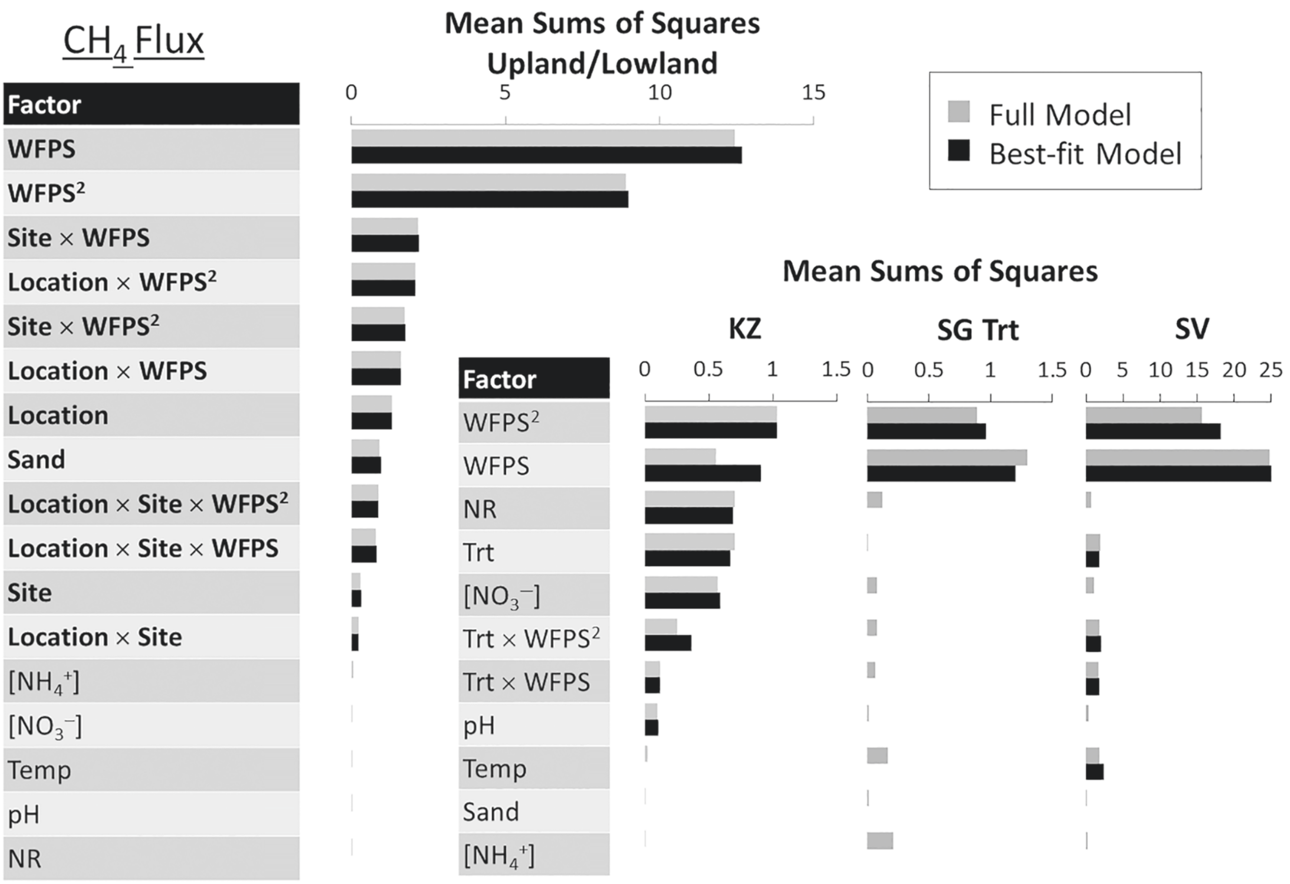


FIGURE 3 Results of mean sums of squares statistics to assess the relative importance of potential environmental factors to influence CH_4 flux rates for the six study sites with the upland/lowland plots (i.e., MA, NM, OR, SG, SH, and SV) and for KA, SG, and SV with the field treatments (Trt). Results of the full and best-fit models are shown. [NH_4^+], soil NH_4^+ concentration; [NO_3^-], soil NO_3^- concentration; Location, upland/lowland; NR, ratio of [NO_3^-] to [total inorganic N]; Sand, sand content (%); Temp, soil temperature; Trt, field treatments; WFPS, water-filled pore space; See Table 1 for an explanation of site abbreviations.

response of diffusivity to WFPS among the sites (Figure 6; Appendix S1: Table S2). In KZ, overall model fitness was poor with R^2 values of 0.115 and 0.119 for the full and best-fit models, respectively. The best-fit KZ model retained only the field treatments (i.e., water addition and fire return intervals, Table 1), and WFPS was a poor predictor for diffusivity (Figures 5a and 6; Appendix S1: Table S2). In the SG field treatment plots, WFPS had the highest MSS values in both full ($R^2 = 0.276$) and best-fit models ($R^2 = 0.270$), followed by the field treatments (i.e., N and water addition, Table 1, Figure 5a). In SV, the field treatments (i.e., water regimes and vegetation types, Table 1) had the highest MSS values in both full ($R^2 = 0.276$) and best-fit models ($R^2 = 0.255$), followed by WFPS (Figure 5a).

Results of MSS statistics to assess environmental factors potentially shaping net methanotroph activity (Figure 5b) were similar to those for net CH_4 fluxes (Figure 3). WFPS had the highest MSS value in both the full ($R^2 = 0.376$) and best-fit models ($R^2 = 0.368$) for the lowland/upland sites, followed by WFPS^2 (Figure 5b). This indicates consistent trends of net methanotroph activity responding to WFPS across the sites (Figure 7; Appendix S1: Table S3). Other environmental factors retained in the best-fit model included interactions among location (i.e., upland/lowland), site, and WFPS/WFPS^2 (Figure 5b). In KZ, [NO_3^-]:[IN] ratio had the highest MSS value, followed by WFPS^2, WFPS, and the field treatments in both full ($R^2 = 0.129$) and best-fit models ($R^2 = 0.126$) (Figure 5b). In the SG field treatment plots, WFPS had the highest MSS value, followed by WFPS^2 and the field treatments in the full ($R^2 = 0.363$) and best-fit models ($R^2 = 0.349$). Similar trends were found in SV where WFPS and WFPS^2 had the highest MSS values in the full ($R^2 = 0.489$) and best-fit models ($R^2 = 0.484$). In addition, the SV best-fit model suggested soil temperature and [NO_3^-] were important predictors for net methanotroph activity (Figure 5b).

