Mitigating the impacts of arsenic on human health and rice yield in Bangladesh

by

Brittany Lynn Huhmann

B.A. in Earth and Planetary Sciences, Washington University in St. Louis, 2010
M.S. in Civil and Environmental Engineering, University of Iowa, 2013

Submitted to the Department of Civil and Environmental Engineering in partial fulfillment of the requirements for the degree of

Doctor of Philosophy in Environmental Engineering

at the

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

September 2018

Author ....
Department of Civil and Environmental Engineering
August 17, 2018

Certified by ....
Charles F. Harvey
Professor of Civil and Environmental Engineering
Thesis Supervisor

Accepted by...
Heidi Nepf
Professor of Civil and Environmental Engineering
Chair, Graduate Program Committee

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ABSTRACT
Naturally-occurring groundwater arsenic can threaten human health and food security. In Bangladesh, >50 million people are estimated to have chronically consumed water with arsenic above the World Health Organization (WHO) guideline of 10 µg/L, which can contribute to cancer, cardiovascular disease, and reproductive and developmental effects. Studies relating arsenic exposure to health impacts generally estimate dose based on participants' primary household wells. Using a mass-balance for arsenic and water, we estimate that participants in Araihazar, Bangladesh obtain 37±8% of their water from primary household wells and 31±14% from other wells, and we thus recommend the inclusion of other wells in dose estimation.

Concentrations of arsenic in well water are spatially variable, enabling many exposed households to switch to nearby lower-arsenic wells in response to area-wide well testing. Following well testing and education in Araihazar, arsenic exposure declined and remained lowered for at least eight years. Participants with arsenic-unsafe wells were 6.8 times more likely to switch wells over the first two years and 1.4-1.8 times more likely to switch wells over the ensuing decade.

Rice comprises more than 70% of calories consumed in Bangladesh, and rice yield is negatively impacted by the buildup of arsenic in soil from irrigation with high-arsenic water. We investigated the effect of soil arsenic on yield using a controlled study design where we exchanged the top 15 cm of soil between high-arsenic and low-arsenic plots. Differences in yield were negatively correlated to differences in soil arsenic between adjacent soil replacement and control plots, suggesting that boro rice yield countrywide may be diminished by 7−26% due to arsenic in soil.

Soil testing and removal of high-arsenic soil may enable farmers to mitigate the impacts of arsenic on rice. Twelve measurements made with the ITS Econo-Quick field kit could be used to estimate whether soil arsenic was above or below a 30 mg/kg intervention threshold with 80-90% accuracy. A soil inversion, where deep low-arsenic soil was exchanged with surface high-arsenic soil, decreased soil arsenic, organic carbon, nitrogen, and phosphorus concentrations by about 40% in the top 20 cm of soil and improved rice yield by 15-30%.

Thesis Supervisor: Charles F. Harvey
Title: Professor of Civil and Environmental Engineering

Acknowledgements

I greatly appreciate the opportunity to work with my advisors Charles Harvey and Lex van Geen. I am grateful for their contributions in the form of many hours of fruitful discussions, countless rounds of feedbacks on manuscript drafts, and support and encouragement to share my research in a range of venues. I also appreciate their support of my involvement in science policy activities, including allowing me the flexibility to pursue MIT's graduate certificate in science policy, lead state and local advocacy for MIT's Graduate Student Council, and complete two internships during my time as a graduate student. The opportunity to pursue these professional development activities alongside my research greatly contributed to my post-MIT career goals.

I am grateful to my committee members, Ben Kocar and Dennis McLaughlin, for their insightful questions and feedback over the years. I would like to thank Imtiaz Choudhury, who provided help with technical and logistical aspects of my fieldwork in Bangladesh, and who, knowing my love of fresh fruit, would find coconuts, jackfruit, and lychees for us to enjoy after a long day in the field. I am especially grateful to Anjal Uddin, who has worked tirelessly at our field sites in Faridpur, and is gifted at maintaining and strengthening relationships with local farmers, without which our fieldwork would not have been possible.

I appreciate the warmth and support of so many at the Parsons Laboratory, but especially my research group members Mason Stahl, Alison Hoyt, and Neha Mehta, as well as the members of Parsons Supergroup, who provided feedback on my research and shared with me the amazing work they were doing. I value the contributions of many other research collaborators, including Kazi Matin Ahmed, John Duxbury, Ben Bostick, Ana Navas-Acien, Maria Argos, Jason Gross, Brian Mailloux, and Tyler Ellis. I am also grateful to my undergraduate advisors Ray Arvidson and Jeff Catalano, who first set me on the path of conducting research in the earth sciences, and to my master's advisor Michelle Scherer, who showed me how satisfying conducting research could be and encouraged me to pursue my goals within and beyond the lab.

Finally, I would like to express my deepest appreciation to my friends and family for their support. I am especially grateful to have been a part of MIT's Tech Squares and my housing cooperative BADHouse throughout my five years at MIT. Tech Squares has provided me with a regular outlet for physical activity and with the satisfaction of making clear progress as a square and rounds dancer, including during times of research delays and setbacks. BADHouse has provided me with countless opportunities to care for others and to be cared for, including through cooking meals, playing games, hosting events, and sharing our day-to-day life. These communities have been a powerful source of queer friendships and close connections with wonderful humans.

Table of Contents

Introduction

Mitigating the impacts of arsenic exposure on human health in Bangladesh

Natural contamination of groundwater with arsenic (As) occurs in many regions of the world, including much of South and Southeast Asia. It poses a significant health threat in regions where people rely primarily on well water as their drinking water source. Bangladesh is the country with the highest fraction of the population drinking from wells elevated in As, with more than 50 million people estimated to have been chronically exposed to concentrations above the World Health Organization (WHO) guideline of 10 µg/L (BGS & DPHE, 2001; Brammer & Ravenscroft, 2009).

Chronic exposure to As can produce skin lesions and cancers of the skin, bladder, and lung, but the current leading cause of mortality resulting from As exposure is cardiovascular disease (Argos et al., 2010; Chen et al., 2011; Flanagan, Johnston, & Zheng, 2012; A H Smith, Lingas, & Rahman, 2000; Nazmul Sohel et al., 2009). Exposure of pregnant women to As increases the risk of stillbirth and infant mortality, and exposure of children to As in well water has been shown to reduce their intellectual function (Quansah et al., 2015; A. Rahman et al., 2007; Anisur Rahman et al., 2010; Wasserman et al., 2004). One of every 18 deaths in Bangladesh may be arsenic-related (Flanagan et al., 2012).

Many studies have quantified the relationship between urinary As and health effects and between primary household well As exposure and health effects (Ahsan et al., 2006; Argos et al., 2010; Nazmul Sohel et al., 2009). While urinary As is a good metric of overall As exposure, it is less clear that this is true for primary household well As. Wells other than the primary household well may be a significant source of water for many individuals, including schoolchildren (Allan H. Smith et al., 2013) and men who work away from home (N Sohel et al., 2010). It is plausible that these other wells could make up the majority of As exposure for individuals with low levels of As in their primary household wells. It is important to accurately understand the relationship between well As exposure and health impacts, since this relationship is used to set standards for As concentrations in drinking water in Bangladesh and other As-impacted countries, including the United States.

The Bangladesh government and NGOs have supported a range of methods for reducing As exposure. Many of these interventions have been insufficiently safe, effective, or persistent (Ahmed et al., 2006; Hoque, Yamaura, & Sakai, 2006; Howard, Ahmed, Shamsuddin, Mahmud, & Deere, 2006). A common problem with water filtration, which was the focus of early interventions, is rapid abandonment of filters due to maintenance issues and inconvenience (Ahmed et al., 2006; M. A. Hossain et al., 2005; Sanchez et al., 2016). An alternative approach is blanket well-testing to provide individuals with information about the As concentration of their own wells and nearby wells, thus facilitating switching to drinking from lower-As wells. This approach is made possible by the high spatial variability of well As concentrations, even within a small area. Since well As concentrations are relatively stable, once an As-safe well has been identified, further maintenance is generally not required, in contrast with water filtration.

Mitigating the impacts of arsenic exposure on rice yield in Bangladesh

Rice is the primary crop of Bangladesh in terms of production and caloric consumption, comprising 70% of calories consumed (BBS 2016b; FAO and WHO 2014). Rice is predominantly grown during the boro (dry winter) and aman (monsoon) seasons (BBS 2016a, 2016b). Winter season (boro) rice is the dominant crop in Bangladesh, and high volumes of groundwater are required to maintain the flooded conditions under which boro rice is grown.

Much of the irrigation water in rice-growing regions of Bangladesh is naturally contaminated with high concentrations of As. Among crops, rice is especially impacted by irrigation water As, since it is grown under flooded conditions, resulting in the use of higher volumes of contaminated irrigation water and in a chemically reduced soil environment that enhances As mobility. When rice is irrigated with high-As irrigation water, the As can build up in rice field soil (Dittmar et al. 2010; Hossain et al. 2008; Lu et al. 2009; Neumann et al. 2011; Panaullah et al. 2009; Saha and Ali 2007). Monsoon season (aman) rice is often grown in the same fields where boro rice is cultivated, and although it is primarily rainfed, it is still exposed to the high concentrations of soil As that build up during boro irrigation (A van Geen et al., 2006). Arsenic in soil can be taken up into the rice grain, resulting in human exposure to As and associated health risks (Brammer and Ravenscroft 2009; Duxbury and Panaullah 2007; Heikens 2006), although in high-As regions, drinking water from As-contaminated wells is a much more significant exposure route (Polya et al. 2008).

Elevated As concentrations in irrigation water and soil have been found to decrease boro and aman rice yield in greenhouse studies and pot experiments (M. J. Abedin, Cresser, Meharg, Feldmann, & Cotter-Howells, 2002; Delowar et al., 2005; Iqbal, Rahman, Panaullah, Rahman, & Biswas, 2016; Islam, Islam, Jahiruddin, & Islam, 2004; Khan et al., 2009; Montenegro & Mejia, 2001; M. A. Rahman, Hasegawa, Rahman, Rahman, & Miah, 2007; Williams et al., 2009). A prior field study in Faridpur, Bangladesh found that boro rice yields were 7-9 t/ha where soil As concentrations were low (~10 mg/kg), but were much poorer, 2-3 t/ha, where soil As concentrations were high (~70 mg/kg) (Panaullah et al., 2009).

Various options have been considered to reduce the uptake of soil As by rice and the impacts of soil As on rice yield. These include providing cleaner irrigation water, growing As-resistant rice varieties, and growing rice under conditions that are less conducive to As uptake (Brammer 2009; Polizzotto et al. 2015). Even with these methods, rice yield will likely be negatively impacted by the high levels of legacy As contamination in many rice fields. Removal of the highest-As upper 10-15 cm of soil has been suggested to address this problem, since farmers commonly remove soil for use in brick-making, building houses, and raising infrastructure above monsoon flooding (Brammer 2009). However, the impacts of soil removal on soil As and rice yield have not been documented.

Summary of research

In this thesis, we build on prior research to improve the understanding of how water As in Bangladesh impacts human health and rice yield and to explore options for mitigating those impacts. Specifically, we quantify the sources of As exposure in rural Bangladesh and document the potential of area-wide well-As testing to decrease As exposure. Additionally, we quantify the negative impacts of As on rice yield throughout Bangladesh, and explore the potential of a field kit to identify high-As areas for mitigation and the potential of a soil inversion to improve rice yield in As-impacted areas.

In Chapter 1, we use a mass-balance approach for water and As to estimate how the sources of water consumption and As exposure for an individual are distributed between their primary well and other wells in the area. We use well-water and urinary As data collected between 2000 and 2001 within a 25 km^2 area of Araihazar upazila, Bangladesh for 2,811 participants enrolled in the Health Effects of Arsenic Longitudinal Study (HEALS). We develop a long-term mass-balance for As and water intake and release. Conducting a mass balance involves equating the mass of water entering the body to the mass of water leaving the body and, similarly, the mass of arsenic entering the body to the mass of arsenic leaving the body. We also consider the possibility of some loss of As to a permanent sink in the body.

By measuring or estimating the other parameters in the water and mass balance equations, we solve for the average fraction of total water intake that participants consume from primary household wells and the average fraction of water output that participants lose via urine. The results of the mass balance suggest that HEALS participants obtain 37±8% of their drinking water from their primary household wells and 31±14% from other wells. This suggests that participants with primary well As concentrations less than the area average of about 100 µg/L get one-third or more of their total As exposure from wells other than their primary household wells. The mass balance also suggests that women obtain 8±10% more of their drinking water from primary wells than men do. Wells other than primary household wells are thus a significant source of As exposure in rural Bangladesh. This means that dose-response relationships that aim to understand the relationship between As concentration in drinking water and health outcomes should take both primary household wells and other wells into account.

In Chapter 2, we document changes in behavior and As exposure following interventions to facilitate well-switching in an As-impacted 25 km^2 area of Araihazar, Bangladesh. The interventions began with an initial round of blanket well testing for As, with most wells labeled in January through March of 2001. People drinking from wells with As concentrations of more than 50 µg/L ("As-unsafe wells") were encouraged to switch to As-safe wells, and deep, As-safe community wells were installed in areas with few As-safe wells. Additional blanket well testing in Araihazar was conducted by the government in 2003, after which wells were painted red or green to indicate whether they were above or below the Bangladesh drinking water standard, and by a team of local village-health workers in 2012-2013 (van Geen et al. 2014).

Prior studies have shown positive impacts on well As and urinary As two to three years after blanket well testing (Chen et al., 2007; Madajewicz et al., 2007; Opar et al., 2007). In this chapter, we observe the changes in participants' arsenic exposure over sixteen years starting from the initial round of blanket well-testing in 2000-2002. We use well-water and urinary As data collected between 2000 and 2008, along with household interviews extending through 2016, within a 25 km^2 area of Araihazar upazila for nearly 12,000 participants enrolled in HEALS.

Arsenic exposure for participants most exposed at baseline declined from a mean As concentration of 226 µg/L at baseline to 173 µg/L two years later, and further declined to 139 µg/L over 8 years. Well status with respect to As was predictive of well-switching decisions for at least a decade after the initial testing. Participants with As-unsafe wells were 6.8 times more likely to switch wells over the first two years and 1.4-1.8 times more likely to switch wells over the ensuing decade. The percentage of participants drinking from new wells that were untested at baseline or from wells that had lost their identification tags increased to 27% by about two years after baseline and reached a majority (66%) of participants by about 8 years after baseline. The substantial increase in the number of participants with unknown As concentrations in their primary household wells over time indicates the importance of additional well testing as new wells continue to be installed.

In Chapter 3, published as "Field Study of Rice Yield Diminished by Soil Arsenic in Bangladesh" in *Environmental Science and Technology* (DOI: 10.1021/acs.est.7b01487), we quantify the impacts of As on rice yield in Faridpur, Bangladesh by using a controlled study design. While prior greenhouse and pot studies have shown the negative effects of As on rice yield, these studies do not provide sufficient information to quantify the magnitude of the yield impact of As under field conditions. Furthermore, the only previous field study on the yield effects of As did not include aman-season rice and was conducted in an 8 ha area managed by a single farmer and irrigated by a single high-As well – and thus under much narrower set of conditions.

In our study, to quantify the yield impact of As we exchanged high- and low-As soils at thirteen field sites distributed throughout a 150 km^2 area in Faridpur, Bangladesh and compared these soil replacement plots to adjacent control plots. Our study plots were managed by sixteen different farmers, and these farmers chose to cultivate two boro rice varieties and nine aman rice varieties. We hypothesized that replacing high-As soil with low-As soil would improve yield, and that replacing low-As soil with high-As soil would cause a decline in yield. We tested this hypothesis for rice grown during the 2015 and 2016 boro and aman seasons.

Soil As and rice yields were measured for soil replacement plots where the soil was exchanged and adjacent control plots where the soil was not exchanged. Differences in yield (ranging from +2 to -2 t/ha) were negatively correlated to the differences in soil As (ranging from -9 to +19 mg/kg) between adjacent replacement and control plots during two boro seasons. Using the observed relationship between soil As and yield in combination with shallow well As data from across Bangladesh, we estimate a boro rice yield loss over the entire country of 1.4-4.9 million tons annually, or 7-26% of the annual boro harvest, due to the accumulation of As in soil over the past 25 years.

In Chapter 4, we explore the use of a field kit as a rapid, affordable option for farmers to identify high-As soil for mitigation. We adapted a field kit method for measuring water As to measure soil As. We compared a total of 4592 field kit measurements of soil As concentrations on fresh and oven-dried soil samples with measurements of total soil As by X-ray fluorescence. Kit measurements on fresh soil were more consistent across seasons and were less time- and labor-intensive to make than kit measurements on dried soil.

We compared the use of a linear regression versus a Bayesian approach for estimating total soil As from kit measurements. Using the linear regression approach, averaging the results of 12 soil kit tests from the same 5 × 5 m plot improved the correlation between kit and total soil As to an R^2 of 0.67 compared to an R^2 of 0.40 when single samples were used. The 12-sample average of kit measurements accurately determined whether soil As was above or below a 30 mg/kg threshold in 87% of cases where soil As was above the threshold and in 84% of cases where soil As was below the threshold. Use of a Bayesian approach to estimate the probability that soil As concentration is above a threshold has similar or slightly better accuracy and allowed for additional flexibility in the tradeoff between false positives and false negatives. The results suggest that the use of multiple field kit measurements made on fresh soil can enable farmers to identify high-As soil for mitigation.

In Chapter 5, we explored the possibility of a soil inversion to decrease the As in the soil that rice roots are in contact with and to improve rice yield. Since As concentration in paddy soil decreases with depth, we exchanged the deeper low-As soil with the surface high-As soil, putting the low-As soil in contact with the rice roots. A soil inversion is more versatile than soil removal, since there is no elevation discrepancy between the inversion area and the surrounding paddy, allowing farmers to implement it without disrupting irrigation water management. It additionally does not require disposal of As-contaminated soil.

We compared soil As, soil nutrients, and rice yield in control plots with those in adjacent plots where a soil inversion was implemented. We also estimated the quantity of soil As deposited on a yearly basis via irrigation water, to explore the longevity of a soil inversion to reduce surface As. Soil As, organic carbon, nitrogen, and phosphorus concentrations decreased by about 40% in response to the inversion and remained persistently lower in soil inversion plots compared to adjacent control plots over four seasons of monitoring. Rice yield in inversion plots increased above rice yield in control plots by 15-30% after a one-season lag, but was uncorrelated with soil As and nutrient concentrations.

The results suggest that a soil inversion may be a cost-effective method for farmers to improve rice yield. However, the longevity of the yield gain remains unknown, since the cause could not be identified and since soil As from irrigation water builds up again at a rate of 0.4-1.1 mg/kg per year.

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Chapter 1: A mass-balance model to re-assess arsenic exposure from multiple wells in Araihazar, Bangladesh

Abstract

Background: Many public health studies of arsenic exposure have assigned an arsenic dose based on arsenic concentrations in participants' primary household wells. However, other water arsenic sources beyond the primary household well may be a significant source for individuals including schoolchildren and men who work away from home. We estimate the average fraction of a Bangladesh villager's drinking water that comes from wells other than their primary household well and use this to estimate sources of arsenic exposure for villagers with a range of primary household well arsenic concentrations.

Methods: We use well-water and urinary arsenic data collected between 2000 and 2001 within a 25 km^2 area of Araihazar upazila, Bangladesh for 2,811 participants enrolled in the Health Effects of Arsenic Longitudinal Study (HEALS). We develop a long-term mass-balance for arsenic and water intake and release to estimate the fraction of water that participants drink from their primary household wells.

Findings: The results of the mass balance model suggest that on average HEALS participants obtain 37±8% of their water from their primary household wells and 31±14% from other wells, assuming that the remaining 32±12% comes from food and cellular respiration. We estimate that water arsenic makes up the majority of arsenic exposure for most participants, and that participants with primary well arsenic concentrations less than the area average of about 100 µg/L get one-third or more of their total arsenic exposure from wells other than their primary household wells. A comparison of urinary arsenic variance and use of the mass balance separately on men and women suggest that women obtain about 8±10% more of their drinking water from primary household wells than men do.

Interpretation: Wells other than primary household wells are a significant source of arsenic exposure in rural Bangladesh. Dose-response relationships that aim to understand the relationship between arsenic concentration in drinking water and health outcomes should take both primary household wells and other wells into account.

Introduction

Natural contamination of groundwater with arsenic occurs in many regions of the world, including much of South and Southeast Asia. It poses a significant health threat in regions where people rely primarily on well water as their drinking water source. Bangladesh is the country with the highest fraction of the population drinking from wells elevated in arsenic, with more than 50 million people estimated to have been chronically exposed to concentrations above the World Health Organization (WHO) guideline of 10 µg/L (BGS and DPHE 2001; Brammer and Ravenscroft 2009). Chronic exposure to arsenic can induce skin lesions and cancers of the skin, bladder, and lung, but the current leading cause of death related to arsenic exposure is cardiovascular disease (Argos et al. 2010; Chen et al. 2011; Flanagan et al. 2012; Smith et al. 2000; Sohel et al. 2009). Exposure of pregnant women to arsenic increases the risk of stillbirth and infant mortality, and exposure of children to arsenic in well water has been related to reduced intellectual function (Quansah et al. 2015; Rahman et al. 2007, 2010; Wasserman et al. 2004).

Many studies have quantified the relationship between urinary arsenic and health effects and between primary household well water arsenic exposure and health effects (Ahsan et al. 2006b; Argos et al. 2010; Sohel et al. 2009). While urinary arsenic is a good metric of overall arsenic exposure, it is less clear that this is true for primary well arsenic. Wells other than the primary household well may be a significant source of water for many individuals, including schoolchildren (Smith et al. 2013) and men who work away from home (Sohel et al. 2010). Water from these other wells could contribute the majority of arsenic exposure for individuals with low levels of arsenic in their primary household well.

In this study, we use a mass-balance approach for water and arsenic to estimate how the sources of water consumption and arsenic exposure for an individual are distributed between their primary well and other wells in the area. Conducting a mass balance involves equating the mass of water entering the body to the mass of water leaving the body and, similarly, the mass of arsenic entering the body to the mass of arsenic leaving the body. We also consider the possibility of loss of arsenic to a permanent sink in the body.

The considerable benefit of introducing mass balance as a constraint for interpreting the available data, along with some reasonable assumptions, is that we can solve the equations for the average fraction of total water intake that participants consume from their primary household wells and the average fraction of water output that participants lose via urine. Using this information, we can estimate the fraction of arsenic consumed from the primary household well, other wells, and food for individuals with different primary household well arsenic concentrations. This approach is made possible by a unique data set that includes measurements of urinary arsenic and primary household well arsenic for 2,811 study participants, and the arsenic concentration of all 4,236 wells in the study area. We further explore how the distribution of water sources between primary household wells and other wells varies by gender.

Methods

Health Effects of Arsenic Longitudinal Study (HEALS)

In this paper we use well arsenic data, well location data, urinary arsenic data, and gender information collected for 2,811 participants in the Health Effects of Arsenic Longitudinal Study (HEALS). The collection of the HEALS data used in this paper is briefly summarized here, and a detailed description of the study design is found in Ahsan et al. (2006). Data were collected in a 25 km^2 area in Araihazar, Bangladesh. In 2000-2002, the field team conducted a blanket survey of wells in the study area, recorded their GPS coordinates, and tested them to determine their arsenic concentrations.

HEALS participants were aged 18 years or older and had lived in the study area for 5 or more years. During baseline surveys from October 2000 to May 2002, each participant provided a urine sample and told interviewers which well they used as their primary drinking water source. The participant's gender was also recorded at this time. Many study participants were informed of the status of their well with respect to the Bangladesh standard for arsenic in drinking water with a first set of placards posted on their wells between January and March 2001 (Ahsan et al. 2006a), while baseline surveys and urine collections were still being conducted. A second set of placards listing arsenic concentrations was subsequently attached to the wells after the surveys were completed (van Geen et al. 2003).

We conducted the arsenic mass balance using data from the group of participants who were not informed of their primary household well arsenic status prior to providing urinary arsenic samples (n=2,811, about 25% of the 11,224 participants in the HEALS cohort). Participants belonged to this group if their urinary arsenic was tested before the first round of well labeling began in January 2001 or if they drank from a well with an ID greater than 5000, since these wells were labeled after all urinary arsenic samples had been collected. We additionally demonstrate evidence of behavioral modifications among the remaining 75% of participants who may have been informed of their well arsenic before their urinary arsenic was tested. Only about 3% of participants in HEALS had independently tested their wells for arsenic prior to the beginning of the study, so previous independent well testing is unlikely to substantially impact our results (Parvez et al. 2006).

Arsenic and water mass balance in the bodies of participants

A linear regression model based on the volume balance of water and mass balance of arsenic for study participants is used to estimate the fraction of water excreted via urine and the fraction of water consumed from the primary household well.

The volumetric water balance on an individual can be described by

$$Q_{in} = Q_{out} = Q$$

$$Q_p + Q_o + Q_f + Q_c = Q_u + Q_e + Q_d = Q \tag{1}$$

where $Q_p, Q_o, Q_f,$ and Q_c represent water input from the individual's primary household well, other wells, food, and cellular respiration (a process that converts food molecules into energy for cells and produces water as a byproduct), respectively, and where $Q_u, Q_e,$ and Q_d represent water output through urination, evaporation (perspiration and respiration), and defecation. Rewriting the equation in terms of the fractions of water gained or lost gives

$$(f_p + f_o + f_f + f_c)Q = (f_u + f_e + f_d)Q \tag{2}$$

Assuming negligible arsenic loss through evaporation (Cullen and Reimer 1989), the arsenic mass balance on an individual can be described by

$$f_p Q[As]_p + f_o Q[As]_o + M_f = f_u Q[As]_u + M_d + M_b \tag{3}$$

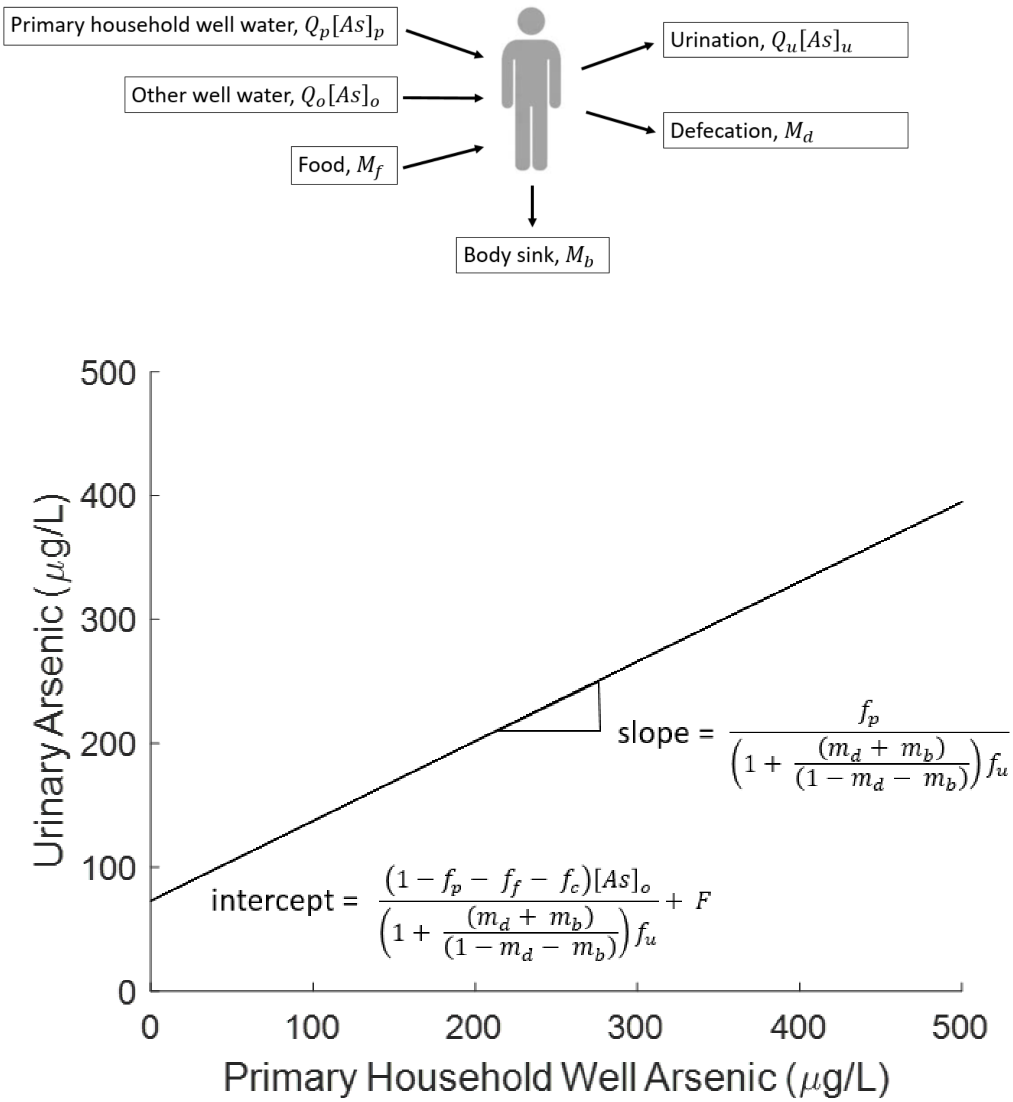

Figure 1. (a) Nomenclature of the terms considered in the water balance for a study participant. (b) Resulting linear model for the relationship between urinary arsenic and well arsenic.

where $[As]_p$ is the arsenic concentration in the primary household well, $[As]_o$ is the arsenic concentration in other wells, $[As]_u$ is the arsenic concentration in urine, M_f is the mass of arsenic consumed per time in food, M_d is the mass of arsenic lost per time to defecation, and M_b is the mass of arsenic lost per time to a sink in the body (Figure 1a). The mass loss of arsenic via defecation M_d and the loss of arsenic to a sink in the body M_b are modeled to be a fixed percentage of the total mass of arsenic input or loss. For loss of arsenic to the body, this represents an upper bound, since it treats the body as an infinite sink for arsenic. The equation then becomes

$$f_p Q[As]_p + (1 - f_p - f_f - f_c)Q[As]_o + M_f = f_u Q[As]_u + \frac{(m_d + m_b)}{(1 - m_d - m_b)} f_u Q[As]_u \tag{4}$$

where m_d is the mass fraction of arsenic lost to defecation and m_b is the mass fraction of arsenic lost to a sink in the body. The expected value for urinary arsenic $[\overline{As}]_{u,i}$ of an individual can be calculated as a function of the primary household well arsenic $[As]_{p,i}$ for that individual *i* by assuming statistical independence with and among the other variables, taking expected values, and rearranging the equation to solve for $[\overline{As}]_{u,i}$. The other variables are now understood to average values across the population.

$$[\overline{As}]_{u,i} = \frac{1}{\left(1 + \frac{(m_d + m_b)}{(1 - m_d - m_b)}\right) f_u} \left(f_p [As]_{p,i} + \left(1 - f_p - f_f - f_c\right)[As]_o\right) + F \tag{5}$$

where F is defined as the urinary arsenic contribution from food

$$F = \frac{1}{\left(1 + \frac{(m_d + m_b)}{(1 - m_d - m_b)}\right) f_u} \frac{M_f}{Q} \tag{6}$$

Once the other variables in the equation are estimated, the observations for $[As]_{u,i}$ and $[As]_{p,i}$ can be used to solve for f_u (the fraction of water excreted as urine) and f_p (fraction of water consumed from primary household wells) (Figure 1b).

The fraction of water consumed via food f_f is estimated as 0.2 ± 0.1 from studies conducted in the United States (Popkin et al. 2011), since no such estimates are available for Bangladesh. The fraction of water produced from cellular respiration f_c is estimated as 0.12 ± 0.06 (Gomella and Haist 2007). The mass fraction of urinary arsenic loss via defecation m_d is estimated to be 6% ± 3% (Pomroy et al. 1980). The loss of urinary arsenic to the body m_b is estimated to be negligible, but the effects of varying amounts of arsenic loss to the body are examined.

The arsenic concentration in other wells is estimated in one of two ways. In the "distributed wells" model, we assume that individuals drink well water from other wells with an expected arsenic concentration equal to the average arsenic in all wells. $[As]_o$ is estimated as the average arsenic in all wells in the study area, 100.50 ± 0.03 µg/L. This model also requires estimation of the contribution to urinary arsenic from food F. As a lower bound, if food arsenic were negligible compared to arsenic intake via water, this term could be set to zero. As an upper bound, if the participants with the lowest-arsenic primary household wells got all of their arsenic from food and none from well water, F would be equal to the average urinary arsenic concentration of this group of participants, 40 µg/L. Since the true value is likely to fall between these two extremes, F is estimated as 20 ± 10 µg/L.

In the "neighboring wells" model, we assume that individuals drink well water from wells other than the primary household well with an expected arsenic concentration equal to the average arsenic in wells within a certain radius of their primary household well, $[As]_{o,i}$, and the equation becomes

$$[\overline{As}]_{u,i} = \frac{1}{\left(1 + \frac{(m_d + m_b)}{(1 - m_d - m_b)}\right) f_u} \left(f_p [As]_{p,i} + \left(1 - f_p - f_f - f_c\right)[As]_{o,i}\right) + F$$

(7)

where $[As]_{o,i}$ is unique to each study participant. An estimate of the arsenic contribution from food F is produced from the observed data in this model and does not need to be independently estimated.

Variance of urinary arsenic

The variance of primary household well arsenic and the variance of urinary arsenic were compared between men and women using a Brown-Forsythe test. This test was used because it is robust for many types of non-normal data.

Results

Water consumption from primary household wells and other wells under the distributed model

The distributed wells model describes participants as drinking from wells throughout the study area. The two mass balance equations for water and arsenic and the observations for $[As]_{u,i}$ and $[As]_{p,i}$ can be used to estimate f_u (the fraction of water excreted as urine) and f_p (the fraction of water obtained from primary household wells).

Conducting a simple linear regression on $[As]_{u,i}$ as a function of $[As]_{p,i}$ (Figure 2a,b) we obtain a slope of 0.64 ± .02 and an intercept of 72.9 ± 3.3. Solving two equations for two unknowns while setting m_b = 0 (no sink of arsenic in the body)

$$0.64 \pm .02 = \frac{f_p}{\left(1 + \frac{(0.06)}{(1-0.06)}\right) f_u}$$

$$72.9 \pm 3.3 \frac{ug}{L} = \frac{1}{\left(1 + \frac{(0.06)}{(1-0.06)}\right) f_u}\left(\left(1 - f_p - 0.2 \pm 0.1 - 0.12 \pm 0.06\right)\left(100.50 \pm .03 \frac{ug}{L}\right)\right) + 20 \pm 10 \frac{ug}{L}$$

(8)

gives $f_u = 0.55 \pm 0.11$ and $f_p = 0.37 \pm 0.08$. Thus participants are estimated on average to lose 44-66% of water via urine and to get 29-45% of total water gain from primary household wells. As previously described, the fractions of water gained from food and from cellular respiration are estimated to be $f_f = 0.20 \pm 0.10$ and $f_c = 0.12 \pm 0.06$ respectively, which results in an estimate of water gain from other wells of $f_o = 0.31 \pm 0.14$. This suggests that water from other wells makes up about 46% of the average participant's drinking water intake (where total drinking water intake is comprised of primary household well water and other well water).

Water consumption from primary household wells and other wells under the neighboring well model

In contrast with the distributed wells model, the neighboring wells model describes participants drinking only from wells within 50 m of their primary household wells. Conducting a multiple regression on $[As]_{u,i}$ as a function of $[As]_{p,i}$ and $[As]_{o,i}$ while setting m_b = 0 (no sink of arsenic in the body)

$$0.64 \pm 0.03 = \frac{f_p}{\left(1 + \frac{(0.06)}{(1-0.06)}\right) f_u}$$

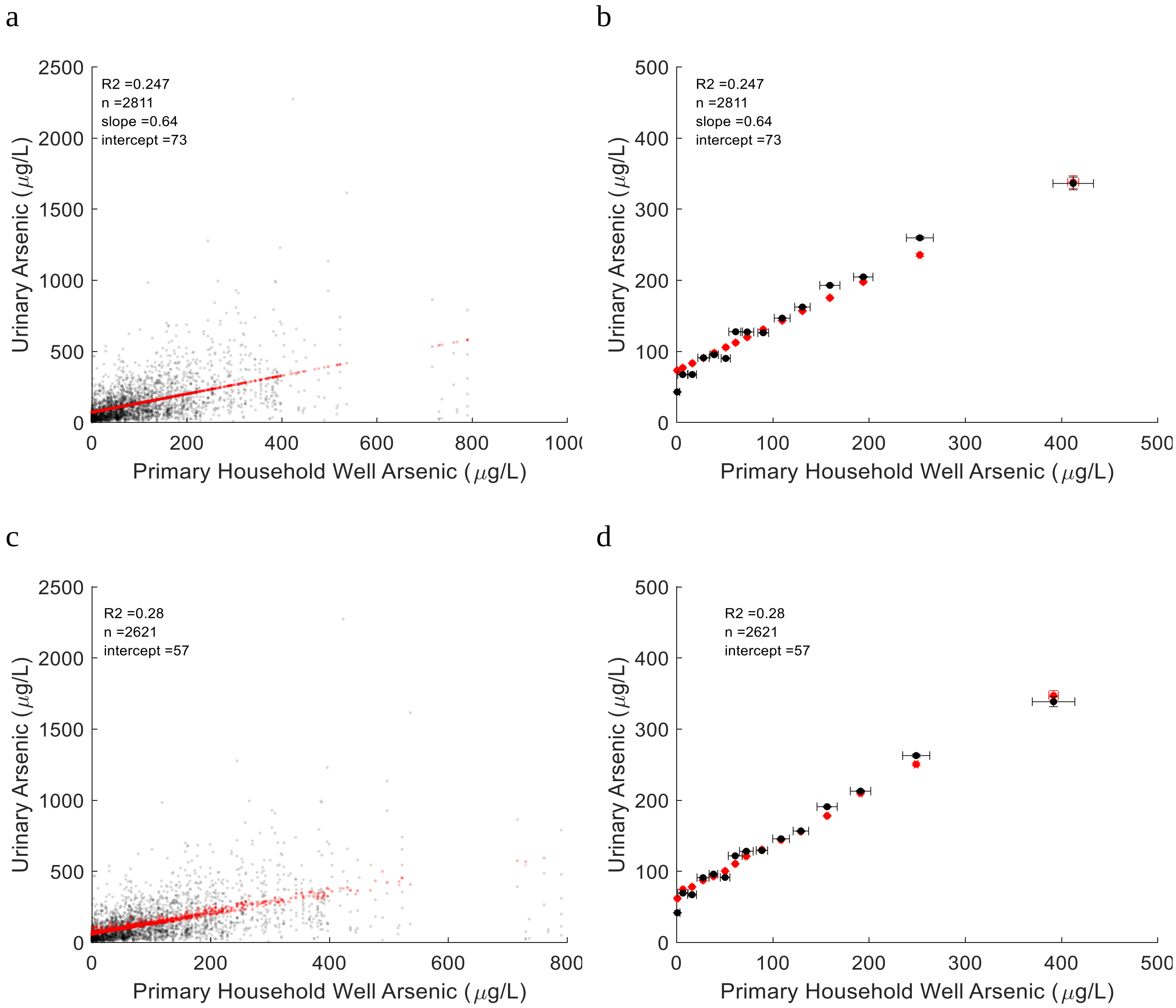

Figure 2. (a) Fitted (red, simple linear regression, $[As]_{u,i} = (0.64 \pm 0.02)[As]_{p,i} + 72.9 \pm 3.3$) and observed (black) values of urinary arsenic as a function of individual well arsenic (b) and average arsenic for equally sized bins of 187 wells. (c) Fitted (red, multiple linear regression, $[As]_{u,i} = (0.64 \pm 0.03)[As]_{p,i} + (0.18 \pm 0.03)[As]_{o,i} + 57.3 \pm 3.8$) and observed (black) values of urinary arsenic as a function of well arsenic (d) and binned into bins of 175.

