

Carbon Isotope Fractionation during Acetoclastic Methanogenesis by *Methanosaeta concilii* in Culture and a Lake Sediment

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The isotope enrichment factors (ϵ) in *Methanosaeta concilii* and in a lake sediment, where acetate was consumed only by *Methanosaeta* spp., were clearly less negative than the ϵ usually observed for *Methanosarcina* spp. The fraction of methane produced from acetate in the sediment, as determined by using stable isotope signatures, was 10 to 15% lower when the appropriate ϵ of *Methanosaeta* spp. was used.

Methane is a major product of the degradation of organic matter in anoxic environments like rice paddies, natural wetlands, and lake sediments. Its production from H_2 - CO_2 and acetate (ac) in natural systems has been quantified by stable isotope modeling (7, 17). One of the input parameters needed is the carbon isotope fractionation of acetoclastic methanogenesis. To determine the fractionation factor (α) in culture or in an environmental system, the $\delta^{13}C$ of the methyl carbon of acetate (ac-methyl) must be known, since CH_4 is produced from the methyl carbon rather than the carboxyl carbon of acetate (5, 19). For *Methanosarcina* spp. isotope fractionation has been determined in pure culture (7, 10, 20) and has been verified in natural environments dominated by this group of acetoclastic methanogens (14). However, detailed data on isotope fractionation by *Methanosaeta* spp. are rare. Valentine et al. (18) reported a rather low fractionation factor ($\alpha = 1.007$) for thermophilic *Methanosaeta thermophila*. Similarly, the fractionation factor for mesophilic *Methanosaeta concilii* also seems to be lower ($\alpha = 1.017$) (A. Chidhaisong, S. C. Tyler, et al., unpublished results) than that for *Methanosarcina* spp. ($\alpha = 1.021$ to 1.027) (for a review see reference 18). A difference in isotope fractionation by the two acetoclastic methanogenic groups is possible, since they differ in their biochemical activation of acetate to acetyl coenzyme A (acetyl-CoA).

To increase our knowledge of isotope fractionation by *Methanosaeta* spp., we grew *M. concilii* under defined conditions and determined the isotope enrichment factor (ϵ). We also determined isotope fractionation by acetoclastic *Methanosaeta* spp. in a natural environment, Lake Dagow sediment, in which only members of the *Methanosaetaceae* were detected (2, 8).

Cultures ($n = 5$) of *M. concilii* DSM 3671 were grown under N_2 - CO_2 (80:20) at 37°C in glass bottles (250 ml; Müller Krempel, Bülach, Switzerland) without shaking using carbonate-buffered (pH 7.1) mineral medium (DSM medium 334). Sodium acetate

was added to an initial concentration of approximately 70 mM. For the total molecule the added acetate (ac) had a $\delta^{13}C_{ac}$ of -24.0‰ , and for the methyl group the $\delta^{13}C_{ac-methyl}$ was -29.4‰ . After inoculation with a 20% bacterial suspension in the late exponential phase (resulting in a final volume of 125 ml), several gas (0.4-ml) and liquid (2-ml) samples were removed and used for analysis of pH, concentration, and the carbon isotope composition of acetate and the products formed.

Sediment samples from eutrophic Lake Dagow (Northern Brandenburg, Germany) were obtained on 9 November 2004 and 17 August 2005 and transported to the laboratory in Marburg as described previously (8). The sediment samples from 2004 (19 ml; depth, 0 to 10 cm) and from 2005 (10 ml; depth, 0 to 5 cm) were placed in 120-ml serum vials and 27-ml pressure tubes, respectively. The glass vessels were closed with butyl rubber stoppers and incubated under an N_2 atmosphere at 10°C without shaking. After preincubation overnight, the appropriate volume of a sodium acetate stock solution (40.6 mM) was added to obtain an initial acetate concentration of 1 mM. The acetate had a $\delta^{13}C_{ac}$ of -32.1‰ and a $\delta^{13}C_{ac-methyl}$ of -36.4‰ . At each time examined during incubation triplicate tubes were analyzed and subsequently sacrificed. In another experiment, using sediment from 2005, CH_4 production was investigated after consumption of the acetate that was added. To do this, the headspace was exchanged with N_2 , which was followed by repeated gas sampling over the next 18 days.

