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Effects of soil structure destruction on methane production and carbon partitioning between methanogenic pathways in tropical rain forest soils

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[1] Controls on methanogenesis are often determined from laboratory incubations of soils converted to slurries. Destruction of soil structure during slurry conversion may disrupt syntrophic associations, kill methanogens, and/or alter the microsite distribution of methanogenic activity, suppressing CH4 production. The effects of slurry conversion on methanogenesis were investigated to determine if disruption of aggregate structure impacted methanogenesis, substrate utilization, and C partitioning between methanogenic pathways. Soils were collected from the tropical rain forest life zone of the Luquillo Experimental Forest, Puerto Rico, and exposed to different physical disturbances, including flooding and physical homogenization. Slurry conversion negatively impacted methanogenesis. Rates of CH4 production declined by a factor of 17 after well-aggregated soils were converted to slurries. Significantly more 13C-acetate was recovered in CO2 compared to CH4 after slurry conversion, suggesting that methanogens consumed less acetate after slurry conversion and may have competed less effectively with other anaerobes for acetate. Isotopic data indicate that the relative partitioning of C between aceticlastic and hydrogenotrophic pathways was unchanged after slurry conversion. These data suggest that experiments which destroy soil structure may significantly underestimate methanogenesis and overestimate the potential for other microorganisms to compete with methanogens for organic substrates. Current knowledge of the factors that regulate methanogenesis in soil may be biased by the findings of slurry-based experiments, that do not accurately represent the complex, spatially heterogeneous conditions found in well-aggregated soils.


1. Introduction

[2] Methane (CH4) is a biogenic trace gas that plays a critical role in the chemistry of the Earth’s atmosphere. Methane is approximately 20 times more effective in retaining heat in the atmosphere than carbon dioxide (CO2), and increases in atmospheric methane since 1850 A.D. account for 25–35% of current climate forcing [Hansen et al., 2000]. Future expansion of anthropogenic activities such as rice cultivation, cattle husbandry, biomass burning, and coal/oil extraction are expected to increase atmospheric CH4 concentrations over the next 30 years, unless mitigation strategies are adopted to reduce emissions [Hansen et al., 2000].

[3] Methane production occurs during the terminal stages of anaerobic decomposition, and is dependent on the obligate relationships formed between fermentative bacteria and methanogenic archaea [Thiele and Zeikus, 1988; Stams, 1994; Bryant et al., 1967]. This syntrophic consortium is necessary for the degradation of complex polymers to CH4 and CO2 under anoxic conditions. Methanogenic archaea lack the enzymatic capacity to breakdown organic macromolecules to CO2/H2 and acetate, and depend on their syntrophic associates to decompose complex polymers to simple substrates [Zinder, 1993]. In return, the syntrophic associates depend on methanogens to consume their metabolic waste products, in order to make their fermentation reactions energetically favorable [Stams, 1994; Bryant et al., 1967].

[4] In highly weathered, well-aggregated, wet tropical soils, anaerobic processes such as dissimilatory nitrate reduction to ammonium [Silver et al., 2001], dissimilatory Fe(III)-reduction [Peretyazhko and Sposito, 2005; Teh, 2005], and methanogenesis [von Fischer and Hedin, 2002; Teh et al., 2005] occur simultaneously owing to microsite-level microbial activity [Anderson et al., 1998;
von Fischer and Hedin, 2002; Teh et al., 2005]. Steep gradients in oxygen (O\textsubscript{2}) and redox potential can occur across very small spatial scales in these well-aggregated soils [Sextone et al., 1985; Tokunaga et al., 2003], leading to spatial segregation of microbiobally driven redox reactions [Berkins et al., 1999; Curtis, 2003]. Disruption of this spatial heterogeneity during soil homogenization may lead to interactions (e.g., substrate competition) between microbial communities that are otherwise spatially isolated from each other in the field.

[5] A common method for determining the CH\textsubscript{4} production potential of a soil or sediment is to incubate field samples under strictly anaerobic conditions, after converting them to slurries. This technique is often used in mechanistic studies to determine seasonal changes in methanogenic activity, the effects of fluctuating redox potential on methanogenesis, the population dynamics of methanogenic archaea, or C partitioning between methanogenic pathways [Kruger et al., 2001, 2002; Amaral and Knowles, 1994; Leuders and Friedrich, 2000; Mayer and Conrad, 1990; Frenzel et al., 1999; Sugimoto and Wada, 1993]. Homogenization of soil by slurry conversion, followed by mechanical shaking or stirring allows substrates to be supplied in excess, and is thought to reduce the effects of mass transfer limitation on the enzymatic reactions associated with methanogenesis, providing investigators with a maximum potential rate of CH\textsubscript{4} production [Bailey and Ollis, 1986]. However, destruction of the soil physical structure during slurry conversion may disrupt syntrophic associations, kill methanogens [Dannenberg et al., 1997], or alter the microsite distribution of anaerobic activity, leading to a reduction in methanogenic activity.

