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## Arsenic Accumulation in Hydroponically Grown *Schizachyrium scoparium* (Little Bluestem) Amended with Root-Colonizing Endophytes

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Supporting Information

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Additional figures and tables (PDF)

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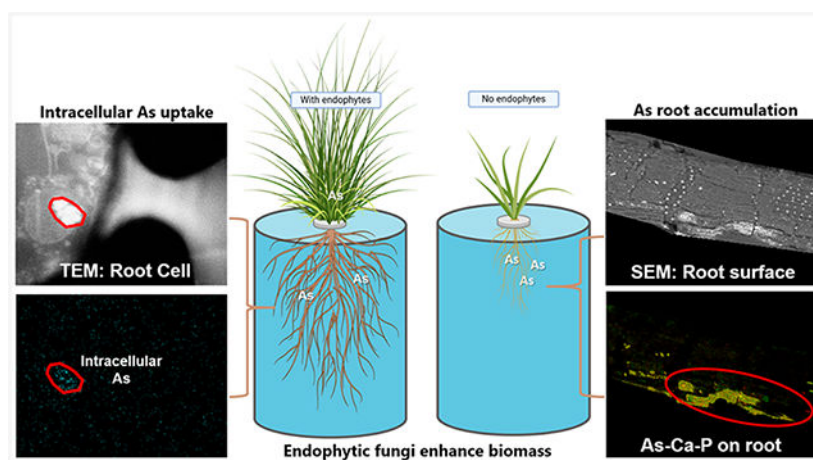
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**Abstract**

We integrated microscopy, spectroscopy, culturing and molecular biology, and aqueous chemistry techniques to evaluate arsenic (As) accumulation in hydroponically grown *Schizachyrium scoparium* inoculated with endophytic fungi. *Schizachyrium scoparium* grows in historically contaminated sediment in the Cheyenne River Watershed and was used for laboratory experiments with As(V) ranging from 0 to 2.5 mg L<sup>-1</sup> at circumneutral pH. Arsenic accumulation in regional plants has been a community concern for several decades, yet mechanisms affecting As accumulation in plants associated with endophytic fungi remain poorly understood. Colonization of roots by endophytic fungi supported better external and vascular cellular structure, increased biomass production, increased root lengths and increased P uptake, compared to noninoculated plants (*p* value <0.05). After exposure to As(V), an 80% decrease of As was detected in solution and accumulated mainly in the roots (0.82–13.44 mg kg<sup>-1</sup>) of noninoculated plants. Endophytic fungi mediated intracellular uptake into root cells and translocation of As. Electron microprobe X-ray mapping analyses detected Ca–P and Mg–P minerals with As on the root surface of exposed plants, suggesting that these minerals could lead to As adsorption on the root surface through surface complexation or coprecipitation. Our findings provide new insights regarding biological and physical–chemical processes affecting As accumulation in plants for risk assessment applications and bioremediation strategies.

**Graphical Abstract**



## Keywords

Endophytes; fungi; arsenic; accumulation; grass; little bluestem

## INTRODUCTION

Widespread arsenic (As) contamination of plants and sediment due to natural and anthropogenic processes has been extensively documented worldwide.<sup>1–8</sup> This study was conducted within the context of previous work, which identified As contamination in sediments from the Cheyenne River Watershed,<sup>9</sup> and there are existing community concerns about As accumulation in local plants. Many As uptake and accumulation studies have focused on rice (*Oryza sativa*) not only because of its agronomic importance, but also because rice is cultivated in regions where soils contain natural As concentrations and in flooded conditions where As can be readily mobilized from soil.<sup>10–13</sup> However, the watershed is characterized by semiarid grassland and 90% of the economy depends on grasses for agricultural and livestock purposes.<sup>14,15</sup> Understanding the potential mechanisms for tolerance and As accumulation in these plants can aid in risk reduction strategies for nearby communities.

Plants support several mechanisms of As tolerance, detoxification, and accumulation, including low rates of As(V) uptake, transformation of inorganic As to less toxic organic forms, and complexation and sequestration to nonreactive locations.<sup>16–20</sup> Additionally, volatilization or efflux of accumulated As can reduce the total As present in a plant.<sup>21–25</sup> Plants growing in heavily As-contaminated soils (such as *Holcus lanatus* and *Cytisus striatus*) can achieve hypertolerance by decreasing the rate of As uptake through suppression of phosphate transporter activity.<sup>26–29</sup> Conversion of inorganic As species to volatile methylated As compounds occur in bacteria and fungi and are proposed to exist in plants as well.<sup>30–33</sup> Microorganisms in plant rhizospheres provide additional pathways for As tolerance, detoxification, and accumulation, but much remains unknown about plant–microbe interactions under As contamination.

The colonization of the rhizosphere by microorganisms represents a natural process commonly found in various types of soils, including those affected by agricultural and mining activities.<sup>34,35</sup> Plants also interact with symbiotic organisms such as mycorrhizal fungi, rhizobial and endophytic bacteria, plant growth promoting rhizobacteria, and endophytic fungi.<sup>36,37</sup> Arbuscular mycorrhizal (AM) symbiosis can mediate the oxidation and reduction of As, transform inorganic As into a less toxic organic form, and enhance plant growth to minimize the concentration of accumulated As in plant tissues.<sup>38,34</sup> Although AM has been the focus of many plant-fungi investigations, endophytic fungi are present in ubiquitous plants in the natural environment and contribute to plant growth and tolerance to environmental stress conditions, such as metal contamination.<sup>39</sup> Endophytic fungi are mutualistic symbionts that grow in the intercellular spaces of plant tissues and are transmitted by seeds.<sup>40</sup> For example, dark septate endophytes are known to colonize the root tissues of plants and belong to a few orders of Ascomycota.<sup>41</sup> These fungi are septate and generally have hyphae that colonize the cortical cells and intercellular regions of roots.<sup>42</sup> Endophytes can provide multiple benefits to tall grasses, such as increased drought tolerance, pathogen resistance, nutrient acquisition, and overall plant vitality.<sup>43–50</sup>

Endophytic fungi in the phylum Ascomycota dominate heavy metal contaminated sites worldwide. For example, Ascomycota was the most abundant fungal phylum in a gold mining site,<sup>34</sup> and ascomycetes generally have higher relative abundances in contaminated sites than uncontaminated sites.<sup>51–53</sup> Several Ascomycota exhibit high tolerance to heavy metals and interact with metals through biosorption, bioprecipitation, or bioreduction and volatilization.<sup>31,54–56</sup> Commonly studied genera in research on As or other metals include *Aspergillus*, *Fusarium*, and *Talaromyces*.<sup>55,57–61</sup> These taxa are also commonly found in symbiosis with plant roots. These endophytes in the seedlings of some grasses have been isolated and examined but not under the environmental stress of metals such as As.

To our knowledge, limited studies exist to evaluate the role of endophytic fungi on As accumulation from grasses grown in the study area, which justifies the need for this study. *Schizachyrium scoparium* was chosen for our hydroponic investigation because of its widespread distribution in the watershed and in arid North American grasslands. Although fungi can associate with many plants, the chemical and biological mechanisms of As uptake in native grasses, such as *Schizachyrium scoparium* (little bluestem) are not well understood. Thus, our objective was to determine the effect of Ascomycota root endophytes on As accumulation using laboratory hydroponic experiments to improve knowledge of interfacial processes that affect As accumulation in little bluestem grass. We predicted that inoculation with endophytes would increase plant growth under As exposure conditions, thereby reducing As toxicity.

## MATERIALS AND METHODS

### Germination and Colonization of Endophytes.

We grew *Schizachyrium scoparium* from seeds obtained from southwestern U.S. regional seed stock (Curtis and Curtis, Clovis, NM; Western Native Seed, Coaldale, CO). We first removed the palea and lemma, which can house native fungal communities from the parent seed source. Seeds were internally sterilized using a 55 °C water bath for 15 min, followed

by external sterilization in 70% ethanol for 2 min and 2% NaClO for 3 min. Seeds were then rinsed four times using autoclaved Milli-Q water before inoculation with select fungal isolates. We plated a subset of seeds on malt extract agar (MEA) to confirm sterilization effectiveness. The seeds were inoculated with one of two fungal species, *Cadophora sp.* or *Talaromyces pinophilus* by placing seeds next to fungal agar in sterile growing chambers. Seedlings were cleared with 1 M KOH and stained with alinine blue according to a previously recorded method.<sup>62</sup> Isolates used in this study were cultured from little bluestem during a latitudinal survey of root endophytes across the central U.S.<sup>48</sup> In addition, a subset of sterile seeds was placed in a sterile growing chamber with MEA as a noninoculated control. Little bluestem is a bunchgrass with extensive root systems requiring soil substrate to support plant growth.<sup>63</sup> Thus, we placed seeds in autoclaved sand for 5 weeks at room temperature and watered with sterile Milli-Q water for an initial germination period.

