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Abundant and diverse arsenic-metabolizing microorganisms in peatlands treating arsenic-contaminated mining wastewaters

Running title: Arsenic-metabolizing microorganisms in peatlands

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Originality significance statement: This study investigates the role of arsenic-metabolizing microorganisms in treatment of mining-affected waters in natural peatlands. A wide range of current methods are combined to address microbial communities, their activity, and possible influences on retention of arsenic in peat. Arsenic-metabolizing microorganisms in peatlands have not been studied in detail so far, especially not in an environment which is additionally challenged by inflow of mining-affected waters.

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Abstract

Mining operations produce large quantities of wastewater. At a mine site in Northern Finland, two natural peatlands are used for the treatment of mining-influenced waters with high concentrations of sulfate and potentially toxic arsenic (As). In the present study, As removal and the involved microbial processes in those treatment peatlands (TPs) were assessed. Arsenic-metabolizing microorganisms were abundant in peat soil from both TPs (up to 10^8 cells g_{dw}^{-1}), with arsenate respirers being about 100 times more abundant than arsenite oxidizers. In uninhibited microcosm incubations, supplemented arsenite was oxidized under oxic conditions and supplemented arsenate was reduced under anoxic conditions, while little to no oxidation/reduction was observed in NaN_3-inhibited microcosms, indicating high As-turnover potential of peat microbes. Formation of thioarsenates was observed in anoxic microcosms. Sequencing of the functional genemarkers *aioA* (arsenite oxidizers), *arrA* (arsenate respirers), and *arsC* (detoxifying arsenate reducers) demonstrated high diversity of the As-metabolizing microbial community. The microbial community composition differed between the two TPs, which may have affected As removal efficiencies. In the present situation, arsenate reduction is likely the dominant net process and contributes substantially to As removal. Changes in TP usage (e.g. mine closure) with lowered water tables and heightened oxygen availability in peat might lead to reoxidation and remobilization of bound arsenite.

Introduction

Mining operations produce large amounts of wastewater, which have to be purified prior to their release into the environment (Ledin & Pedersen 1996). Mining-affected waters contain

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a variety of contaminants. The contaminant content depends on the type of ore mined and on the ore beneficiation processes. Typical contaminants in mining-affected waters include nitrogen compounds (from remnant explosives or the ore beneficiation process), sulfate (from oxidation of sulfidic ores) as well as (potentially toxic) metals and metalloids (Nordstrom 2011). Arsenic (As) minerals often accompany gold and copper ores, since they all share the same chalcophillic behavior, and beneficiation of these ores can release As via process waters into the environment (Matschullat 2000; Bissen & Frimmel 2003; Nordstrom 2011). Here, the mobility, bioavailability, and toxicity of As strongly depends on As speciation. In most terrestrial environments, the oxyanions arsenate ($H_xAs^VO_4^{x-3}$, x=1-3) and arsenite ($H_xAs^{III}O_3^{x-3}$, x=1-3) dominate under oxidizing and reducing conditions, respectively (Bissen & Frimmel 2003).

Wetlands, including natural peatlands, are widely used for treatment of different kinds of runoffs and wastewaters, as they retard the water flow and provide a large active surface area (Ledin & Pedersen 1996; Sheoran & Sheoran 2006; Vymazal 2011). Contaminants are removed from the water by plant uptake, microbially catalyzed redox-processes, precipitation, and adsorption onto plant roots and soil particles (Sheoran & Sheoran 2006). Under oxic conditions, mostly in the upper peat layers, As can adsorb to (oxyhydr-)oxides of iron, manganese, or aluminum (Bissen & Frimmel 2003). Under suboxic to anoxic conditions and in the presence of sulfur, (hydr-)oxides may dissolve. The released or newly formed arsenite can either react with zerovalent sulfur and sulfide to form thioarsenates ($H_xAs^VS^{-II}{}_nO_{4-n}^{x-3}$; n=1-4; x=1-3) at neutral to alkaline pH (Besold et al., 2018; Planer-Friedrich et al., 2015) or bind to natural organic matter (NOM) via sulfhydryl groups at slightly

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acidic pH (Besold et al., 2018; Hoffmann et al., 2012; Langner et al., 2012). At strongly acidic pH and in extremely reducing microenvironments arsenite eventually precipitate as As-sulfides. While at acidic conditions, thiol-binding and As-sulfide formation are considered rather stable, even if the peat gets intermittently oxic (Langner et al., 2012; Langner et al., 2014), thioarsenate formation may lead to (re-)mobilization of As at neutral to alkaline pH in presence of reduced inorganic sulfur (Besold et al. 2018).

Microorganisms can use the oxoacids of As in their metabolism (Oremland & Stolz 2003; Stolz et al., 2006). Arsenite oxidizing microorganisms (e.g. found within the *Proteobacteria, Deinococci,* and *Crenarchaeota*) conserve energy from the oxidation of arsenite to arsenate and can be autotrophs or heterotrophs (Oremland & Stolz 2003). Strains capable of arsenite oxidation have been isolated from soils, sediments, and As contaminated waters (Salmassi et al., 2006; Garcia-Dominguez et al., 2008; Osborne et al., 2010), and arsenite oxidizing bacteria can be used for bioremediation purposes (Battaglia-Brunet et al., 2002). The arsenite oxidase (AioAB) is the key enzyme in arsenite oxidation, and *aioA* has been frequently used as a genetic marker for arsenite oxidation (e.g. Inskeep et al., 2007; Escudero et al., 2013; Hamamura et al., 2013; Zhang et al., 2015). In wetlands, arsenite oxidation is likely restricted to the uppermost layers, as the water saturated conditions prevent diffusion of oxygen into deeper layers.

Under anoxic conditions, microorganisms can conserve energy via dissimilatory reduction of arsenate to arsenite (i.e. arsenate respiration; Oremland & Stolz 2003; Stolz et al., 2006). Microbial arsenate reduction has been observed in many anoxic soils and sediments (Dowdle et al., 1996; Kulp et al., 2006; Hery et al., 2008), and arsenate respirers

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have been isolated from these systems (Hery et al., 2008; Kudo et al., 2013; Abin & Hollibaugh 2017). The dissimilatory arsenate reductase (ArrAB) is the key enzyme in arsenate respiration, and *arrA* has been frequently used as a genetic marker (e.g. Malasarn et al., 2004; Lear et al., 2007; Song et al., 2009; Escudero et al., 2013). Arsenate respiration is feasible in lower layers of wetlands, where oxygen is scarce and reducing conditions prevail. Further, arsenate can be reduced by the detoxifying arsenate reductase ArsC (Oremland & Stolz 2003), and *arsC* has been used as a genetic marker for As detoxification (e.g. Escudero et al., 2013; Costa et al., 2014; Zhang et al., 2015). By catalyzing kinetically limited transformations of As species, microorganisms can control the retention of As in treatment peatlands (TPs) and can thus be an important factor in the As biogeochemistry in such systems.

Efficient removal of As in TPs is observed in the field (Kujala et al., 2018; Palmer et al., 2015), but the role of microorganisms in As removal remains elusive. To fill this knowledge gap, As concentrations and As speciation in surface and porewaters of two TPs treating mine process waters (TP A) and drainage waters (TP B) were assessed in the field to elucidate As transformation processes. Then, the potential of peat soil from different depths to oxidize/reduce arsenite/arsenate was investigated in laboratory incubations in order to elaborate potential links of As field speciation to microbial activity. Moreover, microorganisms capable of As turnover and resistance were quantified and the microbial community composition was determined to see how numerous and diverse As-cycling microorganisms are in TPs and to identify potential key players in As species transformation. The obtained results about the role of microorganisms in As removal provide valuable

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information for the continued use of TPs in the treatment of mining-affected waters.

Results

Total As concentrations and speciation in treatment peatlands

As concentrations in surface water and porewater were assessed in two TPs, one receiving mine process water (TP A) and receiving drainage water (TP B; Figure S1, see Material and Methods for full description of TPs). Total As concentrations (As_{tot}) in surface water were 21-28 and 33 µg L^{-1} near the inlet of TP A and TP B, respectively (Table S1). In TP A, As_{tot} decreased rapidly with increasing distance from the inlet (TP A1 → TP A7; Figure S1) to mostly below 1 µg L^{-1}. In TP B, there was only a slight decrease to 28 µg L^{-1} close to the outlet (TP B3; Figure S1). Arsenate was the dominant As species in all surface water samples for which As speciation was analyzed (TP A1, A2 and TP B1, B2, B3). However, the arsenite contribution increased in TP B with increasing distance from the inlet (TP B1 → TP B3) from below detection limit (TP B1) to 16% of detected species (TP B3; Table S1). Total As concentrations in the porewater at sampling points/depths used for incubations and microbial community studies ranged from 6.1 µg L^{-1} to 132 µg L^{-1} (Table S2). In porewater samples, arsenate was the dominant species at 10 cm depth near the inlet (TP A1), while arsenite was the dominant species at 60 cm depth (Table S2). In addition, inorganic thiolated, methylated and methylthiolated arsenate species were detected in the TP A1 profile, while only methylated As species and arsenite were detected in porewater samples from TP A5 (73-75% and 6-7% of detected As species, respectively).

Arsenic-utilization potential of peat microorganisms

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The potential of peat microorganisms to oxidize and reduce 50 µM supplemented arsenite and arsenate to peat suspensions, respectively, was assessed in microcosm incubations prepared with deionized water. Under oxic conditions, supplemented arsenite was oxidized to arsenate completely within 9 days of incubation in uninhibited microcosms (Figure 1 B). Arsenite oxidation started after 2 days of incubation, indicating a short lag phase in the beginning. In microcosms where sodium azide (NaN_3) had been added as an inhibitor, no arsenite oxidation was observed within the 18-day incubation period (Figure 1 A), indicating that arsenite oxidation was catalyzed by microorganisms. NaN_3 is a common inhibitor for microbial growth and activity (e.g. Cabrol et al., 2017), which has also been used to inhibit As redox transformations in many As-NOM studies (Besold et al., 2018; Buschmann et al., 2006; Hoffmann et al., 2012).

