



The Effects of Soil Microbial Disturbance and Plants on Arsenic Concentrations and Speciation in Soil Water and Soils

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Abstract

Arsenic (As) in soils harms soil organisms and plants, and it can enter the human food chain via the dietary consumption of crops. The mobility, bioavailability and toxicity of As are determined by its concentration and speciation. A greenhouse pot experiment was conducted to study the effects of soil microbial disturbance and maize plants on arsenic concentration and speciation in soil (pore) water and soils. Three soil treatments with varying microbial disturbance were designed for this experiment: native soil, sterilized soil and sterilized soil reconditioned with soil indigenous microbes. The three soil treatments were intersected with three levels of As in soils (0, 100 and 200 mg kg⁻¹ spiked As). Ten pots of each treatment were planted with maize, while three pots were filled with soil without maize. The difference between native and reconditioned soil indicated the abiotic sterilization effect (artifact of the sterilization process), while the difference between sterilized and reconditioned soil showed the microbial disturbance effect. Both effects increased As release into soil water. The microbial disturbance effect was more pronounced for organic As species, showing the influence of soil microbes involved in As methylation. The abiotic sterilization effect was more evident in unplanted pots than planted pots and the microbial disturbance effect was observed only in unplanted pots, suggesting that both effects were mitigated by the presence of maize.

Keywords Metalloids · Soil sterilization · Soil-plant system · Arsenic in soil · Plant-microbe interactions

Introduction

Arsenic (As) is a toxic metalloid that can cause health problems to humans due to long-term exposures through drinking water and food (Shankar et al. 2014; Mandal 2017; Parascandola 2017). Natural As concentrations in the crust usually range from 1.5 to 5 mg kg⁻¹ (Rahaman et al. 2021). In soils, typical background levels of As do not exceed 100 mg kg⁻¹ (Rimondi et al. 2022). Anthropogenic As sources such as industrial and mining activities can increase As concentrations in soils up to 20,000 mg kg⁻¹ (Smith et al. 1998). When crops are grown in As-contaminated soils, As can enter the human food chain. The transfer of As from soils to crops is determined by As concentration and speciation in soil water (Rosas et al. 1999; Prabpai et al. 2009). However, total As concentration has only limited relevance, because As speciation controls the mobility, bioavailability, distribution and toxicity of As in the food chain (Garcia-Manyes et al. 2002).

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The solubility of As to soil water is determined by the chemical speciation of As in soils and by the physicochemical properties of soils. The different As species have different sorption properties to different soil parameters at different pH. The study of As speciation in soils as well as in soil water allows to directly measure the solubility of As. To know the amount of As in soil water is important, because it comprises the fraction of As that is available to plants and other soil organisms and that is crucial for the risk assessment of As in soils (Huang et al. 2011). The two dominant inorganic As species (inAs) in soils are arsenate (As^{V}) and arsenite (As^{III}) (Huang et al. 2011). Arsenate represents the vast majority (70–99%) of inAs in oxic environments and is approximately 2–10 times less-toxic than As^{III} (Hong et al. 2014). In oxic soils, inAs can be converted by microbes to less-toxic organic As species (orgAs), such as methylarsonic acid (MMA^{V}), dimethylarsinic acid (DMA^{V}) and trimethylarsine oxide (TMAO) (Thomas 2021). These orgAs can be transformed to volatile arsine mainly under anaerobic conditions (Huang et al. 2011, 2012; Viacava et al. 2020; Kabiraj et al. 2022). Although MMA^{V} and DMA^{V} are the most abundant orgAs in oxic soil environment (Huang et al. 2011), they occur only in small quantities compared to inAs (Pongratz 1998; Garcia-Manyes 2002; Tlustoš et al. 2002; Dobran and Zagury 2006; Sadee et al. 2016). TMAO is detected only in a few cases with minor concentrations in soil water (Geiszinger et al. 2002). Arsenic speciation in soil water is essential to determine its toxicity and bioavailability to plants and humans.

Higher plants lack the ability to methylate As (Jia et al. 2013b; Zheng et al. 2013), and can instead take up inAs and orgAs from soil water, while orgAs in soil water is produced by soil microbes (Lomax et al. 2012). Thus, soil microbes play a key role in As bioavailability for plants (Turpeinen 2002; Zhao et al. 2013; Kuivenhoven and Mason 2019). Soil microbes can remediate metal(loid) toxicity in the rhizosphere, as they can facilitate the crystallization and precipitation of metal(loid)s (Diels et al. 2003; Ahemad 2019). Many microbes have the potential to conduct microbial methylation, i.e., convert inAs to less-toxic organic forms and eventually to volatile As species, allowing them to be removed from soils (Jia et al. 2013a; Upadhyay et al. 2018). The potential of microbes for As volatilization depends on soil chemistry, As level and organic matter concentrations (Mestrot et al. 2011). It can be significant under anaerobic conditions but the overall contribution of volatilization to the total As loss from soils is relatively low compared to other processes, such as leaching and erosion. Arbuscular mycorrhizal fungi, rhizospheric bacteria, fungi and algae can mitigate As stress in soils through bioaccumulation and biotransformation (Rahimzadeh and Pirzad 2017; Upadhyay et al. 2018). Microalgae can not only adsorb As on their surface, but also extract toxic As species from soil water,

convert them to less-toxic species such as arsenosugars, and store them in their cells (Wang et al. 2015; Danouche et al. 2021).

Arsenic concentration and speciation in the rhizosphere are affected not only by soil microbes but also by plants. When encountering As stresses, plants can interact with soil microbes to mitigate As toxicity. The interactions between plants and soil microbes determine their responses to As contamination (Del Molina et al. 2020). Some plants can convert As^{V} to As^{III} in roots, which is the first step in the major detoxification pathway of As, followed by As methylation by soil microbes (Pickering et al. 2000). The As^{V} can bind to ferric sulfate precipitates on root epidermis and be immobilized in root vacuoles as arsenite-trivalent complexes ($\text{As}^{\text{III}}\text{-(SR)}_3$), effectively limiting As absorption into the aerial tissues of mesquite plants (Hammond et al. 2018). Arsenic can also be adsorbed and sequestered in the Fe(III)-containing plaques of wetland plants and rice, reducing As mobility to groundwater, soil water and wetland soils as well as As amount in root interiors and the As bioavailability to plants (Emerson et al. 1999; Blute et al. 2004; Hu et al. 2015). When plants encounter environmental stresses, such as exposure to toxic metal(loid)s, they can excrete chemicals like root exudates (sugars, amino acids, enzymes, etc.) to reduce metal toxicity. These responses include changes in soil pH and redox potential, increase in root surface area, and the release of anions. The response mechanism can enhance nutrient acquisition by roots and promote microbial activity (Rengel and Marschner 2005; Prasad et al. 2006; Colombo et al. 2014; Seshadri et al. 2015). The carbon sources obtained by photosynthesis can be deposited in the rhizosphere and fed by the plants to their soil microbes (Sasse et al. 2018; Zhahnina et al. 2018). The release of such root exudates can consequently apply a selective pressure to soil microbes (Pantigoso et al. 2022). Under As stress, plants may select As-resistant microbes that are efficiently involved in the oxidation of As^{III} to As^{V} and in the As detoxification/methylation process, thereby functionally reducing As concentrations in soil water and As availability to plants (Kowalczyk and Latowski 2018; Raturi et al. 2023). Such interactions between plants and microbes strongly influence As speciation and thus its toxicity to soils and plants, since various As species differ in their toxicities.