### Hydroponic Experiments.

After germination, the seedlings were moved to a sterile hydroponic system (200 mL) supplied with NPK liquid fertilizer prepared in purified water. Seedlings were grown in these conditions for six months under 25 °C (day) and 21 °C (night) temperature in a 18 h/6 h light/dark cycle. Daylength is a critical factor in growing little bluestem. The 6-month-old seedlings from the hydroponic system were then acclimatized to conditions relevant to the Cheyenne River Watershed for 5 d by placing them separately in 500 mL sterile Nalgene polypropylene (PP) bottles with conditioning solution (Supporting Information, Table S1). Conditioning solutions were freshly prepared in sterile ultrapure water containing simplified Hoagland nutrients (MgSO<sub>4</sub>, 0.5 mM; NH<sub>4</sub>NO<sub>3</sub>, 2 mM; KCl, 1 mM; NaHCO<sub>3</sub>, 5 mM; KH<sub>2</sub>PO<sub>4</sub>, 0.12 mM and CaCl<sub>2</sub>·2H<sub>2</sub>O, 3 mM) at pH 7.5. After 5 d, plants inoculated with *Cadophora sp.* were exposed to 0, 0.5, or 2.5 mg L<sup>-1</sup> Na<sub>3</sub>AsO<sub>4</sub> for 7 d before harvesting for chemical and microscopy analyses. Because of the large number of replicates and similar response to endophytic colonization, only *Cadophora sp.* was chosen for the hydroponic experiments. Aliquots were collected from the hydroponic solution at 0, 12, 24, 48, 96, or 168 h for chemical analyses.

### Chemical Analyses.

Aliquots collected from the hydroponic solution were acidified using 2% ultrahigh purity nitric acid (HNO<sub>3</sub>), and total As concentration was measured with a PerkinElmer NexION 300D (dynamic reaction cell) inductively coupled plasma-mass spectrometer (ICP-MS) with a detection limit <0.5 µg L<sup>-1</sup>. The total phosphorus (P) content in acid-digested root and stems were analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES, PerkinElmer Optima 5300DV). Laboratory blanks and QA/QC measures were taken and checked to ensure quality data. At harvest, plants were separated into roots and stems and washed three times with Ultrapure Milli-Q water. Whole root, and stem fresh weights were recorded before drying plant material at 60 °C for 12 h, weighing dry material, then pulverizing for acid digestions. Roots and stems were acid digested in triplicate under progressive heating using 10 mL reagent-grade HNO<sub>3</sub> using a previously used method.<sup>64</sup> Following acid digestion, samples were diluted and filtered using a 0.45 µm filter for As analyses using ICP-MS.

## Microscopy and Spectroscopy Analyses.

Cross-section samples were prepared for transmission electron microscopy (TEM) and scanning electron microscopy (SEM) from the finer roots of the plants. Plant roots were fixed with osmium tetroxide to preserve membrane structure and increase contrast for imaging. To account for the possible interference of  $\text{PO}_4^{3-}$ , tannic acid was also used as a stain and counterstained with uranyl acetate and lead citrate references. Dried roots were fixed on aluminum stubs and coated with a thin layer of carbon for SEM imaging and microprobe X-ray mapping for the root surface. Microstructural and microanalytical studies were performed using a JEOL 2010F FASTEM field emission gun scanning TEM (FEGSTEM/TEM) instrument equipped with an Oxford AZtec EDS system using an Oxford X-Max N 80 mm<sup>2</sup> ultrathin window SDD EDS detector. The root cross section was characterized using bright-field TEM (BF-TEM), high-angle annular dark-field (HAADF) STEM, electron diffraction, and STEM EDS X-ray mapping. Full spectral X-ray maps of the areas of interest were obtained and EDS spectra of regions were extracted from the X-ray maps using the AZtec software. Digital TEM and STEM images were acquired and processed using GATAN Digital Microscopy Suite (DMS) imaging software. Electron microprobe analyses were conducted using a JEOL JXA-8200 SuperProbe electron probe microanalyzer (EPMA) with wavelength dispersive X-ray spectroscopy (WDS) at an accelerating voltage of 15 kV with a 10  $\mu\text{m}$  probe diameter and 10 nA probe current. The quantitative data was reduced using the Phi-Rho-Z correction method in the Probe for EPMA software (Probe Software, Inc., Eugene, OR).

## Chemical Equilibrium Modeling.

Visual MINTEQ was used to predict the expected aqueous As species and various complexes and precipitates that could form in solution during exposure under atmospheric equilibrium and circumneutral pH. The aqueous As species were calculated using inputs based on the chemical composition of the conditioning and exposure solutions (Supporting Information, Table S1) with 0 mM (Control), 0.667 mM (low), 33.3 mM (high) As at pH 7.5 and 25 °C.

## RESULTS AND DISCUSSION

### Inoculation of Endophytes with Plants.

Fungal colonization was confirmed by microscopy and PCR analysis 14 d after inoculation in plant growing chambers. Aseptate and septate hyphae were observed within finer roots using Brightfield microscopy (Figure 1). Hyphae were the principal fungal structures observed under the cuticle within the epidermal cells. Images also confirmed the localization of endophytes in root vascular cells. (Figure 2). PCR and Illumina sequencing confirmed cultures of the inoculum in plants grown with endophytes (Supporting Information, Table S2).

Statistical difference was detected using a parametric *t* test for biomass measurements in plants grown with fungi and without fungi ( $p < 0.05$ ). Plants inoculated with fungi were 1800% larger than noninoculated plants (Table 1). Statistical difference was also detected for root length using a parametric *t* test in plants grown with fungi and without fungi ( $p < 0.05$ ).



Plant roots inoculated with fungi were 132% longer than plant roots grown without fungi (Table 1). Stem lengths were also statistically different between plants grown with and without fungi ( $P < 0.05$ ) using a parametric  $t$  test. Stem lengths were 71% longer than plant stems grown without fungi. Several studies have illustrated the positive effect of fungal endophyte colonization on increased root-shoot length and biomass production.<sup>37,65,66</sup> It should be noted that the soluble P levels in these experiments are relatively high, compared to several pore water and groundwater P measurements, as reported in other studies.<sup>67–69</sup> However, it has also been reported that some fungi can increase net P uptake under both high and low P levels.<sup>70</sup>

Before exposure to As, inoculated plants with endophytes significantly increased ( $p < 0.05$ ) the amount of P measured in stems compared to noninoculated control plants (Supporting Information, Figure S2A). In non-As control experiments with inoculated plants, the mass percentage of P in the stems was 86.4% (corresponding to  $3228.6 \pm 361 \text{ mg kg}^{-1}$  dry biomass). The mass percentage of P in stems of noninoculated control plants was 53.7% ( $2470.6 \pm 442 \text{ mg kg}^{-1}$  dry biomass), indicating that plants with fungi translocated more P (Supporting Information, Figure S2B). These results suggest that endophytic fungi can help facilitate the uptake of P into the stems. The effects on P with addition of As to noninoculated and inoculated plants is summarized in Supporting Information, Figure S2C,D. The mass percentage of P in the stems of inoculated plants (83%) is similar to noninoculated plants (88%) after As exposure.

### Arsenic Accumulation in Hydroponic Experiments.

Noninoculated plants had significantly higher As in the roots ( $p < 0.05$ ) with less root-to-shoot translocation compared to inoculated plants. Noninoculated plant roots had  $8.03 \pm 4.1 \text{ As mg kg}^{-1}$  dry biomass compared to plant roots with fungi  $4.95 \pm 1.1 \text{ mg kg}^{-1}$ . (Figure 4A). Compared to noninoculated plants, more As was detected in the stems of plants inoculated with fungi, which indicated that translocation occurred. We measured  $3.49 \pm 0.5 \text{ mg kg}^{-1}$  As dry biomass in the stems of plants inoculated with *Cadophora sp.* and the translocation factor in inoculated plants was 0.76 (Figure 4B). The mass of accumulated P and As (Supporting Information, Figure S2 and Supporting Information, Tables S5 and S6) in plants inoculated with fungi suggest that both As and fungi can affect the uptake of P into plants. Future P uptake analyses with As and are needed to understand these specific mechanisms. In addition to plant dry biomass measurements, As concentrations were monitored in the hydroponic system.

An 80% decrease in As concentration was detected in the hydroponic solution within the first 12 h, indicating rapid As removal in all experimental systems (Figure 5). The decrease in As in solution could be due to biologically mediated uptake in plants with or without fungi or chemically mediated adsorption and precipitation of As in the hydroponic system. The pH in hydroponic media ranged between 7.04 and 8.10 throughout the experiment. The most noticeable changes in hydroponic solution pH occurred in noninoculated plants over 168 h, suggesting the influence of a chemical reaction on the decrease of As observed. An abiotic control experiment (without a plant) with As and conditioning solution at pH 7.5 did not result in removal of As from the hydroponic solution. Thus, biotic and abiotic

hydroponic experiments suggested that 80% As removal was due to accumulation in or on exposed plants.

