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Does dissolved organic carbon regulate biological methane oxidation in semiarid soils?

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Abstract

In humid ecosystems, the rate of methane (CH_4) oxidation by soil-dwelling methane-oxidizing bacteria (MOB) is controlled by soil texture and soil water holding capacity, both of which limit the diffusion of atmospheric CH_4 into the soil. However, it remains unclear whether these same mechanisms control CH_4 oxidation in more arid soils. This study was designed to measure the proximate controls of potential CH_4 oxidation in semiarid soils during different seasons. Using a unique and well-constrained 3-million-year-old semiarid substrate age gradient, we were able to hold state factors constant while exploring the relationship between seasonal potential CH_4 oxidation rates and soil texture, soil water holding capacity, and dissolved organic carbon (DOC). We measured unexpectedly higher rates of potential CH_4 oxidation in the wet season than the dry season. Although other studies have attributed low CH_4 oxidation rates in dry soils to desiccation of MOB, we present several lines of evidence that this may be inaccurate. We found that soil DOC concentration explained CH_4 oxidation rates better than soil physical factors that regulate the diffusion of CH_4 from the atmosphere into the soil. We show evidence that MOB facultatively incorporated isotopically labeled glucose into their cells, and MOB utilized glucose in a pattern among our study sites that was similar to wet-season CH_4 oxidation rates. This evidence suggests that DOC, which is utilized by MOB in other environments with varying effects on CH_4 oxidation rates, may be an important regulator of CH_4 oxidation rates in semiarid soils. Our collective understanding of the facultative use of DOC by MOB is still in its infancy, but our results suggest it may be an important factor controlling CH_4 oxidation in soils from dry ecosystems.

Keywords: arid ecosystems, desiccation, facultative, methane-oxidizing bacteria, PLFA, seasonal dynamics

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Introduction

Methane (CH_4) is the second most important greenhouse gas contributing to climate change, and though it occurs at lower concentrations in the atmosphere than carbon dioxide (CO_2), it has 25 times the global warming potential of CO_2 when compared on a molar basis (Shine & Sturges, 2007; Montzka *et al*., 2011). The only known terrestrial biological sink of CH_4 occurs in upland soil as a result of the oxidation of CH_4 by methane-oxidizing bacteria (MOB) that utilize CH_4 as a carbon (C) source (Hanson & Hanson, 1996). Twenty years ago Striegl *et al*. (1992) drew attention to CH_4 oxidation in desert soils, but today we know little more about the contribution of soil CH_4 oxidation in arid and semiarid ecosystems to global CH_4 budgets than we did in 1992 (Dutaur & Verchot, 2007). Arid and semiarid ecosystems cover approximately one third of the earth's land surface (Archibold, 1995), but models of global CH_4 oxidation rates either ignore arid ecosystems (Potter *et al*., 1996) or explicitly call for more assessment in these regions because the few studies in these soils lead to poor estimates of the contribution of dry ecosystems to global fluxes (Dutaur & Verchot, 2007). Therefore, improving our understanding of CH_4 oxidation in soils of dry regions can substantially improve our understanding of the global CH_4 budget, and by extension, the global C budget.

Part of the difficulty in assessing soil CH_4 oxidation in arid ecosystems using modeling approaches stems from our poor understanding of the factors that control it. In temperate and boreal ecosystems, soil texture and water content are consistently the dominant controls over biological CH_4 oxidation because these factors affect the diffusion rate of CH_4 from the atmosphere into soil (Dörr *et al*., 1993; Striegl, 1993; Potter *et al*., 1996; Torn & Harte, 1996; King, 1997; Bowden *et al*., 1998; Gulledge & Schimel, 1998; Del Grosso *et al*., 2000; von Fischer *et al*., 2009). In their seminal article, Striegl *et al*. (1992) found that in a desert ecosystem, CH_4

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oxidation was greater in wet soils than dry soils. They used two *in situ* watering experiments to demonstrate this pattern, which had never been previously observed, was caused by water limitation of CH_4 oxidation. This would be logically consistent with a unimodal response of soil CH_4 oxidation rates to increasing soil water content: MOB shift from physiological limitation by water stress to resource limitation by low atmospheric CH_4 diffusion rates into the soil under more saturated conditions. This unimodal trend has been borne out in a variety of soil types, often from temperate ecosystems, where peak rates of CH_4 oxidation occur at some intermediate level of soil water content (Torn & Harte, 1996; Bowden *et al*., 1998; Gulledge & Schimel, 1998; Del Grosso *et al*., 2000; von Fischer *et al*., 2009; Dijkstra *et al*., 2011). However, recent studies call into question the validity of such seemingly rigorous paradigms when applied to soils from dry environments. Strong seasonal water dynamics in arid and semiarid ecosystems had counterintuitive effects on microbially mediated soil biogeochemical processes (Austin *et al*., 2004; Borken & Matzner, 2009; Parker & Schimel, 2011; Sullivan *et al*., 2012).