To study the microbe-based effects in soils, sterilization of soils is a common technique. The primary consequence of soil sterilization is the elimination of soil indigenous microbes (Blankinship et al. 2014). After soil sterilization, microbes were shown to rapidly acclimate and recolonize the rhizosphere, resulting in a new microbial community with lower diversity (Marschner and Rumberger 2004; Hinsinger et al. 2009; Mahmood et al. 2014; Li et al. 2019). Consequently, sterilized soil has a rather disturbed microbial composition and is referred to as disturbed soil in this study. Soil

sterilization also changes abiotic factors such as accelerating the decomposition of soil organic matter, thereby increasing dissolved organic carbon (DOC) content in soil water (Berns et al. 2008). Dissolved organic carbon can compete with As for adsorption sites on soils (Jackson et al. 2006; Fisher et al. 2015) as well as bind with As to form As-DOC complexes (Buschmann et al. 2006; Williams et al. 2011), leading to As mobilization into soil water. A decrease in soil pH is also an abiotic sterilization effect that results from the dissolution of organic acids (Razavi 2007), which can affect the speciation, mobility and toxicity of As (Masscheleyn et al. 1991). By distinguishing between abiotic sterilization and microbe-based effects, the role of soil indigenous microbes in As concentration and speciation in the soil environment can be better elucidated.

Arsenic uptake in crops and its transfer to the human food chain is a significant problem worldwide, because of its potential toxicity. The problem occurs mostly on soils with high As concentrations, causing high As in crops. This is especially relevant for crops like maize (*Zea mays* L.), which is the most widely grown cereal in the world (with an annual production of more than one billion tons (Rosas-Castor et al. 2014)). However, plant–microbe interactions are known to mitigate As toxicity (Anand et al. 2022; Raturi et al. 2023), but the relative contributions of plant and microbial mechanisms in the As speciation and release to soil water and soil remain largely unknown. Based on these considerations we conducted an experiment growing maize on a soil with three different As concentration levels (0, 100 and 200 mg kg⁻¹ As added) and three different levels of microbial disturbance (natural, sterilized but reconditioned with the indigenous microbiome, sterilized). Rather than analyzing the effects of individual microbes, we aim to examine the global disturbance effects of soil microbes and their interactions with maize plants and answer the following questions: (1) What are the abiotic sterilization effects on As concentration and speciation in soil water and soils? (2) What are the microbial disturbance effects on As concentration and speciation in soil water and soils? (3) What are the effects of the different soil As levels on As speciation? (4) How do maize plants influence As concentration and speciation in soil water and soils? (5) How do the interactions between maize plants and soil microbes affect As concentration and speciation?

Materials and Methods

Greenhouse Pot Experiment

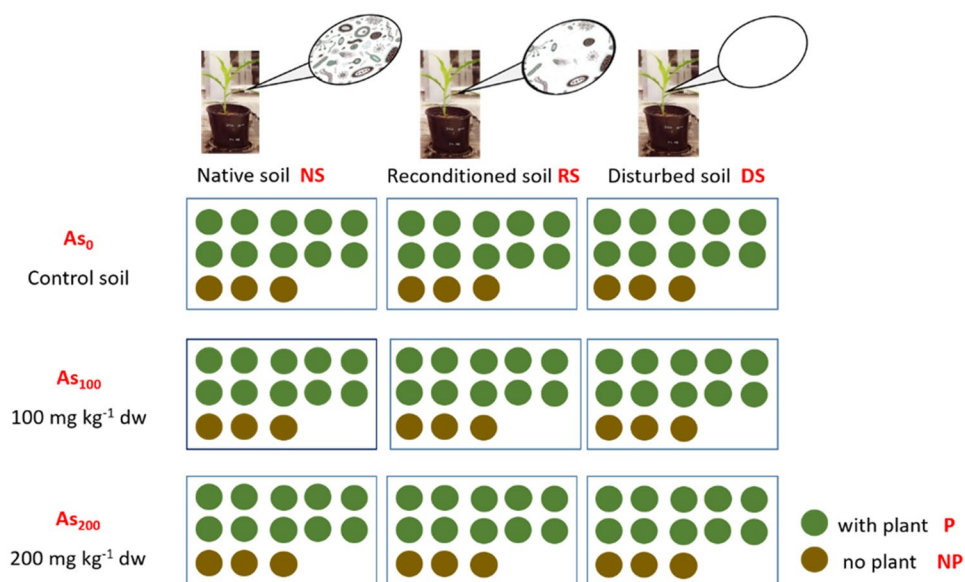
The soil for this experiment (silty loam) was taken from the uppermost 20 cm of an agricultural site in Frauenkappelen, Switzerland. The soil was then stored outside the greenhouse in the Institute of Plant Sciences at the

University of Bern (Ostermundigen, Switzerland). Prior to the experiment, a six-month preliminary experiment was conducted to test the effect of the three As levels on plants. We targeted As concentrations that would show a significant effect on plant health but not kill the plants. Based on similar experiments from the literature, 0, 100 and 250 mg As kg⁻¹ soil were selected for our preliminary experiment. Thus, the soils were spiked with 0, 100 and 250 mg As kg⁻¹ soil, incubated for 2 months and the soil water was sampled regularly. The soil As concentrations stabilized after 2 months. After stabilization of the As concentration maize was grown. The maize plants grown at 100 mg kg⁻¹ soils grew well, showing only minor effects. However, the health of maize grown at 250 mg kg⁻¹ was strongly affected. Some of the plants died and the plant heights were generally very low. As a result, we decided to apply 200 mg kg⁻¹ as the third As group for our main experiment.

For the greenhouse pot experiment, around 800 kg of soil were sampled and sieved to 1 cm. This experiment included a total of 18 different groups: three soil treatments (native soil (NS), reconditioned soil (RS) and disturbed soil (DS)) × three soil As levels (As₀, As₁₀₀ and As₂₀₀, namely addition of 0, 100 and 200 mg As kg⁻¹ soil) × two crop scenarios (with no plant (No-plant) and with plant (Plant)). Three replicates in unplanted pots and ten replicates in planted pots were established (Fig. 1). Soils in the As₀ group were not spiked with As and had a natural concentration of 2.91 ± 0.54 mg kg⁻¹. For As₁₀₀ and As₂₀₀ groups, around 510 kg of soils were spiked with sodium arsenate (Na₂HAsO₄·7H₂O, ≥ 98.0%; Sigma-Aldrich®, CH) to enrich an additional 100 and 200 mg kg⁻¹ of As in soils. The soils were incubated at room temperature for two months at 50% water holding capacity (WHC), simulating soil aging and allowing for As equilibration between soil water and soil phases (Song et al. 2006).

Afterwards, soils in the three As treatments were further subdivided into three subgroups for the three soil treatments. The first subgroup was kept untreated and named native soil (NS). The second and third segments were sterilized by X-ray (25 kGy minimum to 60 kGy maximum at Synergy Health Däniken AG, Switzerland). Due to these concomitant abiotic and microbial changes of soil sterilization, a treatment with reconditioned soil (RS) was performed to disentangle microbial effects on As speciation. A soil microbial extract was applied on the sterilized soils, making the soils independent of abiotic changes caused by soil sterilization. The third part (without microbial reconditioning) was referred to as disturbed soil (DS). Due to the presence of microbes in the greenhouse and potential microbial recolonization, DS was not assumed to be free of microbes, but rather to have a disturbed microbial composition.