We used chemical equilibrium modeling to help understand relevant reactions affecting water chemistry in the hydroponic experiments at circumneutral pH (7.5). A summary of the aqueous chemical speciation and saturation indices using inputs based on the hydroponic solution used is provided in Supporting Information, Table S3. The charge of As is negative given that the predominant As species was  $\text{HAsO}_4^{2-}$  (82.1%), and  $\text{H}_2\text{AsO}_4^-$  (17.9%) was also present at a lower percentage. The charge of Ca, Mg, and K was mainly positive given that  $\text{Ca}^{2+}$  (90.9%),  $\text{Mg}^{2+}$  (92.2%), and  $\text{K}^+$  (99.5%) are predominant. However, aqueous complexes with phosphate, sulfate, carbonate, and chloride were formed. Although the predominant species of phosphate were  $\text{HPO}_4^{2-}$  (49.5%) and  $\text{H}_2\text{PO}_4^-$  (17.4%), the rest of the phosphate species consists of aqueous complexes with Ca (24.2%  $\text{CaHPO}_4$  and 2.3%  $\text{CaPO}_4^-$ ), Mg (5.6%  $\text{MgHPO}_4$ ) and K (0.26%  $\text{KHPO}_4^-$ ).

Several Ca and  $\text{PO}_4$  solids like  $\text{Ca}_3(\text{PO}_4)_2(\text{s})$ ,  $\text{Ca}_3(\text{PO}_4)_2(\text{s})$ , and hydroxyapatite [ $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2(\text{s})$ ] can precipitate at the circumneutral pH range (7.5) of this study (Supporting Information, Table S4). Calcium-apatite minerals can react with metals (Cd, Zn) in solution to form surface complexes, mixed metal phosphate precipitates, and metal coprecipitation.<sup>71,72</sup> Other studies have reported that removal of As from solution can be achieved by precipitation of apatite-like minerals at pH 8–10,<sup>73,74</sup> which is within the pH range investigated in our study. Previous work in a soil environment shows that Ca–P amendments with As-contaminated soil can decrease mobility of As where geochemical modeling confirmed oversaturated soil solutions with respect to several Ca–P–As(V) phases.<sup>74</sup> The presence and distribution of As with Ca and P on root cells caused by exposure to As in the hydroponic solution was confirmed by electron microscopy analyses.

### Microscopy Results.

Grass roots colonized by *Cadophora* sp. were detected in intracellular spaces of plants by TEM (Figure 2A). The TEM analyses of control plants without fungal inoculation also detected the presence of nonfungal microorganisms, indicating that the application of heat, ethanol, and hypochlorite in these control plants did not result in complete microbial sterilization or that plants were colonized during growth in the hydroponic system (Figure 2C). EDS and X-ray STEM mapping detected the intracellular accumulation of As in the cellular structure of inoculated roots (Supporting Information, Figure S1). Electron microprobe analyses conducted on the plant root surface detected the presence of As and Mg–P solid phases in plants inoculated with fungi (Figure 3B). These results suggest that in the presence of fungi As was accumulated both inside the root cells and on the surface of the roots. In experiments without fungi, TEM-EDS analyses did not detect As in the intracellular structure, and the presence of As and Ca–P solid phases were detected on the root surface (Figure 3A). Different sites on the plant roots were analyzed with electron microprobe. However, the detection of As only coincided with the presence of Ca and P precipitates on the surface of roots in noninoculated plants.

The formation of As–P–Ca and As–Mg–P solids can lead to the sequestration of As on the roots of plants by adsorption or coprecipitation. The formation of these solids on the plant



roots can partly explain the decrease of As measured within the first 12 h likely caused by adsorption and chemical precipitation with Ca, Mg, and P solids. The equilibrium with respect to these solids explains why As concentrations remained steady in the hydroponic solution until the end of the experiment. An increase of pH from 7 to 8.10 within the first 48 h of exposure coincides with the removal of <85% As in solution, which is a favorable pH range for Ca and P solid formation in other studies.<sup>74</sup> Additionally, substitutions of  $\text{PO}_4^{3-}$  ions or other anions on the root surface may partly explain the limited As detected inside roots using microscopy. Another plant investigation using the addition of hydroxyapatite as a metal immobilizing additive confirmed similar As sequestration in roots exposed to hydroxyapatite amended contaminated soil.<sup>75</sup> These results indicate that As may be heterogeneously distributed in the plant roots and that limited As made it into the intracellular spaces within the plant.

### Biological and Chemical Considerations.

Our results indicate that biological and chemical processes influenced As accumulation in hydroponic systems. The morphology of intracellular root structures and biomass changes enhanced by the fungi were related to plant As tolerance. Other studies have illustrated the positive effect of fungal associations on increased plant root and biomass production.<sup>50,51</sup> Control plants without endophytes exposed to high and low concentrations of As experienced inhibited root growth and in a few cases, died before harvest. When plants are exposed to toxic metalloids, the most common protection mechanism is limiting transport into the aerial parts of the plant, resulting in accumulation of metals primarily in the root system.<sup>76</sup> Root morphology is also an important health parameter to analyze arsenic toxicity.<sup>60</sup> Arsenic treated plants without fungal inoculum had less root biomass and were the ones that died before harvest compared to inoculated plants. This effect was consistent with As toxicity studies in nontolerant plant species that undergo stress symptoms, which include inhibition of root growth, decreased biomass, and death.<sup>77–80</sup> Further evidence from contaminated field sites suggest that fungal populations can develop tolerance to As, resulting in improved host performance. For instance, fungal isolates of *G. mosseae* and *G. caledonium* associated with velvet grass roots from an As-contaminated site were more As(V) tolerant than those from the non As-contaminated site.<sup>81</sup> As(V) uptake in the tolerant velvet grass growing in the As-contaminated site was reduced by inoculating with the tolerant fungal isolates. Thus, it is necessary to integrate the knowledge of biological processes with chemical speciation to further understand the mechanisms of As uptake mediated by fungi in plants.

Since As and P are chemical analogues and can exhibit similar geochemical behavior,<sup>1</sup> the role of P can play a role on the uptake of As in inoculated plants. Arsenate and phosphate can compete for sites on the root surface for binding and adsorption. In addition, previous work suggests that As uptake can be decreased by increasing external P concentrations.<sup>82</sup> Phosphate transport systems have also been identified in various plants,<sup>83–85</sup> which can partly explain the accumulation of As and P observed in our study. Phosphate transporters also exist in several fungi,<sup>86,87</sup> and indirect intracellular uptake into the cortical tissues of plant roots by fungal hyphae is also possible due to the thinner and finer structure of hyphae.<sup>88</sup> Though it is well-known that P can suppress the uptake of As(V), we cannot draw any

major conclusions about P suppression. The high P concentration in the hydroponic solution selected for this study were based on several years of field measurements (Supporting Information, Figure S1) and typical hydroponic nutrient solution values. Previous research has demonstrated that both high and low P concentrations can increase net P uptake rates in the presence of fungi equipped with P transporters.<sup>70</sup> Thus, using a lower concentration of P in our study can be explored in future research initiatives with endophytic fungi.

Chemical equilibrium modeling indicated that calcium phosphate minerals, such as hydroxyapatite and magnesium minerals like dolomite, were likely to precipitate at the pH range investigated in this study. Oversaturation with respect to Ca–P–As(V) phases has been previously reported when Ca and P have been amended in As-contaminated soil, resulting in decreased mobility of As.<sup>74</sup> In aqueous solutions, hydroxyapatite can lead to an immobilization of Al, Cd, Cu, Fe(II), Ni, and Zn, and several mechanisms have been proposed for immobilization of these metals: ion exchange processes at the surface of hydroxyapatite, surface complexation, and coprecipitation.<sup>72,89,90</sup> For example, immobilization due to adsorption through surface complexation with phosphate, calcium, and hydroxyl surface groups of hydroxyapatite, or coprecipitation of new partially soluble phases were reported.<sup>91,92</sup> Thus, the possible adsorption through surface complexation and coprecipitation on the root surface could affect the accumulation of As in the hydroponic experiments investigated in this study.

