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2 # Fractionation of stable carbon isotopes during formate consumption in
3 # anoxic rice paddy soils and lake sediments

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13 **Running head:** Isotope fractionation by anaerobic formate consumption

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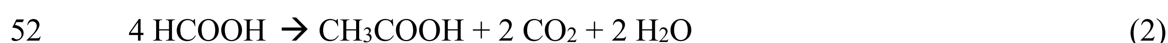
**Abstract.**
Formate is energetically equivalent to hydrogen and thus, is an important intermediate during the breakdown of organic matter in anoxic rice paddy soils and lake sediments. Formate is a common substrate for methanogenesis, homoacetogenesis and sulfate reduction. However, how much these processes contribute to formate degradation and fractionate carbon stable isotopes is largely unknown. Therefore, we measured the conversion of formate to acetate, $CH_4$ and $CO_2$ and the $\delta^{13}C$ of these compounds in samples of paddy soils from Vercelli (Italy) and the International Rice Research Institute (IRRI, the Philippines) and of sediments from the NE and SW basins of Lake Fuchskuhle (Germany). The samples were suspended in phosphate buffer (pH 7.0) both in the absence and presence of sulfate (gypsum) and of methyl fluoride ($CH_3F$), an inhibitor of aceticlastic methanogenesis. In the paddy soils, formate was mainly converted to acetate both under methanogenic and sulfidogenic conditions. Methane was only a minor product and was mainly formed from the acetate. In the lake sediments, the product spectrum was similar, but only under methanogenic conditions. In the presence of sulfate, however, acetate and $CH_4$ were only minor products. The isotopic enrichment factors ($\varepsilon_{form}$) of formate consumption, determined by Mariotti plots, were in the low range of -8‰ to -2.5‰ when sulfate was absent and formate was mainly converted to acetate and $CH_4$. However, no enrichment factor was detectable when formate was degraded with sulfate to mainly $CO_2$. The $\delta^{13}C$ of acetate was by about 25-50‰ more negative than that of formate indicating acetate production by chemolithotrophic homoacetogenesis. Hence, formate seems to be an excellent substrate for homoacetogenesis in anoxic soils and sediments, so that this process is competing well with methanogenesis and sulfate reduction.

## 1 Introduction

Formate is energetically almost equivalent to $H_2$ (Schink et al. 2017) and thus, is an important intermediate in the anaerobic degradation of organic matter. Formate is a product of microbial fermentation, where it is for example produced in pyruvate cleavage by pyruvate formate lyase (Thauer et al., 1977) or by reduction of $CO_2$ (Schuchmann and Müller, 2013). Formate can also be produced in secondary fermentation, such as oxidation of butyrate or propionate (Dong et al., 1994; Sieber et al., 2014). In fact, formate and $H_2$ may equivalently be used as electron shuttles between secondary fermenting bacteria and methanogens (Montag and Schink, 2018; Schink et al., 2017)

Formate can serve alternatively to $H_2$ as a substrate for methanogenesis (Zinder, 1993), (homo)acetogenesis (Drake, 1994) or sulfate reduction (Widdel, 1988), i.e.:

$$4\ HCOOH \rightarrow CH_4 + 3\ CO_2 + 2\ H_2O \qquad (1)$$



$$4\ HCOOH \rightarrow CH_3COOH + 2\ CO_2 + 2\ H_2O \qquad (2)$$

53
$4\ HCOOH + SO_4^{2-} + H^+ \rightarrow HS^- + 4\ CO_2 + 4\ H_2O$ (3)

54 Formate may also be a substrate for syntrophic bacteria, which live from the little Gibbs free
55 energy ($\Delta G^{0\prime} = -3.4$ kJ mol$^{-1}$) that is generated by the conversion of formate to $H_2$ plus $CO_2$
56 (Dolfing et al., 2008; Kim et al., 2010; Martins et al., 2015), i.e.

57
$HCOOH \rightarrow CO_2 + H_2$ (4)

58 Formate can also be enzymatically equilibrated with $H_2$ and $CO_2$ without energy generation.
59 This reaction happens in any organism possessing the suitable enzymes, such as formate
60 hydrogen lyase or hydrogen-dependent carbon dioxide reductase, and in anoxic sediments
61 (DeGraaf and Cappenberg, 1996; Peters et al., 1999; Schuchmann et al., 2018):

62
$HCOOH \leftrightarrow CO_2 + H_2$ (5)

63 Formate has been identified as an important substrate for methanogenesis,
64 homoacetogenesis or sulfate reduction in lake sediments (DeGraaf and Cappenberg, 1996;
65 Lovley and Klug, 1982; Phelps and Zeikus, 1985), soils (Kotsyurbenko et al., 1996; Küsel
66 and Drake, 1999; Rothfuss and Conrad, 1993), mires (Hausmann et al., 2016; Hunger et al.,
67 2011; Liebner et al., 2012; Wüst et al., 2009) and marine sediments (Glombitza et al., 2015).
68 However, it is not very clear to which extent formate-dependent methanogenesis,
69 homoacetogenesis and sulfate reduction are actually operative and to which extent formate
70 affects stable carbon isotope fractionation. The $\delta^{13}C$ values of compounds involved in the
71 degradation process of organic matter provide valuable information on the metabolic
72 pathways involved (Conrad, 2005; Elsner et al., 2005; Hayes, 1993). However, for correct
73 interpretation the knowledge of the enrichment factors ($\varepsilon$) of the major metabolic processes is
74 also important. The $\varepsilon$ values of methanogenesis or homoacetogenesis from $H_2$ plus $CO_2$ are
75 large (Blaser and Conrad, 2016). However, our knowledge of carbon isotope fractionation
76 with formate as substrate is scarce. In cultures of homoacetogenic bacteria the carbon in the
77 acetate produced from formate was strongly depleted in $^{13}C$ ($\varepsilon = -56.5‰$) almost similarly as
78 with $CO_2$ as carbon source (Freude and Blaser, 2016). However, it is not known which
79 enrichment factors operate in methanogenic or sulfidogenic environmental samples.
80 Therefore, we measured isotope fractionation in methanogenic and sulfidogenic rice paddy
81 soils and lake sediments amended with formate. We recorded the consumption of formate
82 along with the production of acetate, $CH_4$ and $CO_2$ and measured the $\delta^{13}C$ of these
83 compounds. We also used the treatment with methyl fluoride ($CH_3F$) to inhibit the
84 consumption of acetate by methanogenic archaea (Janssen and Frenzel, 1997). We used the
85 same environmental samples as for the study of carbon isotope fractionation during
86 consumption of acetate (Conrad et al., 2021) and propionate (Conrad and Claus, 2023), i.e.,
87 rice paddy soils from Vercelli, Italy and the International Rice Research Institute (IRRI, the
88 Philippines) and sediments from the NE and SW basins of Lake Fuchskuhle (Germany). The
89 molecular data characterizing the microbial community compositions in these samples are
90 found in Conrad et al. ( 2021).

91

92 **2 Materials and Methods**

93 *2.1 Environmental samples and incubation conditions*

94 The soil samples were from the research stations in Vercelli, Italy and the International Rice
95 research Institute (IRRI) in the Philippines. Sampling and soil characteristics were described
96 before (Liu et al., 2018). The lake sediments (top 10 cm layer) were from the NE and SW
97 basins of Lake Fuchskuhle, an acidic-bog (pH 4.2 – 4.6) lake in Northeastern Germany
98 (Casper et al., 2003). The lake was artificially divided into four compartments in 1987 and
99 finally in 1991, resulting in four nearly equal sized compartments, each with a different
100 catchment area. The NE basin is characterized by higher biomass and activity throughout all
101 trophic levels in the water column than the SW basin. The lake sediments were sampled in
102 July 2016 using a gravity core sampler as described before (Kanaparthi et al., 2013). The
103 experiments with rice field soil were carried out in 2016, those with sediments of Lake
104 Fuchskuhle in 2017.

105 The experimental setup was exactly the same as during previous studies of acetate
106 consumption (Conrad et al., 2021) and propionate consumption (Conrad and Claus, 2023).
107 For methanogenic conditions, paddy soil was mixed with autoclaved anoxic $H_2O$ (prepared
108 under $N_2$) at a ratio of 1:1 and incubated under $N_2$ at 25°C for 4 weeks. In a second
109 incubation, for sulfidogenic conditions, paddy soil was mixed with autoclaved anoxic $H_2O$ at
110 a ratio of 1:1, was amended with 0.07 g $CaSO_4.2H_2O$, and then incubated under $N_2$ at 25°C
111 for 4 weeks. These two preincubated soil slurries were sampled and stored at -20°C for later
112 molecular analysis (see data in Conrad et al. ( 2021)). The preincubated soil slurries were also
113 used (in 3 replicates) for the following incubation experiments. Two different sets of
114 incubations were prepared. In the first set (resulting in methanogenic conditions), 5 mL soil
115 slurry preincubated without sulfate was incubated at 25°C with 40 mL of 20 mM potassium
116 phosphate buffer (pH 7.0) in a 150-mL bottle under an atmosphere of $N_2$. The bottles were
117 amended with (i) 5 mL $H_2O$; (ii) 5 mL $H_2O$ + 4.5 mL $CH_3F$; (iii) 5 mL 200 mM sodium
118 formate; (iv) 5 mL 200 mM sodium formate + 4.5 mL $CH_3F$. In the second set (resulting in
119 sulfidogenic conditions), 5 mL soil slurry preincubated with sulfate was incubated at 25°C
120 with 40 mL of 20 mM potassium phosphate buffer (pH 7.0) in a 150-mL bottle under an
121 atmosphere of $N_2$. The amendments were the same as above, but with the addition of 200 µl
122 of a $CaSO_4$ suspension corresponding to a concentration of 2.5 M (giving a final
123 concentration of 10 mM sulfate).

