ROBUST SILICON EXTRACTION METHOD AND CYCLING OF ARSENIC IN RICE PADDY SOIL

by

Weida Wu

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

Winter 2019

© 2019 Weida Wu All Rights Reserved

ROBUST SILICON EXTRACTION METHOD AND CYCLING OF ARSENIC IN RICE PADDY SOIL

by

Weida Wu

Approved:Angelia L. Seyfferth, Ph.D.
Professor in charge of thesis on behalf of the Advisory CommitteeApproved:Erik H. Ervin, Ph.D.
Chair of the Department of Plant and Soil SciencesApproved:Mark Rieger, Ph.D.
Dean of the College of Agriculture and Natural ResourcesApproved:Douglas J. Doren, Ph.D.
Interim Vice Provost for Graduate and Professional Education

ACKNOWLEDGMENTS

I would first thank my thesis advisor, Dr. Angelia Seyfferth. The office door of Dr. Seyfferth is always open, and she gives me the best advice whenever I had questions about experiments or writing. I would also thank my committee members, Dr. Yan Jin and Dr. Donald Sparks for their helpful advice along this thesis process.

I would like to thank Seyfferth Lab members for their help with my lab experiments. I would like to thank Joseph Paler, Karen Gartley, Caroline Golt, Chin-Chen Kuo and Gerald Poirier for their help with analyzing my samples. I would like to acknowledge Preeti Krishnamani, a senior student in high school with helping me perform CBD extractions.

I would like to thank my host families, Barbara Boom and Joseph Schwartz. They helped my overcome language problems and helped me adapt to a new environment when I first arrived in the United States.

Finally, I would like to thank my parents and my brother for providing me with unfailing support and continuous encouragement throughout my years of study. Without them, this project will not be possible.

iii

TABLE OF CONTENTS

LIST OF TABLES	v
LIST OF FIGURES	vi
ABSTRACT	ix

Chapter

	1.1	Introduction	1
	1.2	Materials and Methods	3
	1.3	Results	6
	1.4	Discussion	11
	1.5	Conclusion	15
	REF	FERENCES	16
2	REF IMP	FERENCES PACTS OF SILICON AMENDMENTS AND TIME ON ARSENIC	16
2	REF IMP CYC	FERENCES PACTS OF SILICON AMENDMENTS AND TIME ON ARSENIC CLING IN RICE PADDY SOIL	16
2	REF IMP CYC 2.1	FERENCES PACTS OF SILICON AMENDMENTS AND TIME ON ARSENIC CLING IN RICE PADDY SOIL Introduction	16 20 20
2	REF IMP CYC 2.1 2.2	FERENCES PACTS OF SILICON AMENDMENTS AND TIME ON ARSENIC CLING IN RICE PADDY SOIL Introduction Materials and Methods	16 20 20 23
2	REF IMP CYC 2.1 2.2 2.3	FERENCES PACTS OF SILICON AMENDMENTS AND TIME ON ARSENIC CLING IN RICE PADDY SOIL Introduction Materials and Methods Results	16 20 20 23 26
2	REF IMP CYC 2.1 2.2 2.3 2.4	FERENCES PACTS OF SILICON AMENDMENTS AND TIME ON ARSENIC CLING IN RICE PADDY SOIL Introduction Materials and Methods Results Discussion	16 20 20 20 26 37

LIST OF TABLES

Table 1.1:	Regression model statistics between soil extractable Si and Si	
	concentrations in rice husk and rice straw	3

LIST OF FIGURES

Figure 1.1:	A) Average (n=9, \pm SD) extractable Si from flooded paddy soil (post- harvest) that was nonamended (Control) or amended with charred rice husk (Char), rice husk (Husk) or calcium silicate/silicic acid (Silicate) in 2015, 2016 and 2017 with four extraction methods. Different letters denote significant differences (p<0.05) between groups. B) Average (n=2, \pm SD) extractable Si from non-Si amended paddies under a range of redox in 2016 and 2017 (post-harvest) with four extraction methods
Figure 1.2:	Relationship between soil extractable Si and Si concentration in rice husk (A, B, C, D) or rice straw (E, F, G, H). \diamond are samples from 2015. \circ are samples from 2016. Δ are samples from 2017 is the predicted plant Si concentration in 2015 is predicted plant Si concentration in 2016. === is predicted plant Si concentration in 2017 8
Figure 1.3:	Relationship between the robust 16-h CaCl ₂ extraction method with other methods used in this study. \diamond are samples from 2015. \circ are samples from 2016. Δ are samples from 2017
Figure 1.4:	Relationship between 4-h CaCl ₂ extractable soil Si and Si concentration in rice husk (A) or rice straw (B), and the relationship between the robust 16-h CaCl ₂ extraction and 4-h CaCl ₂ extraction (C). \diamond are samples from 2015. \circ are samples from 2016. \triangle are samples from 2017 is the predicted plant Si concentration in 2015 is predicted plant Si concentration in 2016. === is predicted plant Si concentration in 2017 14
Figure 2.1:	Total background As from 2015 pre-rice paddy soil (a). Arsenic fraction F1 (b), F2 (c), F3(d), F4 (e), F5 (f) in 2015 pre-rice and post- harvest soil that was nonamended (Control) or amended with charred rice husk (Char), rice husk (Husk) or calcium silicate/silicic acid (Silicate). Different upper-case letters denote significant differences (p<0.05) between sampling time. Different lower-case letters denote significant differences (p<0.05) between Si treatments

Figure 2.2:	Oxalate-extractable Fe (a) and Mn (b), and CBD-extractable Fe (c) and Mn (d) in 2015 pre-rice and post-harvest soil that was nonamended (Control) or amended with charred rice husk (Char), rice husk (Husk) or calcium silicate/silicic acid (Silicate). Different upper- case letters denote significant differences (p<0.05) between sampling time. Different lower-case letters denote significant differences (p<0.05) between Si treatments.	28
Figure 2.3:	Arsenic fraction F1 (a), F2 (b), F3(c), F4 (d) and F5 (e) in all post- harvest soil that was nonamended (Control) or amended with charred rice husk (Char), rice husk (Husk) or calcium silicate/silicic acid (Silicate). Different upper-case letters denote significant differences (p<0.05) between sampling year. Different lower-case letters denote significant differences (p<0.05) between Si treatments. * denotes that there is significant interaction between sampling year and Si treatments.	29
Figure 2.4:	Oxalate-extractable Fe (a) and Mn (b), and CBD-extractable Fe (c) and Mn (d) in all post-harvest soil that was nonamended (Control) or amended with charred rice husk (Char), rice husk (Husk) or calcium silicate/silicic acid (Silicate). Different upper-case letters denote significant differences (p<0.05) between sampling year. Different lower-case letters denote significant differences (p<0.05) between Si treatments.	31
Figure 2.5:	Arsenic fraction F1 (a), F2 (b), F3(c), F4 (d) and F5 (e) in 2017 soil that was collected during rice growth period. Soil was nonamended (Control) or amended with charred rice husk (Char), rice husk (Husk) or calcium silicate/silicic acid (Silicate). Different upper-case letters denote significant differences (p <0.05) between sampling time. Different lower-case letters denote significant differences (p <0.05) between Si treatments. * denotes that there is significant interaction between sampling year and Si treatments.	32
Figure 2.6:	Oxalate-extractable Fe (a) and Mn (b) in 2017 soil that was collected during rice growth period. Soil was nonamended (Control) or amended with charred rice husk (Char), rice husk (Husk) or calcium silicate/silicic acid (Silicate). Different upper-case letters denote significant differences (p<0.05) between sampling year. Different lower-case letters denote significant differences (p<0.05) between Si treatments.	33

Figure 2.7:	Relationship between organic matter with oxalate-extractable Fe (a) and oxalate-extractable Mn (b) from post-harvest soil. Correlation without fit-line was not significant	34
Figure 2.8:	Relationship between oxalate-extractable Fe and sulfate-extractable As in 2015 pre-rice and post-harvest soil (a), all post-harvest soil (b), and 2017 soil over rice growth period (c). Relationship between oxalate-extractable Mn and sulfate-extractable As in 2015 pre-rice and post-harvest soil (d), all post-harvest soil (e), and 2017 soil over rice growth period (f). In (a) and (f), \bigcirc are samples from pre-rice and \diamond are samples from post-harvest. Correlations without fit-lines were not	
	significant	35
Figure 2.9:	Relationship between oxalate-extractable Fe and porewater As (a) and between oxalate-extractable Mn and porewater As (b) from all post- harvest soil. Relationship between oxalate-extractable Fe and porewater As (c) and between oxalate-extractable Mn and porewater As (d) from 2017 soil over rice growth period. Porewater As in (a) and (b) was the average of porewater As measured from each week at every year. Porewater As in (c) and (d) was the porewater As at each week corresponded with the week of soil collection. The three points in x-axis in (c) and (d) were the weeks of missing porewater As data. Correlation without fit-line was not significant.	36

ABSTRACT

Arsenic contamination is a great concern for many countries in the world due to its toxic effects and natural occurrence. Arsenic tends to accumulate to higher concentration in rice than in other cereals. Extensive research has been conducted to find ways to lower As in rice plants, and studies have found addition of Si into paddy soil increases plant Si and decreases the uptake of As. However, there are numerous methods to estimate plant-available Si and some have limitations when Si has been applied to soil. In addition, very few studies have examined the impact of Si addition on soil As pools. Because As is associated with poorly-crystalline Fe oxides, which are stabilized with increasing Si, it was hypothesized that Si addition would impact soil As pools by increasing the proportion of As associated with poorly-crystalline Fe oxides. This work describes research on 1) five Si extraction methods (1-h CaCl₂, 4-h CaCl₂, 16-h CaCl₂, 1-h CH₃COOH and 24-h PO₄) and relation of soil-extractable Si to plant Si in rice straw and husk, and 2) pools of As, Fe and Mn from soil that was amended with different Si treatments. Samples were collected from a 3-year field study in which rice was grown in soil either under a range of soil redox (by manipulating flooding extent) or receiving different Si treatments (nonamended (Control), charred rice husk (Char), rice husk (Husk) or calcium silicate/silicic acid (Silicate)) under reducing conditions (i.e. continuously flooded). Results show that 1) rice straw better correlated with soil Si than rice husk regardless of soil-extraction method; 2) 16-h CaCl₂ extraction had the highest correlation ($R^2=0.67$) with straw Si followed by PO₄ (R^2 =0.50), CH₃COOH (R^2 =0.26) and 1-h CaCl₂ (R^2 =0.13); 3) both

CH₃COOH and to a less extent PO₄ extraction overestimated Si from silicate-amended soil while the 1-h CaCl₂ extraction correlated poorly with the 16-h CaCl₂ method $(R^2=0.37)$; 4) 4-h CaCl₂ extraction method correlated very well with the 16-h CaCl₂ $(R^2=0.82)$ method and straw Si $(R^2=0.58)$; 5) Si addition had no significant effect on plant-available pools or As pool associated with poorly-crystalline Fe oxides (p>0.05); 6) time played an important role in As, Fe and Mn pools; 7) a significant positive correlation between organic matter and poorly-crystalline Fe oxides ($R^2=0.22$, p=0.004); 8) a significant negative relationship between poorly-crystalline Mn oxides and the plant-available pool ($R^2=0.15$, p=0.02). Data suggest that the 4-h CaCl₂ extraction method is robust to assess plant-available Si in rice paddy soil regardless of agronomic conditions. Moreover, Si addition does not impact the soil As pool associated with poorly-crystalline Fe oxides in rice paddy soil. Thus, the previously described impact of Si addition increasing ferrihydrite on Fe plaque minerals seems to be localized to the rhizosphere.

