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Acetate turnover and methanogenic pathways in Amazonian lake sediments

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Abstract

Lake sediments in Amazonia are a significant source of CH_4, a potential greenhouse gas. Previous studies of sediments using ^{13}C analysis found that the contribution of hydrogenotrophic versus aceticlastic methanogenesis to CH_4 production was relatively high. Here, we determined the methanogenic pathway in the same sediments (n = 6) by applying [^{14}C]bicarbonate or [2-^{14}C]acetate, and confirmed the high relative contribution (50-80%) of hydrogenotrophic methanogenesis. The respiratory index (RI) of [2-^{14}C]acetate, which is $^{14}CO_2$ relative to $^{14}CH_4 + {}^{14}CO_2$, divided the sediments into two categories, i.e., those with an RI < 0.2 being consistent with the operation of aceticlastic methanogenesis, and those with an RI > 0.4 showing that a large percentage of the acetate-methyl was oxidized to CO_2 rather than reduced to CH_4. Hence, part of the acetate was probably converted to CO_2 plus H_2 via syntrophic oxidation, thus enhancing hydrogenotrophic methanogenesis. This happened despite the presence of potentially aceticlastic *Methanosaetaceae* in all the sediments. Alternatively, acetate may have been oxidized with a constituent of the sediment organic matter (humic acid) serving as oxidant. Indeed, apparent acetate turnover rates were larger than CH_4 production rates except in those sediments with a R < 0.2. Our study demonstrates that CH_4 production in Amazonian lake sediments was not simply caused by a combination of hydrogenotrophic and aceticlastic methanogenesis, but probably involved additional acetate turnover.

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1. Introduction

Acetate is an important intermediate in the anoxic degradation of organic matter and is produced by fermentation processes and chemolithotrophic homoacetogenesis (Conrad, 2019; Conrad et al., 2014; Ye et al., 2014). The contribution of these two processes to acetate production is difficult to determine, but seems to be quite different for different environments (Fu et al., 2018; Hädrich et al., 2012; Heuer et al., 2010). The degradation of acetate requires a suitable oxidant such as oxygen, nitrate, ferric iron or sulfate. If such oxidants are not or no longer available, such as in many freshwater environments (e.g., paddy fields, lake sediments, peat) acetate sometimes accumulates until suitable electron acceptors become again available. Temporal accumulation and subsequent oxidative consumption has for example been observed in peatlands during increase and decrease, respectively, of the water table (Duddleston et al., 2002). Normally, however, acetate degradation in the absence of inorganic electron acceptors is accomplished by aceticlastic methanogenesis (Conrad, 2019). If aceticlastic methanogenesis is operative, the methyl group of the acetate is converted to CH_4.

If methanogenesis is the exclusive final step in the anaerobic degradation of organic matter, polysaccharides (one of the most important compounds from primary production) will be dismutated to equal amounts of CH_4 and CO_2. Furthermore, acetate usually accounts for more than two third of total methane production, especially if polysaccharides are the predominant degradable organic matter (Conrad, 2019). However, CO_2 has often been found to be the main product in many anoxic environments despite the absence of inorganic electron acceptors (O_2, nitrate, ferric iron, sulfate) (Keller et al., 2009; Yavitt and Seidmann-Zager, 2006). Such results have been explained by the assumption that organic substances (e.g. humic acids) may also serve as electron acceptors (Gao et al., 2019; Keller et al., 2009; Klüpfel et al., 2014). Organic electron acceptors also allow the oxidation of acetate (Coates et al., 1998; Lovley et al., 1996). The role of organic electron acceptors during anaerobic degradation of organic matter is potentially important, but still not well known (Corbett et al., 2013)

There are also many reports that methane production in lake sediments is dominated by hydrogenotrophic rather than aceticlastic methanogenesis (Conrad et al., 2011; Ji et al., 2016).

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Such observations were explained (1) by incomplete degradation of organic matter producing predominantly H_2 and CO_2 without concomitant acetate production (Conrad et al., 2010; Hodgkins et al., 2014; Liu et al., 2017), (2) by acetate oxidation coupled to the reduction of organic substances (see above), or (3) by syntrophic acetate oxidation coupled with hydrogenotrophic methanogenesis (Lee and Zinder, 1988; Vavilin et al., 2017). If syntrophic acetate oxidation is operative, the methyl group of the acetate is converted to CO_2, similarly as found during acetate oxidation with external inorganic or organic electron acceptors. However, syntrophic acetate oxidation does not require a chemical compound (other than H^+) as electron acceptors, since it is the hydrogenotrophic methanogenesis that eventually accepts the electrons released during acetate oxidation.

