



## The effect of rice straw on the priming of soil organic matter and methane production in peat soils



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### ARTICLE INFO

#### Article history:

Received 23 July 2014

Received in revised form

6 November 2014

Accepted 8 November 2014

Available online 20 November 2014

#### Keywords:

Anaerobic decomposition

CH<sub>4</sub> production

Rice straw

Priming effects

Microbial community composition

### ABSTRACT

Rice residue management often leads to increased methane (CH<sub>4</sub>) emissions but the outcomes of edaphic and management factors are not always predictable. Rice residue can act as a substrate for CH<sub>4</sub> production; however the role it plays in priming (mineralization) of soil organic matter (SOM) to release additional substrates for CH<sub>4</sub> production are not well established. We anaerobically incubated a highly organic soil with <sup>13</sup>C-enriched rice straw for 3 months to investigate its priming effect (PE) on SOM and source of C for CH<sub>4</sub> production. Anaerobic decomposition of SOM was accompanied by iron (Fe) reduction with minimal CH<sub>4</sub> production when straw was absent. Straw addition enhanced Fe reduction and increased CH<sub>4</sub> production concurrently with a clear succession of microbial community structure and function assessed with phospholipid fatty acid (PLFA) profiling. The PE on CH<sub>4</sub> production from SOM was strong and positive during the entire experiment. Overall, PE on SOM (CO<sub>2</sub> plus CH<sub>4</sub> production) was slightly positive at the end of the experiment, associated with only a 32% mineralization of the added straw-C (as CO<sub>2</sub> plus CH<sub>4</sub>). Straw addition also released large amounts of dissolved organic carbon (DOC) from SOM. Our results suggest that straw addition effects on PE of SOM and CH<sub>4</sub> production can last for a long period of time showing that straw will cause non-linear response in CH<sub>4</sub> production and potentially result in significant losses of soil C as DOC by leaching or direct exports in histosols.

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### 1. Introduction

The rewetting of former peat wetlands, such as those used for agriculture production, suppresses nitrous oxide (N<sub>2</sub>O) and carbon dioxide (CO<sub>2</sub>) emissions but likely leads to increased methane (CH<sub>4</sub>) emissions (Couwenberg et al., 2010). Methane production is the terminal step of anaerobic decomposition, which requires sequential cooperation of multiple groups of microorganisms (Conrad, 1999; Megonigal et al., 2004). Substrates for CH<sub>4</sub> production are derived from soil organic matter (SOM) and plant residues that are converted to alcohols, fatty acids, and dihydrogen (H<sub>2</sub>) by fermenting bacteria. Syntrophic bacteria further degrade the alcohols and fatty acids to acetate, H<sub>2</sub>, and CO<sub>2</sub>. Ultimately, the acetate and H<sub>2</sub> are utilized as substrates for microbial respiration with the reduction of a variety of electron acceptors (EAs), such as nitrate, ferric iron, sulfate, and CO<sub>2</sub> (Reddy and DeLaune, 2008). Nitrate is reduced first during anaerobic respiration, followed by ferric iron

and sulfate, while CH<sub>4</sub> production becomes important only after the reduction of EAs is complete. However, under natural conditions, anaerobic decomposition of SOM to CH<sub>4</sub> can be a complex process and easily affected by other edaphic and soil management factors besides the presence of EAs.

Rice paddies are potential management systems that can be used to rewet wetlands but the reintroduced anaerobic conditions can emit significant amounts of CH<sub>4</sub> (Denman et al., 2007; Kögel-Knabner et al., 2010). In paddy soils, rice straw is an important carbon source for microbial respiration and CH<sub>4</sub> production (Watanabe et al., 1998; Yuan et al., 2012) and the incorporations of rice straw can increase CH<sub>4</sub> emissions substantially (Watanabe et al., 1993; Bossio et al., 1999). Moreover, additions of rice straw were also found to induce CH<sub>4</sub> production from SOM (Conrad et al., 2012; Yuan et al., 2014). The stimulatory effects on SOM mineralization are commonly referred as priming effects (PEs) (Kuzakov, 2010), which have been widely observed in soils of various upland ecosystems with exact mechanisms that still need resolving (Kuzakov et al., 2000; Fontaine et al., 2004; Dijkstra and Cheng, 2007; Nottingham et al., 2009; Zhu and Cheng, 2011).