[6] We investigated the effects of slurry conversion on methanogenesis, to determine if disruption of aggregate structure negatively influenced CH\textsubscript{4} production rates, C substrate utilization, and C partitioning between methanogenic pathways. Our experiment consisted of two disturbance treatments. In the first treatment, soil structure was destroyed by converting intact soils to slurries. In the second treatment, water was added in an equivalent amount to the slurry, but the soil itself was not physically homogenized. This allowed us to separate the disturbance effects associated with water addition (e.g., dispersion of soil particles) from that of physical homogenization. In addition, many methanogenic soils and sediments experience periodic destruction of the soil physical structure during natural processes.

2. Methods and Materials

2.1. Study Site

[7] Soils were collected from the 0–15 cm depth of the tropical rain forest life zone of the Luquillo Experimental Forest (LEF), a NSF Long-Term Ecological Research Site in northeastern Puerto Rico (18°30'N, 65°8'W). The forest is located approximately 900–1000 m above sea level. It receives between 4000 and 5000 mm of precipitation per year and an additional 300–500 mm of cloud water input [Weaver and Murphy, 1990]. Mean annual temperature is approximately 19°C. Soils are volcanoclastic in origin, contain approximately 12% soil organic C, >70% clay-sized particles and are mildly acidic, with a pH of 5.41 [Silver et al., 1999]. The mineral fraction is dominated by Al and Fe oxides [Beinroth, 1982]. The soil is classified as a very fine, mixed isomesic Humic Hapludalf in the Dwarf Series [Huffaker, 2002].

2.2. Soil Sampling and Experimental Design

[8] Soils cores were randomly collected from long-term field sites in the tropical rain forest life zone [Teh et al., 2005]. Soils were collected in forty 6-cm-diameter PVC cores and shipped overnight to the University of California, Berkeley (UCB). Upon arrival at UCB, 25-g subsamples were dispensed into forty 100-mL serum bottles and sealed with Geo-Microbial Technologies septa (Geo-Microbial Technologies, Inc., Ochelota, Oklahoma). Gravimetric water content was measured for each core using 10-g soil samples that were dried to a constant weight at 105°C. The initial pH of each core was determined in a 2:1 slurry of deionized H\textsubscript{2}O.

[9] The soils were divided into 8 treatment groups and exposed to different physical disturbances (Table 1). Carbon-13 labeled acetate and bicarbonate were added to six of the eight treatments to determine the effects of different physical manipulations on substrate utilization and C partitioning between different methanogenic pathways. The isotope tracers were applied by injecting 1 mL of a highly concentrated solution containing 1\textsuperscript{3}CH\textsubscript{3}COO\textsuperscript- or H\textsuperscript{13}CO\textsubscript{3} on soils or slurries, increasing initial pore water concentrations to approximately 1 mM for either 1\textsuperscript{3}CH\textsubscript{3}COO\textsuperscript- or H\textsuperscript{13}CO\textsubscript{3}. The relative amount of \textsuperscript{13}C-tracer added to each incubation varied with the total water content of the system. Treatments containing more water received more \textsuperscript{13}C-tracer than those that were drier to compensate for the dilution of the \textsuperscript{13}C-tracer in a larger volume of water. The relative amounts of \textsuperscript{13}C-tracer added to the different treatments are reported in Table 1.

[10] The headspaces of the bottles were purged with ultra-high purity nitrogen (N\textsubscript{2}) at a flow rate of 2000 mL min\textsuperscript{-1} for 1 min to induce anaerobiosis. The soil treatments were not disturbed or physically manipulated after the bottles were purged of room air. Fifty milliliters of deoxygenated, deionized water was injected through the septa of the bottles in the soil + water and slurry treatments. A second vent needle was introduced to allow the bottles to equilibrate to atmospheric pressure. This second needle was only inserted after water was injected into each bottle, to ensure a positive pressure gradient out of each bottle and reduce the risk of room air reentering the headspace. The soil + water treatments were not physically agitated or disturbed after the addition of water. Soil structure in the slurry treatments was destroyed by shaking each bottle vigorously. Oxygen (O\textsubscript{2}) concentrations were determined immediately after the bottles were purged with N\textsubscript{2} and over the course of the incubation. Oxygen concentrations were below the detection limit of the instrument (0.02%) throughout the course of the experiment, suggesting that our method was successful in removing O\textsubscript{2} from the bottles. Analyses were performed with a SRI Instruments gas chromatograph (GC) (SRI Instruments, Torrance, California), equipped with a molecular sieve column and a thermal conductivity detector (TCD).

