Methane Emission Induced by Short-Chain Organic Acids in Lowland Soil

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ABSTRACT: Methane (CH₄) is the second major greenhouse gas after CO₂, exerting a significant influence on the climate and the chemistry of the atmosphere. In lowland soil, acetate and H₂/CO₂ are the most important precursors of CH₄ and formed from organic matter fermentation in an anaerobic environment, giving rise to short-chain organic acids (ethanoic, propanoic, and butanoic), depending on the type of crop residue and the soil management system. Ethanoic acid can be directly converted to CH₄ by methanogenic microorganisms, but propanoic and butanoic acids must be converted to acetate before being converted to CH₄. This study aimed to quantify, in isolation, the dynamics and CH₄ emission potential of the three short-chain organic acids found in flooded lowland soils with rice crops. The study was carried out in a controlled environment using four standard carbon doses (0, 90, 180, and 270 mg kg⁻¹) of ethanoic, propanoic, and butanoic acids. The dynamics and the potential emission of CH₄ from soil were investigated when the acids were applied to flooded soil previously incubated for 20 days. The CH₄ emission dynamics were altered with the application of the three short-chain organic acids to the soil, even using an equal amount of carbon. The faster and more intense emission was achieved with the ethanoic acid application in relation to the other two acids application, while butanoic acid presents slower, delayed, and prolonged dynamics of CH₄ emission. Propanoic acid resulted in the lowest CH₄ emission due to its own stoichiometry and the temperature condition in which the experiment was conducted, which were unfavorable to the hydrogenotrophic bacteria. The addition of short-chain organic acids promoted a priming effect in the soil with conversion values of C to CH₄ above the calculated theoretical values.

Keywords: ethanoic acid, butanoic acid, propanoic acid, methanogenesis, irrigated rice.
INTRODUCTION
Methane (CH₄) is the second major greenhouse gas after CO₂, exerting a significant influence on the climate and the chemistry of the atmosphere. The addition of one mole of CH₄ to the atmosphere is approximately 28 times more effective in the infrared radiation absorption than one mole of CO₂ (IPCC, 2014). A complex microbial community, involving hydrolytic, fermenting, acetogenic, syntrophic, and methanogenic microorganisms, is responsible for the organic matter degradation in flooded soils, from the transformation of composite molecules into simpler forms until the CH₄ production (Liu et al., 2018; Zabranska and Pokorna, 2018). Acetate and H₂/CO₂ are the most important precursors of CH₄ in soils. Theoretically, acetate and H₂/CO₂ contribute about 70 and 30 % to the emission of CH₄, respectively. Acetate is used by the group of methanogenic acetoclastic bacteria, which reduce acetate to CH₄ and CO₂, and hydrogenotrophic bacteria, which reduce CO₂ by using H₂ as an electron donor (Leoncio, 2016).

These CH₄ precursors are formed through the organic matter fermentation, where simpler compounds are fermented intracellularly by fermenting bacteria giving rise to acetate and short-chain organic acids, such as propanoic and butanoic acid, in addition to the production of H₂ and CO₂ in a process called acidogenesis (Costa and Leigh, 2014). Temperature, redox potential, pH, pressure exerted by H₂, and availability of organic substrates are identified as the main factors influencing CH₄ production from these precursors (Cheng et al., 2013, 2014).

Research by Sousa (2001), Sousa et al. (2002), and Bohnen et al. (2005), related to lowland soils in Rio Grande do Sul cultivated with irrigated rice, describe ethanoic, propanoic, and butanoic acids as the three short-chain organic acids found in higher concentrations in lowland soil that accumulate after fermentation processes. These concentrations differ according to the type of crop residue and the soil management system used. These authors report a decrease in total acid concentration after two to four weeks of flooding. This decrease occurs due to the conversion of short-chain organic acids to CH₄ (Sousa et al., 2002). It is known that ethanoic acid can be used directly for conversion to CH₄ by methanogenic bacteria that use the methyl acid group for conversion. However, propanoic and butanoic acids need to be converted to acetate by the acetogenesis process before being converted to CH₄ (Madigan et al., 2016; Bedoya et al., 2019). When using legume residues (vetch) in the soil, Sousa (2001) demonstrated higher concentrations of ethanoic acid in soil solution, while grass residues (ryegrass) provided higher concentrations of propanoic and butanoic acids.