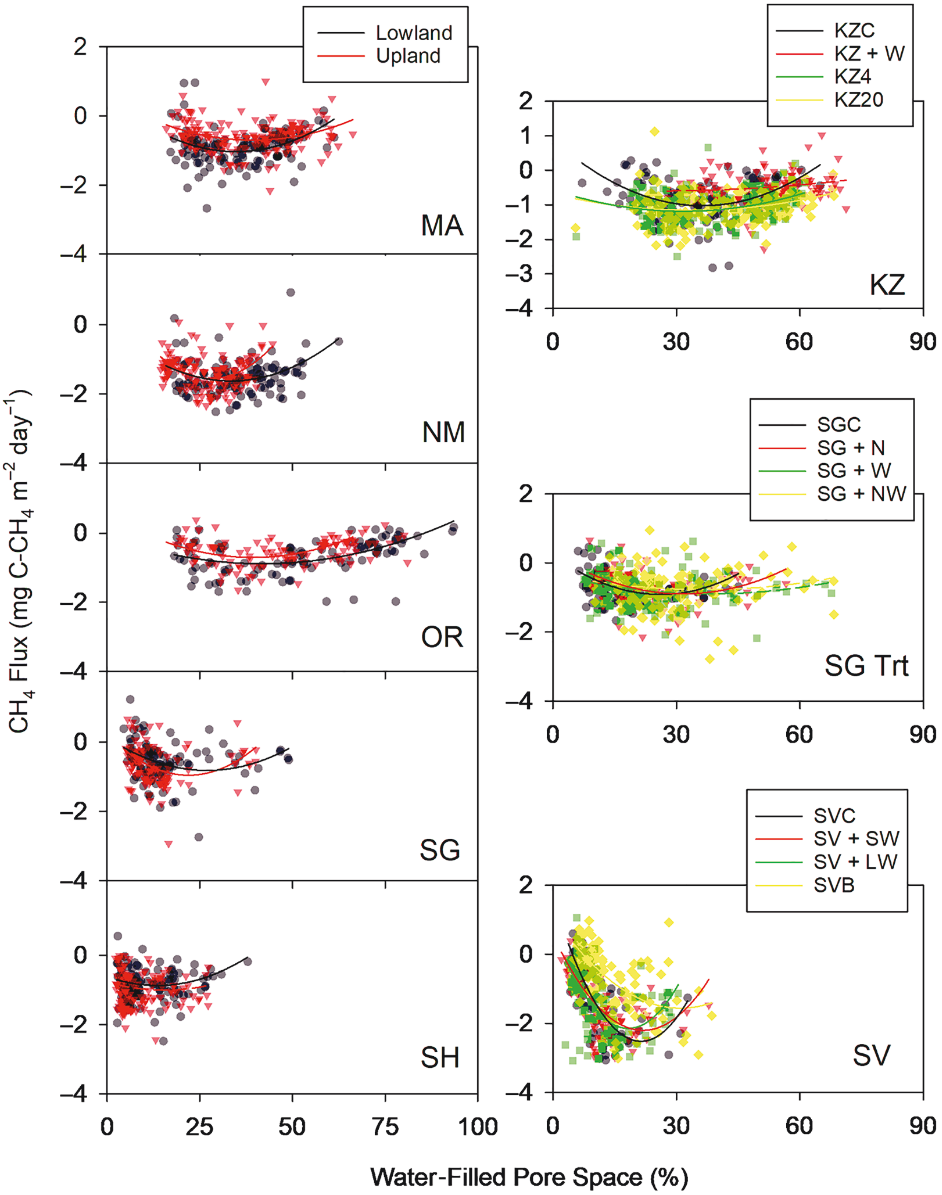
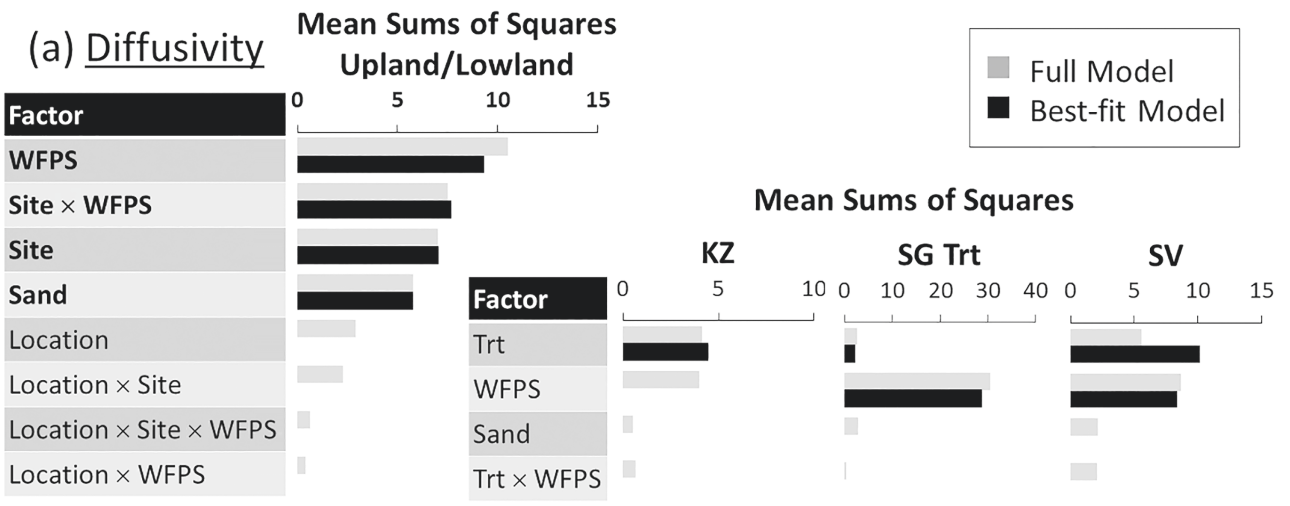


FIGURE 4 Relationship between water-filled pore space (WFPS) and CH_4 flux rates fitted in a quadratic equation for each plot. Solid and dashed lines indicate that fitted equations are statistically significant ($p \leq 0.05$) and not significant ($p > 0.05$, SHU, KZ + W and SG + NW), respectively. Equations and statistical information are found in Appendix S1: Table S1. See Table 1 for an explanation of site abbreviations.

Relative contributions of diffusivity and net methanotroph activity to temporal and spatial variations in net CH_4 uptake (Objective 2)

CVs were used to assess the relative contributions of net methanotroph activity and diffusivity for temporal and spatial variations in net CH_4 uptake (Figure 8). Plot-level CVs for temporal variations varied from 1.0 (SGN) to 2.3 (MAU) for diffusivity ($CV_{\text{diffusivity}}$), and from 1.2 (SGC) to 4.0 (KZ4) for net methanotroph activity (CV_{μ}) (Figure 8a). Ratios of the two CVs within plots varied ranging from 0.7 (SV + SW) to 2.6 (SHU) (Figure 8b). Of the 22 plots, 16 plots had higher than 1 (Figure 8b inset), indicating that net methanotroph activity had more control in temporal net CH_4 uptake variations than diffusivity in majority of the plots. There were no apparent trends in the ratios regarding MAP or the sites (Figure 8b). Site-level means and standard deviations were calculated using 12 chamber-level means from the upland/lowland plots at each site (except KZ where the control and water addition plots were used) (Figure 8c). These site-level values resulted in site-level CVs to assess within-site spatial variations (Figure 8d).



