Effect of CH_4 and O_2 variations on rates of CH_4 oxidation and stable isotope fractionation in tropical rain forest soils

Yit Arn Teh[1], Mark Conrad[2], Whendee L. Silver[1] & Charlotte M. Carlson[3]

[1]Ecosystem Sciences Division, University of California at Berkeley, [2]Center for Isotope Geochemistry, Lawrence Berkeley National Laboratory, [3] Department of Chemistry and Biochemistry, Middlebury College

Abstract

Methane-oxidizing bacteria are the primary sink for CH_4 in reduced soils, and account for as much as 90 % of all CH_4 produced. Methanotrophic bacteria strongly discriminate against the heavy isotopes of carbon, resulting in CH_4 emissions that are significantly more enriched in ^{13}C than the original source material. Previous studies have used an isotope mass balance approach to quantify CH_4 sources and sinks in the field, based on the assumption that the fractionation factor for CH_4 oxidation is a constant. This study quantifies the effect of systematic variations in CH_4 and O_2 concentrations on rates of CH_4 oxidation and stable isotope fractionation in tropical rain forest soils. Soils were collected from the 0-15 cm depth, and incubated with varying concentrations of CH_4 (100 ppmv, 500 ppmv, 1000 ppmv, and 5000 ppmv) or O_2 (3 %, 5 %, 10 %, and 21 %). The isotope fractionation factor for CH_4 oxidation was calculated for each incubation using a Rayleigh fractionation model. Rates of CH_4 oxidation varied significantly between CH_4 treatments, with the 100 ppmv CH_4 treatment showing the lowest rate of CH_4 uptake, and the other 3 treatments showing similar rates of CH_4 uptake. Rates of CH_4 oxidation did not vary significantly between the different O_2 treatments. The fractionation factor for CH_4 oxidation varied significantly between the different CH_4 treatments, with the 5000 ppmv CH_4 treatment showing the largest ^{13}C-enrichment of residual CH_4. In treatments where CH_4 concentration was not rate-limiting (> 500 ppmv CH4), the fractionation factor for CH_4 oxidation was negatively correlated with CH_4 oxidation rate ($P < 0.003$, $r^2 = 0.86$). A multiple regression model that included initial CH_4 concentration and CH_4 oxidation rate as independent variables accounted for 94 % of the variability in the isotope fractionation data, suggesting that both factors are important in determining the extent of isotopic fractionation ($P < 0.002$, $r^2 = 0.94$). The fractionation factor for CH_4 oxidation did not vary significantly between the different O_2 treatments. These results challenge the assumption that the isotope fractionation factor for CH_4 oxidation remains constant, regardless of metabolic activity or CH_4 pool size.

Introduction

Upland, tropical forests are generally characterized as net sinks for atmospheric CH_4 (Keller & Matson 1994), though recent research suggests that humid tropical forests are a net source of CH_4, or a weaker sink than previously suspected (Figure 1; also Silver et al. 1999). The factors that regulate CH_4 production, oxidation, and flux are poorly characterized in tropical environments. Most investigators rely on relatively coarse, surface flux measurements to infer the dynamics of CH_4 in the field. Identifying the principle controls over CH_4 cycling in tropical forests is of critical importance, since our mechanistic understanding of global CH_4 sources extends to only a few ecosystem types, such as rice paddies, northern latitude wetlands, and marine sediments (Fung et al. 1991)

Isotopic analysis of soil CH_4 is a direct, non-invasive method of examining the CH_4 cycle, at a finer scale than is possible by monitoring surface emissions alone (Whiticar et al. 1986). Each component of the CH_4 cycle imparts a unique isotopic "signature" on soil CH_4, enabling investigators to determine the simultaneous effects of CH_4 production and oxidation on CH_4 emissions. For example, methanotrophic bacteria strongly discriminate against the heavy isotopes of C, resulting in CH_4 emissions that are significantly more enriched in ^{13}C than the original source material (Barker & Fritz 1981; Coleman et al. 1981). Previous studies have used an isotope mass balance approach to quantify CH_4 sources and sinks in the field, based on the assumption that the fractionation factor for CH_4 oxidation is a constant (Liptay et al. 1998). This study sought to test this assumption, by examining the effect of systematic variations in CH_4 and O_2 concentrations on rates of CH_4 oxidation and stable isotope fractionation in tropical rain forest soils.

