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Field study of rice yield diminished by soil arsenic in Bangladesh

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Abstract

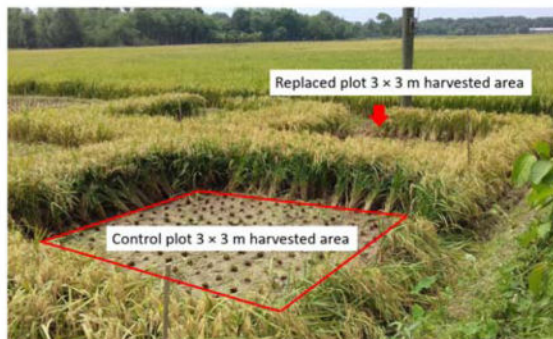
Rice was traditionally grown only during the summer (aman) monsoon in Bangladesh but more than half is now grown during the dry winter (boro) season and requires irrigation. A previous field study conducted in a small area irrigated by a single high-arsenic well has shown that the accumulation of arsenic (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.

Graphical Abstract

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Supporting Information

Figures showing the locations of the study plots, nutrients in replaced versus control plots, yield as a function of soil As, yield difference as a function of soil As difference, and soil arsenic concentrations at different distances from the center of the study plots. Tables showing irrigation water As for the field sites, soil As for the control plots, elemental XRF measurements on standards compared to the reported values, the plots for which yield was measured during each season, and soil nutrient concentrations in replaced versus control plots.



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^{1–4}. Winter season (boro) rice, grown in standing water maintained by groundwater irrigation, is the dominant crop in Bangladesh in both production⁵ and caloric consumption⁶. 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^{7–13}. 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 irrigation¹⁴.

Elevated As concentrations in irrigation water and soil have been found to decrease boro and aman rice yield in greenhouse studies and pot experiments^{8,9,15–20}. 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)²¹.

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 km² 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 $\mu\text{g/L}$ 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-MS²² (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^{12,21,23}.

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 country²⁴. 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 \times 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 \times 5 m soil exchange plots with an adjacent 5 \times 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 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 40°C and homogenized by mortar and pestle before As analysis with XRF, to ensure a moisture content and sample morphology similar to the NIST standards used²⁵.

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 40°C 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 ($+12 \pm 3$ 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 ($+0.8 \pm 0.4$ 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 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^{26–28}. 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 elements²⁹. 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 meters³⁰. 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 of soil types. Since BR 28 and BR 29 make up more than half of the total boro rice cropped area in Bangladesh²⁴, 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 $\mu\text{g/L}$ ³¹. 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 harvest⁸. 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^{32,33}, only 9% of land in Bangladesh is flooded to more than 1.8 m³², and shallowly flooded areas appear to retain their As²¹. 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 $-0.1 \text{ (t/ha)(mg/kg)}^{-1}$. 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 2012–2013² 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 $-0.057 \text{ (t/ha)(mg/kg)}^{-1}$ 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 km² 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^{3,34,35}.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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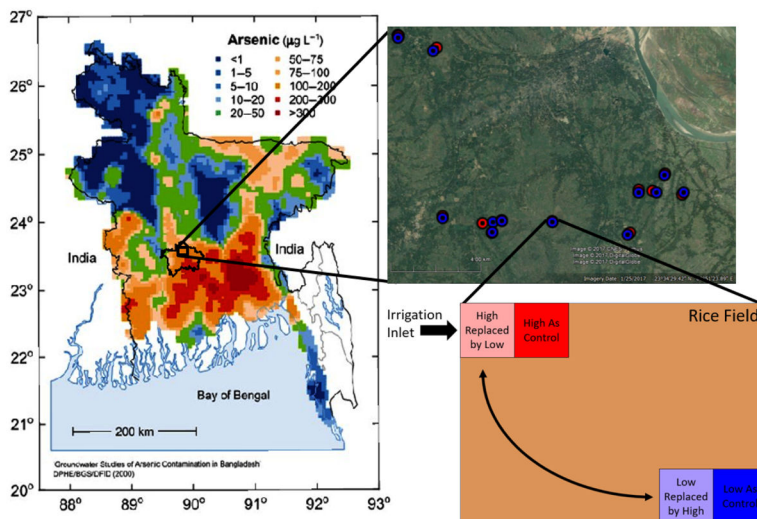


Figure 1. Soil exchange schematic and distribution of the thirteen study sites within a 150 km² area in Faridpur, Bangladesh

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.