124 For lake sediments under methanogenic conditions, 5 ml sediment was incubated in 3
125 replicates at 10°C (which is close to the in-situ temperature) with 40 ml of 20 mM potassium
126 phosphate buffer (pH 7.0) in a 150-ml bottle under an atmosphere of $N_2$. The bottles were
127 amended with (i) 5 ml $H_2O$; (ii) 5 ml $H_2O$ + 4.5 ml $CH_3F$; (iii) 5 ml 200 mM sodium formate;
128 (iv) 5 ml 200 mM sodium formate + 4.5 ml $CH_3F$. For sulfidogenic conditions, lake

129 sediments were preincubated with sulfate by adding 0.1 g $CaSO_4.2H_2O$ (gypsum) to 50 ml
130 sediment and incubating at 10°C for 4 weeks. For sulfidogenic conditions, 5 ml of the
131 preincubated sediment was incubated in 3 replicates at 10°C with 40 ml of 20 mM potassium
132 phosphate buffer (pH 7.0) in a 150-ml bottle under an atmosphere of $N_2$. The bottles were
133 amended as above, but in addition also with 200 µl of a $CaSO_4$ suspension giving a final
134 concentration of 10 mM sulfate. Samples for later molecular analysis were taken from the
135 original lake sediment and from the lake sediment preincubated with sulfate. The samples
136 were stored at -20°C (see data in Conrad et al. ( 2021)).
137
138 *2.2 Chemical and isotopic analyses*
139 Gas samples for analysis of partial pressures of $CH_4$ and $CO_2$ were taken from the
140 headspace of the incubation bottles after vigorous manual shaking for about 30 s using a gas-
141 tight pressure-lock syringe, which had been flushed with $N_2$ before each sampling. Soil
142 slurries were sampled, centrifuged and filtered through a 0.2 µm cellulose membrane filter
143 and stored frozen at -20ºC for later fatty acid analysis. Chemical and isotopic analyses were
144 performed as described in detail previously (Goevert and Conrad, 2009). Methane was
145 analyzed by gas chromatography (GC) with flame ionization detector. Carbon dioxide was
146 analyzed after conversion to $CH_4$ with a Ni catalyst. Stable isotope analyses of $^{13}C/^{12}C$ in gas
147 samples were performed using GC-combustion isotope ratio mass spectrometry (GC-C-
148 IRMS). Formate and acetate were measured using high-performance liquid chromatography
149 (HPLC) linked via a Finnigan LC IsoLink to an IRMS. The isotopic values are reported in the
150 delta notation ($\delta^{13}C$) relative to the Vienna Peedee Belemnite standard having a $^{13}C/^{12}C$ ratio
151 ($R_{standard}$) of 0.01118: $\delta^{13}C = 10^3 (R_{sample}/R_{standard} - 1)$. The precision of the GC-C-IRMS was
152 ± 0.2‰, that of the HPLC-IRMS was ± 0.3‰.
153
154 *2.3 Calculations*
155 Millimolar concentrations of $CH_4$ were calculated from the mixing ratios (1 ppmv = $10^{-6}$
156 bar) measured in the gas phase of the incubation bottles: 1000 ppmv $CH_4$ correspond to 0.09
157 µmol per mL of liquid. Note, that this is the total amount of $CH_4$ in the gas phase relative to
158 the liquid phase.
159 Fractionation factors for reaction A → B are defined after Hayes (Hayes, 1993) as:
160 $\alpha_{A/B} = (\delta_A + 1000)/ (\delta_B + 1000)$ (7)
161 also expressed as $\varepsilon \equiv 1000 (1 - \alpha)$ in permil. The carbon isotope enrichment factor $\varepsilon_{form}$
162 associated with formate consumption was calculated from the temporal change of $\delta^{13}C$ of
163 formate as described by Mariotti et al. (Mariotti et al., 1981) from the residual reactant
164 $\delta_r = \delta_{ri} + \varepsilon [\ln(1- f)]$ (8)
165 where $\delta_{ri}$ is the isotopic composition of the reactant (formate) at the beginning, and $\delta_r$ is the
166 isotopic composition of the residual formate, both at the instant when $f$ is determined. $f_{form}$ is

167 the fractional yield of the products based on the consumption of formate ($0 < f_{form} < 1$).
168 Linear regression of $\delta^{13}C$ of formate against $\ln(1 - f)$ yields $\varepsilon_{form}$ as the slope of best fit lines.
169 The regressions of $\delta^{13}C$ of formate were done for data in the range of $f_{form} < 0.7$. The linear
170 regressions were done individually for each experimental replicate (n = 3) and were only
171 accepted if $r^2 > 0.7$. The $\varepsilon$ values resulting from the replicate experiments were then averaged
172 (± SE).

173 **3 Results**

174 *3.1 Conversion of formate under methanogenic and sulfidogenic conditions*

175 The rice paddy soils were submerged and preincubated to create methanogenic or
176 sulfidogenic conditions. Samples of these soils were suspended in buffer at pH 7 and
177 amended with formate. In the Vercelli soil, formate was consumed after a lag phase of 4 days
178 under methanogenic and 5 days under sulfidogenic conditions (Fig. 1a). During this time the
179 pH increased from pH 7 up to pH 8 despite buffering. Formate consumption was not inhibited
180 by $CH_3F$ (Fig. 1a). Similar results were obtained with IRRI soil (Fig. S1). Acetate was
181 produced concomitantly with formate consumption, again without effect by $CH_3F$ (Fig. 1b).
182 The production of acetate under sulfidogenic conditions was smaller than under
183 methanogenic conditions. Methane was also produced under both methanogenic and
184 sulfidogenic conditions concomitantly with formate consumption (Fig. 1c; S1c). It is
185 noteworthy that $CH_3F$ inhibited the production of $CH_4$ (Fig. 1c; S1c). Finally, $CO_2$ was
186 produced under all conditions without lag phase and without effect by $CH_3F$ (Fig. 1d). In
187 Vercelli soil, $CO_2$ production was about two times larger under sulfidogenic than under
188 methanogenic conditions (Fig. 1d). In IRRI soil, it was only slightly larger (Fig. S1d). The
189 accumulation of acetate plus $CH_4$ was equimolar to the consumption of formate in terms of
190 electron equivalents, while the accumulation of $CH_4$ alone accounted only for <30%, in the
191 presence of $CH_3F$ even less (Fig. 2a; S2a). Hence, acetate was the more important product of
192 formate consumption. Under sulfidogenic conditions, accumulation of acetate plus $CH_4$ was
193 less than equimolar, especially in Vercelli soil (Fig. 2b), probably since formate was instead
194 converted to $CO_2$. However, acetate formation was still substantial accounting for 60-80% of
195 formate consumption (Fig. 2b; S2b).

196 The sediments from Lake Fuchskuhle were methanogenic in-situ so that preincubation of
197 the samples was not required. However, sulfidogenic conditions were created analogously to
198 the paddy soils by preincubtion with sulfate (gypsum). Substantial formate depletion did not
199 start before about 20 days of incubation both in sediments from the NE basin (Fig. 3) and the
200 SW basin (Fig. S3). Again, $CH_3F$ only inhibited the production of $CH_4$ but not that of acetate
201 or $CO_2$ (Fig. 3; S3). The main difference to the paddy soils was that $CH_4$ was not produced
202 concomitantly with formate consumption, but started right from the beginning. However, the
203 amounts of $CH_4$ produced were only small and were apparently due to the little formate that
204 was consumed in the beginning of incubation (i.e., before day 20), as seen by the fact that

205 $CH_4$ production in the water control (not amended with formate) was negligible (Fig. 3c;
206 S3c). Production of $CO_2$ started without lag phase but accelerated together with formate
207 consumption (Fig. 3d; S3d). In the lake sediments, $CH_4$ accounted only for <10% of formate
208 consumption, while acetate was the main product when sulfate was absent (Fig. 4a, S4a). In
209 contrast to the paddy soils, formate consumption in both lake sediments was much slower
210 under sulfidogenic than under methanogenic conditions (Fig. 3a; S3a). In the sediment from
211 SW basin, formate consumption was very slow so that less than half of the formate was
212 consumed during 80 days of incubation and consumption was not completed until the end of
213 the experiment (Fig. S3a). Very little acetate was produced and no $CH_4$ was formed from
214 formate in both lake sediments, when sulfate was present (Fig. 4b, S4b).
215
216 *3.2 Isotope fractionation during formate consumption*
217 In the rice paddy soils, $\delta^{13}C$-values of formate increased when formate was consumed
218 indicating discrimination against the heavy carbon isotope. This process was not affected by
219 $CH_3F$ and was similar without and with sulfate (Fig. 1e; S1e). The same was the case with the
220 sediment from the NE lake basin, but only in the absence of sulfate (Fig. 3e). With sulfate,
221 the $\delta^{13}C$ of formate slowly decreased with time (Fig. 3e). In the sediment from the SW basin,
222 $\delta^{13}C$ of formate slowly decreased (without sulfate) or stayed constant with time (with sulfate)
223 (Fig. S3e). Note that formate was not completely consumed in the SW sediment when sulfate
224 was present (Fig. S3a).
225 Mariotti plots of $\delta^{13}C$ of formate as function of $f_{form}$ resulted in negative slopes (Fig. 4;
226 S5). Hence, the enrichment factors ($\varepsilon_{form}$) for the paddy soils, both without and with sulfate,
227 and for the sediments from the NE basin of Lake Fuchskuhle without sulfate showed that the
228 light isotope of formate carbon was preferred. Values of $\varepsilon_{form}$ were in the range of -8.5 to -
229 2.5‰ (Fig. 6). Under sulfidogenic conditions, however, the Mariotti plots of the sediments
230 from the NE basin (Fig. 5) did not show a negative slope and $\varepsilon_{form}$ could not be determined.
231 The same was the case for the sediments from the SW basin (Fig. 6).
232 The negative $\varepsilon_{form}$ indicates that products of formate should be depleted in $^{13}C$. Indeed the
233 $\delta^{13}C$ of acetate and $CH_4$ were generally more negative than the $\delta^{13}C$ of formate. This was the
234 case in the paddy soils from Vercelli (Fig. 1f) and the IRRI (Fig. S1f) as well as in the
235 sediments from the NE basin (Fig. 3f) and the SW basin (Fig. S3f) of Lake Fuchskuhle. In the
236 sediment of the NE basin, the $\delta^{13}C$ of acetate increased from very low -95‰ to finally about -
237 57‰ in parallel with formate consumption (Fig. 3f). $CO_2$ was also produced during formate
238 degradation to various extent (equ.1, 2 and 3). Since the pH was in a range of pH 7 to pH 8,
239 $CO_2$ was also converted to bicarbonate. The $\delta^{13}C$ of bicarbonate is generally by about 10‰
240 more positive than the $\delta^{13}C$ of $CO_2$ (Stumm and Morgan, 1996). The $\delta^{13}C$ of the gaseous $CO_2$
241 was always close to the $\delta^{13}C$ of formate or was more positive. In the paddy soils and the NE
242 basin of Lake Fuchskuhle, the $\delta^{13}C$ of $CO_2$ increased in parallel with the increasing $\delta^{13}C$ of