The means by which the carbon becomes available to soil microbiota for degradation influences the organic acids production, as well as the CH₄ emission, because the different vegetal residues will affect, in a differentiated way, the dynamics of elements and compounds release to the soil solution due to its chemical composition. The experimental hypotheses are: the main fatty acids (ethanoic, propanoic and butanoic acids) released during the organic matter decay under anaerobic soil conditions potentiate the CH₄ emissions by flooded soil; and the size of the organic chain, at equivalent C availability, determines the dynamic of CH₄ emission, being fastest for the shortest chain. This study aimed to quantify, in isolation, the dynamics and CH₄ emission potential of the three short-chain organic acids found in higher concentrations in flooded lowland soils for irrigated rice.

MATERIALS AND METHODS
Conduction of the study and experimental design
The study was conducted in a controlled environment (BOD type incubator) in the Soil Chemistry and Soil Fertility Laboratory of the Federal University of Santa Maria (Universidade...
Federal de Santa Maria-UFSM), Santa Maria, Rio Grande do Sul State. The soil used was a Planosol (IUSS Working Group WRB, 2015), which corresponds to Planossolo Háplico Hidromórfico típico (Santos et al., 2018), collected in the experimental field of the Soil Science Department of the UFSM (29° 43’ 5” S; 53° 42’ 20” W). This collection was carried out in the 0.00-0.20 m layer and the soil was then air-dried and sieved using a 4.0-mm mesh. Afterwards, a soil sample was sent for chemical analysis with the following results: pH (in water at a ratio of 1:1) = 4.9; table SMP = 5.5; M.O. = 2.8 dag kg⁻¹; clay = 400 g kg⁻¹; Ca²⁺ = 6.1 cmol, dm⁻³; Mg²⁺ = 2.7 cmol, dm⁻³; Al³⁺ = 1.8 cmol, dm⁻³; H+Al = 7.7 cmol, dm⁻³; Cation Exchange Capacity (CEC) effective = 10.8 cmol, dm⁻³; CEC pH₇ = 16.7 cmol, dm⁻³; base saturation = 54.1 %; P-Mehlich = 8.5 mg dm⁻³; K-Mehlich = 64 mg dm⁻³. All the chemical analyses were performed according to the methodology of Tedesco et al. (1995).

The experimental units were composed of glass pots with a capacity of 995 mL with perforated plastic lids, containing a 0.20 m long silicone hose coupled to a three-way valve for sampling the gas in the “free atmosphere” of the bottles. In these units, 300 g of soil and 350 mL of distilled water were accommodated, forming a water layer sheet of 4.0 cm depth. Then, the soil was preincubated in a BOD-type incubator for 20 days keeping the water layer sheet and adopting an incubation temperature of 30±1 °C. The visual organic material previously present in the soil, such as root pieces or crop straw, was removed before weighing to avoid interference with CH₄ emissions. According to the methodology of Aulakh et al. (2001), containing some modifications (an increase in the size of the experimental units, vacuum performed with a syringe, and manual agitation).

After the preincubation period, all the experimental units were subjected to a vacuum using a 60 mL syringe, followed by the injection of N₂ in the “free atmosphere” of the bottles, potentiating the conditions favorable to the reduction processes in the soil. After exactly 24 h of this procedure, the treatments were applied. The treatments involved three different short-chain acids and four standardized doses of carbon that were used for each acid (Table 1), thus constituting a factorial arrangement (3 × 4) in a completely randomized design with three replicates. The three acids used were in the form of reagents for chemical analysis being the ethanoic acid (purity ≥99.5 %), propanoic acid (purity ≥99.0 %), and butanoic acid (purity ≥99.7 %). A 30-mL aliquot corresponding to the treatments was applied to the soil with the aid of a calibrated vacuum system.