[11] The bottles were sampled repeatedly over 10 days for CH\textsubscript{4} and CO\textsubscript{2} using gas-tight syringes. Gas samples were
Table 1. Description of the Different Physical Disturbance Treatments and the 13C-Tracers Added to Determine the Effect of Destroying Soil Structure on Methanogenesis

<table>
<thead>
<tr>
<th>Number</th>
<th>Treatment</th>
<th>Physical Disturbance</th>
<th>13C-Tracer Additiona</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Undisturbed Soil</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>II</td>
<td>Unamended Slurry</td>
<td>soil structure destroyed after addition of deoxygenated, deionized water in a ratio of two parts water to one part soil</td>
<td>none</td>
</tr>
<tr>
<td>III</td>
<td>Soil + H13CO3</td>
<td>none</td>
<td>1 mL of tracer solution added containing 38 mM 2.3 atom % H13CO3</td>
</tr>
<tr>
<td>IV</td>
<td>Soil + 13CH3COO−</td>
<td>none</td>
<td>1 mL of tracer solution added containing 17 mM 2.3 atom % 13CH3COO</td>
</tr>
<tr>
<td>V</td>
<td>Soil + Water + H13CO3</td>
<td>deoxygenated, deionized water added in a ratio of two parts water to one part soil</td>
<td>1 mL of tracer solution added containing 163 mM 2.3 atom % H13CO3</td>
</tr>
<tr>
<td>VI</td>
<td>Soil + Water + 13CH3COO−</td>
<td>deoxygenated, deionized water added in a ratio of 2 parts water to 1 part soil</td>
<td>1 mL of tracer solution added containing 65 mM 2.3 atom % 13CH3COO</td>
</tr>
<tr>
<td>VII</td>
<td>Slurry + H13CO3−</td>
<td>soil structure destroyed after addition of deoxygenated, deionized water in a ratio of two parts water to one part soil</td>
<td>1 mL of tracer solution added containing 65 mM 2.3 atom % H13CO3</td>
</tr>
<tr>
<td>VIII</td>
<td>Slurry + 13CH3COO−</td>
<td>soil structure destroyed after addition of deoxygenated, deionized water in a ratio of two parts water to one part soil</td>
<td>1 mL of tracer solution added containing 65 mM 2.3 atom % 13CH3COO</td>
</tr>
</tbody>
</table>

*aVarying amounts of H13CO3 or 13CH3COO− are added depending on the total volume of water in the incubation bottle. Initial pore water concentration of H13CO3 or 13CH3COO− was approximately 1 mM.

2.4. Isotope Tracer Recovery

[13] The amount of 13C-tracer recovered in CH4 or CO2 (g) was calculated according to the equation

\[
\%R = \left(1 - \frac{r_t}{r_{CONTROL}}\right) \times q_t / q_{TRACER} \times 100%,
\]

where \( \%R \) is percent recovered, \( r_t \) = the atom% of CH4 or CO2 (g) of the treatment, \( r_{CONTROL} \) = the atom% of CH4 or CO2 (g) in the control, \( q_t \) = the quantity of CH4 and CO2 (g) in mmol, and \( q_{TRACER} \) = the quantity of 13C in the tracer added at the start of the experiment. The fraction of 13C-tracer recovered in H2CO3, HCO3−, and CO32− was calculated using the carbonate system equilibria and the known isotopic fractionations between CO2 (g) and aqueous carbonate species [Clark and Fritz, 1997; Langmuir, 1997].