Table 1. Variance in well arsenic and urinary arsenic for women and men at baseline, before being informed of their well arsenic status.

	Primary household well arsenic (µg/L)	Urinary arsenic (µg/L)	N
Women	10,767	24,214	1605
Men	10,576	17,730	1196
p	**0.742**	**<0.001**	

$$0.18 \pm 0.03 \ = \frac{\left(1 - f_p - 0.2 \pm 0.1 - \ 0.12 \pm 0.06\right)}{\left(1 + \frac{(0.06)}{(1-0.06)}\right) f_u}$$

$$57.3 \pm 3.8 \ = \ F \tag{9}$$

gives $f_u = 0.78 \pm 0.14$ and $f_p = 0.53 \pm 0.09$ (Figure 2c,d). These are higher than the values of f_u and f_p estimated using the distributed model. Using radii of 20 m, 100 m, and 200 m to estimate $[As]_{o,i}$ did not produce significantly different estimates of f_u or f_p. Thus the neighboring well model estimates that participants on average lose 64-92% of water via urine and gain 44-62% of water from primary household wells. Since the fractions of water gained from food and from cellular respiration are estimated to be $f_f = 0.20 \ \pm 0.10$ and $f_c = 0.12 \ \pm 0.06$ respectively, this results in an estimate of water gain from other wells of $f_o = 0.15 \ \pm 0.15$. This suggests that water from other wells makes up about 22% of the average participant's drinking water intake (where total drinking water intake is comprised of primary household well water and other well water).

Gender differences in drinking from multiple wells

We also investigated whether the fraction of water a person drinks from primary household wells versus other wells differs by gender. There is no statistically significant difference in the variance of primary household well arsenic for men as compared to women, which is consistent with the fact that most men and women in the study were recruited as married couples and thus generally drink from the same primary household well. However, the variance in urinary arsenic is higher across women than across men (Table 1). These results are consistent with men drinking from more wells (and/or wells with more variable arsenic concentrations) than women do, since drinking from multiple wells tends bring an individual's urinary arsenic closer to the population average and thus reduce variance across a population.

Applying separately for men and for women our mass balance calculations, the distributed wells model estimates that on average men get 33 ± 7% and that women get 41 ± 8% of their water from primary household wells (Table 2). Similarly, the neighboring wells model estimates that on average men get 47 ± 9% and that women get 57 ± 10% of their water from primary household wells (Table 3). Thus both models show a small but not significant gender difference in the proportion of water consumed from primary versus other wells.

Discussion

The distributed wells model of water consumption estimates that people get 37±8% of their water from primary household wells and 31±14% from other wells, assuming that the remaining 32±12% comes from food and cellular respiration. In contrast, the neighboring wells model of water consumption estimates that people get 53±9% of their drinking water from primary household wells and 15±15% from other wells. Evaluating the plausibility of the estimate of water loss to urine from each of these models may enable us to determine which model is most likely. Based on a comparison of urinary arsenic variance and based on the application of the mass balance models to women and men separately, women are estimated to consume about 12% more of their drinking water from primary household wells than men.

Table 2. Simple linear regression parameters for urinary arsenic as a function of well arsenic, and the resulting estimates of the fraction of water gained from primary household wells f_p and fraction of water lost to urine f_u for all participants, male participants, and female participants.

	n	slope	intercept	f_p	f_u
All	2811	0.64±0.02	72.9±3.3	0.39±0.08	0.58±0.11
Male	1201	0.57±0.03	80.8±4.7	0.33±0.07	0.49±0.11
Female	1610	0.70±0.03	67.0±4.6	0.41±0.08	0.49±0.11

Table 3. Multiple linear regression parameters for urinary arsenic as a function of well arsenic and the average arsenic in wells within 50 m of the primary household well, and the resulting estimates of the fraction of water gained from primary household wells f_p and fraction of water lost to urine f_u for all participants, male participants, and female participants.

	n	slope 1	slope 2	intercept	f_p	f_u
All	2621	0.64±0.03	0.18±0.03	57.3±3.8	0.53±0.09	0.78±0.14
Male	1116	0.54±0.04	0.24±0.05	63.1±5.3	0.47±0.09	0.82±0.16
Female	1505	0.71±0.04	0.14±0.05	53.0±5.4	0.57±0.10	0.75±0.14

Accuracy of the assumption of independence

A key assumption of our mass balance model is the independence among terms of the model. In particular, our mass balance model would not be accurate, and we would not expect a linear relationship between urinary arsenic and primary household well arsenic, if the arsenic concentration in individuals' primary household wells affected the amount of water they consumed from other wells.

The participants whose data was used in our model had not been informed of their primary household well arsenic concentrations, and there was a linear relationship between their urinary arsenic and primary household well arsenic as predicted by the mass balance model. In contrast, the urinary arsenic concentrations of participants who may have had access to information about their well arsenic, because their wells were labeled in early 2001 and their urinary arsenic was tested in 2001 or later, deviated from this linear relationship, indicating that one or more variables in the model were not independent for this group.

Specifically, participants in this group with very high or very low primary well arsenic had lower urinary arsenic than predicted by the linear model (Figure 3). This may result from behavioral modifications by participants who have been informed of the arsenic concentrations of their well and the wells around them. National campaigns that preceded this study as well as information provided by field staff during the baseline survey likely motivated participants with high primary well arsenic to modify their behavior (Madajewicz et al., 2007; Chen et al., 2007). Informed participants may therefore have begun to shift their water consumption away from their primary well to other wells with lower arsenic, producing the observed concentrations of urinary arsenic below the linear values for this group. Participants with low primary well arsenic are the most likely to have access to a large selection of low-arsenic wells, which could provide them with many opportunities to adjust their drinking habits and further lower their arsenic exposure, producing the observed concentrations or urinary arsenic below the linear values for this group. The effect of greater discrimination based on arsenic status for secondary wells would be expected to be proportionally the largest at low original exposure levels, as observed in Figure 3.

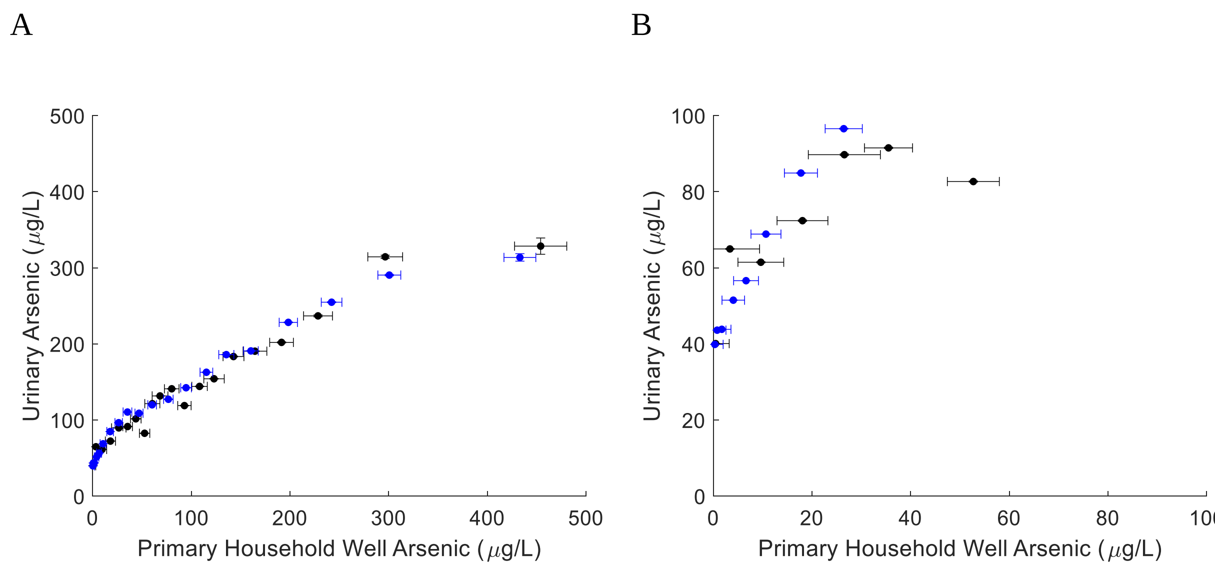

Figure 3. Urinary arsenic as a function of primary household well arsenic for participants who had not been told their primary well arsenic status (black) and participants who may have known their primary well arsenic status (blue) across (a) the full range of concentrations and (b) the low end of the range. Both data sets are binned into twenty bins for comparison purposes, resulting in a bin size of 141 (2811/20) for participants who had not been told their primary well arsenic status (black) and of 421 (8411/20) for participants who may have known their primary well arsenic status (blue). The group of participants who had not been told their primary well arsenic status (black) had a somewhat higher proportion of participants with primary well As above the Bangladesh drinking water standard of 50 ug/L (65% versus 51%), possibly due the geographic distribution of the two groups within the study area.

Including a sink of arsenic in the body

The mass of arsenic flowing to a sink in the body was assumed in the previous models to be negligible compared to other flows in the arsenic mass balance equation. Documented half-lives for arsenic in the body, even in multi-compartment models, are on the order of tens of days or less, suggesting that significant amounts of arsenic are not stored in the body over the long term (Buchet et al. 1981; Byrd et al. 1996; Crecelius 1977; Cullen et al. 1995; Hughes 2006; Johnson and Farmer 1991; Lehmann et al. 2001; Pomroy et al. 1980; Yamauchi and Yamamura 1984). We explore here the potential impacts of a sink of arsenic in the body on the results from the mass balance models.

The mass balance analysis is used to estimate the ratio of water consumed from the primary household well to water consumed from other wells. Because this quantity is a ratio (not the absolute amount of either input) it is independent of sinks of arsenic in the model such as accumulation in the body or loss to defecation. The result that participants get a larger fraction of their water from other wells depends on neither an assumption of a small rate of accumulation nor an assumption that total arsenic in the body reaches an equilibrium when the input and output from the body are balanced.

To illustrate this, we adjust the body sink term in the distributed wells model; analogous results would be observed with the neighboring wells model. Setting the mass fraction of arsenic stored in the body to m_b = 10% gives $f_u = 0.49 \pm 0.10$ and $f_p = 0.37 \pm 0.07$ and increasing it further to m_b = 20% gives $f_u = 0.43 \pm 0.09$ and $f_p = 0.37 \pm 0.06$. Including body storage of arsenic in our mass balance model simply amounts to a scaling factor on the fraction of water lost to urine f_u, producing increasingly small estimates of the fraction of water lost to urine f_u without affecting the estimated fraction of water consumed from primary household wells f_p.

Plausibility of water balance estimates

As a check on whether the estimates produced by the arsenic mass balance models are reasonable, we compare the estimated water loss to urination to prior observations of human water balance. A 70 kg man is estimated to lose 800-1500 mL water to urination, 250 mL to stool, and 600-900 mL to insensible water loss, which does not include sweat (Gomella and Haist 2007). This suggests that around 40-60% of water is lost to urination in the absence of sweat. During exposure to high temperatures, loss of water via sweat can be very large, while loss of water to urine may decline, and thus individuals are likely to lose an even lower fraction of their water to urination (ADOLPH 1947; Mack and Nadel 2011).

The estimated water loss to urination is 55 ± 11% for the distributed model and 78 ± 14% for the neighboring well model. A large portion of the range of the estimate of water lost to urine in the distributed wells model is within the expected range of 40-60% or less suggested in the literature. In contrast, the percent of water loss to urine estimated by the neighboring wells model is higher than the upper end of the expected range from the literature. Since the neighboring wells model gives an estimate for the fraction of water lost to urine f_u that is less likely to be accurate, it is less likely to be the correct model for arsenic exposure in Araihazar. This suggests that the distributed wells model is the more plausible model for participants in our study area.

It is likely that participants drink water from throughout the study area under circumstances such as visiting tea shops, doing day labor away from home, or visiting others in their homes. Another possibility is that a significant non-drinking water source of arsenic exposure exists that is not accounted for in the model, although no evidence of such a source has been observed. Finally, while observed half-lives for arsenic storage in the body suggest that an arsenic sink term should not be included in our mass balance models, inclusion of such a term would reduce the estimates for water loss to urination and could make the neighboring wells model, or something between the neighboring wells and distributed wells models, more plausible.

Implications of arsenic exposure model for sources of arsenic exposure

Given that the distributed wells model fits the data and is physically plausible in terms of its water balance, we can use this model to estimate the degree to which different sources contribute to an individual's arsenic exposure (Figure 4). The model estimates that, on average, individuals with low concentrations of primary household well arsenic get most of their arsenic exposure from water consumed from other wells. For example, an individual with 10 µg/L primary household well arsenic is estimated to get 67 ± 26% of their arsenic exposure from other well water. As primary household well arsenic increases, primary household well water is predicted to make up an increasing fraction of an individual's arsenic exposure. For individuals with 50 µg/L and 100 µg/L primary household well arsenic, respectively, other well arsenic is predicted to comprise 50 ± 25% and 39 ± 19% of arsenic exposure.

These estimated contributions to arsenic exposure represent a population average and do not capture the variability between individuals in the study population. A model of arsenic

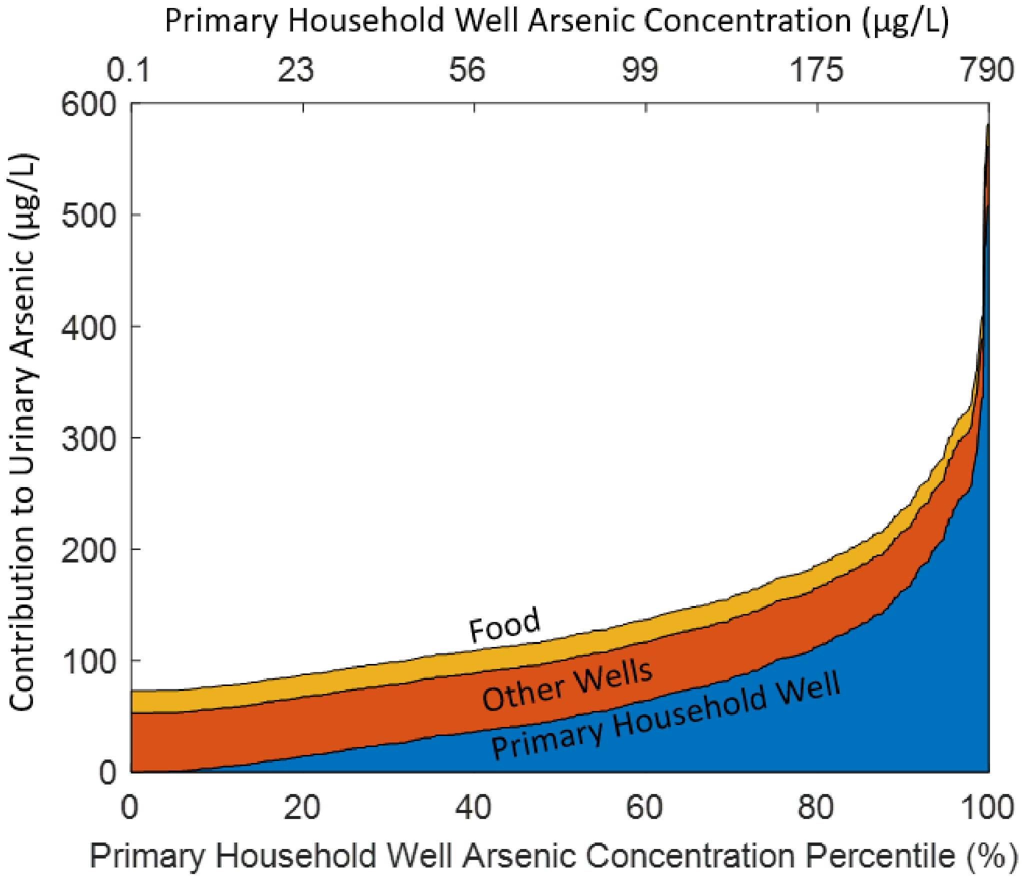

Figure 4. Predicted contribution of primary household well arsenic (blue), other well arsenic (orange), and food arsenic (gold) to total arsenic exposure based on the distributed model as a function of primary household well arsenic concentration. The x-axis has been scaled by primary well arsenic concentration percentile, so that equal numbers of study participants are represented by equal distances along this axis.

exposure at the individual rather than population level would require knowledge of the arsenic content and amount consumed for each water and food source.

Implications of arsenic exposure model for previously reported dose-response relationships

Understanding the relationship between health effects and water arsenic is desirable to inform health standards for arsenic concentrations in drinking water, including in places beyond Bangladesh. Past studies in Bangladesh have generally modeled the relationship between health effects and primary household well arsenic or urinary arsenic, but have not modeled the relationship between health effects and overall water arsenic exposure. One example of such a study is Argos et al. (2010), who present mortality hazard ratios for participants grouped by arsenic (μg/L) in primary household well water, arsenic dose (μg per day), and total arsenic in urine (μg/g Cr).

Based on the proportion of water consumption from primary household wells and other wells estimated in our distributed well model, we show how the relationship between arsenic exposure and mortality in Argos et al. (2010) changes if total arsenic exposure in drinking water replaced primary household well arsenic exposure (Figure 5, Table S1). Using the same data as

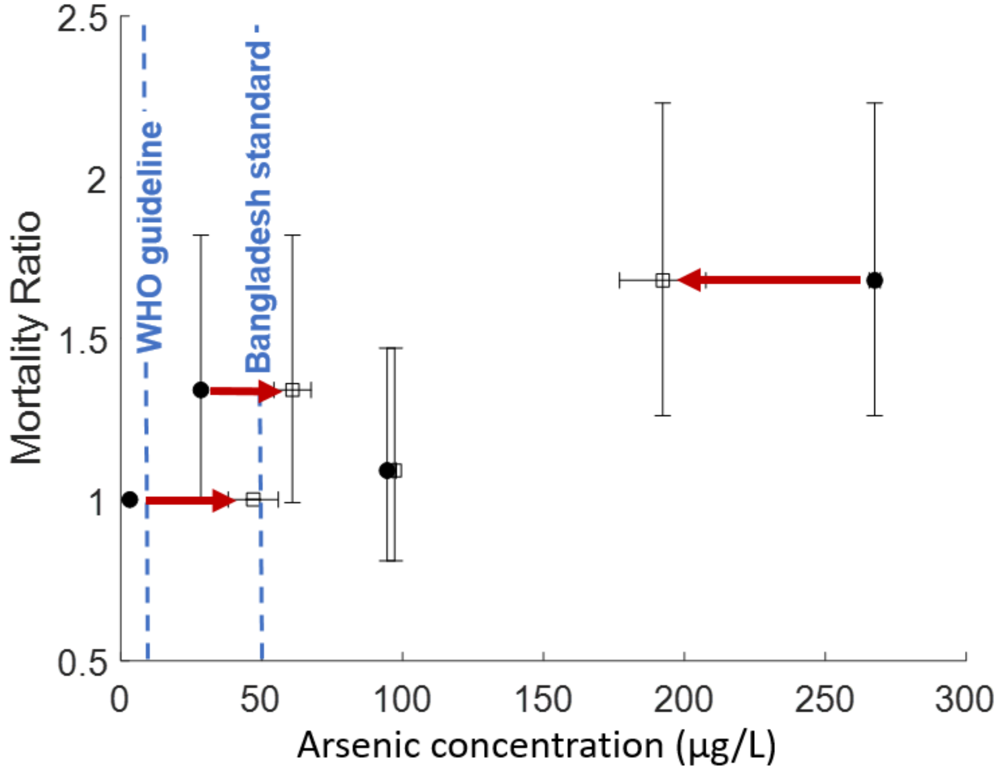

Figure 5. Comparison of mortality ratio as a function of well water arsenic exposure estimated from primary household well arsenic concentration (filled circles; Argos et al. 2010) and well water arsenic exposure estimated when both primary household wells and other wells are included (unfilled squares).

Argos et al. 2010, we compare their exposure estimates based on primary household well arsenic to our exposure estimates based on primary household well and other well arsenic. The well arsenic concentrations listed in the Argos paper are based on the wells that participants were using during the baseline survey and, similarly, our model predicts arsenic exposures for participants who have not yet been informed about their well arsenic concentrations.

For participants with low-arsenic primary household wells, the average concentration of arsenic consumed in drinking water overall is higher than the average concentration of arsenic consumed from their primary household wells. For example, participants in the group with 0.1-10 µg/L (mean of 3.2 µg/L) arsenic in primary household well water in Argos et al. (2010) are estimated to consume an average of 45 µg/L arsenic in well water when other well arsenic is included. Similarly, participants with 10-50 µg/L (mean of 28.4 µg/L) arsenic in primary household well water are estimated to consume water with 59 µg/L arsenic when other well arsenic is included. For participants with high-arsenic primary household wells, the average concentration of arsenic consumed in drinking water is lower than the average concentration of arsenic consumed from their primary household wells. Participants in the group with 150.1-864 µg/L (mean of 267.5 µg/L) arsenic in primary household well water are estimated to consume an average of 196 µg/L in well water once other wells are included.

Overall, the range of water arsenic exposures predicted by a model that includes both primary household well and other well arsenic is smaller than the range of arsenic exposures for primary household wells only, which have generally been the focus in previous water arsenic studies in Bangladesh (Ahsan et al. 2006b; Argos et al. 2010; Sohel et al. 2009). This is because participants with low primary household well arsenic, on average, drink from other wells with higher arsenic concentrations than their primary household wells and, similarly, participants with high primary household well arsenic, on average, drinking from other wells with lower arsenic concentrations than their primary household wells. Overall water arsenic exposure taking into account both primary household well and other well arsenic are thus spread over a narrower range of arsenic levels than the levels based on primary household wells. Indeed, because of the contribution of water arsenic from other wells in Araihazar, Bangladesh, when interpreting findings for the population exposed to low arsenic (e.g. <10 µg/L) exposures from the primary household well, it is important to consider that average water arsenic exposure from all sources, including other wells is higher (estimated mean overall water arsenic when including both primary household wells and other wells is 47 µg/L for the study area in 2000-2001). This source of misclassification in water arsenic exposure is non-differential and could result in an underestimation, but not an overestimation, of the impact of water arsenic exposure levels on mortality.

Gender differences in consumption of water from primary wells

The men in our study cohort were primarily manual laborers, while the women were primarily homemakers (Ahsan et al. 2006a), consistent with the traditional division of labor throughout Bangladesh (Cain et al. 1979). Under this division of labor, we expect men to drink from a wider array of wells beyond their primary household well, and our observations are consistent with this expectation. Our observations are also consistent with the prior findings of Sohel et al. (2010), who observed higher agreement between urinary arsenic and the reported main source of drinking water for women compared to men in Matlab, Bangladesh. They also observed that including the nearest five water sources in a spatial model of arsenic exposure improved the fit only marginally over a model based on the reported main source of drinking water and suggested that information about water sources used by men at work would be useful to better quantify arsenic exposure.

Conclusions

We investigated how much water participants consume from primary household wells and other wells and estimated that, on average, about 46% of participants' drinking water consumption may come from other wells, and thus that other wells may comprise a significant fraction of total arsenic exposure, particularly for participants whose primary household well arsenic concentrations are low. For example, participants with primary household well arsenic concentrations at the Bangladesh drinking water standard of 50 ug/L are estimated to get 50 ± 25% of their arsenic exposure from other wells, with the remainder coming from their primary household wells and food. We also estimated that women likely drink 12% more of their water from primary household wells than men, consistent with the fact that men tend to work outside the family compound and women tend to work within it. Finally, we hypothesize that the use of solely primary household well arsenic concentrations in dose-response curves likely results in non-differential measurement error of the estimated water arsenic dose and a subsequent underestimation of the health impacts of water arsenic.

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Supporting Information

Table S1. Comparison of well water As exposure estimated from primary household wells (Argos et al. 2010) to well water As exposure estimated when other wells are included.

n (Argos)	**n (this paper)**	**As (µg/L) in primary household well water (Argos)**	**Average As (µg/L) in primary household well water**	**Average As (µg/L) in all water consumed**	**All-cause mortality HR (95% CI; Argos)**
2743	2742	0.1-10	3.2±0.1	44.7±12.0	1
2511	2513	10.1-50	28.4±0.2	59.2±8.9	1.34 (0.99-1.82)
3600	3600	50.1-150	94.5±0.5	97.1±0.8	1.09 (0.81-1.47)
2889	2889	150.1-864	267.5±2.0	196.3±20.6	1.68 (1.26-2.23)

Chapter 2: Changes in arsenic exposure in Araihazar, Bangladesh from 2001 through 2015 following a blanket well-testing and education campaign

Abstract

Background: Concentrations of arsenic (As) are elevated in a large proportion of wells in Bangladesh but are spatially variable even within a village. This heterogeneity can enable exposed households to switch to a nearby well lower in As in response to blanket (area-wide) well As testing.

Objectives: We document the evolution of As exposure in Araihazar, Bangladesh following a blanket well-testing and education campaign.

Methods: We use well-water and urinary As data collected between 2000 and 2008, along with household interviews extending through 2016, within a 25 km^2 area of Araihazar upazila for nearly 12,000 participants enrolled in the Health Effects of Arsenic Longitudinal Study (HEALS). We observe changes in participants' well water and urinary As concentrations following a blanket well-testing and education campaign and use logistic regression to determine the factors associated with participants' decisions to switch primary household wells.

Results: Arsenic exposure for participants most exposed at baseline declined from a mean As concentration of 226 μg/L at baseline to 173 μg/L two years later, and further declined to 139 μg/L over 8 years. Well status with respect to As was predictive of well-switching decisions for at least a decade after the initial testing. Participants with As-unsafe wells were 6.8 times more likely to switch wells over the first two years and 1.4-1.8 times more likely to switch wells over the ensuing decade.

Conclusions: Arsenic exposure rapidly declined following a blanket well testing and education campaign, and remained lowered for over a decade. However, the number of participants with unknown As concentrations in their primary household wells increased substantially over time, indicating the importance of additional well testing as new wells continue to be installed.

1. Introduction

Natural contamination of groundwater with arsenic (As) poses a health threat in many regions of the world where people rely on wells for drinking water. In Bangladesh, more than 50 million people are estimated to have been chronically exposed to As concentrations above the World Health Organization (WHO) guideline of 10 µg/L (BGS and DPHE 2001; Brammer and Ravenscroft 2009). Arsenic exposure produces negative health outcomes such as skin lesions, cancers of the skin, bladder, and lung, cardiovascular disease, increased risk of stillbirth and infant mortality, and reduced intellectual function in children (Argos et al. 2010; Chen et al. 2011; Flanagan et al. 2012; Quansah et al. 2015; Rahman et al. 2007; Smith et al. 2000; Sohel et al. 2009; Wasserman et al. 2004).

The Bangladesh government and NGOs have supported a range of methods for reducing As exposure. Many of these interventions have been insufficiently safe, effective, or persistent (Ahmed et al. 2006; Hoque et al. 2006; Howard et al. 2006). A common problem with water filtration, which was the focus of early interventions, is rapid abandonment of filters due to maintenance issues and inconvenience (Ahmed et al. 2006; Hossain et al. 2005; Sanchez et al. 2016). An alternative approach is blanket well-testing to provide individuals with information about the As concentration of their own wells and nearby wells, thus facilitating switching to drinking from lower-As wells. This approach is made possible by the high spatial variability of well As concentrations, even within a small area. Since well As concentrations are generally stable, once an As-safe well has been identified, additional maintenance or monitoring is generally not required, in contrast with water filtration.

In this paper, we report changes in behavior and As exposure following interventions to facilitate well-switching in an As-impacted 25 km^2 area of Araihazar, Bangladesh. The interventions began with an initial round of blanket well testing for As, with most wells labeled in January through March of 2001. People drinking from wells with As concentrations of more than 50 µg/L ("As-unsafe wells") were encouraged to switch to As-safe wells, and deep, As-safe community wells were installed in areas with few As-safe wells. Additional blanket well testing in Araihazar was conducted by the government in 2003, after which wells were painted red or green to indicate whether they were above or below the Bangladesh drinking water standard, and by a team of local village-health workers in 2012-2013 (van Geen et al. 2014). Prior studies have shown positive impacts on well As and urinary As two to three years after blanket well testing (Chen et al. 2007; Madajewicz et al. 2007; Opar et al. 2007). In this paper, we observe the changes in participants' arsenic exposure over sixteen years starting from the initial round of blanket well-testing in 2000-2002.

2. Methods

2.1. Health Effects of Arsenic Longitudinal Study (HEALS)

The study is briefly summarized here, and a detailed description of the study design is found in Ahsan et al. (2006). Data were collected in a 25 km^2 area in Araihazar, Bangladesh. In 2000-2002, the field team conducted a blanket survey of wells in the study area, recorded their GPS coordinates, and tested them to determine their As concentrations.

From October 2000 to May 2002, paired HEALS study teams (each with one physician and one non-physician interviewer) identified and recruited eligible individuals (aged >=18 years and living in the study area for >=5 years) into the cohort. At baseline recruitment, each eligible participant, following informed consent, completed a structured interview including detailed drinking water history, health, household, and demographic information and provided a blood and a urine sample. As part of the baseline interview, each study participant reported all sources of their current and past drinking water including both primary and secondary drinking water sources. For ethical reasons, as soon as the laboratory testing data on arsenic concentration became available all surveyed wells were labeled with placards indicating values of As concentrations (Ahsan et al. 2006; van Geen et al. 2003). However, individual level health education messages, including information and interpretation of arsenic values posted on placards and specific advice to switch wells for those reported using high arsenic wells at baseline interview were given by study physicians after the completion of baseline interview and bio-sample collection. Subsequently, extensive community level health education campaigns as well as physician-led individual-level health education messages were provided to cohort participants during their visits to HEALS study clinic and also during follow-up home visits.

Five follow-up surveys were conducted in 2002-2004, 2004-2006, 2007-2009, 2010-2013, and 2014-2016, respectively. Urinary As was measured during the first three follow-up surveys, i.e. through 2009, but was not measured subsequently due to limited resources. Also due to limited resources, well water As was not tested systematically during surveys after baseline, and thus well As concentrations at follow-up are only known for participants who either remained using their original baseline well or who switched to another baseline well. Well water and urine samples are being collected as part of the ongoing, sixth follow-up cycle.

2.2. Determination of well IDs and well-switching

From the data collected during field surveys, well IDs could be determined for a total of 11744, 8919, 8059, 6671, 5604, and 4838 participants at baseline through follow-up five respectively (Figure S1). Because many wells lost their identifying placards, and because some participants switched to newly-installed wells, a majority of participants were no longer drinking from identifiable primary household wells by the end of the study period.

In this study, when documenting whether a participant switched wells between one survey and the next, we only considered participants with known well IDs at each survey. If the well IDs between one survey and the following survey differed, we concluded that the participant switched wells. If the well IDs for the two surveys matched, we concluded that the participant did not switch wells.

2.3. Logistic regressions

Logistic regression models were used to investigate how well-switching depended on whether a well was above or below the Bangladesh drinking water standard of 50 μg/L (“As-unsafe” or “As-safe”, respectively) and on the As concentration in the well (treated as a continuous variable). Our hypothesis was that participants with As-unsafe wells and participants with higher well As concentrations would be more likely to switch wells at a follow-up visit. The Wald test was used to determine whether individual coefficients were statistically significant and the Pearson chi-square goodness of fit test to determine whether the model was a good fit for the data.

3. Results

3.1. Participant primary household well As exposure over time

The percentage of participants with primary household wells observed to be As-unsafe decreased from 56% to 27% between the baseline survey and the first follow-up survey and continued to decline thereafter, reaching 14% by the third follow-up about 8 years after the initial

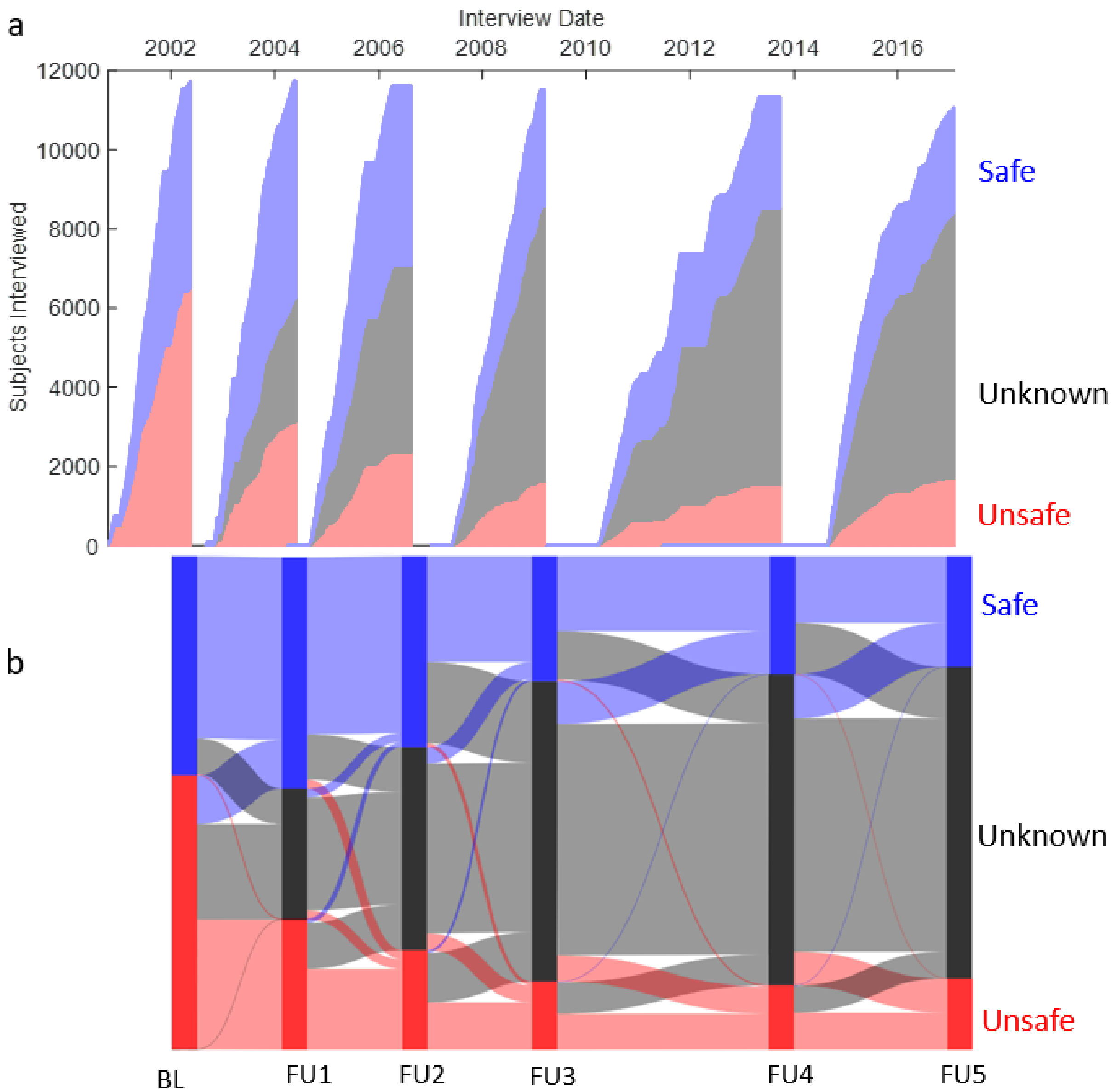

Figure 1. Number of subjects with primary household wells that are As-safe (blue, <50 µg/L As), As-unsafe (red, >50 µg/L As), or of unknown As concentration (black) at baseline and at each follow-up. Collection of well information over time during each interview cycle (top, panel a) and flows of participants from one well category to another between interview cycles (bottom, panel b). BL = baseline, FU = follow-up.

intervention (Figure 1). The percentage of participants with As-safe wells increased slightly from 44% to 47% percent between baseline and the first follow-up survey and then declined, reaching 26% by the third follow-up. The participants with wells that were either newly installed and thus untested as baseline, or wells that had lost their identification tags, increased from <0.1% at baseline to 27% at the first follow-up survey, and continued to increase, reaching 60% by the third follow-up. The number of participants with each type of well held relatively steady between the third follow-up eight years after the initial intervention and the fifth follow-up sixteen years after the initial intervention.