Chapter 1

A QUANTITATIVE ASSESSMENT OF ROBUST PLANT-AVAILABLE SILICON EXTRACTION METHODS IN RICE PADDY SOIL

1.1 Introduction

Although not considered essential for higher plants, Si is beneficial for many grasses such as rice and sugarcane (Savant et al., 1999; Savant et al., 1996). Replete Si can improve plant growth and yield (Gottardi et al., 2012; Teasley et al., 2017), improve water and nutrient use efficiency (Manivannan and Ahn, 2017; Soundararajan et al., 2014), and improve resistance to a range of biotic (pathogens and insect pests) and abiotic (drought, salinity, and metal(loid) toxicities) stresses (Liang et al., 2007; Ma and Yamaji, 2006; Meena et al., 2014). However, many soils used to grow rice and sugarcane are highly weathered and therefore Si-depleted (Savant et al., 1997). Even though soil contain ca. 30% SiO₂, soil weathering promotes desilication whereby soluble silicic acid is leached from soil as primary minerals weather to secondary minerals. In natural ecosystems, phytogenic pools are important sources of Si for plants (Derry et al., 2005; Frings et al., 2014) because Si in plant phytoliths is more soluble than the structural Si within soil (Savant et al., 1997) minerals.

Addition of Si to soil has proven an effective way to increase plant Si and decrease uptake of excess metal(loid)s in rice. Increasing Si decreases Cd uptake due to interactions of Cd and Si in the cell wall (Liu et al., 2013), Al uptake due to Al-Si complexing in soil solution (Ma et al., 1997) and formation of hydroxyaluminosilicates within cell wall (Cocker et al., 1998), and As uptake because

Si and arsenite (the major form of arsenic in rice paddies) share the same uptake pathway into rice plants (Ma et al., 2008). Si is also effective at decreasing the uptake and yield loss induced by DMA (Limmer et al., 2018b). In Japan, Si has been applied to paddy soil since 1995 and has increased rice grain yields (Takahashi et al., 1990). Seyfferth et al. (2018) emphasized that the source of Si matters from both a practical and chemical point of view. For example, addition of Si-rich rice residues to Ascontaminated soil improves yield and decreases inorganic As in rice grain, but calcium silicate fertilizer had no effect and this was attributed to differences in the solubility of each Si source over the duration of plant growth. A variety of Si sources with different solubilities have been added to soil including silicate fertilizer (Limmer et al., 2018a; Pereira et al., 2004), silica gel (Fleck et al., 2013; Seyfferth and Fendorf, 2012) and rice crop residues (Limmer et al., 2018a; Ma et al., 2014; Seyfferth et al., 2016; Teasley et al., 2017). As plant-available Si becomes increasing important for rice production, it is critical that estimations of Si plant-availability are robust, independent of Si amendment and other agronomic factors.

Various methods have been used to measure plant-available silicon in soil (Korndörfer et al., 2001; Savant et al., 1999; Snyder, 2001); for a detailed review see Sauer et al. (2006). Most commonly, calcium chloride or acetic acid extractions have been used to estimate plant-available Si (Haysom and Chapman, 1975; Miles et al., 2014). Korndörfer et al. (1999) found that 0.5M acetic acid was the best extractant for determining plant-available Si in upland rice soil of Minas Gerais, Brazil. However, Nonaka and Takahashi (1988) found that acetate extraction was too strong for soil previously fertilized with calcium silicate, because acetate extraction removed more Si from the fertilizer than would normally be found in the soil. Another study compared

0.01M calcium chloride, 0.5M ammonium acetate, and 0.005 M sulfuric acid and found that Si extracted by calcium chloride showed the greatest correlation to sugarcane yield in Australia (Haysom and Chapman, 1975). Despite the various methods used worldwide, very few have successfully quantified plant-available Si in soil after Si amendments have been applied.

The aim of this study was to quantitatively assess methods used to predict plant-available Si in rice paddy soil and their performance under different Si-rich soil amendments or flooding conditions. We compared four different extraction procedures and related extractable Si to plant levels of Si rich rice plant parts (straw and husk) over a 3-year study.

1.2 Materials and Methods

Rice was grown at The University of Delaware's Rice Investigation, Communication, and Education (RICE) Facility over 3 years (2015 to 2017) under different Si amendments compared to nonamended controls in 2x2 m mesocosms where each treatment is conducted in triplicate in the same soil type. The soil is classified as an Ultisol/Acrisol and has a silty clay loam texture, pH of 7.8 and 1.33% organic matter (Limmer et al., 2018a). In 2015, 12 paddies were established with four treatments: control, rice husk, charred rice husk, and calcium silicate/silicic acid. Rice husk, charred rice husk, and calcium silicate/silicic acid were applied at the start of the growing season in May 2015 at a rate of 5Mg Si/ha before rice had been planted (Limmer et al., 2018a), and no additional Si amendments have been applied since. All paddies were flooded during the growing season and drained 1-2 weeks before harvest. In 2016 six additional paddies were added that exhibited a range of water management from flooded to non-flooded conditions during rice growth and these

additional 6 paddies did not receive Si amendments. Each year, we germinated rice seeds (*Oryza sativa L. cv.* Jefferson) were germinated in a greenhouse, transplanted 50-70 plants per paddy at the 3-4 leaf stage in late May, and harvested straw, husk, and grain in early September (Limmer et al., 2018a).

The details for plant collection can be found in Limmer et al. (2018a). Briefly, plants were harvested ~100 days after transplanting, and were separated into ripe panicles, unripe panicles, ripe flag leaves and straw. Panicles were dried at room temperature for one week and grain were separated from the panicle. Rice husk was then separated from grain using a laboratory dehusker. Straw was dried at 50 °C for 7 days. Husk and straw were finely ground for later chemical analyses (Limmer et al., 2018a). Five soil samples were collected from each paddy at harvest from the 0-10 cm depth in a dual diagonal pattern across the paddy and then composited it into one sample per paddy per sampling time point in 2015, 2016 and 2017. All soil was air-dried and ground to pass through a 2-mm sieve for chemical analyses. We collected porewater weekly from six additional paddies that were established in 2016 using rhizons about 15cm deep, and measured the redox in field with calibrated redox probe. We then averaged the redox from each of six paddies over two years as a measure of the redox gradient experienced by the six paddies.

Four Si extraction methods were tested to assess plant-available Si: 0.5 M acetic acid (1:10 w:v, 1-h, "CH₃COOH" hereafter) (Snyder, 2001), 0.01 M calcium chloride (1:10 w:v, 1-h, "1-h CaCl₂" hereafter and 1:20 w:v, 16-h, "16-h CaCl₂" hereafter) (Haysom and Chapman, 1975; Snyder, 2001), and 0.04 M phosphate buffer at pH 6.2 (1:10 w:v, 24-hr, "PO₄" hereafter) (Sauer et al., 2006). After shaking for the aforementioned times at 120 rpm, samples were centrifuged at 1000 rpm for 10min

and the supernatants were poured into 50mL conical centrifuge tubes and used for Si analysis. Si concentration in extraction solutions was determined by a modified molybdenum blue colorimetric method using UV-VIS spectrophotometer (Evolution 60S) at 630nm (Kraska and Breitenbeck, 2010). Concentrated acetic acid was used rather than 20% acetic acid to decrease sample dilution thus increasing sensitivity.

Detailed procedures for the Si extraction in rice plants can be found in Seyfferth et al. (2016). Briefly, 200mg of ground husk or straw harvested in 2015, 2016, and 2017 were microwave digested separately with 7ml of trace metal grade HNO₃ in Teflon vessels. Digested, diluted solutions were then separated into the acid fraction, which was analyzed for Si using ICP-OES, and undigested silica gel. After decanting the acid, the remaining silica-rich gel was washed with 50mL of DDI water, centrifuged and decanted three times. Silica gel was dissolved in 15mL of 2M NaOH and the concentration of Si in the base digestion was analyzed colorimetrically after complexation with molybdenum blue using a UV-VIS spectrophotometer (Evolution 60S) at 630nm (Kraska and Breitenbeck, 2010). The concentration of Si in the base digestion was added to the concentration in the acid digestion for total Si in straw and husk.

To test the effect of Si treatments on the amount of Si in paddy soil extracted by different extraction methods, we performed repeated measures ANOVA because samples were taken from the same paddies in different years. If significant differences (p<0.05) were identified, a Tukey post hoc test was used to distinguish differences among groups. A multiple regression was performed to compare the correlation between Si in soil extractions and Si in husk or straw. The multiple regression model used the equation $Y=b+a_1X_1 + a_2X_2 + a_3X_2^2$, where Y is the plant Si, X₁ is the soil Si

extracted by each method, X_2 is the sampling year and lowercase letters are fitted coefficients. Sampling year is the year samples were collected, with 2015 set as 0, 2016 as 1 and 2017 as 2. In many cases, the response was not linear with time thus a quadratic time term was added to the model. Type II, partial R^2 values were used to assess the amount of variance explained by each term. All statistical analyses were performed with SAS 9.4 using PROC MIXED, PROC REG, or PROC UNIVARIATE (The SAS Institute).

