

**IMPACT OF INORGANIC AND PLANT-BASED SILICON AMENDMENTS  
ON ARSENIC ACCUMULATION, YIELD, AND GREENHOUSE GAS  
EMISSIONS IN RICE (ORYZA SATIVA L.) UNDER ELEVATED SOIL  
ARSENIC CONDITIONS**

by

William Alfred Teasley IV

A thesis submitted to the Faculty of the University of Delaware in partial  
fulfillment of the requirements for the Master of Science in Plant and Soil Sciences

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## ABSTRACT

Arsenic (As) is a ubiquitous contaminant in the world's rice supply due to the practice of paddy (flooded) agriculture and addition of As to paddy soils through irrigation water and pesticide use. Both inorganic and organic forms of As contaminate rice, but inorganic As is a recognized human carcinogen and threatens human health accordingly. While organic As species are less toxic to humans, they are suspected agents of Straighthead disorder in rice, which reduces yield. Silicon (Si), a beneficial nutrient for rice that increases yield and decreases inorganic As accumulation in grain, could mitigate the negative impacts of As on rice production and human health. Although Si fertilization could be accomplished by using rice straw as a soil amendment, labile C additions (i.e., straw) to rice paddies have been implicated with increased grain As concentrations and CH<sub>4</sub> emissions. Recent work has demonstrated the potential of fresh rice husk (FH) as a Si-rich amendment that decreases grain As and without increasing CH<sub>4</sub> emissions. It is unknown, however, how FH and other Si-rich amendments would perform in a soil elevated in As. In this experiment, we evaluated FH, rice husk ash (RHA), and calcium silicate (CaSiO<sub>3</sub>) as soil amendments for their effects on Si fertilization, grain-As accumulation, rice yield, and emissions of CO<sub>2</sub> and CH<sub>4</sub> in a pot experiment utilizing a well-weathered (i.e., Si-depleted soil) contaminated with As and irrigated with As-contaminated water to simulate locales in South and Southeast Asia (e.g., Bangladesh). Using synchrotron techniques, we examined the impacts of the amendments on root Fe-plaque as a driver for differences between treatments in terms of As uptake. The results indicate that

using FH as an amendment holds promise for decreasing grain As and increasing rice yield in As-contaminated soils.

## **Chapter 1**

### **INTRODUCTION**

Arsenic (As) is a ubiquitous contaminant in the world's rice supply. Analyses of rice sourced from the global market indicate that contamination is not only widespread, but poses a risk to human health<sup>1-3</sup>. The underlying cause of contamination is the practice of paddy (flooded) cultivation, which mobilizes soil As through the reductive dissolution of Fe minerals<sup>4</sup> and the subsequent reduction of inorganic (i) pentavalent As (iAs(V)) to the more mobile trivalent As (iAs(III)), which ultimately increases As bioavailability in rice paddies. The problem is exacerbated in locales where groundwater contaminated with inorganic As (iAs) is used to irrigate rice, which results in elevated As in paddy porewaters<sup>5, 6</sup>. While both iAs species are observed in paddy porewaters and are taken up by rice roots, iAs(III) is predominant<sup>7</sup>. In addition to the inorganic forms, organic As species dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) may arise in rice paddy porewaters due to microbial methylation and be taken up by rice roots. These four forms are the dominant As species detected in paddy porewaters and rice grain.

Inorganic and organic As species vary in their toxicity to humans and plants, and their relative importance varies geographically. Inorganic As is a class 1 non-threshold carcinogen and is more toxic to human health than DMA and MMA (hereafter referred to collectively as oAs). Rice grown in Asia is predominantly contaminated by iAs, while grain from the United States is higher in DMA<sup>1, 8, 9</sup>; MMA is regarded as a minor constituent by comparison to iAs(III), iAs(V), and DMA<sup>10</sup>.

While iAs is more toxic to humans, it is not clear what the relative phytotoxicities of As species are<sup>11</sup>. In a hydroponic experiment comparing the relative toxicities of DMA and iAs(V) to rice, DMA accumulated in reproductive tissues causing deformation of florets and lower seed setting, while iAs(V) accumulated in vegetative tissue and did not affect yield<sup>12</sup>. In general, oAs is a suspected cause of Straighthead disease, a disorder of rice causing sterility and low grain yield<sup>10, 12</sup>. Straighthead is most prevalent in the south-central U.S., where As is thought to reside as a legacy contaminant from past use of As-based pesticides<sup>1, 10</sup>. Although oAs has been implicated as a contributing factor, Straighthead can occur without As contamination<sup>1, 10, 13, 14</sup>. Given rice's importance as a staple crop, strategies that improve yield and decrease risk to human health are critically needed.

Silicon (Si), which improves yield and decreases iAs uptake in rice, may alleviate the risk to human health and impact yield under elevated As conditions<sup>15-20</sup>. The beneficial effect of Si with respect to As accumulation in rice is attributed to a shared root-uptake pathway (i.e., Lsi1 and Lsi2) between the predominant form of Si (as silicic acid,  $\text{H}_4\text{SiO}_4^0$ ) and iAs(III) (as arsenous acid,  $\text{H}_3\text{AsO}_3^0$ ) in paddy porewaters<sup>21</sup>. Consequently, increasing plant-available Si in the soil solution will result in decreased iAs(III) uptake due to competition with Si. In addition, increasing Si will result in the down-regulation of Lsi transporters, which should decrease the uptake of oAs as well<sup>22, 23</sup>.

The effects of Si extend beyond internal plant processes. Altered Si can impact Fe (oxyhydr)oxide minerals that comprise root Fe-plaque, possibly affecting As retention in paddy soils<sup>24</sup>. While root Fe-plaque is comprised of many different Fe minerals, ferrihydrite is predominant and has a high sorption capacity for As relative

to higher-ordered phases (e.g. goethite, lepidocrocite, etc.)<sup>25-28</sup>. Overall, root Fe-plaques have the potential to immobilize As and occlude plant uptake<sup>26, 27</sup>. However, the availability of Fe<sup>2+</sup> under reducing conditions can induce progressive crystallization of Fe-plaque minerals to higher ordered phases, resulting in a loss of As sorption capacity<sup>29</sup>. Dissolved Si has been shown to stabilize ferrihydrite, preventing the development of higher ordered phases<sup>29-31</sup>, and thus could be an agent in enhancing As immobilization in rice paddy soil, preventing plant uptake.

Rice paddy soils in South and Southeast Asia are typically depleted in plant-available Si due to extensive weathering and rice cultivation with removal of Si-rich crop residues<sup>32, 33</sup>, which may impact As cycling. Rice straw and rice husk are Si-rich residues that could be used as Si sources, particularly for smallholder farmers, but addition of these materials to paddy soil results in different outcomes. Rice straw increases both grain iAs and DMA content due to increased microbial activity facilitating the mobilization and methylation of As<sup>19, 20, 34, 35</sup>, and also increases CH<sub>4</sub> emissions<sup>19, 36, 37</sup>. In contrast, amending rice paddies with fresh rice husk (FH) or rice husk ash (RHA) decreases inorganic As in grain by 25 – 50 % without affecting yield or CH<sub>4</sub> production in non-contaminated soil<sup>19, 20</sup>. However, the impact of FH and RHA on rice growing in contaminated soil (> 20 mg kg<sup>-1</sup> As) and receiving contaminated irrigated water has not been evaluated. Furthermore, it is unknown how the addition of FH and RHA compares to an inorganic Si source such as calcium silicate (CaSiO<sub>3</sub>), the principal Si component of furnace slags<sup>33, 38</sup>, which is an effective Si fertilizer of rice but has not been evaluated for its impact on As uptake<sup>15, 33, 39</sup>.

The objective of this study was to evaluate the impact of different Si-rich amendments on rice yield, As cycling, uptake and grain speciation, and greenhouse gas emissions under elevated soil As conditions. We hypothesized that a) the amendment which most effectively increases plant available Si will decrease plant accumulation of As, b) increased Si in porewater (soil solution) will stabilize ferrihydrite formation on rice roots and increase the sorption capacity of root Fe-plaque for As, and c) the emissions of CH<sub>4</sub> will increase with amendment C content. To test these hypotheses, rice was grown in a Si-depleted, As-contaminated soil to which FH, RHA, or CaSiO<sub>3</sub> were added and irrigated with As-contaminated water in a pot study in quadruplicate. Porewater chemistry and gas fluxes were monitored weekly during rice growth, after which plants were harvested at maturity and tissues were quantified for As species and plant nutrients. In addition, root Fe-plaque was analyzed for quantity and Fe mineralogy. We show that each Si amendment has varying effects on Fe plaque mineralogy, CH<sub>4</sub> production, and plant uptake of As.

## Chapter 2

### MATERIALS AND METHODS

#### 2.1 Soil Collection and Characterization.

A well-weathered soil depleted in plant-available Si was collected from the University of Delaware Research Farm in Newark, Delaware. The sampling site had been used as an orchard prior to 1950 and has been fallow for at least the past five years, allowing for the establishment of native grasses including N-fixing clover. The soil is classified as a fine-loamy, mixed, semiactive, mesic Typic Hapludult in the Elsinboro series by the U.S. taxonomic system<sup>40</sup> or as an Acrisol according to the Food and Agriculture Organization system. The soil was cleared of sod and sampled to a depth of 30 cm by digging 10 shallow pits. After collection, soil was stored in high-density polyethylene tubs and gently hand mixed, taking care to preserve soil structure as much as possible, over several weeks to allow for air-drying and ensure soil uniformity.

Subsamples of collected soil were analyzed for chemical characteristics. Soil was air dried and sieved (2 mm) to measure pH in a 1:1 soil-water slurry and ground for total C and N analysis (Elementar CHNS Cube). The soil had a pH (1:1) of 5.2 and total C and N of 1.4 and 0.13 %, respectively

To approximate plant available elements in soil,  $\text{CaCl}_2$ ,  $\text{BaCl}_2$ , acetic acid, and Mehlich-3 extractions were performed according to standard methods<sup>41, 42</sup> and analyzed with Inductively Coupled Plasma – Optical Emission Spectrometry and Mass spectrometry (ICP-OES and ICP-MS), the results of which are presented in Table 1.