Fig. 1 Overview of the experimental design



The microbial extracts for the RS treatment were obtained by mixing 70 kg of native soils entirely with 70 L of Milli-Q water ($> 18.2 \text{ M}\Omega \text{ cm}$ at 25°C) in a pre-sterilized concrete mixer (pre-sterilized with ethanol and a gas burner) (Fig. S1). The solutions were left to stand for 2 h and filtered through a $250 \mu\text{m}$ stainless sieve and $25 \mu\text{m}$ filter papers (Whatman®, CH). Lastly, 800 mL of the microbial extracts were added sequentially to RS. This method was adopted from the literature (Hu et al. 2018), allowing us to achieve an approximate microbial structure in RS as in NS. The microbial extracts still contained nematodes, arbuscular mycorrhizal spores and suspended microbes after filtration (Hu et al. 2018). The detailed characterizations of NS and DS can be found in Table S1.

The abiotic sterilization effect was the same between RS and DS, while the microbial disturbance by soil sterilization was partly eliminated in the RS treatment due to the reconditioning with microbial extracts. Therefore, it was assumed that the difference between RS and DS showed the microbial disturbance effect, and the difference between NS and RS reflected the abiotic sterilization effect. All soils were adequately homogenized. Each pot (7 L) was filled with 6.5 kg of soils and reached the same height to ensure a uniform bulk density of soils. In the end, 90 pots with maize plants and 27 pots without maize were cultivated from April to September 2019.

Maize Cultivation

Maize seeds (*Zea mays* L., W22 genotype) were soaked for 6 min in a commercial bleach containing 5% active hypochlorite (Potz Javel-Wasser Natur, Migros, Switzerland) followed by six washes and an 8 h soak in autoclaved

MilliQ-water ($> 18.2 \text{ M}\Omega \text{ cm}$ at 25°C). Before sowing, one week after soil sterilization, seeds were placed overnight in plastic Petri plates (Petri dish $94 \times 16 \text{ mm}$, without vents, sterile, Greiner Bio-One, Switzerland) with moist filter papers (Rundfilter Sorte 1 Whatman, 90 mm, Huberlab, Switzerland). Each pot was initially sown with three pre-sterilized maize kernels and only the best performing seedling was kept per pot for further growth. To minimize the difference in growth conditions among treatments, all pots were initially randomly placed in the greenhouse. In the beginning, maize plants were watered weekly by weighing pots and adjusting the WHC to 50%. From the third month of growth, maize was watered more frequently as they needed more water for growth. The weekly fertilization in both No-plant and Plant pots started with 100 mL of 2 g L^{-1} complex fertilizer (Plantaktiv Starter 151, Hauert®) plus a 0.25 g of low iron ingredient (Sequestrene Rapid, Maag®), increasing to 200 mL complex fertilizer with a 0.5 g of high iron ingredient after one month. The complex fertilizer mainly contained 52% phosphate (P_2O_5), 10% total nitrogen (8.4% $\text{NH}_3\text{-N}$ and 1.4% $\text{NO}_3\text{-N}$) and 10% potassium oxide (K_2O).

Additionally, a side experiment was conducted to estimate the fresh biomass of maize during growth, while maintaining the same WHC in the soil (50%) by controlling the weight of all pots. In this experiment, 60 maize plants were grown for five months and three of them were harvested weekly to determine their fresh biomass. Maize images were simultaneously recorded to derive the green pixels area of leaves. Therefore, a linear model could be built between the calculated biomass and the leaf area to estimate the maize's actual fresh biomass (Fig. S2) (Neumann et al. 2015; Valasek and Thomasson 2016). The estimated fresh biomass was then

applied to calculate the amount of irrigation water to correct pot weight to retain 50% of WHC.

Sample Preparations and As Analysis

The soil pore water sampler (0.15 μm pore size, Rhizosphere Research Products) was installed in a hole located 2 cm above the level of the pot saucer (details see Fig. S3). The tip of the sampler reached the center of the pot close to the rhizosphere. 30 mL syringes were connected to the samplers and fixed with a wooden stick to suck the soil water overnight at a low pressure. The soil water was sampled biweekly and divided into four sets of aliquots. In the first set of aliquots, pH was immediately measured using a WTW SenTix® Mic pH micro combination electrode (pH electrode; Xylem™, Rye Brook, NY). In the second set of aliquots, major cations and anions were analyzed by the Dionex™ Aquion™ Ion Chromatography System (IC; Thermo Fisher Scientific, Waltham, MA), including Na^+ , NH_4^+ , K^+ , Mg^{2+} , Ca^{2+} , F^- , Cl^- , NO_2^- , NO_3^- , PO_4^{3-} and SO_4^{2-} . The third set was analyzed for DOC concentrations by the vario TOC cube (TOC analyzer; Elementar, Langenselbold, DE).

The last set of aliquots was spiked with 1% (v/v) of 14.65 M nitric acid (HNO_3 ; VWR®, Switzerland) and stored at 4 °C prior to the multielement analysis by inductively coupled plasma mass spectrometry (ICP-MS; 7700× Agilent Technologies, Santa Clara, CA). The multielement analysis with ICP-MS concluded totAs, B, Al, V, Cr, Mn, Co, Ni, Cu, Zn, Ga, Se, Rb, Ag, Cd, Cs, Ba, Ti, Pb and U. In the As speciation analysis, 250 μL soil water was spiked with 50 μL H_2O_2 and 200 μL 1% (v/v) of 14.65 M HNO_3 (VWR®, Switzerland), and stored at 4 °C maximum for 1 week before the analysis by high-performance liquid chromatography (HPLC; 1200 Infinity, Agilent Technologies, Santa Clara, CA) coupled to ICP-MS. Due to the addition of H_2O_2 and HNO_3 , all trivalent As species were oxidized and all determined As species were pentavalent. Arsenic species were separated into inorganic As species (inAs or As^{V}) and organic As species (orgAs, including MMA^{V} , DMA^{V} , TMAO and unknown species) using a Hamilton PRP-X100 anion-exchange column (4.1 × 50 mm, 5 μm). An example chromatogram for soil water is given in Fig. S4. The operating parameters for HPLC are listed in Table S2 and adapted from the literature (Jackson 2015).

Bulk soils (3.6 g) were taken monthly from pot edges with a small auger to measure their As speciation. The soils were air-dried at room temperature, sieved to 2 mm, and ground into powders by a Retsch MM400 Mixer Mill (Retsch GmbH, Haan, D). Afterwards, 0.2 g of ground soil powder was mixed with 4.8 mL of 1% (w/w) subboiled HNO_3 and 0.2 mL of 30% (w/w) peroxide (Suprapur H_2O_2 ; Sigma-Aldrich®, CH), left for at least 30 min at room temperature before conducting an

open-vessel microwave digestion (95 °C for 30 min) (Microwave Digestion System MARS™ 6; CEM GmbH, Kamp-Lintfort, DE) (Norton et al. 2013). After extraction, the solutions were centrifuged at 2500 RPM for 5 min, filtered with a 0.22 μm hydrophilic Polytetrafluoroethylene Filter (13 mm syringe filter, BGB®, CH), diluted if needed, and stored at 4 °C for less than one week before the analysis with HPLC-ICP-MS. The column recovery for bulk soils was $91 \pm 15\%$ ($n=28$). Triplicates of certified reference materials (CRMs) and blanks were extracted and measured together with the soil samples. The CRM ERM®-BC211 Rice was utilized and the percentage recoveries of acid extraction for inAs and DMA^{V} in CRMs were $70 \pm 8\%$ ($n=12$) and $100 \pm 3\%$ ($n=12$), respectively.

