The Role of Soil Indigenous Microbes and Their Interactions with Maize Plants in Arsenic Uptake, Translocation, Speciation and Detoxification in the Soil-Plant System

Inaugural dissertation

of the Faculty of Science,

University of Bern

presented by

Hang Guan

from China

Supervisor of the doctoral thesis

Prof. Dr. Moritz Bigalke & Prof. Dr. Adrien Mestrot

Institute of Geography

source: https://doi.org/10.48549/3840 | downloaded: 17.4.2024

The role of soil indigenous microbes and their interactions with maize plants in arsenic uptake, translocation, speciation and detoxification in the soil-plant system

Inaugural dissertation

of the Faculty of Science,

University of Bern

presented by

Hang Guan

from China

Supervisor of the doctoral thesis

Prof. Dr. Moritz Bigalke & Prof. Dr. Adrien Mestrot

Institute of Geography

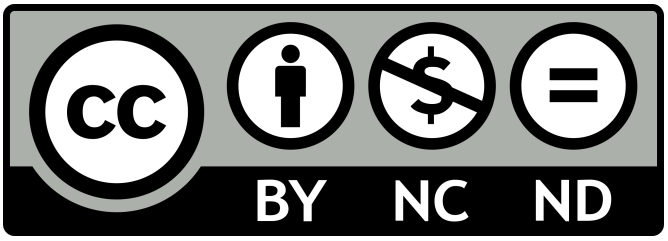
Accepted by the Faculty of Science.

Bern, 25. August 2022

The Dean

Prof. Dr. Marco Herwegh

License



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

"***If I have seen further it is by standing on the shoulders of Giants.***"

by Isaac Newton in 1675

Contents

Summary ..... **xviii**

Acknowledgement ..... **xxi**

Chapter I Summarizing Overview **1**

1 Introduction **2**

2 Research Objectives and Thesis Structure **7**

3 Materials and Methods **10**

3.1 Greenhouse Pot Experiment 10

3.2 Preparation of Soil Water, Soil, and Plant Samples and Analysis of Their As Concentration, Speciation and Other Parameters 12

3.3 Plant Health Parameters 14

3.4 Statistical Analysis 15

4 General Results and Discussion **17**

4.1 Total As Concentrations in Soil Water and Maize 17

4.2 Arsenic Speciation in Soils, Soil Water and Maize Tissues 21

4.3 Answers to The Research Questions 24

5 General Conclusion **26**

Reference **28**

Chapter II Maize plants and soil microbes interact to reduce arsenic concentrations and influence arsenic speciation in the soil water **38**

Abstract **39**

1 Introduction **40**

2 Materials and Methods **43**

3 Results **48**

3.1 Total As and Inorganic As Species in Soil Water 48

3.2 Organic As Species in Soil Water 50

3.3 Arsenic Species in Soils 53

3.4 Comparison between Arsenic Speciation in Soil Water and Soils 55

3.5 Other Chemical Parameters in Soil Water 56

4 Discussion **58**

4.1 Microbial Disturbance Effects on As in the Soil Environment 58
4.2 Sterilization Effects on As in the Soil Environment 59
4.3 Maize Plant Effects on As Release in Soil Water 60
4.4 Maize and Soil Microbes Reduced As Concentrations in Soil Water 62
5 Conclusion 65
Associated Content 66
Supporting Information 66
Acknowledgements 66
Author Contributions 67
Reference 68
Chapter III Maize plants in high-arsenic soils interact with soil microbes to limit the translocation of inorganic arsenic species to maize upper tissues 76
Abstract 77
1 Introduction 79
2 Materials and Methods 82
3 Results 87
3.1 Total As and As Translocation in Maize 87
3.2 Arsenic Speciation in Maize Tissues 89
3.3 Arsenic Correlations in Soil Water and Maize and Other Chemical Parameters 94
3.4 Dry Biomass and Its Correlation with As Species in Maize 96
4 Discussion 99
4.1 Differential As Translocation in Maize at Different Soil As Concentrations 99
4.2 Microbial Disturbance Effects on As in Plants 102
4.3 Sterilization Effects on As in Plants 103
4.4 Correlation Between As in Soil Water and in Maize 105
4.5 Environmental Relevance and Implications 105
5 Conclusion 107
Associated Content 108
Supporting Information 108
Acknowledgements 108
Author Contributions 109
Reference 110
Chapter IV Soil indigenous microbes protect maize plants cultivated in soils with varying arsenic levels 119

Contents

Abstract **120**
1 Introduction **121**
2 Materials and Methods **125**
3 Results **129**
4 Discussion **135**
4.1 Plant Health Responses to Microbial Disturbance 135
4.2 Microbial disturbance Effects in Different As-containing Soils 136
4.3 Sterilization Effects in Different As-containing Soils 138
4.4 Correlation Between As in Maize Tissues and Plant Health 140
5 Conclusion **141**
Associated Content **142**
Supporting Information 142
Acknowledgements **142**
Author Contributions **143**
Reference **144**
Chapter I Appendix **151**
1 Materials and Methods (detailed information) **152**
1.1 Soil Incubation 152
1.2 Preparation of Microbial Extracts 152
1.3 Chemical Characterizations of Soils 153
1.4 Greenhosue Conditions 153
1.5 A Model to Estimate Maize Biomass 154
1.6 Soil Water Sampling 155
1.7 HPLC Operating Parameters 156
1.8 Determination of the Maximum WHC of the Soil 157
1.9 Determination of Dry Matter and Water Content on a Mass Basis 158
Chapter II Appendix **160**
1 Results **161**
1.1 Three-way Interactions on Soil Water and Soils 161
1.2 Time-series Plots 165
Chapter III Appendix **174**
1 Materials and Methods (detailed information) **175**
1.1 Plant Cultivation 175
1.2 Plant Harvest 175

2 Results 178
Chapter IV Appendix 185
Appendix Reference 195

List of Figures

Figure I-1. The five As species investigated in soil water, soil, and maize samples of our experiment 4
Figure I-2. Overview of the experimental design 10
Figure I-3. Changes in the concentrations of total As (totAs) and inorganic As species (inAs) in soil water and maize plants under soil treatments (native soil (NS), reconditioned soil, and disturbed soil (DS)) and As treatments (As_0, As_{100} and As_{200}, the addition of 0, 100 and 200 mg As kg^{-1} soil, respectively) 18
Figure I-4. The increase in As concentrations in maize tissues as well as the decrease of plant height with the increasing levels of As in soils (from As_0, As_{100}, to As_{200} group) and of soil microbial disturbance (from NS to RS to DS) 20
Figure I-5. The changes in As species with the increasing levels of soil As and the increased soil microbial disturbance from the soil to the soil water and to maize plants 22

Figure II-1. Overview of the experimental design 44
Figure II-2. The concentration of (a) total As (totAs) and (b) inorganic As species (inAs) in soil water 49
Figure II-3. The concentration of (a) sum of organic As species (orgAs) and (b) percentage of orgAs (orgAs%) in soil water 52
Figure II-4. The plot of (a) sum of organic As species (orgAs) concentration and (b) percentage of orgAs (orgAs%) in soils 54
Figure II-5. The changes in As species in soil water and in soils of the Plant pots with the increasing levels of soil As concentrations and the increased soil microbial disturbance 55
Figure II-6. Redundancy analysis (RDA) triplot showing samples as dots with a) experimental factors (microbial disturbance, plants and As treatments); b) corresponding changes in response variables (soil water chemistry parameters) in the system 57

Figure III-1. Overview of the experimental design..... 82
Figure III-2. Total As concentrations (totAs) (a) in the entire maize; and (b) in maize tissues 88
Figure III-3. The concentrations of inorganic As species (inAs) in maize tissues 90
Figure III-4. The values of (a) sum of organic As species (orgAs) and (b) orgAs% in maize tissues 92
Figure III-5. The changes in As species in maize tissues with the increasing soil As levels (from the As_0 group to As_{200} group) and the increasing soil microbial disturbance (from NS to DS), i.e., MMA^V, DMA^V, and TMAO) 93
Figure III-6. Redundancy analysis (RDA) triplot showing samples as dots with a) experimental factors (microbial disturbance and As treatments), b) corresponding changes in response variables (plant chemistry parameters) in the system 95
Figure III-7. The dry biomass (a) in the entire maize; and (b) in maize tissues under microbial disturbance effect and As treatments 97

Figure IV-1. Overview of the experimental design 125
Figure IV-2. Changes in (a) plant height; (b) fresh biomass over time under soil sterilization and As treatments..... 129
Figure IV-3. Changes in (a) BBCH-scale and (b) leaf numbers under soil sterilization and As treatments 131
Figure IV-4. The effects of soil sterilization and As treatments on (a) chlorophyll content; (b) leaf spot (foliar diseases) 133

Figure S1.1. (a) Preparation of microbial extracts in a concrete mixer and (b) sieving of their supernatant..... 152
Figure S1.2 (a) An example of maize photos at A: 0 degree, B: 90 degrees, C: top view (Ge et al. 2016); (b) the derived maize biomass estimation model 155
Figure S1.3. An example of a soil water sampler in a 7 L pot 156

Figure S2.1. The totAs concentration in soil water of (a) NP_ As_0 group; (b) P_ As_0 group; (c) NP_ As_{100} group; (d) P_ As_{100} group; (e) NP_ As_{200} group; and (f) P_ As_{200} group..... 166
Figure S2.2. The inAs concentration in soil water of (a) NP_ As_0 group; (b) P_ As_0 group; (c) NP_ As_{100} group; (d) P_ As_{100} group; (e) NP_ As_{200} group; and (f) P_ As_{200} group..... 167

List of Figures

Figure S2.3. The orgAs concentration in soil water of (a) NP_ As_0 group; (b) P_ As_0 group; (c) NP_ As_{100} group; (d) P_ As_{100} group; (e) NP_ As_{200} group; and (f) P_ As_{200} group……168
Figure S2.4. The concentration of the individual orgAs: (a) MMA^V; (b) DMA^V; and (c) TMAO in soil water..... 169
Figure S2.5. The concentration of the individual orgAs: (a) MMA^V; (b) DMA^V; and (c) TMAO in soils 170
Figure S2.6. The concentration of DOC in soil water of (a) NP_ As_0 group; (b) P_ As_0 group; (c) NP_ As_{100} group; (d) P_As_{100} group; (e) NP_ As_{200} group; and (f) P_ As_{200} group..... 171
Figure S2.7. The positive correlations of DOC concentration with (a) totAs concentration and (b) orgAs concentration in soil water of As_0 group..... 172
Figure S2.8. The soil pH in (a) NP_ As_0 group; (b) P_ As_0 group; (c) NP_ As_{100} group; (d) P_ As_{100}; (e) NP_As_{200} group; and (f) P_ As_{200} group 173

Figure S3.1. The concentrations of individual orgAs (a) MMA^V; (b) DMA^V; and (c) TMAO in maize tissues..... 179
Figure S3.2. Pearson correlations of (a) totAs in maize roots; (b) inAs in maize roots; (c) totAs in maize stem; (d) inAs in maize stem; (e) totAs in maize leaves; and (f) inAs in maize leaves with the respective concentrations in soil water 180
Figure S3.3. The concentrations of total P in maize leaves under microbial disturbance and As treatments..... 184

Figure S4.1. Changes in plant height under microbial disturbance and As treatments 186
Figure S4.2. Changes in fresh biomass under microbial disturbance and As treatments... 187
Figure S4.3. Changes in BBCH-scale of maize over time under microbial disturbance and As treatments 187
Figure S4.4. Changes in the leaf numbers over time under microbial disturbance and As treatments 188
Figure S4.5. Changes in the chlorophyll content in maize leaves over time under microbial disturbance and As treatments..... 188
Figure S4.6. Damage scale of leaf spot under microbial disturbance and As treatments. Red dots represent the outliers 189
Figure S4.7. The concentrations of total K in maize leaves under microbial disturbance and As treatments..... 191

Figure S4.8. The concentrations of essential nutrients (a) P; (b) Mn in maize stem under microbial disturbance and As treatments..... 192

List of Tables

Table S1.1. Characterizations of native soil (NS) and disturbed soil (DS). 152
Table S1.2. The operating parameters for HPLC 155

Table S2.1 The *p* values of univariate ANOVA statistical analysis on totAs and As species in soil water 162
Table S2.2. The estimated marginal means (emmeans) of totAs and As species concentrations in soil water 163
Table S2.3. The *p* values of MANOVA statistical analysis on As species in soil water and in soils 164
Table S2.4. The *p* values of univariate ANOVA statistical analysis on totAs and As species in soils 164
Table S2.5. The emmeans of totAs and As species concentrations in soils 165

Table S3.1. The CRMs data on the total As and As speciation analysis in maize tissues 176
Table S3.2. The emmeans of totAs concentration and dry biomass in the entire maize 176
Table S3.3. The *p* values of ANOVA on total As and As species concentrations and dry biomass in maize tissues 177
Table S3.4. The bioconcentration factor (BCF), bioaccumulation coefficient (BAC), and translocation factor (TF) in maize tissues under microbial disturbance and As treatments 177
Table S3.5. The *p* values of the multivariate analysis of variance (MANOVA) on As species in maize tissues 178
Table S3.6. The estimated marginal means (emmeans, R Package) of dry biomass in maize tissues 181
Table S3.7. Conventional Pearson correlation coefficients r and partial correlation coefficients r′ between the concentrations of As species in the entire maize and its dry biomass 181
Table S3.8. The emmeans of total As concentration and As species concentrations in maize roots 182
Table S3.9. The emmeans of total As concentration and As species concentrations in maize stem 182

Table S3.10. The emmeans of total As concentration and As species concentrations in maize leaves 183
Table S3.11. The emmeans of total As concentration and As species concentrations in maize grain 183

Table S4.1. The *p* values of univariate ANOVA statistical analysis on plant health parameters 186
Table S4.2. The correlation coefficient between As species in maize root and plant health parameters..... 189
Table S4.3. The correlation coefficient between As species in maize stem and plant health parameters..... 190
Table S4.4. The correlation coefficient between As species in maize leaf and plant health parameters..... 190
Table S4.5. The correlation coefficient between As species in maize grain and plant health parameters..... 190
Table S4.6. The estimated marginal means (emmeans, R Package) of totAs concentration and As species concentrations in maize roots..... 193
Table S4.7. The emmeans of totAs concentration and As species concentrations in maize stem 193
Table S4.8. The emmeans of totAs concentration and As species concentrations in maize leaves 194
Table S4.9. The emmeans of totAs concentration and As species concentrations in maize grains..... 194

List of Abbreviations

ANOVA	=	univariate analysis of variance
arsM	=	arsenic methylating gene
As	=	arsenic
As_0	=	uncontaminated soils
As_{100}	=	moderate-arsenic soils (addition of 100 mg As kg^{-1} soil)
As_{200}	=	high-arsenic soils (addition of 200 mg As kg^{-1} soil)
As_2O_3-NP	=	oxidative damage caused by As oxide nanoparticles
As^{III}	=	arsenite
As^{III}-$(SR)_3$	=	arsenite-trivalent complexes
As^{V}	=	arsenate
BAC	=	bioaccumulation coefficient
BCF	=	bioconcentration factor
CLD	=	compact letter display
CRMs	=	certified reference materials
DMA^{V}	=	dimethylarsinic acid
DOC	=	dissolved organic carbon
DS	=	disturbed soil
emmeans	=	estimated marginal means
EPA	=	U.S. Environmental Protection Agency
GSH	=	glutathione
HNO_3	=	nitric acid
HPLC	=	high-performance liquid chromatography
IARC	=	International Agency for Research on Cancer
IC	=	ion chromatography
ICP-MS	=	inductively coupled plasma mass spectrometry
inAs	=	inorganic arsenic species
K	=	potassium
K_2O	=	potassium oxide

LOD	=	limit of detection
MANOVA	=	multivariate analysis of variance
MMA^V	=	methylarsonic acid
Mn	=	manganese
$Na_2HAsO_4 \cdot 7H_2O$	=	sodium arsenate
NH_3-N	=	ammonium nitrogen
NO_3-N	=	nitrate nitrogen
NS	=	native soil
orgAs	=	organic arsenic species
orgAs%	=	percentage of the sum of organic arsenic species
P	=	phosphorous
P_2O_5	=	phosphate
PCs	=	phytochelatins
PGPMs	=	plant growth-promoting microorganisms
RDA	=	redundancy analysis
ROS	=	reactive oxygen species
RS	=	reconditioned soil
- SH	=	sulfhydryl groups
SPAD	=	Soil Plant Analysis Development chlorophyll meter
Sqrt	=	square root transformation
TF	=	translocation factor
TMAO	=	trimethylarsine oxide
totAs	=	total arsenic
WHC	=	water holding capacity

Summary

Arsenic (As) is a metalloid that is classified as a Class 1 carcinogen. Due to its high toxicity, As can cause a variety of human diseases such as anemia, leucopenia, and skin cancer. Arsenic is persistent, non-biodegradable, and bio-accumulative, and it persists in soils for extended periods of time and has negative effects on soil organisms. Since As is readily absorbed by plants, it can harm crop plants and result in a reduction in crop development and yields. Once these plants are consumed by animals or humans, As can enter the human food chain and poses a serious health risk to both animals and humans. To avoid such harmful repercussions, it is necessary to understand the uptake, translocation, speciation, and detoxification of As in the systemic soil-plant system. The overall goal of my research is to investigate the role of soil indigenous microbes to immobilize As in soils and decrease its bioavailability to plants. Specifically, there are three questions to be answered: (i) What are the effects of microbial disturbance, plant growth, and As treatments on the concentration and speciation of As in soil water and in soils? (ii) How do microbial disturbance and As treatments affect the concentration and speciation of As in maize (*Zea mays* L.) plants? And (iii) what effects do microbial disturbance and As treatments have on the health of maize plants?

To answer these research questions, a greenhouse pot experiment was conducted to investigate the transformation of As in the soil-maize system and its influence on plant health. Three soil treatments with varying levels of soil microbial disturbance were performed in the experiment: native soil (NS, control soils), reconditioned soil (RS, sterilized soils and reconditioned with native soil microbes), and disturbed soil (DS, sterilized soil before planting). The DS and RS treatments were introduced to differentiate between biotic (microbial disturbance) and abiotic (soil sterilization) effects. The sterilization effect was the same in DS and RS, while the microbial disturbance effect was partly eliminated in the RS treatment due to the microbial reconditioning. Therefore, it is assumed that the difference between RS and DS showed the microbial disturbance effect, and the difference between NS and RS reflected the abiotic effect. The three soil treatments were intersected with three As treatments (uncontaminated soils (As_0), moderate-As soils (As_{100}, addition of 100 mg As kg^{-1} soil), and high-As soils (As_{200}, addition of 200 mg As kg^{-1}soil)). There were three replicates without maize (No-plant) and ten replicates with maize (Plant). This experiment

thus comprised a total of 18 treatment groups. Arsenic concentration and speciation were analyzed in soil water, in soils, and in different maize tissues (roots, stem, leaves, and grains). Arsenic speciation was categorized into inorganic As species (inAs, i.e., arsenate (As^{V}) and arsenite (As^{III})) and organic As species (orgAs, including methylarsonic acid (MMA^{V}), dimethylarsinic acid (DMA^{V}), and trimethylarsine oxide (TMAO)). Various plant health parameters were also measured on a regular basis to examine the physiological responses of maize to microbial disturbance and As exposure, including plant height, fresh and dry biomass, BBCH-scale, leaf numbers per plant, leaf chlorophyll content, and damage scale of leaf spot.

In soil water, total As (totAs) and As species followed a general concentration pattern of NS < RS ≤ DS, owing to the release of As into soil water caused by both the microbial disturbance and sterilization effects. Both effects played a greater role in the concentration of orgAs compared to that of inAs in soil water, implying that microbial disturbance may have influenced the methylation process of As, which converts inAs to organic forms. The microbial disturbance effect (difference RS-DS) is defined as the difference between RS and DS and is caused by the presence of soil indigenous microbes in RS. While the sterilization effect (difference NS-RS) is due to physicochemical changes and nutrient release after soil sterilization. For instance, the increased concentration of dissolved organic carbon (DOC) in soil water and lowered soil pH could mobilize As in soil water. Interestingly, the presence of maize plants mitigated both the microbial disturbance and sterilization effects, possibly helping soil microbes to recover from soil sterilization and favoring beneficial microbes in coping with As stress jointly (Chapter II).

The concentrations of totAs and inAs in maize tissues followed the same order of NS < RS ≤ DS as in soil water. Among different maize tissues, totAs and inAs showed a concentration consequence of roots > leaves > stem > grains in uncontaminated soils, while in contaminated soils, the position of stem and leaves changed, indicating lower translocation of As into the maize leaves and grains. Moreover, the simultaneous presence of microbial disturbance and sterilization effects could exaggerate the adverse effects of As on plant health. Without added As (As_0), both effects had no effect on dry biomass, which is one of the most critical indicators of plant growth and health. In the presence of As, however, the loss in dry biomass was more pronounced in maize grown in sterilized soils (RS and DS) than

in unsterilized soils (NS) due to the sterilization effect. Furthermore, inAs and MMA^V were revealed to be the species responsible for the loss in dry biomass, probably due to the high abundance and toxicity of inAs and the efficient translocation of MMA in maize (Chapter III).

As with dry biomass, plant height and BBCH-scale were not affected by both the microbial disturbance and sterilization effects in maize grown on uncontaminated soils, implying that plants are capable to buffer both effects. In contrast, the effects of microbial disturbance on contaminated soils resulted in a reduction in plant height, leaf numbers, and chlorophyll content as well as an increase in the damage scale of leaf spot. Even at a high As concentration in soils, these affected health parameters were not or only slightly retarded in maize in NS indicating the resilience of an undisturbed soil-microbe-plant system. The sterilization effect caused phosphorus (P) and manganese (Mn) deficiencies in maize grown on high-As soils, which hampered plant growth and may have indirectly led to increased As accumulation in maize plants (Chapter IV).

Overall, this research highlights the importance of soil indigenous microbes and their potential interaction with plants in their common resistance to the detrimental effects of As, which may improve the knowledge of As uptake, translocation, speciation, and detoxification in the soil-maize system and reduce As inputs into the food chain. This could also help to ensure food safety, food security, and sustainable food production as well as the protection of animal and human health. Further studies on soil microbial diversity, communities, and functioning (e.g., enzyme activity) as well as plant microbial communities, would be beneficial to learn more about As effects on soil health and plant health.

Acknowledgement

For data protection reasons, this section can only be seen on the printed version.

Acknowledgement

Chapter I Summarizing Overview

Hang Guan

A brief introduction to the research background and the greenhouse pot experiment is provided. The research objectives, thesis structure and general materials and methods are given. At the end, the general discussion and conclusion of the work are presented.

Chapter I Summarizing Overview

1 Introduction

Arsenic (As) has been classified as a Group 1 human carcinogen by the International Agency for Research on Cancer (IARC). It is a natural constituent of the Earth's crust and the 20th most abundant element with an approximate amount of 4.01×10^{16} kg (Matschullat 2000). Arsenic originates from the parent rock and is present in over 500 minerals (Jacks et al. 2002). More than 90% of As pollution is presumed to be geogenic and the main sources of geogenic As are minerals of diverse classes such as As sulfides (Hug et al. 2020). Arsenic can be released into the environment through geogenic processes such as weathering and volcanic activities and thus causes contamination (Basel Landschaft 2018). Arsenic contamination of groundwater is a major public health concern worldwide with nearly 108 countries affected (Shaji et al. 2021). Over 200 million people are exposed to As-contaminated water with levels above the WHO guideline value (10 µg L^{-1}) (Shakoor et al. 2015). Arsenic is also introduced into the environment from anthropogenic sources, including metal mining and smelting activities, fossil fuel processing and combustion, wood preservation, pesticide production and application, etc. (Wang and Mulligan 2006b; Han et al. 2003; Bhattacharya et al. 2002).

Arsenic is considered persistent, non-biodegradable, and bio-accumulative and remains persistent in soils for a long period of time (Maji et al. 2016). Once As is adsorbed in plant roots, it can be translocated to plant aerial tissues, thereby entering the food chain, exerting hazardous impacts on animal and human health (Roychowdhury et al. 2018a). High As levels in the arial tissues of crop plants have been assessed as human exposure pathways (WHO 2018), representing the main routes of As exposure in humans (> 90%) (Anjum et al. 2017a). Plant growth can be rigorously constrained by arsenic by reducing plant reproductivity and yield as well as decreasing fertility and inhibiting the development of reproductive organs (Flora 2011). Arsenic exposure has an adverse effect on the morphological (e.g., chlorosis), physiological (e.g., growth processes inhibition), and biochemical (e.g., formation of reactive oxygen species (ROS)) responses of plants (Zemanová et al. 2021; Alam et al. 2019; Gulz et al. 2005). The ROS can cause oxidative damage to biomolecules such as lipids and proteins

and causes plant physiological disorders and eventually result in cell death (Garg and Singla 2011; Finnegan and Chen 2012b).

Maize (*Zea mays* L.) has become a staple food in many regions of the world, with more than one billion tons produced annually, surpassing wheat or rice. All components of maize can be used for food, with straw and husk serving as cattle feed as well as for non-food products such as ethanol and starch (IITA 2009). Maize is grown in many countries with As-contaminated water or soils, including Afghanistan, Argentina, Bangladesh, Cambodia, Chile, China, India, Mexico, Mongolia, Myanmar, Nepal, Pakistan, the USA, and Vietnam (Brinkel et al. 2009). Arsenic accumulation in maize poses a higher health risk to people with high maize consumption and vulnerable populations e.g., pregnant women and infants (Zheng and Ayotte 2015). Some countries and authorities, such as China, WHO, and the EU, have established limits for inorganic arsenic (inAs) in food (0.2 mg kg^{-1}). For infants and young children, the EU advised an even lower inAs concentration (0.1 mg kg^{-1}) (WHO 2018). The immediate symptoms of As acute poisoning are vomiting, abdominal pain and diarrhea, followed by numbness and tingling in the extremities, muscle cramping and, in an extreme cases, death. While As chronic poisoning is much more insidious in nature, resulting in anemia, leucopenia, skin cancer, and other internal cancers (Hong et al. 2014; WHO 2011; Wang and Mulligan 2006b).

Maize is generally classified as a tolerant plant to heavy metals and as an As excluder with a low capacity to translocate metals (Abbas and Abdelhafez 2013; Fellet et al. 2007; Rosas-Castor et al. 2014b; Armienta et al. 2020). Arsenic concentrations in maize roots have been reported to range from 5.32 to 237% of the As concentration in soils (0.2 - 718 mg kg^{-1}), indicating that As accumulation in maize differs in various soils and among maize varieties. Similar, As concentrations are observed in the stem and leaves, being 0.16 - 5.71% of the soil As concentrations, while in a few cases higher As concentrations are detected in the stem (up to 23.33% of soil As concentration). The translocation of As to grains is lowest and As concentrations in grains range from 0.04 to 5.00% of the concentration in soils (Neidhardt et al. 2012; Cao et al. 2019; Rosas-Castor et al. 2014b). Arsenic concentration and speciation in soil water are deterministic for its transfer from soils to crop plants (Prabpai et al. 2009; Rosas et al. 1999).

Arsenic speciation in soil water is important to elucidate its bioavailability and toxicity in plants, as individual As species differs in terms of their solubilities and mobilities and thus bioavailability to plants (Tu and Ma 2002). Once As is taken up by plants, it is stored primarily as inAs. The inAs is the predominant species in maize roots, stem, and leaves, whereas orgAs represent only small amounts (< 1%) (Yu et al. 2009) or below the respective limit of detection (LOD) (Rosas-Castor et al. 2014b). Arsenate (As^{V}) and arsenite (As^{III}) are the two predominant inAs species. Arsenate predominates in aerobic soils, while As^{III} prevails in flooded soils. Maize plants are grown in an aerobic environment, where inAs is primarily present as As^{V}. Arsenate enters plants through phosphate channels in the roots, where it is reduced to As^{III} *via* complexation with phytochelatins and subsequently stored as an As^{III}-tristhiolate complex in vacuoles. A small portion of inAs can be transported *via* the xylem and stored in the stem as an As^{III}-tris glutathione complex (Patra et al. 2004). The inAs can be converted in biota to less-toxic orgAs, such as methylarsonic acid (MMA^{V}), dimethylarsinic acid (DMA^{V}), and trimethylarsine oxide (TMAO) (Figure I-1) (Kuivenhoven and Mason 2021). According to in vivo studies, the toxicities of As species are as follows: inAs > MMA^{V} , DMA^{V} > TMAO (Khairul et al. 2017; Di et al. 2019). OrgAs are translocated to the grains *via* phloem more readily than inAs in crop plants, although roots absorb inAs much faster than orgAs (Li et al. 2009b; Carey et al. 2011; Awasthi et al. 2017; Raab et al. 2007a).

