Impacts of soil incorporation of pre-incubated silica-rich rice residue on soil biogeochemistry and greenhouse gas fluxes under flooding and drying

Madison Y. Gutekunst, Rodrigo Vargas, Angelia L. Seyfferth *

Department of Plant and Soil Sciences, University of Delaware, 531 S. College Avenue, 152 Townsend Hall, Newark, DE 19716, USA

HIGHLIGHTS

• Methods to attenuate arsenic impacts on rice through soil incorporation of silica-rich residues may affect GHG emissions.
• We monitored GHG and biogeochemical impacts of pre-incubated rice residue incorporation to soil during flooding and drying.
• Soils pre-incubated with rice husk had 2-4 fold higher pore water Si than control and soils pre-incubated with rice straw.
• GHG fluxes from straw-amended soils were 2-3 fold higher than control and ash- and husk-amended soils due mainly to N₂O.

GRAPHICAL ABSTRACT

ABSTRACT

Incorporation of silica-rich rice husk residue into flooded paddy soil decreases arsenic uptake by rice. However, the impact of this practice on soil greenhouse gas (GHG) emissions and elemental cycling is unresolved particularly as amended soils experience recurrent flooding and drying cycles. We evaluated the impact of pre-incubated silica-rich rice residue incorporation to soils on pore water chemistry and soil GHG fluxes (i.e., CO₂, CH₄, N₂O) over a flooding and drying cycle typical of flooded rice cultivation. Soils pre-incubated with rice husk had 4-fold higher pore water Si than control and 2-fold higher than soils pre-incubated with rice straw, whereas the pore water As and Fe concentrations in soils amended with pre-incubated straw and husk were unexpectedly similar (maximum ~0.85 μM and ~450 μM levels, respectively). Pre-incubation of residues did not affect Si but did affect the pore water levels of As and Fe compared to previous studies using fresh residues where straw amended soils had higher As and Fe in pore water. The global warming potential (GWP) of soil GHG emissions decreased in the order straw (612 ± 76 g CO₂-eq m⁻²) > husk (367 ± 42 g CO₂-eq m⁻²) > ashed husk = ashed straw (251 ± 26 and 278 ± 28 g CO₂-eq m⁻²) > control (186 ± 23 g CO₂-eq m⁻²). The GWP increase due to pre-incubated straw amendment was due to: a) larger N₂O fluxes during re-flooding; b) smaller contributions from larger CH₄ fluxes during flooded periods; and c) higher CH₄ and CO₂ fluxes at the onset of drainage. In contrast, the GWP of the husk amendment was dominated by CO₂ and CH₄ emissions during flooded and drainage periods, while ashed amendments increased CO₂ emissions particularly during drainage. This experiment shows that ashed residues and husk addition minimizes GWP of flooded soils and enhances pore water Si compared to straw addition even after pre-incubation.

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* Corresponding author.
E-mail addresses: gute@udel.edu (M.Y. Gutekunst), rvargas@udel.edu (R. Vargas), angelias@udel.edu (A.L. Seyfferth).

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1. Introduction

The management of rice paddy soils influences global greenhouse gas (GHG) emissions (Delwiche and Cicerone, 1993; Neue, 1997), nutrient dynamics (Kae wpradit et al., 2008; Penido et al., 2016; Seyfferth et al., 2013), and arsenic (As) availability to rice (Arao et al., 2009; Li et al., 2009; Linquist et al., 2015; Seyfferth and Fendorf, 2012; Seyferth et al., 2016). As a staple food for over 50% of the global population, rice yield is directly linked to global food security. The highest rice yield is typically obtained under flooded paddy cultivation in which soils are flooded and remain so until grain filling. The timing of the drainage impacts both As uptake by rice and soil GHG emissions. (Adviento-Borbe et al., 2015; Kim et al., 2012; Linquist et al., 2012; Linquist et al., 2015). While rice paddy flooding is beneficial for yield, it exacerbates both CH4 emissions and As mobilization in soils due to anaerobic metabolic reactions under reduced soil conditions (Linquist et al., 2012; Linquist et al., 2015). The mobilized As, in turn, may be taken up by rice (Ma et al., 2008) and transferred to grain (Carey et al., 2010), where it can compromise yield (Duxbury et al., 2003; Panaullah et al., 2009) and impact human health (Banerjee et al., 2013; Williams et al., 2005) upon consumption. Decreasing As uptake by rice is a primary local-to-global food security goal, but sustainable solutions must consider its impacts on yield and its biogeochemical implications such as short- and long-term GHG emissions.

Soil incorporation of silica is an emerging method to decrease toxic As uptake by rice and storage in grain (Seyfferth et al., 2016). Silica affects rice As concentrations because dissolved silica (silicic acid) and reduced arsenic (arsenous acid) are chemically similar and share root transporters (i.e., Lsi1 and Lsi2) in rice (Ma et al., 2008). Due to weathering and leaching, many rice soils are depleted in plant-available silica (Savant et al., 1997a), which leads to upregulation of Si transporters in rice roots and inadvertently increases the potential for As uptake. Addition of silica to soil may decrease As uptake by downregulation of Si transporters and competition between Si and As for uptake (Ma et al., 2008). However, Si addition may also cause As desorption from soil solids (Luxton et al., 2006), stabilization of poorly crystalline Fe (oxyhydr)oxides in soil and on root-bound Fe (oxyhydr)oxide plaques (Schwertmann and Thalmann, 1976), and co-precipitation with As-adsorbing Fe (oxyhydr)oxides in bulk soil and in plaque (Sweedlund and Webster, 1999). The overall impact of Si on As in rice reflects an interplay of the aforementioned plant physiological and soil chemical processes.

