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Effect of Temperature and Oxidation Rate on Carbon-isotope Fractionation during Methane Oxidation by Landfill Cover Materials

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The quantification of methane oxidation is one of the major uncertainties in estimating CH_4 emissions from landfills. Stable isotope methods provide a useful field approach for the quantification of methane oxidation in landfill cover soils. The approach relies upon the difference between the isotopic composition of oxidized gas at the location of interest and anaerobic zone CH_4 and knowledge of α_{ox}, a term that describes the isotopic fractionation of the methanotrophic bacteria in their discrimination against $^{13}CH_4$. Natural variability in α_{ox} in different landfill soils and the effect of temperature and other environmental factors on this parameter are not well defined. Therefore, standard determinations of α_{ox}, batch incubations of landfill cover soils with CH_4, were conducted to determine α_{ox} under a variety of conditions. When these results were combined with those of previous landfill incubation studies, the average α_{ox} at 25 °C was 1.022 ± 0.0015. α_{ox} decreased with increasing temperature ($-0.00039\ \alpha_{ox}\ °C^{-1}$) over the temperature range of 3−35 °C. α_{ox} was found to be higher when determined after CH_4-free storage and declined following CH_4 pretreatment. α_{ox} declined nonlinearly with increasing methane oxidation rate, V_{max}.

Introduction

Globally, the overall waste sector is responsible for less than 5% of total greenhouse gas emissions including CO_2, CH_4, N_2O, and other gases (*1*). Landfilling of solid waste is responsible for about 3−7% of global CH_4 emissions (*2, 3*) and is considered to be the largest anthropogenic CH_4 source in the U.S. (*4*). As landfills are engineered facilities, they offer excellent opportunities for CH_4 emission mitigation. At large modern facilities gas capture for power generation or flaring reduces emissions significantly. Fugitive emissions from all landfills and total emissions from older and smaller landfills without gas collection systems can be minimized by methanotrophic bacteria in soil layers and biofilters (*5−12*).

Enhancing landfill cover CH_4 oxidation with biological systems is an approach that is being utilized by a number of research groups across the world (*13−17*). In a broader context, lack of knowledge about the extent of methane oxidation is one of the "soft spots" in the global methane budget (*18*).

While soil incubations and models can be used to constrain estimates of cover oxidation (*19−22*), the stable isotope approach offers a noninvasive measurement technique for determination of CH_4 oxidation (*23, 24*). Methanotrophic bacteria consume $^{12}CH_4$ at a slightly faster rate than $^{13}CH_4$ (*12, 25*). This results in a shift in the isotopic ratio, or fractionation. From the shifts in isotopic composition of CH_4 before (δ_{anox}) and following CH_4 oxidation (δ_z) and the degree of isotopic fractionation by the microbial population (α_{ox}) it is possible to calculate the fraction of methane oxidized (*24*),

$$f_{ox} = \frac{\delta_z - \delta_{anox}}{1000(\alpha_{ox} - \alpha_{trans})} \quad (1)$$

where f_{ox} is the fraction of methane oxidized in the passage of methane through the aerobic layer of the soil, δ_{anox} and δ_z are $\delta^{13}C$ values for anoxic zone CH_4 and CH_4 sampled at depth z (within the oxidation zone) or emitted CH_4 (that has passed through the oxidation zone), α_{ox} is the isotope fraction factor due to oxidation, and α_{trans} is the isotope fraction factor due to transport. The term α_{trans} is assumed to be 1 for purely advective systems and is >1 where diffusion is important (*26*). Landfills have been considered to be sites where gas exchange is dominated by advection. Liptay et al. (*24*) argued that landfill gas transport is dominated by advection because of the significant gas production within the landfill and changes in methane emission observed due to variations in atmospheric pressure. If diffusion is important in a system, neglecting it will result in this approach yielding conservative estimates (*12, 26*).