Chemical and isotopic analyses were performed as described by Penning and Conrad (13). A stable isotope analysis of $^{13}C/^{12}C$ in gas samples was performed using a gas chromatograph-combustion-isotope ratio mass spectrometer system (Thermoquest, Bremen, Germany) (13). Measurement of isotopes and quantification of acetate were performed with a high-performance liquid chromatography system coupled to Finnigan LC IsoLink (Thermo Electron Corporation, Bremen, Germany). Off-line pyrolysis was performed to determine $\delta^{13}C_{ac-methyl}$ contents (1, 3).

The amount of total inorganic carbon (TIC) produced by *M. concilii* was calculated (13) and was expressed as the difference between the amount of TIC at any time and the initial amount of TIC. The isotope enrichment factor associated with aceto-

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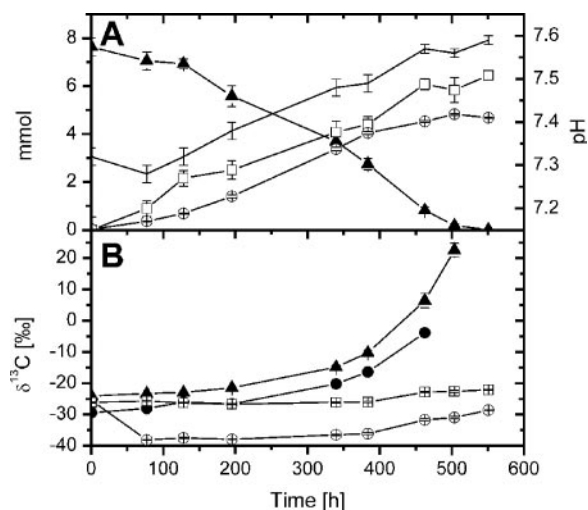


FIG. 1. Catabolism of acetate in a pure culture of *M. concilii*. (A) Acetate consumption, product formation, and pH. (B) Isotope signatures of total acetate, ac-methyl, CO₂, and CH₄. ▲, ac; ●, ac-methyl; □, CO₂; ○, CH₄; line with no symbols, pH. The values are means ± standard errors ($n = 5$).

clastic methanogenesis was determined as described by Mariotti et al. (11) from the residual reactant

$$\delta_r = \delta_{ri} + \epsilon[\ln(1 - f)] \quad (1)$$

and from the product formed

$$\delta_p = \delta_{ri} - \epsilon(1 - f)[\ln(1 - f)]/f \quad (2)$$

where δ_{ri} is the isotope composition of the reactant (either ac or ac-methyl) at the beginning, δ_r and δ_p are the isotope compositions of the residual ac and the pooled CH₄, respectively, at the instant when f was determined, and f is fractional yield of the products based on the consumption of ac ($0 < f < 1$). Linear regression of δ_r against $\ln(1 - f)$ and of δ_p against $(1 - f)[\ln(1 - f)]/f$ gives ϵ as the slope. The enrichment factor was converted to the fractionation factor: $\epsilon = 10^3 \times (1 - \alpha)$.