1.3 Results

Extraction method, Si treatments (F=22.46 to 104.88, p<0.0001) and sampling year (F=3.38 to 24.69, p=<0.0001 to 0.05) affected the amount of extractable Si from flooded paddy soil (Figure 1.1A),



Figure 1.1: A) Average (n=9, ±SD) extractable Si from flooded paddy soil (post-harvest) that was nonamended (Control) or amended with charred rice husk (Char), rice husk (Husk) or calcium silicate/silicic acid (Silicate) in 2015, 2016 and 2017 with four extraction methods. Different letters denote significant differences (p<0.05) between groups. B) Average (n=2, ±SD) extractable Si from non-Si amended paddies under a range of redox in 2016 and 2017 (post-harvest) with four extraction methods.

but no obvious trend was found in the amount of extractable Si from non-flooded paddy soil that varied in redox (Figure 1.1B). Regardless of Si treatment, the amount of Si extracted from flooded paddy soil increased in the order 1-h CaCl₂ < 16-h CaCl₂ < 1-h CH₃COOH < 24-h PO₄. There was less extractable Si in nonamended Control soil than Si amended soil and this effect was significant for 1-h CaCl₂ (p=0.002 to 0.04), 16-h CaCl₂ (p= 0.0008 to 0.002), PO₄ (p= <0.0001 to 0.0008), and CH₃COOH (p= <0.0001 to 0.01) extractions. There was no significant difference in amounts of Si among Si-amended soil when extracted by calcium chloride at either duration (p= 0.52 to 0.99), but CH₃COOH (p= 0.003 and p=0.0006) and PO₄ (p= 0.02 and p= 0.004) extracted significantly more Si from silicate-amended soil than from char and huskamended soil. Amounts of extractable Si from flooded paddy soil tended to be highest in 2016, and lowest in 2015.

Regardless of extraction method, Si concentration in straw was more robustly correlated with plant-available Si than Si in husk (Figure 1.2).



Figure 1.2: Relationship between soil extractable Si and Si concentration in rice husk (A, B, C, D) or rice straw (E, F, G, H). ◊ are samples from 2015. ○ are samples from 2016. △ are samples from 2017. ---- is the predicted plant Si concentration in 2015. _____ is predicted plant Si concentration in 2016. === is predicted plant Si concentration in 2017.

The correlation between extractable soil Si and plant Si was highest for straw ($R^2 = 0.36$ to 0.60) and lowest for husk ($R^2 = 0.08$ to 0.20) (Table 1.1).

	Si in soil vs Si in husk		Si in soil vs Si in straw	
Model Terms	p-value	Partial R ²	p-value	Partial R ²
1-h CaCl ₂ extraction	0.005	0.03	<.0001	0.13
Sampling Year	<.0001	0.26	0.009	0.71
Sampling Year ²	<.0001	0.65	0.76	0.002
16-h CaCl ₂ extraction	0.0022	0.20	<.0001	0.67

Table 1.1:Regression model statistics between soil extractable Si and Si
concentrations in rice husk and rice straw.

Table1.1 continued

Sampling Year	<.0001	0.08	0.58	0.29
Sampling Year ²	<.0001	0.67	0.43	0.01
CH ₃ COOH extraction	0.02	0.08	<0.0001	0.24
Sampling Year	<.0001	0.19	0.13	0.52
Sampling Year ²	<.0001	0.64	0.67	0.004
PO ₄ extraction	0.006	0.10	<.0001	0.50
Sampling Year	< 0.0001	0.13	0.91	0.39
Sampling Year ²	< 0.0001	0.67	0.13	0.05

Si in husk also varied significantly and nonlinearly across years (Year: $R^2 = 0.08$ to 0.26, p<0.0001; Year²: $R^2 = 0.64$ to 0.67, p<0.0001) and therefore had a wider range of values (Figure 1.2 A-D) than Si in straw (Figure 1.2 E-F). In contrast for straw, sampling year was only significant for 1-h CaCl₂ (p=0.009) (Table 1.1), and none of the extraction was affected by sampling year² (p= 0.12 to 0.76).

Among the four extraction methods tested, 16-h CaCl₂ was the best indicator of plant-available Si. The highest correlation was found between 16-h CaCl₂ extractable Si and husk Si (R^2 =0.20, p<0.01) or straw Si (R^2 =0.60, p<0.0001). The next highest correlation was found between PO₄ extractable Si and husk Si (R^2 =0.10, p=0.03) or straw Si (R^2 =0.54, p<0.0001). While neither of these extractions were affected by time (for straw Si), PO₄ extraction appeared to overestimate plant– available Si for Silicate-amended soil as observed with CH₃COOH (Figure 1.1).

Among the other three extraction methods used, PO₄ extractable Si strongly and significantly correlated with 16-h CaCl₂ extractable Si (R^2 =0.77, p<0.0001, Figure 1.3A).



Figure 1.3: Relationship between the robust 16-h CaCl₂ extraction method with other methods used in this study. \diamond are samples from 2015. \circ are samples from 2016. Δ are samples from 2017.

However, the overestimation of silicate-treated soil is apparent with PO₄ extraction. Acetic acid extraction also overestimated plant-available Si for soil treated with Silicate but to a greater extent than for PO₄ extraction, and had a lower but significant correlation coefficient (R^2 =0.53, p<0.0001 Figure 1.3B). Acetic acid extraction only agreed well with the 16-h CaCl₂ method for non-Si treated soil (Figure 1.3B). Extraction with the 1-h CaCl₂ method was weakly but significantly correlated with 16h CaCl₂ (R^2 =0.37, p<0.0001 Figure 1.3C).

1.4 Discussion

Straw is more stable over time than husk to correlate with plant-available Si in soil regardless of extraction methods as indicated by higher R² and smaller variations across years. We expected to observe higher Si in rice plants from 2015 because that was the year when Si amendments were added. After 2015, without any Si replenishment along with the removal of aboveground plant parts, there should be less plant-available Si for rice to uptake. However, plant Si in 2015 should not be significantly than other years because as Si was taken up, more Si would be released from Si pools. Plant parts responded differently to this phenomenon as there were large variations in husk Si across years compared with the small variation in straw Si. We think the difference could be due to two reasons. First, straw accumulate Si over the entire growth period, whereas husk accumulate Si only during the reproduction period. There might be less change in the amount of Si in straw compared with those in husk thus better represent how much was taken up by plants. Second, the fact that extractable Si in flooded soil was highest in 2016 regardless of extraction methods indicated there was a delay of Si releasement from added amendments. This might explain why husk Si was measured highest in 2016.

Despite numerous studies on developing Si extraction methods (Haysom and Chapman, 1975; Korndörfer et al., 1999; Snyder, 2001), little is known how well they correlate to each other and how well they predict plant Si levels under a variety of agronomic conditions. Our results show that 16-h CaCl₂ extraction is the best predictor of plant-available Si compared to other commonly used methods. 1-h CaCl₂ extracted little Si and was weakly correlated with plant Si whether using husk or straw. CH₃COOH and PO₄ over-extracted Si when soil is amended with silicate, and the amount of Si extracted by 1-h CaCl₂ and CH₃COOH varied with time since Si

application. Of the methods tested, only 16-h CaCl₂ was robust regardless of agronomic conditions or time since Si application. Each tested extraction method targets different Si pools, and this helps to explain the differences observed with extraction method. Extraction with CaCl₂ targets the easily soluble Si (Berthelsen et al., 2001), CH₃COOH extracts both soluble Si and exchangeable Si from soil (Sauer et al., 2006), and PO₄ displaces adsorbed Si (Snyder 2001). Consistent with Nonaka and Takahashi (1988), we observed that CH₃COOH over-extracted Si from Silicate amended soil likely because CH₃COOH aggressively removed too much Si from silicate fertilizer that would normally not be taken up by plants. In addition, we observed higher soil pH (silicate pH 6.8; control pH 6.1; char pH 5.8; husk pH 5.6) due to Silicate amendment in 2016 that coincided with the over-extraction of Si from Silicate (Figure 1.2G). Increased soil pH due to Silicate could have promoted polarization of Si and the formation of alumino-silicate compounds, thus limiting the solubility and availability of these Si for plants (Snyder, 2001). Camargo et al. (2007) reported a positive relationship between CH₃COOH extractable Si and soil pH. Therefore, CH₃COOH extraction is not robust against different agronomic conditions. While we observed that PO₄ extraction also over-estimated plant-available Si in Silicate amended soil, it was a better predictor than CH₃COOH (Table 1.1). Kato (2000) did not observe that PO₄ over-extracted Si from Silicate amended soil, and Snyder (2001) assumed PO₄ exchanged with adsorbed silicic acid rather than dissolved residual calcium silicate fertilizer. The overestimation of plant-available Si with PO₄ in our study is negligible when compared with the correlation between 16-h CaCl₂ extractable Si and straw Si, as they agreed well with the exception silicate

treatment (partial R^2 =0.50 for PO₄, Figure 1.2 H; partial R^2 =0.67 for 16-h CaCl₂, Figure 1.2 F).

Despite both used 0.01M CaCl₂ as extractants, 1-h shaking method correlated poorly with 16-h shaking method. While 1-h shaking is better for lab use, it is inferior to the 16-h shaking in terms of the correlation between Si in soil and Si in plants. In an South Africa study, Miles et al. (2014) also used 0.01M CaCl₂ to extract soil Si, and compared 30-min shaking with 16-h shaking, and he found the latter performed better. One disadvantage of 16-h CaCl₂ extraction is the requirement for overnight shaking. For this reason, 4-h CaCl₂ (1:20 w:v) was tested for evaluation here, because it required less shaking time thus server a quicker method for laboratory use. We found 4-h CaCl₂ extraction correlated significantly with 16-h CaCl₂ extraction (R^2 =0.82, p<0.0001) (Figure 1.4C),



Figure 1.4: Relationship between 4-h CaCl₂ extractable soil Si and Si concentration in rice husk (A) or rice straw (B), and the relationship between the robust 16-h CaCl₂ extraction and 4-h CaCl₂ extraction (C). ◊ are samples from 2015. ○ are samples from 2016. △ are samples from 2017. ---- is the predicted plant Si concentration in 2015. _____ is predicted plant Si concentration in 2016. === is predicted plant Si concentration in 2017

with slightly higher R^2 than the correlation between PO₄ extraction and 16-h CaCl₂ extraction (R^2 =0.77, p<0.0001). For the 4-h CaCl₂ extraction, sampling year (p<0.0001) and sampling year² (p<0.0001) were significant for husk, but not for straw (p=0.15 for sampling year and p=0.89 for sampling year²), which is consistent with our finding that straw is a better indicator to correlate with plant-available Si in soil. The correlation between 4-h CaCl₂ extractable soil Si and plant Si (R^2 =0.21, p=0.003

for husk; $R^2=0.58$, p<0.0001 for straw) (Figure 1.4 A, B) is very close to the 16-h CaCl₂ extraction method ($R^2=0.20$, p=0.0022 for husk; $R^2=67$, p<0.0001 for straw), yet markedly superior than the 1-h CaCl₂ extraction method ($R^2=0.03$, p=0.005 for husk; $R^2=0.13$, p<0.001 for straw). Thus, assessing plant-available Si is a function of both extractant and extraction time. When extracting Si, silicates in the soil, particularly clay minerals are attacked both chemically and physically (Sauer et al., 2006). As weak salt solution was not designed to extract exchangeable Si from soil, particularly the exchangeable Si not be taken up by rice plants, a short shaking time could not physically remove all the most readily available Si. 4-h CaCl₂ have both characterizes of enough shaking time and weak salt solution thus successfully quantified plant-available Si in paddy soil. No literature has reported using 4-h CaCl₂ as extraction method to determine plant-available Si in rice paddy soil so far. We suggest there is a great potential of using 4-h CaCl₂ over 16-h CaCl₂, which takes longer shaking time but generate the same results with 4-h CaCl₂.