Under anoxic conditions, supplemented arsenate was reduced to arsenite completely within 9 days of incubation in uninhibited microcosms with peat from both tested depths (Figure 1 D, F). Arsenate reduction started after a lag phase of 2 days. Only minor arsenate reduction was observed in NaN_3 supplemented microcosms with peat soil from 0-10 cm depth, while about half of the supplemented arsenate was reduced to arsenite in NaN_3 supplemented microcosms with peat soil from 60-70 cm depth (Figure 1 C, E). This finding indicates that arsenate reduction was not completely inhibited by NaN_3 in peat soil from 60-70 cm depth, which might be due to (i) incomplete inhibition caused by rather low NaN_3 concentrations, (ii) microorganisms that were not affected by NaN_3, or (iii) abiotic arsenate reduction with considerably lower reaction rates. Moreover, the inhibitory effect of NaN_3 was not permanent: After 167 days of incubation arsenate had been reduced to arsenite in the NaN_3

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supplemented incubations of both depths (Figure 1 C, E).

Sulfide production potential of peat soil microcosms

Sulfide production potentials were determined in anoxic microcosms with 0 to 10 cm peat soil from TP A5 prepared with deionized water and additionally in incubations prepared with mine process water. Initial sulfide concentrations in mine process water were below the detection limit of the assay used for determination of sulfide concentrations. Sulfide accumulated only in microcosms without supplemented NaN_3 (Figure 2), indicating that NaN_3 almost completely inhibited sulfate reduction in peat soil.

Using deionized water, 270 and 210 µM sulfide accumulated within 167 days of incubation in microcosms with 0 to 10 cm and 60 to 70 cm peat, respectively (Figure 2 A and B). In microcosms set up with mine process water, 265 µM sulfide had accumulated after 173 days and 330 µM after 433 days of incubation (Figure 2 C). Accumulation of sulfide was faster in microcosms with mine process water than in microcosms with deionized water. This difference occurred possibly due to the more readily available sulfate in incubations with mine process water (as mine process water contains 2,100 mg L^{-1} sulfate). In fact, 91 µM sulfide accumulated within 11 days in microcosms with mine process water, while only 7 µM sulfide accumulated within 16 days in microcosms with deionized water (Figure 2 A, C). However, sulfide accumulation in microcosms with mine process water started to level out at later time points, indicating lower sulfate reduction rates.

Thioarsenate production potential of peat soil microcosms

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Thioarsenate formation was assessed in anoxic microcosms prepared with deionized water and with mine process water. Deionized water was used to avoid possible interference of mine process water contaminants with the formation process and to allow comparison to the arsenite oxidation and arsenate reduction potentials assessed earlier, while mine process water was used to assess the influence of increased sulfate concentrations (2,100 mg L^{-1} in mine process water) on thioarsenate formation. Thioarsenates started to accumulate in uninhibited anoxic microcosms with increasing incubation time. After 167 days of incubation, more than half of the detected As was in the form of thioarsenates in microcosms set up with deionized water, with trithioarsenate being the most abundant species (Figure 1 B, C). In uninhibited anoxic microcosms with mine process water, thioarsenates likewise accumulated with time (Figure 3 B). 68% of the detected As species were thioarsenates after 420 days of incubation, and trithioarsenate was the dominant species as well. No thioarsenates were detected in the NaN_3 inhibited incubations with deionized or mine process water (Figure 1 C, E; Figure 3 A).

Enumeration of As-metabolizing microorganisms

As-tolerating aerobic/aerotolerant microorganisms were abundant in TP A and TP B based on MPN counts (Figure 4). Up to 3.8 x 10^7 cells g_{DW}^{-1} were detected in 0-10 cm peat soil. The cell numbers obtained were slightly (but not significantly) higher with TSB than with NB growth medium. MPNs of arsenite-oxidizing microorganisms were about 2 orders of magnitude lower than for As-tolerating microorganisms and approximated 1.5 x 10^5 cells g_{DW}^{-1} in 0-10 cm peat soil. MPNs of arsenate-respiring microorganisms ranged from 1.6 x

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10^6 cells g_{DW}^{-1} to 8.6 x 10^6 cells g_{DW}^{-1} (Figure 4), indicating that arsenate-respiring microorganisms were more abundant than arsenite-oxidizing microorganisms in peat soil of both layers. MPNs of As-tolerating and arsenite-oxidizing microorganisms were slightly (but not significantly) lower in 60-70 cm peat soil, while this trend was not observed for arsenate-respiring microorganisms.

Functional genes involved in As metabolism in treatment peatlands

Amplicon sequence libraries were obtained from four samples (TP A and TP B, 2 depths per TP) in four replicates (i.e., total number of 16 amplicon libraries per primer set) for bacterial 16S rRNA genes and genes involved in As turnover (*aioA, arrA, arsC*) with eight different primer sets (Table 1). Since two nested approaches for *arrA* (As1f/r + As2f/r and As1f/2r + As 2f/r) used the same primers in the second PCR step, the sequences from those approaches could not be distinguished after sequencing and are thus combined in the same library. The percentage of correct sequences (i.e., sequences that match the targeted gene) were on average 50%, 93%, and 97% for *aioA, arrA* and *arsC*, respectively. The dada2 algorithm used for quality filtering created high-quality amplicon sequence variants (ASVs). For most of the following analyses, these ASVs were used unclustered. Amplicon sequencing of genes involved in As turnover was most successful with the two primersets targeting *arsC* (arsC1 and arsC2), which yielded a total of 178,470 sequences and 895 unclustered ASVs for the non-rarefied dataset (arsC1+arsC2) after quality filtering and removal of non-target sequences. For *aioA* (aioA1 and aioA2) and *arrA* (arrA1 and arrA2), less sequences were obtained, with 39,691 sequences in 582 ASVs for *aioA* (aioA1+aioA2)

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and 65,839 sequences in 418 ASVs for *arrA* (arrA1+arrA2). PERMANOVA analysis indicated differences in community composition obtained with different primer sets for each gene ($p < 0.001$), indicating that the use of two different primer systems per gene likely captured a higher diversity than the use of a single primer system.

For the estimation of diversity indicators which are comparable between different samples, genes and primer sets, ASV were tables rarified to a depth of 600 sequences. In the rarified ASV tables, the number of observed ASVs was highest for *arsC* (Table 2). While for *aioA* the number of detected ASV was only slightly lower, significantly less ASV were detected for *arrA* ($p = 0.04$; Table 2). Similarly, Faith's PD was significantly higher for *aioA* and *arsC* than for *arrA* ($p < 0.01$), indicating that the phylogenetic diversity recovered was higher for *aioA* and *arsC.* Shannon diversity was only slightly higher for *arrA* and *arsC* than for *aioA* (not significant), while Evenness was significantly higher for *arrA* and *ar*sC than for *aioA* ($p < 0.05$).

Phylogenetic analysis indicated five, seven, and eleven groups for *aioA, arrA* and *arsC*, respectively, with individual groups containing 2-44 ASVs or 0.5-34% of all sequences per gene (Figure 5). The *aioA* ASVs were related to *Burkholderia multivorans* and *Sinorhizobium* sp. (group 1; 34%), *Thiobacillus* sp. and *Bradyrhizobium* sp. (group 3; 8.2%), *Pseudomonas arsenicoxydans, Thiomonas* sp. and *Ralstonia syzygii* (group 4; 3.7%) as well as to *Limnobacter* sp. and *Hydrogenophaga* sp. (group 5; 1.1%; Figure 5 A). The *arrA* ASVs were related to *arrA* of *Sulfuritalea hydrogenivorans* and *Exiguobacterium* sp. (group 1 and 4; Figure 5 B), *Geobacter* spp. and *Desulfuromonas* sp. (group 3), *Geobacter uraniireducens, Citrobacter* sp. and *Aeromonas* sp. (group 6), or were not closely related to

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arrA of cultured organisms (groups 2, 5 and 7). The *arsC* ASVs were related to *Syntrophobacter* sp., *Syntrophus* spp. and *Smithella* sp. (groups 1 and 5; Figure 5 C), *Sedimentisphaera* spp. and *Chitinispirillum alkaliphilum* (group 2), fungal *arsC* (group 3), *Bosea thiooxidans* (group 6), *Bacillus* spp. and *Lactobacillus* spp. (group 7) and *Geobacillus pelophilus* (group 8). Sequences of group 4 were not closely related to *arsC* of cultured organisms. 16S rRNA gene amplicons were dominated by Chloroflexi, Proteobacteria and Acidobacteria (Figure 6).

For all As genes and primer sets, PERMANOVA analysis indicated significant differences in community composition between the two TPs ($p < 0.05$; Figure 6). For *aioA* and *arrA*, depth did not affect community composition ($p \geq 0.1$), while for *arsC* communities differed between depths ($p < 0.01$). ANCOM did not indicate any specific ASVs characteristic for either one of the TPs or depth, indicating that the differences in community composition were not caused by single ASVs but rather by groups of ASVs. The *aioA* groups 1 and 2 were on average more abundant in TP B, while *aioA* group 4 was on average more abundant in TP A (Figure 6). The *arrA* groups 3 and 5 were more prominent in TP A, while *arrA* groups 1, 4 and 6 were more prominent in TP B. The *arsC* groups 1, 7 and 8 were on average more abundant in TP A, while *arsC* groups 2 and 6 were on average more abundant in TP B (Figure 6). Based on 16S rRNA gene analysis, bacterial phyla were of similar relative abundance in both TPs (Figure 6).

Network analysis: This paragraph has been removed.

Discussion

Wetlands including peatlands are widely used for treatment of metal-/metalloid-

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contaminated waters (Sheoran & Sheoran 2006; Vymazal 2011). The studied peatlands have been rather effectively removing As from mining-affected water for up to 10 years and have accumulated As in the peat (Palmer et al., 2015). High retention of As was confirmed in the present study, since most of the As was retained near the inlet in mine process water receiving TP A (less in TP B; Table S1), and most of the As accumulated in 0-10 cm peat. Natural peatlands have been reported to accumulate very high concentrations of As of more than 3,000 mg kg_{dw}^{-1} (González et al., 2006; Bauer et al., 2008), indicating the potential of the TPs to accumulate even more As in future years and thus allowing for their continued use as a purification system for mining-affected waters.