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The PE in anaerobic systems, such as rice paddies, has received much less attention than for upland soils (Conrad et al., 2012; Yuan et al., 2014). It is well known that rice residue can act as a substrate for CH<sub>4</sub> production, but the role it plays in priming SOM to produce substrates for CH<sub>4</sub> are not defined. Given that CH<sub>4</sub> is a potent greenhouse gas (GHG), a better understanding of the PE is needed to design management outcomes to mitigate its emissions and improve process-based modeling that predict emissions from rice fields particularly when rice is used as a management practice to restore wetlands. In the present study, we incubated a peat soil that was formally drained for agricultural production and converted to rice paddies with <sup>13</sup>C-enriched rice straw to determine the substrates contributing to CH<sub>4</sub> production. The main objective was to determine the timing, magnitude, and duration of the PE from rice straw on SOM and CH<sub>4</sub> production in peat soil where rice cropping is being implemented to restore degraded peat soils.

## 2. Materials and methods

### 2.1. <sup>13</sup>C-enriched rice straw

Rice plants were grown to maturity in the greenhouse using labeling chamber with <sup>13</sup>C–CO<sub>2</sub> as the source of <sup>13</sup>C enrichment (Bird et al., 2003). After harvest, the rice straw was dried at 60 °C and stored at room temperature (20 ± 1 °C) until used. The straw was cut into small pieces (<1 cm) and used for the laboratory incubation.

### 2.2. Soil preparation and laboratory incubation

In October 2012, 21 soil cores were randomly taken with PVC pipes (5 cm diameter) to a depth of 15 cm from a 30 by 30 m area of a rice field at Twitchell Island, California, USA (38°06' N, 121°39' W). The field was in rice production for 4 years prior to sampling. Samples were taken before harvest when soils were drained. Soils were transported on ice to the University of California Davis and stored at 4 °C for 7 days until used. Selected soil properties are listed on Table 1.

In the laboratory, the 21 soil cores were randomly composited into three replicate soil samples. Following the removal of vegetation, visible roots, and dead plant residues, soils were homogenized by hands. A subsample was collected and dried at 60 °C for 3 days to determine moisture content. Subsamples were also used to quantify extractable NO<sub>3</sub><sup>-</sup>, Fe<sup>3+</sup>, and SO<sub>4</sub><sup>2-</sup> (described below). A straw amended treatment consisted of 10 g of soil placed into a 160 mL serum bottle and amended with 50 mg of <sup>13</sup>C-enriched straw (see Table 1 for <sup>13</sup>C enrichment). The soil/straw mixture was homogenized using a spatula, followed by additions of 10 mL deionized (DI) water to surpass 100% of water holding capacity. Control samples were prepared similarly without straw amendments. The serum bottles were capped with butyl rubber septum, crimped with aluminum seals, and purged with pure N<sub>2</sub> gas for 10 min, followed by incubations at 20 ± 1 °C in dark for 3 months. Three sets of multiple parallel samples were prepared. One was destructively sampled on days 6, 22, and 41 for phospholipid fatty acid analysis

(PLFA). A second was destructively sampled on days 4, 6, 10, 15, 22, 32, 41, and 99 for the determinations of iron (Fe) species, while the last set was destructively sampled on days 2, 4, 6, 10, 15, 22, 41, and 71 for the quantification of dissolved organic carbon (DOC). Head-space gas analysis was frequently done on the second set of the samples for Fe analysis before they were destructively sampled.

### 2.3. Soil and water chemistry

Total C and N, and δ<sup>13</sup>C values of soil and rice straw were analyzed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 IRMS (Sercon Ltd., Cheshire, UK).