## CONCLUSION

Our findings provide new information on the accumulation of As and P in plants amended with endophytic fungi. We observed As accumulation under all experimental conditions in this study; however, the degree of accumulation differed between inoculated and noninoculated plants. Arsenic mainly accumulated in or on the roots of noninoculated plants by adsorption and precipitation with Ca–P minerals on the root surface, as observed by microprobe X-ray mapping. Plants with endophytic fungi translocated As concentrations to the stems and intracellular As were detected by STEM analysis. The mass percentage of As in the stems was also significantly greater in inoculated plants. These results suggest that fungi increased As penetration into the root cells and enhanced root-to-shoot translocation. Additionally, P uptake significantly increased in plants inoculated compared to control plants without fungi. Both As and fungi affected the distribution of P in our plants, but further studies are needed to better understand these results. Endophyte-colonized roots were also the healthiest at the end of the experiment, compared to many defunct noninoculated plants. Endophytic fungi significantly enhanced root length and biomass production, which also enabled the inoculated plants to better tolerate As. The increased biomass and length of endophyte colonized roots could provide sites for adsorption and precipitation reactions with Ca–P and Mg–P minerals that could accumulate As. The results from this study provide information that can be useful for remediation applications that make use of the interaction of fungi and plants for uptake and accumulation of As. Future studies are necessary to better understand relevant conditions that would favor translocation of As mediated by fungi and P for the development of these bioremediation strategies. It is also important to consider that chemical processes, such as adsorption and precipitation to solid mineral phases as reported in this study, can be integrated to ensure immobilization of As and other metals.

Additionally, As accumulation in native grasses like *Schizachyrium scoparium* with endophytic fungi can affect community and agricultural practices. The simplified conditions represented in this hydroponic study represent an initial effort to understand As accumulation in *Schizachyrium scoparium* and more work is needed to elucidate the combined roles of As, fungi, and macronutrients (P, Mg, Ca) on As uptake. Because the root zone is the first plant contact with potentially harmful elements in the rhizosphere, the effects of soil organic matter and redox conditions on As accumulation also remain subject to future investigations. The results from this study have important implications for risk assessments and future studies are necessary to investigate the role of plants and organic matter in soils to better understand pathways of exposure to As and potential bioremediation applications with fungi.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## REFERENCES

- (1). Smith E; Naidu R; Alston A Arsenic in the soil environment: A review. *Adv. Agron* 1998, 64, 149–195.
- (2). Fendorf S; Herbel M; Tufano KJ; Kocar BD Biophysico Chem. Proc. of Heavy Metals and Metalloids in Soil Environments. In *Biogeochemical Processes Controlling the Cycling of Arsenic in Soils and Sediments*; Wiley-Interscience: Hoboken, NJ, 2007; pp 313–338
- (3). Fendorf SNPS; Kocar BD; Masue Y; Tufano KJ Arsenic chemistry in soils and sediments. In *Dev. in Soil Science* 2010, 34, 357–378.
- (4). Seyffert AL; Fendorf S Silicate Mineral Impacts on the Uptake and Storage of Arsenic and Plant Nutrients in Rice (*Oryza sativa* L.). *Environ. Sci. Technol* 2012, 46 (24), 13176–13183. [PubMed: 23153302]
- (5). Koch I; Wang L; Ollson C; Cullen W; Reimer K The predominance of inorganic arsenic species in plants from Yellowknife, Northwest Territories, Canada. *Environ. Sci. Technol* 2000, 34 (1), 22–26.
- (6). La Force M; Hansel C; Fendorf S Arsenic speciation, seasonal transformations, and co-distribution with iron in a mine waste-influenced palustrine emergent wetland. *Environ. Sci. Technol* 2000, 34 (18), 3937–3943.
- (7). Filippi M; Drahota P; Machovic V; Bohmova V; Mihaljevic M Arsenic mineralogy and mobility in the arsenic-rich historical mine waste dump. *Sci. Total Environ* 2015, 536, 713–728. [PubMed: 26254072]
- (8). Nghiem AA; Shen Y; Stahl M; Sun J; Haque E; DeYoung B; Nguyen KN; Thi Mai T; Trang PTK; Pham HV; Mailloux B; Harvey CF; van Geen A; Bostick BC Aquifer-Scale Observations of Iron Redox Transformations in Arsenic-Impacted Environments to Predict Future Contamination. *Environ. Sci. Technol. Lett* 2020, 7, 916. [PubMed: 33604397]
- (9). DeVore CL; Rodriguez-Freire L; Mehdi-Ali A; Ducheneaux C; Artyushkova K; Zhou Z; Latta DE; Lueth VW; Gonzales M; Lewis J; Cerrato JM Effect of bicarbonate and phosphate on arsenic

release from mining-impacted sediments in the Cheyenne River watershed, South Dakota, USA. *Environ. Sci.-Proc. Imp* 2019, 21, 456.

- (10). Verma S; Verma P; Meher A; Bansiwali A; Tripathi R; Chakrabarty D A novel fungal arsenic methyltransferase, WaarsM reduces grain arsenic accumulation in transgenic rice (*Oryza sativa* L.). *J. Hazard. Mater* 2018, 344, 626–634. [PubMed: 29112921]
- (11). Ghosh P; Maiti T; Pramanik K; Ghosh S; Mitra S; De T The role of arsenic resistant *Bacillus aryabhattai* MCC3374 in promotion of rice seedlings growth and alleviation of arsenic phytotoxicity. *Chemosphere* 2018, 211, 407–419. [PubMed: 30077937]
- (12). Dittmar J; Voegelin A; Maurer F; Roberts L; Hug S; Saha G; Ali M; Badruzzaman A; Kretzschmar R Arsenic in Soil and Irrigation Water Affects Arsenic Uptake by Rice: Complementary Insights from Field and Pot Studies. *Environ. Sci. Technol* 2010, 44 (23), 8842–8848. [PubMed: 21043519]
- (13). Seyfferth A; McCurdy S; Schaefer M; Fendorf S Arsenic Concentrations in Paddy Soil and Rice and Health Implications for Major Rice-Growing Regions of Cambodia. *Environ. Sci. Technol* 2014, 48 (9), 4699–4706. [PubMed: 24712677]
- (14). Heakin AJ; USGS Water quality trends for the Cheyenne and Moreau Rivers, Cheyenne River Indian Reservation, South Dakota, 1972–94; U.S. Geological Survey: Rapid City, SD, 1998.
- (15). Mulkey V; Owens V; Lee D Management of warm-season grass mixtures for biomass production in South Dakota USA. *Bioresour. Technol* 2008, 99 (3), 609–617. [PubMed: 17349789]
- (16). Zhao FJ; Ma JF; Meharg AA; McGrath SP Arsenic Uptake and Metabolism in Plants. *New Phytol.* 2009, 181 (4), 777. [PubMed: 19207683]
- (17). Clemens S Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. *Biochimie* 2006, 88 (11), 1707–1719. [PubMed: 16914250]
- (18). Raab A; Schat H; Meharg A; Feldmann J Uptake, translocation and transformation of arsenate and arsenite in sunflower (*Helianthus annuus*): formation of arsenic-phytochelatin complexes during exposure to high arsenic concentrations. *New Phytol.* 2005, 168 (3), 551–558. [PubMed: 16313638]
- (19). Meng X; Qin J; Wang L; Duan G; Sun G; Wu H; Chu C; Ling H; Rosen B; Zhu Y Arsenic biotransformation and volatilization in transgenic rice. *New Phytol.* 2011, 191 (1), 49–56. [PubMed: 21517874]
- (20). Huang Z; Chen T; Lei M; Liu Y; Hu T Difference of toxicity and accumulation of methylated and inorganic arsenic in arsenic-hyperaccumulating and -hypertolerant plants. *Environ. Sci. Technol* 2008, 42 (14), 5106–5111. [PubMed: 18754355]
- (21). Chen Y; Xu W; Shen H; Yan H; Xu W; He Z; Ma M Engineering Arsenic Tolerance and Hyperaccumulation in Plants for Phytoremediation by a PvACR3 Transgenic Approach. *Environ. Sci. Technol* 2013, 47 (16), 9355–9362. [PubMed: 23899224]
- (22). Duan G; Kamiya T; Ishikawa S; Arao T; Fujiwara T Expressing ScACR3 in Rice Enhanced Arsenite Efflux and Reduced Arsenic Accumulation in Rice Grains. *Plant Cell Physiol.* 2012, 53 (1), 154–163. [PubMed: 22107880]
- (23). Ali W; Isner J; Isayenkov S; Liu W; Zhao F; Maathuis F Heterologous expression of the yeast arsenite efflux system ACR3 improves *Arabidopsis thaliana* tolerance to arsenic stress. *New Phytol.* 2012, 194 (3), 716–723. [PubMed: 22380876]
- (24). Verma S; Verma P; Pande V; Tripathi R; Chakrabarty D Transgenic *Arabidopsis thaliana* expressing fungal arsenic methyltransferase gene (WaarsM) showed enhanced arsenic tolerance via volatilization. *Environ. Exp. Bot* 2016, 132, 113–120.
- (25). Jia Y; Huang H; Sun G; Zhao F; Zhu Y Pathways and Relative Contributions to Arsenic Volatilization from Rice Plants and Paddy Soil. *Environ. Sci. Technol* 2012, 46 (15), 8090–8096. [PubMed: 22724924]
- (26). Porter E; Peterson P Arsenic tolerance in grasses growing on mine waste. *Environ. Pollut* 1977, 14 (4), 255–265.
- (27). Meharg A; Macnair M An altered phosphate uptake system in arsenate tolerant *Holcus Lanatus* L. *New Phytol.* 1990, 116 (1), 29–35.
- (28). Bleeker PM; Schat H; Vooijs R; Verkleij JAC; Ernst WHO Mechanisms of Arsenate Tolerance in *Cytisus striatus*. *New Phytol.* 2003, 157 (1), 33. [PubMed: 33873707]