243 formate (Fig. 1h, 3h; S1h). The $\delta^{13}C$ of the gaseous $CO_2$ produced from the formate-amended
244 samples was initially more negative than that from the unamended samples, but eventually
245 the $\delta^{13}C$ increased above these values when formate was completely consumed (Fig. 1h, 3h;
246 S3h).
247 The $\delta^{13}C$ values of the initial formate were about -24‰ (Fig. 5). When formate was
248 completely consumed, the $\delta^{13}C$ values of the products acetate and $CH_4$ were always more
249 negative. The average $\delta^{13}C$ values of the products after complete consumption of formate are
250 shown in Fig. 7. In the absence of sulfate, $\delta^{13}C$ of acetate was in a range of -51‰ to -49‰
251 and -70‰ to -63‰, in the paddy soils and lake sediments, respectively (Fig. 7). In the
252 presence of sulfate, $\delta^{13}C$ of acetate was in a range of -57‰ to -52‰ and -78‰ to -72‰, in
253 the paddy soils and lake sediments (only NE basin), respectively (Fig. 7). The $\delta^{13}C$ of $CH_4$
254 was in a range of -70‰ to -54‰ and -60‰ to -54‰, in the absence and presence of sulfate,
255 respectively (Fig. 7). The $\delta^{13}C$ of gaseous $CO_2$ (for bicarbonate plus 10‰) was in a range of -
256 23‰ to -11‰ and -24‰ to -19‰, in the absence and presence of sulfate, respectively (Fig.
257 7).
258
259 **4 Discussion**
260 *4.1 Formate degradation under acetogenic/methanogenic conditions*
261 In rice paddy soils formate was consumed within <10 days. The absence of sulfate did not
262 allow sulfidogenic (equ.3) degradation, but allowed the operation of methanogenic (equ.1),
263 homoacetogenic (equ.2) or syntrophic (equ.4) degradation. Syntrophic degradation is still
264 disputed, since many microorganisms are able to enzymatically equilibrate $H_2$ and formate
265 and thus prohibit exploitation of the difference in the energy content (Montag and Schink,
266 2018; Schink et al., 2017). Syntrophic formate degradation is exergonic by only a few
267 kilojoules of Gibbs free energy per mole and requires the coupling with methanogenesis or
268 other efficient hydrogen (electron) scavengers. Although formate-driven $CH_4$ production was
269 observed in our study, the production was sensitive to inhibition by $CH_3F$ indicating that $CH_4$
270 was predominantly produced from acetate rather than from $H_2$. Therefore, syntrophic formate
271 oxidation coupled to $CH_4$ production was probably not a major pathway.
272 Acetate was the most important product of formate degradation in the paddy soils as well
273 as in the lake sediments. Methane also was a product, but was much less important than
274 acetate. Furthermore, it was predominantly produced from acetate as shown by the inhibition
275 by $CH_3F$ and the concomitant decrease of $\delta^{13}C$ of $CH_4$, which is characteristic for
276 hydrogenotrophic methanogesis that is not inhibited by $CH_3F$ (Conrad et al., 2010). Hence,
277 formate was apparently primarily degraded by homoacetogenesis (equ.1). Only part of the
278 produced acetate was immediately used by aceticlastic methanogenesis generating $CH_4$ as
279 secondary product. Although formate is a perfect substrate for homoacetogenic bacteria
280 operating the Wood-Ljungdahl pathway (WLP) (Drake, 1994), the yield of Gibbs free energy

281 per mole formate is less for homoacetogenic than for methanogenic degradation (Dolfing et
282 al., 2008). Thus, it is surprising that formate-driven homoacetogenesis prevailed over
283 methanogenesis. Nevertheless, simultaneous operation of homoacetogenesis and
284 methanogenesis from formate has been observed before in a fen soil (Hunger et al., 2011).
285 Homoacetogenesis prevailing over methanogenesis has also frequently been observed with
286 $H_2/CO_2$ as substrate (Conrad et al., 1989; Nozhevnikova et al., 1994), indicating that
287 homoacetogens can take particular advantage from low temperatures (Conrad, 2023) or the
288 availability of secondary substrates (Peters et al., 1998). It is noteworthy that homoacetogens
289 have to invest ATP for fixation of formate, while methanogens are able to bypass this step
290 (Lemaire et al., 2020). Perhaps it is such energy investment which makes the homoacetogens
291 to competitive formate utilizers.
292 Formate consumption was recorded upon addition of formate to initial concentrations of
293 about 15 mM, which was much higher than the in-situ concentration being typically on the
294 order of a few micromolar (Montag and Schink, 2018). However, the increased concentration
295 allowed stable isotope fractionation, which would not occur under formate limitation. The
296 $\delta^{13}C$ of the produced acetate was by about 24-33‰ lower than that of formate. This isotopic
297 discrimination between formate and acetate is similar to that measured in a culture of the
298 homoacetogen *Thermoanaerobacter kivui* (Freude and Blaser, 2016). However, this
299 discrimination is much larger than the isotopic enrichment factors ($\varepsilon_{form}$ of -8‰ to -2.5‰)
300 determined from the change of $\delta^{13}C$ during formate consumption. There are two conceivable
301 explanations for this observation. (1) Formate is disproportionated to $CO_2$ and acetate. In the
302 WLP three formate are oxidized to $CO_2$, one formate is reduced to the methyl group of
303 acetate and one of the produced $CO_2$ is reduced to the carboxyl group of acetate. The
304 disproportionation of formate to acetate and 2 $CO_2$ is possibly a branch point (Fry, 2003;
305 Hayes, 2001), at which the carbon flow is split into the production of $^{13}C$-enriched $CO_2$ and
306 $^{13}C$-depleted acetate, which together result in the $\varepsilon_{form}$ observed. (2) Formate first is
307 completely converted to $CO_2$ plus $H_2$ (equ.5) or other electron equivalents. This reaction
308 displays the $\varepsilon_{form}$ determined by the Mariotti plots. Acetate is then produced via the WLP by
309 the chemolithotrophic reduction of 2 $CO_2$ to acetate, of which the isotopic enrichment factor
310 is typically on the order of about -55‰ (Blaser and Conrad, 2016). In any case, it is plausible
311 to assume that acetate was formed via the WLP. In the WLP, oxidation of formate is
312 catalyzed by a formate dehydrogenase, which provides $CO_2$ to the carboxyl branch of the
313 WLP. The methyl branch of the WLP normally starts with formate being converted to
314 formyl-THF. However, it can also start with the reduction of $CO_2$ to formate with a
315 hydrogen-dependent carbon dioxide reductase (HDCD). Homoacetogens (e.g., *Acetobacter*
316 *woodii, T. kivui*) contain such a HDCD, which allows the interconversion of formate and $H_2$
317 plus $CO_2$ (Jain et al., 2020; Schuchmann et al., 2018). The isotope discrimination in our
318 experiments indicates that the $CO_2$ produced from formate has been enriched in $^{13}C$ rather

319 than depleted, thus supporting the first explanation. The $\delta^{13}C$ of $CO_2$ produced from formate
320 was initially lower than that of the unamended soil or sediment being on the order of -20‰ to
321 -10‰ (Fig. 1h, 3h, S1h, S3h). Eventually, however, $\delta^{13}C$ of $CO_2$ reached values of -25‰ to -
322 10‰ (Fig. 7). The $\delta^{13}C$ of bicarbonate is 10‰ more positive than that of $CO_2$. This mixed
323 inorganic carbon would be the $CO_2$ substrate for WLP, which together with formate generates
324 the acetate having a $\delta^{13}C$ of about -70‰ to -50‰ (Fig. 7).
325 Methane was a minor product of formate degradation in all soils and sediments. Since $CH_4$
326 formation was strongly inhibited by $CH_3F$, it was most likely produced from acetate by
327 aceticlastic methanogens. Since $CH_4$ production from the soils or sediments was much lower
328 without formate amendment, the $CH_4$ must have primarily been produced from the acetate
329 that was generated from formate. The $\delta^{13}C$ of $CH_4$ in the soil incubations was more negative
330 than that of acetate (Fig. 7). The difference between the $\delta^{13}C$ of $CH_4$ and the $\delta^{13}C$ of acetate
331 indicated an isotopic enrichment factor of $\varepsilon_{ac\text{-}CH4}$ = -10‰ to -8‰, which is close to the
332 enrichment factor of aceticlastic *Methanosaeta (Methanothrix) concilii* (Penning et al., 2006).
333 In the lake sediments, the $\delta^{13}C$ of $CH_4$ and acetate were not much different indicating that
334 acetate was instantaneously consumed by methanogens as it was produced by homoacetogens
335 so that carbon isotopes were not discriminated. Both, paddy soils and lake sediments
336 contained *mcrA* genes (coding for a subunit of methyl CoM reductase) of *Methanosaetaceae*
337 *(Methanotrichaceae)* (Conrad et al., 2021).
338
339 *4.2 Formate degradation under sulfidogenic conditions*
340 In the rice paddy soils, formate was consumed within ten days when sulfate was present,
341 not quite as fast as without sulfate. In the lake sediments, however, sulfidogenic formate
342 consumption was much slower. Formate degradation by sulfate reduction normally results in
343 complete oxidation to $CO_2$ (equ.3). In the lake sediments, $CO_2$ was indeed the main
344 degradation product. However, in the paddy soils substantial amounts of acetate and even
345 $CH_4$ were also produced. The homoacetogenic bacteria in these soils apparently competed
346 well with the sulfate reducing bacteria, although the soils had been adapted by preincubation
347 in the presence of sulfate. The production of acetate and $CH_4$ was dependent on formate
348 degradation, since no production was observed in the unamended control. Production of $CH_4$
349 was inhibited by $CH_3F$ indicating that aceticlastic methanogenesis was the main process of
350 $CH_4$ production. The carbon isotope fractionation of formate was similar as under non-
351 sulfidogenic conditions, exhibiting a small $\varepsilon_{form}$ of -8‰ to -3.5‰ (Fig. 5) and displaying a
352 strong isotope effect with the formation of acetate ($\delta^{13}C$ = -57--52‰) and $CH_4$ ($\delta^{13}C$ = -60--
353 58‰). The mechanism of fractionation is probably the same (see above).
354 In the lake sediments, however, sulfidogenic degradation of formate was much slower
355 than methanogenic/acetogenic degradation. In the sediment of the SW basin, formate was not
356 even completely degraded within 80 days. In the sediments of both lake basins, neither