Syntrophic acetate oxidation can replace aceticlastic methanogenesis and thus, has been found when aceticlastic methanogenic archaea were not present in the microbial community of lake sediment (Nüsslein et al., 2001). This may also happen in other anoxic environments when conditions are not suitable for aceticlastic methanogens, e.g., at elevated temperatures (Conrad et al., 2009; Liu and Conrad, 2010; Liu et al., 2018), in the presence of high concentrations of ammonium (Müller et al., 2016; Schnürer et al., 1999; Zhang et al., 2014), or phosphate (Conrad et al., 2000). However, syntrophic acetate oxidation has also been found in lake sediments that contained populations of putatively aceticlastic methanogens (Vavilin et al., 2017). It is presently unkown under which conditions syntrophic acetate oxidizers can successfully compete with aceticlastic methanogens and co-occur with acetate oxidation that is coupled to the reduction of organic substances.

As a further step in understanding the ecology of syntrophic acetate oxidizers versus aceticlastic methanogens, we attempted to document their coexistence by studying lake sediments, which had been reported containing 16S rRNA genes of putatively aceticlastic *Methanosaetaceae* (*Methanotrichaceae* (Oren, 2014)) (Ji et al., 2016). We used these sediments and measured the fractions of hydrogenotrophic methanogenesis and of the methyl group of acetate being oxidized to CO_2 rather than reduced to CH_4, and compared the turnover of acetate to the production rate of CH_4.

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2. Materials and Methods

The sediment samples were obtained from floodplain lakes in the Amazon region and have already been used for a study on structure and function of methanogenic microbial communities (Ji et al., 2016). In particular, these sediment have been assayed for the percentage of hydrogenotrophic methanogenesis using values of $\delta^{13}C$ of CH_4, CO_2 and acetate-methyl, and for the percentage contribution of putatively aceticlastic methanogens to the total archaeal community (Ji et al., 2016). Here, we used six of these sediments for incubation experiments with radioactive tracers. These sediments are identical to those listed in our previous publication (Ji et al., 2016). The identity of the lake sediments and the percentage content of putatively aceticlastic methanogens is summarized in Table 1.

The experiments were carried out at the same time as those in our previous publication and were basically using the same incubation techniques. Briefly, for determination of CH_4 production rates and the fractions of hydrogenotrophic methanogenesis about 10-15 ml of each replicate (n =3) were filled into 27-ml sterile tubes, flushed with N_2, closed with butyl rubber stoppers, and incubated at 25ºC. After preincubation for 12 days (in order to deplete eventually present inorganic oxidants), 0.5 ml of a solution of $NaH^{14}CO_3$ (about 1 µCi) was added, the tubes flushed again with N_2, and incubation was continued at 25ºC for about 100 days. Partial pressures of CH_4 and CO_2 as well as their contents of ^{14}C were measured at different time points after mixing the slurries by heavy manual shaking. The gas partial pressures were measured by gas chromatography with a flame ionization detector (Ji et al., 2016), the radioactivities were analyzed with a radiodetector (RAGA) (Conrad et al., 1989). For determination of acetate turnover, the same conditions were used, except that preincubation was for 25 days, 0.5 ml of a solution of carrier-free Na[2-^{14}C]acetate (about 2 µCi) was added, and incubation was continued for about 8 h. During this time gas samples were repeatedly taken and the radioactivities in CH_4 and CO_2 were analyzed in a gas chromatograph with a radiodetector (RAGA) (Conrad et al., 1989). In the end, the sediment samples were acidified with 1 ml of 1M H_2SO_4 to liberate CO_2 from carbonates, and the radioactivities in CH_4 and CO_2 were analyzed again.

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The data were used to calculate the fractions of hydrogenotrophic methanogenesis (f_{H2}), the acetate turnover rate constants (k_{ac}) and the respiratory index (RI) values as described by Schütz et al. (1989). The RI is defined as RI = $^{14}CO_2/(^{14}CO_2 + ^{14}CH_4)$. The acetate turnover rates were calculated as the product of k_{ac} time the acetate concentration, which was analyzed in the sediments at the end of the incubation using high pressure liquid chromatography. The acetate concentrations are summarized in Table 1.