Soil silica amendments can affect grain As concentrations and GHG emissions, but the impacts depend on the type of silica added (Ma et al., 2014; Penido et al., 2016; Seyfferth and Fendorf, 2012; Seyfferth et al., 2016). Under typical flooded paddy cultivation, soil incorporation of silica gel decreases grain As concentrations without affecting soil CH4 production or rice yield (Li et al., 2009; Seyfferth and Fendorf, 2012), whereas silica-rich diatomaceous earth increases grain As concentrations (Seyfferth and Fendorf, 2012). Soil incorporation of silica-rich rice straw increases grain As (Ma et al., 2014) and exacerbates soil CH4 production (Penido et al., 2016) and emissions (Liu et al., 2014; Vibol and Towprayoon, 2010; Wang et al., 2012; Wassmann et al., 2000). In contrast, soil incorporation of silica-rich rice husk, a holistic yet seldom used soil amendment, decreases inorganic grain As levels by 25–50% without affecting yield or CH4 production (Seyfferth et al., 2016). Fewer studies have focused on multiple GHG emissions and As cycling due to rice husk incorporation than due to rice straw incorporation. To evaluate the impact of husk residue incorporation on GHG emissions and compare it to other silica-rich amendments, research efforts need to consider the impact of husk amendment on soil biogeochemistry for multiple flooding and drying events and CH4, CO2, and N2O emissions simultaneously.

Factors that promote CH4 emissions include strongly reduced soil conditions and labile carbon availability, whereas N2O emissions are promoted under suboxic soil conditions and in response to NO3-fertilization (Zou et al., 2005). Organic residue incorporation such as rice straw is known to increase CH4 production and efflux (Conrad et al., 2012; Weber et al., 2001). Flooded rice fields, particularly those amended with organic residues, are thus major sources of CH4 emissions and comprise between 9 and 13% of global anthropogenic emissions (Pedersen et al., 2006). While CH4 emissions tend to dominate in flooded rice production (Wassmann et al., 2000), periods of suboxic soil conditions such as those that occur during drainage may increase N2O emissions (Adviento-Borbe et al., 2015). Moreover, the management history (e.g., incorporation of organic matter such as plant residues) of the soil also affects the microbial community structure, which in turn affects GHG production and fluxes (Lagomarsino et al., 2016).

We previously reported that rice husk incorporation to flooded soil led to higher dissolved silica, lower dissolved As, and lower dissolved CH4 concentrations than rice straw incorporation in a six-week flooded incubation study without plants (Penido et al., 2016). That study utilized a 1% residue:soil ratio, which was designed to provide silica benefits for multiple growing seasons and thus recurrent flooding and drying cycles. The findings from that work open the following question: To what extent does pre-incubation under flooded conditions affect biogeochemical cycling and GHG emissions when amended soils are re-flooded and again dried? This question is relevant to evaluate how biogeochemical processes of amended soils respond to multiple flooding-drying cycles, and to inform long-term soil management practices.

To address that question, we experimentally tested the influence of soil incorporation of pre-incubated silica-rich rice residues of straw, husk, ashed husk and ashed straw over dynamic soil moisture conditions. We simulated a flooded rice cultivation cycle that consists of a wetting-up-flood-drainage cycle of the approximate length of a growing season. We conducted the experiment under laboratory conditions (i.e., controlled temperature) without plants to isolate the amended soil impacts and avoid confounding effects. We monitored biogeochemical dynamics in pore water and CO2, CH4, and N2O fluxes, and calculated the 100-year global warming potential (GWP) of these GHG emissions. We hypothesized that soil incorporation of pre-incubated 1) rice husk would lead to the highest pore water Si concentrations and lower dissolved As and GWP than rice straw; 2) rice straw would lead to the highest pore water As concentrations and GWP, mainly as CH4 due to strongly reduced soil conditions; and 3) ashed residues would lead to the lowest GWP because these residues have more recalcitrant carbon.

2. Materials and methods

2.1. Soil sampling and experimental set-up

Soils and treatments were identical to those described in Penido et al. (2016). Briefly, air-dried soil collected from 2 to 30 cm depth (to avoid collection of overlying grass vegetation) at the University of Delaware’s Newark farm, Newark DE, USA was used for the experiment. This soil was chosen because of its similar weathering extent, plant-available silica and arsenic concentration to rice paddy soils in Cambodia (Seyfferth et al., 2014). Soil characteristics were reported in Penido et al. (2016) and are briefly summarized in Table 1. During collection, care was taken to remove vegetation and roots while keeping the soil structure as intact as possible. Approximately 3.5 kg soil was added to each of 15 acid-washed, 4 L high-density polyethylene pots.

For treatments, 1% (w/w) of rice residues were incorporated by hand into the soil; these consisted of rice husk, rice straw, ashed husk, or ashed straw. Both husk and straw were ground to a powder using a Wiley Mill prior to soil incorporation. The total As, Si, Fe and P was ~0.6% for straw (Kajiura et al., 2015). Ashed husk residues were obtained from a mill in Battambang, Cambodia where fresh husk is used as a...
fuel source and incompletely combusted at ~450 °C as is done in some rice-growing countries (Haefele et al., 2009; Savant et al., 1997b). Ashed straw was created in the laboratory by combustion of the fresh straw at 450 °C in a muffle furnace. The creation of the ashes concentrated As, Si, Fe, and P by at least a factor of two, but it should be noted that these are not true ashes as they represented incompletely combusted materials that were a mixture of ash and charred material. Each treatment and non-amended control was replicated three times for a total of 15 pots. Prior to the start of the experiment soils were amended, flooded for 6 weeks (Penido et al., 2016), and then allowed to air-dry at room temperature (22 °C) for over two months. This allowed us to test our main question on amended soils that have been exposed to flooding and drainage for more than 1 cycle (i.e., pre-incubation) as expected in actively managed rice paddies.

