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Carbon kinetic isotope effect accompanying microbial oxidation of methane in boreal forest soils

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Abstract—Atmospheric methane (CH_4) oxidation occurs in soils at sites in the Bonanza Creek L.T.E.R. near Fairbanks, Alaska, USA, at rates $\leq$2 mg CH_4 m^{-2} d^{-1}; the maximum CH_4 oxidizing activity is located in loess at a depth of ~15 cm. Methane, carbon dioxide, and stable isotope (δ^{13}C-CH_4, δ^{13}C-CO_2) depth distributions were measured at two sites: South facing Aspen (AS2) and North facing Black Spruce (BS2).

The combined effects of diffusion and oxidation are similar at both sites and result in a CH_4 concentration decrease (1.8–0.1 ppm) and a δ^{13}C-CH_4 increase (−48‰ to −43‰) from the soil surface to 60–80 cm depth. Isotope flux ratio and diffusion-consumption models were used to estimate the kinetic isotope effect (KIE); these results agree with the observed top-to-bottom difference in δ^{13}C-CH_4, which is the integrated result of isotope fractionation due to diffusion and oxidation. The KIE for CH_4 oxidation determined from these measurements is 1.022–1.025, which agrees with previous KIE determinations based on changes in headspace CH_4 concentration and δ^{13}C-CH_4 over time.

A much lower soil respiration rate in the North facing Black Spruce soils is indicated by fivefold lower soil CO_2 concentrations. The similarity in CH_4 oxidation at the two sites and the differences in inferred soil respiration at the two sites suggest that soil CH_4 oxidation and soil respiration are independent processes. The soil organic matter responsible for the CO_2 flux has a δ^{13}C estimated to be −27 to −28‰.

1. INTRODUCTION

Microbially mediated CH_4 oxidation serves as an important control on CH_4 emitted to the atmosphere; its magnitude is estimated to be about 200 Tg yr^{-1} larger than the 500 Tg yr^{-1} emitted to the atmosphere (Reeburgh et al., 1993). Much of this oxidation occurs in zones below soil and sediment surfaces, where it affects both the flux and isotope composition of CH_4 emitted to the atmosphere.

Oxidation of atmospheric CH_4 in aerobic soils is an important secondary sink for atmospheric CH_4. Born et al. (1990) have estimated a soil sink magnitude of 10–58 Tg CH_4 yr^{-1}, or 3–15‰ of the net atmospheric budget. Uptake and oxidation of atmospheric CH_4 by soils has been observed in a wide range of agricultural, tundra, grassland, desert, boreal forest, deciduous forest, and landfill cover soils (see Reeburgh et al., 1993 for summary). The process is mediated by an unidentified community of aerobic methanotrophic bacteria (Conrad, 1996) operating at low, diffusion-limited rates (Born et al., 1990; Striegl, 1993) and at low concentration (0.1 ppm CH_4) thresholds (Whalen et al., 1992).

Microbially-mediated CH_4 oxidation is accompanied by a kinetic isotope effect (KIE). A summary of estimates of the KIE for CH_4 oxidation (Reeburgh, 1996: Table 2) shows a large range of values. The existing data suggest that fractionation factors are system- and experiment-specific and may not be suitable for general application in global models of CH_4 sources and sinks. Estimates of the CH_4 budget which rely on stable carbon isotope data to balance sources and sinks require information on the isotope fractionation effect of the major sinks, oxidation by OH and Cl, and the soil sink. The model study of Gupta et al. (1996) shows that uptake of CH_4 by the soil sink can enrich the isotopic composition of atmospheric CH_4 by about 1.2‰. Only two estimates of the KIE for oxidation of CH_4 in soils were available, highlighting the need for a better understanding of isotope fractionation associated with microbial oxidation.

Previous estimates of the carbon isotopic fractionation factors for CH_4 oxidation in soils were based on analyses of samples collected from the headspaces of chambers placed above methane-consuming soils. King et al. (1989) collected large (40 L) samples from the headspace of a large (300 L) static chamber deployed at two tundra sites observed to consume methane and used changes in the chamber methane concentration and $\delta^{13}CH_4$ with the relationship for Rayleigh fractionation under equilibrium conditions to calculate a kinetic isotope effect [k_{12}/k_{13} = 1.026 (14°C); 1.016 (4°C)] for soil oxidation. Tyler et al. (1994) used a smaller (152 L) chamber deployed on mixed deciduous hardwood forest soils and applied corrections to account for outside air drawn into the static chamber during isotope sampling. This work involved seasonal field measurements over 2 yr as well as laboratory incubations. The Rayleigh fractionation relationship was used to calculate a KIE of $k_{12}/k_{13} = 1.022 \pm 0.004$. A slight temperature effect opposite that of King et al. (1989) was noted, as was the similarity of the KIE to that expected for diffusion, 1.0195 (Mason and Marrero, 1970; Tyler et al., 1994).