357 acetate nor $CH_4$ was a major product of sulfidogenic formate degradation. Hence, formate
358 was apparently degraded according to equ.3 forming $CO_2$ as main carbon product. This
359 formation process displayed no depletion of the heavy carbon isotope, as the Mariotti plots of
360 $\delta^{13}C$ of formate did not exhibit a negative slope. The $\delta^{13}C$ of the $CO_2$ slowly decreased with
361 increasing fraction of formate consumed (Fig. 3h; 5c), probably involving isotope exchange
362 between formate and $CO_2$ (DeGraaf and Cappenberg, 1996). The little acetate, which was
363 formed, displayed a $\delta^{13}C$ of -77‰ (Fig. 7b) indicating that it was produced by a similar
364 mechanism as in the absence of sulfate, presumably via the WLP.
365 The strong differences between rice paddy soils and lake sediments were possibly caused
366 by their different microbial communities (Conrad et al., 2021). The differences were seen in
367 the composition of the *mcrA* and *dsrB* genes coding for methyl CoM reductase and
368 dissimilatory sulfate reductase, respectively, as well as the gene coding for the bacterial 16S
369 rRNA (data are shown in Conrad et al. (2021)) . The microbial community structures based
370 on these genes were similar whether the soils and sediments were amended with sulfate or
371 not. However, they were strongly different between soils and sediments (Conrad et al., 2021).
372 Unfortunately, these data do not allow to discriminate for particular taxa of homoacetogenic
373 bacteria. Nevertheless, it is possible that formate-consuming homoacetogens were more
374 prevalent in the soils than in the sediments and accordingly competed more or less with the
375 formate-consuming sulfate reducers.
376
377 *4.3 Conclusions*
378 Formate was found to be an excellent substrate for acetate formation in the paddy soils as
379 well as in the lake sediments, confirming and extending similar observations in a fen soil
380 (Hunger et al., 2011). In the anoxic soils, acetate was the major product even in the presence
381 of sulfate, which would have allowed sulfate reduction. The acetate was strongly depleted in
382 $^{13}C$ relative to formate, but the consumption of formate itself displayed only a small isotopic
383 enrichment factor. Therefore, it is likely that formate was disproportionated to $^{13}C$-depleted
384 acetate and $^{13}C$-enriched $CO_2$. The $\delta^{13}C$ of $CO_2$ was indeed slightly higher than that of
385 formate. Acetate was most likely produced by homoacetogenesis via the WLP. The produced
386 acetate was then used by aceticlastic methanogens (probably by *Methanothrix*), but only to
387 minor extent, resulting in further depletion of $^{13}C$. The homoacetogenic bacteria in the paddy
388 soils apparently competed well with both methanogenic and sulfate-reducing
389 microorganisms, when formate was the substrate. The preference of homoacetogenesis as
390 degradation pathway is unexpected, since other substrates, such as acetate and propionate, are
391 degraded in these paddy soils by methanogenesis or sulfate reduction (Conrad et al., 2021)
392 (Conrad and Claus, 2023). Only in the lake sediments, formate oxidation by sulfate reduction
393 was more prevalent than homoacetogenesis.
394

**Supplement link**

**Author contribution:** RC designed the experiments, evaluated the data and wrote the manuscript. PC conducted the experiments.

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408 References

409


410 Blaser, M. and Conrad, R.: Stable carbon isotope fractionation as tracer of carbon
411 cycling in anoxic soil ecosystems, Curr. Opinion Biotechnol., 41, 122-129,
412 2016.
413 Casper, P., Chan, O. C., Furtado, A. L. S., and Adams, D. D.: Methane in an
414 acidic bog lake: The influence of peat in the catchment on the biogeochemistry
415 of methane, Aquat. Sci., 65, 36-46, 2003.
416 Conrad, R.: Quantification of methanogenic pathways using stable carbon
417 isotopic signatures: a review and a proposal, Org. Geochem., 36, 739-752,
418 2005.
419 Conrad, R.: Complexity of temperature dependence in methanogenic microbial
420 environments, Front. Microbiol., 14, 1232946-
421 doi:10.3389/fmicb.2023.1232946, 2023.
422 Conrad, R., Bak, F., Seitz, H. J., Thebrath, B., Mayer, H. P., and Schütz, H.:
423 Hydrogen turnover by psychrotrophic homoacetogenic and mesophilic
424 methanogenic bacteria in anoxic paddy soil and lake sediment, FEMS
425 Microbiol. Ecol., 62, 285-294, 1989.
426 Conrad, R. and Claus, P.: Fractionation of stable carbon isotopes during microbial
427 propionate consumption in anoxic rice paddy soils, Biogeosciences, 20, 3625-
428 3635, 2023.
429 Conrad, R., Klose, M., Claus, P., and Enrich-Prast, A.: Methanogenic pathway,
430 $^{13}C$ isotope fractionation, and archaeal community composition in the sediment
431 of two clearwater lakes of Amazonia, Limnol. Oceanogr., 55, 689-702, 2010.
432 Conrad, R., Liu, P., and Claus, P.: Fractionation of stable carbon isotopes during
433 acetate consumption by methanogenic and sulfidogenic microbial communities
434 in rice paddy soils and lake sediments, Biogeosciences, 18, 6533-6546, 2021.
435 DeGraaf, W. and Cappenberg, T. E.: Evidence for isotopic exchange during
436 metabolism of stable- isotope-labeled formate in a methanogenic sediment,
437 Appl. Environ. Microbiol., 62, 3535-3537, 1996.
438 Dolfing, J., Jiang, B., Henstra, A. M., Stams, A. J. M., and Plugge, C. M.:
439 Syntrophic growth on formate: a new microbial niche in anoxic environments,
440 Appl. Environ. Microbiol., 74, 6126-6131, 2008.
441 Dong, X. Z., Plugge, C. M., and Stams, A. J. M.: Anaerobic degradation of
442 propionate by a mesophilic acetogenic bacterium in coculture and triculture
443 with different methanogens, Appl. Environ. Microbiol., 60, 2834-2838, 1994.
444 Drake, H. L.: Acetogenesis, acetogenic bacteria, and the acetyl-CoA
445 "Wood/Ljungdahl" pathway: past and current perspectives, in: Acetogenesis,
446 edited by: Drake, H. L., Chapman & Hall, New York, 3-60, 1994.
447 Elsner, M., Zwank, L., Hunkeler, D., and Schwarzenbach, R. P.: A new concept
448 linking observable stable isotope fractionation to transformation pathways of
449 organic pollutants [review], Environ. Sci. Technol., 39, 6896-6916, 2005.
450 Freude, C. and Blaser, M.: Carbon sotope fractionation during catabolism and
451 anabolism in acetogenic bacteria growing on different substrates, Appl.
452 Environ. Microbiol., 82, 2728-2737, 2016.
453 Glombitza, C., Jaussi, M., Roy, H., Seidenkrantz, M. S., Lomstein, B. A., and
454 Joergensen, B. B.: Formate, acetate, and propionate as substrates for sulfate
455 reduction in sub-arctic sediments of Southwest Greenland, Frontiers
456 Microbiol., 6, 846-doi: 10.3389/fmicb.2015.00846 , 2015.

457 Goevert, D. and Conrad, R.: Effect of substrate concentration on carbon isotope
458 fractionation during acetoclastic methanogenesis by *Methanosarcina barkeri*
459 and *M. acetivorans* and in rice field soil, Appl. Environ. Microbiol., 75, 2605-
460 2612, 2009.
461 Hausmann, B., Knorr, K. H., Schreck, K., Tringe, S. G., DelRio, T. G., Loy, A.,
462 and Pester, M.: Consortia of low-abundance bacteria drive sulfate reduction-
463 dependent degradation of fermentation products in peat soil microcosms,
464 ISME J., 10, 2365-2375, 2016.
465 Hayes, J. M.: Factors controlling $^{13}C$ contents of sedimentary organic
466 compounds: principles and evidence, Mar. Geol., 113, 111-125, 1993.
467 Hunger, S., Schmidt, O., Hilgarth, M., Horn, M. A., Kolb, S., Conrad, R., and
468 Drake, H. L.: Competing formate- and carbon dioxide-utilizing prokaryotes in
469 an anoxic methane-emitting fen soil, Appl. Environ. Microbiol., 77, 3773-
470 3785, 2011.
471 Jain, S., Dietrich, H. M., Müller, V., and Basen, M.: Formate is required for
472 growth of the thermophilic acetogenic bacterium *Thermoanaerobacter kivui*
473 lacking hydrogen-dependent carbon dioxide reductase (HDCR), Frontiers
474 Microbiol., 11, 59-doi: 10.3389/fmicb.2020.00059, 2020.
475 Janssen, P. H. and Frenzel, P.: Inhibition of methanogenesis by methyl fluoride -
476 studies of pure and defined mixed cultures of anaerobic bacteria and archaea,
477 Appl. Environ. Microbiol., 63, 4552-4557, 1997.
478 Kanaparthi, D., Pommerenke, B., Casper, P., and Dumont, M. G.:
479 Chemolithotrophic nitrate-dependent Fe(II)-oxidizing nature of actinobacterial
480 subdivision lineage TM3, ISME J., 7, 1582-1594, 2013.
481 Kim, Y. J., Lee, H. S., Kim, E. S., Bae, S. S., Lim, J. K., Matsumi, R.,
482 Lebedinsky, A. V., Sokolova, T. G., Kozhevnikova, D. A., Cha, S. S., Kim, S.
483 J., Kwon, K. K., Imanaka, T., Atomi, H., Bonch-Osmolovskaya, E. A., Lee, J.
484 H., and Kang, S. G.: Formate-driven growth coupled with H2 production,
485 Nature, 467, 352-U137, 2010.
486 Kotsyurbenko, O. R., Nozhevnikova, A. N., Soloviova, T. I., and Zavarzin, G. A.:
487 Methanogenesis at low temperatures by microflora of tundra wetland soil, Ant.
488 Leeuwenhoek, 69, 75-86, 1996.
489 Küsel, K. and Drake, H. L.: Microbial turnover of low molecular weight organic
490 acids during leaf litter decomposition, Soil Biol. Biochem., 31, 107-118, 1999.
491 Lemaire, O. N., Jespersen, M., and Wagner, T.: CO2-fixation strategies in energy
492 extremophiles: What can we learn from acetogens?, Frontiers Microbiol., 11,
493 2020.
494 Liebner, S., Schwarzenbach, S. P., and Zeyer, J.: Methane emissions from an
495 alpine fen in central Switzerland, Biogeochem., 109, 287-299, 2012.
496 Liu, P. F., Klose, M., and Conrad, R.: Temperature effects on structure and
497 function of the methanogenic microbial communities in two paddy soils and
498 one desert soil, Soil Biol. Biochem., 124, 236-244, 2018.
499 Lovley, D. R. and Klug, M. J.: Intermediary metabolism of organic matter in the
500 sediments of a eutrophic lake, Appl. Environ. Microbiol., 43, 552-560, 1982.
501 Mariotti, A., Germon, J. C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A., and
502 Tardieux, P.: Experimental determination of nitrogen kinetic isotope
503 fractionation: some principles; illustration for the denitrification and
504 nitrification processes, Plant and Soil, 62, 413-430, 1981.