To monitor pore water chemistry, Rhizon pore water sampling devices (Eijkelkamp Soil & Water, The Netherlands) were inserted into a pre-drilled hole approximately 1 cm from the top rim of each pot. The 10-cm long Rhizon samplers, with 0.2 μm pore size, penetrated the soil at a 45-degree angle to allow for weekly pore water sampling (Penido et al., 2016; Seyfferth and Fendorf, 2012; Seyfferth et al., 2016). To ensure a tight seal for GHG measurements, each port was sealed with silicone adhesive and allowed to dry prior to manipulation of soil moisture.

### 2.2. Soil moisture manipulation

Soil moisture was manipulated to approximate the dynamic conditions experienced during traditional flooded rice cultivation, which lasts approximately 90–120 days from seed to harvest. The soil moisture regime consisted of three distinct phases: wetting up (Phase I, initial dry down over another six-week period. To maintain water levels during the last approximately 90–120 days from seed to harvest. The soil moisture regime consisted of three distinct phases: wetting up (Phase I, initial dry 60 days) and dry down (Phase III, soil moisture decreasing over 25 days). In Phase I, the pots were wet up (Phase I, initial dry down over another six-week period. To maintain water levels during

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Pots remained flooded for six weeks, and were then allowed to dry down over another six-week period. To maintain water levels during the six-week flooded period accounting for evaporation, DDI water was added weekly (<100 mL) after GHG flux measurements, moisture measurements, and pore water samples were taken. After six weeks of flooding, no additional water was added during the six-week dry down period. The experiment was carried out at room temperature (i.e., 22 °C) to avoid confounding effects with changes in air temperature.

### 2.3. Pore water sampling and analysis

To measure impacts of amendment and soil moisture on pore water chemistry and controls on gas fluxes, pore water was collected 1–2 times per week through Rhizom samplers into acid-washed vials via a needle stop-cock assembly (Penido et al., 2016; Seyfferth and Fendorf, 2012; Seyfferth et al., 2016). Briefly, empty and pre-cleaned vials were capped with septa and crimp sealed in an oxygen-free atmosphere and evacuated with a hand-held vacuum pump just prior to sampling. The septa of evacuated vials were pierced with needles that were attached to the Rhizom samplers with luer fittings. Once the stop-cock was opened, pore water flowed into the vial under an inert N2 atmosphere. Pore water samples were taken after each gas flux and soil moisture measurement and before the water additions. Each pore water sample was immediately analyzed for Eh using a platinum electrode (Thermo/Orion) with a built-in silver/silver chloride reference and Eh values are reported relative to the standard hydrogen electrode. The sample was then analyzed for pH using a calibrated probe (Thermo/Orion), total Fe, Si, and P using ICP-OES (Thermo) and As using ICP-MS (Agilent 7500) against known standards after acidification to 2% acid with trace metal grade HNO3. For metal and nutrient analysis, quality assurance and quality control protocols included duplicate measurements, blanks, and standard checks every 20 samples.

### 2.4. Soil gas flux, temperature and moisture measurements

Soil gas fluxes of CO2, CH4, and N2O were measured using a closed dynamic flux chamber (Pumphen et al., 2004) coupled with a Picarro G2508 analyzer (Picarro Inc., Sunnyvale, CA, USA) (Petakis et al., 2017). The Picarro G2508 analyzer measures gas concentrations for CO2 (0.02–2 μmol mol−1 ± 0.05%), CH4 (0.5–15 μmol mol−1 ± 0.05%), and N2O (0–400 μmol mol−1 ± 0.05%). The instrument was calibrated using factory standards that included high precision gases and tests against other similar units (Picarro Inc., Sunnyvale, CA, USA). Prior to each gas flux measurement, surface soil/water temperature was obtained using an infrared handheld thermometer (5500H, NuBee, USA). During each gas flux measurement, each pot was sealed to create a closed dynamic loop system to measure changes in gas concentration for a total of 5 min, which included a dead band of the first 2 min and a 3 min observation time for calculations (see the following section). After each measurement, the instrument was allowed to re-equilibrate with laboratory atmospheric concentrations between 2 and 5 min (i.e., CO2 < 450 ppm; CH4 < 2 ppm; and N2O < 1 ppm) prior to measuring the next pot. Following each gas flux measurement, soil moisture was measured using a Delta-T SM150 sensor (Delta-T Devices, Cambridge, UK); however, when soils were completely flooded, 100% soil moisture was assumed.

Gas flux measurements were taken throughout Phases I, II, and III, with more frequent measurements during Phases I and III when rapid changes in soil moisture occurred due to soil wetting up and drying down (Kim et al., 2012). During Phase I, an initial measurement was taken on dry soil prior to any water additions, and then every 2–3 h during days 1–2 in the rewetting period. During Phase II, measurements were taken three times per day on day 3 (once flooding was achieved), once per day on days 4 through 6 (first days of completely flooded conditions), and weekly thereafter (constant flooding). During the latter part of Phase II and during Phase III, gas fluxes were measured twice per week for the last 4 weeks of the drying phase.