The relative contribution of acetate- and CO₂-derived CH₄ to total CH₄ was determined as follows (4):

$$f_{mc} = (\delta_{CH_4\text{-new}} - \delta_{ma}) / (\delta_{mc} - \delta_{ma}) \quad (3)$$

where f_{mc} is the fraction of CH₄ formed from H₂-CO₂, δ_{ma} and δ_{mc} are the isotope ratios of CH₄ derived from either ac or H₂-CO₂, and $\delta_{CH_4\text{-new}}$ is the isotopic signature calculated for the methane formed since the last measurement (13). δ_{ma} was calculated from $\delta_{ac\text{-methyl}}$ using α_{ma} (experimentally determined in this study and from previously published data). δ_{mc} was calculated using the isotope fractionation factor for hydrogenotrophic methanogenesis ($\alpha_{CO_2/CH_4} = 1.085$) determined previously for Lake Dagow sediment (2). For calculation of δ_{ma} , $\delta_{ac\text{-methyl}}$ was assumed to be equal to $\delta^{13}C$ of organic matter ($\delta_{org} = -30.4 \pm 0.4$) (2).

During growth experiments with *M. concilii* acetate was consumed within 550 h to threshold concentrations and was converted to CO₂ and CH₄ (Fig. 1A). The amount of CO₂ and CH₄ accounted for approximately 73% of the initial amount of acetate. As expected for a closed-system approach, the $\delta^{13}C$

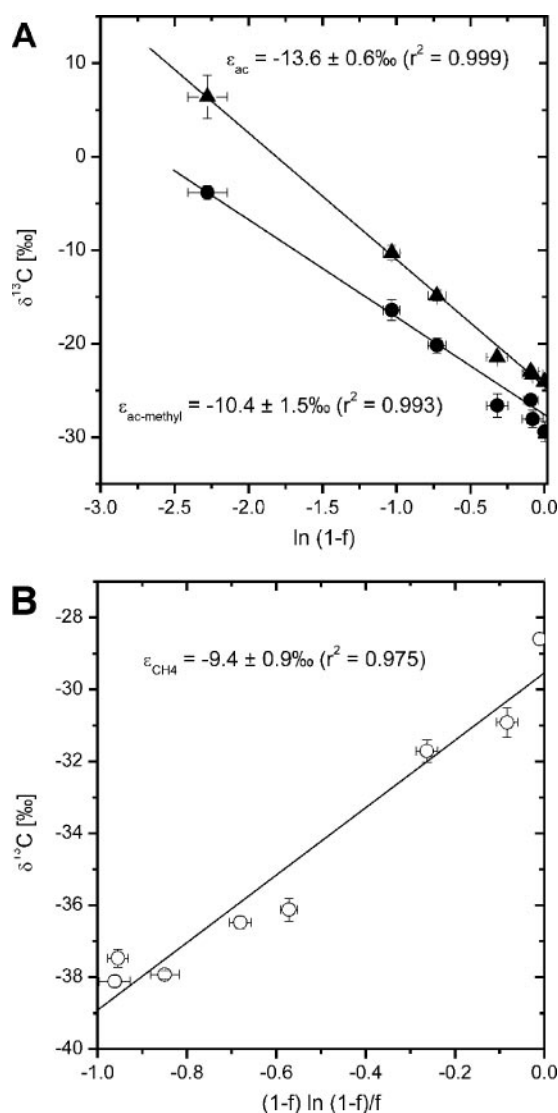


FIG. 2. Isotope enrichment during acetoclastic methanogenesis by *M. concilii*. The plots are based on equations derived by Mariotti et al. (11). (A) ▲, ac; ●, ac-methyl. (B) CH₄. The values are means ± standard errors ($n = 5$).

values for both the substrate and the products increased due to continuous preferential consumption of the [¹²C]acetate (Fig. 1B). The initial high $\delta^{13}C$ value of methane ($\delta^{13}C_{CH_4}$) was due to transfer of dissolved CH₄ by inoculation. $\delta^{13}C_{CO_2}$ increased with time but was not used for determination of isotope fractionation, since the high background level of the bicarbonate buffer did not allow precise quantification of the $\delta^{13}C$ of the newly formed TIC. Isotope fractionation during CH₄ formation from acetate was determined from data for acetate, ac-methyl, and CH₄ using equations 1 and 2 (Fig. 2). The isotope enrichment factors determined for ac-methyl ($\epsilon_{ac\text{-methyl}} = -10.4\text{‰} \pm 1.5\text{‰}$) and CH₄ ($\epsilon_{CH_4} = -9.4\text{‰} \pm 0.9\text{‰}$) agreed within error, whereas the isotope enrichment factor for acetate (both carbon atoms) was more negative ($\epsilon_{ac} = -13.6\text{‰} \pm 0.6\text{‰}$). The good correlation fit indicates that even for a wide range of concentrations (70 mM to the thresh-