### Table 1. Treatments with the addition of different doses of soil carbon via different short-chain organic acids

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
<th>Amount of carbon</th>
<th>Amount of acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T₂</td>
<td>90 via ethanoic acid</td>
<td>225.0</td>
<td></td>
</tr>
<tr>
<td>T₃</td>
<td>180 via ethanoic acid</td>
<td>450.0</td>
<td></td>
</tr>
<tr>
<td>T₄</td>
<td>270 via ethanoic acid</td>
<td>675.0</td>
<td></td>
</tr>
<tr>
<td>T₅</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T₆</td>
<td>90 via propanoic acid</td>
<td>183.6</td>
<td></td>
</tr>
<tr>
<td>T₇</td>
<td>180 via propanoic acid</td>
<td>370.0</td>
<td></td>
</tr>
<tr>
<td>T₈</td>
<td>270 via propanoic acid</td>
<td>555.1</td>
<td></td>
</tr>
<tr>
<td>T₉</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T₁₀</td>
<td>90 via butanoic acid</td>
<td>164.9</td>
<td></td>
</tr>
<tr>
<td>T₁₁</td>
<td>180 via butanoic acid</td>
<td>329.9</td>
<td></td>
</tr>
<tr>
<td>T₁₂</td>
<td>270 via butanoic acid</td>
<td>494.9</td>
<td></td>
</tr>
</tbody>
</table>

Dose values were based on preliminary tests and values found by Sousa et al. (2002).
syringe and the three-way valve coupled to the silicone hose attached to the lid of the experimental units.

**Sampling and quantification of CH₄ gas emission**

The CH₄ gas samples were always taken every 24 h after the treatments were applied for 15 consecutive days. The experimental period was defined by relevant literature results and pilot tests performed before this experiment. Samples were collected using a polypropylene syringe (BD®), previously evacuated and treated with N₂ gas, coupled to the three-way valve. Before each collection, the experimental units underwent vigorous manual agitation to homogenize the CH₄ gas which could be trapped in bubbles formed at aqueous layer. After the daily gas collection, a new vacuum and N₂ injection procedure were performed on the experimental units.

Samples contained in the syringes were transferred to pre-evacuated glass vials (Exetainer® vials, Labco Limited, UK) with a capacity of 12 mL for analysis by gas chromatography. The gas chromatograph (GC-2014, model Greenhouse, Shimadzu) was equipped with a flame ionization detector (FID) and analysis conducted at 250 °C with direct injection of 1 mL of sample. The production rate of CH₄ was calculated according to the methodology of Aulakh et al. (2001) using equation 1.

\[
TP_{CH₄} = \frac{dc}{dt} \times \frac{V_{hs} \times MW}{W_s \times MV}
\]

Eq. 1

In which: \(TP_{CH₄}\) is the rate of methane production (µg g⁻¹ day⁻¹); \(dc/dt\) represents the change in CH₄ concentration in the “free atmosphere” of the flask measured in the apparatus (µmol day⁻¹) converted to µg; \(V_{hs}\) is the volume of the free atmosphere of the experimental unit (L); \(MV\) is the molecular volume of CH₄ at 30 °C (24.88 L mol⁻¹); \(W_s\) is the dry weight of the soil (g); and \(MW\) is the molecular weight of CH₄ (16 g mol⁻¹). The accumulated CH₄ production was calculated by adding the emission obtained during 24 h with the emission obtained the previous day, and so on.

The CH₄ produced from experimental units with addition of organic acids was subtracted from the CH₄ produced in the experimental units without addition of acids (dose of 0 mg kg⁻¹), using equation 2, to obtain the net production of CH₄ (\(P_{netCH₄}\)).

\[
P_{netCH₄} = PCH₄sa - PCH₄sc
\]

Eq. 2

In which: \(P_{netCH₄}\) is the net CH₄ production from the added organic acids (µg g⁻¹ day⁻¹); \(PCH₄sa\) is CH₄ produced in soil altered with organic acids (µg g⁻¹ day⁻¹); and \(PCH₄sc\) is the CH₄ produced in the control soil (µg g⁻¹ day⁻¹).

**Statistical analysis**

Means and standard deviations (SD) of the data from each treatment were presented. For total CH₄ emission, analysis of variance (ANOVA) and F test (p<0.05) were performed. Subsequently, according to ANOVA results, regression analysis was performed to evaluate the effect of acid dose and the Tukey test (p<0.05) was performed to verify the difference between the kind of acid. The graphs were made using SigmaPlot, version 12.0 (SigmaPlot, 2012).