The rate of oxidation is dependent on the volumetric concentration (X), and the first-order rate constant (k):

$$\frac{dX_{12}}{dt} = -k_{12}X_{12} \quad (2)$$

$$\frac{dX_{13}}{dt} = -k_{13}X_{13} \quad (3)$$

k for $^{12}CH_4$ is greater than that for $^{13}CH_4$, and the ratio k_{12}/k_{13} is the kinetic isotope effect or fractionation factor α_{ox}. In practice, α_{ox} is determined from a batch incubation of landfill soil under air with methane (*24, 27, 28*) and is obtained by finding the slope of the regression for ln X on the y-axis and ln (δ + 1000) on the x-axis as described by Mahieu et al. (*22, 26*):

$$\alpha_{ox} = \frac{\text{slope}}{1 + \text{slope}} \quad (4)$$

The δ ‰ notation represents the stable isotopic ratio as follows.

$$\delta = 1000\left(\frac{R_{sam}}{R_{std}} - 1\right) \quad (5)$$

where R_{sam} is the $^{13}C/^{12}C$ ratio of the sample and R_{std} is the ratio for standard Vienna Peedee Belemnite (0.01124).

The isotope technique is increasingly being utilized worldwide (*29*), and was recently described as "a promising approach and currently one of the most precise methods available for directly determining methane oxidation in landfill cover soils" (*30*). This group further stated that the

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TABLE 1. Intercepts and Slopes for Landfill Soil Incubation A_{0x} versus Temperature (°C) and α_{ox} at 25° C

material	temperature °C	observations *n*	intercept α_{ox}	slope α_{ox} °C^{1-}	std. err. of slope σ_{xi}	std.err of regression σ	$\hat{\alpha}_{ox}$[a] at 25 °C α_{ox}
Leon County, Florida, clay[b]	8−35	8	1.041	-4.07×10^{-4}	1.79×10^{-4}	5.36×10^{-3}	1.031
Leon County, Florida, mulch[b]	8−35	8	1.041	-4.30×10^{-4}	6.08×10^{-5}	1.82×10^{-3}	1.030
Falkoping, Sweden, sandy soil[c]	4−25	11	1.028	-1.71×10^{-4}	8.34×10^{-5}	2.89×10^{-3}	1.023
Hokhuvud, Sweden, sandy soil[c]	4−25	6	1.039	-4.48×10^{-4}	3.28×10^{-5}	7.95×10^{-4}	1.028
Filborna, Sweden, soil[d]	5−20	4	1.020	-9.80×10^{-5}	2.26×10^{-4}	2.53×10^{-3}	1.017
Hagby, Sweden, soil[d,d]	3−20	4	1.027	-3.04×10^{-4}	3.40×10^{-5}	4.27×10^{-4}	1.017
Haljestorp, Sweden, soil[d,d]	3−20	3	1.036	-6.64×10^{-4}	1.58×10^{-5}	1.91×10^{-4}	1.019
Hogbytorp, Sweden, soil[d,d]	3−20	4	1.023	-2.20×10^{-4}	8.36×10^{-5}	1.05×10^{-3}	1.016
Sundsvall, Sweden, soil[d,d]	3−20	4	1.024	-3.53×10^{-4}	8.80×10^{-5}	1.11×10^{-3}	1.015
Visby, Sweden, soil[d,d]	3−20	4	1.021	-2.39×10^{-4}	4.16×10^{-5}	5.23×10^{-4}	1.015
Outer Loop, KY, mulch BC[e]	10−22	4	1.028	-5.98×10^{-4}	3.28×10^{-5}	3.64×10^{-4}	1.013
Outer Loop, KY, FBC[e]	10−22	4	1.041	-4.87×10^{-4}	1.64×10^{-4}	1.82×10^{-3}	1.029
Outer Loop, KY, sandy soil, SC[e]	10−22	4	1.038	-9.01×10^{-4}	2.78×10^{-5}	3.08×10^{-4}	1.016
Outer Loop, sandy soil 5[e]	25	5				$2.92 \times 10-3$[f]	1.020
Outer Loop, sandy soil 7[e]	25	6				$4.98 \times 10-3$[f]	1.023
Springhill 10−20 cm, sandy soil[g]	6−33	8	1.029	4.00×10^{-6}	1.55×10^{-4}	4.47×10^{-3}	1.029
Springhill 20−30 cm, sandy soil[g]	6 − 33	8	1.040	-4.81×10^{-4}	1.08×10^{-4}	3.11×10^{-3}	1.028
				statistics			
mean				-3.9×10^{-4}			1.022
standard error				6.2×10^{-5}			0.0015