2.5. Fraction of CH4 From Acetate or CO2

[14] The fraction of CH4 derived from acetate or CO2 was estimated for the first 10 days of each experiment using an isotope mass balance model,

\[
\delta^{13}CH_4 = \delta^{13}CH_4(ACETATE) \times FACETATE + \delta^{13}CH_4(CO2/H2) \times (1 - FACETATE),
\]

where \( \delta^{13}CH_4 \) = the isotope value of the headspace CH4, \( \delta^{13}CH_4(ACETATE) \) = the isotope value of CH4 derived from acetate, \( FACETATE = \frac{CH_4 \text{ derived from acetate}}{CH_4 \text{ derived from CO2/H2}} \), and \( \delta^{13}CH_4(CO2/H2) \) = the isotope value of CH4 derived from CO2/H2, and \( (1 - FACETATE) = \frac{CH_4 \text{ derived from CO2/H2}}{CH_4 \text{ derived from CO2/H2}} \).
The $\delta^{13}C$ value of CH$_4$ derived from acetate or CO$_2$/H$_2$ was calculated using the equation [Kruger et al., 2002]

$$\delta^{13}CH_4(\text{SUBSTRATE}) = \left[\frac{(\delta^{13}C_{\text{SUBSTRATE}} + 1000)/\alpha}{C_0/C_1}ight] - 1000,$$

where $\alpha$ = the isotopic fractionation factor for the conversion of the substrate (CH$_3$COO$^-$ or HCO$_3^-$) to CH$_4$, $\delta^{13}C_{\text{SUBSTRATE}}$ = the C isotopic value of the substrate, and $\delta^{13}CH_4(\text{SUBSTRATE})$ = the C isotopic value of CH$_4$ formed from the C substrate.

We did not extract pore water acetate for isotope analysis because a previous study suggested that acetate concentrations in tropical rain forest soils were extremely low (data not shown). In an earlier experiment, soils were collected from the same field site and incubated under anaerobic conditions for 7 days, to determine the net acetate production potential of the soil. Attempts to measure pore water acetate using ion chromatography indicated that acetate concentrations were below the minimum detection limit for the instrument (0.05 ug L$^{-1}$ = 8.47 x 10$^{-7}$ m$M$).

Addition of 1 m$M$ $^{13}$CH$_3$COO$^-$ should have increased background pools by approximately 6 orders of magnitude. Thus we assumed that the $^{13}$CH$_3$COO$^-$ tracer dominated the acetate pool during the 10-day incubation in the $^{13}$CH$_3$COO$^-$ treatments. The $\delta^{13}CH_4(\text{ACETATE})$ in the $^{13}$CH$_3$COO$^-$ tracer experiments was estimated on the basis of the isotope value of the $^{13}$C-tracer (calculated to be 1110%), and fractionation factors ($\alpha_{\text{ACETATE}}$) of 1.021 or 1.032, which represent the extremes observed in nature [Blair and Carter, 1992; Gelwicks et al., 1994]. The $\delta^{13}CH_4(\text{ACETATE})$ value in the H$^{13}$CO$_3^-$ tracer experiment was approximated using the $\delta^{13}C$ of soil organic matter and an $\alpha_{\text{ACETATE}}$ of 1.021 or 1.032. Prior research suggests that the methyl group of acetate bears a similar C isotopic composition to bulk organic matter [Blair et al., 1985]. Thus we assumed that the $\delta^{13}C$ value of the methyl group was similar to that of rain forest soil organic matter, determined to be -27.7%o [von Fischer and Tieszen, 1995]. The $^{13}$CH$_4$(CO$_2$/H$_2$) was calculated on the basis of the instantaneous $\delta^{13}C$ value of headspace CO$_2$ (g), and fractionation factors ($\alpha_{\text{CO2/H2}}$) of 1.045 or 1.07 [Conrad et al., 2002].

### 2.6. 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. Means comparisons were performed using Fisher’s Least Significant Difference (LSD) test.

### 3. Results

The addition of 1 m$M$ $^{13}$CH$_3$COO$^-$ to the soil, soil + water, and slurry treatments resulted in a significant increase in headspace CH$_4$ concentrations relative to the undisturbed soil and the unamended slurry (Figure 1a). In the soil + $^{13}$CH$_3$COO$^-$ treatment, CH$_4$ concentrations increased linearly in the first 4 days of the experiment, and began to plateau after this point (Figure 1a). In contrast, CH$_4$ concentrations in the soil + water + $^{13}$CH$_3$COO$^-$ treatment increased more slowly in the first 4 days, and then more rapidly in the 5- to 10-day period. Methane concentrations in the slurry + $^{13}$CH$_3$COO$^-$ treatment increased linearly over 10 days.

