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# *Pteris vittata* Arsenic Accumulation Only Partially Explains Soil Arsenic Depletion during Field-Scale Phytoextraction

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**Abstract:** Soil arsenic heterogeneity complicates our understanding of phytoextraction rates during arsenic phytoextraction with *Pteris vittata*, including in response to rate stimulation with nutrient treatments. In a 58-week arsenic phytoextraction field study, we determined the effects of soil arsenic concentrations, fertilizer application, and mycorrhizal fungi inoculation on *P. vittata* arsenic uptake rates, soil arsenic depletion, and arsenic soil–plant mass balances. Initial soil arsenic concentrations were positively correlated with arsenic uptake rates. Soil inoculation with mycorrhizal fungus *Funneliformis mosseae* led to 1.5–2 times higher fern aboveground biomass. Across all treatments, ferns accumulated a mean of 3.6% of the initial soil arsenic, and mean soil arsenic concentrations decreased by up to 44%. At depths of 0–10 cm, arsenic accumulation in *P. vittata* matched soil arsenic depletion. However, at depths of 0–20 cm, fern arsenic accumulation could not account for 61.5% of the soil arsenic depletion, suggesting that the missing arsenic could have been lost to leaching. A higher fraction of arsenic (III) (12.8–71.5%) in the rhizosphere compared to bulk soils suggests that the rhizosphere is a distinct geochemical environment featuring processes that could solubilize arsenic. To our knowledge, this is the first mass balance relating arsenic accumulation in *P. vittata* to significant decreases in soil arsenic concentrations under field conditions.

**Keywords:** *Pteris vittata*; arsenic; soil; remediation; mass balance; leaching; field study; XANES

## 1. Introduction

Anthropogenic activities and geogenic processes lead to elevated concentrations of arsenic in soil, soil porewater, and plant tissue, increasing human exposure to this carcinogen [1–7]. Globally, arsenic occurs at a background mean of <10 mg/kg, but in contaminated soils, arsenic levels are elevated typically in the $10^2$–$10^3$ mg/kg range, with values of up to $10^4$ mg/kg [1,8] having been reported. The chemical form of arsenic controls its solubility, bioavailability, and toxicity [9]. In soils and aqueous environments, arsenic occurs mainly as arsenic(V) ($H_2AsO_4^-$ and $HAsO_4^{2-}$ in a pH of range 2–11) in oxidizing conditions, and arsenic(III) ($H_3AsO_3$ for pH below 9) [10] in moderately reducing conditions. Both arsenic(V) and arsenic(III) can be water-soluble in soils depending on the redox conditions and pH [10,11]. Toxicity occurs as arsenic(V) (taken up in organisms through the phosphate intake pathway) substitutes for phosphate in ATP processes, or as arsenic(III) binds to sulfhydryl groups to damage enzymes [10], among other processes. During detoxification, arsenic(V)

is reduced to arsenic(III) and then methylated, with some controversy regarding which species is most toxic to animals and plants [10,12,13].

Plant-based removal of arsenic from soil, or phytoextraction, with the fern *Pteris vittata* is an in situ technology which is potentially suitable to remediate moderately contaminated areas where soil excavation is cost-prohibitive and risk is less acute [14]. Phytoextraction with *P. vittata* is theoretically promising but faces practical limitations, especially under field conditions where heterogeneous soil arsenic concentrations lead to slow and variable arsenic uptake rates [15–17]. Remediation time estimates derived from some of the few published field studies [16–18] range from 10 to 78 years to remove 390 kg As/ha (100 mg As/kg) from soils.

Soil fertilization and inoculation with mycorrhizal fungi have been investigated in container and hydroponic systems to increase arsenic uptake rates, either through direct effects on plant biomass or indirect effects on arsenic availability in soil and/or effects on the volume of soil accessed by roots. Nitrogen fertilization led to higher fern biomass, and therefore, higher arsenic accumulation per fern [19]. Fertilization with calcium phosphate increased fern arsenic concentrations and biomass more effectively than fertilization with soluble or acidic phosphorus, which could have competed with arsenic for uptake into the fern [18,20,21]. However, in other cases, calcium phosphorus [22–24] or soluble phosphorus [24–26] did not affect or even decreased arsenic uptake in *P. vittata*. Additionally, soluble phosphorus applied to *P. vittata* led to higher arsenic concentrations in porewater in soils contaminated with arsenic and metals, because phosphorus competitively desorbed arsenic from the soil [27]. The competitive desorption that occurred during phytoextraction was likely due to the chemical similarity of phosphate and arsenic(V) [28], and has been reported during arsenic phytoextraction [27,29] and in mining [30] and orchard soils [31,32], where it has led to arsenic leaching [33]. Inoculation with the mycorrhizal fungus *Funneliformis mosseae* led to a larger *P. vittata* biomass [34,35], but did not affect arsenic accumulation per fern [34], especially in moderately contaminated soils [35]. Most studies investigating the effects of nutrient application on arsenic uptake in *P. vittata*, with one exception [21], were performed under hydroponic and greenhouse conditions or in outdoor container studies. It is unclear whether those findings obtained in simplified conditions might apply to in situ conditions [36], where plant roots and water movement are not confined to a limited volume of growth medium [37].

Furthermore, *P. vittata* nutrient acquisition could affect arsenic speciation, availability, transport in the rhizosphere, and uptake by the fern [13,38,39]. Phosphorus and arsenic are commonly found in soils associated with iron oxide minerals [40,41]. *P. vittata* releases root exudates [18,42–44] that solubilize phosphorus, iron, and arsenic through processes including ligand-enhanced dissolution of iron minerals [44–46]. Rhizosphere dissolved organic carbon (DOC), including root exudates, can also fuel microbial activity that couples DOC oxidation to iron and, in some cases, arsenic reduction [47,48], releasing iron and arsenic from iron minerals into solution through reductive dissolution. If *P. vittata* does not take up arsenic solubilized in the rhizosphere, the arsenic could leach, potentially beyond the root zone.

Therefore, in the context of phytoextraction, soil–plant mass balances that compare arsenic stocks in soil to that accumulated in plants are important, especially under field conditions [15]. Mass balances can indicate the existence of leaching [49] and other processes in addition to expected plant uptake that deplete contaminants from soils. However, field-scale mass balances are rarely calculated [15]. Complete mass balances are very challenging to obtain in the field, due to the infrastructure required to fully capture leaching [50] and volatilization [51]. Moreover, the high spatial variability in soil arsenic distribution can mask changes in arsenic levels [15,16,36]. Using only plant uptake-based remediation rates to quantify phytoextraction effectiveness [16,36] circumvents soil arsenic variability but does not truly assess the extent to which arsenic phytoextraction, defined as the transfer of arsenic from soil to plant aboveground biomass [52], works. A partial mass balance [53–55] equating arsenic depletion from soil with accumulation in plant tissue can determine phytoextraction effectiveness and indicate the existence of other arsenic input/output processes and stocks which are more difficult to quantify.

We present a 58-week field study investigating how soil arsenic concentrations, fertilizer application, and mycorrhizal fungi inoculation control arsenic uptake rates in *P. vittata*. We calculate the first, to our knowledge, soil–plant mass balance in the *P. vittata* literature linking fern arsenic accumulation to significant decreases in soil arsenic concentrations during phytoextraction under field conditions. We evaluate temporal changes in arsenic concentrations in *P. vittata* from transplant to harvest, and characterize arsenic speciation in the rhizosphere at harvest to hypothesize mechanisms for arsenic mobilization from soil. Unexpectedly, we found that soil arsenic depletion exceeded fern arsenic accumulation across all treatments, suggesting that fern growth processes might solubilize more arsenic than what is taken up in the fern.

## 2. Materials and Methods

### 2.1. Study Site and Experimental Design

A field site (81 $m^2$) was established at a former industrial property on the San Francisco Bay shore (Richmond, CA, USA) under remediation order from the State of California for the removal of pyrite roasting residues [56,57]. Soil arsenic, lead, copper, zinc, and iron concentrations were elevated and heterogeneously distributed, with mean arsenic levels per plot ranging from 23.5 to 118.6 mg/kg (Table 1), above background (11 mg As/kg) [58] and soil screening levels (0.36–4.2 mg As/kg) [59]. Major soil minerals identified with X-ray diffraction (PANalytical) following USGS protocols [60] included quartz, albite, hematite, and clays including nontronite, trioctahedral montmorillonite, and/or vermiculite.

**Table 1.** Soil characteristics before phytoextraction.

| Soil Characteristic | Unit | Value | Range |
|---|---|---|---|
| pH [1] before liming | | 5.5 ± 0.02 | *n*/a |
| pH after liming | | 6.1 ± 0.22 | *n*/a |
| Total concentrations [2] | | | |
| As | (mg/kg) | 78.3 ± 4.47 | 23.5–118.6 |
| P | (mg/kg) | 290.3 ± 20.2 | 98.0–735.5 |
| Fe | % | 3.4 ± 0.12 | 1.9–4.6 |
| Pb | (mg/kg) | 143.9 ± 5.23 | 95.2–223.0 |
| Cu | (mg/kg) | 550.6 ± 11.75 | 404.5–772.0 |
| Zn | (mg/kg) | 401.5 ± 9.96 | 293.6–562.3 |
| Modified Morgan extractable concentrations [3] | | | |
| P | (mg/kg) | 2.7 ± 0.04 | *n*/a |
| K | (mg/kg) | 129.0 ± 0.58 | *n*/a |
| Ca | (mg/kg) | 2,479.1 ± 0.16 | *n*/a |
| Mg | (mg/kg) | 341.2 ± 2.96 | *n*/a |
| Zn | (mg/kg) | 38.1 ± 0.05 | *n*/a |
| B | (mg/kg) | 0.3 ± 0 | *n*/a |
| Mn | (mg/kg) | 44.7 ± 0.11 | *n*/a |
| Cu | (mg/kg) | 18.6 ± 0.13 | *n*/a |
| Fe | (mg/kg) | 24.4 ± 0.3 | *n*/a |
| Pb | (mg/kg) | 4.4 ± 0.02 | *n*/a |
| Al | (mg/kg) | 15.2 ± 0.15 | *n*/a |
| Na | (mg/kg) | 32 ± 0.4 | *n*/a |
| S | (mg/kg) | 46.3 ± 0.01 | *n*/a |
| CEC [3] | meq/100g | 23.7 ± 0.22 | *n*/a |
| Organic matter [3] | % | 8.6 ± 0.17 | *n*/a |
| Bulk density [4] | $g/cm^3$ | 1.1 ± 0.01 | *n*/a |
| Sand content [4] | % | 53.6 ± 0.16 | *n*/a |
| Silt content [4] | % | 29.8 ± 0.29 | *n*/a |
| Clay content [4] | % | 16.6 ± 0.45 | *n*/a |
| Texture [4] | | Sandy loam | |

[1] Mean ± standard error of the mean of three replicates, in water; [2] Field mean ± standard error of the mean and range of plot means in 36 plots, after soil treatments applied; [3] Mean ± standard error of the mean of two replicates, before soil treatments applied; [4] Mean ± standard error of the mean of three replicates.