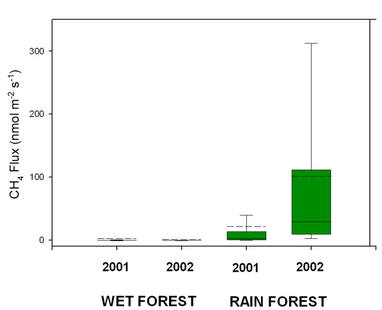

Figure 1. Methane flux for tropical wet forest and rain forest in the Luquillo Experiment Forest, Puerto Rico, during two separate field seasons. Broken lines represent means, solid lines represent median values and bars represent standard errors.

Methods

STUDY SITE

Soils were collected from the montane tropical rain forest life zone of the Luquillo Experimental Forest (LEF), a NSF Long-Term Ecological Research Site in northeastern Puerto Rico (18°18' N, 65°50' W). The rain forest receives between 4000-5000 mm of precipitation per year and approximately 300-500 mm of cloud water input, with little variation throughout the year (Weaver & Murphy 1990). Soils are volcanoclastic in origin, contain 25 % soil organic matter, >70 % clay, and are mildly acidic, with a pH of 5.41 (Silver et al. 1999). The mineral phase is dominated by Al and Fe oxides (Beinroth 1982).

SOIL INCUBATIONS

Soil was collected in 45 6 cm diameter PVC cores from the 0-15 cm depth and shipped immediately to the University of California, Berkeley. Sodium 2-bromoethanesulfonate (BES) was added to each soil sample to inhibit methanogenesis (Chidthaisong & Conrad 2000). One hundred grams of soil was then transferred from each PVC core to 45 1000 mL Bellco bottles (headspace ~930.2 mL), fitted with Geo-Microbial Technologies rubber septa. The headspace of each bottle was then adjusted to 1 of 4 different CH_4 or O_2 treatments, each containing 5 replicates. A ninth treatment group constituted a negative control. The 4 CH_4 treatments contained (I) 100 ppmv CH_4, (II) 500 ppmv CH_4, (III) 1000 ppmv CH_4, or (IV) 5000 ppmv CH_4, and an initial O_2 concentration of 21 %. The 4 O_2 treatments contained (I) 3 % O_2, (II) 5 % O_2, (III) 10 % O_2, or (IV) 21 % O_2, and an initial CH_4 concentration of 1000 ppmv. The negative control contained difluoromethane (DFM), a specific inhibitor of CH_4 oxidation (Miller et al. 1998), at an initial concentration of 1000 ppmv, ~2 ppmv CH_4 and 21 % O_2. Gas leakage was monitored in all bottles by adding sulfur hexafluoride (SF_6) at an initial concentration of 1000 ppmv. The headspace of each bottle was sampled repeatedly over the course of the experiment, with a minimum of 5 samples collected for each bottle.

ANALYTICAL TECHNIQUES

Methane and CO_2 concentrations were measured using a SRI Instruments 8610 gas chromatograph (GC) fitted with a Hysep-Q column, a flame ionization detector (FID) for hydrocarbon analysis, and a thermal conductivity detector (TCD) for CO_2 quantification. Oxygen and SF_6 analysis was performed on a Hewlett-Packard 6890 GC equipped with a Varian CP-Molsieve 5A column and a Valco Instruments pulsed discharge detector (PDD). Methane and CO_2 isotope analysis was conducted using a Micromass Isoprime continuous flow isotope ratio mass spectrometer, equipped with an in-line trace gas module and a combustion furnace (GC/C/IRMS) at the Center for Isotope Geochemistry, E.O. Lawrence Berkeley National Laboratory.