**RESULTS**

**Dynamic and cumulative CH₄ emission**

The addition of short-chain organic acids stimulated CH₄ production in the flooded Planosol, but with different emission dynamics between the three acids, at the same
carbon dose (Figure 1). With the ethanoic acid addition in the soil, there was a \( \text{CH}_4 \) emission peak on the third day after acid application, presenting values of 19.3, 34.5, and 49.4 \( \mu g \text{ g}^{-1} \text{ day}^{-1} \) at the carbon doses of 90, 180, and 270 mg kg\(^{-1} \) soil, respectively (Figures 1a, 1b, and 1c). A second peak of \( \text{CH}_4 \) emission was observed on the sixth day after ethanoic acid application, with values of 70.2, 82.4, and 94.0 \( \mu g \text{ g}^{-1} \text{ day}^{-1} \), respectively, for the three carbon doses of 90, 180, and 270 mg kg\(^{-1} \) soil, respectively. It can be seen on the sixth day that, although the peaks are higher than at three days, the difference between the doses is lower; with 270 mg kg\(^{-1} \) there was 1.3-fold higher emission than with 90 mg kg\(^{-1} \), while at three days this difference was 2.6 fold larger. Soon after this second peak, the emission rates of \( \text{CH}_4 \) with the ethanoic acid application decreased, presenting only more expressive emission at the tenth day. It is possible to observe that \( \text{CH}_4 \) emissions with ethanoic acid application were higher than the emissions obtained with another two acid application, except at the dose of 180 mg kg\(^{-1} \) where the accumulated \( \text{CH}_4 \) emission values of butanoic acid exceeded the values obtained by ethanoic acid. With respect to \( \text{CH}_4 \) emissions when propanoic acid is applied (Figures 1a, 1b, and 1c), the first emission peak occurred later in relation to ethanoic acid, namely on the forth day after application of propanoic acid, with emissions of 21.5, 24.4, and 26.6 \( \mu g \text{ g}^{-1} \text{ day}^{-1} \) for the three doses of 90, 180, and 270 mg kg\(^{-1} \), respectively. The use of this acid also resulted in a second peak of emission that was more pronounced to the sixth day after application of the treatments representing three carbon doses, with values of 23.8, 35.5, and 55.8 \( \mu g \text{ g}^{-1} \text{ day}^{-1} \), respectively.

Similar to ethanoic acid, this second \( \text{CH}_4 \) emission peak was followed by a decrease in emissions, but a new emission peak occurred on the ninth day after the acid application with the three doses used, with values similar to the second peak. For the dose of 270 mg kg\(^{-1} \), a fourth emission peak could still be observed on the 11th day after the propanoic acid application, which did not occur with the other doses.

The accumulated emissions of \( \text{CH}_4 \) for this acid were less than the emissions when compared with the emissions obtained with the ethanoic acid application, at doses of 90 and 270 mg kg\(^{-1} \), but without differing from the intermediate dose. Compared with butanoic acid, emissions were similar at doses of 90 and 270 mg kg\(^{-1} \) and lower at the dose of 180 mg kg\(^{-1} \) (Figures 1d, 1e, and 1f).

Butanoic acid, on the other hand, was the organic acid with the later and more prolonged emission peaks (Figure 1). At the dose of 90 mg kg\(^{-1} \), the first \( \text{CH}_4 \) emission peak occurred only on the 11th day after the acid application, presenting an emission value of 37.9 \( \mu g \text{ g}^{-1} \text{ day}^{-1} \). At doses of 180 and 270 mg kg\(^{-1} \), the emissions were initially more similar to each other, with a first emission peak occurring on the 6th day after application of the acid. At the end of the evaluation period, the \( \text{CH}_4 \) production rate using the three organic acids decreased steadily with increasing carbon doses, reaching values similar to the rates observed with the control soil (Figures 1a, 1b, and 1c). From the results reported, it can be inferred that the greater stimulation of methanogenesis by addition of short-chain organic acids to the soil was restricted to a relatively short period lasting a maximum of 15 days.