For extractable Fe<sup>2+</sup>, 90 mL of 0.5 M HCl was directly injected into the serum bottle, shaken on a reciprocal shaker for 1 h, followed by centrifugation at 3000 rpm for 10 min. An aliquot of the supernatant was further centrifuged at 10,000 rpm for 10 min. Approximately, 0.25 mL of the final centrifuged samples was added to a mixture of 0.5 mL DI water and 0.25 mL of 0.25 M acetic acid (pH 4) with 4 g L<sup>-1</sup> of ferrozine, followed by measuring Fe<sup>2+</sup> concentration at A<sub>562</sub> with a UV mini 1240 spectrophotometer (Shimadzu) (Gibbs, 1976; Lovley and Phillips, 1987). After the measurement, 50 μL of hydroxylamine hydrochloride in 0.25 M HCl was added to the mixture and allowed to sit overnight to reduce any Fe<sup>3+</sup>. The amounts of total extractable Fe (Fe<sup>2+</sup> plus Fe<sup>3+</sup>) were determined by measuring A<sub>562</sub> of the mixture. The extractable Fe<sup>3+</sup> was defined as the difference between the total extractable Fe and Fe<sup>2+</sup>.

Dissolved organic carbon was quantified by UV-persulfate oxidation (Teledyne-Tekmar Phoenix 8000) after extracting the soils with 0.5 M K<sub>2</sub>SO<sub>4</sub> for 1 h, followed by centrifugation at 3000 rpm for 15 min and filtration through a Walkman 57 filter paper. Subsamples of the filtrates were also used to measure extractable NO<sub>3</sub><sup>-</sup> (Doane and Horwath, 2003). The δ<sup>13</sup>C values of DOC was determined on an O.I. Analytical Model 1030 TOC Analyzer (OI Analytical, College Station, TX) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) utilizing a GD-100 Gas Trap Interface (Graden Instruments).

For extractable SO<sub>4</sub><sup>2-</sup>, 4 g soil was extracted with 40 mL DI for 30 min, followed by centrifugation at 3000 rpm for 10 min and filtration through a Walkman GA55 glass-fiber filters. The concentration of SO<sub>4</sub><sup>2-</sup> was determined by ion chromatography (EPA, 1993).

### 2.4. CO<sub>2</sub> and CH<sub>4</sub> measurements

Methane and CO<sub>2</sub> were quantified on a gas chromatograph (GC) system (GC-2014, Shimadzu), equipped with a thermal conductivity detector (for CO<sub>2</sub>) and a flame ionization detector (for CH<sub>4</sub>). Total CO<sub>2</sub> and CH<sub>4</sub> production was calculated from both gas and liquid phases, adjusting for solubility, temperature, and pH (Stumm and Morgan, 1995). Isotopic composition of CO<sub>2</sub> and CH<sub>4</sub> was analyzed at the Stable Isotope Facility of the University of California Davis with a Thermo GC combustion isotope ratio mass spectrometer (GC/C-IRMS) consisting of a Trace GC Ultra gas

**Table 1**  
Selected properties of the peat soil and rice straw (means ± standard error, n = 3).

|       | C (%)    | C/N ratio | δ <sup>13</sup> C | <sup>13</sup> C (atom %) | NO <sub>3</sub> <sup>-</sup><br>(mg g <sup>-1</sup> ) | Fe <sup>3+</sup> | SO <sub>4</sub> <sup>2-</sup> |
|-------|----------|-----------|-------------------|--------------------------|---|------------------|-------------------------------|
| Straw | 34 ± 0.1 | 23 ± 0.1  | 636 ± 0.8         | 1.81 ± 0.00              | N/A   | N/A              | N/A                           |
| Soil  | 15 ± 0.0 | 14 ± 0.1  | -27 ± 0.0         | 1.07 ± 0.00              | 0.01 ± 0.00   | 8 ± 0.2          | 0.13 ± 0.02                   |

Note: N/A, not available.

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