[a] α_{ox} estimated from regression (averages for Outer Loop 5 and 7). [b] Florida (Chanton and Liptay (*30*). [c] Sweden (Borjesson et al. (*32*)). Slope and standard error estimated from summary statistics. [d] Sweden (Borjesson et al. (*33*)). Standard error estimated from graphed data. [e] Kentucky (this study). For Outer Loop 5 and 7 α_{ox} was only measured at 25 °C. [f] Standard error of the mean. [g] Florida (this study). 43 °C data excluded.

advantages of the isotope approach are that the method is noninvasive and allows direct measurement of methane oxidation and that sample collection is easy. However it was noted that a current drawback is the lack of knowledge of the fractionation factor, α_{ox}. Possible factors that influence this term include temperature, oxidation rate, microbial population, number, type and their physiological state, moisture content, soil properties, and nutrient status.

As recently described in detail (*22*), standard practice is to determine α_{ox} in closed system batch incubations of landfill soil. The fractionation factor so determined is then applied to field measurements of anoxic zone (δ_{anox}) and oxidized (δ_z)methane to determine the fraction of methane oxidized in situ (f_{ox}) (*8, 11*−*13, 16, 24, 26*−*28, 31*).

The relationships between α_{ox} and temperature reported in the literature are contradictory. Coleman et al. (*25*) found that α_{ox} increased with increasing temperature from 1.0130 at 11.5 °C to 1.0200 at 26 °C in liquid cultures. Tyler et al. (*32*) used field measurements of f_{ox} and δ and the Rayleigh approach for forest soils to calculate α_{ox}; and using the temperature of the most active layer, they found that α_{ox} decreased with increasing temperature from 1.023 at 5.6 °C to 1.021 at 16.6 °C. Bergamaschi et al. (*23*) also used the Rayleigh method to calculate α_{ox}, and found that it increased slightly with temperature, from 1.005 at 8.1 °C to 1.009 at 11.1 °C and at 26.9 °C. Liptay et al. (*24*) incubated samples of landfill cover material and found that the different sand and clay contents of the samples did not affect α_{ox}, which averaged 1.022 at 25 °C. Chanton and Liptay (*31*) incubated landfill clay and mulch, and found that for both materials α_{ox} decreased linearly with increasing temperature from an average of 1.039 at 8 °C to 1.027 at 35 °C with r^2 values of 0.96 and 0.92. Snover and Quay (*33*) found the combined kinetic isotope effect of oxidation and diffusion (α_{soil}) in the field to average 1.0173 for grassland soil (17.4 °C) and 1.0181 for forest soil (21.3 °C). Borjesson et al. (*27*) incubated two landfill cover soils and found that α_{ox} for one soil declined with increasing temperature from 1.0270 at 4 °C to 1.0234 at 25 °C, and for the other soil it declined from 1.0375 at 4 °C to 1.0281 at 25 °C. Borjesson et al. (*28*) reported that α_{ox} decreased with increasing temperature in six landfill soils in Sweden.

Cell density may also influence α_{ox} as shown by Templeton et al. (*34*), who found that in liquid culture (22−24 °C) low cell densities resulted in α_{ox} greater than 1.030, whereas higher cell densities resulted in α_{ox} as low as 1.003. α_{ox} varied over time in their experiments as the cell density in their liquid cultures increased by a factor of 10. Templeton et al. (*34*) postulated that isotopic measurements could not be used to estimate CH_4 oxidation because of this temporal variability in cell density and associated variation in α_{ox}.

This study was conducted to compile the data from the literature on the effect of temperature on landfill soil α_{ox} and to report additional measurements conducted in our laboratory. A second objective of this study was to determine the variability in α_{ox} from various landfill cover materials and to examine the postulation of Templeton et al. (*34*) that the isotopic signature of residual CH_4 cannot be used to determine methane oxidation. Our third objective was to examine the variation of α_{ox} with the maximum oxidation rate (V_{max}).

