Arsenic contamination of rice (*Oryza sativa* L.) endangers global food security and human health (Meharg et al., 2009; Panaullah et al., 2009; Gilbert-Diamond et al., 2011; Banerjee et al., 2013; Seyfferth et al., 2014). Although knowledge of the extent and implications of arsenic contaminated rice is growing, it is unclear how the problem, and its associated threats, will change in the future under altered climate conditions.

Arsenic uptake by rice is directly related to concentration and speciation of arsenic available to roots, which is controlled by rhizosphere processes. In flooded paddy soil, arsenic is mobilized through reductive dissolution of arsenic-bearing iron(III) oxides (Ponnamperuma, 1972; Weber et al., 2010). Arsenic also enters rice paddies through the use of arsenic-based pesticides (Reed and Sturgis, 1936) or arsenic-contaminated irrigation water, as documented in Bangladesh (Roberts et al., 2007; Panaullah et al., 2009; Dittmar et al., 2010; Khan et al., 2010). Rice roots leak oxygen transported through aerenchyma into the rhizosphere (Colmer, 2003; Larsen et al., 2015). This oxygen can oxidize iron(II) and form iron(III)-oxide plaques that scavenge arsenic near or on roots (Hu et al., 2005; Frommer et al., 2010; Seyfferth et al., 2010; Williams et al., 2014). Roots take up arsenic from solution or desorbed from plaques. Arsenate enters roots through phosphate transporters (Abedin et al., 2002), while arsenite enters through aquaglyceroporins (Ma et al., 2008). Arsenic is then transported to grains and deposited mostly in the bran layer (Lombi et al., 2009; Carey et al., 2010; Seyfferth et al., 2011).

Rhizosphere processes are putatively sensitive to environmental conditions. Thus, arsenic availability to and uptake by rice could change as atmospheric CO₂ concentrations increase and the climate warms. By 2100, surface temperatures are expected to increase in rice growing regions by 1 to 5°C (Stocker et al., 2013), which will increase floodwater and soil temperatures. Floodwater

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**Abstract:** Arsenic uptake by rice (*Oryza sativa* L.) threatens yield and contaminates grain. Climate warming could affect these hazards. We tested the effect of elevated soil temperature on arsenic availability to and uptake by rice plants. Rice was grown in arsenic-amended soil in rhizoboxes that facilitated porewater sampling and synchrotron X-ray fluorescence (XRF) imaging of the rhizosphere. Plants were subjected to similar atmospheric conditions but different soil temperatures. The XRF imaging revealed greater arsenic sequestration in root iron plaques with a warmer soil temperature. Mean and median arsenic concentrations in porewater and root, straw, and husk tissue were positively correlated with average daily maximum soil temperature. Grain arsenic concentrations did not change. Warmer soil temperatures likely increased plant-available arsenic by increasing reductive dissolution of arsenic-bearing iron minerals, but the plants effectively regulated grain arsenic. The impacts of changing environmental conditions on arsenic contamination of rice should be further explored.

**Abbreviations:** cps, counts per second; ICP-MS, inductively coupled plasma mass spectrometry; XRF, X-ray fluorescence.
arsenic concentrations match those in Bangladeshi paddy soils and irrigation water (Roberts et al., 2007; Panaullah et al., 2009; Dittmar et al., 2010; Khan et al., 2010).

Plants grew for ~4.5 mo under similar atmospheric conditions (~30.5°C day/~23.5°C night), but in soils with different temperatures. Soil temperature was controlled by placing rhizoboxes in water baths and heating baths with hot plates. Two growth chambers were used. Each chamber contained three plants in an “ambient” and three plants in an “elevated” soil-temperature treatment (Supplemental Fig. S2), but one plant in an “elevated” treatment died (11 plants total at harvest). On Day 82, an elevated treatment experienced a transient temperature spike (1.5 d long) due to malfunctioning of a hot plate (Supplemental Fig. S3). While unplanned, the temperature spike facilitated a wider soil temperature gradient. Average daily maximum soil temperatures in the four treatments were 25.4, 26.1, 30.5, and 31.4°C (Supplemental Table S2), with the 31.4°C treatment experiencing the temperature spike. Atmospheric and ambient soil temperatures mimic current rice growth temperatures in Bangladesh (Supplemental Fig. S4), and elevated soil temperatures were ~5°C higher, the upper end of expected end-of-century warming for rice regions (Stocker et al., 2013).

During active tillering, panicle formation, heading, and post-grain-filling, three porewater samples (0.5–1.5 mL) were collected from each plant, acidified, and analyzed for arsenic via inductively coupled plasma mass spectrometry (ICP–MS; PerkinElmer Elan DRCe Quadrupole). Standards, internal standards, blanks, and spikes were run for quality control.

Approximately 5 wk after heading, plant stems were cut and rhizoboxes were moved into an anaerobic chamber. Two boxes, one each from the 26.1 and 30.5°C treatments, were prepared for synchrotron X-ray fluorescence (XRF) imaging of solid-phase arsenic and iron (see Supplemental Material, Section 1 for imaging details). Arsenic was desorbed from roots collected from remaining rhizoboxes using 1 M phosphate (Keon et al., 2001). Aboveground plant tissue and post-extraction roots treated with 0.05 M titanium-citrate-EDTA-bicarbonate to remove iron plaque (Keon et al., 2001) were oven dried (65°C), weighed, ground, and microwave digested in HNO3/H2O2. Solutions were analyzed for arsenic via ICP–MS.