### Table 1

<table>
<thead>
<tr>
<th>Soil characteristics of the Elsinboro silt loam.</th>
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<td>pH</td>
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<td>P (mg kg⁻¹)</td>
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<td>Extractable (mg kg⁻¹)</td>
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*aHOAc Si* 13.5

*bCBD Fe* 18,715.3

^cAAO Fe* 17,409.6

^dCBD As* 16.7

^eAAO As* 1.2

a Aceric acid.
b Citrate-bicarbonate-dithionite.
c Acid ammonium oxalate.

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**Note:** The table includes soil characteristics and extractable elements for the Elsinboro silt loam soil, measured using various methods such as Acid ammonium oxalate (HOAc), Citrate-bicarbonate-dithionite (CBD), and Acid ammonium oxalate (AAO). The concentrations are given in mg kg⁻¹.

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**Conclusion:** The experimental setup and procedures for soil moisture manipulation and pore water sampling are described, focusing on the dynamic conditions experienced during traditional flooded rice cultivation. Pore water chemistry and soil moisture impacts on gas fluxes were measured and analyzed using a Picarro G2508 analyzer, with careful attention to quality assurance and quality control protocols. The measurements were taken throughout Phases I, II, and III, with more frequent measurements during Phases I and III when rapid changes in soil moisture occurred due to soil wetting up and drying down.
2.5. Soil gas flux calculations

Measured gas concentrations were used to calculate gas fluxes of CO₂, CH₄, and N₂O (in μmol m⁻² s⁻¹), using the following equation (Steduto et al., 2002):

\[
\text{Soil GHG Flux} = \frac{6Ct \cdot V}{\delta \cdot S \cdot RT}
\]

where \( C \) is the mole fraction of CO₂, CH₄, or N₂O in μmol mol⁻¹, \( t \) is the observation time of 180 s (3 min after removing a dead band of 2 min), \( V \) is the total volume of the closed system (0.00163 m³), \( S \) is the surface area of the soil in the pot (0.03 m²), \( P_a \) is the atmospheric pressure inside the chamber (100.96 kPa), \( R \) is the universal gas constant \( (8.3 \times 10^{-3} \text{ m}^3 \text{ kPa} \text{ mol}^{-1} \text{ K}^{-1}) \), \( T \) is the surface water or surface soil temperature (°K), and we applied a linear fit to calculate \( \delta C/\delta t \) (Pearson et al., 2016). For QC, only slopes from the linear regression that were significantly different from zero \((P < 0.05)\) were used to compute fluxes (Petrikis et al., 2017).

2.6. Data analysis

We report instantaneous GHG emissions and GHG emissions for the entire length of the experiment (i.e., 90 days) to describe instantaneous and then cumulative treatment effects. We calculated the GWP for each treatment and control over the entire experiment using the average of individual (CO₂, CH₄, and N₂O) GHGs and the conservative 100-yr GWP of 265 for N₂O and 28 for CH₄ relative to CO₂ that do not include the influence of climate-carbon feedbacks (Myhre et al., 2013) to express emissions as g CO₂-equivalent units per m² (g CO₂-eq m⁻²). Consequently, the GWP for each treatment and control was computed as described above but for each individual phase.

We analyzed GHG fluxes and the GWP per phase (i.e., wetting up, flood, and drying down) to provide information on temporal dynamics and influence of wetting-drying conditions. These analyses only included data obtained and duration of time for individual phases, where Phase I included data from \( t = 0–0.14 \) weeks, Phase II included data from \( t = 0.154–8.32 \) weeks, and Phase III included data from \( t = 9.32–12.74 \) weeks. The GWP for each treatment and control was computed as described above but for each individual phase.

We verified that all data from each treatment and control met quality control protocols, were normally distributed, and variance was homogeneous. One-way ANOVAs and post-hoc Tukey tests were performed to find differences in the overall GWP among treatments. Two-way repeated measurements ANOVAs were conducted to evaluate treatment and temporal (i.e., Phase) effects and their interaction on response variables i.e., GHG fluxes, GWP, pore water Eh and pH, and pore water concentrations of Si, As, Fe, and P. All statistical tests were performed with SPSS v. 23 software.

3. Results

3.1. Soil moisture, redox and pH dynamics

Soil moisture, Eh, and pH were similar among treatments and control and changed through time due to dynamic water regime across Phases I, II, and III (Fig. 1). Soil moisture sharply increased from 0% to 100% within the first two days during Phase I and remained at 100% through Phase II and the first part of Phase III until it decreased to 15–20% (Fig. 1A). Eh showed an opposite pattern to soil moisture and decreased from an initial 120–180 mV during Phase I to a minimum of 40–100 mV, during Phase II before increasing to levels approaching 200 mV during Phase III (Fig. 1B); however, no significant differences were observed among treatments. pH values did not significantly differ among treatments and control but differed with Phase, ranging from 6.8 to 7.2 for Phases I and II before dropping to significantly lower values (\( F = 26.6; \ p < 0.001 \)) as low as 6.3 as the soils dried (Fig. 1C).

3.2. Pore water Fe, As, Si and P dynamics

Statistically significant differences in pore water chemistry due to treatment were evident with Fe, As, Si, and P concentration dynamics in pore water (Fig. 2). While the pattern of an initial increase during Phase I, leveling off during Phase II, and decrease during Phase III was consistent for all four solutes, the magnitude of change differed among the treatments for each solute.