3. Results

Six different lake sediments from Amazonia were incubated in the absence and the presence of $H^{14}CO_3$. Although the rates of CH_4 production were different in the two different incubations, the orders of magnitude were similar for the different lake sediments (Fig. 1A). The incubations in the presence of $H^{14}CO_3$ were used to follow the specific radioactivities of CH_4 (Fig. 2A) and CO_2 (Fig. 2B) over the incubation time. The specific radioactivities of CH_4 changed only little but were slightly different for the different lake sediments. The specific radioactivities of CO_2 decreased with time as expected due to the production of non-radioactive CO_2. Both specific radioactivities were used to calculate the fractions of hydrogenotrophic methanogenesis (f_{H2}), which increased with incubation time and eventually reached a plateau. The values of f_{H2} averaged between 30 and 60 d of incubation are summarized in Fig. 1B. Only the incubations of sediment “Grande” did not reach a plateau but still increased after 260 d of incubation due to the continuously decreasing specific radioactivities of CO_2 (data not shown). Averaging these values over the 4 data points between 160 and 260 d resulted in f_{H2} of about 60% (Fig. 1B). The thus determined values of f_{H2} were comparable to those determined in the absence of $H^{14}CO_3$ using values of $\delta^{13}C$, which have already been published (Ji et al. 2016) (Fig.1B).

The same sediments were used to determine the turnover of [2-^{14}C]acetate by measuring the increase of radioactive CH_4 (Fig. 3A) and CO_2 (Fig. 3B). These data were used to determine the rate constants of acetate turnover (Fig. 3C), which ranged between 0.02 and 1.7 h^{-1}. The respiratory indices (RI) were generally larger than 0.2 except those of the sediments Tapari and Verde, which were smaller than 0.2 (Fig. 4A). The RI values and the acetate

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turnover rate constants were used to calculate the rates of CH_4 production from acetate in comparison to the rates of total CH_4 production (Fig. 4B). Interestingly, acetate-dependent CH_4 production was always larger than total CH_4 production, except in those sediments exhibiting a RI <0.2.

4. Discussion

The RI value quantifies the fraction of the methyl group of acetate that is oxidized to CO_2 rather than reduced to CH_4. Since some oxidation of acetate methyl is also happening in pure cultures of aceticlastic methanogens (Weimer and Zeikus, 1978), and since a RI of around 0.2 has often been found in environments where acetate turnover was dominated by aceticlastic methanogenesis (Phelps and Zeikus, 1984; Rothfuss and Conrad, 1993; Winfrey and Zeikus, 1979), an RI value of 0.2 may in practice be used as the threshold for the change of methanogenic to oxidative acetate turnover. Based on this criterion, i.e. RI < 0.2, the lake sediments of Tapari and Verde behaved as when acetate turnover was exclusively caused by aceticlastic methanogenesis. The percentage of acetate-dependent CH_4 production was fairly consistent with the fraction of hydrogenotrophic methanogenesis, which made up the remainder of total CH_4 production. In conclusion, the acetate turnover and CH_4 production in these lake sediments behaved as expected as when aceticlastic methanogenesis was the sole process of acetate consumption (Fig. 5).

However, the sediments of Jua and in particular those of Jupinda, Cataldo, and Grande exhibited RI values >0.2, showing that a substantial fraction of the acetate-methyl was oxidized to CO_2. Hence, acetate was not exclusively consumed by aceticlastic methanogenesis, but it was oxidized, for example by syntrophic acetate oxidation producing H_2 and CO_2. The H_2 and CO_2 may subsequently have been used as methanogenic substrates, thus supporting CH_4 production (Fig. 5). Such support would be consistent with the relatively high fractions (f_{H2}) of hydrogenotrophic methanogenesis observed in these sediments. However, it would not explain why acetate turnover rates were higher than necessary for supporting the observed rates of total CH_4 production. A possible conclusion is that acetate was converted to CO_2 without concomitant production of H_2. Possibly, electrons from acetate

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were transferred to organic electron acceptors (Fig. 5), such as suggested in the literature (Coates et al., 1998; Lovley et al., 1996). In conclusion, these lake sediments behaved as when acetate consumption was accomplished not only by aceticlastic methanogenesis, but also by oxidative consumption.