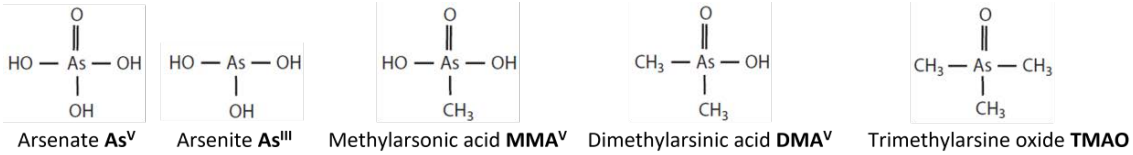


Figure I-1. The five As species investigated in soil water, soil and maize samples of our experiment

Soil microbes have been widely reported to remediate heavy metal toxicity in the rhizosphere (Jing and Kjellerup 2018; Ko et al. 2017). The addition of soil microbes promotes maize growth and reduces As concentration in roots and stem by a maximum of 27% and 48%, respectively (Natasha et al. 2021). They can facilitate the crystallization and precipitation of heavy metals (Ahemad 2019; Diels et al. 2003). Some prokaryotic (bacteria, archaea) and eukaryotic (algae, fungi) microbes can excrete extracellular polymeric substances such as polysaccharides and glycoproteins, which possess abundant functional groups with biosorption and metal binding properties that can immobilize metal(loid)s

(Seshadri et al. 2015; Pal and Paul 2008). On the other hand, soil microbes, e.g., rhizospheric fungi, bacteria, and microalgae, can mitigate As stress in soils through bioaccumulation and biotransformation. Rhizospheric fungi can transform As species from inorganic to less-toxic organic forms and eventually to volatile As species to remove them from soils (Upadhyay et al. 2018). The actinomycete strain can synthesize stress-alleviating metabolites to recover maize growth, photosynthesis inhibition, and oxidative damage caused by As oxide nanoparticles (As_2O_3-NP) (Selim et al. 2021). It can also reduce As bioaccumulation in maize roots and stem and promoted biomass gain despite high As levels in maize tissues, due to its general growth-promoting effect and its increased stress tolerance of the plants (AbdElgawad et al. 2021). Microalgae can not only adsorb As on their surface, also extract As species from soil water and convert them into low toxic species such as arsenosugars and arsenolipids to be stored in the cell (Danouche et al. 2021; Wang et al. 2015).

Arsenic concentrations in the rhizosphere can also be influenced by plants. Plants can increase their root surface area and release organic acids to improve nutrient availability in the root environment (Colombo et al. 2014; Prasad et al. 2006; Rengel and Marschner 2005). Root exudations contain important components to attract beneficial microbes (e.g. phenolics, organic acids, and sugars), restrict the passage of toxic metals across the roots (López-Bucio et al. 2000), and prevent heavy metals from entering the cell symplast by chelating metal(loid)s in the rhizosphere or apoplast (Magdziak et al. 2011). Root exudations may favor bacteria that synthesize efficient exopolysaccharides and increase the soil aggregation around roots, which promotes plant growth under As stress (Mahmood et al. 2014). Moreover, as plants encounter environmental stress, such as exposure to heavy metals, they can excrete chemicals through roots that trigger cascade responses to reduce metal toxicity. These responses include changes in soil pH and redox potential, the release of anions, and nutrient acquisition by roots that can enhance microbial activity (Seshadri et al. 2015). Plant roots play an important role in altering As speciation in soils and they can convert As^{V} in soils to As^{III}, which is considered the first step in the As methylation pathway for plant detoxification (Turpeinen 2002; Pickering et al. 2000). Arsenic methylation can be catalyzed by the homologs of *Streptomyces* As methylating gene (arsM) in bacteria, archea, and fungi (Chen and Rosen 2020; Jia et al. 2013a).

Plant-microbe interactions have reciprocal effects on both partners and play an important role in their adaptation and survival in stressed environments (Berg 2009). In response to environmental stressors, plants frequently increases the excretion of root exudates to recruit beneficial rhizosphere communities (Yuan et al. 2018; Bauer and Mathesius 2004; López-Bucio et al. 2000). Soil microbes can produce volatile organic compounds that can be sensed by plants to alter their morphogenesis or trigger their defense and stress responses (Ortíz-Castro et al. 2009). They can also secrete antioxidant enzymes or induce plants to synthesize antioxidant enzymes to reduce oxidative damage (Kavita et al. 2008). Altogether, plant-microbe interactions are beneficial for plant survival under As stress (Del Molina et al. 2020). Bacteria and fungi in the roots have an intimate interaction with their host plants and can promote plant growth as well as suppress plant pathogens (Whipps 2001; Berg 2009). This interaction can be potentially disrupted by soil sterilization, as the balance between pathogen and beneficial microbes can be destroyed by biotic and abiotic stressors, which influences the growth and health of host plants (Li et al. 2019).

Soil sterilization may cause both biotic and abiotic effects on soils. The foremost consequence of soil sterilization is the elimination of soil indigenous microbes, including both pathogens and beneficial microbes in soils (Li et al. 2019). Some studies have reported positive responses of plants to soil sterilization (Moreira et al. 2019; Mahmood et al. 2014; CI et al. 2012). Soil sterilization can increase plant growth by removing pathogens and promoting rapid changes in microbial communities to reach a healthier rhizosphere microbiome (Li et al. 2019), increase nutrient availability from the decomposition of soil biota, and decrease microbial competitors for inorganic nutrients (Zhang et al. 2011). However, other studies have reported contradictory results (Lu et al. 2022; Ochieno 2022; Yu et al. 2019). Soil sterilization eliminates beneficial microbes, which removes healthy competition between plant-parasitic nematodes and impairs natural ecosystem services for pest suppression in banana plants (Ochieno 2022). It also downregulates the expression of related biosynthesis genes, decreasing plant photosynthesis and growth (Yu et al. 2019). Furthermore, the sterilization effect can modify soil physicochemical properties by increasing dissolved organic carbon (DOC) concentration *via* the decomposition of soil organic matter (Boyd 1971; Berns et al. 2008), decreasing soil pH owing to released organic acids (Skipper and Westermann 1973; Razavi and Lakzian 2007), causing P deficiency by killing of symbiotic mycorrhizae involved in P absorption (Wallace et al. 1973), and mobilizing As by altering its sorption behavior on soils (Dao et al. 1982; Razavi and Lakzian 2007). The

enhanced DOC can compete with As for adsorption sites on soils (Jackson et al. 2006; Fisher et al. 2015) and bind with As to form As-DOC complexes (Williams et al. 2011; Buschmann et al. 2006), leading to As mobilization into soil water.

2 Research Objectives and Thesis Structure

Interest in the remediation of As contamination of plants has been growing, and progressive attempts should be taken to immobilize available As in soils to limit As uptake by plants, followed by As translocation in their essential aerial tissues, to minimize the health hazards of As on animals and humans. Most published studies focused on inAs in soils (Patrick et al. 1991; Dobran and Zagury 2006; Yuan et al. 2020) or As in plants (Larios et al. 2012; Ruiz-Chancho et al. 2008; Punshon et al. 2017; Mir et al. 2007). Systemic research on the presence of As in soil water, soils, and crop plants is needed to evaluate their interrelationship and combined effects on soil health and plant health. Therefore, this interdisciplinary study was conducted to clarify the potential roles of soil indigenous microbes and maize plants in the uptake, translocation, speciation, and detoxification of As in the soil-maize system. In this work, a greenhouse pot experiment with undisturbed, disturbed, and reconditioned soil microbes × three different As treatments was conducted to investigate the interactions among soil microbes, plant growth, and As treatments on the concentrations and speciation of As in the soil-maize system. These specific scientific questions are aimed to be answered:

i) What are the effects of microbial disturbance, plant growth, and As treatments on the As concentration and speciation in soil water and soils?
ii) How do microbial disturbance and As treatments affect the concentration and speciation of As in maize tissues?
iii) What effects do microbial disturbance and As treatments in soils have on the health of maize plants?

This dissertation is divided into three main chapters to answer these questions: Arsenic in the soil environment (Chapter II), As in maize plants (Chapter III), and the health of maize plants (Chapter IV). A brief summary of each chapter is presented below:

Chapter II: Arsenic in the Soil Environment

This chapter focuses on the investigation of the effects of microbial disturbance, plant growth, and As treatments on As concentration and speciation in soil water and in soils. Soil water was sampled biweekly, and soils were sampled monthly during the entire growth period of maize plants. Arsenic concentrations and speciation were determined in all soil water and soil samples. Apart from As, the concentrations of multielement, major cations and anions, and DOC in soil water samples as well as soil pH were analyzed to investigate the influence of microbial disturbance, plant growth, and As treatments. To elucidate the uptake, translocation, speciation, and toxicity of As in the systemic soil-plant system, the next chapter continues the research on As in maize plants.

Chapter III: Arsenic in Maize Plants

In this chapter, the focus is on the effects of microbial disturbance and As treatments on As concentration and speciation in maize tissues. Arsenic concentration and speciation were investigated in four maize tissues: roots, stem, leaves, and grains. A correlation analysis was conducted between As in soil water and As in maize plants to determine if there was a correlation between them. Moreover, dry biomass is one of the most important indicators of plant growth and health, so the dry biomass of the four tissues was determined at the end of the experiment when we harvested them. To investigate the responsible As species for the reduction in dry biomass, a further correlation analysis was performed between the concentrations of As species in maize and its dry biomass. Since other parameters of plant health besides dry biomass can also be significantly influenced by As, further parameters of plant health are examined in the next chapter.

Chapter IV: Health of Maize Plants

This chapter focuses on the effects of microbial disturbance and As treatments on the health of maize plants. Plant height, fresh biomass, and leaf chlorophyll content were measured biweekly to assess their health status. BBCH-scale and leaf numbers per plant were measured

monthly due to their less frequent changes. The damage scale of leaf spot was recorded once in the middle of plant growth. Furthermore, a correlation analysis between As species in maize tissues and plant health parameters was conducted to determine which maize tissue was most affected by As and whether there were correlations between them.

3 Materials and Methods

3.1 Greenhouse Pot Experiment

The soil (silty loam) was sampled from the uppermost 20 cm of an agricultural site in Frauenkappelen, Switzerland by a company. The soil was then stored outside the greenhouse in the Institute of Plant Sciences at the University of Bern (Ostermundigen, Switzerland). For this greenhouse pot experiment, around 800 kg of soils were sampled from both sides of the soil pile for homogeneity and sieved to 10 mm. This experiment had a total of 18 different groups: three soil treatments (native soil (NS), reconditioned soil (RS), and disturbed soil (DS)) × two crop scenarios (No-plant and Plant) × three As treatments (As_0, As_{100}, and As_{200} mg As kg^{-1} dw). Three replicates of No-plant pots and ten replicates of Plant pots were established (Figure I-2). The As_0 group had a natural As concentration of 2.91 ± 0.54 mg kg^{-1} without the addition of As. For the As_{100} and As_{200} groups, around 510 kg of soils were spiked with sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$, ≥ 98.0%; Sigma-Aldrich®, CH) to enrich the soils with an additional 100 and 200 mg kg^{-1} As. The soils were incubated at room temperature for two months at 50% water holding capacity (WHC), allowing for As equilibration between soil water and soil phases (data not shown) and simulating aging (Song et al. 2006).

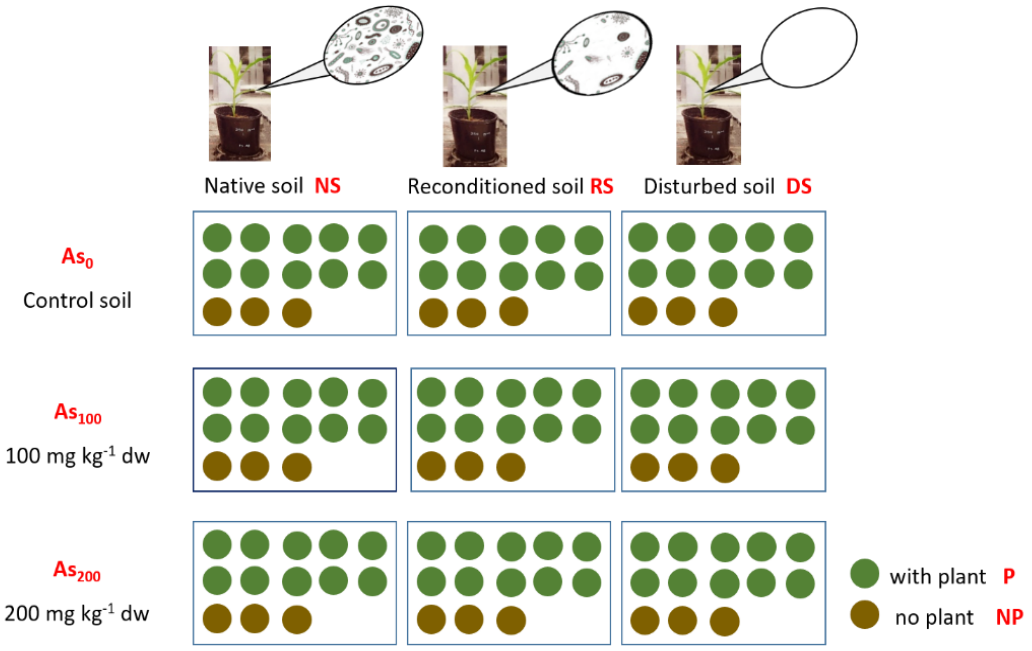


Figure I-2. Overview of the experimental design

Soils from the three As treatments were further subdivided into three subgroups for the three soil treatments (NS, RS, and DS). The first treatment was kept untreated and named as NS. The second and third segments were sterilized by X-ray (25 kGy minimum to 60 kGy maximum at Synergy Health Däniken AG, Switzerland). The second segment was reconditioned with microbial extracts from NS after microbial disturbance and designated as RS. The third segment was left without reconditioning and referred to as DS. The microbial extracts for the RS treatment were obtained by entirely mixing 70 kg of native soils with 70 L of Milli-Q water (> 18.2 MΩ·cm at 25°C) in a pre-sterilized concrete mixer (sterilized with ethanol and a gas burner) (Figure S1.1). The solutions were left to stand for 2 h and filtered through a 250 µm stainless sieve and 25 µm 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) and allowed 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). Due to the presence of microbes in the greenhouse, DS was not assumed to be free of microbes but to have a disturbed microbial composition.

Due to the presence of microbes in the greenhouse, DS was not assumed to be free of microbes but to have a disturbed microbial composition. The sterilization effect was the same in DS and RS, while the microbial disturbance effect was partly eliminated in the RS treatment due to the reconditioning of microbial extracts. Therefore, it is assumed that the difference between RS and DS showed the microbial disturbance effects, and the difference between NS and RS reflected the abiotic effects. The detailed characterizations of NS and DS can be found in Table S1.1. All soils were adequately homogenized and decanted into 117 pots. Each pot (7 L) was filled with 6.5 kg of soils and reached the same height to ensure 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 seeds (*Zea mays* L., W22 genotype) were sown one week after soil sterilization. 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, the 117 pots were randomly placed in the greenhouse. In the beginning, plants were watered weekly by weighing pots and adjusting the WHC to 60%. From the third month of growth, they were watered

more frequently. The weekly fertilization of all pots (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 supplement (Sequestrene Rapid, Maag®), increasing to 200 mL complex fertilizer with a 0.5 g of high iron supplement after one month. The complex fertilizer mainly contains 52% phosphate (P_2O_5), 10% total nitrogen (8.4% NH_3-N and 1.4% NO_3-N), and 10% potassium oxide (K_2O). The maize plants were cultivated in the greenhouse with 14 h of light each day and a temperature of 18 - 26°C during the day and 16 - 24°C at night. The greenhouse cabin is heated in case of temperatures below 18°C during the day and below 16°C at night. The cooling system automatically turns on if the temperature exceeds 26°C. The ventilation system turns on once the temperature is over 22°C in the daytime or over 20°C at night. The humidity ranged from 30 to 60%.

Additionally, a side experiment was conducted to estimate the fresh biomass of maize during growth while maintaining the same WHC in the soil by controlling the weight of the pots. In this experiment, 60 maize plants were grown for five months and three of them were harvested weekly to determine their fresh biomass. Plant images were simultaneously recorded to derive the green pixels area of plant leaves. Therefore, a linear model could be built between the calculated biomass and the leaf area to estimate the plant's actual fresh biomass (Figure S1.2) (Neumann et al. 2015; Valasek and Thomasson 2016). The estimated fresh biomass was then applied to calculate the amount of irrigation water and correct the weight of pots in order to retain a 50% WHC.

3.2 Preparation of Soil Water, Soil and Plant Samples and Analysis of Their As Concentration, Speciation and Other Parameters

Multielement was analyzed in soil water and plant samples, while As speciation analysis was conducted for soil water, soil, and plant samples. In soil water, pH, major ions and DOC were also determined. The soil 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 Figure S1.3). The tip of the sampler reached the pot center close to the rhizosphere. 30mL syringes were connected to the samplers and a low pressure was established by pulling out the syringe and fixing them in a position to suck soil water overnight. The soil water was sampled biweekly and divided into four sets of aliquots (Chapter II).

In the first set of aliquots, pH was measured using a WTW SenTix® Mic pH micro combination electrode (pH electrode; Xylem™, Rye Brook, NY). The second set of aliquots was analyzed by the Dionex™ Aquion™ Ion Chromatography System (IC; Thermo Fisher Scientific, Waltham, MA) for major cations and anions, 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 with the vario TOC cube (TOC analyzer; Elementar, Langenselbold, DE). To the last set of aliquots, it was added with 1% (v/v) of 14.65 M nitric acid (HNO_3; VWR®, Switzerland) and stored at 4°C before the multielement analysis by inductively coupled plasma mass spectrometry (ICP-MS; 7700x Agilent Technologies, Santa Clara, CA). Multielement analysis by ICP-MS included totAs or As, B, Al, V, Cr, Mn, Co, Ni, Cu, Zn, Ga, Se, Rb, Ag, Cd, Cs, Ba, Ti, Pb, and U. Triplicates of certified reference material (CRM) and blank were digested and measured together with the plant samples. The CRM ERM®- CD281 Rye grass and the Standard Reference Material® 1573a Tomato leaves were used in multielement analysis with certified As concentrations of 0.042 ± 0.010 mg kg^{-1} and 0.112 ± 0.004 mg kg^{-1}, respectively.

In the As speciation analysis of soil water (Chapter II), 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 maximum for one week at 4°C 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 the determined As species were all pentavalent. Arsenic species were separated into inorganic As species (inAs or As^V) and organic As species (orgAs, i.e., MMA^V, DMA^V and TMAO) using a Hamilton PRP-X100 anion-exchange column (4.1 × 50 mm, 5 µm). The operating parameters for HPLC are listed in Table S1.2 and adapted from the literature (Jackson 2015).

Bulk soils (3.6 g) were taken monthly from pot edges with a small auger to measure As speciation. The soils were air-dried at room temperature, sieved to 2 mm, and ground into powders by a Retsch MM400 Mixer Mill (Fisherbrand™, Waltham, MA). In As speciation analysis of soils (Chapter II), 0.2 g of ground soils were mixed with 4.8 mL of 1% (w/w) HNO_3 and 0.2 mL of 30% (w/w) Suprapur H_2O_2 (Sigma-Aldrich®, CH), and left for at least 30 min at room temperature before conducting open-vessel microwave digestion (Microwave Digestion System MARS™ 6; CEM GmbH, Kamp-Lintfort, DE) (Norton et al. 2013). The temperature program was as follows: ramp from room temperature to 50°C, hold at 50°C for

10 min, ramp again to 95°C, and hold at 95°C for 30 min. After extraction, soil samples were centrifuged at 2500 rpm for 5 min (Multifuge™ X1 Centrifuge Series, Thermo Scientific™, Reinach, CH), filtered with a 0.22 µm hydrophilic Polytetrafluoroethylene Filter (13mm syringe filter, BGB®, CH), diluted if needed, and stored at 4°C (less than one week) before the analysis with HPLC-ICP-MS. Information on the column and operating parameters were described above. The column recovery was 91 ± 15% (n = 28) for soil samples. Triplicates of CRMs and blanks were extracted and measured together with the soil samples. The CRM ERM®- BC211 Rice was utilized with a certified DMA^{V} concentration of 119 ± 13 µg kg^{-1} and a certified sum concentration of As^{III} and As^{V} of 124 ± 11 µg kg^{-1}.

After a half year of growth, plants were harvested individually as roots, stem, leaves, and cob. The root samples were carefully dug out from soils, washed with Milli-Q water, air-dried, and stored at room temperature. Grains were peeled from the cob to be determined their totAs concentration and As speciation. All plant material was oven-dried at 70°C and ground to powder in a Retsch MM400 Mixer Mill (Fisherbrand™, Waltham, MA). In the multielement analysis of plants (Chapter III), 0.25 g of ground plant powder was mixed with 4 mL of 65% (w/w) nitric acid (HNO_3; VWR®, FR) 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 the open-vessel microwave digestion described above. After digestion, the solutions were diluted to 50 mL with Milli-Q water and stored at 4°C and centrifuged at 2500 rpm for 5 min before ICP-MS analysis. The same multielement and the same CRM were digested together as written above. In the As speciation analysis of plants by HPLC-ICP-MS (Chapter III), the same extraction method as well as the same HPLC column and operating parameters were applied as described above. The column recovery was 96 ± 17% (n = 153) for plant samples. Triplicates of blanks and the same CRM ERM®- BC211 Rice were extracted and measured together with the plant samples.

3.3 Plant Health Parameters

The dry biomass of the four tissues was weighted after drying in the oven at 70°C to a constant weight. Plant height, fresh biomass, and leaf chlorophyll content were recorded biweekly, while BBCH-scale and leaf numbers per plant were measured monthly. Plant height was measured from the base to the tip of the plant with a carpenter's ruler. Leaf chlorophyll content was assessed by averaging three readings from three positions between the base

and the apex of a leaf with the Soil Plant Analysis Development chlorophyll meter (SPAD-502, Minolta Camera CO., LTD., Japan). BBCH-scale is a universal scale using a decimal code to describe the growth stages of most agricultural crops and weeds (Lancashire et al. 1991), where similar growth stages of plants were given the same code. The lower the code, the slower the development of the plant. Leaf numbers on the entire plant were counted visually. The damage scale of the leaf spot was also visually inspected once in the middle of the plant growth.

3.4 Statistical Analysis

All the statistical analysis were performed in R software (version 1.2.5033) including the following packages: car, multcomp, emmeans and vegan. The univariate analysis of variance (ANOVA) was applied to the concentrations of totAs and As species in soil water and As species in soils, investigating the interaction effects and individual effects of the four experimental factors (microbial disturbance, plants, As treatments, and time) (Chapter II). The ANOVA was also applied for the concentration of totAs, As species, and different plant health parameters in maize tissues, exploring the interaction and individual effects of the four experimental factors (microbial disturbance, As treatments, tissues, and time) (Chapter III). 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 response variables: inAs, MMA^V, DMA^V and TMAO), studying the interaction effects and individual effects of the four experimental factors on individual As species.

In all three chapters, the three-way or two-way ANOVA was performed to explore the interaction effects using the three-way or two-way comparisons among experimental factors (microbial disturbance, plants, and As treatments). The reported estimated marginal means (emmeans) were produced from the fitted models of the original data and used for the post-hoc analysis (R Package *emmeans*). 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. Moreover, all As data were Log10-transformed to improve normality and analyzed using linear mixed effects models. For the same reason, the square root transformation (Sqrt) was applied for the dry biomass

data. In addition, the conventional correlation analysis was performed to investigate the relation between the concentrations of individual As species in maize with all the plant health parameters (Chapter III & IV). As damage scale is a categorical variable, it was analyzed with Spearman rank correlation, while all other plant health parameters were analyzed with the Pearson correlation analysis. Since As may have caused a reduction in BBCH-scale and leaf numbers, the percentages of BBCH-scale loss (BBCH-scale/the highest BBCH-scale * 100) and leaf loss (leaf numbers/the highest leaf numbers * 100) were calculated for their correlation analyses. In addition, the partial correlation was applied for the pairwise correlation between each individual As species and the total dry biomass, aiming to find the responsible As species for the reduction in dry biomass (Chapter III).

4 General Results and Discussion

To better understand the interconnections between the three chapters, Figure I-3, Figure I-4, and Figure I-5 are presented to provide additional information on As in the soil-plant system, showing the consequence of As in soils on the concentration and speciation of As in soils, soil water, and plants as well as associated plant health effects.

4.1 Total As Concentrations in Soil Water and Maize

From NS to RS to DS, the soil microbes in these three soils were increasingly disturbed (Figure I-3). In RS and DS, after soil sterilization, they can rapidly recolonize and recruit a new microbial community with lower diversity (Mahmood et al. 2014; Marschner and Rumberger 2004). Due to the microbial reconditioning with some indigenous microbes in RS, its microbial disturbance was less than that of DS. We discovered that totAs and inAs concentrations in both soil water and maize tissues generally showed a concentration trend of NS < RS ≤ DS, indicating the presence of the microbial disturbance (difference RS-DS) and/or sterilization effects (difference NS-RS). While higher quantities of orgAs were occasionally seen in maize grown in RS than those in DS, likely due to the reconditioned soil indigenous microbes actively converting As from inorganic to organic forms (Ultra et al. 2007).

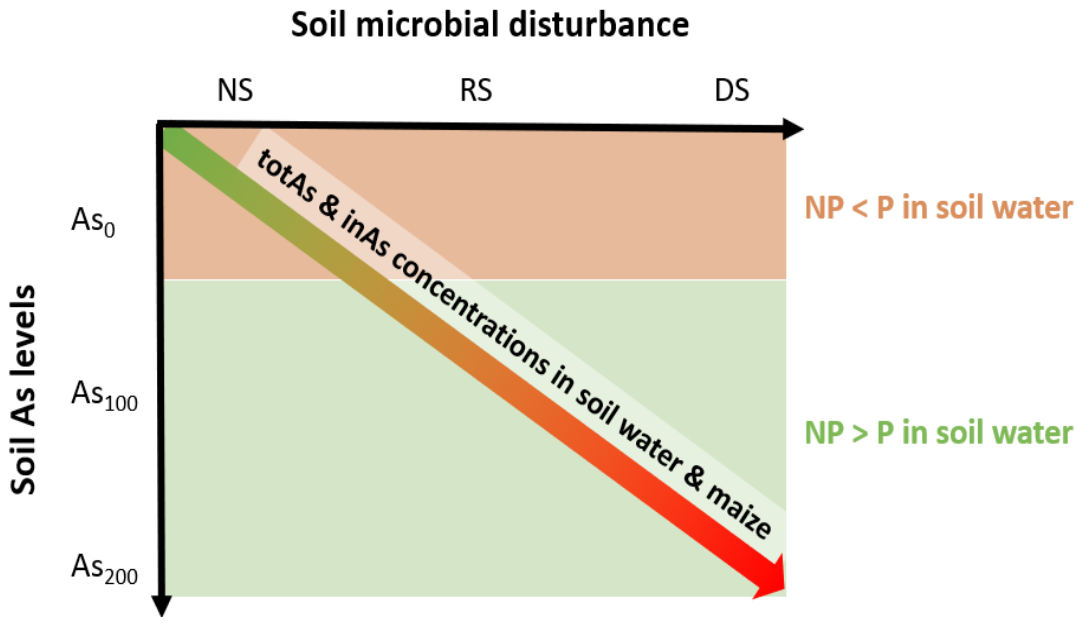


Figure I-3. Changes in the concentrations of total As (totAs) and inorganic As species (inAs) in soil water and maize plants under soil treatments (native soil (NS), reconditioned soil, and disturbed soil (DS)) and As treatments (As_0, As_{100}, and As_{200}, the addition of 0, 100, and 200 mg As kg^{-1} soil, respectively). No-plant > Plant means that As concentrations in the soil water were higher in No-plant pots than in Plant pots, and vice versa.

