Effects of ferric iron reduction and regeneration on nitrous oxide and methane emissions in a rice soil

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Abstract

A laboratory soil slurry experiment and an outdoor pot experiment were conducted to study effects of ferric iron (Fe(III)) reduction and regeneration on nitrous oxide (N₂O) and methane (CH₄) emissions in a rice (Oryza sativa L.) soil. The anoxic slurry experiment showed that enhancing microbial Fe(III) reduction by ferrihydrite amendment (40 mol Fe g⁻¹) transitionally stimulated N₂O fluxes in the days following flooding. The Fed reduction suppression on CH₄ emission was obscured in the early season but became significant upon reflooding in the mid- and late-seasons. As a result, seasonal CH₄ emission in Fe-amended pots was 26% lower than the control with a single 2-day drainage and 69% lower with a double 2-day drainage. The reduction in CH₄ emission upon reflooding from the Fe-amended pots was mainly attributed to the increased Fe(III) regeneration during drainage showing a mechanism of Fe(III) regeneration in mitigating CH₄ emission by short-term drainage in flooded soils.

1. Introduction

Flooded rice (Oryza sativa L.) fields are a major biogenic source of methane (CH₄), a potent greenhouse gas second only to carbon dioxide (CO₂) contributing to the current enhanced global warming (Intergovernmental Panel on Climate Change, 2001). Methane emission from flooded rice fields, as the net result of production and oxidation, is regulated by various factors including soil properties, fertilization, water management, and rice cultivars (Wang et al., 1993; Sigren et al., 1997; Yagi et al., 1997; Watanabe and Kimura, 1999; Huang et al., 2002).

The level of microbially reducible ferric iron (Fe(III)) was found to be a major soil characteristic regulating CH₄ emission from rice soils (Watanabe and Kimura, 1999; Cheng et al., 2007). The pool of reducible Fe(III) is comprised of various Fe compounds with various reducibilities (Van Bodegom et al., 2003). Water soluble Fe(III) and exchangeable Fe(III) are reducible but generally low in concentration. Amorphous Fe(III) oxides like ferrihydrite are partly reducible and presumably the dominant component of reducible Fe(III) (Lovley, 1991). Crystalline Fe(III) oxides like magnetite and goethite have much less reducibilities (Lovley and Phillips, 1988). Nevertheless, due to a high abundance of iron, microbial reducible Fe(III) is normally the dominant alternative electron acceptor (AEA) in rice soil (Yao et al., 1999). More importantly, it is capable of regeneration by chemical and microbial oxidation at the soil–water interface and rice plant rhizosphere (Frenzel et al., 1999; Neubauer et al., 2007, 2008), and during soil drainage (Ratering and Conrad, 1998). Microbial Fe(III) reduction competitively suppresses CH₄ production to regulate CH₄ emission from flooded rice fields (Wang et al., 1993; Frenzel et al., 1999).

Increasing the microbially reducible Fe level by amending with Fe materials has been used to assess the effect of Fe(III) reduction on reducing CH₄ emission in flooded rice fields (Furukawa and Inubushi, 2002). A few pot studies and one field study have shown that the influence of Fe amendment on lowering CH₄ emission in rice soils, mostly investigated under continuously flooded conditions, varied from being effective in Fe-low rice soils to being neglibile in Fe-rich soils (Furukawa and Inubushi, 2002, 2004; Jäckel et al., 2005).

Ferric iron regeneration during drainage has been suggested to be important in reducing CH₄ emission upon reflooding (Ratering and Conrad, 1998), but not substantially effective in some treatments in two Fe amendment experiments (Furukawa and Inubushi, 2002, 2004). More studies are needed for evaluating the role of Fe(III) regeneration during drainage in reducing CH₄ emission and in determination of suitable drainage durations to reduce CH₄ emission without increasing N₂O emission and reducing rice...
yield (Yagi et al., 1997; Cai et al., 1999). In this study, we focused on the effect of Fe(III) reduction and regeneration on CH₄ emission from both soil slurries and rice pots. The effect of Fe(III) reduction and regeneration on N₂O flux in the soil was also investigated.