The addition of 1 m$M$ H$^{13}$CO$_3^-$ resulted in different patterns of CH$_4$ increase in the soil + H$^{13}$CO$_3^-$ and soil + water + H$^{13}$CO$_3^-$ relative to the other treatments (Figure 1b). In the soil + H$^{13}$CO$_3^-$ and soil + water + H$^{13}$CO$_3^-$ treatment, CH$_4$ increased more slowly during the first 4 days of the experiment, and then more rapidly during the remaining 5–10 days. In contrast, the slurry + H$^{13}$CO$_3^-$, undisturbed soil, and unamended slurry showed a linear increase in CH$_4$ concentrations over time.
Methane production in the intact soils was significantly greater than in the slurry treatments (Table 2). Methanogenesis in the undisturbed soil exceeded the unamended slurry by 17 times, while CH₄ production in the soil + ¹³CH₃COO⁻ and soil + water + ¹³CH₃COO⁻ treatments exceeded the slurry + ¹³CH₃COO⁻ treatment by a factor of 4 and 5, respectively. Likewise, CH₄ production in the soil + H¹³CO³⁻ and soil + water + H¹³CO³⁻ treatments exceeded the slurry + H¹³CO³⁻ treatment by a factor of 30 and 26, respectively. Bicarbonate addition did not cause a statistically significant increase in CH₄ production in the soil + H¹³CO³⁻ and soil + water + H¹³CO³⁻ treatments relative to the undisturbed soil, although the averages and variances for CH₄ production increased in these treatments. Carbon dioxide production in the soil + ¹³CH₃COO⁻, soil + water + H¹³CO³⁻, slurry + H¹³CO³⁻, and slurry + ¹³CH₃COO⁻ were significantly greater than the undisturbed soil or unamended slurry.

The isotope mass balance calculations are a measure of the relative amount of ¹³C-tracer used by aceticlastic or CO₂-reducing methanogens. In contrast, the tracer recovery data indicate the total amount of ¹³C-tracer converted to CH₄ or CO₂, and reflect the total amount of ¹³C-tracer used during the course of the experiment. Isotope mass balance calculations suggest that approximately 56% of the CH₄ formed over the 10-day experiment came from acetate (Table 3). The fraction of CH₄ derived from acetate or CO₂ did not vary significantly among treatments. In contrast, there were significant differences in the total amount of ¹³C-tracer recovered in CH₄ or CO₂. In the ¹³CH₃COO⁻ treatments, the amount of ¹³C-tracer recovered in CH₄ and CO₂ was significantly higher in the soil + ¹³CH₃COO⁻ treatment compared to the others (Table 4). Approximately 30 times more ¹³C-tracer was recovered as CH₄, and 6 times more tracer was recovered as CO₂ in the soil + ¹³CH₃COO⁻ relative to the slurry + ¹³CH₃COO⁻ treatment. There were no significant differences for ¹³C-tracer recovered as CH₄ in the H¹³CO³⁻ treatments. The total amount of H¹³CO³⁻ recovered was significantly higher in the soil + H¹³CO³⁻ treatment compared to the other treatments (Table 4). Most of the difference in recovery was due to more ¹³C recovered as CO₂ in the soil + H¹³CO³⁻ treatment compared to the others. The low recovery of the H¹³CO³⁻ tracer in the soil + water and slurry treatments may be due to carbonate precipitation [Duckworth and Martin, 2004; Peretyazhko and Sposito, 2005]. We found significant differences in the ratio of % Tracer in CH₄:% Tracer in CO₂ among ¹³CH₃COO⁻

### Table 2. Methane and CO₂ Production Rates After Addition of ¹³CH₃COO⁻ or H¹³CO³⁻ Tracers in Different Soil Disturbance Treatments

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>CH₄ Production, nmol d⁻¹ g dry soil⁻¹</th>
<th>Total CO₂ Production, umol d⁻¹ g dry soil⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undisturbed soil</td>
<td>6.39 ± 2.80 a</td>
<td>1.01 ± 0.11 a</td>
</tr>
<tr>
<td>Unamended slurry</td>
<td>0.38 ± 0.12 b</td>
<td>1.07 ± 0.14 a, b</td>
</tr>
<tr>
<td>Soil + H¹³CO³⁻</td>
<td>47.83 ± 28.28 a</td>
<td>1.23 ± 0.17 a, b, d</td>
</tr>
<tr>
<td>Soil + ¹³CH₃COO⁻</td>
<td>103.68 ± 22.70 c</td>
<td>1.81 ± 0.13 c</td>
</tr>
<tr>
<td>Soil + water + H¹³CO³⁻</td>
<td>42.28 ± 25.13 a</td>
<td>1.53 ± 0.11 c</td>
</tr>
<tr>
<td>Soil + water + ¹³CH₃COO⁻</td>
<td>125.84 ± 18.21 c</td>
<td>1.33 ± 0.11 b, c, d</td>
</tr>
<tr>
<td>Slurry + H¹³CO³⁻</td>
<td>1.57 ± 0.94 a, b</td>
<td>1.54 ± 0.21 c</td>
</tr>
<tr>
<td>Slurry + ¹³CH₃COO⁻</td>
<td>25.33 ± 9.34 a</td>
<td>2.14 ± 0.32 c</td>
</tr>
</tbody>
</table>