The microbial disturbance effect resulted in lower As concentrations in the soil water of RS than in DS, suggesting that the elimination or disturbance of soil indigenous microbes promoted As release into soil water. Concurrent findings indicate that the elimination of oxidizing bacteria by soil sterilization slows down iron oxidation, leading to insufficient sorptive sites for As and higher As leaching in sterilized soils (Kumpiene et al. 2007). In contrast to RS and DS, no As was released into NS soil water without soil sterilization, highlighting the role of soil indigenous microbes in immobilizing and regulating As in the soil environment. Some prokaryotic and eukaryotic microbes, for example, can excrete extracellular polymeric substances that possess abundant functional groups with biosorption and metal binding properties, immobilizing metal(loid) ions (Seshadri et al. 2015; Pal and Paul 2008). Beneficial microbes, such as auxin-producing bacteria, may have been killed by soil sterilization, disrupting their interactions with the host plant and negating their beneficial functions in promoting plant development and resistance to As stress (Li et al. 2019).

In contaminated soil water, As concentrations were always lower in No-plant than in Plant pots (Figure I-3). Although plants absorb As from soil water, this may not be the primary cause of the higher As levels in the soil water of No-plant pots, as the proportions of As taken up by plants (< 1.34%) were too low to adequately explain the concentration difference between No-plant and Plant pots. This contrasted with our findings in uncontaminated soils, where No-plant pots had lower As levels in their soil water than in Plant pots. Lower As levels in uncontaminated soil water of No-plant pots could be due to the competition between As and phosphate on soil adsorption sites (Lambkin and Alloway 2003), resulting in less As in soil water being taken up by plants and higher As levels in the soil water of Plant pots. In contaminated soils, however, larger As concentrations were found in soil water of No-plant than in Plant pots. The same results have been reported in a previous study showing that rice planting reduces the concentrations of As species in soil water by more than 30% than non-planting, because the presence of maize roots increases the copy numbers of the gene arsM, which is responsible for As methylation and volatilization from bacteria (Afroz et al. 2019). Based on this, we came up with two hypotheses to explain why As levels in contaminated soil water were higher in No-plant pots than in Plant pots. On one hand, plants can operate as a filter for their own microbiome following soil sterilization and reshape their rhizosphere microbes by helping microbes recover from sterilization (Li et al. 2019; Zhalnina et al. 2018; Reinhold-Hurek et al. 2015). Plants can, on the other hand, favor beneficial soil microbes by altering root exudations, which may aid them in coping with As stress (Broeckling et al. 2008). By changing the chemical composition of the rhizosphere, plants can establish various microhabitats and improve their adaptability to As stress (Zhalnina et al. 2018). With the help of soil microbes and their potential interactions with maize plants, they could lower As concentrations in soil water and As bioavailability to themselves as a survival mechanism in response to the As stressor.

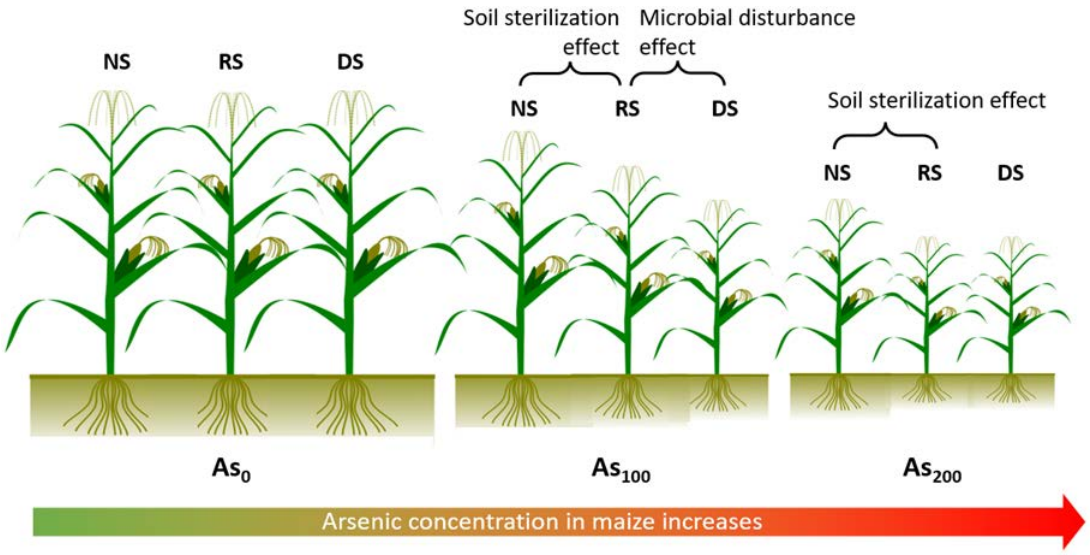


Figure I-4. The increase in As concentrations in maize tissues as well as the decrease of plant height with the increasing levels of As in soils (from As_0, As_{100} to As_{200} group) and of soil microbial disturbance (from NS, RS to DS)

In the current study, an increase in DOC concentration, a decrease in soil pH, and P deficiency were all detected as one of the sterilization effects. Meanwhile, P is known to usually reduce As uptake, mainly As^V, in plant and microbial systems as well as to interfere with As biotransformation by competing for As transporters (Wu et al. 2022). When maize plants are under P deficiency stress, it might affect their growth by either a decrease in photosynthesis or an increase in energy investment (Malhotra et al. 2018). This could explain why the maize plants grown in RS and DS were in poorer health than those grown in NS. The sterilization effect, for instance, may have caused P deficiency in maize of both the As_{100} and As_{200} groups (Figure I-4), resulting in a reduction in their plant height compared to the maize in NS. In the absence of As, the sterilization effect did not reduce dry biomass, plant height, and BBCH-scale. Plants can adapt to P deficiency in a variety ways, including morphological, physiological, and biochemical changes (Malhotra et al. 2018). However, our maize plants appeared to be resistant to P deficiency in uncontaminated soils, with no alterations in some of their morphological traits.

4.2 Arsenic Speciation in Soils, Soil Water, and Maize Tissues

Organic As species in plants are known to be originated from soil microbes and can be taken up by plants from soil water (Lomax et al. 2012). Due to the rapid translocation of orgAs in plants, their proportions increased from 2.8% in roots (Figure I-5a) to 35.6% in leaves (Figure I-5c) in NS. This is because that orgAs are more readily translocated to grains in crop plants via phloem than inAs, although roots absorb inAs much faster than orgAs (Li et al. 2009b; Carey et al. 2011; Awasthi et al. 2017). Furthermore, microbial disturbance and As treatments increased inAs concentrations in maize tissues while 5edecreasing the levels of orgAs. In maize roots, the proportion of orgAs was exceedingly low, with less than 1% in DS of the As_{200} group (Figure I-5d). In maize leaves, orgAs% decreased greatly to 10.5% in DS of the As_{200} group (Figure I-5b), due to the increase in soil As levels rather than microbial disturbance. The increase in soil microbial disturbance had no effect on orgAs% in maize plants, suggesting that the plant uptake of As was not affected by the microbial disturbance effect. In addition, the primary orgAs differed amongst maize tissues. The levels of DMA^V were usually higher than that of MMA^V and TMAO in maize roots. Trimethylarsine oxide was the primary species in maize stem and leaves but had the lowest proportion in grains. Due to their frequent presence in soils (Bissen and Frimmel 2000; Bowell 1994), most research has so far focused only on the uptake and translocation of MMA^V and DMA^V in soils and plants (Carey et al. 2011; Awasthi et al. 2017; Raab et al. 2007a). Similar research on TMAO is awaited, for instance, to explain why TMAO accumulates primarily in maize leaves rather than being translocated further into grains.

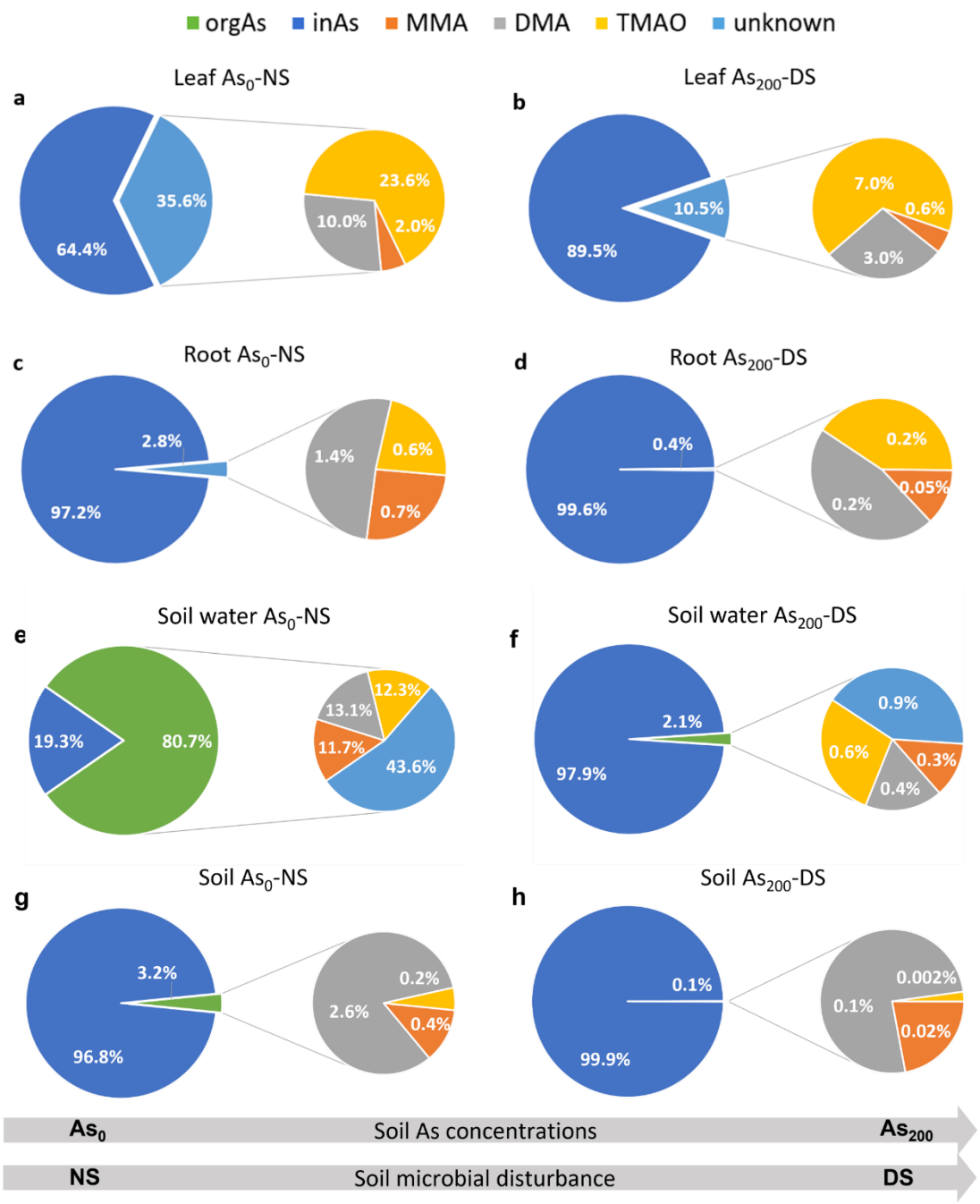


Figure I-5. The changes in As species with the increasing levels of soil As and the increased soil microbial disturbance from the soil to the soil water and to maize plants, presenting the percentages of inorganic As species (inAs%) and organic As species (orgAs%, i.e., MMA^V, DMA^V, and TMAO) in (a) maize leaves in native soil (NS) of the As_0 group; (b) maize leaves in disturbed soil (DS) of the As_{200} group; (c) maize roots in NS of the As_0 group; (d) maize roots in DS of the As_{200} group; (e) soil water of NS of the As_0 group; (f) soil water of DS of the As_{200} group; (g) NS of the As_0 group; and (h) DS of the As_{200} group.

Organic As species made up to 80.7% of the sum of all As species in uncontaminated soil water (Figure I-5e). This supported the prior research indicating nearly all microbes have the ability to undergo microbial methylation, i.e., convert inorganic As species to less-toxic organic forms and then to volatile As species (Upadhyay et al. 2018; Jia et al. 2013b). In contrast to As species in soil water, inAs predominated in our soils (> 96.8%) (Figure I-5g and 1-5h), which did not change with the increasing As levels or microbial disturbance in soils. In maize roots, the proportion of orgAs was exceedingly low, with less than 1% in the DS of the As_{200} group (Figure I-5d). Because of the dual influence or interactions of microbial disturbance and As treatments, orgAs% decreased greatly to 10.5% in leaves in the DS of the As_{200} group (Figure I-5b). This reduction in orgAs% in high-As soils might be because that the higher As levels in soil water inhibit microbial growth responsible for methylation of inAs in soils (Abedin et al. 2002). The levels of orgAs% were also decreased by both the micorbial disturbance and sterilization effects, which could be explained by the elimination and disturbance of soil indigenous microbes as well as the halt of enzyme and microbial activities (Blankinship et al. 2014; Xun et al. 2015; Gianfreda 2015). These disadvantages might have inhibited the roles of soil microbes in the As methylation process, lowering the synthesis of orgAs and lower proportions in RS and DS.

Organic As species were substantially less abundant in maize roots than in soil water, as seen in Figures I-5e and I-5c. This could be explained by the lower uptake of orgAs as compared to inAs. The uptake of orgAs in plants is known to be much lower than inAs (Rahman et al. 2007b; Raab et al. 2007a; Rahman et al. 2011). Arsenate enters the cell cytoplasm of plants *via* high-affinity phosphate transporters, while As^{III} is taken primarily *via* aquaglyceroporins e.g., water and glycerin or sugar permeases (Garbinski et al. 2019; Mitra et al. 2017). DMA^{V} and MMA^{V} can be taken up by rice roots at a slow rate *via* aquaglyceroporins (Abedin et al. 2002), with MMA^{V} uptake being slightly higher than DMA^{V} (Carbonell-Barrachina et al. 1998). InAs, on the other hand, have limited mobility in most plants (Finnegan and Chen 2012b), which may result in a high proportion of inAs accumulation in plant roots. The low uptake of orgAs by maize roots observed in our study could be explained by low root uptake of orgAs and primary accumulation of inAs in roots.

4.3 Answers to The Research Questions

More specifically, the three outlined research questions are answered as follows:

i) What are the effects of microbial disturbance, plant growth, and As treatments on As concentration and speciation in soil water and soils (Chapter II)?

Higher concentrations of totAs and As species were observed in sterilized soils (RS and DS) than in unsterilized soils (NS). This was due to both the microbial disturbance and sterilization effects that mobilized As in soil water. The observed microbial disturbance effect suggested that the elimination or disturbance of soil indigenous microbes is capable of immobilizing As in soils. While the sterilization effect increased DOC concentration and lowered soil pH and mobilized As into the soil water. The changes in DOC and pH were more pronounced in uncontaminated than in contaminated soil water, probably due to the overprinting effect of As. When the As level was high in soils, As treatments played a more important role in DOC and pH than microbial disturbance. The presence of maize plants mitigated both the microbial disturbance and sterilization effects, possibly assisting the recolonization of microbes following soil sterilization and favoring beneficial microbes in coping with As stress.

ii) How do microbial disturbance and As treatments affect the concentration and speciation of As in maize tissues (Chapter III)?

Higher levels of totAs and inAs were detected in maize grown in sterilized than unsterilized soils, following the same order of NS < RS ≤ DS as in soil water, as both the microbial disturbance and sterilization effects mobilized As in soil water. The microbial disturbance effect indicated that the disturbance or elimination of soil indigenous microbes resulted in higher As accumulation in maize grown in sterilized soils. The abiotic effect caused P deficiency in maize plants, resulting in less competition with As, enabling plants to absorb more As. The simultaneous presence of microbial disturbance and sterilization effects could exaggerate the adverse effects of As on plant health. Both effects were observed on the stem, leading to a stronger reduction of its dry biomass than in other tissues. Interestingly, the totAs

and inAs showed a concentration consequence of roots > leaves > stem > grains in uncontaminated soils, whereas in contaminated soils, less As could be translocated into the aerial maize tissues, with a different pattern of roots > stem ≥ leaves > grains.

iii) What effects do microbial disturbance and As treatment have on the health of maize plants (Chapter IV)?

At a soil background level of As, microbial disturbance had no effect on dry biomass, plant height, and BBCH-scale, implying that plants can buffer the effects of microbial disturbance. However, at high As concentrations, all plant health parameters were hampered by As, with symptoms being more prominent in maize grown in sterilized soils. Nevertheless, thanks to the undisturbed soil indigenous microbes in NS, even at a high As level, plant health parameters were not or only slightly retarded in maize grown in NS. Meanwhile, P and Mn deficiencies were induced in maize grown on high-As soils by the sterilization effect, which hindered plant growth and health, potentially leading to enhanced As accumulation in maize.

5 General Conclusion

Overall, the concentration of totAs and all determined As species in soil water followed a general concentration consequence of NS < RS ≤ DS, while totAs and inAs also showed the same consequence, but higher levels of orgAs were occasionally observed in maize grown in RS than in DS. Due to the microbial methylation by soil microbes, the highest proportion of orgAs was observed in soil water (65.8%), while decreased to no more than 2.8% in maize roots. This decrease in orgAs% could be attributed, on one hand, to the lower uptake of orgAs than inAs by roots and, on the other hand, to the high accumulation of inAs in roots due to their low translocation efficiency in plants. Plants can take up As from soil water and accumulate it primarily in their roots, with inAs being the predominant species (> 97.2%) and orgAs accounting for less than 2.8%. Since orgAs can be translocated more readily than inAs in maize plants, a higher level of orgAs% was observed in maize leaves (< 35.6%) than in roots (< 2.8%). In maize tissues, inAs% increased while orgAs% decreased as soil microbial disturbance increased caused by soil sterilization, probably due to a disruption in As methylation process resulting from the elimination and disturbance of soil indigenous microbes as well as a halt in microbial and enzyme activities. In addition, the sterilization effect influenced not only As concentrations and speciation in soil water and maize plants but also plant health. In the absence of As, both the microbial disturbance and sterilization effects had no effect on plant height, dry biomass, and BBCH-scale. When As was present in the soil environment, however, both the biotic and abiotic effects were observed in various plant health parameters. Because of their interactions, microbial disturbance exaggerated the harmful effects of As on plant health.

Through answering the three specific research questions, we found that plant-microbe interactions reduced the As concentrations in soil water, resulting in lower available As and less transfer into plants. Maize plants might have assisted soil microbes to recolonize after soil sterilization and soil microbes, in return, promote the growth and fitness of the host plant and improve its resistance to As stress. The interactions between maize plants and soil microbes could be interpreted as a self-defense strategy or a survival mechanism in response to the As stressor in the environment (Chapter II). With a potential mutual attempt with soil microbes, maize plants in high-As soils tend to limit the translocation of inAs to the essential upper tissues, leading to even higher As concentrations in the stem than in the leaves.

Conversely, in uncontaminated soils, inAs can be readily translocated into the leaves with higher concentrations than in the stem. The simultaneous presence of both the microbial disturbance and sterilization effects in the stem aggravated the adverse effects of As and resulted in a greater reduction in the stem dry biomass than in the other tissues (Chapter III). Maize plants can buffer both effects in the absence of As. However, when As is present, the interaction effects of microbial disturbance and As treatments can seriously impair plant growth and health. Nevertheless, the presence of soil indigenous microbes can ameliorate this impairment, resulting in less damage to plants grown in unsterilized soils and sterilized soils reconditioned with soil microbes (Chapter IV).

Reference

Abbas, Mohamed H. H.; Abdelhafez, Ahmed A. (2013): Role of EDTA in arsenic mobilization and its uptake by maize grown on an As-polluted soil. In *Chemosphere* 90 (2), pp. 588–594. DOI: 10.1016/j.chemosphere.2012.08.042.

AbdElgawad, Hamada; Zinta, Gaurav; Abuelsoud, Walid; Hassan, Yasser M.; Alkhalifah, Dalal Hussien M.; Hozzein, Wael N. et al. (2021): An actinomycete strain of Nocardiopsis lucentensis reduces arsenic toxicity in barley and maize. In *Journal of Hazardous Materials* 417, p. 126055. DOI: 10.1016/j.jhazmat.2021.126055.

Abedin, Mohammed Joinal; Feldmann, Jörg; Meharg, Andy A. (2002): Uptake kinetics of arsenic species in rice plants. In *Plant physiology* 128 (3), pp. 1120–1128. DOI: 10.1104/pp.010733.

Afroz, Hasina; Su, Shiming; Carey, Manus; Meharg, Andy A.; Meharg, Caroline (2019): Inhibition of Microbial Methylation via arsM in the Rhizosphere: Arsenic Speciation in the Soil to Plant Continuum. In *Environmental science & technology* 53 (7), pp. 3451–3463. DOI: 10.1021/acs.est.8b07008.

Ahemad, Munees (2019): Remediation of metalliferous soils through the heavy metal resistant plant growth promoting bacteria: Paradigms and prospects. In *Arabian Journal of Chemistry* 12 (7), pp. 1365–1377. DOI: 10.1016/j.arabjc.2014.11.020.

Alam, Mohammad Zahangeer; Hoque, Md Anamul; Ahammed, Golam Jalal; McGee, Rebecca; Carpenter-Boggs, Lynne (2019): Arsenic accumulation in lentil (Lens culinaris) genotypes and risk associated with the consumption of grains. In *Sci Rep* 9 (1), p. 9431. DOI: 10.1038/s41598-019-45855-z.

Anjum, Naser A.; Gill, Sarvajeet Singh; Tuteja, Narendra (2017): Enhancing Cleanup of Environmental Pollutants. Cham: Springer International Publishing.

Armienta, María Aurora; Beltrán, Margarita; Martínez, Sarayth; Labastida, Israel (2020): Heavy metal assimilation in maize (Zea mays L.) plants growing near mine tailings. In *Environmental geochemistry and health* 42 (8), pp. 2361–2375. DOI: 10.1007/s10653-019-00424-1.

Awasthi, Surabhi; Chauhan, Reshu; Srivastava, Sudhakar; Tripathi, Rudra D. (2017): The Journey of Arsenic from Soil to Grain in Rice. In *Frontiers in plant science* 8, p. 1007. DOI: 10.3389/fpls.2017.01007.

Basel Landschaft (2018): Geminde Liesberg: Arsen in Landwirtschaftsböden und Futterpflanzen.

Bauer, Wolfgang D.; Mathesius, Ulrike (2004): Plant responses to bacterial quorum sensing signals. In *Current opinion in plant biology* 7 (4), pp. 429–433. DOI: 10.1016/j.pbi.2004.05.008.

Berg, Gabriele (2009): Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. In *Applied microbiology and biotechnology* 84 (1), pp. 11–18. DOI: 10.1007/s00253-009-2092-7.

Berns; Philipp, H.; Narres, H.-D.; Burauel, P.; Vereecken, H.; Tappe, W. (2008): Effect of gamma-sterilization and autoclaving on soil organic matter structure as studied by solid state NMR, UV and fluorescence spectroscopy. In *Eur J Soil Science* 59 (3), pp. 540–550. DOI: 10.1111/j.1365-2389.2008.01016.x.

Bhattacharya, P.; MUKHERJEE, A.; JACKS, G.; NORDQVIST, S. (2002): Metal contamination at a wood preservation site: characterisation and experimental studies on remediation. In *Science of The Total Environment* 290 (1-3), pp. 165–180. DOI: 10.1016/S0048-9697(01)01073-7.

Bissen, M.; Frimmel, F. H. (2000): Speciation of As(III), As(V), MMA and DMA in contaminated soil extracts by HPLC-ICP/MS. In *Fresenius' journal of analytical chemistry* 367 (1), pp. 51–55. DOI: 10.1007/s002160051597.

Blankinship, Joseph C.; Becerra, Caryl A.; Schaeffer, Sean M.; Schimel, Joshua P. (2014): Separating cellular metabolism from exoenzyme activity in soil organic matter decomposition. In *Soil Biology and Biochemistry* 71, pp. 68–75. DOI: 10.1016/j.soilbio.2014.01.010.

Bowell, R. J. (1994): Sulphide oxidation and arsenic speciation in tropical soils. In *Environ Geochem Health* 16 (2), p. 84. DOI: 10.1007/BF00209833.

Boyd, H. W. (1971): Manganese toxicity to peanuts in autoclaved soil. In *Plant Soil* 35 (1-3), pp. 133–144. DOI: 10.1007/BF01372638.

Brinkel, Johanna; Khan, Mobarak H.; Kraemer, Alexander (2009): A systematic review of arsenic exposure and its social and mental health effects with special reference to Bangladesh. In *International journal of environmental research and public health* 6 (5), pp. 1609–1619. DOI: 10.3390/ijerph6051609.

Broeckling, Corey D.; Broz, Amanda K.; Bergelson, Joy; Manter, Daniel K.; Vivanco, Jorge M. (2008): Root exudates regulate soil fungal community composition and diversity. In *Applied and environmental microbiology* 74 (3), pp. 738–744. DOI: 10.1128/AEM.02188-07.

Buschmann, Johanna; Kappeler, Alexandra; Lindauer, Ursula; Kistler, David; Berg, Michael; Sigg, Laura (2006): Arsenite and arsenate binding to dissolved humic acids: influence of pH, type of humic acid, and aluminum. In *Environmental science & technology* 40 (19), pp. 6015–6020. DOI: 10.1021/es061057.

Cao, Xiaoxia; Bai, Lingyu; Zeng, Xibai; Zhang, Junzheng; Wang, Yanan; Wu, Cuixia; Su, Shiming (2019): Is maize suitable for substitution planting in arsenic-contaminated farmlands? In *Plant Soil Environ.* 65 (No. 9), pp. 425–434. DOI: 10.17221/155/2019-PSE.

Carbonell-Barrachina, A. A.; Aarabi, M. A.; DeLaune, R. D.; Gambrell, R. P.; Patrick Jr., W. H. (1998): The influence of arsenic chemical form and concentration on Spartina patens and Spartina alterniflora growth and tissue arsenic concentration. In *Plant Soil* 198 (1), pp. 33–43. DOI: 10.1023/A:1004285625998.

Carey, Anne-Marie; Norton, Gareth J.; Deacon, Claire; Scheckel, Kirk G.; Lombi, Enzo; Punshon, Tracy et al. (2011): Phloem transport of arsenic species from flag leaf to grain during grain filling. In *New Phytologist* 192 (1), pp. 87–98. DOI: 10.1111/j.1469-8137.2011.03789.x.

Chen, Jian; Rosen, Barry P. (2020): The Arsenic Methylation Cycle: How Microbial Communities Adapted Methylarsenicals for Use as Weapons in the Continuing War for Dominance. In *Front. Environ. Sci.* 8. DOI: 10.3389/fenvs.2020.00043.

CI, Xiao-ke; LIU, Hua-lin; HAO, Yu-bo; ZHANG, Ji-wang; LIU, Peng; DONG, Shu-ting (2012): Arsenic Distribution, Species, and Its Effect on Maize Growth Treated with Arsenate. In *Journal of Integrative Agriculture* 11 (3), pp. 416–423. DOI: 10.1016/S2095-3119(12)60026-4.

Colombo, Claudio; Palumbo, Giuseppe; He, Ji-Zheng; Pinton, Roberto; Cesco, Stefano (2014): Review on iron availability in soil: interaction of Fe minerals, plants, and microbes. In *J Soils Sediments* 14 (3), pp. 538–548. DOI: 10.1007/s11368-013-0814-z.

Danouche, Mohammed; El Ghachtouli, Naïma; El Arroussi, Hicham (2021): Phycoremediation mechanisms of heavy metals using living green microalgae: physicochemical and molecular approaches for enhancing selectivity and removal capacity. In *Heliyon* 7 (7), e07609. DOI: 10.1016/j.heliyon.2021.e07609.