old concentration) the magnitude of isotope fractionation was basically unchanged. Isotope enrichment in the carboxyl carbon of ac (ac-carboxyl) was calculated from $\delta^{13}\text{C}_{\text{ac-carboxyl}} = 2\delta^{13}\text{C}_{\text{ac}} - \delta^{13}\text{C}_{\text{ac-methyl}}$ using equation 1, resulting in an $\epsilon_{\text{ac-carboxyl}}$ value of -16.0% .

Our study shows that the isotopic fractionation factor of acetate methyl to CH_4 was much less in *M. concilii* ($\epsilon = \sim -10\%$) than in *Methanosarcina barkeri* ($\epsilon = \sim -20\%$) (7). The agreement of isotope enrichment in ac-methyl ($\epsilon_{\text{ac-methyl}}$) (Fig. 2A) and CH_4 (ϵ_{CH_4}) (Fig. 2B) is a reasonable result, since in *M. concilii* the methyl group should be almost exclusively (98%) used for methane production (12). After complete consumption of acetate, the intercept of the regression line of CH_4 ($\delta_{\text{r,i}}$) (Fig. 2B) was -29.5% , which is almost identical to $\delta^{13}\text{C}_{\text{ac-methyl}}$ (-29.4%), as expected from equation 2. The $\delta^{13}\text{C}$ of the biomass of *M. concilii* was not determined in the experiment reported here, but previous studies showed that the biomass was slightly ^{13}C enriched with respect to the initial $\delta^{13}\text{C}_{\text{ac}}$ ($\epsilon_{\text{biomass}} = 6.0\% \pm 0.7\%$) (unpublished data). We assumed that due to the relatively low level of biomass formation in anaerobic metabolism $\delta^{13}\text{C}_{\text{CH}_4}$ was probably not significantly affected, but this might have resulted in slightly enhanced depletion of ^{13}C in the catabolic product CH_4 (e.g., a lower ϵ for CH_4 than for ac and ac-methyl).

The depletion of ^{13}C in the ac-carboxyl ($\epsilon_{\text{ac-carboxyl}} = -16.0\%$) was greater than that in the ac-methyl, as observed previously for *M. barkeri* (7). The stronger fractionation in ac-carboxyl could theoretically have been caused by reversible exchange of the carbonyl carbon of acetyl-CoA with the ^{13}C -enriched CO_2 in the growth medium. This exchange reaction was observed in *M. barkeri* (6). However, when we considered such an exchange reaction using a $\delta^{13}\text{C}_{\text{ac-carboxyl}}$ value of -18.7% of the initial acetate and a $\delta^{13}\text{C}_{\text{CO}_2}$ value of $\leq -24\%$ (Fig. 1B), we expected ^{13}C depletion leading to weaker isotope fractionation in $\delta^{13}\text{C}_{\text{ac-carboxyl}}$, which was not the case. Therefore, the exchange reaction either might not be active in *M. concilii* or might be catalyzed to only a minor extent. As an alternative explanation we suggest that the stronger isotope fractionation in the ac-carboxyl was caused by ^{13}C fractionation during conversion of acetate via acetyl phosphate to acetyl-CoA. The actual bond breaking of this reaction occurs at the carboxyl carbon (5), and therefore the carboxyl carbon might experience a stronger isotope effect.