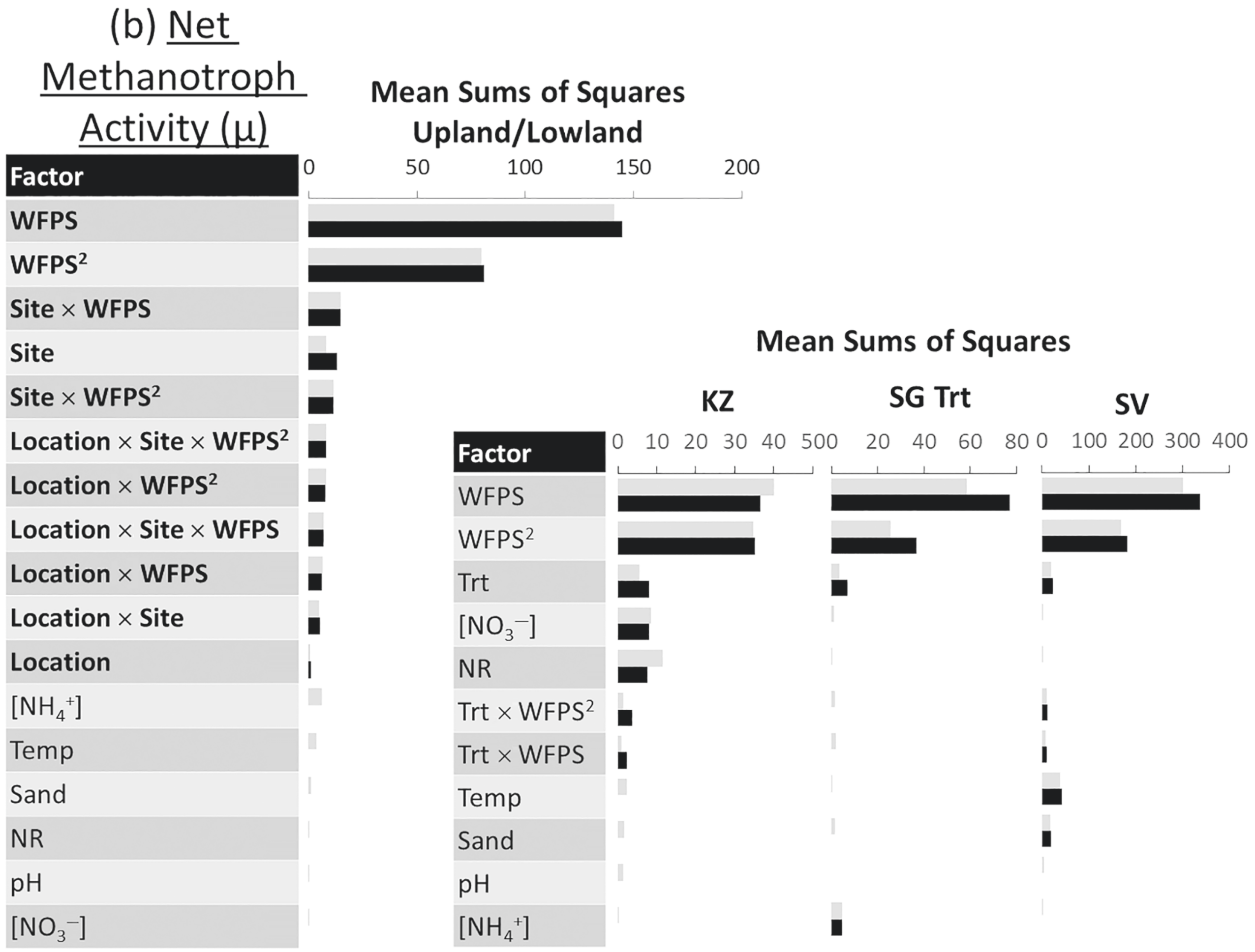


FIGURE 5 Results of mean sums of squares statistics to assess the relative importance of potential environmental factors to influence (a) diffusivity and (b) net methanotroph activity for the six study sites with the upland/lowland plots (i.e., MA, NM, OR, SG, SH, and SV) and for KA, SG, and SV with the field treatments. Results of the full and best-fit models are shown. The factors in boldface for the upland/lowland data set are in the best-fit model. [NH_4^+], soil NH_4^+ concentration; [NO_3^-], soil NO_3^- concentration; Location, upland/lowland; NR, ratio of [NO_3^-] to [total inorganic N]; Sand, sand content (%); Temp, soil temperature; Trt, field treatments; WFPS, water-filled pore space. See Table 1 for an explanation of site abbreviations.

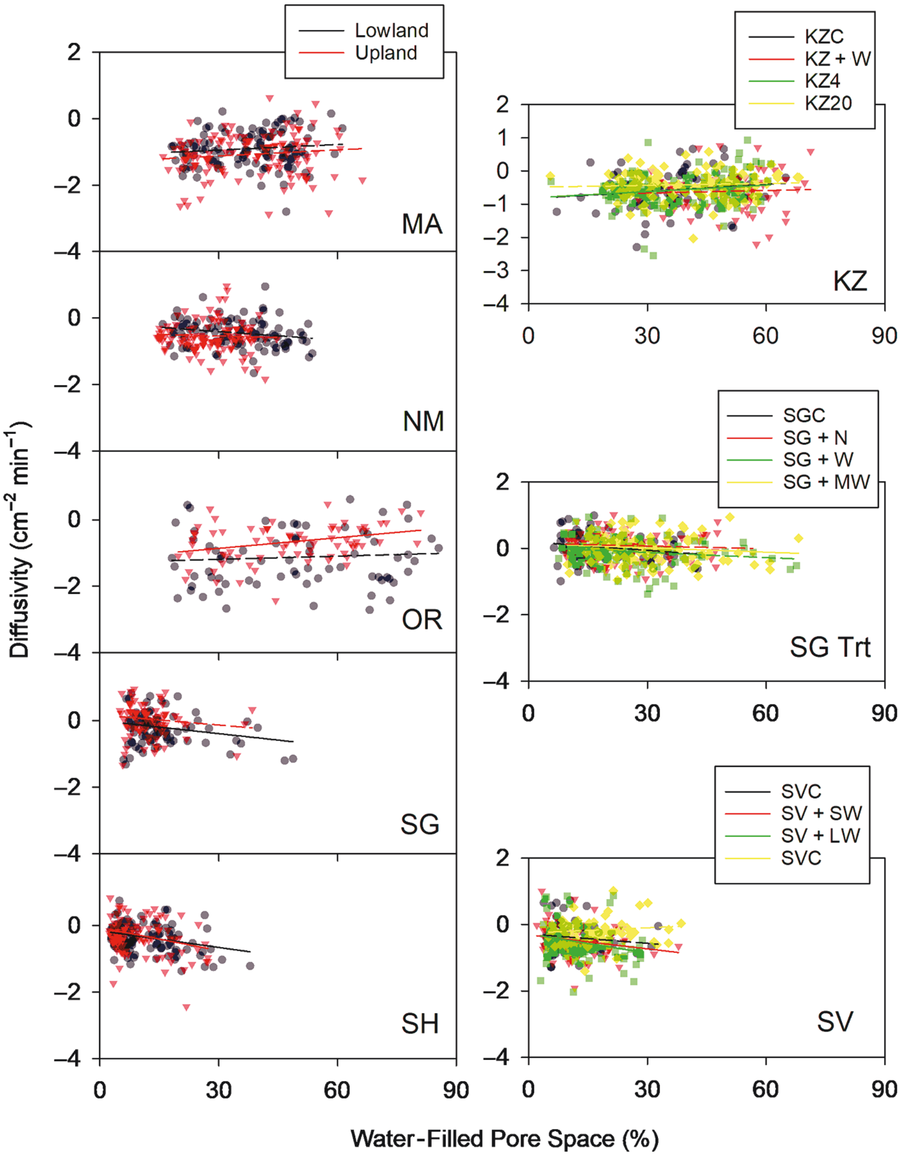


FIGURE 6 Relationship between water-filled pore space (WFPS) and CH_4 flux rates fitted in a linear equation for each plot. Solid and dashed lines indicate that fitted equations are statistically significant ($p \leq 0.05$) and not significant ($p > 0.05$), respectively. Equations and statistical information are found in Appendix S1: Table S2. See Table 1 for an explanation of site abbreviations.