Microorganisms are capable of using different As species in their energy metabolism or when detoxifying As (Stolz et al., 2006) and may affect As speciation in peat. Changes in As speciation within the TPs and/or along depth profiles can thus indicate microbial As turnover in peat. This was indeed observed in the field: As enters the TPs mainly as arsenate due to the strongly oxidizing nature of the gold extraction process (cyanide leaching), and mainly arsenate was found in surface waters as arsenate reduction was likely prevented in most parts by the rather oxic conditions in those waters. However, a higher percentage of arsenite was found near the outlet of TP B, indicating ongoing arsenate reduction even in rather oxic surface waters. Moreover, arsenite was detected in all profiles and was the dominant As species in the TP A1 profile in 50 and 60 cm depth (Table S2), which shows that most of the arsenate reduction in TPs is likely found in deeper, anoxic peat layers.

The high potential of the TP peat for microbially-catalyzed arsenate reduction under anoxic conditions was demonstrated also in microcosm incubations (Figure 1), and arsenate

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respirers as well as As-tolerating microorganisms were abundant in TPs (10^6-10^7 and ~10^7 cells g_{dw}^{-1}, respectively) and accounted for about 36% and 50% of the general aerobic heterotrophs (Figure 4). The high abundance of arsenate respirers and As-tolerators is likely responsible for fast arsenate reduction in deeper peat layers and might thus contribute to efficient As removal in the TPs (see below for discussion of removal processes).

Arsenate reduction has been reported from a variety of anoxic soils and sediments (Dowdle et al., 1996; Oremland & Stolz 2003; Kulp et al., 2006), and arsenate respirers have been isolated from many anoxic As-contaminated environments (Oremland & Stolz 2003). However, limited information is available on arsenate reduction and arsenate reducers in peatlands (natural or disturbed). Thus, the present study contributes to a better understanding of arsenate reducers in peatlands. Arsenate can be reduced by arsenate respiring as well as arsenate detoxifying microorganisms. The ability to detoxify As is important for the survival and functioning of microorganisms in environments with elevated As concentrations such as the studied TPs, since As can be toxic at rather low concentrations (Paez-Espino et al., 2009). Moreover, soil processes such as respiration, microbial biomass as well as functioning of enzymes like alkaline phosphatase are inhibited by As concentrations as low as 40 to 100 mg As kg_{DW}^{-1} (Bhattacharyya et al., 2008; Ghosh et al., 2004; Liu et al., 2019), which is much lower than the concentrations that were detected in TP peat (around 200 mg kg_{DW}^{-1}). As-tolerating bacteria have been detected and isolated from a variety of As-contaminated soils and can tolerate up to 15 g L^{-1} As (Villegas-Torres et al., 2011; Escudero et al., 2013; Xiao et al., 2017). On the other hand, As-sensitive microorganisms such as *Aliivibrio fischeri* are inhibited already by As concentrations < 2 mg

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L^{-1} (Fulladosa et al., 2005). Thus, there is a high need to detoxify As in TPs, which would explain the high observed diversity and abundance of As-tolerating microorganisms. Sequences of *arsC* from TPs were related to *arsC* from bacteria, archaea, and fungi (Figure 5 C), highlighting the importance of an arsenic detoxification mechanism for microorganisms in TPs. Arsenic detoxification moreover contributes to arsenate reduction and might be in part responsible for arsenate reduction observed in microcosm studies or in the field.

Arsenate respirers from TPs as assessed by *arrA* sequencing were mostly not related to *arrA* of cultured microorganisms (Figure 5 B), indicating a high potential of yet unknown arsenate respirers in TPs. These hitherto uncharacterized arsenate respirers are likely well adapted to the conditions in the TPs, including cold climate conditions and high contaminant loads. Some detected groups of *arrA* were related to *Geobacter* spp. and *Sulfuritalea hydrogenivorans*, species which are known to reduce arsenate under anoxic conditions and have been detected in many As-contaminated environments (Lear et al., 2007; Hery et al., 2008; Ohtsuka et al., 2013; Watanabe et al., 2017). However, based on 16S rRNA gene sequencing, the overall abundance of *Geobacter* and *Sulfuritalea* is rather low in the studied TPs (0.5-0.9% relative abundance; Kujala et al., 2018, this study), highlighting the importance of the yet unknown arsenate respirers in the system and their possible contribution to As removal. Even though arsenate respirers were almost equally abundant in TP A and TP B (Figure 4), the arsenate respirer community differed strongly between the two TPs (Figure 6). Different kinds of arsenate respirers likely differ in their habitat preferences and arsenate turnover rates. Differences in TP usage (e.g. different flow regimes, different inflow water composition, different TP ages) as well as initial differences

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between the TPs (e.g. peat type, peat depth) might have selected for different microbial communities which in turn might contribute to the observed differences in As removal efficiencies. Arsenate respirers of *arrA* group 3 (related to *Geobacter* spp. and *Desulfuromonas* sp.) and arsenate tolerators of group 1 (related to *Syntrophobacter* sp.*,* *Syntrophus* spp. and *Smithella* sp.) were more abundant in TP A (receiving mine process water), indicating that those groups might be in part responsible for the higher As removal in TP A. However, microbial arsenic reduction is likely not the only factor determining removal efficiencies of As in TPs, since other factors like hydraulic load and water residence time also contribute. Since hydraulic load is much higher for TP B than for TP A (38 vs. 6.1 mm d^{-1}; Palmer et al., 2015), this also negatively affects As removal in TP B.

Oxic microcosm incubations with supplemented arsenite demonstrated the potential for arsenite oxidation in peat soil (Figure 1) and the arsenite oxidizer community was rather diverse (Figure 5 A, Figure 6). The potential arsenite oxidation rate was comparable to the potential arsenate reduction rate assessed in anoxic microcosm incubations with supplemented arsenate (all arsenite/arsenate oxidized/reduced within 9 days, Figure 1), indicating a similar process potential for both arsenite oxidation and arsenate reduction. However, arsenite oxidizers were less abundant in the studied TPs (10^5 cells g_{dw}^{-1}, ~0.5% of general aerobic heterotrophs; Figure 4). As the TPs are permanently water-saturated, oxygen availability in the peat is likely low, which would restrict the growth of arsenite oxidizers. Abundant *aioA* were related to *Hydrogenophaga* sp., *Thiocapsa marina*, and *Bradyrhizobium* sp. (Figure 5 A). These organisms are known arsenite oxidizers and have been detected and/or isolated from a variety of As-contaminated environments including

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soils, sediments, and Hot Creeks (Salmassi et al., 2006; Garcia-Dominguez et al., 2008). However, many ASVs were not closely related to known *aioA*, indicating phylogenetic novelty of arsenite-oxidizers in the studied TPs. These novel arsenite-oxidizers are likely well-adapted to the conditions in the TPs.

Both arsenite oxidation and arsenate reduction are feasible processes in the studied TPs. Moreover, it is conceivable that As cycling occurs within the peatlands, leading to reduction of arsenate in the lower, anoxic peat layers or anoxic microenvironments in the upper layers followed by oxidation of arsenite in the upper, oxic layers. Such internal cycling might even occur repeatedly. Arsenite oxidizers, arsenate respirers and arsenic-tolerating microorganisms were detected in both layers based on MPN counts and functional gene analysis (Figures 4, 6), indicating that those functional groups are present in the same layers, which might allow for switching between oxidizing and reducing processes even within the same layer with possible changes of environmental parameters. However, it was not possible to determine if and how often oxidation-reduction cycling occurred *in situ,* and arsenate reduction to arsenite was the observed net process. Thus, for net As cycling, arsenate reduction is likely the dominating process as (i) arsenate is the dominating As species in the inflow, (ii) anoxic conditions prevail through most of the peat which would support arsenate reduction, (iii) arsenite is found in the porewater likely as a result of arsenate reduction, and (iv) arsenite oxidizers are less abundant in TPs than arsenate reducers (arsenate respirers and arsenate tolerators). However, the contribution arsenite oxidation to net As cycling might increase when changes in the water table become more prominent, as might be the case when the use of the TPs for water treatment is discontinued

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(e.g. due to mine closure or changes in the water treatment process). Lowered water tables would lead to enhanced diffusion of oxygen into the peat and might thus allow for the reoxidation and remobilization of bound arsenite (Langner et al., 2014) which might in turn lead to leaching of bound As from the TPs.

In systems with high concentrations of arsenite and reduced sulfur species, thioarsenate formation may occur. Thioarsenates have been studied extensively in extreme environments like hot springs (e.g. Planer-Friedrich et al., 2007; Ullrich et al., 2013) or in groundwaters (Wallschläger & Stadey 2007; Planer-Friedrich et al., 2018), while peatlands have received less attention. However, thioarsenates have been detected in a natural As-enriched peatland just recently (Besold et al., 2018), indicating that they may be indeed widespread in anoxic, sulfidic environments. In the studied TPs, sulfate concentrations are very high, especially in TP A which receives mine process waters (Palmer et al., 2015). Anoxic conditions in deeper peat layers allow for microbial sulfate reduction to elemental sulfur and further on to sulfide. Indeed, sulfide accumulation was observed in anoxic microcosms (Figure 2). After microbially catalyzed arsenate reduction to arsenite (Figures 1, 3), high sulfide concentrations can then lead to an abiotic formation of thioarsenates at neutral to alkaline pH (Planer-Friedrich et al., 2010) and a subsequent mobilization of As in soils (Sun et al., 2016; ThomasArrigo et al., 2016). Abiotic reduction of arsenate with sulfide can be neglected in our microcosms (pH 6-7) as it becomes kinetically relevant only at much lower pH (Rochette et al. 2000).