The isotope fractionation during acetoclastic methanogenesis in *Methanosaeta* spp. (*M. concilii* in this study and *M. thermophila* in the study of Valentine et al. [18]) is apparently weaker than that in *Methanosarcina* spp., in which fractionation factors typically range from 1.021 to 1.027, equivalent to enrichment factors of -21 to -27% (7, 10, 20). Gelwicks et al. (7) proposed that the carbon monoxide dehydrogenase (CODH) enzyme complex catalyzes the rate-limiting step of the overall reaction and therefore is responsible for the observed fractionation. Yet this does not explain the difference in isotope fractionation caused by the two groups of acetoclastic methanogenic archaea. Although the CODHs of *Methanosarcina* spp. and *Methanosaeta* spp. differ to some extent, the enzymes catalyze the same principal reactions, which determine the magnitude of the isotope effect. Thus, a priori it is not expected that the two CODHs would exhibit very different isotope effects. We therefore speculated that the dif-

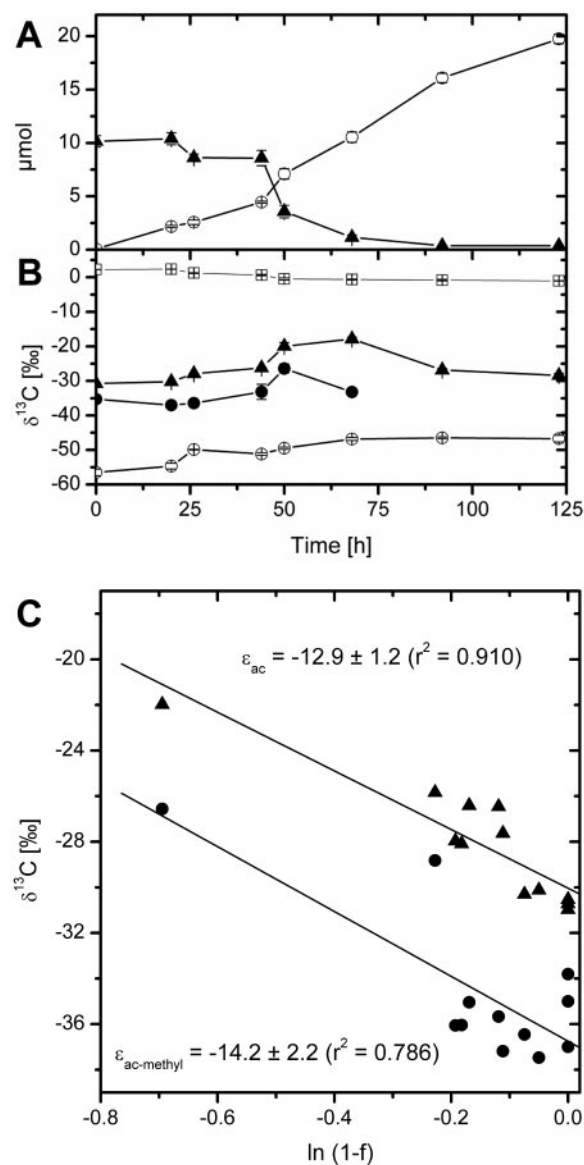


FIG. 3. Acetate consumption in Lake Dagow sediment (obtained in 2004) amended with acetate. (A) Acetate consumption (▲) and CH_4 production (○). (B) Isotope ratios of total ac (▲), ac-methyl (●), CO_2 (□), and CH_4 (○). (C) The plots are based on equations derived by Mariotti et al. (11). The values in panels A and B are means \pm standard errors ($n = 3$).

ference in the overall isotope fractionation is caused by the initial activation of acetate. In this reaction the two acetoclastic genera differ biochemically. While *Methanosarcina* spp. activate acetate in two steps (acetate kinase and phosphotransacetylase) at the expense of one high-energy phosphate bond, *Methanosaeta* spp. activate acetate using the acetyl-CoA synthetase at the expense of two high-energy phosphate bonds (9). Activation by *Methanosarcina* spp., which is driven only by the energy from the breakage of one high-energy bond, may have greater reversibility. This could explain why the isotope effect of the later C—C bond cleavage of acetyl-CoA is expressed more strongly.