This work extends these two previous studies on decreases in headspace CH_4 by presenting $\delta^{13}CH_4$ measurements on CH_4 samples collected from a soil profile. The soils considered in this study produce no CH_4 internally and are net CH_4

consumers (Whalen et al., 1992). All CH_4 is supplied by diffusion from the atmosphere, and all but a small quantity is microbially oxidized in the soil. Diffusion and biological oxidation are the major processes altering the isotopic composition of soil CH_4. An advantage of the approach used in this study is the fact that the samples were collected in the soil from separate probes, so that the soil methane gradient was essentially undisturbed, and the measurements likely represent in situ values. We show here that changes with depth in the soil are analogous to changes with time in chamber headspaces.

Carbon dioxide concentration and isotope measurements are an additional result of this study. Similar measurements of isotopic variations in CO_2 from soils have been presented by Cerling et al. (1991); results from Bonanza Creek soils are presented to demonstrate the similarity of Bonanza Creek soils to that study and to estimate $\delta^{13}C$ of the source material for respiration, the soil organic matter.

2. EXPERIMENTAL METHODS

2.1. Field Sites

Samples were taken at two previously studied sites in the Bonanza Creek Long Term Ecological Research (L.T.E.R.) site, near Fairbanks, AK. Methane flux studies at these sites have documented a seasonal record of negative methane fluxes (Whalen et al., 1991), the location of the oxidation maximum and soil physical characteristics (Whalen et al., 1992), and the temperature-moisture response of CH_4 oxidation (Whalen and Reeburgh, 1996). Seasonal and site-to-site differences in CH_4 flux and dark-chamber carbon dioxide fluxes (gross system respiration) are reported by Barber (1995). Soils at AS2, a south facing Aspen site, have a thin layer of litter and decomposed organic matter that grades directly into loess. Soils at BS2, a north facing Black Spruce site, are covered with a moss layer that grades into decomposed organic matter overlying loess. Temperature profiles to 15 cm were measured during time-series studies; October 1990 measurements to 60 cm show that BS2 soils are slightly cooler (2.5°C) than AS2 soils (5.5°C; Whalen et al., 1992). Maximum methane CH_4 rates occur below the surface ($\sim$15 cm) in the loess at both sites (Whalen et al., 1992). Methane is consistently consumed at both sites at rates of $\leq$2 mg m^{-2} d^{-1}; integrated growing season CH_4 consumption averages 83 and 73 mg m^{-2} for AS2 and BS2, respectively (Barber, 1995). Dark chamber carbon dioxide fluxes (gross system respiration) reflect the differences in overall productivity at the two sites; they are maximum in July with averages of the 1991 and 1992 median values of 8.2 and 3.9 g m^{-2} d^{-1} at AS2 and BS2, respectively.

2.2. Gas Sampling

Soil air samples were collected using permanently installed gas sampling probes. The probes were made from 1/2" o.d. stainless steel tubes perforated over the bottom 5 cm. A pointed driving rod, which fitted snugly inside the sampling probe and extended 1 cm beyond the sampling tube, prevented plugging during driving and was withdrawn when the perforated end of the sampling probe was driven to the desired depth. Arrays of gas sampling probes spaced 30 cm apart were deployed at each site; probe depths were adjusted so that soil gases could be sampled at 5 cm intervals. After driving, the top of each tube was capped with a 1/2–1/4" Swagelok reducing union. The 1/4" end was fitted with a silicone rubber septum for syringe sampling. These probes had been in place for over 2 yr when the samples treated in this paper were collected.

The gas samples for concentration analysis were collected with syringes. Approximately three sample probe volumes of gas were withdrawn with large syringes prior to sampling with 10 cc glass syringes. The syringes were sealed by inserting the hypodermic needle tips into a butyl rubber stopper. The samples for isotope analysis were collected by pumping through 1/4" Teflon tubing into evacuated 6 L electropolished stainless steel canisters with a battery-operated diaphragm pump (KNF Neuberger, Trenton, NJ). To avoid isotope fractionation, the canister valve was opened only slightly so that it remained under positive pressure as the initially evacuated canister was filled to a final pressure of $\sim$27 psig. The canister samples were pumped from probes with a lateral separation of at least 1 m. Average agreement between CH_4 measurements on samples withdrawn from the pressurized canisters and the concentration profiles collected by syringes at the same soil depths was 0.1 ppmv. Only one instance (BS2, see Fig 2) of contamination of the samples by air channelling along the probes in the upper portion of the profile is evident.