The time is ripe to reexamine the factors that control soil CH_4 oxidation. For years, it has been widely accepted that MOB use CH_4 as their sole C source, and that CH_4 supply limits CH_4 oxidation. This information is often repeated in current literature on CH_4 dynamics. However, recent investigations into the metabolism and functionality of MOB have used molecular and isotopic methods to show that MOB are actually facultative and can utilize organic C sources other than CH_4 such as dissolved soil organic C (Dunfield, 2007; Conrad, 2009; Aronson & Helliker, 2010; Dunfield *et al*., 2010; Belova *et al*., 2011; Im & Semrau, 2011; Pratscher *et al*., 2011; Wieczorek *et al*., 2011). This information seemingly complicates the paradigm that CH_4 oxidation is governed by substrate supply or water limitation, and has implications for our understanding and predictions of soil CH_4 oxidation rates. The facultative use of organic C by MOB, and its effect on CH_4 oxidation rates, warrants consideration in arid and semiarid ecosystems that experience strong seasonal dynamics of water availability. Because litter and root decomposition rates in dry ecosystems are relatively slow (Classen *et al*., 2007), dissolved organic carbon (DOC) inputs to these soils are limited to periods of high soil water content when decomposition and root exudation rates are at their peak.

Here, we present a series of studies that suggest that DOC is an important mechanism controlling CH_4 oxidation in semiarid soils. We sought to isolate the effects of soil texture and soil water holding capacity on the seasonal dynamics of soil CH_4 oxidation using a series of sites previously shown to have strong gradients of soil particle size and water holding capacity (Selmants & Hart, 2008; Fig. 1). This naturally occurring semiarid gradient (the Substrate Age Gradient of Arizona; SAGA) was caused by 3 million years of soil development; our sites ranged in age from 1 to 3000 ky, but were well constrained with respect to other soil forming factors (Jenny, 1941) that can all affect soil CH_4 oxidation directly or indirectly. Therefore, we were able to isolate the effects of variation in soil texture and water holding capacity on CH_4 oxidation in two different seasons: an early-summer dry season and a late-summer wet season. The SAGA provided an opportunity to address the following questions: (i) what are the dynamics of CH_4 oxidation and MOB community size between dry and wet seasons; (ii) what are the proximate controls of the observed seasonal dynamics in CH_4 oxidation rates; and (iii) what is the nature of the relationship between DOC and CH_4 oxidation in semiarid soils?

Materials and methods

Study Sites

The SAGA is located within the San Francisco Volcanic Field, a 5000 km^2 area near the southern extent of the Colorado Plateau in Arizona, USA. Volcanic activity has generated >600 monogenetic basaltic cinder cones, and has migrated in an east-northeasterly direction as the North American plate moves over a 'hot spot' in the Earth's crust (Tanaka *et al*., 1986).

The SAGA consists of four sites with distinctly different substrate ages: 1 ky, 55 ky, 750 ky, and 3 000 000 ky. Each site has a slope of less than 1%. Each of the four sites is dominated by two tree species: piñon pine (*Pinus edulis* Engelm.) and juniper (*Juniperus monosperma* Engelm.) (Looney *et al*., 2012). At the three oldest sites, areas between trees (intercanopy spaces) are dominated by Blue gramma

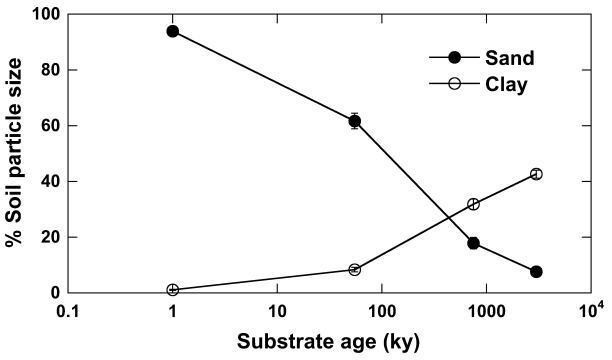

Fig. 1 Effect of substrate age on soil texture, as indicated by soil clay and sand content (adapted from Selmants & Hart, 2008). Error bars indicate one standard error of the mean (n = 4).

(*Bouteloua gracilis* (Wild. ex Dunth) Lag. ex Griffiths) grass. Intercanopy spaces at the youngest site are dominated by woody shrubs [Fallugia paradoxa (D. Don) Endl. ex Torr. and Rhus trilobata Nutt.] or entirely lack vegetation.

The climate is similar at each of the four SAGA sites (Selmants & Hart, 2008; Looney *et al*., 2012). Mean annual precipitation is ~340 mm and mean annual temperature is ~11 °C (Selmants & Hart, 2008). Like many other arid or semiarid regions, northern Arizona has distinct dry and wet seasons. Typically, early summers (late April through early July) are hot and dry, and are followed by a wet and warm late summer (July–September) 'monsoon' precipitation pattern (Sheppard *et al*., 2002).