Thioarsenate formation was observed in anoxic, uninhibited microcosms, and thioarsenates contributed to up to 80% of the detected As species (Figure 1, 3). Di- and

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trithioarsenate were the dominating thioarsenates formed in the microcosms. Earlier studies have shown that high S:As ratios favor the formation of di- and trithioarsenate rather than monothioarsenate (Planer-Friedrich et al., 2010). S:As ratios in the microcosm incubations were >10 in all cases, thus the observed dominance of di- and trithioarsenate is in line with the earlier studies.

Even though the results obtained from the microcosm incubations cannot be transferred directly to the situation *in situ*, (re-)mobilization of bound arsenite due to thioarsenate formation is feasible in the TPs. This study found first indications that in peat layers where (microbially formed) arsenite coincides with high microbial sulfate reduction rates, thioarsenate formation may occur.

Conclusions and outlook

The present study characterized the microbial community composition within two TPs receiving mining-affected waters using functional gene analysis and their As oxidation and reduction potential. The most important findings are summarized in Figure 7. Both TPs efficiently removed As, even though removal efficiencies were lower in TP B. In TP A, total As concentrations were high and arsenate was the major As species in the inflow water, while total As concentrations were low in the outflow. In TP B, differences in the total As concentration between inflow and outflow were low, reflecting the smaller removal efficiencies of TP B. Like in TP A, arsenate was the dominant As species, however, the contribution of arsenite increased in TP B with increasing distance from the inflow.

Peat microorganisms showed the potential to oxidize and reduce arsenite and arsenate,

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respectively, and especially arsenate reducers were abundant in peat. Based on the analysis of functional genes, As-metabolizing microbes in TPs were diverse and many detected ASVs were not closely related to cultured microorganisms. Sulfide and thioarsenate production were observed in anoxic microcosms, and formation of thioarsenates might lead to lowered As removal or even As remobilization. Thus, the collective data indicate that (i) net arsenate reduction occurs *in situ* and is likely catalyzed by As-metabolizing microorganisms, (ii) hitherto uncultivated arsenic-metabolizing microorganisms contribute to As removal in TPs, (iii) thioarsenate formation might lead to remobilization of bound As, and (iv) changes in water table (e.g. after mine closure) might lead to oxidation of bound arsenite by hitherto uncultured arsenite oxidizers and subsequent remobilization of bound As.

Even though the present study gives a good insight into the As-metabolizing potential of peat microorganisms, open questions remain that should be addressed in future research. Future approaches might include (i) the isolation and characterization of As-metabolizing microorganisms from peat soil, (ii) metagenomic analyses to overcome the issue of primer bias encountered in amplicon-based approaches, (iii) *in situ* detection and quantification of thioarsenates in TPs, and (iv) investigation of *in situ* binding mechanisms and As speciation in TP peat.

Experimental procedures

Sampling site and peat sampling

Samples were taken from two peatlands treating mining affected waters near a gold mine in

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Finnish Lapland (about 68° northern latitude; Figure S1). Treatment peatland A (TP A) is used for purification of pretreated process waters, while treatment peatland B (TP B) is used for the purification of drainage water. Average inflow concentrations of As were 1.3 μM and 0.4 μM to TP A and TP B, respectively, and average removal efficiencies were 98% and 57% in TP A and TP B, respectively (average 2013 to 2017, obtained from monitoring data provided by the mining company). The pH in surface water and porewater of both TPs varied from 5.0 to 7.5 and the redox potential varied from -150 mV to 0 mV in TP A and -100 mV to 100 mV in TP B. In TP A, porewater sulfide concentrations were 2.0 μM and 11 μM in 10 cm and 60 cm depth, respectively, while porewater Fe(II) concentrations were 2.9 μM and 113 μM in 10 cm and 60 cm depth, respectively. In TP B, porewater sulfide concentrations were 0.2 μM and 1.0 μM in 10 cm and 60 cm depth, respectively, while porewater Fe(II) concentrations were 226 μM and 1190 μM in 10 cm and 60 cm depth, respectively. A more detailed site description is provided in Palmer et al., 2015. Peat samples were taken on several occasions (MPN counts and molecular work September 2015, microcosm incubations March 2016) with a soil corer from 0-10 cm peat and 60-70 cm peat. Peat As concentrations were 196 mg kg^{-1} and 5.1 mg kg^{-1} in 0-10 cm and 60-70 cm peat, respectively, at sampling point TP A1, and 65.7 mg kg^{-1} and 31.0 mg kg^{-1} in 0-10 cm and 60-70 cm peat, respectively, at point TP B2 (Table S2). Samples intended for DNA extraction and subsequent analysis of the As metabolizing microbial community were flash-frozen on dry ice in the field and stored at -20°C. Samples intended for determination of cell numbers and for incubation experiments were stored at 4°C in the dark until sample processing. Incubation experiments were started within 2-3 weeks after sampling to prevent impact of

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storage on process potentials.

Determination of aqueous total As concentrations and speciation in treatment peatlands

Surface water was sampled in summer 2017 at 7 sampling points along TP A and 3 sampling points along TP B (Figure S1). For total As determination, samples were filtered through 0.2 µm filters (cellulose acetate, CA; Macherey-Nagel), stabilized in 0.5% H_2O_2 and 0.8% HNO_3, and analyzed by inductively coupled mass spectroscopy (ICP-MS). Surface water samples for As speciation were filtered through 0.2 µm filters and acidified with 0.2 % (v/v) HCl to avoid precipitation of Fe mineral phases and As loss due to co-precipitation or sorption. All samples with total As concentrations > 0.5 µg L^{-1} were analyzed for their As speciation using anion exchange chromatography with a 20-100 mM gradient NaOH eluent and suppressor coupled to ICP-MS (AEC-ICP-MS) as described earlier (Planer-Friedrich et al., 2007). Additionally, pore water samples were obtained at sampling points TP A1 and TP A5 at the depths where samples were collected for incubation experiments and microbial community studies (see below). Since potential occurrence of thioarsenates was expected in these pore water samples and acidification leads to their precipitation as AsS mineral phases (Smieja & Wilkin 2003), filtration and on-site flash-freezing on dry ice for species preservation was used instead of HCl acidification as described earlier (Planer-Friedrich et al., 2007). Total As for these two samples was analyzed as described above. All samples with total As concentrations > 0.1 µg L^{-1} were analyzed for their As speciation using AEC-ICP-MS as well but with a 2.5-100 mM gradient NaOH eluent leaving out the suppressor, to enable detection of methylated species, as described earlier (Planer-Friedrich et al., 2007).

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Incubation experiments

Soil slurry incubations were set up with field-fresh peat soil from sampling point TP A5 (0-10 cm depth) and TP A1 (60-70 cm depth) to test for the potential of peat microorganisms to oxidize and reduce arsenite or arsenate, respectively. 0-10 cm peat from TP A5 was chosen to prevent masking of target processes by potential leaching of the high initial As content of surface peat from TP A1. 1 g wet peat soil was diluted 1:60 with deionized water in 125 ml incubation bottles. Incubations were set up in triplicate. Oxic and anoxic incubations supplemented with 50 µM arsenite and arsenate, respectively, were set up with and without inhibitor. Incubations with inhibitor were supplemented with 0.75 mmol mol_{carbon}^{-1} sodium azide (NaN_3) to inhibit biological arsenite oxidation/arsenate reduction (controls). The concentration of NaN_3 was chosen based on previous studies investigating As binding and transformation in organic-rich environments (Besold et al., 2018; Buschmann et al., 2006; Hoffmann et al., 2012). For oxic incubations, slurries were prepared with ambient air in the headspace. Bottles were closed with rubber stoppers, and stoppers were removed regularly for aeration (1-2 times per day) as well as for sampling. For anoxic incubations, slurries were prepared in a glovebag (Coy Laboratory Products) which was operated with a gas mix of 95% N_2 and 5% H_2. Bottles were closed with butyl-rubber stoppers and crimp-sealed to preserve anoxic conditions. Anoxic incubations were sampled inside the glovebag using a syringe. Peat soil slurries were incubated at room temperature on a shaker in the dark, and sampled after 0, 1, 2, 5, 9, 12, 19, and 167 days. Concentrations of produced sulfide were determined after 16 and 167 days using the methylene blue method (Cline

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1969). Additional incubations were set up with peat soil and mine process water (sulfate = 2,100 mg L^{-1}) to assess the effect of increased sulfate concentrations on thioarsenate formation. These incubations were set up with 0-10 cm peat soil under anoxic conditions with and without NaN_3 and were supplemented with 50 µM arsenate. Samples were taken at least 6 times from 2 to 433 days for determination of As speciation and sulfide concentration. Samples were filtered (0.2 µm, Chromafil CA; Macherey-Nagel, Germany) and flash-frozen immediately to prevent changes in As speciation. After thawing the flash-frozen samples in a glovebag, aqueous As speciation was analyzed by AEC-ICP-MS as described in Planer-Friedrich et al., 2007.