This work involved isotope measurements on very low concentrations of CH_4 (0.1 ppmv) in the soil gas. We anticipated that large soil gas samples would be required for reliable isotope measurements on CH_4 with conventional techniques and collected large ($\sim$18 L) samples. Following sample collection we learned of advances in gas chromatography combustion isotope ratio monitoring (irm GC/MS) techniques (Sansone et al., 1997) and arranged to collaborate in the isotope analyses with colleagues at the University of Hawaii.

2.3. Gas Concentration Analyses

Gas samples from the syringes and canisters were analyzed within hours of collection by gas chromatography. A Shimadzu GC mini-2 gas chromatograph equipped with a flame ionization detector (FID) and a Molecular Sieve 5A column (60/80 mesh, 1 m x 1/8" o.d.) operated at 70°C with N_2 (33 mL min^{-1}) carrier was used for the methane analyses. The carbon dioxide measurements were performed with a Shimadzu GC-8A equipped with a thermal conductivity detector and a Porapak Q column (50/80 mesh, 1 m x 1/8" o.d.) column operated at 50°C with He carrier (33 mL min^{-1}). Both gas chromatographs were equipped with sampling valves and calibrated sample loops. Standards from the National Institute for Technology and Standards (NIST) or NIST-relatable standards were used for calibration.

2.4. Carbon Isotope Analyses

2.4.1. $\delta^{13}C$-CH_4

The carbon isotopic composition of CH_4 was determined by irm-GC/MS using the method of Sansone et al. (1997), which incorporates modifications of methods by Merritt et al. (1995a) and Popp et al. (1995). Gases were transferred into a 50 mL sample loop at 60 mL min^{-1} using the pressure in the sample canister. Carbon dioxide was removed by passing the sample after injection through a 6 cm x 6.4 mm o.d. polypropylene column packed with Ascarite. The remaining gases were trapped on a 15 cm x 3.2 mm o.d. stainless steel column packed with Porapak-Q (80–120 mesh) at liquid nitrogen temperature. Rapid heating of the Porapak-Q column with boiling water desorbed the gases onto a 2.4 m x 6.4 mm o.d. column packed with Porapak-Q (80–100 mesh) held at subambient temperatures (0 to −6°C). This column allowed baseline separation of N_2 and O_2 from CH_4 in $\sim$4.5 min at a carrier flow rate of 60 mL min^{-1}. Methane was trapped by diverting the CH_4 peak to a second 15 cm x 3.2 mm o.d. stainless steel column packed with Porapak-Q (80–120 mesh) held at liquid nitrogen temperature. Methane and any remaining gases were subsequently transferred to a cryofocusing segment prior to final separation of CH_4 from residual N_2, O_2, and CO_2 using a PoroPLOT-Q column (0.32 mm o.d. x 25 m) held at subambient temperature (−25°C; see Popp et al., 1995 for procedural details). The CH_4 separated on the PoroPLOT-Q column was combusted (1150°C, NiO_2/Pt oxidant), water from combustion was removed, and isotopic composition was determined using a MAT 252 irm-GC/MS system previously described by Hayes et al. (1990) and Merritt et al. (1995b). Accuracy and precision of this method was determined to be ± 0.3‰ by analysis of 2.5–5 nmoles of CH_4 from a tank of a laboratory standard containing 100 ppm CH_4 in He. The size and isotopic composition of the analytical blank was determined using the techniques of Gelwicks and Hayes (1990). The analytical blank ranged from 2–30‰ of the total concentration (sample

+ blank) of the samples analyzed. The d-value of the blank (−44.1‰ *vs.* PDB) was close to that of the samples and resulted in a small correction to the isotopic composition of the samples (up to 0.6‰, but typically less than 0.1‰).

2.4.2. $\delta^{13}C$-CO_2

The carbon isotopic composition of CO_2 was determined by irm-GC/MS using a modification of the headspace method of Popp et al. (1995). Briefly, gas was transferred to a sample loop using the pressure in the sample container. The sample loops ranged in size from 50–500 μL depending on the concentration of CO_2 in the gas analyzed. Gases were cryofocused on a 2 cm Porapak-Q (80–120 mesh) segment located within a capillary precolumn which was cooled to −75°C using a dry ice-pentane slush. Separation of N_2, O_2, and CO_2 was accomplished on a 25 m x 0.32 mm PoraPLOT-Q (Chrompack Inc.) analytical capillary column held at 30°C. Accuracy and precision was determined to be better than 0.2‰ by repeated analysis of a laboratory standard containing 100 ppm CO_2 in He. The analytical blank was found to be negligible for these samples.