Table 1: Average (+/- standard deviation, n = 3) extractable nutrients of untreated soil. Only one replicate used for Mehlich-3 extraction.

|           | <b>CaCl<sub>2</sub></b><br><b>(mmol kg<sup>-1</sup>)</b> | <b>BaCl<sub>2</sub></b><br><b>(mmol kg<sup>-1</sup>)</b> | <b>Acetic acid</b><br><b>(mmol kg<sup>-1</sup>)</b> | <b>Mehlich-3</b><br><b>(mmol kg<sup>-1</sup>)</b> |
|-----------|----------------------------------------------------------|----------------------------------------------------------|-----------------------------------------------------|---------------------------------------------------|
| <b>B</b>  | 0.24 ±0.01                                               | 0.27 ±0.006                                              | 0.54 ±0.03                                          | n.m.                                              |
| <b>Ca</b> | n.m. <sup>a</sup>                                        | 12 ±0.1                                                  | 12 ±0.2                                             | 11                                                |
| <b>Cu</b> | BDL <sup>b</sup>                                         | BDL                                                      | BDL                                                 | 0.04                                              |
| <b>Fe</b> | BDL                                                      | BDL                                                      | 0.03 ±0.001                                         | n.m.                                              |
| <b>K</b>  | 0.78 ±0.05                                               | 2.30 ±0.06                                               | 2.4 ±0.06                                           | 2.4                                               |
| <b>Mg</b> | 4.6 ±0.1                                                 | 5.20 ±0.2                                                | 5.6 ±0.06                                           | 5.3                                               |
| <b>Mn</b> | 0.08 ±0.002                                              | 0.15 ±0.004                                              | 0.26 ±0.005                                         | 0.54                                              |
| <b>Na</b> | 2.9 ±0.4                                                 | 4.60 ±0.6                                                | 3.0 ±0.2                                            | n.m.                                              |
| <b>P</b>  | BQL <sup>c</sup>                                         | BDL                                                      | 0.31 ±0.01                                          | 2.5                                               |
| <b>S</b>  | 0.41 ±0.02                                               | 0.37 ±0.01                                               | 0.38 ±0.02                                          | n.m.                                              |
| <b>Si</b> | 0.12 ±0.004                                              | 0.11 ±0.002                                              | 0.46 ±0.01                                          | n.m.                                              |
| <b>Zn</b> | 0.02 ±0.003                                              | 0.09 ±0.003                                              | 0.18 ±0.01                                          | 0.07                                              |

<sup>a</sup> n.m. - Not measured

<sup>b</sup> BDL - Below detection limit

<sup>c</sup> BQL - Below quantification limit

Table 2. Average (+/- standard deviation, n = 3) ammonium oxalate (AAO) and citrate-bicarbonate-dithionite (CBD) extractable Si, As, and Fe. Samples were analyzed by ICP-OES unless otherwise noted.

|           | <b>AAO (mmol kg<sup>-1</sup>)</b> | <b>CBD (mmol kg<sup>-1</sup>)</b> |
|-----------|-----------------------------------|-----------------------------------|
| <b>Si</b> | 2.11 ± 0.27                       | 4.33 ± 0.50                       |
| <b>Fe</b> | 28.2 ± 1.0                        | 148 ± 6.1                         |
| <b>As</b> | 0.0188 ± 0.0005 <sup>a</sup>      | 0.047 ± 0.002                     |

<sup>a</sup> analyzed by ICP-MS

Acid ammonium oxalate (AAO) and citrate-bicarbonate-dithionite (CBD) extractions were conducted according to standard methods<sup>42</sup> and analyzed via ICP-OES to quantify short-ordered and crystalline fractions of Fe, As, and Si and the results are reported in Table 2. In addition, untreated soil underwent a sequential extraction procedure (SEP)<sup>43</sup> to quantify As-soil phase associations prior to the addition of As.

## **2.2 Soil Spiking and Si Amendments.**

To simulate an As contaminated soil typical of South and Southeast Asia<sup>5, 6</sup>, soil was spiked according to the results of an adsorption isotherm (Figure 1) to achieve an initial porewater As level of 4  $\mu\text{M}$ . Arsenic, as Na-salts, was prepared as a solution with iAs(III) and iAs(V) in a molar ratio of 80:20, mixed into the soil, and allowed to equilibrate for several weeks, hand-mixing intermittently to ensure uniformity, per previous work<sup>16</sup>. An As loading rate of 0.125  $\mu\text{mol g}^{-1}$  was determined to yield an initial porewater concentration of 4  $\mu\text{M}$  at the beginning of the experiment.

Each treatment received one type of silicon-rich amendment: ground and fresh rice husk (FH), rice husk ash (RHA), or  $\text{CaSiO}_3$ . The control group was spiked with As but had no added Si. For comparison of yield and biomass, a set of plants was grown in untreated soil irrigated with deionized water in quadruplicate. Both FH and RHA had been obtained from a rice milling factory in Battambang, Cambodia and each were applied at a rate of 1% (w/w) amendment/soil per previous work, which demonstrated equivalent porewater concentrations of amendment-derived As and Si at that loading<sup>19, 20</sup>.  $\text{CaSiO}_3$  was amended at 0.46% (w/w), a rate equivalent to the Si in the FH treatment (Table 3); this rate equates to 5  $\text{Mg ha}^{-1}$ , an application rate previously shown to benefit rice plants<sup>15</sup>. After mixing each Si amendment

thoroughly, the soil in each group was divided equally by weight into 4 L pots that each received 3.5 kg dry weight (d.w.) of As-spiked soil with 4 replicate pots per Si treatment group and +As control.

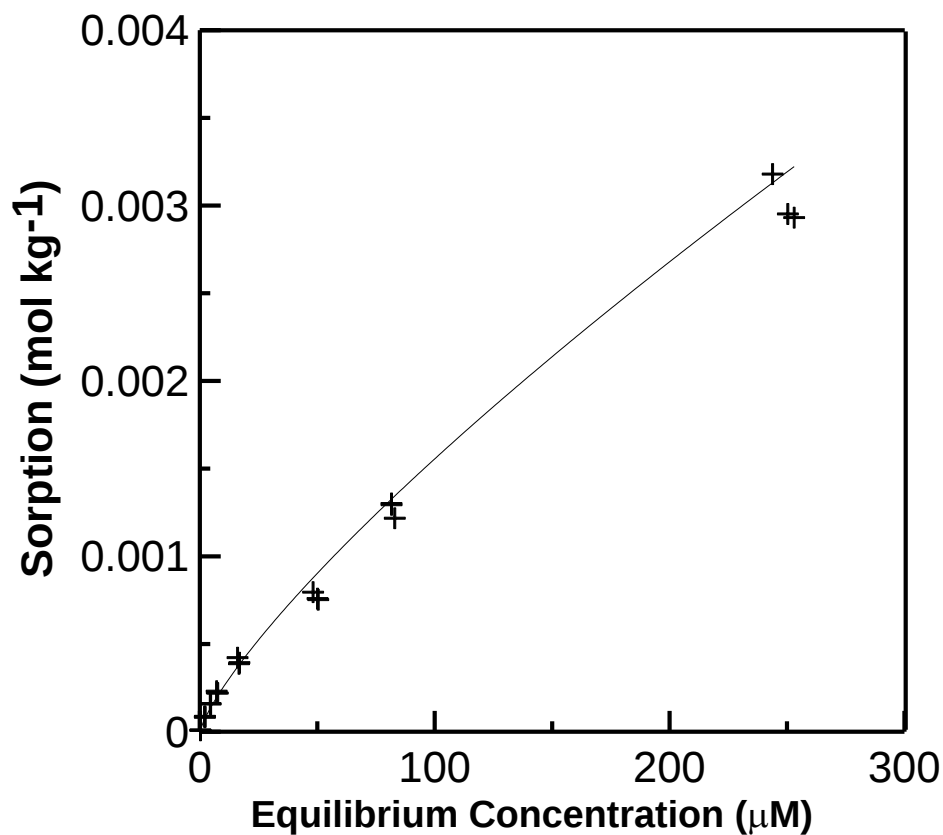


Figure 1: Adsorption isotherm of untreated soil to determine appropriate As loading for soil spiking. Solutions of 0.5 – 350  $\mu\text{M}$  As (80:20 As(III):As(V) molar ratio) were mixed 30:1 (w/w) with soil in an acetate buffered solution (pH 6) for 24 h.

Table 3 Elemental analysis of fresh husk (FH) and rice husk ash (RHA) used as amendments. Average of 3 replicates (unless otherwise indicated) and standard deviation are reported when applicable. Samples were acid digested and analyzed by ICP-OES unless specified otherwise.

|                       | Fresh Husk (FH)          | Rice Husk Ash (RHA)      |
|-----------------------|--------------------------|--------------------------|
|                       | (mmol kg <sup>-1</sup> ) | (mmol kg <sup>-1</sup> ) |
| <b>As<sup>a</sup></b> | 0.0035 ± 0.0001          | 0.0067 ± 0.0003          |
| <b>Ca</b>             | 15.4 ± 2.6               | 45.5 ± 2.3               |
| <b>Fe<sup>b</sup></b> | 6.7 n/a                  | 14.0 n/a                 |
| <b>K</b>              | 44.7 ± 5.5               | 196 ± 7.1                |
| <b>Mg</b>             | 12.4 ± 1.8               | 36.7 ± 2.1               |
| <b>Mn</b>             | 3.8 ± 0.5                | 8.2 ± 0.4                |
| <b>Na</b>             | 1.3 ± 0.2                | 4.5 ± 0.4                |
| <b>P</b>              | 6.0 ± 0.4                | 47.3 ± 2.6               |
| <b>S</b>              | 14.5 ± 0.7               | 9.2 ± 0.2                |
| <b>Zn</b>             | 0.20 ± 0.02              | 0.28 ± 0.01              |
| <b>Si<sup>b</sup></b> | 4020 (n/a)               | 13200 (n/a)              |

<sup>a</sup>analyzed by ICP-MS

<sup>b</sup>analyzed by XRF (n=1)

### 2.3 Plant Growth and Porewater Monitoring.

Plants were grown in a climate controlled growth chamber using LED growth lights (Lumigrow, Novato, CA) set to a 16 hour photoperiod. Humidity was set at 70% and temperature was set to a 28°/ 22°C day/night cycle. Rice seeds (*Oryza sativa* L., cv. M206, medium grain Calrose rice, *japonica*) were sterilized in a dilute bleach solution, rinsed, and germinated for 10 days in untreated soil. Ten day old seedlings of a similar height were transplanted into the pots, three seedlings per pot, and each pot was fertilized with 120 mg nitrogen (as 0.4 M NH<sub>4</sub>NO<sub>3</sub>) and immediately flooded with deionized water to a height of 5 cm above the soil surface. After 1 week, DI water was replaced by 4 µM As solution (80:20 iAs(III)/ iAs(V)) to simulate

contaminated irrigation water of a moderately contaminated rice paddy in South Asia<sup>5, 16</sup>. The plants were grown to maturity and harvested after 120 days.

Porewater was drawn from soil weekly using Rhizon samplers (10 cm, 0.2  $\mu\text{m}$  pore size, Rhizosphere Research Products, Wageningen, The Netherlands) inserted in the soil at a 45° angle fixed to a needle and stop-cock assembly per previous work<sup>16, 19, 20</sup>. Porewater was collected into acid-washed, crimp-sealed vials that were flushed with  $\text{N}_2$  and evacuated immediately prior to sampling. Aliquots of porewater were used to measure pH, Eh, and concentrations of  $\text{Fe}^{2+}$ , As species, and total elements (ICP-MS and ICP-OES). All analyses and sample processing occurred within an hour of sampling. Samples for As speciation were frozen, while samples for total element analysis were acidified with trace-metal grade (TMG)  $\text{HNO}_3$  and stored until analysis.  $\text{Fe}^{2+}$  was determined using the colorimetric ferrozine method<sup>44</sup>.

Arsenic speciation was determined for select porewater samples that had been frozen just after collection. Prior to analysis, samples were thawed at room temperature, acidified 1:1 with 2% TMG  $\text{HNO}_3$ , filtered through a 0.2  $\mu\text{m}$  nylon syringe filter, and analyzed within 24 hours. An Agilent 1200 HPLC and PRP-X100 column was used to separate As species that were detected with an Agilent 7500cx Series ICP-MS. Matrix-matched standards, blanks, and duplicates were included to ensure quality assurance. Only two samples per treatment per time point were analyzed and were selected to capture time-series of As dynamics in porewater based on results from higher-throughput total As analyses.



Figure 2: A dark gas flux chamber was constructed using 8 inch diameter PVC pipe, a PVC pipe cap, and push-connect fittings (McMaster-Carr, Robbinsville, NJ). Three 12-volt computer fans were secured to the inside of the chamber arranged in an X-Y orientation and powered by an AC adapter. Junctions were sealed with neoprene and silicone sealant.