The maximum pore water Fe in husk and straw was as high as ~450 μM and was 2-fold higher than the other treatments and control (Fig. 2A). These results are supported by a two-way repeated measurements ANOVA that showed significant differences by treatment (\( F = 9.7, P < 0.0001 \)) and their interaction (\( F = 5.4, P < 0.0001 \)). Post hoc tests revealed that both straw and husk treatments had significantly higher \((p < 0.05)\) average dissolved Fe in pore water compared to ashed husk, ashed straw and non-amended treatments (Phases I, II, and III) periods.

Fig. 1. Soil moisture (A) and pore water redox (B) and pH (C) in pre-incubated non-amended soil or pre-incubated soil amended with rice straw, straw ash, rice husk or husk ash during wetting up (Phase I), flooded (Phase II, gray shading), and drying (Phase III) periods.
control. However, the husk treatment lagged behind the straw treatment during both the increase in Phase I and the decrease to nondetectable levels in Phase III (Fig. 2A).

Pore water As concentrations were initially ~0.2 μM for amended soils and non-amended controls, but thereafter deviated from each other (Fig. 2B). Pore water As in non-amended soil increased to 0.4 μM during Phase II before decreasing to <0.05 μM during Phase III. Both ashed straw and ashed husk had similar patterns of pore water As, which showed an increase to the highest levels of ~0.65 μM by week 2 before slowly decreasing throughout the experiment. Straw μM during Phase II before decreasing to ~0.65 μM by week 2 before slowly decreasing throughout the experiment. Straw amendment also showed a rapid increase to ~0.65 μM during the first 2 weeks, but remained at this level to week 7 and then increased to the highest value of ~0.85 μM by week 9 before decreasing rapidly to <0.05 μM during Phase III. In contrast, pore water As in husk amended soils exhibited a gradual increase to ~0.85 μM from weeks 1 to 8 and after which lagged behind straw as it decreased to <0.05 μM in Phase III (Fig. 2B). These results are supported by a two-way repeated measures ANOVA that showed significant differences by treatment (F = 5.5, P < 0.0001), phase (F = 45.1, P < 0.0001), and their interaction (F = 2.7, P = 0.032). Post hoc tests revealed that the average pore water As was significantly higher (p < 0.05) in husk and straw treatments compared to control.

Pore water Si concentrations varied considerably with treatment (Fig. 2C). Non-amended control soils had the lowest Si levels of ~200 μM throughout the experiment. Amended soils had similar patterns of an initial increase in pore water Si during Phase I and a relatively steady level during Phase II before dropping off during the latter part of Phase III, but varied in magnitude among treatments. Pore water Si was the highest in husk amended soils, ranging ~600–900 μM, followed by ashed husk, ranging 320–500 μM, while straw and ashed straw had similar low levels of between 250 and 350 μM (Fig. 2C). These results are supported by a two-way repeated measures ANOVA that showed significant differences by treatment (F = 258.4, P < 0.0001) and phase (F = 485.9, P < 0.0001) but not their interaction (F = 1.6, P = 0.163). Post hoc tests revealed that the average pore water Si as decreased in the following order husk > ashed husk > straw = ashed straw > non-amended control.

Pore water P initially increased during Phase I, reached a relatively steady level during Phase II and a sharply decreased during Phase III in all treatments. Similar to Fe and As trends, pore water P in husk amended soil showed a lag compared to straw amended soil in which levels were lower than in straw amended soils until week 4 and then increased to higher levels in weeks 8–10 before returning to low levels at the end of Phase III (Fig. 2D). However, in contrast to Fe, As and Si levels, pore water P concentrations were higher in ashed amendments than in fresh amendments (Fig. 2D). Ashed husk and ashed straw had similar and relatively high initial concentrations of 40 μM that increased to nearly 60 μM by week 4 before they slowly decreased to ~45 μM by week 10 and sharply decreased to ~25 μM at the end of Phase III. Non-amended control and straw amended soils had similar pore water P levels that were initially 25 μM and increased to ~40 μM by week 4 and remained steady until they sharply decreased at the end of Phase III. These results are supported by a two-way repeated measures ANOVA that showed significant differences by treatment (F = 19.6, P < 0.0001) and phase (F = 25.2, P < 0.0001), but not their interaction (F = 2.2, P = 0.075). Post hoc tests revealed that the average pore water P was significantly higher (p < 0.05) in ashed straw and ashed husk treatments.

3.3. Soil GHG fluxes

Moisture phases and silica-rich rice residue treatments impacted soil GHG fluxes. Soil CO2 fluxes were the most variable and were relatively high during Phase I (maximum 4 μmol CO2 m−2 s−1 for straw–amended soils) before leveling off to a near zero flux during Phase II and then increasing during Phase III (maximum 2 μmol CO2 m−2 s−1 in ashed husk– and straw-amended soils) as the soils dried (Fig. 3A). These results are supported by a two-way repeated measures ANOVA that showed significant differences by treatment (F = 5.5, P < 0.0001) and phase (F = 137.6, P < 0.0001), and their interaction (F = 2.0, P —
0.042). Post hoc tests revealed significant differences (p < 0.05) showing that the average CO₂ efflux was highest from straw- and husk-amended soils, and was highest in Phase I and lowest in Phase II.