3. RESULTS AND DISCUSSION

The methane and $\delta^{13}C$-CH_4 depth distributions at sites AS2 and BS2 are shown in Figs. 1 and 2. The carbon dioxide and $\delta^{13}C$-CO_2 distributions for sites AS2 and BS2 are shown in Figs. 3 and 4. Note that the points plotted at 0 cm are air samples collected at 1 m height above the forest floor.

The kinetic isotope effect (KIE) due to microbially-mediated methane oxidation can be inferred from our data by three approaches: a flux ratio model, a simple steady-state diffusion-consumption model, and by the difference between the $\delta^{13}C$ values at the top and bottom of the soil profile.

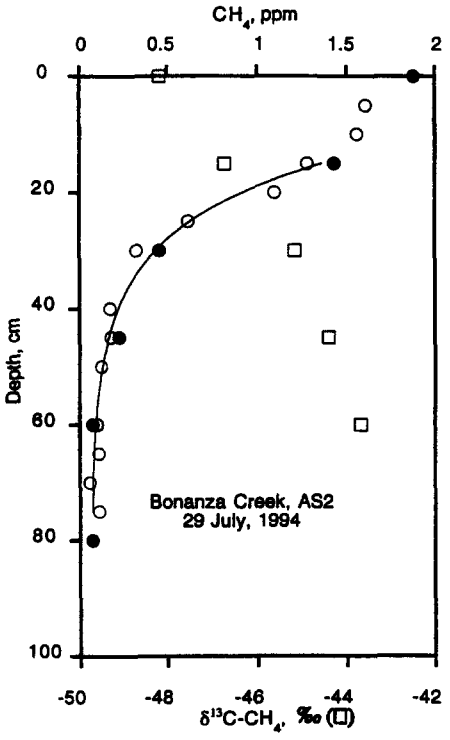

Fig. 1. Soil methane concentration and $\delta^{13}C$-CH_4 depth distributions at the AS2 (South-facing Aspen) site in the Bonanza Creek L.T.E.R., 29 July 1994. Circles are methane concentrations from syringe (○) and canister (●) samples, squares (□) are $\delta^{13}C$-CH_4. The 0 cm points are air samples collected at 1 m height. The CH_4 depth distribution (syringe samples: ○) was fitted using Eqn. 9.

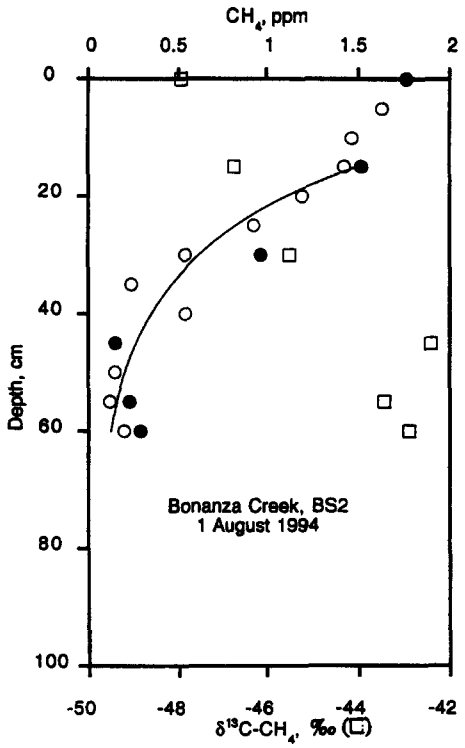

Fig. 2. Soil methane concentration and $\delta^{13}C$-CH_4 depth distributions at the BS2 (North-facing Black Spruce) site in the Bonanza Creek L.T.E.R, 1 August 1994. Circles are methane concentrations from syringe (○) and canister (●) samples; squares (□) are $\delta^{13}C$-CH_4. The 0 cm points are air samples collected at 1 m height. The CH_4 depth distribution [syringe samples (○)] was fitted using Eqn. 9.