505 Martins, M., Mourato, C., and Pereira, I. A.: *Desulfovibrio vulgaris* growth
506 coupled to formate-driven H2 production, Environ. Sci. Technol., 49, 14655-
507 14662, 2015.
508 Montag, D. and Schink, B.: Formate and hydrogen as electron shuttles in terminal
509 fermentations in an oligotrophic freshwater lake sediment, Appl. Environ.
510 Microbiol., 84, e01572-18-https://doi.org/10.1128/AEM .01572-18, 2018.
511 Nozhevnikova, A. N., Kotsyurbenko, O. R. and Simankova, M. V.: Acetogenesis
512 at low temperature, in: Acetogenesis, edited by: Drake, H. L., Chapman &
513 Hall, New York, 416-431, 1994.
514 Penning, H., Claus, P., Casper, P., and Conrad, R.: Carbon isotope fractionation
515 during acetoclastic methanogenesis by *Methanosaeta concilii* in culture and a
516 lake sediment, Appl. Environ. Microbiol., 72, 5648-5652, 2006.
517 Peters, V., Janssen, P. H., and Conrad, R.: Efficiency of hydrogen utilization
518 during unitrophic and mixotrophic growth of Acetobacterium woodii on
519 hydrogen and lactate in the chemostat, FEMS Microbiol. Ecol., 26, 317-324,
520 1998.
521 Peters, V., Janssen, P. H., and Conrad, R.: Transient production of formate during
522 chemolithotrophic growth of anaerobic microorganisms on hydrogen, Curr.
523 Microbiol., 38, 285-289, 1999.
524 Phelps, T. J. and Zeikus, J. G.: Effect of fall turnover on terminal carbon
525 metabolism in Lake Mendota sediments, Appl. Environ. Microbiol., 50, 1285-
526 1291, 1985.
527 Rothfuss, F. and Conrad, R.: Vertical profiles of $CH_4$ concentrations, dissolved
528 substrates and processes involved in $CH_4$ production in a flooded Italian rice
529 field, Biogeochem., 18, 137-152, 1993.
530 Schink, B., Montag, D., Keller, A., and Müller, N.: Hydrogen or formate:
531 Alternative key players in methanogenic degradation [review], Environ.
532 Microbiol. Reports, 9, 189-202, 2017.
533 Schuchmann, K., Chowdhury, N. P., and Müller, V.: Complex multimeric [FeFe]
534 hydrogenases: biochemistry, physiology and new opportunities for the
535 hydrogen economy [review], Frontiers Microbiol., 9, 2911-doi:
536 10.3389/fmicb.2018.02911, 2018.
537 Schuchmann, K. and Müller, V.: Direct and reversible hydrogenation of CO2 to
538 formate by a bacterial carbon dioxide reductase, Science, 342, 1382-1385,
539 2013.
540 Sieber, J. R., Le, H. M., and McInerney, M. J.: The importance of hydrogen and
541 formate transfer for syntrophic fatty, aromatic and alicyclic metabolism,
542 Environ. Microbiol., 16, 177-188, 2014.
543 Stumm, W. and Morgan, J. J.: Aquatic Chemistry, 3. ed. Wiley, New York,1996.
544 Thauer, R. K., Jungermann, K., and Decker, K.: Energy conservation in
545 chemotrophic anaerobic bacteria, Bacteriol. Rev., 41, 100-180, 1977.
546 Widdel, F.: Microbiology and ecology of sulfate- and sulfur-reducing bacteria, in:
547 Biology of Anaerobic Microorganisms, edited by: Zehnder, A. J. B., Wiley,
548 New York, 469-585, 1988.
549 Wüst, P. K., Horn, M. A., and Drake, H. L.: Trophic links between fermenters
550 and methanogens in a moderately acidic fen soil, Environ. Microbiol., 11,
551 1395-1409, 2009.
552 Zinder, S. H.: Physiological ecology of methanogens, in: Methanogenesis.
553 Ecology, Physiology, Biochemistry and Genetics, edited by: Ferry, J. G.,
554 Chapman & Hall, New York, 128-206, 1993.

555

556

557 **Figure legends**

558

559 **Figure 1.** Formate conversion to acetate, $CH_4$ and $CO_2$ in suspensions of paddy soil from
560 Vercelli (Italy) after addition of formate without sulfate (blue squares) or formate plus sulfate
561 (gypsum) (red triangles) without $CH_3F$ (open symbols) or with $CH_3F$ (closed symbols).
562 Controls with addition of only water (blue or red X crosses) are only shown occasionally. The
563 panels show the temporal change of (a) concentrations of formate, (b) concentrations of
564 acetate, (c) mixing ratios of $CH_4$ (1 ppmv = $10^{-6}$ bar), (d) mixing ratios of $CO_2$, (e) $\delta^{13}C$ of
565 formate, (f) $\delta^{13}C$ of acetate, (g) $\delta^{13}C$ of $CH_4$, and (h) $\delta^{13}C$ of $CO_2$. Means ± SE.

566 **Figure 2.** Balance of produced acetate plus $CH_4$ (blue symbols) and of only $CH_4$ (red
567 symbols) against the consumed formate in (a) the absence and (b) the presence of sulfate in
568 paddy soil from Vercelli (Italy). Acetate and $CH_4$ are each equivalent to 4 $H_2$, formate to 1
569 $H_2$. The open and closed symbols denote conditions in the absence and the presence of $CH_3F$,
570 respectively. The different symbols indicate three different replicates. The line indicate
571 equimolarity (in terms of reducing equivalents between substrate and product.

572 **Figure 3.** Formate conversion to acetate, $CH_4$ and $CO_2$ in suspensions of sediment from the
573 NE basin of Lake Fuchskuhle after addition of formate without sulfate (blue squares) or
574 formate plus sulfate (gypsum) (red triangles) without $CH_3F$ (open symbols) or with $CH_3F$
575 (closed symbols). Controls with addition of only water (blue or red X crosses) are only shown
576 occasionally. The panels show the temporal change of (a) concentrations of formate, (b)
577 concentrations of acetate, (c) mixing ratios of $CH_4$ (1 ppmv = $10^{-6}$ bar), (d) mixing ratios of
578 $CO_2$, (e) $\delta^{13}C$ of formate, (f) $\delta^{13}C$ of acetate, (g) $\delta^{13}C$ of $CH_4$, and (h) $\delta^{13}C$ of $CO_2$. Means ±
579 SE.

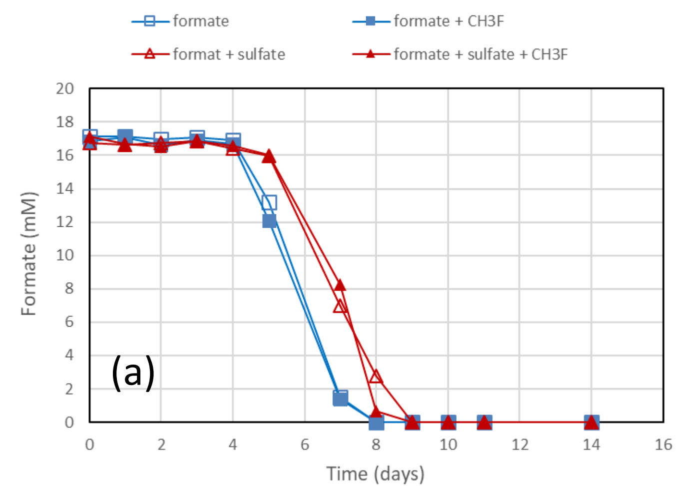
580 **Figure 4.** Balance of produced acetate plus $CH_4$ (blue symbols) and of only $CH_4$ (red
581 symbols) against the consumed formate in (a) the absence and (b) the presence of sulfate in
582 sediment from the NE basin of Lake Fuchskuhle. Acetate and $CH_4$ are each equivalent to 4
583 $H_2$, formate to 1 $H_2$. The open and closed symbols denote conditions in the absence and the
584 presence of $CH_3F$, respectively. The different symbols indicate three different replicates. The
585 line indicate equimolarity (in terms of reducing equivalents between substrate and product.