Materials and Methods

Soil Material. Soils were collected from a variety of landfills and treated one of two ways. Samples listed in Table 1, with the exception of those from Springhill, were immediately refrigerated and incubations commenced within 5 days. Samples listed in Table 2 were shipped to our laboratory in 20 L lidded plastic buckets by ground transportation which resulted in their being held at room temperature from 1−2 weeks. These samples were pretreated with 6% CH_4 for at least 9 days before starting the measurments, with the exception of the first run on soil from the Florida Landfill (Springhill) that was also pretreated for 1 day. The preincubation period was performed to "reconstitute" the microbes to conditions they likely experienced in situ in the landfill soils from which they were sampled.

TABLE 2. Incubation Conditions and Results for Each Flask

soil material	temperature °C	water g g^{-1}	nmol g^{-1} h^{-1} V_{max}	std. err.	ln *X*/ ln (δ + 1000) regression slope	std. err.	R^2	α_{ox}
Iowa	23.7	0.382	248	31	−33.06	0.56	0.997	1.031
Iowa	23.7	0.382	206	25	−29.94	0.55	0.996	1.035
Outer Loop Unit 5	24.6	0.185	269	34	−38.67	0.93	0.997	1.027
Outer Loop Unit 5	24.6	0.185	275	24	−38.95	1.39	0.995	1.026
Peoria 1[a]	25.6	0.329	1750	747	−42.11	1.36	0.999	1.024
Peoria 1[a]	25.6	0.329	1967	627	−39.45	1.05	0.999	1.026
Peoria 1[a]	25.6	0.329	4804	320	−44.31	4.12	0.991	1.023
Peoria 2[a]	23.9	0.303	310	24	−21.23	0.71	0.989	1.049
Peoria 2[a]	23.9	0.303	386	32	−21.95	0.76	0.993	1.048
Peoria 2[a]	23.9	0.303	252	14	−21.64	0.68	0.993	1.048
Springhill 10−20 cm	6.0	0.108	40	12	−29.53	1.90	0.960	1.035
Springhill 10−20 cm	6.0	0.108	13	5	−45.91	5.84	0.873	1.022
Springhill 10−20 cm	15.0	0.108	496	59	−32.21	0.99	0.991	1.032
Springhill 10−20 cm	15.0	0.108	90	9	−39.07	0.79	0.996	1.026
Springhill 10−20 cm 1[b]	25.0	0.108	256	40	−32.42	0.53	0.997	1.032
Springhill 10−20 cm 1[b]	25.0	0.108	218	35	−32.79	0.40	0.999	1.031
Springhill 10−20 cm 2[b]	25.0	0.108	2365	465	−39.69	0.99	0.997	1.026
Springhill 10−20 cm 2[b]	25.0	0.108	928	92	−36.17	0.73	0.997	1.028
Springhill 10−20 cm	33.0	0.108	3026	199	−37.85	1.95	0.990	1.027
Springhill 10−20 cm	33.0	0.108	1413	151	−32.27	1.61	0.980	1.032
Springhill 10−20 cm	43.0	0.108	442	75	−32.88	1.37	0.981	1.031
Springhill 10−20 cm	43.0	0.108	371	43	−33.28	1.30	0.973	1.031
Springhill 20−30 cm	6.0	0.112	21	8	−28.72	3.24	0.897	1.036
Springhill 20−30 cm	6.0	0.112	51	9	−24.90	1.10	0.981	1.042
Springhill 20−30 cm	15.0	0.112	113	11	−34.98	1.15	0.989	1.029
Springhill 20−30 cm	15.0	0.112	585	51	−33.60	1.41	0.991	1.031
Springhill 20−30 cm 1[b]	25.0	0.112	565	140	−30.63	0.54	0.997	1.034
Springhill 20−30 cm 1[b]	25.0	0.112	503	88	−32.27	0.99	0.992	1.032
Springhill 20−30 cm 2[b]	25.0	0.112	2402	195	−39.48	0.39	1.000	1.026
Springhill 20−30 cm 2[b]	25.0	0.112	3704	407	−40.63	0.29	1.000	1.025
Springhill 20−30 cm	33.0	0.112	3644	55	−38.25	1.38	0.996	1.027
Springhill 20−30 cm	33.0	0.112	6657	846	−40.79	1.41	0.998	1.025
Springhill 20−30 cm	43.0	0.112	733	43	−33.50	0.92	0.993	1.031
Springhill 20−30 cm	43.0	0.112	1266	165	−39.86	1.27	0.993	1.026

[a] Peoria 2 was conducted 59 days after Peoria 1, and during the interim the soil material was stored moist at room temperature with air but without CH_4. [b] Springhill 1 was pretreated with CH_4 for 1 day and Springhill 2 was pretreated for 9 days. The other soil materials in this table were pretreated for at least 9 days.