3.1. Determination of KIE Using Flux Ratios

The most straightforward way to calculate the isotopic signature of the methane flux into the soil is to take the ratio of the $^{13}CH_4$ and the $^{12}CH_4$ fluxes into the soil. This approach also allows direct comparison with previous studies which used chamber headspace changes over time (King et al., 1989; Tyler et al., 1994). We assume that the fluxes (F) obey Fick's first law, $F = D_{eff} d[CH_4]/dz$, so the ratio of fluxes (using the ratio of diffusivities) is:

$$F_{13}/F_{12} = (1/1.0195)([^{12}CH_4]_{15cm} - [^{12}CH_4]_{0cm})/ ([^{13}CH_4]_{15cm} - [^{13}CH_4]_{0cm}) \quad (1)$$

Note that using this expression, we do not need to know the actual values for the effective diffusivities for $^{12}CH_4$ and $^{13}CH_4$; we know their ratio, which is 1.0195, the ratio of their reduced masses (Tyler et al., 1994). For the AS2 site, we calculate a $\delta^{13}CH_4$ value of −70.88‰, and for the BS2 site, we calculate −73.87‰. These values are 23 and 26‰ lighter than the atmospheric value for the two sites; that is,

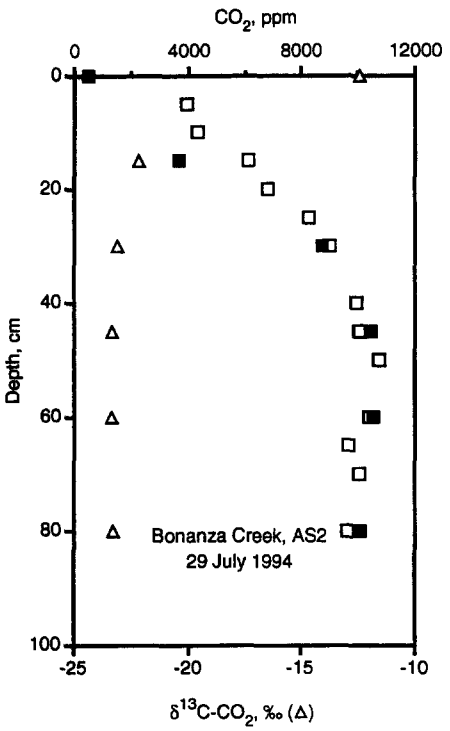

Fig. 3. Soil carbon dioxide concentration and $\delta^{13}C\text{-}CO_2$ depth distributions at the AS2 (South-facing Aspen) site in the Bonanza Creek L.T.E.R., 29 July 1994. Squares are CO_2 concentrations from syringe (○) and cannister (■) samples, triangles (△) are $\delta^{13}C\text{-}CO_2$. The 0 cm points are air samples collected at 1 m height.

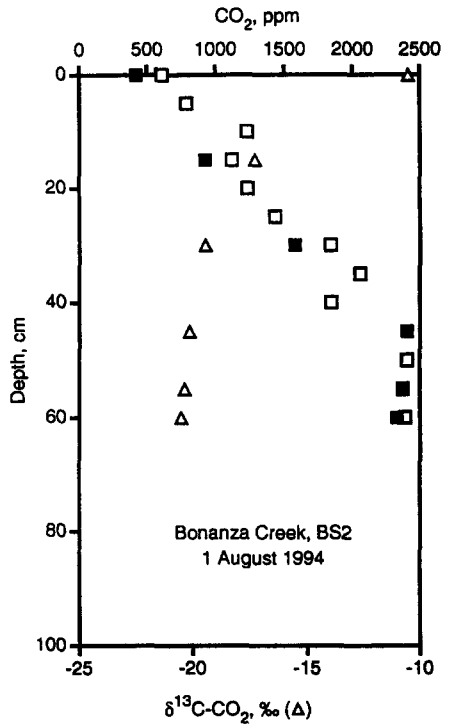

Fig. 4. Soil carbon dioxide concentration and $\delta^{13}C\text{-}CO_2$ depth distributions at the BS2 (North facing Black Spruce) site in the Bonanza Creek L.T.E.R., 1 August 1994. Squares are CO_2 concentrations from syringe (○) and cannister (■) samples; triangles (△) are $\delta^{13}C\text{-}CO_2$. The 0 cm points are air samples collected at 1 m height. Note that maximum BS2 CO_2 concentrations are fivefold lower than AS2, indicating a lower soil respiration rate.

we can say the KIE for soil uptake is 1.023 for the AS2 site and 1.026 for the BS2 site. The isotopic gradient in the soil at both sites is set by the balance of diffusion and fractionation by microbial oxidation. However, the flux into the soil will be lighter than that simply determined by the isotopic gradient because $^{12}CH_4$ will diffuse more quickly along its $^{12}CH_4$ gradient than will $^{13}CH_4$ along its gradient (by a factor of 1.0195). That is, we are seeing the effect of diffusion into the soil along a gradient set by the net effect of diffusion (a = 1.0195) and microbial discrimination (a = 1.022–1.025) in the soil.

