Phosphate enhanced abiotic and biotic arsenic mobilization in the wetland rhizosphere

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HIGHLIGHTS

• Increased PO4^3-- loading to wetlands stimulates abiotic and biotic As mobilization.
• Increased PO4^3-- loading results in more As-reducing bacteria in wetland sediments.
• Increased PO4^3-- loading results in more As reduction in wetland sediments.
• Increased PO4^3-- loading results in more As uptake by wetland plants.
• Increased PO4^3-- loading to wetlands results in lower ORP and less Fe reduction.

ABSTRACT

Although abiotic process of competitive sorption between phosphate (P) and arsenate (As(V)), especially onto iron oxides, are well understood, P-mediated biotic processes of Fe and As redox transformation contributing to As mobilization and speciation in wetlands remain poorly defined. To gain new insights into the effects of P on As mobility, speciation, and bioavailability in wetlands, well-controlled greenhouse experiments were conducted. As expected, increased P levels contributed to more As desorption, but more interestingly the interactions between P and wetland plants played a synergistic role in the microbially-mediated As mobilization and enhanced As uptake by plants. High levels of P promoted plant growth and the exudation of labile organic carbon from roots, enhancing the growth of heterotrophic bacteria, including As and Fe reducers. This in turn resulted in both, more As desorption into solution due to reductive iron dissolution, and a higher fraction of the dissolved As in the form of As(III) due to the higher number of As(V) reducers. Consistent with the dissolved As results, arsenic-XANES spectra from solid medium samples demonstrated that more As was sequestered in the rhizosphere as As(III) in the presence of high P levels than for low P levels. Hence, increased P loading to wetlands stimulates both abiotic and biotic processes in the wetland rhizosphere, resulting in more As mobilization, more As reduction, as well as more As uptake by plants. These interactions are important to be taken into account in As fate and transport models in wetlands and management of wetlands containing As.

1. Introduction

Arsenic (As) release in response to biogeochemical perturbation and its mobility in groundwater and saturated soils is a major health concern since it may lead to As contamination of groundwater and food crops (Harvey et al., 2002; Polizzotto et al., 2008; Rango et al., 2013). Chronic exposure to As contaminated groundwater and food may result in various diseases and a high risk of
arsenopyrite (FeAsS) in anoxic soils and sediments (Saatmagnetite) also play an important role in As mobilization (Tufano et al., 2012), or the hyper-accumulation of As in plants (e.g. soils and sediments have mainly focused either on the competitive sorption and concomitant release of sorbed As to the aqueous phase. Moreover, the reductive iron-mineral transformations toward more reduced and more stable iron phases with less As sorption affinity and capacity (e.g. from poorly crystalline ferrihydrite to more crystalline goethite, lepidocrocite and/or magnetite) also play an important role in As mobilization (Tufano and Fendorf, 2008; Johnston et al., 2011; Rawson et al., 2016). Second, the heterotrophic bacteria-mediated reduction of As(V) to As(III) also contributes to the enhanced As mobility in the anoxic conditions and arsenite (As(III)) in anaerobic soils and sediments (Saafield and Bostick, 2009).

Numerous studies have demonstrated As mobilization from the As-enriched soils and sediments during a transition from oxidizing to reducing conditions (Beauchemin and Kwong, 2006; Borch et al., 2010; Lizama et al., 2011; Gorny et al., 2015). Several underlying mechanisms have been elucidated to explain As mobility, toxicity, and bioavailability, which determines the release of As into groundwater and accumulation in plant tissue. First, the microbially mediated reductive dissolution of iron oxides leads to the loss of sorption sites and concomitant release of sorbed As to the aqueous phase. Moreover, the reductive iron-mineral transformations toward more reduced and more stable iron phases with less As sorption affinity and capacity (e.g. from poorly crystalline ferrihydrite to more crystalline goethite, lepidocrocite and/or magnetite) also play an important role in As mobilization (Tufano and Fendorf, 2008; Johnston et al., 2011; Rawson et al., 2016). Second, the heterotrophic bacteria-mediated reduction of As(V) to As(III) also contributes to the enhanced As mobility in the anoxic sediments (Kocar et al., 2006; Tufano et al., 2008; Wang et al., 2012). Finally, the loading of other competing sorbates than phosphate (e.g. bicarbonate and dissolved organic matter) may also promote As release from the solid phase to the dissolved phase (Zeng et al., 2008; Hartland et al., 2015; Mladenov et al., 2015).