2. Materials and methods

2.1. Soil

A silt loam soil (fine, smectitic, hyperthermic Typic Albaqualfs) was used in this study. The soil was taken from the field top layer (0–15 cm) at the Rice Experiment Station, Crowley, Louisiana, United States (30°14.4' N, 92°20.4' W). The soil was air-dried, and stored at room temperature (20 °C). The soil sample was passed through a 1-mm mesh sieve for the slurry experiment and through a 4-mm mesh sieve for the pot experiment. The soil pH was 7.3 (soil: water = 1:1), organic matter 1.67%, total nitrogen 0.07%, extractable Mn (with diethylene triamine pentaacetic acid) 19.5 mg kg⁻¹, extractable Fe (with diethylene triamine pentaacetic acid) 68.2 mg kg⁻¹, and extractable S (with ammonium acetate and acetic acid) 10.7 mg kg⁻¹.

2.2. Slurry experiment

The microcosm technique of Patrick et al. (1973) was used in the slurry experiment with modification (Yu and Patrick, 2003). A homogenized soil slurry was formed with 1600 mL deionized water and 400 g air-dried soil in a 2300 mL Erlenmeyer flask under constant stirring. The slurry was amended with ferrihydrite at 0 or 40 μmol Fe g⁻¹ of soil (n = 2). The preparation of ferrihydrite was according to the procedure described by Schwertmann and Cornell (1991). After a 4-day aerobically pre-incubation at 25 °C, the slurry soil was amended with 4 g ground rice straw and KNO₃ at 8 μmol N g⁻¹, and then anaerobically incubated by bubbling the slurry with pure N₂ at a rate of ~10 mL min⁻¹.

Nitrate contents and N₂O production in the slurries were measured at 6, 24, 48 and 72 h after initiation of the anaerobic incubation. Measurements of redox potential (Eh), pH and CH₄ flux in the slurries were conducted on days one, two, four, six and once every three days afterwards until day 52, except when the slurry was aerated from day 33 to 37. On each gas sampling date, the microcosm (n = 2) was purged with N₂ at a rate of ~30 mL min⁻¹ for 2 h and gas samples were taken from the headspace at 0, 20 and 40 min after stopping purging.

2.3. Pot experiment

The outdoor pot experiment was carried out between 3 June and 8 October, 2003. Three kilograms of soil (dry weight) per pot (diameter 15 cm; height 20 cm) were flooded by mixing with 1000 mL fertilizer solution containing 0.98 g KH₂PO₄, 0.48 g urea and 0 or 10.50 g ferrihydrite-Fe. The fertilizer solution was prepared by mixing the fertilizers with ferrihydrite suspension and mixed well with the soil. The pot was planted with three 21-day-old rice seedlings 2 days after flooding (5 June), maintaining a 2–4 cm layer of overlaying water in the first month and a 5–7 cm flooding depth afterwards. Weeds in the pots were manually removed weekly. Urea (0.32 g) was applied twice as top dressing on 10 July and 9 August. The pots (n = 3) were drained for 2 days either once (on 28 July) or twice (on 28 July and 27 August). The selection of drainage dates was based on CH₄ emissions from the pots and rice growth stage. All pots were drained on 24 September and harvested on 8 October.

Nitrous oxide and CH₄ fluxes in the pots were measured by a closed chamber method. An equilateral triangle-shape frame made of plastic rods was permanently positioned at the soil surface as the base for holding a transparent chamber (height 100 cm; inner diameter 12.5 cm) during gas sampling. The chamber was fitted with a small battery-operated fan for gas mixing and a rubber septum for gas sampling. Gas samples were taken from the chamber by a 10 mL air-tight syringe at 0, 10, 20 and 30 min after closing the chamber. Gas samples were taken between 9:30 and 11:00 AM on days four, six and ten after flooding, and weekly or bi-weekly afterwards until 23 September.