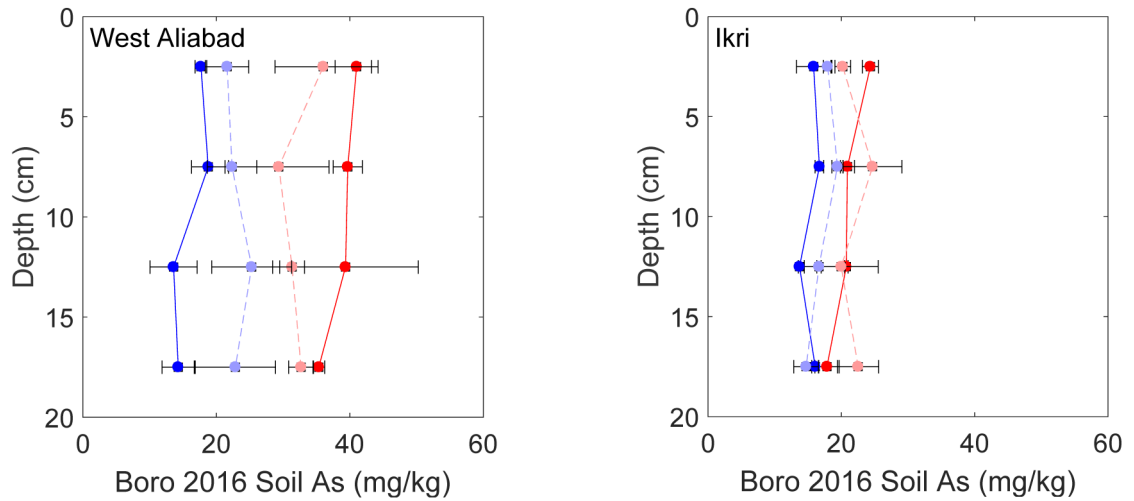


Figure 2. Depth profiles over the top 20 cm from two representative study sites

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.