The site-level CVs varied from 1.1 (SG) to 1.7 (NM) for diffusivity, and from 1.7 (NM) to 3.8 (KZ) for net methanotroph activity (Figure 8d). Ratios of the two CVs within sites varied ranging from 0.8 (NM) to 2.7 (SH) (Figure 8e), and most of the ratios were higher than 1 (Figure 8e inset). This indicates that net methanotroph activity had more control in spatial variabilities in net CH_4 uptake than diffusivity in the majority of the sites at the local scale. CVs calculated using site-level averages (Figure 8c) were similar between diffusivity (0.60) and net methanotroph activity (0.56) (Figure 8f), resulting in 0.93 in their ratio (Figure 8g). This indicates that spatial variations in net CH_4 uptake across the US Great Plains were controlled by diffusivity and net methanotroph activity at a similar magnitude.

Relationship between WFPS and net methanotroph activity at the regional scale (Objective 3)

To further assess how net methanotroph activity was controlled by WFPS, a quadratic equation was fitted for each plot (Figure 7; Appendix S1: Table S3). This analysis was based on the MSS statistics in which significant WFPS^2 in best-fit models suggested nonlinear relationships between WFPS and net methanotroph activity

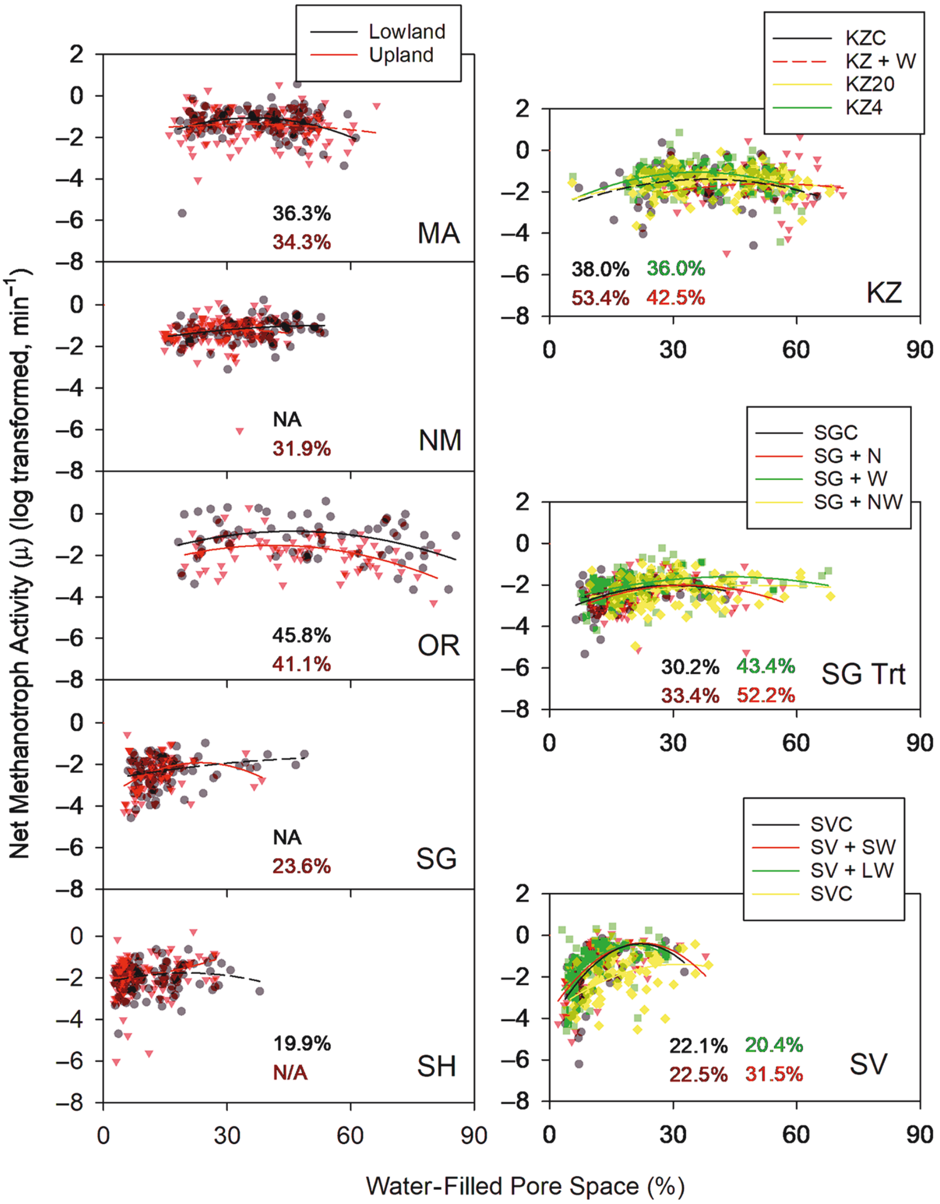


FIGURE 7 Relationship between water-filled pore space (WFPS) and net methanotroph activity (μ) fitted in a quadratic equation for each plot. Quadratic equations were successfully fitted in 19 out of the total of 22 plots. In three plots (SHU, SGU, and SGC), quadratic equations do not have vertexes within observed ranges of WFPS. Values of WFPS (%) associated with peak net methanotroph activity estimated by the quadratic equations are shown in colors corresponding to data points and fitted parabolas. Solid and dashed lines indicate that fitted equations are statistically significant ($p \leq 0.05$) and not significant ($p > 0.05$), respectively. Equations and statistical information are found in Appendix S1: Table S3. See Table 1 for an explanation of site abbreviations.

(Figure 5b). All the plots, except three (NML, SGL, and SHU), showed concaved-down, parabolic relationships between WFPS and net methanotroph activity where, in a given plot, predicted peak net methanotroph activity occurred within an observed range of WFPS even at SV, the driest site (Figure 7).

Relationships between characteristics of the plot-level mean WFPS (Appendix S1: Figure S6) and the quadratic equations (Figure 6; Appendix S1: Table S3) were assessed (Figure 9). Plot-level WFPS means were a significant predictor for modeled WFPS values when net methanotroph activity was optimal (Figure 9a). The slope of regression was significantly different from 1 ($p = 0.004$), and modeled WFPS values at peak net methanotroph activity in the relatively dry sites (i.e., SG, SV, and SH) tended to be higher than the plot-level mean