Our conclusions are mainly based on radiotracer measurements, which may be biased. For example, acetate turnover rate constants are calculated from acetate concentrations and turnover rate constants. Acetate concentrations were only measured at the end of incubation and thus, may not have been representative for much of the incubation time. Furthermore, acetate in the sediment may occur in several pools with different turnover (Christensen and Blackburn, 1982). Therefore, acetate turnover rates and acetate-dependent CH_4 production rates may be overestimated, if the actual acetate turnover depends on a pool size that is smaller than that analyzed. Such overestimation in sediments of Jua, Jupinda, Cataldo and Grande cannot be completely excluded, although rates in sediments of Tapari and Verde were in a realistic range.

The determination of fractions of hydrogenotrophic methanogenesis (f_{H2}) depends on the specific radioactivity of the dissolved CO_2 pool that is involved in CH_4 production. However, it is the pool of gaseous CO_2 that is analyzed in the assay, assuming that its specific radioactivity is identical to that of the active dissolved pool. Since non-radioactive CO_2 is permanently produced from oxidation of organic matter, there may be disequilibrium. Nevertheless, determinations of f_{H2} using radioactive bicarbonate exhibited the same tendencies as those based on $\delta^{13}C$ values, and thus are probably quite reliable.

Despite these reservations, our results collectively demonstrated that acetate turnover in tropical lake sediments did not necessarily follow a canonical pattern with aceticlastic methanogenesis as sole or predominant process of acetate turnover, despite the fact that all these sediments contained populations of putative aceticlastic methanogenic archaea. Acetate consumption in *Methanosaeta* species is known to have a relatively high affinity and a low threshold for acetate (Jetten et al., 1992). Therefore, the question arises why oxidative processes, including syntrophic acetate oxidation, could successfully compete with aceticlastic methanogenesis.

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5. Author contribution

RC designed the experiments, evaluated the data and wrote the manuscript; MK did the experiments; AEP provided the samples and contributed to the discussion of the data.

6. Competing interests

The authors declare that they have no conflict of interest.

7. Acknowledgements

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309 Table 1: Identity of sediment samples (same as those in Ji et al. (2016)),
310 percentage content of putatively aceticlastic methanogens (*Methanosaetaceae*)
311 relative to total archaea, and concentrations of acetate; mean ± SE.
312

Lake #	Name	Type	*Methanosaetaceae* (%)	Acetate (nmol g^{-1} dry weight)
P1	Jua	clear water	21 ± 1	93 ± 5
P8	Tapari	clear water	19 ± 3	261 ± 39
P9	Verde	clear water	19 ± 11	126 ± 12
P10	Jupinda	clear water	27 ± 4	110 ± 6
A1	Cataldo	white water	42 ± 1	50 ± 3
A2	Grande	white water	36 ± 3	35 ± 1

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315 **Figure captions**

316 Fig. 1: Methane production in sediments of different Amazonian lakes: (A) rates of CH_4
317 production, and (B) fractions of hydrogenotrophic methanogenesis, both determined in the
318 absence and the presence of radioactive bicarbonate. The data in the absence of radioactive
319 bicarbonate are the same as published in Ji et al. (2016), when f_{H2} was determined from
320 values of $\delta^{13}C$; mean ±SE.

321 Fig. 2: Conversion of radioactive bicarbonate in sediments of different Amazonian lakes: (A)
322 specific radioactivities in CH_4; (B) specific radioactivities in gaseous CO_2; and (C)
323 fractions (f_{H2}) of hydrogenotrophic methanogenesis; mean ±SE.

324 Fig. 3: Conversion of [2-^{14}C]acetate in sediments of different Amazonian lakes: (A)
325 accumulation of radioactive CH_4; (B) accumulation of radioactive gaseous CO_2; and (C)
326 acetate turnover rate constants; mean ±SE.

327 Fig. 4: (A) Rates of total and acetate-derived CH_4 production in sediments of different
328 Amazonian lakes and (B) respiratory indices (RI) of the turned over [2-^{14}C]acetate; mean
329 ±SE.