In contrast to CO₂, CH₄ fluxes were near zero during Phase I and the latter part of Phase III for all treatments, but deviated by treatment during Phase II and the start of Phase III (e.g., >50 nmol CH₄ m⁻² s⁻¹ from straw-amended soils) (Fig. 3B). During this time, straw amendment led to occasionally high soil CH₄ fluxes up to 200 nmol m⁻² s⁻¹ that were highly variable and were likely due to ebullition. In contrast, husk amendment led to low fluxes similar to ashed husk and ashed straw amendments and control until week 6 when a steady increase in soil CH₄ flux up to 50 nmol CH₄ m⁻² s⁻¹ was observed that lasted until the week 10, the beginning of Phase III (Fig. 3B). These results are supported by a two-way repeated measures ANOVA that showed significant differences by treatment (F = 2.4, P = 0.49) and the interaction between treatment and phase (F = 2.2, P = 0.028), but not with phase (F = 1.382, P = 0.252). Post hoc tests revealed that the average soil CH₄ flux was significantly higher (p < 0.05) in straw treatments than in ashed straw treatments, but did not significantly differ with phase.

Soil N₂O fluxes were variable and relatively high for straw amendments up to 22 nmol m⁻² s⁻¹ during Phase I and the beginning of Phase II, but for all amendments were low during the remainder of Phase II and increased to ca. 10 nmol m⁻² s⁻¹ during Phase III (Fig. 3C). A two-way repeated measures ANOVA showed significant differences by treatment (F = 4.8, P < 0.0001) and phase (F = 5.1, P = 0.006), but not their interaction (F = 1.4, P = 0.183). Post hoc tests revealed significant differences (p < 0.05) and show that the average soil N₂O efflux was highest in straw treatments compared to all other treatments and control, and was highest in Phase III.

3.4. Treatments and control GWP

Considering the sum of all three measured GHGs together over the entire experiment on a CO₂-equivalent basis, straw amendment led to the highest GWP observed in this study of 612 ± 76 g CO₂-eq m⁻², which was 228% higher than control (Fig. 4). Husk amendment led to GWP of 367 ± 42 g CO₂-eq m⁻², which was 40% less than straw but was 96% higher than control. Ashed husk and ashed straw amendments led to ca. 40% higher GWP than control but lower than fresh residues and were 251 ± 26 and 278 ± 28 g CO₂-eq m⁻², respectively (Fig. 4).

To capture the dynamics of GWP on a temporal basis, we report results per Phase. Using a two-way repeated measures ANOVA, significant differences were observed by treatment (F = 9.7, P < 0.0001), Phase (F = 37.7, P < 0.0001), and their interaction (F = 2.2, P = 0.028). Post hoc tests showed that GWP was highest in Phases II and III and was significantly higher in straw treatments than all other treatments and control. The increase in GWP relative to control due to straw was mostly as enhanced N₂O and CH₄ emissions during Phase II, but CO₂ emissions during Phases II and III also contributed (Table 2). In contrast, the increase in GWP due to husk was a result of enhanced CO₂ and CH₄ emissions during Phases II and III (Table 2). Contributions of N₂O and CH₄ for GWP was about an order of magnitude less from husk-amended soils compared to straw amendments during Phase II (Table 2). The increases in GWP for ashed husk and ashed straw amendments compared to control were mostly as increased CO₂ during Phases II and III and these amendments did not exhibit the increase in CH₄ emissions observed with the fresh residues.

4. Discussion

We sought to understand how soil incorporation of rice husk and rice straw residues would affect pore water chemistry and GHGs when amended soils that had been previously incubated under flooded conditions (Penido et al., 2016) are re-flooded and again dried. Incorporation of residues at levels used in these experiments (1% w/w) was
designed to provide soluble silica that could benefit plants for multiple growing seasons; thus, it is imperative to evaluate the impacts of that practice on pore water chemistry and GHG emissions over multiple wet-dry cycles.

Our results support our first hypothesis as rice husk incorporation led to the highest average pore water Si, which was 4-fold higher than control and 2-fold higher than straw incorporation (Fig. 2C). These pore water Si results are consistent with the findings reported in the initial incubation study (Penido et al., 2016). Taken together, these results indicate that soils amended with rice husk will have significantly higher concentrations of pore water Si available for plant uptake after a series of wet-dry cycles, more so than straw- or ash-amended soils. Soil incorporation of fresh husk into flooded rice soils has also resulted in a 25–50% decrease in inorganic As in rice grain without affecting dissolved CH4 levels in 3 rice cultivars (Seyffarth et al., 2016).