Incubations. Following standard protocol for determination of α_{ox} (*22*) batch incubations were performed in close system flasks. About 60 g (moist weight) of soil material was weighed and placed in each 1 L flask, and a sample of soil was oven-dried to determine the water content. An incubation was initiated by flushing the flask with air and then replacing sufficient air with an equal volume of pure methane. Initial concentrations were generally 6% while final concentrations were about 0.5% A homemade balloon manometer was used to prevent large declines in pressure due to consumption of CH_4 and O_2 (*12*). Gas subsamples were taken after O_2 was injected (generally around 20 mL) to return the manometer water level to the original mark. By this means the flask gas pressure and volume were constant for each sample, and O_2 limitation was prevented. The O_2 was mixed in the flask before sampling by attaching the sampling syringe and repeatedly extracting and injecting 30 mL. Twenty mL samples were taken and stored in pre-evacuated 10 mL vials for CH_4 concentration and isotope measurements. Samples were collected twice a day unless the manometer indicated rapid oxidation, in which case samples were taken more frequently. Incubations were conducted for 3−15 days.

Methane Concentration and Isotope Measurements. The sample methane concentration (X_s) was determined with a Shimadzu GC-8A gas chromatograph equipped with a Carbosphere 80/100 column and a flame-ionization detector (FID-GC). The stable C-isotope ratio (δ) was determined by direct injection into a Hewlett-Packard 5890 gas chromatograph coupled via a combustion interface to a Finnigan Mat Delta S isotope ratio mass spectrometer (GCC-IRMS).

***X* and V_{max} Calculations.** After the first sample X_s was corrected for the loss of CH_4 due to sampling to determine what the volumetric concentration of methane would have been from oxidation alone (*X*):

$$X_m = X_{s,m} + \frac{V_s}{V_f}\sum_{i=1}^{m} X_{s,i-1} \quad (6)$$

where *m* is the sample number, V_s is the sample volume and V_f is the flask gas volume. V_f was obtained from the volume of CH_4 injected in the flask and the initial concentration measured.

The maximum oxidation rate V_{max} (nmol g^{-1}_{soil} h^{-1}) and the Michaelis constant K_m (nM, not reported) were obtained by least-squares fitting of the Michaelis−Menten equation to the data:

$$V = \frac{V_{max}S}{K_m + S} \quad (7)$$

where *V* is the rate of CH_4 oxidation (nmol g^{-1}_{soil} h^{-1}) and *S* is the aqueous CH_4 concentration (nM). *V* was obtained from the change in amount of CH_4 in the flask (nmol) from one sample to the next, the oven-dry mass of soil in the flask (g), and the time between samples (h). The amount of CH_4 was calculated from the universal gas law using the volume of

CH_4 in the flask (XV_f) and the temperature. S was obtained from X by

$$S = \frac{10^9 X}{V_m H} \approx 1.5 \times 10^6 X \quad (8)$$

where V_m is the molar volume at 25 °C (24.47 L mol^{-1}) and H is the Henry's constant for CH_4 at 25 °C (27.2 L_{water} L^{-1}_{air} (*35*)).

Results and Discussion

Isotopic fractionation, α_{ox} decreased with increasing temperature at an extent of −0.00039 α_{ox} °C^{-1} over the temperature range of 3−35 °C (Table 1). The Table represents results compiled from the literature and reports new measurements conducted in our laboratory. In all of the soils except for one, α_{ox} decreased as temperature increased. This indicates that at higher oxidation rates, associated with higher temperatures, there is less discrimination against $^{13}CH_4$. The soil with a postive slope, Springhill 10−20 cm, was influenced by a single outlying measurement. The standard error of the mean slope was 6.2×10^{-05}. We suggest that this slope (−0.00039 α_{ox} °C^{1-}) may be used as a correction factor in future studies if the determination of α_{ox} for a soil is only conducted at a single laboratory temperature other than what was measured in the field.

$$\alpha_{ox} = \alpha_{ox,measured} - 0.00039(^oC - ^oC_{measured}) \quad (9)$$

Where α_{ox} represents the fractionation factor at the temperature of interest, °C, and $\alpha_{ox,measured}$ is the fractionation factor measured at $^oC_{measured}$.