**\( \text{CH}_4 \) emission potential**

The total \( \text{CH}_4 \) emissions after 15 days of short-chain organic acids application showed some statistically significant differences between the acids when the same dose of carbon was applied (Table 2). At 90 mg kg\(^{-1} \), the value found for ethanoic acid differed from the value found for butric acid, while propanoic acid was similar to ethanoic and butanoic acids. Only at the dose of 180 mg kg\(^{-1} \), there was a distinction between the three acids, with the highest \( \text{CH}_4 \) emission value occurring with butanoic acid application and the lowest value with propanoic acid. At 270 mg kg\(^{-1} \), ethanoic acid returned to have a higher total \( \text{CH}_4 \) emission than propanoic and butanoic acids, which had no statistical
Figure 1. Flow of CH₄ after application of short-chain organic acids (a, b, and c). Vertical bars indicate the standard deviation. Accumulated net emission of CH₄ after application of short-chain organic acids (d, e, and f). The vertical bars indicate a significant difference between the means of the treatments using Tukey’s test (p<0.05).
difference between themselves (Table 2). In this table, it is also possible to observe that the organic acids application raised the CH$_4$ emission above the values of carbon added in the soil. Propanoic acid reached the lowest values in the total CH$_4$ emission at the end of the experiment, at doses of 180 and 270 mg kg$^{-1}$ (Table 2), as shown in the cumulative emission graphs (Figures 1e and 1f), although CH$_4$ emission exceeds the amount of carbon added to the soil.

The CH$_4$ emission data obtained with the application of ethanoic, propanoic, and butanoic acids were adjusted to a quadratic regression model as a function of the carbon doses (Figure 2). It can be seen from figure 2 and table 2 that the conversion rate of carbon applied as ethanoic acid is higher than the conversion rate of propanoic acid, resulting in lower emission with the last acid with increasing carbon doses. With respect to the behavior of butanoic acid, it can be inferred that there a saturation point was reached in the total emission of CH$_4$ with the dose of 180 mg kg$^{-1}$. Conversion of butanoic acid to CH$_4$ yielded almost the same ratio as for ethanoic acid, although the former was converted to more acetates. However, this conversion is slower, which delays the CH$_4$ emission when butanoic acid is applied to the soil.

**Table 2.** Average values (n = 36) obtained for the emission of total CH$_4$, after application of different short-chain organic acids under controlled laboratory conditions

<table>
<thead>
<tr>
<th>Doses (mg kg$^{-1}$)</th>
<th>Total CH$_4$-C emission</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanoic</td>
<td>Propanoic</td>
</tr>
<tr>
<td>0</td>
<td>27.77 a</td>
<td>26.06 a</td>
</tr>
<tr>
<td>90</td>
<td>287.12 a</td>
<td>229.36 ab</td>
</tr>
<tr>
<td>180</td>
<td>382.41 b</td>
<td>287.72 c</td>
</tr>
<tr>
<td>270</td>
<td>511.65 a</td>
<td>386.06 b</td>
</tr>
</tbody>
</table>

Means followed by the same letter in the line do not differ by Tukey’s test (p<0.05). CV: coefficient of variation.

**Figure 2.** Regression of total CH$_4$ emission as a function of carbon doses from three short-chain organic acids. **: significant at 1 % using the F test.
DISCUSSION

Although occurring simultaneously, the sequence for the \( \text{CH}_4 \) generation follows the steps of hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Dong et al., 2019). With ethanoic acid being readily available in the soil, the acetogenesis and methanogenesis stages were anticipated and, consequently, there was faster \( \text{CH}_4 \) emission compared to the other two acids and at all carbon doses, interfering in this way in the dynamics emissions of \( \text{CH}_4 \) (Figure 1). In previous studies which investigated the \( \text{CH}_4 \) emission in soils incubated under anaerobic conditions, Fey and Conrad (2000) and Kotsyurbenko et al. (2004) also reported a short time for conversion of acetate to \( \text{CH}_4 \), taking about three days. The microorganisms that participate in the methanogenesis process belong to the hydrolytic, fermentative, syntrophic, acetogenic, and methanogenic groups, which are stimulated by the introduction of substrates with different characteristics in the microbial system (Liu et al., 2018). This probably favored the group of methanogens that, consequently, allowed the highest \( \text{CH}_4 \) emission on the sixth day when ethanoic acid was applied.

The delay in the first \( \text{CH}_4 \) emission peak appearance, which occurred with the application of propanoic acid in relation to the application of ethanoic acid, can be explained by the degradation of propanoic acid starting only after the degradation of ethanoic acid present in the soil (Figure 1). In some studies using ethanoic and propanoic acids as the carbon source when evaluating the specific methanogenic activity in biological sewage sludge treatment reactors, Zhang et al. (2008) and Zhao et al. (2010) found that the propanoic acid degradation began only when the ethanoic acid, already contained in the sludge, was completely degraded. In figure 1, it can be observed that in the control treatment there is a small amount of \( \text{CH}_4 \) emission on the third day, similar to the first emission peak when ethanoic acid was used. This emission may be from endogenous ethanoic acid in the soil.