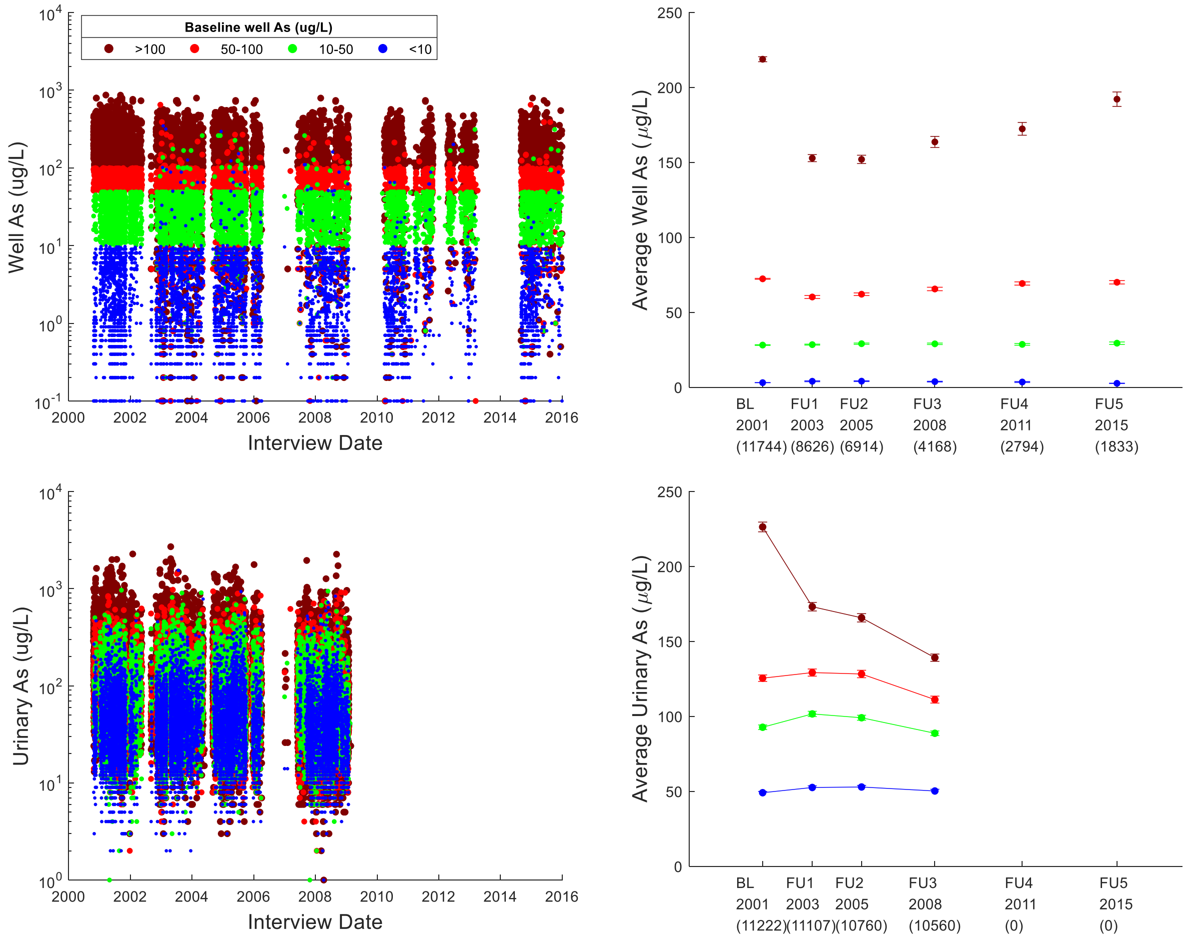

Figure 2. (a) Primary household well As, (b) average primary household well As, (c) urinary As, and (d) average urinary As over time after the intervention for subjects drinking from baseline wells with <10 μg/L, 10-50 μg/L, 50-100 μg/L, and >100 μg/L As. The x-axis labels in (b) and (d) list the median year of data collection and the number of participants included in each average (in brackets). The averages in (b) are unconnected by lines since the number of individuals whose well As is known substantially declines between follow-ups, whereas the averages in (d) are connected by lines because the same group of people is tracked across all follow-ups. BL = baseline, FU = follow-up.

Within the diminishing subset of participants drinking from wells tested at baseline, the proportion of participants with wells observed to be As-unsafe decreased, and the proportion of participants with wells observed to be As-safe increased, from baseline to the first follow up. Beyond the first follow up the proportion of participants drinking from each type of well remained largely constant for sixteen years after the initial intervention (Figure 1, Figure S1).

We can further break out the impact of the intervention on well As according to the As concentrations in participants' wells at baseline (Figure 2a,b). Changes in primary household well As result from participants switching wells between surveys, and are only reported for the subgroup of participants drinking from household wells tested at baseline that had retained their ID tags. Between the baseline and first follow-up survey two years later, there was a steep decline in average well As (219 to 153 μg/L) for participants who started with wells with >100 µg/L As, and a lesser decline (72 to 60 µg/L) for participants who started with wells with 50-100 µg/L As. In contrast, little change in well As occurred for the participants who started with As-safe wells <50 µg/L As. After this initial effect of the intervention, well As held roughly steady across all groups through the sixteen years of monitoring after the initial intervention.

3.2. Participant urinary As over time

Urinary As was measured for eight years after the initial intervention compared to sixteen years of participants reporting primary household well IDs. Urinary As is more representative of the overall population exposure, since it integrates all sources of exposure and is not limited to participants whose primary household wells have known As concentrations. We consider changes in urinary As following the initial intervention for participants who had baseline wells with As concentrations >100, 50-100, 10-50, and <10 µg/L (Figure 2c,d). For the group of participants drinking from baseline wells >100 µg/L As, there was a large decline in average urinary As from 226 to 173 µg/L between the initial intervention and the first follow-up two years later. At later times, average urinary As continued to decline for this group, but more gradually, reaching 139 µg/L by the third follow-up eight years after the initial intervention.

In contrast, the participants drinking from wells with 50-100, 10-50, and 0-10 µg/L As showed no average decline in urinary As in the two years after the initial intervention. These groups had relatively stable urinary As concentrations throughout the eight years of monitoring, although all groups had a slight decline in urinary As between the second and third follow-ups. For participants with either As-safe or As-unsafe primary household wells at baseline, those with As-unsafe wells at the third follow-up eight years later had the highest urinary arsenic concentrations, and those with As-safe wells had the lowest urinary arsenic concentrations (Figure 3). More than 50% of participants relied on primary wells that were not tested at baseline or had missing labels by the third follow-up, and these participants had intermediate urinary arsenic concentrations.

3.3. Factors influencing well-switching

To explore how the persistent lowering of household well As and urinary As may have been facilitated by well testing and education, we used a logistic regression to determine how well status (As-safe or As-unsafe), well As concentration (posted on a placard on each tested well), age, and sex were related to participants' decisions to switch wells at baseline and at later times (Table S1). Sex and age were not consistently related to well-switching. The most consistent factor associated with participants' decisions to switch wells was well status (Figure 4a). Participants with As-unsafe wells were 6.8 times more likely to have switched wells between baseline and first follow-up compared to participants with As-safe wells. Between the first and second, second and third, and third and fourth follow-up surveys, participants with As-unsafe wells were 1.4-1.8 times more likely to switch wells, with well status becoming negligible after the fourth follow-up. Thus the relationship between well status and well switching was weaker at later times but well status continued to be related to participants' switching decisions more than a decade after the intervention.

Well As concentration remained a significant factor after controlling for well status, indicating that providing participants with information about well As concentration in addition to well As status informed their behavior. However, well As concentration was only related to participant well switching between baseline and the first follow-up and between the first and

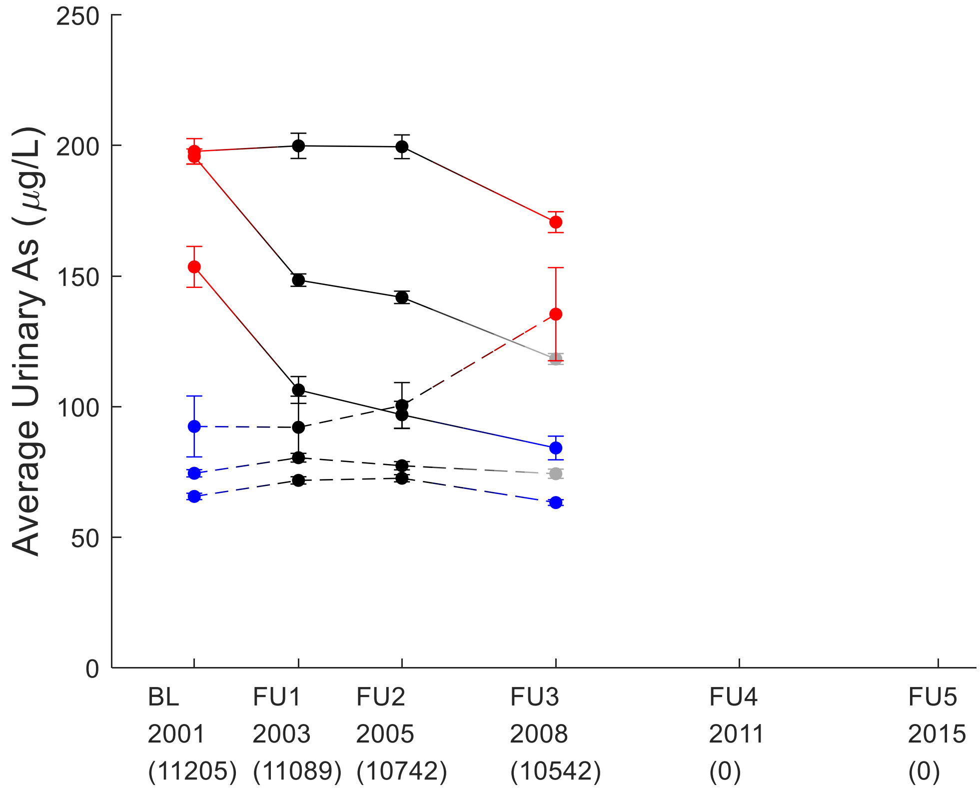

Figure 3. Average urinary As over time for subjects with As-safe (blue) and As-unsafe (red) baseline wells who were drinking from As-safe wells (blue), As-unsafe wells (red), or wells not tested at baseline or with missing labels (gray) by the third follow-up. The colors of the data points represent the well type used by participants. Since each series is based on the well type used at baseline and at the third follow-up, the data points for the first and second follow-ups are black, representing participants using a mix of well types. The x-axis labels list the median year of data collection and the number of participants included in each average (in brackets). BL = baseline, FU = follow-up.

second follow-ups. At both times, each 100 µg/L increase in As concentration led to participants being about $1.003^{100} = 1.35$ times more likely to switch wells (Figure 4b).

Comparing the well-switching behavior of participants drinking from As-safe versus As-unsafe wells suggests that participants drinking from As-safe wells may more strongly and persistently take their well As concentrations into account when deciding whether to switch wells (Figure 5). For participants drinking from As-safe wells, at times from baseline through the third follow-up, a 10 µg/L increase in As concentration led to about a $1.008^{10} = 1.08$ times higher likelihood of switching wells, with diminishing effects at times after that.

3.4. Impacts of switching away from As-safe and As-unsafe wells

We investigate how primary household well As changes for participants who switch away from As-safe wells and from As-unsafe wells. Participants who switched away from an As-unsafe well between the baseline and first follow-up had a mean decrease in well-water As of 106 µg/L (Figure 6). At later times after the intervention, participants who switched away from As-unsafe wells had a lesser mean decrease in well As, with a mean decline of only 47 µg/L

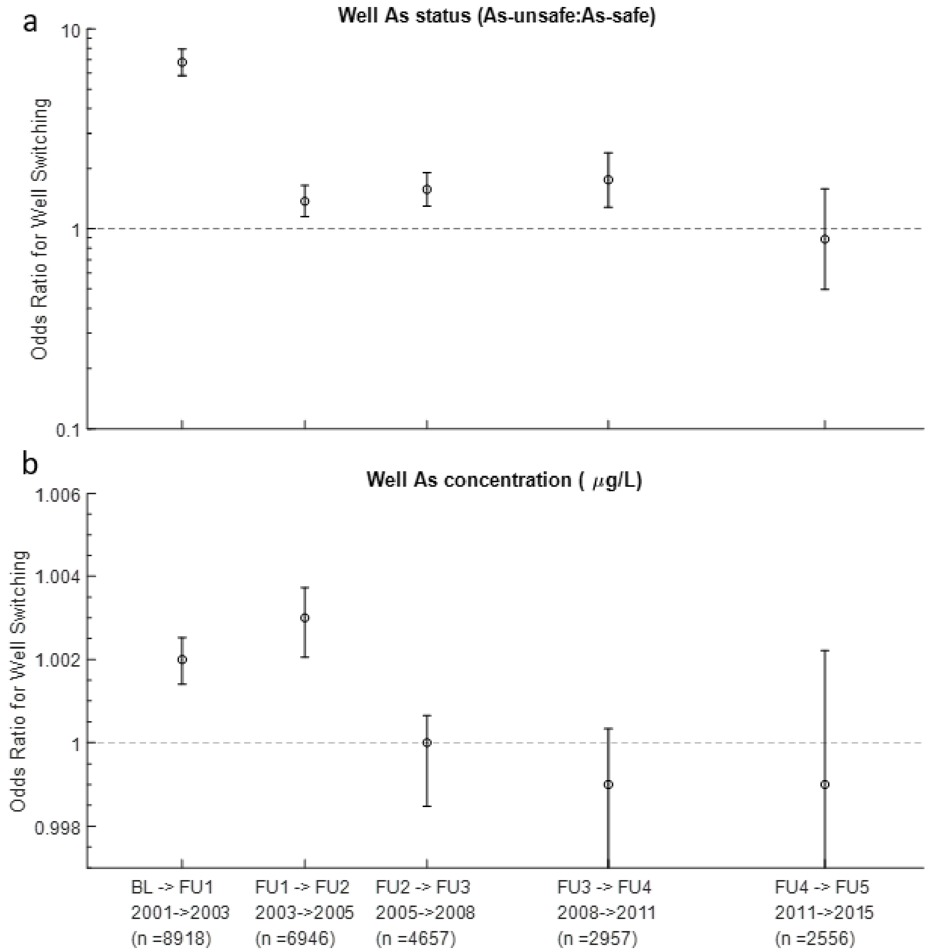

Figure 4. Odds ratio of well-switching as a function of (a) well As concentration (µg/L) and (b) well As status (As-unsafe:As-safe). The x-axis labels list the median years of data collection and the number of participants included in each regression. BL = baseline, FU = follow-up.

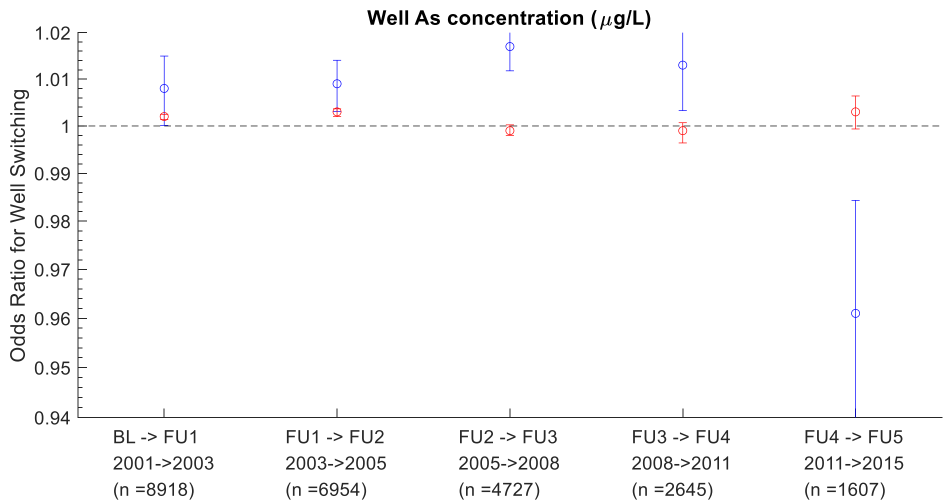

Figure 5. Odds ratio of switching away from an As-safe (blue) or As-unsafe (red) well as a function of As concentration (µg/L). The x-axis lists the median years of data collection and the number of participants included in each regression. BL = baseline, FU = follow-up.

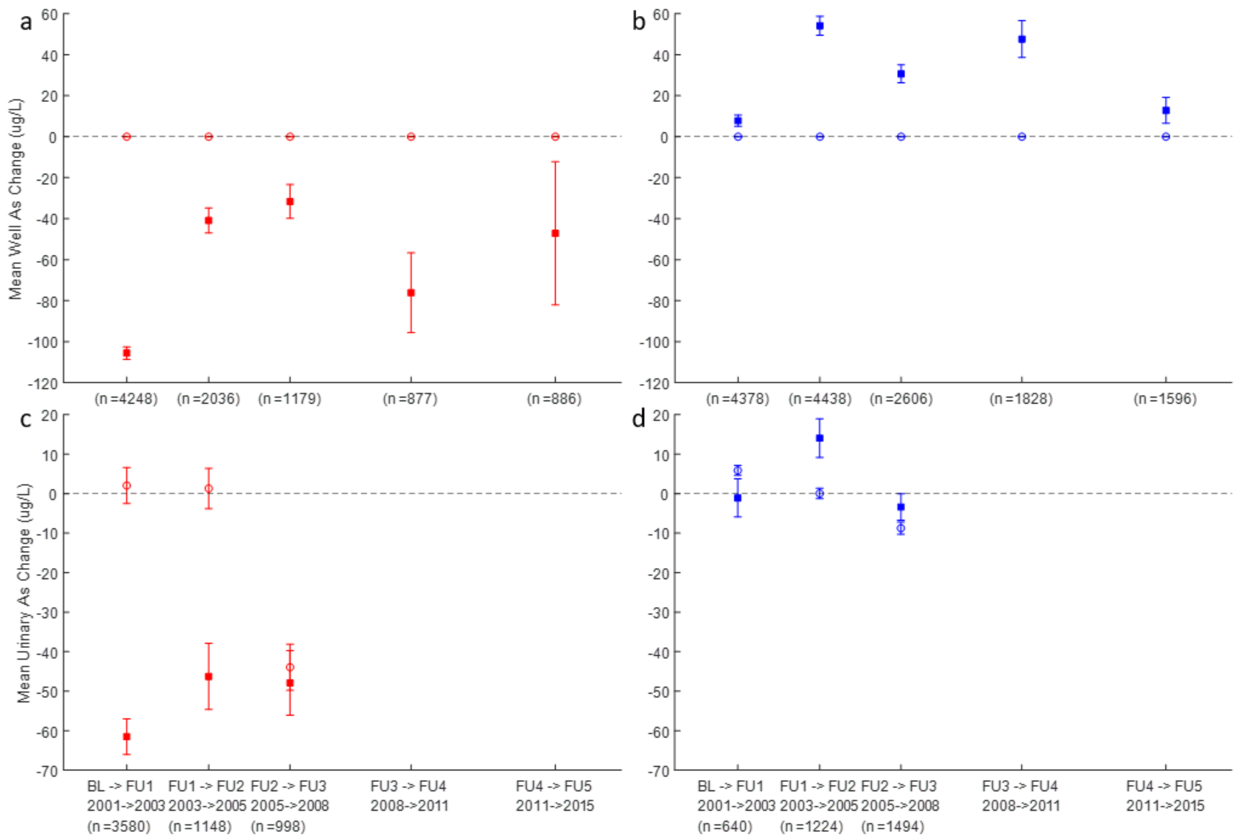

Figure 6. Mean difference in well As concentrations for participants who switched away from (a) As-unsafe and (b) As-safe primary household wells and mean difference in urinary As concentration for participants who switched away from (c) As-unsafe and (d) As-safe primary household wells in the interval between each survey. Filled squares represent participants who switched wells and hollow circles represent participants who did not switch. The x-axes list the median years of data collection and the number of participants included from each time period. BL = baseline, FU = follow-up.

between the fourth and fifth follow-up. Similarly, users of As-safe wells who switched wells between the baseline and first follow-up had only a slight increase in mean primary household well As of 8 µg/L. However, users of As-safe wells who switched wells at later times had larger increases in mean primary household well As of 30-50 µg/L.

We also look at changes in urinary As over time for people who switched and did not switch primary household wells. Changes in urinary As can occur with or without changes in primary household well As, since urinary As provides a comprehensive measure of As exposure through drinking water from primary household wells and other wells, in addition to As exposure from food and other sources. Participants drinking from As-unsafe wells who switched wells between the baseline and first follow-up and first and second follow-up had declines in urinary As over those intervals of 62 µg/L and 45 µg/L (Figure 6). In contrast, urinary As did not change for participants drinking from As-unsafe wells who did not switch wells between baseline and the first follow-up or between the first and second follow-ups.

Urinary As concentrations for participants drinking from As-safe wells also did not change much between baseline and the first follow-up or between the first and second follow-ups, regardless of whether those participants switched wells between follow-ups (Figure 6).

Urinary As declined for all participants between the second and third follow-ups (Figure 2, Figure 7), and it declined most strongly (by more than 40 µg/L) for participants drinking from As-unsafe primary household wells, regardless of whether those participants switched wells during that interval.

4. Discussion

4.1. Summary of intervention impacts

We investigated how behavior and As exposure changed in a 25 km^2 area of Araihazar, Bangladesh following blanket well testing and education conducted by HEALS beginning in 2001 and blanket well testing by the Bangladesh government in 2003. Within the first two years after the initial HEALS intervention, there was a steep decline in primary household well As and in urinary As for the individuals with the highest exposure at baseline. Beyond these two years, significant further gains were not realized, but urinary As and primary household well As remained at the new, lowered level for at least eight years, and an additional slight decline in urinary As occurred about 6 to 8 years after the initial intervention. Participants with As-unsafe wells remained more likely than participants with As-safe wells to switch wells for at least ten years after the initial well testing, and, among participants drinking from As-safe wells, those with higher well As concentrations remained more likely to switch.

4.2. Comparison with prior studies of blanket well-testing

The impacts we observed were broadly consistent with previously reported impacts of the 2003 government blanket well testing in other areas of Bangladesh. In a nearby area of Araihazar, 27% of households with unsafe wells (and only 2% of households with safe wells) had switched wells within 2 years of government well testing, although average As concentrations in primary household wells only declined from 109 to 93 µg/L (Pfaff et al. 2017). At a follow-up survey in the same area three years later, no households had switched back to their unsafe wells and additional households had switched away from unsafe wells (Balasubramanya et al. 2013). In Matlab, Bangladesh, following the government well testing, mean primary household well As evolved from 93 µg/L in 2003 to 55 µg/L in 2008 and 60 µg/L in 2013 while the percentage of individuals with unsafe primary household wells declined from 58% to 28% to 27% over the same time period (Kippler et al. 2016). Lower percentages of well-switching were observed in these studies as compared to HEALS, possibly due to the fact that HEALS involved an arsenic education component in addition to well testing. However, these studies reflect a similar trend of an initial decrease followed by a plateau in As exposure in response to blanket well testing, as was observed in the HEALS cohort.

We additionally compare our observations of well-switching with those reported previously for HEALS. We recorded a well switch or lack of switch between each pair of surveys only for participants with a known well ID before and after the switch. At the first follow-up survey only, participants additionally directly reported whether they had switched wells, and we can compare our estimates to this direct measure. We measured less switching away from both safe (7% versus 14-17%) and unsafe (42% versus 58-65%) wells between baseline and first follow-up than HEALS studies that used the direct report of switching (Chen et al. 2007; Madajewicz et al. 2007; Opar et al. 2007). This indicates that our measure underestimated well switching by not capturing switching to wells with unknown well IDs (i.e. new wells or wells that had lost their labels).

Similarly, our metric of well switching indicated that participants with As-unsafe wells were 6.8 times more likely to switch than participants with As-safe wells between baseline and first follow-up, higher than the ratio of 4-to-1 based on participants' direct reports of well-switching (Chen et al. 2007). This suggests that our odds ratios may be skewed by the fact that participants drinking from As-unsafe wells may more often switch to known wells than participants drinking from As-safe wells. This could occur if participants switch from As-unsafe wells primarily in order to lower their As exposure while participants switch from As-safe wells primarily due to external factors such as well failures that require the installation of a new well.

4.3. Decline in urinary As between six and eight years after the initial intervention

An overall decline in urinary As was observed throughout the study area from the second to the third follow-up, that is, about six to eight years after the initial intervention (Figure 2). Given that this decline was observed for participants who switched primary household wells and participants who did not switch wells (Figure 5b), this suggests that many participants lowered their As exposure from other sources. One possibility is that participants lowered their As exposure by drinking less from their primary wells and more from non-primary wells with low As concentrations. Another possibility is that the proportion of water that participants consumed from non-primary wells stayed the same, but that participants switched to using non-primary wells with lower arsenic concentrations. These behavior changes could occur due to increased availability of information about As-safe drinking water sources, increased installation of As-safe drinking water sources, or an increased interest in drinking As-safe water.

4.4. Protective effect of the interventions for participants drinking from As-safe wells

If the interventions had not taken place, we would expect participants drinking from As-safe wells at baseline to switch wells just as frequently as participants drinking from As-unsafe wells and on average to increase their As exposure. Instead, we observe that participants who start out drinking from As-safe wells maintain roughly constant average concentrations of primary household well As and urinary As over time (Figure 2).

This is at least partly attributable to the fact that participants drinking from As-safe wells are less likely to switch wells than participants drinking from As-unsafe wells (Figure 4b). Participants drinking from As-safe wells also appear to take their well As concentrations into account more strongly and persistently when making decisions about well switching compared with participants drinking from As-unsafe wells (Figure 5). Furthermore, even those participants drinking from As-safe wells who do switch wells have little increase in their urinary As (Figure 7), whereas if participants were switching wells randomly rather than strategically, we would expect a larger negative impact of switching. Thus, in addition to lowering As exposure for the study participants drinking from As-unsafe wells, the interventions had a protective effect on participants drinking from As-safe wells at baseline.

4.5. Limitations of a well testing and education campaign

Our study reveals a significant limitation of one-time blanket well testing: with the rapid increase in the number of new and unlabeled wells, participants had diminishing access to information about well As at later times. Immediately after the blanket survey of well As in 2000-2002, essentially all subjects knew the As concentration in their primary household wells, allowing them to use this information when deciding whether to switch wells (Figure 1). However, over the ensuing years, the proportion of participants drinking from wells that were not tested at baseline or had lost their labels increased rapidly (Figure 1). Our observation of 27% unlabeled wells by the first follow-up is consistent with the observations of Opar et al. (2007) that two to four years after the initial round of well testing, new labels could be attached to only 68% of the previously tested wells because the well had moved or its identification tag was missing. Our observation of 61% unknown wells by the fourth follow-up (2010-2013) is comparable to the 58% unknown wells observed in 2014 (van Geen et al. 2014).

It has previously been reported after blanket well-testing in nearby areas of Araihazar that the proportion of As-safe newly installed wells was not any higher than in older wells (Pfaff et al. 2017). Additionally, households who no longer knew their well As 5 years after the blanket well-testing were more likely to switch away from safe wells than from unsafe wells, opposite of the trend observed for households that did know their well As concentrations (Balasubramanya et al. 2013). This suggests that follow-up well testing should be done frequently so that people can continue to incorporate information about well As into their decision-making. One way to accomplish this would be to test all wells for arsenic at the time that they are drilled, using a field kit that provides rapid measurements of water As.

Additionally, within the HEALS cohort, participant well-switching decisions were more strongly predicted by well arsenic concentrations and well status within the first few years after wells were labeled by HEALS staff in 2001. The importance of this information then appeared to fade over time, becoming negligible by about fourteen years after the initial intervention (Figure 4,5). Overall, the increase in the proportion of unlabeled wells along with the diminished impact of well As status and well As concentration on well switching decisions appear to have resulted in diminishing benefits in terms of As exposure for participants switching away from As-unsafe wells at later times after the intervention (Figure 6). This suggests that additional well testing and education may be needed to make further gains.

4.6. Limitations of this study

The impacts of well testing that occurred after baseline in our study area are not consistently captured in this analysis. The Bangladesh government conducted a blanket well-testing campaign throughout the study area in 2003, and a small number of additional wells were tested by HEALS field staff after the baseline survey. Another round of blanket well testing was conducted in the study area in 2012-2013. The results of any additional well testing that occurred before the end of urinary As testing in 2008 may have contributed to the observed decline in urinary As documented in this paper. Any re-testing of wells tested and labeled by HEALS staff at baseline could have reinforced this information and contributed to the well-switching decisions documented in this paper. However, the impacts of testing new wells on participant well-switching decisions is not captured, since these new wells are not among the wells tracked in the HEALS surveys.

5. Conclusions

Following a blanket well testing and education campaign in 2000-2002, As exposure substantially decreased for individuals with As-unsafe primary household wells. These individuals maintained the new, lower levels of As exposure, and individuals with As-safe wells also maintained low As exposures, for more than 8 years after the well-testing intervention. However, the number of participants with wells that were untested at baseline or had lost their labels increased substantially over time, and participants appeared to decreasingly take their well

As status and concentration into account when switching wells at later times, highlighting the need for continued well-testing and education campaigns.

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Supporting Information

Table S1. Logistic regression of well-switching as a function of well status (As-safe/unsafe), well arsenic concentration, age, and sex.

	Coefficient	p	Odds ratio	Odds ratio CI
Baseline -> FU1 (n = 8918)				
Intercept	-2.532	<0.001	---	---
Well Status	1.917	<0.001	6.799	[5.84, 7.916]
Arsenic Concentration (µg/L)	0.002	<0.001	1.002	[1.001, 1.003]
Age (y)	-0.058	0.327	0.944	[0.841, 1.059]
Sex	0	0.946	1	[0.995, 1.006]
FU1 -> FU2 (n = 6946)				
Intercept	-1.838	<0.001	---	---
Well Status	0.318	<0.001	1.375	[1.15, 1.64]
Arsenic Concentration (µg/L)	0.003	<0.001	1.003	[1.002, 1.004]
Age (y)	0.125	0.081	1.133	[0.985, 1.304]
Sex	-0.004	0.248	0.996	[0.989, 1.002]
FU2 -> FU3 (n = 4657)				
Intercept	-1.070	<0.001	---	---
Well Status	0.453	<0.001	1.573	[1.300, 1.904]
Arsenic Concentration (µg/L)	-0.000	0.433	1.000	[0.998, 1.001]
Age (y)	0.048	0.510	1.049	[0.909, 1.212]
Sex	-0.002	0.484	0.998	[0.991, 1.005]
FU3 -> FU4 (n = 2957)				
Intercept	-1.738	<0.001	---	---
Well Status	0.563	<0.001	1.755	[1.289, 2.399]
Arsenic Concentration (µg/L)	-0.001	0.109	0.999	[0.997, 1.000]
Age (y)	-0.021	0.862	0.980	[0.776, 1.237]
Sex	-0.006	0.319	0.994	[0.983, 1.006]
FU4 -> FU5 (n = 2556)				
Intercept	-3.038	<0.001	---	---
Well Status	-0.122	0.679	0.885	[0.496, 1.578]
Arsenic Concentration (µg/L)	-0.001	0.520	0.999	[0.996, 1.002]
Age (y)	-0.090	0.646	0.914	[0.623, 1.341]
Sex	-0.007	0.450	1.001	[0.988, 1.027]

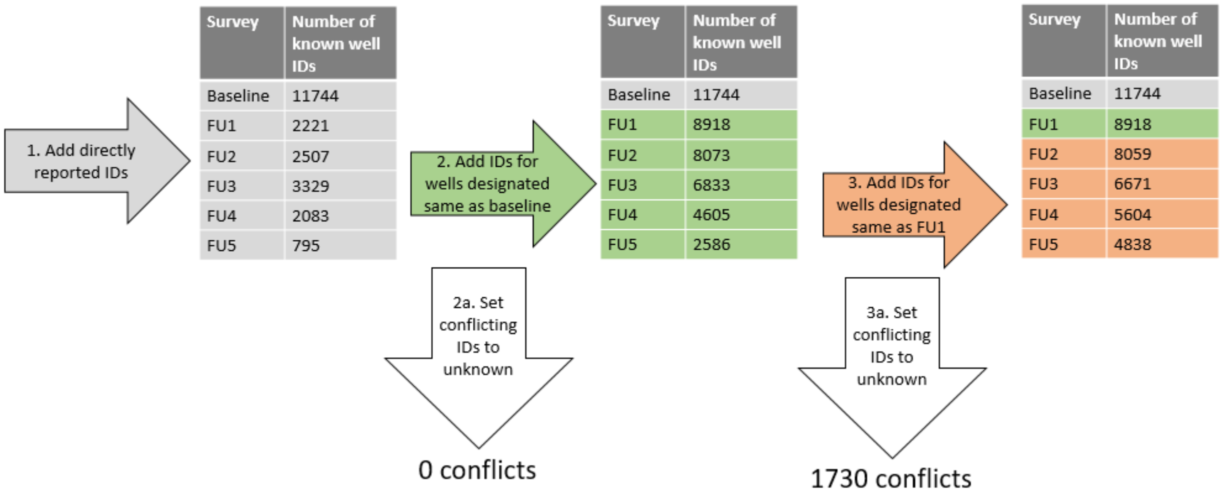

Figure S1. Well IDs were directly recorded by field staff during participant interviews for 11744 participants at baseline and for 2221, 2507, 3329, 2083, and 795 participants (generally those who had switched from their baseline wells to other identifiable wells) at follow-ups one through five respectively. Additionally, many participants were designated as drinking from their baseline wells or the wells they used at the first follow-up. Using these designators in addition to the directly recorded well IDs, well IDs could be determined for a total of 8919, 8059, 6671, 5604, and 4838 participants at follow-ups one through five respectively.

In 1730 cases, a participant was recorded as drinking from the same well that they used during the first follow-up, but had a directly recorded well ID that was different from their well ID at first follow-up. When such a conflict occurred, the participant's well ID was marked as unknown. Because of the existence of these conflicts, we also completed the analyses in the paper without conducting the third step in the flow chart, and results were generally similar within error to those presented in the paper.

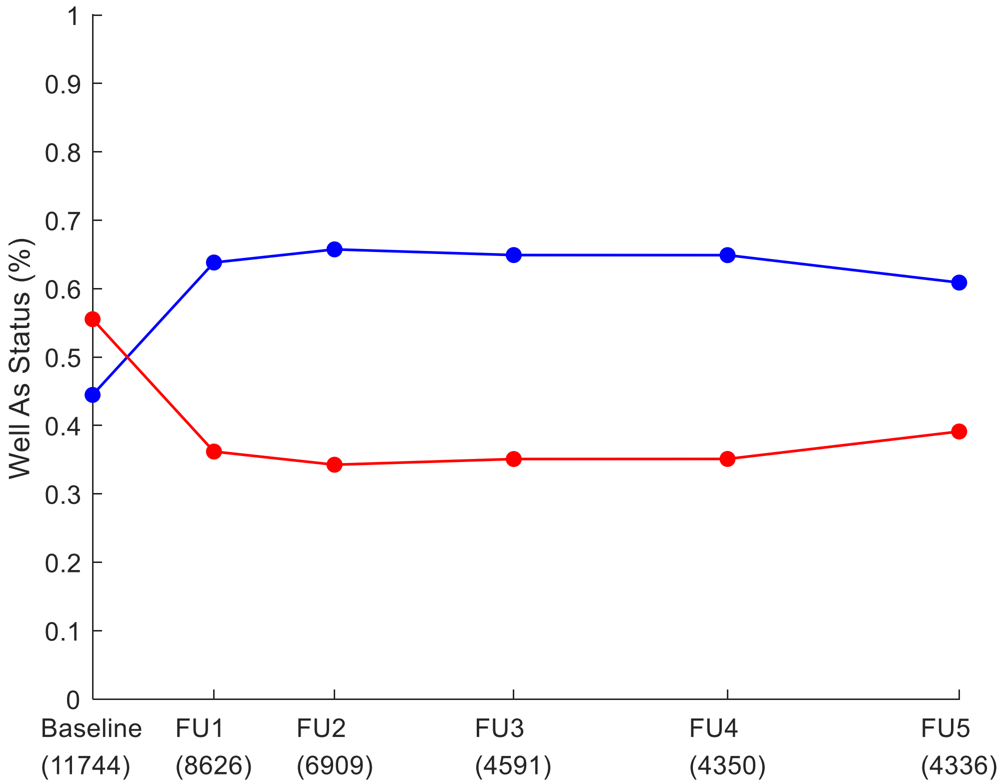

Figure S2. Of the people drinking from wells with known arsenic concentrations, proportion of people drinking from As-safe and As-unsafe wells at baseline and each follow-up survey.

Chapter 3: Field study of rice yield diminished by soil arsenic in Bangladesh

Huhmann BL, Harvey CF, Uddin A, Choudhury I, Ahmed KM, Duxbury JM, et al. 2017. Field Study of Rice Yield Diminished by Soil Arsenic in Bangladesh. Environ Sci Technol acs.est.7b01487; doi:10.1021/acs.est.7b01487.