*Lower case letters indicate significant differences between treatments (Fisher’s LSD, n = 5, P < 0.05).
treatments, with a significantly lower ratio in the slurry + $^{13}\text{CH}_3\text{COO}^-$ treatment, compared to the soil + $^{13}\text{CH}_3\text{COO}^-$ and soil + water + $^{13}\text{CH}_3\text{COO}^-$ treatments (Figure 2). The C isotope composition was $-65.4 \pm 1.3\%$ in the undisturbed soil and $-70.1 \pm 2.3\%$ in the unamended slurry.

4. Discussion

[22] Conversion of tropical rain forest soils to slurries significantly reduced methanogenic activity. Methane production in the undisturbed soil was approximately 17 times greater than in the unamended slurry. This pattern was repeated for the $^{13}$C-tracer treatments. Methane production in the soil + $^{13}\text{CH}_3\text{COO}^-$ and soil + water + $^{13}\text{CH}_3\text{COO}^-$ treatments exceeded the slurry + $^{13}\text{CH}_3\text{COO}^-$ treatment by a factor of four and five, respectively. Likewise, methanogenesis in the soil + $^3\text{HCO}_3^-$ and soil + water + $^{13}\text{CO}_2^-$ treatments were 26–30 times greater then in the slurry + $^3\text{HCO}_3^-$ treatment.

[23] Patterns of $^{13}$C-acetate utilization support the notion that slurry conversion suppressed methanogenesis, as both the soil and soil + water treatments showed a more vigorous response to acetate addition than the slurry. Headspace CH$_4$ showed a small linear increase over time in the slurry + $^{13}\text{CH}_3\text{COO}^-$ treatment (Figure 1a). In contrast, the soil + $^{13}\text{CH}_3\text{COO}^-$ and soil + water + $^{13}\text{CH}_3\text{COO}^-$ treatments showed large, non-linear increases in CH$_4$ over time. For example, in the soil + $^{13}\text{CH}_3\text{COO}^-$ treatment, CH$_4$ increased rapidly in the first 4 days of the experiment, during which time most of the $^{13}\text{CH}_3\text{COO}^-$ was converted to CH$_4$ and CO$_2$ (Table 4). The absence of excess water may have resulted in methanogenic populations close to the point of tracer injection receiving a higher concentration of $^{13}\text{CH}_3\text{COO}^-$, stimulating an immediate response in the microbial community. In the soil + water + $^{13}\text{CH}_3\text{COO}^-$ treatment, CH$_4$ increased more slowly in the first 4 days of the experiment, followed by a more rapid rise in the 5- to 10-day period. This pattern suggests that the excess water may have diluted the tracer available to microbes, resulting in a more gradual uptake of $^{13}\text{CH}_3\text{COO}^-$ by the microbial community in the first 4 days. Although patterns of acetate utilization may differ slightly between the soil + $^{13}\text{CH}_3\text{COO}^-$ and soil + water + $^{13}\text{CH}_3\text{COO}^-$ treatments, the net result is a significant increase in CH$_4$ production relative to the slurry, implying that methanogenic populations in these treatments were more active and/or grew more vigorously than their slurry counterparts.

[24] The isotopic measurements are also supportive of this interpretation of these data. More $^{13}\text{CH}_3\text{COO}^-$ was converted to CH$_4$ in the soil + $^{13}\text{CH}_3\text{COO}^-$ and soil + water + $^{13}\text{CH}_3\text{COO}^-$ treatments compared to the slurry + $^{13}\text{CH}_3\text{COO}^-$ treatment (Table 4), indicating that aceticlastic methanogens were unable to consume as much tracer as in the intact soils. These data imply that aceticlastic methanogens may have competed less effectively with other anaerobic heterotrophs in the slurries, as a larger proportion of the tracer was converted to CO$_2$ rather than to CH$_4$. Carbon dioxide-reducing methanogens were probably negatively affected by slurry conversion as well, as implied by the isotope mass balance data. These data indicate that relative partitioning of C between aceticlastic and CO$_2$-reducing pathways was not significantly affected by slurry conversion; thus any reduction in CH$_4$ production rate between the intact soil and slurry treatments was probably due to an over all reduction in methanogenesis, rather than due to a decrease in aceticlastic methanogenesis alone.