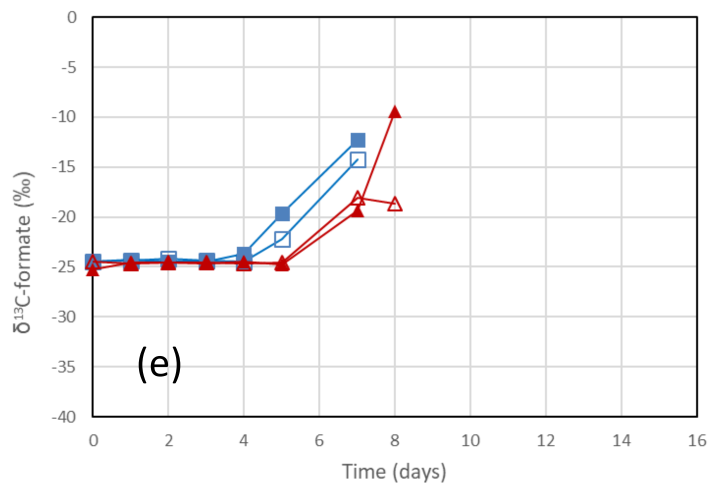
586 **Figure 5.** Mariotti plots of formate consumption in (a, b) paddy soil from Vercelli and (c, d)
587 sediment from the NE basin of Lake Fuchskuhle under methanogenic (a, c, blue symbols) and
588 sulfidogenic (b, d, red symbols) conditions both in the absence (open symbols) and in the
589 presence (closed symbols) of $CH_3F$. Three different replicates.

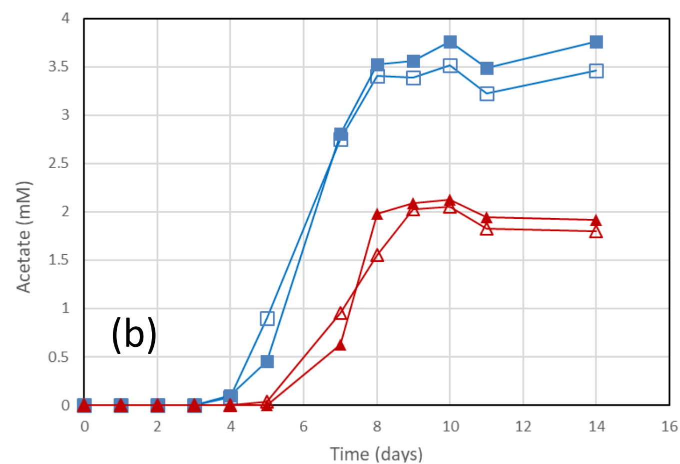
590 **Figure 6.** Isotopic enrichment factors ($\varepsilon_{form}$, given as negative values) in paddy soils from
591 Vercelli and the IRRI (the Philippines) and in lake sediments from the NE and SW basins of

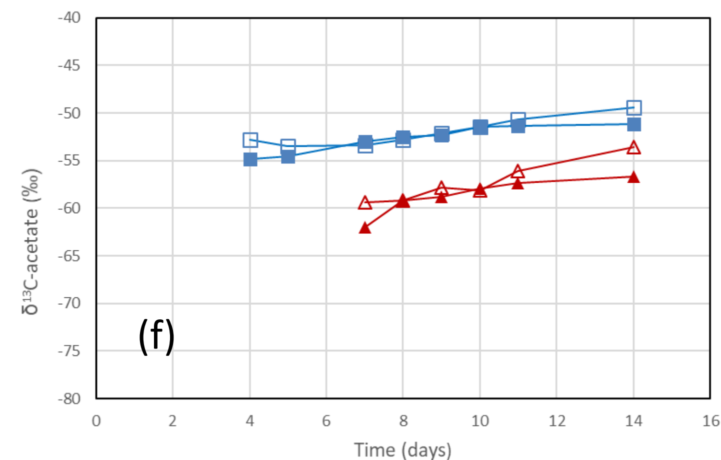
592 Lake Fuchskuhle without (left panel) and with (right panel) addition of sulfate (gypsum) and
593 $CH_3F$. Means ± SE.

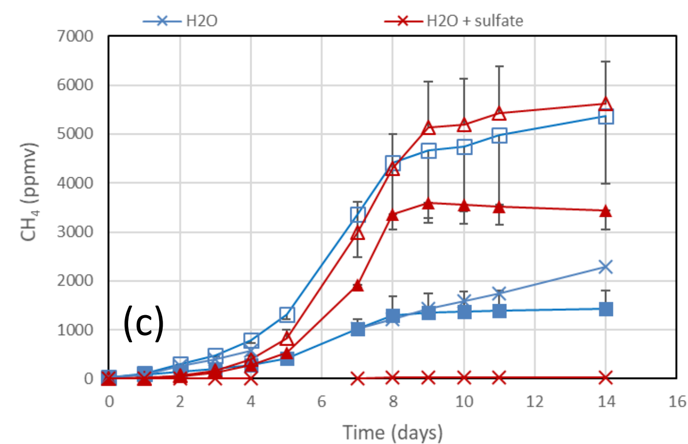
594 **Figure 7.** Average $\delta^{13}C$ of formate (at the beginning of incubation) and of $CO_2$, acetate and
595 $CH_4$ (after the depletion of formate) in paddy soils from Vercelli (blue) and the IRRI (green),
596 and in sediments from the NE basin (red) and the SW basin (yellow) of Lake Fuchskuhle in
597 the absence (filled bars) and the presence (dotted bars) of $CH_3F$. Means ± SE.
598