Statistical Analysis

All the statistical analysis was performed in R software (version 1.2.5033) including the following packages: car, multcomp, emmeans and vegan. The concentrations of total As (totAs) and As species in soil water (Table S3) and in soils (Table S6) were Log10-transformed to improve normality and analyzed using linear mixed effects models. The experimental factors were soil sterilization (three levels: NS, DS and RS), As treatments (three levels: As_0 , As_{100} and As_{200}), crop scenarios (two levels: No-plant and Plant) and time as well as their interactions. The interactions stand for the combined effects of the experimental factors on the response variables, e.g., totAs concentrations in soil water. The estimated marginal means (in the *emmeans* package) were calculated for the post-hoc analysis. The *emmeans*, formerly known as least-squares means in the context of traditional regression models, are derived to make predictions using a model. These predictions are typically averaged with equal weights across one or more predictors. Such marginally averaged predictions are helpful in describing the results of fitting a model, particularly when presenting factor effects. The compact letter display (CLD; in the *multcomp* package) was used to visually report the pairwise comparisons. Groups with the same CLD letters did not differ significantly, whereas groups that significantly differed had different CLD letters. For multiple As species (multiple-dependent variables), the multivariate analysis of variance (MANOVA) was applied to the comparison of multivariate sample means in soil water and in soils, studying interaction effects and single effects of the four experimental factors on single As species (Table S5). The original data *emmeans* are listed in the supplementary document (Tables S4 and S7).

Results

Total As and Inorganic As Species in Soil Water

Significant interactions among the three experimental factors (microbial disturbance, soil As levels and plant scenarios) were observed in six out of our eight cases (details see Tables S3, S5 and S6). The interactions among the three experimental factors significantly affected total As (totAs) concentrations in soil water ($F_{4, 587} = 6.506$, $p < 0.001$) (Table S3). Regarding the effects of single experimental factors, the abiotic sterilization effect could be observed by the difference between NS and RS, resulting in higher totAs concentrations in the soil water of RS than of NS in the As₀ group and in No-plant pots of the As₁₀₀ group. The microbial disturbance effect increased totAs concentrations in soil water ($F_{2, 587} = 105.286$, $p < 0.001$). As the microbial disturbance increased, totAs concentrations in soil water increased following the pattern NS < RS ≤ DS (Fig. S5 and Table S4). In uncontaminated soils (As₀ group), totAs concentrations in the soil water was lower in No-plant than in Plant pots. Conversely, in contaminated soils (As₁₀₀ and As₂₀₀ group), totAs concentrations were consistently higher in No-plant than in Plant pots, i.e., Plants decreased totAs concentrations in soil water ($F_{1, 587} = 3.97$, $p = 0.047$). The totAs levels were higher in the soil water of DS than of RS in No-plant pots of the As₀ group (Fig. 2a). The totAs and inAs concentrations in the soil water of RS and DS decreased in the first 2 months in all three As groups ($F_{11, 587} = 67.4$, $p < 0.001$) (Fig. S5 and S6). In contrast, totAs concentrations in the soil water of NS were temporarily stable in the absence of microbial disturbance.

The inAs levels in soil water changed over time ($F_{11, 545} = 8.170$, $p < 0.001$). Microbial disturbance increased inAs concentrations in the soil water of contaminated RS and DS. Their concentrations increased in the first two months of the experiment and then decreased, while inAs levels in the soil water of NS remained stable over time (Fig. S6). In uncontaminated soils, No-plant and Plant pots had a similar range of inAs concentrations in soil water, whereas inAs levels in contaminated soils were sometimes lower in the presence of plants.

Organic As Species in Soil Water

The abiotic sterilization effect resulted in higher orgAs concentrations in the soil water of RS than of NS, and still affected orgAs but not inAs at the high soil As level (200 mg kg⁻¹). The microbial disturbance increased orgAs concentrations (sum of MMA^V, DMA^V, TMAO and

three unknown species) in soil water ($F_{2, 545} = 87.929$, $p < 0.001$). Due to the microbial disturbance effect, orgAs concentrations were higher in the soil water of RS than of DS in No-plant pots (Fig. 3a). While NS had the lowest orgAs concentrations in the soil water of both No-plant and Plant pots. The orgAs concentrations decreased over time in contaminated soil water, while it remained stable in uncontaminated soil water (Fig. S7). In both the uncontaminated and contaminated soils, the presence of plants decreased orgAs concentrations in soil water (Plant ≤ No-plant) ($F_{1, 545} = 7.432$, $p = 0.007$). Moreover, the microbial disturbance effect increased orgAs% in soil water ($F_{2, 545} = 47.777$, $p < 0.001$), leading to higher orgAs% in the soil water of RS than DS in No-plant pots of the As₂₀₀ group (Fig. 3b). In contaminated soils, RS soil water had higher orgAs% than that of NS. Moreover, orgAs% in soil water decreased with the increasing As levels in soils. It ranged from 26.8 to 91.7% in the As₀ group and was lower in the As₁₀₀ group (0.12–31.3%) and lowest in the As₂₀₀ group (0.10–8.67%).

In addition, all effects were examined on the three single orgAs (MMA^V, DMA^V and TMAO) in soil water. The interactions between microbial disturbance and As levels or between As levels and plant scenarios significantly affected the concentrations of inAs, DMA^V and TMAO ($p < 0.001$), but not of MMA^V (Table S3). Only MMA^V concentrations were affected by the interactions between microbial disturbance and plant scenarios ($p < 0.001$). Organic As species showed a concentration trend of MMA^V < DMA^V < TMAO in soil water, increasing from NS to RS to DS (Fig. S8). The abiotic sterilization effect was significant in both No-plant and Plant pots for TMAO as well as in No-plant pots of MMA^V and DMA^V, whereas the microbial disturbance effect was not observed in Plant pots for single orgAs.

Arsenic Speciation in Soils

As in the soil water, the same three orgAs i.e., MMA^V, DMA^V and TMAO were found in soils. The three-way interactions among the microbial disturbance, soil As levels and plant scenarios were insignificant for all As species in soils (Fig. 4a and Table S5). Only MMA^V concentrations were affected by the interactions between microbial disturbance and As levels ($F_{4, 294} = 2.945$, $p = 0.021$) (Table S6), showing higher concentrations in RS than in NS ($F_{2, 294} = 3.935$, $p = 0.021$) (Fig. S9). The orgAs% in soils decreased with the increasing As levels in soils (Fig. 4b).

The orgAs% in soil water and soils varied only slightly between No-plant and Plant pots (Figs. 3b and 4b) and is thus not discussed separately here. OrgAs were the dominant form of As in uncontaminated soil water, with unknown species being the main composition and the three organic species i.e., MMA^V, DMA^V and TMAO accounting