CARBON ISOTOPE FRACTIONATION

The isotope fractionation factors associated with bacterial degradation of CH_4 were calculated from the gradient (b) of the regression of the isotope value of the reactant ($_^{13}C\text{-}CH_4$) against the natural logarithm of the fraction of reactant remaining (ln *F*) (Miller et al. 2001):

$$b = (_^{13}C\text{-}CH_{4\,F} - _^{13}C\text{-}CH_{4\,0}) / \ln F$$

where $_^{13}C\text{-}CH_{4\,F}$ refers to the isotope value of CH_4 at *F* and $_^{13}C\text{-}CH_{4\,0}$ to the initial isotope value of CH_4. The isotope fractionation factor ($_{OXIDATION}$ = k13/k12) is thus:

$$_{OXIDATION} = (b + 1000) / 1000$$

Isotope analysis was conducted on gas samples collected from 3 of 5 replicates in each treatment. Isotope fractionation factors were calculated for each incubation, rather than pooling the samples from each treatment.

STATISTICS

Statistical analyses were performed using JMP IN Version 4.0.2 (SAS Institute Inc.) software. The data were log transformed where appropriate to meet the assumptions of analysis of variance (ANOVA). Residuals from all analyses were checked for normality and homogeneity of variances. Statistical significance was determined at the P < 0.05 level, unless otherwise stated. The effect of variations in CH_4 and O_2 concentrations on rates of CH_4 oxidation and stable isotope fractionation were tested using ANOVA. Means comparisons were performed using Fisher's Least Significant Difference (LSD) test.

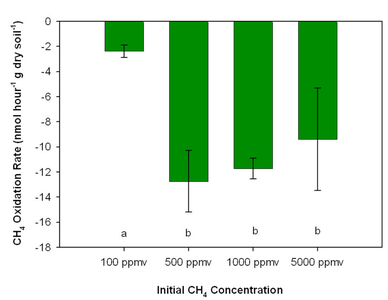

Figure 2. CH_4 oxidation rate for different initial CH_4 concentrations in 21% O_2 headspace

Results I

•Rates of CH_4 oxidation varied significantly between CH_4 treatments (ANOVA, $P < 0.0105$; see Figure 2), with the 100 ppmv CH_4 treatment showing the lowest rate of CH_4 uptake (Fisher's LSD, $P < 0.05$).

•Rates of CH_4 oxidation did not vary significantly between the different O_2 treatments (Figure 3).

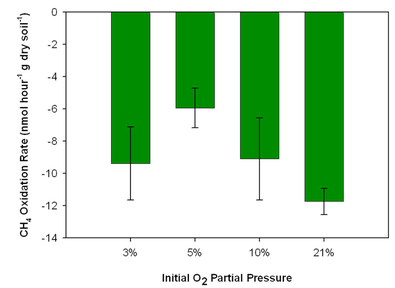

Figure 3. CH_4 oxidation rate for 1000 ppmv CH_4 with different initial O_2 concentrations

CH_4 Treatment	$_{OXIDATION}$	Standard Error	
100 ppmv	1.01023	0.00013	A
500 ppmv	1.01273	0.00168	A
1000 ppmv	1.0126	0.00187	A
5000 ppmv	1.0225	0.00548	B

Table 1. Isotope fractionation factor for CH_4 oxidation in different CH_4 treatments.

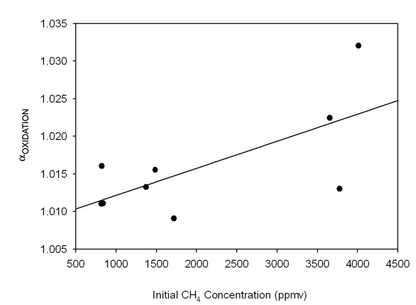

Figure 4. Regression of the isotope fractionation factor for CH_4 oxidation on initial CH_4 concentration for saturating CH_4 concentrations ($P < 0.044$, $r^2 = 0.46$)

Results II

•The isotope fractionation factor ($_{OXIDATION}$) for CH_4 oxidation factor varied significantly between CH_4 treatments at the _ = 0.10 level (ANOVA, $P < 0.0809$; see Table 1). The fractionation factor for CH_4 oxidation was greater in the 5000 ppmv CH_4 treatment than in the 100 ppmv CH_4 or 1000 ppmv CH_4 treatments (Fisher's LSD, $P < 0.05$).

•Initial CH_4 concentrations were positively correlated with $_{OXIDATION}$ ($P < 0.044$, $r^2 = 0.44$; see Figure 4). Rates of CH_4 oxidation were negatively correlated with $_{OXIDATION}$ ($P < 0.0003$, $r^2 = 0.86$; see Figure 5).