Enumeration of As-utilizing prokaryotes by most probable number (MPN) counts

Most probable number counts were set up targeting arsenite-oxidizers, aerobic As-tolerating microorganisms, and arsenate respirers. Incubations were set up with soil from TP A1 and TP B2 (0-10 cm and 60-70 cm depth). An artificial porewater solution containing minerals, trace elements and vitamins (modified from Balch et al., 1979; Kuhner et al., 1996) was prepared with the following concentrations (in mg L^{-1}): $(NH_4)_2$ SO_4, 12.6; Na_2SO_4, 13.5; $CaCl_2$ * 2 H_2O, 10.0; $MgCl_2$ * 2 H_2O, 10.0; $FeCl_2$ * 4 H_2O, 10; KH_2PO_4, 0.4; $MnSO_4$ * 1 H_2O, 5; $FeSO_4$ * 7 H_2O, 1; $CoCl_2$ * 6 H_2O, 1; $CaCl_2$ * 2 H_2O, 1; $ZnSO_4$ * 7 H_2O, 1; $CuSO_4$ * 5 H_2O, 0.1; $AlK(SO_4)_2$ * 12 H_2O, 0.2; H_3BO_3, 0.1; Na_2MoO_4 * 2 H_2O, 0.1; and nitrilotriacetic acid, 15; biotin, 0.004; folic acid, 0.004; pyridoxine-HCl 0.02; thiamine-HCl, 0.01; riboflavin, 0.01; niacin,0.01; DL-Ca-pantothenic acid, 0.01; vitamin B12, 0.0002; p aminobenzoic acid, 0.01; lipoic acid, 0.01. Growth medium was prepared using artificial porewater solution

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supplemented with 2 mM arsenite and 2 mM HCO_3^- for arsenite-oxidizers, with 2 mM arsenite, 2 mM arsenate and nutrient broth (NB, Sigma-Aldrich; to a final concentration of 2.5 g L^{-1}) or tryptic soil broth (TSB, Sigma-Aldrich; to a final concentration of 3 g L^{-1}) for aerobic As-tolerating microorganisms, with 2 mM arsenate, 2 mM lactate, and 2 mM acetate for arsenate respirers, and with NB (at a final concentration of 2.5 g L^{-1}) and TSB (at a final concentration of 3 g L^{-1}) for general aerobic heterotrophs. The pH of all growth media was adjusted to 7.0. Incubations for arsenite-oxidizers and As-tolerating microorganisms were conducted in 96-well plates with 8 replicates per dilution step. Incubations for arsenate respirers were conducted in triplicates in 50 mL serum bottles with sterile N_2 in the headspace. Plates and bottles were incubated at 20 °C for 3 months in the dark. Production of arsenate or arsenite was qualitatively determined in the arsenite oxidizer and arsenate respirers MPN, respectively, using a $KMnO_4$ screening technique (Salmassi et al., 2002; Salmassi et al., 2006). 40 µl or 60 µl of 0.01 M $KMnO_4$ were added to 300 µl or 500 µl of an arsenite oxidizer or arsenate respirer incubation, respectively. A purple color indicates the presence of arsenate, while an orange color indicates the presence of arsenite. Arsenite-oxidizer incubations were scored positive, when the $KMnO_4$ test indicated mainly arsenate in the growth medium, arsenate respirer incubations were scored positive, when the $KMnO_4$ test indicated mainly arsenite in the growth medium, and As-tolerating and general aerobic heterotrophic incubations were scored positive when growth was observed in the wells by visual inspection.

DNA extraction and barcoded amplicon sequencing

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DNA was extracted from three replicate samples per site (TP A1 and TP B2) and depth (0-10 cm and 60-70 cm) using the PowerLyzer PowerSoil DNA isolation kit (MoBio Laboratories). Samples were freeze-dried, homogenized, and rewetted (0.1 g of dried soil + 150 µL water) prior to extraction. In total, 17 previously published primersets for genes involved in microbial As metabolism were tested, and functional genes were successfully amplified and sequenced with two (aioA), two (arsC), and three (arrA) primer sets (Table 2). Moreover, 16S rRNA genes were amplified from all samples as described earlier (Kujala et al., 2018). PCRs were set up in 25 µL reactions with the following composition: Maxima SYBR Green/Fluorescein qPCR 2x Master Mix (Thermo Scientific), 0.4 µM of each primer (Biomers), and 0.02% Bovine Serum Albumin (Thermo Scientific), 1-5 ng template DNA. Primers and PCR conditions are given in Table 2. Additional primersets were tested (Table S4), but no PCR product was obtained with those despite thorough testing, or no correct sequences were obtained from the PCR products. Following the initial PCR, barcodes and sequencing primers were added in a second PCR (Table 2). PCR products from the second PCR were purified and pooled in equimolar concentrations prior to sequencing on the Ion Torrent PGM. Sequencing templates were prepared using the Ion PGM Hi-Q OT2 Kit. For sequencing, the Ion PGM Hi-Q OT2 Kit and an Ion 316 Chips v2 (Thermo Fisher) were used as described earlier (Mäki et al., 2016; Kujala et al., 2018).

Sequence analysis

Sequence analysis was done in Qiime2 (Boylen et al., 2019). After demultiplexing, during which sequences with incorrect primer (>1 mismatch) or barcode (>1 mismatch) were

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removed, sequences were quality-filtered using the dada2 plugin for IonTorrent sequences ("denoise-pyro") with the following modifications to the default parameters: At the 5' end of the sequences, primers were removed ("--p-trim-left"; 41-44 bp, depending on analyzed gene) and sequences were trimmed at the 3' end to achieve equal lengths for all sequences of a gene ("--p-trun-len"; 261-314 bp depending on gene). The length for trimming was selected to retain a reasonable number of sequences with the fragments being as long as possible. This resulted in final sequence lengths after using dada2 were 220 bp, 220 bp and 270 bp for *aioA, arrA* and *arsC*, respectively. Amplicon sequence variants (ASV) obtained after dada2 quality filtering were used without further clustering unless indicated otherwise. Feature classifiers were trained using *aioA*, *arrA* and *arsC* sequences of pure cultures (114, 35 and 600 sequences, respectively) with the Qiime2 plugin "feature-classifier". For classifier training, only the part of the sequence corresponding to the amplified genes were used. Classifiers were used mainly to identify and remove non-target sequences, which accounted for 50%, 7% and 3% of all *aioA, arrA* and *arsC* amplicons. While trained classifiers allowed the identification of amplicon sequences on phylum level, they did not allow for classification at lower taxonomic levels. Taxonomic affiliations of amplicon sequences on lower taxonomic level were obtained by BLAST search of sequences and by construction of phylogenetic trees. Closely-related reference sequences to ASV representative sequences were identified by BLAST (Altschul et al., 1990; Table S5). For construction of phylogenetic trees, the same collection of pure culture sequences was used as for the classifier training, albeit with full-length sequences. Alignment of *in silico* translated reference and own sequences as well as construction of phylogenetic trees was done in ARB (Ludwig et al., 2004). ARB

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allows for the construction of trees based on full-length reference sequences, thus creating more robust phylogenetic trees. Frequency-based position filters were applied for construction of reference phylogenies, i.e. only alignment positions in which the majority of the sequences had an amino acid were considered. This resulted in the use of 390, 410, and 115 alignment positions for *aioA, arrA,* and *arsC*, respectively. Individual shorter sequences (i.e., own sequences from the present study and related environmental sequences) were added to the existing tree using ARB parsimony using a frequency-based position filter for those shorter sequences (resulting in the use of 73, 72, and 86 alignment positions for *aioA, arrA*, and *arsC*, respectively). The addition of shorter sequences does not affect the overall tree topology. This approach of adding shorter sequencing to a preexisting tree has been frequently used in studies of ribosomal RNA as well as functional genes (see e.g. Glöckner & Meyerdierks, 2006; Gutierrez et al., 2013; Pester et al., 2012; Pjevac et al., 2017). Own sequences were grouped based on their positioning in the phylogenetic trees. A heatmap showing the occurrence of the aforementioned clusters of *aioA, arrA* and *arsC* as well as the major phyla (>1% relative abundance) based on 16S rRNA gene sequencing were constructed in R using "annHeatmap2" of the Heatplus package.

ASV tables were rarefied, i.e., subsampled at a depth of 600 sequences for all genes to allow for comparison of diversity in the two TPs and depths and between the three genes. Alpha diversity measures (number of observed ASVs, Faith's phylogenetic diversity (PD), Shannon diversity index *H'*, Pielou's Evenness) were calculated based on these rarified ASV tables. Library coverage and richness estimators were not calculated, since the dada2 algorithm removes singletons during its chimera detection step. The nonrarefied libraries

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were tested for community differences between peatlands, depths and the two primer sets per gene using adonis PERMANOVA on weighted unifrac distance matrices (Anderson 2001; Oksanen et al., 2018). ANCOM (Mandal et al., 2015) was used to identify ASVs that differed in abundance between the two TPs or depths. Sequences were deposited in the NCBI sequence read archive under accession number SRP158809.

Co-occurrence network

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Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

Supplementary material is available online.

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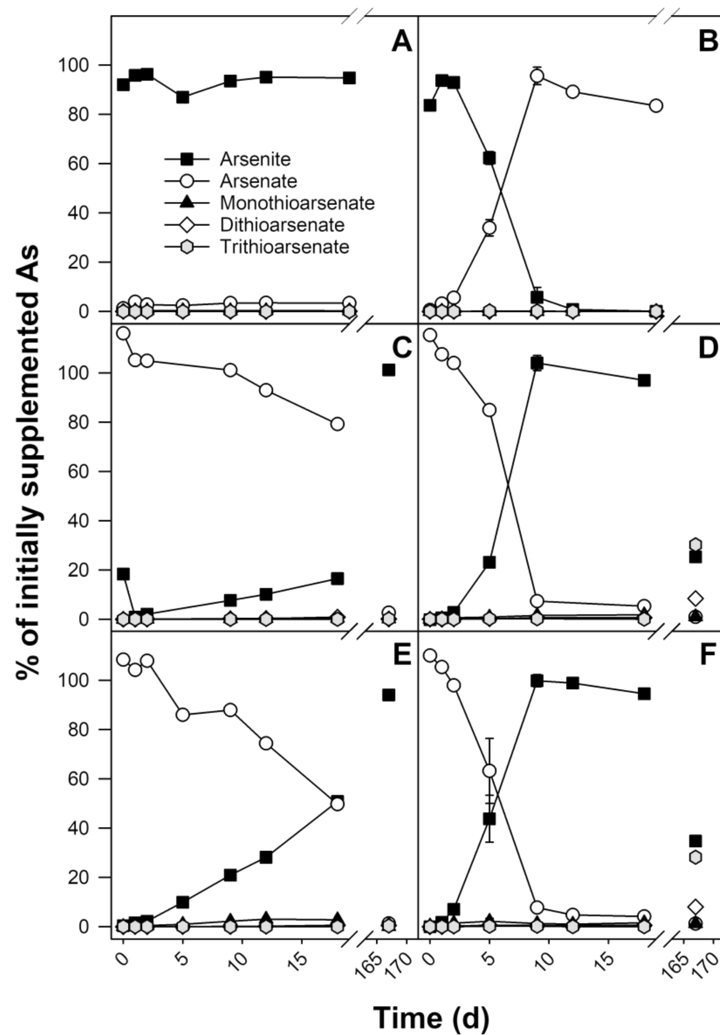

Figure 1: Arsenite-oxidation (A, B) and arsenate-reduction (C-F) potential of peat microorganisms. Incubations were conducted under oxic (A, B) or anoxic (C-F) conditions with peat from 0 to 10 cm (A-D) and 60 to 70 cm (E, F) depth. Microcosms were supplemented with NaN_3 to inhibit microbial activity (A, C, E) or left without inhibitor (B, D, F) and 50 µM arsenite (A, B) or arsenate (C-F). Incubations were done in triplicate. One replicate was analyzed at all sampling timepoints, triplicates were analyzed at selected timepoints. Error bars indicate standard errors in cases in which triplicates were analyzed.