According to the stoichiometry of propanoic acid (Table 3), the amount of mol equivalent of \( \text{CH}_4 \) produced from this acid is 1.75, with a yield of 3 mols equivalent of \( \text{H}_2 \) and 1 mol equivalent of acetate in the acetogenesis process. The more \( \text{H}_2 \) there is in the medium, the more this favors the activity of methanogenic bacteria that use \( \text{H}_2 \) as an electron donor, reducing the \( \text{CO}_2 \) for the formation of \( \text{CH}_4 \); these are bacteria classified as hydrogenotrophic. However, the activity of these bacteria is most benefited by temperatures below 20 °C because they are classified as psychrophiles (Guneratnam et al., 2017).

However, in the present study, the temperature was maintained at 30 °C to benefit the acetoclastic bacteria that are classified as mesophilic (Junicke et al., 2016). Methanogenesis, from \( \text{H}_2/\text{CO}_2 \), occurs in small proportions under average ambient temperature of 25 to 30 °C for thermodynamic reasons. There are two possible explanations for this event. Firstly, the composition of the methanogenic microbial community changes with temperature and the same occurs with the relative activity of different physiological groups of microorganisms which depends on their classification, such as psychrophiles, mesophiles or thermophiles. Secondly, the fluidity of the microbial cytoplasmic membrane changes with temperature.

| Table 3. Molecular hydrogen (\( \text{H}_2 \)), acetate, and methane (\( \text{CH}_4 \)) equivalent from the anaerobic degradation of different compounds |
|-----------------------------|------|-------|------|
| **Compound**                | **\( \text{H}_2 \)** | **Acetate** | **\( \text{CH}_4 \)** |
| Ethanoic acid: \( \text{CH}_3\text{COO}^-+\text{H}^+ = \text{CH}_4+\text{CO}_2 \) | 0    | 1     | 1.00 |
| \( \text{H}_2 \): \( \text{H}_2+0.25\text{CO}_2=0.25\text{CH}_4+0.5\text{H}_2\text{O} \) | 1    | 0     | 0.25 |
| Propanoic acid: \( \text{CH}_3\text{CH}_2\text{COO}^-+3\text{H}_2\text{O}=\text{CH}_3\text{COO}^-+\text{HCO}_3^-+\text{H}^++3\text{H}_2 \) | 3    | 1     | 1.75 |
| Butanoic acid: \( \text{CH}_3\text{(CH}_2)_2\text{COO}^-+2\text{H}_2\text{O}=2\text{CH}_3\text{COO}^-+\text{H}^++2\text{H}_2 \) | 2    | 2     | 2.50 |

Adapted from Kotsyurbenko et al. (2004).
differently affecting the use of acetate and H₂ (Fey and Conrad, 2000). The decrease in temperature often results in a decrease in the efficiency of the transport of substances by the plasma membrane and decrease in the use of the substrate. In this way, the acetate must be transported through the membrane, while the H₂ is freely diffusible. When an inhibition occurs in the use of acetate in relation to H₂ with the decrease of temperature, as in high temperatures, there may be a limitation to the solubility of gaseous substrates, such as H₂ and CO₂ in the aqueous phase (Adams et al., 2010; Dong et al., 2018). This may explain the finding that when propanoic acid, which gives higher concentrations of H₂ to the system, was added, the CH₄ emissions were lower.

Before being transformed to CH₄, all short-chain organic acids are first degraded to H₂ and acetate (Wang et al., 2009). This sequence of processes clarifies the delay in the appearance of and the prolongation of the CH₄ emission peaks with the application of butanoic acid. In the processes of acetogenesis and methanogenesis, butanoic acid results in two mols equivalent of H₂ and two mols equivalent of acetate, resulting in 2.5 mols equivalent of CH₄ (Table 3). Thus, endogenous acetogenic microorganisms in the soil, besides converting the short-chain organic acids that were already present in the medium, must have converted a large amount of H₂ and acetate to CH₄ from the application of the butanoic acid, thus prolonging the peak CH₄ emissions.