1.5 Conclusion

We tested five extraction methods and found 16-h CaCl₂ was the best indicator of plant-available Si. Both CH₃COOH and PO₄ over extracted Si from silicateamended soil, but overall PO₄ extractable soil Si correlated much better with plant Si than CH₃COOH. 1-h CaCl₂ correlated poorly with 16-h CaCl₂ due to the short shaking time. We tested a new extraction method 4-h CaCl₂ to save shaking time, and found it correlated significantly well with 16-h CaCl₂. In the future, we recommend using 4-h or 16-h CaCl₂ as extraction method to assess plant-available Si in rice paddy soil under different agronomic or flooding conditions.

REFERENCES

- Berthelsen, S., Noble, A. D., and Garside, A. L. (2001). Chapter 15 Silicon research down under: Past, present, and future. *In* "Studies in Plant Science" (L. E. Datnoff, G. H. Snyder and G. H. Korndörfer, eds.), Vol. 8, pp. 241-255. Elsevier.
- Camargo, M. S. d., Pereira, H. S., Korndörfer, G. H., Queiroz, A. A., and Reis, C. B. d. (2007). Soil reaction and absorption of silicon by rice. *Scientia Agricola* **64**, 176-180.
- Cocker, K. M., Evans, D. E., and Hodson, M. J. (1998). The amelioration of aluminium toxicity by silicon in higher plants: Solution chemistry or an in planta mechanism? *Physiologia Plantarum* **104**, 608-614.
- Derry, L. A., Kurtz, A. C., Ziegler, K., and Chadwick, O. A. (2005). Biological control of terrestrial silica cycling and export fluxes to watersheds. *Nature* **433**, 728.
- Fleck, A. T., Mattusch, J., and Schenk, M. K. (2013). Silicon decreases the arsenic level in rice grain by limiting arsenite transport. *Journal of Plant Nutrition and Soil Science* 176, 785-794.
- Frings, P. J., Clymans, W., Jeppesen, E., Lauridsen, T. L., Struyf, E., and Conley, D. J. (2014). Lack of steady-state in the global biogeochemical Si cycle: emerging evidence from lake Si sequestration. *Biogeochemistry* 117, 255-277.
- Gottardi, S., Iacuzzo, F., Tomasi, N., Cortella, G., Manzocco, L., Pinton, R., Römheld, V., Mimmo, T., Scampicchio, M., Dalla Costa, L., and Cesco, S. (2012).
 Beneficial effects of silicon on hydroponically grown corn salad (*Valerianella locusta (L.) Laterr*) plants. *Plant Physiology and Biochemistry* 56, 14-23.
- Gwon, H. S., Khan, M. I., Alam, M. A., Das, S., and Kim, P. J. (2018). Environmental risk assessment of steel-making slags and the potential use of LD slag in mitigating methane emissions and the grain arsenic level in rice (*Oryza sativa L.*). Journal of Hazardous Materials **353**, 236-243.
- Haysom, M. B. C., and Chapman, L. S. (1975). Some aspects of the calcium silicate trials at Mackay. *Australian Society of Sugar Cane Technologists*. 42, 117-122.

- Kato, S. (2000). Evaluation of silicon availability in paddy soil by an extraction using a pH 6.2 phosphate buffer solution. *Annual report of the Tohoku Agricultural Experiment Station for 1999*, 73-5.
- Korndörfer, G. H., Coelho, N. M., Snyder, G. H., and Mizutani, C. T. (1999). Avaliação de métodos de extração de silício em solos cultivados com arroz de sequeiro. *Revista Brasileira de Ciência do Solo* 23, 101-106.
- Korndörfer, G. H., Snyder, G. H., Ulloa, M., Powell, G., and Datnoff, L. E. (2001). Calibration of soil and plant silicon analysis for rice production. *Journal of Plant Nutrition* 24, 1071-1084.
- Kraska, J. E., and Breitenbeck, G. A. (2010). Simple, Robust Method for Quantifying Silicon in Plant Tissue. *Communications in Soil Science and Plant Analysis* **41**, 2075-2085.
- Liang, Y., Sun, W., Zhu, Y.-G., and Christie, P. (2007). Mechanisms of siliconmediated alleviation of abiotic stresses in higher plants: A review. *Environmental Pollution* 147, 422-428.
- Limmer, M. A., Mann, J., Amaral, D. C., Vargas, R., and Seyfferth, A. L. (2018a). Silicon-rich amendments in rice paddies: Effects on arsenic uptake and biogeochemistry. *Science of The Total Environment* 624, 1360-1368.
- Limmer, M. A., Wise, P., Dykes, G., and Seyfferth, A. L. (2018b). Silicon decreases dimethylarsinic acid concentration in rice grain and mitigates straighthead disorder. *Environmental Science & Technology* **52**, 4809-4816.
- Liu, J., Ma, J., He, C. W., Li, X. L., Zhang, W. J., Xu, F. S., Lin, Y. J., and Wang, L. J. (2013). Inhibition of cadmium ion uptake in rice (*Oryza sativa*) cells by a wall-bound form of silicon. *New Phytologist* 200, 691-699.
- Ma, J. F., Sasaki, M., and Matsumoto, H. (1997). Al-induced inhibition of root elongation in corn, Zea mays L. is overcome by Si addition. *Plant and Soil* 188, 171-176.
- Ma, J. F., and Yamaji, N. (2006). Silicon uptake and accumulation in higher plants. *Trends in Plant Science* **11**, 392-397.
- Ma, J. F., Yamaji, N., Mitani, N., Xu, X.-Y., Su, Y.-H., McGrath, S. P., and Zhao, F.-J. (2008). Transporters of arsenite in rice and their role in arsenic accumulation in rice grain. *Proceedings of the National Academy of Sciences* **105**, 9931-9935.

- Ma, R., Shen, J. L., Wu, J. S., Tang, Z., Shen, Q. R., and Zhao, F. J. (2014). Impact of agronomic practices on arsenic accumulation and speciation in rice grain. *Environmental Pollution* 194, 217-223.
- Manivannan, A., and Ahn, Y. K. (2017). Silicon Regulates Potential Genes Involved in Major Physiological Processes in Plants to Combat Stress. *Frontiers in Plant Science* **8**.
- Meena, V. D., Dotaniya, M. L., Coumar, V., Rajendiran, S., Ajay, Kundu, S., and Subba Rao, A. (2014). A case for silicon fertilization to improve crop yields in tropical soil. *Proceedings of the National Academy of Sciences, India Section* B: Biological Sciences 84, 505-518.
- Miles, N., Manson, A. D., Rhodes, R., van Antwerpen, R., and Weigel, A. (2014). Extractable silicon in soil of the South African sugar industry and relationships with crop uptake. *Communications in Soil Science and Plant Analysis* 45, 2949-2958.
- Nonaka, K., and Takahashi, K. (1988). A method of measuring available silicate in paddy soil. *Jarq-Japan Agricultural Research Quarterly* **22**, 91-95.
- Pereira, H. S., Korndorfer, G. H., Vidal, A. D., and de Camargo, M. S. (2004). Silicon sources for rice crop. *Scientia Agricola* **61**, 522-528.
- Sauer, D., Saccone, L., Conley, D. J., Herrmann, L., and Sommer, M. (2006). Review of methodologies for extracting plant-available and amorphous Si from soil and aquatic sediments. *Biogeochemistry* **80**, 89-108.
- Savant, N. K., Korndörfer, G. H., Datnoff, L. E., and Snyder, G. H. (1999). Silicon nutrition and sugarcane production: A review. *Journal of Plant Nutrition* 22, 1853-1903.
- Savant, N. K., Snyder, G. H., and Datnoff, L. E. (1996). Silicon management and sustainable rice production. *In* "Advances in Agronomy" (D. L. Sparks, ed.), Vol. 58, pp. 151-199. Academic Press.
- Savant, N. K., Snyder, G. H., and Datnoff, L. E. (1997). Silicon management and sustainable rice production. *In* "Advances in Agronomy, Vol 58" (D. L. Sparks, ed.), Vol. 58, pp. 151-199. Elsevier Academic Press Inc, San Diego.
- Seyfferth, A. L., and Fendorf, S. (2012). Silicate mineral impacts on the uptake and storage of arsenic and plant nutrients in rice (*Oryza sativa L.*). *Environmental Science & Technology* 46, 13176-13183.

- Seyfferth, A. L., Limmer, M. A., and Dykes, G. E. (2018). Chapter Two On the use of silicon as an agronomic mitigation strategy to decrease arsenic uptake by rice. *In* "Advances in Agronomy" (D. L. Sparks, ed.), Vol. 149, pp. 49-91. Academic Press.
- Seyfferth, A. L., Morris, A. H., Gill, R., Kearns, K. A., Mann, J. N., Paukett, M., and Leskanic, C. (2016). Soil incorporation of silica-rich rice husk decreases inorganic arsenic in rice grain. *Journal of Agricultural and Food Chemistry* 64, 3760-3766.
- Snyder, G. H. (2001). Chapter 11 Methods for silicon analysis in plants, soil, and fertilizers. *In* "Studies in Plant Science" (L. E. Datnoff, G. H. Snyder and G. H. Korndörfer, eds.), Vol. 8, pp. 185-196. Elsevier.

Chapter 2

IMPACTS OF SILICON AMENDMENTS AND TIME ON ARSENIC CYCLING IN RICE PADDY SOIL

2.1 Introduction

Arsenic is a ubiquitous toxin in the environment, and can be present in rocks, soil, waters, air and biological tissues (Nriagu and Pacyna, 1988). Arsenic is naturally present in rice paddy soil and becomes mobile or plant-available under flooded (i.e. paddy) conditions due to the reductive dissolution of As-bearing Fe and Mn oxides (Takahashi et al., 2004). When soil is flooded, adsorbed As(V) is desorbed from Fe and Mn oxides and reduced to As(III) and therefore becomes more mobile, because unlike As(V) which adsorbs to Fe and Mn oxides and clays, As(III) can only adsorb to Fe oxides (Goldberg and Johnston, 2001). Under aerobic soil conditions, As(V) dominates, whereas in submerged soil condition the predominate species is As(III) (Masscheleyn et al., 1991). Inorganic As can be further methylated to MMA or DMA or TMAO by soil microbes (Stolz et al., 2006). Once mobilized, arsenic can be taken up by rice roots and stored in rice bran and grain due to different pathways depending on the As species. As(V) share the transporter with P (Meharg and Macnair, 1992); As(III), DMA and MMA share the transporter with Si (Li et al., 2009; Ma et al., 2008). Rice is therefore a source of human As exposure, and compared to other crops, rice accumulates As 10 folds higher than those of wheat and barley (Williams et al., 2007). Chronic exposure to As increases rate of bladder, skin and long cancers (Smith et al., 1992). Therefore, mitigating As in rice is an active area of research.