Methane oxidation potentials

We sampled soil from the middle of 12 intercanopy spaces (minimum of 10 m diameter) at the four SAGA sites once during the dry early summer season (June 15th) and once during the wet late summer season (August 15th). We selected intercanopy spaces because they represent a substantial portion of the landscape, lack potentially deep rooting profiles that could be associated with hydraulic lift of water from deep in the soil profile (which would complicate soil water availability and, in turn, CH_4 diffusion), and because previous research has shown biogeochemical differences among sites to be most strong in this canopy type (e.g., soil total C and N; Selmants & Hart, 2008). We collected intact soil cores (0–15 cm mineral soil) using a 4.8 cm diameter slide hammer (AMS Incorporated, American Falls, ID, USA). The top 15 cm of the mineral soil is consistently the depth of the A horizon at all four of the SAGA sites (Emerson, 2010). The intact cores were kept cool (4 °C) and transferred to the laboratory for analysis of potential CH_4 oxidation rates.

We measured potential CH_4 oxidation rates, as opposed to *in situ* or laboratory analyses of ambient CH_4 oxidation, because potential assays provide two unique advantages. First, potential assays measure the maximum functional capacity of the associated microbial community, as long as the microbial community is slow growing (Hart *et al*., 1994). Methane-oxidizing bacteria have been demonstrated to be relatively slow growing (Priemé *et al*., 1996), and so differences between seasons and among sites in potential CH_4 oxidation rates should reflect changes in the relative size of the MOB community. Second, potential assays minimize limiting factors that could obscure otherwise important constraints on a biogeochemical process. Therefore, we measured potential CH_4 oxidation rates using a modification of the procedure described by Blankinship *et al*. (2010). We collected sixty grams of unsieved, field-moist soil evenly from the 15 cm length of the soil core, placed the soil in specimen cups, sealed the specimen cups in 1 l Mason jars, and increased the CH_4 concentration of the jar headspace to 10 times that of ambient (18 μmol mol^{-1}). By increasing headspace CH_4 concentrations above ambient levels, we reduced substrate limitation to high-affinity MOB, the group responsible for atmospheric CH_4 consumption in soil. High-affinity MOB have half-saturation constants between 10 and 80 μmol mol^{-1} (Bender & Conrad, 1993; Benstead & King, 1997; Gulledge *et al*., 2004). Therefore, an 18 μmol mol^{-1} headspace provides abundant substrate for high-affinity MOB, but does not strongly stimulate low-affinity MOB (Bender & Conrad, 1992). We incubated the soil contained in the Mason jars in the dark at 22 °C. We collected 15 ml headspace gas samples 2, 10, 60 and 72 h after sealing the jars. We used a gas chromatograph equipped with a flame-ionization detector and a Porapak N 80/100 column (Shimadzu 8A, Kyoto, Japan) to measure CH_4 concentrations. Check standards were measured once for every 10 samples, and the coefficient of variation for both time periods was less than 5%. After 72 h, the soil was wet sieved through 2 mm mesh to determine the actual amount of fine earth soil (<2 mm diameter within each specimen cup.

Characterization of glucose uptake by MOB using ^{13}C-PLFA

In a separate experiment, performed on the SAGA soil sampled during the wet season (early August), we added uniformly labeled ^{13}C-glucose to soil. Using ^{13}C-glucose, we were able to measure the incorporation of glucose into phospholipid fatty acid (PLFA) biomarker (18:1ω7c) to demonstrate that MOB use other forms of C as substrates. Other studies have used isotopically labeled $^{13}CH_4$ to demonstrate that the PLFA biomarker 18:1ω7c incorporates atmospheric CH_4 (Knief *et al*., 2003; Maxfield *et al*., 2006; Dunfield *et al*., 2010). Utilization of ^{13}C-glucose was measured in soil from three canopy interspaces collected during the wet season at each of the four SAGA sites. We used glucose because it is an energy rich, low-molecular weight compound that is easily metabolized by the microbial community (Jones *et al*., 2004) and is a derivative of other, more complex C substrates (Hättenschwiler & Vitousek, 2000; Rivas-Ubach *et al*., 2012). Furthermore, it is often added to soil, either under laboratory or *in situ* conditions, to measure the use of C by microbes (e.g., Dalenberg & Jager, 1981; Brooks *et al*., 2004). Each replicate was divided into 90 g pairs, one of which received 99 atom% uniformly labeled ^{13}C-glucose equivalent to 42% of the microbial biomass C at each site (Selmants & Hart, 2008). Soils were incubated in the dark at 20 °C for 112 days. Phospholipids were extracted from 5 g freeze-dried soil using chloroform and methanol. The fatty acids were then separated into glycolipids, neutral lipids, and phospholipids using silicic acid chromatography. Phospholipids were dissolved in hexane and methylated to convert the PLFAs into fatty acid methyl esters (FAMEs). The abundance and isotope ratios of the 18:1ω7c compound were measured at the University of California-Davis Stable Isotope Facility using a Varian gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) at the Stable Isotope Facility with a factorFOUR VF-5ms column (30 m × 0.25 mm ID, 0.25 μm film thickness). Once separated, FAMEs were quantitatively converted into CO_2 in an oxidation reactor at 950 °C. The resultant CO_2 was then analyzed with isotope ratio mass spectrometry (ThermoScientific Delta V Plus isotope ratio mass spectrometer, Bremen, Germany).