330 Fig. 5: Scheme of the pathways involved in acetate turnover in sediments of Amazonian
331 lakes; (1) aceticlastic methanogenesis; (2) syntrophic acetate oxidation; (3)
332 hydrogenotrophic methanogenesis; (4) acetate oxidation with organic electron acceptors.
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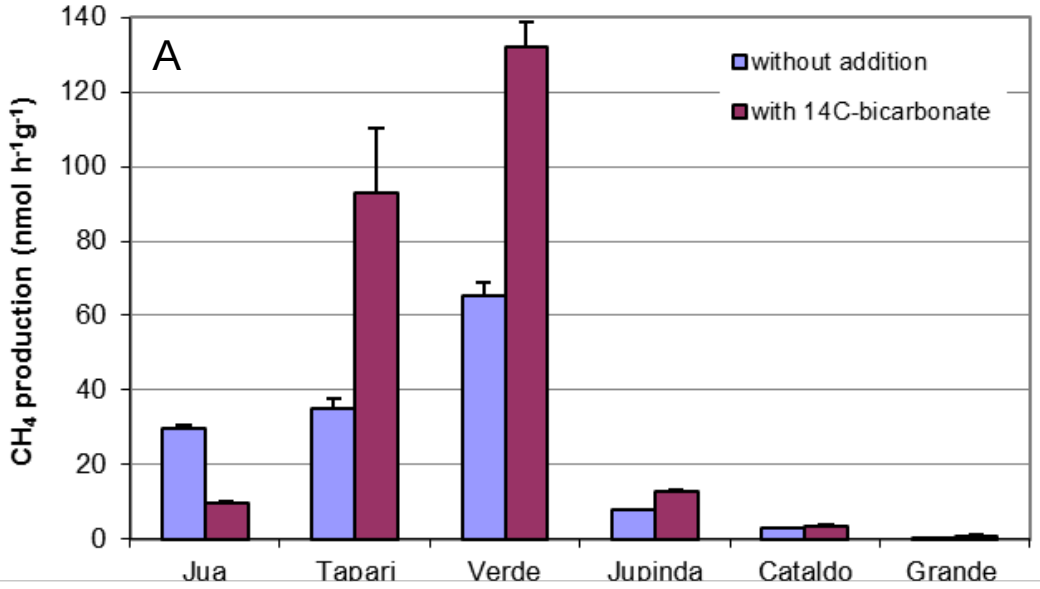