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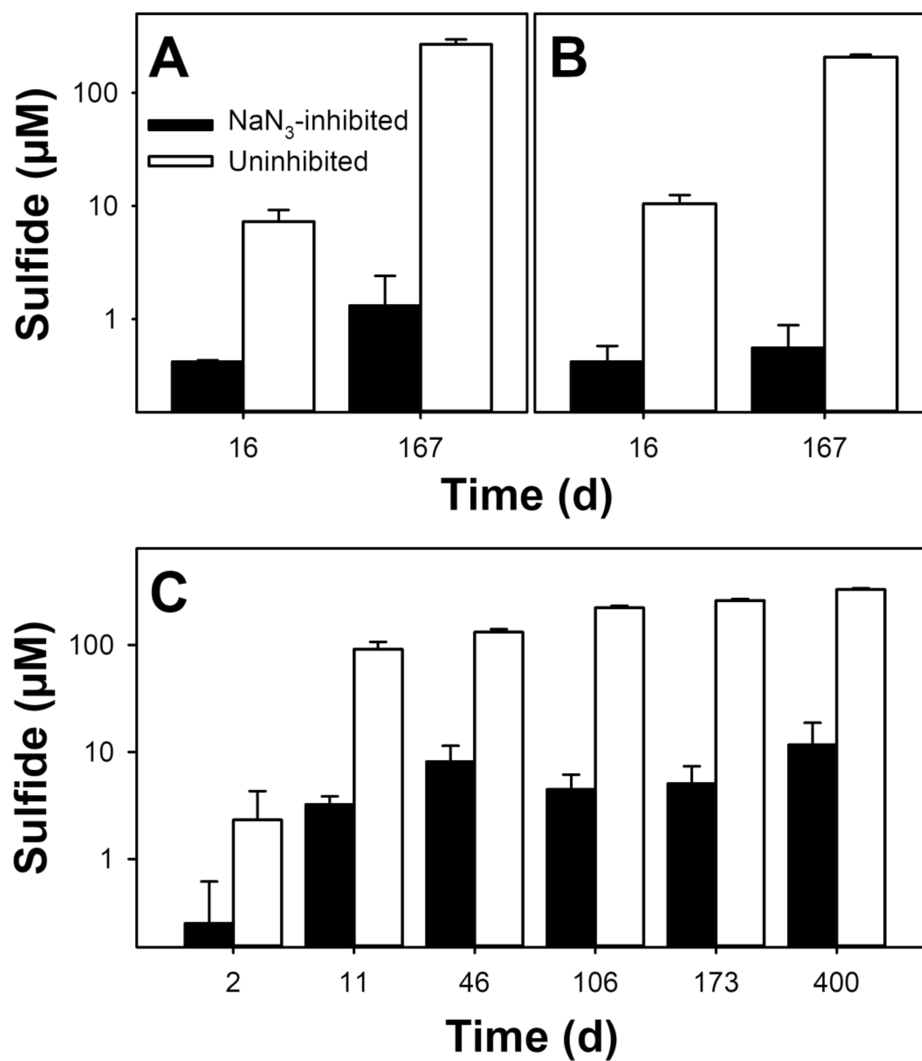

Figure 2: Sulfide production in anoxic microcosms with peat soil from TP A1. Incubations were set up in triplicate with peat from 0 to 10 cm (A, C) and 60 to 70 cm (B). Microcosms were prepared with deionized water (A, B) or mine process water (C). Mean values with standard errors are displayed.

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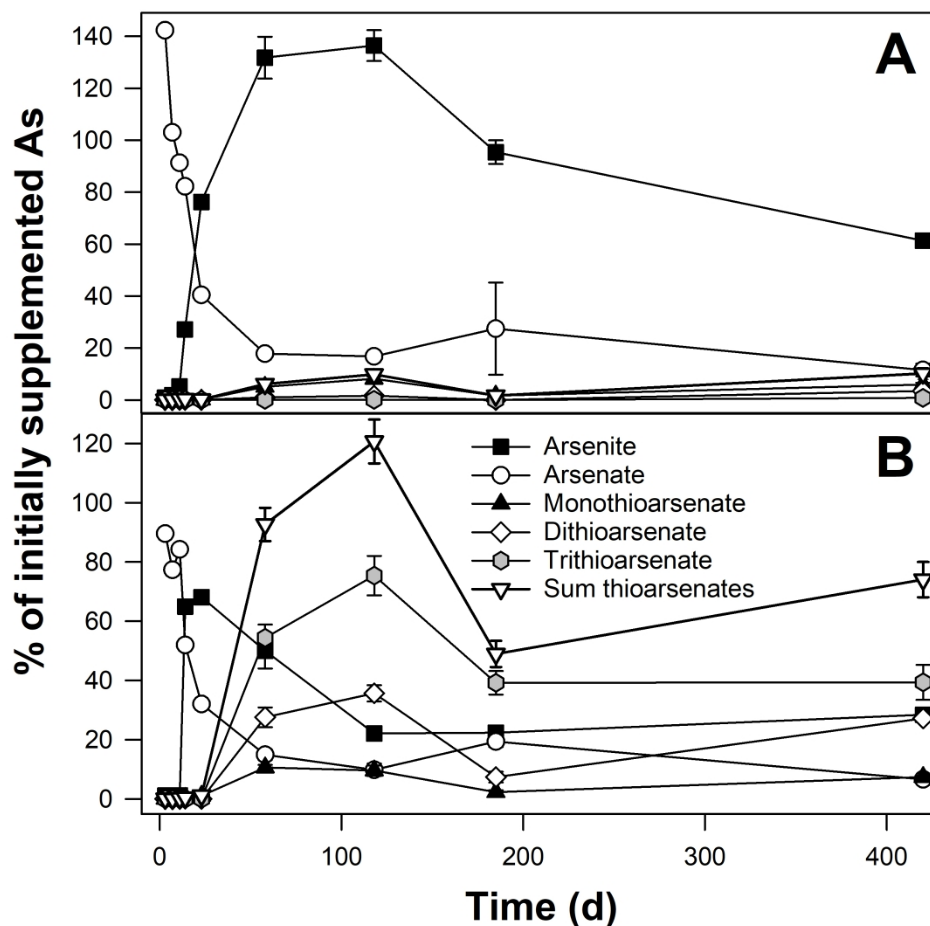

Figure 3: Influence of mine process water on thioarsenate production in anoxic peat microcosms. Triplicate incubations were prepared with 0 to 10 cm peat soil (TP A1) and mine process water and were supplemented with 50 µM arsenate. Microcosms were supplemented with NaN_3 to inhibit microbial activity (A) or left without inhibitor (B). One replicate was analyzed at all sampling timepoints, triplicates were analyzed at selected timepoints. Error bars indicate standard errors in cases in which triplicates were analyzed. At day 185, small amounts of MMA(V) and DMDTA(V) were detected in microcosms without NaN_3.

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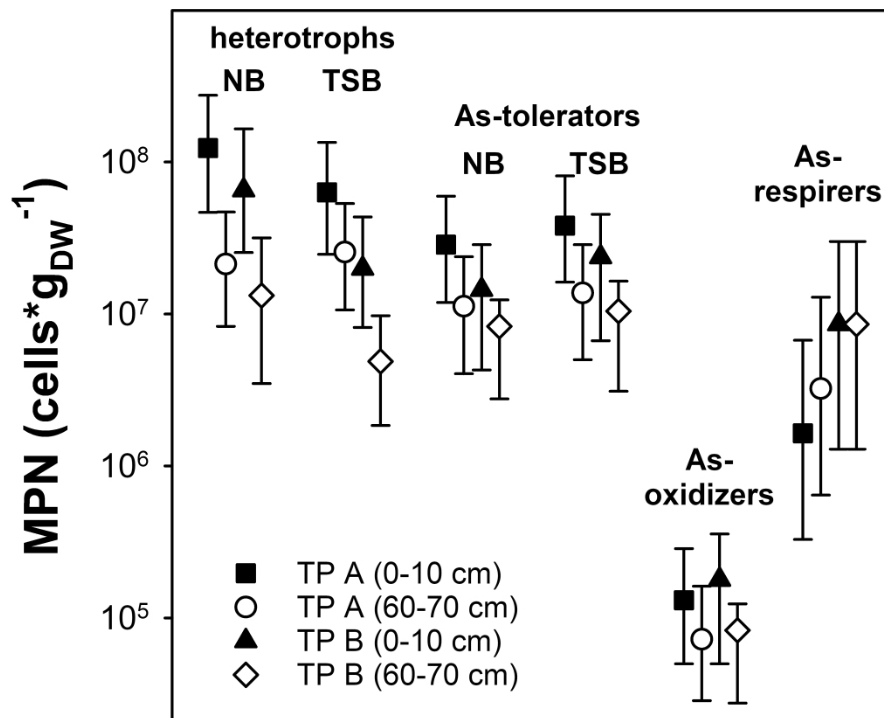

Figure 4: Most probable numbers (MPNs) of aerobic heterotrophic, arsenic-tolerating, arsenite-oxidizing, and arsenate-reducing prokaryotes in peat soil from TP A1 and TP B2. MPNs and 95% confidence intervals are shown. Incubations were conducted under oxic (aerobic heterotrophs, arsenic-tolerating, arsenite-oxidizing) or anoxic (arsenate-reducing) conditions. Eight replicates were used for MPNs of aerobic heterotrophs, arsenic-tolerating microorganisms and arsenite-oxidizing microorganism, three replicates were used for MPNs of arsenate-reducers. Aerobic heterotrophs and arsenic-tolerating MPNs were estimated using two different growth media (nutrient broth = NB and tryptic soy broth = TSB).