Soil physical and chemical characteristics

We measured soil gravimetric water content for each sample at each measurement period using a ~20 g subsample of soil dried at 105 °C to a constant mass. We used these gravimetric water content data, in association with bulk density measurements (P.C. Selmants, unpublished results), to calculate soil air-filled pore space in each sample. We report soil particle size distribution (percentage sand, silt, and clay) for the four SAGA sites previously published by Selmants & Hart (2008). We measured site-specific soil water potentials at known gravimetric water contents using a WP4 Dewpoint Potentiometer (Decagon Devices, Pullman, WA, USA) to develop site-specific soil water release curves. We used these site-specific curves to convert gravimetric water content values measured at each site and sample date to equivalent soil water potentials. Finally, we measured DOC of late wet season soil (October) by extraction in solution using the procedure described by Vance *et al.* (1987). Ten grams of fresh soil was added to 50 ml 0.5 M K_2SO_4 and shaken for 1 h, then allowed to settle overnight. Samples were then filtered through Whatman #1 paper. The concentration of DOC was measured using a TOC-Vcsh total organic carbon analyzer (Shimadzu, Kyoto, Japan). Salt-extractable DOC is often used as an indicator of the C readily available to the heterotrophic microbial community (Chantigny *et al.*, 2008). Soil water contents were not significantly different between the wet-season sampling periods (early August, mid-August, and October) for CH_4 oxidation, PLFA analysis, and DOC concentration (data not shown).

Statistical analysis

The goal of our statistical analysis was to determine if the response of CH_4 oxidation was different between the dry and wet seasons, and if rates were correlated with soil physical and chemical properties. Although site ages across the SAGA are unreplicated, the use of unreplicated substrate age gradients nonetheless provides important opportunities for the study of soil and ecosystem development and associated biogeochemical processes (Vitousek, 2002; Wardle *et al.*, 2004). Sites were carefully chosen to ensure all factors of soil formation (climate, vegetation, topography, and parent material) were held constant with the exception of time (Jenny, 1941). We used repeated measures analysis of variance (RMANOVA) to determine within-site differences in soil water characteristics and CH_4 uptake between seasons, indicated by a significant effect of time on the factor of interest. We used linear regression and Pearson's Correlation to test for relationships between CH_4 oxidation rates and soil texture, soil water characteristics, and DOC. We used JMP software (v8.0.1), SAS Institute, Cary, NC USA) for all statistical analysis. For all statistical tests, alpha was set *a priori* at 0.05.

Results

Wet season potential rates of CH_4 oxidation were much higher than in the dry season for the three older sites, but there was no difference in potential CH_4 oxidation between the dry and wet seasons at the youngest (1-ky-old) site, which had the coarsest soil texture (Fig. 2a, Table 1). Gravimetric soil water

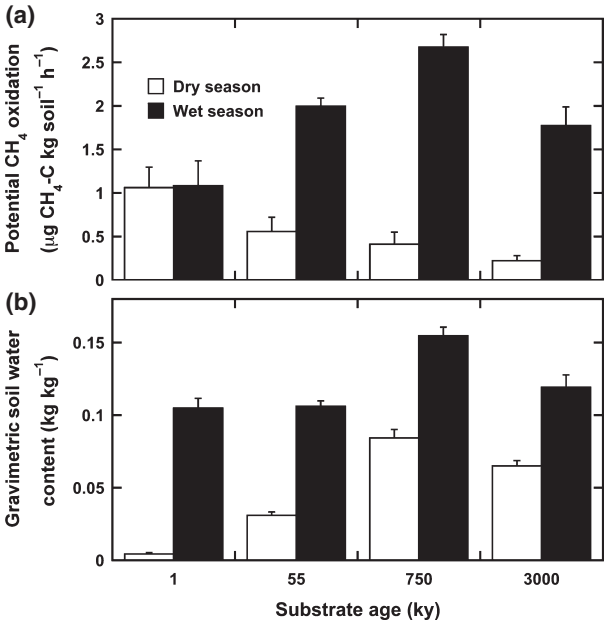

Fig. 2 Effect of substrate age on (a) mean potential methane (CH_4) oxidation rates and (b) mean gravimetric water content in the dry and wet seasons. Error bars indicate one standard error of the mean (n = 12).