2.4. Measurements

The redox potential in the slurries was measured with a pH/ISE meter (Orion, Model 290A) using a platinum electrode and a saturated calomel reference electrode. The readings were corrected against the standard hydrogen electrode by adding 242 mV. The soil slurry samples (10 mL) taken at 12, 24 and 48 h during the anaerobic incubation were centrifuged at 2000 g for 5 min, the supernatant filtered (<0.45 μm) and stabilized for N₂O determination with a pH/ISE meter (Orion, Model 290A) using a combination N₂O selective electrode (Orion, 9707BN) (American Public Health Association, 1995). Accumulated microbially reduced Fe during the anaerobic soil slurry incubation was measured on day 33 before the starting of aeration. A soil slurry sample (0.5 mL) was anaerobically taken, extracted with 0.5 N HCl (12 mL) for 2 h at room temperature (Lovley and Phillips, 1986) and centrifuged at 2000 g for 5 min. The supernatant (0.1 mL) was added to 1 g L⁻¹ Ferrozin solution (0.9 mL) in 50 mM N-2-hydroxyl-ethylpipera- zin-N’-2-ethane sulfate buffer at pH 4.0 for measurement of Fe(II) with a spectrometer at 562 nm. Gas samples from the slurry and pot experiments were analyzed within 2 days after sampling, using a Tremetrics 9001 GC with an electron capture detector (ECD) for determining N₂O concentrations and a flame ionization detector (FID) for determining CH₄ concentrations. Gas samples from the pot experiment were analyzed for N₂O during the first 22 days and one day after reflooding of the drained pots, and for CH₄ throughout the study.

2.5. Calculation and statistical analysis

Gas flux was calculated by linear regression of gas concentrations at sampling intervals (Yu and Patrick, 2003). The cumulative gas emission from the soil slurry or rice pot was computed as the sum of the flux on each sampling date multiplied by the interval from the midpoints of two neighboring sampling dates.

Statistical analysis was conducted using SAS (SAS Institute Inc., 1999–2001). Methane and N₂O emissions at sampling dates from the control and ferrihydrite amendment treatments were compared using the Student t-test for both slurry and pot experiments. The Student t-test was also used for comparison of rice yields in the control and ferrihydrite amendment pots. Temporal variations of CH₄ emission in the control and ferrihydrite amendment treatments were evaluated with one-way repeated measures ANOVA for both slurry and pot experiments. A simple linear regression was conducted between potential of drainage-induced Fe(III) regeneration and cumulative CH₄ emission in the rice pots. The statistical level of significance was chosen at α = 0.05 for all statistical analysis.

3. Results

3.1. Redox potential and pH change in soil slurries

When the anaerobic incubation started, the control slurry remained weakly reducing (above 200 mV) for 2 days. Highly reduc-
ing conditions (<–290 mV) developed by day nine through 33 (Fig. 1). The slurry Eh increased to >500 mV when the slurry was aerated for 4 days starting on day 33 and decreased to <–280 mV in six days after restarting the anaerobic incubation. The ferrihydrite amendment slightly delayed the redox potential change from being aerobic to being highly reducing (and vice versa) and slightly lowered the Eh under highly reducing conditions. The pH in the control and Fe-amended slurries varied between 7.2 and 7.7 during the study. The pH was more variable under conditions of rapid Eh change than under highly reducing conditions. Ferrihydrite amendment increased pH about 0.1 unit under highly reducing conditions.

3.2. Denitrification, Fe(III) reduction and CH₄ production in soil slurries

The amount of microbially reducible Fe(III) in the soil slurry increased by 53% with Fe amendment, changing from 63.3 ± 1.8 to 97.0 ± 0.42 μmol g⁻¹ (mean ± SD, n = 2). In the control soil slurry, nitrate declined linearly with time and was depleted 48 h after anaerobic incubation was initiated (Fig. 2). Ferrihydrite amendment significantly increased N₂O fluxes before nitrate depletion (Fig. 2). The N₂O fluxes in the control and Fe-amended treatments were below detection after day two. Significant CH₄ production (>10 nmol g⁻¹ d⁻¹) in the two treatments was observed on day six and declined after peaking on day 24 through day 33. After a 4-day aeration treatment from day 33 to day 37, significant CH₄ production in the two treatments occurred again on day 43 and peaked on day 49 (Fig. 3). Iron amendment significantly changed the temporal variation of CH₄ flux in the slurry (p < 0.05). Methane fluxes in the Fe-amended slurry were significantly lower than in the control slurry on days 15, 18, 24, 47 and 50 (p < 0.05), with a cumulative reduction of 15% in the initial 33 days, 30% in the subsequent 15 days after aeration, and 21% in the whole 52 day incubation.