Because several forms of As are taken up by the Si transport pathway (Li et al., 2009; Ma et al., 2008), researchers have investigated the impacts of adding Si to soil to lower As in rice grain (Bogdan and Schenk, 2008; Fleck et al., 2013; Limmer et al., 2018a; Seyfferth and Fendorf, 2012; Seyfferth et al., 2016; Teasley et al., 2017; Wu et al., 2016). Bogdan and Schenk (2008) found there was a strong negative correlation between the Si in porewater and As in rice grain in Italian soil. Increased dissolved Si is also known to downregulate the expression of Si transporters (Ma et al., 2006; Ma et al., 2007), which will in turn lower the potential for As uptake. Addition of Si was recently shown to decrease DMA uptake into rice and reverse the DMA-induced yield loss (Limmer et al., 2018b). Due to the difference in solubility of Si amendments over rice growth period, the Si source matters in terms of lowering As uptake in rice (Seyfferth et al., 2018; Seyfferth et al., 2016; Teasley et al., 2017). Seyfferth's group has shown that rice husk incorporation into soil can decrease either total or inorganic As in rice grain, but calcium silicate was not effective (Seyfferth et al., 2016; Teasley et al., 2017). However, the fate of As in soil after soil-incorporation of silicon-rich materials is unresolved.

In flooded soil, the fate and bioavailability of As is mostly controlled by Fe and Mn oxides (Suda and Makino, 2016; Xu et al., 2017). Under oxic conditions, arsenic is strongly retained on Fe oxides in soil (Yang et al., 2002). As soil are flooded for paddy rice cultivation, soil become more reduced and reductive dissolution of Fe oxides takes place resulting in a subsequent release of sorbed As into the solution phase (Takahashi et al., 2004), therefore making As more plant-available. In the rhizosphere, rice plants release oxygen via aerenchyma, which oxidize Fe(II) in porewater and induce the precipitation of Fe oxides (Fe plaque) onto rice roots (Chen

et al., 1980). The major components of Fe plaque is ferrihydrite with lesser amounts of lepidocrocite, goethite and siderite (Amaral et al., 2017; Liu et al., 2006; Seyfferth et al., 2011). Studies have found that Fe plaques are important for As sequestration and may limit the amount of As taken up by rice plants (Liu et al., 2004; Syu et al., 2013). On the other hand, Fe plaques may also be a source of As to rice plants (Yamaguchi et al., 2014). Mn oxides also play an important role in As cycling by oxidizing As(III) to As(V) (Lafferty et al., 2010; Manning et al., 2002; Ying et al., 2012), and the reactivity of Mn oxides depend on their crystallinity, surface area and presence of vacancy sites (Fischel et al., 2015). For example, birnessite, one of the most common Mn oxides in soil, can oxidize As(III) to As(V) (Manning et al., 2002). The mechanism is that at the surface of birnesssite, As(III) is first oxidized to As(V) along with the reduction of Mn(V) to Mn(II), then As(V) is adsorbed onto the surface of birnessite. Co-existing ions such as PO_4^{3-} and Ca^{2+} can displace As(V), and desorbed As(V) can be further sorbed onto Fe oxides (Lafferty et al., 2011; Manning et al., 2002; Ying et al., 2012). Therefore, this As oxidation by Mn oxides may limit the amount of As that is plant-available.

In addition to competing with As for plant uptake, adding Si to paddy soil affects the fate of As and Fe. Due to the chemical similarity between dissolved Si and As(III), Si and As(III) compete for adsorption sites of Fe oxides and adding Si will desorb As(III) from those minerals (Luxton et al., 2006). Increasing dissolved Si also retard the transformation of Fe oxides to a higher order, more crystalline phase (Jones et al., 2009; Schwertmann and Taylor, 1972; Schwertmann and Thalmann, 1976). Poorly-crystalline Fe oxide such as ferrihydrite can transform into well-crystalline Fe oxides such as goethite (Pedersen et al., 2005). The mechanism is that electron transfer

between Fe(II) and Fe(III) result in reduction and subsequent dissolution of the Fe(III) minerals, which allows released Fe(II) to recrystallize into more stable species than initial Fe(III) minerals (Jones et al., 2009; Yee et al., 2006). The presence of dissolved Si can 1) compete with Fe(II) on Fe(III) mineral sorption sites, which interfere the electron transfer between Fe(II) and Fe(III) and 2) prevent the polymerization of thermodynamically stable Fe(III) minerals (Jones et al., 2009). By retarding the transformation of Fe minerals, Si addition into soil is expected to create more adsorption sites for As (Ona-Nguema et al., 2005). Previous studies have found that Si amendments increased the fraction of ferrihydrite on Fe plaque onto rice roots compared to nonamended controls (Amaral et al., 2017; Limmer et al., 2018a; Teasley et al., 2017). If this process happens in bulk paddy soil amended with Si, then As retention onto Fe oxides may increase with Si amendment. However, this has not been directly evaluated.

The aim of this study is to understand how different Si amendments affect soil As, Fe and Mn pools in rice paddy soil. It was hypothesized that Si addition would impact the soil As pools by increasing the proportion of As associated with poorly crystalline Fe oxides.

2.2 Materials and Methods

Twelve rice paddy mesocosms were established in 2015 with four different Si treatments (nonamended (Control), charred rice husk (Char), rice husk (Husk) and calcium silicate/silicic acid (Silicate)), 3 paddies per treatment. Rice paddies are located in the University of Delaware farm, DE, USA, where the soil has a silty clay loam texture, pH of 7.8, 1.33% organic matter and is classified as Ultisol/Acrisoil (Limmer et al., 2018a). Before soil was amended and rice was planted in 2015, soil

samples were obtained from each paddy (see next section). Si amendments were then applied in a rate of 5 Mg Si/ha. Rice seeds were germinated in greenhouse and transplanted into paddies at the 3 to 4 leaf stage, grown under flooded conditions until 2 weeks before harvest when paddies were drained (Limmer et al., 2018a). Rice was harvested in early September and soil was collected the day after harvest. In 2016 and 2017, rice was planted and harvested the same way, but no additional Si amendments were added.

Soil samples were collected before Si amendments had been added in 2015 (hereafter referred as pre-rice), at post-harvest in 2015, 2016, 2017 (hereafter referred as post-harvest) and four times throughout the growing season in 2017. The four time points during the growing season in 2017 were 56, 78, 87 and 109 days after transplant. For those collected at pre-rice and post-harvest in 2015, at post-harvest in 2016 and 2017, five soil samples were collected in diagonal from each paddy and composited into one sample at the 0-10 cm depth. For those collected during the growing season in 2017, one soil sample was collected from each paddy at the 0-10 cm depth. All the soil samples we collected were air dried and passed through 2mm sieve for chemical analysis. 15mL of porewater was collect weekly using rhizon samplers at around 15cm deep in paddy soil and saved in transport tube. Five drops of concentrated nitric acid were added immediately after porewater were added in transport tube. Saved porewater was further analyzed for total As concentration with inductively coupled plasma-mass spectrometer (ICP-MS).

2mm sieved soil was subjected to a sequential extraction developed by Wenzel et al. (2001). This procedure uses progressively stronger extractants to target different As pools (0.05 M ammonium sulfate for non-specifically sorbed As (F1 fraction); 0.05

M ammonium phosphate for specifically sorbed As (F2 fraction); 0.2 M ammonium oxalate buffer for As associated with amorphous and poorly-crystalline Fe/Al oxides (F3 fraction); 0.2 M ammonium oxalate and ascorbic acid for As associated with well-crystalline Fe/Al oxides (F4 fraction); nitric acid/hydrogen peroxide for residual As phases (F5 fraction)). After centrifugation, supernatants were syringe filtered through 0.45 μ m filters into acid-washed polyproelene tubes and acidified with 2% nitric acid. Elemental analysis for each extractant was conducted using matrix-matched standards. Blanks and checks were added every 20 samples for QA/QC. NIST 2711 (certified As level 107 \pm 5 mg/kg) was included during the last step of As extraction. The recovery for As in the reference soil was 86% \pm 23%. All the samples were analyzed with ICP-MS.

Poorly-crystalline Fe and Mn oxides were assessed through ammonium oxalate extraction, as part of As sequential extraction. Total reducible Fe and Mn oxides were assessed through citrate-bicarbonate-dithionite (CBD) extraction (Loeppert and Inskeep, 1996). For this extraction, 2.5 g of 53µm sieved soil was mixed with 20 mL of 0.3 M sodium citrate and 2.5 mL of 0.1 M sodium bicarbonate in a 50mL polyproelene tube. Tubes were transferred into a water bath that was preheated to 75 to 80°, and once the temperature of the soil suspension has risen to 75 to 80 °C, 0.5 g of sodium dithionite was added and stirred for 6 mins. A second 0.5 g of sodium dithionite was added and stirred for another 10 mins. Samples were centrifuged at 1000 rpm for 15 mins and supernatant were poured off into a 100mL volumetric flask. The remaining soil was washed with 10 mL of 0.3 M sodium citrate, centrifuged at 1000 rpm for 15 mins, and the supernatant was combined with previous solution. All

samples were analyzed with inductively coupled plasma-optical emission spectroscopy (ICP-OES) using matrix-matched standards.

We performed three models to study the effect of Si treatments and time on the pools of As, Fe-, and Mn- oxides (variables). Models were performed with repeated measures ANOVA because samples were taken from the same paddies at each year. These models consider treatments, time and interaction between time and treatments for factors, and compared all the variables in 2015 pre-rice and post-harvest, all the post-harvest in 2015, 2016, and 2017 throughout growth season (including post-harvest) respectively for each model. We used CS (compound symmetry) for repeated statement. If significant differences (p<0.05) were identified, a Tukey post post-harvest hoc test was used to distinguish differences among groups. A multiple regression model was performed to compare the correlation between oxalate-extractable Fe and Mn with sulfate-extractable As (F1) and porewater As. We used R² to assess the amount of variance explained by each term. All statistical analyses were performed with SAS 9.4 (The SAS Institute).

2.3 Results

Si treatment had no significant effect on As pools in pre-rice and post-harvest soil from 2015, but conversion from grassland to rice paddy was a significant factor (Figure 2.1).