Dao, T. H.; Marx, D. B.; Lavy, T. L.; Dragun, J. (1982): Effect, and Statistical Evaluation, of Soil Sterilization on Aniline and Diuron Adsorption Isotherms. In *Soil Science Society of America Journal* 46 (5), pp. 963–969. DOI: 10.2136/sssaj1982.03615995004600050016x.

Del Molina, María Carmen; White, James F.; García-Salgado, Sara; Quijano, M. Ángeles; González-Benítez, Natalia (2020): A Gnotobiotic Model to Examine Plant and Microbiome Contributions to Survival under Arsenic Stress. In *Microorganisms* 9 (1). DOI: 10.3390/microorganisms9010045.

Di, Xuerong; Beesley, Luke; Zhang, Zulin; Zhi, Suli; Jia, Yan; Ding, Yongzhen (2019): Microbial Arsenic Methylation in Soil and Uptake and Metabolism of Methylated Arsenic in Plants: A Review. In *International journal of environmental research and public health* 16 (24). DOI: 10.3390/ijerph16245012.

Diels, L.; Spaans, P. H.; van Roy, S.; Hooyberghs, L.; Ryngaert, A.; Wouters, H. et al. (2003): Heavy metals removal by sand filters inoculated with metal sorbing and precipitating bacteria. In *Hydrometallurgy* 71 (1-2), pp. 235–241. DOI: 10.1016/S0304-386X(03)00161-0.

Fellet, G.; Marchiol, L.; Perosa, D.; Zerbi, G. (2007): The application of phytoremediation technology in a soil contaminated by pyrite cinders. In *Ecological Engineering* 31 (3), pp. 207–214. DOI: 10.1016/j.ecoleng.2007.06.011.

Finnegan, Patrick M.; Chen, Weihua (2012): Arsenic toxicity: the effects on plant metabolism. In *Frontiers in physiology* 3, p. 182. DOI: 10.3389/fphys.2012.00182.

Fisher, Daniel J.; Yonkos, Lance T.; Staver, Kenneth W. (2015): Environmental concerns of roxarsone in broiler poultry feed and litter in Maryland, USA. In *Environmental science & technology* 49 (4), pp. 1999–2012. DOI: 10.1021/es504520w.

Flora, Swaran J. S. (2011): Arsenic-induced oxidative stress and its reversibility. In *Free radical biology & medicine* 51 (2), pp. 257–281. DOI: 10.1016/j.freeradbiomed.2011.04.008.

Garbinski, Luis D.; Rosen, Barry P.; Chen, Jian (2019): Pathways of arsenic uptake and efflux. In *Environment International* 126, pp. 585–597. DOI: 10.1016/j.envint.2019.02.058.

Garg, Neera; Singla, Priyanka (2011): Arsenic toxicity in crop plants: physiological effects and tolerance mechanisms. In *Environ Chem Lett* 9 (3), pp. 303–321. DOI: 10.1007/s10311-011-0313-7.

Ge, Yufeng; Pandey, Piyush; Bai, Geng (2016): Estimating fresh biomass of maize plants from their RGB images in greenhouse phenotyping. In John Valasek, J. Alex Thomasson (Eds.): Autonomous Air and Ground Sensing Systems for Agricultural Optimization and Phenotyping. SPIE Commercial + Scientific Sensing and Imaging. Baltimore, Maryland, United States, Sunday 17 April 2016: SPIE (SPIE Proceedings), p. 986605.

Gianfreda, L. (2015): Enzymes of importance to rhizosphere processes. In *J. Soil Sci. Plant Nutr.* (ahead), p. 0. DOI: 10.4067/S0718-95162015005000022.

Gulz, Petra Angela; Gupta, Satish-Kumar; Schulin, Rainer (2005): Arsenic accumulation of common plants from contaminated soils. In *Plant Soil* 272 (1-2), pp. 337–347. DOI: 10.1007/s11104-004-5960-z.

Han, Fengxiang X.; Su, Yi; Monts, David L.; Plodinec, M. John; Banin, Amos; Triplett, Glover E. (2003): Assessment of global industrial-age anthropogenic arsenic contamination. In *Die Naturwissenschaften* 90 (9), pp. 395–401. DOI: 10.1007/s00114-003-0451-2.

Hong, Young-Seoub; Song, Ki-Hoon; Chung, Jin-Yong (2014): Health effects of chronic arsenic exposure. In *Journal of preventive medicine and public health = Yebang Uihakhoe chi* 47 (5), pp. 245–252. DOI: 10.3961/jpmph.14.035.

Hu, Lingfei; Robert, Christelle A. M.; Cadot, Selma; Zhang, Xi; Ye, Meng; Li, Beibei et al. (2018): Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. In *Nature communications* 9 (1), p. 2738. DOI: 10.1038/s41467-018-05122-7.

Hug, Stephan J.; Winkel, Lenny H.E.; Voegelin, Andreas; Berg, Michael; Johnson, C. Annette (2020): Arsenic and Other Geogenic Contaminants in Groundwater – A Global Challenge. In *Chimia* 74 (7-8), pp. 524–537. DOI: 10.3929/ethz-b-000431950.

IITA (2009): Maize. Available online at https://www.iita.org/cropsnew/maize/, updated on 5/13/2021, checked on 9/29/2021.

Jacks; Frisbie; Smith; Naidu; Sarkar (2002): Arsenic in the environment: a global perspective. New York: Marcel Dekker.

Jackson, B. P.; Seaman, J. C.; Bertsch, P. M. (2006): Fate of arsenic compounds in poultry litter upon land application. In *Chemosphere* 65 (11), pp. 2028–2034. DOI: 10.1016/j.chemosphere.2006.06.065.

Jackson, Brian (2015): Fast ion chromatography-ICP-QQQ for arsenic speciation. In *Journal of analytical atomic spectrometry* 30 (6), pp. 1405–1407. DOI: 10.1039/C5JA00049A.

Jia, Yan; Huang, Hai; Zhong, Min; Wang, Feng-Hua; Zhang, Li-Mei; Zhu, Yong-Guan (2013a): Microbial arsenic methylation in soil and rice rhizosphere. In *Environmental science & technology* 47 (7), pp. 3141–3148. DOI: 10.1021/es303649v.

Jia, Yan; Sun, Guo-Xin; Huang, Hai; Zhu, Yong-Guan (2013b): Biogas slurry application elevated arsenic accumulation in rice plant through increased arsenic release and methylation in paddy soil. In *Plant Soil* 365 (1-2), pp. 387–396. DOI: 10.1007/s11104-012-1391-4.

Jing, Ran; Kjellerup, Birthe V. (2018): Biogeochemical cycling of metals impacting by microbial mobilization and immobilization. In *Journal of Environmental Sciences* 66, pp. 146–154. DOI: 10.1016/j.jes.2017.04.035.

Kavita, B.; Shukla, Sapna; Naresh Kumar, G.; Archana, G. (2008): Amelioration of phytotoxic effects of Cd on mung bean seedlings by gluconic acid secreting rhizobacterium Enterobacter asburiae PSI3 and implication of role of organic acid. In *World J Microbiol Biotechnol* 24 (12), pp. 2965–2972. DOI: 10.1007/s11274-008-9838-8.

Khairul, Islam; Wang, Qianqian; Jiang, Yuhan; Chao, Wang; Naranmandura, Hua (2017): Metabolism, toxicity and anticancer activities of arsenic compounds 8, pp. 23905–23926.

Ko, Myoung-Soo; Park, Hyun-Sung; Lee, Jong-Un (2017): Influence of indigenous bacteria stimulation on arsenic immobilization in field study. In *CATENA* 148, pp. 46–51. DOI: 10.1016/j.catena.2016.08.022.

Kuivenhoven; Mason (2021): Arsenic Toxicity. In Matthew Kuivenhoven, Kelly Mason (Eds.): StatPearls [Internet]: StatPearls Publishing. Available online at https://www.ncbi.nlm.nih.gov/books/NBK541125/.

Kumpiene, Jurate; Castillo Montesinos, Isaac; Lagerkvist, Anders; Maurice, Christian (2007): Evaluation of the critical factors controlling stability of chromium, copper, arsenic and zinc in iron-treated soil. In *Chemosphere* 67 (2), pp. 410–417. DOI: 10.1016/j.chemosphere.2006.08.031.

Lambkin, D. C.; Alloway, B. J. (2003): Arsenate-induced Phosphate Release from Soils and its Effect on Plant Phosphorus. In *Water Air Soil Pollut* 144 (1/4), pp. 41–56. DOI: 10.1023/A:1022949015848.

Lancashire, Peter D.; H. Bleiholder; T. van den Boom; P. Langelüddeke; R. Stauss; Elfriede Weber; and A. Witzenberger (1991): A uniform decimal code for growth stages of crops and weeds. In *Ann Applied Biology* 119 (3), pp. 561–601. DOI: 10.1111/j.1744-7348.1991.tb04895.x.

Larios, Raquel; Fernández-Martínez, Rodolfo; Lehecho, Isabelle; Rucandio, Isabel (2012): A methodological approach to evaluate arsenic speciation and bioaccumulation in different plant species from two highly polluted mining areas. In *The Science of the total environment* 414, pp. 600–607. DOI: 10.1016/j.scitotenv.2011.09.051.

Li, Kun; DiLegge, Michael J.; Minas, Ioannis S.; Hamm, Alison; Manter, Daniel; Vivanco, Jorge M. (2019): Soil sterilization leads to re-colonization of a healthier rhizosphere microbiome. In *Rhizosphere* 12, p. 100176. DOI: 10.1016/j.rhisph.2019.100176.

Li, Ren-Ying; Ago, Yukiko; Liu, Wen-Ju; Mitani, Namiki; Feldmann, Jörg; McGrath, Steve P. et al. (2009): The rice aquaporin Lsi1 mediates uptake of methylated arsenic species. In *Plant Physiol* 150 (4), pp. 2071–2080. DOI: 10.1104/pp.109.140350.

Lomax, Charlotte; Liu, Wen-Ju; Wu, Liyou; Xue, Kai; Xiong, Jinbo; Zhou, Jizhong et al. (2012): Methylated arsenic species in plants originate from soil microorganisms. In *New Phytologist* 193 (3), pp. 665–672. DOI: 10.1111/j.1469-8137.2011.03956.x.

López-Bucio, José; Nieto-Jacobo, María Fernanda; Ramírez-Rodríguez, Verenice; Herrera-Estrella, Luis (2000): Organic acid metabolism in plants: from adaptive physiology to transgenic varieties for cultivation in extreme soils. In *Plant Science* 160 (1), pp. 1–13. DOI: 10.1016/S0168-9452(00)00347-2.

Lu, Yuqiu; Wang, Enzhao; Tang, Zhiyu; Rui, Junpeng; Li, Yanling; Tang, Zhixi et al. (2022): Roots and microbiome jointly drive the distributions of 17 phytohormones in the plant soil continuum in a phytohormone‐specific manner. In *Plant Soil* 470 (1-2), pp. 153–165. DOI: 10.1007/s11104-021-04898-w.

Magdziak, Z.; Kozlowska, M.; Kaczmarek, Z.; Mleczek, M.; Chadzinikolau, T.; Drzewiecka, K.; Golinski, P. (2011): Influence of Ca/Mg ratio on phytoextraction properties of Salix viminalis. II. Secretion of low molecular weight organic acids to the rhizosphere. In *Ecotoxicology and environmental safety* 74 (1), pp. 33–40. DOI: 10.1016/j.ecoenv.2010.09.003.

Mahmood, T.; Mehnaz, S.; Fleischmann, F.; Ali, R.; Hashmi, Z. H.; Iqbal, Z. (2014): Soil sterilization effects on root growth and formation of rhizosheaths in wheat seedlings. In *Pedobiologia* 57 (3), pp. 123–130. DOI: 10.1016/j.pedobi.2013.12.005.

Maji, Chinmoy; Sarkar, Samar; Biswas, Suman; Patra, Pabitra Hriday; Datta, Bakul Kumar; Bandyopadhyay, Samiran et al. (2016): Experimental assessment of arsenic toxicity in garole sheep in India. In *Emerging Contaminants* 2 (3), pp. 128–134. DOI: 10.1016/j.emcon.2016.04.002.

Malhotra, Hina; Vandana; Sharma, Sandeep; Pandey, Renu (2018): Phosphorus Nutrition: Plant Growth in Response to Deficiency and Excess. In : Plant Nutrients and Abiotic Stress Tolerance: Springer, Singapore, pp. 171–190. Available online at https://link.springer.com/chapter/10.1007/978-981-10-9044-8_7.

Marschner, Petra; Rumberger, Angelika (2004): Rapid changes in the rhizosphere bacterial community structure during re-colonization of sterilized soil. In *Biol Fertil Soils* 40 (1), pp. 1–6. DOI: 10.1007/s00374-004-0736-4.

Matschullat, Jörg (2000): Arsenic in the geosphere — a review. In *Science of The Total Environment* 249 (1-3), pp. 297–312. DOI: 10.1016/S0048-9697(99)00524-0.

Mir, Kalam A.; Rutter, Allison; Koch, Iris; Smith, Paula; Reimer, Ken J.; Poland, John S. (2007): Extraction and speciation of arsenic in plants grown on arsenic contaminated soils. In *Talanta* 72 (4), pp. 1507–1518. DOI: 10.1016/j.talanta.2007.01.068.

Mitra, Anindita; Chatterjee, Soumya; Gupta, Dharmendra K. (2017): Uptake, Transport, and Remediation of Arsenic by Algae and Higher Plants. In Dharmendra Kumar Gupta, Soumya Chatterjee (Eds.): Arsenic contamination in the environment. The issues and solutions / Dharmendra Kumar Gupta, Soumya Chatterjee, editors. Cham: Springer, pp. 145–169.

Moreira, Helena; Pereira, Sofia I.A.; Marques, Ana P.G.C.; Rangel, António O.S.S.; Castro, Paula M.L. (2019): Effects of soil sterilization and metal spiking in plant growth promoting rhizobacteria selection for phytotechnology purposes. In *Geoderma* 334, pp. 72–81. DOI: 10.1016/j.geoderma.2018.07.025.

Natasha; Bibi, Irshad; Hussain, Khalid; Amen, Rabia; Hasan, Israr Masood Ul; Shahid, Muhammad et al. (2021): The potential of microbes and sulfate in reducing arsenic phytoaccumulation by maize (Zea mays L.) plants. In *Environmental geochemistry and health*. DOI: 10.1007/s10653-021-00902-5.

Neidhardt, H.; Norra, S.; Tang, X.; Guo, H.; Stüben, D. (2012): Impact of irrigation with high arsenic burdened groundwater on the soil-plant system: Results from a case study in the Inner Mongolia, China. In *Environmental pollution (Barking, Essex : 1987)* 163, pp. 8–13. DOI: 10.1016/j.envpol.2011.12.033.

Neumann, Kerstin; Klukas, Christian; Friedel, Swetlana; Rischbeck, Pablo; Chen, Dijun; Entzian, Alexander et al. (2015): Dissecting spatiotemporal biomass accumulation in barley under different water regimes using high-throughput image analysis. In *Plant, cell & environment* 38 (10), pp. 1980–1996. DOI: 10.1111/pce.12516.

Norton, Gareth; Deacon, Claire; Mestrot, Adrien; Feldmann, Joerg; Jenkins, Paul; Baskaran, Christina; Meharg, Andrew A. (2013): Arsenic speciation and localization in horticultural produce grown in a historically impacted mining region. In *Environmental science & technology* 47 (12), pp. 6164–6172. DOI: 10.1021/es400720r.

Ochieno, Dennis M. W. (2022): Soil Sterilization Eliminates Beneficial Microbes That Provide Natural Pest Suppression Ecosystem Services Against Radopholus similis and Fusarium Oxysporum V5w2 in the Endosphere and Rhizosphere of Tissue Culture Banana Plants. In *Front. Sustain. Food Syst.* 6. DOI: 10.3389/fsufs.2022.688194.

Ortíz-Castro, Randy; Contreras-Cornejo, Hexon Angel; Macías-Rodríguez, Lourdes; López-Bucio, José (2009): The role of microbial signals in plant growth and development. In *Plant signaling & behavior* 4 (8), pp. 701–712. DOI: 10.4161/psb.4.8.9047.

Pal, Arundhati; Paul, A. K. (2008): Microbial extracellular polymeric substances: central elements in heavy metal bioremediation. In *Indian J Microbiol* 48 (1), pp. 49–64. DOI: 10.1007/s12088-008-0006-5.

Patra, Manomita; Bhowmik, Niladri; Bandopadhyay, Bulbul; Sharma, Archana (2004): Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. In *Environmental and Experimental Botany* 52 (3), pp. 199–223. DOI: 10.1016/j.envexpbot.2004.02.009.

Pickering, I. J.; Prince, R. C.; George, M. J.; Smith, R. D.; George, G. N.; Salt, D. E. (2000): Reduction and coordination of arsenic in Indian mustard. In *Plant Physiol* 122 (4), pp. 1171–1177. DOI: 10.1104/pp.122.4.1171.

Prabpai, S.; Charerntanyarak, L.; Siri, B.; Moore, M. R.; Noller, Barry N. (2009): Effects of residues from municipal solid waste landfill on corn yield and heavy metal content. In *Waste management (New York, N.Y.)* 29 (8), pp. 2316–2320. DOI: 10.1016/j.wasman.2009.02.009.

Prasad, M. N. V.; Sajwan, Kenneth S.; Naidu, R. (2006): Trace elements in the environment. Biogeochemistry, biotechnology, and bioremediation. Boca Raton: CRC/Taylor and Francis.

Punshon, Tracy; Jackson, Brian P.; Meharg, Andrew A.; Warczack, Todd; Scheckel, Kirk; Guerinot, Mary Lou (2017): Understanding arsenic dynamics in agronomic systems to predict and prevent uptake by crop plants. In *The Science of the total environment* 581-582, pp. 209–220. DOI: 10.1016/j.scitotenv.2016.12.111.

Raab; Williams, Paul N.; Meharg, Andrew; Feldmann, Jörg (2007): Uptake and translocation of inorganic and methylated arsenic species by plants. In *Environ. Chem.* 4 (3), p. 197. DOI: 10.1071/EN06079.

Rahman, M. A.; Hasegawa, H.; Ueda, K.; Maki, T.; Okumura, C. (2007): Arsenic adsorption on iron plaque and uptake in duckweed (Spirodela polyrhiza L.) affected by chemical species. With assistance of S. K. Starrett, J. Hong, R. J. Wilcock, Q. Li, J. H. Carson, S. Arnold: American Science Press. Available online at https://opus.lib.uts.edu.au/handle/10453/14253.

Rahman, M. Azizur; Kadohashi, K.; Maki, T.; Hasegawa, H. (2011): Transport of DMAA and MMAA into rice (Oryza sativa L.) roots. In *Environmental and Experimental Botany* 72 (1), pp. 41–46. DOI: 10.1016/j.envexpbot.2010.02.004.

Razavi; Lakzian (2007): Evaluation of chemical and biological consequences of soil sterilization methods 5, pp. 87–91.

Reinhold-Hurek, Barbara; Bünger, Wiebke; Burbano, Claudia Sofía; Sabale, Mugdha; Hurek, Thomas (2015): Roots shaping their microbiome: global hotspots for microbial activity. In *Annual review of phytopathology* 53, pp. 403–424. DOI: 10.1146/annurev-phyto-082712-102342.

Rengel, Z.; Marschner, P. (2005): Nutrient availability and management in the rhizosphere: exploiting genotypic differences. In *The New phytologist* 168 (2), pp. 305–312. DOI: 10.1111/j.1469-8137.2005.01558.x.

Rosas, I.; Belmont, R.; Armienta, A.; Baez, A. (1999): Arsenic concentrations in water, soil, milk and forage in Comarca Lagunera, Mexico. In *Water Air Soil Pollut* 112 (1/2), pp. 133–149. DOI: 10.1023/A:1005095900193.

Rosas-Castor, J. M.; Guzmán-Mar, J. L.; Hernández-Ramírez, A.; Garza-González, M. T.; Hinojosa-Reyes, L. (2014): Arsenic accumulation in maize crop (Zea mays): a review. In *The Science of the total environment* 488-489, pp. 176–187. DOI: 10.1016/j.scitotenv.2014.04.075.

Roychowdhury, Rajib; Khan, Md. Hussain; Choudhury, Shuvasish (2018): Arsenic in Rice: An Overview on Stress Implications, Tolerance and Mitigation Strategies. In : Plants Under Metal and Metalloid Stress: Springer, Singapore, pp. 401–415. Available online at https://link.springer.com/chapter/10.1007/978-981-13-2242-6_15.

Ruiz-Chancho, Maria José; López-Sánchez, José Fermín; Schmeisser, Ernst; Goessler, Walter; Francesconi, Kevin A.; Rubio, R. (2008): Arsenic speciation in plants growing in arsenic-contaminated sites. In *Chemosphere* 71 (8), pp. 1522–1530. DOI: 10.1016/j.chemosphere.2007.11.054.

Selim, Samy; AbdElgawad, Hamada; Alsharari, Salam S.; Atif, Muhammad; Warrad, Mona; Hagagy, Nashwa et al. (2021): Soil enrichment with actinomycete mitigates the toxicity of arsenic oxide nanoparticles on wheat and maize growth and metabolism. In *Physiologia Plantarum*. DOI: 10.1111/ppl.13496.

Seshadri, B.; Bolan, N. S.; Naidu, R. (2015): Rhizosphere-induced heavy metal(loid) transformation in relation to bioavailability and remediation 15, pp. 524–548.

Shaji, E.; Santosh, M.; Sarath, K. V.; Prakash, Pranav; Deepchand, V.; Divya, B. V. (2021): Arsenic contamination of groundwater: A global synopsis with focus on the Indian Peninsula. In *Geoscience Frontiers* 12 (3), p. 101079. DOI: 10.1016/j.gsf.2020.08.015.

Shakoor, Muhammad Bilal; Niazi, Nabeel Khan; Bibi, Irshad; Rahman, Mohammad Mahmudur; Naidu, Ravi; Dong, Zhaomin et al. (2015): Unraveling Health Risk and Speciation of Arsenic from Groundwater in Rural Areas of Punjab, Pakistan. In *IJERPH* 12 (10), pp. 12371–12390. DOI: 10.3390/ijerph121012371.

Skipper, Horace D.; Westermann, Dale T. (1973): Comparative effects of propylene oxide, sodium azide, and autoclaving on selected soil properties. In *Soil Biology and Biochemistry* 5 (4), pp. 409–414. DOI: 10.1016/0038-0717(73)90067-9.

Song, Jing; Zhao, Fang-Jie; McGrath, Steve P.; Luo, Yong-Ming (2006): Influence of soil properties and aging on arsenic phytotoxicity. In *Environmental toxicology and chemistry* 25 (6), pp. 1663–1670. DOI: 10.1897/05-480r2.1.

Tu, Cong; Ma, Lena Q. (2002): Effects of Arsenic Concentrations and Forms on Arsenic Uptake by the Hyperaccumulator Ladder Brake. In *J. environ. qual.* 31 (2), pp. 641–647. DOI: 10.2134/jeq2002.6410.

Turpeinen, Riina (2002): Interactions between metals, microbes-bioremediation of As and Pb contaminated soils. Available online at https://helda.helsinki.fi/bitstream/handle/10138/22027/interact.pdf?sequence=2.

Ultra, Venecio U. Y.; Tanaka, Sota; Sakurai, Katsutoshi; Iwasaki, Kōzō (2007): Arbuscular mycorrhizal fungus (Glomus aggregatum) influences biotransformation of arsenic in the rhizosphere of sunflower (Helianthus annuus L.). In *Soil Science and Plant Nutrition* 53 (4), pp. 499–508. DOI: 10.1111/j.1747-0765.2007.00143.x.

Upadhyay, Munish K.; Yadav, Poonam; Shukla, Anurakti; Srivastava, Sudhakar (2018): Utilizing the Potential of Microorganisms for Managing Arsenic Contamination: A Feasible and Sustainable Approach. In *Front. Environ. Sci.* 6. DOI: 10.3389/fenvs.2018.00024.

Valasek, John; Thomasson, J. Alex (Eds.) (2016): Autonomous Air and Ground Sensing Systems for Agricultural Optimization and Phenotyping. SPIE Commercial + Scientific Sensing and Imaging. Baltimore, Maryland, United States, Sunday 17 April 2016: SPIE (SPIE Proceedings).

Wallace, A.; Frolich, E. F.; Alexander, G. V. (1973): Effect of steam sterilization of soil on two desert plant species. In *Plant Soil* 39 (2), pp. 453–456. DOI: 10.1007/BF00014815.

Wang, Suiling; Mulligan, Catherine N. (2006): Occurrence of arsenic contamination in Canada: sources, behavior and distribution. In *Science of The Total Environment* 366 (2-3), pp. 701–721. DOI: 10.1016/j.scitotenv.2005.09.005.

Wang, Ya; Wang, Shu; Xu, Pingping; Liu, Cong; Liu, Misha; Wang, Yulan et al. (2015): Review of arsenic speciation, toxicity and metabolism in microalgae. In *Rev Environ Sci Biotechnol* 14 (3), pp. 427–451. DOI: 10.1007/s11157-015-9371-9.

Whipps, J. M. (2001): Microbial interactions and biocontrol in the rhizosphere. In *Journal of experimental botany* 52 (Spec Issue), pp. 487–511. DOI: 10.1093/jexbot/52.suppl_1.487.

WHO (2011): Guidelines for drinking-water quality.

WHO (2018): Arsenic Primer: guidance on the investigations & mitigation of arsenic contamination.

Williams, Paul N.; Zhang, Hao; Davison, William; Meharg, Andrew A.; Hossain, Mahmud; Norton, Gareth J. et al. (2011): Organic matter-solid phase interactions are critical for predicting arsenic release and plant uptake in Bangladesh paddy soils. In *Environmental science & technology* 45 (14), pp. 6080–6087. DOI: 10.1021/es2003765.

Wu, Jingwen; Liang, Jieliang; Björn, Lars Olof; Li, Jintian; Shu, Wensheng; Wang, Yutao (2022): Phosphorus-arsenic interaction in the 'soil-plant-microbe' system and its influence on arsenic pollution. In *The Science of the total environment* 802, p. 149796. DOI: 10.1016/j.scitotenv.2021.149796.

Xun, Weibing; Huang, Ting; Zhao, Jun; Ran, Wei; Wang, Boren; Shen, Qirong; Zhang, Ruifu (2015): Environmental conditions rather than microbial inoculum composition determine the bacterial composition, microbial biomass and enzymatic activity of reconstructed soil microbial communities. In *Soil Biology and Biochemistry* 90, pp. 10–18. DOI: 10.1016/j.soilbio.2015.07.018.

Yu, Meng; Xie, Wei; Zhang, Xin; Zhang, Shubin; Wang, Youshan; Hao, Zhipeng; Chen, Baodong (2019): Arbuscular Mycorrhizal Fungi Can Compensate for the Loss of Indigenous Microbial Communities to Support the Growth of Liquorice (Glycyrrhiza uralensis Fisch.). In *Plants* 9 (1), p. 7. DOI: 10.3390/plants9010007.

Yu, Yang; Zhang, Shuzhen; Huang, Honglin; Luo, Lei; Wen, Bei (2009): Arsenic accumulation and speciation in maize as affected by inoculation with arbuscular mycorrhizal fungus Glomus mosseae. In *Journal of agricultural and food chemistry* 57 (9), pp. 3695–3701. DOI: 10.1021/jf900107y.

Yuan, Jun; Zhao, Jun; Wen, Tao; Zhao, Mengli; Li, Rong; Goossens, Pim et al. (2018): Root exudates drive the soil-borne legacy of aboveground pathogen infection. In *Microbiome* 6 (1), p. 156. DOI: 10.1186/s40168-018-0537-x.

Zemanová, Veronika; Pavlíková, Daniela; Hnilička, František; Pavlík, Milan (2021): Arsenic Toxicity-Induced Physiological and Metabolic Changes in the Shoots of Pteris cretica and Spinacia oleracea. In *Plants (Basel, Switzerland)* 10 (10). DOI: 10.3390/plants10102009.