## 2.4 Gas Flux Measurements.

An Ultraportable Los Gatos Greenhouse Gas Analyzer (LGR, Los Gatos Research, St Clara, CA) was used in conjunction with a custom-built dynamic flux chamber (Figure 2) to monitor weekly CO<sub>2</sub> and CH<sub>4</sub> flux in the dark. The LGR can measure CH<sub>4</sub> concentrations from 0.01 to 100 ppm ( $\pm 0.002$  ppm) and CO<sub>2</sub> concentrations from 1 to 20,000 ppm ( $\pm 0.3$  ppm) at 1 Hz. The pot-chamber-LGR formed a closed system where accumulating gases within the chamber were measured in real-time by the LGR over 3 minutes. Due to a large chamber size (0.045 m<sup>3</sup>), a 45 s deadband was removed and the remaining 135 s was used to calculate flux with the following equation<sup>45</sup>:

$$fCx = \left(\frac{dC}{dt}\right) \left(\frac{V_c}{A_c}\right) \left(\frac{P}{R * (T_s + 273.15)}\right)$$

where  $fCx$  is the flux of  $CO_2$  or  $CH_4$ ,  $dC/dt$  is the change in concentration with time measured by the LGR ( $ppm\ s^{-1}$ ),  $V_c$  is the system volume ( $0.045\ m^3$ ),  $A_c$  is the area of the pot soil ( $0.0248\ m^2$ ),  $P$  is atmospheric pressure ( $101.325\ kg\ m\ s^{-2}$ ),  $R$  is the ideal gas law constant ( $0.00831447\ kg\ m^2\ \mu mol^{-1}\ K^{-1}\ s^{-2}$ ), and  $T_s$  is the temperature of standing water within the pots in  $^{\circ}C$ . Flux values were calculated using a python script. Linear regressions were fit to the flux data and were utilized only when  $R^2$  was at least 0.95 and  $P < 0.05$ ; following approaches used previously<sup>46</sup>. Using this approach, 95% of the measurements were utilized throughout the 120-day experiment.  $CH_4$  and  $CO_2$  emissions were calculated over 120 days of rice growth by linear interpolation of the sampled emissions assuming emissions followed a linear trend between measurements.

## 2.5 Plant Harvest and Analysis.

At grain maturity, rice was harvested by cutting straw above the water inundation line approximately 5 cm from the soil surface. Panicles were cut from stems, and the rough rice (grain + husk) was removed and weighed. Root systems were manually separated from the bulk soil, taking care to leave roots intact, then carefully washed to remove soil particles and allowed to air dry. Dry weights of straw and roots were obtained. Root systems were then separated longitudinally in half: one half underwent a DCB extraction to remove Fe plaques and plaque-free roots utilized for total elemental analysis, and the other half was utilized for Fe mineral characterization (described in the next section) according to previous work<sup>24, 27, 47</sup>.

After DCB extraction, plaque-free roots were rinsed clean and were oven dried along with straw prior to homogenization and elemental analyses.

Rough rice was removed from panicles, weighed, and de-husked using a bench-top de-husker, leaving unpolished grain and husk to be analyzed separately. Unpolished grain, husk, straw, and plaque-free roots were ground in stainless steel grinders and microwave digested in concentrated TMG HNO<sub>3</sub> and analyzed for total elemental concentrations<sup>16, 20</sup>. Arsenic was quantified using ICP-MS while plant nutrients (Fe, P, etc.) were quantified using ICP-OES. Undigested Si gels that formed after straw, husk, and root digestions were triple washed with DI water, dissolved in 2 M NaOH, and quantified for Si concentration using a colorimetric method<sup>20, 48, 49</sup>. Grain As species were extracted using the methods of Maher et al.<sup>50</sup>, which involved microwave-assisted extraction in 2% TMG HNO<sub>3</sub> at 100° C. Straw C and N were quantified using an Elementar Variomax CN analyzer (Elementar Americas, Mt. Holly, NJ). In addition to plant analyses, soil samples were taken from the pots at harvest and air-dried in an anaerobic chamber. A sequential extraction procedure (SEP) was performed to quantify specific phase associations of soil As post-harvest<sup>43</sup>.

Sample sets were accompanied by a National Institute of Standards and Technology standard reference material (SRM) rice flour (1568a) for which ICP-OES recoveries for Ca, K, P, S were 105%, 93%, 100% and 95%, respectively, and ICP-MS recoveries for total As were within 5% of the certified values (Table 4). While certified speciation results are not reported for SRM 1568a, recoveries for iAs(III), DMA, and iAs(V) were 116, 124, and 42%, respectively of the values reported in Maher et al.<sup>50</sup>. Despite poor comparison of our iAs(V) to that of Maher et al.<sup>50</sup>, species sums of grain samples from our study were within +/- 10% of total As values,

and because our total grain As in the SRM had excellent recoveries of 95-105% and most of the grain As in our samples were comprised of DMA and iAs(III), robust data quality in As speciation was achieved.

Table 4: Average recoveries (+/- standard deviation) of National Institute of Standards and Technology (NIST) standard reference material 1568a (rice flour) using trace metal grade HNO<sub>3</sub> followed by analysis with ICP-OES (unless specified otherwise).

| <b>Element</b> | <b>mg kg<sup>-1</sup></b>  | <b>Recovery (%)</b> | <b>n</b> |
|----------------|----------------------------|---------------------|----------|
| <i>As</i>      | 0.292 ± 0.011 <sup>a</sup> | 100                 | 8        |
| <i>Ca</i>      | 124 ± 1.1                  | 105                 | 2        |
| <i>K</i>       | 1180 ± 30                  | 93                  | 2        |
| <i>Mg</i>      | 482 ± 2.4                  | 86                  | 2        |
| <i>Mn</i>      | 18.7 ± 0.16                | 93                  | 2        |
| <i>P</i>       | 1530 ± 23                  | 100                 | 2        |
| <i>S</i>       | 1140 ± 31                  | 95                  | 2        |
| <i>Zn</i>      | 18.5 ± 1.2                 | 95                  | 2        |

<sup>a</sup> analyzed with ICP-MS

## 2.6 Iron Plaque and Synchrotron Analyses.

To investigate the role of Fe-plaque as a driver for As uptake dynamics, DCB extractions were performed on one half of the root system to quantify elemental concentrations of Fe, As, Si, and P associated with Fe-plaque<sup>51</sup>, and the other half was utilized for Fe mineral and As species characterizations with synchrotron-based Fe and As X-ray absorption spectroscopy (XAS) and X-ray diffraction (XRD)<sup>47</sup>. To prepare samples for synchrotron analysis, root halves from each pot were sonicated in cool water (ca. 20 kHz and 20°C) and the resulting solution was vacuum filtered through a 0.2 µm nitro cellulosic filter membrane to collect and concentrate intact Fe (oxyhydr)oxide plaques so they could be directly positioned in front of the X-ray

beam. Samples were analyzed at the Stanford Synchrotron Radiation Lightsource (SSRL). XRD (beamline 11-3) was used to investigate mineral composition, using an incident energy of 12700 eV ( $\lambda = 0.976 \text{ \AA}$ ) and a MAR 345 Image Plate Detector. Detector parameters were calibrated with lanthanum hexaboride and the resulting diffractograms were integrated in Q space between 0 and 6  $\text{\AA}$  at with the wxdiff software. Nitro cellulosic filter paper was used as a blank. Match! software<sup>52</sup> was used to subtract the signal of the blank from the samples and to find peak correlations. In this way, several Fe mineral phases were identified to constrain fitting parameters for X-ray absorption spectroscopy analysis.

To further elucidate the mineral composition of Fe-plaque, samples were characterized by Extended X-ray Absorption Fine Structure (EXAFS) spectroscopy at SSRL on beamlines 11-2 and 4-3. The monochromator was calibrated with an Fe foil and the first inflection point was assigned to 7112.0 eV. Iron k-edge spectra were obtained with a Lytle detector from 200 eV below the absorption edge to k values of 15  $\text{\AA}^{-1}$ . Three spectra were collected and averaged per sample. These averaged spectra were background-subtracted, normalized, and transformed to chi space ( $k$ -weight = 3) using Sixpack software<sup>53</sup>. Linear combination fitting of  $k^3$  weighted EXAFS spectra in the range of 2-12  $\text{\AA}^{-1}$  was conducted utilizing results from XRD analysis including ferrihydrite, which is poorly resolved via XRD analysis due to its nanocrystalline structure<sup>54</sup>. Information regarding standard preparation can be found in Hansel et al<sup>55</sup>.

In addition to Fe mineral composition, As speciation in plaque samples was determined using X-ray absorption near edge structure (XANES) on beamline 11-2 at SSRL. An ACS certified sodium arsenate standard diluted in boron nitride was used

to calibrate the incident energy of the beam and was assigned its first inflection point at 11874.0 eV. XANES spectra were obtained from 200 eV below the absorption edge to 350 eV above the edge in 8 minute scans and fluorescence signal intensity was monitored with a 100-element Germanium detector. Three repeated scans showed no signs of beam damage; the collected XANES spectra were averaged, background-subtracted, normalized and analyzed by linear combination fitting in the edge region 11800-12000 eV using Sixpack software<sup>53</sup>. Standards used for linear combination fitting included iAs(III) as NaAsO<sub>2</sub> and iAs(V) as Na<sub>3</sub>AsO<sub>4</sub>.

## **2.7 Statistical Analyses.**

SAS 9.4 was used to test for significant differences between treatments in elemental concentrations of plant-digests, porewater, and DCB extracts as well as biomass, grain yield, CH<sub>4</sub>, and CO<sub>2</sub> flux using Proc GLM. If significant differences ( $P < 0.05$ ) were identified, a Tukey or Tukey-Kramer post-hoc test was used to distinguish differences between the treatments. In addition, the univariate procedure was used to verify the data for satisfying the assumptions of ANOVA (i.e., normality and homoscedasticity).

## Chapter 3

### RESULTS

#### 3.1 Grain Yield and Plant Biomass.

Grain yield was significantly different between treatments ( $F = 38.08$ ,  $p < 0.0001$ ). Spiking soil with As resulted in a 89% yield loss in the control compared to the untreated soil, which was not alleviated by the  $\text{CaSiO}_3$  amendment (Figure 3). In contrast, the FH and RHA treatments improved yields to untreated levels despite As addition (Figure 3). The control and  $\text{CaSiO}_3$  treatment showed symptoms of Straighthead to a greater degree, with average % (+/- standard deviation) of unripe grain at 37 (+/- 25) and 73 (+/- 14) %, respectively, compared to the FH (4 +/- 2%) and RHA (13 +/- 10%) treatments.

Straw biomass differed between treatments ( $F = 26.44$ ,  $p < 0.0001$ ), with the  $\text{CaSiO}_3$  treatment having more straw biomass than the other treatments and control (Figure 4). Root biomass did not differ between treatments and control (data not shown).

#### 3.2 Porewater pH, Eh, and Fe.

The amendments had varying effects on porewater pH, Eh, and Fe concentrations (Figure 5). With the onset of irrigation, all pots exhibited a spike in pH before stabilizing to near neutral levels by week 5 (Figure 5A). From week 10-onward, the  $\text{CaSiO}_3$  treatment exhibited an aberrant trend, increasing in pH until plant maturity. Eh potentials dropped with the onset of flooding to ca. 100 mV by week 4

and remained within 100-150 mV until a spike at week 13 (Figure 5B). The  $\text{CaSiO}_3$  treatment had higher pH and lower Fe ( $\text{Fe}^{2+}$  and total) relative to the other treatments throughout rice growth.