Table 1 Results of repeated measures analysis of variance (RM ANOVA) for the effects of season, or ANOVA for the effect of carbon (C) addition on four dependent variables at each site at the Substrate Age Gradient of Arizona (SAGA)

Dependent variable	Test	Effect	Substrate age (kyr)	F	P
CH_4 oxidation	RM ANOVA	Season	1	<0.01	0.95
			55	31.3	<0.01
			750	147.3	<0.01
			3000	60.0	<0.01
Water content	RM ANOVA	Season	1	226.2	<0.01
			55	244.2	<0.01
			750	38.6	<0.01
			3000	53.5	<0.01
PLFA enrichment	ANOVA	C addition	1	57.6	<0.01
			55	106.4	<0.01
			750	276.9	<0.01
			3000	80.5	<0.01
MOB biomass	ANOVA	C addition	1	0.85	0.41
			55	3.15	0.15
			750	0.97	0.38
			3000	<0.01	0.95

PLFA, phospholipid fatty acid; MOB, methane-oxidizing bacteria.

Table 2 Pearson correlation coefficients between soil CH_4 oxidation and soil water availability [gravimetric water content (GWC) and soil water potential (SWP)], air-filled pore space (AFPS), percent clay, and dissolved organic carbon (DOC) concentration in both the dry and wet season. Data were pooled among all sites that comprise the Substrate Age Gradient of Arizona (SAGA)

Variable	Dry season CH_4 oxidation	Wet season CH_4 oxidation
Dry season GWC	−0.40**	–
Wet season GWC	–	0.38*
Dry season SWP	0.04	–
Wet season SWP	–	0.19
Dry season AFPS	0.34*	–
Wet season AFPS	–	−0.33*
%Clay	−0.45	−0.14
DOC	–	0.76**

*Significant at $P < 0.05$ level and **0.01 level.

content increased significantly between the dry and wet seasons; it increased the most at the youngest (1-ky-old) site, which had no seasonal difference in potential CH_4 oxidation, and the least at the 750-ky-old site (Fig. 2b, Table 1).

Most soil physical attributes were poorly correlated with potential CH_4 oxidation (Table 2). Soil texture (percent clay) was not significantly related to potential CH_4 oxidation rates in either the dry season ($r^2 = 0.21$, $P = 0.08$) or the wet season ($r^2 = 0.02$, $P = 0.62$; Fig. 3a). Gravimetric soil water content explained a statistically significant but small amount of variation in dry ($r^2 = 0.16$, $P < 0.01$) and wet season potential CH_4 oxidation rates ($r^2 = 0.14$, $P = 0.02$), and the directionality of these relationships changed between seasons (Fig. 3b, Table 2). Similarly, soil air-filled pore space explained a significant but small amount of variation in dry season potential CH_4 oxidation ($r^2 = 0.12$; $P = 0.02$) and wet season potential CH_4 oxidation ($r^2 = 0.11$; $P = 0.04$); the directionality of these relationships also changed between seasons (Table 2). There was no significant relationship between potential CH_4 oxidation rates and soil water potential in either the dry ($r^2 = 0.01$; $P = 0.49$) or wet season ($r^2 = 0.04$; $P = 0.28$; Table 2). However, there was a significant, positive linear relationship between wet season DOC concentrations and potential CH_4 oxidation ($r^2 = 0.58$; $P < 0.01$; Fig. 4; Table 2).

Methane-oxidizing bacteria, identified using the 18:1ω7c phospholipid fatty acid (PLFA) biomarker (e.g., Maxfield *et al.*, 2006), utilized the added ^{13}C-glucose; MOB were enriched relative to controls (Table 1). Carbon utilization rates increased between the 1 ky and 750 ky sites before decreasing at the 3000 ky site (Fig. 5a) in a manner similar to wet season CH_4 oxidation (Fig. 2a). Although C from the added glucose was utilized by MOB, the glucose addition had no significant effect on MOB biomass at any of the SAGA sites (Fig. 5b, Table 1).