3.2. KIE Using Steady-State Diffusion-Consumption Model

3.2.1. Bottom [CH_4] approaches zero

The second approach involves evaluating the KIE profile using a simple diffusion-consumption model. If we assume that only diffusion and oxidation are influencing CH_4, we can represent the time dependence of methane in the soil by the following differential equation:

$$d[CH_4]/dt = D_{eff}d^2[CH_4]/dz^2 - L[CH_4] \quad (2)$$

where D_{eff} is the effective diffusivity for CH_4, and L (sec^{-1}) is a first order loss frequency or rate constant describing consumption. If we assume steady-state, ($d[CH_4]/dt = 0$),

$$d^2[CH_4]/dz^2 - (L/D_{eff})[CH_4] = 0 \quad (3)$$

This equation has the solution (for now approximating by assuming that $[CH_4]$ goes to 0 at depth; see discussion below):

$$[CH_4]_z = [CH_4]_0 e^{-[z\sqrt{(L/D_{eff})}]} \quad (4)$$

which can be solved for L, yielding

$$L = D_{eff}[\ln([CH_4]_0/[CH_4]_z)]^2 \quad (5)$$

This expression for L holds true for both $^{12}CH_4$ and $^{13}CH_4$, and we can take the ratio of L_{12} to L_{13} to find the KIE due to bacterial CH_4 consumption

$$L_{12}/L_{13} = \text{KIE} = (D_{eff,12}/D_{eff,13})$$
$$[\ln([^{12}CH_4]_z/[^{12}CH_4]_0)/\ln([^{13}CH_4]_z/[^{13}CH_4]_0)]^2 \quad (6)$$

The KIE values calculated for various depth intervals at AS2 and BS2 are shown in Table 1.

The term $\sqrt{D_{eff}/L}$ (Eqn. 3) is commonly referred to as the relaxation depth (Dörr and Munnich, 1990) and can be thought of as the average distance methane will diffuse into the soil before being consumed. Denoting the relaxation depth as z*, we can rearrange Eqn. 6 to give the following:

$$z_{13}^*/z_{12}^* = \sqrt{(\text{KIE}/1.0195)}$$
$$= \ln([^{12}CH_4]_z/[^{12}CH_4]_0)/\ln([^{13}CH_4]_z/[^{13}CH_4]_0) \quad (7)$$

Table 1. Biological KIE for Bonanza Creek soil methane consumption.

Depth interval (cm)	Biological KIE	
	AS2	BS2
0–15	(a)	(a)
15–30	1.022	1.026
15–45	1.022	1.023
15–60	1.022	—

(a) Consumption does not occur in the upper 10 cm of these soils (Whalen et al., 1992), so equation (5) cannot be used for the 0–15 cm depth interval.

Substituting $^{13}CH_4 = R\,^{12}CH_4$ in Eqn. 7, where R is the $^{13}C/^{12}C$ ratio at a given depth gives

$$= \ln([^{12}CH_4]_z/[^{12}CH_4]_0)/[\ln(R_z/R_0) + \ln([^{12}CH_4]_z/[^{12}CH_4]_0)] \quad (8)$$

and

$$= 1/\{1 + \ln[(\delta C_z + 1000)/(\delta C_0 + 1000)]/\ln F\} \quad (9)$$

where δC is the $\delta^{13}C$ value for CH_4 in ‰ at a given depth, and F is the fraction of the initial concentration remaining at depth z.

The right-hand side of Eqn. 8 is used in many different systems to calculate the fractionation factor associated with Rayleigh distillation (Hoefs, 1987). Tyler et al. (1994) used this expression to calculate the KIE of methane oxidation by observing the isotopic enrichment of methane in a chamber headspace over time. Here we can think of the isotopic enrichment of CH_4 as it diffuses through the soil as a Rayleigh distillation process and can calculate the KIE by measuring the enrichment in ^{13}C with distance into the soil. The ^{13}C enrichment with depth can also be envisioned to occur because $^{13}CH_4$ is likely to diffuse deeper into the soil than $^{12}CH_4$ before being consumed (as shown in the left-hand side of Eqn. 6).