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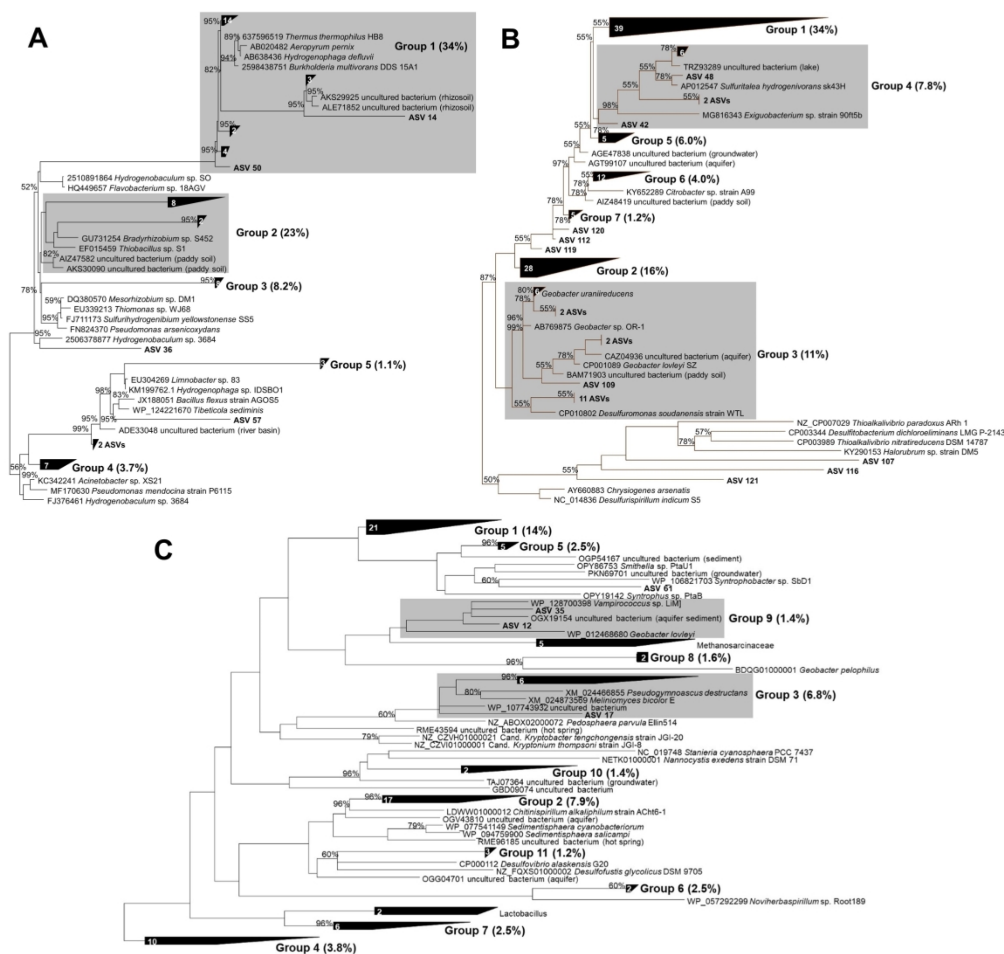

Figure 5: Phylogenetic trees of *aioA* (A), *arrA* (B), and *arsC* (C) representative sequences detected in TPs (TP A1 and TP B2). Reference sequences from cultured species and uncharacterized microorganisms were obtained from public databases. Neighbor Joining trees were constructed in ARB from translated amino acid sequences of full-length references sequences using frequency-based position filters (390, 410, and 115 alignment positions used for *aioA*, *arrA*, and *arsC*, respectively), and own sequences were added to the reference trees using ARB parsimony and frequency-based position filters tailored to the length of the added sequences. ASVs generated with the two primer sets per gene were combined after normalization to relative abundances. Bootstrap values (1000 replications) are indicated, bootstrap values < 50% have been omitted. Reference sequences not closely related to TP ASVs were omitted from the final tree representation to improve readability.

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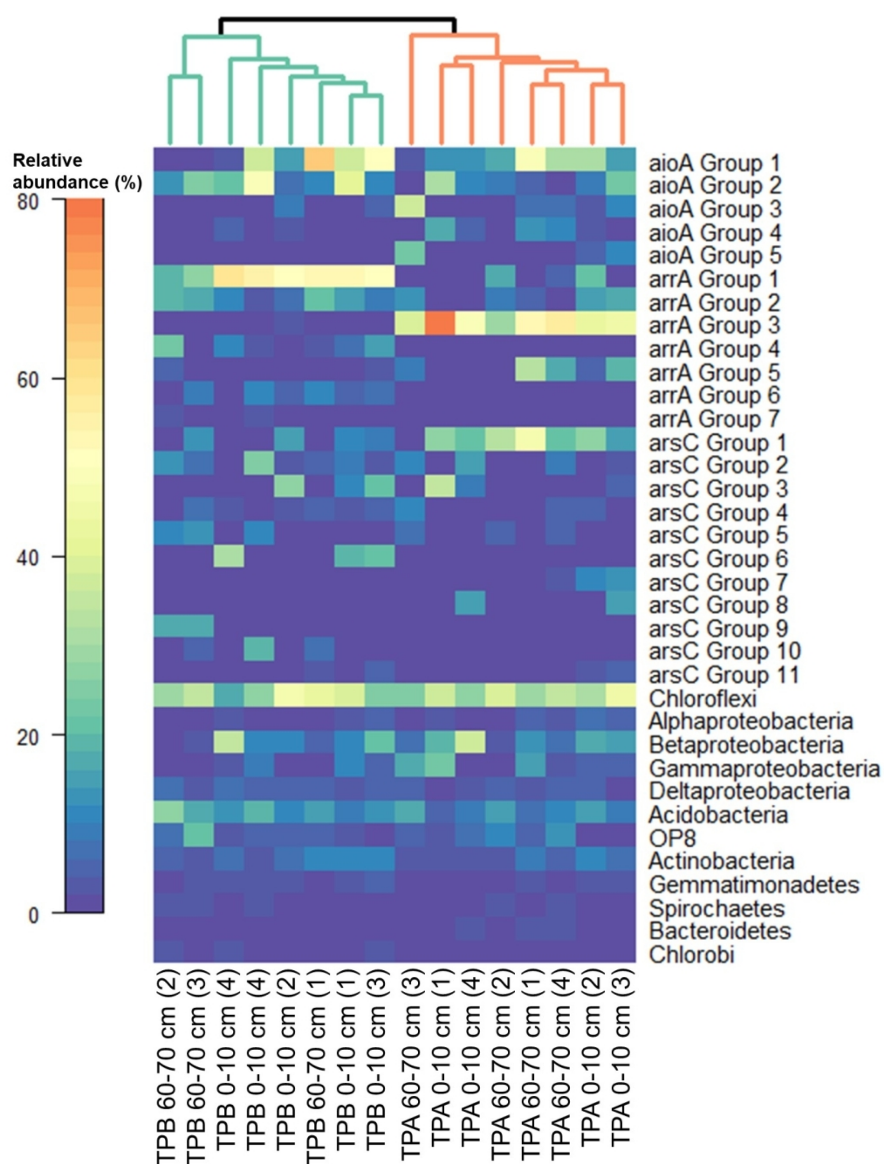

Figure 6: Heatmap showing the relative abundance of *aioA*, *arrA*, and *arsC* groups as well as 16S rRNA gene phyla in TPs receiving mining affected waters (TP A1 and TP B2). The heatmap was generated in R ("annHeatmap2") from relative abundance data of the observed groups. Average relative abundances per group were calculated from relative abundances obtained for the two primer sets per gene. Columns were clustered using average linkage hierarchical clustering based on the Bray-Curtis dissimilarity matrix of the dataset ("vegdist").

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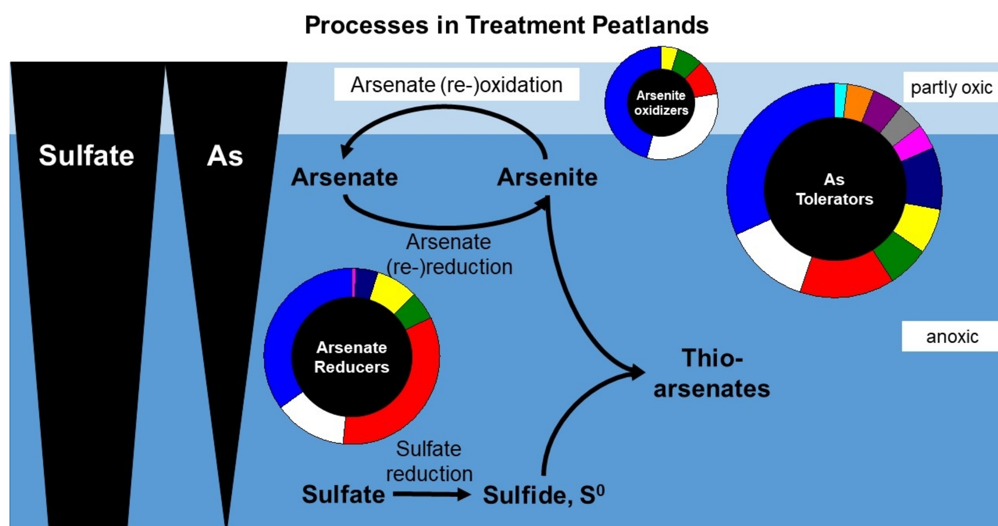

Figure 7: Conceptual model of microbial As turnover in TPs. Processes are indicated by arrows. Microbial groups contributing to As turnover are represented as spheres. The relative abundance of an As-metabolizing group is indicated by the size of the sphere, while the diversity of the group is indicated by the number of pie sectors. The peat is water-saturated and thus mainly anoxic. Arsenic and sulfate pore water concentrations decrease with increasing peat depth. Under anoxic conditions, arsenate is reduced to arsenite. Repeated As cycling, i.e. (re-)reduction and (re-)oxidation might occur. Sulfate is reduced in anoxic peat layers, leading to the production of reduced sulfur species and the subsequent formation of thioarsenates.