Zhalnina, Kateryna; Louie, Katherine B.; Hao, Zhao; Mansoori, Nasim; Da Rocha, Ulisses Nunes; Shi, Shengjing et al. (2018): Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. In *Nature microbiology* 3 (4), pp. 470–480. DOI: 10.1038/s41564-018-0129-3.

Zhang, Gui-Yun; Zhang, Li-Ping; Wei, Ming-Feng; Liu, Zhen; Fan, Qiao-Lan; Shen, Qi-Rong; Xu, Guo-Hua (2011): Effect of arbuscular mycorrhizal fungi, organic fertilizer and soil sterilization on maize growth. In *Acta Ecologica Sinica* 31 (4), pp. 192–196. DOI: 10.1016/j.chnaes.2011.04.005.

Zheng, Yan; Ayotte, Joseph D. (2015): At the crossroads: Hazard assessment and reduction of health risks from arsenic in private well waters of the northeastern United States and Atlantic Canada. In *The Science of the total environment* 505, pp. 1237–1247. DOI: 10.1016/j.scitotenv.2014.10.089.

Chapter II Maize plants and soil microbes interact to reduce arsenic concentrations and influence arsenic speciation in the soil water

Hang Guan[†], Veronica Caggìa[‡], Andrea Gómez-Chamorro[¶], Daniela Fischer[†], Miquel Coll-Crespí[†], Xiaowen Liu[¤], Teresa Chávez-Capilla[†], Klaus Schlaeppi[‡§], Alban Ramette[¶], Adrien Mestrot[†] and Moritz Bigalke[†*]

[†] Institute of Geography, University of Bern, Hallerstrasse 12, CH-3012 Bern, Switzerland

[‡] Institute of Plant Sciences, University of Bern, Altenbergrain 21, CH-3013 Bern, Switzerland

[¶] Institute for Infectious Diseases, University of Bern, Friedbühlstrasse 51, CH-3001 Bern, Switzerland

¤ Center of Deep Sea Research, Institute of Oceanology, Center for Ocean Mega-Science, Chinese Academy of Sciences, CN-266071 Qingdao, China

§ Department of Environmental Sciences, University of Basel, CH-4056 Basel, Switzerland

* Institute of Applied Geoscience, Technical University Darmstadt, Schnittspahnstrasse 9, D-64287 Darmstadt, Germany

This is a preprint version of the manuscript to be submitted for publication.

Abstract

Arsenic (As) in soils can harm soil organisms and plants, and it enters the human food chain *via* the dietary consumption of crops. Measures of total As concentration are of limited usability because the mobility, bioavailability and toxicity of As are determined by its speciation. But little is known about how microbes and plants interact to change As speciation in the soil-maize system. Therefore, we performed a greenhouse pot experiment with maize plants to study the interactions of soil microbes, plants and As treatments on total concentration and speciation of As in the soil water and the soils. The experiment had three soil treatments: native soil (NS), reconditioned soil (RS, sterilized soils and reconditioned with native soil microbes), and disturbed soil (DS, sterilized soils before planting). Because soil sterilization may casue both biotic and abiotic changes, DS and RS treatments were introduced to differentiate between the biotic (microbial disturbance) and abiotic (soil sterilization) effects. The three soil treatments were intersected without maize (No-plant) and with maize (Plant) at three As treatments (uncontaminated soils (As_0) and contaminated soils (As_{100} and As_{200}, addition of 100 and 200 mg As kg^{-1} soil) in a full factorial design. Due to both microbial disturbance and sterilization effects, As was more mobile in the soil water of DS and RS than of NS with the increasing concentration trend of NS < RS ≤ DS. The observed microbial disturbance effect (difference between RS and DS) indicated the roles of soil microbes in the amount of As released into soil water. The microbial disturbance effect was more pronounced for organic As species than for inorganic species, implying a more prominent influence from the soil microbes involved in As methylation. Meanwhile, the microbial disturbance effect (difference between NS and RS) induced an increase in dissolved organic carbon (DOC) and a decrease in soil pH. The induced changes in DOC and pH were more pronounced in uncontaminated than in contaminated soil water, as indicated by a stronger correlation between the concentrations of DOC and organic As species in uncontaminated soil water. In addition, the microbial disturbance effect was observed only in the No-plant pots and the sterilization effect was more evident in the No-plant than in the Plant pots, indicating that both microbial disturbance and sterilization effects were mitigated by plants. We hypothesize that maize presumably directly reduced As levels in soil water *via* border cells while also indirectly helping soil microbes to recover from microbial disturbance by soil sterilization, such that maize plants and soil microbes interacted to minimize As concentrations in soil water for self-protections.

1 Introduction

Arsenic (As) is a toxic metalloid that can cause health problems in humans upon long-term exposure through drinking water and food (John Parascandola 2017; Mandal 2017; Shankar et al. 2014). Natural As concentrations in soils usually range from 1 to 40 mg kg^{-1} with an average of 5 mg kg^{-1} (Toxicological Profile for Arsenic 2007). These concentrations can increase up to 20,000 mg kg^{-1} in soils subjected to the anthropogenic As sources from industrial and mining activities (Smith et al. 1998). When crops are grown in As-contaminated soils, As can enter the human food chain through livestock feed or direct consumption, where the As concentration and speciation in soil water are deterministic for its transfer from soils to crops (Prabpai et al. 2009; Rosas et al. 1999).

Total As concentration has only limited usability, because its speciation controls the mobility, bioavailability, distribution and toxicity of As in the food chain (Garcia-Manyes et al. 2002). The two dominant inorganic As species (inAs) in soils are arsenate (As^{V}) and arsenite (As^{III}). Arsenate represents the vast majority (70 - 99%) of inAs and is approximately 2-10 times less toxic than As^{III} (Hong et al. 2014). Inorganic As species can be converted in biota to less-toxic organic As species (orgAs), such as methylarsonic acid (MMA^{V}), dimethylarsinic acid (DMA^{V}), and trimethylarsine oxide (TMAO) (Kuivenhoven and Mason 2021). Although MMA^{V} and DMA^{V} are the most abundant orgAs in the soil environment (Huang et al. 2011), they occur only in small quantities compared to inAs (Pongratz 1998; Dobran and Zagury 2006; Sadee et al. 2016; Garcia-Manyes 2002; Tlustoš et al. 2002). TMAO is detected only in a few cases and in minor concentrations in soil water (Cattani et al. 2015; Geiszinger et al. 2002).

Higher plants appear to lack the ability to methylate As (Zheng et al. 2013; Jia et al. 2013a), and instead take up orgAs produced by soil microbes in soil water (Lomax et al. 2012). Thus, soil microbes play a key role in As bioavailability for plants (Zhao et al. 2013; Turpeinen 2002; Kuivenhoven and Mason 2021). Soil microbes can remediate heavy metal toxicity in the rhizosphere, as they can facilitate the crystallization and precipitation of heavy metals (Ahemad 2019; Diels et al. 2003). Nearly all 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 (Upadhyay et al. 2018; Jia et al. 2013b). Arbuscular mycorrhizal fungi, rhizospheric bacteria, fungi, and algae can mitigate As stress in soils through bioaccumulation and biotransformation (Upadhyay et al. 2018; Rahimzadeh and Pirzad 2017). Microalgae can not only adsorb As on their surface, they also extract toxic As species from soil water, converting them into less-toxic species such as arsenosugars and storeing them in the cell (Danouche et al. 2021; Wang et al. 2015).

Arsenic concentrations in the rhizosphere are not only affected by soil microbes, can also be influenced by plants. The interactions between plants and soil microbes determine their responses to As contamination (Del Molina et al. 2020). Plant roots can convert As^{V} to As^{III} in soils, which is the first step in the major As detoxification pathway in plants (As methylation) (Turpeinen 2002; 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 (As^{III}–$(SR)_3$), effectively limiting As absorption into the aerial tissues of mesquite plants (Hammond et al. 2018). Plants can further modify their root environment by increasing root surface area and releasing organic acids in root exudations to enhance nutrient availability (Colombo et al. 2014; Prasad et al. 2006; Rengel and Marschner 2005). The release of such root exudates can consequently apply a selective pressure to soil microbes, so that interactions between plants and microbes ultimately determines As speciation and therefore its toxicity for soils (Hinsinger et al. 2009). Simple carbon sources, such as photosynthesis-derived carbon deposited to the rhizosphere, plants feed their soil microbes (Zhalnina et al. 2018; Sasse et al. 2018). When plants encounter environmental stress, such as exposure to heavy metals, they can excrete chemicals through roots to reduce metal toxicity. These responses include changes in soil pH and redox potential, the release of anions, and nutrient acquisition by roots that can enhance microbial activity (Seshadri et al. 2015). Until now most As studies focused only on the plant compartment (Larios et al. 2012; Ruiz-Chancho et al. 2008; Punshon et al. 2017; Mir et al. 2007) or on inAs in soils (Patrick et al. 1991; Dobran and Zagury 2006; Yuan et al. 2020). However, the interactions and relative contributions of plant and microbial mechanisms in As speciation in soil and soil water remain largely unknown.

To study the microbe-based effects on As speciation, we sterilized our experimental soils using X-ray. Although the primary consequence of soil sterilization is the elimination of soil indigenous microbes (Blankinship et al. 2014), it also changes abiotic factors. After soil

sterilization, microbes were shown to rapidly acclimate and recolonize the rhizosphere, resulting in a new microbial community with lower diversity (Mahmood et al. 2014; Marschner and Rumberger 2004; Li et al. 2019; Hinsinger et al. 2009). Abiotically, soil sterilization accelerated the decomposition of soil organic matter and increased DOC concentrations 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 (Williams et al. 2011; Buschmann et al. 2006), leading to As mobilization into soil water. A decrease in soil pH due to the dissolution of organic acids is also soil sterilization effect (Razavi and Lakzian 2007).

Because of this concomitant biotic and abiotic changes of soil sterilization, we included a further treatment in our study to allow to disentangle microbe-based effects on As speciation: we applied a soil microbial extract on the disturbed soils, which was independent of abiotic changes casued by soil sterilization. With this greenhouse pot experiment we set out to clarify the main knowledge gap on As speciation in the soil environment of a soil-plant system: 1) What are the microbial disturbance effect on the concentration and speciation of As in soil water and in soils at different As levels? 2) What are the soil sterilization effect on the concentration and speciation of As in soil water and in soils at different As levels? 3) How do plants influence the concentration and speciation of As in soil water and in soils with varying As levels? And 4) How the interactions between soil microbes and plants change with varying As levels in soils?

2 Materials and Methods

The soil (silty loam) was taken from the uppermost 20 cm of an agricultural site in Frauenkappelen, Switzerland. The soil pile was then stored outside the greenhouse in the Institute of Plant Sciences at the University of Bern (Ostermundigen, Switzerland). For this greenhouse pot experiment, around 800 kg of soils were sampled from both sides of the soil pile to reach homogeneity and sieved to 1 cm. This experiment had in total of 18 different groups: three soil treatments (native soil (NS), reconditioned soil (RS), and disturbed soil (DS)) × two crop scenarios (with no plant (No-plant) and with plant (Plant)) × three As treatments (As_0, As_{100}, and As_{200}, addition of 0, 100, and 200 mg As kg^{-1} soil). Three replicates in No-plant pots and ten replicates in Plant pots were established (Figure II-1). The soils in the As_0 group have a naturally occurring concentration of 2.91 ± 0.54 mg kg^{-1} without the addition of As. For As_{100} and As_{200} groups, around 510 kg of soils were spiked with sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$, ≥ 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), allowing for As equilibration between soil water and soil phases (data not shown) and simulating soil aging (Song et al. 2006).

Afterwards, soils in the three As treatments were further subdivided into three subgroups for the three soil treatments (NS, RS, and DS). The first subgroup was kept untreated and named as NS. The second and third segments were sterilized by X-ray (25 kGy minimum to 60 kGy maximum at Synergy Health Däniken AG, Switzerland). The second segment was reconditioned with microbial extracts from NS after soil sterilization and designated as RS. The third part was referred to as DS without microbial reconditioning. The microbial extracts for the RS treatment were obtained by entirely mixing 70 kg of native soils with 70 L of Milli-Q water (> 18.2 MΩ·cm at 25°C) in a pre-sterilized concrete mixer (sterilized with ethanol and a gas burner) (Figure S1.1). The solutions were left to stand for 2 h and filtered through a 250 µm stainless sieve and 25 µm 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) and allowed 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 S1.1. Due to the presence of microbes in the greenhouse, DS was not assumed to be free of microbes but rather to have a disturbed microbial composition.

The sterilization effect was the same between DS and RS, while the microbial disturbance by soil sterilization was partly eliminated in the RS treatment due to the reconditioning of 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 sterilization effect. All soils were adequately homogenized and decanted into 117 pots. Each pot (7 L) was filled with 6.5 kg of soils and reached the same height to ensure an 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.

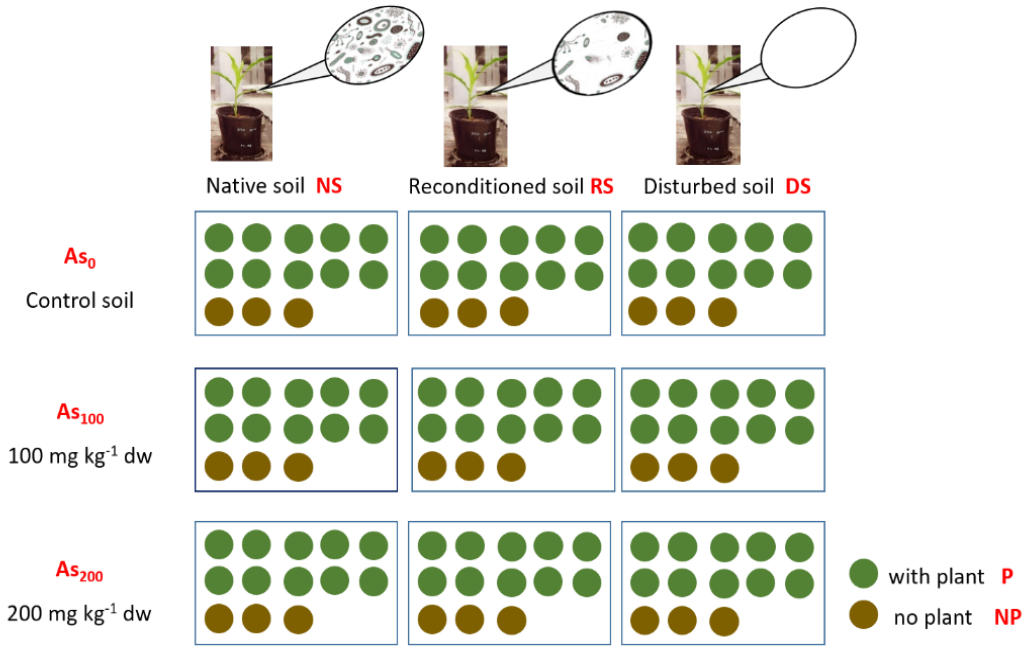


Figure II-1. Overview of the experimental design

Maize seeds (*Zea mays* L., W22 genotype) were were soaked for 6 minutes in a commercial bleach solution followed by 6 washes and an 8-hour soak in autoclaved MilliQ-water (> 18.2 MΩ·cm at 25°C). Before sowing, one week after soil sterilization, seeds were placed overnight in plastic Petri plates with moist filter papers. 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, the 117 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, plants were 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 contains 52% phosphate (P_2O_5), 10% total nitrogen (8.4% NH_3-N and 1.4% NO_3-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 the pots. In this experiment, 60 maize plants were grown for five months and three of them were harvested weekly to determine their fresh biomass. Plant images were simultaneously recorded to derive the green pixels area of plant leaves. Therefore, a linear model could be built between the calculated biomass and the leaf area to estimate the plant's actual fresh biomass (Figure S1.2) (Neumann et al. 2015; Valasek and Thomasson 2016). The estimated fresh biomass was then applied to calculate the amount of irrigation water and correct pot weights to retain a 50% WHC.

The soil 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 Figure S1.3). The tip of the sampler reached the center of the pot close to the rhizosphere. 30mL syringes were connected to the samplers and fixed with a wooden stick to suck the soil water overnight at 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 concentration 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; 7700x Agilent Technologies, Santa Clara, CA). The multielement analysis by ICP-MS concluded As, 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 one 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). The operating parameters for HPLC are listed in Table S1.2 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 (Fisherbrand™, Waltham, MA). Afterwards, 0.2 g of ground soil powder was mixed with 4.8 mL of 1% (w/w) HNO_3 (VWR®, FR) 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 (Microwave Digestion System MARS™ 6; CEM GmbH, Kamp-Lintfort, DE) (Norton et al. 2013). The temperature ramp program was as follows: ramp from room temperature to 50°C, hold at 50°C for 10 min, ramp again to 95°C, and hold at 95°C for 30 min. After extraction, the solutions were centrifuged at 2500 rpm for 5 min, filtered with a 0.22 µm hydrophilic Polytetrafluoroethylene Filter (13mm 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 ± 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 has a certified sum concentration of As^{III} and As^V of 124 ± 11 µg kg^{-1} and a certified DMA^V concentration of 119 ± 13 µg kg^{-1}. The percentage recoveries of acid extraction for inAs and DMA^V in CRMs were 70 ± 8% (n = 12) and 100 ± 3% (n = 12), respectively.

All the statistical analysis were 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 S2.2) and in soils (Table S2.5) were Log10-transformed to improve normality and analyzed using linear mixed effects models. The experimental factors were soil sterilization (three levels: NS, DS, RS), plants (two levels: No-plant and Plant), As treatments (three levels: As_0, As_{100}, As_{200}), and time as well as their

interactions. The interactions stand for the combined effects of the experimental factors on the response variable, e.g. totAs concentration in soil water. 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 response variables), the multivariate analysis of variance (MANOVA) was applied to the comparison of multivariate sample means in soil water and in soils, studying the interaction effects and individual effects of the four experimental factors on individual As species (Table S2.3). The original data, emmeans, are listed in the supplementary document (Table S2.2 and S2.5).

3 Results

3.1 Total As and Inorganic As Species in Soil Water

Overall, we found significant interactions among the three experimental factors (microbial disturbance, plants and As treatments) in six out of eight cases, due to our intersectional experimental design (details see Table S2.1, S2.3, and S2.4). The interactions among microbial disturbance, plants, and As treatments significantly affected total As (totAs) concentration in soil water ($F_{4, 587}$ = 6.506, p < 0.001) (Table S2.1). Regarding the effects of individual experimental factors, microbial disturbance increased totAs concentration in soil water ($F_{2, 587}$ = 105.286, p < 0.001). As the microbial disturbance increased, the totAs concentration in soil water increased following the pattern NS < RS ≤ DS (Figure S2.1 and Table S2.2). The microbial disturbance effect can be observed between RS and DS, which resulted in higher totAs concentration in the soil water of DS than of RS in No-plant pots of As_0 group (Figure II-2a). While the sterilization effect can be observed by the difference between NS and RS, resulting in higher totAs concentrations in the soil water of RS than of NS in As_0 group and in No-plant pots of As_{100} group. Moreover, plants decreased totAs concentration in soil water ($F_{1, 587}$ = 3.97, p = 0.047). In uncontaminated soils (As_0 group), totAs concentration in soil water was lower in No-plant than in Plant pots. Conversely, in contaminated soils (As_{100} and As_{200} group), totAs concentration was consistently higher in No-plant than in Plant pots. The totAs concentration in the soil water of RS and DS decreased in the beginning two months in all three As groups ($F_{11, 587}$ = 67.4, p < 0.001) (Figure S2.1). In contrast, As concentrations in NS were temporarily stable without microbial disturbance.

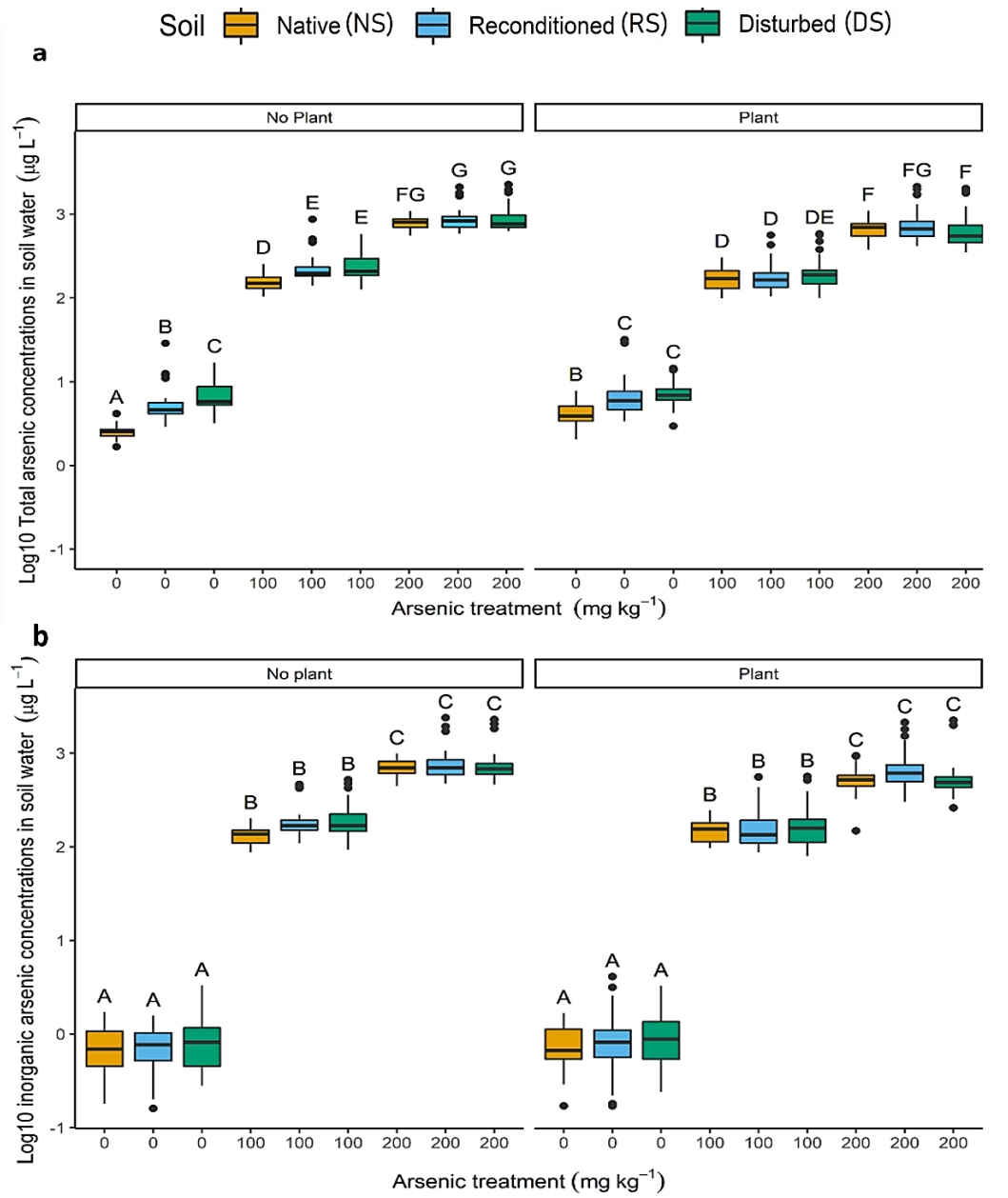


Figure II-2. The concentration of (a) total As (totAs) and (b) inorganic As species (inAs) in soil water. Data are the estimated marginal means (emmeans) ± standard error. Pairwise comparisons were explored and reported using CLD letters. Different letters indicated a statistically significant difference between emmeans ($p < 0.05$).

We also investigated the effects of microbial disturbance, plants and As treatments on the inAs concentration in soil water. In uncontaminated soils, No-plant and Plant pots had a similar range of inAs concentrations in soil water, whereas inAs concentration in contaminated soils was always lower in the presence of plants (this effect of plants was marginally significant and was not consistent in the pairwise comparisons as shown in Figure II-2b). We also noticed that inAs concentration in soil water changed over time ($F_{11, 545} = 8.170$,

$p < 0.001$). Microbial disturbance mobilized inAs into the soil water of contaminated RS and DS. This concentration increased in the first two months of the experiment and then decreased over time, while inAs in the soil water of NS remained stable over time (Figure S2.2). Collectively, totAs and inAs concentrations were decreased by the presence of plants in contaminated soil water.

3.2 Organic As Species in Soil Water

The sum of orgAs was also performed the same statistical analyses to investigate its responses to microbial disturbance, plants and As treatments. The sum of orgAs represented the sum of the six individual orgAs (MMA^V, DMA^V, TMAO, and three unknown species) detected in soil water. Microbial disturbance increased orgAs concentration in soil water ($F_{2, 545} = 87.929$, $p < 0.001$). The microbial disturbance effect (difference RS-DS) caused higher orgAs concentration in the soil water of RS than of DS in No-plant pots (Figure II-3a), whereas NS had the lowest orgAs concentration in the soil water of both No-plant and Plant pots. Meanwhile, the sterilization effect (difference NS-RS) was shown in both No-plant and Plant pots, where orgAs concentrations were higher in the soil water of RS than of NS. Interestingly, the presence of plants decreased orgAs concentration in soil water (Plant ≤ No-plant) ($F_{1, 545} = 7.432$, $p = 0.007$). Furthermore, the orgAs concentration in soil water decreased over time only in contaminated soil water, while it remained stable in uncontaminated soil water (Figure S2.3).

We were also interested in the changes in the percentage of orgAs (orgAs%), because it showed variations in the As methylation process. Microbial disturbance increased orgAs% in soil water ($F_{2, 545} = 47.777$, $p < 0.001$) (Figure II-3b). The microbial disturbance effect resulted in higher orgAs% in the soil water of RS than in DS in No-plant pots of the As_{200} group. In contaminated soils, RS soil water had higher concentrations than that of NS due to the sterilization effect. Moreover, orgAs% in soil water decreased with the increasing As levels in soils. It ranged from 26.8% to 91.7% in the As_0 group, was lower in the As_{100} group (0.12% - 31.3%), and was lowest in the As_{200} group (0.10% - 8.67%).

In addition, we also examined the effects of microbial disturbance, plants and As treatments on the three individual orgAs (MMA^V, DMA^V, and TMAO) in soil water. The interactions

between microbial disturbance and As or between As and plants significantly affected the concentrations of inAs, DMA^V, and TMAO ($p < 0.001$), but not of MMA^V (Table S2.1). Only MMA^V concentration was affected by the interactions between microbial disturbance and plants ($p < 0.001$). This implies that the interactions between microbial disturbance and plants played a role in the first step of As methylation process in soil water (inAs -> MMA -> DMA -> TMAO). Moreover, this reaction pathway was followed by the abundance of our orgAs, showing a concentration trend of $MMA^V < DMA^V <$ TMAO in soil water. Furthermore, the concentrations of MMA^V, DMA^V, and TMAO in soil water were lowest in NS (Figure S2.4), and they displayed a general concentration pattern of NS < RS ≤ DS. The microbial disturbance effect was not observed in Plant pots for individual orgAs, suggesting that the microbial disturbance effect was probably mitigated by the presence of plants. Whereas the sterilization effect was significant in both No-plant and Plant pots of TMAO as well as in No-plant pots of MMA^V and DMA^V.