The acids were applied using the same carbon doses but differed in the amounts of acetate, H₂ and CO₂ formed, which in turn determined the different amounts of CH₄ emitted in the experiment. In general terms, the conversion rates of butanoic acid to CH₄ were very similar to the conversion rates of ethanoic acid and higher than the conversion rates of propanoic acid to CH₄, especially at the two highest doses. This is in agreement with previous studies conducted by Ren et al. (2003) and Wang et al. (2009), in which reported conversion rates of short-chain organic acids to CH₄ varied in the following order: ethanoic acid > butanoic acid > propanoic acid.

When the mean values for the three evaluated acids are examined (Table 2), the average methane emission was greater than 300 or 400 mg kg⁻¹ in the last two doses for ethanoic and butanoic acids, whereas for propanoic acid the emission was less than 300 or 400 mg kg⁻¹, indicating the closest methanogenic response between the first two and lower in the third. Results by Barredo and Evison (1991) and Demirel and Yenigün (2002) suggest that a large amount of propanoic acid results in a failure in the methanogenic process, leading to inhibition of methanogenic bacteria activity, whereas butanoic acid improves the conversion rates of short-chain organic acids to CH₄, increasing the yield of this acid by stimulating the activity of methanogenic bacteria.

Investigating CH₄ emission in lowland soils incubated anaerobically under various temperatures, (Fey and Conrad, 2000) reported that the concentrations of ethanoic, propanoic, and butanoic acids in the incubated soil were zero after one month of incubation at all temperatures, proving that the processes of acidogenesis, acetogenesis, and methanogenesis under these conditions are very rapid. The results of the present study are in agreement with the literature since the higher stimulation of the methanogenesis by addition of short-chain organic acids to the soil was restricted to a relatively short period of 15 days.

The sequential reduction process and the different phases of CH₄ production have been characterized in previous anaerobic incubation studies using lowland soils cultivated with rice. However, published data on the dynamics of CH₄ involving the application of three different short-chain organic acids as the exogenous carbon source in soil are scarce, but only in the evaluation of the specific methanogenic activity in UASB reactors (Upflow Anaerobic Sludge Blanket) for biological treatment of sewage (Zhang et al., 2008; Wang et al., 2009; Schneiders et al., 2013; De Sá et al., 2014). Thus, the present study can elucidate some aspects of the dynamics of CH₄ emission from the degradation of short-chain organic acids that accumulate in the soil, resulting from the fermentation
of organic matter or organic residues at the beginning of the flooding period for the cultivation of irrigated rice.

In previous studies, Xu and Hosen (2010) and Zschornack et al. (2018) reported that the presence of ryegrass straw influenced soil CH\textsubscript{4} emissions in irrigated rice cultivation, with an increase of up to 25 %. The presence of residues in the soil from a crop used as winter cover can increase CH\textsubscript{4} emissions in flooded soil, increasing the supply of C to the methanogens present in the soil, as a result of a greater oxidation process (Kim et al., 2013). With regard to the soil solution, Sousa (2001) demonstrated higher ethanoic acid concentrations when vetch residues were present in rice cultivation, while ryegrass residues provided higher concentrations of propanoic and butanoic acids. Thus, the emission of CH\textsubscript{4} is not only related to the carbon content that is available to the microbiota, but also to the types of acids that are produced from the fermentation of different organic residues in anaerobic conditions.

The emission of CH\textsubscript{4} from the soil in quantities exceeding the carbon that was applied to the soil can be explained by the displacement of populations or the increase in the number of organisms active in the soil, resulting in a conversion of CH\textsubscript{4} from other soil carbon sources with values superior to what had been applied as a substrate for acetogenic and methanogenic bacteria. This could be called a priming effect, although the phenomenon is still unclear and more research is needed to increase understanding of its effect (Kuzyakov, 2010).

A factor that may favor this high emission was the constant use of a temperature suitable for the optimal activity of acetoclastic methanogenic bacteria, which may have exaggerated the emission of CH\textsubscript{4} because, in the field, such as in irrigated rice crops, daily variations in temperature occur which balance the activities of different groups of microorganisms involved in the whole hydrolysis process until the methanogenesis takes place. Applying glucose and acetate as an exogenous carbon source in anaerobically incubated soils, Lu et al. (2000) also found a conversion of these compounds that was greater than expected. Chidthaisong et al. (1999) observed that glucose and acetate supplementation of the soil increased the CH\textsubscript{4} emission by 1.6 to 500 times the expected level, depending on the type of fertilizer applied to the soil in the field before incubation. Lu et al. (2000), although they standardized glucose and acetate to the same carbon dose, also found that the conversion rate and conversion efficiency of the substrate to CH\textsubscript{4} largely exceeded the theoretical maximum values expected.