Before starting experiments, the soil was moistened and well-mixed with repeated tillage and by hand to a depth of 20 cm, where a clay pan occurred. Soil (initial pH 5.5, Table 1) was limed (16,000 kg/ha) because *P. vittata* grows well in soils with higher pH [61]. After liming, the soil pH was 6.1. We compared six soil treatments: (i) organic fertilization with compost (1% N, 0.21% P; 1,374 kg N/ha, 283 kg P/ha), inorganic fertilization with (ii) nitrogen ($(NH_4)_2SO_4$; 50 kg N/ha) or (iii–iv) phosphorus (CalPhos$^{TM}$, 7.9% P, 18% Ca; 85 kg P/ha or 737 kg P/ha), (v) inoculation with the fungus *F. mosseae* (INVAM; 44 mL inoculum/fern), and (vi) controls without amendments. Each treatment was applied to individual square plots (1.5 $m^2$), with six replicates per treatment. Powdered fertilizers and compost were tilled into plots to 20 cm, and were resupplied via top dressing (at the same rate) at 45 weeks. Mycorrhizal fungal inoculant was applied directly to each hole at planting. Fungus-treated plots were surrounded in aluminum sheeting to 30 cm depth to prevent hyphae migration to adjacent plots.

Twenty-five young ferns with three to seven fronds and bare roots were planted (30 cm spacing) in each of the 36 plots in November 2016. There were no negative (no fern) control plots. Ferns were watered via drip irrigation (mean 7 L/day). The study field was protected by hoophouses (mean high temperature 27 ± 0.4 °C, mean low temperature 9 ± 0.2 °C, mean relative humidity 94 ± 0.4%) so the only water input was from irrigation. Experiments lasted 58 weeks.

### 2.2. Soil, Fern, and Porewater Sample Collection

At the beginning and end of the experiments, triplicate soil samples (approximately 1.5–2 L) were collected using an incremental sampling method [62], where each plot was divided into 49 equally sized increments. Because the soil had been mixed well, initial soil samples were collected from the surface (0–3 cm) only and assumed to represent the 0–20 cm depth interval. Final soil samples were collected from two depth intervals, i.e., 0–10 cm and 10–20 cm, after rhizome removal and without regard to fern location. Soil samples were air-dried and ground (2 mm) before analyses.

Pinnae (1 middle pair from each frond) from 4–9 ferns were sampled every six weeks and were washed three times with deionized water [63]. At the end of the experiments, the aboveground biomass of each fern was cut above the rhizome. Fronds living at harvest (hereafter, fronds) were separated from senesced fronds, which were not analyzed because biomass was much smaller and arsenic concentrations have been shown to be lower in senesced fronds [17,64]. Samples were dried at 55 °C in an oven (pinnae) or 37 °C in a large drying room (fronds). Four fern samples per plot were randomly selected and each was ground (20 gauge mesh) separately for analysis.

Soil porewater was sampled every six weeks from the 5–10 cm depth interval using Rhizon porewater samplers inserted between fern crowns. Porewater was vacuum-extracted using acid-washed syringes and acidified to approximately 120 mM with HCl. Samples were stored at −18 °C in the dark until analyses.

Root and soil samples for microfocused and bulk X-ray absorption spectroscopy (XAS) were collected at the end of the experiments from soil adjacent to fern crowns 0–10 cm deep in triplicate control plots and immediately stored at 4 °C. Samples were dried, processed, and maintained under anoxic conditions [65]. Freeze-drying the samples was avoided, as it has been shown to affect chemical speciation [66].

### 2.3. Fern and Soil Sample Analyses

Dry biomass was measured on both pinnae and final frond samples. Plant tissue and soil samples were digested following a modified EPA 3050B protocol in a MARS5 microwave digester (CEM, Matthews, NC, USA). Approximately 100 mg plant tissue was digested in 5 mL concentrated (69%) $HNO_3$ and 2 mL 30% $H_2O_2$, or approximately 1 g soil was digested in 10 mL concentrated (69%) $HNO_3$. Reference materials and duplicates were digested in each batch of test samples to evaluate quality of digestion and analytical procedures. For soil, the reference material was NIST 2711a-Montana II soil. For plant tissue, no standard reference material exists in the appropriate concentration range, so arsenic-containing plant tissue from a prior study [64] analyzed by an outside certified laboratory (Brookside Laboratories, New Bremen, OH, USA) using the same protocols was used. Total arsenic, phosphorus, iron, copper, zinc, and lead concentrations of soil and fern digests were determined using

inductively coupled plasma optical emission spectroscopy (ICP-OES). Recovery of reference materials was within 10%. Extractable soil nutrient concentrations were analyzed following Modified Morgan extraction [67], but extractable arsenic was not analyzed because *P. vittata* growth has been shown to affect arsenic availability in soil [68]. Total arsenic concentrations in filtered porewater samples (0.45 μm) was analyzed using hydride-generation-ICP-OES after addition of 0.8 mL of 40% KI/8% ascorbic acid to 4 mL sample and 2.7 mL 1.1 M HCl.

Samples of roots with rhizospheric soil and of soil aggregates (5–6 mm diameter) were embedded in EPO-TEK 301 epoxy under anoxic conditions [65] and prepared as thin sections (30 μm; Spectrum Petrographics) with roots sliced longitudinally for X-ray microprobe analyses. For bulk XAS, the rhizosphere soil was sampled with a small brush. Aggregates with no visible roots were considered bulk soil. Bulk soil, rhizosphere soil, and roots were finely ground and mounted on a 0.22 μm nitrocellulose filter under anoxic conditions. X-ray microprobe analyses were performed at the Advanced Light Source (ALS) XFM beamline 10.3.2 [69], Lawrence Berkeley National Laboratory (LBNL, Berkeley, CA, USA). Briefly, arsenic and iron spatial distribution in the samples was determined using micro-focused X-ray fluorescence (μXRF) elemental mapping using a 12 keV incident beam. In sample regions of interest, arsenic speciation was determined using arsenic K-edge X-ray absorption near edge structure (μXANES) spectroscopy. Bulk XANES spectra were collected at Stanford Synchrotron Radiation Laboratory (SSRL, Menlo Park, CA, USA) beamline 7.3. Filter membranes were sealed under anoxic conditions and mounted on sample holders with Kapton tape. Micro and bulk XANES spectra were plotted using the Athena software (Demeter package version 0.9.25) [70] and least-square linear combination fitting was performed with customized LabVIEW software and XAS databases of Fe and As compounds available at beamline 10.3.2. Details of the spectroscopy measurements are available in the Supplemental Information.

### *2.4. Calculations*

Arsenic accumulated per fern (mg/fern) was calculated by multiplying fern arsenic concentration by the dry fern aboveground biomass. Arsenic uptake rate (kg/ha/y) was calculated by dividing the arsenic accumulated per fern by the soil surface area per fern and study duration. A remediation time estimate (y) to remove all soil arsenic in the 0–20 cm depth interval was calculated by dividing the mass of soil arsenic per unit surface area by the arsenic uptake rate, assumed to be constant [71]. A partial mass balance approach [53–55] was used to equate output of arsenic from soil with input of arsenic to ferns. In this arsenic soil–plant mass balance, the mean arsenic accumulation per fern, normalized to mass soil per depth, was compared to the difference in mean initial and mean final soil arsenic concentrations per depth (i.e., soil arsenic depletion) (Equation (1)):

$$\text{mass As}_{\text{fern}}/\text{mass soil} = \text{mass As}_{\text{soil,init}}/\text{mass soil} - \text{mass As}_{\text{soil, final}}/\text{mass soil} \quad (1)$$

The mass of soil in a 30 cm × 30 cm × 10 cm volume for the 0–10 cm depth or 10–20 cm depth, or 30 cm × 30 cm × 20 cm volume for the 0–20 cm depth, was used to normalize the mass arsenic accumulated per fern or depleted from soil. An inequality in Equation (1) would indicate the existence of output/input processes other than fern uptake of arsenic from soil, for example depletion of soil arsenic through loss of arsenic to leaching, or the input of arsenic to aboveground plant tissue or soil via aerial deposition [72].

### *2.5. Statistical Analysis*

Statistical analysis was performed with R [73]. Because initial soil arsenic concentrations were found to affect some response variables, data were not normalized to initial soil arsenic concentrations. Instead, to determine this effect, initial soil arsenic concentrations were included in linear models as a covariate, as appropriate. However, normalizing data to initial soil arsenic concentrations was required in some cases to determine response variable means by treatment.

Analyses of covariance with initial soil arsenic concentrations as a covariate were performed on linear models to analyze effects of treatment on fern arsenic concentrations, biomass, arsenic accumulation, arsenic uptake rate, and changes in soil arsenic concentrations during phytoextraction, though initial soil arsenic concentration was not included as a covariate in the analysis of variance for remediation time. Regression summaries were used to compare effects of treatments to the control, and differences in means were determined with Tukey's Honestly Significant Difference test on means normalized to soil arsenic concentration where appropriate. A paired t test was used to determine the mass balance across all plots, comparing mean soil arsenic depletion per plot to mean arsenic accumulation per fern per plot (Equation (1)).

## 3. Results

### *3.1. Temporal Evolution of Fern Biomass and Arsenic Uptake*

Time after transplanting affected fern pinnae arsenic concentrations ($p < 0.001$; Figure 1A) and biomass ($p < 0.001$; Figure 1B). Pinnae arsenic concentrations and biomass did not change between 12 and 18 weeks after transplanting (Figure 1A,B), but increased significantly between 18 and 30 weeks after transplanting (Figure 1A,B). The increase was an order of magnitude for pinnae arsenic concentrations (Figure 1A). For all soils with amendments, pinnae arsenic concentrations reached the hyperaccumulation threshold (1000 mg/kg [74]) and stabilized after 30 weeks. Pinnae biomass did not stabilize but continued increasing until 48 weeks after transplanting (Figure 1B).