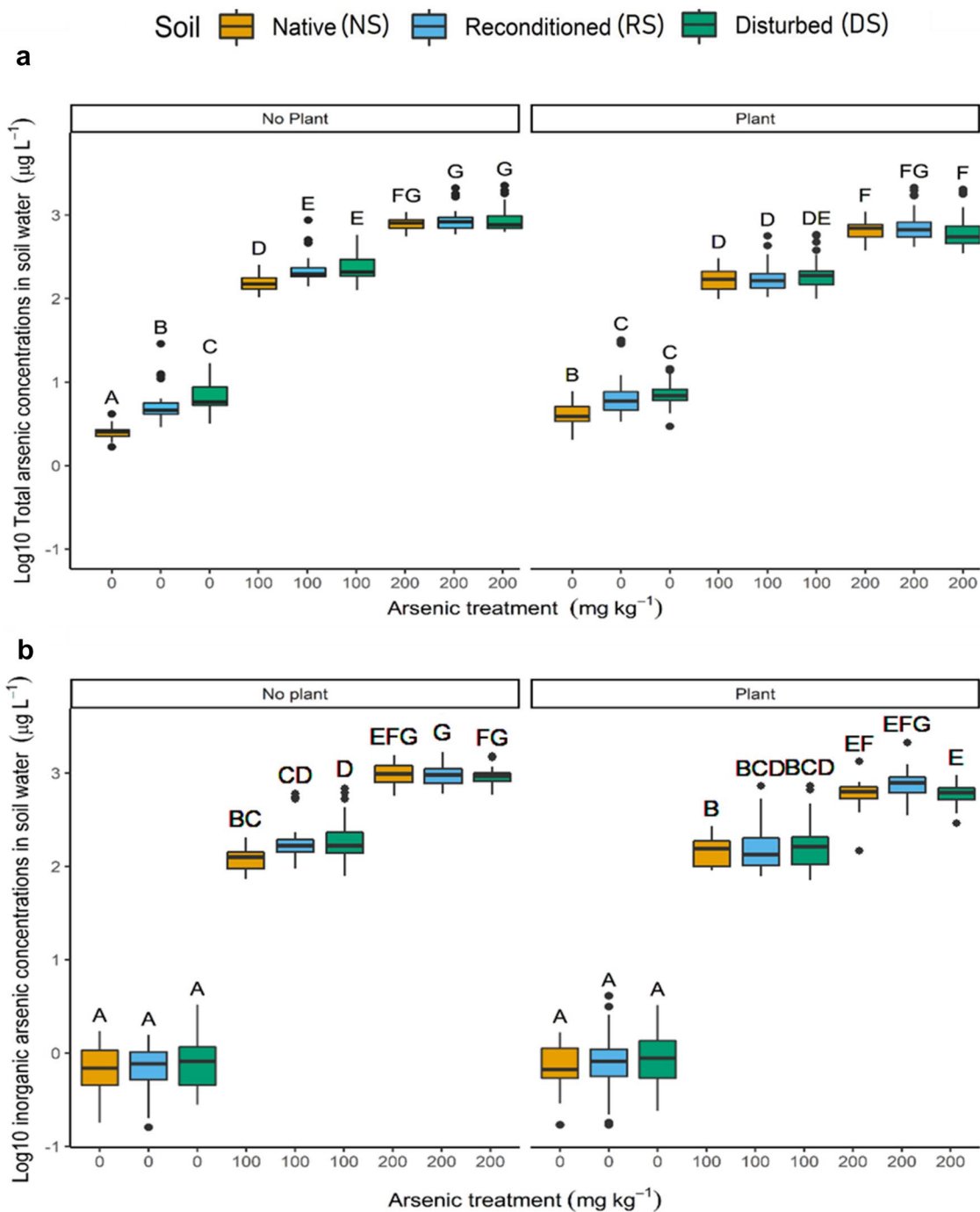


Fig. 2 The concentrations of **a** total As (totAs) and **b** inorganic As species (inAs) in soil water. Data were the estimated marginal means (emmeans) ± standard error. Pairwise comparisons were explored and

reported using CLD letters. Different letters indicated a statistically significant difference between emmeans ($p < 0.05$)

for similar proportions (Fig. 5a). OrgAs decreased with rising As levels in both soil water and soils (Fig. 5b). In soils, inAs were the predominant species (> 96.8%) with DMA^V as the major orgAs (Fig. 5c and d).

Other Chemical Parameters in Soil Water

The redundancy analysis (RDA) was applied to explore the effects of experimental factors (microbial disturbance, soil As levels and plants scenarios) (Fig. 6a) on

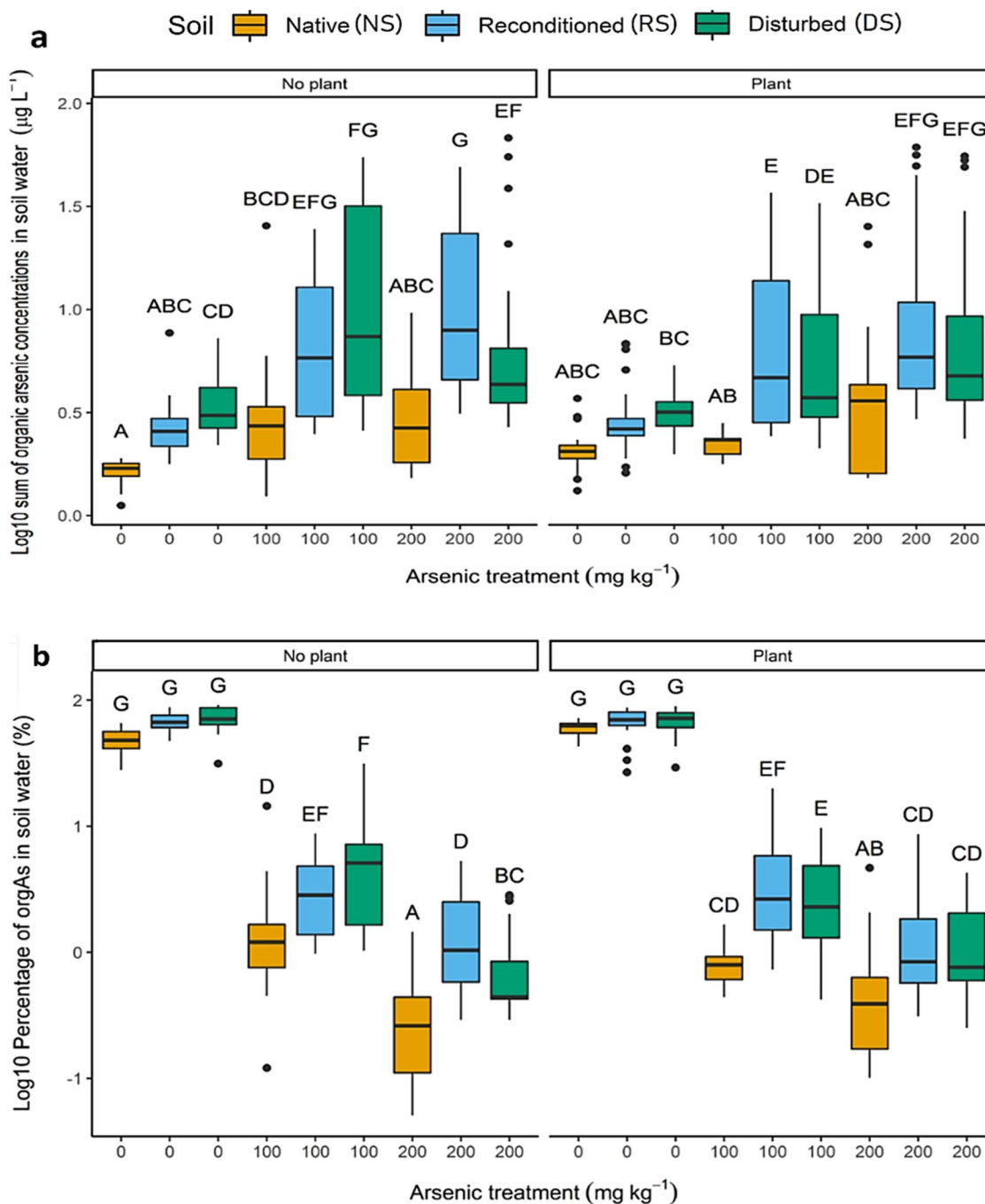


Fig. 3 The plot of **a** concentrations of the sum of organic As species and **b** percentage of orgAs (orgAs%) in soil water. Data were emmeans \pm standard error. Pairwise comparisons were explored and

reported using CLD letters. Different CLD letters indicated a statistically significant difference between emmeans ($p < 0.05$)

the corresponding changes in response variables. Response variables comprised soil water chemistry parameters, i.e., pH, DOC, major cations and anions as well as some major and trace elements (Fig. 6b). The RDA model explained 35% of the variations in soil water chemistry data, with RDA1 and RDA2 explaining 28% of the data. The three

experimental factors, i.e., microbial disturbance, soil As levels and plants scenarios, all had a significant effect on the multiple response variables ($F_{10, 241} = 14.680$, $p < 0.001$) with adjusted R^2 values of 8.95%, 5.37% and 11.85%, respectively. Parameters with arrows pointing in the same direction in an RDA graph indicate positive