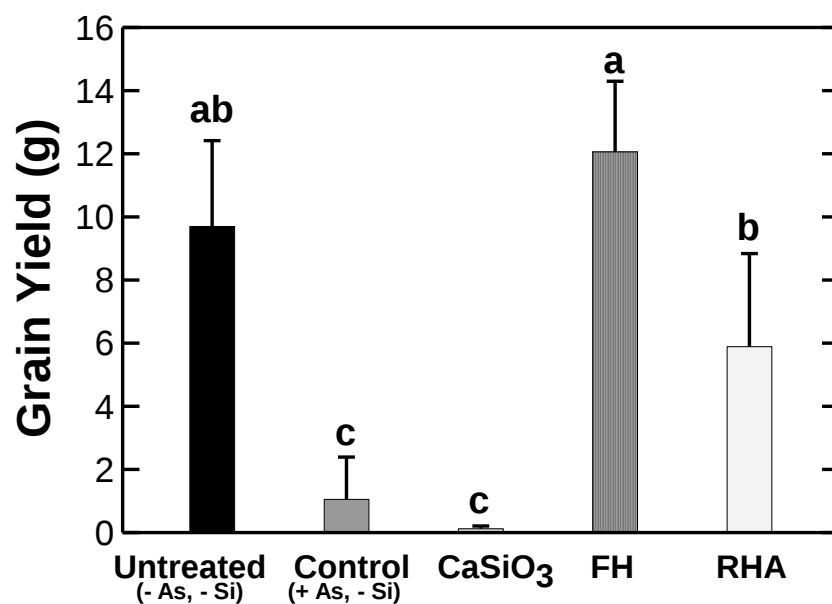


Figure 3: Average (+/- standard deviation (SD)) rough grain yield per pot (weight of ripe grains + husk) of rice grown in untreated (no added As or Si), control (added As but no Si amendment),  $\text{CaSiO}_3$  amended, fresh husk (FH) amended, or rice husk ash (RHA) amended soil under elevated As conditions. Rice growth (M-206 variety) occurred over the full 120 day lifecycle. Letters denote significant differences ( $p < 0.05$ ) between treatments.

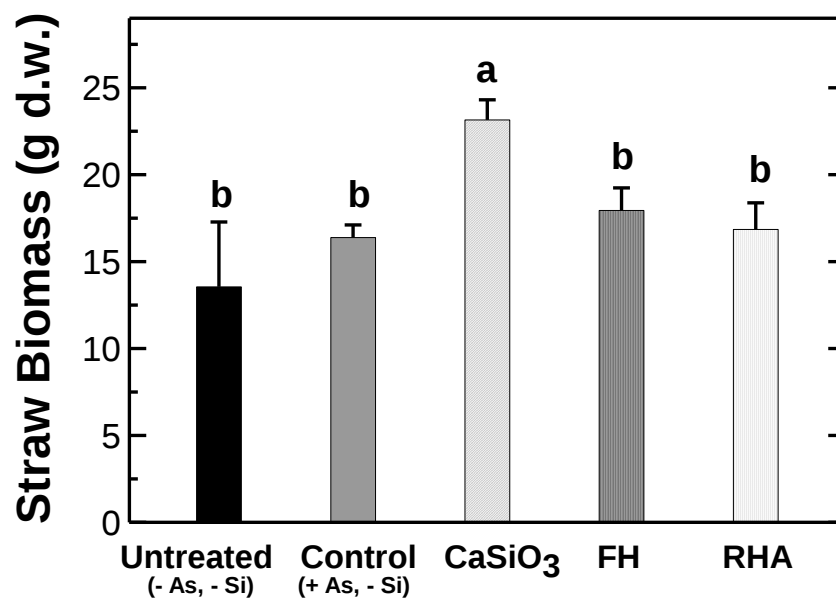


Figure 4: Average ( $\pm$  SD,  $n=4$ ) straw biomass (per pot) of rice grown in untreated (no added As or Si), control (added As but no Si amendment),  $\text{CaSiO}_3$  amended, fresh husk (FH) amended, or rice husk ash (RHA) amended soil under elevated As conditions. Letters denote significant differences ( $p < 0.05$ ) between treatments.

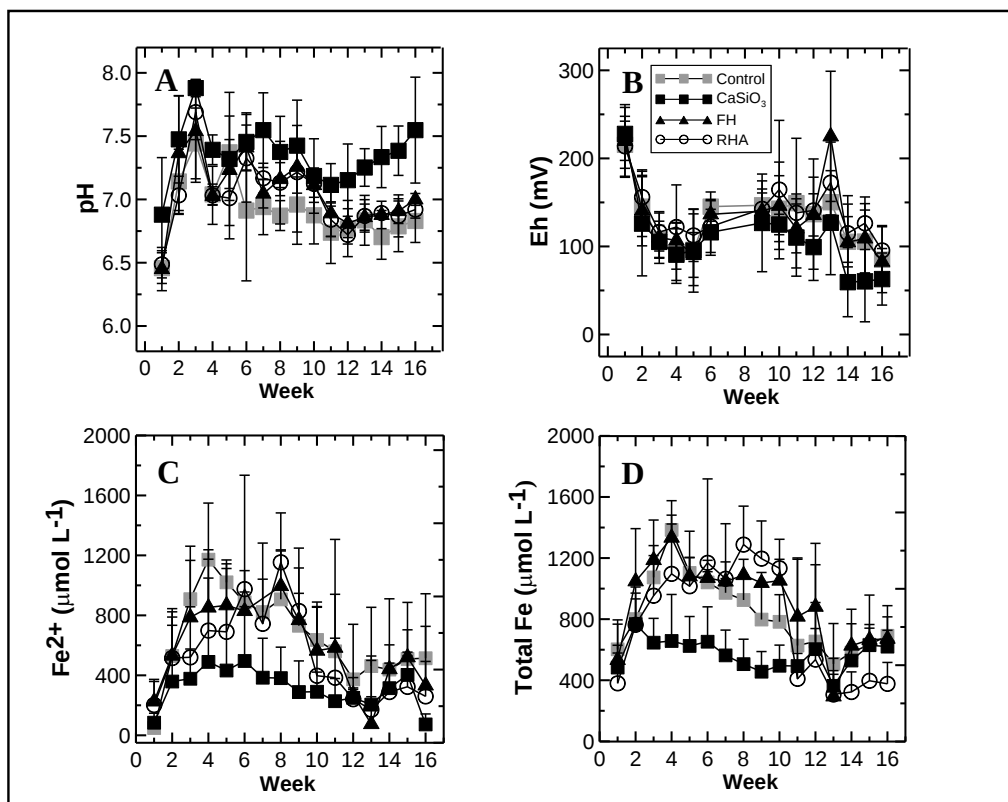


Figure 5: Average ( $\pm$  95% confidence intervals (CI),  $n = 4$ ) porewater pH (A), redox (B), and concentrations of  $\text{Fe}^{2+}$  (C) and total Fe (D) for paddy rice exposed to different Si treatments under elevated soil As.

### 3.3 Plant and Porewater Si.

Clear differences in porewater Si were observed between treatments and control (Figure 6A). The  $\text{CaSiO}_3$  and FH treatments exhibited elevated Si levels, which differed on a temporal basis. The  $\text{CaSiO}_3$  treatment exhibited a quick-release behavior, with initially the highest Si concentrations of 750  $\mu\text{M}$  that plummeted to ca. 175  $\mu\text{M}$  by week 5. In contrast, porewater Si in the FH treatment slowly increased from an initial ca. 200  $\mu\text{M}$  to a high of 400  $\mu\text{M}$ , and remained elevated until week 10,

after which porewater Si concentrations dropped to near control levels. The drop in Si coincided temporally with a drop in porewater pH. Despite higher concentrations of Si in the RHA amendment than FH (Table 3), RHA porewater Si levels were indiscernible from the control and were both low throughout the experiment.

The treatments significantly affected the plant concentrations of Si in husk ( $F = 17.6$ ,  $p = 0.0002$ ) and straw ( $F = 139$ ,  $p < 0.0001$ ) but not roots. The FH treatment led to highest concentrations of Si in husk and straw, followed by the  $\text{CaSiO}_3$  treatment, which were both significantly higher than the control and RHA treatment (Figure 6B).

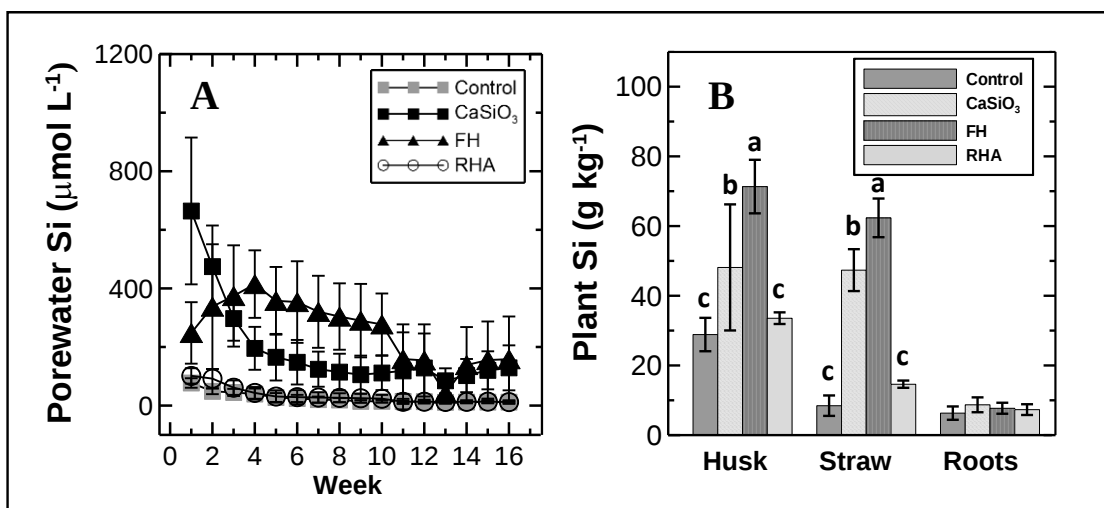


Figure 6: Average ( $\pm$  95% CI,  $n = 4$ ) porewater Si concentrations (A) throughout the growing period for paddy rice exposed to different Si treatments under elevated soil As. Plant Si concentrations ( $\pm$  SD,  $n=4$ ) in husk, straw, and roots (B). Letters denote significant differences ( $P < 0.05$ ) between treatments.

### 3.4 Soil, Porewater, and Plant As.

A post-harvest SEP indicated that the majority of spiked As was associated with poorly crystalline Al/Fe-oxides followed by specifically and non-specifically

sorbed phases, which increased in As content by 3.4-3.6, 5.7-6.7, and 16-18-fold relative to the untreated soil, respectively (Figure 7). Soil-spiking increased crystalline Al/Fe-oxide phase As only slightly by comparison, and residual phases were unchanged. Significant differences were found between post-experiment soils in the specifically sorbed ( $F = 10.2$ ,  $p = 0.001$ ) and crystalline Al/Fe-oxide phases ( $F = 5.71$ ,  $p = 0.01$ ), with the  $\text{CaSiO}_3$  treatment having higher crystalline Al/Fe-oxide phase As compared to RHA, and higher specifically sorbed phase As than the FH and RHA treatments.