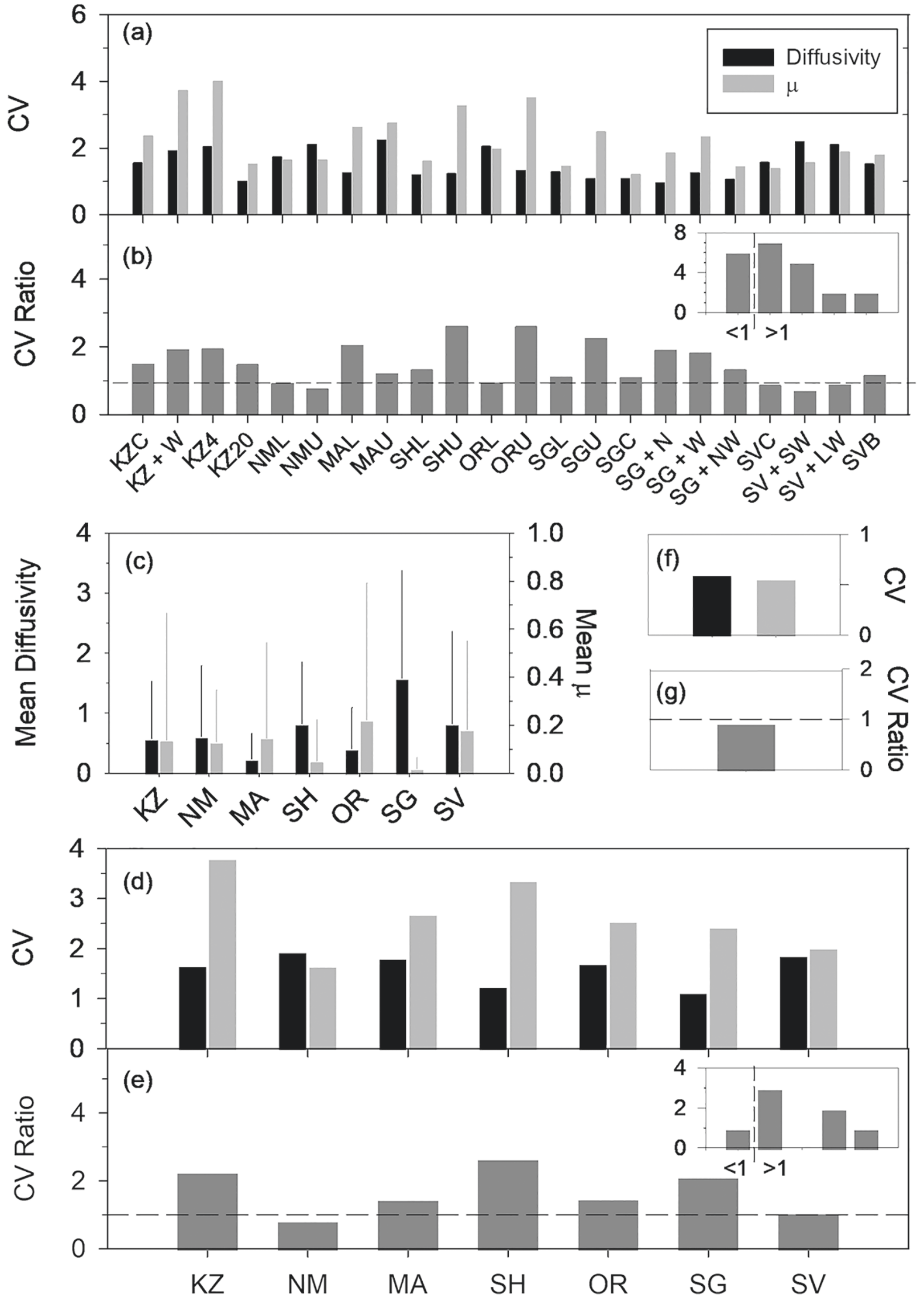


FIGURE 8 (a) CVs of diffusivity and net methanotroph activity (μ) and (b) their ratio to assess their relative importance to control temporal variations for CH_4 uptake at each plot. The dotted line indicates the 1:1 ratio. Inset shows a histogram of the CV ratios across the 22 plots. (c) Site-level means and standard deviations for diffusivity and μ based on chamber-level means at each site. (d) Site-level CVs for diffusivity and μ calculated based on Figure 5c and (e) their ratios to assess their relative importance to control spatial variation within sites for CH_4 uptake. Inset shows a histogram of the CV ratios across the seven sites (Figure 5e). (f) CV across the seven sites calculated based on Figure 5c and (g) their ratio to assess their relative importance to control spatial variations for CH_4 uptake. See Table 1 for an explanation of site abbreviations.

WFPS values (i.e., above the 1:1 line, Figure 9a). However, this was not the case for the relatively wet sites (e.g., KZ and OR) whose values were close to the 1:1 line (Figure 9a). Absolute values of coefficients of second-degree terms of quadratic equations were negatively correlated with plot-level mean WFPS (Figure 9b). This indicates that, in plots where overall soil moisture availability was low due to low precipitation or sandy soils, net methanotroph activity tended to respond dramatically to soil water availability.

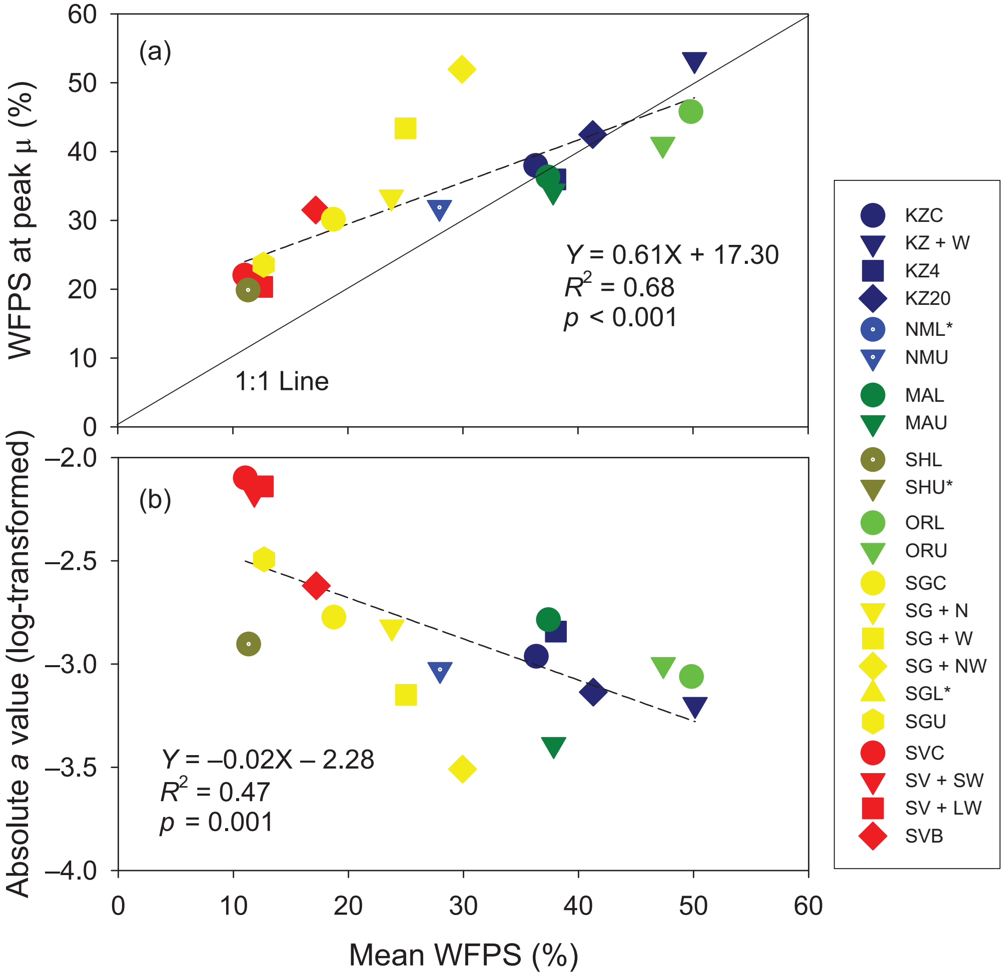


FIGURE 9 Relationship between plot-level mean water-filled pore space (WFPS) (Appendix S1: Figure S5) and characteristics of the quadratic equations (Figure 6); (a) WFPS associated with peak net methanotroph activity (μ) estimated by the quadratic equations and (b) the absolute value of the coefficient of the second-degree term (i.e., a in $Y = aX^2 + bX + c$). *In NML, SHU, and SGL, quadratic equations do not have vertexes within observed ranges of WFPS. See Table 1 for an explanation of site abbreviations.