3.2.2. *Bottom $[CH_4] \neq$ zero*

The equations derived above for KIE are only approximations because they assume that CH_4 concentration goes to zero deep in the soil. The CH_4 profile can be modeled more accurately by taking the bottom boundary ($d[CH_4]/dz = 0$) into account

$$[CH_4]_z = [CH_4]_\infty + ([CH_4]_0 - [CH_4]_\infty)e^{-z/z^*} \quad (10)$$

where $[CH_4]_\infty$ is the steady-state concentration at depth (Dörr and Munnich, 1990). Figures 1 and 2 show $[CH_4]$ from syringe samples fitted using Eqn. 9. Again we can solve for L_{12}/L_{13} (KIE)

$$\text{KIE} = 1.0195\,\{\ln[([^{12}CH_4]_0 - [^{12}CH_4]_\infty)/([^{12}CH_4]_z - [^{12}CH_4]_\infty)]/\ln[([^{13}CH_4]_0 - [^{12}CH_4]_\infty)/([^{13}CH_4]_z - [^{13}CH_4]_\infty)]\}^2 \quad (11)$$

We can also fit curves to the CH_4 profiles to obtain $^{12}[CH_4]_\infty$ and $^{13}[CH_4]_\infty$. Because there is no oxidation and a change in soil properties (moss/litter vs. loess) above 10 cm at both AS2 and BS2 (Whalen et al., 1992), only data below 15 cm yields a good fit to Eqn. 9. Fitting the AS2 data gives the values 0.065 ppmv for $^{12}[CH_4]_\infty$ and 0.00073 ppmv for $^{13}[CH_4]_\infty$.There were only three usable points above 45 cm for the BS2 site, which did not allow us to fit the curve with Eqn. 9. The error associated with the $[CH_4]_\infty$ terms is approximately equal to their magnitudes, which means that using Eqn. 10 will not yield meaningful results; more measurements along the profile and extending deeper in the soil will make Eqn. 10 more useful. The overall error in using Eqn. 5 instead of Eqn. 9 will be $(1 - [^{12}CH_4]_\infty/[^{12}CH_4]_0)/(1-[^{13}CH_4]_\infty/[^{13}CH_4]_0)$, which is the ratio of the errors in the second derivatives of the $^{12}CH_4$ and $^{13}CH_4$ profiles (which are similar and almost cancel). Using the above values, Eqn. 5 underestimates the KIE by 1.7‰, which gives a corrected KIE of 1.024.

3.3. KIE Using Top-to-Bottom $\delta^{13}C$-CH_4 Difference

Since CH_4 is almost completely (95‰) oxidized in the upper 45–60 cm of these soils, we can determine an approximate KIE by taking the difference of the $\delta^{13}C$ values at the top and bottom of the soil profile. This difference represents the integration of the net isotopic effects of diffusion (making CH_4 isotopically lighter) and consumption (making CH_4 isotopically heavier) with depth. This approach is an approximation, because the absolute lowest CH_4 concentration (maximum effect of both bacterial CH_4 oxidation and diffusion) may lie below our deepest sampling point. The CH_4 profiles from the AS2 and BS2 sites become isotopically heavier with depth, indicating that the kinetic isotope effect due to bacterial CH_4 oxidation must predominate over the diffusional isotopic effect (i.e., exceed 19.5‰). Comparing 0 cm and 60 cm at the AS2 site, we calculate a value of 4.48‰. Accounting for the isotopic effect of diffusion (add 19.5‰) gives a value of 24‰ for the bacterial oxidation effect, a KIE of 1.024. Comparing 0 cm and 45 cm at the BS2 site yields a value of 5.52‰, a bacterial KIE of 1.025. All of the approaches used for estimating the KIE for bacterial oxidation of CH_4, the isotope flux ratio model, the diffusion-consumption model, and finally, the inspection approach described above, give the same result.

3.4. Carbon Dioxide

Carbon dioxide concentration and isotope measurements are an additional result of this study. Similar measurements of isotopic variations in carbon dioxide from soils have been presented by Cerling (1984) and Cerling et al. (1991). These results are presented to demonstrate the similarity of Bonanza Creek soils to those studies. Carbon dioxide is produced in the soil by root respiration and soil organic matter oxidation. Cerling et al. (1991) distinguished between the isotopic composition of soil CO_2, which is the gas occupying pore space in the soil, and soil respired-CO_2, which represents the flux through a soil. The isotopic differences observed between soil CO_2 and soil respired-CO_2 reflect isoto-

pic fractionation of respired CO_2 due to diffusion. Samples collected in surface chambers have the same isotopic composition as the organic matter respired at depth in the soil. The isotope ratio and soil distribution of CO_2 are determined by the soil respiration rate and diffusion, and model results are presented graphically in Cerling et al. (1991). The processes producing the CH_4 profiles presented here are opposite in sense to those for CO_2; the flux of CH_4 is from the atmosphere into the soils, where oxidation occurs, while CO_2 is produced in the soil by respiration and diffuses to the atmosphere. The large concentration differences between CO_2 and CH_4 make detection of CO_2 produced from CH_4 oxidation unlikely.