For example, if an α_{ox} of 1.025 is measured at 25 °C, then α_{ox} at 15 °C is predicted to be 1.0229 ± 0.0006. The associated error is (°C − $^oC_{measured}$) × $6.2\ 10^{-05}$. A 10‰ shift in $\delta^{13}C$ between anoxic zone and oxidized CH_4, ($(\delta_z - \delta_{anox})$, eq 1) using $\alpha_{ox} = 1.0229$ and neglecting the effects of diffusion for this calculation ($\alpha_{trans} = 1$) will result in $f_{ox} = 0.437$ or 43.7% oxidation. The error in α_{ox} of ±0.0006 translates into an error in % oxidation of ±1.1%.

Also reported (Table 1) for comparison purpose are two cases where α_{ox} was determined from repeated measurements at 25 °C. All of the values of α_{ox} at 25 °C (Table 1) ranged from 1.013 to 1.031 with an average of 1.022 ± 0.0015. Factoring in this α_{ox} mean and standard error results in a range of percent oxidation calculated from eq 1 of 45.5% ± 3.2% for a 10‰ shift in $\delta^{13}CH_4$ from anoxic to oxidized values. The variability in α_{ox} is a source of concern in the application of the isotope approach (*30*). For the same 10‰ shift in $\delta^{13}C$ between anoxic and oxidized CH_4 described above, the variability in percent oxidation ranges from 77 to 32% as a result of varying α_{ox} from 1.013 to 1.031. Note this variation in α_{ox} at 25 °C is compiled from 17 landfills across two continents. Clearly in applying the isotopic method to determine fraction oxidized it is important to have measurements of the isotopic fraction factor α_{ox} at each site and within each soil type at a site.

However, the variability in α_{ox} is not so great as to make estimation of methane oxidation by isotope fractionation useless. The experiments reported by Templeton et al. were conducted under nonsteady-state conditions. The microbial populations increased over 10-fold in their liquid cultures over the time-course of their studies. The fraction of CH_4 oxidized (f_{ox}) increased over their experiments and appears to have been controlled by this increase in cell density. Associated with increasing cell density was a decrease in α_{ox}. Thus α_{ox} varied with the fraction of methane oxidized because both were related to increasing cell density. An additional concern is that these experiments were conducted in aqueous medium and boundary effects would also limit isotopic fractionation relative to landfill soil incubations which are conducted under air.

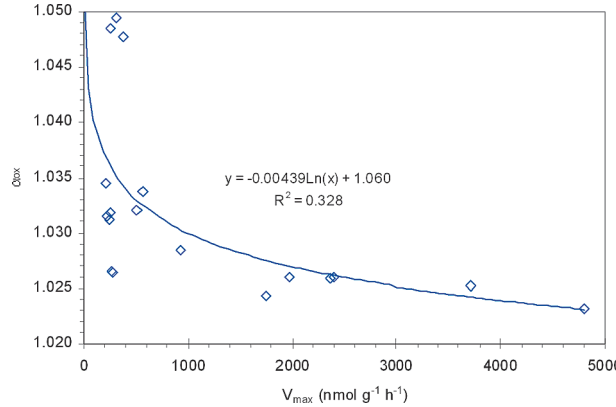

FIGURE 1. α_{ox} versus V_{max} and fitted logarithmic curve for all soils incubated at 24−26 °C. The fit of the function was significant, $p = 0.005$.

In a landfill soil, f_{ox} is controlled by a number of factors in addition to methanotroph cell density. One important factor is the time of exposure of the methane to the soil oxidation zone (*11*), which is governed by soil permeability, moisture content, and gas advection rates. Other factors that control f_{ox} include the supply of methane and oxygen and the methane oxidation rate. Integrating over the entire 30−50 cm thick oxidation zone of the landfill soil, we suggest that the microbial population is quite likely at steady state, so the problems associated with the isotope method described by Templeton et al. should not be so severe. Additionally, in addition to being controlled by reactant concentrations and cell number, the methane oxidation rate itself is controlled by the physiological state of the microbial population, which is governed by a host of factors including moisture, temperature, and nutrient status.