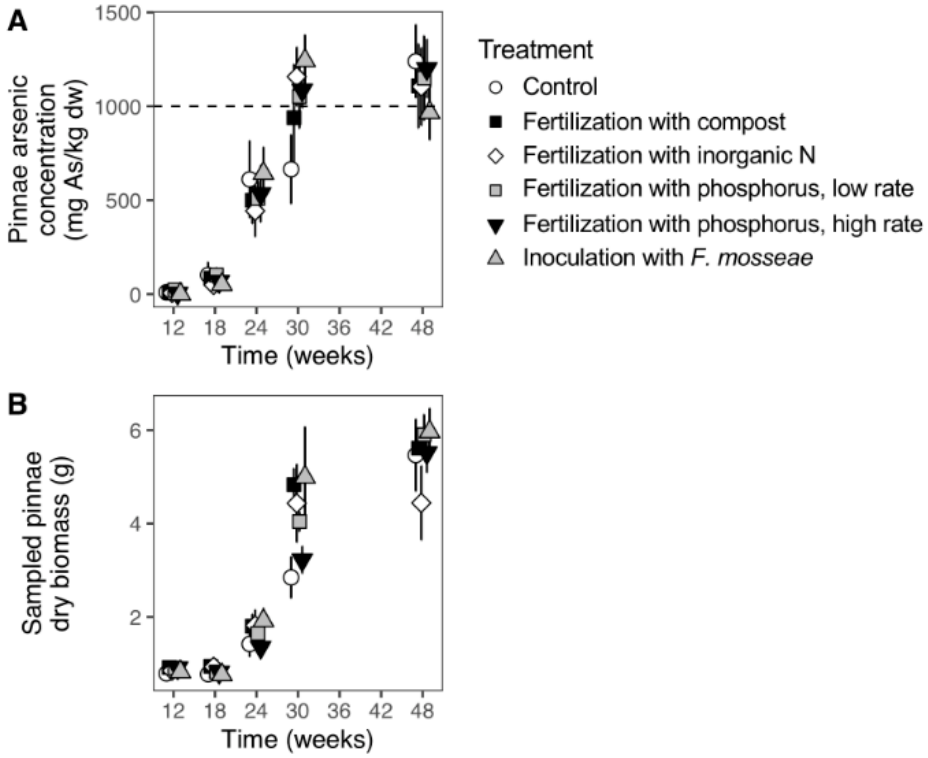


**Figure 1.** Temporal evolution of (**A**) mean dry sampled pinnae arsenic concentrations and (**B**) mean dry sampled pinnae biomass starting 12 weeks after fern transplant into arsenic-contaminated soils. Dotted line in panel A indicates hyperaccumulation threshold. Symbols represent mean values per treatment, and error bars represent standard error of the mean. $n = 6$ plots/treatment. Points are slightly shifted on the X axis for clarity.

Final fern frond arsenic concentrations ($p < 0.001$) and biomass ($p < 0.01$), and hence arsenic accumulation per fern ($p < 0.001$) and arsenic uptake rates ($p < 0.001$), increased with initial soil arsenic concentrations and ranged over 1–2 orders of magnitude (Figure 2A–D).

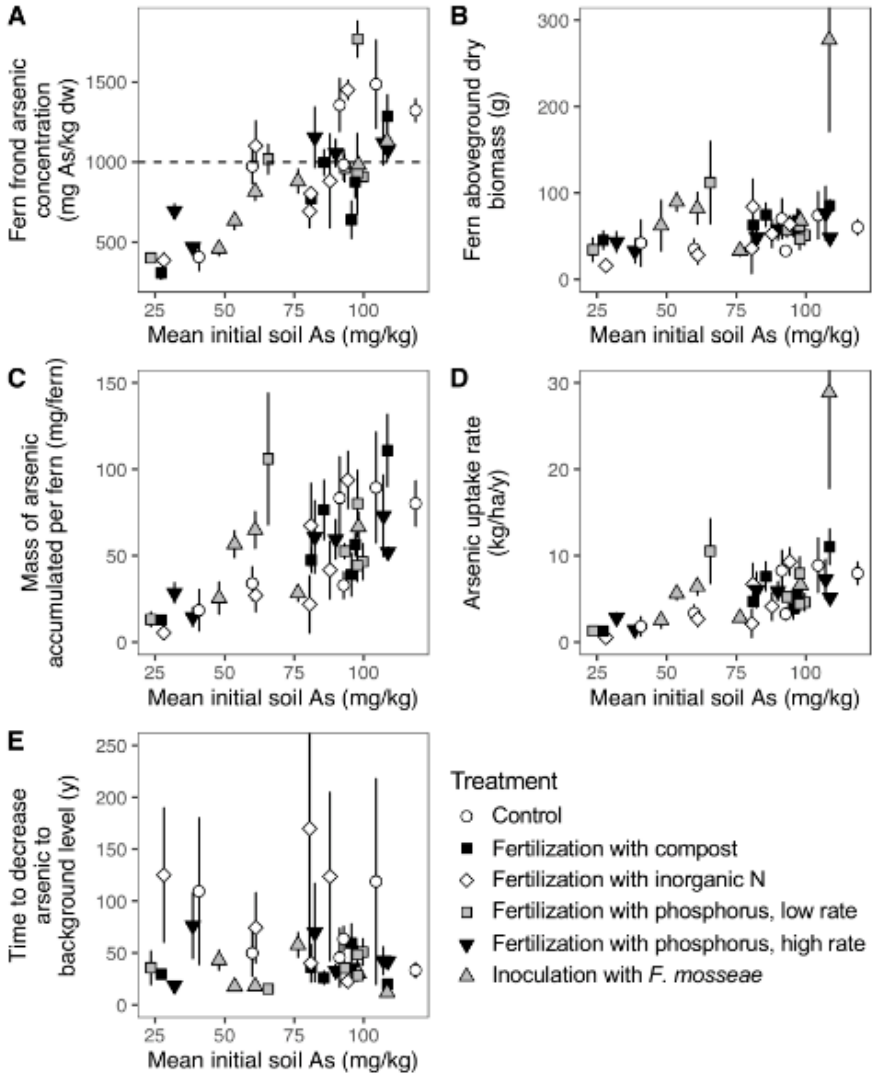


**Figure 2.** Mean (**A**) fern arsenic concentrations, (**B**) fern aboveground dry biomass, (**C**) fern accumulated arsenic mass, (**D**) arsenic uptake rates, and (**E**) remediation times at final harvest, 58 weeks after fern transplanting in arsenic-contaminated soils. $n = 4$. Dotted line in panel A indicates hyperaccumulation threshold. Symbols represent mean values per plot, and error bars represent standard error of the mean. See Figure S1 for mean arsenic concentrations and biomass per treatment.

### *3.2. Effects of Soil Fertilization and Inoculation with F. mosseae on Fern Arsenic Uptake*

When effects of initial soil arsenic concentrations were also considered, treatment did not affect pinnae arsenic concentrations nor biomass (Figure 1A,B), but it significantly affected frond arsenic concentrations ($p < 0.05$, Figure 2A) and biomass ($p < 0.05$, Figure 2B). More specifically, when considering initial soil arsenic concentrations, frond arsenic concentrations were significantly lower in ferns grown in compost- ($p < 0.01$) and fungi- ($p < 0.05$) treated soils than in the control ferns, while biomass of ferns grown in fungi-treated soils ($p < 0.01$) was significantly higher than in the control soils.

The effect of treatment on arsenic accumulation per fern (Figure 2C) and arsenic uptake rate (Figure 2D) was only significant at $p < 0.1$, with ferns in fungi-treated soils removing significantly more arsenic ($p < 0.05$) than in the control plots, when initial soil arsenic concentrations were considered. Treatment significantly affected remediation time (Figure 2E) ($p < 0.05$), with remediation times for fungi-treated ferns significantly lower ($p < 0.05$) than for the control. When frond arsenic concentrations were normalized to initial soil arsenic concentrations, treatment did not significantly affect frond arsenic concentrations (Figure S1).

In ferns grown in compost-treated soils, mean phosphorus concentrations were significantly higher than in control plots (Figure S2), while arsenic concentrations were lower (Figure 2A). Likewise, soil arsenic concentrations ($p < 0.001$) and treatment ($p < 0.001$) also significantly affected fern phosphorus concentrations. Soil arsenic concentrations were negatively correlated with fern phosphorus concentrations. However, compost application did not affect arsenic accumulated per fern (Figure 2C), due to a slight increase in biomass in the presence of compost.

In contrast, calcium phosphate, regardless of application rate, did not affect *P. vittata* biomass (Figure 2B), nor arsenic (Figure 2A) or phosphorus concentrations (Figure S2) in *P. vittata*.

### 3.3. Soil Arsenic Depletion Compared to Fern Arsenic Accumulation

After phytoextraction, soil arsenic concentrations were significantly lower than initial values, both in 0–10 cm ($p < 0.001$) and 10–20 cm ($p < 0.001$) depth intervals, with significantly lower final values in the deeper depth interval ($p < 0.001$; Table 2). Over the 58-week experiment, mean arsenic concentrations in the 0–10 cm depth interval decreased by up to 20.5% (field mean 9.8%) in 34 of 36 plots (Table 2), whereas in the 10–20 cm depth interval, they decreased by up to 43.6% (field mean 18.5%) in 35 of the 36 plots (Table 2). Treatment had no effect ($p = 0.1997$) on final soil arsenic concentrations. Arsenic concentrations in porewater at a depth of 5–10 cm were <6 µg/L for the study duration (Figure S3).

Over 58 weeks, ferns accumulated a mean of 3.6% of initial soil arsenic (Table 2). The amount of arsenic depleted from the 0–10 cm depth interval was not significantly different from the amount of arsenic accumulated in ferns (Table 2 and Figure S4; $p = 0.1007$). However, in the overall 0–20 cm depth interval, the amount of arsenic depleted from soil was significantly larger than the amount of arsenic accumulated in ferns (Table 2 and Figure S4; $p < 0.001$). Fern arsenic accumulation accounted for a mean of 38.5% of the arsenic depleted from soil across 0–20 cm depth, in the 35 plots where depletion was observed (Table 2).

### 3.4. Arsenic Speciation in Soil and Fern Roots

Bulk XANES spectra showed that most arsenic in bulk soil in the 0–10 cm depth was present as arsenic(V) (Figure 3D and Table S1). Bulk and micro XANES spectra showed that arsenic in the rhizosphere soil was present as mixed arsenic(III) (12.8–71.5%) and arsenic(V) (29.1–89.8%). Furthermore, bulk XANES spectra collected from whole root samples showed high abundance of arsenic(III) (75.5–101.8%) with limited arsenic(V) (6.1–26.3%) (Figure 3, Figure S5, Figure S6). Bulk and micro spectra showed abundant iron oxides and silicates (Table S2).

Micro XANES spectra showed more arsenic(III) on the exterior (49.1 to 64.8%) than interior (27.9 to 40.0%) of the soil aggregate (Figure 3C,D and Table S1).