DISCUSSION

Physical versus biotic contributions to net CH_4 uptake

To our knowledge, this is the first study to assess the relative contributions of physical (i.e., diffusivity) and biotic (i.e., combination of methanotrophic and methanogenic activity; Dutaur & Verchot, 2007) components for net CH_4 uptake in response to various environmental factors in well-drained soils in situ across diverse grasslands over the course of multiple years. Soil gas diffusivity and methanotroph/methanogen activities are the two primary properties controlling CH_4 uptake in well-drained soils (Brumme & Borken, 1999; Smith et al., 2003). Many past studies suggested that soil CH_4 uptake rates were controlled primarily by gas diffusivity (e.g., Born et al., 1990; Curry, 2007; Dörr et al., 1993; Keller & Reiners, 1994; Kruse et al., 1996; Striegl, 1993). Our findings, however, indicate that biotic activity was often more important than diffusivity for temporal and local spatial variations for soil CH_4 uptake rates. In addition, at a large spatial scale across the US Great Plains, the biotic component was as important as diffusivity to drive spatial variability of soil CH_4 uptake. These findings emphasize the critical roles of methanotrophs and, potentially, methanogens in controlling CH_4 uptake in response to environmental variation in grassland soils across the US Great Plains.

Control of soil water conditions on CH_4 fluxes, diffusivity, and net methanotroph activity

Overall, WFPS was the key environmental control, being the strongest predictor for CH_4 flux and net methanotroph activity. Our finding that plot-level CH_4 uptake was greatest at soil moisture (i.e., WFPS) that fell in the middle of the 3-year moisture range indicates that methanotroph communities are adapted to local soils and climate. This adaptation likely occurs through a combination of habitat selection in the pore spaces and eco-physiological responses to local climate. Peak methanotrophy occurring within the local range of soil moisture (Figures 5 and 7) is consistent with an array of studies including lab incubations using intact soil cores from forests (Bowden et al., 1998; Saari et al., 1998), field-based observations in SG (Chen et al., 2011; Dijkstra et al., 2011, 2013; Mosier et al., 1996; Nichols et al., 2016), a heather grassland (van den Pol-van Dasselaar et al., 1998), and pasture and pine plantations (Tate et al., 2007), and a modeling study in diverse vegetation types (Curry, 2007). In previous simulations (Curry, 2007), CH_4 uptake always decreased with increasing soil water contents in three simulated sites with varying soil texture and vegetation types, and the reduction was mainly attributed to decreasing gas diffusivity. However, our findings indicate that the net methanotroph activity can also contribute to decreasing CH_4 uptake under a regime of increasing soil water content.

The reduction in net methanotroph activity with increasing WFPS observed in this study is consistent with the results of several laboratory studies where artificial destruction of physical structure of soil was performed prior to the experiment so that the impact of gas diffusivity was minimized, effectively making measurement of net CH_4 uptake equivalent to measurement of net methanotrophy. When soils were manipulated this way and placed under atmospheric CH_4 concentrations, net methanotroph activity decreased when water availability was manipulated to extremely high and low contents (Borken et al., 2006; Gulledge & Schimel, 1998; Reay, Nedwell, & McNamara, 2001; Reay, Radajewski, et al., 2001; Schnell & King, 1996; van den Pol-van Dasselaar et al., 1998; Whalen et al., 1990). Surprisingly, we observed such a reduction even at SV, the driest site in this study. The reduced net methanotroph activity with increasing soil water content should be caused by reduced methanotrophy and/or stimulated methanogenesis. Relative contributions of these two processes can co-vary with site characteristics controlling local soil water conditions. In grasslands where WFPS can occasionally exceed 60%, such as MA, OR, and KZ, due to relatively high precipitation and water-holding capacity of soils, it is reasonable to assume that WFPS in soils would create an anoxic environment which would suppress methanotrophy and stimulate methanogenesis (Nazaries et al., 2013; Reay, Nedwell, & McNamara, 2001; Reay, Radajewski, et al., 2001). On the other hand, this may not be the case in certain grasslands where WFPS rarely exceeds 30%, such as SG (upland/lowland) and SV due to low precipitation, or SH due to soil texture (>90% sand). In these sites, it is likely that anoxic microsites were created in part by high oxygen demand associated with stimulated heterotrophic and root respiration, which in turn suppressed methanotrophy (Steinkamp et al., 2001) and enhanced methanogenesis (Brewer et al., 2018). Nevertheless, at sites SG, SH, and SV, which had overall low soil water availability, the simulated WFPS values at the peak net methanotroph activity tended to be higher than mean WFPS, indicating that methanotrophy was limited by soil water deficiency most of the time. This observation is consistent with a finding that the two plots (SHU and SGC) where the WFPS at peak net methanotroph activity appears to be higher than WFPS observed at the plots were found at sites SG and SH.

Semiarid grasslands, such as SV and SG, are characterized as pulse-driven systems where occasional precipitation events induce disproportionally high activities in soil biota and plants (Collins et al., 2008). Typically, a precipitation event induces an immediate spike of soil respiration, followed by a short-lived boost of photosynthesis by plants (Huxman et al., 2004). Both responses would contribute to decreasing soil oxygen concentrations which would be adverse for methanotrophy (Steinkamp et al., 2001) and promote anoxic microsites favorable for CH_4 production by methanogens. This phenomenon can be present even in arid environments and can occur rapidly under anoxic conditions (Angel et al., 2012). Following a precipitation pulse, high oxygen demand caused by a combination of increased root respiration, root exudation, and soil heterotrophic respiration would increase the prevalence of anoxic microsites and, in turn, stimulate methanogenesis (Brewer et al., 2018). These mechanisms likely contributed to the nonlinear responses of CH_4 fluxes and net methanotroph activity to WFPS observed at semiarid SG and SV in this study.

Reduction of CH_4 uptake in low soil water availability was likely caused by dormancy, reduced population sizes, and/or changes in methanotroph community structure. Rates of methanotrophic CH_4 oxidation quickly decreases in response to desiccation (Schnell & King, 1996) as methanotrophs are relatively intolerant to desiccation stress compared with some microbes such as gram-negative, heterotrophic eubacteria and fungi (Brown, 1990). However, methanotrophs can be highly resilient from desiccation events likely due to their ability to produce desiccation-resistant exospores or cysts (Bowman et al., 1993; Collet et al., 2015; Dedysh & Knief, 2018; Ho et al., 2011; Whittenbury et al., 1970). High resilience in soil CH_4 uptake was observed in a controlled lab experiment where CH_4 uptake was reduced following soil drying but recovered upon rewetting within days even after repeated exposures to multiple drying and rewetting events (van Kruistum et al., 2018). In the same experiment, abundances of methane monooxygenase gene (*pmoA*) showed a similar trend with the multiple drying and rewetting events, but CH_4 uptake and *pmoA* abundances were not significantly correlated, suggesting that observed changes in methanotroph community structure affected CH_4 uptake (van Kruistum et al., 2018).

It is noteworthy that we observed consistent parabolic relationships between WFPS and CH_4 fluxes where CH_4 uptake rates were highest at plot-level intermediate WFPS values. This trend was found across the seven sites, even in the driest SV site where net CH_4 emission was frequently observed when WFPS was lowest. Although a few past studies reported parabolic relationships between soil water content and CH_4 fluxes like this study (e.g., Curry, 2007; Li et al., 2023), the majority of grassland studies showed linear relationships where decreasing soil water content increased CH_4 uptake (e.g., Brachmann et al., 2020; Grover et al., 2012; Liu et al., 2021; Menyailo et al., 2008; Wang et al., 2012). One possible explanation for the discrepancy is that, compared with most of the past studies, this study had more frequent measurements, thus we likely observed CH_4 fluxes when WFPS was extremely low to the extent that methanotrophic CH_4 oxidation was regulated by desiccation in each plot. A meta-analysis shows that soil CH_4 sink capacity can increase when precipitation is reduced in grasslands (Guo et al., 2023). However, our study suggests that drought conditions caused by reduced precipitation, which is predicted to occur more often under warmer climate in the future (Knapp et al., 2015; Stocker et al., 2013), may weaken CH_4 uptake by grassland soils.