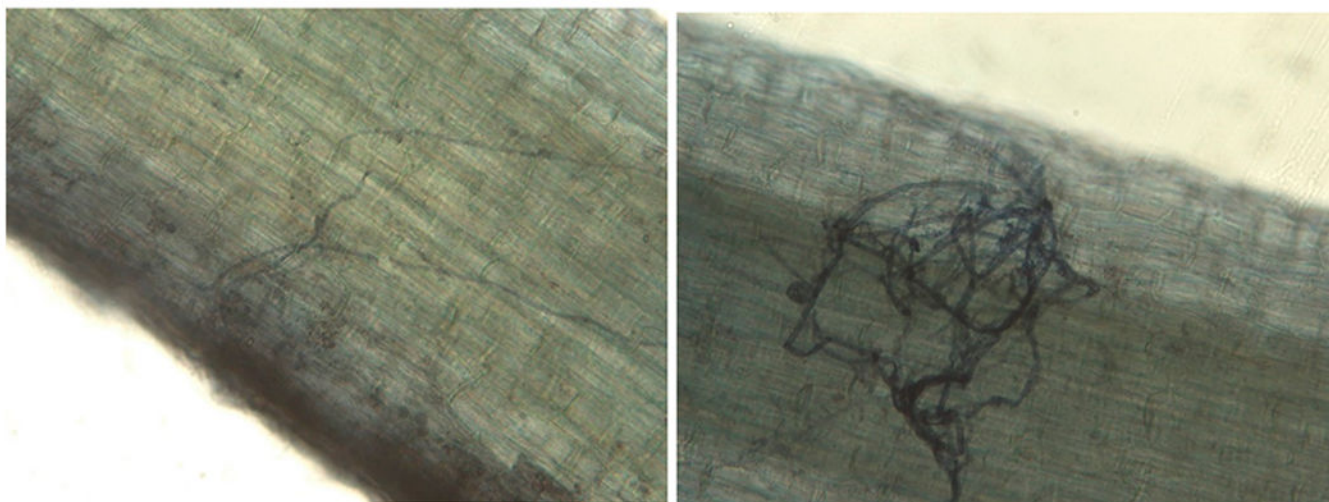
- (29). Bleeker P; Assuncao A; Teiga P; de Koe T; Verkleij J Revegetation of the acidic, As contaminated Jales mine spoil tips using a combination of spoil amendments and tolerant grasses. *Sci. Total Environ* 2002, 300 (1–3), 1–13. [PubMed: 12685466]
- (30). Lomax C; Liu WJ; Wu L; Xue K; Xiong J; Zhou J; McGrath SP; Meharg AA; Miller AJ; Zhao FJ Methylated arsenic species in plants originate from soil microorganisms. *New Phytol.* 2012, 193 (3), 665–72. [PubMed: 22098145]
- (31). Cernansky S; Urik M; Sevc J; Khun M Biosorption and biovolatilization of arsenic by heat-resistant fungi. *Environ. Sci. Pollut. Res* 2007, 14, 31–35.
- (32). Li J; Sun Y; Zhang X; Hu Y; Li T; Zhang X; Wang Z; Wu S; Wu Z; Chen B A methyltransferase gene from arbuscular mycorrhizal fungi involved in arsenic methylation and volatilization. *Chemosphere* 2018, 209, 392–400. [PubMed: 29935468]
- (33). Huang K; Chen C; Zhang J; Tang Z; Shen Q; Rosen BP; Zhao F-J Efficient Arsenic Methylation and Volatilization Mediated by a Novel Bacterium from an Arsenic-Contaminated Paddy Soil. *Environ. Sci. Technol* 2016, 50, 6389. [PubMed: 27258163]
- (34). Crognale S; D'Annibale A; Pesciaroli L; Stazi S; Petruccioli M Fungal Community Structure and As-Resistant Fungi in a Decommissioned Gold Mine Site. *Front. Microbiol* 2017, 8, 2202. [PubMed: 29170658]
- (35). Johansson JF; Paul LR; Finlay RD Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS Microbiol. Ecol* 2004, 48 (1), 1–13. [PubMed: 19712426]
- (36). Vandenkoornhuyse P; Quaiser A; Duhamel M; Le Van A; Dufresne A The importance of the microbiome of the plant holobiont. *New Phytol.* 2015, 206 (4), 1196. [PubMed: 25655016]
- (37). Rodriguez RJ; White JF Jr.; Arnold AE; Redman RS Fungal Endophytes: Diversity and Functional Roles. *New Phytol.* 2009, 182 (2), 314. [PubMed: 19236579]
- (38). Christophersen HM; Smith FA; Smith SE Arbuscular mycorrhizal colonization reduces arsenate uptake in barley via downregulation of transporters in the direct epidermal phosphate uptake pathway. *New Phytol.* 2009, 184 (4), 962–74. [PubMed: 19754635]
- (39). Domka A. M. Ig.; Rozpaadek P; Turnau K Are Fungal Endophytes Merely Mycorrhizal Copycats? The Role of Fungal Endophytes in the Adaptation of Plants to Metal Toxicity. *Front. Microbiol* 2019, 10, 371. [PubMed: 30930857]
- (40). Schardl CL; Leuchtmann A; Spiering MJ Symbioses of grasses with seedborne fungal endophytes. *Annu. Rev. Plant Biol* 2004, 55 (1), 315. [PubMed: 15377223]
- (41). Andrade-Linares D; Franken P; Aroca R Fungal Endophytes in Plant Roots: Taxonomy, Colonization Patterns, and Functions. *Soil Biol.* 2013, 37, 311–334.
- (42). Knapp D; Pintye A; Kovacs G The Dark Side Is Not Fastidious - Dark Septate Endophytic Fungi of Native and Invasive Plants of Semiarid Sandy Areas. *PLoS One* 2012, 7 (2), e32570. [PubMed: 22393417]
- (43). Malinowski DP; Belesky DP Ecological importance of Neotyphodium spp. grass endophytes in agroecosystems. *Grassl. Sci* 2006, 52 (1), 1–14.
- (44). Gwinn KD; Gavin AM Relationship between endophyte infestation level of tall fescue seed lots and rhizotonia-zeae seedling disease. *Plant Dis.* 1992, 76, 911–914.
- (45). Siddiqui ZA; Mahmood I Some Observations on the Management of the Wilt Disease Complex of Pigeonpea by Treatment with a Vesicular Arbuscular Fungus and Biocontrol Agents for Nematodes. *Bioresour. Technol* 1995, 54, 227.
- (46). Malinowski DP; Belesky DP Neotyphodium coenophialum-Endophyte Infection Affects the Ability of Tall Fescue to Use Sparingly Available Phosphorus. *J. Plant Nutr* 1999, 22, 835.
- (47). Rahman MH; Saiga S Endophytic fungi (Neotyphodium coenophialum) affect the growth and mineral uptake, transport and efficiency ratios in tall fescue (*Festuca arundinacea*). *Plant Soil* 2005, 272 (1-2), 163.
- (48). Rudgers JA; Fischer S; Clay K Managing plant symbiosis: fungal endophyte genotype alters plant community composition. *J. Appl. Ecol* 2010, 47, 468.
- (49). MARKS S; CLAY K Physiological Responses of *Festuca arundinacea* to Fungal Endophyte Infection. *New Phytol.* 1996, 133 (4), 727.