3.3. N₂O and CH₄ emissions from pots

Ferrihydrite amendment stimulated N₂O emissions in the early days upon flooding (Fig. 4). Nitrous oxide fluxes became small in all pots after flooding for eight days. No significant N₂O fluxes were observed in the first day after reflooding of the drained pots in the mid- and late-seasons (data not shown).

The initiation of CH₄ emissions from the pots was slow. Methane was emitted at <1.5 nmol CH₄ m⁻³ d⁻¹ in all pots until day 40 after flooding (Fig. 5). Iron amendment had little effect on CH₄ emission before the midseason drainage on day 55. Compared to
the control pots, CH₄ emissions upon reflooding in the Fe-amended pots were significantly lower on days 63 and 77 ($p < 0.05$) after the pots were drained once on day 55; after day 77 there was no significant difference. When the pots were drained once more on day 85, CH₄ emission upon reflooding was significantly lower on two of four sampling dates (days 99 and 106, $p < 0.05$). As a result, the seasonal CH₄ emission from the rice pots (113 days of measurement) was significantly lowered by Fe amendment when the rice pots were drained once or twice (Table 1) ($p < 0.05$). Regression analysis showed that the cumulative CH₄ emission across the rice pot in the whole measurement period was significantly related to

![Fig. 4. Nitrous oxide emissions (mean ± SD, n = 3) from control and Fe-amended rice pots.](image)

![Fig. 5. Methane emission (mean ± SD, n = 3) from control and Fe-amended rice pots. The arrows show the dates when 2-day drainage was conducted.](image)

### Table 1

A summary of studies on CH₄ emissions in soil–rice systems as affected by amendment of Fe materials.