α_{ox} was strongly affected by V_{max}. When a nonlinear curve was fit to individual values of α_{ox} and V_{max} the result was significant (Figure 1, $p = 0.0046$, $n = 19$). The decline in α_{ox} with increasing V_{max} indicates that more rapid CH_4 oxidation is associated with less isotopic discrimination at constant temperature (*34*). This finding is consistent with the temperature α_{ox} relationship reported in Table 1. Temperature also had a significant nonlinear affect on α_{ox} over the entire temperature range 6−44 °C for the Springhill soil, 20−30 cm depth (Figure 2, $p < 0.0001$). This effect may be related to the V_{max} effect because the temperature for the lowest α_{ox} (25 °C) was close to the temperature for maximum V_{max} (33 °C, Table 2). The temperature effect on α_{ox} for Springhill 10−20 cm soil was not significant because of one outlier at 6 °C, but otherwise the relationship was similar to Springhill 20−30 cm (Table 2).

The CH_4 exposure history appeared to alter α_{ox}. The second Peoria run was conducted 59 days after the first; and during this time the soil material was stored moist and aerobic but without added CH_4. This storage resulted in an increase in α_{ox} from 1.025 to 1.049 (Table 2). The length of pretreatment with CH_4 before starting the experiment may also have affected α_{ox}. All samples in Table 2 were pretreated for at least 9 days except Springhill 1, which was pretreated for only one day. When the Springhill 25 °C incubation was repeated with the same samples after the soil had been exposed to 6% CH_4 for 9 days, α_{ox} for all four flasks declined (Table 2). These results are consistent with the idea that cell density is inversely related to α_{ox} (*34*) because it is likely that methanotroph cell density was greater before 59 days of CH_4 starvation, and the density was greater after 9 days of exposure to CH_4. Nontheless, the cell densities and α_{ox} apparently remained

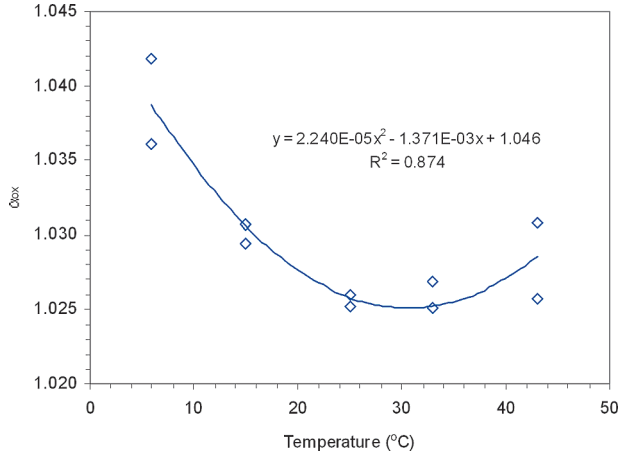

FIGURE 2. α_{ox} versus temperature and fitted polynomial equation for Springhill cover soils for 20−30 cm depth. The equation had minimum α_{ox} in the midrange of temperature: 1.025 at 30.6 °C. The incubations reported in Table 1 were conducted over the temperatures range of 3−35C.

at quasi-steady state over the course of our incubations as evidenced by the good linear fits we obtained (Table 2). Alternatively, these results may indicate that presence of abundant CH_4 alters the methanotrophic populations by promoting the growth of species with a lower preference for $^{12}CH_4$ and resulting in lower α_{ox}. More research is needed to understand this effect, however, Templeton et al. did not observe differences in the isotopic fractionation in the uptake of CH_4 by different types of bacteria.

The impact of water content was further examined for a single landfill soil amended to 9 water contents ranging from 0.105 to 0.401 g g^{-1}. The results indicated no significant relationship: $\alpha_{ox} = -0.018\ \theta_g + 1.023$, $R^2 = 0.142$, $p = 0.317$, but more work is required to thoroughly investigate this parameter.

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