- (50). Hiruma K; Kobae Y; Toju H Beneficial associations between Brassicaceae plants and fungal endophytes under nutrient-limiting conditions: evolutionary origins and host-symbiont molecular mechanisms. *Curr. Opin. Plant Biol* 2018, 44, 145–154. [PubMed: 29738938]
- (51). Simonovicova A; Krakova L; Pauditsova E; Pangallo D Occurrence and diversity of cultivable autochthonous microscopic fungi in substrates of old environmental loads from mining activities in Slovakia. *Ecotoxicol. Environ. Saf* 2019, 172, 194–202. [PubMed: 30708231]
- (52). Jaswal R; Pathak A; Chauhan A Metagenomic Evaluation of Bacterial and Fungal Assemblages Enriched within Diffusion Chambers and Microbial Traps Containing Uraniferous Soils. *Microorganisms* 2019, 7, 324.
- (53). Stepniewska H; Uzarowicz Ł; Błońska E; Kwasowski W; Ślodziński Z; Gałka D; Hebda A Fungal abundance and diversity as influenced by properties of Technosols developed from mine wastes containing iron sulphides: A case study from abandoned iron sulphide and uranium mine in Rudki, south-central Poland. *Appl. Soil Ecol* 2020, 145, 103349.
- (54). Srivastava PK; Vaish A; Dwivedi S; Chakrabarty D; Singh N; Tripathi RD Biological removal of arsenic pollution by soil fungi. *Sci. Total Environ* 2011, 409 (12), 2430–2442. [PubMed: 21459413]
- (55). Govarthanan M; Mythili R; Kamala-Kannan S; Selvankumar T; Srinivasan P; Kim H In-vitro bio-mineralization of arsenic and lead from aqueous solution and soil by wood rot fungus, *Trichoderma* sp. *Ecotoxicol. Environ. Saf* 2019, 174, 699–705. [PubMed: 30878010]
- (56). Button M; Koch I; Watts MJ; Reimer KJ Arsenic speciation in the bracket fungus *Fomitopsis betulina* from contaminated and pristine sites. *Environ. Geochem. Health* 2020, 42, 2723. [PubMed: 31897873]
- (57). Ayad F; Matallah-Boutiba A; Rouane-Hacene O; Bouderbala M; Boutiba Z Tolerance of *Trichoderma* sp. to Heavy Metals and its Antifungal Activity in Algerian Marine Environment. *J. Pure Appl. Microbiol* 2018, 12 (2), 855–870.
- (58). Khan AR; Mahmood-Ul-Hassan M; Ahmad R; Munir A Arsenic (III) tolerance potential of fungi isolated from polluted and non-polluted soils of Pakistan. *Pak. J. Agr. Res* 2017, 30 (1), 18–29.
- (59). Yang S; Hai FI; Nghiem LD; Price WE; Roddick F; Moreira MT; Magram SF Understanding the factors controlling the removal of trace organic contaminants by white-rot fungi and their lignin modifying enzymes: A critical review. *Bioresour. Technol* 2013, 141, 97–108. [PubMed: 23499178]
- (60). Mohd S; Shukla J; Kushwaha AS; Mandrah K; Shankar J; Arjaria N; Saxena PN; Narayan R; Roy SK; Kumar M Endophytic Fungi *Piriformospora indica* Mediated Protection of Host from Arsenic Toxicity. *Front. Microbiol* 2017, 8, 754 DOI: 10.3389/fmicb.2017.00754. [PubMed: 28539916]
- (61). Segura FR; Paulelli ACC; Braga GÚL; dos Reis Pedreira Filho W; Silva FF; Batista BL Promising filamentous native fungi isolated from paddy soils for arsenic mitigation in rice grains cultivated under flooded conditions. *J. Environ. Chem. Eng* 2018, 6 (4), 3926–3932.
- (62). Hood M; Shew H Applications of KOH-aniline blue fluorescence in the study of plant-fungal interactions. *Phytopathology* 1996, 86 (7), 704–708.
- (63). Boe A; Bortnem R Morphology and Genetics of Biomass in Little Bluestem. *Crop Science* 2009, 49 (2), 411–418.
- (64). El Hayek E; Torres C; Rodriguez-Freire L; Blake J; DeVore C; Brearley A; Spilde M; Cabaniss S; Ali A; Cerrato J Effect of Calcium on the Bioavailability of Dissolved Uranium(VI) in Plant Roots under Circumneutral pH. *Environ. Sci. Technol* 2018, 52 (22), 13089–13098. [PubMed: 30412391]
- (65). Bilal L; Asaf S; Hamayun M; Gul H; Iqbal A; Ullah I; Lee I-J; Hussain A Plant growth promoting endophytic fungi *Aspergillus fumigatus* TS1 and *Fusarium proliferatum* BRL1 produce gibberellins and regulates plant endogenous hormones. *Symbiosis* 2018, 76 (2), 117–127.
- (66). Khan A; Ullah I; Waqas M; Shahzad R; Hong S-J; Park G-S; Jung B; Lee I-J; Shin J-H Plant growth-promoting potential of endophytic fungi isolated from *Solanum nigrum* leaves. *World J. Microbiol. Biotechnol* 2015, 31 (9), 1461–1466. [PubMed: 26081602]
- (67). Hens M; Merckx R Functional characterization of colloidal phosphorus species in the soil solution of sandy soils. *Environ. Sci. Technol* 2001, 35 (3), 493–500. [PubMed: 11351719]