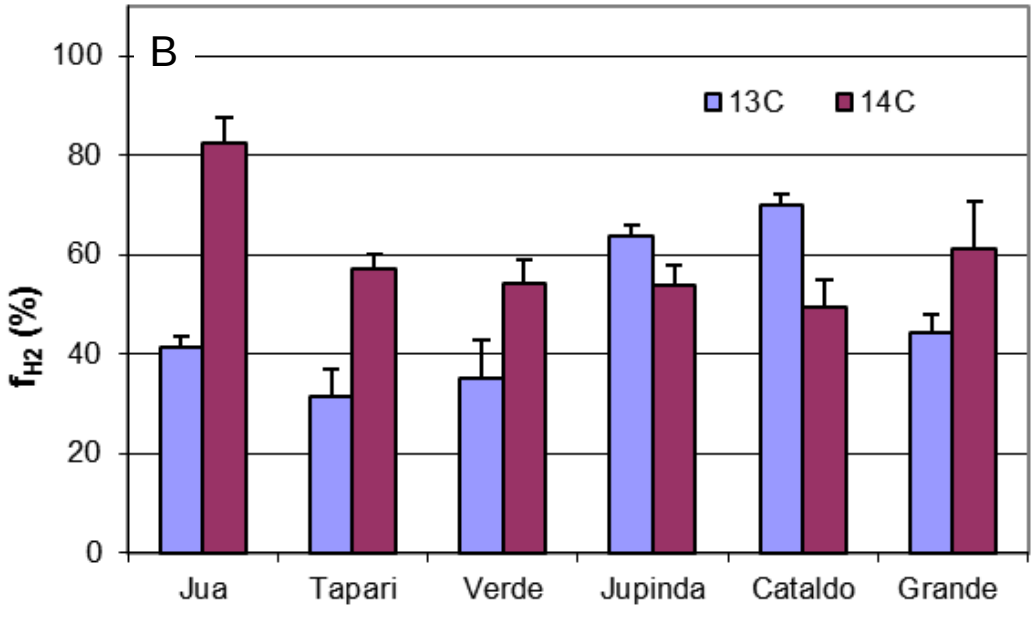

Fig. 1

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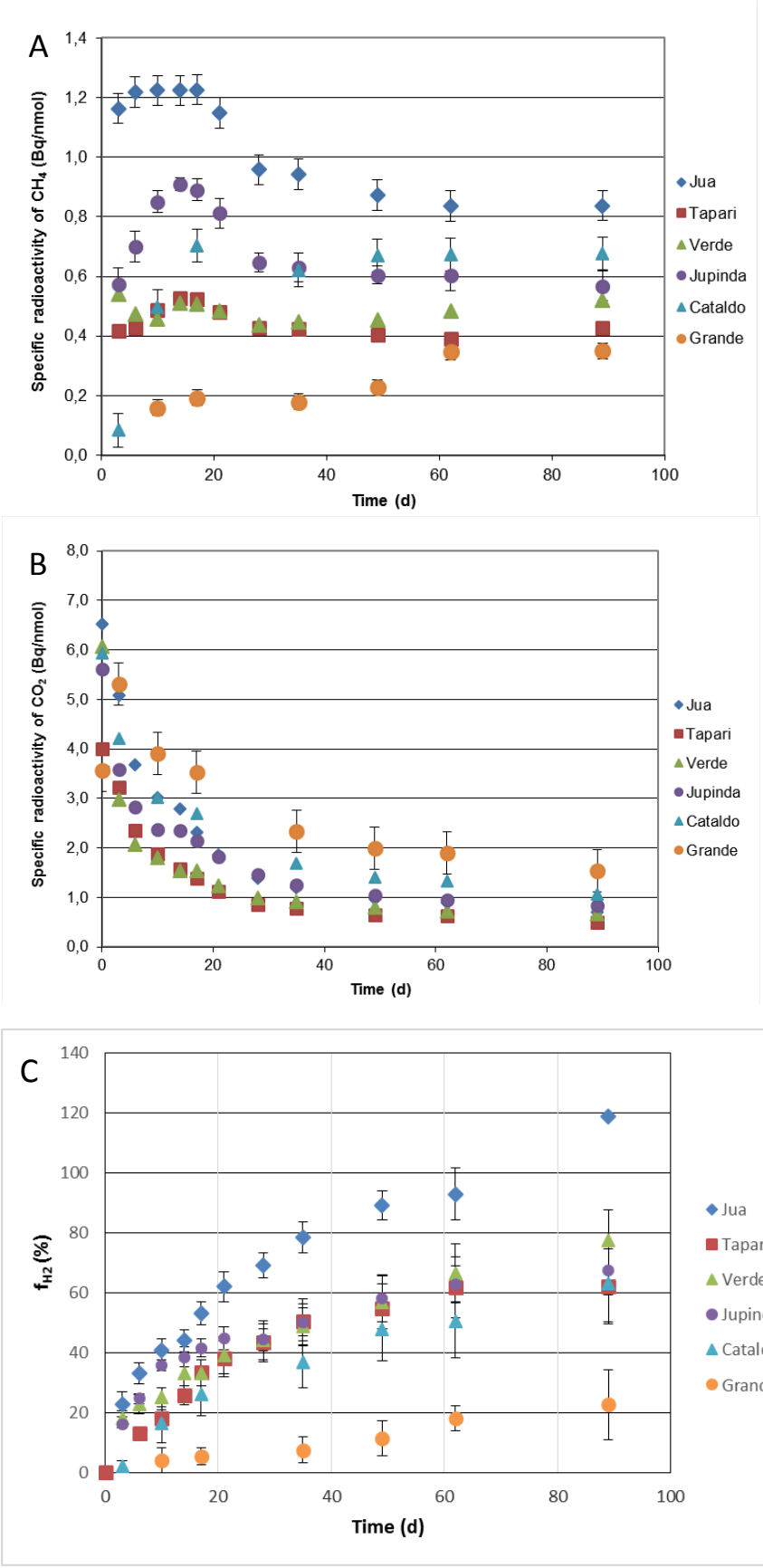