For all treatments and control, pore water Fe and As increased as soils were flooded due to reductive dissolution of Fe (oxyhydr)oxides and As release, and decreased as soils dried due to Fe oxidative precipitation and As adsorption (Fig. 2A–B) (Pedersen et al., 2006; Smedley and Kinniburgh, 2002). While it is possible that some Fe(III)–OM complexes contribute to the Fe signal (Steinmann and Sotyk, 1997), Fe(II) is the dominant form and thus high pore water Fe are an indication of reductive Fe dissolution (Ponnampерuma, 1972). In contrast to our hypotheses, average pore water As and Fe was unexpectedly similar when fresh husk- and rice straw-amended soils were re-flooded (Fig. 2A and B). In our previous work, Penido et al. (2016) observed 200–1600 μM Fe and 1–1.6 μM As in pore water upon straw incorporation and <100 μM Fe and 0.25 to 0.45 μM As upon husk incorporation. After the re-flooding and drying reported in the present study, pore water Fe and As reached a maximum of ~400 μM and ~0.8 μM, respectively, for both husk and straw amendments (Fig. 2A–B). These values are generally lower for straw amendment and higher for husk amendment than during the first flooding cycle (Penido et al., 2016). Similar to results from husk, incorporation of ashed husk and ashed straw also led to higher pore water Fe and As during re-flooding (average ~100 μM Fe and ~0.4 μM As, Fig. 2A–B) than the initial incubation (average ~ ~50 μM Fe and ~0.2 μM As, Penido et al., 2016). These data suggest that during the first incubation, the strong reducing conditions due to fresh straw amendment (Penido et al., 2016) drove Fe reduction, As release and reduction, and formation of volatile As compounds that likely left the system via outgassing (Huang et al., 2012). This resulted in lower pore water As concentrations than expected for straw-amended soils during re-flooding (Fig. 2B). However, the modest reducing conditions observed in the first incubation with husk or ash amended soils (Penido et al., 2016) did not favor As volatilization (Huang et al., 2012) and upon drying favored retention of As (adsorption/co-precipitation) onto neoformed Fe (oxyhydr)oxides that were readily released upon the re-flooding (Neubauer et al., 2008) in the present study (Fig. 2A–B). The lag in maximum pore water Fe and As in husk amended soils relative to straw amended soils may indicate differences in neoformed Fe minerals and/or mechanisms of As retention upon drying arising from differences in silica and organic matter (Jones et al., 2009) between husk and straw after the first incubation (Penido et al., 2016), but further research is needed in order to confirm that claim.

Our results partially support our second hypothesis as incorporation of rice straw led to the highest GHG emissions and consequently the largest GWP (Fig. 4). However, our observations do not support that the rice straw GWP increase would be mainly an effect of enhanced CH4 emissions due to strongly reducing conditions (Fig. 1, Table 2). The reducing conditions due to pre-incubated straw amendment reported here were not as strong as those observed in Penido et al. (2016). In fact, no significant differences in average Eh between treatments were observed in the present study (Fig. 1). This lack of strongly reducing conditions favored less CH4 production in straw-amended soils than was hypothesized. While CH4 emissions were important, N2O emissions contributed more to GWP for straw-amended soils in the present study (Table 2). Rice straw is known to have a high labile C content and upon soil incorporation and flooding results in a drop in soil redox potential (Wang et al., 1992). These strongly reducing conditions due to straw amendment typically increase CH4 emissions under continuous flooding (Bronson et al., 1997; Kimura et al., 1992; Kludze and Delaune, 1995; Wang et al., 1992; Wassmann et al., 2000; Wassmann et al., 2000; Yagi and Minami, 1990). However, our data (Figs. 1B and 4; Table 2) in conjunction with that of Penido et al. (2016) indicates that much of the labile C in fresh straw that typically results in strongly reducing conditions after soil incorporation and flooding is oxidized to CO2 and/or outgassed, particularly upon drying/drainage. This results in C loss from the soil. Noteworthy, rice farmers in Northern California, USA who incorporate fresh straw and flood fields during the winter fallow season observe rapid decomposition of the material when flooded soils are drained for field preparation (Fitzgerald et al., 2000). When these dried and pre-incubated soils are re-flooded, soil microorganisms have access to less labile pools of organic carbon, similar to those encountered when soils are amended with husk. This labile carbon limitation could also explain the similar extent of reductive
dissolution of Fe oxyhydr(oxides) and As release observed in pre-incubated straw and husk amended soils in the present study.

Regardless of the similar average Eh observed (Fig. 1B), we observed the largest cumulative GWP from straw-amended soils, which were 40% higher than those from husk-amended soils, 60% higher than those from ash-amended soils, and 70% higher than those from non-amended control soils (Fig. 4). The increase in overall GWP due to straw amendment was mainly due to larger N₂O fluxes during re-flooding (early Phase II), as well as smaller components from larger CH₄ fluxes during flooded periods and CH₄ and CO₂ fluxes at the onset of drainage (Table 2; Fig. 3). Recent work has highlighted the importance of measuring GHG emissions during end-of-season drainage, which shows that enhanced N₂O emissions occur when the percentage of water-filled pore spaces (WFPS) decrease (Adviento-Borbe et al., 2015). Because of a similar decrease in WFPS, mid-season drainage events also result in higher N₂O emissions (Towprayoon et al., 2005). Our data show that when pre-incubated soils are re-flooded, N₂O emissions are enhanced particularly within the first 1–2 weeks for soils amended with straw. Rice straw also has a higher N content and increases soluble organic N within the first 5 days of incubation, which stimulates microbial activity, N mineralization, and N₂O flux within the first 10 days of incubation (Luo et al., 2007). Some prior work has shown enhanced N₂O upon N fertilizer application, which was not as great as with straw amendment (Bronson et al., 1997; Sander et al., 2014). However, the frequency of sampling (i.e., once every one to two weeks) and the late initiation of sampling (i.e., typically after rice transplantation) may have missed spikes in N₂O emissions due to straw amendment as soils are re-flooded in prior work (Towprayoon et al., 2005). Similar to our findings, Linquist et al. (2015) observed elevated N₂O emissions before fields were flooded in Arkansas, USA rice soils into which the preceding crop residues had been incorporated.