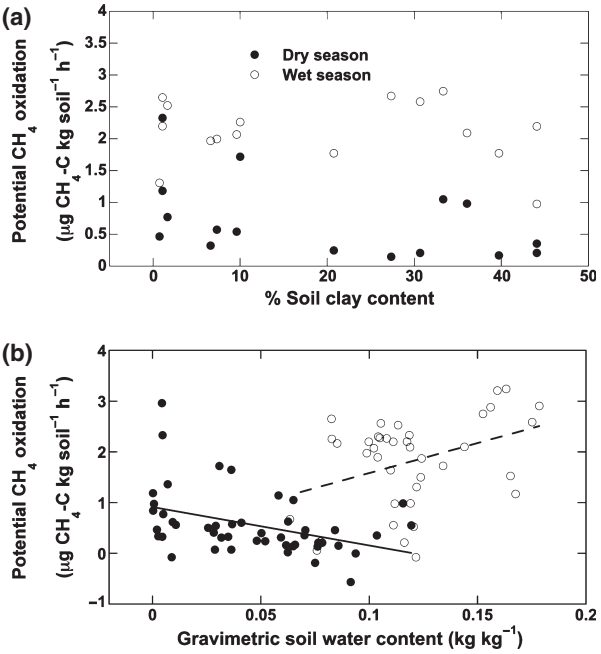

Fig. 3 Relationships between potential methane (CH_4) oxidation rates and (a) soil clay content and (b) gravimetric soil water content across the Substrate Age Gradient of Arizona (SAGA). Regression lines indicate significant linear relationships within a season ($P < 0.05$; $n = 16$ and 48 for soil clay content and gravimetric soil water content, respectively).

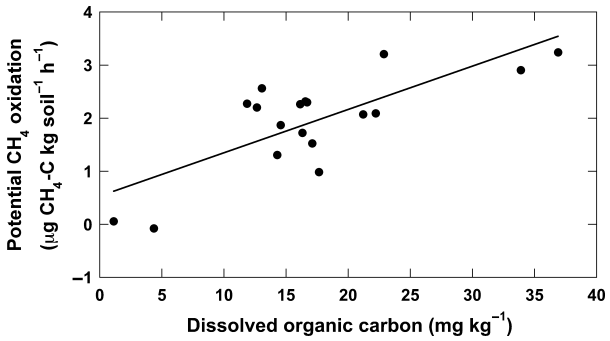

Fig. 4 Relationship between potential methane (CH_4) oxidation rates and soil dissolved organic carbon concentration pooled across the Substrate Age Gradient of Arizona (SAGA) during the wet season. The regression line indicates a significant positive linear correlation ($P < 0.01$; $r^2 = 0.58$; $n = 18$).

Discussion

The results of this study elucidate the potential mechanisms that control CH_4 oxidation in soils of dry ecosystems, and raise several intriguing possibilities that

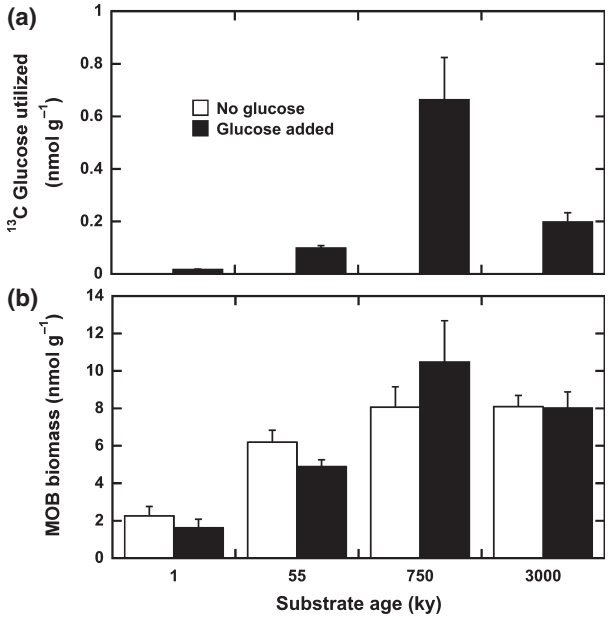

Fig. 5 Effect of substrate age on (a) Methane-oxidizing bacteria (MOB) utilization of labeled ^{13}C-glucose and (b) MOB biomass measured by phospholipid fatty acid (biomarker 18:1ω7c) concentration. Error bars indicate one standard error of the mean (n = 3).

warrant further investigation. The relationships we measured between soil physical properties and potential CH_4 oxidation among seasons and substrate ages did not match the paradigm established in more humid ecosystems. In temperate forests and grasslands, CH_4 oxidation increases as soils dry out; more air-filled pore space results in greater CH_4 substrate diffusing into the soil from the atmosphere. At some point, soils become sufficiently dry that MOB become water limited and CH_4 oxidation rates decrease.