In the sites with sandy soils (i.e., SH, SG, and SV), WFPS was a significant predictor for diffusivity. This is consistent with the theoretical relationship between increasing soil water contents and decreasing gas diffusivity demonstrated in artificial soil environments in the lab (Kruse et al., 1996; Rolston et al., 1991) and in the field (e.g., Ball et al., 1994; De Bernardi et al., 2019; Grover et al., 2012). However, most of the other sites with lower sand content in soils, WFPS was a poor predictor for diffusivity. A similar phenomenon was observed by Ball et al. (1994): Air-filled pore space (the inverse of WFPS) was a poor predictor for gas diffusivity in a clay loam soil (28%–40% clay). It is likely that macropores, such as cracks originated from shrink-swell dynamics and channels made by biotic agents such as earthworms and roots (Beven & Clarke, 1986), play a major role in gas diffusion in clay-dominated soils, leaving soil micropores less control in gas diffusivity. In two cases (KZ4 and ORU), WFPS and diffusivity showed significant positive correlations. Given the relatively large sample sizes (123 and 74 for KZ4 and ORU, respectively), it is likely that these relationships were not spurious, and further study is needed to investigate the mechanisms behind these observations.

Conceptual model for control of soil water conditions on grassland CH_4 dynamics

Based on the relationship between net methanotroph activity and WFPS observed in this study, we propose a conceptual model for grassland CH_4 dynamics (Figure 10). Here, we envision a soil matrix composed of macropores, through which the bulk of gas diffusion occurs, and micropores, which branch off the macropores and hold most of the soil's moisture during drier conditions. Following physical principles (e.g., Fick's first law), the diffusive movement of CH_4 through micropores is slower than through macropores. We envision that methanotrophs are generally less abundant in more arid grasslands (e.g., SG and SV, Figure 10a–c) than more mesic grassland (e.g., KZ and NM, Figure 10d–f) because of overall water availability in soil matrix. Low moisture in the macropores of arid soils causes most of the methanotrophs in arid grasslands to reside in micropores (Figure 10a–c) whereas methanotrophs reside in both macropores and micropores in mesic grasslands (Figure 10d–f). A small number of methane-producing (methanogenic) microbes reside in spaces where oxygen availability is frequently limited (e.g., within soil aggregates), and we expect their total abundance will be greater in mesic grasslands than in arid grasslands (Figure 10). In the dry grassland, under a drought, most of the methanotrophs are dormant due to desiccation, but some may be active in micropores where water is still available (Figure 10a). Under optimal water availability where peak net methanotroph activity occurs, all the methanotrophs are active, except for those in or near the drier macropores (Figure 10b). After a substantial precipitation event, most of the methanotrophs in micropores become dormant due to oxygen limitation caused by water in micropores and high respiration from plant roots and heterotrophic microbes (Figure 10c). Under this condition, the few methanotrophs close to macropores can be active, and methanogens in micropores can produce CH_4, offsetting part of the methanotrophy (Figure 10c). This shifted balance of methanotrophy and methanogenesis along the water availability gradient in the dry grassland creates a large difference in rates of CH_4 uptake (i.e., at the soil surface) between dry and wet

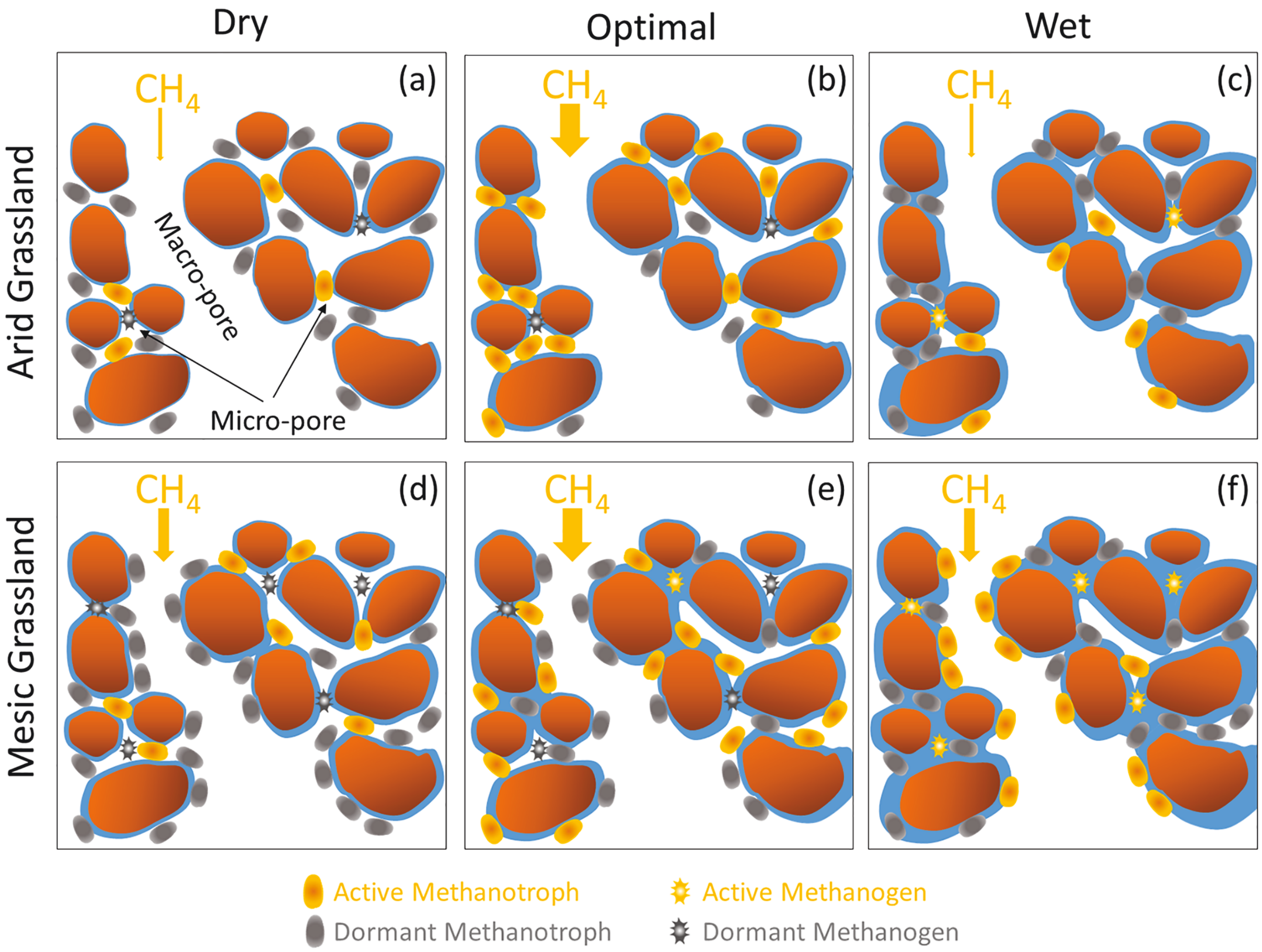


FIGURE 10 Conceptual diagram depicting methanotroph and methanogen activities along the soil moisture gradient (dry, optimal, and wet conditions) in soil matrix in dry (e.g., SG and SV; Figure 8a–c) and wet sites (KZ and NM; Figure 8d–f). See Table 1 for an explanation of site abbreviations.

conditions (Figure 10a–c). The mesic grassland also shows a parabolic relationship in net CH_4 uptake along the soil water availability gradient, but have a more modest fluctuation in net CH_4 uptake between dry and wet conditions because of abundant methanotrophs along macropores (Figure 10d–f).