Since Cerling et al. (1991) have previously modeled CO_2 isotope distributions, we restrict attention to estimating the isotopic composition of the soil organic matter (SOM) substrate for CO_2 production. No measurements of the $\delta^{13}C$ of soil organic matter are available, but it can be inferred from the soil CO_2 isotopic and concentration profiles. Cerling (1984) modeled $\delta^{13}CO_2$ in soils, assuming that soil respiration is distributed equally over a depth (L) so that the rate of CO_2 production at a given depth is

$$F^* = (C_s^* - C_a^*)D_s^*(Lz - z^2/2) \quad (12)$$

where C_s^* is the soil CO_2 concentration, C_a^* is the atmospheric concentration, and D_s^* is the effective diffusivity of CO_2 in the soil, where the asterisk (*) denotes bulk CO_2 values. If we assume that microbes do not fractionate the SOM substrate, we can take the ratio of $^{12}F^*$ and $^{13}F^*$ to calculate the $^{13}C/^{12}C$ ratio of the SOM, by the expression

$$^{13}C/^{12}C = (D_s^{13}/D_s^{12})(^{13}C_s - {}^{13}C_a)/(^{12}C_s - {}^{12}C_a) \quad (13)$$

and can then convert this ratio to a $\delta^{13}C$ value. Again, we do not need to know the absolute values for the two effective diffusivities: we know that D_s^{13}/D_s^{12} is the ratio of the reduced masses of $^{12}CO_2$ and $^{13}CO_2$, or 1.0044 (Mason and Marrero, 1970). Calculated values for SOM over several depth intervals are given in Table 2. For both sites, the calculated $\delta^{13}C$ values are close to the measured values for C_3 vegetation (approximately −27‰).

4. CONCLUSIONS AND FUTURE WORK

Isotope fractionation by both diffusion and microbial oxidation occurs in soils that consume atmospheric CH_4. The similarity in CH_4 oxidation at the two sites and the differences in inferred soil respiration at the two sites suggests that soil CH_4 oxidation and soil respiration are independent processes. The soils considered here to estimate the biological KIE produce no CH_4 internally and are net consumers of atmospheric CH_4. The overall KIE in net CH_4-consuming systems depends on the balance of two factors: the ratio of $^{12}CH_4$ and $^{13}CH_4$ effective diffusivities, 1.0195, and a factor related to the biological consumption of CH_4, estimated here to be 1.022–1.025.

Table 2. Bonanza Creek soil organic matter $\delta^{13}C$.

Depth (cm)	AS2	BS2
15	−28.0	−27.3
30	−28.0	−27.0
45	−28.2	−26.5
60	−28.2	—

Three approaches were used to estimate the biological KIE. Simple steady-state diffusion-consumption models that assume $[CH_4]$ goes to zero with depth or evaluate $[CH_4]$ at depth, and a flux ratio model give similar KIE's and agree very well with two previous determinations derived from headspace $\delta^{13}CH_4$ changes over time (Tyler et al., 1994; King et al., 1989). A first-order estimate of the biological KIE can be obtained in systems where methane is consumed to low levels by examining the top-to-bottom differences in $\delta^{13}CH_4$ over a soil profile. Isotope ratio monitoring gas chromatography/mass spectrometry techniques require much smaller samples than collected here, so much greater depth resolution than reported here is possible in future work. Model studies evaluating the role of soil oxidation on atmospheric $\delta^{13}CH_4$ (e.g., Gupta et al., 1996) are correct in using the biological KIE derived from this study and the two previous ones.

The suggestions of Tyler et al. (1994) that the biological KIE may vary with temperature and CH_4 concentration implies that there will be natural variations in the KIE of CH_4 oxidation. An approach to understanding the global range of the KIE for CH_4 oxidation is to make seasonal measurements of the biological KIE from several globally important CH_4-consuming site types (e.g. grasslands, desert, forests), much like the the Tyler et al. (1994) study, which reported a range in bacterial KIE of 1.017–1.029. Extending this approach to other sites, relationships can be developed between soil characteristics and temperature, microbial characteristics, and the KIE of soil CH_4 consumption, and applied in models similar to those of Gupta et al. (1996).

Future stable isotope work should also address systems where both CH_4 production and consumption occur. Oxidation in these systems (wetlands, rice paddies, landfills) not only modulates the flux of CH_4 to the atmosphere but also modifies the isotopic composition of the emitted CH_4.

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