Phosphate (P) is extensively used as fertilizers in agriculture, and leaches into groundwater and surface water from agricultural land runoff and/or from wastewaters. Since P is a similar structural analog to As(V) and has a higher electric density, the loading of P effectively competes with As(V) for sorption onto Fe oxyhydroxides (Zeng et al., 2008). A positive correlation has been found between P and As concentrations in groundwater in Bangladesh (Aziz et al., 2002). Furthermore, P can regulate microbially mediated Fe and As redox transformation to influence the mobility of As (Zhang et al., 2014b).

Previous studies investigating the impact of P on As dynamics in soils and sediments have mainly focused either on the competitive exchange between P and As in soils and sediments (Dong et al., 2012), or the hyper-accumulation of As in plants (e.g. Pteris vititata) (Xie et al., 2009; Wang et al., 2011). Although the processes of competitive sorption and microbially mediated reductive dissolution have been well documented individually, the As mobility in wetlands and how it responds to increasing P loads for soils with different Fe(III) contents still remains poorly understood. P loading to wetlands affects As mobility, given that as described above P and As compete for the same sorption sites onto Fe oxyhydroxides and accumulation in wetland plants (Blute et al., 2004; Liu et al., 2006; Yamaguchi et al., 2014). High levels of P can also promote the growth of wetland plants, which has a significant impact on the biogeochemical dynamics of the sediment redoxcline and subsequently determines As mobility. The impact of the presence of wetland plants on As mobility have been confounding until now.

On the one hand, root exudates from plants can be used as labile organic carbon source for microorganisms to establish anaerobic environments, promote Fe and As reduction, and consequently enhance As release into water. On the other hand, wetland plants supply oxygen to roots and transfer oxygen to the rhizosphere, resulting in the re-oxidation of aqueous Fe(II) to generate Fe(III) plaques on the roots surface, leading to the attenuation of aqueous As (Jia et al., 2014). Additionally, plants can translocate As from pore water into plant roots/shoots (Lyubenova et al., 2013). Hence, the As mobility in the wetland rhizosphere with high P and its bio-accumulation in plants are determined by a combination of multiple physical, biochemical and phyto-accumulation processes.

This study focuses on the confounding impact of P on As mobility in the wetland rhizosphere, via a systematic comparison among a series of well-controlled greenhouse mesocosms with various P and Fe levels in the presence or absence of wetland plants (Scirpus actus). The objective of this study was to gain novel insights into the interactions between P, Fe, and wetland plants and their influence on As dynamics via biotic and abiotic processes.

2. Materials and methods

2.1. Mesocosm experiment

A series of greenhouse mesocosms (low P (10 μM) or high P (100 μM), low Fe(III) (no external ferrihydrite added) or high Fe(III) (25 μmol ferrihydrite/g solid medium added), in the presence or absence of plants (Scirpus actus) were constructed. Six individual cylinder-shaped plastic containers (15.6 cm internal diameter in the bottom, 17.1 cm internal diameter at the top, 15.6 cm in the depth) were placed in each of two large rectangular plastic containers (65.5 × 40.0 × 35.0 cm) to keep all the mesocosms in the same container immersed and exposed to the same levels of As(V) (~50 μM) and P (10 μM or 100 μM) over the duration of the experiments (Fig. S1). The solutions containing As and the two different P levels were continuously recycled using a pump through the containers to keep all the mesocosms flooded, eliminate any concentration gradient between mesocosms in each treatment, and maintain water levels constant. For each P treatment, because the solid medium was fully mixed and homogenous, only one low Fe mesocosm and one high Fe mesocosm without plants were set up, and each mesocosm was large enough to allow for the collection of replicate samples. Because of heterogeneity in the wetland rhizosphere, two mesocosms with plants were built for each treatment (Fig. S1). Another four mesocosms with plants (low P low Fe, low P high Fe, high P low Fe, and high P high Fe) were set up as blank controls and maintained under identical experimental conditions without the addition of As. Four sampling ports were drilled along the side-walls of the plastic containers at different depths (5, 8, 11 and 14 cm below water surface) to allow for the collection of pore water samples along the longitudinal flow axis using syringes.