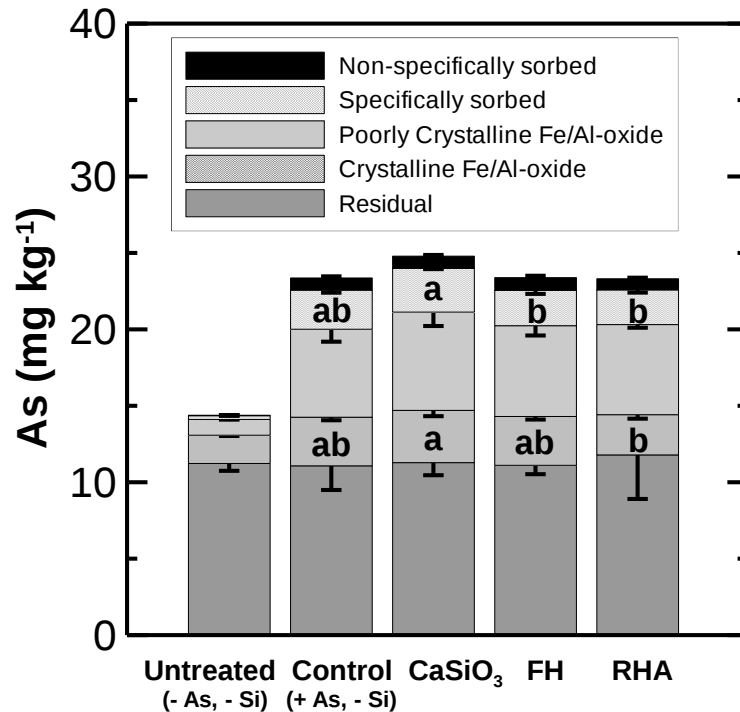


Figure 7: Average extractable As ( $\pm$  SD,  $n = 2$ ) from different soil-As phases of untreated (no added As) soil and post experiment soil for each treatment group. Significant differences ( $p < 0.05$ ) between treatments for specific soil phases are indicated by letters and were tested only for post-experiment soils. As-phases with no lettering were found to have no significant differences between treatments.

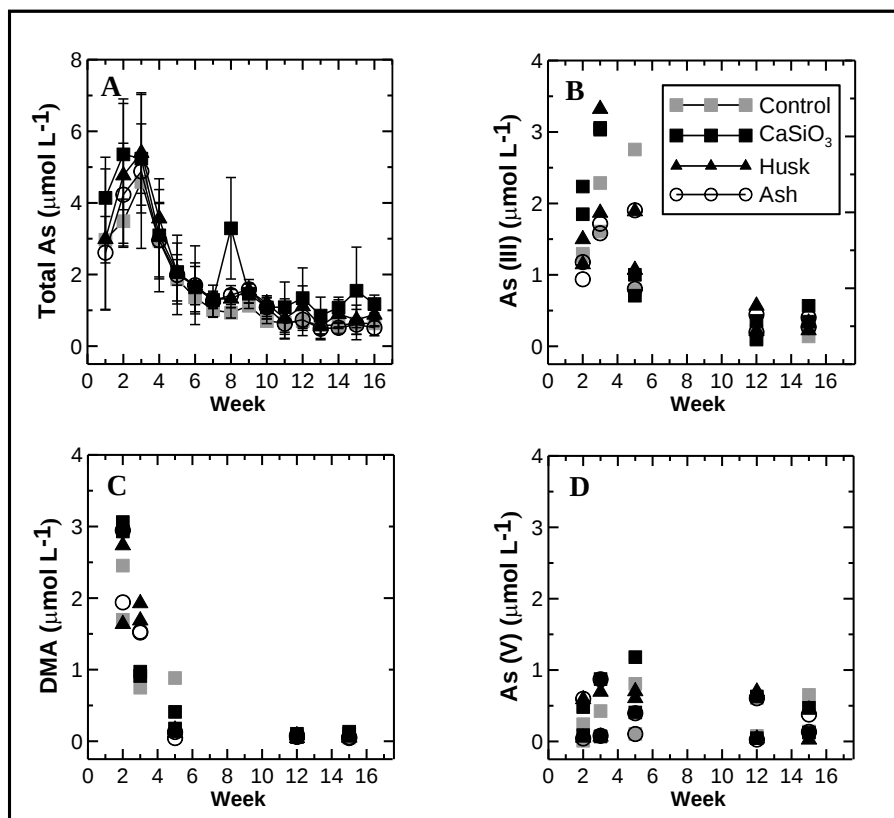


Figure 8: Porewater concentrations of total As (A), iAs(III) (B), DMA (C), and iAs(V) (D). Total As expressed as average of 4 replicates per treatment group ( $\pm$  95% CI). As species expressed as individual measurements ( $n$  = 2 per treatment per sampling date).

Porewater As was similar between treatments (Figure 8). Spiking soil with As was intended to yield an initial porewater As concentration of ca. 4  $\mu$ M, which was observed for all treatments within  $\pm$ 1  $\mu$ M in the initial four weeks (Figure 8A). Total As concentrations dropped to ca. 1  $\mu$ M by week 6 and remained at that level until harvest, despite irrigation with 4  $\mu$ M As. DMA and iAs(III) were predominant initially, while iAs(III) and iAs(V) dominated in the latter part of the experiment

(Figure 8B-D). MMA was detected only in the  $\text{CaSiO}_3$  treatment and only at weeks 3 and 5 at low levels (ca.  $0.05 \mu\text{M}$ , data not shown).

Soil amendments had contrasting effects on As storage in plant organs. Total As in grain was significantly different ( $F = 22.03$ ,  $p < 0.0001$ ) among treatments with the FH treatment having 40% lower As in grain compared to control (Figure 9A). In addition, the  $\text{CaSiO}_3$  treatment had higher grain As than the RHA treatment. Significant differences in As concentrations were also observed in husk ( $F = 14.65$ ,  $p = 0.01$ ), straw ( $F = 18.34$ ,  $p < 0.0001$ ) and plaque-free roots ( $F = 25.29$ ,  $p < 0.0001$ ) between treatments and the control (Figure 9B). Total As in husk was 60% lower in the FH treatment relative to the control. The RHA treatment had significantly higher straw As than the FH and  $\text{CaSiO}_3$  treatments, and the control roots had ca. 50% higher root As relative to the average concentration of the other treatments.

Similar to total As, each Si amendment had different impacts on grain As speciation, with significant differences in concentrations of DMA ( $F = 8.40$ ,  $p = 0.01$ ) and iAs(III) ( $F = 7.71$ ,  $p = 0.01$ ) (Table 5). The majority of the decrease in FH grain total As was due to a decrease in DMA. Although FH-treated plants had lower grain iAs(III) than control, this was not statistically significant. In contrast, the RHA-treatment led to the highest grain iAs(III). For all treatments, speciation analysis of unpolished grain indicated DMA as the predominant species (74-87%), followed by iAs(III) (12-25%) and iAs(V) (ca. 1%, not shown).

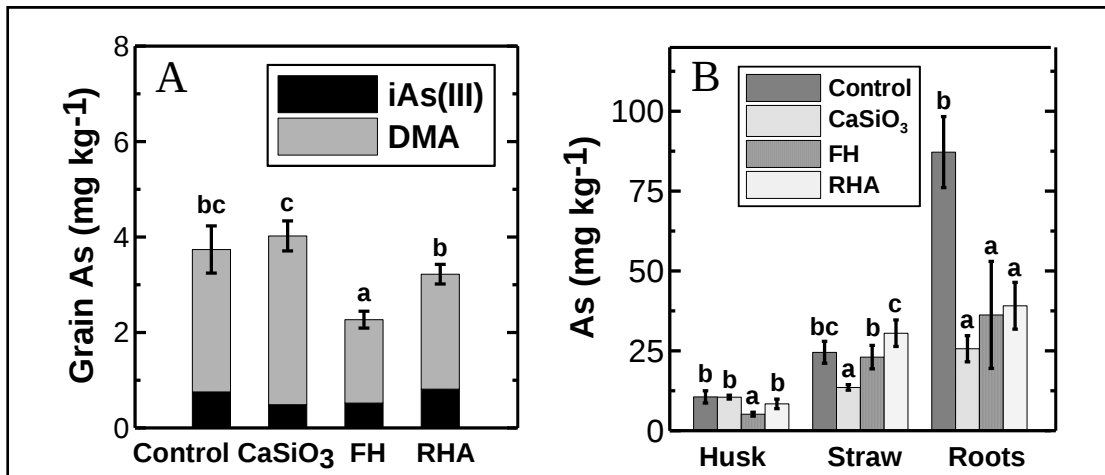


Figure 9: Average total As concentrations (+/- SD, n = 4) in unpolished grain (A) and husk, straw, and roots (B). Grain yield in one CaSiO<sub>3</sub> replicate was so low that it could not be included in the analysis, and thus the CaSiO<sub>3</sub> average only includes 3 measurements. Grain As bar series indicate average As species concentration determined for each treatment and the control (see Table 5 for averages and error associated with species determinations). Letters denote significant differences (p < 0.05) between treatments.

Table 5: Average grain As species (+/- SD) of rice grown under elevated As conditions in soils amended with either CaSiO<sub>3</sub>, fresh husk (FH), rice husk ash (RHA), or a non Si-amended control. Low grain yield in the control and CaSiO<sub>3</sub> treatment led to some replicates not being represented (indicated by n).

| Treatment          | iAs(III) mg kg <sup>-1</sup> | DMA mg kg <sup>-1</sup>    | n |
|--------------------|------------------------------|----------------------------|---|
| Control            | 0.70 (± 0.16) <sup>ab</sup>  | 2.72 (±0.58) <sup>b</sup>  | 3 |
| CaSiO <sub>3</sub> | 0.52 (n/a)                   | 3.69 (n/a)                 | 1 |
| FH                 | 0.49 (± 0.08) <sup>a</sup>   | 1.67 (± 0.14) <sup>a</sup> | 4 |
| RHA                | 0.87 (± 0.17) <sup>b</sup>   | 2.50 (± 0.34) <sup>b</sup> | 4 |

### 3.5 Fe-Plaque Quantity, Mineral Composition, and Associated Elements.

Significant differences were found in concentrations of DCB extractable Fe ( $F = 3.59$ ,  $p = 0.047$ ), As ( $F = 4.49$ ,  $p = 0.025$ ), and Si ( $F = 47.65$ ,  $P < 0.0001$ ) among treatments and the control (Figure 10). The  $\text{CaSiO}_3$  treatment had lower DCB extractable Fe concentrations than the control. The FH treatment had higher DCB extractable As concentrations than the control. The FH and  $\text{CaSiO}_3$  treatments had significantly higher DCB extractable Si than RHA and the control.

The results of Fe EXAFS analysis revealed significant differences in percent ferrihydrite ( $F = 5.73$ ,  $p = 0.02$ ) with a higher proportion in the FH treatment relative to the control (Table 6; Figure 11A). In addition, goethite differed between treatments ( $F = 7.78$ ,  $p = 0.009$ ), with the control having a higher proportion than the FH and RHA treatments. Significant differences were not found for hematite, siderite, and lepidocrocite. Arsenic K-edge XANES spectra indicated a higher proportion of  $\text{iAs(III)}$  in root Fe-plaque in the FH treatment relative to the control (Table 7; Figure 11B).

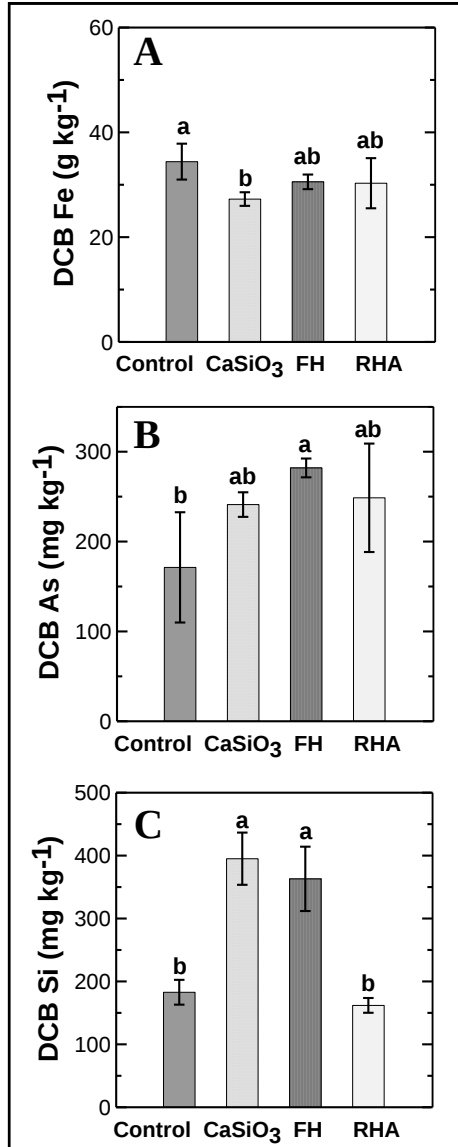


Figure 10: Average DCB extractable Fe (A), As (B) and Si (C) (+/- SD, n = 4) from root Fe-plaque. Letters denote significant differences ( $p < 0.05$ ) between treatments.