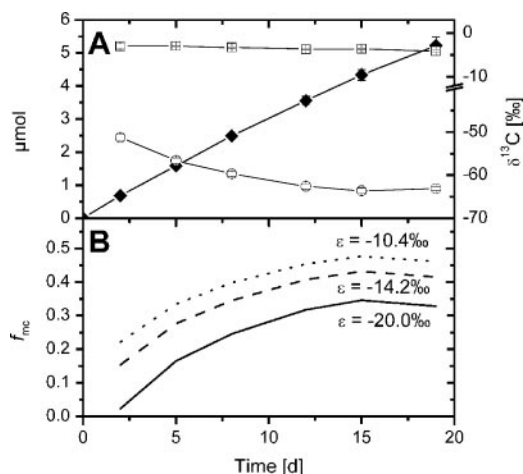


FIG. 4. Effect of $\epsilon_{\text{ac-methyl}}$ on quantification of the fraction of CH_4 originating from $\text{H}_2\text{-CO}_2$ (f_{mc}). (A) Production of CH_4 (♦) and isotope ratios of the newly formed CH_4 (○) and of the CO_2 (□) during incubation of Lake Dagow sediment (obtained in 2005). The values are means \pm standard errors ($n = 3$). (B) f_{mc} calculated using different enrichment factors ($\epsilon_{\text{ac-methyl}}$) (an $\epsilon_{\text{ac-methyl}}$ of -14.2‰ is the value determined in this study for Lake Dagow sediment).

Lake Dagow sediment (obtained in 2004) was incubated anoxically after addition of acetate (initial concentration, 1 mM). Under these conditions CH_4 was produced from $\text{H}_2\text{-CO}_2$, from acetate that is naturally produced in the sediment, and from the acetate added (Fig. 3A). Within 92 h, the elevated concentrations caused by addition of acetate decreased to constant low concentrations ($93 \pm 18 \mu\text{M}$). Quantification of acetate and CH_4 showed that in the sediment samples 52% of the total CH_4 produced was derived from the additional acetate. Note that acetoclastic methanogenesis was the only acetate-consuming process in the sediment, which was devoid of sulfate or other inorganic electron acceptors (2). Due to preferential consumption of ^{12}C for CH_4 production, ac, as well as ac-methyl, became enriched in ^{13}C with time (Fig. 3B). Simultaneously, CH_4 also became isotopically enriched, but to a minor extent. After 50 h, $\delta^{13}\text{C}$ again decreased due to dilution of the residual ^{13}C -enriched acetate by acetate produced from anaerobic degradation of sediment organic matter. Since CH_4 was also produced from $\text{H}_2\text{-CO}_2$, isotope enrichment by acetoclastic methanogenesis was calculated only from the isotope data for acetate. Only data for f values that were ≥ 0 and ≤ 0.5 ($\ln[1 - f] \geq -0.7$) were analyzed, since acetate was still being produced in the sediment. At a higher f value the continuously produced isotopically relatively light acetate would dilute $\delta^{13}\text{C}_{\text{ac-methyl}}$ and $\delta^{13}\text{C}_{\text{ac}}$ (Fig. 3) and therefore bias the quantification of ϵ . The values of ϵ_{ac} and $\epsilon_{\text{ac-methyl}}$ determined were $-12.9\text{‰} \pm 1.2\text{‰}$ and $-14.2\text{‰} \pm 2.2\text{‰}$, respectively. The data point at a $\ln(1 - f)$ value of 0.69 influenced the determination of ϵ . Removal of this data point altered ϵ_{ac} to -20.3‰ , yet $\epsilon_{\text{ac-methyl}}$ changed only slightly to -11.6‰ . We also studied sediment obtained in 2005. Although the ϵ values determined were somewhat different ($\epsilon_{\text{ac}} = -17.2\text{‰} \pm 1.2\text{‰}$ with $r^2 = 0.928$; $\epsilon_{\text{ac-methyl}} = -7.8\text{‰} \pm 1.8\text{‰}$ with $r^2 = 0.566$), they clearly confirmed the tendency of ϵ to be greater than -20‰ . Notably, the results for Lake Dagow sediment con-

firmed that the ϵ values for *Methanosaeta* spp. were clearly less than those for *Methanosarcina* spp. (7, 10, 20).