[25] More $^{13}$C was also recovered as CH$_4$ in the soil + water + $^{13}\text{CH}_3\text{COO}^-$ treatment relative to the soil + $^{13}\text{CH}_3\text{COO}^-$ treatment, indicating that flooding the soil improved the ability of methanogens to acquire the tracer. Methanogenic archaea may have competed more effectively with other anaerobes for acetate under water-saturated

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Fraction of CH4 from Aceticlastic Methanogenesis</th>
<th>Fraction of CH4 from Aceticlastic Methanogenesis</th>
<th>Fraction of CH4 from Aceticlastic Methanogenesis</th>
<th>Fraction of CH4 from Aceticlastic Methanogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil + $^{13}\text{HCO}_3^-$</td>
<td>$0.60 \pm 0.05$</td>
<td>$0.58 \pm 0.04$</td>
<td>$0.55 \pm 0.05$</td>
<td>$0.53 \pm 0.05$</td>
</tr>
<tr>
<td>Soil + $^{13}\text{CH}_3\text{COO}^-$</td>
<td>$0.57 \pm 0.10$</td>
<td>$0.59 \pm 0.10$</td>
<td>$0.58 \pm 0.10$</td>
<td>$0.60 \pm 0.10$</td>
</tr>
<tr>
<td>Soil + Water + $^3\text{HCO}_3^-$</td>
<td>$0.71 \pm 0.01$</td>
<td>$0.70 \pm 0.01$</td>
<td>$0.73 \pm 0.04$</td>
<td>$0.67 \pm 0.01$</td>
</tr>
<tr>
<td>Soil + Water + $^{13}\text{CH}_3\text{COO}^-$</td>
<td>$0.43 \pm 0.07$</td>
<td>$0.44 \pm 0.07$</td>
<td>$0.53 \pm 0.10$</td>
<td>$0.46 \pm 0.07$</td>
</tr>
<tr>
<td>Slurry + $^{13}\text{CH}_3\text{COO}^-$</td>
<td>$0.60 \pm 0.24$</td>
<td>$0.59 \pm 0.24$</td>
<td>$0.57 \pm 0.27$</td>
<td>$0.55 \pm 0.26$</td>
</tr>
<tr>
<td>Slurry + $^{13}\text{CH}_3\text{COO}^-$</td>
<td>$0.46 \pm 0.21$</td>
<td>$0.47 \pm 0.21$</td>
<td>$0.47 \pm 0.20$</td>
<td>$0.48 \pm 0.21$</td>
</tr>
</tbody>
</table>

Table 4. Percent Recovery of $^{13}$C-Tracers After Addition of $^{13}\text{CH}_3\text{COO}^-$ or $^3\text{HCO}_3^-$ in Different Soil Disturbance Treatments

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Percent Tracer in CH4</th>
<th>Percent Tracer in CO2 Total Percent Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil + $^{13}\text{HCO}_3^-$</td>
<td>$0.973 \pm 0.520$</td>
<td>$89.648 \pm 10.992$</td>
</tr>
<tr>
<td>Soil + $^{13}\text{CH}_3\text{COO}^-$</td>
<td>$25.098 \pm 5.160$</td>
<td>$86.236 \pm 3.462$</td>
</tr>
<tr>
<td>Soil + Water + $^3\text{HCO}_3^-$</td>
<td>$0.293 \pm 0.165$</td>
<td>$12.464 \pm 1.191$</td>
</tr>
<tr>
<td>Soil + Water + $^{13}\text{CH}_3\text{COO}^-$</td>
<td>$6.890 \pm 1.007$</td>
<td>$7.175 \pm 1.268$</td>
</tr>
<tr>
<td>Slurry + $^{13}\text{CH}_3\text{COO}^-$</td>
<td>$0.015 \pm 0.005$</td>
<td>$17.725 \pm 2.955$</td>
</tr>
<tr>
<td>Slurry + $^{13}\text{CH}_3\text{COO}^-$</td>
<td>$0.752 \pm 0.365$</td>
<td>$14.176 \pm 0.794$</td>
</tr>
</tbody>
</table>

*Lower case letters represent significant differences between disturbance treatments within each tracer treatment (Fisher’s LSD, n = 5, P < 0.05).
conditions. Alternatively, these data may indicate that addition of water distributed the $^{13}$CH$_3$COO$^-$/CO$_2$ tracer more effectively among methanogenic microsites in the soil + water + $^{13}$CH$_3$COO$^-$/CO$_2$ treatment relative to the soil + $^{13}$CH$_3$COO$^-$/CO$_2$ treatment.