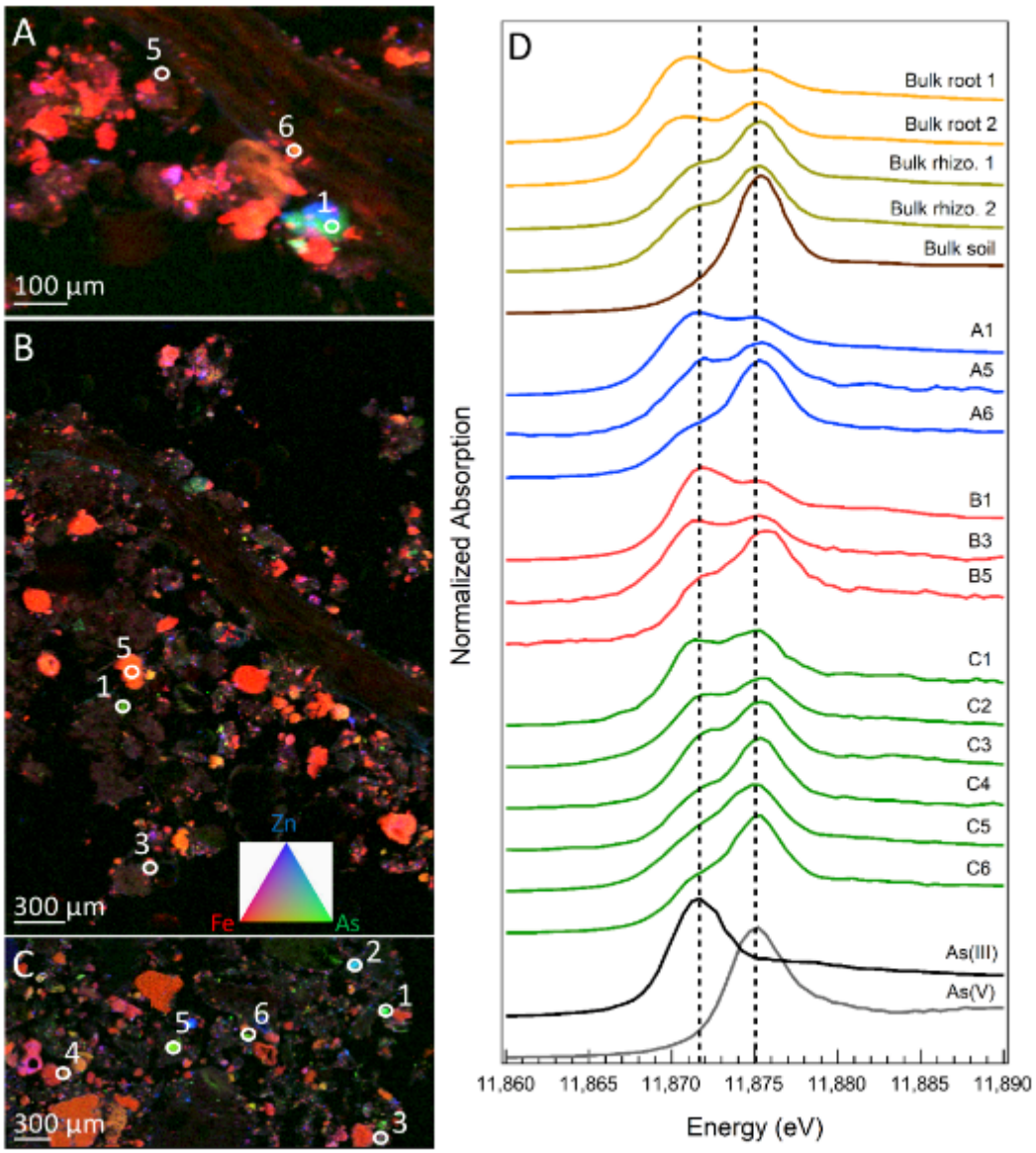


**Figure 3.** Tricolor-coded micro- X-ray fluorescence maps of As, Fe, and Zn in (**A**,**B**) untreated (control) roots with rhizosphere soil and (**C**) untreated (control) soil aggregate. The tri-color shield indicates color in panels A to C. Within an image, brighter colors indicate higher fluorescence signal. (**D**) Selected arsenic K-edge bulk and micro XANES data: bulk spectra from powdered untreated (control) roots, rhizosphere soil, and bulk soil; micro spectra for three spots across root cross-section A; three spots across root cross-section B; and six spots across aggregate cross-section C (spots 1–3 are on aggregate edge; spot four is approximately aggregate center). Spectra for additional spots in panels A to C can be found in the Supplemental Information. As(III) and As(V) standard spectra that were included in the best-fit results from LCF analysis are shown in panel D for reference. The complete library of XAS standards is available in the Supplemental Information. Vertical lines denote energies at 11,871.7 and 11,875 eV that were used to differentiate As(III) and As(V) species, respectively.

**Table 2.** Soil–plant mass balance comparing soil arsenic depletion to fern arsenic accumulation after 58 weeks of phytoextraction. Plots are listed in order of decreasing initial soil arsenic. Mass balance compares mean fern arsenic accumulation ($n$ = 4) per kg soil (per 1 depth)/2 to mean soil arsenic depletion ($n$ = 3) across 0–20 cm, or mean fern arsenic accumulation ($n$ = 4) per kg soil (per 1 depth) to mean soil arsenic depletion ($n$ = 3) in either 0–10 cm or 10–20 cm depth. Initial soil arsenic values are the mean ± standard error of the mean ($n$ = 3).

| | | | | 0–20 cm | | | 0–10 cm | | 10–20 cm | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Plot** | **Treatment** | **Fern As Accumulation per kg Soil (per 1 Depth)** | **Initial Soil As** | **% of Initial Soil As Accumulated in Fern after 58 Weeks** | **Soil As Depletion after 58 Weeks** | **% of Depleted Soil As Accumulated in Fern after 58 Weeks** | **Soil As Depletion after 58 Weeks** | | **Soil As Depletion after 58 Weeks** | |
| | | **mg As/kg Soil** | **mg/kg** | **%** | **mg/kg** | **%** | **mg/kg** | **% decr** | **mg/kg** | **% decr** |
| 16 | Control | 8.1 | 118.6 ± 4.0 | 3.4 | 1.4 | 281.4 | −2.4 | −2.0 | 5.3 | 4.4 |
| 11 | P—high | 5.3 | 108.7 ± 3.6 | 2.4 | 31.0 | 8.5 | 15.3 | 14.1 | 46.6 | 42.9 |
| 17 | Compost | 11.2 | 108.6 ± 5.0 | 5.2 | −2.5 | NA | −2.3 | −2.2 | −2.7 | −2.5 |
| 21 | Fungi | 29.3 | 108.4 ± 1.7 | 13.5 | 14.0 | 104.6 | 20.6 | 19.0 | 7.4 | 6.9 |
| 18 | P—high | 7.4 | 107.1 ± 3.5 | 3.4 | 11.9 | 31.1 | 10.0 | 9.3 | 13.8 | 12.8 |
| 9 | Control | 9.0 | 104.4 ± 4.9 | 4.3 | 10.0 | 45.3 | 8.1 | 7.7 | 11.9 | 11.4 |
| 3 | P—low | 4.7 | 99.6 ± 2.3 | 2.4 | 24.0 | 9.8 | 7.0 | 7.0 | 41.1 | 41.2 |
| 8 | Fungi | 6.7 | 98.1 ± 1.3 | 3.4 | 6.2 | 54.6 | 3.8 | 3.8 | 8.6 | 8.7 |
| 25 | P—low | 8.1 | 97.8 ± 4.7 | 4.1 | 15.0 | 26.9 | 16.9 | 17.2 | 13.2 | 13.5 |
| 5 | P—low | 4.5 | 97.7 ± 0.9 | 2.3 | 30.3 | 7.4 | 17.9 | 18.4 | 42.6 | 43.6 |
| 6 | Compost | 5.7 | 97.0 ± 1.9 | 2.9 | 21.2 | 13.5 | 9.4 | 9.7 | 33.0 | 34.1 |
| 2 | Compost | 3.9 | 95.6 ± 0.6 | 2.1 | 12.4 | 15.9 | 8.5 | 8.9 | 16.2 | 17.0 |
| 24 | Nitrogen | 9.5 | 94.2 ± 6.1 | 5.0 | 13.0 | 36.6 | 14.2 | 15.1 | 11.7 | 12.4 |
| 14 | P—low | 5.3 | 93.2 ± 3.3 | 2.9 | 11.0 | 24.2 | 9.0 | 9.7 | 13.0 | 13.9 |
| 36 | Control | 3.3 | 92.7 ± 6.1 | 1.8 | 12.0 | 13.9 | 14.7 | 15.8 | 9.4 | 10.1 |
| 23 | Control | 8.4 | 91.3 ± 7.6 | 4.6 | 13.4 | 31.5 | 12.1 | 13.2 | 14.7 | 16.1 |
| 7 | P—high | 6.0 | 90.0 ± 2.7 | 3.3 | 11.7 | 25.6 | 4.7 | 5.3 | 18.7 | 20.8 |
| 10 | Nitrogen | 4.2 | 87.9 ± 3.1 | 2.4 | 16.3 | 12.9 | 3.8 | 4.4 | 28.9 | 32.8 |
| 35 | Compost | 7.7 | 85.6 ± 2.4 | 4.5 | 5.8 | 66.4 | 4.2 | 4.9 | 7.4 | 8.7 |
| 22 | P—high | 6.2 | 82.4 ± 4.4 | 3.7 | 4.4 | 70.2 | 3.2 | 3.8 | 5.6 | 6.8 |

**Table 2.** *Cont.*

| | | | | 0–20 cm | | | 0–10 cm | | 10–20 cm | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Plot** | **Treatment** | **Fern As Accumulation per kg Soil (per 1 Depth)** | **Initial Soil As** | **% of Initial Soil As Accumulated in Fern after 58 Weeks** | **Soil As Depletion after 58 Weeks** | **% of Depleted Soil As Accumulated in Fern after 58 Weeks** | **Soil As Depletion after 58 Weeks** | | **Soil As Depletion after 58 Weeks** | |
| | | **mg As/kg Soil** | **mg/kg** | **%** | **mg/kg** | **%** | **mg/kg** | **% decr** | **mg/kg** | **% decr** |
| 15 | Compost | 4.8 | 81.1 ± 1.0 | 3.0 | 10.5 | 22.8 | 5.8 | 7.1 | 15.2 | 18.8 |
| 19 | Nitrogen | 6.8 | 81.0 ± 0.9 | 4.2 | 6.8 | 50.0 | 4.5 | 5.5 | 9.2 | 11.3 |
| 1 | Nitrogen | 2.2 | 80.6 ± 2.6 | 1.4 | 14.7 | 7.5 | 11.5 | 14.3 | 18.0 | 22.3 |
| 4 | Fungi | 2.9 | 76.3 ± 2.1 | 1.9 | 13.9 | 10.3 | 3.6 | 4.8 | 24.2 | 31.7 |
| 33 | P—low | 10.7 | 65.6 ± 2.0 | 8.2 | 9.6 | 55.5 | 10.8 | 16.4 | 8.5 | 13.0 |
| 34 | Nitrogen | 2.7 | 61.2 ± 2.8 | 2.2 | 6.6 | 20.6 | 10.9 | 17.8 | 2.4 | 4.0 |
| 20 | Fungi | 6.6 | 61.0 ± 0.5 | 5.4 | 2.9 | 114.6 | 2.7 | 4.5 | 3.0 | 4.9 |
| 12 | Control | 3.4 | 59.9 ± 0.2 | 2.9 | 10.2 | 16.7 | 12.3 | 20.5 | 8.2 | 13.7 |
| 32 | Fungi | 5.7 | 53.6 ± 1.8 | 5.3 | 8.4 | 34.2 | 8.0 | 14.9 | 8.8 | 16.4 |
| 27 | Fungi | 2.6 | 48.0 ± 1.7 | 2.7 | 7.1 | 18.3 | 5.4 | 11.3 | 8.7 | 18.1 |
| 28 | Control | 1.9 | 40.7 ± 1.8 | 2.3 | 9.4 | 10.0 | 4.7 | 11.6 | 14.0 | 34.4 |
| 26 | P—high | 1.5 | 38.5 ± 0.9 | 1.9 | 6.9 | 10.7 | 2.8 | 7.1 | 11.0 | 28.6 |
| 13 | P—high | 2.9 | 32.0 ± NA | 4.5 | 5.8 | 24.8 | 5.6 | 17.4 | 6.1 | 18.9 |
| 30 | Nitrogen | 0.5 | 28.1 ± 0.4 | 1.0 | 4.6 | 6.0 | 0.3 | 1.1 | 8.9 | 31.6 |
| 31 | Compost | 1.3 | 27.2 ± 0.3 | 2.4 | 6.4 | 10.1 | 3.6 | 13.3 | 9.3 | 34.1 |
| 29 | P—low | 1.3 | 23.5 ± 1.1 | 2.8 | 1.2 | 55.7 | 0.2 | 1.0 | 2.2 | 9.2 |