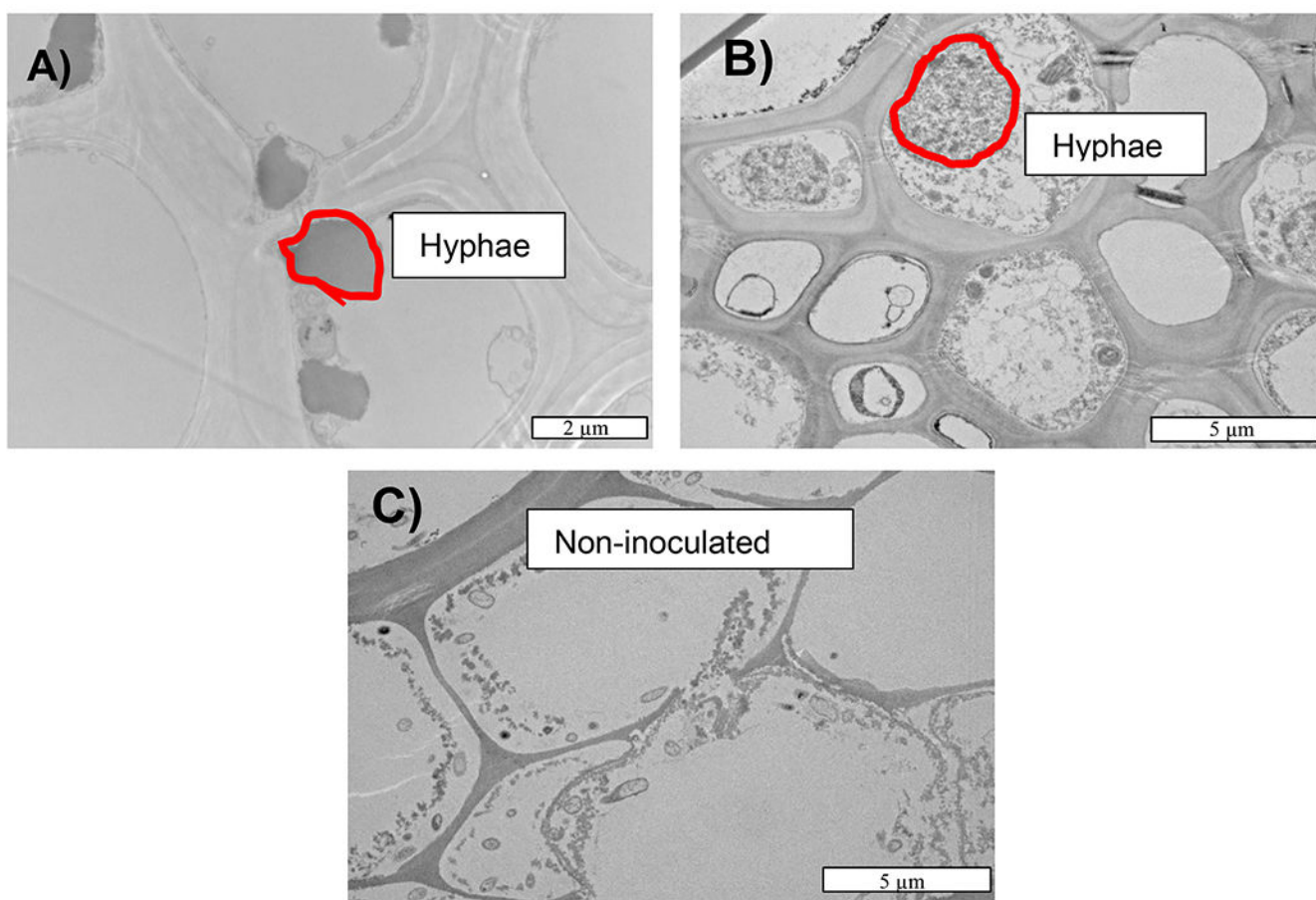


- (68). Young EO; Ross DS Phosphate release from seasonally flooded soils: a laboratory microcosm study. *J. Environ. Qual* 2001, 30 (1), 91–101. [PubMed: 11215671]
- (69). Meyer G; Bell MJ; Doolette CL; Brunetti G; Zhang Y; Lombi E; Kopittke PM Plant-Available Phosphorus in Highly Concentrated Fertilizer Bands: Effects of Soil Type, Phosphorus Form, and Coapplied Potassium. *J. Agric. Food Chem* 2020, 68 (29), 7571–7580. [PubMed: 32657588]
- (70). Tatry MV; El Kassis E; Lambilliotte R; Corratge C; van Aarle I; Amenc LK; Alary R; Zimmermann S; Sentenac H; Plassard C Two differentially regulated phosphate transporters from the symbiotic fungus *Hebeloma cylindrosporum* and phosphorus acquisition by ectomycorrhizal *Pinus pinaster*. *Plant J.* 2009, 57, 1092. [PubMed: 19054369]
- (71). Xu Y; Schwartz F; Traina S Sorption of  $Zn^{2+}$  and  $Cd^{2+}$  on hydroxyapatite surface. *Environ. Sci. Technol* 1994, 28 (8), 1472–1480. [PubMed: 22165931]
- (72). Ma QY.; Traina SJ; Logan TJ; Ryan J. A. Effects of aqueous Al, Cd, Cu, Fe(II), Ni and Zn on PB immobilization by Hydroxyapatite. *Environ. Sci. Technol* 1994, 28 (7), 1219. [PubMed: 22176311]
- (73). Twidwell LG; Plessas KO; Comba PG; Dahnke DR In Removal of arsenic from wastewaters and stabilization of arsenic bearing waste solids: Summary of experimental studies, Netherlands; Elsevier Science Division: Netherlands, 1994; p 69.
- (74). Neupane G; Donahoe RJ Calcium-phosphate treatment of contaminated soil for arsenic immobilization. *Appl. Geochem* 2013, 28, 145–154.
- (75). Boisson J; Ruttens A; Mench M; Vangronsveld J Evaluation of hydroxyapatite as a metal immobilizing soil additive for the remediation of polluted soils. Part 1. Influence of hydroxyapatite on metal exchangeability in soil, plant growth and plant metal accumulation. *Environ. Pollut* 1999, 104, 225–233.
- (76). Lemos Batista B; Nigar M; Mestrot A; Alves Rocha B; Barbosa Junior F; Price AH; Raab A; Feldmann J Identification and quantification of phytochelatins in roots of rice to long-term exposure: evidence of individual role on arsenic accumulation and translocation. *J. Exp. Bot* 2014, 65 (6), 1467–1479. [PubMed: 24600019]
- (77). MacNair MR; Cumbes Q Evidence that arsenic tolerance in *Holcus Lanatus L.* is caused by an altered phosphate uptake system. *New Phytol.* 1987, 107 (2), 387. [PubMed: 33873845]
- (78). Meharg AA; Macnair MR The Mechanisms of Arsenate Tolerance in *Deschampsia cespitosa (L.) Beauv.* and *Agrostis capillaris L.* Adaptation of the Arsenate Uptake System. *New Phytol.* 1991, 119 (2), 291. [PubMed: 33874130]
- (79). Georgia P; Thomas CH Arsenic, Cobalt and Nickel Tolerances in Two Populations of *Silene vulgaris (Moench) Garcke* from Ontario, Canada. *New Phytol.* 1991, 117 (3), 449. [PubMed: 33874306]
- (80). Carbonell-Barrachina AA; Aarabi MA; DeLaune RD; Gambrell RP; Patrick WH Jr. The influence of arsenic chemical form and concentration on *Spartina patens* and *Spartina alterniflora* growth and tissue arsenic concentration. *Plant Soil* 1998, 198, 33.
- (81). Gonzalez-Chavez C; Harris P; Dodd J; Meharg A Arbuscular mycorrhizal fungi confer enhanced arsenate resistance on *Holcus lanatus*. *New Phytol.* 2002, 155 (1), 163–171. [PubMed: 33873289]
- (82). Wang L; Duan G Effect of external and internal phosphate status on arsenic toxicity and accumulation in rice seedlings. *J. Environ. Sci. (Beijing, China)* 2009, 21 (3), 346–51.
- (83). Cao Y; Sun D; Chen JX; Mei H; Ai H; Xu G; Chen Y; Ma LQ Phosphate Transporter PvPht1;2 Enhances Phosphorus Accumulation and Plant Growth without Impacting Arsenic Uptake in Plants. *Environ. Sci. Technol* 2018, 52 (7), 3975–3981. [PubMed: 29539263]
- (84). Sun D; Feng H; Li X; Ai H; Sun S; Chen Y; Xu G; Rathinasabapathi B; Cao Y; Ma LQ Expression of New *Pteris vittata* Phosphate Transporter PvPht1;4 Reduces Arsenic Translocation from the Roots to Shoots in Tobacco Plants. *Environ. Sci. Technol* 2020, 54, 1045. [PubMed: 31825207]
- (85). Feng H; Li X; Sun D; Chen Y; Xu G; Cao Y; Ma LQ Expressing Phosphate Transporter PvPht2;1 Enhances P Transport to the Chloroplasts and Increases Arsenic Tolerance in *Arabidopsis thaliana*. *Environ. Sci. Technol* 2021, 55 (4), 2276–2284. [PubMed: 33497189]
- (86). Burns DJ; Beever RE Kinetic characterization of the two phosphate uptake systems in the fungus *Neurospora crassa*. *J. Bacteriol* 1977, 132 (2), 511–9. [PubMed: 144113]

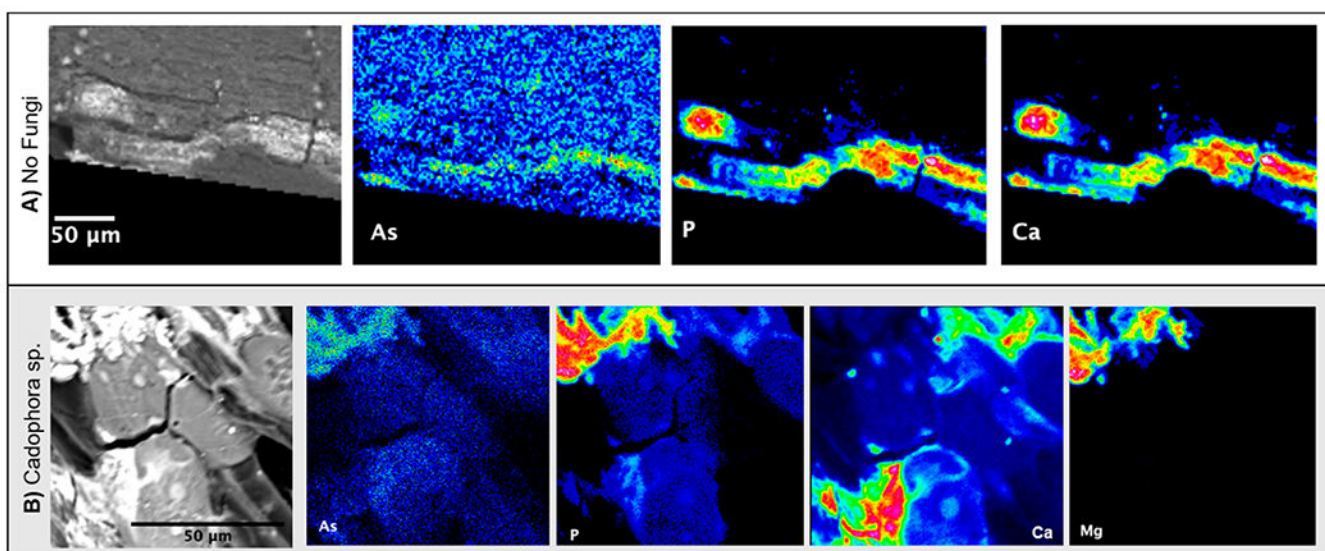
- (87). Yadav V; Kumar M; Deep DK; Kumar H; Sharma R; Tripathi T; Tuteja N; Saxena AK; Johri AK A phosphate transporter from the root endophytic fungus *Piriformospora indica* plays a role in phosphate transport to the host plant. *J. Biol. Chem* 2010, 285 (34), 26532–44. [PubMed: 20479005]
- (88). Johri AK; Oelmüller R; Dua M; Yadav V; Kumar M; Tuteja N; Varma A; Bonfante P; Persson BL; Stroud RM Fungal association and utilization of phosphate by plants: success, limitations, and future prospects. *Front. Microbiol* 2015, 984 DOI: 10.3389/fmicb.2015.00984. [PubMed: 26528243]
- (89). Suzuki T; Hatsushika T; Hayakawa Y Synthetic hydroxyapatites employed as inorganic cation-exchangers. *J. Chem. Soc., Faraday Trans. 1* 1981, 77, 1059–1062.
- (90). Guan D; Ren C; Wang J; Zhu Y; Zhu Z; Li W Characterization of Lead Uptake by Nano-Sized Hydroxyapatite: A Molecular Scale Perspective. *ACS Earth Sp. Chem* 2018, 2 (6), 599–607.
- (91). Bostick WD; Stevenson RJ; Jarabek RJ; Conca JL Use of Apatite and Bone Char for the Removal of Soluble Radionuclides in Authentic and Simulated DOE Groundwater. *Adv. Environ. Res* 1999, 488.
- (92). Czerniczyniec M; Farías S; Magallanes J; Cicerone D Arsenic(V) Adsorption onto Biogenic Hydroxyapatite: Solution Composition Effects. *Water, Air, Soil Pollut.* 2007, 180 (1–4), 75–82.