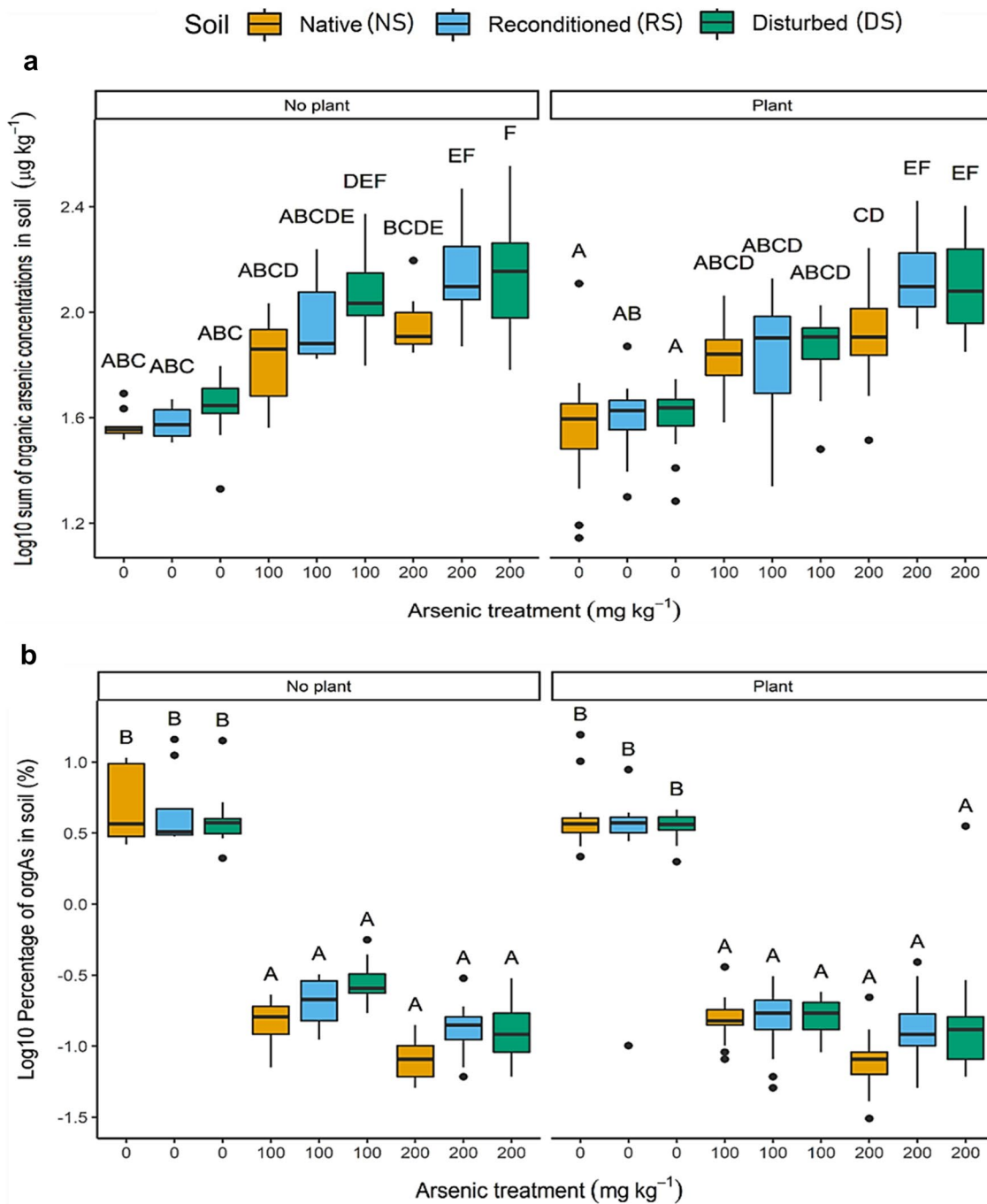


Fig. 4 The plot of **a** concentrations of the sum of organic As species and **b** percentage of orgAs (orgAs%) in soils. Data were emmeans ± standard error. Pairwise comparisons were explored and

reported using CLD letters. Different CLD letters indicated a statistically significant difference between emmeans ($p < 0.05$)

associations, and arrows pointing in opposite directions indicate negative associations between them. The RS and DS and As₁₀₀ and As₂₀₀ groups pointed in the same direction as the concentrations of DOC, V, Ba, Na⁺, NO₃⁻, K⁺ and Mg²⁺ on the RDA plot, which can be interpreted in a way that soil disturbance by sterilization or high As

increased the concentrations of these parameters in soil water. In the opposite, RS and DS and As₁₀₀ and As₂₀₀ groups showed a negative association with the values of pH, Zn, Cr, Al, Cu, Ni, Ca²⁺, Cl⁻, SO₄²⁻ and U in soil water, indicating a decrease of these parameters in the soil solution as a result of disturbance. Meanwhile, the

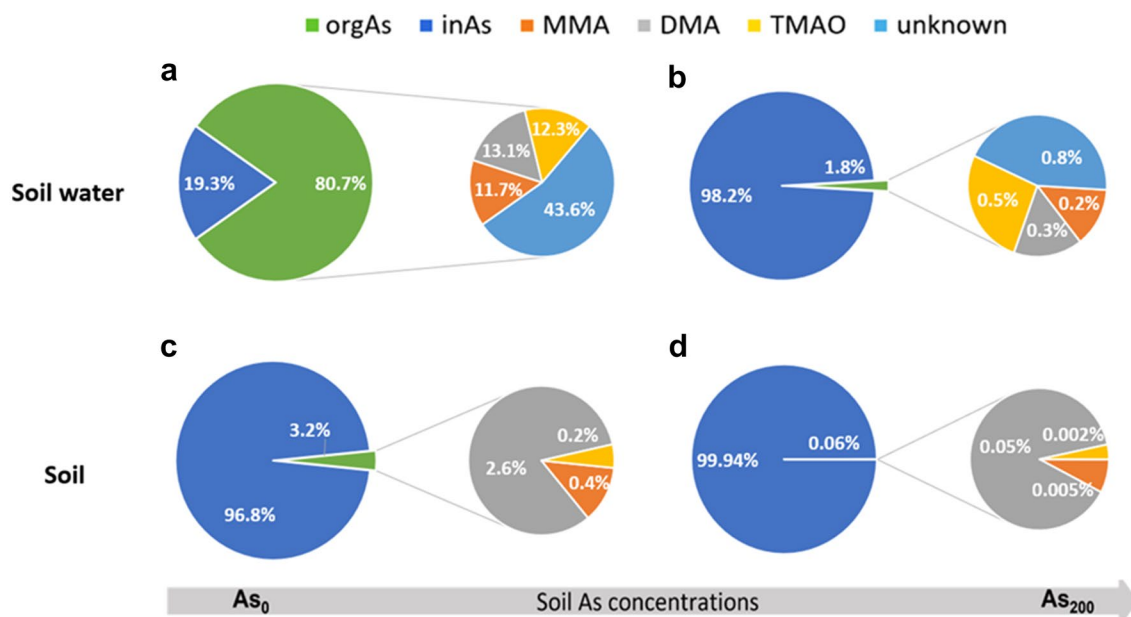


Fig. 5 The changes in As species in soil water and in soils of the Plant pots with the increasing As levels in soils, presenting the percentages of inorganic As species (inAs%) and orgAs (orgAs%, i.e.,

MMA^V, DMA^V, TMAO and unknown species) in **a** soil water of NS of the As_0 group; **b** soil water of NS of the As_{200} group; **c** NS of the As_0 group; and **d** NS of the As_{200} group

experimental factor plants (P_or_NP) pointed in the opposite direction than the microbial disturbance and As levels.