## 4. Discussion

### 4.1. Fern Arsenic Uptake Varies with Soil Arsenic Content

We found that *P. vittata* phytoextracted arsenic under field conditions, with or without soil amendments. Others reported similar arsenic uptake rates (4.3 kg/ha/yr) [16], similar (674–1500 mg/kg) [16,75] or higher (3881 mg/kg) [17] frond arsenic concentrations, and slightly higher (81 g/fern) biomass [16] for *P. vittata* in arsenic phytoextraction field studies. Soil arsenic concentrations were positively correlated with frond arsenic concentrations and biomass, so lower mean soil arsenic concentrations reported here than in previous studies [16,17,75] could explain the lower frond arsenic concentrations we measured. Consistent with previous studies [18,27,68], we found *P. vittata* did not hyperaccumulate arsenic from soils with arsenic concentrations lower than 60 mg/kg. Our report of *P. vittata* arsenic uptake on a continuum of soil arsenic concentrations fill in gaps in previous reports of higher *P. vittata* biomass in the presence of low to moderate soil arsenic concentrations compared to arsenic-free soils [76]. This positive correlation suggests that arsenic hyperaccumulation from soils with low to moderate arsenic concentrations stimulates growth instead of costing metabolic energy.

Our results suggest the onset of phytoextraction could be delayed in sub-ideal field conditions and triggered by specific climate conditions. In soils with similar arsenic concentrations to ours, but in a pot study, arsenic concentrations in *P. vittata* fronds passed the hyperaccumulation threshold before 8–12 weeks of growth [23,77]. Our findings suggest that frond harvest could occur as early as 12 weeks after frond growth commences (i.e., at 30 weeks here) for efficient field-scale phytoextraction.

### 4.2. Soil Treatment Did Not Affect Fern Arsenic Accumulation

Phosphorus application via compost and fungi inoculation both lead to lower fern arsenic concentrations, but contrary to expectations, we found treatment did not strongly affect fern arsenic accumulation, due to slight or significant biomass increases.

Our findings suggest that phosphorus supplied via compost is more available to *P. vittata* than when supplied as phosphate rock, and can interfere with arsenic uptake on a mole-for-mole basis under field conditions. Similar interference from phosphorus with arsenic uptake in *P. vittata* has been observed on time scales of hours to months, over wide phosphorus concentration ranges [26,63,78]. Despite a high phosphorus rock application rate that supplied approximately twice the available phosphorus as compost, we saw an increase in fern phosphorus content only with compost application. Calcium phosphate could have reacted with the lead in the pyrite cinders (mean 143.9 mg Pb/kg; Table 1) to form insoluble lead-phosphates [79] and therefore not been available for fern uptake. In contrast, compost application has been shown to decrease phosphorus sorption and increase phosphorus solubility in soil [80,81], so could have made pre-existing soil phosphorus more available and added phosphorus.

Our results contrast with recent observations that phosphate rock application at rates similar to ours led to higher *P. vittata* frond arsenic concentrations, biomass, and phosphorus accumulation than when phosphorus was applied as soluble form, or not applied at all [18,20]. In those previous reports, ferns died without supplemental phosphorus, implying that soil available phosphorus (0.38 mg/kg) was too low to maintain fern survival [18]. In contrast, in our study available phosphorus (2.7 mg/kg) appears to have been sufficient for fern biomass production and concomitant arsenic uptake, such that phosphorus application was not needed to maximize fern arsenic accumulation. Phosphorus application might be necessary to support *P. vittata* growth and increase arsenic uptake only in soils with extremely low (<< 2.7 mg/kg) available phosphorus.

Furthermore, our field-observations confirm previous findings in controlled greenhouse conditions of higher *P. vittata* biomass upon inoculation with *F. mosseae* [34,35,82,83]. In some cases [35,83] this biomass increase led to higher arsenic accumulation per fern even when frond arsenic concentrations did not increase, though in our study inoculation did not significantly affect arsenic accumulation. *F. mosseae*

could increase fern growth by increasing transport of phosphorus to the fern [34,35,82]. However, we found significantly lower phosphorus concentrations in ferns grown in *F. mosseae*-inoculated soils. This could be due to repression of the arbuscular mycorrhizal fungi phosphorus uptake pathway, though such downregulation was reported in high phosphorus status plants [84] and *P. vittata* phosphorus status has not been considered high [13,85]. Our results suggest that fungi inoculation can increase *P. vittata* growth through processes other than nutrient transport, perhaps through effects on arsenic reductase activity [82] to increase arsenic tolerance, rendering metabolic energy (otherwise used to support arsenic tolerance) available for growth. *F. mosseae* inoculation could be especially important to support growth of *P. vittata* under field stress, even if it does not always increase fern arsenic accumulation.

### *4.3. Fern Arsenic Accumulation Is Less than Soil Arsenic Depletion*

Soil arsenic concentrations decreased significantly over the phytoextraction period. We observed greater depletion of soil arsenic at deeper depth intervals, similar to field observations with *P. vittata* [17] and *Pityrogramma calomelanos*, another arsenic-hyperaccumulating fern [16].

When considering only the 0–10 cm depth, our results suggest *P. vittata* took up all arsenic depleted from the soil, an outcome promising for practical use of phytoextraction. The abundance of *P. vittata* roots in this depth interval would support this interpretation. *P. vittata* roots have been reported to grow mainly (up to 84% [86]) in the 0–10 cm soil depth interval [17,86], which we confirmed in our field study.

Surprisingly, over the 0–20 cm depth, we found that sequestration of arsenic in fern fronds accounted only for 39.5% of the soil arsenic depletion. This mass balance is to our knowledge the first relating arsenic phytoextraction with *P. vittata* to significant decreases in soil arsenic concentrations under field conditions and revealed a major gap in our understanding of arsenic cycling during phytoextraction.

Our findings for phytoextraction with *P. vittata* are consistent with those for another arsenic hyperaccumulating fern, *P. calomelanos*. Similarly, although soil arsenic concentrations decreased significantly during phytoextraction under field conditions with *P. calomelanos*, fern arsenic accumulation alone could not account for that decrease [16]. The discrepancy was attributed to soil sampling error induced by high heterogeneity in soil arsenic concentrations [16], a problem masking changes in soil metal concentrations in other phytoextraction studies [87]. However, similar discrepancies, with *P. vittata* accumulation accounting for only 13–22% of soil arsenic depletion, have occurred in pot studies where soil is presumably well-mixed [68].

Here, we calculated a mass balance based on a robust experimental design to show that the discrepancy in frond arsenic accumulation and soil arsenic depletion is not an artifact of sampling error, but indicates the possibility of other processes leading to loss of soil arsenic. We used a well-replicated study design, in contrast to previous first attempts at mass balances where all replicates were at the fern scale [16,17]. We extensively mixed the soil before planting the ferns to decrease within-plot heterogeneity and we collected incremental, representative soil samples [62,88]. Although a pot or closed system study would permit tighter quantification of arsenic stocks and flows [68], we worked under field conditions with realistic movement of water and dissolved arsenic, to more closely approximate practical applications.

In addition to fronds, rhizomes (though not roots [89]) can be a secondary storage organ for arsenic in *P. vittata* [86]. Rhizome arsenic concentrations, biomass, and arsenic accumulation were 50–60% that of pinnae in a naturally occurring *P. vittata* population [86], though arsenic concentrations in rhizomes were only 19–29% of frond concentrations in cultivated ferns [90]. Here, we calculated that the mass of arsenic contained in ferns, even including an additional 60% arsenic to account for possible rhizome accumulation as observed in well-established populations [86], was still significantly lower ($p < 0.001$) than soil arsenic depletion across both depths (0–20 cm). In another study with the same ferns, we found arsenic accumulation in rhizomes was 1–2 orders of magnitude less than

in fronds [64]. Processes other than fern arsenic accumulation likely lead to arsenic loss from soils during phytoextraction.

We found no treatment effect on fern arsenic accumulation and soil arsenic depletion, contrary to observations that compost and phosphorus soil treatments can lead to arsenic desorption and increase arsenic concentrations in porewater [27,47] and therefore potentially increase arsenic uptake in the fern and/or arsenic leaching from soil. Importantly, this lack of treatment effect suggests arsenic depletion across all treatments is due to fern growth, even though it cannot be explained entirely by accumulation of arsenic in fern fronds.

### 4.4. Leaching Could Explain Discrepancy in Soil–Plant Mass Balance

Arsenic loss by leaching could explain the arsenic not accounted for by plant tissue accumulation in our mass balance. The low arsenic concentrations we found in porewater extracted from bulk soils in the 5–10 cm depth suggest that if arsenic leached, leaching was spatiotemporally heterogeneous and not well represented in our samples. Indeed, at pH 6.1 we expected arsenic to be present in aqueous solution as $H_2AsO_4^-$ [10]. We therefore expected, with up to 4.6% iron in soil, that arsenic would sorb strongly to soil iron(III) oxides and clays [40,91], limiting the potential for arsenic leaching from soil during phytoextraction [16]. Our porewater results are consistent with low leaching of arsenic in the 0–10 cm depth from bulk soil. Alternately, leachable arsenic could have had been depleted by the time porewater measurements commenced, or arsenic reduction and therefore mobility could have increased in the 10–20 cm depth interval due to poor drainage above the clay pan [92].

We hypothesize that arsenic mobilization processes in the fern rhizosphere lead to spatiotemporally heterogeneous arsenic leaching. In a closed-system, soil column arsenic phytoextraction study with *P. vittata*, we found that arsenic concentrations in leachate increased in the presence of *P. vittata* [64]. In this field study, the high fraction of arsenic(III) we found in rhizospheric compared to bulk soils suggests the rhizosphere is a reactive zone with arsenic mobilization processes distinct from bulk soil.