Even exceeding the expected theoretical maximum values were observed lower values in the total emission of CH\textsubscript{4} at the end of the experiment with the application of propanoic acid. This may have been a consequence of the high concentrations of H\textsubscript{2} present in the medium as a result of the degradation of this acid and according to its stoichiometry (Table 3). As the temperature at which the experiment was conducted did not benefit the activity of hydrogenotrophic bacteria, an accumulation of H\textsubscript{2} could have occurred in the system, thus inhibiting the activity of methanogenic acetoclastic bacteria. Temperature is a key factor that regulates CH\textsubscript{4} production in soils used for cultivation of irrigated rice (van Groenigen et al., 2013; Yvon-Durocher et al., 2014) as it affects, not only the production rate of CH\textsubscript{4}, but also the methanogenesis-acetoclastic or hydrogenotrophic pathway (Lu et al., 2015). In the present study, the temperature may not have favored the hydrogenotrophic bacteria and their activities may have been compromised; this may have caused an accumulation of hydrogen in the system.

The concentration of hydrogen must be “controlled” during the methanogenic phase to maintain the equilibrium of the process at this stage; the ideal is a low hydrogen pressure level of around 5.82 Pa under standard conditions. A high concentration of hydrogen does not provide the necessary environmental conditions for the acetogenic bacteria to convert the organic acids generated to the acetate (Thauer, 2012). For this reason,
it is extremely important to reduce the amount of hydrogen in the mixture, where this fundamental task is carried out precisely by bacteria from the methanogenesis process, transforming hydrogen and carbon dioxide into methane (Baldacin and Pinto, 2015). In a previous study (Guiot et al., 2011; Bassani et al., 2015), a reduction in the activity of acetogenic bacteria in reactors used to treat effluents was observed when external H$_2$ was added, both in mesophilic and thermophilic conditions.

Based on thermodynamic considerations, specifically with regard to the Gibbs free energy ($\Delta G$) resulting from fatty acid oxidation, it is predicted that acetoclastic methanogenic bacteria will be able to grow only in environments with low hydrogen pressures. This condition is achieved when hydrogen-consuming microorganisms are present in the system, such as hydrogenotrophic methanogenic arrays or sulfate-reducing bacteria (Karadagli and Rittmann, 2005). Most of the methanogenic environments maintain an H$_2$ concentration low enough to stimulate the growth of acetoclastic bacteria, avoiding the accumulation of acids. However, with the application of propanoic acid readily available in the soil, a high H$_2$ load was added to the system, which caused a decrease in the efficiency of the propanoic acid to be transformed to CH$_4$.

There is a great uncertainty in the values generated by the theoretical models of CH$_4$ emission since they do not take into consideration important factors that influence the process of anaerobic digestion, such as the physicochemical properties of the compounds, the biological inhibitors and the interactions and requirements among the different groups of bacteria involved in the process. Studies involving specific methanogenic activity have generally been carried out under sterile conditions in mini-reactors for effluent treatment, domestic or industrial, where standard organic acids are commonly used as the only source of carbon (Holmes et al., 2017; Li et al., 2018). However, the measurement of this activity in soil samples is somewhat more complex. Therefore, there is a need for more experiments and adjustments in methodologies to achieve more effective and more accurate results. Understanding the CH$_4$ emission dynamics, separately studying each short-chain organic acid that is produced by fermenting of different residues incorporated into the soil, can help in a future mitigation strategy for this greenhouse gas.

**CONCLUSIONS**

The dynamics of CH$_4$ emission are different by the application of three short-chain organic acids to the soil (ethanoic, propanoic, and butanoic) even the same amount of carbon was added. The CH$_4$ emission is faster and more intense when ethanoic acid is applied in relation to the other two acids application, while butanoic acid presents a slower, delayed, and prolonged CH$_4$ emission dynamics. Propanoic acid results in lower CH$_4$ emission values due to its own stoichiometric CH$_4$ conversion and the temperature condition under which the experiment was conducted, which was unfavorable for hydrogenotrophic bacteria. The addition of short-chain organic acids promotes a priming effect on soil with conversion values of C to CH$_4$ above the calculated theoretical values.

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