Abstract

Rice was traditionally grown only during the summer (aman) monsoon in Bangladesh but more than half is now grown during the dry winter (boro) season and requires irrigation. A previous field study conducted in a small area irrigated by a single high-arsenic well has shown that the accumulation of arsenic (As) in soil from irrigating with high-As groundwater can reduce rice yield. We investigated the effect of soil As on rice yield under a range of field conditions by exchanging the top 15 cm of soil between 13 high-As and 13 low-As plots managed by 16 different farmers, and we explore the implications for mitigation. Soil As and rice yields were measured for soil replacement plots where the soil was exchanged and adjacent control plots where the soil was not exchanged. Differences in yield (ranging from +2 to -2 t/ha) were negatively correlated to the differences in soil As (ranging from -9 to +19 mg/kg) between adjacent replacement and control plots during two boro seasons. The relationship between soil As and yield suggests a boro rice yield loss over the entire country of 1.4-4.9 million tons annually, or 7-26% of the annual boro harvest, due to the accumulation of As in soil over the past 25 years.

Introduction

Much of the groundwater in Bangladesh is contaminated with high levels of arsenic (As) that harm human health when this water is used for drinking and, to a lesser extent, when groundwater is used for irrigating rice and As is taken up by the rice grain (Brammer & Ravenscroft, 2009; Duxbury & Panaullah, 2007; Heikens, Panaullah, & Meharg, 2007; Polya, Berg, Gault, Takahashi, & Mondal, 2008). Winter season (boro) rice, grown in standing water maintained by groundwater irrigation, is the dominant crop in Bangladesh in both production (BBS, n.d.) and caloric consumption (Food and Agriculture Organization of the United Nations, 2015). The groundwater used for irrigation often contains high concentrations of As that accumulates in soil over time and can be taken up by rice plants, elevating As concentrations in rice straw, husk, and grain (M. Abedin, Feldmann, & Meharg, 2002; Adomako et al., 2009; Azizur Rahman, Hasegawa, Mahfuzur Rahman, Mazid Miah, & Tasmin, 2008; M. B. Hossain et al., 2008; Khan et al., 2009; Meharg & Rahman, 2003; Williams et al., 2009). Monsoon season (aman) rice is often grown in the same fields where boro rice is cultivated, and although it is primarily rainfed during the monsoon, it is still exposed to the high concentrations of soil As that build up during boro irrigation (van Geen et al., 2006).

Elevated As concentrations in irrigation water and soil have been found to decrease boro and aman rice yield in greenhouse studies and pot experiments (M. J. Abedin, Cresser, Meharg, Feldmann, & Cotter-Howells, 2002; Delowar et al., 2005; Iqbal, Rahman, Panaullah, Rahman, & Biswas, 2016; Islam, Islam, Jahiruddin, & Islam, 2004; Khan et al., 2009; Montenegro & Mejia, 2001; Rahman, Hasegawa, Rahman, Rahman, & Miah, 2007; Williams et al., 2009). A prior field study in Faridpur, Bangladesh found that boro rice yields were 7-9 t/ha where soil As

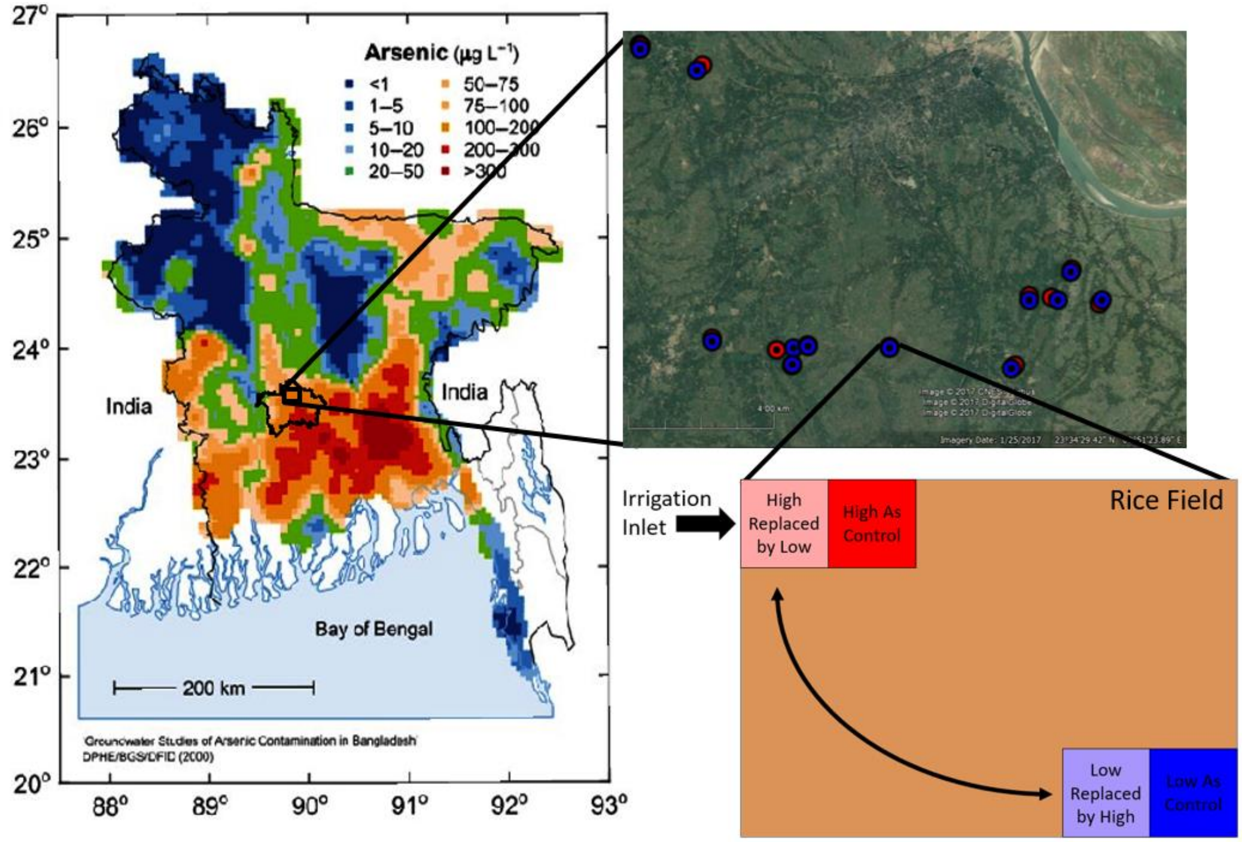

Figure 1. Soil exchange schematic and distribution of the thirteen study sites within a 150 km^2 area in Faridpur, Bangladesh. The top 15 cm of soil was exchanged between a 5×5 m high-arsenic plot near the irrigation inlet and a 5×5 m low-arsenic plot far from the irrigation inlet. Adjacent 5×5 m control plots remained undisturbed and were managed identically to the soil replacement plots. Heat map of As in groundwater is from BGS and DPHE 2001.[36] Map data is from Google, CNES/Airbus, and DigitalGlobe.

concentrations were low (~10 mg/kg), but were much poorer, 2-3 t/ha, where soil As concentrations were high (~70 mg/kg) (Panaullah et al., 2009).

While greenhouse and pot studies have shown the negative effects of As on rice yield, these studies do not provide sufficient information to quantify the magnitude of the yield impact of As under field conditions. Furthermore, the only previous field study on the yield effects of As did not include aman-season rice and was conducted in an 8 ha area managed by a single farmer and irrigated by a single high-As well – and thus under a relatively narrow set of conditions. The goal of our study was to quantify the yield impacts of As under a broader array of field conditions by using a controlled study design. To quantify the yield impact of As we exchanged high- and low-As soils at thirteen field sites distributed throughout a 150 km^2 area in Faridpur district, Bangladesh and compared these soil replacement plots to adjacent control plots. Our study plots were managed by sixteen different farmers, and these farmers chose to cultivate two boro rice varieties and nine aman rice varieties. We hypothesized that replacing high-As soil with low-As soil would improve yield, and that replacing low-As soil with high-As soil would cause a decline in yield. We tested this hypothesis for rice grown during the 2015 and 2016 boro and aman seasons.

Materials and Methods

Experimental Site and Design

The study was conducted in fields irrigated by high-As wells in Faridpur district, Bangladesh (Figure 1). The wells drew water from 40 to 100 m in depth, ranged from 5 to 43 years in age, and had As concentrations of 100 to 400 µg/L as measured by the ITS Econo-Quick field kit and converted based on a prior intercalibration of field kit As concentrations with As concentrations measured by ICP-MS (George et al., 2012) (Table S1). As has been observed at other field sites, soil As tended to decrease away from the irrigation inlet, which is where much of the reduced iron in the irrigation water precipitates to form iron oxides that adsorb or coprecipitate As (Dittmar et al., 2007; M. B. Hossain et al., 2008; Panaullah et al., 2009).

Up to two rice crops are grown at our study sites each year. Boro rice is grown during the dry season, from mid-January through May, and is irrigated with groundwater. During the 2015 and 2016 boro seasons, farmers at our study sites grew two rice varieties, BRRI dhan 28 (BR 28) and BRRI dhan 29 (BR 29). These are also the predominant rice varieties grown across Bangladesh, and were estimated in 2005 to be grown in nearly 60% of the total boro rice cropped area in the country (M. Hossain, Jaim, Alam, & Rahman, 2013). Aman rice is traditionally grown during the monsoon season, from June through mid-November, and is primarily rainfed, with supplemental groundwater irrigation if needed. While boro rice is always transplanted, aman rice may be transplanted or broadcast sown. During the aman season, farmers grow a larger number of rice varieties. At our study sites, farmers grew nine different varieties during the aman 2015 season and five different varieties during the aman 2016 season. Many of these were local varieties, but the dominant variety grown during both aman seasons was BR 39.

In January 2015 before the fields were transplanted with boro rice, we exchanged the top fifteen centimeters of soil between thirteen 5×5 m high-As (near the irrigation inlet) and low-As (far from the irrigation inlet) plots (Figure 1). Each plot where soil was replaced was paired with an adjacent control plot where the soil remained undisturbed and no changes were made. Soil was swapped within a field in four cases, and swapped between nearby fields in nine cases (Figure S1). By pairing each 5×5 m soil exchange plots with an adjacent 5×5 control plot, we implemented a study design that controls for the management of the plots (the plots in each pair are managed by the same farmer, fertilized and irrigated in the same way, and planted with the same rice variety) and for the environmental conditions (air temperature, relative humidity, sunlight, rainfall). We then measured soil As concentrations and rice yields in the soil replacement and control plots during the 2015 and 2016 boro and aman rice seasons.

Soil As Measurements

Total soil As concentrations were measured using an Innov-X Delta Premium field X-ray fluorescence (XRF) spectrometer in the soil mode for a total counting time of 35-150 s. Soil standards 2709 and 2711 from the National Institute of Standards and Technology (NIST) were analyzed at the beginning and end of each day and periodically during longer sample runs. The measured average and standard deviation for standard 2711 of 103 ± 7 ($n = 39$) matched the reference value of 105 ± 8 mg/kg. The measured average and standard deviation for standard 2709 of 16.4 ± 1.9 ($n = 19$) matched the reference value of 17.7 ± 0.8 mg/kg. All soil As concentrations were above the detection limit of the XRF.

At harvest time, twelve 20-cm deep soil cores (diameter of 3 cm) were collected from each plot where rice yield was measured. Three soil cores were collected at a distance of 1 m

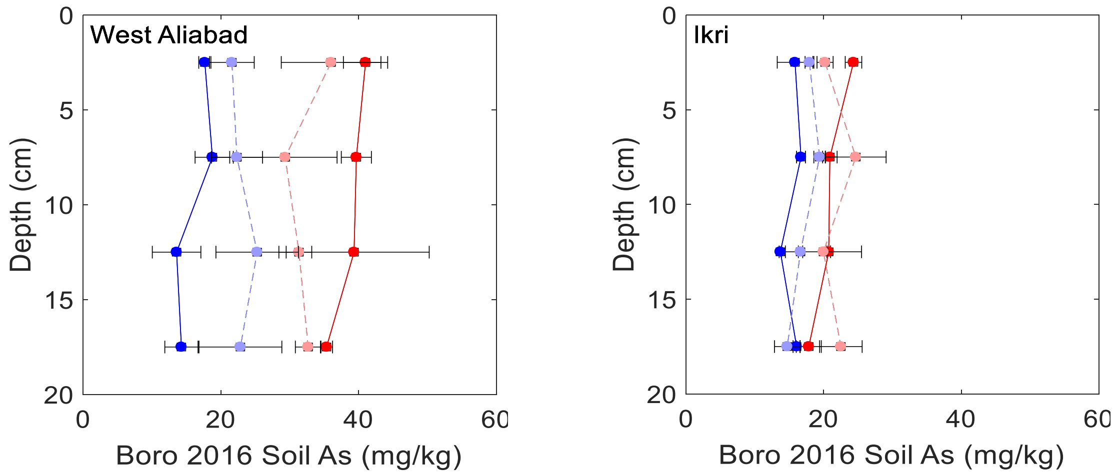

Figure 2. Depth profiles over the top 20 cm from two representative study sites. Arsenic profiles measured over the top 20 cm of soil for the low As control (dark blue), low-replaced-by-high (light blue), high-replaced-by-low (light red), and high As control (dark red) plots during the boro 2016 season for **a.** West Aliabad and **b.** Ikri. These figures represent the average across monthly samples taken four times from each plot during the growing season. Error bars represent standard deviation divided by the square root of the number of samples.

inward from each of the four sides of a 5 × 5 m plot and combined, for a total of four composited soil samples from each plot. The average and standard error of these four samples were used to represent the soil arsenic concentration in each plot. The soil samples were dried in an oven at 40ºC and homogenized by mortar and pestle before As analysis with XRF, to ensure a moisture content and sample morphology similar to the NIST standards used (Kalnicky & Singhvi, 2001).

Single 20-cm deep soil cores (diameter of 3 cm) were also collected monthly from each plot from anywhere in the 5×5 m area throughout each growing season. These soil samples were dried in an oven at 40ºC and homogenized by mortar and pestle in 5 cm increments from 0 to 20 cm, and those from the boro 2016 and aman 2016 growing seasons were analyzed by XRF to provide depth profiles of soil As, two examples of which are shown in Figure 2.

Measurements of Additional Soil Element Concentrations

Concentrations of K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Rb, Sr, and Zr were also measured by XRF during the course of the As measurements. The measured averages for the NIST 2709 standard were within 12% of the reported values (Table S3).

Soil Nutrient Measurements

Samples from the single 20 cm cores collected monthly from each plot during the boro 2015 season were also sent to the BRAC soil laboratory in Gazipur, Bangladesh, for measurement of electrical conductivity (measured on a 1:1 mixture of soil and distilled water), N (total Kjeldahl nitrogen), organic carbon (Walkley-Black method), P (modified Olsen method), K (ammonium acetate extraction), S (calcium hydrogen phosphate extraction), and Zn (diethylenetriaminepentaacetic acid extraction). Nutrients were measured similarly during the subsequent boro and aman seasons, except that sets of three 20 cm cores (rather than a single core) were collected monthly from each plot to ensure sufficient soil for nutrient analyses.

Rice Yield Measurements

Rice yields were measured for a 3×3 m area in the center of each 5×5 m plot. The rice was threshed immediately after harvest, its weight and moisture content were recorded, and yield values were adjusted to 14% moisture content. During the boro 2016 season, we obtained an estimate of the error on yield by dividing each 3×3 m plot along the diagonal and making a separate measurement of the yield for each half of the 3×3 plot.

Due to miscommunication with the farmers and other farmer decisions, yield measurements were obtained for only a subset of the 26 plots at the end of each growing season. We obtained yield measurements for 13 pairs of soil replacement and control plots during the boro 2015 season, 24 pairs during the aman 2015 season, 17 pairs during the boro 2016 season, and 19 pairs during the aman 2016 season (Table S4).

Results

Effect of the Soil Exchange on Soil As and Soil Nutrients

The soil exchanges had a large effect on soil As concentrations at some study sites (Figure 2a) and a minimal effect at others (Figure 2b). In some of the cases where the effect was small, the soil exchange was conducted after some initial irrigation and because the soil was very wet it was hard to ensure that precisely the top 15 cm of soil were exchanged. For the boro 2015 growing season, we observed that the soil replacement on average decreased soil As for the high-As plots (-4.0 ± 3.5 mg/kg) and increased soil As for the low-As plots (+12 ± 3 mg/kg) compared to the adjacent control plots (Figure 3a). This represents an average 8% decrease in soil As concentration for the high-replaced-by-low plots and a 65% increase in As concentration for the low-replaced-by-high plots compared to their respective control plots. We did not observe a significant difference between replaced and control plots for OC, N, P, K, S, Zn, or EC during the boro 2015 growing season or thereafter (Figure S2, Table S5).

The effect of the soil replacement on soil As remained significant for plots observed during the aman 2015 growing season, with a -4 ± 3 mg/kg (11%) decrease observed in the high-As plots and a 4 ± 1 mg/kg (20%) increase observed in the low-As plots. However, the difference in soil As between high-As and low-As pairs of plots was no longer detectable during the boro and aman 2016 seasons (Figure 3a).

Effect of the Soil Exchange on Rice Yield

For the boro 2015 growing season, the soil replacement increased yield for the high-As plots (+0.8 ± 0.4 t/ha) and decreased yield for the low-As plots (-0.47 ± 0.45 t/ha) compared to the adjacent control plots (Figure 3b). This represents a 16% increase in yield for the high-replaced-by-low plots and a 6.6% decrease in yield for the low-replaced-by-high plots as compared with their respective control plots. The average yield differences between pairs of high-As soil replacement and control plots and pairs of low-As soil replacement and control plots significantly differed from each other at the $p = 0.05$ level.

Unlike with soil As, the effect of the soil replacement on yield remained significant for plots observed during both the aman 2015 and boro 2016 seasons, with the high-replaced-by-low plots continuing to show an increase in yield and the low-replaced-by-high plots a decrease in yield compared to their control plots. During the aman 2015 season, the replacement of high-As

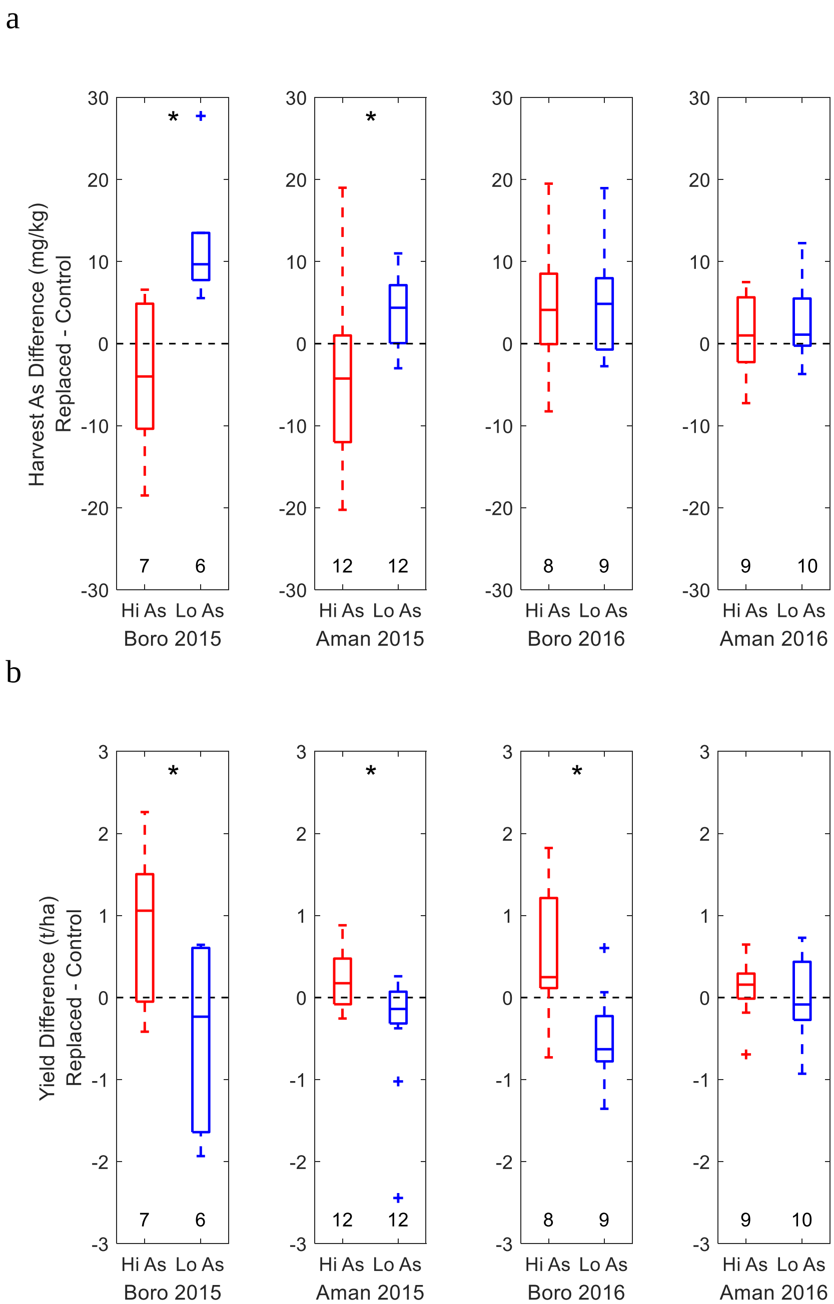

Figure 3. Soil As and yield differences between replacement and control plots. a. Differences in soil As between the replaced and adjacent control plots over the top 20 cm as measured by XRF on cores collected at harvest. **b.** Differences in rice yield between the replaced and adjacent control plots. Data are shown for all plots where yield was measured in each growing season, and the numbers below each box indicate the number of pairs of plots that box represents. The tops and bottoms of each box are the 25th and 75th percentiles. The line in the middle of the box shows the sample median. Outliers are values that are more than 1.5 times the interquartile range beyond the edge of the box. Asterisks denote a significant difference in medians at p = 0.05 according to an unequal variance t test.

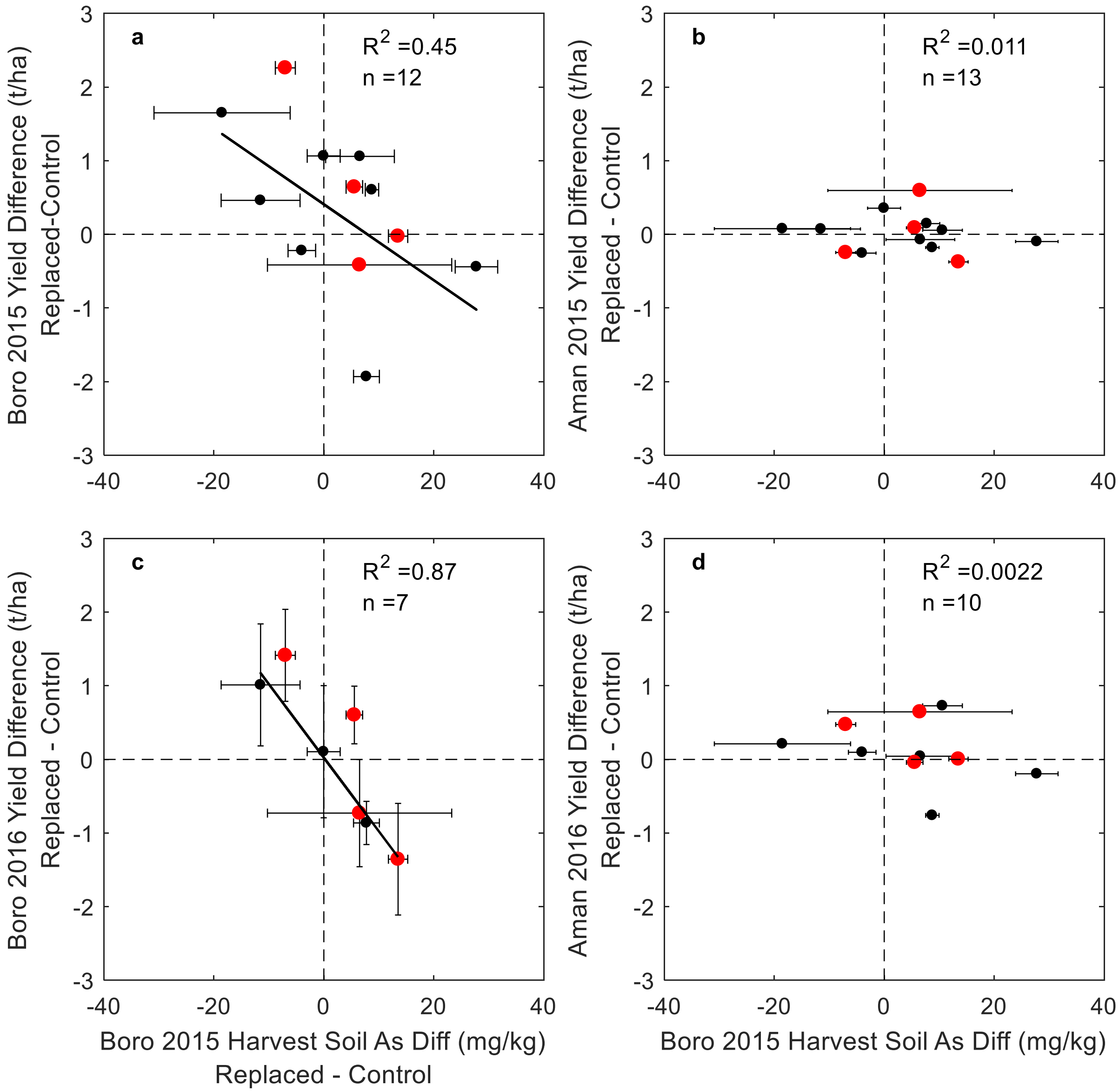

Figure 4. Rice yield difference as a function of soil As difference between replacement and control plots. a. Boro 2015 yield difference correlates with boro 2015 soil As difference (y = -0.06±0.02x + 0.3±0.2, R^2 = 0.45, p-value = 0.011, n = 13). **b.** Aman 2015 yield difference does not correlate with boro 2015 soil As difference (R^2 = 0.011, p-value = 0.73, n = 13). **c.** Boro 2016 yield difference correlates with boro 2015 soil As difference (y = -0.10±0.02x + 0.02±0.14, R^2 = 0.87, p-value = 0.002, n = 7). **d.** Aman 2016 yield difference does not correlate with boro 2015 soil As difference (R^2 = 0.002, p-value = 0.90, n = 10). Data are shown for all study plots for which yield was measured that season and for which soil arsenic was measured in boro 2015. Red symbols represent the four pairs of plots where soil As and yield were measured in all four seasons. Soil As was measured by XRF on the cores collected at rice harvest. Slopes and intercepts are listed with 95% confidence intervals. Error bars represent standard deviation divided by the square root of the number of samples, and regressions are weighted by the error in soil As.

soil with low-As soil achieved on average a yield increase of 8% (yield difference of 0.2 ± 0.1 t/ha and average high-As control plot rice yield of 2.59 t/ha). During the boro 2016 season, the replacement of high-As soil with low-As soil achieved a yield increase of 17% (yield difference of 0.5 ± 0.3 t/ha and average high-As control plot yield of 3 t/ha), which is similar to the 16% increase observed in boro 2015. We did not observe a significant difference in yield between high-As and low-As pairs of plots during the aman 2016 season (Figure 3b).

Yield as a function of soil As

There is no direct correlation between yield and soil As for our study plots (Figure S3); other factors that affect yield evidently conceal the effect of soil As. Our experimental design, where each soil replacement plot is paired with an adjacent unaltered control plot, allows us to control for many of these other factors. Computing the difference in soil As and the difference in rice yield between each soil replacement and adjacent control plot holds constant fertilizer and pesticide use, farmer care (e.g. weeding), transplanting and harvesting dates, irrigation water source, rice variety, and other variations in local conditions (e.g. air temperature, relative humidity, sunlight, rainfall).

When difference in yield is plotted as a function of difference in soil As between adjacent plots for the boro 2015 season, there is a negative linear relationship with a slope of -0.06 $(t/ha)(mg/kg)^{-1}$ in which soil As accounts for 45% of the variance in yield (Figure 4a). A similar relationship exists between boro 2016 yield difference and boro 2015 soil As difference (R^2 = 0.87, slope = -0.10) (Figure 4c). During the 2015 boro season the difference in rice yield ranged from +2.3 to -1.9 t/ha (average yield of 6.3 t/ha), and during the boro 2016 season the difference in rice yield ranged from +1.8 to -1.4 t/ha (average yield of 3.8 t/ha). This indicates that exchanging soil caused an increase or decrease by about a third of the average rice yield.

In contrast, there is no correlation of yield differences observed in aman 2015 or aman 2016 with soil As differences observed in boro 2015 (Figure 4d). Additionally, when using soil As difference from any season after boro 2015, there is little-to-no correlation with difference in rice yield for any season (Figure S4). As shown by the four pairs of plots where soil As was measured in all four growing seasons (red symbols in Figure S4), this is at least in part because of the decline in the effect of the soil exchange on soil As after the boro 2015 season.

Yield difference as a multivariable function of As and nutrient concentrations

We used a stepwise linear model with a threshold p-value of 0.05 to test whether a number of other factors were significant when added to the regression. These included the differences in OC, N, P, K, S, Zn, and EC between the replaced and control plots as measured in the BRAC lab. These also included differences in total K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Rb, Sr, and Zr concentrations as measured by XRF. We also tested the significance of rice variety and, during the aman seasons, whether rice was transplanted or broadcast sown. Finally, we included whether the soil in the replacement plot was swapped from a plot in the same field or a plot in a different field as a binary variable (Figure S5). When we tested these factors across all four seasons, occasionally a factor was significant, but none of the factors were statistically significant during more than one season, and thus none of these factors had a reproducible effect on the observed yield differences between the soil replacement plots and the adjacent control plots. Furthermore, including the other significant factors did not change whether the difference in As was significantly related to difference in yield between pairs of plots. None of these additional measured variables were therefore confounding factors that could explain the observed correlation between soil As difference and yield difference.

Discussion

Effect of soil replacement on soil As

Replacing the soil in high-As plots with low-As soil decreased the soil As concentrations, and replacing the soil in low-As plots with high-As soil increased the soil As concentrations, consistent with expectations (Figure 3). Some of the exchanges were more successful than others, however (Figure 2), resulting in an overall modest effect of the exchange on soil arsenic concentrations.

Additionally, the effect of the soil exchange on soil arsenic declined over time. We initially hypothesized that this was due to lateral mixing between the soil replacement plots and their surroundings over the course of the two-year experimental study. However, the arsenic content was identical in soil cores taken 1 m from the outer edge and 1 m from the center of the soil replacement plots at the end of the 2017 boro season (Figure S6). Thus, the replacement plots lacked the soil arsenic gradient from edge to center that would be expected if mixing between the study plots and the surrounding soil had occurred. Other possible explanations for the decline in the effect of the exchange on soil arsenic concentrations over time include vertical mixing (since only the top 15 cm of soil were exchanged) or, for the high-arsenic plots, rapid buildup of arsenic in the soil near the irrigation inlet during boro 2015 and boro 2016 irrigation.

Relationship between soil As difference and yield difference

Yield difference between the replacement and control plots in boro 2015 and boro 2016 had a strong negative linear dependence on soil As difference as measured in boro 2015 (Figure 4). The two rice varieties grown in our study plots during these seasons, BR 28 and BR 29, did not statistically differ in their response to soil As. Panuallah et al. reported slopes of -0.09 and -0.11 t/ha in 2006 and 2007 for BR 29 respectively, which are comparable but slightly higher than the slopes of -0.06 and -0.10 that we observed in 2015 and 2016.

In contrast with boro rice, aman rice yield differences did not correlate strongly with soil As differences (Figure 4). A possible explanation is the larger number of rice varieties that were grown in our study plots during the aman seasons as compared with the boro seasons. Different rice varieties may have different responses to As, and with only a few measurements for each variety, we do not have sufficient information to determine these distinct relationships. Additionally, aman yields are generally lower than boro yields, and our study plots had average aman yields of 2.7 t/ha in 2015 and 2.9 t/ha in 2016, compared to average boro yields of 6.3 t/ha and 3.8 t/ha, respectively. Lower overall yields mean that the same proportional change in yield will be smaller and thus harder to detect. Despite the lack of correlation between aman yield differences and soil As differences, we did observe an increase in aman yields in the high-replaced-by-low plots and a decrease in aman yields in the low-replaced-by-high plots compared to their control plots in first aman season after the soil was exchanged (Figure 3).

Different duration of impact of exchange on soil As and rice yield

We observe a strong relationship between boro 2015 soil As differences and yield differences in boro 2015 and boro 2016. Surprisingly, the effect of the soil exchange on yield persisted into 2016 even after soil As concentrations had increased in the high-arsenic plots and the effect of the exchange on soil As was no longer observable (Figure 3).

This suggests that some factor other than bulk soil As may mediate the effect of the soil exchange on arsenic available to the rice plants and thus on rice yield. One possible factor is the interplay between soil As and iron oxides, since the presence of As can affect iron oxide formation and transformation (Auffan et al., 2008; Das, Hendry, & Essilfie-Dughan, 2011; Masue-Slowey, Loeppert, & Fendorf, 2011). In general, lowering the concentration of As in a system is expected to result in faster transformation (from less crystalline to more crystalline) and recrystallization of the iron oxides that form as iron from irrigation water precipitates in our study plots. These transformation and recrystallization processes could allow for As uptake and sequestration by the iron minerals, similar to what has been observed for other elements (Latta, Gorski, & Scherer, 2012). As arsenic is added to the soil again over time, the rate of iron oxide recrystallization could slow, potentially decreasing the uptake and sequestration of newly added As, but also inhibiting the release of As that has already been incorporated. Overall, this could result in a lag between the increase in bulk soil As and the increase in plant-available As, thus resulting in a lag in the effect of the increase in bulk soil As on rice yield. Our study was not designed to examine the relationship between iron oxides and As in these systems, but our results suggest that tracking porewater arsenic and investigating the relationship between soil As and iron oxides may be valuable avenues of future research.

Countrywide impact of As on rice yield

The rice-growing regions of Bangladesh are all composed of soils derived from relatively recent sediments delivered by rivers, and the variability in these sediments occurs on the scale of hundreds of meters (Weinman et al., 2008). Our sites span tens of kilometers and contain soils of a range of grain sizes, and yet we have observed a consistent relationship between yield difference and soil arsenic difference across these sites, suggesting that this relationship is generalizable across a variety soil types. Since BR 28 and BR 29 make up more than half of the total boro rice cropped area in Bangladesh (M. Hossain et al., 2013), we can use the relationship we observe between soil As and yield in our study to estimate the overall boro rice yield loss in Bangladesh due to the buildup of irrigation water As. Wells shallower than 100 m in depth have an average As content of 61 µg/L (BGS & DPHE, 2001). Assuming that paddy soil has a bulk density of 1 kg/L, that boro rice has been irrigated with 1 m of water annually for 25 years, and that all As is retained in the top 15 cm of soil, an estimated average of 10.2 mg/kg of As has been added to paddy soil in Bangladesh since the Green Revolution when boro rice irrigation became widespread.

As an upper bound estimate, we assume no As loss from the soil, since As concentrations in rice plants are low enough to result in negligible removal with the rice harvest (Williams et al., 2009). Furthermore, while measurements at a site flooded to 4.5 m during the monsoon season suggest that it may lose 13-46% of the As deposited each year (Dittmar et al., 2010; Roberts et al., 2010), only 9% of land in Bangladesh is flooded to more than 1.8 m (Dittmar et al., 2010), and shallowly flooded areas appear to retain their As (Panaullah et al., 2009). The 10.2 mg/kg increase in total soil As corresponds to a change in yield of -0.58 t/ha using our boro 2016 slope of -0.1 $(t/ha)(mg/kg)^{-1}$. Since the observed relationship between soil As and yield is linear, this estimate of the impact of soil As on yield holds true regardless of how the total mass of soil As is distributed across the rice-growing areas. Boro rice was grown on 4.8×10^6 ha in 2012-2013[2] and the corresponding loss attributable to the build-up of As is 4.9×10^6 tons, or 26% of total boro yield. As a lower bound estimate, we assume that only 50% rather than all of the soil As is retained and use our lower observed slope, from boro 2015, of -0.057 (t/ha)(mg/kg)$^{-1}$ resulting in a loss of 1.4×10^6 tons or 7.4% of total boro yield.

Mitigating the impact of As on rice agriculture

Our study conducted in multiple fields across a 150 km^2 area shows that soil As negatively impacts boro rice yield. Given that the buildup of irrigation water As in soils may already have substantially reduced rice yield and that the trend is set to continue unless farmers find a source of low-As irrigation water, it will be important to continue to explore options to address this problem.

In our study area, we have occasionally observed farmers removing the topsoil from their rice fields to build up land for houses and other infrastructure. In highly arsenic contaminated fields, targeted removal of the upper 15 cm of soil where the majority of the As buildup occurs could reduce soil As and improve yields. A potential concern with this approach is that the surface soil generally has the highest nutrient concentrations and best soil structure, and thus its removal might cause a substantial enough yield loss to offset yield gains from decreasing the soil As concentration. Further research could be done to better understand these potential impacts of soil removal.

We have also observed farmers in our study area switching away from rice to other crops that require less water and are grown under more oxidizing conditions, and thus are less impacted by As. In places where farmers continue to grow rice, other options for reducing the impacts of As include soil amendments, improved water management or treatment, or growing different rice cultivars that are more resistant to the effects of As (Brammer, 2009; Duxbury & Panaullah, 2007; Spanu, Daga, Orlandoni, & Sanna, 2012).

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Supporting Information

Table S1. Irrigation water arsenic as measured by field kit for all 13 study sites.