Methane oxidation in arid ecosystems appears to function differently. Similar to patterns found by Striegl *et al.* (1992), our results indicate that wet soils have the capability to oxidize more CH_4 than dry soils. Striegl *et al.* (1992) used a watering experiment to suggest that water limitation to MOB limited their activity, and hence CH_4 oxidation rates. But three factors in this study suggest it is unlikely that low rates of potential CH_4 oxidation during the dry season were caused by water limitation of MOB. First, in the dry season, the youngest site had the lowest gravimetric soil water content (<0.01 kg kg^{-1}), yet had the highest rates of CH_4 oxidation of all four sites (Fig. 2a). If soil water limited CH_4 oxidation by MOB, we should have observed little to no CH_4 oxidation at the 1 ky site. Second, the 750-ky-old site had the greatest increase in potential CH_4 oxidation rates between the dry and wet season, despite having the smallest seasonal increase in gravimetric soil water content of the four SAGA sites (Fig. 2b). Such a disproportionate increase would argue against desiccation as a limiting factor of CH_4 oxidation. Third, the lack of a significant relationship between soil water potential and potential CH_4 oxidation among sites in either season would suggest that CH_4 oxidation is decoupled from water stress. These results are notably different from a desert ecosystem in Israel where most soils showed no net CH_4 consumption under laboratory and *in situ* conditions (Angel & Conrad, 2009). The two sites measured by Angel & Conrad (2009) experienced nearly an order of magnitude less precipitation (mean annual precipitation = 89.5 and 22.9 mm) than the four sites in this study (340 mm). Such a discrepancy indicates diverse responses to soil moisture in arid ecosystems. It remains to be determined at what soil moisture status an arid region ceases to be a sink for atmospheric CH_4.

It is evident that something other than soil water content and soil texture explains the CH_4 oxidation patterns we observed. Particularly curious was the unimodal, retrogressive (Selmants & Hart, 2008; Peltzer *et al.*, 2010) pattern of potential CH_4 oxidation among the sites in the wet season, which is a trend repeatedly exhibited in pools and fluxes of C and N (Selmants & Hart, 2008; Sullivan *et al.*, 2012). Nitrogen, especially ammonium (NH_4^+) has been shown to have a diverse and complex interaction with CH_4 oxidation, but most often NH_4^+ inhibits CH_4 oxidation (Aronson & Helliker, 2010). Because NH_4^+ concentrations follow the same unimodal pattern as wet season potential CH_4 oxidation among the SAGA sites, NH_4^+ would not explain either the inter- or intraseasonal patterns of potential CH_4 oxidation we measured. Instead, we focused on organic C, which varies substantially across the SAGA in the same unimodal trend among sites as wet season potential CH_4 oxidation (Selmants & Hart, 2008). Evidence is rapidly mounting that MOB facultatively use organic C sources in addition to CH_4 (Conrad, 2009; Aronson & Helliker, 2010; Dunfield *et al.*, 2010; Belova *et al.*, 2011; Im & Semrau, 2011; Pratscher *et al.*, 2011; Wieczorek *et al.*, 2011).

We present two lines of evidence that indicate DOC may be an important mechanism regulating CH_4 oxidation in these soils. First, DOC explained more of the variation in wet season potential CH_4 oxidation when all SAGA sites were pooled (r^2 = 0.58, P < 0.01; Fig. 4) than gravimetric soil water content (r^2 = 0.14), soil water potential (r^2 = 0.04), or soil texture (r^2 = 0.02). To our knowledge, this represents the first correlative evidence from arid ecosystems that DOC was strongly related to CH_4 oxidation. Second, our laboratory incubation provided experimental evidence that MOB, as denoted by the 18:1ω7c fatty acid, utilized ^{13}C-labeled

glucose in a unimodal pattern similar to wet season CH_4 oxidation rates.

While not all organisms with the 18:1ω7c fatty acid are MOB (Frostegård & Bååth, 1996), and not all MOB have the 18:1ω7c fatty acid, the 18:1ω7c fatty acid was common to MOB in a variety of studies (Knief *et al*., 2003; Maxfield *et al*., 2006; Dunfield *et al*., 2010). These studies have identified MOB using ^{13}C-PLFA methods; for instance, by exposing soil to ^{13}C–CH_4 and measuring isotopic enrichment of the 18:1ω7c fatty acid (Knief *et al*., 2003; Maxfield *et al*., 2006). Such previous labeling experiments would not have detected incorporation of other sources of organic C into MOB biomass, but by adding ^{13}C-glucose, rather than ^{13}C–CH_4, we were able to evaluate this C flux. While an important organic molecule, glucose does not reflect the broad suite of organic C in DOC, though, like other forms of DOC (Chantigny *et al*., 2008), it is easily utilized by most aerobic soil heterotrophs. It is also possible that the ^{13}C enrichment of MOB in this study was the result of MOB utilizing derivatives of glucose, after decomposition by soil heterotrophs, in addition to glucose itself. In fact, MOB recently have been found to utilize other organic C sources including acetate (Belova *et al*., 2011; Pratscher *et al*., 2011) and ethanol (Im & Semrau, 2011). Further studies will be required to determine the nature of the C compounds utilized by MOB in arid ecosystems, but currently our results are the first to indicate that MOB in arid ecosystems may use other sources of C than CH_4. Unfortunately, our study design prevents us from eliminating the possibility that methanogens used labeled glucose to produce CH_4, which was then oxidized by MOB. However, this is an unlikely scenario, for the glucose incubation was carried out near field capacity, so the anaerobic conditions ideal for methanogens would have been rare, and the isotopic enrichment of the 18:1ω7c fatty acid indicated that a substantial amount of labeled C was incorporated into cells (Table 1). We encourage future investigators to utilize technologies such as stable isotope probing of RNA and DNA (Pratscher *et al*., 2011) and fluorescence spectroscopy (McKnight *et al*., 2001; Cory *et al*., 2011) to address these unknowns.