Figure 2.1: Total background As from 2015 pre-rice paddy soil (a). Arsenic fraction F1 (b), F2 (c), F3(d), F4 (e), F5 (f) in 2015 pre-rice and post-harvest soil that was nonamended (Control) or amended with charred rice husk (Char), rice husk (Husk) or calcium silicate/silicic acid (Silicate). Different upper-case letters denote significant differences (p<0.05) between sampling time. Different lower-case letters denote significant differences (p<0.05) between Si treatments.

Arsenic in the F1 fraction in post-harvest soil was 7-fold (range: 0.78 to 46x, p=0.004) higher than those in pre-rice soil. Arsenic in the F5 fraction in post-harvest soil was also higher than pre-rice but not as drastically as in F1 (1.5-fold, range: 0.91 to 2.2x, p<0.0001). Si treatments had no significant effect on F1 and F5 fraction (p=0.92 and

p=0.08). Neither sampling time nor Si treatments had significant effect on F2, F3, and F4 fractions (p=0.16 to p=0.57).

Si treatment had no significant effect on Fe and Mn pools in pre-rice and postharvest soil from 2015, but time was a significant factor (Figure 2.2).



Figure 2.2: Oxalate-extractable Fe (a) and Mn (b), and CBD-extractable Fe (c) and Mn (d) in 2015 pre-rice and post-harvest soil that was nonamended (Control) or amended with charred rice husk (Char), rice husk (Husk) or calcium silicate/silicic acid (Silicate). Different upper-case letters denote significant differences (p<0.05) between sampling time. Different lowercase letters denote significant differences (p<0.05) between Si treatments.

Oxalate-extractable Mn experienced a 0.55-fold change (range: 0.23 to 1.11x) from pre-rice to post-harvest soil (p<0.0001). Si treatments had no significant effect on the amount of oxalate-extractable Fe and Mn oxides (p=0.32 and p=0.19). CBD-

extractable Fe and Mn experienced no significant change neither from time nor with Si treatments (p=0.26 to p=0.88).

Sampling year played a larger role in As fractions from all post-harvest soil than Si treatments (Figure 2.3).



Figure 2.3: Arsenic fraction F1 (a), F2 (b), F3(c), F4 (d) and F5 (e) in all post-harvest soil that was nonamended (Control) or amended with charred rice husk (Char), rice husk (Husk) or calcium silicate/silicic acid (Silicate). Different upper-case letters denote significant differences (p<0.05) between sampling year. Different lower-case letters denote significant differences (p<0.05) between Si treatments. * denotes that there is significant interaction between sampling year and Si treatments.

In the F1 fraction, arsenic from 2017 was 1.75-fold higher (range: 1.11 to 2.50x, p<0.0001) than those from 2016 and 1.55-fold (range: 0.38 to 3.74x, p<0.0001) higher than those from 2015 (Figure 2.3a). In the F2 fraction, arsenic from 2016 was 1.43-fold (range: 1.22 to 1.69x, p<0.0001) higher than those from 2015 and 1.11-fold (range: 0.93 to 1.28x, p=0.01) higher than those from 2017 (Figure 2.3b). Sampling year had no significant effect on As in the F3 fraction (p=0.23) (Figure 2.3c). In the F4 faction, arsenic in 2016 and 2017 post-harvest soil was not significantly different from As in 2015 post-harvest soil (p=0.07 and p=0.66), but As in 2016 was 1.25-fold higher (range: 0.80 to 1.71x, p=0.01) than As in 2017 (Figure 2.3d). Si treatment had no significant effect on the amount of As in F1, F2, F3 and F4 fractions (p=0.45 to p=0.84). In the F5 fraction, arsenic experienced a 0.79-fold change (range: 0.35 to 1.46x, p=0.004) from 2015 to 2016 (Figure 2.3e).

Si treatment had no significant effect on Fe and Mn pools from all post-harvest soil, but sampling year was a factor (Figure 2.4).



Figure 2.4: Oxalate-extractable Fe (a) and Mn (b), and CBD-extractable Fe (c) and Mn (d) in all post-harvest soil that was nonamended (Control) or amended with charred rice husk (Char), rice husk (Husk) or calcium silicate/silicic acid (Silicate). Different upper-case letters denote significant differences (p<0.05) between sampling year. Different lowercase letters denote significant differences (p<0.05) between Si treatments.

The increase for oxalate-extractable Fe was 1.56-fold (range: 0.82 to 2.14x, p<0.0001) from 2015 to 2016 and was 1.48-fold (range: 0.99 to 2.08x, p=0.0001) from 2015 to 2017. The increase for oxalate-extractable Mn was 2.29-fold (range: 0.55 to 3.46x, p=0.004) from 2015 to 2016 and 1.29-fold (range: 0.46 to 2.05x, p=0.07) from 2015 to 2017 (Figure 2.4b). CBD-extractable Fe in 2015 experienced a 0.77-fold change from 2015 to 2016 (range: 0.69 to 0.85x, p<0.0001) and a 1.18-fold change from 2016 to 2017 (range: 0.88 to 1.31x, p=0.0002) (Figure 2.4c) No significant change was found in CBD-extractable Mn among years (p=0.70) (Figure 2.4d).

Both sampling time and Si treatments affect As pools in all 2017 soil (Figure

2.5).





Figure 2.5: Arsenic fraction F1 (a), F2 (b), F3(c), F4 (d) and F5 (e) in 2017 soil that was collected during rice growth period. Soil was nonamended (Control) or amended with charred rice husk (Char), rice husk (Husk) or calcium silicate/silicic acid (Silicate). Different upper-case letters denote significant differences (p<0.05) between sampling time. Different lowercase letters denote significant differences (p<0.05) between Si treatments. * denotes that there is significant interaction between sampling year and Si treatments. In the F1 fraction, neither Si treatment (p=0.32) nor sampling time (p=0.32) had significant effect on As pools (Figure 2.5a). In the F2 fraction, significant interaction between Si treatment and sampling time (p=0.0006) was found and the trend of As with time was not obvious (Figure 2.5b). Both F3 and F4 fractions experienced an increase of As from 56 days after transplant to 87 days after transplant and then started to decrease (Figure 2.5c). In the F4 fraction, arsenic from Char-amended soil was 1.16-fold higher (range: 1.00 to 1.35x, p=0.04) than As from Silicate-amended soil (Figure 2.4d). In the F5 fraction, amount of As was significantly higher in 109 days after transplant than rest of the collection time points (p<0.0001 to p=0.0005) (Figure 2.5e).

Both sampling time and Si treatment affected amount of poorly-crystalline Fe and Mn oxides from all 2017 soil (Figure 2.6).



Figure 2.6: Oxalate-extractable Fe (a) and Mn (b) in 2017 soil that was collected during rice growth period. Soil was nonamended (Control) or amended with charred rice husk (Char), rice husk (Husk) or calcium silicate/silicic acid (Silicate). Different upper-case letters denote significant differences (p<0.05) between sampling year. Different lower-case letters denote significant differences (p<0.05) between Si treatments.

Although sampling time had no significant effect on oxalate-extractable Fe (p=0.06), oxalate-extractable Fe in Silicate-amended soil was 1.23-fold higher (range: 0.63 to 1.99x, p=0.002) than those in Control soil, 1.05-fold higher (range: 0.83 to 1.47x, p=0.02) than those in Char-amended soil, and 1.03-fold higher (range: 0.60 to 1.47x, p=0.009) than those in Husk-amended soil (Figure 2.6a). Oxalate-extractable Mn followed similar trend with As in F3 and F4 (Figure 2.5c, d) as the amount of oxalate-extractable Mn first increased and then decreased with time (Figure 2.6b).

Organic matter was positively correlated with oxalate-extractable Fe (R^2 =0.22, p=0.0004) but not with oxalate-extractable Mn (R^2 =0.04, p=0.23) (Figure 2.7).



Figure 2.7: Relationship between organic matter with oxalate-extractable Fe (a) and oxalate-extractable Mn (b) from post-harvest soil. Correlation without fit-line was not significant.

Oxalate-extractable was negatively correlated with sulfate-extractable As for all post soil($R^2=0.15$, p=0.02) or soil collected over rice growth period ($R^2=0.13$, p=0.004) but no obvious relationship was found between oxalate-extractable Fe and

sulfate extractable As for all post soils ($R^2=0.02$, 0.47) or soil collected over rice growth period ($R^2=0.007$, p=0.54). (Figure 2.8b, c, e, f).



Figure 2.8: Relationship between oxalate-extractable Fe and sulfate-extractable As in 2015 pre-rice and post-harvest soil (a), all post-harvest soil (b), and 2017 soil over rice growth period (c). Relationship between oxalate-extractable Mn and sulfate-extractable As in 2015 pre-rice and post-harvest soil (d), all post-harvest soil (e), and 2017 soil over rice growth period (f). In (a) and (f), ○ are samples from pre-rice and ◊ are samples from post-harvest. Correlations without fit-lines were not significant.

2015 pre-rice soil showed no significant relationship between oxalate-extractable Fe, Mn and sulfate-extractable As (R^2 =0.02, p=0.70 for Fe; R^2 =0.04, p=0.54 for Mn), whereas in post-harvest soil significant positive relationship was found between oxalate-extractable Fe and sulfate-extractable As ($R^2=0.50$, p=0.01) but not between oxalate-extractable Mn and sulfate-extractable As ($R^2=0.08$, p=0.37) (Figure 2.8a, b).

Across three years, oxalate-extractable Fe but not Mn was significantly and positively related with porewater As ($R^2=0.32$, p=0.0003) (Figure 2.9a-b).



Figure 2.9: Relationship between oxalate-extractable Fe and porewater As (a) and between oxalate-extractable Mn and porewater As (b) from all post-harvest soil. Relationship between oxalate-extractable Fe and porewater As (c) and between oxalate-extractable Mn and porewater As (d) from 2017 soil over rice growth period. Porewater As in (a) and (b) was the average of porewater As measured from each week at every year. Porewater As in (c) and (d) was the porewater As at each week corresponded with the week of soil collection. The three points in x-axis in (c) and (d) were the weeks of missing porewater As data. Correlation without fit-line was not significant.

However, for samples collected over the growing season, both oxalate-extractable Fe (R^2 =0.15, p=0.009) and oxalate-extractable Mn (R^2 =0.11, p=0.03) were positively correlated with porewater As (Figure 2.9c, d).

2.4 Discussion

We evaluated As, Fe and Mn pools to examine the impact of Si addition on As cycling in rice paddy soil. We hypothesized that Si addition would impact the soil As pools by increasing the proportion of As associated with poorly-crystalline Fe oxides because Si addition has been observed to stabilize ferrihydrite (Amaral et al., 2017; Schwertmann and Taylor, 1972; Schwertmann and Taylor, 1976). However, our results showed that Si addition had no significant effect on bulk soil As pools associated with poorly-crystalline Fe oxides (p>0.05) (Figure 2.1d, 2.3c, 2.5c) and therefore our hypothesis was not supported. This finding suggests the increase of the proportion of poorly crystalline Fe minerals due to Si addition previously observed (Amaral et al., 2017; Limmer et al., 2018a; Teasley et al., 2017) is restricted to the rhizosphere in rice paddies.