The difference between the two acetoclastic methanogenic genera is important for correct computation of the amount of CH_4 that is produced from acetate compared with the amount of CH_4 that is produced from CO_2 (equation 3). A lower $\epsilon_{\text{ac-methyl}}$ value leads to a higher value of f_{mc} . Thus, application of the fractionation factor of *Methanosarcina* spp. to an environment that is dominated by *Methanosaeta* spp. would result in underestimation of the relative contribution of $\text{H}_2\text{-CO}_2$ -dependent CH_4 production. We tested the influence of $\epsilon_{\text{ac-methyl}}$ on this quantification using data from anoxic incubation of Lake Dagow sediment. We used sediment samples from 2005 and calculated the relative fraction of CH_4 produced from $\text{H}_2\text{-CO}_2$ (f_{mc}) (equation 3) during the incubation while CH_4 was produced at a constant rate (Fig. 4A). $\delta^{13}\text{C}_{\text{CO}_2}$ in the carbonate-buffered system stayed fairly constant, while $\delta^{13}\text{C}_{\text{CH}_4}$ decreased, reaching stable values around -63‰ . The sensitivity of f_{mc} to $\epsilon_{\text{ac-methyl}}$ was calculated using the enrichment factors either for *Methanosarcina* spp. (7), for *M. concilii* (Fig. 2A), or for Lake Dagow sediment (Fig. 4B). Comparison of calculated f_{mc} values showed that f_{mc} was on average larger by 0.11 units ($\epsilon_{\text{ac-methyl}} = -14.2\text{‰}$) and 0.15 units ($\epsilon_{\text{ac-methyl}} = -10.4\text{‰}$) using enrichment factors obtained in this study rather than using previously published values typical for acetoclastic *Methanosarcina* spp. ($\epsilon_{\text{ac-methyl}} = -20\text{‰}$). The final f_{mc} values were 0.33, 0.42, and 0.46 for $\epsilon_{\text{ac-methyl}}$ values of -20‰ , -14.2‰ , and -10.4‰ , respectively. The f_{mc} values of 0.2 to 0.3 calculated for an previous incubation experiment performed with Lake Dagow sediment (2) might have been biased by such an inappropriate fractionation factor, so that the real values probably are slightly higher (0.3 to 0.4), as suggested by Fig. 4.

Our results show that two functionally equivalent groups of acetoclastic methanogens, whose biochemical pathways differ, exhibit different isotope fractionation and consequently affect isotope modeling. Hence, microbial community structure can affect biogeochemical fluxes and thus provides an example of the hypothesis that the composition of environmental microbial communities does affect ecosystem function (15, 16).

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REFERENCES

- Blair, N., A. Leu, E. Munoz, J. Olsen, E. Kwong, and D. DesMarais. 1985. Carbon isotopic fractionation in heterotrophic microbial metabolism. Appl. Environ. Microbiol. 50:996-1001.
- Chan, O. C., P. Claus, P. Casper, A. Ulrich, T. Lueders, and R. Conrad. 2005. Vertical distribution of structure and function of the methanogenic archaeal community in Lake Dagow sediment. Environ. Microbiol. 7:1139-1149.
- Chidthaisong, A., K. J. Chin, D. L. Valentine, and S. C. Tyler. 2002. A comparison of isotope fractionation of carbon and hydrogen from paddy field rice roots and soil bacterial enrichments during CO_2/H_2 methanogenesis. Geochim. Cosmochim. Acta 66:983-995.
- Conrad, R. 2005. Quantification of methanogenic pathways using stable carbon isotopic signatures: a review and a proposal. Org. Geochem. 36:739-752.
- Ferry, J. G. 1992. Methane from acetate. J. Bacteriol. 174:5489-5495.
- Fischer, R., and R. K. Thauer. 1990. Methanogenesis from acetate in cell extracts of *Methanosarcina barkeri*: isotope exchange between CO_2 and the carbonyl group of acetyl-CoA, and the role of H_2 . Arch. Microbiol. 153:156-162.
- Gelwicks, J. T., J. B. Risatti, and J. M. Hayes. 1994. Carbon isotope effects