The solid medium for the mesocosm study consisted of ASTM 20/30 silicon sand (US Silica, Ottawa, USA), peat moss (Premier Horticulture Inc., Quebec, Canada) and soil collected at the Charles H. Rogers wildlife wetland (Princeton, USA) at a weight ratio of 1:1:1.05, respectively. Ferrihydrite (synthesized as described by Schwertman and Cornell (Schwertmann and Cornell, 2007)) was used as the sediment iron phase, because it is a common Fe oxy-hydroxide found in soils and sediments at various sites, including wetlands. Moreover, iron precipitates/iron plaques on root surfaces are usually ferrihydrite (Borch et al., 2007). Scirpus actus (Pinelands Nursery and Supply, Columbus, USA) was chosen for this study because it is a typical wetland plant commonly found in natural wetlands, grows fast, and is easily grown in wetland mesocosms (Reid and Jaffé, 2012). Scirpus actus was transplanted to the
“planted” mesocosms to monitor the effect of plants on the As dynamics. All the mesocosms were operated in a greenhouse plant growth chamber (Environmental Growth Chamber, USA) under well controlled temperature, light intensity, and humidity, simulating typical New Jersey temperature, humidity and light conditions during mid-Summer (Table S1).

A modified Hoagland nutrient solution, containing (NH₄)₂SO₄ 10.1 g L⁻¹, Ca(NO₃)₂·4H₂O 23.6 g L⁻¹, Fe(NO₃)₃·9 mg L⁻¹, MgCl₂·6H₂O 9.26 g L⁻¹, NH₄Cl 1.07 g L⁻¹, H₂BO₃ 57.2 mg L⁻¹, MnCl₂·4H₂O 36.2 mg L⁻¹, ZnSO₄·7H₂O 4.4 mg L⁻¹, CuSO₄·1.02 mg L⁻¹, Na₂MoO₄·2H₂O 0.24 mg L⁻¹, KH₂PO₄ 1.36 g L⁻¹) was freshly prepared, diluted 200 times, and used as plant nutrient solution, which was pumped into the container for each P treatment (Xu and Jaffe, 2006). The pH of the nutrient solution was adjusted to 6.8–7.0 with 1 M HCl or NaOH. Sodium acetate at a concentration of 2 mM was added as a labile carbon source to establish the redox transition from oxic conditions at the top of the mesocosms to anoxic conditions deeper into the mesocosms. The water level was kept at 3 cm above the solid medium, and nutrient solution in each container containing the mesocosms for a specific P treatment (low P (10 μM) or high P (100 μM)) was re-circulated with a pump to maintain fully mixed conditions in the container. The mesocosms were run for about four months and monitored regularly along the depth profile for dissolved As, Fe, and P species as well as total organic carbon. Initially, after the mesocosms were setup and flooded with the As(V) enriched solutions for the low and high P conditions, the solutions were pumped through the mesocosms from the bottom upwards for two days to develop an initial homogenous dissolved concentration depth. Chloride (Cl⁻) was also monitored as a non-reactive dissolved species.

2.2. Pore water, solid medium, and plant sampling

Porewater samples were intermittently collected at different depth at one/two-week intervals during daytime. For each mesocosm without plants, two replicate samples were collected from the mesocosm at each depth every time samples were taken, whereas for each duplicate mesocosm with plants, one sample was taken from each depth. At each depth, approximately 15 mL of pore water was collected. Redox potential (ORP) and pH values were measured immediately after sample collection. Part of the pore water samples were centrifuged at 2000 rpm, filtered with 0.22 μm PVDF filters ( Fisher Scientific, USA) and stored at 4 °C for Fe, anions (e.g. PO₄³⁻ and Cl⁻) and total organic carbon (TOC) analysis. This sampling strategy was shown to be reliable to help account for possible variations of chemical species in the horizontal dimension in the same mesocosms (Xu and Jaffe, 2006).

After four months of operation, the mesocosms were dismantled in an anaerobic chamber to avoid the oxidation of reduced species. Solid medium samples from the four layers corresponding to pore water sampling depths were collected into glass vials immediately after sample collection. Part of the pore water samples was determined using a Dionex Ion Chromatograph (IC) (Model LC20, Dionex Corp., USA) with conductivity detector (Dionex CD25) and columns which is Dionex IonPak AS-14 and AG14, both with a diameter of 4 mm. The injection loop was 25 μL and the columns were Dionex IonPak AS-14 and AG14, both with a diameter of 4 mm. The eluent consisted of 4.5 mM Na₂CO₃ and 1.4 mM NaHCO₃. The flow rate was set at 1.2 mL min⁻¹. HCl-extractable Fe(II) and Fe (III) were analyzed using the ferrozine assay method (Lovley and Phillips, 1987). Total dissolved organic carbon (TOC) content in the filtered pore water samples was determined using a TOC analyzer ( Shimadzu Scientific Instruments, Japan).