The strength of positive monotonic correlation between both mean and median arsenic concentrations and soil temperature was measured with Kendall’s Tau test, and strength of positive linear correlation was measured with Pearson’s test and fit with a linear regression model (fitlm in Matlab). Statistical significance was accepted for p values ≤0.05. The mean is traditionally used to summarize data, but the median is more appropriate for small datasets because it is not strongly influenced by a few extreme observations (Helsel and Hirsch, 1991).

## Results

Arsenic concentrations measured in porewater and plant tissue systemically responded to soil temperature (Fig. 1), whereas plant growth, including grain yield and above- and belowground biomass, did not systematically respond (Supplemental Fig. S5). Variability within temperature treatments was, in many instances, large enough such that the interquartile range of measured arsenic concentrations in porewater and plant tissue in different treatments overlapped. But mean and median arsenic concentrations in porewater, root tissue, straw tissue, and husk tissue statistically increased with average daily maximum soil temperature (Fig. 1b,c,d,f; Supplemental Table S3). Additionally, median porewater arsenic, mean phosphate-extracted arsenic on roots, mean root tissue concentrations, and both mean and median straw tissue arsenic concentrations had statistically significant positive linear correlations with average daily maximum soil temperature (Fig. 1c–f; Supplemental Table S3).

Grain arsenic concentrations did not change with soil temperature (Fig. 1a). Median grain arsenic concentrations had a statistically significant linear correlation with average daily maximum soil temperature, but the change in median concentration across treatments was negligible (0.0038 μg g⁻¹ °C⁻¹). Further, Kendall’s test did not detect a positive temperature correlation for either mean or median arsenic concentrations in grain (Supplemental Table S3).

Although not necessarily statistically significant, linear fits suggest that within the plant, the concentration response to soil temperature decreased with increasing distance from the rhizosphere. Fitted slopes were greatest for mean and median phosphate-extracted arsenic on roots (34 and 37 μg g⁻¹°C⁻¹), followed by mean and median root tissue arsenic concentrations (4.1 and 3.8 μg g⁻¹°C⁻¹), mean and median straw tissue...
concentrations (0.55 and 0.63 \( \mu g \) g\(^{-1}\)°C\(^{-1}\)), mean and median husk tissue concentrations (0.07 \( \mu g \) g\(^{-1}\)°C\(^{-1}\)), and mean and median grain tissue concentrations (0.0038 and 0.013 \( \mu g \) g\(^{-1}\)°C\(^{-1}\)) (Fig. 1; Supplemental Table S3).

The XRF imaging showed that the plant from the 30.5°C soil-temperature treatment accumulated more solid-phase iron and arsenic at the root base and along the length of a few roots relative to the plant from the 26.1°C soil-temperature treatment (Fig. 2). Integrating counts per second (cps) from the images quantified that although the two plants had similar bulk solid-phase iron mass, the plant from the 30.5°C soil-temperature treatment accumulated 91% more iron mass as hotspots (i.e., pixels with >1000 cps iron) and 52% more solid-phase arsenic mass in the rhizosphere.

**Discussion**

We aimed to assess the impact of elevated soil temperatures on arsenic availability to and uptake by rice. Although natural variability and sample-size constraints limited treatment differences, mean and median arsenic concentrations in porewater and root, straw, and husk tissues were positively correlated with average daily maximum soil temperature. The temperature response was greater for plant tissue compartments closer to the rhizosphere. Grain arsenic concentrations did not change with soil temperature. These data signify that soil warming increased rhizosphere arsenic availability and that plants effectively controlled translocation and grain loading of additional arsenic (Song et al., 2014). Excess arsenic was sequestered in root-iron plaque (Fig. 2) and stored in other plant tissue (Fig. 1). From a human-health perspective, the negligible response of grain arsenic concentrations to soil temperature was fortunate.

Our experiment suggests that arsenic-relevant biogeochemical processes in the rice rhizosphere were sensitive to soil temperature. Warmer soil temperatures likely facilitated increased rates of microbially mediated reductive dissolution of arsenic-bearing iron oxides, releasing more arsenic from soil (Cho and Ponnamperuma, 1971; Weber et al., 2010). As the climate system warms and soil temperatures increase, rates and interactions of rhizosphere processes could change, altering availability and uptake of arsenic.
While we chose to isolate soil temperature, we posit that predicted future changes in other climate variables such as precipitation patterns, air temperatures, and atmospheric CO₂ concentrations could also affect plant and rhizosphere processes that govern arsenic availability to and uptake by rice. Future research should focus on gaining mechanistic understanding of how climate variables, operating in isolation and in combination, will affect arsenic cycling at the plant–soil nexus for a range of rice varieties growing in different soil types. Such understanding is required to evaluate future yield and human-health impacts of arsenic contamination and in combination, will affect arsenic cycling at the plant–soil nexus for a range of rice varieties growing in different soil types. Such understanding is required to evaluate future yield and human-health impacts of arsenic contamination.

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