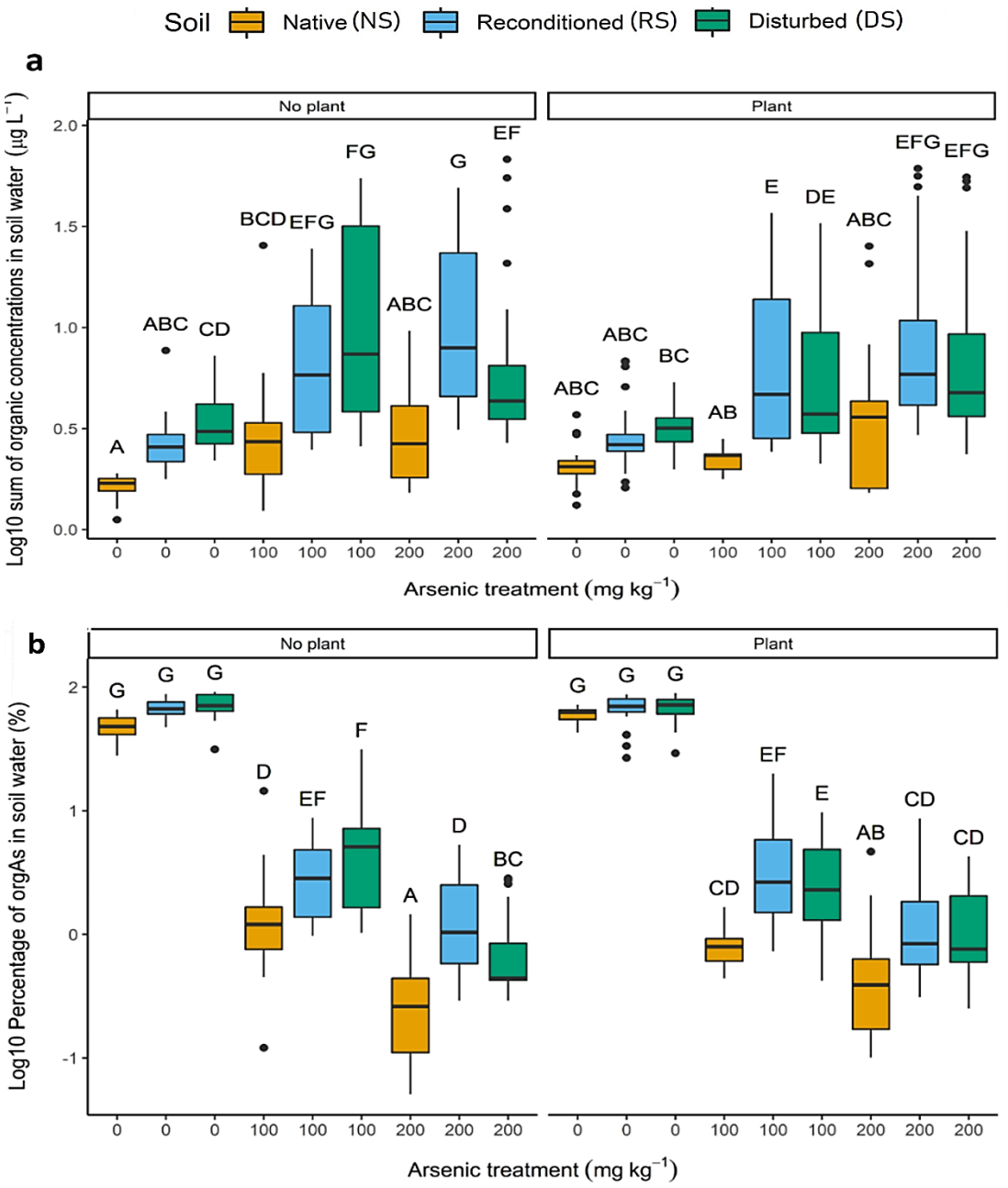


Figure II-3. The concentration of (a) sum of organic As species (orgAs) and (b) percentage of orgAs (orgAs%) in soil water. Data are emmeans ± standard error. Pairwise comparisons were explored and reported using CLD letters. Different CLD letters indicated a statistically significant difference between emmeans ($p < 0.05$).

3.3 Arsenic Species in Soils

We found the same three orgAs (MMA^V, DMA^V, and TMAO) in soil as in soil water. These As species were also examined their responses to the effects of the three experimental factors. Compared to soil water, less significant interactions among the three experimental factors were observed in soils (Table S2.3). The interactions among the microbial disturbance, plants and As treatments were insignificant for all As species in soils. Moreover, MMA^V concentration was affected by the interactions between microbial disturbance and As treatments ($F_{4, 294} = 2.945$, $p = 0.021$) (Table S2.4). Its concentration was higher in RS than in NS due to the sterilization effect ($F_{2, 294} = 3.935$, $p = 0.021$) (Figure S2.5). The sum of the three orgAs concentrations in soils was marginally affected by the presence of plants ($F_{1, 294} = 4.028$, $p = 0.046$; marginally significant and not visible in Figure II-4a). Same as orgAs% in soil water, orgAs% in soils decreased with the increasing As levels in soils (Figure II-4b).

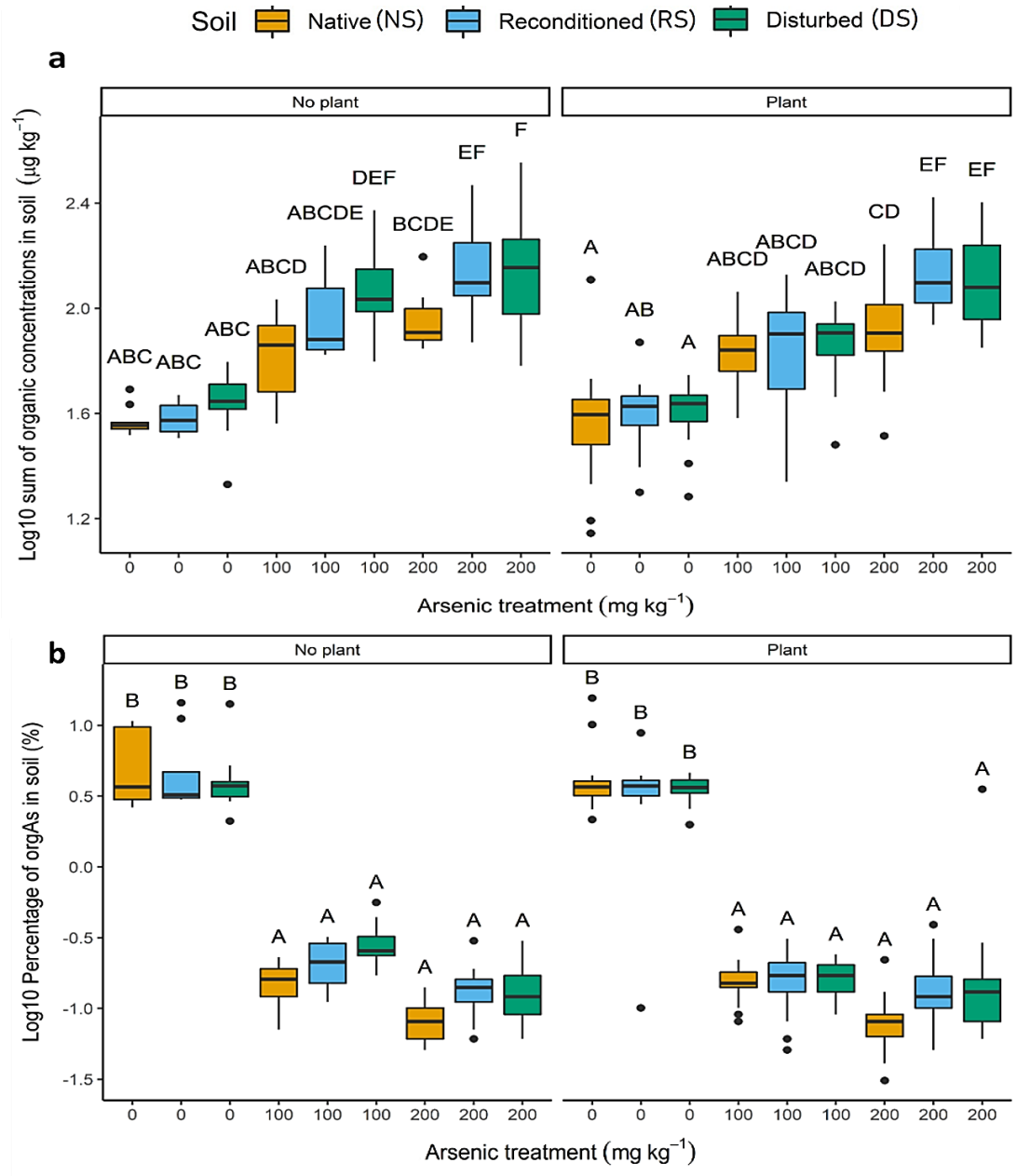


Figure II-4. The plot of (a) sum of organic As species (orgAs) concentration and (b) percentage of orgAs (orgAs%) in soils. Data are emmeans ± standard error. Pairwise comparisons were explored and reported using CLD letters. Different CLD letters indicated a statistically significant difference between emmeans ($p < 0.05$).

3.4 Comparison between Arsenic Speciation in Soil Water and Soils

As shown in Figure II-3b and II-4b, orgAs% in soil water and soils varied only slightly between No-plant and Plant pots, we therefore focused on the comparison between orgAs % and inAs% in soil water and soils of Plant pots in response to microbial disturbance and As treatments. The orgAs% in soil water appeared to rise with the increasing microbial disturbance in contaminated soils, possibly due to the concurrent influence or interactions of microbial disturbance and As treatments. OrgAs made up to 80.7% of the sum of all As species in uncontaminated soil water, with the three orgAs accounting for a similar proportion (11.7 - 13.1%) (Figure II-5a). inAs% in soil water also increased as the As level in soils increased by 200 mg kg^{-1}, whereas orgAs% decreased (Figure II-5b). When soil As levels increased to 200 mg kg^{-1}, TMAO in soil water constituted a larger proportion in soil water than MMA^V and DMA^V (Figure II-5b). inAs predominated in our soils (> 96.8%) (Figure II-5c and II-5d), with DMA^V as the major orgAs. The percentage of orgAs in soils did not change as the microbial disturbance increased, but it decreased strongly as soil As levels increased (Figure II-5d).

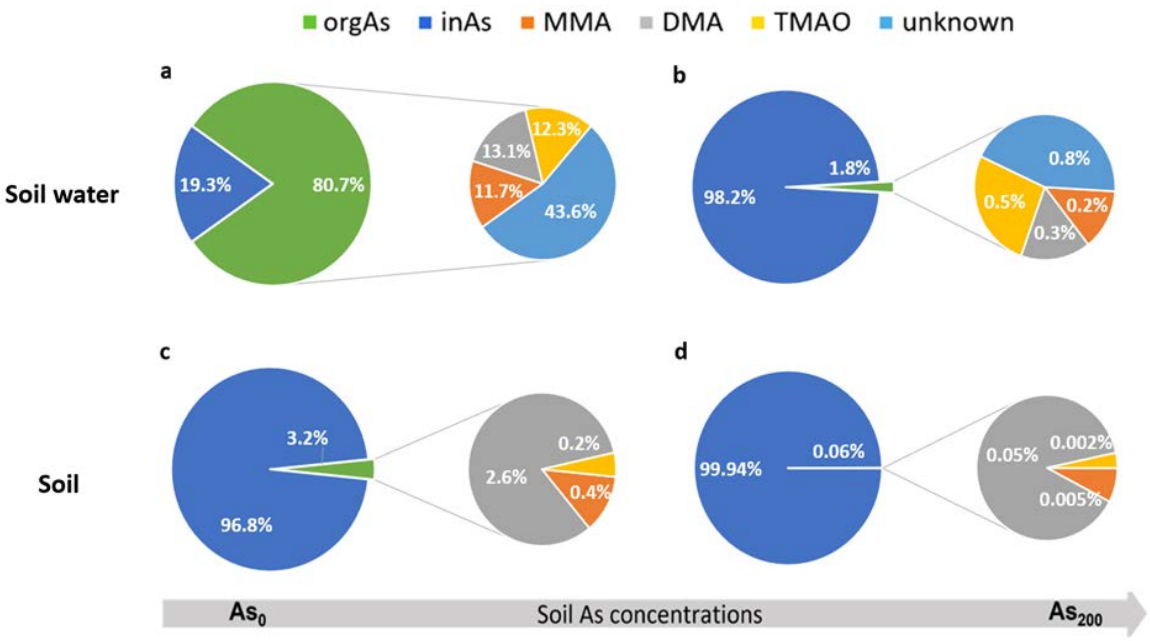


Figure II-5. The changes in As species in soil water and in soils of the Plant pots with the increasing levels of soil As concentrations, presenting the percentages of inorganic As species (inAs%) and orgAs (orgAs%, i.e., MMA^V, DMAV, TMAO, and unknown species)

in (a) soil water of NS of the As_0 group; (b) soil water of DS of the As_{200} group; (c) NS of the As_0 group; and (d) DS of the As_{200} group.

3.5 Other Chemical Parameters in Soil Water

The redundancy analysis (RDA) was applied to explore the effects of experimental factors (microbial disturbance, plants and As treatments) (Figure II-6a) on the corresponding changes in response variables (soil water chemistry parameters, i.e., pH, DOC, major cations and anions as well as some major and trace elements) (Figure II-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, plants and As treatments, 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. This indicated that e.g., microbial disturbance explained 8.95% of the variations in chemistry data. Parameters whose arrows point in the same direction in an RDA plot indicate positive associations and arrows pointing to opposite directions indicate negative associations between them. The RS and DS and As_{100} and As_{200} groups pointed in the same direction as the concentrations of DOC, V, Ba, Na^+, NO_3^-, K^+, and Mg^{2+} on the RDA plot, demonstrating their positive associations. Similarly, the RS and DS and As_{100} and As_{200} groups showed a negative association with the values of pH, Zn, Cr, Al, Cu, Ni, Ca^{2+}, Cl^-, SO_4^{2-}, and U in soil water. Moreover, the experimental factor plants (P_or_NP) pointed in the opposite direction than the microbial disturbance and As treatments, which showed positive associations with most soil water parameters, e.g., the presence of maize plants had a positive association with DOC concentration in soil water and soil pH.

In all the three As groups, DOC concentration in soil water from NS was lower than those from DS and RS (Figure S2.6). In uncontaminated soils, DOC strongly correlated with totAs ($R = 0.82$, $p < 0.001$) and orgAs ($R = 0.69$, $p < 0.001$) in soil water (Figure S2.7). In contaminated soils, DOC slightly correlated with orgAs in As_{100} ($R = 0.19$, $p = 0.015$) and As_{200} group ($R = 0.26$, $p = 0.020$), but not with totAs. Besides, soils in both No-plant and Plant pots had a nearly neutral pH (6.8 - 8.2) (Figure S2.8). In uncontaminated soils, the 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 As_{100} group ($p = 0.003$).

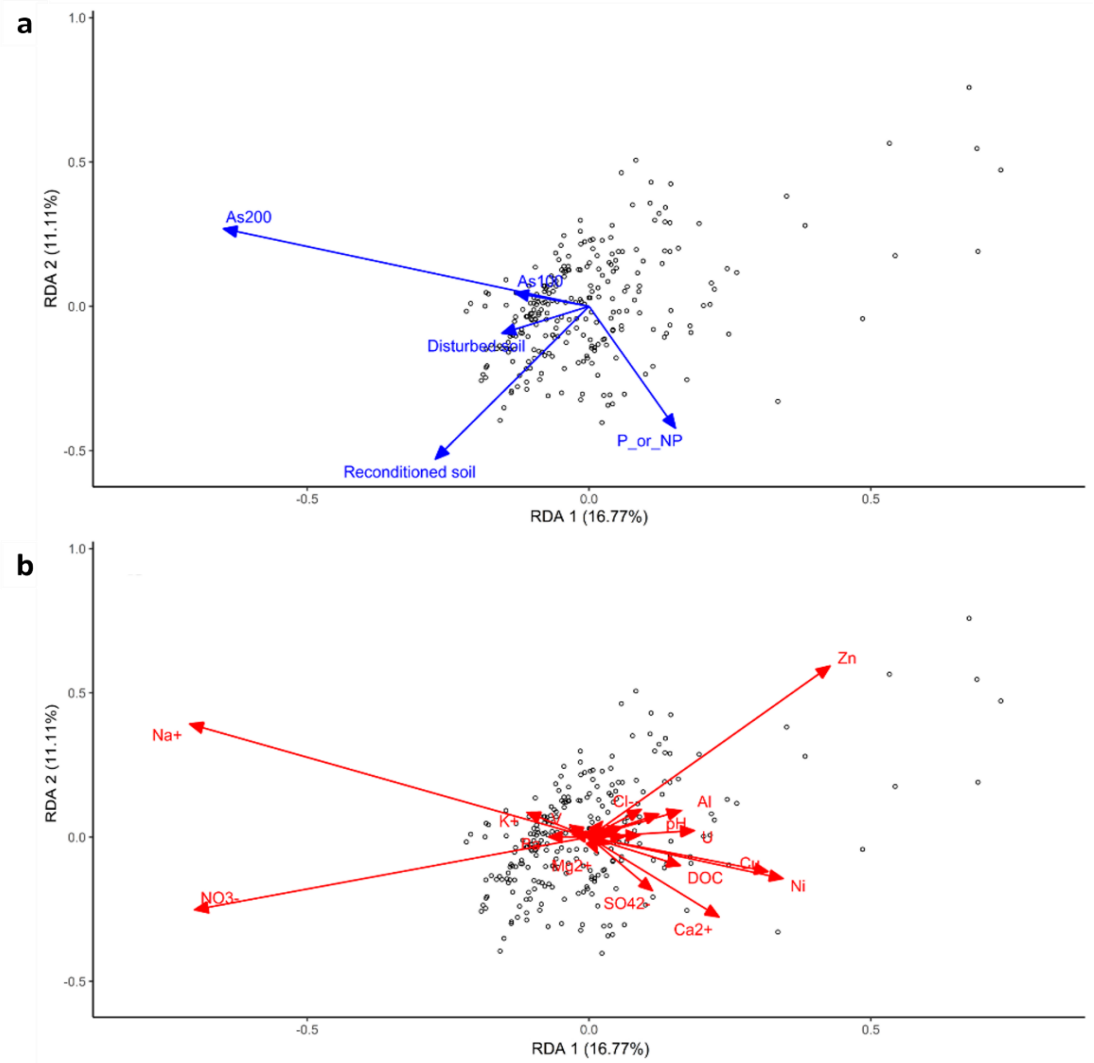


Figure II-6. Redundancy analysis (RDA) triplot showing samples as dots with a) experimental factors (microbial disturbance, plants and As treatments); b) corresponding changes in response variables (soil water chemistry parameters) in the system. The percentage of explained variance was indicated on each axis.

4 Discussion

4.1 Microbial Disturbance Effects on As in the Soil Environment

In the present study, we aim to differentiate between the microbial disturbance effect and the sterilization effect. Regarding the microbial disturbance effect, we found that it resulted in higher concentrations of totAs and orgAs in the soil water of DS than RS (Figure II-2). This suggested that the levels of totAs and orgAs in soil water might have risen with the elimination and disturbance of soil indigenous microbes. Concurrent results have been announced that the elimination of oxidizing bacteria by soil sterilization slows down iron oxidation, leading to insufficient sorptive sites for As and higher As leaching in sterilized soils (Kumpiene et al. 2007). However, due to the reconditioning of some indigenous microbes in RS, the levels of totAs and orgAs in the soil water of RS were lower than that of DS. Interestingly, the concentrations of totAs and As species in soil water of unsterilized soils showed no temporal changes irrespective of the As addition to soils, providing evidence of the role of soil indigenous microbes in immobilizing As in the soil environment. This is in agreement with existing evidence providing that some prokaryotic (bacteria, archaea) and eukaryotic (algae, fungi) microbes can excrete extracellular polymeric substances such as polysaccharides and glycoproteins, which possess abundant functional groups with biosorption and metal binding properties that can immobilize metal(loid) ions (Seshadri et al. 2015; Pal and Paul 2008). Soil indigenous microbes can also catalyze the transformation or mediate redox reactions of As species, thereby controlling As mobility in soils (Wang and Mulligan 2006) and its bioavailability to plants (Upadhyay et al. 2018). This finding is supported in our study by the observation that the microbial disturbance effect affected only orgAs but not inAs, which implies that the microbial disturbance effect i.e., the elimination and disturbance of soil indigenous microbes, might be related to the transformation of inAs to orgAs. Future work determining the microbial species in DS and RS soils is needed to uncover the microbial dynamics in soils after soil sterilization.

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 recovered soils were lower than those of unsterilized soils, including the activities of catalase, invertase, urease, protease, acid phosphatase, and phytase (Xun et al. 2015). These enzymes are involved in the hydrolysis of carbon substrates

and organic nutrients, which alters nutrient availability in the rhizosphere, and changes in their activities can affect microbial composition and activities (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). Taken together, our results are in line with the strong evidence described above, i.e., soil sterilization not only eliminates or disturbs soil indigenous microbes and inhibits their roles in As methylation process, thereby increasing orgAs levels in soil water. Soil sterilization also halts enzyme and microbial activities that are important for As immobilization in soils, which may explain higher As concentrations in the soil water of sterilized soils.

4.2 Sterilization Effects on As 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). However, the observed sterilization effects need to be resolved to identify the microbial disturbance effect. In this study, the concentrations of totAs and As species in soil water showed a general concentration trend of NS < RS ≤ DS, suggesting that microbial disturbance promoted As release into soil water. The concentrations of totAs, inAs, and orgAs were largely enhanced in the soil water of contaminated RS and DS. This is because that 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). Arsenic concentration mainly declined in the first month of our experiment, which could be due to the re-equilibrium of As adsorption between the soil water and the soil phases.

Soil sterilization can alter the sorptive behavior of As due to ion competition with e.g. P for sorption sites (Tiberg et al. 2020; Hongshao and Stanforth 2001), and alter As reactivity to organic matter owing to changes in DOC and/or soil pH (Dao et al. 1982; Razavi and Lakzian 2007). Our findings agree with the aforementioned literatures that the sterilization modified soil physicochemical properties with increased organic carbon content because of the nutrient release. The sterilized soils (DS and RS) had higher DOC levels than unsterilized soils (NS) in our study, showing the same concentration trend NS < RS ≤ DS as with the concentrations of totAs and orgAs in soil water. Since microbes in soil sterilization could not resist the leaching of their cellular compounds as soil indigenous microbes can, DOC

concentrations increased. Soil sterilization also decreased soil adsorption capacity due to the competition between DOC and As for sorptive sites, leading to enhanced As remobilization (Schaller et al. 2011).

The DOC has been observed to be positively correlated with orgAs concentration in soil water up to 20 ug L^{-1} (Zhao et al. 2013; Williams et al. 2011), which was close to the orgAs level in our uncontaminated soils (< 8 ug L^{-1}). In our uncontaminated soil water, DOC strongly correlated with totAs and orgAs concentration, whereas in contaminated soil water, it only slightly correlated with orgAs concentration. 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 water probably due to the overprint effect of high As. The positive correlation with DOC is consistent with previous reports that organic matter can stimulate As methylation and the volatilization of methylated As species (Huang et al. 2012). This is because the mobilized As in soils is more bioavailable to As-methylating microbes, which can thrive in soils with DOC of different derivations serving as nutrients for their growth (Huang et al. 2012; Yan et al. 2020).

Soil pH also influences As concentration and speciation. Under weakly alkaline conditions, As^{III} in the form of $H_2AsO_3^-$ is less mobile than H_3AsO_3 in acidic soils (Wei et al. 2016; Marin et al. 1993). Whereas at low pH, the dissolution of Fe-oxy-hydroxides releases bound As and mobilizes it into soil water (Marin et al. 1993). Our soil pH is negatively correlated with orgAs concentration in contaminated soils, presenting an opposite pattern DS ≤ RS < NS with As concentrations in soil water. The same negative correlation has been found in previous research because the activity of As methylation is higher in acidic soils (Zhao et al. 2013). In our study, the negative correlation with pH accounted for more mobile As in sterilized than in unsterilized soils, but the pH difference was insignificant between No-plant and Plant pots in contaminated soils. Conversely, in uncontaminated soils, soil pH was higher in the presence of plants, although both No-plant and Plant pots were given the same fertilizer containing 8.4% NH_3-N and 1.4% NO_3-N. Ammonium-based fertilizers can acidify the soil by producing two H^+ ions per each NH_4^+ molecule oxidized to NO_3^-, but the final acidification level in soils is determined by whether the NO_3^- is taken up by plants. For each NO_3^- taken up by plants, a H^+ ion is consumed or OH^- is expelled, so its net acidification is only half that without plants, resulting in pH rises in the rhizosphere (Mike McLaughlin 2009; Smiley and

Cook 1973). Meanwhile, when plants take up nitrate-based fertilizers, they release OH^- ions that react with the H^+ ions produced during nitrification. The overall influence on soil pH should be virtually neutral, which is consistent with our results on soil pH (Figure S2.8).

4.3 Maize Plant Effects on As Release in Soil Water

In our uncontaminated soils, totAs concentration in soil water was higher in the presence of plants (Figure II-2a). We hypothesize that less As was taken up by plants as a result of As-phosphate competition, leading to higher As concentrations in the soil water with plant cultivation. Low As levels have been reported to increase the bioavailability of inorganic phosphate in soil water by competing for soil adsorption sites (Lambkin and Alloway 2003), and the increased P bioavailability and uptake can promote maize plant growth and increase their biomass (Sillen et al. 2020). Contrarily, in our contaminated soils, lower concentrations of totAs and As species were found in the soil water with plant cultivation, which could not be attributed solely to As uptake by plants that reduced As concentrations in soil water. The maximum percentage of As uptake by plants from soils (totAs in plants/in soils) was highest in the As_0 group (1.34%), lower in the As_{100} group (0.66%), and lowest in the As_{200} group (0.28%). If plant uptake was the reason that As concentration was lower in the presence of plants, this would be clearly observed in the As_0 group. However, totAs concentration in soil water of As_0 group was higher in Plant than in No-plant pots. This implied that As uptake by plants (< 1.34%) could not account for lower As levels in the contaminated soil water with plant cultivation. Nevertheless, such proportions of As taken up from soils into plants were too low to fully explain the concentration differences between No-plant and Plant pots. Higher percentages of water extractable As (6.1 - 12.0%) in soils than in our case have been reported, ranging from 51 to 1860 mg kg^{-1} As (Francesconi et al. 2002). Even in As-rich soils, extractable As accounts for only a minor proportion, 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}) (Itabashi et al. 2019).

4.4 Maize and Soil Microbes Reduced As Concentrations in Soil Water

In this study, changes in As speciation with the increasing soils As levels were evident in soil water but not in soils. The three orgAs (MMA^V, DMA^V, and TMAO) had similar proportions in uncontaminated soil water, while TMAO had a larger percentage in contaminated soil water than MMA^V and DMA^V. The higher percentage of TMAO in contaminated soil water revealed that an in-depth As methylation process has occurred. Moreover, we discovered that orgAs% in both soil water and soils decreased with the increasing As levels in soils. The same phenomenon has been observed in a previous study suggesting that the higher As levels in soils inhibite microbial growth responsible for the methylation of inAs in soils, resulting in lower production of orgAs in the soil environment (Abedin et al. 2002). However, TMAO predominated in our contaminated soil water and As concentrations increased as soil As levels increased, indicating that As methylation was active even in contaminated soil water. Since inAs are the most common As species in the soil environment, we believe this is more likely due to the fact that with a higher background inAs level in soils, the fraction of orgAs in contaminated soil water can be reduced. Unlike in soil water, DMA^V was always the primary As species in both un- and contaminated soils. MMA^V and DMA^V are mainly associated with Fe-oxyhydroxides in soils as sorption complexes. But the sorption of DMA^V is lower than that of MMA^V as it is substituted by an additional methyl group (Shimizu et al. 2011; Huang et al. 2011). The additional methyl group not only removes a deprotonation site from DMA^V, making it less negatively charged and less electrostatic attracted, but also makes the DMA^V molecule larger and occupies more space (Shimizu et al. 2011). Thus, fewer potential As-binding sites are available for complex formation in DMA^V than MMA^V. TMAO has three methyl groups, which makes it even more mobile in soils than DMA^V and therefore was not dominant in our soils. Furthermore, as with the orgAs concentration in soil water, orgAs% increased in sterilized contaminated soils, which could be due to the nutrient release from soil sterilization and more carbon sources available for As-methylating microbes (Huang et al. 2012; Yan et al. 2020).

In addition, as the As level in soils increased from 100 to 200 mg kg^{-1}, the abiotic sterilization effect still affected orgAs but not totAs and inAs. Therefore, both microbial disturbance and sterilization effects had a greater impact on orgAs than on inAs, i.e., on the

transformation process from inAs to orgAs in soil water. Similar findings have been reported that DMA^V and TMAO are detected only in unsterilized soils but not in sterilized soils, because the microbial activity of soil indigenous microbes in unsterilized soils is stimulated by a symbiotic association between plant roots and fungi, which is involved in the transformation of inAs into orgAs (Ultra et al. 2007). Such plant-microbe interactions may have been destroyed in sterilized soils, disrupting the As methylation pathway and reducing the production of orgAs, so that the orgAs than inAs in soil water were more affected by the microbial disturbance effect in our study.