Fig. 2

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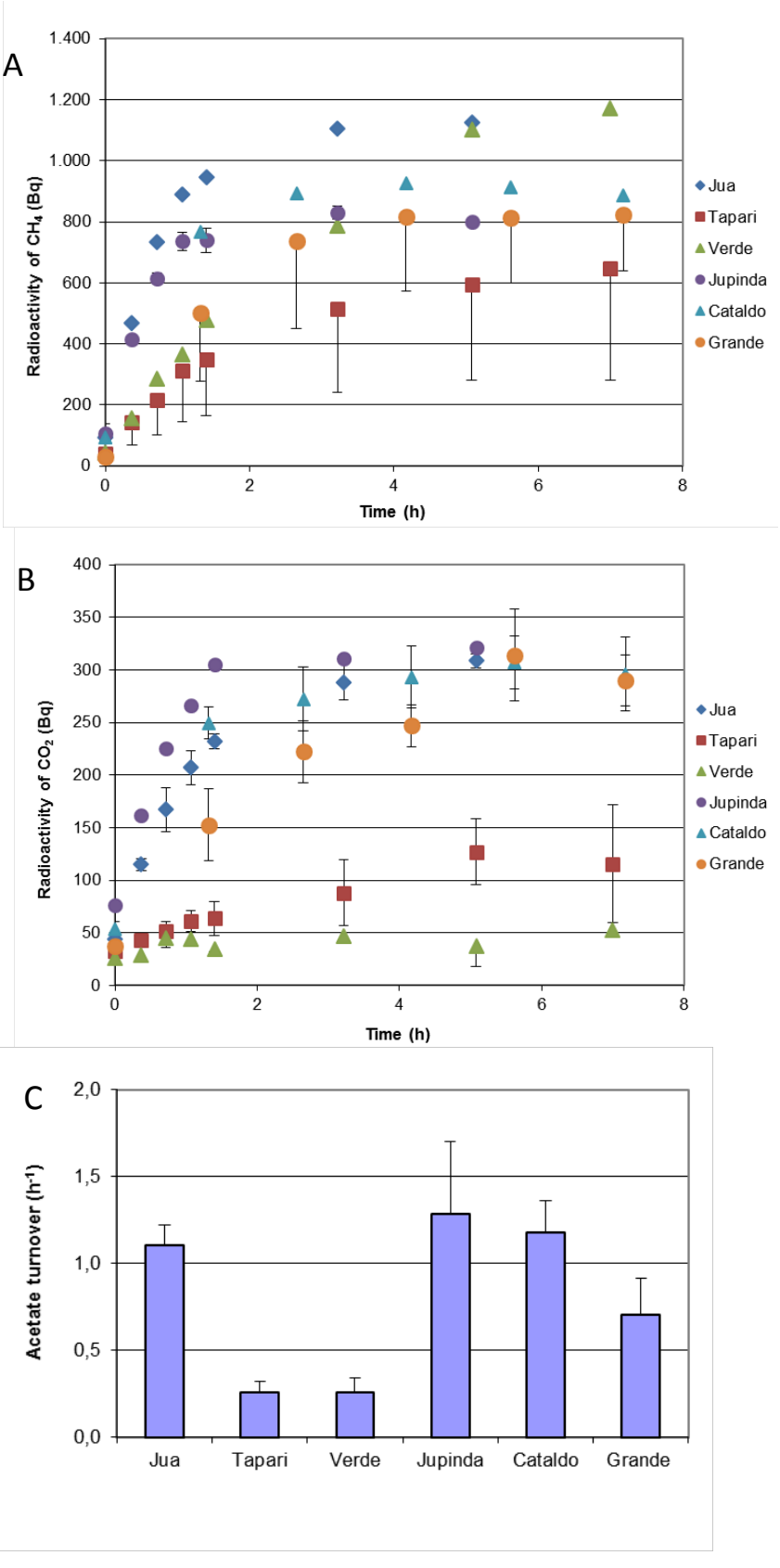

Fig. 3

https://doi.org/10.5194/bg-2019-411
Preprint. Discussion started: 23 October 2019