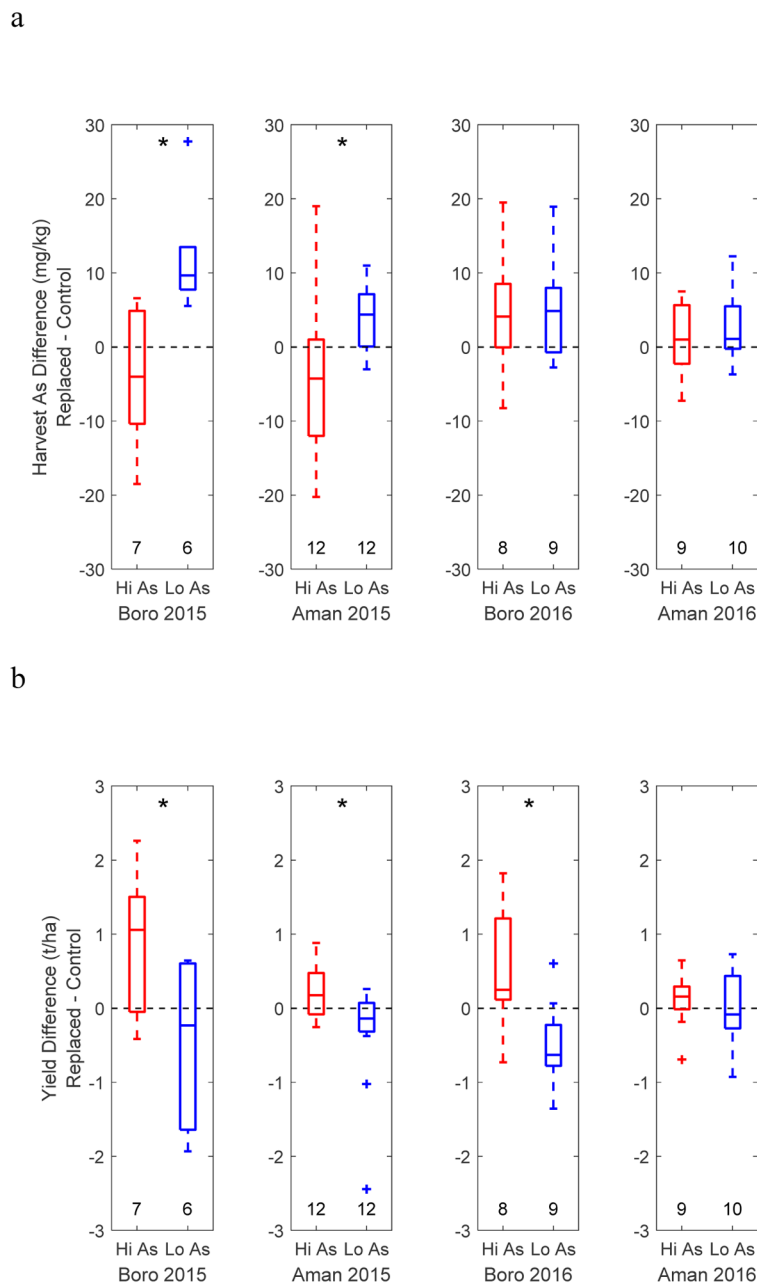


Figure 3. Soil As and yield differences between replacement and control plots
a. Differences in soil As between the replaced and adjacent control plots over the top 20 cm as measured by XRF on cores collected at harvest. **b.** Differences in rice yield between the replaced and adjacent control plots. 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.

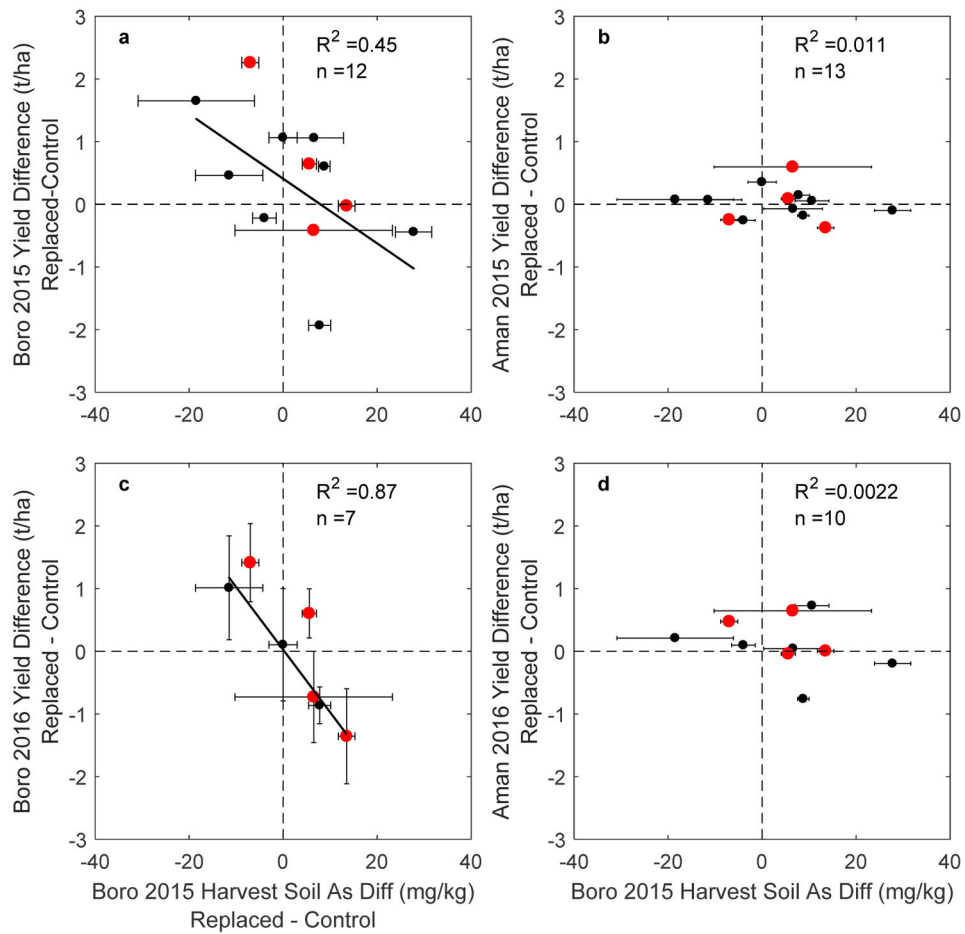


Figure 4. Rice yield difference as a function of soil As difference between replacement and control plots

a. Boro 2015 yield difference correlates with boro 2015 soil As difference ($y = -0.06 \pm 0.02x + 0.3 \pm 0.2$, $R^2 = 0.45$, 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 ($y = -0.10 \pm 0.02x + 0.02 \pm 0.14$, $R^2 = 0.87$, 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.