<table>
<thead>
<tr>
<th>Cumulative CH₄ emission relative to control (%)</th>
<th>Drainage</th>
<th>Soil texture</th>
<th>Soil Fe content (g kg⁻¹)</th>
<th>Amended Fe material</th>
<th>Fe amendment rate (g kg⁻¹)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>52</td>
<td>No</td>
<td>Sandy clay loam</td>
<td>3.9⁹</td>
<td>Fe(OH)₃</td>
<td>6.4</td>
<td>Watanabe and Kimura (1999)</td>
</tr>
<tr>
<td>50</td>
<td>No</td>
<td>Clay loam</td>
<td>3.8⁹</td>
<td>Fe(OH)₃</td>
<td>3.3</td>
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<td>90</td>
<td>No</td>
<td>Coarse medium gley</td>
<td>18.5⁹</td>
<td>Furnace slag</td>
<td>1.5 (490 g m⁻²)</td>
<td>Furukawa and Inubushi (2002)</td>
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<tr>
<td>90</td>
<td>No</td>
<td>Same as above</td>
<td>18.5⁹</td>
<td>Furnace slag</td>
<td>4.2 (980 g m⁻²)</td>
<td>Same as above</td>
</tr>
<tr>
<td>89</td>
<td>No</td>
<td>Same as above</td>
<td>18.5⁹</td>
<td>Furnace slag</td>
<td>6.3 (1960 g m⁻²)</td>
<td>Same as above</td>
</tr>
<tr>
<td>86</td>
<td>No</td>
<td>Same as above</td>
<td>18.5⁹</td>
<td>Furnace slag</td>
<td>14 (4900 g m⁻²)</td>
<td>Same as above</td>
</tr>
<tr>
<td>93</td>
<td>No</td>
<td>Same as above</td>
<td>18.5⁹</td>
<td>Body warmer</td>
<td>4.6 (550 g m⁻²)</td>
<td>Same as above</td>
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<tr>
<td>94</td>
<td>4-day</td>
<td>Same as above</td>
<td>18.5⁹</td>
<td>Furnace slag</td>
<td>1.5 (490 g m⁻²)</td>
<td>Same as above</td>
</tr>
<tr>
<td>66</td>
<td>4-day</td>
<td>Same as above</td>
<td>18.5⁹</td>
<td>Furnace slag</td>
<td>4.2 (980 g m⁻²)</td>
<td>Same as above</td>
</tr>
<tr>
<td>76</td>
<td>4-day</td>
<td>Same as above</td>
<td>18.5⁹</td>
<td>Furnace slag</td>
<td>6.3 (1960 g m⁻²)</td>
<td>Same as above</td>
</tr>
<tr>
<td>81</td>
<td>4-day</td>
<td>Same as above</td>
<td>18.5⁹</td>
<td>Furnace slag</td>
<td>14 (4900 g m⁻²)</td>
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</tr>
<tr>
<td>93</td>
<td>4-day</td>
<td>Same as above</td>
<td>18.5⁹</td>
<td>Body warmer</td>
<td>4.6 (550 g m⁻²)</td>
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<td>77</td>
<td>2-day</td>
<td>Same as above</td>
<td>18.5⁹</td>
<td>Furnace slag</td>
<td>2.3 (490 g m⁻²)</td>
<td>Furukawa and Inubushi (2004)</td>
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<tr>
<td>75</td>
<td>2-day</td>
<td>Same as above</td>
<td>18.5⁹</td>
<td>Furnace slag</td>
<td>7.3 (490 g m⁻²)</td>
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<tr>
<td>92</td>
<td>2-day</td>
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<td>28.5⁹</td>
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<td>490 g m⁻²</td>
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</tr>
<tr>
<td>99</td>
<td>2-day</td>
<td>Same as above</td>
<td>28.5⁹</td>
<td>Body warmer</td>
<td>550 g m⁻²</td>
<td>Same as above</td>
</tr>
<tr>
<td>50</td>
<td>No</td>
<td>Same as above</td>
<td>18.5⁹</td>
<td>Furnace slag</td>
<td>2.3 (490 g m⁻²)</td>
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<td>45</td>
<td>No</td>
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<td>7.3 (980 g m⁻²)</td>
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<tr>
<td>111</td>
<td>No</td>
<td>Same as above</td>
<td>28.5⁹</td>
<td>Furnace slag</td>
<td>490 g m⁻²</td>
<td>Same as above</td>
</tr>
<tr>
<td>115</td>
<td>No</td>
<td>Same as above</td>
<td>28.5⁹</td>
<td>Body warmer</td>
<td>550 g m⁻²</td>
<td>Same as above</td>
</tr>
<tr>
<td>50</td>
<td>No</td>
<td>Sandy loam</td>
<td>2.51</td>
<td>Ferricydrite</td>
<td>1045 g m⁻²</td>
<td>Jäckel et al. (2005)</td>
</tr>
<tr>
<td>74</td>
<td>2-day</td>
<td>Silt loam</td>
<td>3.5⁹</td>
<td>Ferricydrite</td>
<td>3.5</td>
<td>This study</td>
</tr>
<tr>
<td>31</td>
<td>2-day</td>
<td>Silt loam</td>
<td>3.5⁹</td>
<td>Ferricydrite</td>
<td>3.5</td>
<td>This study</td>
</tr>
</tbody>
</table>

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⁹ Amorphous Fe.
⁸ Total Fe.
⁷ Reducible Fe.
⁴ Measured increases on soil weight basis in subsequent years after initial Fe amendments on area basis (values in parenthesis.)
the potential of Fe(III) regeneration during drainage (Fig. 6) ($p < 0.01$).

3.4. Rice growth

Iron amendment delayed the growth of rice seedlings after transplanting in the first four weeks. No observable differences were found in rice growth between the control and Fe-amended pots in the remaining periods. Rice yields from the control and Fe-amended pots were 11.6 ± 1.4 (mean ± SD, $n = 3$) and 9.9 ± 0.7 with single drainage, and 9.6 ± 1.9 and 9.4 ± 0.7 g per pot with double drainage, respectively. Iron amendment showed no significant effect on rice yield ($p > 0.05$) in either single or double drainage treatments.

4. Discussion

In the soil slurry experiment, nitrate measurement showed a rapid depletion of nitrate in 2 days. Ferric iron amendment showed a small effect on nitrate reduction but transitionally promoted N₂O depletion of nitrate in 2 days. Ferric iron amendment showed a slight delay in Eh change and a slight increase in pH, but an effective reduction in CH₄ emission in the soil slurry (Fig. 3). Also importantly, this suppressing effect on CH₄ emission remained in the subsequent anaerobic incubation after Fe(III) was regenerated from oxidation of ferrous Fe (Fe(II)) through aeration (Fig. 3).