Site	**Arsenic concentration measured by field kit (mg/kg)**	**Estimated arsenic concentration based on intercalibration with ICP-MS (mg/kg)**
Aliabad	1000	422±80
Bokile	500	306±25
Dhuldi	200	129±14
Dhuldi Rajapur 1	300	188±20
Dhuldi Rajapur 2	300	188±20
Gerda	300	188±20
Ikri	200	129±14
Middle Tambulkhana	300	188±20
Mirgi	200	129±14
Pearpur	300	188±20
Sachia	300	188±20
Tambulkhana	500	306±25

Table S2. Soil arsenic measured by XRF on cores taken at harvest for all 13 study sites.

	High Arsenic Control (mg/kg)			**Low Arsenic Control (mg/kg)**		
Site	**Mean**	**Standard Error**	**Number of Samples**	**Mean**	**Standard Error**	**Number of Samples**
Aliabad	68.8	1.8	16	19.3	0.5	16
Bokile	28.9	1.8	12	19.8	1.6	8
Dhuldi	55.3	3.2	12	14.7	1.3	12
Dhuldi Rajapur 1	36.1	5.4	12	20.2	0.9	12
Dhuldi Rajapur 2	26.4	1.8	12	10.8	0.7	4
Gerda			0	25.4	1.4	16
Ikri	23.9	1.0	16	16.1	0.8	16
Middle Tambul.	63.1	2.6	16	23.2	1.5	16
Mirgi	19.0	0.8	16	12.8	0.6	12
Pearpur	52.3	4.3	12	22.9	2.0	12
Sachia	52.3	6.1	16	26.5	1.4	12
Tambulkhana	30.0	2.6	12	23.5	2.1	12

Table S3. Observed concentration and standard error compared to reported concentration and reported uncertainty for several elements for NIST Standard Reference Materials 2709 and 2711. Elements without a reported uncertainty are noncertified values reported by NIST.

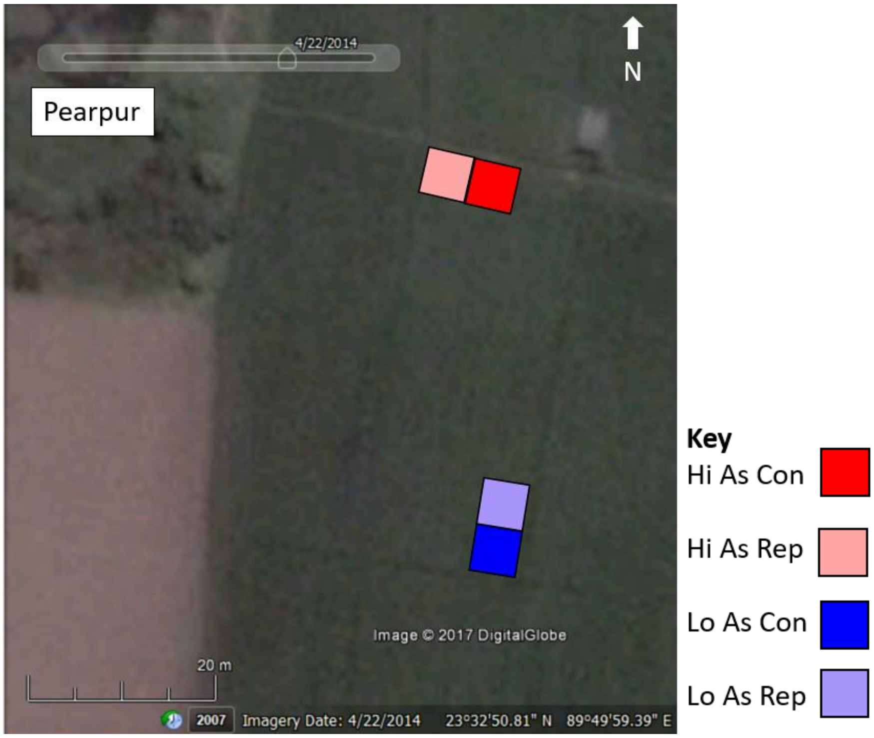
	NIST 2709 Observed		**NIST 2709 Reported**		**NIST 2711 Observed**		**NIST 2711 Reported**		
Element	**Mean (μg/g)**	**SE (μg/g)**	**Conc. (μg/g)**	**Uncertainty (μg/g)**	**Mean (μg/g)**	**SE (μg/g)**	**Conc. (μg/g)**	**Uncertainty (μg/g)**	**Range in field samples (μg/g)**
K	19020	154	20300	600	24196	1092	24500	800	22379-33822
Ca	17733	160	18900	500	27342	1771	28800	800	6901-59718
Ti	3308	39	3420	240	2761	192	3060	230	3720-6404
Cr	124	5	130	4	59	12	47		0-188
Mn	518	5	538	17	580	29	638	28	376-1098
Fe	34459	236	35000	1100	26662	1565	28900	600	30861-71438
Ni	80	2	88	5	23	12	20.6	1.1	24-87
Cu	34	1	34.6	0.7	107	12	114	2	21-70
Zn	102	1	106	3	320	32	350.4	4.8	70-257
Rb	99	1	96		119	4	110		143-223
Sr	230	1	231	2	249	5	245.3	0.7	69-144
Zr	179	1	160		374	29	230		167-354

Table S4. Plots for which yield measurements were made during each growing season.

Site		2015 Boro	2015 Aman	2016 Boro	2016 Aman
Aliabad	Hi As		X	X	X
	Lo As	X	X	X	X
Bokile	Hi As	X	X		X
	Lo As		X		X
Dhuldi	Hi As		X		X
	Lo As	X	X		X
Dhuldi Rajapur 1	Hi As	X	X	X	
	Lo As	X	X	X	
Dhuldi Rajapur 2	Hi As	X	X	X	
	Lo As			X	
Gerda	Hi As				
	Lo As		X	X	X
Ikri	Hi As		X	X	X
	Lo As	X	X	X	X
Middle Tambulkhana	Hi As		X	X	X
	Lo As		X	X	X
Mirgi	Hi As	X	X	X	X
	Lo As		X	X	X
Pearpur	Hi As	X	X		X
	Lo As	X	X		X
Sachia	Hi As	X	X	X	X
	Lo As		X	X	X
Tambulkhana	Hi As	X	X		X
	Lo As	X	X		X
West Aliabad	Hi As		X	X	
	Lo As		X	X	

Table S5. Soil nutrient concentrations are similar between control and soil replacement plots during the boro 2015 growing season.

	Control		**Replaced**		
	Mean	Variance	Mean	Variance	P(T<=t) two-tail
OC	1.02	0.0444	1	0.0372	0.80
N	0.0879	0.00359	0.0866	0.00309	0.87
P	24.4	2.47	24.9	2.32	0.93
K	0.127	0.00581	0.13	0.00529	0.85
S	11.7	0.697	11.9	0.844	0.77
Zn	0.43	0.0215	0.459	0.0239	0.26
EC	0.219	0.0137	0.217	0.0122	0.94

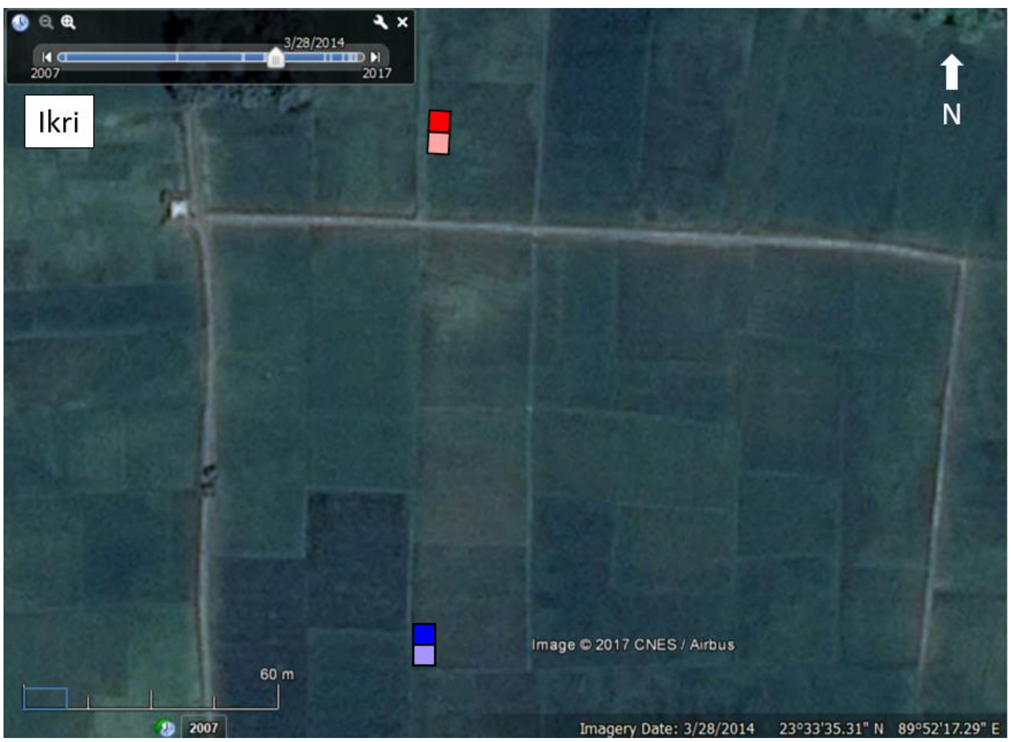

4/22/2014
N
Pearpur
Image © 2017 DigitalGlobe
20 m
2007
Imagery Date: 4/22/2014
23°32'50.81" N 89°49'59.39" E
Key
Hi As Con
Hi As Rep
Lo As Con
Lo As Rep

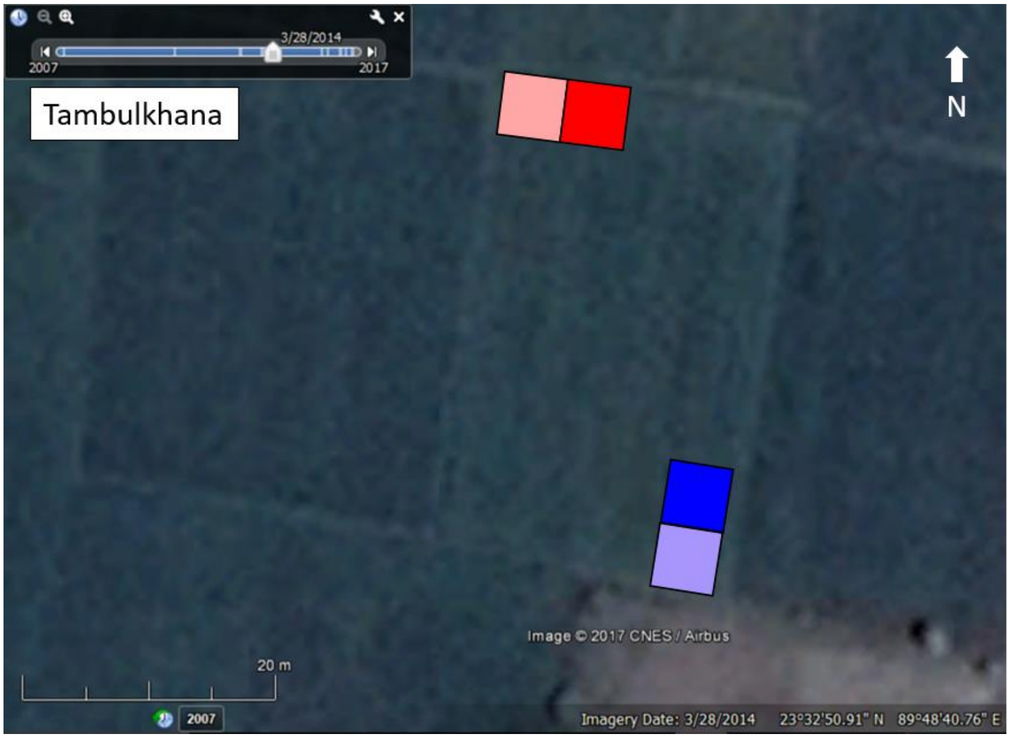

3/28/2014
N
Bokile
Image © 2017 CNES / Airbus
40 m
2007
Imagery Date: 3/28/2014
23°32'33.58" N 89°52'01.54" E


3/28/2014
2007
2017
Aliabad
N
Image © 2017 CNES / Airbus
40 m
2007
Imagery Date: 3/28/2014
23°33'59.35" N 89°52'56.05" E


3/28/2014
2006
2017
West Aliabad
N
Image © 2017 CNES / Airbus
40 m
2006
Imagery Date: 3/28/2014
23°33'32.07" N 89°53'25.44" E

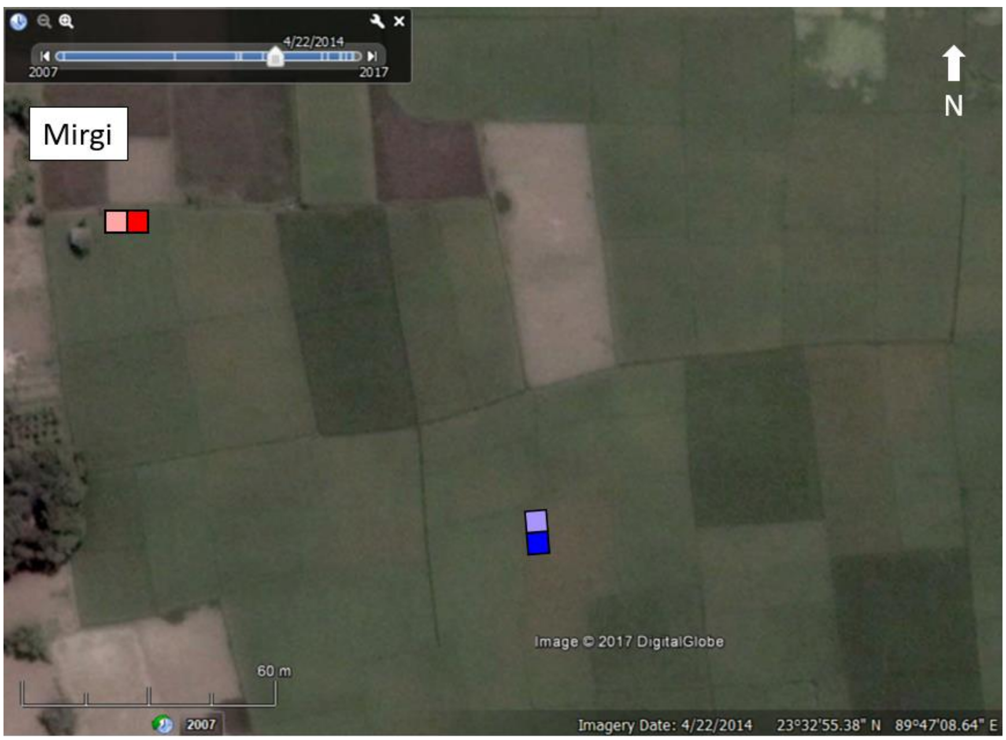

3/28/2014
Gerda
N
Image © 2017 CNES / Airbus
40 m
2007
Imagery Date: 3/28/2014 23°33'34.66" N 89°52'41.75" E

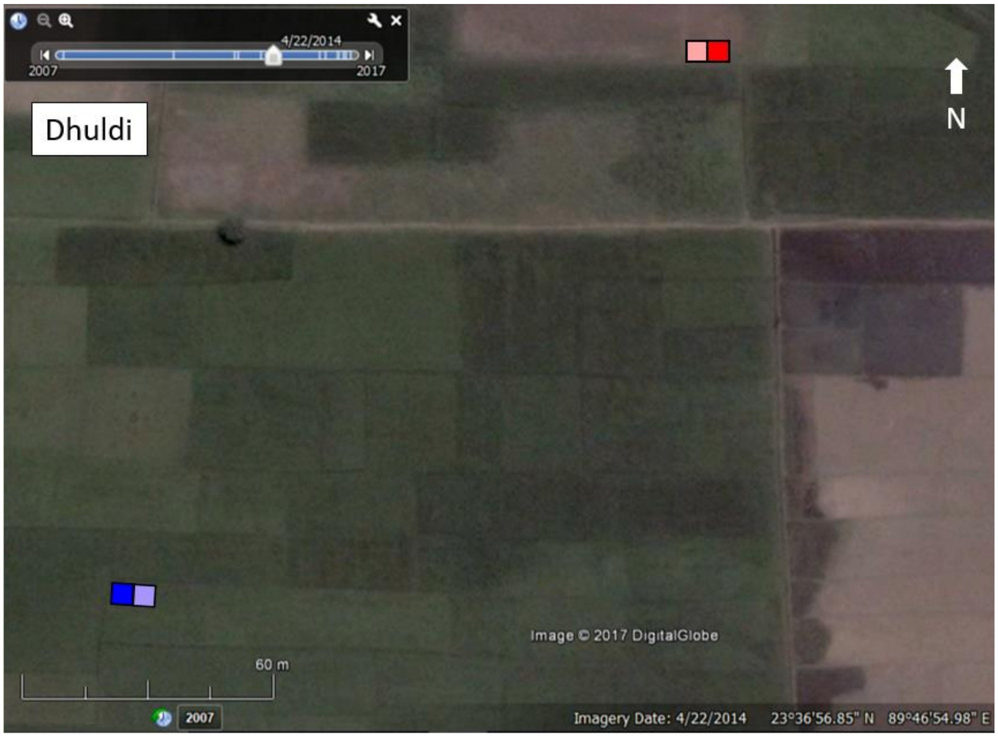

3/28/2014
2007
2017
Ikri
N
Image © 2017 CNES / Airbus
60 m
2007
Imagery Date: 3/28/2014 23°33'35.31" N 89°52'17.29" E


3/28/2014
2007
2017
Tambulkhana
N
Image © 2017 CNES / Airbus
20 m
2007
Imagery Date: 3/28/2014 23°32'50.91" N 89°48'40.76" E


3/28/2014
Sachia
N
Image © 2017 CNES / Airbus
20 m
2007
Imagery Date: 3/28/2014 23°32'33.80" N 89°48'25.71" E


3/28/2014
2007
2017
N
Middle Tambulkhana
Image © 2017 CNES / Airbus
125 m
2007
Imagery Date: 3/28/2014
23°32'48.94" N 89°48'21.24" E


4/22/2014
2007
2017
N
Mirgi
Image © 2017 DigitalGlobe
60 m
2007
Imagery Date: 4/22/2014
23°32'55.38" N 89°47'08.64" E



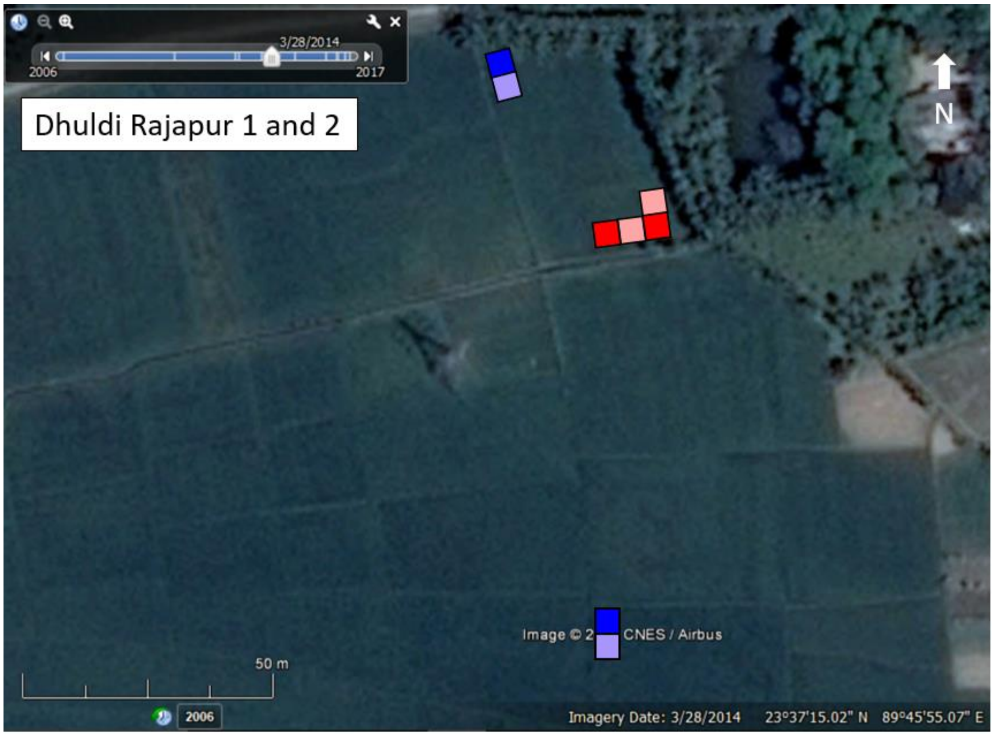
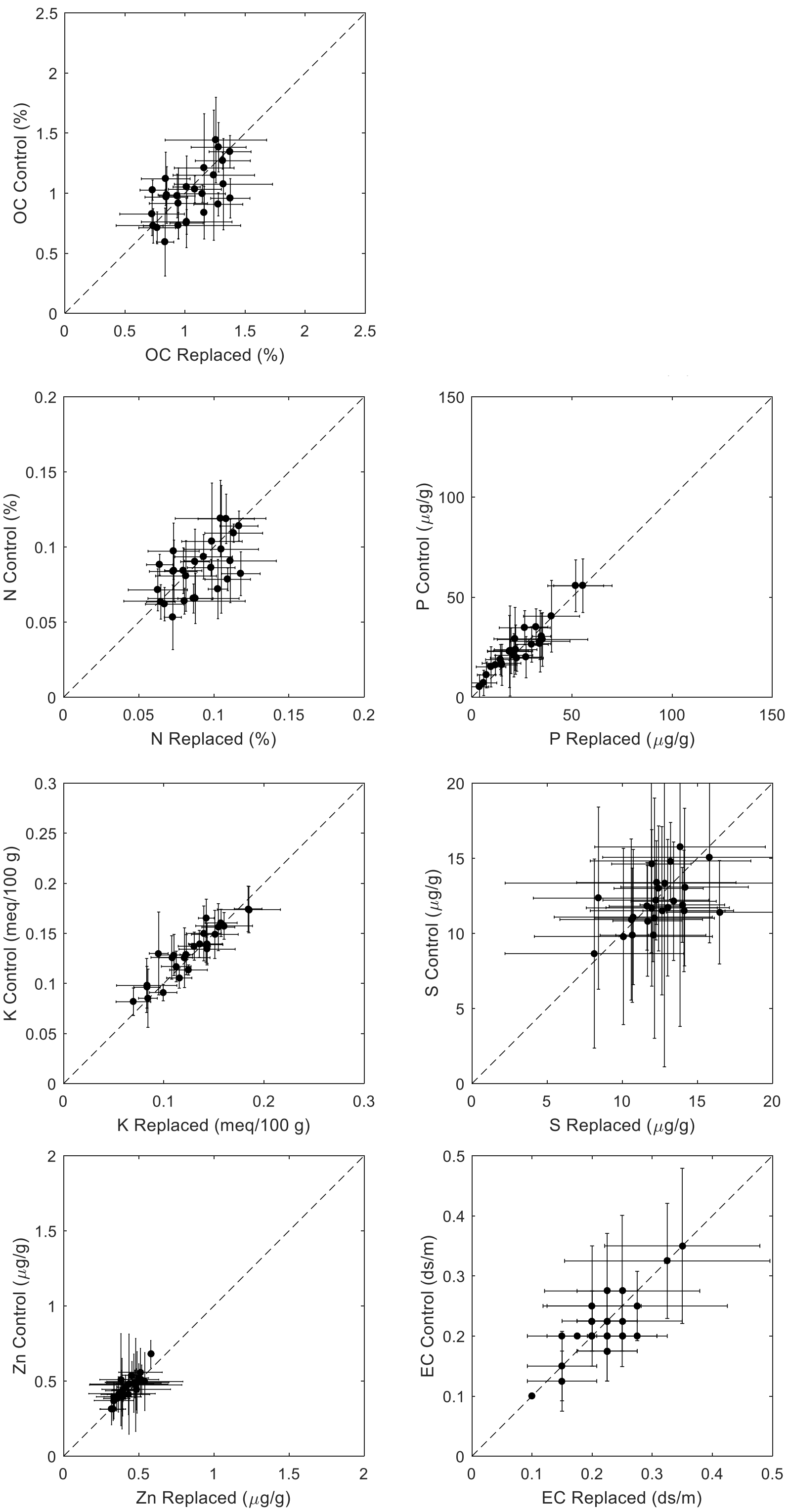
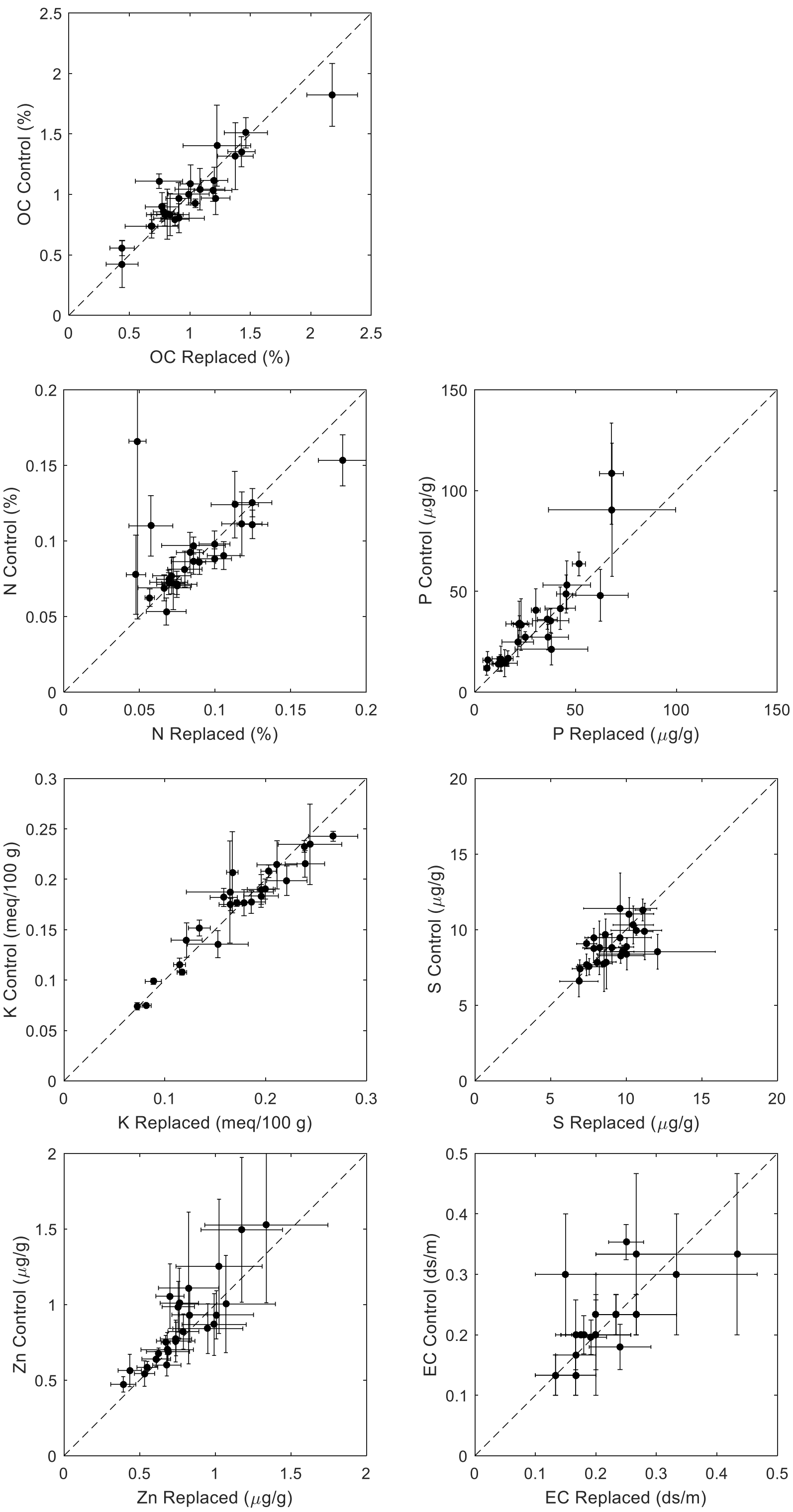


Figure S1. Locations of each of the 13 pairs of plots where soil was exchanged. Map data is from Google, CNES/Airbus, and Digital Globe.

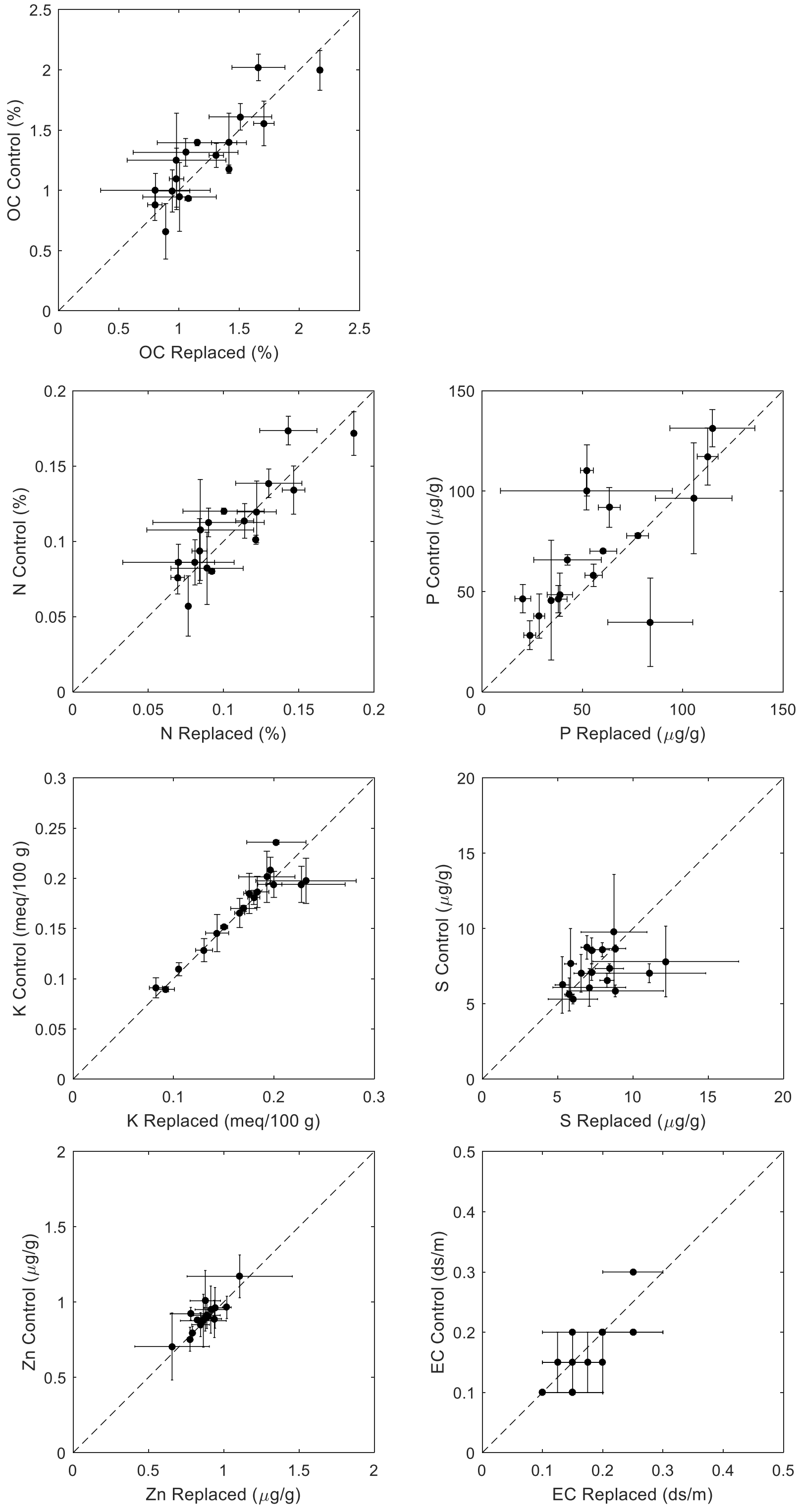
a


OC Control (%)
OC Replaced (%)
N Control (%)
N Replaced (%)
P Control (μg/g)
P Replaced (μg/g)
K Control (meq/100 g)
K Replaced (meq/100 g)
S Control (μg/g)
S Replaced (μg/g)
Zn Control (μg/g)
Zn Replaced (μg/g)
EC Control (ds/m)
EC Replaced (ds/m)

b


OC Control (%)
OC Replaced (%)
N Control (%)
N Replaced (%)
P Control (μg/g)
P Replaced (μg/g)
K Control (meq/100 g)
K Replaced (meq/100 g)
S Control (μg/g)
S Replaced (μg/g)
Zn Control (μg/g)
Zn Replaced (μg/g)
EC Control (ds/m)
EC Replaced (ds/m)

c


OC Control (%)
OC Replaced (%)
N Control (%)
N Replaced (%)
P Control (μg/g)
P Replaced (μg/g)
K Control (meq/100 g)
K Replaced (meq/100 g)
S Control (μg/g)
S Replaced (μg/g)
Zn Control (μg/g)
Zn Replaced (μg/g)
EC Control (ds/m)
EC Replaced (ds/m)

d

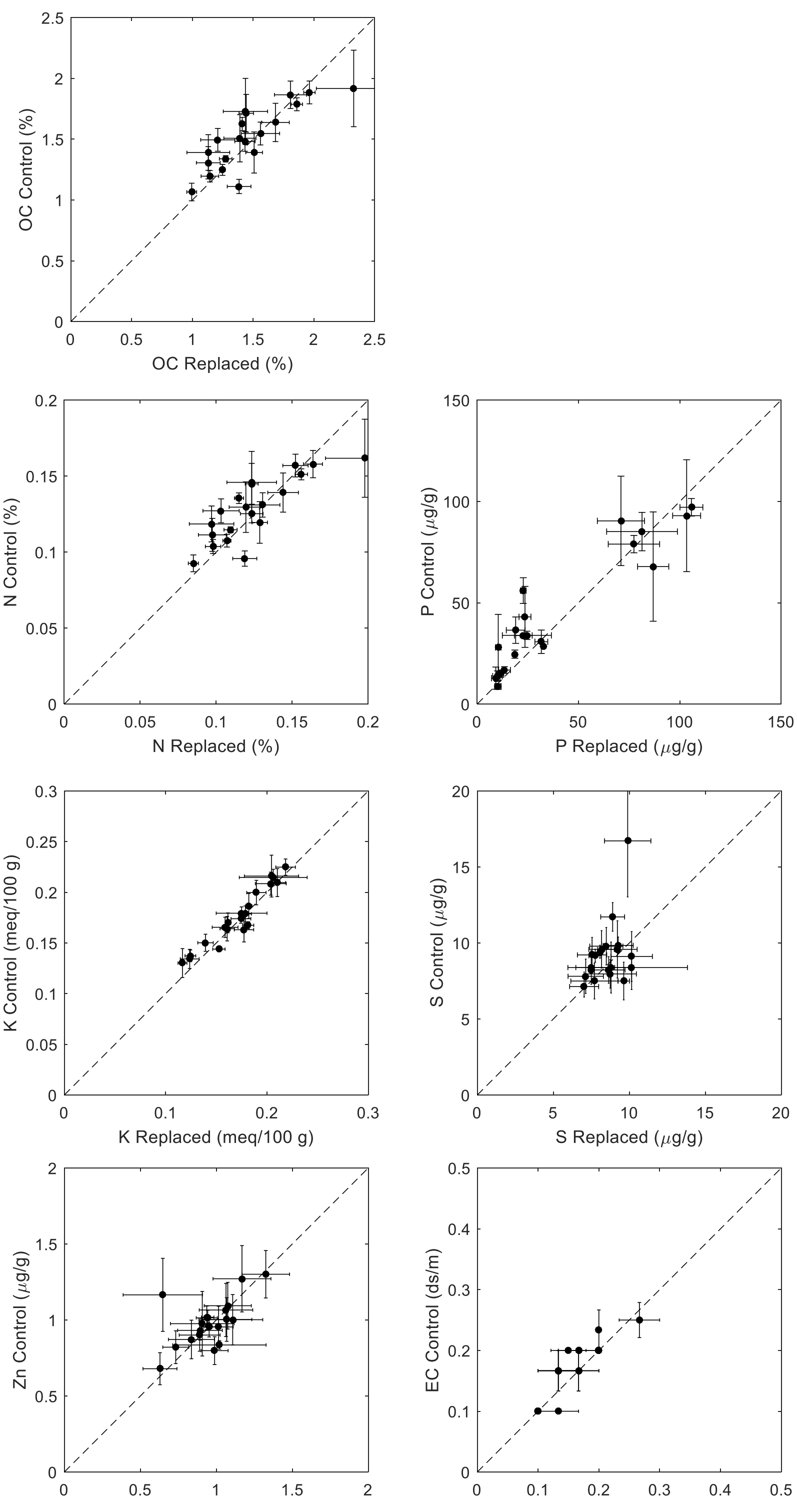
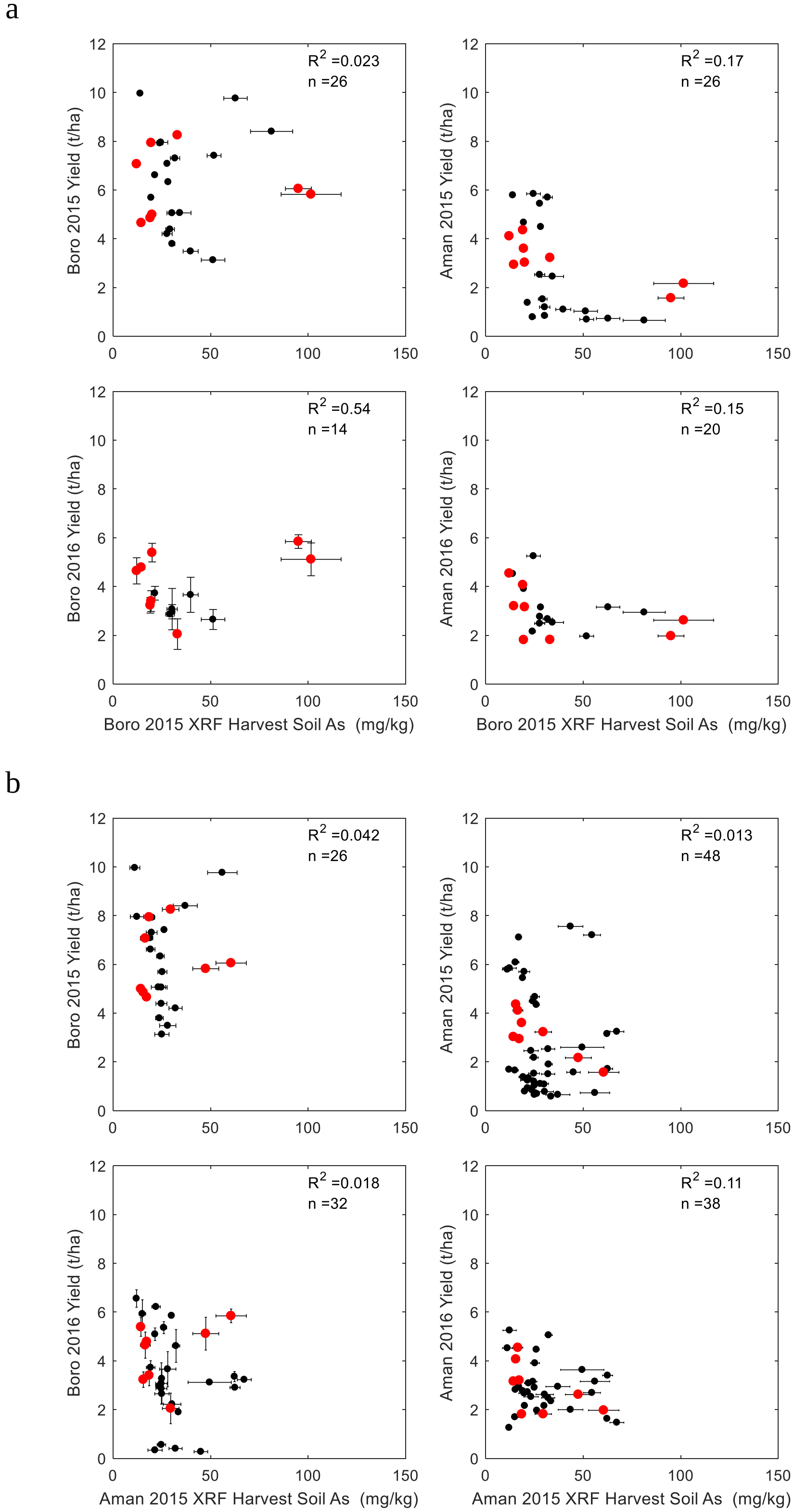
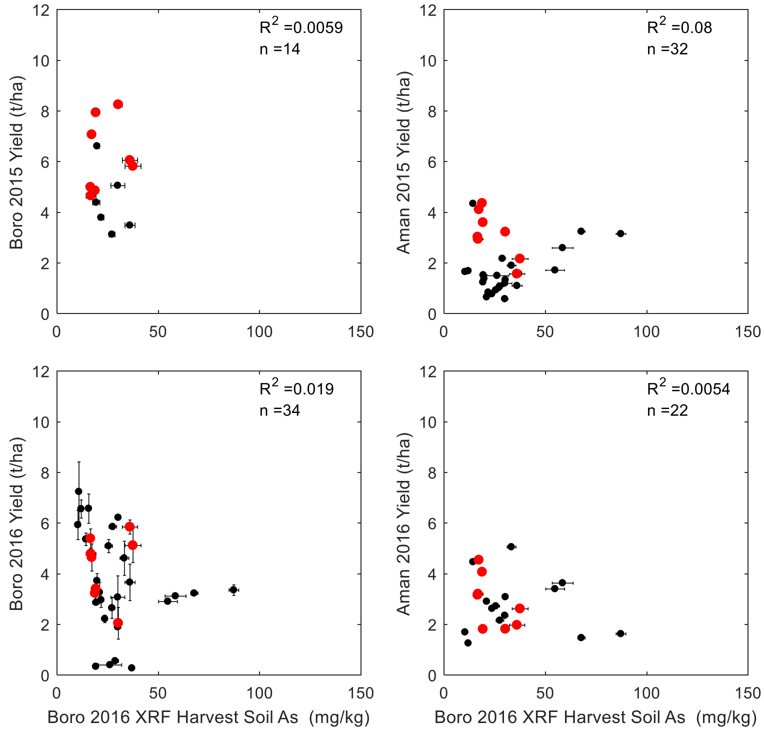