Interestingly, both microbial disturbance and sterilization effects were more significant in the soil water of No-plant than of Plant pots. The microbial disturbance effect was observed only in No-plant pots for totAs and orgAs concentrations. The sterilization effects played a significant role in all groups of No-plant pots, but only in some groups of Plant pots. As a result, the concentration differences among the three soils were generally smaller in Plant than in No-plant pots, suggesting that microbial disturbance effects were less significant in Plant than in No-plant 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 Plant pots. Same as our findings, maize planting has been found to reduce the concentrations of As species (inAs, MMA^V, DMA^V, and TMAO) in soil water by more than 30% compared to non-planted soil water, because the presence of maize roots decreased the copy number of Streptomyces As methylating gene (arsM) that is responsible for the methylation and volatilization of As from bacteria (Afroz et al. 2019). The lower As concentrations in soil water under maize cultivation can be attributed to both the direct impact of maize plants through root border cells and the indirect influence of maize. The latter may help soil microbes to recover from soil sterilization (Li et al. 2019; Zhalnina et al. 2018), while reshaping their communities and favoring beneficial soil microbes (Broeckling et al. 2008).

Border cells surrounding plant roots can regulate microbial interactions by avoiding pathogens and favoring association with beneficial soil microbes (Haldar and Sengupta 2015). They can attract and immobilize nematodes, repel bacteria, resist fungal infection, and avoid soil microbes that compete with the host plant for nutrients (Hawes et al. 1998; Yan et al. 2014). Root border cells can also protect against heavy metal toxicity such as As (Forino et al. 2012; Kopittke et al. 2012), lead (Nriagu et al. 2007), and aluminum (Miyasaka and Hawes

2001; Hawes et al. 2000). A layer of mucilage around the border cells can decrease plant sensitivity to aluminum by increasing its surface area and possibly binding and immobilizing charged aluminum molecules to prevent further cellular damage (Hawes et al. 2000). Border cells also contribute to the maize plants' ability to tolerate excess As(V) by accumulating As and limiting its movement into the main maize root system (Kopittke et al. 2012). These earlier reports might provide an explanation for why the As levels in soil water were lower in the presence of maize plants, which could be due to the direct accumulation of As in border cells of maize roots.

On the other hand, we hypothesize that maize plants have the ability to help microbes to recover from soil sterilization and recruit beneficial soil microbes *via* root exudations to fulfill their demands, which might have helped them 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. 2020). By altering the chemical composition of the rhizosphere, plants can create diverse microhabitats and enhance their adaptability to environmental stressors (Zhalnina et al. 2018). Root exudations of an As-hyperaccumulator Pteris vittate 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. 2020). Taken together, 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 might be considered as a survival mechanism for both maize plants and their associated soil microbes.

5 Conclusion

This unique intersectional experimental design allowed us to investigate the interactions among microbial disturbance, plants and As treatments in terms of the concentration and speciation of As in both soil water and soils. The concentrations of totAs and As species followed a general pattern of NS < RS ≤ DS in soil water, implying that both microbial disturbance and sterilization effects promoted the release of As from soils to soil water. Meanwhile, both effects played a greater role in orgAs than inAs concentrations in soil water, which was in line with the potential influence of microbial As methylation from inorganic to organic forms of As. Interestingly, maize plants could not only accumulate and immobilize As in root border cells (Kopittke et al. 2012), also potentially interacted with soil microbes to lower As concentrations in soil water. Both microbial disturbance and sterilization effects were mitigated by plants to offset the increased As levels in soil water caused by microbial disturbance in RS and DS. Maize plants probably reshaped their growth environment by assisting the recolonization of soil microbes after soil sterilization and by favoring or disfavoring soil microbes to mitigate microbial disturbance effects. Overall, this study highlighted the role of maize planting and its interaction with soil microbes in lowering As concentrations in soil water and As bioavailability to themselves as a survival mechanism in response to As stress in the soil environment. This might provide a reference value for As bioremediation technology to ensure the sustainability of agricultural production, food safety and security as well as the protection of human and animal health. To complete the systemic research of As in the soil-plant system, the research on the uptake, translocation and speciation of As in maize plants are awaited.

Associated Content

Supporting Information

Additional information: *I Materials and Methods*, including the preparation of microbial extracts (Figure S1.1); the details of NS and DS properties (Table S1.1); the maize biomass estimating model (Figure S1.2); an example of water sampler in pot (Figure S1.3); the operating parameters of HPLC (Table S1.2). *II Result* concludes two parts. Part I: *Interaction Effects on Soil Water and on Soil*, including the *p* values of ANOVA and MANOVA statistical results as well as the estimated marginal means (emmeans) of totAs and As species concentrations in soil water and in soils (Table S2.1-S2.5). Part II: *Time-series Plots*, including linear time series plot on totAs, inAs, and orgAs concentration in soil water (Figure S2.1-S2.3); individual orgAs, e.g., MMA^V, DMA^V and TMAO in soil water (Figure S2.4); individual orgAs in soils (Figure S2.5); linear time series on DOC concentration (Figure S2.6); positive correlation of DOC content with totAs and orgAs concentration in soil water of the As_0 group (Figure S2.7); linear time series on soil pH (Figure S2.8).

Acknowledgements

This research was financially supported by the University of Bern through the Interfaculty Research Cooperation One Health (https://www.onehealth.unibe.ch/). We especially thank Patrick Neuhaus for his support in the laboratory as well as Pia Bergamaschi and Andrea Weber for their help with data collection. We would also like to thank Dr. Suyi Hu for his help with data organisation.

Author Contributions

Hang Guan: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - Original draft, Visualization, Project administration. Veronica Caggìa: Investigation, Writing - Review and Editing. Andrea Gómez-Chamorro: Formal analysis, Writing - Review and Editing. Daniela Fischer: Methodology. Miquel Coll Crespi and Xiaowen Liu: Investigation. Teresa Chávez-Capilla: Methodology, Writing - Review and Editing. Alban Ramette: Conceptualization, Formal analysis, Writing - Review and Editing. Klaus Schlaeppi: Conceptualization, Resources, Writing - Review and Editing. Adrien Mestrot: Methodology, Conceptualization, Supervision, Funding acquisition, Writing - Review and Editing. Moritz Bigalke: Conceptualization, Supervision, Writing - Review and Editing, Funding acquisition, Project administration.

Reference

Abedin, Mohammed Joinal; Feldmann, Jörg; Meharg, Andy A. (2002): Uptake kinetics of arsenic species in rice plants. In *Plant physiology* 128 (3), pp. 1120–1128. DOI: 10.1104/pp.010733.

Afroz, Hasina; Su, Shiming; Carey, Manus; Meharg, Andy A.; Meharg, Caroline (2019): Inhibition of Microbial Methylation via arsM in the Rhizosphere: Arsenic Speciation in the Soil to Plant Continuum. In *Environmental science & technology* 53 (7), pp. 3451–3463. DOI: 10.1021/acs.est.8b07008.

Ahemad, Munees (2019): Remediation of metalliferous soils through the heavy metal resistant plant growth promoting bacteria: Paradigms and prospects. In *Arabian Journal of Chemistry* 12 (7), pp. 1365–1377. DOI: 10.1016/j.arabjc.2014.11.020.

Berns; Philipp, H.; Narres, H.-D.; Burauel, P.; Vereecken, H.; Tappe, W. (2008): Effect of gamma-sterilization and autoclaving on soil organic matter structure as studied by solid state NMR, UV and fluorescence spectroscopy. In *Eur J Soil Science* 59 (3), pp. 540–550. DOI: 10.1111/j.1365-2389.2008.01016.x.

Blankinship, Joseph C.; Becerra, Caryl A.; Schaeffer, Sean M.; Schimel, Joshua P. (2014): Separating cellular metabolism from exoenzyme activity in soil organic matter decomposition. In *Soil Biology and Biochemistry* 71, pp. 68–75. DOI: 10.1016/j.soilbio.2014.01.010.

Broeckling, Corey D.; Broz, Amanda K.; Bergelson, Joy; Manter, Daniel K.; Vivanco, Jorge M. (2008): Root exudates regulate soil fungal community composition and diversity. In *Applied and environmental microbiology* 74 (3), pp. 738–744. DOI: 10.1128/AEM.02188-07.

Buschmann, Johanna; Kappeler, Alexandra; Lindauer, Ursula; Kistler, David; Berg, Michael; Sigg, Laura (2006): Arsenite and arsenate binding to dissolved humic acids: influence of pH, type of humic acid, and aluminum. In *Environmental science & technology* 40 (19), pp. 6015–6020. DOI: 10.1021/es061057.

Cattani, I.; Beone, G. M.; Gonnelli, C. (2015): Influence of Rhizophagus irregularis inoculation and phosphorus application on growth and arsenic accumulation in maize (Zea mays L.) cultivated on an arsenic-contaminated soil. In *Environmental science and pollution research international* 22 (9), pp. 6570–6577. DOI: 10.1007/s11356-014-3837-0.

Colombo, Claudio; Palumbo, Giuseppe; He, Ji-Zheng; Pinton, Roberto; Cesco, Stefano (2014): Review on iron availability in soil: interaction of Fe minerals, plants, and microbes. In *J Soils Sediments* 14 (3), pp. 538–548. DOI: 10.1007/s11368-013-0814-z.

Danouche, Mohammed; El Ghachtouli, Naïma; El Arroussi, Hicham (2021): Phycoremediation mechanisms of heavy metals using living green microalgae: physicochemical and molecular approaches for enhancing selectivity and removal capacity. In *Heliyon* 7 (7), e07609. DOI: 10.1016/j.heliyon.2021.e07609.

Dao, T. H.; Marx, D. B.; Lavy, T. L.; Dragun, J. (1982): Effect, and Statistical Evaluation, of Soil Sterilization on Aniline and Diuron Adsorption Isotherms. In *Soil Science Society of America Journal* 46 (5), pp. 963–969. DOI: 10.2136/sssaj1982.03615995004600050016x.

Del Molina, María Carmen; White, James F.; García-Salgado, Sara; Quijano, M. Ángeles; González-Benítez, Natalia (2020): A Gnotobiotic Model to Examine Plant and Microbiome Contributions to Survival under Arsenic Stress. In *Microorganisms* 9 (1). DOI: 10.3390/microorganisms9010045.

Di, Xuerong; Beesley, Luke; Zhang, Zulin; Zhi, Suli; Jia, Yan; Ding, Yongzhen (2019): Microbial Arsenic Methylation in Soil and Uptake and Metabolism of Methylated Arsenic in Plants: A Review. In *International journal of environmental research and public health* 16 (24). DOI: 10.3390/ijerph16245012.

Diels, L.; Spaans, P. H.; van Roy, S.; Hooyberghs, L.; Ryngaert, A.; Wouters, H. et al. (2003): Heavy metals removal by sand filters inoculated with metal sorbing and precipitating bacteria. In *Hydrometallurgy* 71 (1-2), pp. 235–241. DOI: 10.1016/S0304-386X(03)00161-0.

Dobran, Simona; Zagury, Gérald J. (2006): Arsenic speciation and mobilization in CCA-contaminated soils: influence of organic matter content. In *The Science of the total environment* 364 (1-3), pp. 239–250. DOI: 10.1016/j.scitotenv.2005.06.006.

Fisher, Daniel J.; Yonkos, Lance T.; Staver, Kenneth W. (2015): Environmental concerns of roxarsone in broiler poultry feed and litter in Maryland, USA. In *Environmental science & technology* 49 (4), pp. 1999–2012. DOI: 10.1021/es504520w.

Forino, Laura Maria Costantina; Ruffini Castiglione, Monica; Bartoli, Giacomo; Balestri, Mirko; Andreucci, Andrea; Tagliasacchi, Anna Maria (2012): Arsenic-induced morphogenic response in roots of arsenic hyperaccumulator fern Pteris vittata. In *Journal of Hazardous Materials* 235-236, pp. 271–278. DOI: 10.1016/j.jhazmat.2012.07.051.

Francesconi, Kevin; Visoottiviseth, Pornsawan; Sridokchan, Weeraphan; Goessler, Walter (2002): Arsenic species in an arsenic hyperaccumulating fern, Pityrogramma calomelanos: a potential phytoremediator of arsenic-contaminated soils. In *Science of The Total Environment* 284 (1-3), pp. 27–35. DOI: 10.1016/s0048-9697(01)00854-3.

Garcia-Manyes, S. (2002): Arsenic speciation in contaminated soils. In *Talanta* 58 (1), pp. 97–109. DOI: 10.1016/S0039-9140(02)00259-X.

Garcia-Manyes, S.; Jiménez, G.; Padró, A.; Rubio, Roser; Rauret, G. (2002): Arsenic speciation in contaminated soils. In *Talanta* 58, pp. 97–109.

Geiszinger, Anita; Goessler, Walter; Kosmus, Walter (2002): Organoarsenic compounds in plants and soil on top of an ore vein. In *Appl. Organometal. Chem.* 16 (5), pp. 245–249. DOI: 10.1002/aoc.294.

Gianfreda, L. (2015): Enzymes of importance to rhizosphere processes. In *J. Soil Sci. Plant Nutr.* (ahead), p. 0. DOI: 10.4067/S0718-95162015005000022.

Haldar, Shyamalina; Sengupta, Sanghamitra (2015): Plant-microbe Cross-talk in the Rhizosphere: Insight and Biotechnological Potential. In *The open microbiology journal* 9, pp. 1–7. DOI: 10.2174/1874285801509010001.

Hammond, Corin M.; Root, Robert A.; Maier, Raina M.; Chorover, Jon (2018): Mechanisms of Arsenic Sequestration by Prosopis juliflora during the Phytostabilization of Metalliferous

Mine Tailings. In *Environmental science & technology* 52 (3), pp. 1156–1164. DOI: 10.1021/acs.est.7b04363.

Hawes, M. C.; Brigham, L. A.; Wen, F.; Woo, H. H.; Zhu, Y. (1998): Function of root border cells in plant health: pioneers in the rhizosphere. In *Annual review of phytopathology* 36, pp. 311–327. DOI: 10.1146/annurev.phyto.36.1.311.

Hawes, Martha C.; Gunawardena, Uvini; Miyasaka, Susan; Zhao, Xiaowen (2000): The role of root border cells in plant defense. In *Trends in Plant Science* 5 (3), pp. 128–133. DOI: 10.1016/S1360-1385(00)01556-9.

Hinsinger, Philippe; Bengough, A. Glyn; Vetterlein, Doris; Young, Iain M. (2009): Rhizosphere: biophysics, biogeochemistry and ecological relevance. In *Plant Soil* 321 (1-2), pp. 117–152. DOI: 10.1007/s11104-008-9885-9.

Hong, Young-Seoub; Song, Ki-Hoon; Chung, Jin-Yong (2014): Health effects of chronic arsenic exposure. In *Journal of preventive medicine and public health = Yebang Uihakhoe chi* 47 (5), pp. 245–252. DOI: 10.3961/jpmph.14.035.

Hongshao, Z.; Stanforth, R. (2001): Competitive adsorption of phosphate and arsenate on goethite. In *Environmental science & technology* 35 (24), pp. 4753–4757. DOI: 10.1021/es010890y.

Hu, Lingfei; Robert, Christelle A. M.; Cadot, Selma; Zhang, Xi; Ye, Meng; Li, Beibei et al. (2018): Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. In *Nature communications* 9 (1), p. 2738. DOI: 10.1038/s41467-018-05122-7.

Huang, Hai; Jia, Yan; Sun, Guo-Xin; Zhu, Yong-Guan (2012): Arsenic speciation and volatilization from flooded paddy soils amended with different organic matters. In *Environmental science & technology* 46 (4), pp. 2163–2168. DOI: 10.1021/es203635s.

Huang, Jen-How; Hu, Kan-Nian; Decker, Berryinne (2011): Organic Arsenic in the Soil Environment: Speciation, Occurrence, Transformation, and Adsorption Behavior. In *Water Air Soil Pollut* 219 (1-4), pp. 401–415. DOI: 10.1007/s11270-010-0716-2.

Itabashi, Tomohiko; Li, Jining; Hashimoto, Yohey; Ueshima, Masato; Sakanakura, Hirofumi; Yasutaka, Tetsuo et al. (2019): Speciation and Fractionation of Soil Arsenic from Natural and Anthropogenic Sources: Chemical Extraction, Scanning Electron Microscopy, and Micro-XRF/XAFS Investigation. In *Environmental science & technology* 53 (24), pp. 14186–14193. DOI: 10.1021/acs.est.9b03864.

Jackson, B. P.; Seaman, J. C.; Bertsch, P. M. (2006): Fate of arsenic compounds in poultry litter upon land application. In *Chemosphere* 65 (11), pp. 2028–2034. DOI: 10.1016/j.chemosphere.2006.06.065.

Jackson, Brian (2015): Fast ion chromatography-ICP-QQQ for arsenic speciation. In *Journal of analytical atomic spectrometry* 30 (6), pp. 1405–1407. DOI: 10.1039/C5JA00049A.

Jia, Yan; Huang, Hai; Zhong, Min; Wang, Feng-Hua; Zhang, Li-Mei; Zhu, Yong-Guan (2013a): Microbial arsenic methylation in soil and rice rhizosphere. In *Environmental science & technology* 47 (7), pp. 3141–3148. DOI: 10.1021/es303649v.

Jia, Yan; Sun, Guo-Xin; Huang, Hai; Zhu, Yong-Guan (2013b): Biogas slurry application elevated arsenic accumulation in rice plant through increased arsenic release and

methylation in paddy soil. In *Plant Soil* 365 (1-2), pp. 387–396. DOI: 10.1007/s11104-012-1391-4.

John Parascandola (2017): The King of Poisons: A History of Arsenic.

Kopittke, Peter M.; Jonge, Martin D. de; Menzies, Neal W.; Wang, Peng; Donner, Erica; McKenna, Brigid A. et al. (2012): Examination of the distribution of arsenic in hydrated and fresh cowpea roots using two- and three-dimensional techniques. In *Plant Physiol* 159 (3), pp. 1149–1158. DOI: 10.1104/pp.112.197277.

Kuivenhoven; Mason (2021): Arsenic Toxicity. In Matthew Kuivenhoven, Kelly Mason (Eds.): StatPearls [Internet]: StatPearls Publishing. Available online at https://www.ncbi.nlm.nih.gov/books/NBK541125/.

Kumpiene, Jurate; Castillo Montesinos, Isaac; Lagerkvist, Anders; Maurice, Christian (2007): Evaluation of the critical factors controlling stability of chromium, copper, arsenic and zinc in iron-treated soil. In *Chemosphere* 67 (2), pp. 410–417. DOI: 10.1016/j.chemosphere.2006.08.031.

Lambkin, D. C.; Alloway, B. J. (2003): Arsenate-induced Phosphate Release from Soils and its Effect on Plant Phosphorus. In *Water Air Soil Pollut* 144 (1/4), pp. 41–56. DOI: 10.1023/A:1022949015848.

Larios, Raquel; Fernández-Martínez, Rodolfo; Lehecho, Isabelle; Rucandio, Isabel (2012): A methodological approach to evaluate arsenic speciation and bioaccumulation in different plant species from two highly polluted mining areas. In *The Science of the total environment* 414, pp. 600–607. DOI: 10.1016/j.scitotenv.2011.09.051.

Li, Kun; DiLegge, Michael J.; Minas, Ioannis S.; Hamm, Alison; Manter, Daniel; Vivanco, Jorge M. (2019): Soil sterilization leads to re-colonization of a healthier rhizosphere microbiome. In *Rhizosphere* 12, p. 100176. DOI: 10.1016/j.rhisph.2019.100176.

Lomax, Charlotte; Liu, Wen-Ju; Wu, Liyou; Xue, Kai; Xiong, Jinbo; Zhou, Jizhong et al. (2012): Methylated arsenic species in plants originate from soil microorganisms. In *New Phytologist* 193 (3), pp. 665–672. DOI: 10.1111/j.1469-8137.2011.03956.x.

Mahmood, T.; Mehnaz, S.; Fleischmann, F.; Ali, R.; Hashmi, Z. H.; Iqbal, Z. (2014): Soil sterilization effects on root growth and formation of rhizosheaths in wheat seedlings. In *Pedobiologia* 57 (3), pp. 123–130. DOI: 10.1016/j.pedobi.2013.12.005.

Mandal, Paramita (2017): An insight of environmental contamination of arsenic on animal health. In *Emerging Contaminants* 3 (1), pp. 17–22. DOI: 10.1016/j.emcon.2017.01.004.

Marin, A. R.; Masscheleyn, P. H.; Patrick, W. H. (1993): Soil redox-pH stability of arsenic species and its influence on arsenic uptake by rice. In *Plant Soil* 152 (2), pp. 245–253. DOI: 10.1007/BF00029094.

Marschner, Petra; Rumberger, Angelika (2004): Rapid changes in the rhizosphere bacterial community structure during re-colonization of sterilized soil. In *Biol Fertil Soils* 40 (1), pp. 1–6. DOI: 10.1007/s00374-004-0736-4.

McNamara, N. P.; Black, H.I.J.; Beresford, N. A.; Parekh, N. R. (2003): Effects of acute gamma irradiation on chemical, physical and biological properties of soils. In *Applied Soil Ecology* 24 (2), pp. 117–132. DOI: 10.1016/S0929-1393(03)00073-8.

Mike McLaughlin (2009): Technical Bulletin: Fertilizer and soil acidity. Available online at https://sciences.adelaide.edu.au/fertiliser/system/files/media/documents/2020-01/factsheet-fertilizers-and-soil-acidity.pdf.

Mir, Kalam A.; Rutter, Allison; Koch, Iris; Smith, Paula; Reimer, Ken J.; Poland, John S. (2007): Extraction and speciation of arsenic in plants grown on arsenic contaminated soils. In *Talanta* 72 (4), pp. 1507–1518. DOI: 10.1016/j.talanta.2007.01.068.

Miyasaka, S. C.; Hawes, M. C. (2001): Possible role of root border cells in detection and avoidance of aluminum toxicity. In *Plant physiology* 125 (4), pp. 1978–1987. DOI: 10.1104/pp.125.4.1978.

Neumann, Kerstin; Klukas, Christian; Friedel, Swetlana; Rischbeck, Pablo; Chen, Dijun; Entzian, Alexander et al. (2015): Dissecting spatiotemporal biomass accumulation in barley under different water regimes using high-throughput image analysis. In *Plant, cell & environment* 38 (10), pp. 1980–1996. DOI: 10.1111/pce.12516.

Norton, Gareth; Deacon, Claire; Mestrot, Adrien; Feldmann, Joerg; Jenkins, Paul; Baskaran, Christina; Meharg, Andrew A. (2013): Arsenic speciation and localization in horticultural produce grown in a historically impacted mining region. In *Environmental science & technology* 47 (12), pp. 6164–6172. DOI: 10.1021/es400720r.

Nriagu, J. O.; Bhattacharya, P.; Mukherjee, A. B.; Bundschuh, J.; Zevenhoven, R.; Loeppert, R. H. (2007): Arsenic in soil and groundwater: an overview. In *Trace Metals and other Contaminants in the Environment* 9, pp. 3–60. DOI: 10.1016/S1875-1121(06)09001-8.

Pal, Arundhati; Paul, A. K. (2008): Microbial extracellular polymeric substances: central elements in heavy metal bioremediation. In *Indian J Microbiol* 48 (1), pp. 49–64. DOI: 10.1007/s12088-008-0006-5.

Pickering, I. J.; Prince, R. C.; George, M. J.; Smith, R. D.; George, G. N.; Salt, D. E. (2000): Reduction and coordination of arsenic in Indian mustard. In *Plant Physiol* 122 (4), pp. 1171–1177. DOI: 10.1104/pp.122.4.1171.

Pongratz, Richard (1998): Arsenic speciation in environmental samples of contaminated soil. In *Science of The Total Environment* 224 (1-3), pp. 133–141. DOI: 10.1016/S0048-9697(98)00321-0.

Prabpai, S.; Charerntanyarak, L.; Siri, B.; Moore, M. R.; Noller, Barry N. (2009): Effects of residues from municipal solid waste landfill on corn yield and heavy metal content. In *Waste management (New York, N.Y.)* 29 (8), pp. 2316–2320. DOI: 10.1016/j.wasman.2009.02.009.

Prasad, M. N. V.; Sajwan, Kenneth S.; Naidu, R. (2006): Trace elements in the environment. Biogeochemistry, biotechnology, and bioremediation. Boca Raton: CRC/Taylor and Francis.

Punshon, Tracy; Jackson, Brian P.; Meharg, Andrew A.; Warczack, Todd; Scheckel, Kirk; Guerinot, Mary Lou (2017): Understanding arsenic dynamics in agronomic systems to predict and prevent uptake by crop plants. In *The Science of the total environment* 581-582, pp. 209–220. DOI: 10.1016/j.scitotenv.2016.12.111.

Rahimzadeh, Saeedeh; Pirzad, Alireza (2017): Arbuscular mycorrhizal fungi and Pseudomonas in reduce drought stress damage in flax (Linum usitatissimum L.): a field study. In *Mycorrhiza* 27 (6), pp. 537–552. DOI: 10.1007/s00572-017-0775-y.

Razavi; Lakzian (2007): Evaluation of chemical and biological consequences of soil sterilization methods 5, pp. 87–91.

Reinhold-Hurek, Barbara; Bünger, Wiebke; Burbano, Claudia Sofía; Sabale, Mugdha; Hurek, Thomas (2015): Roots shaping their microbiome: global hotspots for microbial activity. In *Annual review of phytopathology* 53, pp. 403–424. DOI: 10.1146/annurev-phyto-082712-102342.

Rengel, Z.; Marschner, P. (2005): Nutrient availability and management in the rhizosphere: exploiting genotypic differences. In *The New phytologist* 168 (2), pp. 305–312. DOI: 10.1111/j.1469-8137.2005.01558.x.

Rosas, I.; Belmont, R.; Armienta, A.; Baez, A. (1999): Arsenic concentrations in water, soil, milk and forage in Comarca Lagunera, Mexico. In *Water Air Soil Pollut* 112 (1/2), pp. 133–149. DOI: 10.1023/A:1005095900193.

Ruiz-Chancho, Maria José; López-Sánchez, José Fermín; Schmeisser, Ernst; Goessler, Walter; Francesconi, Kevin A.; Rubio, R. (2008): Arsenic speciation in plants growing in arsenic-contaminated sites. In *Chemosphere* 71 (8), pp. 1522–1530. DOI: 10.1016/j.chemosphere.2007.11.054.

Sadee, Bashdar A.; Foulkes, Mike E.; Hill, Steve J. (2016): A study of arsenic speciation in soil, irrigation water and plant tissue: A case study of the broad bean plant, Vicia faba. In *Food chemistry* 210, pp. 362–370. DOI: 10.1016/j.foodchem.2016.04.066.

Sasse, Joelle; Martinoia, Enrico; Northen, Trent (2018): Feed Your Friends: Do Plant Exudates Shape the Root Microbiome? In *Trends in Plant Science* 23 (1), pp. 25–41. DOI: 10.1016/j.tplants.2017.09.003.

Schaller, Jörg; Weiske, Arndt; Dudel, E. Gert (2011): Effects of gamma-sterilization on DOC, uranium and arsenic remobilization from organic and microbial rich stream sediments. In *The Science of the total environment* 409 (17), pp. 3211–3214. DOI: 10.1016/j.scitotenv.2011.05.014.

Seshadri, B.; Bolan, N. S.; Naidu, R. (2015): Rhizosphere-induced heavy metal(loid) transformation in relation to bioavailability and remediation 15, pp. 524–548.

Shankar, Shiv; Shanker, Uma; Shikha (2014): Arsenic contamination of groundwater: a review of sources, prevalence, health risks, and strategies for mitigation. In *TheScientificWorldJournal* 2014, p. 304524. DOI: 10.1155/2014/304524.