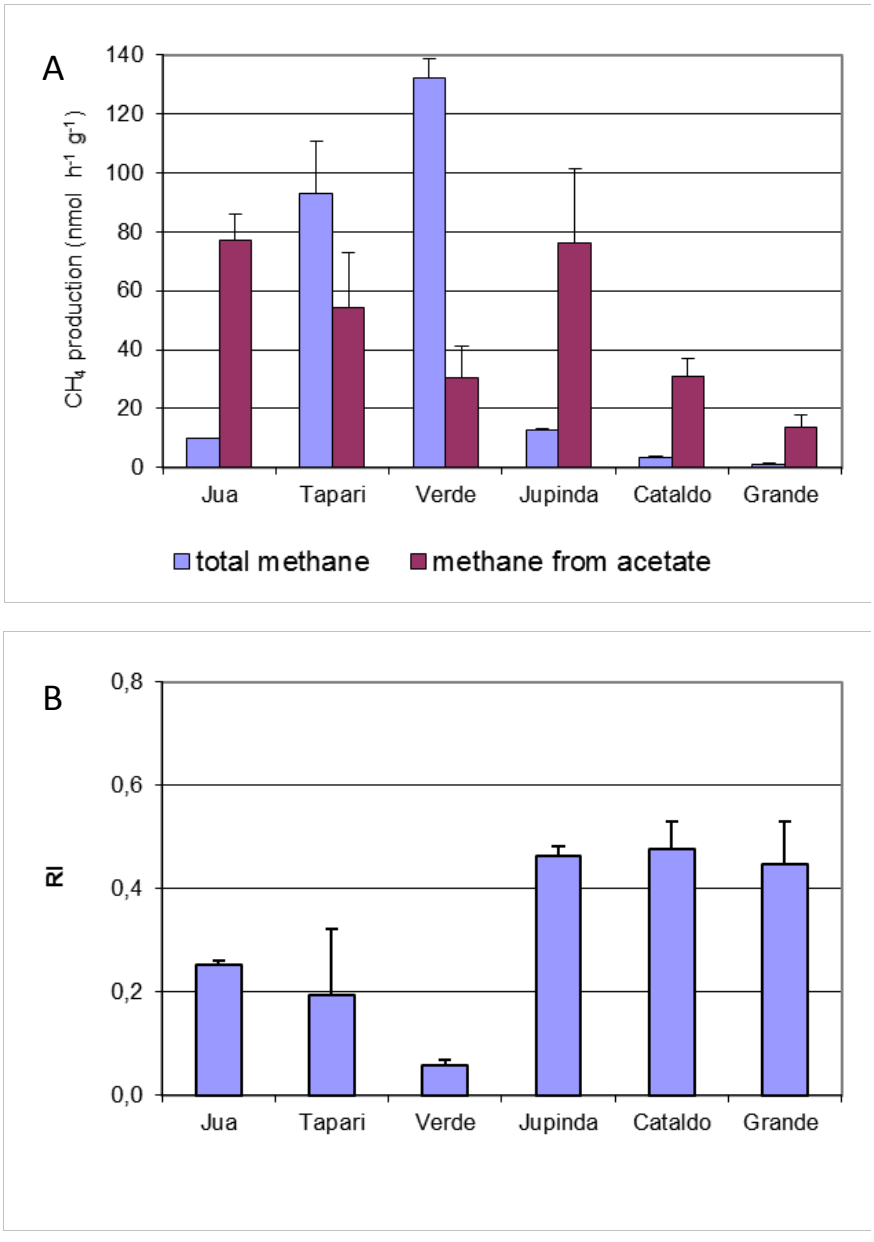

Fig. 4

https://doi.org/10.5194/bg-2019-411
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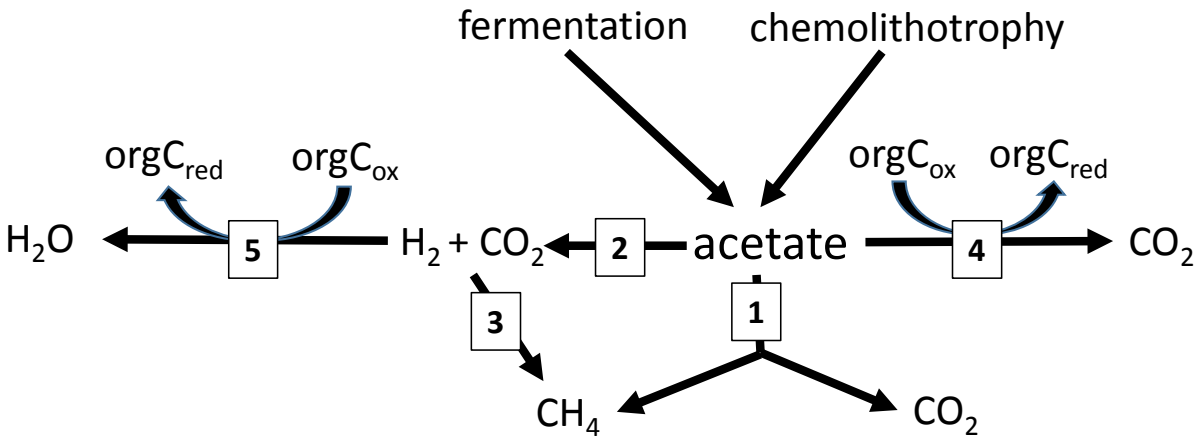

Fig. 5