The mixed redox status of rhizosphere solid-phase arsenic we observed could be coupled to dissolved organic carbon (DOC) mobilization and/or oxidation in the *P. vittata* rhizosphere. Root exudates have been suggested to play an important role in arsenic release from soil solid surfaces for uptake into *P. vittata* [42,93]. Arsenic and DOC cycles in *P. vittata* rhizosphere soil could intersect through a combination of processes including: (1) release of arsenic(V) from iron oxide surfaces due to ion exchange or ligand-enhanced dissolution with DOC [44,47], leading to fern uptake and/or leaching of soluble arsenic(V) [47,92,94,95] and/or DOC [96,97]; (2) enhanced weathering of sulfide residues in pyrite cinders [98], decreasing pH locally [75] and solubilizing arsenic(III) and/or arsenic(V); (3) reduction of arsenic(V) within roots [99]; (4) microbially-mediated arsenic reduction in the rhizosphere coupled to DOC oxidation [47,48]; (5) fern uptake of soluble arsenic(III) [100]; and/or (6) replenishment of soil arsenic through leaching from foliage [101] or excretion of soluble arsenic(III) from roots to soil [102,103] and subsequent sorption to soil.

Arsenic reduction coupled to DOC oxidation could increase leaching of arsenic from rhizosphere and bulk soils, particularly if low pH microsites exist near pyrite cinders [75]. With decreasing pH, arsenic(III) sorption decreases, in contrast to arsenic(V) sorption [11]. The greater abundance of arsenic(III) we found on the surface of a bulk root zone soil aggregate could indicate transport of arsenic(III) from rhizosphere to bulk soil. This redox gradient is contrary to expected reducing conditions typically found in the aggregate interior, not exterior [104]. Soluble species, including arsenic(III) and DOC, could be flushed from rhizosphere to bulk soils when gravitational flow exceeds transpiration flux, for example during irrigation events.

However, at pH 6, similar amounts of arsenic(III) and (V) sorb to iron oxides [11], indicating that both species are equally leachable at the soil pH we studied. The pH increase we observed upon liming is expected to slightly decrease arsenic(V) and increase arsenic(III) sorption to soil iron oxide minerals [11]. Others observed arsenic(V) leaching from oxic soils [92,94,95], especially upon addition of organic matter [47]. Here, arsenic(V) could be mobilized from rhizosphere and bulk soil

due to ion exchange with leaching of root-derived organic carbon from the rhizosphere to lower soil horizons [96,97]. The leaching of arsenic(III) and (V) from soils below the root zone could help explain the greater depletion in arsenic concentrations in the 10–20 cm than in the 0–10 cm depth. Our findings suggest the 10–20 cm soil depth is not a sink for arsenic, but instead is a zone of active arsenic mobilization processes coupled to *P. vittata* growth, even if below the root zone. Moreover, our results confirm other reports that arsenic leached from soil after *P. vittata* planting and irrigation [105].

## 5. Conclusions

We hypothesized that arsenic leaches from the top 20 cm of soil during phytoextraction. The possibility of leaching is a liability for applied phytoextraction and must be investigated further. Our work suggests that the decrease in soil arsenic concentrations observed across treatments of this soil–plant system was due to fern cultivation/growth. Future work must pinpoint the specific aspects of fern growth and cultivation that contribute to arsenic mobilization and transport during phytoextraction, from root exudation and nutrient acquisition to gravitational flow. Higher resolution field data on arsenic mobilization and transport from root zone to bulk soil, particularly at deeper depths, is needed. To rigorously quantify arsenic loss to leaching during *P. vittata* growth, studies should include controls without ferns, use representative soil sampling methods, calculate mass balances, and be compared to work in closed systems. Mass balances should quantify the mass of arsenic in soil and plant, and, if possible and applicable, the mass of the leached arsenic. More broadly, field-scale work with *P. vittata* should be expanded to determine the extent to which arsenic leaching occurs under other climate conditions, in naturally occurring populations, and with other hyperaccumulator species. Ultimately, this work speaks to the challenge of obtaining a 1:1 relationship between the solubilization and removal of strongly sorbed contaminants in soils, especially when working with biological systems.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2571-8789/4/4/71/s1, Micro-focused and bulk X-ray spectromicroscopy method details, Figure S1: Mean fern arsenic concentrations and fern aboveground biomass by treatment, Figure S2: Mean fern phosphorus concentrations by treatment, Figure S3: Porewater arsenic concentrations, Figure S4: Fern arsenic accumulation plotted by soil arsenic depletion, Figure S5: Micro X-ray fluorescence maps showing all spots, Figure S6: Valence state scatter plot of arsenic and iron in root and soil samples, Figure S7: Standard spectra used in LCF fitting of arsenic K-edge spectra, Figure S8: Standard spectra used in LCF fitting of iron K-edge spectra, Table S1: Linear combination fits for arsenic K-edge spectra, Table S2: Linear combination fits for iron K-edge spectra.

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## References

1. Singh, R.; Singh, S.; Parihar, P.; Singh, V.P.; Prasad, S.M. Arsenic contamination, consequences and remediation techniques: A review. *Ecotoxicol. Environ. Saf.* **2015**, *112*, 247–270. [CrossRef] [PubMed]

2. Wang, S.; Mulligan, C.N. Occurrence of arsenic contamination in Canada: Sources, behavior and distribution. *Sci. Total Environ.* **2006**, *366*, 701–721. [CrossRef] [PubMed]
3. Crecelius, E.A.; Johnson, C.J.; Hofer, G.C. Contamination of soils near a copper smelter by arsenic, antimony and lead. *Water Air Soil Pollut.* **1974**, *3*, 337–342.
4. Colbourn, P.; Alloway, B.J.; Thornton, I. Arsenic and heavy metals in soils associated with regional geochemical anomalies in South-West England. *Sci. Total Environ.* **1975**, *4*, 359–363. [CrossRef]
5. Moreno-Jimenez, E.; Esteban, E.; Penalosa, J.M. The fate of arsenic in soil-plant systems. In *Reviews of Environmental Contamination and Toxicology*; Whitacre, D.M., Ed.; Springer: New York, NY, USA, 2012; Volume 225, pp. 1–37.
6. Teixeira, M.C.; Santos, A.C.; Fernandes, C.S.; Ng, J.C. Arsenic contamination assessment in Brazil – Past, present and future concerns: A historical and critical review. *Sci. Total Environ.* **2020**, *730*, 138–217. [CrossRef]
7. Upadhyay, M.K.; Shukla, A.; Yadav, P.; Srivastava, S. A review of arsenic in crops, vegetables, animals and food products. *Food Chem.* **2019**, *276*, 608–618. [CrossRef]
8. Kabata-Pendias, A. *Trace Elements in Soils and Plants*, 4th ed.; CRC Press/Taylor and Francis: Boca Raton, FL, USA, 2011; pp. 1–548.
9. Mandal, B.K.; Suzuki, K.T. Arsenic around the world: A review. *Talanta* **2002**, *58*, 201–235. [CrossRef]
10. Bissen, M.; Frimmel, F.H. Arsenic-a review. Part I: Occurrence, toxicity, speciation, mobility. *Acta Hydrochim. Hydrobiol.* **2003**, *31*, 9–18. [CrossRef]
11. Dixit, S.; Hering, J.G. Comparison of arsenic(V) and arsenic(III) sorption onto iron oxide minerals: Implications for arsenic mobility. *Environ. Sci. Technol.* **2003**, *37*, 4182–4189. [CrossRef]
12. Mitchell, V.L. Health risks associated with chronic exposures to arsenic in the environment. *Rev. Miner. Geochem.* **2014**, *79*, 435–449. [CrossRef]
13. Meharg, A.A.; Hartley-Whitaker, J. Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. *New Phytol.* **2002**, *154*, 29–43. [CrossRef]
14. McGrath, S.P.; Zhao, F.J.; Lombi, E. Phytoremediation of metals, metalloids, and radionuclides. *Adv. Agron.* **2002**, *75*, 1–56.
15. Robinson, B.H.; Anderson, C.W.N.; Dickinson, N.M. Phytoextraction: Where's the action? *J. Geochem. Explor.* **2015**, *151*, 34–40. [CrossRef]
16. Niazi, N.K.; Singh, B.; Van Zwieten, L.; Kachenko, A.G. Phytoremediation of an arsenic-contaminated site using *Pteris vittata* L. and *Pityrogramma calomelanos* var. austroamericana: A long-term study. *Environ. Sci. Pollut. Res.* **2012**, *19*, 3506–3515. [CrossRef]
17. Kertulis-Tartar, G.M.; Ma, L.Q.; Tu, C.; Chirenje, T. Phytoremediation of an arsenic-contaminated site using *Pteris vittata* L.: A two-year study. *Int. J. Phytoremediat.* **2006**, *8*, 311–322. [CrossRef]
18. Lessl, J.T.; Ma, L.Q. Sparingly-soluble phosphate rock induced significant plant growth and arsenic uptake by *Pteris vittata* from three contaminated soils. *Environ. Sci. Technol.* **2013**, *47*, 5311–5318. [CrossRef]
19. Liao, X.-Y.; Chen, T.-B.; Xiao, X.-Y.; Xie, H.; Yan, X.-L.; Zhai, L.-M.; Wu, B. Selecting appropriate forms of nitrogen fertilizer to enhance soil arsenic removal by *Pteris vittata*: A new approach in phytoremediation. *Int. J. Phytoremediat.* **2007**, *9*, 269–280. [CrossRef]
20. da Silva, E.B.; Lessel, J.T.; Wilkie, A.C.; Liu, X.; Liu, Y.; Ma, L.Q. Arsenic removal by As-hyperaccumulator *Pteris vittata* from two contaminated soils: A 5-year study. *Chemosphere* **2018**, *206*, 736–741. [CrossRef]
21. Yang, J.; Guo, Y.; Yan, Y.; Yang, J.; Wan, X.; Guo, J.; Guo, J.; Chen, T.; Lei, M. Phytoaccumulation of As by *Pteris vittata* supplied with fertilizers under different soil moisture regimes—A field case. *Ecol. Eng.* **2019**, *138*, 274–280. [CrossRef]
22. Fayiga, A.O.; Ma, L.Q. Using phosphate rock to immobilize metals in soil and increase arsenic uptake by hyperaccumulator *Pteris vittata*. *Sci. Total Environ.* **2006**, *359*, 17–25. [CrossRef]
23. Cao, X.; Ma, L.Q.; Shiralipour, A. Effects of compost and phosphate amendments on arsenic mobility in soils and arsenic uptake by the hyperaccumulator, *Pteris Vittata* L. *Environ. Pollut.* **2003**, *126*, 157–167. [CrossRef]
24. Hua, C.-Y.; Chen, J.-X.; Cao, Y.; Li, H.-B.; Chen, Y.; Ma, L.Q. *Pteris vittata* coupled with phosphate rock effectively reduced As and Cd uptake by water spinach from contaminated soil. *Chemosphere* **2020**, *247*, 1–7. [CrossRef]
25. Chen, T.; Fan, Z.; Lei, M.; Huang, Z.; Wei, C. Effect of phosphorus on arsenic accumulation in As-hyperaccumulator *Pteris vittata* L. and its implication. *Chin. Sci. Bull.* **2002**, *47*, 1876–1879. [CrossRef]