Our results and those of others illustrate conflicting reports of the impact of Si on bulk soil As pools, which is likely due to different Si amendment properties (e.g., Si solubilities (Seyfferth et al., 2018), pH and other elements). Wu et al. (2016) found that Si addition increased the amount of plant-available As and decreased the amount of As from both poorly-crystalline and well-crystalline Fe oxides pools. In contrast, Gwon et al. (2018) found that Si addition increased the amount of As associated with poorly-crystalline Fe oxides and decreased the amount of plant-available As. Wu et al. (2016) used Si gel, Gwon et al. (2018) used silicate slag, and the present study used husk based Si and calcium silicate mixed with silicic acid for Si amendments. High

levels of Fe in silicate slag may serve as additional pools for As sorption. On the other hand, Teasley et al. (2017) found that Si addition had no significant effect on plantavailable As or As from poorly-crystalline Fe oxides compared to control using Si treatments similar to those in the present study, which corroborate our findings. However, Teasley et al. 2017 observed that Silicate-amended soil had significantly higher plant-available As or As from poorly-crystalline Fe oxides than those in Huskamended soil, which contrast with our findings where no difference between Si amendments was observed (Figure 2.3a-c). Results from the present study contrast with others for several reasons. First, the total soil As in the present study was at background levels of ~4 mg/kg (Figure 2.1a), whereas soil used in Wu et al. (2016), Gwon et al. (2018) and Teasley et al. (2017) were artificially spiked with As to 60 mg/kg, 40 mg/kg and 24 mg/kg of As respectively. Arsenic that is spiked into soil tends to artificially increase the proportion of As in the plant-available and poorlycrystalline pools (Teasely et al. 2017). Dittmar et al. (2010) compared As levels in rice straw and grain grown under high As soil versus low As soil but spiked with As to reach the same As level with high As soil, and they found rice grown in As spiked soil had higher straw As and grain As suggesting the freshly added As was highly bioavailable. Second, studies by Wu et al. (2016), Gwon et al. (2018) and Teasley et al. (2017) were conducted in pots, which have limitations, whereas our study was conducted in a field setting. These differences in spiking vs. nonspiking coupled with different Si amendments used likely contribute to the discrepancies among studies.

Although studies have found that Si addition increased the crystallinity of Fe oxides (Anderson and Benjamin, 1985; Schwertmann and Taylor, 1972), our results indicated that Fe and Mn pools from post-harvest soil were not significantly affected

by Si treatment but by sampling year (Figure 2.4a-c). The significant positive correlation between organic matter and poorly-crystalline Fe oxides ($R^2=0.22$, p=0.004) (Figure 2.7a) suggests organic matter limits the transformation of poorlycrystalline Fe oxides to more crystalline forms and thus increases the amount of poorly-crystalline Fe oxides. This is consistent with a recent study where they found organic matter inhibited the transformation of poorly-crystalline Fe oxides to more crystalline phases (ThomasArrigo et al., 2018). However, the non-significant correlation between organic matter and poorly-crystalline Mn oxides (p=0.23) suggests that organic carbon might not be the cause of the increasing amount of poorly-crystalline Mn oxides. Total reducible Fe oxides in 2016 post-harvest soil were also significantly lower than those in 2015 post-harvest soil (p<0.0001). The loss of Fe oxides in 2016 could be due to leaching. During the 2017 rice growth period, we found that Silicate amendments significantly increased the amount of poorlycrystalline Fe oxides than those in Control (p=0.002), Char- (p=0.02) and Husk-(p=0.009) amended soil. This might be explained by the difference in the solubility of Si amendments over the duration of plant growth. Teasley et al. (2017) found that silicate amendment led to a quicker release of Si compared with husk amendment. We suggest that the transformation of poorly-crystalline Fe oxides to more-crystalline form might occur early during rice growth and only encounter Si from Silicate amendments.

We found that the change of As in F1 and F2 fractions over time corresponded more with the change of poorly-crystalline Mn oxides over time than those with poorly-crystalline Fe oxides over time. This which suggests that Mn oxides played a more important role than Fe oxides on plant-available As pools. In addition, we

observed this significant negative relationship between poorly-crystalline Mn oxides and the plant-available As pool from all post-harvest soil (R^2 = 0.15, p=0.02). The increasing amount of poorly-crystalline Mn oxides not only offers adsorption sites for As, but also oxidizes As from porewater thus lower the amount of As that are plantavailable. It has been well established that Mn oxides are strong oxidants for As (Chiu and Hering, 2000; Manning et al., 2002; Lafferty et al., 2010). As a consequence of As oxidization by Mn oxides, arsenic may have shifted to less plant-available pools in our study. This As oxidation explained the negative correlation of oxalate-extractable Mn and As in the F1 fraction over years from our field study (Figure 2.8d).

Across years, the stronger correlation between porewater As and oxalateextractable Fe (R^2 =0.32, p=0.0003) than those between porewater As and oxalateextractable Mn (R^2 =0.09, p=0.08) suggests most As in porewater are from the poorlycrystalline Fe oxides pools (Figure 2.9a, b). This result is consistent with reductive dissolution of Fe oxides being the main cause for As mobility in flooded soil (Takahashi et al., 2004; Weber et al., 2010; Yamaguchi et al., 2011). Although poorlycrystalline Fe oxides had no or little effect on the plant-available soil As pools, reductive dissolution of poorly-crystalline Fe oxides led to the high mobility of As in porewater. However, during the growth period of rice, similar correlation between porewater As with oxalate-extractable Fe (R^2 =0.11, p=0.03) and with oxalateextractable Mn (R^2 =0.15, p=0.009) (Figure 2.9c, d) suggested both poorly-crystalline Fe and Mn oxides contributed to the release of As into porewater.

Rice cultivation led to more plant-available As and that is most likely due the change in poorly-crystalline Mn oxides. Plant-available As in soil became 2.5-fold higher after rice had been planted along with a 0.55-fold change from poorly-

crystalline Mn oxides (Figure 2.1, 2.2). This agreed well with our previous finding that plant-available As was negatively correlated with poorly-crystalline Mn oxides (Figure 2.8d). After rice planting, the flooding of soil led to more reductive dissolution of Mn oxides thus decreased the amount of poorly-crystalline Mn oxides. Therefore, not only did As lose sorbent on which to adsorb, but also less As was oxidized thus rendering more plant-available As.

2.5 Conclusion

We studied As, Fe and Mn pools over three years in rice paddy soil with different Si treatments. We found that Si addition had no significant effects on the As pool associated with poorly-crystalline Fe oxides and As pools that are plant-available; however, Silicate treatment did increase the proportion of poorly crystalline Fe oxides in soil collected over rice growth period. Overall, time was more impactful than Si treatment on changes in As, Fe and Mn pools. Conversion of grassland to rice paddies decreased the amount of poorly-crystalline Mn oxides and likely mobilized As thus become plant-available. Organic matter played an import role in affecting the crystallinity of Fe oxides while Mn oxides played an important role in controlling the plant-available As pool. Our study provides valuable information on understanding As dynamics in rice paddy soil.

REFERENCES

- Amaral, D. C., Lopes, G., Guilherme, L. R. G., and Seyfferth, A. L. (2017). A new approach to sampling intact Fe plaque reveals Si-induced changes in Fe mineral composition and shoot As in rice. *Environmental Science & Technology* 51, 38-45.
- Anderson, P. R., and Benjamin, M. M. (1985). Effects of silcion on the crystallization and adsorption properties of ferric oxides. *Environmental Science & Technology* 19, 1048-1053.
- Bogdan, K., and Schenk, M. K. (2008). Arsenic in rice (*Oryza sativa L.*) related to dynamics of arsenic and silicic acid in paddy soil. *Environmental Science & Technology* 42, 7885-7890.
- Chen, C. C., Dixon, J. B., and Turner, F. T. (1980). Iron coatings on rice rootsmorphology and models of development *Soil Science Society of America Journal* 44, 1113-1119.
- Chiu, V. Q., and Hering, J. G. (2000). Arsenic adsorption and oxidation at manganite surfaces. 1. Method for simultaneous determination of adsorbed and dissolved arsenic species. *Environmental Science & Technology* **34**, 2029-2034.
- Dittmar, J., Voegelin, A., Maurer, F., Roberts, L. C., Hug, S. J., Saha, G. C., Ali, M. A., Badruzzaman, A. B. M., and Kretzschmar, R. (2010). Arsenic in soil and irrigation water affects arsenic uptake by rice: complementary insights from field and pot studies. *Environmental Science & Technology* 44, 8842-8848.
- Fischel, M. H. H., Fischel, J. S., Lafferty, B. J., and Sparks, D. L. (2015). The influence of environmental conditions on kinetics of arsenite oxidation by manganese-oxides. *Geochemical Transactions* **16**.
- Fleck, A. T., Mattusch, J., and Schenk, M. K. (2013). Silicon decreases the arsenic level in rice grain by limiting arsenite transport. *Journal of Plant Nutrition and Soil Science* 176, 785-794.