Figure S2. Soil nutrient concentrations in soil replacement and control plots. OC, N, P, K, S, Zn, and EC during the **a.** boro 2015, **b.** aman 2015, **c.** boro 2016, and **d.** aman 2016 growing seasons. Error bars represent standard deviation divided by the square root of the number of samples. Dashed lines are one-to-one lines.


a
Boro 2015 Yield (t/ha)
R² =0.023
n =26
Aman 2015 Yield (t/ha)
R² =0.17
n =26
Boro 2016 Yield (t/ha)
R² =0.54
n =14
Aman 2016 Yield (t/ha)
R² =0.15
n =20
Boro 2015 XRF Harvest Soil As (mg/kg)
Boro 2015 XRF Harvest Soil As (mg/kg)
b
Boro 2015 Yield (t/ha)
R² =0.042
n =26
Aman 2015 Yield (t/ha)
R² =0.013
n =48
Boro 2016 Yield (t/ha)
R² =0.018
n =32
Aman 2016 Yield (t/ha)
R² =0.11
n =38
Aman 2015 XRF Harvest Soil As (mg/kg)
Aman 2015 XRF Harvest Soil As (mg/kg)

c



d

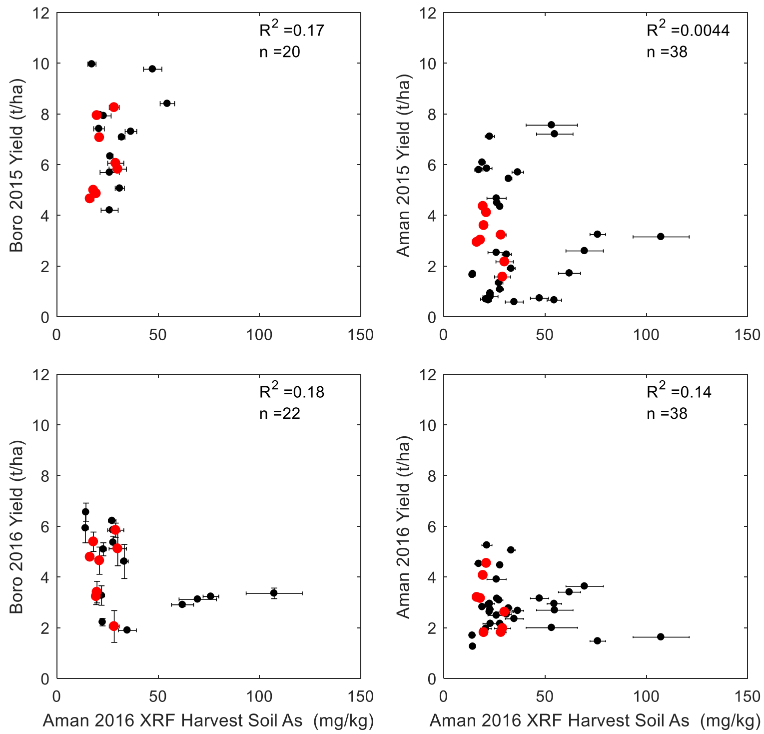
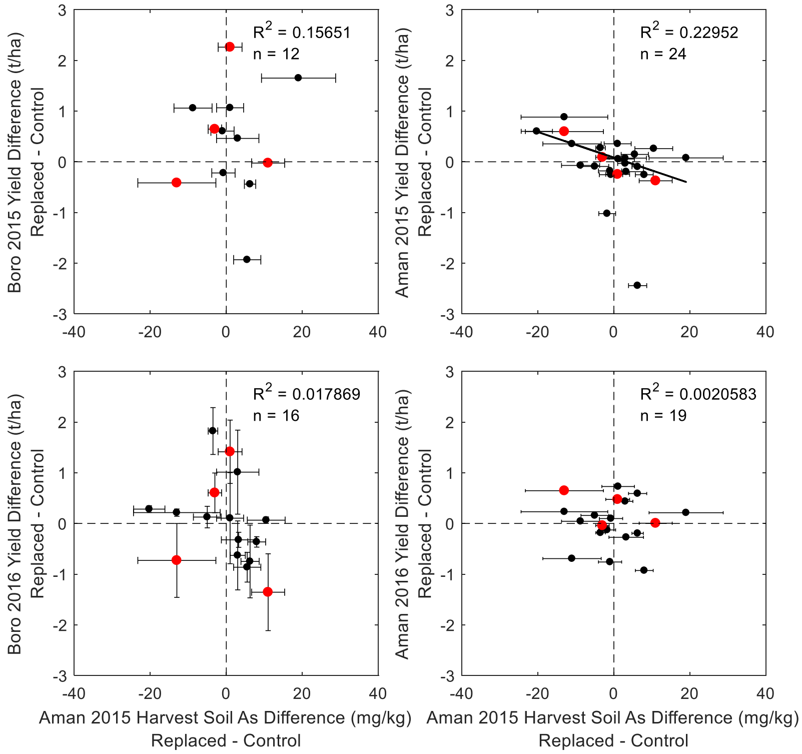


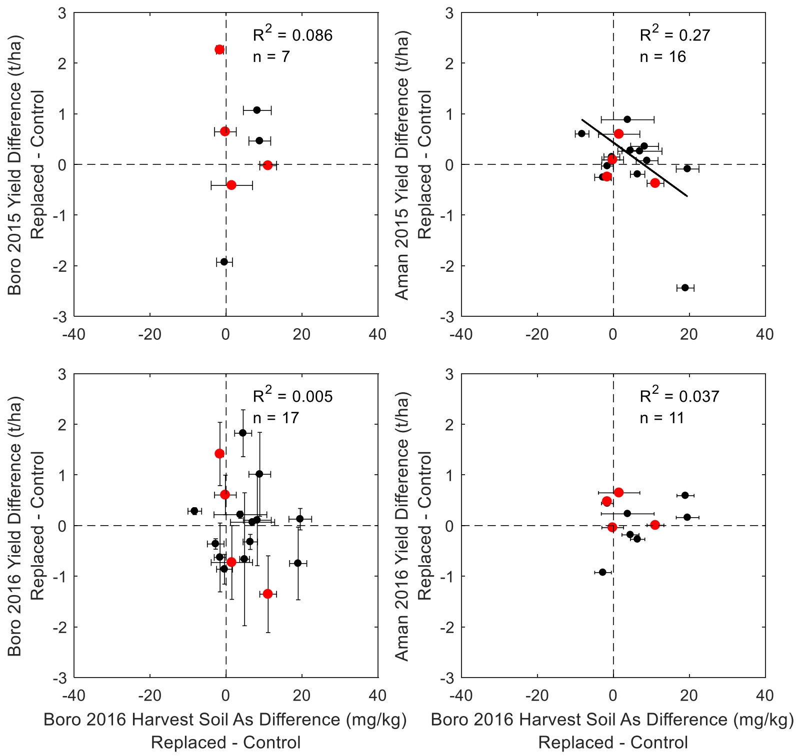
Figure S3. Rice yield as a function of soil arsenic for replacement and control plots. Yield correlates weakly or not at all with soil arsenic measured at **a.** boro 2015, **b.** aman 2015, **c.** boro 2016, and **d.** aman 2016 harvest. Red symbols represent the four pairs of plots where soil arsenic and yield were measured in all four seasons. Slopes and intercepts are listed with 95% confidence intervals. Error bars represent standard deviation divided by the square root of the number of samples, and regressions are weighted by the error in soil arsenic.

a



R^2 = 0.15651
n = 12
R^2 = 0.22952
n = 24
R^2 = 0.017869
n = 16
R^2 = 0.0020583
n = 19
Boro 2015 Yield Difference (t/ha)
Replaced - Control
Aman 2015 Yield Difference (t/ha)
Replaced - Control
Boro 2016 Yield Difference (t/ha)
Replaced - Control
Aman 2016 Yield Difference (t/ha)
Replaced - Control
Aman 2015 Harvest Soil As Difference (mg/kg)
Replaced - Control
Aman 2015 Harvest Soil As Difference (mg/kg)
Replaced - Control

b



R^2 = 0.086
n = 7
R^2 = 0.27
n = 16
R^2 = 0.005
n = 17
R^2 = 0.037
n = 11
Boro 2015 Yield Difference (t/ha)
Replaced - Control
Aman 2015 Yield Difference (t/ha)
Replaced - Control
Boro 2016 Yield Difference (t/ha)
Replaced - Control
Aman 2016 Yield Difference (t/ha)
Replaced - Control
Boro 2016 Harvest Soil As Difference (mg/kg)
Replaced - Control
Boro 2016 Harvest Soil As Difference (mg/kg)
Replaced - Control

c

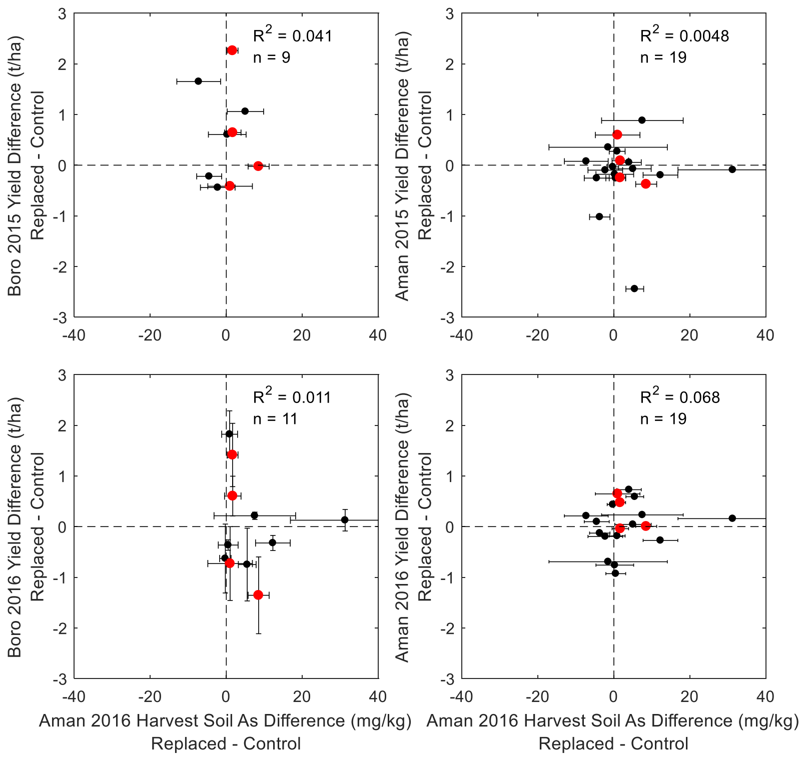


Figure S4. Rice yield difference as a function of soil arsenic difference between replacement and control plots. a. Yield differences correlate weakly or not at all with soil arsenic differences measured in aman 2015. **b.** Yield differences correlate weakly or not at all with soil arsenic differences measured in boro 2016. **c.** Yield differences correlate weakly or not at all with soil arsenic differences measured in aman 2016. Red symbols represent the four pairs of plots where soil arsenic and yield were measured in all four seasons. Soil arsenic was measured by XRF on the cores collected at rice harvest. Slopes and intercepts are listed with 95% confidence intervals. Error bars represent standard deviation divided by the square root of the number of samples, and regressions are weighted by the error in soil arsenic.

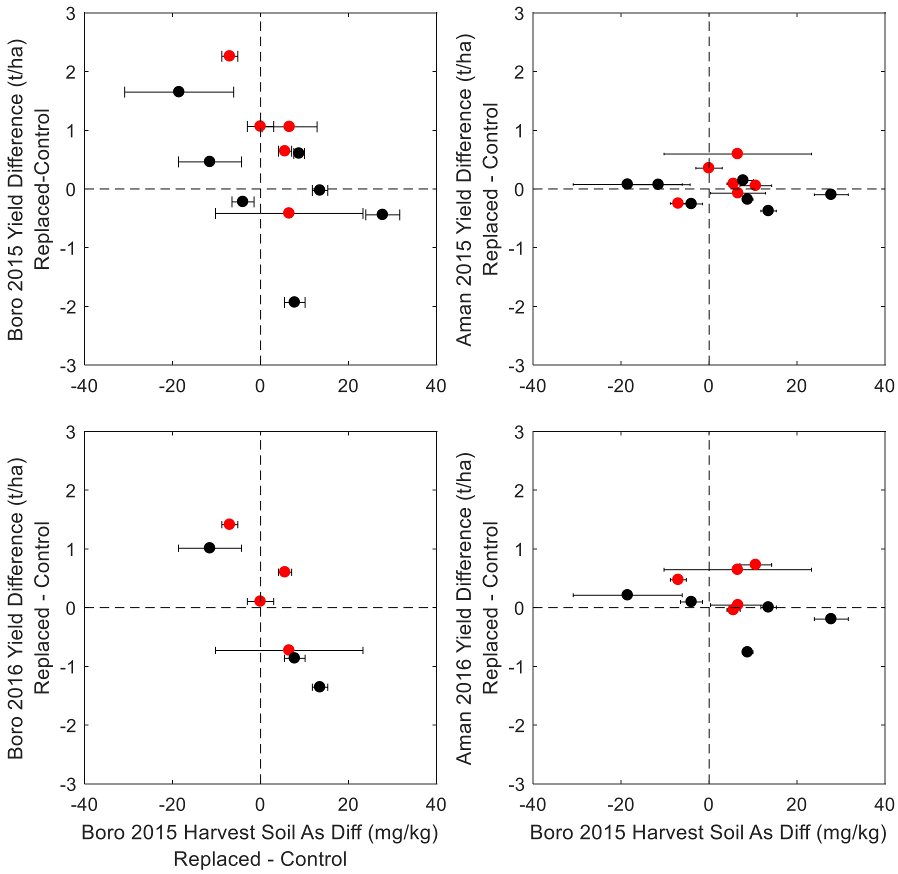


Figure S5. Rice yield difference as a function of boro 2015 soil As difference between replacement and control plots. Data are shown for all study plots for which yield was measured that season and for which soil arsenic was measured in boro 2015. Red symbols represent the pairs of plots subject to between-field swaps and black symbols represent the plots subject to within-field swaps.

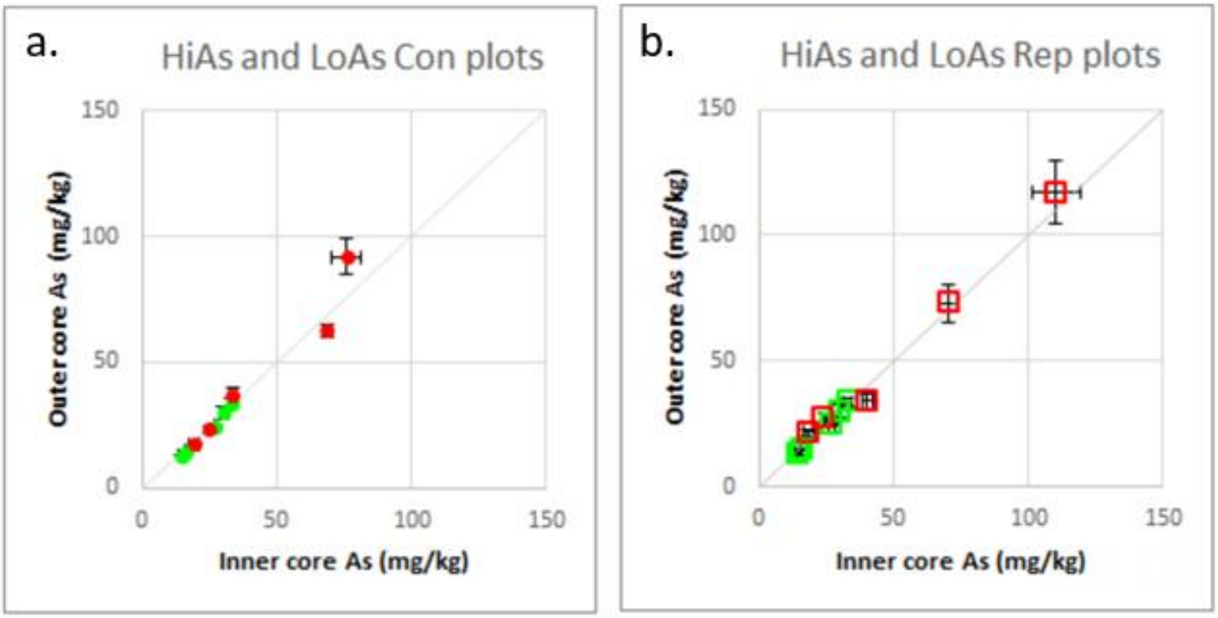


Figure S6. Arsenic concentration of cores taken 1 m from the edges ("outer core As") and 1 m from the center ("inner core As") during the boro 2017 season on (a) control plots where the soil was not altered and (b) replacement plots where the soil was exchanged between high-arsenic and low-arsenic plots. The gray diagonal line represents the one-to-one line. The red and green symbols correspond to the high-arsenic and low-arsenic plots, respectively. Soil arsenic was measured by XRF on sets of 3 composited cores 20 cm in length and 3 cm in diameter. The error bars indicate the standard deviation of 4 measurements obtained for each set of composited cores collected from each side of the square plot and 4 measurements closer to the center. As expected, there is no marked deviation from the one-to-one line for (a) the control plots that were not manipulated. Similarly small deviations for (b) the plots with replaced soil suggest lateral mixing by ploughing did not reduce the difference in soil As between control and intervention plots.

Chapter 4: Evaluation of a field kit for testing arsenic in paddy soil of Bangladesh

Abstract

Rice is the primary crop in Bangladesh, and rice yield is diminished due to the buildup of arsenic (As) in soil from rice irrigation with high-As groundwater. Soil testing could help farmers to target high-As soil for mitigation. We compared a total of 4592 field kit measurements of soil As concentrations on fresh and oven-dried soil samples with measurements of total soil As by X-ray fluorescence. Kit measurements on fresh soil were more consistent across seasons and are less time- and labor-intensive to make than kit measurements on dried soil. We compared the use of a linear regression versus a Bayesian approach for estimating total soil As from kit measurements. Using the linear regression approach, averaging the results of 12 soil kit tests from the same 5 × 5 m plot improved the correlation between kit and total soil As to an R^2 of 0.69 compared to an R^2 of 0.43 when single samples were used. The 12-sample average of kit measurements accurately determined whether soil As was above or below a 30 mg/kg threshold in 86% of cases where soil As was above the threshold and in 79% of cases where soil As was below the threshold. Use of a Bayesian approach to estimate the probability that soil As concentration is above a threshold has similar or slightly better accuracy and allowed for additional flexibility in the tradeoff between false positives and false negatives. The results suggest that the use of multiple field kit measurements made on fresh soil can enable farmers to identify high-As soil for mitigation.

Introduction

Much of the irrigation water in rice-growing regions of Bangladesh is naturally contaminated with high concentrations of arsenic (As). When rice is irrigated with this water, the As can build up in rice field soil (Dittmar et al. 2010; Hossain et al. 2008; Lu et al. 2009; Neumann et al. 2011; Panaullah et al. 2009; Saha and Ali 2007). Arsenic in soil can be taken up into the rice grain, resulting in human exposure to As and associated health risks (Brammer and Ravenscroft 2009; Duxbury and Panaullah 2007; Heikens 2006), although in high-As regions, drinking water from As-contaminated wells is a much more significant exposure route (Polya et al. 2008; van Geen et al. 2006). Soil As also decreases rice yield, and the build up of irrigation water As in soil is estimated to reduce boro rice yield by 7-26% across Bangladesh (Abedin et al. 2002; Huhmann et al. 2017; Panaullah et al. 2009).

Various options have been considered to reduce the uptake of soil As by rice and the impacts of soil As on rice yield. These include providing cleaner irrigation water, growing As-resistant rice varieties, growing rice under conditions that are less conducive to As uptake, and removing the upper layer of As-contaminated soil (Brammer 2009; Heikens 2006; Polizzotto et al. 2015). However, farmers lack a rapid, affordable method to identify high-As soil in order to target these interventions. To provide farmers with the means to identify high-As soil, we adapted a field kit method for measuring water As to measure soil As. We then validated the kit measurements against X-ray florescence (XRF) measurements of soil As.

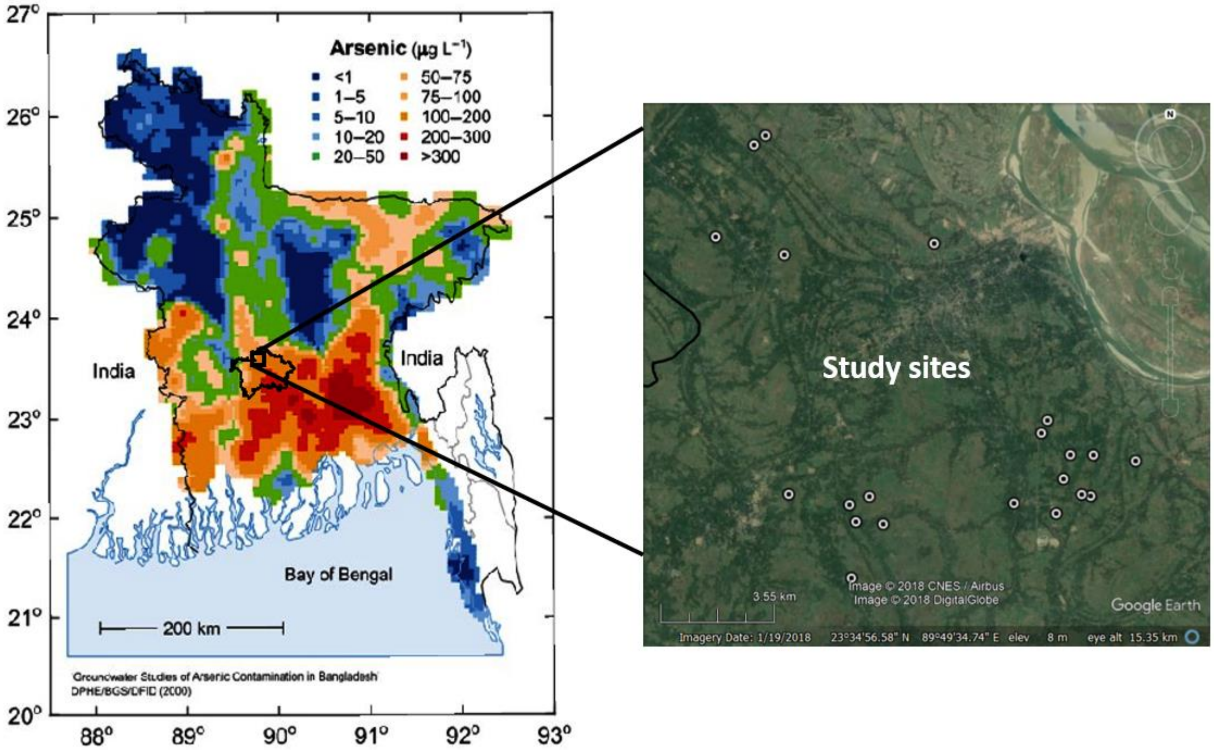


Figure 1. Location of study sites in Faridpur, Bangladesh. Heat map of As in groundwater is from BGS and DPHE 2001.(BGS and DPHE 2001) Map data is from Google, CNES/Airbus, and DigitalGlobe.

Materials and Methods

Experimental Sites

The study was conducted in fields irrigated by high-As wells in Faridpur district, Bangladesh (Figure 1). The wells drew water from 85 to 400 ft in depth, ranged from 15 to 46 years in age, and had As concentrations of 200-500 µg/L as measured by the ITS Econo-Quick field kit, which tends to overestimate water As by about a factor of two (George et al. 2012) (Table S1). Measurements were made over the course of three years within 5 × 5 m study plots in which rice was grown. Up to two rice crops – boro and aman – were grown at the study sites each year. During some seasons crops other than rice were grown and during some seasons no crops were grown at some of the study sites, and at these times soil As measurements were not made.

Field Kit Soil As Measurements

Soil cores of 20 cm depth were collected monthly during each growing season at the study sites where rice was grown. These cores were separated into 5 cm deep subsample increments. Each subsample was measured using the ITS Econo-Quick field kit, generally in the field, but sometimes later the same day after returning to the lab.

Four sets of three 20 cm deep soil cores were collected at the harvest during each growing season at the study sites where rice was grown. Each set of three cores was dried in an oven at 40ºC and homogenized by mortar and pestle before As analysis with the kit.

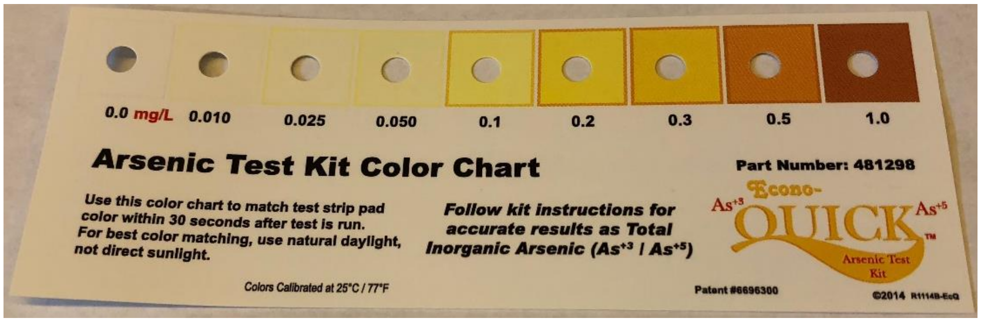


Figure 2. Color categories and the matching numerical arsenic values identified by the ITS Econo-Quick Field Kit.

Table 1. Probabilities of occurrence for each soil As bin measured by the field kit overall, and when soil As is greater than, or less than or equal to, an example 20 mg/kg threshold.

bin_i	$P(bin_i)$	$P(bin_i \mid [\mathrm{As}]_{XRF} > 20\ \mathrm{mg/kg})$	$P(bin_i \mid [\mathrm{As}]_{XRF} \leq 20\ \mathrm{mg/kg})$
0.01	0.088	0.022	0.175
0.025	0.127	0.049	0.229
0.05	0.235	0.167	0.325
0.1	0.245	0.279	0.2
0.2	0.178	0.269	0.058
0.3	0.102	0.171	0.011
0.5	0.024	0.042	0.001
1	0.001	0.001	0.001
Sum	**1**	**1**	**1**

The field kit relies on the generation of arsine gas and visual detection on a strip impregnated with mercuric bromide. We adapted the standard procedure for analyzing water As to measure soil As by adding 0.5 g of soil in 50 mL of bottled (low-As) water. The 2nd reagent of the kit, an oxidant to suppress potential interference by hydrogen sulfide, was not added and the standard reaction time was maintained at 10 min. A soil kit test results in a color on the test strip that is matched to one of nine possible color bins, each of which is identified with a particular arsenic concentration (Figure 2).

XRF Soil As Measurements

All soil samples were dried in an oven at 40ºC and homogenized by mortar and pestle for As analysis with XRF. Total soil As concentrations were measured using an Innov-X Delta Premium field X-ray fluorescence (XRF) spectrometer in the soil mode for a total counting time of 35-150 s. Soil standards 2709 and 2711 from the National Institute of Standards and Technology (NIST) were analyzed at the beginning and end of each day and periodically during longer sample runs. The measured average and standard deviation for standard 2711 of 110 ± 8 (n = 50) matched the reference value of 105 ± 8 mg/kg. The measured average and standard deviation for standard 2709 of 16.8 ± 1.6 (n = 27) matched the reference value of 17.7 ± 0.8 mg/kg. All soil As concentrations were above the detection limit of the XRF. Additionally, while high Pb concentrations can interfere with As measurements, we generally did not observe Pb concentrations higher than 50 mg/kg, and we observed accurate As concentrations when making XRF measurements on NIST standard 2711 which contains more than 1000 mg/kg Pb. Since

XRF measurements of soil As concentrations are a previously validated method (US Environmental Protection Agency 2006), in this paper we treat the XRF measurements as the ground truth by which we benchmark estimates of soil As made from soil kit measurements.

Application of Bayes' Theorem

We apply Bayes' Theorem to calculate the expected proportion of XRF soil As measurements in a plot above a chosen intervention threshold, based the results of m kit soil As measurements made in that study plot. Bayes' theorem estimates the probability of an event, such as plot As being above an intervention threshold, based on prior knowledge of conditions related to that event, such as specific color observations made with the soil kit. For this application, Bayes' theorem can be written as

$$P([\mathrm{As}]_{XRF} > \text{ threshold} \mid bin_1, \ldots, bin_m) = \frac{P(bin_1, \ldots, bin_m \mid [\mathrm{As}]_{XRF} > \text{ threshold}) \times P([\mathrm{As}]_{XRF} > \text{ threshold})}{P(bin_1, \ldots, bin_m)} \tag{1}$$

where $[\mathrm{As}]_{XRF}$ is the soil arsenic concentration measured by XRF, bin_i is the color bin observed from a single kit measurement, and m is the total number of kit measurements made in a study plot.

To apply Bayes' theorem, we need to calculate several intermediate probabilities based on the observed XRF and kit soil As measurements. First, for each kit color bin we calculate $P(bin_i \mid [\mathrm{As}]_{XRF} > \text{ threshold})$, the probability of getting that bin if soil As measured by XRF is above the threshold, and $P(bin_i \mid [\mathrm{As}]_{XRF} \leq \text{ threshold})$, the probability of getting that bin if soil As measured by XRF is less than or equal to the threshold (Table 1). Using this information we can calculate the probability of getting a particular set of m bins given that soil As measured by XRF is above the threshold as

$$P(bin_1, \ldots, bin_m \mid [\mathrm{As}]_{XRF} > \text{ threshold}) = P(bin_1 \mid [\mathrm{As}]_{XRF} > \text{ threshold}) \times \ldots \times P(bin_m \mid [\mathrm{As}]_{XRF} > \text{ threshold}) \tag{2}$$

and similarly we can calculate the probability of getting a particular set of m bins given that soil As measured by XRF is below the threshold as

$$P(bin_1, \ldots, bin_m \mid [\mathrm{As}]_{XRF} \leq \text{ threshold}) = P(bin_1 \mid [\mathrm{As}]_{XRF} \leq \text{ threshold}) \times \ldots \times P(bin_m \mid [\mathrm{As}]_{XRF} \leq \text{ threshold}) \tag{3}$$.

We also calculate $P([\mathrm{As}]_{XRF} > \text{ threshold})$, the overall probability that As measured by XRF is above the threshold, and $P([\mathrm{As}]_{XRF} \leq \text{ threshold})$ the overall probability that As measured by XRF is below the threshold. We can then find the overall probability of each set of m bins occurring as

$$P(bin_1, \ldots, bin_m) = P(bin_1, \ldots, bin_m \mid [\mathrm{As}]_{XRF} > \text{ threshold}) \times P([\mathrm{As}]_{XRF} > \text{ threshold}) + P(bin_1, \ldots, bin_m \mid [\mathrm{As}]_{XRF} \leq \text{threshold}) \times P([\mathrm{As}]_{XRF} \leq \text{threshold})$$

Table 2. Steps in applying Bayes’ theorem to calculate the probability that soil As is above a 20 mg/kg threshold given 12 As bins observed with the field kit, for three example series of 12 bins.

$\boldsymbol{bin_1, \ldots, bin_{12}}$	$\boldsymbol{P(bin_1, \ldots, bin_{12} \mid [\mathrm{As}]_{XRF} > 20\ \mathrm{mg/kg})}$	$\boldsymbol{P(bin_1, \ldots, bin_{12} \mid [\mathrm{As}]_{XRF} \leq 20\ \mathrm{mg/kg})}$	$\boldsymbol{P(bin_1, \ldots, bin_{12})}$	$\boldsymbol{P([\mathrm{As}]_{XRF} > 20\ \mathrm{mg/kg} \mid bin_1, \ldots, bin_{12})}$	$\boldsymbol{P([\mathrm{As}]_{XRF} \leq 20\ \mathrm{mg/kg} \mid bin_1, \ldots, bin_{12})}$
0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01	1.53×10^{-20}	8.04×10^{-10}	3.48×10^{-10}	8.66×10^{-21}	2.49×10^{-11}
0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.025	3.35×10^{-20}	1.05×10^{-09}	4.56×10^{-10}	1.90×10^{-20}	4.17×10^{-11}
0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.05	1.14×10^{-19}	1.50×10^{-09}	6.48×10^{-10}	6.47×10^{-20}	9.98×10^{-11}

(4).

We now have all of the probabilities we need to apply Bayes' theorem. We substitute these probabilities into Equation 1 to find $P([\mathrm{As}]_{XRF} > \text{ threshold} \mid bin_1, \ldots, bin_m)$, the probability that As measured by XRF is above the threshold, given a set of m color bins observed using the kit (Table 2).

Results

Regression of kit As vs XRF As

There was a significant correlation between total soil As and kit As measurements on the 5 cm soil subsamples collected during the growing season (Figure 3a) and on the dried and homogenized cores collected at harvest (Figure 3b). There was variation in the regressions across seasons, but no consistent seasonal trends were observed.