•A multiple regression model that included initial CH_4 concentration and CH_4 oxidation rate as independent variables accounted for 94 % of the variability in the isotope fractionation data ($P < 0.002$, $r^2 = 0.94$).

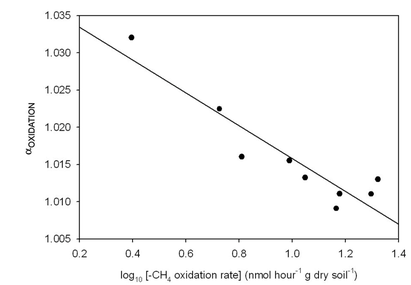

Figure 5. Regression of the isotope fractionation factor for CH_4 oxidation on the common log of the CH_4 oxidation rate under saturating CH_4 concentrations ($P < 0.0003$, $r^2 = 0.86$)

Conclusions

•The isotope fractionation factor for CH_4 oxidation ($_{OXIDATION}$) was not constant.

•The degree of isotopic fractionation was best predicted by the rate of the process (methanotrophic biomass?) and by the initial concentration of substrate.

•Isotope mass balance models cannot be quantitatively applied without knowledge of the total activity of the methanotrophic population.

Acknowledgements

This research was supported by a NASA Earth System Science Fellowship (NGT5-50331) and NSF Doctoral Dissertation Improvement Grant (DEB-0206547) to Y.A.T., a DOE Summer Undergraduate Laboratory Internship award to C.M.C. (operated under the DOE Office of Science contract no. DE-AC03-76SF00098), the NSF LTER Program, and the A.W. Mellon Foundation. Many thanks to M.K. Firestone for her kind words of advice. This work would not be possible without the technical assistance of M. Machavaram and A.W. Thompson. Thanks to E. Mann-Spiotta, B. Gorajski, K. Shamch & C. Torrens for help with soil sample collection.

References

Barker, J. F., and Fritz, P. (1981). "Carbon isotope fractionation during microbial methane oxidation." Nature 293: 289-291.

Beinroth, F. H. (1982). "Some highly weathered soils of Puerto Rico: I. Morphology, formation and classification." Geoderma 27: 1-74.

Chidthaisong, A., and Conrad, R. (2000). "Specificity of chloroform, 2-bromoethanesulfonate and fluoroacetate to inhibit methanogenesis and other anaerobic processes om anoxic ric field soil." Soil Biology and Biochemistry 32: 977-986.

Coleman, D. D., Risatti, J.B. and Schoell, M. (1981). "Fractionation of carbon and hydrogen isotopes by methane-oxidizing bacteria." Geochimica et Cosmochimica Acta 45: 1033-1037

Fung, I., John, J., Lerner, J., Matthews, E., Prather, M., Steele, L.P. and Fraser, P.J. (1991). "Three-Dimensional Model Synthesis of the Global Methane Cycle." Journal of Geophysical Research 96: 13033-13065.

Keller, M., and Matson, P.A. (1994). Biosphere-Atmosphere exchanges of trace gases in the tropics: Evaluating the effects of land use changes. Global Atmospheric-Biospheric Chemistry. R. G. Prinn. New York, Plenum Press: 103-117.

Liptay, K., Chanton, J., Czepiel, P., and Mosher, B. (1998). "Use of stable isotopes to determine methane oxidation in landfill cover soils." Journal of Geophysical Research 103 (D): 8243-8250.

Miller, L. G., Kalin, R.M., McCauley, S.E., Hamilton, J.T.G., Harper, D.B., Miller, D.B., Oremland, R.S., and Goldstein, A.H. (2001). "Large carbon isotope fractionation associated wit oxidation of methyl halides by methylotrophic bacteria." Proceedings of the National Academy of Science 98: 5833-5937.

Miller, L. G., Sasson, C., and Oremland, R.S. (1998). "Difluoromethane, a New and Improved Inhibitor of Methanotrophy." Applied and Environmental Microbiology 64: 4357-4362

Silver, W. L., Lugo, A.E. and Keller, M. (1999). "Soil oxygen availability and biogeochemistry along rainfall and topographic gradients in upland wet tropical forest soils." Biogeochemistry 44: 301-328.