- Goldberg, S., and Johnston, C. T. (2001). Mechanisms of arsenic adsorption on amorphous oxides evaluated using macroscopic measurements, vibrational spectroscopy, and surface complexation modeling. *Journal of Colloid and Interface Science* **234**, 204-216.
- Gwon, H. S., Khan, M. I., Alam, M. A., Das, S., and Kim, P. J. (2018). Environmental risk assessment of steel-making slags and the potential use of LD slag in mitigating methane emissions and the grain arsenic level in rice (*Oryza sativa L.*). *Journal of Hazardous Materials* **353**, 236-243.
- Henry Oppong Tuffour, I. B. Y., Mensah Bonsu, Thomas Adjei-Gyapong, Abdul Aziz Khalid, Awudu Abubakar, Caleb Melenya, Pearl Kpotor (2014). Soil organic carbon: relating the walkley-black wet oxidation method to loss on ignition and clay content. *International Journal of Scientific Research in Knowledge* 2, 249-256.
- Jones, A. M., Collins, R. N., Rose, J., and Waite, T. D. (2009). The effect of silica and natural organic matter on the Fe(II)-catalysed transformation and reactivity of Fe(III) minerals. *Geochimica et Cosmochimica Acta* **73**, 4409-4422.
- Lafferty, B. J., Ginder-Vogel, M., and Sparks, D. L. (2010). Arsenite oxidation by a poorly crystalline manganese-oxide 1. Stirred-flow experiments. *Environmental Science & Technology* **44**, 8460-8466.
- Lafferty, B. J., Ginder-Vogel, M., and Sparks, D. L. (2011). Arsenite oxidation by a poorly-crystalline manganese oxide. 3. Arsenic and manganese desorption. *Environmental Science & Technology* 45, 9218-9223.
- Li, R., Ago, Y., Liu, W., Mitani, N., Feldmann, J., McGrath, S. P., Ma, J., and Zhao, F. (2009). The rice aquaporin Lsi1 mediates uptake of methylated arsenic species. *Plant Physiology* **150**, 2071-2080.
- Limmer, M. A., Mann, J., Amaral, D. C., Vargas, R., and Seyfferth, A. L. (2018a). Silicon-rich amendments in rice paddies: Effects on arsenic uptake and biogeochemistry. *Science of The Total Environment* **624**, 1360-1368.
- Limmer, M. A., Wise, P., Dykes, G., and Seyfferth, A. L. (2018b). Silicon decreases dimethylarsinic acid concentration in rice grain and mitigates straighthead disorder. *Environmental Science & Technology* **52**, 4809-4816.
- Liu, W. J., Zhu, Y. G., Hu, Y., Williams, P. N., Gault, A. G., Meharg, A. A., Charnock, J. M., and Smith, F. A. (2006). Arsenic sequestration in iron plaque, its accumulation and speciation in mature rice plants (*Oryza Sativa L.*). *Environmental Science & Technology* **40**, 5730-5736.

- Liu, W. J., Zhu, Y. G., Smith, F. A., and Smith, S. E. (2004). Do iron plaque and genotypes affect arsenate uptake and translocation by rice seedlings (*Oryza sativa L.*) grown in solution culture? *Journal of Experimental Botany* 55, 1707-1713.
- Loeppert, R. H., and Inskeep, W. P. (1996). Iron. *In* "methods of soil analysis part 3 chemical methods" (D. L. Sparks, A. L. Page, P. A. Helmke and R. H. Loeppert, eds.), pp. 639-664. *Soil Science Society of America, American Society of Agronomy*, Madison, WI.
- Luxton, T. P., Tadanier, C. J., and Eick, M. J. (2006). Mobilization of arsenite by competitive interaction with silicic acid. *Soil Science Society of America Journal* **70**, 204-214.
- Ma, J. F., Tamai, K., Yamaji, N., Mitani, N., Konishi, S., Katsuhara, M., Ishiguro, M., Murata, Y., and Yano, M. (2006). A silicon transporter in rice. *Nature* **440**, 688.
- Ma, J. F., Yamaji, N., Mitani, N., Tamai, K., Konishi, S., Fujiwara, T., Katsuhara, M., and Yano, M. (2007). An efflux transporter of silicon in rice. *Nature* **448**, 209.
- Ma, J. F., Yamaji, N., Mitani, N., Xu, X.-Y., Su, Y.-H., McGrath, S. P., and Zhao, F.-J. (2008). Transporters of arsenite in rice and their role in arsenic accumulation in rice grain. *Proceedings of the National Academy of Sciences* **105**, 9931-9935.
- Manning, B. A., Fendorf, S. E., Bostick, B., and Suarez, D. L. (2002). Arsenic(III) oxidation and arsenic(V) adsorption reactions on synthetic birnessite. *Environmental Science & Technology* 36, 976-981.
- Masscheleyn, P. H., Delaune, R. D., and Patrick, W. H. (1991). Effect of redox potential and pH on arsenic speciation and solubility in a contaminated soil. *Environmental Science & Technology* **25**, 1414-1419.
- Meharg, A. A., and Macnair, M. R. (1992). Suppression of the high-affinity phosphateuptake system - A mechanism of arsenate to tolerance in *Holcus-Lanatus L.*. *Journal of Experimental Botany* **43**, 519-524.
- Nriagu, J. O., and Pacyna, J. M. (1988). Quantitative assessment of worldwide contamination of air, water and soil by trace metals. *Nature* **333**, 134.
- Ona-Nguema, G., Morin, G., Juillot, F., Calas, G., and Brown, G. E. (2005). EXAFS analysis of arsenite adsorption onto two-line ferrihydrite, hematite, goethite, and lepidocrocite. *Environmental Science & Technology* **39**, 9147-9155.

- Pedersen, H. D., Postma, D., Jakobsen, R., and Larsen, O. (2005). Fast transformation of iron oxyhydroxides by the catalytic action of aqueous Fe(II). *Geochimica et Cosmochimica Acta* **69**, 3967-3977.
- Schulte, E. E. (1995). Recommended soil organic matter tests. J. Thomas Sims and A. Wolf (eds.) Recommended Soil Testing Procedures for the Northeastern United States 47-56.
- Schwertmann, U., and Taylor, R. M. (1972). Influence of silicate on transformation of lepidocrocite to goethite. *Clays and Clay Minerals* **20**, 159-+.
- Schwertmann, U., and Thalmann, H. (1976). Influence of Fe(II), Si, and pH on formation of lepriocrocite and ferrihydrite during oxidation of aqueous FeCl₂ solutions. *Clay Minerals* **11**, 189-200.
- Seyfferth, A. L., and Fendorf, S. (2012). Silicate mineral impacts on the uptake and storage of arsenic and plant nutrients in rice (*Oryza sativa L.*). *Environmental Science & Technology* **46**, 13176-13183.
- Seyfferth, A. L., Limmer, M. A., and Dykes, G. E. (2018). On the use of silicon as an agronomic mitigation strategy to decrease arsenic uptake by rice. *In* "Advances in Agronomy, Vol 149" (D. L. Sparks, ed.), Vol. 149, pp. 49-91. Elsevier Academic Press Inc, San Diego.
- Seyfferth, A. L., Morris, A. H., Gill, R., Kearns, K. A., Mann, J. N., Paukett, M., and Leskanic, C. (2016). Soil incorporation of silica-rich rice husk decreases inorganic arsenic in rice grain. *Journal of Agricultural and Food Chemistry* 64, 3760-3766.
- Seyfferth, A. L., Webb, S. M., Andrews, J. C., and Fendorf, S. (2011). Defining the distribution of arsenic species and plant nutrients in rice (*Oryza sativa L.*) from the root to the grain. *Geochimica Et Cosmochimica Acta* **75**, 6655-6671.
- Smith, A. H., Hopenhayn-Rich, C., Bates, M. N., Goeden, H. M., Hertz-Picciotto, I., Duggan, H. M., Wood, R., Kosnett, M. J., and Smith, M. T. (1992). Cancer risks from arsenic in drinking water. *Environmental Health Perspectives* 97, 259-267.
- Stolz, J. E., Basu, P., Santini, J. M., and Oremland, R. S. (2006). Arsenic and selenium in microbial metabolism. *In* "Annual Review of Microbiology", Vol. 60, pp. 107-130. Annual Reviews, Palo Alto.

- Suda, A., and Makino, T. (2016). Functional effects of manganese and iron oxides on the dynamics of trace elements in soil with a special focus on arsenic and cadmium: A review. *Geoderma* 270, 68-75.
- Syu, C. H., Jiang, P. Y., Huang, H. H., Chen, W. T., Lin, T. H., and Lee, D. Y. (2013). Arsenic sequestration in iron plaque and its effect on As uptake by rice plants grown in paddy soil with high contents of As, iron oxides, and organic matter. *Soil Science and Plant Nutrition* 59, 463-471.
- Takahashi, Y., Minamikawa, R., Hattori, K. H., Kurishima, K., Kihou, N., and Yuita, K. (2004). Arsenic behavior in paddy fields during the cycle of flooded and non-flooded periods. *Environmental Science & Technology* 38, 1038-1044.
- Teasley, W. A., Limmer, M. A., and Seyfferth, A. L. (2017). How rice (*Oryza sativa L.*) responds to elevated As under different Si-rich soil amendments. *Environmental Science & Technology* **51**, 10335-10343.
- ThomasArrigo, L. K., Byrne, J. M., Kappler, A., and Kretzschmar, R. (2018). Impact of organic matter on iron(II)-catalyzed mineral transformations in ferrihydrite– organic matter coprecipitates. *Environmental Science & Technology* 52, 12316-12326.
- Weber, F. A., Hofacker, A. F., Voegelin, A., and Kretzschmar, R. (2010). Temperature dependence and coupling of iron and arsenic reduction and release during flooding of a contaminated soil. *Environmental Science and Technology* 44, 116-122.
- Wenzel, W. W., Kirchbaumer, N., Prohaska, T., Stingeder, G., Lombi, E., and Adriano, D. C. (2001). Arsenic fractionation in soil using an improved sequential extraction procedure. *Analytica Chimica Acta* 436, 309-323.
- Williams, P. N., Villada, A., Deacon, C., Raab, A., Figuerola, J., Green, A. J., Feldmann, J., and Meharg, A. A. (2007). Greatly enhanced arsenic shoot assimilation in rice leads to elevated grain levels compared to wheat and barley. *Environmental Science & Technology* **41**, 6854-6859.
- Wu, C., Zou, Q., Xue, S. G., Pan, W. S., Yue, X., Hartley, W., Huang, L., and Mo, J. Y. (2016). Effect of silicate on arsenic fractionation in soil and its accumulation in rice plants. *Chemosphere* 165, 478-486.
- Xu, X., Chen, C., Wang, P., Kretzschmar, R., and Zhao, F.-J. (2017). Control of arsenic mobilization in paddy soil by manganese and iron oxides. *Environmental Pollution* **231**, 37-47.

- Yamaguchi, N., Nakamura, T., Dong, D., Takahashi, Y., Amachi, S., and Makino, T. (2011). Arsenic release from flooded paddy soil is influenced by speciation, Eh, pH, and iron dissolution. *Chemosphere* 83, 925-932.
- Yamaguchi, N., Ohkura, T., Takahashi, Y., Maejima, Y., and Arao, T. (2014). Arsenic distribution and speciation near rice roots influenced by iron plaques and redox conditions of the soil matrix. *Environmental Science & Technology* 48, 1549-1556.
- Yang, J. K., Barnett, M. O., Jardine, P. M., Basta, N. T., and Casteel, S. W. (2002). Adsorption, sequestration, and bioaccessibility of As(V) in soil. *Environmental Science & Technology* 36, 4562-4569.
- Yee, N., Shaw, S., Benning, L. G., and Nguyen, T. H. (2006). The rate of ferrihydrite transformation to goethite via the Fe(II) pathway. *American Mineralogist* **91**, 92-96.
- Ying, S. C., Kocar, B. D., and Fendorf, S. (2012). Oxidation and competitive retention of arsenic between iron- and manganese oxides. *Geochimica et Cosmochimica Acta* **96**, 294-303.