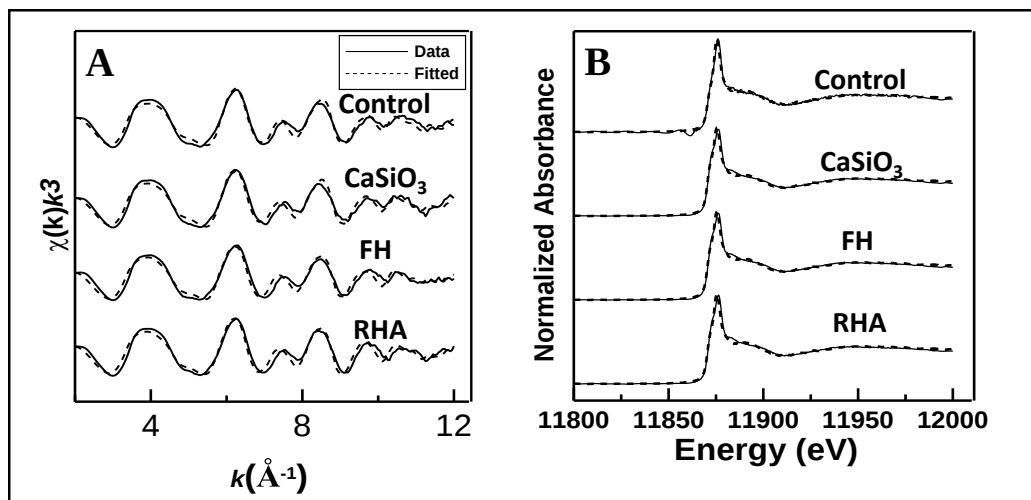


Figure 11: Linear combination fits of  $k^3$  weighted Fe EXAFS spectra (A) and As k-edge spectra (B) of root Fe-plaque that was removed from roots via sonication and concentrated on a nitrocellulosic filter membrane. Each fit is one replicate chosen as a representative sample from the control and treatment groups.

Table 6: Results of linear combination fitting of first shell Fe-EXAFS spectra and goodness of fits (R value) obtained on root Fe plaque concentrated on a nitrocellulosic filter membrane. Three spectra were taken for each replicate and averaged for background subtraction and fitting. Significant differences ( $p < 0.05$ ) denoted by letters.

| Treatment          | Average percent of fitted species                  |                       |                                |                       |                 | Average R value |
|--------------------|----------------------------------------------------|-----------------------|--------------------------------|-----------------------|-----------------|-----------------|
|                    | $\text{Fe}(\text{OH})_3 \cdot n\text{H}_2\text{O}$ | $\alpha\text{-FeOOH}$ | $\alpha\text{-Fe}_2\text{O}_3$ | $\gamma\text{-FeOOH}$ | $\text{FeCO}_3$ |                 |
| Control            | $64 \pm 6^b$                                       | $23 \pm 3^a$          | $4.6 \pm 0.9^a$                | $8.1 \pm 4^a$         | n.d.            | 0.082           |
| CaSiO <sub>3</sub> | $73 \pm 2^{ab}$                                    | $14 \pm 1^{ab}$       | $3.7 \pm 0.6^a$                | $6.6 \pm 1^a$         | $2.4 \pm 1^a$   | 0.067           |
| FH                 | $74 \pm 0.7^a$                                     | $16 \pm 3^b$          | n.d.                           | $4.2 \pm 0.7^a$       | $5.4 \pm 3^a$   | 0.077           |
| RHA                | $65 \pm 4^{ab}$                                    | $20 \pm 2^b$          | $1.5 \pm 2^a$                  | $8.9 \pm 2^a$         | $4.9 \pm 4^a$   | 0.057           |

n.d. – not detected

Table 7: Results of linear combination fitting for normalized As K-edge XANES spectra and goodness of fits (R value) obtained on root Fe plaque concentrated on a nitrocellulosic filter membrane. Fits are average of 3 spectra taken for one replicate per treatment.

| Treatment                | Percent of fitted species |        | R value |
|--------------------------|---------------------------|--------|---------|
|                          | iAs(III)                  | iAs(V) |         |
| <i>Control</i>           | 28                        | 71     | 0.0027  |
| <i>CaSiO<sub>3</sub></i> | 31                        | 69     | 0.0028  |
| <i>FH</i>                | 38                        | 62     | 0.0023  |
| <i>RHA</i>               | 35                        | 65     | 0.0025  |

### 3.6 Plant Nutrients.

Straw nutrient concentrations differed significantly in terms of N ( $F = 27.8$ ,  $p < 0.0001$ ), P ( $F = 8.71$ ,  $p = 0.002$ ), S ( $F = 6.52$ ,  $p = 0.007$ ), and K ( $F = 4.59$ ,  $p = 0.023$ ). Straw of the FH treatment generally had lower nutrient concentrations compared to the other treatments, with the exception of K (Figure 12). Conversely, the FH treatment accumulated more P and S by mass in aboveground tissues (grain, husk, and straw), in addition to having elevated K (Figure 13).

The  $\text{CaSiO}_3$  treatment had elevated levels of porewater  $\text{Ca}^{2+}$  compared to the other treatments (Figure 14A), which led to significantly elevated Ca concentrations in roots ( $F = 54.05$ ,  $p < 0.0001$ ) and Ca mass in aboveground tissues ( $F = 4.08$ ,  $p = 0.03$ ) (Figure 14B, 14C). Straw Ca concentrations were not significantly different among treatments and the control (data not shown) due to the larger straw biomass and consequent dilution effect on straw Ca in the  $\text{CaSiO}_3$  treatment.

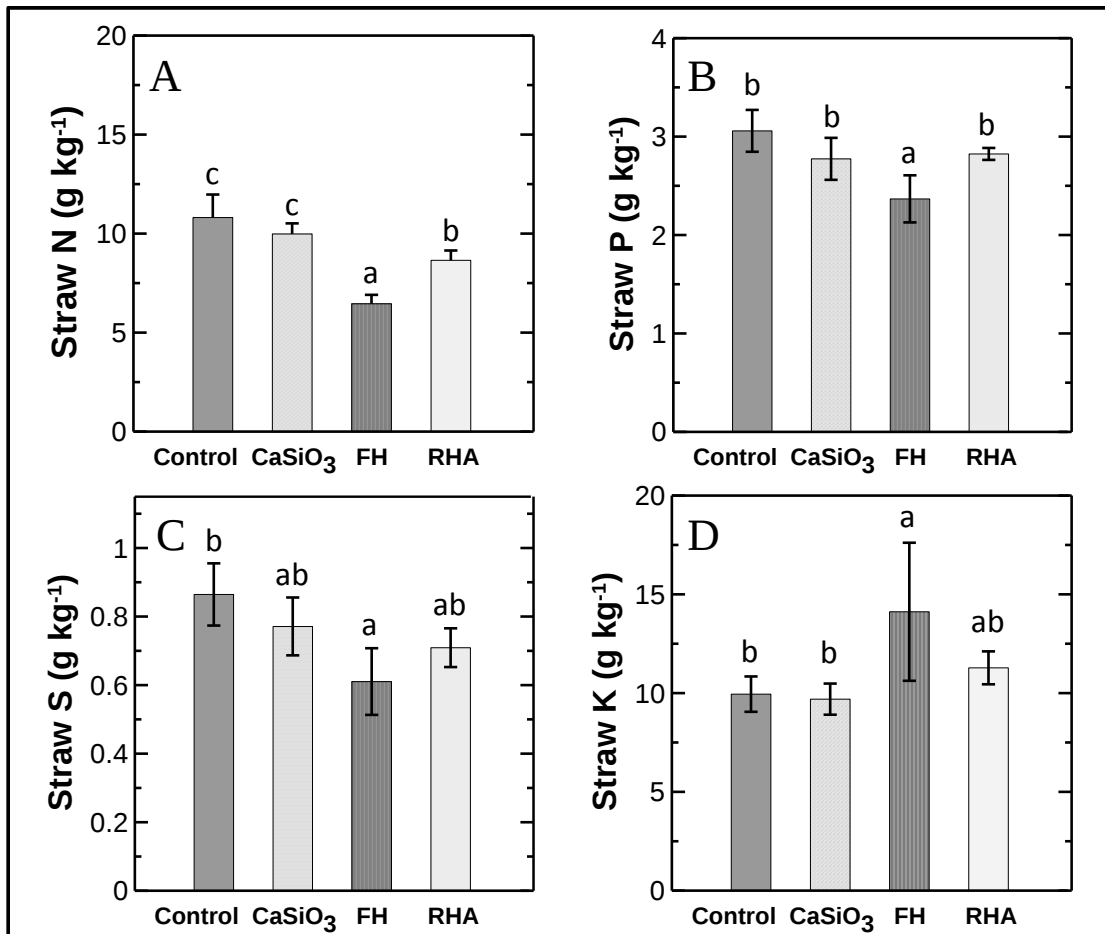


Figure 12: Average concentration (+/- SD, n=4) of N (A), S (B), P (C), and K (D) in rice straw. Significant differences (p < 0.05) between treatments are indicated by letters.

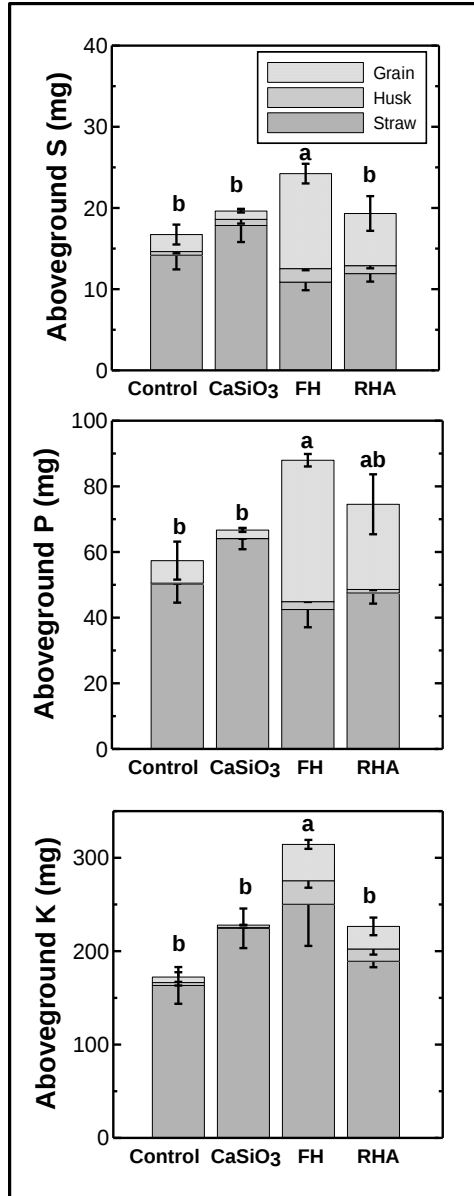


Figure 13: Average mass (+/- SD) of S (A), P (B), and K (C) in aboveground plant organs (straw, husk, and grain). Significant differences ( $p < 0.05$ ) between treatments indicated by letters and were only tested for total aboveground nutrients (sum of nutrient masses in grain, husk, and straw).