Other environmental factors for CH_4 fluxes and net methanotroph activity

The addition of ammonia-based N fertilizer often decreases soil CH_4 uptake in grasslands (e.g., Mosier et al., 1991, 1996; Tlustos et al., 1998; Willison et al., 1995) and forests (e.g., Castro et al., 1995; Martins et al., 2015; Steudler et al., 1989; Willison et al., 1995). However, soil [NH_4^+] was a poor predictor for net CH_4 fluxes and net methanotroph activity at the SG treatment plots even though N fertilization treatments substantially increased soil inorganic N contents. Potential explanations include N limitation for methanotrophy (Aronson et al., 2012, 2013) and alleviated ammonium inhibition on methanotrophy via plant uptake (Stiehl-Braun et al., 2011). Indeed, N fertilization does not always reduce CH_4 uptake in grasslands and forests (reviewed by Bodelier & Laanbroek, 2004). For instance, NH_4^+-based N fertilization caused no consistent effects in a semiarid grassland (Aronson et al., 2019) and a temperate grassland (Kammann et al., 2001), and small and transient effects in temperate grasslands (Hartmann et al., 2011). Using a meta-analysis, Aronson and Helliker (2010) suggested that high N availability from N fertilization (>100 kg N $kg^{-1}\cdot year^{-1}$) most likely reduces soil CH_4 uptake via ammonium inhibition for methanotrophy, but smaller N addition may not due to N limitation for methanotrophy.

Soil [NO_3^-] and [NO_3^-]:[inorganic N] ratio were significantly positively correlated with net methanotroph activity at KZ. However, it is challenging to elucidate the possible mechanisms behind the correlation because even N fertilization, which is often substantially higher than background N deposition, does not always result in consistent effects on CH_4 uptake (Aronson & Helliker, 2010). As expected, past studies reported varying results, including positive correlation between soil [NH_4^+] and CH_4 uptake in forests along a rural to urban transect (Goldman et al., 1995) and old-field grasslands (Priemé et al., 1997), negative correlation between soil [NO_3^-] and CH_4 uptake in forests (Reay, Nedwell, & McNamara, 2001; Reay, Radajewski, et al., 2001) and a grassland (Tate et al., 2007), and no significant correlation between soil [inorganic N] and CH_4 uptake in grasslands (Tate et al., 2006, 2007).

Soil temperature was a significant predictor for net methanotroph activity only at SV, where the relationship was positive. The same relationships have been observed in grasslands (Chen et al., 2011; van den Pol-van Dasselaar et al., 1998) and forests (Hart, 2006; Nakano et al., 2004; Priemé & Christensen, 1997). Some studies reported nonlinear relationships; soil temperature and CH_4 oxidation rates were significantly correlated only when temperature was lower than 10°C in temperate forests (Castro et al., 1995; Steinkamp et al., 2001). However, field soil temperature can covary with other environmental factors, such as soil moisture, which can have greater influence on soil CH_4 dynamics (Bradford et al., 2001; Whalen & Reeburgh, 1996). For instance, soil temperature was a poor predictor for CH_4 uptake after accounting for soil water contents in two temperate grasslands (Hartmann et al., 2011) and a temperate forest (Borken et al., 2006). Under controlled environments in the lab, increasing temperature induced only small stimulation of CH_4 uptake in sieved homogenized soils (Crill et al., 1994; Whalen & Reeburgh, 1996) and soil cores (King & Adamsen, 1992) under the natural atmospheric CH_4 concentration. In addition, methanotrophy is less sensitive to temperature change than methanogenesis (Dunfield et al., 1993), which can further obscure the relationship between temperature and net methanotroph activity when methanogenesis plays an important role in soil CH_4 dynamics under high water availability. Our results emphasize the challenge in using soil temperature to predict CH_4 dynamics in grassland soils. We note that no clear temperature effects on methanotroph activity in this study could be due to the lack of measurements during the nongrowing seasons.

Implications of grassland CH_4 dynamics in the future under climate change

It is essential to accurately model CH_4 fluxes in terrestrial ecosystems, including grasslands, to assess current and future greenhouse gas dynamics, since this allows us to predict the magnitude of global warming. Our results showed that soil water content was the strongest predictor for soil CH_4 uptake in grasslands across the US Great Plains, while other environmental factors, such as temperature and inorganic N contents in soils, were relatively minor and/or inconsistent predictors. This indicates that CH_4 fluxes in grasslands may be modeled effectively using local precipitation data and soil texture.

Based on rates of net methanotrophy, methanotroph communities appear to be adapted to local soils and climate, a combination of habitat selection in the pore spaces and ecophysiological responses, indicated by the peak CH_4 sink falling in the middle of the typical soil moisture range for each site. Shifts in climate could move the interannual range of soil moisture, changing the microbial soil habitat, thus ultimately pushing soil ecosystems away from the current optimal WFPS for CH_4 uptake and decreasing the ecosystem CH_4 sink. We anticipate that methanotroph communities would adapt to the changes in soil habitat, but there may be a significant temporal lag in this adaptation to the new climate. Such a lag could be responsible for the consistent reduction in CH_4 sink observed in forest soils with greater soil moisture. Ni and Groffman (2018) found a global decrease in CH_4 uptake with increasing precipitation even when the increased soil moisture appeared to be well below saturation. Such a response would be expected if the methanotroph communities were adapted to drier conditions and had not yet adapted to new, wetter soil conditions caused by increased precipitation patterns.

Climate change is expected to increase the frequency of extreme inter- and intra-annual precipitation events globally, and this tendency will be more pronounced in drier ecosystems (Stocker et al., 2013), including grasslands (Knapp et al., 2015). Under such precipitation regimes, soils in a given grassland would more frequently experience extreme soil water conditions (i.e., dry and wet in Figure 10), reducing the period under soil moisture that provide optimal CH_4 uptake conditions (i.e., optimal in Figure 10). This will likely result in a reduced CH_4 sink in diverse grassland ecosystems across the globe.

AUTHOR CONTRIBUTIONS

Joseph C. von Fischer and Colleen T. Webb conceived and designed the study and acquired data. Akihiro Koyama and Nels G. Johnson analyzed the data.

Akihiro Koyama, Paul Brewer, and Joseph C. von Fischer interpreted the data. Akihiro Koyama wrote the first draft, and all wrote the manuscript.

ACKNOWLEDGMENTS

We thank the support staff, lab members, and assistants at Colorado State University, including Daniel Ruess, Colin Pinney, Charlotte Alster, Sam Dunn, Jackie Sitko, Claire Squires (Gribenow), Evan Rosenlieb, Ariel Carmichael, Rachael Reddin (Jorgensen), Travis Green, Shreya Pokharel, and Kelsey Thompson. David Wedin, Steven Matzner, and Mike Chapman provided vital site access and logistic support. This research was supported by NSF DEB 1054956 to Joseph von Fischer and Colleen Webb.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data (Koyama et al., 2023) are available from Dryad: 10.5061/dryad.wdbrv15tp.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Koyama, Akihiro, Nels G. Johnson, Paul Brewer, Colleen T. Webb, and Joseph C. von Fischer. 2024. “Biological and Physical Controls of Methane Uptake in Grassland Soils across the US Great Plains.” *Ecosphere* 15(9): e4955. https://doi.org/10.1002/ecs2.4955