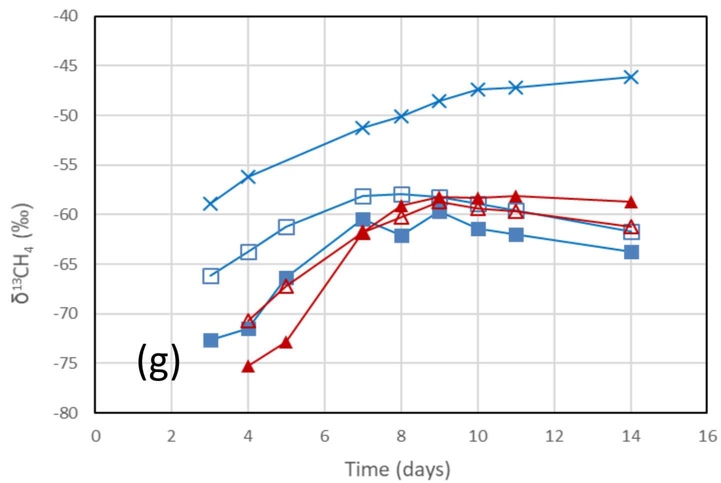


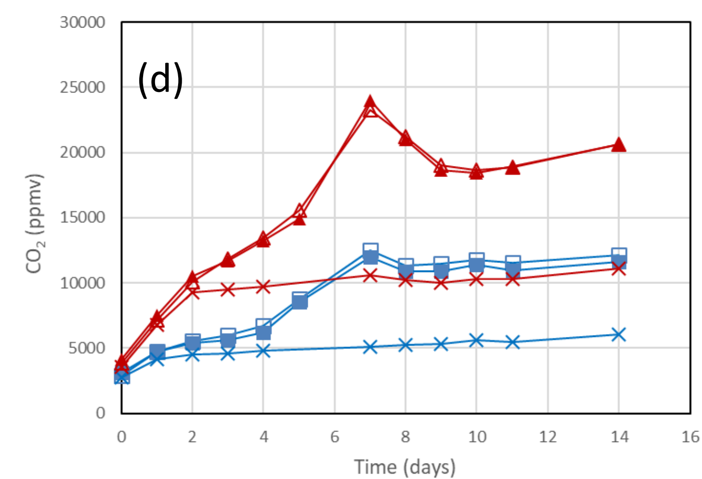



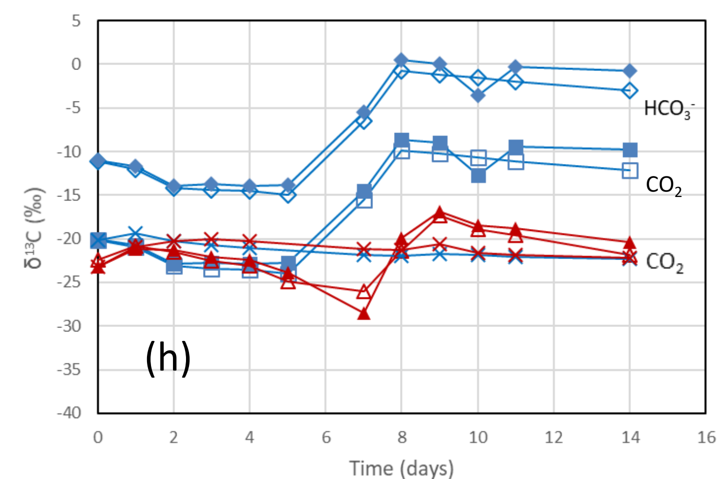




Fig. 1

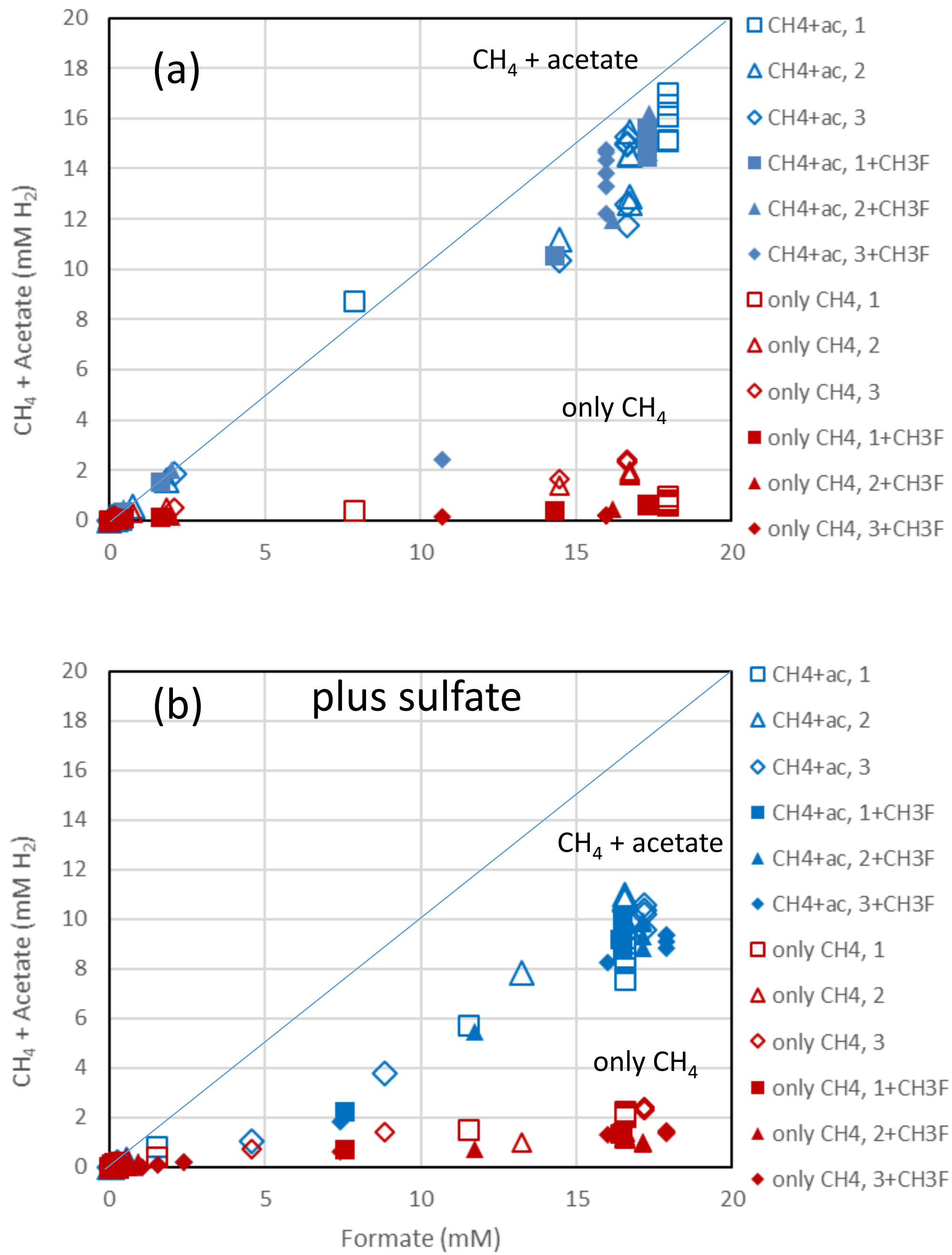



Fig. 2

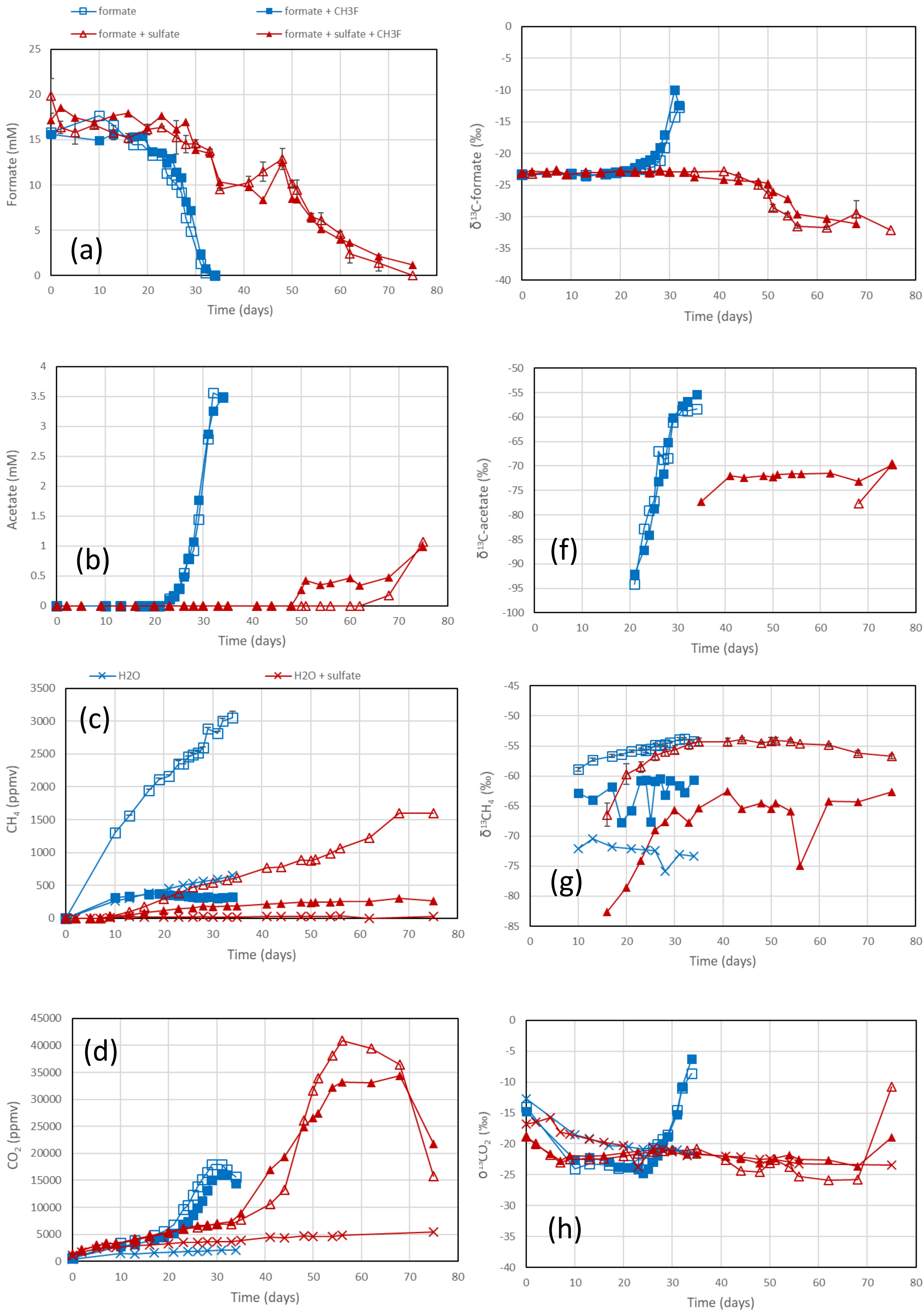


Fig. 3

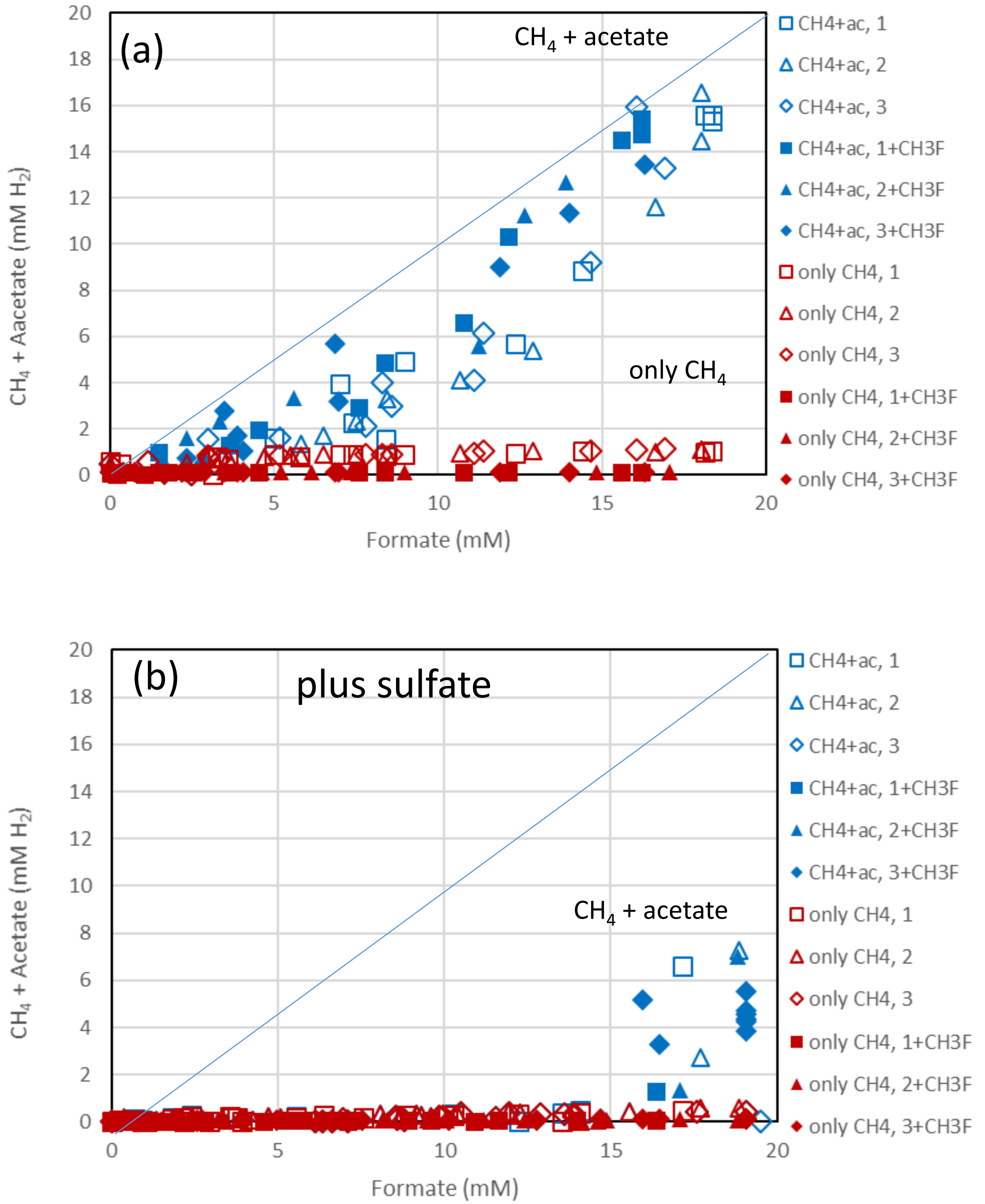
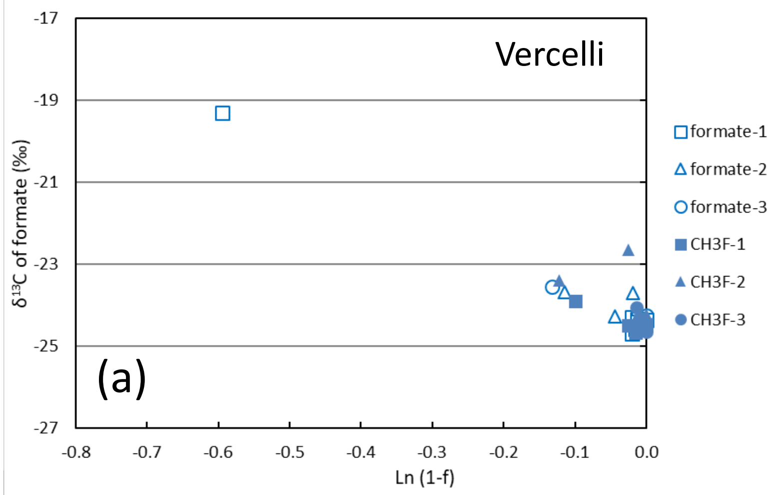
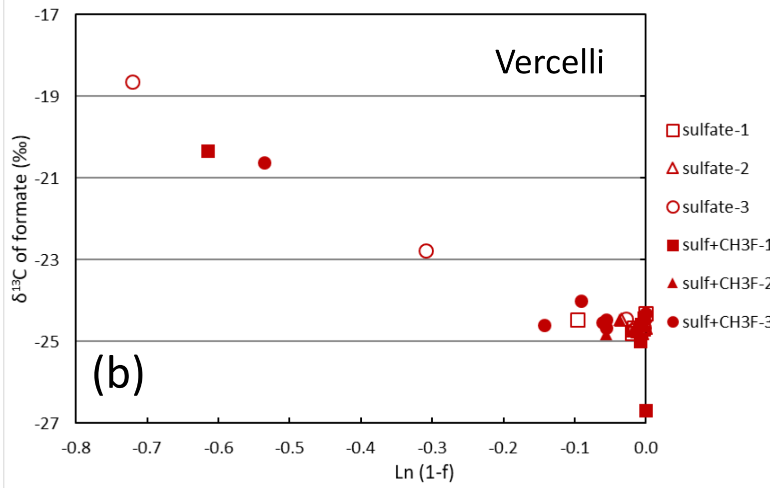
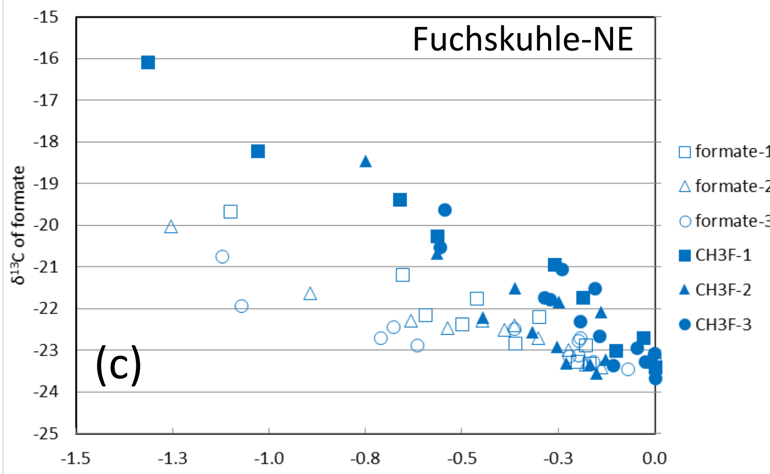