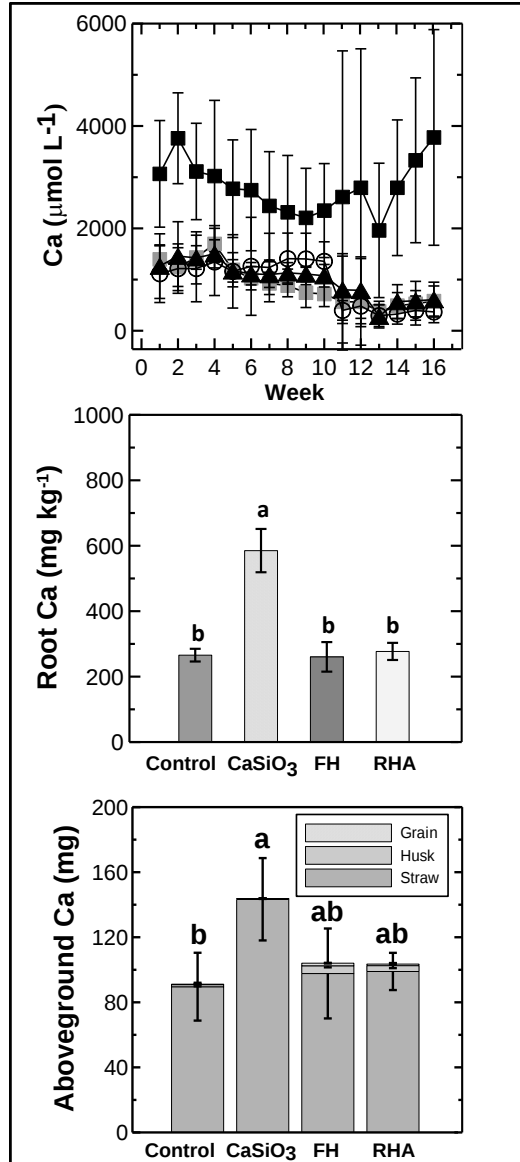


Figure 14: Porewater  $\text{Ca}^{2+}$  ( $\pm$  95% CI,  $n = 4$ ) over rice growth (A). Root Ca concentrations (B) and aboveground Ca mass (C) (average  $\pm$  SD,  $n = 4$ ). The larger straw biomass of the  $\text{CaSiO}_3$  treatment led to similar concentrations of Ca in straw, but elevated Ca on a mass basis

### 3.7 CH<sub>4</sub> and CO<sub>2</sub> Flux.

CO<sub>2</sub> flux rates increased during the tillering stage, peaked during the reproductive stage and decreased thereafter (Figure 15A). CH<sub>4</sub> flux was low at week 3 for all treatments and then increased at week 4 indicating the onset of methanogenesis (Figure 15B). CH<sub>4</sub> flux rates were relatively steady at ca. 0.400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for the duration of rice growth and were generally an order of magnitude lower than CO<sub>2</sub> fluxes. CH<sub>4</sub> emissions were converted to CO<sub>2</sub> equivalents using the 100-yr global warming potential (GWP)<sup>56</sup>.

CH<sub>4</sub> emissions were significantly different between treatments ( $F = 6.34$ ,  $p = 0.008$ ), with the FH treatment emitting more CH<sub>4</sub> than the control and RHA treatment, but were not significantly different from the CaSiO<sub>3</sub> treatment (Table 8). The mean flux rate (time-weighted average) and cumulative emissions of the FH treatment was ca. 25% higher than control. CO<sub>2</sub> emissions were significantly different ( $F = 4.50$ ,  $p = 0.025$ ) with the CaSiO<sub>3</sub> and FH treatments having 12 and 11% higher seasonal emissions, respectively, than the control (Table 8).

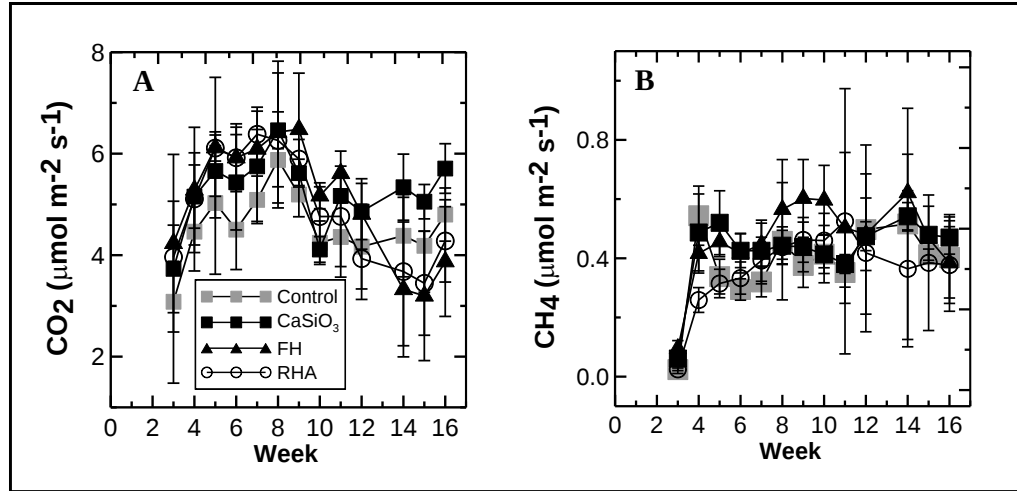


Figure 15: Average flux ( $\pm$  95% CI,  $n = 4$ ) of CO<sub>2</sub> (A) and CH<sub>4</sub> (B) measured weekly over 120 days of rice growth. Flux was measured dynamically and calculated using 135 second of gas concentrations measured at 1 Hz.

Table 8: Average of time-weighted average flux rates ( $\pm$  SD,  $n=4$ ) and average of cumulative emissions (120 day) of CH<sub>4</sub> and CO<sub>2</sub>. Cumulative (120 day) emissions were calculated by taking the area under the curve of measured flux rates for each replicate, using linear interpolation to estimate missing data points. CH<sub>4</sub> was converted to CO<sub>2</sub> equivalents using the 100 year GWP. Letters denote significant differences ( $p < 0.05$ ) between treatments.

| Treatment          | Average<br>CH <sub>4</sub> flux<br>( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) | Cumulative<br>CH <sub>4</sub> Flux<br>( $\text{g m}^{-2} 120 \text{d}^{-1}$ ) | Cumulative<br>CH <sub>4</sub> (g CO <sub>2</sub> eq.<br>$\text{m}^{-2} 120 \text{d}^{-1}$ ) | Average<br>CO <sub>2</sub> flux<br>( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) | Cumulative<br>CO <sub>2</sub> Flux<br>( $\text{g m}^{-2} 120 \text{d}^{-1}$ ) |
|--------------------|-----------------------------------------------------------------------------|-------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|-------------------------------------------------------------------------------|
| Control            | 0.375 ( $\pm 0.049$ ) <sup>b</sup>                                          | 51.7 ( $\pm 7.4$ ) <sup>b</sup>                                               | 3980 ( $\pm 570$ ) <sup>b</sup>                                                             | 4.56 ( $\pm 0.28$ ) <sup>b</sup>                                            | 1690 ( $\pm 99$ ) <sup>b</sup>                                                |
| CaSiO <sub>3</sub> | 0.424 ( $\pm 0.025$ ) <sup>ab</sup>                                         | 58.4 ( $\pm 3.1$ ) <sup>ab</sup>                                              | 4500 ( $\pm 240$ ) <sup>ab</sup>                                                            | 5.29 ( $\pm 0.15$ ) <sup>a</sup>                                            | 1900 ( $\pm 91$ ) <sup>a</sup>                                                |
| FH                 | 0.465 ( $\pm 0.019$ ) <sup>a</sup>                                          | 64.3 ( $\pm 2.4$ ) <sup>a</sup>                                               | 4950 ( $\pm 180$ ) <sup>a</sup>                                                             | 5.12 ( $\pm 0.20$ ) <sup>a</sup>                                            | 1880 ( $\pm 72$ ) <sup>a</sup>                                                |
| RHA                | 0.360 ( $\pm 0.041$ ) <sup>b</sup>                                          | 48.5 ( $\pm 7.4$ ) <sup>b</sup>                                               | 3740 ( $\pm 570$ ) <sup>b</sup>                                                             | 4.98 ( $\pm 0.23$ ) <sup>ab</sup>                                           | 1790 ( $\pm 94$ ) <sup>ab</sup>                                               |

## Chapter 4

### DISCUSSION

#### 4.1 Effect of As and Treatments on Yield and Plant As.

In this proof of concept study, we pushed our system to the extreme case of As contamination to determine impacts of Si amendments on rice yield and As accumulation. Our data show that FH, and to a lesser extent RHA, reverse the negative As-induced yield impacts to levels on par with non-contaminated soils. In contrast, an inorganic Si amendment was not effective at improving yield under As stress.

The large yield losses we observed were accompanied by symptoms of Straighthead, with missing and malformed spikelets, which resulted in “straight” panicles. A substantially larger percentage of grain was unfilled in the +As control and  $\text{CaSiO}_3$  treatment relative to the FH and RHA treatments. Observing symptoms of Straighthead was not surprising as both iAs and oAs are suspected agents of the disorder<sup>10, 57</sup>, but it was surprising that both FH and RHA amendments to As-treated soils reversed the condition. FH and RHA treatments did not substantially impact yield in plants grown with “background” levels of  $16 \text{ mg kg}^{-1}$  As that were irrigated with As-free water<sup>20</sup>, but here we show that FH and RHA amendment have a positive yield impact under elevated As.

The effect of As on rice yield in this study appears to more pronounced compared to other studies that were conducted under elevated As conditions. Rahman et al.<sup>58</sup> found that significant rice yield losses only occurred when soil As

concentrations reached  $60 \text{ mg kg}^{-1}$  under flooded conditions. However, their soils were treated with iAs(V) only, whereas here we utilized iAs(III) as the major component in soil spikes and in irrigation water. Our results are more similar to those reported in a Bangladeshi field study in which yield losses up to 66%<sup>59</sup> were reported for rice grown along a contamination gradient with up to  $70 \text{ mg kg}^{-1}$  As and porewater concentrations up to  $30 \text{ }\mu\text{M}$ , mostly as iAs(III). Syu et al.<sup>60</sup> found that rice grown under excessively high soil As of  $500 \text{ mg kg}^{-1}$  and over  $50 \text{ }\mu\text{M}$  porewater As mostly as iAs(III)) resulted in yield losses of 37-63% in several rice genotypes. Given the relatively lower As levels in porewater (ca.  $4 \text{ }\mu\text{M}$ ) and soil ( $24 \text{ mg/kg}$ ) in this study, the large yield response may be due to the relatively high sensitivity of the M206 cultivar to As stress compared to cultivars that have been growing in As-affected areas. In addition, high initial porewater DMA concentrations may have affected yield, as DMA directly affects reproductive organs and can reduce yield<sup>12, 61</sup>.

In addition to negating the effect of As on yield, the FH treatment also significantly decreased total As concentrations in grain and husk, similar to the findings of Seyfferth et al.<sup>20</sup>. While grain iAs(III) and straw total As concentrations of rice grown in FH treated soil were not significantly different from those of the control, it's possible that the increased toxicity of the control treatment (as indicated by the decreased yield) inhibited plant uptake of solutes, including As, due to plant toxicity. Previous research has indicated that increased soil As can decrease grain As concentrations, indicating a plant threshold for As past which plant uptake processes are affected<sup>60</sup>. The RHA treatment, which decreased apparent As toxicity through higher yields, had significantly higher concentrations of DMA and iAs(III) in grain, and straw total As relative to the FH treatment. Several properties of the FH

amendment may explain its success in increasing yield and decreasing grain As concentrations.