The kit measurements on fresh soil had substantially less variability in the regression parameters between seasons compared to those on dried soil, meaning that kit calibrations conducted on fresh soil in past seasons can be applied more effectively to future seasons. Given that seasonal variability was lowest for kit measurements on fresh soil, we use these soil samples for calibration and evaluation of the field kit. Kit measurements made on fresh soil across all seasons were combined to develop a single calibration curve (Figure 4). There was a correlation between total soil As and kit As measurements with a reasonably high R^2 of 0.40 (Figure 4a), even though the kit measurements are binned to a set of only nine values by visually matching the test strip to a color chart.

The correlation improved when the average of the subsamples taken at each depth interval across three months of monitoring was taken (3-sample average; $R^2 = 0.57$; Figure 4b) or when the average of the 5 cm subsamples across the full 20 cm soil core was taken (4-sample average; $R^2 = 0.52$; Figure 5c). The correlation further improved ($R^2 = 0.67$; Figure 4d) when the average was taken across all depths and all months for a study plot during a growing season (12-sample average). This suggests that farmers should take multiple kit measurements to improve the accuracy of their results.

Using estimated average soil As as a decision rule for intervention

A primary goal of testing soil As with the field kit is to make a recommendation about whether farmers should intervene to mitigate the impact of soil As on their rice. One possible decision rule for intervention is to average the kit As concentrations and convert them to an average XRF concentration using the slope from a linear regression as shown in Figure 5. If the average XRF As is estimated to be above a certain threshold, intervention is recommended.

We choose 20 mg/kg, 30 mg/kg, and 40 mg/kg average soil As as measured by XRF as possible thresholds for intervention. Overall, among the 12-sample averages of soil As measured by XRF on our study plots, which were selected for being As-impacted, 61% were above the 20 mg/kg threshold, 31% were above the 30 mg/kg threshold, and 19% were above the 40 mg/kg threshold. Average boro rice yield in Bangladesh is around 4 t/ha (BBS 2016), and with each 10 mg/kg increase in soil As, boro rice yield is expected to decrease by 0.6-1.1 t/ha (Huhmann et al. 2017; Panaullah et al. 2009). Farmers surveyed in our study area in 2016 reported receiving 16.44 taka per kilogram for their rice. This implies that if farmers could fully mitigate the negative impact of 30 mg/kg soil As on rice yield, their rice yield would improve by 1.8 t/ha and their earnings would increase by 29,592 tk/ha.

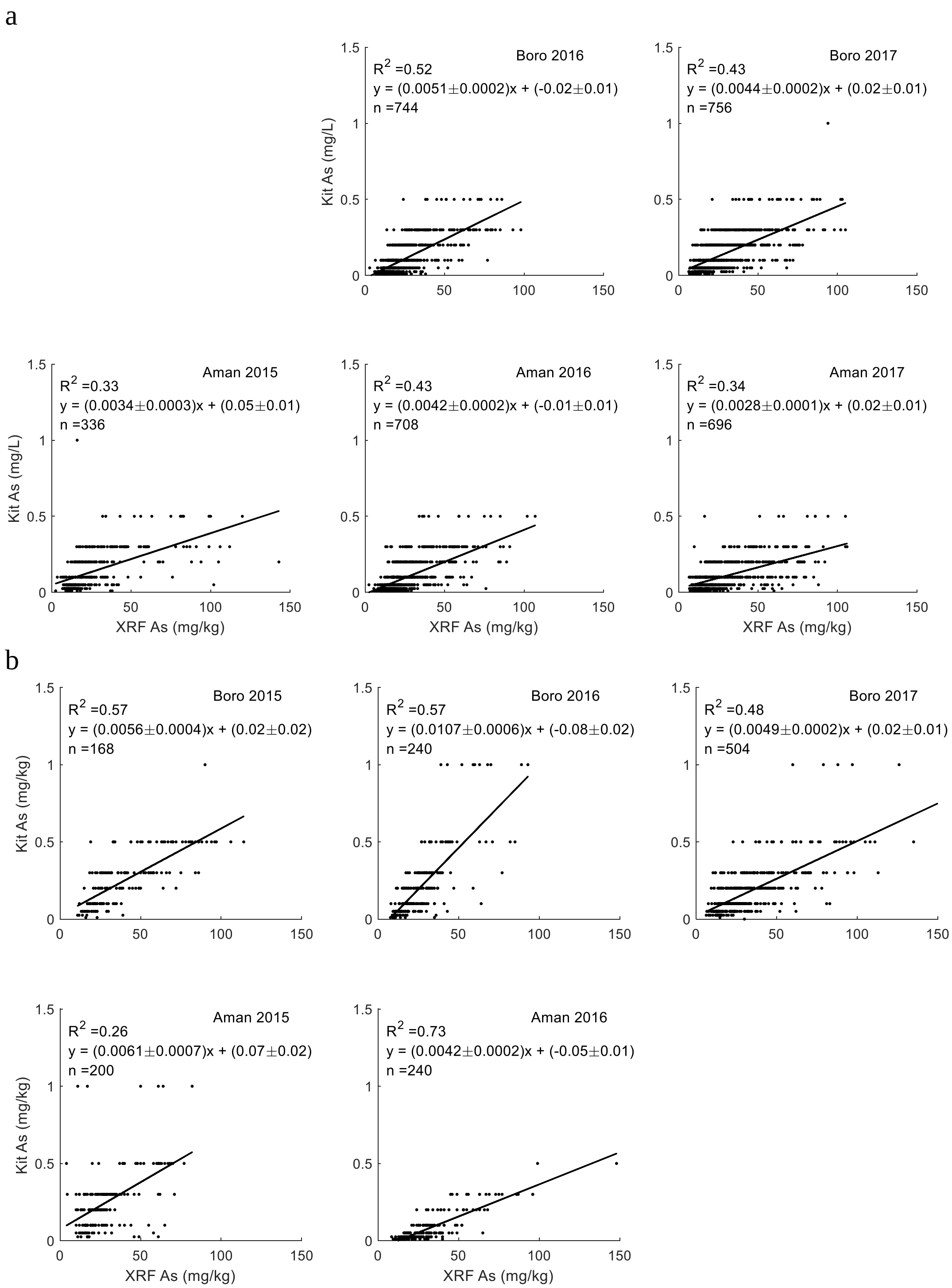


Figure 3. Regression between As as measured by the ITS Econo-Quick Field Kit and total soil As as measured by XRF for (a) fresh soil samples collected monthly during the first three months of the aman 2015, boro 2016, aman 2016, boro 2017, and aman 2017 growing seasons and (b) dried and homogenized soil samples collected at harvest during the boro 2015, aman 2015, boro 2016, aman 2016, and boro 2017 growing seasons.

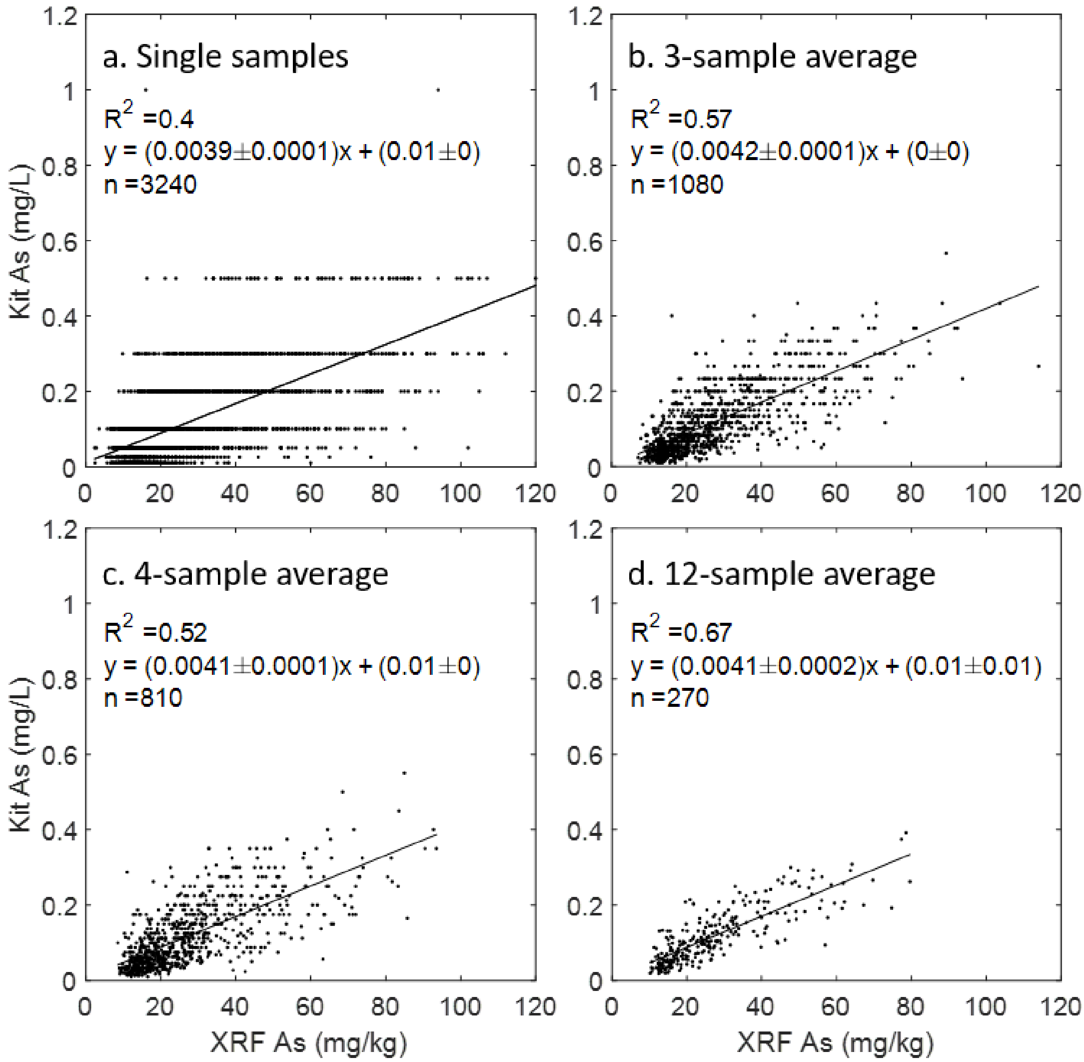


Figure 4. Correlation between As measured by the ITS Econo-Quick Field Kit and total soil As measured by XRF for fresh soil samples collected monthly during the first three months of the aman 2015, boro 2016, aman 2016, boro 2017, and aman 2017 growing seasons for (a) subsamples at 5 cm intervals from 0 to 20 cm depth, (b) the average of the four 5 cm subsamples for each 20 cm core, (c) the average across the three monthly cores collected during a season for each depth interval, and (d) the average across subsamples and across three months for each site during each season.

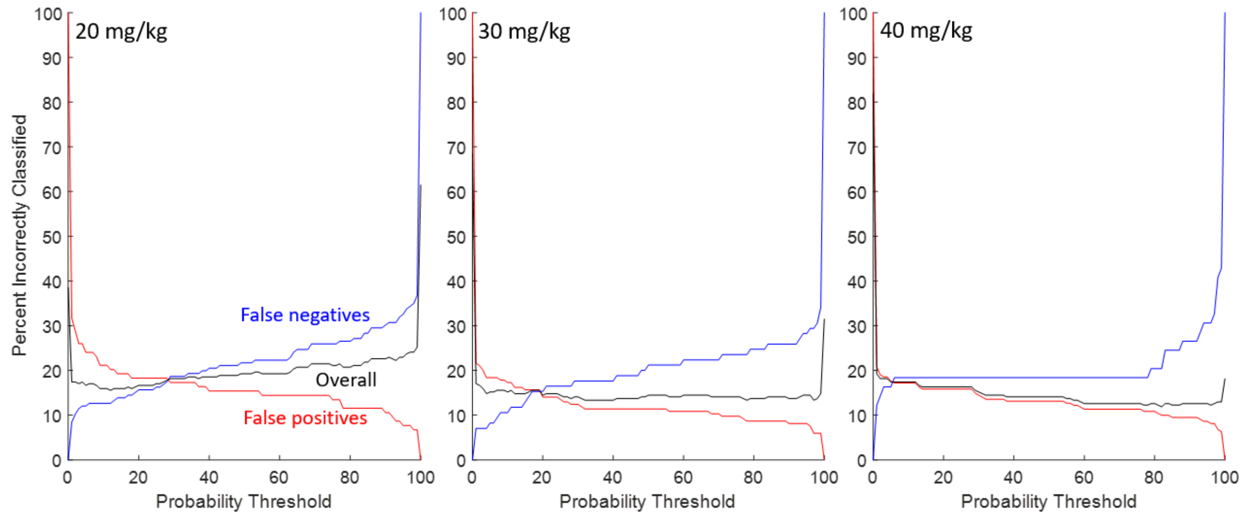


Figure 5. Percent of samples incorrectly classified when the Bayesian method was used with the sets of 12 kit measurements to determine whether to intervene at different probability thresholds. False positives are samples where the probability was above the threshold but the average XRF As was not. False negatives are samples where the probability was below the threshold but the average XRF As was not. Overall is the sum of false positives and false negatives divided by the total number of sets of 12 kit measurements.

We check how accurately the 12-sample average of kit measurements on fresh soil predicts whether a soil's As concentration is above or below these thresholds, using the regression equation relating the kit and XRF measurements. For a threshold of 20 mg/kg average As measured by XRF, the kit has 85% sensitivity (true positive rate) and 81% specificity (true negative rate). That is, of the 166 study plots with more than 20 mg/kg average soil As measured by XRF, 141 (85%) are accurately recommended for intervention by the kit, and 25 (15%) are inaccurately not recommended for intervention. Of the 104 study plots with less than 20 mg/kg average soil As measured by XRF, 84 (81%) are accurately not recommended for intervention by the kit, and 20 (19%) are inaccurately recommended for intervention (Table 1).

The kit similarly had about 80-90% accuracy when used to recommend intervention for average XRF soil As thresholds of 30 mg/kg and 40 mg/kg. The lowest threshold (20 mg/kg) resulted in more false positives (lower specificity), where the kit incorrectly recommended intervention. The highest threshold (40 mg/kg) resulted in more false negatives (lower sensitivity), where the kit incorrectly did not recommend intervention.

Using the probability that soil As is above a threshold as a decision rule for intervention

Another possible decision rule uses Bayes' theorem to calculate the probability that soil As measured by XRF is above a certain threshold (e.g. 20, 30 or 40 mg/kg) based on a set of field kit measurements. If this probability is above a certain value, intervention is recommended.

For each soil As threshold, this approach allows for the selection of a probability threshold between 0 and 1, resulting in different tradeoffs between over-intervening and under-intervening. If our ideal is to intervene in plots that have an average XRF As above the chosen soil As threshold, we can compare the number of correct and incorrect recommendations about intervention made by the kit at different probability thresholds for soil As thresholds of 20, 30, and 40 mg/kg (Figure 5). We can then use this information about the tradeoffs to choose a preferred probability threshold for intervention.

One possibility is to select a probability threshold that minimizes the overall percentage of incorrect classifications. This probability threshold is 0.12 for 20 mg/kg (15.6% incorrect), 0.32 for 30 mg/kg (13.3% incorrect), and 0.82 for 40 mg/kg (11.9% incorrect).

Another possibility is to select a probability threshold that results in the most similar percentage of false positives and false negatives. This probability threshold is 0.28 for 20 mg/kg (17.5% false positive, 17.8% false negative), 0.19 for 30 mg/kg (15.3% false positive, 15.6% false negative), and 0.06 for 40 mg/kg (18.4% false positive, 17.4% false negative).

Different value judgments could lead to still other choices of probability thresholds. If farmers are willing to accept the extra effort that comes with a false positive, so long as it is very unlikely that they miss an opportunity to intervene, a lower probability threshold might be preferred. Alternatively, if farmers are okay with missing an opportunity to benefit from an intervention, as long as they do not waste resources on a false positive, a higher probability threshold could be more ideal. The chosen probability threshold will ultimately depend on a farmer's assessment of the costs and benefits of intervening.

Discussion

Kit Measurements on Fresh versus Dried Soil

The regressions between soil As measured by the field kit and by XRF varied between seasons, especially so for the kit measurements made on dried and homogenized soil. Variability between seasons could be due to factors related to the kit, the personnel, or the paddy soil. The test strip in the kit is sensitive to humidity, and thus results may be affected by how often and under what conditions the jar containing the test strips is left open. Personnel testing the soil may exhibit a learning curve across seasons of using the kit, and the processing and collection of the soil or use of the kit may vary across personnel. The characteristics of the rice paddy soil may also vary across time and location, resulting in soil As being more or less available to the kit. The higher variability between seasons observed for the kit measurements on dried soil could be due to variations in the degree to which soil was dried or homogenized from one season to the next, or the duration for which the soil was stored before kit As testing, all of which might affect As availability to the kit.

The kit measurements on fresh soil have two clear benefits over measurements on dried soil. They are more consistent across seasons, perhaps due to introducing fewer opportunities for methodological variation. They also provide farmers with test results immediately in the field, without the time- and labor-intensive process of soil drying and homogenization. A potential advantage of the measurements on dried soil is that larger amounts of soil were combined in each sample, which may result in measurements that are more representative of the sampling area as a whole. In the future it may be worth exploring whether mixing larger volumes of wet soil in the field before testing with the soil kit could capture the benefits of creating a more representative sample, without the drawbacks of the increased variability that was observed for the dried and homogenized soil in this study.

Comparing the linear regression and Bayesian decision rules

In this paper we described a linear regression and a Bayesian approach for determining when to intervene in a high-As plot. Both approaches involve a calibration step. In the linear regression approach, this calibration involved determining the slope of a regression between 12-

sample averages of kit As and 12-sample averages of XRF As. In the Bayesian approach, this step involved calculating the probability of soil As being above an intervention threshold for every possible set of 12 kit measurements. Each intervention soil As threshold of interest required a different table matching kit measurements to probabilities.

In the application phase, the information from the calibration phase is applied to new kit measurements collected in the field. For the regression approach, a set of 12 kit measurements are collected and averaged, and the regression equation is then applied to them to estimate the average XRF As for a study plot. This average is compared to the soil As threshold to determine if intervention is recommended. For the Bayesian approach, a set of 12 kit measurements is matched to a pre-calculated probability of As being above or below an intervention threshold given those measurements. If this probability is above a selected threshold probability, intervention is recommended.

Comparing the calibration and application phases across the methods, we see that the Bayesian method presents two unique challenges. First, unlike the regression method, the Bayesian method requires the As intervention threshold to be known during the calibration phase. Second, during the application phase, the Bayesian method requires use of a lookup table to match a set of kit observations to a probability that soil As is above the threshold. In contrast, the regression method only requires knowledge of the regression slope.

We can also compare the amount of error in the recommendations from the two methods at threshold of 20 mg/kg, 30 mg/kg, and 40 mg/kg (Table 3). The Bayesian approach is the theoretically optimal approach, giving the best results possible under the set of assumptions we have made. It fully utilizes the available information and derives from scratch the relationship between kit observations and XRF measurements of As, whereas the linear regression approach starts with the values of As concentration assigned by the kit to each bin and scales them up to get estimates of XRF As. Therefore, we compare the results of the two methods to see how close the linear regression approach comes to the accuracy of the Bayesian approach.

Since the errors for the Bayesian method represent different tradeoffs at different probability thresholds, for the purposes of this comparison, we choose the probability threshold for the Bayesian method that gives the same number of false negatives as given by the linear regression method. This probability threshold is 19% at 20 mg/kg, 15% at 30 mg/kg, and 82% at 40 mg/kg soil As. Comparing the number of false positives, we find that the two methods give identical numbers of false positives at 30 mg/kg and 40 mg/kg, while the Bayesian method gives a slightly lower number of false positives (18% versus 19%) at 20 mg/kg.

In summary, we find that the Bayesian method is slightly more complicated to apply, and gives a similar or slightly lower percentage of false positives to the linear regression method, when the number of false negatives is held constant. Additionally, a key advantage of the Bayesian method is that it allows the selection of a probability threshold, enabling an explicit choice of tradeoffs between false positives and false negatives. If this additional advantage of the Bayesian method is not desired, the simpler linear regression method appears to provide sufficient guidance for or against intervention.

Table 3. Accuracy of kit for predicting whether soil As is above a 20 mg/kg, 30 mg/kg, and 40 mg/kg threshold for the regression method compared with the Bayesian method calibrated and tested on plots from the aman 2015 through aman 2017 growing seasons.

		XRF				XRF	
		<20 mg/kg	>20 mg/kg			<20 mg/kg	>20 mg/kg
Kit (12-sample average)	<20 mg/kg	84 (81%)	25 (15%)	Kit (Probability threshold)	<19%	85 (82%)	25 (15%)
	>20 mg/kg	20 (19%)	141 (85%)		>19%	19 (18%)	141 (85%)
		<30 mg/kg	>30 mg/kg			<30 mg/kg	>30 mg/kg
	<30 mg/kg	156 (84%)	11 (13%)		<15%	156 (84%)	11 (13%)
	>30 mg/kg	29 (16%)	74 (87%)		>15%	29 (16%)	74 (87%)
		<40 mg/kg	>40 mg/kg			<40 mg/kg	>40 mg/kg
	<40 mg/kg	198 (90%)	10 (22%)		<82%	199 (90%)	10 (22%)
	>40 mg/kg	23 (10%)	39 (78%)		>82%	22 (10%)	39 (78%)

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Supporting Information

Table S1. Year installed, pump depth, and water arsenic for the twenty-two wells irrigating the fields where the field kit was tested.

Site	Year pump installed	Pump depth (ft)	Arsenic concentration measured by field kit (μg/L)	Estimated arsenic concentration based on intercalibration with ICP-MS (mg/kg)
Aliabad	1985	200	500	306±25
Banumatbordangi	1995	180	200	129±14
Bilmamudpur	1990	200	500	306±25
Bokile	2001	245	300	188±20
Choradampur 1 & 2	1995	100	300	188±20
Chornosipur 1 & 3	1985	240	500	306±25
Chornosipur 2	1970	400	300	188±20
Dhuldi	1972	280	500	306±25
Dhuldi Rajapur 1 & 2	1985	300	300	188±20
Doyarampur	1988	205	500	306±25
Gerda	1979	320	500	306±25
Ikri 1 & 2	1996	250	300	188±20
Kujurdia	1999	85	500	306±25
Middle Tambulkhana	1989	370	500	306±25
Mirgi 1 & 2	1985	350	300	188±20
Pearpur	1998	300	300	188±20
Purbopara	1976	250	500	306±25
Sachia	1990	275	300	188±20
Tambulkhana	1994	228	300	188±20
West Aliabad	1995	300	500	306±25
West Ikri	1995	195	500	306±25
West Sachia	1996	150	200	129±14

Chapter 5: Inversion of high-arsenic soil for improved rice yield in Bangladesh

Abstract

Background and aims
Rice is the primary crop in Bangladesh, and rice yield is diminished due to the buildup of arsenic (As) in soil from rice irrigation with high-As groundwater. Implementing a soil inversion, where deeper low-As soil is exchanged with the surface high-As soil in contact with rice plant roots, may mitigate the negative impacts of As on rice yield.

Methods
We compared soil As, soil nutrients, and rice yield in control plots with those in adjacent plots where a soil inversion was implemented. We also estimated the quantity of soil As deposited on a yearly basis via irrigation water, to explore the longevity of a soil inversion to reduce surface As.

Results
Soil As, organic carbon, nitrogen, and phosphorus concentrations decreased by about 40% in response to the inversion and remained persistently lower in soil inversion plots compared to adjacent control plots over four seasons of monitoring. Rice yield in inversion plots increased above rice yield in control plots by 15-30% after a one-season lag, but was uncorrelated with soil As and nutrient concentrations.

Conclusions
The results suggest that a soil inversion may be a cost-effective method for farmers to improve rice yield. However, the longevity of the yield gain remains unknown, since the cause could not be identified and since soil As from irrigation water builds up again at a rate of about 1 mg/kg per year.

Introduction

Rice is the primary crop of Bangladesh in terms of production and caloric consumption, comprising 70% of calories consumed (BBS 2016b; FAO and WHO 2014). Rice is predominantly grown during the boro (dry winter) and aman (monsoon) seasons (BBS 2016a, 2016b). High volumes of groundwater are required to maintain the flooded conditions under which boro rice is grown, whereas aman rice is primarily rainfed, with occasional supplemental groundwater irrigation (FAO 2002).

Much of the irrigation water in rice-growing regions of Bangladesh is naturally contaminated with high concentrations of arsenic (As). When rice is irrigated with this water, the As can build up in rice field soil (Dittmar et al. 2010; Hossain et al. 2008; Lu et al. 2009; Neumann et al. 2011; Panaullah et al. 2009; Saha and Ali 2007). Among crops, rice is especially impacted by irrigation water As, since it is grown under flooded conditions, resulting in the use of higher volumes of As-contaminated irrigation water and in a chemically reduced soil environment that enhances As mobility. Soil As decreases rice yield, and the build up of irrigation water As in soil is estimated to reduce boro rice yield by 7-26% across Bangladesh (Abedin et al. 2002; Huhmann et al. 2017; Panaullah et al. 2009).

Various options have been considered to reduce the uptake of soil As by rice and the impacts of soil As on rice yield. These include providing cleaner irrigation water, growing As-resistant rice varieties, and growing rice under conditions that are less conducive to As uptake (Brammer 2009; Polizzotto et al. 2015). Even with these methods, rice yields will likely be negatively impacted by the high levels of legacy As contamination in many rice fields. Removal of the highest-As upper 10-15 cm of soil has been suggested to address this problem, since farmers commonly remove soil for use in brick-making, building houses, and raising infrastructure above monsoon flooding (Brammer 2009). However, the impacts of soil removal on soil As and rice yield have not been documented.

Building on the idea of soil removal to improve rice yield, we conducted a soil inversion. Since As concentration in paddy soil decreases with depth, we exchanged the deeper low-As soil with the surface high-As soil, putting the low-As soil in contact with the rice roots. We then compared As concentrations, nutrient concentrations, and rice yields in 5×5 meter control plots to those in the soil inversion plots. A soil inversion is more versatile than soil removal, since there is no elevation discrepancy between the inversion area and the surrounding paddy, allowing farmers to implement it without disrupting irrigation water management. It additionally does not require disposal of As-contaminated soil. To investigate the longevity of the inversion's impact on soil As, we measured irrigation water use and As concentrations to estimate deposition rates of As in paddy soil.

This paper builds on a prior research study in the same region, where we exchanged soil between high- and low-As areas of farmers' fields and compared those soil exchange plots with adjacent control plots to document the impact of soil As on rice yield (Huhmann et al. 2016). This paper extends that work by assessing the effectiveness of a soil inversion to decrease surface soil As concentrations and improve rice yield.

Materials and Methods

Experimental Site and Design

The study was conducted in fields irrigated by high-As wells in Faridpur district, Bangladesh (Figure 1). The wells drew water from 85 to 400 ft in depth, ranged from 17 to 46 years in age, and had As concentrations of 200-500 µg/L as measured by the ITS Econo-Quick field kit, which tends to overestimate water As by about a factor of two (George et al. 2012) (Table S1).

Up to two rice crops – boro and aman – are grown at our study sites each year. The boro rice is transplanted, and the aman rice is transplanted or broadcast sown. The predominant rice varieties that farmers grew at our study plots during the 2016, 2017, and 2018 boro seasons were BRRI dhan 28 (BR 28) and BRRI dhan 29 (BR 29). These are also the predominant rice varieties grown across Bangladesh, and were estimated in 2005 to be grown in nearly 60% of the total boro rice cropped area in the country (Hossain et al. 2013). Farmers chose to grow other rice varieties in a few study plots, which they reported as BR 50, Banglamoti, Basmoti, and hybrid. The predominant rice variety that farmers grew at our study plots during the 2016 and 2017 aman seasons was BRRI dhan 39 (BR 39). Farmers chose to grow other rice varieties in a few study plots, which they reported as BR 51, Sisumoti, Chini Atop, and Hijol Deegha.

In January 2016 before the fields were transplanted with boro rice, soil inversions were conducted on twenty-one 5×5 m plots. To conduct the inversion, soil was excavated in three

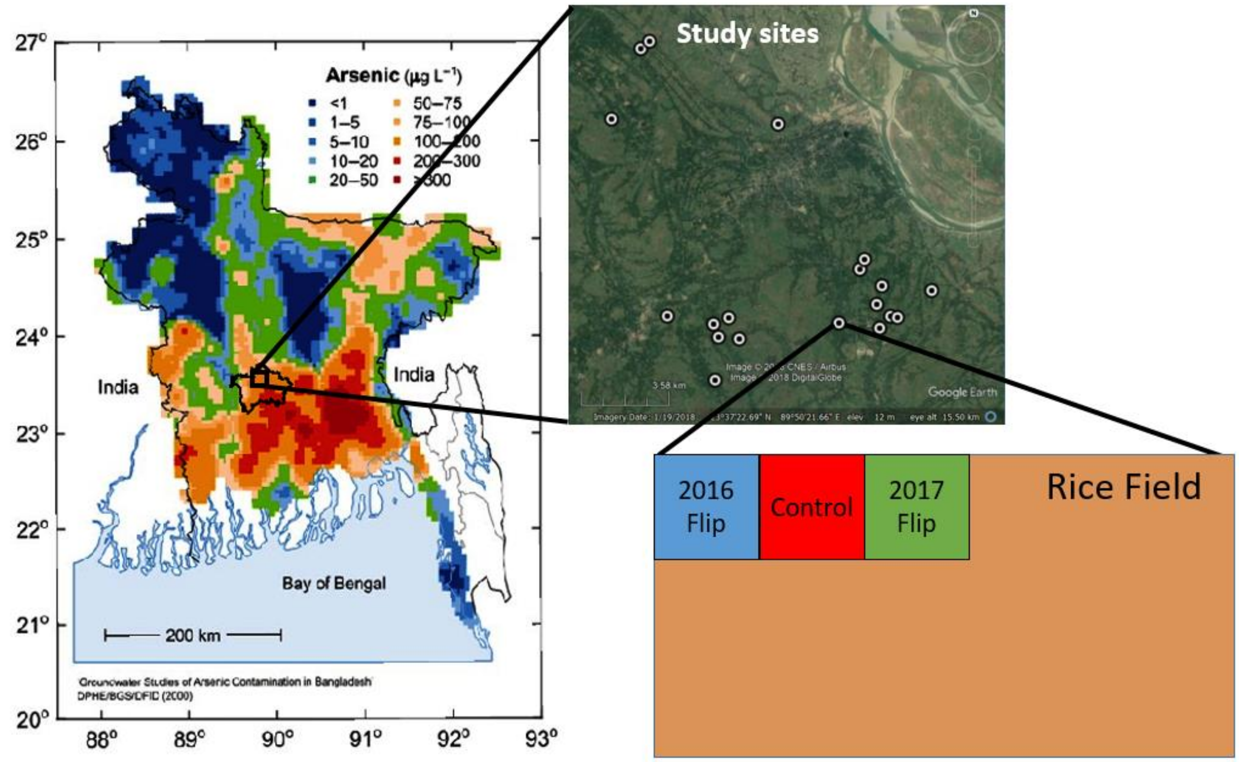


Figure 1. Layout and distribution of study sites in Faridpur, Bangladesh. Heat map of As in groundwater is from BGS and DPHE 2001.(BGS and DPHE 2001) Map data is from Google, CNES/Airbus, and DigitalGlobe.

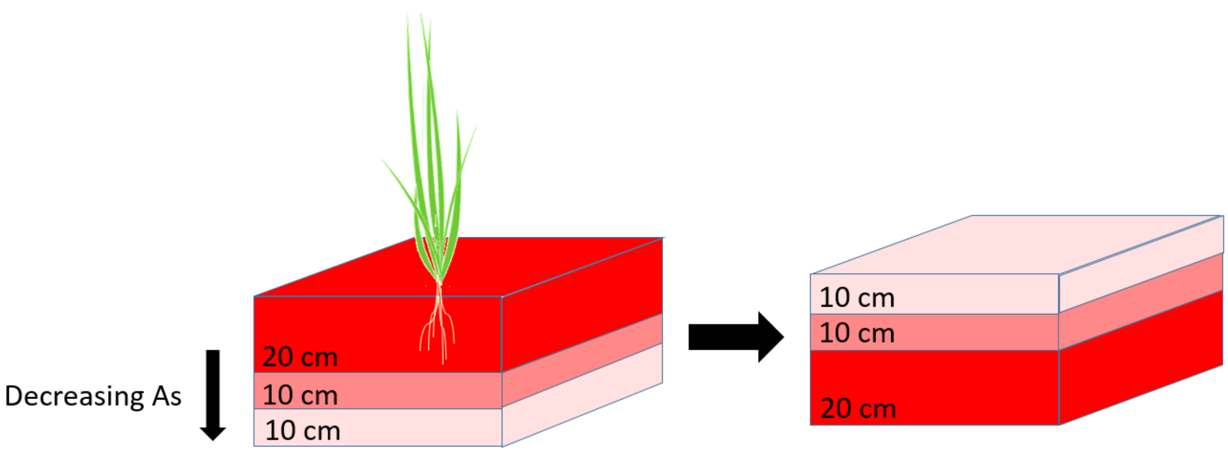


Figure 2. Soil inversion schematic The top 40 cm of soil were removed in three layers and replaced in reverse order within 5×5 m plots, such that arsenic concentrations were lowered in the top 20 cm of soil where rice roots are primarily located. Adjacent 5×5 m control plots remained undisturbed and were managed identically to the soil inversion plots.

layers: a top 20 cm layer, followed by two 10 cm layers. The layers were then replaced in the excavated area in reverse order, such that the lowest As soil was at the top, where the rice plant roots are primarily located (Figure 2) (Henry et al. 2012; Kostopoulou et al. 2015). Each soil inversion plot was paired with an adjacent 5×5 m control plot where no changes were made.

Another twenty soil inversions were conducted in January 2017. For the 2017 soil inversions, we conducted two inversions adjacent to each control plot and, at the recommendation of some farmers who had experience supplementing paddy soil after soil removal, we added 2.5 kg of cow manure and 1.2 kg of mustard cake to one of the two inversion plots at each study site. We measured soil As concentrations and nutrient concentrations in the soil inversion and control plots during the 2016-2017 boro and aman seasons. We measured rice yield in the soil inversion and control plots during the 2016-2018 boro seasons and 2016-2017 aman seasons.

Soil As Measurements

Soil cores of 20 cm depth were collected monthly during the boro 2016 growing season (three total cores per plot) and aman 2016 growing season (three total cores per transplanted plot and 5 total cores per broadcast sown plot). However, only four of the soil cores from each of the broadcast sown plots was analyzed by XRF (months 1-4 for two of the plots and months 1-3 and 5 for the third plot). During the boro 2017 and aman 2017 growing seasons, soil cores were collected monthly for most plots (three total cores per plot) but twice-monthly for the 2016 and 2017 soil inversion and control plots at Aliabad, Ikri, and Middle Tambulkhana.

The 20 cm cores were separated into 5 cm deep subsample increments to provide depth profiles of soil As. The soil subsamples were dried in an oven at 40ºC and homogenized by mortar and pestle for As analysis with XRF. Total soil As concentrations were measured using an Innov-X Delta Premium field X-ray fluorescence (XRF) spectrometer in the soil mode for a total counting time of 35-150 s. Soil standards 2709 and 2711 from the National Institute of Standards and Technology (NIST) were analyzed at the beginning and end of each day and periodically during longer sample runs. The measured average and standard deviation for standard 2711 of 108 ± 7 ($n = 19$) matched the reference value of 105 ± 8 mg/kg. The measured average and standard deviation for standard 2709 of 16.7 ± 1.6 ($n = 20$) matched the reference value of 17.7 ± 0.8 mg/kg. All soil As concentrations were above the detection limit of the XRF analyzer.

Soil Nutrient Measurements

Three sets of 20 cm deep soil cores were taken from each plot during the boro 2016, aman 2016, boro 2017, and aman 2017 seasons at the same times as the cores for soil As measurement were collected. The cores were dried in an oven at 40ºC and sent to the BRAC soil laboratory in Gazipur, Bangladesh, for measurement of electrical conductivity and pH (measured on a 1:1 mixture of soil and distilled water), N (total Kjeldahl nitrogen), organic carbon (Walkley-Black method), P (modified Olsen method), K (ammonium acetate extraction), S (calcium hydrogen phosphate extraction), and Zn (diethylenetriaminepentaacetic acid extraction).

Rice Yield Measurements

Rice yields were measured for a 3×3 m area in the center of each 5×5 m plot. The rice was threshed immediately after harvest, its weight and moisture content were recorded, and yield values were adjusted to 14% moisture content. In the 2016-2017 boro and aman seasons, we obtained an estimate of the error on yield by dividing each 3×3 m plot along the diagonal and making a separate measurement of the yield for each half of the 3×3 plot. In some study plots farmers chose to switch away from rice, to plant no crops, or to abandon their rice during some seasons, resulting in differences in which plots we obtained yield measurements for from season to season. For the 2016 soil inversions, we obtained yield measurements for 19 pairs of inversion and control plots during the boro 2016 season, 16 pairs during the aman 2016 season, 12 pairs during the boro 2017 season, 11 pairs during the aman 2017 season, and 12 pairs during the boro 2018 season. For the 2017 soil inversions, we obtained yield measurements for 20 pairs during the boro 2017 season, 18 pairs during the aman 2017 season, and 18 pairs during the boro 2018 season.