Fig. 4



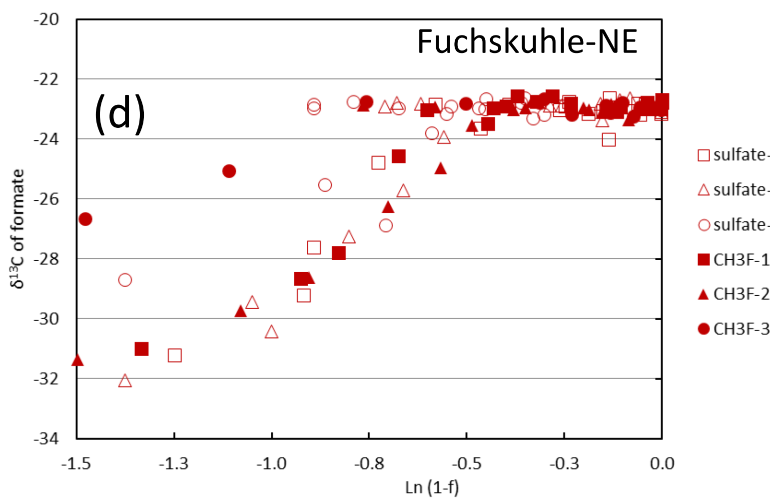




Fig. 5

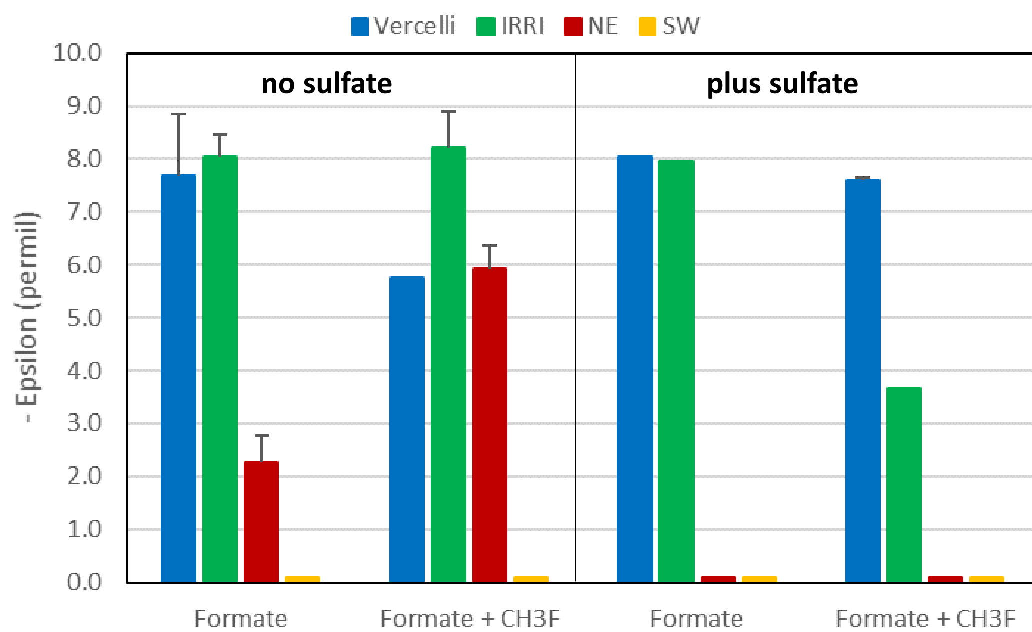



Fig. 6

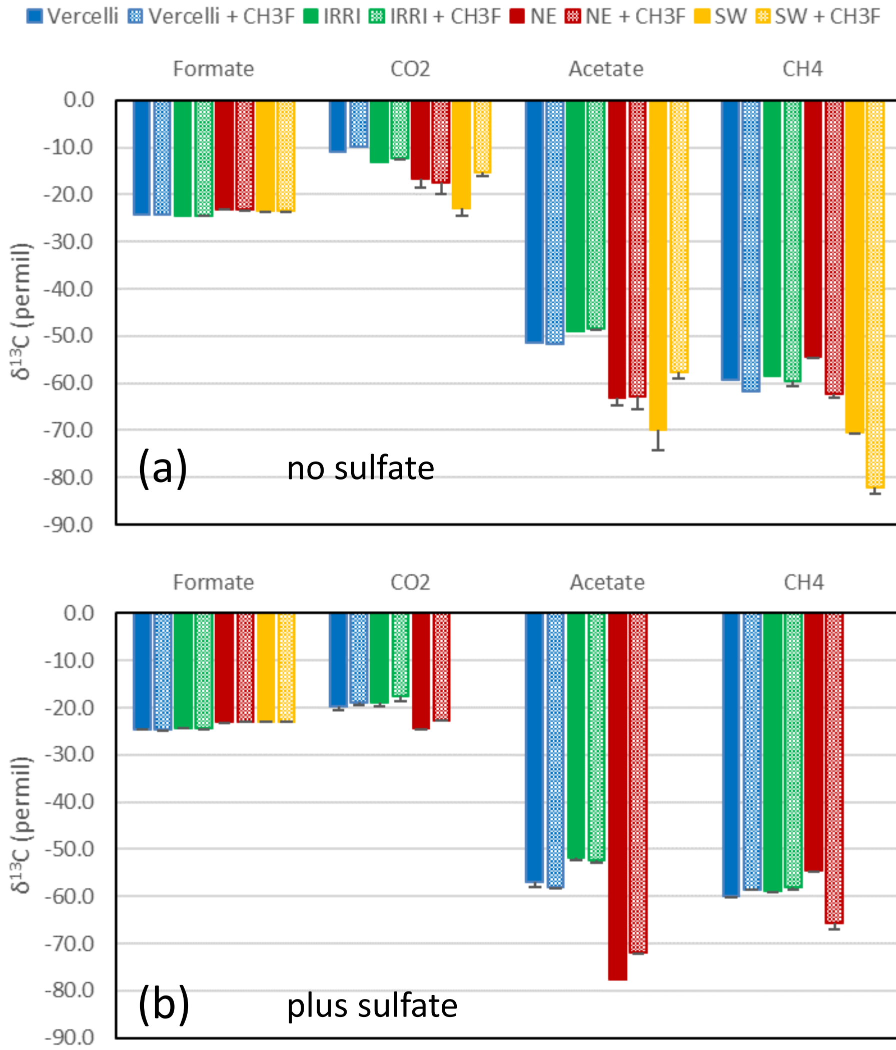



Fig. 7