Laboratory-based methods like potential assays are useful for their ability to isolate mechanisms that control a biogeochemical process, but a limitation of their approach is the inability to extrapolate to realistic *in situ* fluxes. Yet *in situ* CH_4 oxidation has been characterized in northern Arizona soils, and it is interesting to reconsider two studies, performed in close proximity to the SAGA, in light of our results. In an experiment that measured *in situ* CH_4 oxidation in soils at multiple positions across an elevational gradient in northern Arizona, correlations between *in situ* CH_4 oxidation and *in situ* CO_2 efflux were stronger than correlations between *in situ* CH_4 oxidation and temperature or soil water content (Hart, 2006). This trend was attributed to similar responses of both MOB and the broader heterotrophic community to environmental conditions that favor growth and activity, which would be consistent with the availability of DOC. On the same elevation gradient, another study found environmental variables poorly explained *in situ* CH_4 oxidation, but *in situ* CH_4 oxidation rates of soil from dry ecosystems were higher in the wet season than the dry season, and experimental water addition substantially increased soil CH_4 oxidation rates in most sites (Blankinship *et al*., 2010). Furthermore, the effect of water addition on soil CH_4 oxidation rates increased with time (up to 8 h) since water was added. The authors interpreted this result as evidence of water stress, but it is possible that the pulse water additions actually caused several major changes in microbial C dynamics that increased C availability, a phenomenon known as the 'Birch effect' (Birch, 1958; Jarvis *et al*., 2007).

It appears that in semiarid soils, DOC stimulates CH_4 oxidation. But generally, the directionality and magnitude of the relationship between CH_4 oxidation and DOC remains unclear. The positive correlation between DOC and CH_4 oxidation we observed (Fig. 4) stands in contrast to recent evidence from a mire in central Europe indicating that MOB may preferentially utilize organic C over CH_4 as a substrate, resulting in lower overall rates of CH_4 oxidation (Wieczorek *et al*., 2011). Similarly, a laboratory experiment showed that glucose addition to soil suppressed CH_4 oxidation by 83% relative to unamended soil from a temperate forest in Germany (Fender *et al*., 2012). We speculate that the different effect of organic C on CH_4 oxidation rates between these studies may be due to differences in CH_4 concentrations and seasonal dynamics of DOC concentrations between relatively humid and arid ecosystems. High CH_4 concentrations in wetter environments, especially mires, likely support low-affinity MOB. When these low-affinity MOB oxidize DOC, they oxidize less CH_4. In contrast, high-affinity MOB in arid soils may opportunistically oxidize DOC during brief periods of high DOC concentrations, thereby subsidizing the production and activity of methane monooxygenase enzymes. Subsequently, CH_4 oxidation rates are higher during periods of high DOC availability.

Nearby northern Arizona piñon-juniper woodlands and desert grasslands both had higher mean annual *in situ* CH_4 oxidation rates than the global mean values for deserts, grasslands, chapparal ecosystems, and temperate forests (Dutaur & Verchot, 2007; Blankinship *et al*., 2010). In a meta-analysis of CH_4 oxidation studies

designed to quantify the size of the global soil CH_4 sink, dry ecosystems were the least studied on Earth, with only five studies in deserts and three studies in chapparal ecosystems (Dutaur & Verchot, 2007). The soil physical characteristics that often adequately predict CH_4 oxidation in humid environments regularly fail to explain much variation of CH_4 oxidation in semiarid forests and woodlands of northern Arizona (Hart, 2006; Sullivan *et al*., 2008; Blankinship *et al*., 2010; Sullivan *et al*., 2011; this study). Given that potential CH_4 oxidation rates were more than five times higher in wet soil than dry soil at one of our sites, DOC has the potential to strongly influence annual CH_4 budgets in this semiarid ecosystem. Furthermore, our estimates of the effects of DOC may actually be conservative because we collected our samples in the grassy canopy interspaces, rather than under tree canopies, where organic C values were consistently and significantly higher (Selmants & Hart, 2010). Because arid and semiarid ecosystems comprise a substantial portion of the Earth's land surface (Archibold, 1995; Dutaur & Verchot, 2007), it is entirely possible that CH_4 oxidation in dry ecosystems during periods of relatively high DOC availability may increase the size of the global soil CH_4 sink above previous estimates.

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