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Table 1. Primers, PCR reactions, and PCR conditions used to PCR amplify fragments of *aioA, arsC*, *arrA* and 16S rRNA genes for IonTorrent sequencing. All amplifications were run in a CFX96 touch qPCR cycler (Biorad).

Gene	PCR-type	Primer set	Name for primer set in this study	Forward primer	Reverse primer	PCR conditions	PCR product length (bp)	References
aioA	Initial amplification	M13-aroA #1F/ aroA #1R	aioA1	TGTAAAACGACGGCCAGTGTSGGBTGYGGMTAYCABGYCTA	TTGTASGCBGGNCGRTTRTGRAT	95 °C 10min, 35 cycles (95°C/45s, 50°C/45s, 72°C/90s) 72°C/5min.	500	Inskeep et al., 2007
	Initial amplification	M13-aroA #2F/ aroA #2R	aioA2	TGTAAAACGACGGCCAGTGTCGGYYGYGGMTAYCAYGYYTA	YTCDGARTTGTAGGCYGGBCG	95 °C 10min, 35 cycles (95°C/45s, 50°C/45s, 72°C/90s) 72°C/5min.	500	Inskeep et al., 2007
arsC	Initial amplification	M13-arsC-4f/ arsC-4r	arsC1	TGTAAAACGACGGCCAGTTCHTGYCGHAGYCAAATGGCHGAAG	GCCATGCACCWCCTCT	95 °C/5min, 35 cycles (95°C/90s, 46°C/90s, 72°C/3min), 72°C/5min.	300-400	Escudero et al., 2013
	Initial amplification	M13-arsC-5f/arsC-5r	arsC2	TGTAAAACGACGGCCAGTGGHAAYTCHTGYCGNAGYCAAATGGC	GCNGGATCVTCRAAWCCCCARNWC	95 °C/5min, 35 cycles (95°C/90s, 58°C/90s, 72°C/3min), 72°C/5min.	300-400	Escudero et al., 2013
arrA	Initial amplification	AS1f/AS1r	arrA1	CGAAGTTCGTCCCGATHACNTGG	GGGGTGCGGTCYTTNARYTC	95 °C 10min, 35 cycles (95°C/45s, 50°C/45s, 72°C/90s) 72°C/5min.		Lear et al., 2007
	Nested amplification	M13-AS2f/AS1r	arrA1	TGTAAAACGACGGCCAGTGTCCCNATBASNTGGGANRARGCNMT	GGGGTGCGGTCYTTNARYTC	95 °C 10min, 30 cycles (95°C/45s, 55°C/45s, 72°C/90s) 72°C/5min.	625	Lear et al., 2007
	Initial amplification	AS1f/AS2r	arrA1	CGAAGTTCGTCCCGATHACNTGG	ATANGCCCARTGNCCYTGNG	95 °C 10min, 35 cycles (95°C/45s, 55°C/45s, 72°C/2min) 72°C/5min.		Song et al., 2009
	Nested amplification	M13-AS2f/AS2r	arrA1	TGTAAAACGACGGCCAGTGTCCCNATBASNTGGGANRARGCNMT	ATANGCCCARTGNCCYTGNG	95 °C 10min, 30 cycles (95°C/45s, 55°C/45s, 72°C/90s) 72°C/5min.	625	Song et al., 2009
	Initial amplification	M13-ArrPSRfwd/ ArrPSRrev	arrA2	TGTAAAACGACGGCCAGTAGTTCGTSCCSATCWSSTGGGAC	ACTCSGGSGTSYKGTCCTTSAG	95 °C 5min, 35 cycles (95°C/60s, 59°C/60s, 72°C/90s) 72°C/5min.	550-600	Kudo et al., 2013
all functional genes	Addition of sequencing adapters	IonA_IonXpress Barcode_M13/ P1_reverse primer		CCATCTCATCCCTGCGTGTCTCCGAC-barcode-TAAAACGACGGCCAGT	CCTCTCTATGGGCAGTCGGTGAT- reverse primer	Same temperatures as for initial PCR, 10 cycles		Kujala et al., 2018; Mäki et al., 2016
Bacterial 16S rRNA gene	Initial amplification	27f/ 338r	16S	AGAGTTTGATCMTGGCTCAG	TGCTGCCTCCCGTAGGAGT	95 °C 10min, 30 cycles (95°C/30sec, 52°C/30sec, 72°C/60s) 72°C/10min.	300	universal primers
	Addition of barcodes and sequencing adapters	IonA_IonXpress Barcode_27f/ P1_338r		CCATCTCATCCCTGCGTGTCTCCGAC-barcode-AGAGTTTGATCMTGGCTCAG	CCTCTCTATGGGCAGTCGGTGAT TGCTGCCTCCCGTAGGAGT	Same temperatures as for initial PCR, 10 cycles		Kujala et al., 2018; Mäki et al., 2016

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Table 2: Diversity of different microbial groups along the gradients in TP A and TP B. Number of sequences are obtained from the original OTU tables, while all other diversity indicators are based on rarified OTU tables (rarefied at a depth of 600 sequences). Average values of 1-4 replicates per sampling point are given.

			No. of sequences	OTUs (observed)	Faith PD	Shannon	Evenness
Arsenite oxidase (*aioA*)	aioA1 (220) [a]	TP A (0-10 cm)	3 102 (1 901-3 696)	38 ± 15	7.33 ± 2.25	3.29 ± 1.03	0.63 ± 0.13
		TP A (60-70 cm)	2 298 (398-3 497) [b]	29 ± 13	7.93 ± 3.08	2.43 ± 0.92	0.50 ± 0.12
		TP B (0-10 cm)	1 746 (951-3 570)	34 ± 5	6.58 ± 1.06	4.12 ± 0.75	0.81 ± 0.16
		TP B (60-70 cm)	1 025 (197-2 964) [b]	25	8.23	2.13	0.46
	aioA2 (220)	TP A (0-10 cm)	729 (263-1 145) [b]	24 ± 1	7.05 ± 1.38	3.99 ± 0.11	0.87 ± 0.01
		TP A (60-70 cm)	887 (167-2 897) [c]	20	3.58	2.27	0.52
		TP B (0-10 cm)	1 853 (346-3 341) [b]	21 ± 5	7.12 ± 2.04	2.3 ± 0.82	0.54 ± 0.25
		TP B (60-70 cm)	767 (106-1 847) [c]	9 ± 6	6.32 ± 1.99	0.51 ± 0.31	0.16 ± 0.05
Dissimilatory arsenate reductase (*arrA*)	arrA1 (220)	TP A (0-10 cm)	1 944 (768-4 362)	12 ± 3	4.36 ± 2.32	2.45 ± 0.94	0.69 ± 0.21
		TP A (60-70 cm)	1 626 (764-2 245)	11 ± 3	4.29 ± 0.78	2.32 ± 0.49	0.67 ± 0.08
		TP B (0-10 cm)	5 476 (4 769-6 050)	29 ± 5	4.68 ± 2.07	3.96 ± 0.33	0.82 ± 0.03
		TP B (60-70 cm)	3 408 (2 057-4 509)	19 ± 4	3.67 ± 0.98	3.4 ± 0.36	0.8 ± 0.04
	arrA2 (220)	TP A (0-10 cm)	488 (231-856) [d]	13	4.34	2.96	0.80
		TP A (60-70 cm)	635 (444-977) [c]	21	4.36	3.74	0.85
		TP B (0-10 cm)	1 821 (1 493-2 153)	17 ± 5	2.51 ± 0.43	3.23 ± 0.34	0.8 ± 0.03
		TP B (60-70 cm)	1 353 (1 016-2 042)	21 ± 7	4.71 ± 2.25	3.54 ± 0.57	0.81 ± 0.04
Detoxifying arsenate reductase (*arsC*)	arsC1 (270)	TP A (0-10 cm)	4 857 (4 455-5 279)	23 ± 5	6.41 ± 0.92	3.15 ± 0.13	0.70 ± 0.02
		TP A (60-70 cm)	5 672 (4 349-6 855)	26 ± 2	8.24 ± 0.85	3.36 ± 0.28	0.71 ± 0.04
		TP B (0-10 cm)	3 587 (2 544-4 149)	21 ± 7	6.79 ± 1.72	2.77 ± 0.51	0.64 ± 0.04
		TP B (60-70 cm)	3 647 (2 320-5 191)	17 ± 5	6.33 ± 1.04	2.80 ± 0.20	0.69 ± 0.05
	arsC2 (270)	TP A (0-10 cm)	6 470 (2 092-8 350)	25 ± 12	7.71 ± 5.15	3.36 ± 1.22	0.74 ± 0.16
		TP A (60-70 cm)	6 022 (4 454-7 721)	30 ± 6	13.75 ± 3.29	3.90 ± 0.30	0.80 ± 0.04
		TP B (0-10 cm)	10 446 (8 961-12 068)	48 ± 11	13.11 ± 4.90	4.34 ± 0.55	0.78 ± 0.05
		TP B (60-70 cm)	6 385 (3 445-8 165)	27 ± 8	7.93 ± 2.23	3.79 ± 0.37	0.81 ± 0.03

[a] Number in paranthesis indicates sequence length after dada2.
[b] 3 samples used for diversity calculations.
[c] 2 sample used for diversity calculations.
[d] 1 sample used for diversity calculations.

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