Compared to straw, far fewer studies have been conducted on rice husk residue incorporation into paddy soil. Our previous work showed that soil incorporation of husk did not result in as strong of reducing conditions as straw incorporation in flooded soils, with consequently higher Eh and lower dissolved CH₄ levels (Penido et al., 2016). However, when those soils are re-flooded (in the present study), no difference in total CH₄ fluxes was observed between husk- and straw-amended soils (Fig. 4). Instead, higher N₂O and to a lesser extent CO₂ were responsible for the 2-fold higher GWP from straw amended soils compared to husk amended soils (Fig. 4). As discussed above, the partial decomposition of straw during the initial incubation and subsequent drying (during which labile C was likely converted to CO₂ and outgassed) rendered straw-amended soils more labile C-limited in the present study and on par with husk-amended soils. As a result, reducing conditions were similar between husk and straw amended soils in the present study (Fig. 1B). Thus, a similar extent methanogenesis was observed in husk- and straw-amended soils (Table 2, Fig. 3B).

Our results provide support for our third hypothesis that ashed residues would lead to the lowest GWP of amended soils (Fig. 4). Burning of rice straw and rice husk residues for an energy source is practiced commonly in Asia (Savant et al., 1997b). The burned residues that remain that we termed “ashes” are not true ashes, but incompletely combusted material that is concentrated in Si, As, and P, depleted in labile C, and do not affect soil pH upon incorporation (Fig. 1C). Burning of residues undoubtedly leads to increased emissions of C as CO₂ during incomplete combustion and leaves behind recalcitrant C (Gustafsson et al., 2009). These C emissions due to burning are not accounted for in the GWP estimates presented in this study. However, we can estimate C losses upon burning by using our measurements that burning 15 kg of fresh husk to make ash results in 9.35 kg of mass loss. Then, by applying the SOM = 1.72 × %TOC conversion factor used for soils, approximately 36% of the original 15 kg was lost as C. Thus, including the C lost during husk burning would yield C emissions that are roughly 36% higher than we measured here. Moreover, it is estimated that burning straw to make ashed straw results in 10% higher C emissions than burning husk to make ashed husk (Kajiura et al., 2015). Due to the loss of labile C during burning, it is no surprise that ashed amendments led to the lowest GWP of amended soils that we measured (Fig. 4). If these burning C losses were included, we might expect GWP from ashed residues to be comparable to fresh residues, but we would also need to account for C losses during the first incubation (Penido et al., 2016) and other C costs associated with transportation of the materials, and the impact that plants would have (e.g., root C exudation, C fixation) on the C cycle in these systems for a full life cycle assessment. Noteworthy, incorporation of these burned materials increased CO₂ emissions from soils compared to control (Fig. 4). We also observed higher dissolved P concentrations in ashed materials (Fig. 2D), which may have provided an advantage for CO₂-respiring aerobic and anaerobic microorganisms in ash-amended soils compared to control, and could also be plant-available (Ehlers et al., 2010). Finally, to fully address the long-term implications for GWP we must consider multiple flooding-drying cycles where the impact of initial C emissions by combustion of ashes are likely to be compensated by lower long-term GHG emissions as result of the chosen management practice.

While Ash was also concentrated in ashed residues, the rate of release was fastest during re-flooding to a maximum of ~0.6 to 0.7 µM As and then pore water As slowly declined throughout the flooded period, and was decoupled from pore water Fe (Fig. 2A and B). These observations indicate that a retention mechanism other than adsorption onto reducible Fe (oxyhydr)oxides was responsible for the retention of As in ash-amended soils upon drying in the initial study (Penido et al., 2016). This As was readily released upon initial re-flooding, but was slowly retained in the soil over time. These results will require further investigation across multiple rewetting and drying cycles to evaluate their persistence and the underlying mechanisms. Furthermore, burning of silica-rich rice residues, particularly at higher temperatures, enables crystallization (Savant et al., 1997b), which is less soluble than the poorly-crystalline opaline Si that forms in fresh tissues. This practice thus leads to a lower concentration of pore water Si compared to fresh residues upon incorporation (Fig. 2C and Seyfferth et al., 2016). Taken together, despite its ability to concentrate and release higher pore water P, the low pore water Si concentrations and decrease in air quality (Gustafsson et al., 2009) from rice residue burning released ashed residues not ideal to sustainably enhance Si for rice plants; however, it may be an effective P fertilizer. Furthermore, the ashed residues to decrease pore water As over time (Fig. 2B) may be due to adsorption onto the burned material and should be further explored.

5. Conclusions

While the results reported here are focused on soils without plants to avoid confounding effects, these data show that major biogeochemical differences exist among rice residues in terms of their potential for GHG emissions and cycling of arsenic and nutrients over recurrent flooding and drying cycles. Pre-incubation of straw appears to minimize As release and CH₄ emissions upon re-flooding likely because of As and C outgassing during the initial incubation, yet pre-incubation of husk does affect Si release. Husk amendment could thus be beneficial for plants even after recurrent flooding and drying cycles. Plants will undoubtedly affect GHG fluxes due to plant-mediated gas exchange through aerenchyma and C exudation into the rhizosphere, and will directly influence cycling of As and nutrients (i.e., Si) via plant-uptake. It is likely that increased plant nutrition due to increased Si from husk amendment will increase plant growth and thus the rate of plant-mediated gas exchange and C exudation from roots and plant uptake. Future research on rice husk incorporation should focus on plant-based studies at the field scale under natural weather variability with measurements of multiple GHGs. In particular, studies should focus on the wetting-up and flooded periods prior to transplanting, which we show is important for N₂O fluxes even when plants are absent and especially where pre-
incubated straw has been incorporated to test for long-term effects of this management practice.

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