Total As was measured using a digital arsenic test kit (Palintest Ltd, UK). The method uses strong reducing reagents to reduce the total As (As(V) and As(III)) to AsH₃, which reacts with the chromogenic reagent to form a yellow color. Phosphate does not interfere with colorimetric assays (www.palintest.com/en/products/digital-arsenic-test-kit). As(V) and As(III) standard solutions in de-ionized water were prepared and analyzed using the test kit to show that the method detected total As accurately (limit of detection: 3 μg L⁻¹), independent of As speciation. Therefore, the total dissolved As (As(V) + As(III)) in the samples was measured by mixing filtered pore water with reagents according to the manual instruction. Then, the same samples were filtered through As speciation cartridges (Metalsoft Center Inc., USA) which can specifically sequester As(V) (Meng et al., 2001). The filtrates were collected and analyzed with the arsenic test kit to determine the levels of dissolved As(III).

For total As analysis in the solid medium, plant roots, and shoots, we used a modified digestion procedure previously developed for As analysis in sea grass (Cai et al., 2000). Briefly, 0.2–0.5 g samples (solid medium or plants) were digested in open vessels with 8 mL of concentrated nitric acid in a heater at 150 °C for 1 h. Then, 2 mL hydrogen peroxide was added into the sample vessel and the sample was digested at 150 °C for an additional 30 min. After cooling, the samples were filled to 15 mL with de-ionized water. These solutions were diluted with de-ionized water to their appropriate detection range, prior to analysis using the digital arsenic test kit.

2.4. Quantitative PCR analysis

Total bacterial abundance, dissimilatory iron-reducing bacteria, sulfate-reducing bacteria (SRB), and dissimilatory arsenate-respiring bacteria (DARB) in the solid medium were quantified using quantitative PCR (qPCR) assays performed on a Light Cycler480 (Applied Biosystems, USA). DNA was extracted from 0.5 g of solid medium with a FastDNA SPIN soil kit (MP Biomedicals, USA), following the protocol described by the manufacturer. The concentrations of extracted DNA were measured using a Nano-drop 2000 spectrophotometer (ThermoFisher Scientific, USA). DNA from the collected solid medium were diluted 10-fold with UltraPure™ DNase/RNase-Free Distilled Water (ThermoFisher Scientific, USA) and analyzed via qPCR to determine different bacterial abundance. Total bacterial abundance were represented by the number of copies of 16S rRNA genes when qPCR was performed using a primer set of 1055F (ATGCGCTGTGCTGACT) and 1392R (AAGGGGCGGTGTCGACT) (Harms et al., 2003). Arsenate reducing bacteria were enumerated by qPCR using a primer set of arrfAF1 (CAGAGTTCGCTCCGATHCN) and arrfAR1 (GGGGCTGGCTTCYTNTNCT) to target arsenic respiratory reductase gene sequences (arra) (Song et al., 2009). Geobacteraceae, a dissimilatory iron-reducing bacteria, was enumerated by qPCR using a primer set of 561F (GCCTGACGGCTTGTTCTTA) and 852R (TACCAGACACCTAGTTCT) (Stults et al., 2001). Sulfate-reducing bacteria (SRB) were enumerated by qPCR using a primer set of DSR1F (ACS CAC TGG AAC CAC C) and DSR-R (GTG GMR CCG TGC
AKR TTG G) (Kondo et al., 2004). All qPCR experiments were carried out using a StepOnePlus™ Real-Time PCR System (Life Technologies, USA). For DNA amplification, each 20 μL qPCR mixture was composed of 10 μL of SYBR Premix Ex Taq™ II (Takara, Japan), 0.8 μL of 10 μM of each primer, and ~10 ng DNA template. The optimized PCR thermal cycling parameters for the total bacteria, arsenic reductase (arra), Geobacteraceae, SRB were as follows: 94 °C denatured 30s, followed by 40 cycles at 94 °C for 5 s, 60 °C for 30 s, 70 °C for 30 s; after these cycles, the reaction mixtures were further incubated at 72 °C for 10 min. Each assay contained a standard produced by serial dilution of plasmids containing specific target genes, independent duplicate templates for each solid medium sample, and duplicate no template controls (NTC). Standards for arra, Geobacter and SRB, were not available to provide actual bacterial numbers. Given that there are differences between the mesocosms and natural wetlands (see TOC values), it was felt that comparing relative bacterial numbers by normalizing with respect to those of the low P, low Fe without plants as the reference condition is more meaningful for this study than focusing on actual numbers, especially since the actual bacterial numbers in these mesocosms are likely atypical for natural conditions.