In all the three As groups, DOC levels in soil water from NS were lower than those from DS and RS (Fig. S10). Only in uncontaminated soil water, DOC levels correlated strongly with totAs ($R=0.82$, $p<0.001$) and orgAs ($R=0.69$, $p<0.001$) (Fig. S11). In uncontaminated soils, No-plant pots had a lower pH than Plant pots in NS and RS ($p<0.05$). In contaminated soils, the pH difference between No-plant and Plant pots was less prominent and only significant in NS of the As_{100} group ($p=0.003$).

Discussion

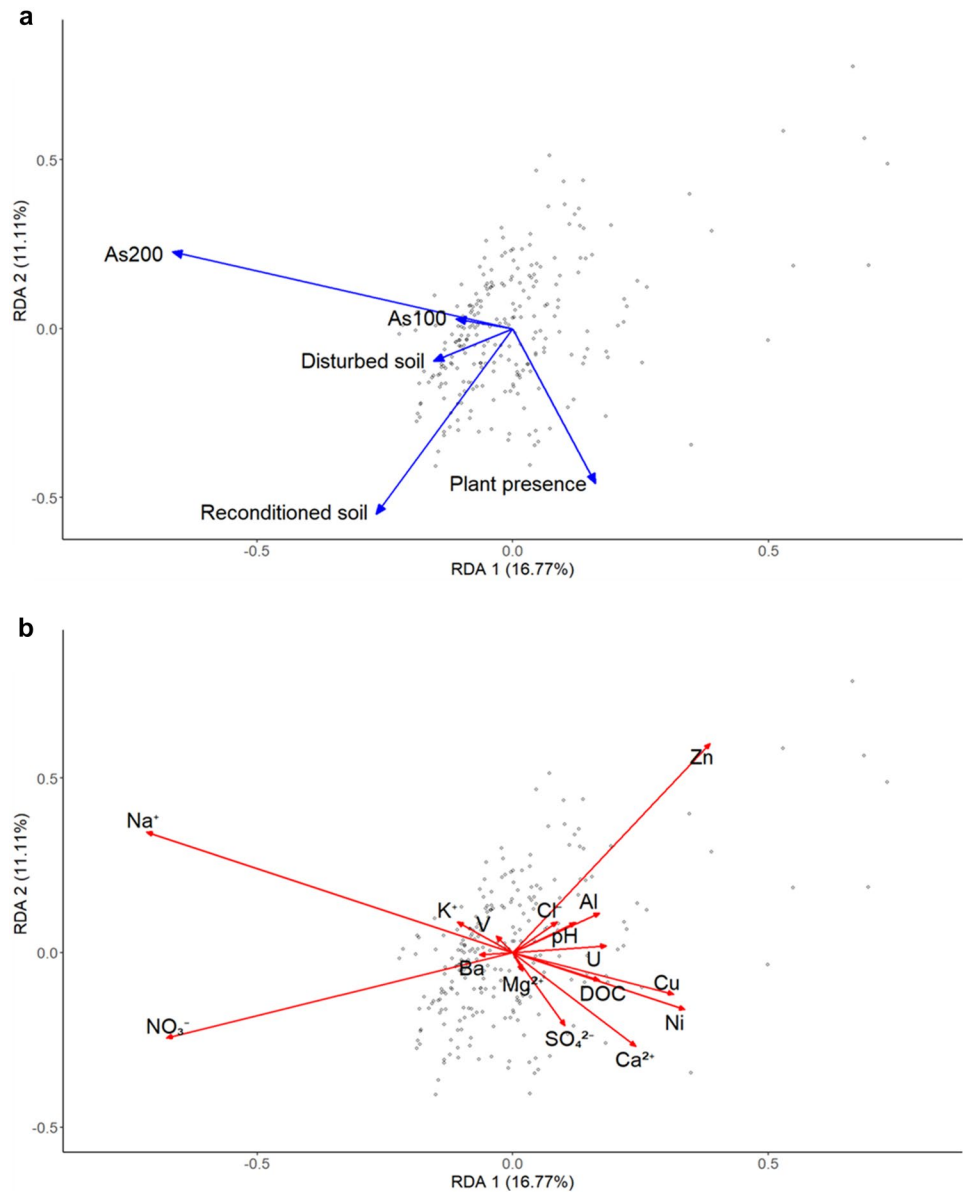
Abiotic Sterilization Effect on As Concentrations and Speciation in the Soil Environment

The abiotic effect of soil sterilization is a side effect of our experimental design, because it is impossible to sterilize soils without abiotic effects (McNamara et al. 2003). Thus, the observed sterilization effects need to be resolved to differentiate them from the microbial disturbance effect. In this study, totAs and As species in soil water showed higher concentrations in RS than in NS, suggesting that the abiotic sterilization effect promoted As release into soil water. This is because the immobilization of As by sorption on soils is reversible and the remobilization of adsorbed As may occur when the physicochemical conditions of soils are changed

by sterilization (Wang and Mulligan 2006). Soil sterilization can also alter the sorption behavior of As due to changed soil pH (Dao et al. 1982; Razavi 2007) and ion competition e.g., with nutrients like phosphorus for sorption sites (Hongshao and Stanforth 2001; Tiberg et al. 2020). The DOC and other nutrients increased in our soil water, as microbes released their cellular compounds during soil sterilization. Increased DOC in soil water can compete with As for sorption sites on soils or complex As, leading to enhanced As mobilization (Schaller et al. 2011). Arsenic concentrations in the soil water declined in the first month of our experiment, which could be due to the re-equilibrium of As adsorption on soils after the initial sterilization.

The DOC concentrations have been observed to be positively correlated with orgAs concentrations in soil water up to $20 \mu\text{g L}^{-1}$ (Williams et al. 2011; Zhao et al. 2013), which is close to our orgAs levels in uncontaminated soils ($<10.10 \mu\text{g L}^{-1}$). Indeed, in our uncontaminated soil water, DOC concentrations strongly correlated with orgAs concentrations. The positive correlation with DOC concentrations was consistent with reports that organic matter can stimulate As methylation and the volatilization of methylated As species, because DOC can serve as energy and carbon source for the growth of As-methylating microbes (Huang et al. 2012; Yan et al. 2020). In contrast, in our contaminated soil water, DOC levels correlated only slightly with orgAs concentrations. Given that DOC levels were similar between un- and contaminated soil water, DOC might have played a minor role in As availability in contaminated soil

Fig. 6 Redundancy analysis (RDA) triplot showing samples as dots with **a** experimental factors (microbial disturbance, soil As levels and plants scenarios); **b** corresponding changes in response variables (soil water chemistry parameters) in the system. The percentages of explained variance were indicated on each axis



water probably due to the overprint effect of highly spiked As. The soil pH was negatively correlated with orgAs concentrations in contaminated soils, presenting an opposite pattern $DS \leq RS < NS$. The same negative correlation has been found in a previous research because the activity of As methylation is higher in acidic soils (Zhao et al. 2013).