**Figure 1.**  
Light microscope image of hyphae of *Cadophora sp.* in little bluestem grass root cleared with 1 M KOH and stained with blue alanine (20× magnification).



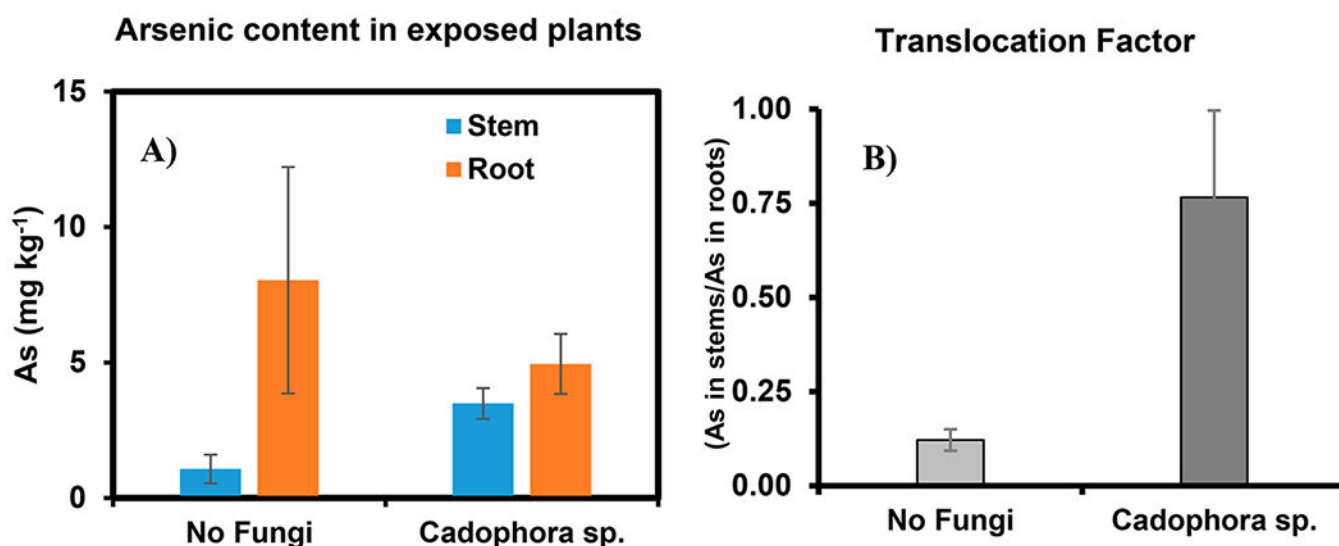
**Figure 2.** TEM image of hyphae (dark spots, red outline) in root cross sections of little bluestem using (A) Os stain; (B) tannic acid counterstained with uranyl acetate and lead citrate; (C) noninoculated using tannic acid counterstained with uranyl acetate and lead citrate.



	C Wt%	O Wt%	Mg Wt%	P Wt%	Ca Wt%	As Wt%	Other elements
<b>No Fungi</b>	25.20	36.26	0.37	7.88	12.89	0.59	17.3
<b>Fungi</b>	10.94	57.83	11.69	14.94	3.12	0.47	1.01

**Figure 3.**

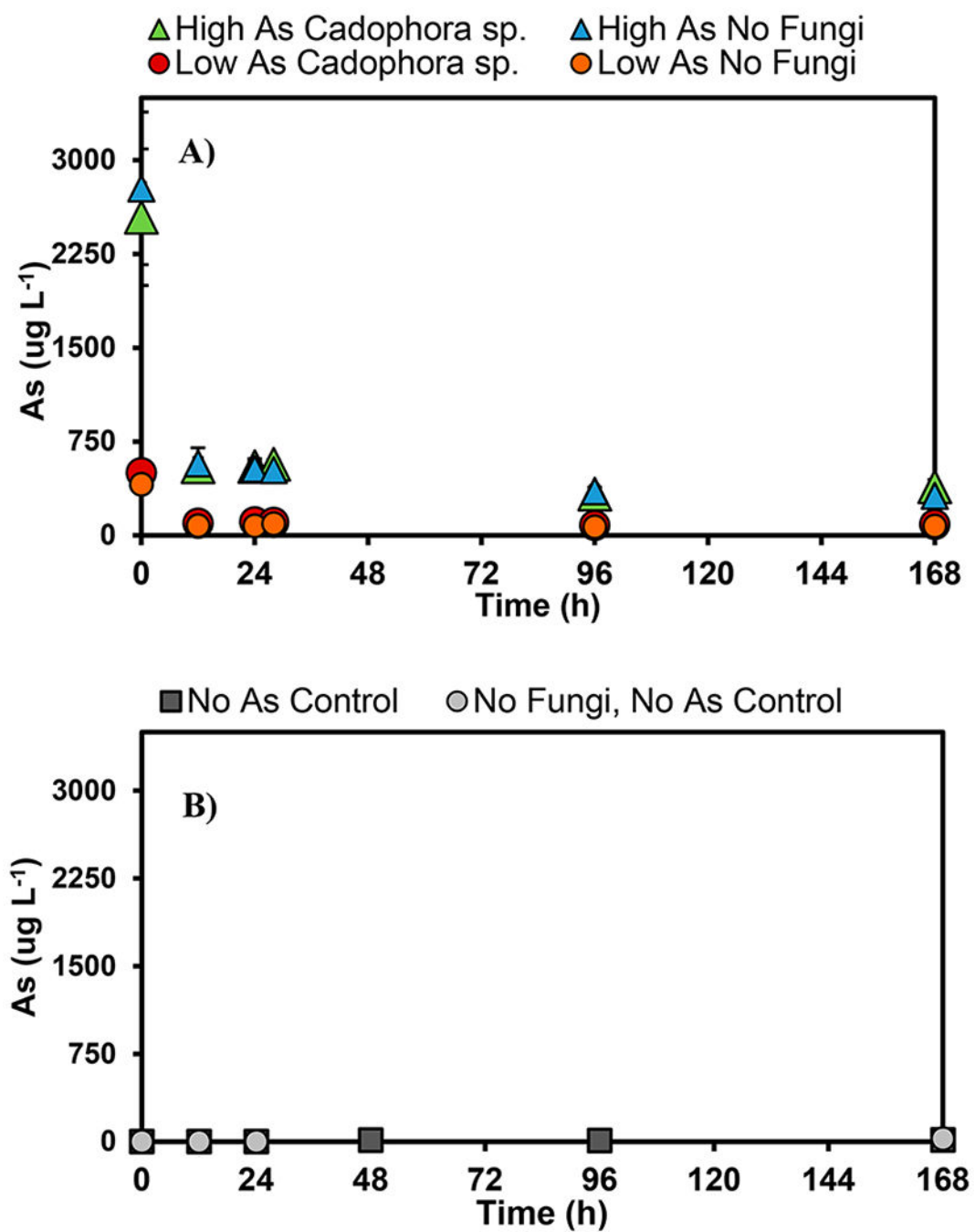
Accumulation of As and quantitative weight percentage on the surface of plant roots was detected by SEM microprobe X-ray mapping for plants exposed to  $2.5 \text{ mg L}^{-1}$  of (A) noninoculated (control) plant and (B) inoculated plant.



**Figure 4.**

(A) Average As content ( $\text{mg kg}^{-1}$ ) in grasses exposed to  $2.5 \text{ mg L}^{-1}$  without endophytic fungi and inoculated with *Cadophora sp.* fungi. (B) Translocation factor calculated as the ratio of As content in the stems to that of the roots. Standard deviation was determined from  $n = 5$  replicates.





**Figure 5.**

Arsenic concentrations measured in hydroponic solution over 168 h from plants exposed to (A) high As 2.5  $\text{mg L}^{-1}$  and low 0.5  $\text{mg L}^{-1}$  As(V); (B) 0  $\text{mg L}^{-1}$  controls.

**Table 1.**Summary of Mean and Standard Deviation ( $n = 9$ ) for Plant Root, Stem, and Biomass Measurements<sup>a</sup>

	root (cm)	stem (cm)	total biomass (g)
<i>Cadophora sp.</i>	74.89 ± 25.21	14.00 ± 5.01	14.97 ± 9.72
<i>Talaromyces</i>	99.50 ± 32.21	20.33 ± 5.54	29.75 ± 21.02
no fungi control	32.33 ± 10.48	4.63 ± 0.77	1.51 ± 0.77

<sup>a</sup>Grown in hydroponic system alone or with each fungus ( $p$ -value <0.05).