2.5. Pyrosequencing analysis

The extracted DNA was amplified using the bar-coded fusion primers targeting the V1 to V3 regions of the 16S rRNA gene. The forward sequence of the 9F primer was ‘5’- CTTACCTCCCTGTTGCTGTAGCTCAGTC-AC-AGAGTTT-GATCMTGGCTCAG-3’ and the reverse sequence 514R was ‘5’- CACTTCATACGGCTGTGTCCTACG-X-AC-ATTACCAGGCTGTCCTG-3’, where X represents a unique barcode sequence designed to differentiate the sequencing reads from different samples (http://www.ezbiocloud.net/olkb/b101). The PCR process was performed as follows: 95 °C denatured 5 min, followed by 30 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s; after these cycles, the reaction mixtures were further incubated at 72 °C for 5 min. The PCR products were purified using the QiAquick PCR purification kit (Qiagen, USA) and quantified using a PicoGreen dsDNA Assay kit (Invitrogen, USA). Equimolar concentrations of each amplicon from different samples were pooled and purified using an AMPure bead kit (Agencourt Bioscience, USA) and were subsequently amplified on sequencing beads by emulsion PCR. The beads recovered from the emulsion PCR were deposited on a 454 Picotiter Plate and sequenced with a Roche/454 GS Junior System at Chunlab, Inc. (Seoul, Korea). The raw pyrosequencing reads from the samples were sorted by their barcode sequences. The barcoded fusion primers were trimmed, and the low-quality sequences (e.g., ambiguous base cell ≥ 2, read length < 300 bp, or average quality < 25) were eliminated. Only PCR amplicons that showed a high match (e.g., an E-value > 10^{-5}) were used for the analyses.

The individual reads were assigned to their taxonomic positions according to the highest pair-wise similarity among the top five BLASTN hits against the EzTaxon-e database. The diversity indices and species richness were calculated using the Cluster Database at High Identity with Tolerance (CD-HIT) method. The compositions and proportions of the bacterial species that were shared among sets of multiple samples were calculated using the CLcommunity software (ChunLab, Inc., Seoul, Korea).

2.6. XANES spectroscopy analysis

Arsenic XANES spectroscopy studies were conducted at the Stanford Synchrotron Radiation Lightsource (SSRL) on beamline 9-3. The XANES spectral measurements were made using Si(220) monochromator, and a 100 element Ge fluorescence detector. The incident beam was detuned by 30% above the absorption edge to reject higher-order harmonics. Some of the bad channels (10–13) in the fluorescence detector were deleted before the spectra were processed. All the spectra were calibrated with ferric arsenate standard, with the white line of this sample spectrum set at 11.875 eV. The fraction of As(III) and As(V) were obtained by fitting the sample XANES spectra with Na-As(V) and Na-As(III) standards. Ferric arsenate was run often to check for any energy drifts during the run, which suggested that the incident beam energy was stable during the entire data collection. For each sample, 2–10 spectral scans were collected to evaluate the reproducibility. Samples with As(III) showed beam induced redox transformations. In such cases, we exposed fresh samples to the beam, and the first scan was considered for further analysis.

3. Results and discussion

3.1. Pore water As dynamics during the redox transition from oxidizing to reducing conditions

Pore water As dynamics during the redox transition from oxidizing to reducing conditions are shown in Fig. 1. Initially, all the mesocosms showed relatively homogenous vertical profiles of dissolved As, which were consistent with the almost fully uniform depth profiles of Cl− (Fig. S2) and P (Fig. S3) for both P treatments, suggesting that the pore water chemical constituents were initially well mixed. This confirms that changes in the chemical vertical profiles observed during the operation of the mesocosms were mainly due to a combination of physicochemical sorption and redox transformation, microbially mediated transformation, plant uptake, and/or transport/concentration effects due to plant transpiration rather than physical diffusion limitation. Over the experimental time (from day 0–118), redox-potential-depth profiles that were initially relatively uniform and oxidizing (140 mV–200 mV), ended up ranging from oxidizing conditions (80 mV–160 mV) at the top/surface of the mesocosms to reducing conditions (−140 mV–−20 mV) at the bottom of the mesocosms (Fig. 2).

For the low P treatments, the vertical concentration profiles of As progressively shifted to lower concentration values except for the condition of low P, high Fe, with plants (Fig. 1). However, for the high P treatments without plants, the aqueous As levels initially increased a little before decreasing, while the trend was shown to be opposite for the high P with plants. Consistently, r-pair tests including all experimental conditions indicated that P treatment and plant treatment, rather than Fe treatment, are key factors regulating As mobilization in this study (Table S2). The specific effects of P, Fe, and wetland plants on pore water As dynamics are discussed below.