The effect of Si on rice yield and grain accumulation of As is dependent upon several factors including plant-availability, relative concentrations in the soil solution, and temporal dynamics. The  $\text{CaSiO}_3$  treatment, which yielded poorly, resulted in a quick burst of porewater Si initially which plummeted in the following weeks to levels similar to control and RHA treatment (Figure 6A). The FH treatment, on the other hand, provided a slow-release of Si, which dropped at weeks 10-11 (ca. 1 week prior to the observed 50% heading date). We argue that the slow release behavior of FH is critical to its success as a Si amendment. Plant demand for Si is highest during the reproductive phase, and a previous study demonstrated that Si levels at this stage are critical for yield<sup>62, 63</sup>. While the  $\text{CaSiO}_3$  amendment effectively increased plant Si, lower levels of Si at the reproductive phase would not have facilitated high yields.

The slow release behavior of the FH amendment is also important for decreasing grain As concentrations. Under flooded conditions, the grain filling phase of the rice life cycle marks an important point for uptake of As<sup>64</sup>. At this point, As may be taken up by roots and deposited in grain via xylem transport. Arsenite, as arsenous acid, shares an uptake pathway with silicic acid and competes for uptake via the Lsi pathway<sup>21</sup>. As a result, increased Si during grain filling can suppress As uptake and subsequent grain loading. In addition, elevated Si can lead to downregulation of the Lsi transporters which in turn would decrease As uptake<sup>22</sup>.

Soil and porewater As do not explain As accumulation and yield differences we observed because those parameters were similar between treatments. While the  $\text{CaSiO}_3$  treatment had significantly higher soil As in the specifically-sorbed and

crystalline Fe/Al-oxide phases, this difference was not apparent in plant As concentrations. However, the treatments affected other aspects of soil and porewater chemistry that altered interactions at the plant-soil interface.

#### **4.2 Role of Root Fe-Plaque.**

Multiple lines of evidence highlight the potential role of alterations in root Fe-plaque quantity and mineralogy in paddy soil As dynamics. Higher root and lower DCB-extractable As concentrations in the control relative to the FH treatment indicate the decreased capacity of control root Fe-plaque for As sorption. Porewater and Fe-plaque of the FH-treatment was enriched in Si, which previous research has shown to stabilize ferrihydrite and prevent progressive crystallization to higher-ordered phases (i.e., goethite)<sup>29-31</sup>. The results of EXAFS analysis of root Fe-plaque confirmed a significantly higher percentage of ferrihydrite in the FH treatment and goethite in the control (Table 6). Ferrihydrite has been shown to have increased sorption capacity for iAs(III) relative to goethite in batch experiments<sup>25</sup>, so it is expected that the treatment which promoted ferrihydrite (via stabilization through increased porewater Si) would in turn promote As sorption on root Fe-plaque. Although the CaSiO<sub>3</sub> treatment was also elevated in Si, relatively low porewater Fe levels (likely due to increased pH) resulted in significantly less DCB-extractable Fe compared to the control – evidence that the treatment hindered the development of root Fe-plaque. The results of XANES analysis indicate an increased proportion of iAs(III) on FH root plaque, further supporting the notion that Fe-plaque of the FH treatment most effectively immobilized free iAs.

### 4.3 Role of Plant Nutrients.

In addition to FH being a superior Si fertilizer, the FH treatment tended to be enriched in plant P, K, and S (Figure 13). While P and K improve yield, S is important because it is the main constituent of glutathione (GSH) and phytochelatins (PC) which are involved in plant detoxification of As<sup>65, 66</sup>. These biomolecules contain multiple thiol groups and can detoxify iAs through chelation and vacuolar sequestration<sup>67, 68</sup>. Although DMA does not bind with thiol groups directly, when activated by sulfide, DMA can bind to GSH to form the dimethylarsinothioglutathione (DMAS-GS) complex, which has been identified *in planta* and could play a role in detoxifying DMA<sup>66, 69</sup>. Sulfur (S) was the only nutrient more concentrated in FH than RHA (Table 3), and thus the FH treatment received the highest application of S. The role of the other plant nutrients cannot be ignored in light of the success of the FH treatment.

Plant nutrition may also explain the yield improvements of the RHA treatment. In general, the RHA amendment was more concentrated in plant nutrients than the FH treatment (Table 3), although this was not always evident from plant nutrient analyses (Figure 13). The improved yields of the RHA treatment came about despite the amendment not affecting porewater Si levels, and would suggest that Si is not solely responsible for increased plant tolerance to As. RHA levels of porewater K were significantly higher than the other treatments for the first 3 weeks of rice growth. Potassium (K) is an essential macronutrient involved in photosynthesis and osmoregulation<sup>65</sup>, but it's not clear how K would affect plant health under conditions of As toxicity. Studies which examined plant and soil mineral concentrations rice affected by naturally-occurring and induced Straighthead did not find K to be a significant factor<sup>70, 71</sup>. Yan et al.<sup>72</sup>, in a study examining nutrient concentrations in

panicles and flag leaves of different cultivars affected by Straighthead, found elevated K in panicles of a tolerant variety, but this was not observed for other varieties. Regardless, RHA shows promise as a soil amendment to increase yield in As-contaminated soils.

In contrast to the FH and RHA amendments, the  $\text{CaSiO}_3$  treatment performed very poorly in terms of yield and grain As concentrations. In addition to elevating Si, the  $\text{CaSiO}_3$  amendment increased plant and porewater Ca levels. This may have impacted the plant response to As, as intracellular Ca plays an important role in biochemical signaling during As exposure and elevated Ca from the  $\text{CaSiO}_3$  amendment may interfere with plant coping mechanisms to As stress<sup>73-75</sup>. It is important to note that our study used a very fine, highly soluble  $\text{CaSiO}_3$  powder, which would contrast chemically to industrial slags which are coarse, heterogeneous materials that have lower solubility and exhibit slow release characteristics, making them suitable agricultural amendments<sup>38</sup>. Our findings are in contrast with those of field experiments which determined  $\text{CaSiO}_3$  confers beneficial effects to rice<sup>15, 39, 76</sup>. However, the above mentioned studies did not examine the impacts of  $\text{CaSiO}_3$  in As-contaminated soils. Given the observations of this study, amendments which contribute significant Ca may be poor choices in As amended soils.

#### **4.4 Impacts of Si-Rich Amendments on C Emissions.**

While the FH amendment was found to improve yield and decrease grain As, its incorporation to soil led to higher C fluxes relative to the control and other treatments. The FH treatment emitted 25% more  $\text{CH}_4$  over the rice growing period relative to the control. Despite this increase, FH is a better choice than straw as a paddy Si fertilizer, as straw return is associated with elevating  $\text{CH}_4$  emissions by 110%

as indicated by a meta-analysis study<sup>36</sup>. Similarly, Penido et al.<sup>19</sup> demonstrated that dissolved CH<sub>4</sub> concentrations in porewater are lower in FH amended soil than rice straw-amended soil, but higher than ash amended soil. This was attributed to the higher lignin content of FH relative to rice straw, which decreases its potential as a substrate for methanogens<sup>77, 78</sup>. This study utilized a 1% application rate for FH, which would be unsustainable given world production rates<sup>19</sup>. In a realistic scenario, farmers would amend FH at lower application rates which in turn would result in decreased emissions, further making FH an optimal Si amendment for decreasing CH<sub>4</sub> emissions relative to the practice of straw return.

The primary concern of this study was CH<sub>4</sub> emissions, as the net exchange of CO<sub>2</sub> in the biosphere is approximately balanced, and thus agricultural CO<sub>2</sub> emissions are not recognized as contributing to climate change<sup>79</sup>. However, we did observe higher CO<sub>2</sub> fluxes from FH and CaSiO<sub>3</sub> treated soils compared to the control which may be related to plant processes. The plants grown with FH and CaSiO<sub>3</sub> treatments were elevated in plant Si, an important mineral for aerenchyma development<sup>65</sup>, and thus these plants may have had better-developed aerenchyma for gas exchange. Higher gas exchange could have promoted more CO<sub>2</sub> release, but also more O<sub>2</sub> into the rooting zone, thus potentially stimulating more aerobic heterotrophic respiration. In the case of the CaSiO<sub>3</sub> treatment, plants had higher straw biomass, which may have also led to more aerenchyma and thus a larger conduit for gas exchange. In addition, FH is a carbonaceous amendment which may have stimulated heterotrophic respiration in soil. These possibilities are outside of the scope of the present study, but pave the way for future research into these mechanisms.

## Chapter 5

### CONCLUSIONS

In this proof of concept study, we pushed our system to an extreme case of As contamination to determine impacts of Si amendments on rice yield and As accumulation. Our data show that FH, as a comprehensive plant fertilizer rich in available Si and other nutrients, allowed rice to tolerate the toxic effects of As and avoid yield losses. Burning FH to make RHA provided nutrients that helped to increase plant tolerance to As and increase yield, yet locked Si in insoluble forms unavailable for plant uptake and consequently As uptake was not restricted. In contrast,  $\text{CaSiO}_3$ , an inorganic Si amendment was not effective at improving yield or decreasing grain As levels in rice grown in As impacted soils.

$\text{CaSiO}_3$  increased porewater Si levels, but lacked a diverse nutritional profile and contributed high levels of  $\text{Ca}^{2+}$  to porewater, which may have disrupted plant processes and led to very low yields. Although previous studies show  $\text{CaSiO}_3$  to be beneficial for rice<sup>15, 39, 76</sup>, these studies were not conducted under conditions of elevated soil As. Amendments rich in calcium may be poor choices for As contaminated soils, as the results of this experiment indicate Ca may interfere with the plant's ability to cope with As stress. While our results do not disprove the benefits of slags and  $\text{CaSiO}_3$  as amendments for Si fertilization, they demonstrate that their potential for Si fertilization cannot be matched by FH, as FH can be applied at much greater rates without the possibility of excess calcium, increased pH, or, in the case of industrial slags, introducing toxic metals<sup>80</sup>. However, the benefits of FH may come at

the expense of increased CH<sub>4</sub> emissions. Nevertheless, the increase in C emissions are not nearly as great as those reported for straw incorporation, and may simply be a manifestation of healthier plants with stronger aerenchyma.

We initially hypothesized that a) the amendment which most effectively increases plant available Si will decrease plant accumulation of As, b) increased Si in porewater (soil solution) will stabilize ferrihydrite formation on rice roots and increase the sorption capacity of root Fe-plaque for As, and c) the emissions of CH<sub>4</sub> will increase with amendment C content. We found that the FH treatment, which led to the greatest release of Si throughout rice growth, decreased plant accumulation of As, which supports our hypothesis. In support of our second hypothesis, the FH treatment fostered the development and stabilization of ferrihydrite on root plaque due to a slow release of Si. In contrast, CaSiO<sub>3</sub> hindered root Fe-plaque development due its effect on porewater/soil pH decreasing porewater Fe levels despite high Si levels in pore water. The differences in root Fe-plaque mineralogy coincided with differences in plaque/root As partitioning that showed the root plaque of the FH treatment to more effectively adsorb As. In support of our third hypothesis, we showed that the amendment with the highest C content, FH, led to increase CH<sub>4</sub> emissions by 25%.

FH is a feasible field amendment to combat As stress in both industrialized and developing nations. In the United States, where most farms are industrial operations, straw incorporation is commonly practiced<sup>81</sup>. Not only does this increase CH<sub>4</sub> emissions, but it is a cost consideration for farms and problems can occur due to straw interfering with machinery<sup>81</sup>. FH could be applied at only the cost of broadcasting, as it would not need to be chopped or undergo tillage as is necessary during straw-incorporation. In addition, the FH offers an economically and environmentally

sustainable alternative to purchasing mineral nutrients to help combat As stress and boost yields, especially to small holder farms in South and Southeast Asia where soils may be compromised by As.

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