Several possible mechanisms may explain the suppression of methanogenesis arising from slurry conversion. One potential explanation is that the physical disturbance caused by slurry conversion may have reduced CH$_4$ production by disrupting syntrophic associations and killing aceticlastic methanogens [Dannenberg et al., 1997]. Another possible explanation is that disruption of soil structure may have altered the redox environment experienced by methanogenic archaea. Studies indicate that CH$_4$ production in soils from these field sites occur in anoxic microenvironments that are physically protected or isolated from the rest of the bulk soil [Teh et al., 2005]. Disruption of soil aggregate structure due to slurry conversion may have resulted in the intermingling of anaerobes (e.g., methanogenic archaea, dissimilatory Fe(III)-reducing bacteria, sulfate-reducing bacteria) that are otherwise spatially isolated from one another [Sextone et al., 1985; Berkins et al., 1999; Curtis, 2003; Tokunaga et al., 2003], leading to substrate (i.e., H$_2$, acetate) competition between methanogenic archaea and other microorganisms. Dissimilatory Fe(III)-reducing bacteria (DFRB) are the most likely competitors for these substrates, as previous experiments have demonstrated that DFRB are very active in these soils and capable of suppressing methanogenesis [Peretyazhko and Sposito, 2005; Teh, 2005; Chacon et al., 2005]. For example, in a slurry experiment exploring acetate competition between DFRB and methanogenic archaea, we found that DFRB were able to reduce methanogenesis by a factor of 3, relative to treatments where competition was alleviated by the addition of excess acetate [Teh, 2005]. Ferric iron reduction rates were between 5.75 ± 2.93 to 14.08 ± 2.36 μmol g dry soil$^{-1}$ day$^{-1}$ and inversely related to CH$_4$ production.

5. Conclusions and Implications

Conversion of intact, well-aggregated soils to slurries significantly reduced methanogenic activity. Methanogenesis declined by a factor of 17 after undisturbed soils were converted to slurries. Likewise, CH$_4$ production in soils amended with $^{13}$C-acetate or $^{13}$C-bicarbonate exceeded amended slurries by a factor of 4 and 26, respectively. Slurry conversion reduced the capacity for aceticlastic methanogens to acquire acetate, with 30 times more $^{13}$C-tracer recovered as CH$_4$ in intact soils relative to slurries. More of the $^{13}$C-acetate was recovered as CH$_4$ rather than as CO$_2$ in the soil treatments, indicating that soil structure destruction may have reduced the capacity for methanogens to compete for C substrates with other anaerobes. Possible mechanisms for suppression of methanogenic activity include disruption of syntrophic associations, killing of methanogens, and/or increased competition from other anaerobes, such as dissimilatory Fe(III)-reducing bacteria, due to loss of microsite structure.

The implications of these results are threefold. First, rates of CH$_4$ production determined from slurries may significantly underestimate the methanogenic potential of a soil or sediment. Second, slurry-based experiments investigating substrate competition between methanogens and Fe(III)- or sulfate-reducing bacteria may significantly overestimate the capacity of these organisms to suppress CH$_4$ production, as slurry conversion may have a disproportionately large negative effect on methanogenic archaea relative to other anaerobes. The loss of spatial organization during physical homogenization may also result in “artificial” groupings or associations of microorganisms that do not normally interact. Last, slurry conversion itself may have
little or no effect on the partitioning of C between methanogenic pathways, which suggests that it is possible to derive representative pathway data from slurry experiments. Our findings suggest that intact soil incubations may be a better means of determining the CH$_4$ production potential of soils, or exploring the mechanistic controls on methanogenesis in natural environments, owing to the artifacts associated with slurry-based experiments. Our current knowledge of the factors that influence CH$_4$ production in soil may be biased by the findings of slurry-based experiments, which do not accurately represent the complex, spatially heterogeneous nature of well-aggregated soils. Future process-based studies should take into account the importance of soil structure and syntrophic associations for methanogenesis.

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References


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


Weaver, P. L., and P. G. Murphy (1990), Forest structure and productivity in Puerto Rico’s Luquillo Mountains, Biotropica, 22, 69–82.