26. Tu, S.; Ma, L.Q. Interactive effects of pH, arsenic and phosphorus on uptake of As and P and growth of the arsenic hyperaccumulator *Pteris vittata* L. under hydroponic conditions. *Environ. Exp. Bot.* **2003**, *50*, 243–251. [CrossRef]
27. Caille, N.; Swanwick, S.; Zhao, F.J.; McGrath, S.P. Arsenic hyperaccumulation by *Pteris vittata* from arsenic contaminated soils and the effect of liming and phosphate fertilisation. *Environ. Pollut.* **2004**, *132*, 113–120. [CrossRef]
28. Wenzel, W.W.; Kirchbaumer, N.; Prohaska, T.; Stingeder, G.; Lombi, E.; Adriano, D.C. Arsenic fractionation in soils using an improved sequential extraction procedure. *Anal. Chim. Acta* **2001**, *436*, 309–323. [CrossRef]
29. Cao, X.; Ma, L.Q.; Tu, C. Antioxidative responses to arsenic in the arsenic-hyperaccumulator Chinese brake fern (*Pteris vittata* L.). *Environ. Pollut.* **2004**, *128*, 317–325. [CrossRef]
30. Zafeiriou, I.; Gasparatos, D.; Kalyvas, G.; Ioannou, D.; Massas, I. Desorption of arsenic from calcareous mine affected soils by phosphate fertilizers application in relation to soil properties and As partitioning. *Soil Syst.* **2019**, *3*, 54. [CrossRef]
31. Codling, E.E.; Dao, T.H. Short-term effect of lime, phosphorus, and iron amendments on water-extractable lead and arsenic in orchard soils. *Commun. Soil Sci. Plant Anal.* **2007**, *38*, 903–919. [CrossRef]
32. Peryea, F.J. Phosphate-induced release of arsenic from soils contaminated with lead arsenate. *Soil Sci. Soc. Am. J.* **1991**, *55*, 1301–1306. [CrossRef]
33. Davenport, J.R.; Peryea, F.J. Phosphate fertilizers influence leaching of lead and arsenic in a soil contaminated with lead arsenate. *Water. Air. Soil Pollut.* **1991**, *57–58*, 101–110. [CrossRef]
34. Trotta, A.; Falaschi, P.; Cornara, L.; Minganti, V.; Fusconi, A.; Drava, G.; Berta, G. Arbuscular mycorrhizae increase the arsenic translocation factor in the As hyperaccumulating fern *Pteris vittata* L. *Chemosphere* **2006**, *65*, 74–81. [CrossRef] [PubMed]
35. Liu, Y.; Zhu, Y.G.; Chen, B.D.; Christie, P.; Li, X.L. Influence of the arbuscular mycorrhizal fungus *Glomus mosseae* on uptake of arsenate by the As hyperaccumulator fern *Pteris vittata* L. *Mycorrhiza* **2005**, *15*, 187–192. [CrossRef] [PubMed]
36. Gerhardt, K.E.; Huang, X.D.; Glick, B.R.; Greenberg, B.M. Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges. *Plant Sci.* **2009**, *176*, 20–30. [CrossRef]
37. Neugschwandtner, R.W.; Tlustoš, P.; Komárek, M.; Száková, J. Phytoextraction of Pb and Cd from a contaminated agricultural soil using different EDTA application regimes: Laboratory versus field scale measures of efficiency. *Geoderma* **2008**, *144*, 446–454. [CrossRef]
38. Audet, P. Examining the ecological paradox of the mycorrhizal metal hyperaccumulators. *Arch. Agron. Soil Sci.* **2012**, *59*, 549–558. [CrossRef]
39. Marschner, H.; Romheld, V. Strategies of plants for acquisition of iron. *Plant Soil* **1994**, *165*, 261–274. [CrossRef]
40. Lombi, E.; Sletten, R.S.; Wenzel, W.W. Sequentially extracted arsenic from different size fractions of contaminated soils. *Water Air Soil Pollut.* **2000**, *124*, 319–332. [CrossRef]
41. Smith, E.; Smith, J.; Naidu, R. Distribution and nature of arsenic along former railway corridors of South Australia. *Sci. Total Environ.* **2006**, *363*, 175–182. [CrossRef]
42. Gonzaga, M.I.; Santos, J.A.G.; Ma, L.Q. Arsenic chemistry in the rhizosphere of *Pteris vittata* L. and *Nephrolepis exaltata* L. *Environ. Pollut.* **2006**, *143*, 254–260. [CrossRef]
43. Tu, S.; Ma, L.; Luongo, T. Root exudates and arsenic accumulation in arsenic hyperaccumulating *Pteris vittata* and non-hyperaccumulating *Nephrolepis exaltata*. *Plant Soil* **2004**, *258*, 9–19. [CrossRef]
44. Fitz, W.J.; Wenzel, W.W.; Zhang, H.; Nurmi, J.; Stipek, K.; Fischerova, Z.; Schweiger, P.; Köllensperger, G.; Ma, L.Q.; Stingeder, G. Rhizosphere characteristics of the arsenic hyperaccumulator *Pteris vittata* L. and monitoring of phytoremoval efficiency. *Environ. Sci. Technol.* **2003**, *37*, 5008–5014. [CrossRef] [PubMed]
45. Reichard, P.U.; Kretzschmar, R.; Kraemer, S.M. Dissolution mechanisms of goethite in the presence of siderophores and organic acids. *Geochim. Cosmochim. Acta* **2007**, *71*, 5635–5650. [CrossRef]
46. Furrer, G.; Stumm, W.; Zinder, B. The coordination chemistry of weathering: II. Dissolution of Fe(III) oxides. *Geochim. Cosmochim. Acta* **1986**, *50*, 1861–1869. [CrossRef]
47. Dobran, S.; Zagury, G.J. Arsenic speciation and mobilization in CCA-contaminated soils: Influence of organic matter content. *Sci. Total Environ.* **2006**, *364*, 239–250. [CrossRef] [PubMed]

48. Das, S.; Chou, M.L.; Jean, J.S.; Yang, H.J.; Kim, P.J. Arsenic-enrichment enhanced root exudates and altered rhizosphere microbial communities and activities in hyperaccumulator *Pteris vittata*. *J. Hazard. Mater.* **2017**, *325*, 279–287. [CrossRef]
49. Madrid, F.; Liphadzi, M.S.; Kirkham, M.B. Heavy metal displacement in chelate-irrigated soil during phytoremediation. *J. Hydrol.* **2003**, *272*, 107–119. [CrossRef]
50. Martin del Campo, M.V.; Esteller, M.V.; Morell, I.; Expósito, J.L.; Bandenay, G.L.; Díaz-Delgado, C. A lysimeter study under field conditions of nitrogen and phosphorus leaching in a turf grass crop amended with peat and hydrogel. *Sci. Total Environ.* **2019**, *648*, 530–541. [CrossRef]
51. Meredith, L.K.; Boye, K.; Savage, K.; Vargas, R. Formation and fluxes of soil trace gases. *Soil Syst.* **2020**, *4*, 22. [CrossRef]
52. Salt, D.E.; Smith, R.D.; Raskin, I. Phytoremediation. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1998**, *49*, 643–668. [CrossRef]
53. Mlakar, T.L.; Horvat, M.; Vuk, T.; Stergaršek, A.; Kotnik, J.; Tratnik, J.; Fajon, V. Mercury species, mass flows and processes in a cement plant. *Fuel* **2010**, *89*, 1936–1945. [CrossRef]
54. Shi, J.; Thompson, V.S.; Yancey, N.A.; Stavila, V.; Simmons, B.A.; Singh, S. Impact of mixed feedstocks and feedstock densification on ionic liquid pretreatment efficiency. *Biofuels* **2013**, *4*, 63–72. [CrossRef]
55. Burns, K.; Villeneuve, J.-P. Chlorinated hydrocarbons in the open Mediterranean ecosystem and implications for mass balance calculations. *Mar. Chem.* **1987**, *20*, 337–359. [CrossRef]
56. Tetra Tech EM. *Final Current Conditions Report, University of California, Berkeley Richmond Field Station, Richmond*; Tetra Tech EM, Inc.: San Francisco, CA, USA, 2008; pp. 1–193.
57. Department of Toxic Substances Control, California Environmental Protection Agency, State of California. *State of California Department of Toxic Substances Control Site Investigation and Remediation Order, Docket No. I/SE-RAO 0607-004*; State of California: Sacramento, CA, USA, 2006; pp. 1–47.
58. Duvergé, D.J. *Establishing Background Arsenic in Soil of the Urbanized San Francisco Bay Region*; San Francisco State University: San Francisco, CA, USA, 2011.
59. *Human Health Risk Assessment (HHRA) Note Number 3, DTSC-Modified Screening Levels (DTSC-SLs)*; California Department of Toxic Substance Control (Cal DTSC): Sacramento, CA, USA, 2020; pp. 1–46.
60. Poppe, L.J.; Paskevich, V.F.; Hathaway, J.C.; Blackwood, D.S. *A Laboratory Manual for X-ray Powder Diffraction*; United States Geological Survey: Reston, VA, USA, 2002.
61. Chen, T.; Wei, C.; Huang, Z.; Huang, Q.; Quanguo, L.; Fan, Z. Arsenic hyperaccumulator *Pteris vittata* L. and its arsenic accumulation. *Chin. Sci. Bull.* **2002**, *47*, 902–905. [CrossRef]
62. Brewer, R.; Peard, J.; Heskett, M. A critical review of discrete soil sample data reliability: Part 1—Field study results. *Soil Sediment Contam.* **2017**, *26*, 1–22. [CrossRef]
63. Wang, J.; Zhao, F.-J.; Meharg, A.A.; Raab, A.; Feldmann, J.; McGrath, S.P. Mechanisms of arsenic hyperaccumulation in *Pteris vittata*. Uptake kinetics, interactions with phosphate, and arsenic speciation. *Plant Physiol.* **2002**, *130*, 1552–1561. [CrossRef]
64. Matzen, S.L. *Effects of Soil Biogeochemical and Physical Characteristics on Arsenic Hyperaccumulation in Pteris vittata L.*; University of California: Berkeley, CA, USA, 2020.
65. Arai, Y.; Dahle, J.T. Redox-ligand complexation controlled chemical fate of ceria nanoparticles in an agricultural soil. *J. Agric. Food Chem.* **2018**, *66*, 6646–6653. [CrossRef]
66. Sarret, G.; Willems, G.; Isaure, M.-P.; Marcus, M.A.; Fakra, S.C.; Frérot, H.; Pairis, S.; Geoffroy, N.; Manceau, A.; Saumitou-Laprade, P. Zinc distribution and speciation in *Arabidopsis halleri* × *Arabidopsis lyrata* progenies presenting various zinc accumulation capacities. *New Phytol.* **2009**, *184*, 581–595. [CrossRef]
67. McIntosh, J.L. Bray and Morgan soil extractants modified for testing acid soils from different parent materials 1. *Agron. J.* **1969**, *61*, 259–265. [CrossRef]
68. Gonzaga, M.I.S.; Santos, J.A.G.; Ma, L.Q. Phytoextraction by arsenic hyperaccumulator *Pteris vittata* L. from six arsenic-contaminated soils: Repeated harvests and arsenic redistribution. *Environ. Pollut.* **2008**, *154*, 212–218. [CrossRef]
69. Marcus, M.A.; MacDowell, A.A.; Celestre, R.; Manceau, A.; Miller, T.; Padmore, H.A.; Sublett, R.E. Beamline 10.3.2 at ALS: A hard X-ray microprobe for environmental and materials sciences. *J. Synchrotron Radiat.* **2004**, *11*, 239–247. [CrossRef]
70. Ravel, B.; Newville, M. ATHENA, ARTEMIS, HEPHAESTUS: Data analysis for X-ray absorption spectroscopy using IFEFFIT. *J. Synchrotron Radiat.* **2005**, *12*, 537–541. [CrossRef]