3.1.1. Effect of P on pore water As dynamics

Whereas in the absence of plants the vertical P concentration profile remained constant with time, dissolved P gradually increased with depth for both high and low P treatment in the presence of plants, which is attributed in part to the increased flux of P to the bottom of the mesocosms, which was induced by increased water flow due to plant transpiration as well as the enhanced iron reduction at the bottom of the mesocosms which results in fewer P sorption sites (Fig. S3). The highest levels of pore water As (50.0–57.3 μM) at the end of the experiment were observed at the bottom of the mesocosm with high P, high Fe, and in the presence of plants (Fig. 3). In the presence of plants, which results in more iron reduction, the changes in absorbed P onto Fe oxides is significantly correlated with the changes in desorbed As from Fe oxides (R² = 0.98, p = 0.01), with a slope of ~1.02 (Fig. S4).
indicating competitive adsorption/desorption between the two species for surface sites on Fe oxides in the solid medium. This is consistent with other studies which have shown that the presence of P can substantially desorb As(V) and As(III) from soils and Fe oxides through ion exchange reaction and/or competitive sorption, especially around pH 7–8 (Jain and Loeppert, 2000; Gao and Mucci, 2001).

The higher levels of dissolved As in the high P treatment mesocosms were mainly in the form of As(III) rather than As(V) (Fig. 3). About 80% total dissolved As was As(III) for the high P treatment, while As(III) contributed to about 50% of the total As in the corresponding low P treatments. This is consistent with As(III) as the main As species in response to input of excess P fertilizers in a West Bengal soil under anaerobic conditions (Signes-Pastor et al., 2007). This effect is discussed further below in terms of differences in iron reduction and the bacterial community.

3.1.2. Effect of Fe on pore water As dynamics
Initially there was less As in solution in the high Fe treatment mesocosms, for both low and high P treatments in the presence of

Fig. 1. Evolution of pore water As depth profiles for low P (LP) and high P (HP) treatments. The initial treatments of LP and HP are 10 μM and 100 μM P, respectively. The low Fe (LFe) and high Fe (HFe) consisted of no external ferrihydrite addition and 25 μM ferrihydrite/g solid medium addition, respectively. Planted mesocosms were planted with Scirpus actus.

Fig. 2. Redox potential depth profiles in (a) low P and (b) high P treatments on day 0 and day 118. (±Plt: with plant, – Plt: without plant).
plants, indicating that more As was sequestered into the solid medium in these mesocosms (Fig. 1). As discussed above, amorphous ferrihydrite plays a critical role as an As retention reservoir, because ferrihydrite has a high sorption capacity for As. However, in the presence of labile carbon, ferrihydrite is easily transformed by bacteria during reductive dissolution, releasing sorbed As and Fe(II) into the aqueous phase (Tadanier et al., 2005). A smaller amount of Fe(II) (~10^7 M) was produced in the absence of plants (Fig. 4), indicating less Fe(III) reduction under these conditions, presumably due to the lower levels of labile organic carbon (Fig. 5). No significant difference in As levels between low Fe (As = 6.3 ± 3.4 μM) and high Fe (As = 5.8 ± 2.0 μM) was observed (p = 0.71) for the low P treatments, while a significant difference was observed (p = 0.04) for the high P treatments at the end of the mesocosm operation (Fig. 3), indicating that in the presence of plants, the addition of ferrihydrite has a strong impact on As dynamics for the high P levels, but not for low P levels, as will be discussed in more detail below.

In the presence of plants, the release of root exudates drives Fe reduction, and hence As release into pore water (Fig. 3), which was observed via the high levels of Fe(II) at the bottom of the planted mesocosms, especially those with high Fe treatment (Fig. 4). Therefore, as soils with high iron content and sorbed As become more reducing, As is released into solution, resulting in a positive correlation between changes in As and changes in Fe(II) in solution (Fig. S5).