Irrigation Water Measurements

For a subset of 10 wells that irrigate the study sites, well water As concentrations were measured using inductively-coupled plasma mass spectrometry (ICP-MS). Irrigation water was collected in 20 mL polyethylene scintillation vials with a PolySeal-lined cap (Wheaton no. 986706). Samples were acidified to 1% high-purity HCl (Fisher Scientific Optima) at least one week before analysis with a Thermo-Finnigan Element2 high-resolution inductively-coupled plasma mass spectrometer (Cheng et al. 2004). This procedure has been shown to ensure redissolution of any arsenic associated with precipitated iron oxides (van Geen et al. 2007). An in-house consistency standard of artificial groundwater containing 430 μg/L As and reference materials NIST1640a (8.2 ± 0.3 μg/L As) and NIST1643f (58.6 ± 0.5 μg/L As) were included with every run to verify accuracy and precision of the method to within <5% of expected values.

Additionally, well flow rate was estimated by timing with a stop watch the number of seconds it took for water from the pump to fill a 120 L container. Two such measurements were made to provide an error estimate on the flow rate. Throughout the boro 2017 season, the manager of each well recorded each day whether the well was used and, if so, the time at which the pump was turned on and turned off. Well managers also reported the total area of rice fields irrigated by each well.

Results

Effect of the Soil Inversion on Soil As Concentrations

Within the upper 20 cm of soil, where the rice plant roots are primarily located, the boro 2016 soil inversions decreased soil As by an average of 12.1 ± 2.3 mg/kg (40%) compared to the adjacent control plots during the growing season immediately after the inversion (Figure 3). Similarly, the boro 2017 soil inversions decreased soil As by an average of 18.0 ± 3.0 mg/kg (39%) compared to the control plots (Figure 3).

The effect of the soil inversion on soil As remained significant for plots observed during the aman 2016, boro 2017, and aman 2017 growing seasons (Figure 3). However, the magnitude of the difference decreased over time following the inversions. The soil As difference between inversion and control plots for the boro 2016 inversions decreased from 12.1 ± 2.3 mg/kg during the boro 2016 growing season to 6.4 ± 2.1 mg/kg during the aman 2017 growing season (Figure 3). A similar trend is observed in the data for the subset of 10 plots where As was measured in all growing seasons (Figure S1). Soil As did not differ between the 2017 inversions with added cow manure and mustard cake and the inversions without these soil amendments, so the data for the two were combined in the box plot.

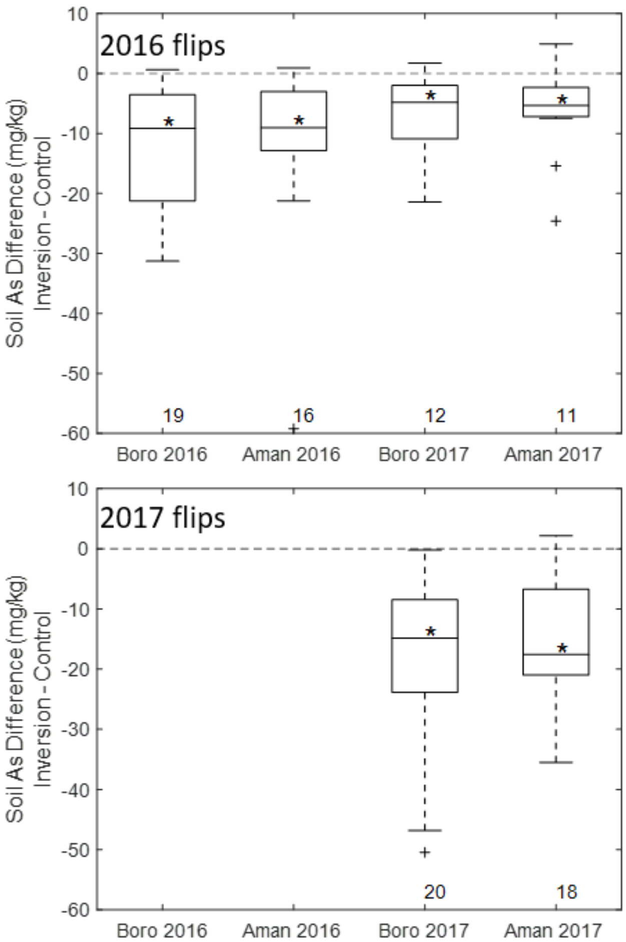


Figure 3. Soil As differences between soil inversion and control plots. Differences in soil As between inversion and adjacent control plots over the top 20 cm as measured by XRF on samples collected monthly during the growing season for soil inversions conducted in 2016 (top) and 2017 (bottom). Data are shown for all plots where yield was measured in each growing season, and the numbers below each box indicate the number of pairs of plots that box represents. The tops and bottoms of each box are the 25th and 75th percentiles. The line in the middle of the box shows the sample median. Outliers are values that are more than 1.5 times the interquartile range beyond the edge of the box. Asterisks denote that the mean significantly differs from zero at $p = 0.05$ according to a one-sample t test.

Based on the depth profiles, the soil As decrease was concentrated in the top 15 cm of inverted soil, with similar soil As concentrations observed between inversion and control plots over the 15-20 cm depth interval at the base of the upper layer of inverted soil (Figure 4).

Effect of the Soil Inversion on Soil Nutrient Concentrations

The inversion also decreased the concentrations of some nutrients in the upper 20 cm of soil. The boro 2016 soil inversions decreased organic carbon, nitrogen, and phosphorus to about 60% of their concentrations in the adjacent control plots (Figure 5). Organic carbon decreased from an average of 1.21% to 0.69%, nitrogen from 0.10% to 0.06%, and phosphorus from 64.0 µg/g to 40.1 µg/g. The inversion also produced a small but significant 8% decline in zinc. The

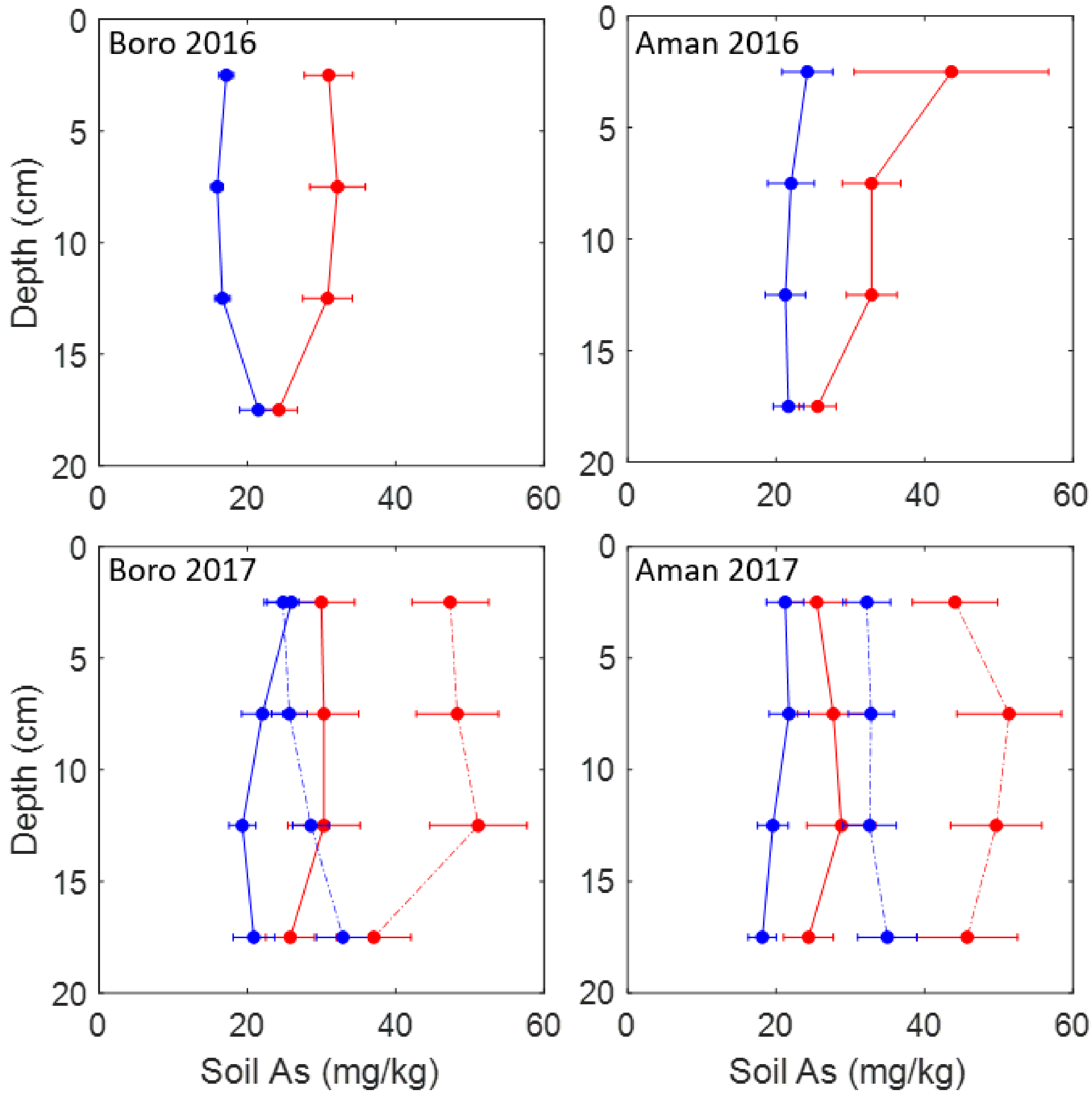
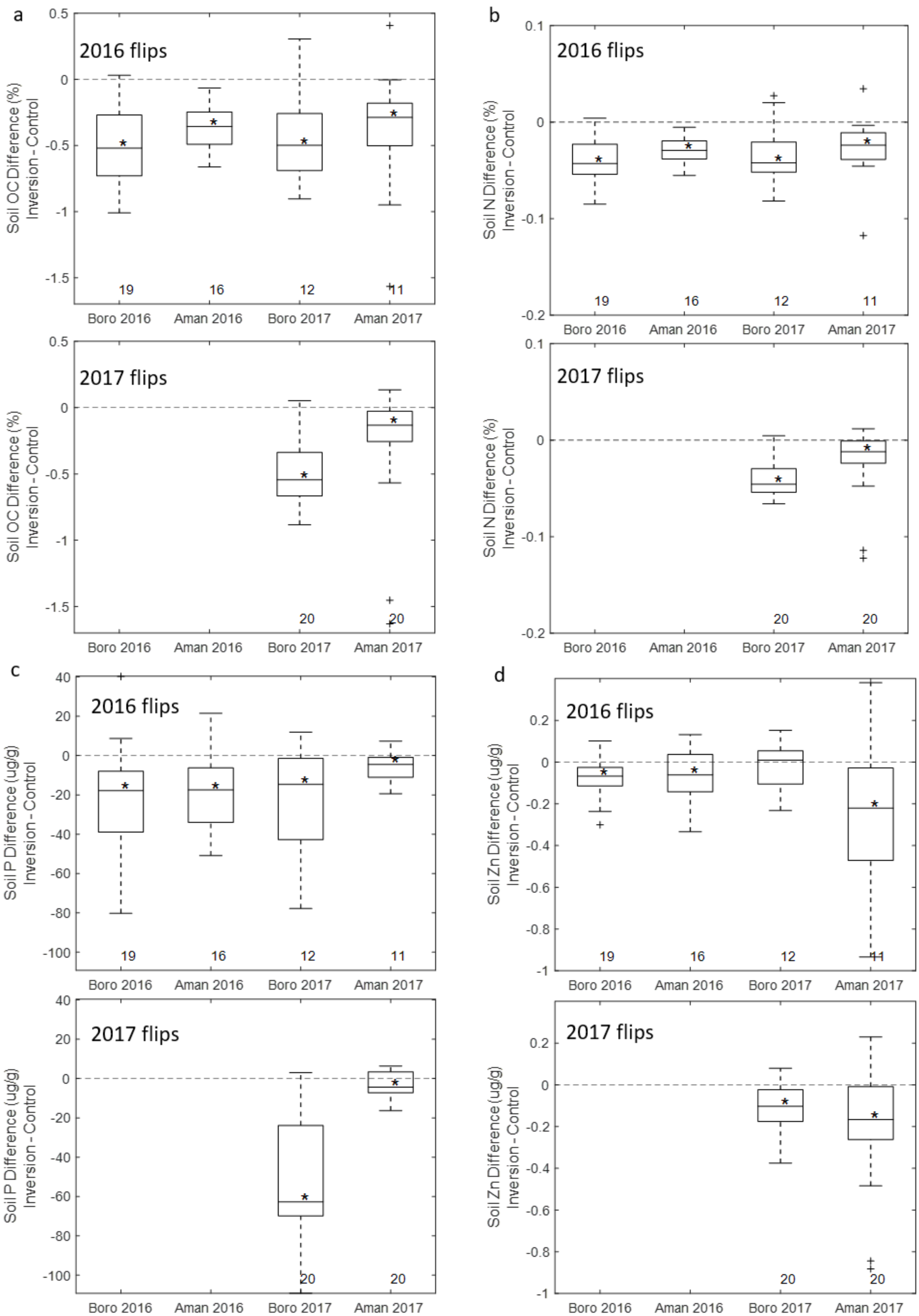


Figure 4. Soil As depth profiles in soil inversion and control plots. As profiles measured over the top 20 cm of soil for the inversion (blue) and control (red) plots for 2016 inversions (solid lines) and 2017 inversions (dashed lines) during boro 2016, aman 2016, boro 2017, and aman 2017. These figures represent the average across study plots and across monthly samples taken three to six times from each plot during the growing season. Error bars represent standard deviation divided by the square root of the number of samples.

boro 2017 inversion similarly decreased the concentrations of these nutrients in the topsoil (Figure 5). The inversions did not significantly affect soil potassium or sulfur concentrations (Figure 5).

Similar to soil As, soil nutrient concentrations in the inversion plots began to rebound at later times. By the aman 2017 growing season, organic carbon, nitrogen, and phosphorus in the 2016 inversion plots had recovered to about 70% of their original concentrations. No difference in soil nutrients was observed between the 2017 inversions with added cow manure and mustard cake and the inversions without these soil amendments, so the data were combined in the box


a
2016 flips
2017 flips
Soil OC Difference (%)
Inversion - Control
0.5
0
-0.5
-1
-1.5
19
16
12
11
20
20
Boro 2016
Aman 2016
Boro 2017
Aman 2017
b
Soil N Difference (%)
Inversion - Control
0.1
0
-0.1
-0.2
c
Soil P Difference (ug/g)
Inversion - Control
40
20
0
-20
-40
-60
-80
-100
d
Soil Zn Difference (ug/g)
Inversion - Control
0.2
0
-0.2
-0.4
-0.6
-0.8
-1

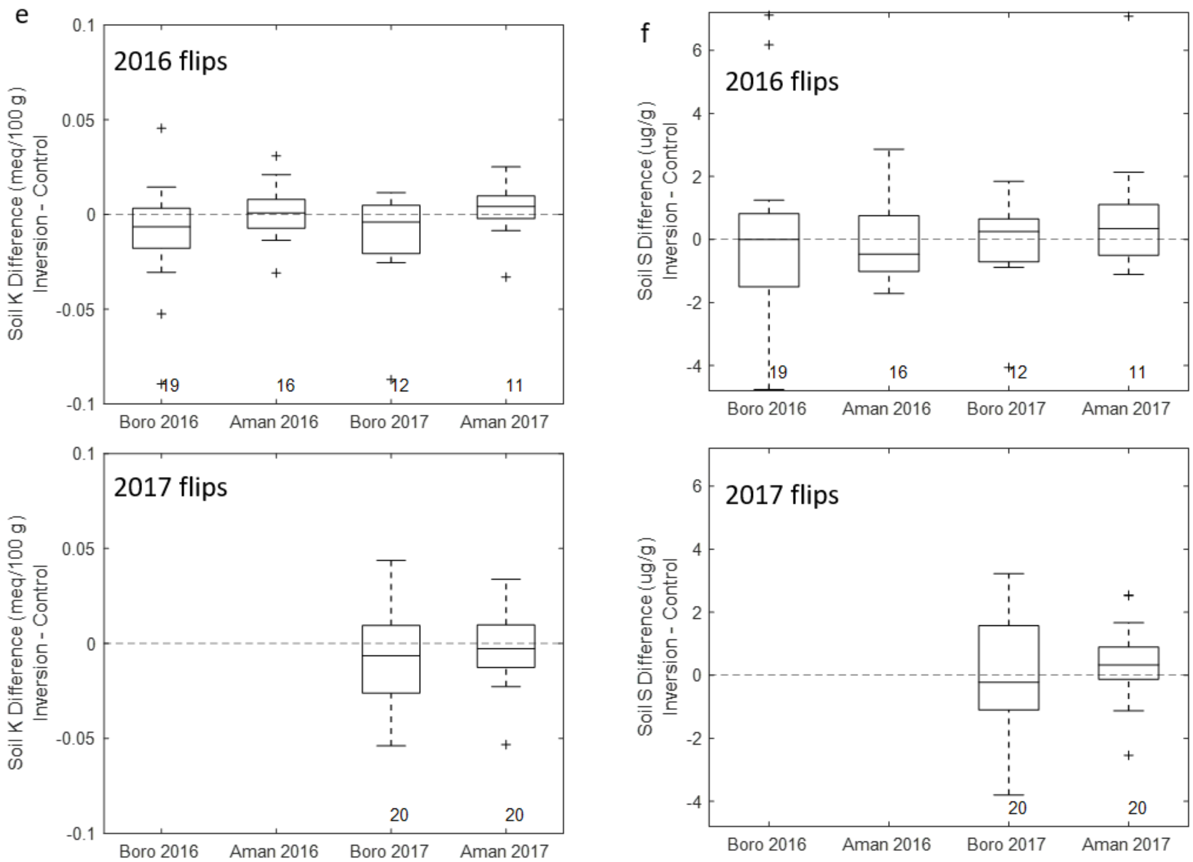


Figure 5. Soil nutrient differences between soil inversion and control plots. Differences in (a) organic carbon, (b) nitrogen, (c) phosphorus, (d) zinc, (e) potassium, and (f) sulfur between inversion and adjacent control plots over the top 20 cm as measured by XRF on samples collected monthly during the growing season for soil inversions conducted in 2016 (top) and 2017 (bottom). Data are shown for all plots where yield was measured in each growing season, and the numbers below each box indicate the number of pairs of plots that box represents. The tops and bottoms of each box are the 25th and 75th percentiles. The line in the middle of the box shows the sample median. Outliers are values that are more than 1.5 times the interquartile range beyond the edge of the box. Asterisks denote that the mean significantly differs from zero at p = 0.05 according to a one-sample t test.

plot. Back-of-the-envelope calculations based on reported concentrations of N and P in manure and mustard cake (EcoChem; Ghosh 2006) suggest that the amendments would at most increase P by 4 µg/g and N by 0.002%, differences that would not be large enough to detect.

Effect of the Soil Inversion on Rice Yield

The soil inversion improved rice yield with a one-season lag between inversion implementation and impact on yield (Figure 6). At the boro 2016 harvest, inversion and control plot yields were statistically indistinguishable, but at the aman 2016 harvest, the rice yield in the inversion plots was greater by 0.70 ± 0.15 t/ha (28% ± 6%) compared to the adjacent control plots. Yields in the inversion plots remained significantly higher (by 15-20%) than those in the control plots at the boro 2017, aman 2017, and boro 2018 harvests. Similarly, at the boro 2017 harvest, the yields in the newly implemented 2017 inversion plots were indistinguishable from

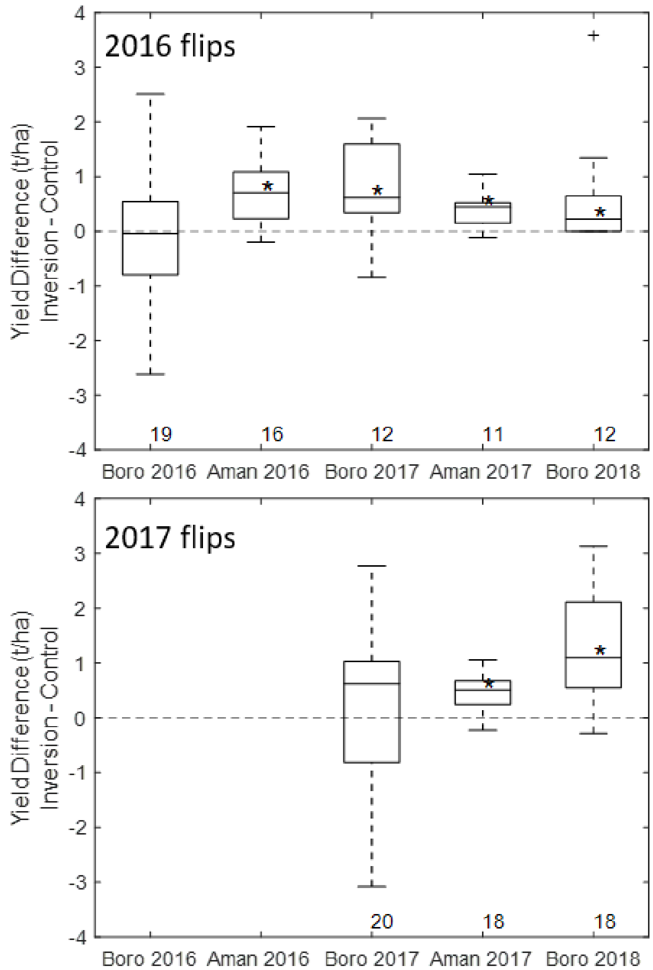


Figure 6. Yield differences between soil inversion and control plots. Differences in yield between inversion and adjacent control plots for soil inversions conducted in 2016 (top) and 2017 (bottom). Data are shown for all plots where yield was measured in each growing season, and the numbers below each box indicate the number of pairs of plots that box represents. The tops and bottoms of each box are the 25th and 75th percentiles. The line in the middle of the box shows the sample median. Outliers are values that are more than 1.5 times the interquartile range beyond the edge of the box. Asterisks denote that the mean significantly differs from zero at $p = 0.05$ according to a one-sample t test.

those in the control plots, but at the aman 2017 harvest inversion plot yields were higher by 0.47 ± 0.08 t/ha (18 ± 3%) and at the boro 2018 harvest inversion plot yields were higher by 1.10 ± 0.24 (26 ± 6%) than those in the control plots. Yield did not differ between the 2017 inversions with added cow manure and mustard cake and the inversions without these soil amendments, so the data were combined in the box plot.

Multiple Linear Regression on Rice Yield as a Function of Soil As and Nutrients

We expected that lowered soil As concentrations in response to the soil inversion would correlate with higher rice yields, while lowered nutrient concentrations would correlate with lower rice yields. However, in a stepwise linear regression of rice yield difference between each

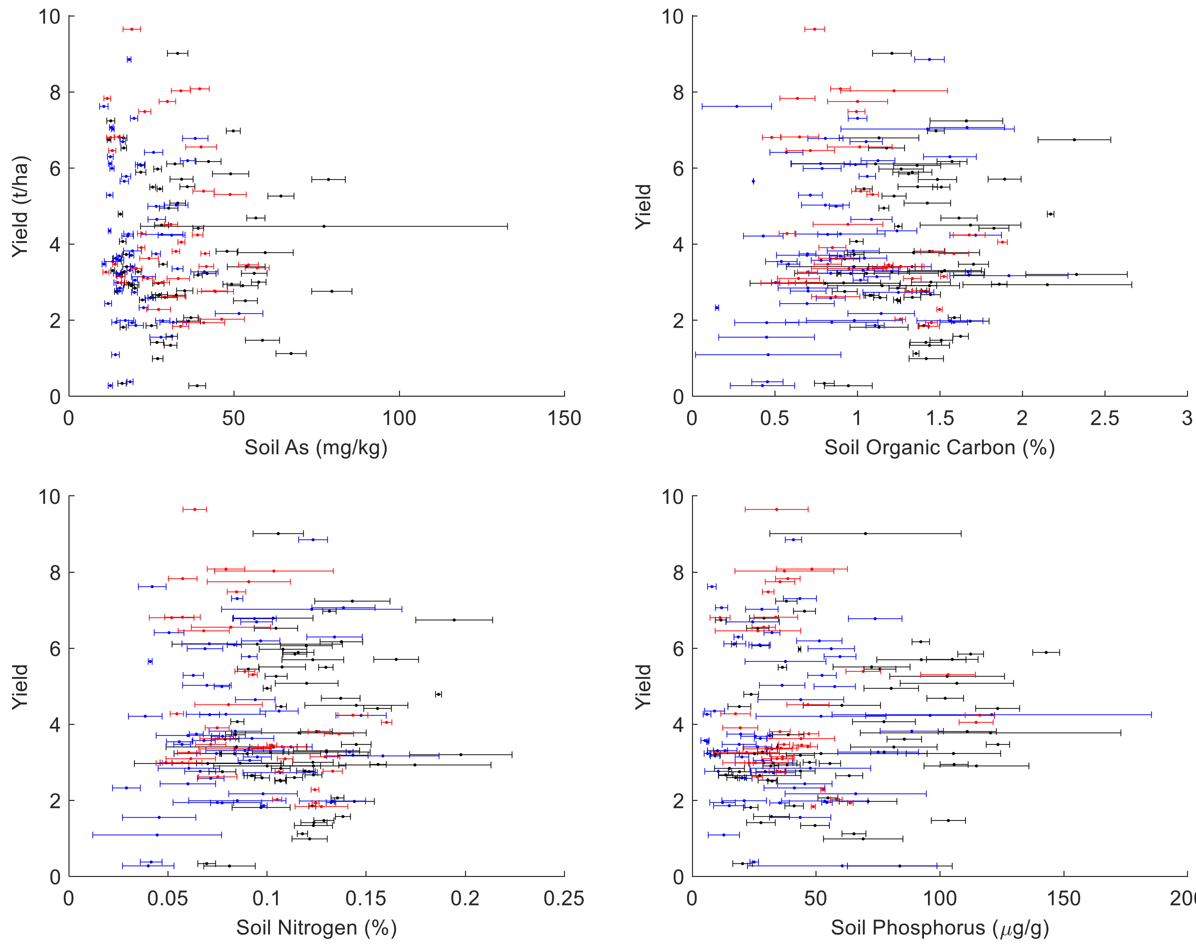


Figure 7. Yield as a function of soil As and nutrients. Yield in control (black), 2016 inversion (blue) and 2017 inversion (red) plots as a function of (a) soil As (mg/kg), (b) soil organic carbon (%), (c) soil nitrogen (%), and (d) and phosphorus (ug/g). Error bars are the standard error of the mean. Data are shown for all plots where yield was measured in each growing season.

inversion plot and its adjacent control plot as a function of soil As difference, nutrient differences, the year the inversion was conducted, and the growing season, no variable was a significant predictor of the rice yield difference at the $p = 0.05$ level. Furthermore, there were no visually identifiable relationships between rice yield and soil As, organic carbon, nitrogen, or phosphorus (Figure 7) or between the differences (inversion – control) for these parameters (Figure 8). Thus the differences in As and soil nutrients that we measured were unable to explain the one season lag followed by improvement in rice yield resulting from the soil inversion.

Irrigation Water Addition and Soil As Deposition

The amount of irrigation water added to rice field soil during the boro 2017 growing season at the monitored irrigation wells ranged from 0.4 to 1.6 m, with an average of 0.8 ± 0.1 m (Table 1). This estimate is close to the values of 0.8-1.5 m per season estimated with limited reference to data in Bhuiyan (Bhuiyan 1992) and is also close to the 1 m per year commonly cited without reference to a primary source (e.g. Heikens 2006; Meharg and Zhao 2012).

Table 1. Irrigation water and added and As deposited for 10 selected irrigation well command areas.

Site	Year pump installed	Pump depth (ft)	As concentration measured by ICP-MS (μg/L)	Pump rate (m^3/h)	Hours pumped during boro 2017 growing season	Paddy area irrigated (m^2)	Irrigation water applied (cm)	As added to soil (mg/kg)
Choradampur	1995	100	198.77	54.0 ± 0.1	523	52,000	54.4 ± 0.1	0.540 ± 0.001
Choradampur 2	2002	120	185.39	35.7 ± 1.0	201	16,640	43.1 ± 1	0.40 ± 0.01
Chornosipur 1 & 3	1985	240	150.3	35.0 ± 0.0	895	37,440	83.7 ± 0	0.63 ± 0
Doyarampur	1988	205	276.98	42.6 ± 0.3	570	60,320	40.3 ± 0.3	0.558 ± 0.004
Ikri 1 & 2	1996	250	209.87	52.4 ± 0.6	830	93,600	46.5 ± 0.6	0.488 ± 0.006
Middle Tambulkhana	1989	370	219.71	185.7 ± 8.1	871	166,400	97.1 ± 4	1.07 ± 0.05
Purbopara	1976	250	161.87	49.3 ± 1.3	822	62,400	64.9 ± 2	0.53 ± 0.01
Sachia	1990	275	207.66	168.5 ± 11.5	747	124,800	101 ± 7	1.05 ± 0.07
West Ikri	1995	195	260.42	39.3 ± 0.5	596	35,360	66.2 ± 0.9	0.86 ± 0.01
West Sachia	1996	150	101.44	56.3 ± 2.9	923	33,280	156 ± 8	0.79 ± 0.04

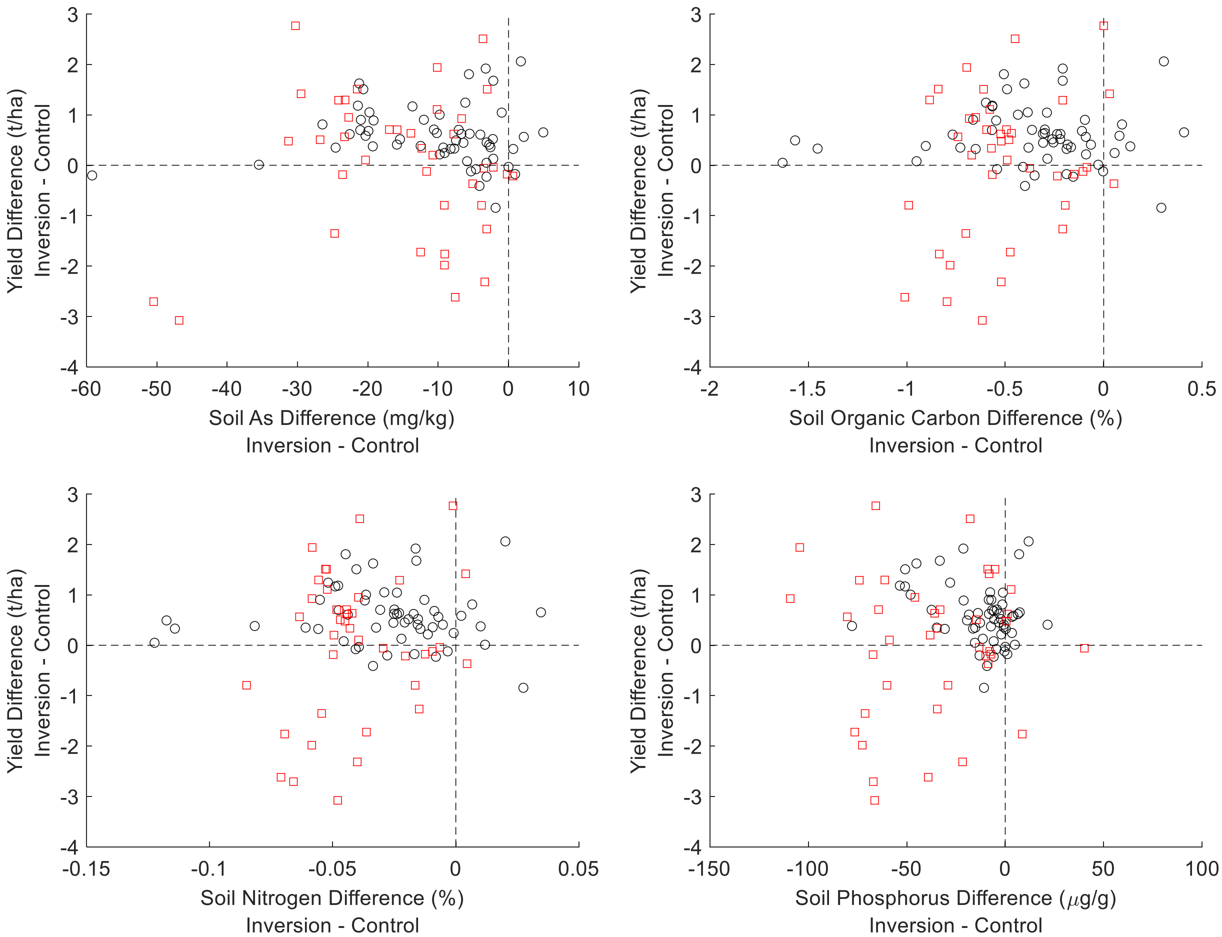


Figure 8. Yield difference between inversion and control plots as a function of soil As and nutrient differences. Yield difference in the first season after the soil inversion (red) and subsequent seasons after the soil inversion (black) as a function of (a) soil As difference (mg/kg), (b) soil organic carbon difference (%), (c) soil nitrogen difference (%), and (d) and phosphorus difference (ug/g). Data are shown for all plots where yield was measured in each growing season.

From the volume of irrigation water applied and the water As concentration, rates of As deposition can be estimated. Assuming a 1 kg/L soil density, even distribution of As across all rice fields irrigated by a well, and deposition of all irrigation water As within the top 20 cm of soil, an estimated 0.4-1.1 mg/kg As is added during a single growing season to the rice fields irrigated by these ten wells.

Discussion

Impact of the Soil Inversion on Rice Yield

The soil inversion decreased soil As concentrations and, after a one season lag, increased rice yield, but yield differences between inversion and control plots were not correlated with the soil As differences between those pairs of plots. Prior studies conducted on rice in Bangladesh have demonstrated a linear relationship between soil As concentrations and rice yield (Huhmann et al. 2017; Panaullah et al. 2009). However, in our prior study in this area, we did not observe a direct correlation between rice yield and soil As, but rather a correlation between soil As and yield *differences* between pairs of plots that had no systematic differences in parameters other than As (Huhmann et al. 2017).

The lack of a directly observed correlation between soil As and rice yield in our prior study indicates that other environmental variables can easily obscure the relationship between rice yield and soil As. In contrast with our prior study, where nutrients did not systematically differ between soil replacement and control plots, in this study we observed differences between soil inversion and control plots with respect to multiple soil nutrients. In addition to differences in the variables we measured, there were likely also differences in variables we did not measure, such as soil structure or microbial community. For example, the farmers reported that the soil in the flip plots was much softer than the soil in the adjacent control plots and difficult to plow during the first season after the flip. These unmeasured variables may have contributed to obscuring the relationship between soil As and yield and to the one-season lag in rice yield improvement following the soil inversions.

Another possible explanation for the lack of correlation between soil As difference and rice yield difference between inversion and control plots is that in addition to directly affecting rice yield, soil As may indirectly affect rice yield through its impacts on other soil characteristics. For example, lowering soil As concentrations may create an environment more conducive to soil pests such as nematodes (Hua et al. 2009), which are present in our study area

Longevity of the Soil Inversion Impact on Soil As

Even if the positive impacts of the soil inversion are related to factors other than soil As, it is valuable to understand the buildup of soil As in the inversion plots over time, since increasing soil As concentrations have negative yield effects. In our study plots over the two years of monitoring after the inversions, the soil As difference between control and inversion plots rapidly diminished. This may be because the soil inversions were conducted over a relatively small 5×5 m area, which may permit lateral mixing from surrounding high As soil over time. However, in our prior study conducted on 5×5 m plots in rice fields in the area, we did not observe evidence of substantial lateral mixing between plots over two years of monitoring (Huhmann et al. 2017). Another possibility is that, since the high As layer of soil remains present below the low As layer, there may be vertical mixing or diffusion of As between the high As and low As layers (Roberts et al. 2009). A soil removal, rather than inversion, conducted over a larger area would minimize (in the case of lateral mixing) or eliminate (in the case of vertical mixing or diffusion) these effects.

The buildup of As added to the soil via irrigation water is also likely to impact the longevity of a soil inversion. In contrast with the rebound of soil As in the inversion plots described above, As deposition from irrigation water should affect both inversion and control plots similarly and thus should not affect the As difference between the two. We estimated that 0.4 to 1.1 mg/kg soil As is deposited on average in the top 20 cm of soil around our high-As wells each year. We reached this estimate based on measuring As in irrigation water, since changes of this magnitude are too small to be distinguished based on our soil As measurements (Figure S2). Given that the soil inversions decreased As in the top 20 cm by about 12 mg/kg (2016 inversions) and 18 mg/kg (2017 inversions) on average, these As deposition rates suggest that boro rice irrigation alone could erase the impacts of a soil inversion or removal as quickly as about a decade or – in areas with a greater lowering of As from soil removal or lower rates of soil As buildup – as slowly as three to four decades.

This estimate does not take into account the varying spatial distribution of As or loss of As to monsoon flooding (Roberts et al., 2007). Incorporating the varying spatial distribution of As shortens the time estimate for the rebound, since soil As removal would most likely be targeted at the most contaminated rice fields, and these are often the fields closest to an irrigation well where soil As builds up the fastest (Panaullah et al. 2009; Roberts et al. 2007). Thus, localized rates of soil As buildup in intervention areas are likely to be faster than rates of soil As buildup averaged over the full irrigated area.

Incorporating loss of As to monsoon flooding lengthens the time estimate, since 13-46% of soil As may be lost during monsoon flooding rather than remaining in the paddy soil (Dittmar et al. 2010; Roberts et al. 2010). Collectively, then, these two factors partially balance each other out, and the exact rate of As buildup will depend on the specifics of each intervention. However, the fact that soil As does eventually build up again suggests that interventions to lower soil As are best used in conjunction with interventions to reduce the future buildup of soil As.

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