71. Niazi, N.K.; Singh, B.; Van Zwieten, L.; Kachenko, A.G. Phytoremediation potential of *Pityrogramma calomelanos* var. austroamericana and *Pteris vittata* L. grown at a highly variable arsenic contaminated site. *Int. J. Phytoremediat.* **2011**, *13*, 912–932. [CrossRef]
72. Lei, M.; Wan, X.; Guo, G.; Yang, J.; Chen, T. Phytoextraction of arsenic-contaminated soil with *Pteris vittata* in Henan Province, China: Comprehensive evaluation of remediation efficiency correcting for atmospheric depositions. *Environ. Sci. Pollut. Res.* **2018**, *25*, 124–131. [CrossRef]
73. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2019.
74. Ent, A.; Van, D.; Baker, A.J.M. Hyperaccumulators of metal and metalloid trace elements: Facts and Fiction. *Plant Soil* **2013**, 319–334. [CrossRef]
75. Ciurli, A.; Lenzi, L.; Alpi, A.; Pardossi, A. Arsenic uptake and translocation by plants in pot and field experiments. *Int. J. Phytoremediat.* **2014**, *16*, 804–823. [CrossRef]
76. Tu, C.; Ma, L. Effects of arsenic concentrations and forms on arsenic uptake by the hyperaccumulator ladder brake. *J. Environ. Qual.* **2002**, *31*, 641–647. [CrossRef]
77. Fayiga, A.O.; Ma, L.Q.; Cao, X.; Rathinasabapathi, B. Effects of heavy metals on growth and arsenic accumulation in the arsenic hyperaccumulator *Pteris vittata* L. *Environ. Pollut.* **2004**, *132*, 289–296. [CrossRef]
78. Natarajan, S.; Stamps, R.H.; Saha, U.K.; Ma, L.Q. Effects of nitrogen and phosphorus levels, and frond-harvesting on absorption, translocation and accumulation of arsenic by Chinese brake fern (*Pteris vittata* L.). *Int. J. Phytoremediat.* **2009**, *11*, 313–328. [CrossRef]
79. Ma, Q.Y.; Traina, S.J.; Logan, T.J.; Ryan, J.A. In situ lead immobilization by apatite. *Environ. Sci. Technol.* **1993**, *27*, 1803–1810. [CrossRef]
80. Mkhabela, M.S.; Warman, P.R. The influence of municipal solid waste compost on yield, soil phosphorus availability and uptake by two vegetable crops grown in a Pugwash sandy loam soil in Nova Scotia. *Agric. Ecosyst. Environ.* **2005**, *106*, 57–67. [CrossRef]
81. Giusquiani, P.L.; Marucchini, C.; Businelli, M. Chemical properties of soils amended with compost of urban waste. *Plant Soil* **1988**, *109*, 73–78. [CrossRef]
82. Leung, H.M.; Leung, A.O.W.; Ye, Z.H.; Cheung, K.C.; Yung, K.K.L. Mixed arbuscular mycorrhizal (AM) fungal application to improve growth and arsenic accumulation of *Pteris vittata* (As hyperaccumulator) grown in As-contaminated soil. *Chemosphere* **2013**, *92*, 1367–1374. [CrossRef]
83. Leung, H.M.; Wu, F.Y.; Cheung, K.C.; Ye, Z.H.; Wong, M.H. Synergistic effects of arbuscular mycorrhizal fungi and phosphate rock on heavy metal uptake and accumulation by an arsenic hyperaccumulator. *J. Hazard. Mater.* **2010**, *181*, 497–507. [CrossRef]
84. Nagy, R.; Drissner, D.; Amrhein, N.; Jakobsen, I.; Bucher, M. Mycorrhizal phosphate uptake pathway in tomato is phosphorus-repressible and transcriptionally regulated. *New Phytol.* **2009**, *184*, 1029. [CrossRef]
85. Zhao, F.J.; Dunham, S.J.; McGrath, S.P. Arsenic hyperaccumulation by different fern species. *New Phytol.* **2002**, *156*, 27–31. [CrossRef]
86. Liao, X.-Y.; Chen, T.-B.; Lei, M.; Huang, Z.-C.; Xiao, X.-Y.; An, Z.-Z. Root distributions and elemental accumulations of Chinese brake (*Pteris vittata* L.) from As-contaminated soils. *Plant Soil* **2004**, *261*, 109–116. [CrossRef]
87. Wilde, E.W.; Brigmon, R.L.; Dunn, D.L.; Heitkamp, M.A.; Dagnan, D.C. Phytoextraction of lead from firing range soil by Vetiver Grass. *Chemosphere* **2005**, *61*, 1451–1457. [CrossRef]
88. Hadley, P.W.; Petrisor, I.G. Incremental sampling: Challenges and opportunities for environmental forensics. *Environ. Forensics* **2013**, *14*, 109–120. [CrossRef]
89. Ma, L.Q.; Komar, K.M.; Tu, C.; Zhang, W.; Cai, Y.; Kennelley, E.D. A fern that hyperaccumulates arsenic. *Nature* **2001**, *409*, 579. [CrossRef]
90. Zhang, W.; Cai, Y.; Tu, C.; Ma, L.Q. Arsenic speciation and distribution in an arsenic hyperaccumulating plant. *Sci. Total Environ.* **2002**, *300*, 167–177. [CrossRef]
91. Lin, Z.; Puls, R.W. Adsorption, desorption and oxidation of arsenic affected by clay minerals and aging process. *Environ. Geol.* **2000**, *39*, 753–759. [CrossRef]
92. Roberts, L.C.; Hug, S.J.; Voegelin, A.; Dittmar, J.; Kretzschmar, R.; Wehrli, B.; Saha, G.C.; Badruzzaman, A.B.M.; Ali, M.A. Arsenic dynamics in porewater of an intermittently irrigated paddy field in Bangladesh. *Environ. Sci. Technol.* **2011**, *45*, 971–976. [CrossRef]

93. Liu, X.; Fu, J.-W.; Guan, D.-X.; Cao, Y.; Luo, J.; Rathinasabapathi, B.; Chen, Y.; Ma, L.Q. Arsenic induced phytate exudation, and promoted $FeAsO_4$ dissolution and plant growth in As-hyperaccumulator *Pteris vittata*. *Environ. Sci. Technol.* **2016**, *50*, 9070–9077. [CrossRef]
94. Hutton, C.; Bryce, D.W.; Russeau, W.; Glass, H.J.; Jenkin, L.E.T.; Corns, W.T.; Stockwell, P.B. Aqueous and solid-phase speciation of arsenic in Cornish soils. *Mineral. Mag.* **2005**, *69*, 577–589. [CrossRef]
95. Masscheleyn, P.H.; Delaune, R.D.; Patrick, W.H. Effect of redox potential and pH on arsenic speciation and solubility in a contaminated soil. *Environ. Sci. Technol.* **1991**, *25*, 1414–1419. [CrossRef]
96. Vinther, F.P.; Hansen, E.M.; Eriksen, J. Leaching of soil organic carbon and nitrogen in sandy soils after cultivating grass-clover swards. *Biol. Fertil. Soils* **2006**, *43*, 12–19. [CrossRef]
97. Uselman, S.M.; Qualls, R.G.; Lilienfein, J. Contribution of root vs. leaf litter to dissolved organic carbon leaching through soil. *Soil Sci. Soc. Am. J.* **2007**, *71*, 1555–1563. [CrossRef]
98. Tiberg, C.; Bendz, D.; Theorin, G.; Kleja, D.B. Evaluating solubility of Zn, Pb, Cu and Cd in pyrite cinder using leaching tests and geochemical modelling. *Appl. Geochem.* **2017**, *85*, 106–117. [CrossRef]
99. Duan, G.-L.; Zhu, Y.-G.; Tong, Y.-P.; Cai, C.; Kneer, R. Characterization of arsenate reductase in the extract of roots and fronds of Chinese brake fern, an arsenic hyperaccumulator. *Plant Physiol.* **2005**, *138*, 461–469. [CrossRef]
100. Wang, X.; Ma, L.Q.; Rathinasabapathi, B.; Cai, Y.; Liu, Y.G.; Zeng, G.M. Mechanisms of efficient arsenite uptake by arsenic hyperaccumulator *Pteris vittata*. *Environ. Sci. Technol.* **2011**, *45*, 9719–9725. [CrossRef] [PubMed]
101. Yan, X.-L.; Liao, X.-Y.; Chen, T. Leaching potential of arsenic from *Pteris vittata* L. under field conditions. *Sci. Total Environ.* **2009**, *408*, 425–430. [CrossRef] [PubMed]
102. Chen, Y.; Fu, J.W.; Han, Y.H.; Rathinasabapathi, B.; Ma, L.Q. High As exposure induced substantial arsenite efflux in As-hyperaccumulator *Pteris vittata*. *Chemosphere* **2016**, *144*, 2189–2194. [CrossRef] [PubMed]
103. Wagner, S.; Hoefer, C.; Puschenreiter, M.; Wenzel, W.; Oburger, E.; Hann, S.; Robinson, B.; Kretzschmar, R.; Santner, J. Arsenic redox transformations and cycling in the rhizosphere of *Pteris vittata* and *Pteris quadriaurita*. *Environ. Exp. Bot.* **2020**, *177*, 1–12. [CrossRef]
104. Masue-Slowey, Y.; Ying, S.C.; Kocar, B.D.; Pallud, C.E.; Fendorf, S. Dependence of arsenic fate and transport on biogeochemical heterogeneity arising from the physical structure of soils and sediments. *J. Environ. Qual.* **2013**, *42*, 1119–1129. [CrossRef] [PubMed]
105. Yang, Q.; Tu, S.; Wang, G.; Liao, X.; Yan, X. Effectiveness of applying arsenate reducing bacteria to enhance arsenic removal from polluted soils by *Pteris vittata* L. *Int. J. Phytoremediation* **2012**, *14*, 89–99. [CrossRef]