Less dissolved Fe(II) was detected in the high P treatment mesocosms (Fig. 4) than in the low P treatment. One possible explanation for the lower Fe(II) levels is that dissolved Fe(II) might react with the excess P to precipitate out as Fe₃(PO₄)₂ (vivianite). MINTEQ calculations confirm that Fe₃(PO₄)₂ is over-saturated in the system when interactions with dissolved organic matter are not considered, but under-saturated in the system when these interactions are considered (Table S3). This suggests that dissolved soil organic matter (e.g. humic and fulvic acids) that leaches out from peat moss and is released from root/microbial turnover and root exudates, is likely to have formed complexes with Fe(II), which has been observed for other trace metals in the wetland rhizosphere (ElBishlawi and Jaffe, 2015) and could also affect As mobility. Since the precipitation of vivianite in the mesocosms is unlikely, a more likely explanation for the lower Fe(II) levels for the high P treatment is that P sorbed onto the ferrihydrite makes the Fe(III) less bioavailable (Borch et al., 2007). Consistent with this explanation are the relative numbers of Geobacter gene copies, which for the planted mesocosms are much higher in the mesocosms with low phosphate, high iron
Sorption of P on ferrihydrite would have resulted in more As(V) desorption, which would lead to more As(V) reduction to As(III) under the more reducing conditions observed for the higher P levels (Fig. 3). This is consistent with the relatively higher number of arsenic reducers as discussed in Section 3.3 below.

3.1.3. Effect of wetland plants on pore water As dynamics

Compared with the mesocosms without plants, the mesocosms with plants have much more dissolved total organic carbon (TOC), which is attributed to the release of root exudates (Fig. 5). The TOC concentrations increased with depth of the mesocosms, which contributed to the establishment of the more reducing conditions at deeper locations (Fig. 2). In the presence of plants, lower TOC was observed in the mesocosms with high P treatment than in those with low P treatment, possibly because more TOC was consumed by the higher number of heterotrophic microorganisms in the mesocosms with high P treatment with plants (Fig. 7).

3.2. As distribution in the solid medium, plant roots, and shoots

The As concentrations in the solid phase (e.g. solid medium, plants roots, and shoots) were measured after the mesocosms were dismantled on day 118 (Table 1). For all the treatments, most of the As mass (>68%) was sequestered in the solid phase including solid medium, plant shoots and roots. Compared to the mesocosms without plants, a significantly higher mass of As was sequestered in the solid medium (p < 0.05) in the mesocosms with plants, indicating that wetland plant plays a key role in the enhanced As sequestration. This is attributed, at least in part, due to the enhanced As transported from the overlaying water into the rhizosphere of mesocosms with plants as a results of wetland-plant transpiration.

The high P loading enhanced the growth of wetland plants (especially the mass of shoots, see Table 1) and consequently increased the total As mass in plants, whereas high P attenuated the As sequestration in the solid medium (Table 1), which is attributed to the competitive sorption between As and P. Of the total mass of As immobilized, the fraction taken up by plants is mainly located in plant roots (8.55 ± 0.69%–31.24 ± 2.47%), when compared to plant shoots (1.16 ± 0.33%–6.03 ± 0.42%). It is interesting to note that for the low P treatment, the As mass in plant roots is negatively correlated to that in the solid medium, however, the opposite trend is observed for the high P treatment. This is because the loading of high P enhances As desorption from Fe oxides into dissolved phase. Meanwhile, high P promotes about 30% more As(V) reduction to As(III) with a higher bioavailability for plant root uptake (Fig. 3). A previous study with identical operational conditions showed that the main As species accumulated in plant roots is As(III) (Zhang et al., 2017).

Arsenic-XANES spectra showed that about 80% of total As in the solid medium samples is in the form of As(III) for high P treatment, while about 20% is in the form of As(III) for the low P treatment (Fig. 6), which is consistent with pore water As speciation (Fig. 3). The reason why the ratio of As(III) to As(V) in the solid medium samples in the low P treatment is lower than in the pore water samples is because of the lower sorption affinity of As(III) onto the solid medium compared to that of As(V) (Dixit and Hering, 2003).

3.3. Effect of treatment on the bacterial population

The numbers of total bacteria in the low and high P treatments were on the same order of magnitude (10^8 bacteria cells/g of solid medium, data not shown). However, the relative number of dissimilatory arsenate-reducing bacteria (arrA) in the high P treatment was 1.2–2 times of that under the corresponding low P treatment (Fig. 7), indicating that high P loading stimulates either directly or indirectly the growth of dissimilatory As reducing bacteria. The relatively higher As reducing bacterial population for high P treatments, especially in the presence of plants, is in agreement with the higher As reduction, resulting in a higher fraction of the dissolved As as As(III) (Fig. 3). Since P results in less As(V) sorption and since in the absence of sulfides As(III) is more mobile, the combined effect explains the significantly higher As mobilization.