Microbial Disturbance Effect on As Concentrations and Speciation in the Soil Environment

In the present study, the microbial disturbance effect resulted in higher concentrations of totAs and orgAs in the soil water of DS than of RS (Fig. 2a). This is in line with a publication that reported the elimination of oxidizing bacteria by soil sterilization slows iron oxidation, leading to insufficient

sorptive sites for As and higher As leaching in sterilized soils (Kumpiene et al. 2007). Soil sterilization causes damage to proteins by ionizing radiation, which disrupts enzyme activity and halts microbial exoenzyme production (Blankinship et al. 2014). The enzyme activities of both sterilized and reconditioned soils were lower than those of unsterilized soils (Xun et al. 2015; Gianfreda 2015). Microbial activity has been found to be negatively correlated with As mobility, demonstrating the importance of microbial activity in As immobilization in soils (Kumpiene et al. 2007). Soil sterilization might have halted enzyme and microbial activities in our experiment that are important for As immobilization in soils, which may explain higher As concentrations in soil water from the sterilization soil treatment (DS). Soil sterilization might also has disturbed or eliminated soil indigenous

microbes and inhibited their roles in the demethylation process of As, thereby increasing orgAs levels in our soil water.

Moreover, as orgAs concentrations increased in soil water and orgAs% increased in sterilized contaminated soils, together with an increase in DOC concentration because of the abiotic sterilization effect. The observed trends pointed to a higher available carbon supply for As-methylating microbes in sterilized soils (Huang et al. 2012; Yan et al. 2020). However, the microbial disturbance affected only orgAs in RS, implying that the recolonized soil microbes in RS promoted As methylation compared to the DS treatment. Other studies reported that DMA^V and TMAO are detected only in unsterilized soils but not in sterilized soils, because microbes were eliminated during soil sterilization (Ultra et al. 2007). However, in our experiment, the soils might have been recolonized fast by microbes after sterilization and the increased availability of DOC might have caused increased orgAs. Taken together, our results showed that the microbial disturbance effect led to lower totAs and orgAs concentrations in sterilized soil water.

As Level Effect on As Speciation

In this study, the orgAs% in both soil water and soils were observed to decrease with the increasing As levels in soils. This could be explained by dilution with the high amount of spiked inAs to the soils. The changes in As speciation with the increasing soil As levels were evident in soil water but not in soils. The three orgAs species (MMA^V, DMA^V and TMAO) had similar proportions in uncontaminated soil water. While in contaminated soil water, TMAO was higher than MMA^V and DMA^V, indicating that As was more methylated or that less demethylation occurred. Two unknown As species occurred in the soil water but were not detected in the soil. In both un- and contaminated soils, DMA^V was always the primary orgAs. While TMAO was predominant in the contaminated soil water, but not in soils. This might be because TMAO has three methyl groups, rendering it more mobile in soils than DMA^V. The additional methyl groups not only remove deprotonation sites from TMAO, making it less negatively charged and less electrostatic attracted, but also cause the TMAO molecule to become larger and occupy more space (Shimizu et al. 2011). Thus, As-binding in soil was weaker and more TMAO remained in soil water.

Maize Plant Effect on As Concentrations and Speciation

The maximum percentage of As uptake by plants from soils (totAs concentrations in plants/in soils) was highest in our As₀ group (1.34%), lower in the As₁₀₀ group (0.66%), and lowest in the As₂₀₀ group (0.28%). These low percentages were not surprising because extractable As has been

known to account for only a minor proportion even in As-rich soils, as evidenced by the poor correlation ($r=0.38$) between water extractable As (mean = 0.019 mg L⁻¹) and soil As (mean = 57.8 mg kg⁻¹) levels (Itabashi et al. 2019). In our uncontaminated soils, totAs concentrations in soil water were higher in the presence of plants (Fig. 2a), probably due to the higher pH in planted pots compared to unplanted pots (Fig. S12). Since totAs concentration has been found positively correlated with soil pH (Katsoyiannis and Katsoyiannis 2006; Podgorski et al. 2017). However, in our contaminated soils, lower concentrations of totAs were found in the soil water with plant cultivation. This could not be attributed to As uptake by plants alone, because such a low percentage of As uptake by plants could not explain the lower As levels in contaminated soil water with plant cultivation. Instead, the presence of maize plants limited the As mobilization into soil water through potential interactions with soil microbes. The details are described in the following chapter.

Plant-Microbe Interactions Reduce As Concentrations in the Soil Water

Both abiotic sterilization and microbial disturbance effects were more significant in the soil water of unplanted than of planted pots. The abiotic sterilization effects played a significant role in all groups of unplanted pots, but only in some groups of planted pots. The microbial disturbance effect was observed only in unplanted pots for totAs and orgAs concentrations. As a result, the concentration differences among the three soil treatments were generally smaller in planted than in unplanted pots, suggesting that both abiotic sterilization and microbial disturbance effects were less significant in planted than in unplanted pots. This might be explained by the mitigation effect of maize plants, which reduced As concentrations in the soil water of RS and DS, resulting in indifferent As levels among the three soils in planted pots.

Maize may help soil microbes to recover from soil sterilization (Zhalnina et al. 2018; Li et al. 2019), while reshaping their communities and favoring beneficial soil microbes (Broeckling et al. 2008). We hypothesize that maize plants can help microbes to recover from soil sterilization and recruit beneficial soil microbes via root exudations to fulfill their demands. This might have helped soil microbes to cope with As stress, leading to lower As concentrations in soil water with the presence of maize. After soil sterilization, plants can act as a filter for their own microbiome and reshape their rhizosphere microbes by helping them to recover from soil sterilization (Reinhold-Hurek et al. 2015). Depending on their structural and functional diversity in soils, plants can recruit beneficial rhizosphere communities through root exudations to adapt to environmental stress, such as aboveground pathogens (Yuan et al. 2018), plant herbivores (Hu et al. 2018) and As stress (Xiao et al. 2021). By

altering the chemical composition of the rhizosphere, plants can create diverse microhabitats and enhance their adaptability to environmental stressors (Zhalnina et al. 2018). For instance, root exudations of the As-hyperaccumulator *Pteris vittata* can mediate root microbes that play an important role in As requisition, which promotes the growth and fitness of the host plant and improve plant capability to adapt to As stress in the environment (Xiao et al. 2021). Comprehensively, the existing evidence supports our hypothesis that the presence of maize plants might have assisted soil microbes in recovering from soil sterilization, while recruiting beneficial microbes via root exudations. In return, maize plants may benefit from their recruited soil microbes in coping with As stress together. This can be considered as a survival mechanism for both maize plants and their associated soil microbes.

Conclusion

The concentrations of totAs and As species in the soil water showed that both abiotic sterilization and microbial disturbance effects enhanced the release of As from soils into soil water. Both effects had a greater impact on orgAs concentrations than inAs concentrations in soil water, which is consistent with the potential role of microbes involved in promoting As methylation. Maize plants reduced the impacts of both abiotic sterilization and microbial disturbance effects to offset rising As levels in the soil water of RS and DS. Maize grown in soils with natural As levels increased totAs concentrations in the soil water. However, when soil As levels were elevated, maize interacted with soil microbes to lower totAs concentrations in the soil water. To mitigate the impacts of microbial disturbance, maize plants likely reshaped their growing environment by assisting the recolonization of soil microbes after soil sterilization and by favoring or disfavoring soil microbes to mitigate the microbial disturbance effects. Overall, this study highlights the role of maize plants and their interactions with soil microbes in lowering As concentrations in the soil water and As bioavailability to themselves as a survival mechanism in response to As stress in the soil environment.

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Data Availability The data used in the manuscript will be made available from the corresponding author of the manuscript on request.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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