- associated with acetoclastic methanogenesis. *Appl. Environ. Microbiol.* **60**:467–472.
8. **Glissmann, K., K. J. Chin, P. Casper, and R. Conrad.** 2004. Methanogenic pathway and archaeal community structure in the sediment of eutrophic Lake Dagow: effect of temperature. *Microb. Ecol.* **48**:389–399.
 9. **Jetten, M. S. M., A. J. M. Stams, and A. J. B. Zehnder.** 1990. Acetate threshold and acetate activating enzymes in methanogenic bacteria. *FEMS Microbiol. Ecol.* **73**:339–344.
 10. **Krzycki, J. A., W. R. Kenealy, M. J. DeNiro, and J. G. Zeikus.** 1987. Stable carbon isotope fractionation by *Methanosarcina barkeri* during methanogenesis from acetate, methanol, or carbon dioxide-hydrogen. *Appl. Environ. Microbiol.* **53**:2597–2599.
 11. **Mariotti, A., J. C. Germon, P. Hubert, P. Kaiser, R. Letolle, A. Tardieux, and P. Tardieux.** 1981. Experimental determination of nitrogen kinetic isotope fractionation: some principles; illustration for the denitrification and nitrification processes. *Plant Soil* **62**:413–430.
 12. **Patel, G. B.** 1984. Characterization and nutritional properties of *Methanothrix concilii* sp. nov., a mesophilic, acetoclastic methanogen. *Can. J. Microbiol.* **30**:1383–1396.
 13. **Penning, H., and R. Conrad.** 2006. Carbon isotope effects associated with mixed acid fermentation of saccharides by *Clostridium papyrosolvans*. *Geochim. Cosmochim. Acta* **70**:2283–2297.
 14. **Penning, H., S. C. Tyler, and R. Conrad.** 2006. Determination of isotope fractionation factors and quantification of carbon flow by stable carbon isotope signatures in a methanogenic rice root model system. *Geobiology* **4**:109–121.
 15. **Schimel, J.** 1995. Ecosystem consequences of microbial diversity and community structure. *Ecol. Stud.* **113**:239–254.
 16. **Schimel, J. P., and J. Gullede.** 1998. Microbial community structure and global trace gases. *Global Change Biol.* **4**:745–758.
 17. **Tyler, S. C., R. S. Bilek, R. L. Sass, and F. M. Fisher.** 1997. Methane oxidation and pathways of production in a Texas paddy field deduced from measurements of flux, $\delta^{13}\text{C}$, and dD of CH_4 . *Global Biogeochem. Cycles* **11**:323–348.
 18. **Valentine, D. L., A. Chidthaisong, A. Rice, W. S. Reebergh, and S. C. Tyler.** 2004. Carbon and hydrogen isotope fractionation by moderately thermophilic methanogens. *Geochim. Cosmochim. Acta* **68**:1571–1590.
 19. **Weimer, P. J., and J. G. Zeikus.** 1978. Acetate metabolism in *Methanosarcina barkeri*. *Arch. Microbiol.* **119**:175–182.
 20. **Zyakun, A. M., V. A. Bondar, K. S. Laurinavichus, O. V. Shipin, S. S. Belyaev, and M. V. Ivanov.** 1988. Fractionation of carbon isotopes under the growth of methane-producing bacteria on various substrates. *Mikrobiol. Zh.* **50**:16–22.