Our study suggests that the effects of Fe amendment on CH₄ emissions are more complex in soil–rice systems than simple inhibition. Methane emission from the soil–rice system is the net result of production and oxidation and greatly mediated by the rice plant and soil water conditions. (Neue, 1993; Yagi et al., 1997; Aulakh et al., 2000; McLain and Ahmann, 2008). Much of CH₄ produced in the soil–rice system would be microbially oxidized at the oxygenated soil–water interface and rice plant rhizosphere before being emitted to the atmosphere (Neue, 1993). Although Fe amendment could lower the level of acetate to subsequently affect CH₄ oxidation (van Bodegom et al., 2001), its suppression on CH₄ production, as found in the slurry experiment, was expected to be dominant to affect CH₄ emission in the rice–soil system.

In the early rice growing season, the suppression of Fe amendment on CH₄ emission was observed in the study by Furukawa and Inubushi (2002) but obscured in our pot experiment due to low CH₄ emissions in both treatments. With no organic amendment and a relatively small effect of early season rice on CH₄ emission (Aulakh et al., 2000, 2001), the soil from our pot study appeared not to provide sufficient energy source to support strong methanogenic activity, while CH₄ oxidation was expected to be active in the early season (Krüger et al., 2002). Further, the extent of increase of the soil reducible Fe through Fe amendment has been found to be important in reducing CH₄ emissions in soil–rice systems. Studies summarized in Table 1 suggest that the reduction of CH₄ emission by Fe amendment may be significant in low-Fe soils but not in Fe-rich soils. Soil texture could also influence the effect of Fe amendment on reducing CH₄ emission through affecting Fe(II) oxidation, especially during drainage (Sigren et al., 1997).

Results from our pot experiment show the importance of Fe(III) regeneration during drainage on CH₄ emission in soil–rice systems. Similar to the effect of Fe(III) regeneration on CH₄ emission after aeration found in the slurry experiment, Fe amendment in the rice pots enhanced the level of reducible Fe(III) by 84% that was subject to regeneration during drainage, leading to effective reductions in CH₄ emission upon reflooding (Fig. 5). In the study by Furukawa and Inubushi (2002), the contribution of Fe(III) regeneration during drainage to reducing CH₄ emission was obvious only in some Fe amendement treatments, possibly due to the offsetting effect on CH₄ emission of carbon contained in amended Fe materials (Furukawa and Inubushi, 2004). The contribution could also be obscured when CH₄ emissions were greatly lowered for a prolonged time after drainage in all treatments (Furukawa and Inubushi, 2004). The significance and consistency of the contribution of Fe(III) regeneration during drainage to reducing CH₄ emission should be validated in the future on a range of other periodically flooded soils.

Also, the results from our pot experiment on CH₄ emissions after single and double drainage treatments (Fig. 5) showed the importance of Fe(III) regeneration in determination of suitable drainage durations required for reducing CH₄ emission in flooded rice soils. Short-term drainage is desirable if effective CH₄ mitigation is obtained. Long-term drainage, while effective in reducing CH₄ emission, may increase N₂O emission (Cai et al., 1999) and reduce rice yield (Yagi et al., 1997). Drainage could be shortened in reducible Fe-rich rice soils to effectively reduce CH₄ emission. Amendment of Fe materials could be an option in low reducible Fe rice soils to improve short-term drainage-based CH₄ mitigation.

5. Conclusions

Enhanced Fe(III) reduction by ferrihydrite amendment temporarily stimulated N₂O production and effectively suppressed CH₄ emission in the anoxic soil slurry. The suppression on CH₄ emission
remained after Fe(III) regeneration through aeration. Ferrihydrite amendment significantly increased N2O emission in the rice pot in the early days after flooding. Ferrihydrite amendment effectively reduce CH4 emission through increasing Fe(III) regeneration during drainage in the mid- and late-seasons, showing the importance of Fe(III) regeneration in short-term drainage-based options for effective CH4 mitigation in flooded rice fields. The achieved results should be validated on a range of other periodically flooded soils in the future.

Acknowledgements

This research is supported by the USDA National Research Initiative Competitive Grants Program. This paper is in dedication to Dr. William H. Patrick, Jr. (1925–2004) who was the principle investigator of this project.

References