Fig. 5. Dissolved organic carbon depth profiles for (a) low P and (b) high P treatments on day 118. (+Plt: with plant, – Plt: without plant).

Fig. 6. As XANES spectra of solid medium in the treatments of low P and high Fe, high P and high Fe, and high P and low Fe in the presence of plants.
observed in the treatment of high P, high Fe, with plants.

The fraction of Geobacter was higher in the planted mesocosms than the corresponding unplanted mesocosms, and the highest Geobacter numbers (Fig. 7) were measured in the planted mesocosms with high Fe treatment which also exhibit the highest Fe(II) concentrations (Fig. 4), indicating higher Fe reduction. As mentioned above, P can decrease the bioavailability of Fe(III) oxyhydroxides, and a lower fraction of Geobacter was observed in the planted mesocosms with high Fe and high P than for the same mesocosms with low P treatment (Fig. 7). The same effect can also be seen in the controls without As (Fig. S6). The addition of As exhibits toxicity for the growth of total bacteria and Fe reducers to be seen in the controls without As (Fig. S6). The addition of As concentrations (Fig. 4), indicating higher Fe reduction. As cosms with high Fe treatment which also exhibit the highest Fe(II) oxyhydroxides, and a lower fraction of mentioned above, P can decrease the bioavailability of Fe(III) oxyhydroxides, and a lower fraction of Geobacter was observed in the treatment of high P, high Fe, with plants.

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Interestingly, the profile of the microbial community structure at the Genus level for the high P, high Fe treatment is distinct from the other treatments (e.g. high P and low Fe, low P and high Fe) in the planted mesocosm (Fig. 8), showing more Fe oxidizers which have been previously detected in As-rich environments and are known as As-resistant bacteria (e.g. Genera Sediminibacterium and Gallionella) (Reis et al., 2016) and As-resistant and As oxidizers (e.g. Genus Flavobacterium) (Escalante et al., 2009). However, the relative abundance of As-reducing bacteria as quantified in terms of arrA, shown in Fig. 7 was highest and almost identical for both, the high P, high Fe treatment and the high P, low Fe treatment, which correspond to the highest and second highest levels of dissolved As in the pore-water of the mesocosms, respectively (Fig. 3).

4. Conclusions

The P-enhanced As mobilization and release into pore water during a transition from oxidizing to reducing conditions when upland soils are flooded, poses a considerable health risk through possible exposure to the contaminated water. The competitive sorption between P and As(V) is commonly thought as the primary reason for the elevated As mobilization as As(V) caused by the loading of P. However, as shown here, P loading can also enhance biotic processes that are critical for the mobilization of As and its reduction to As(III) in the wetland rhizosphere followed by plant uptake. High P levels stimulate wetland plants growth and their release of root exudates, which in turn stimulates bacterial growth. More root exudates and bacterial activity results in more reducing conditions. Higher numbers of Fe- and As-reducing bacteria were
observed in response to the high P loadings. Hence, these conditions did not only result in more As(V) desorption due to the competitive sorption between As(V) and P, but also in As(V) mobilization due to reductive iron dissolution. Higher As levels in solution, coupled to higher number of As reducers resulted in more As reduction to As(III), with has a higher bioavailability for wetland plant uptake into both plant roots and shoots. These interactions should be taken into account in As fate and transport models in wetlands, and perhaps more importantly, in the management of wetlands containing As.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2017.08.096.

References


Dong, H., Guan, X., Lo, I.M.C., 2012. Fate of As(V)-treated nano zero-valent iron: determination of arsenic desorption potential under varying environmental conditions by phosphate extraction. Water Res. 46, 4071–4080.


Kocar, B.D., Herbel, M.J., Tutano, K.J., Fendorf, S., 2006. Contrasting effects of...
dissimilatory iron(III) and arsenic(V) reduction on arsenic retention and transport. Environ. Sci. Technol. 40, 6715–6721.
Lizama, A.K., Fletcher, T.D., Sun, G., 2011. Removal processes for arsenic in con-
surface wetland sediments as a source of arsenic release to ground water in Asia. Nature 454, 505–508.
sphere 161, 266–273.
Tufano, K.J., Reyes, C., Saltikov, C.W., Fendorf, S., 2008. Reductive processes con-
Yamaguchi, N., Ohkura, T., Takahashi, Y., Maejima, Y., Arai, T., 2014. Arsenic distri-
bution and speciation near rice roots influenced by iron plaques and redox conditions of the soil matrix. Environ. Sci. Technol. 48, 1549–1556.