Shimizu, Masayuki; Arai, Yuji; Sparks, Donald L. (2011): Multiscale assessment of methylarsenic reactivity in soil. 2. Distribution and speciation in soil. In *Environmental science & technology* 45 (10), pp. 4300–4306. DOI: 10.1021/es103577e.

Sillen, Wouter M. A.; Thijs, Sofie; Abbamondi, Gennaro Roberto; La Torre Roche, Roberto de; Weyens, Nele; White, Jason C.; Vangronsveld, Jaco (2020): Nanoparticle treatment of maize analyzed through the metatranscriptome: compromised nitrogen cycling, possible phytopathogen selection, and plant hormesis. In *Microbiome* 8 (1), p. 127. DOI: 10.1186/s40168-020-00904-y.

Smiley; Cook (1973): Relationship between Take-all of wheat and rhizosphere pH: Phytopathology. Available online at https://www.apsnet.org/publications/phytopathology/backissues/documents/1973articles/phyto63n07_882.pdf.

Smith; Naidu; Alston (1998): Advances in agronomy. Arsenic in the soil enviromnet: a review. 64 volumes. San Diego, Calif., London: Academic Press (Advances in Agronomy, v. 64).

Song, Jing; Zhao, Fang-Jie; McGrath, Steve P.; Luo, Yong-Ming (2006): Influence of soil properties and aging on arsenic phytotoxicity. In *Environmental toxicology and chemistry* 25 (6), pp. 1663–1670. DOI: 10.1897/05-480r2.1.

Tiberg, Charlotta; Sjöstedt, Carin; Eriksson, Ann Kristin; Klysubun, Wantana; Gustafsson, Jon Petter (2020): Phosphate competition with arsenate on poorly crystalline iron and aluminum (hydr)oxide mixtures. In *Chemosphere* 255, p. 126937. DOI: 10.1016/j.chemosphere.2020.126937.

Tlustoš, Pavel; Goessler, Walter; Száková, Jiřina; Balík, Jiři (2002): Arsenic compounds in leaves and roots of radish grown in soil treated by arsenite, arsenate and dimethylarsinic acid. In *Appl. Organometal. Chem.* 16 (4), pp. 216–220. DOI: 10.1002/aoc.282.

Toxicological Profile for Arsenic (2007): U.S. Department of Health and Human Services.

Turpeinen, Riina (2002): Interactions between metals, microbes-bioremediation of As and Pb contaminated soils. Available online at https://helda.helsinki.fi/bitstream/handle/10138/22027/interact.pdf?sequence=2.

Ultra, Venecio U. Y.; Tanaka, Sota; Sakurai, Katsutoshi; Iwasaki, Kōzō (2007): Arbuscular mycorrhizal fungus (Glomus aggregatum) influences biotransformation of arsenic in the rhizosphere of sunflower (Helianthus annuus L.). In *Soil Science and Plant Nutrition* 53 (4), pp. 499–508. DOI: 10.1111/j.1747-0765.2007.00143.x.

Upadhyay, Munish K.; Yadav, Poonam; Shukla, Anurakti; Srivastava, Sudhakar (2018): Utilizing the Potential of Microorganisms for Managing Arsenic Contamination: A Feasible and Sustainable Approach. In *Front. Environ. Sci.* 6, Article 24. DOI: 10.3389/fenvs.2018.00024.

Valasek, John; Thomasson, J. Alex (Eds.) (2016): Autonomous Air and Ground Sensing Systems for Agricultural Optimization and Phenotyping. SPIE Commercial + Scientific Sensing and Imaging. Baltimore, Maryland, United States, Sunday 17 April 2016: SPIE (SPIE Proceedings).

Wang, Suiling; Mulligan, Catherine N. (2006): Natural attenuation processes for remediation of arsenic contaminated soils and groundwater. In *Journal of Hazardous Materials* 138 (3), pp. 459–470. DOI: 10.1016/j.jhazmat.2006.09.048.

Wang, Ya; Wang, Shu; Xu, Pingping; Liu, Cong; Liu, Misha; Wang, Yulan et al. (2015): Review of arsenic speciation, toxicity and metabolism in microalgae. In *Rev Environ Sci Biotechnol* 14 (3), pp. 427–451. DOI: 10.1007/s11157-015-9371-9.

Wei, Zhigang; Liang, Kai; Wu, Yang; Zou, Yandi; Zuo, Junhui; Arriagada, Diego Cortés et al. (2016): The effect of pH on the adsorption of arsenic(III) and arsenic(V) at the TiO2 anatase 101 surface. In *Journal of colloid and interface science* 462, pp. 252–259. DOI: 10.1016/j.jcis.2015.10.018.

Williams, Paul N.; Zhang, Hao; Davison, William; Meharg, Andrew A.; Hossain, Mahmud; Norton, Gareth J. et al. (2011): Organic matter-solid phase interactions are critical for predicting arsenic release and plant uptake in Bangladesh paddy soils. In *Environmental science & technology* 45 (14), pp. 6080–6087. DOI: 10.1021/es2003765.

Xun, Weibing; Huang, Ting; Zhao, Jun; Ran, Wei; Wang, Boren; Shen, Qirong; Zhang, Ruifu (2015): Environmental conditions rather than microbial inoculum composition determine the bacterial composition, microbial biomass and enzymatic activity of reconstructed soil microbial communities. In *Soil Biology and Biochemistry* 90, pp. 10–18. DOI: 10.1016/j.soilbio.2015.07.018.

Yan, Mengmeng; Zeng, Xibai; Wang, Ji; Meharg, Andy A.; Meharg, Caroline; Tang, Xianjing et al. (2020): Dissolved organic matter differentially influences arsenic methylation and volatilization in paddy soils. In *Journal of Hazardous Materials* 388, p. 121795. DOI: 10.1016/j.jhazmat.2019.121795.

Yan, Zhang; Bo, Chen; Shibin, Gao; Tingzhao, Rong (2014): Biological Characters of Root Border Cells Development in Maize (Zea mays). In *Biotechnology* 13 (3), pp. 89–98. DOI: 10.3923/biotech.2014.89.98.

Yuan, Jun; Zhao, Jun; Wen, Tao; Zhao, Mengli; Li, Rong; Goossens, Pim et al. (2018): Root exudates drive the soil-borne legacy of aboveground pathogen infection. In *Microbiome* 6 (1), p. 156. DOI: 10.1186/s40168-018-0537-x.

Zhalnina, Kateryna; Louie, Katherine B.; Hao, Zhao; Mansoori, Nasim; Da Rocha, Ulisses Nunes; Shi, Shengjing et al. (2018): Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. In *Nature microbiology* 3 (4), pp. 470–480. DOI: 10.1038/s41564-018-0129-3.

Zhao, Fang-Jie; Harris, Eleanor; Yan, Jia; Ma, Jincai; Wu, Liyou; Liu, Wenju et al. (2013): Arsenic methylation in soils and its relationship with microbial arsM abundance and diversity, and as speciation in rice. In *Environmental science & technology* 47 (13), pp. 7147–7154. DOI: 10.1021/es304977m.

Zheng, RuiLun; Sun, GuoXin; Zhu, YongGuan (2013): Effects of microbial processes on the fate of arsenic in paddy soil. In *Chin. Sci. Bull.* 58 (2), pp. 186–193. DOI: 10.1007/s11434-012-5489-0.

Chapter III Maize plants in high-arsenic soils interact with soil microbes to limit the translocation of inorganic arsenic species to maize upper tissues

Hang Guan[†], Veronica Caggìa[‡], Andrea Gómez-Chamorro[¶], Miquel Coll-Crespí[¤], Teresa Chávez-Capilla[†], Klaus Schlaeppi[‡§], Alban Ramette[¶], Adrien Mestrot[†]and Moritz Bigalke[†*]

[†]Institute of Geography, University of Bern, Hallerstrasse 12, CH-3012 Bern, Switzerland

[‡]Institute of Plant Sciences, University of Bern, Altenbergrain 21, CH-3013 Bern, Switzerland

[¶]Institute for Infectious Diseases, University of Bern, Friedbühlstrasse 51, CH-3001 Bern, Switzerland

§Department of Environmental Sciences, University of Basel, CH-4056 Basel, Switzerland

*Institute of Applied Geoscience, Technical University of Darmstadt, Schnittspahnstrasse 9, D-64287 Darmstadt, Germany

This is a preprint version of the manuscript to be submitted for publication.

Abstract

Arsenic (As) is a toxic metalloid, and it can enter the food chain through plant uptake from soils and consumption of the plants by animals and humans. The mobility, bioavailability and toxicity of As are controlled by its speciation, but little is known about how microbes and plants interact to change As speciation in the soil-maize system. Therefore, we performed a greenhouse pot experiment with maize (*Zea mays* L.) plants to study the interactions of soil microbes and plants on the accumulation and translocation of total As (totAs) and different As species in plants. A total of 90 maize pots were prepared with three soil treatments: native soil (NS), reconditioned soil (RS, sterilized soils and reconditioned with soil indigenous microbes), and disturbed soil (DS, sterilized soils before planting) × three As groups (uncontaminated soils (As_0) and contaminated soils (moderate-As soils (As_{100}, 100 mg kg^{-1} As) and high-As soils (As_{200}, 200 mg kg^{-1} As)). Because soil sterilization may cause both biotic and abiotic changes in soils, DS and RS treatments were introduced to differentiate between biotic (microbial disturbance) and abiotic (soil sterilization) effects. The maize plants were harvested after six months of growth, separated into four tissues (roots, stem, leaves, and grains), dried, and weighted. The concentrations of totAs, inorganic As species (inAs), and three organic As species (orgAs), i.e., methylarsonic acid (MMA^V), dimethylarsinic acid (DMA^V) and trimethylarsine oxide (TMAO) were determined in the four maize tissues. In uncontaminated soils, the concentrations of totAs and inAs followed a pattern of roots > leaves > stem > grains, while showing a different consequence of roots > stem ≥ leaves > grains in contaminated soils. These two concentration patterns were consistent with the sequences of metal bioaccumulation coefficient (BAC) and translocation factor (TF). The BAC and TF of maize upper tissues were lower in contaminated than uncontaminated soils, probably due to the increasing chelating agents and antioxidants in roots and stem, which complexed and sequestered inAs in the vacuoles, inhibiting the translocation of inAs. Moreover, the simultaneous presence of both microbial disturbance and sterilization effects increased the levels of inAs and orgAs in maize stem, which aggravated the adverse effects of As exposure and resulted in a greater reduction in dry biomass of the stem than of the other tissues. In uncontaminated soils, both microbial disturbance and sterilization effects did not affect the total dry biomass. Whereas in contaminated soils, the total dry biomass was more reduced in maize grown in sterilized soil than in unsterilized soils, because the sterilization effect caused phosphorus deficiency in maize. In addition, the partial correlation

analysis showed that inAs and MMA^V were the responsible As species for the reduction in the total dry biomass of maize plants.

1Introduction

The consumption of crops grown on As-contaminated soils raises serious concerns for human and animal health (Punshon et al. 2017; Ruiz-Chancho et al. 2008). The transfer of arsenic (As) from soils to plants is an agronomic problem as it negatively affects plant development and leads to yield losses (Srivastava 2020). Arsenic in crops has been studied mainly in rice (Meharg and Zhao 2012; Zhao et al. 2013b), but there is very little work on maize plants. Maize (*Zea mays* L.) is the most widely grown cereal in the world with an annual production of more than one billion tons. It is also an important animal feed or a staple food for many people in South America, Africa, and Asia (Rosas-Castor et al. 2014). In the maize producing countries, As in some soils have significantly exceeded the global average background (10.0 mg kg^{-1}) and the maximum allowable limit for agricultural soils (20.0 mg kg^{-1}) recommended by the U.S. Environmental Protection Agency (EPA) (Rosas-Castor et al. 2014; Kabir et al. 2016). The main pathway of As exposure in humans (> 90%) is the dietary consumption of contaminated foodstuffs and drinking water, making As poisoning a global health issue affecting tens of millions of people worldwide (Anjum et al. 2017). Arsenic study in edible plant parts is crucial as it helps to assess the risks posed by As in plants.

Understanding As speciation is essential because it controls the bioavailability, mobility, and (phyto)toxicity of As, and thus the health consequences for soils, plants, and humans (Rosas-Castor et al. 2014). Arsenic occurs in a variety of different inorganic and organic species. Arsenate (As^{V}) and arsenite (As^{III}), the two most predominant inorganic As species (inAs) in soil and aquatic environments, can be methylated by soil microbes to organic As species (orgAs), e.g., methylarsonic acid (MMA^{V}), dimethylarsinic acid (DMA^{V}), and trimethylarsine oxide (TMAO), which are about 10 to 60 times less toxic than inAs (Thirunavukkarasu et al. 2002). Redox transformation and cycling among different As species characterize its toxicity (Wagner et al. 2020; Borch et al. 2010). In general, the toxicity of As compounds decreases as follows: inAs > DMA^{V}, MMA^{V} > TMAO (Khairul et al. 2017; Di et al. 2019). Arsenic speciation has been studied in numerous terrestrial plants, with inAs being the most abundant species also in maize roots, stem, and leaves (Ruiz-Chancho et al. 2008; Yu et al. 2009). Whereas orgAs represent only small amounts (< 1%) (Yu et al. 2009) or are below the respective limit of detection (LOD) (Rosas-Castor et al. 2014). However, orgAs were the major As species identified in maize

grains, accounting for 61% of total As (totAs) concentration in the grains in a pot experiment with As^{V} treated soils (CI et al. 2012).

Soil microbes can help to mitigate the toxic effects of As in soils (Hasanuzzaman op. 2018; Li et al. 2018; Afroz et al. 2019; Cavalca et al. 2019; Li et al. 2009a; Turpeinen et al. 2002; Pandey et al. op. 2018) by influencing the reduction, oxidation and methylation reactions that leads to the formation of different As species (Jia et al. 2013). The soil indigenous microbes can remediate As toxicity by reducing As^{V} in their cells to As^{III}, which is removed from soil water *via* anaerobic bioprecipitation as sulfide minerals (Wang et al. 2020; Rios-Valenciana et al. 2017). Soil microbes, such as plant growth-promoting microorganisms (PGPMs), can promote As removal from contaminated soils through bioleaching and volatilization to arsines and subsequently reduce its accumulation in plants (Roychowdhury et al. 2018; Tran et al. 2020; Turpeinen et al. 2002). The PGPMs indirectly enhance the biomass production of plant roots and stem and the fitness of host plants through various mechanisms, including solubilizing metal phosphates, increasing root surface area, ameliorating heavy metal stress, and increasing the release of root exudates (Rajkumar et al. 2012; Xiao et al. 2020; Etesami 2018). Root exudates contain components that can attract beneficial microbes (e.g., phenolics, organic acids, and sugars), restrict the transport of toxic metals through roots (e.g., small peptides) (López-Bucio et al. 2000), and prevent toxic metals from entering the cell symplast of roots by chelating them in the rhizosphere or apoplast (Arora and Jha 2019). Root exudations may favor bacteria that synthesize efficient exopolysaccharides that increase soil aggregations around roots, which in return increases the rhizosphere soil mass, promoting plant health under As stress (Mahmood et al. 2014).

Soil sterilization can inhibit plant growth for both biotic and abiotic reasons with the presence of heavy metals in the environment. The primary consequence of soil sterilization is the elimination of soil indigenous microbes including PGPMs, which can reduce the number of viable microbial cells by two to three orders of magnitude (Blankinship et al. 2014). Nonetheless, after soil sterilization, they can rapidly recolonize and recruit a new microbial community with a lower diversity (Mahmood et al. 2014; Marschner and Rumberger 2004). Soil sterilization can also modify soil physicochemical properties by increasing organic carbon content *via* nutrient release (Boyd 1971), decreasing soil pH due

to the release of organic acids (Skipper and Westermann 1973; Razavi and Lakzian 2007) (Chapter II), causing phosphorus (P) deficiency by eliminating symbiotic mycorrhizaeinvolved in P absorption (Wallace et al. 1973), and mobilizing As by altering its sorption behavior on soils (Dao et al. 1982; Razavi and Lakzian 2007).

Metal accumulation in plants is expressed by the bioconcentration factor (BCF) and the bioaccumulation coefficient (BAC). In the context of this study, BCF represents the As concentration in roots relative to that in soils. The BAC describes the ratio of As concentration in the harvestable plant part to that in soils. Additionally, the translocation factor (TF) describes As translocation in plants, i.e., the As concentration in the aerial tissues compared to that in roots. Tolerant plant species tend to restrict As transfers through soil-root and root-stem, resulting in significantly less As accumulation in plants (Finnegan and Chen 2012), whereas hyperaccumulators actively take up and translocate metals into the aerial tissues (Yoon et al. 2006). Maize is generally classified as a tolerant plant to heavy metals (BAC < 1) and as an As excluder with a low capacity to translocate metals (TF < 1) (Abbas and Abdelhafez 2013; Fellet et al. 2007; Rosas-Castor et al. 2014; Armienta et al. 2020). Arsenic is known to be mainly accumulated in maize root once taken up (Yang et al. 2021; Zhao et al. 2018; CI et al. 2012). Similar As concentrations are observed in maize stem and leaves, being 0.16 - 5.71% of the As concentration in soils, while in a few cases higher As concentrations are detected in the stem (up to 23.33% of soil As concentration). The translocation of As to grains is lowest and As concentrations in maize grains range from 0.04 to 5.00% of the concentration in soils (Neidhardt et al. 2012; Cao et al. 2019; Rosas-Castor et al. 2014).

In our previous study (Chapter II), the same greenhouse pot experiment was conducted to analyze the interaction effects of microbial disturbance, plants and As treatment on the concentration and speciation of As in the soil water. Since As uptake to crops is of high relevance for As exposure to humans, we aimed to further study the interaction effects of microbial disturbance and As treatment on the bioavailability and speciation of As in the plants of a soil-plant system. With this greenhouse pot experiment we intend to answer the following research questions: 1) What are the differences in the accumulation and translocation of As species in maize tissues at different soil As concentrations (As treatment)? 2) How do the microbial disturbance and sterilization effects influence the accumulation and translocation of As species in maize tissues? 3) If we observe a reduction in dry biomass, which As species in maize would be responsible for this? 4) What would be the environmental relevance for this study?

2 Materials and Methods

The soil (silty loam) was taken from the uppermost 20 cm of an agricultural site in Frauenkappelen, Switzerland by a soil recycling company (Kästli Bau AG). The soil pile was then stored outside the greenhouse (Ostermundigen, Switzerland) of the Institute of Plant Sciences at the University of Bern. For this greenhouse pot experiment, about 800 kg of soils were sampled from both sides of the pile to ensure homogeneity and sieved to 1 cm. This experiment comprised nine experimental groups: three soil treatments (native soil (NS), reconditioned soil (RS) and disturbed soil (DS)) × three As treatments (As_0, As_{100} and As_{200}, addition of 0, 100 and 200 mg As kg^{-1} soil), with ten replicates in each group (Figure III-1). The soils in the As_0 group were without the addition of As and had a natural As concentration of 2.91 ± 0.54 mg kg^{-1}. For As_{100} and As_{200} groups, around 400 kg of soils were spiked with sodium arsenate ($Na_2HAsO_4{\cdot}7H_2O$, ≥ 98.0%; Sigma-Aldrich®, CH) to enrich an additional 100 and 200 mg kg^{-1} As in soils. The soils were incubated at room temperature for two months at 50% water holding capacity (WHC), allowing for As equilibration between soil water and soil phases and simulating soil aging (Song et al. 2006).

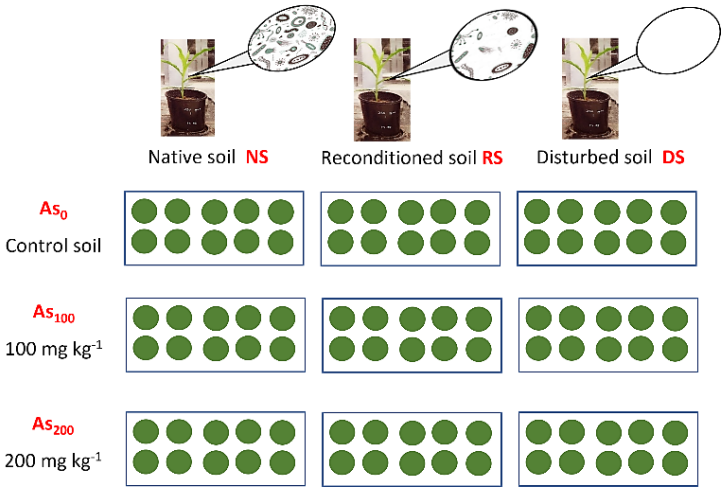


Figure III-1. Overview of the experimental design.

Soils in the three As treatments were then subdivided into three subgroups for the three soil treatments (NS, RS, and DS). The first subgroup was kept untreated and named as NS. The second and third subgroups were sterilized by X-ray (25 kGy minimum to 60 kGy maximum at Synergy Health Däniken AG, Switzerland). After soil sterilization, the second subgroup was reconditioned with microbial extracts from NS and designated as RS. The third subgroup was left without microbial reconditioning and referred to as DS. The microbial extracts for the RS treatment were obtained by entirely mixing 70 kg of native soils with 70 L of Milli-Q water (>

18.2 MΩ·cm at 25°C) in a pre-sterilized concrete mixer (sterilized with ethanol and a gas burner) (Figure S1.1). The solutions were left to stand for 2 h and filtered through a 250 µm stainless sieve and 25 µm 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) and allowed 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). Due to the presence of microbes in the greenhouse, DS was not assumed to be free of microbes but to have a disturbed microbial composition.

The sterilization effect was the same in DS and RS, while the microbial disturbance was partly eliminated in the RS treatment due to the reconditioning of microbial extracts. Therefore, it is assumed that the difference between RS and DS showed the microbial disturbance effect, and the difference between NS and RS reflected the sterilization effect. The detailed characterizations of NS and DS can be found in Table S1.1. All soils were adequately homogenized and decanted into 117 pots. 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 were cultivated from April to September 2019. More information on the sampling and analysis of soil water can be found in our previous study (Chapter II).

Maize seeds (*Zea mays* L., W22 genotype) were were soaked for 6 minutes in a commercial bleach solution followed by 6 washes and an 8-hour soak in autoclaved MilliQ-water (> 18.2 MΩ·cm at 25°C). Before sowing, one week after soil sterilization, seeds were placed overnight in plastic Petri plates with moist filter papers. 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 randomly placed in the greenhouse. In the beginning, plants were watered weekly by weighing pots and adjusting the WHC to 60%. From the third month of growth, they were watered more frequently. The weekly fertilization of maize plants started with 100 mL of 2 g L^{-1} complex fertilizer (Plantaktiv Starter 151, Hauert®) plus a 0.25 g of low iron supplement (Sequestrene Rapid, Maag®), increasing to 200 mL complex fertilizer after one month with a 0.5 g of high iron supplement. The complex fertilizer mainly contains 52% phosphate (P_2O_5), 10% total nitrogen (8.4% NH_3-N and 1.4% NO_3-N), and 10% potassium oxide (K_2O). Maize plants were cultivated in the greenhouse with 14 h of light each day and a temperature of 18 - 26°C during the day and 16 - 24°C at night. The greenhouse cabin is

heated in case of temperatures below 18°C during the day and below 16°C at night. The cooling system automatically turns on if the temperature exceeds 26°C. The ventilation system turns on once the temperature is over 22°C in the daytime or over 20°C at night. The humidity ranged from 30 to 60%.

Additionally, a side experiment was conducted to estimate the fresh biomass of maize during growth while maintaining the same WHC in soils by controlling the weight of the pots. In this experiment, 60 maize plants were grown for five months and three of them were harvested weekly to determine their fresh biomass. Plant images were simultaneously recorded to derive the green pixels area of plant leaves. Therefore, a linear model could be built between the calculated biomass and the leaf area to estimate the plant's actual fresh biomass (Figure S1.2) (Neumann et al. 2015; Valasek and Thomasson 2016). The estimated fresh biomass was then applied to calculate the amount of irrigation water and correct pot weight to retain 50% of WHC.

After a half year of growth, plants were harvested individually as roots, stem, leaves, and cob. The root samples were carefully dug out from the soils, washed with Milli-Q water, air-dried, and stored at room temperature. Grains were peeled from the cob to be analyzed for As concentration and speciation. All plant material was oven-dried at 70°C and then weighted for their dry biomass. Afterwards, the plant tissues were ground to powder in a Retsch MM400 Mixer Mill (Fisherbrand™, Waltham, MA). 0.25 g of ground plant powder was mixed with 4 mL of 65% (w/w) nitric acid (HNO_3; VWR®, FR) and 2mL 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 (Microwave Digestion System MARS™ 6; CEM GmbH, Kamp-Lintfort, DE) (Norton et al. 2013). The temperature program was as follows: ramp from room temperature to 50°C, hold at 50°C for 10 min, ramp again to 95°C, and hold at 95°C for 30 min. After digestion, the solutions were diluted to 50 mL with Milli-Q water and stored at 4°C and centrifuged at 2500 rpm for 5 min (Multifuge™ X1 Centrifuge Series, Thermo Scientific™, Reinach, CH) before transfer to 15 mL centrifuge tubes for the analysis by inductively coupled plasma mass spectrometer (ICP-MS; 7700x Agilent Technologies, Santa Clara, CA). The multielement analysis included totAs or As, B, Al, V, Cr, Mn, Co, Ni, Cu, Zn, Ga, Se, Rb, Ag, Cd, Cs, Ba, Ti, Pb, U. Triplicates of certified reference material (CRM) and blank were digested and measured together with the plant samples. In multielement analysis, the CRM ERM®- CD281 Rye grass and the Standard Reference Material® 1573a Tomato

leaves were used (certified As concentrations of 0.042 ± 0.010 mg kg^{-1} and 0.112 ± 0.004 mg kg^{-1}, respectively). The recovery percentages of acid digestion were 82 ± 13% (n = X) for Rye grass and 124 ± 15% for Tomato leaves (n = X, Table S3.1).

In As speciation analysis, 0.2 g of ground plant tissues were mixed with 4.8 mL of 1% (w/w) nitric acid (HNO_3; VWR®, FR) and 0.2mL of 30% (w/w) Suprapur H_2O_2 (Sigma-Aldrich®, CH), left for at least 30 min at room temperature before conducting the open-vessel microwave digestion described above (Norton et al. 2013). After extraction, samples were centrifuged at 2500 rpm for 5 min, filtered with a 0.22 µm hydrophilic Polytetrafluoroethylene Filter (13mm syringe filter, BGB®, CH), diluted if needed, and stored at 4°C (less than one week) before the analysis with high-performance liquid chromatography (HPLC; 1200 Infinity, Agilent Technologies, Santa Clara, CA) coupled to ICP-MS (HPLC-ICP-MS). The separation of As species was achieved using a Hamilton PRP-X100 anion-exchange column (4.1 × 50 mm, 5 µm). The column recovery was 96 ± 17% (n = 153). The operating parameters for HPLC were adapted from the literature (Jackson 2015) and listed in Table S1.2. Due to the addition of H_2O_2 and HNO_3, all trivalent As species were oxidized and the determined As species were all pentavalent. They included As^V as inAs and MMA^V, DMA^V, and TMAO as orgAs. The identification of TMAO was later demonstrated by the cation exchange HPLC-ICP-MS (Figure S1.3). Triplicates of CRMs and blank were digested together with the plant samples. The CRM ERM®- BC211 Rice was utilized in As speciation analysis with a certified DMA^V concentration of 119 ± 13 µg kg^{-1} and a certified sum concentration of As^{III} and As^V of 124 ± 11 µg kg^{-1}. The recovery of these As species after acid extraction of the CRM Rice were 101 ± 17% for inAs (n = 26) and 108 ± 9% for DMA^V (n = 26) (Table S3.1).

In this study, all data were processed on a dry weight basis and their statistical analysis were performed in R software (version 1.2.5033) including the following packages: car, multcomp, emmeans and vegan. The concentrations of total As (totAs) and total dry biomass (Table S3.2) were Log10-transformed and square-root transformed, respectively, in order to improve normality and analyzed using linear mixed effects models. The effects of experimental factors: microbial disturbance (three levels: NS, DS, RS), As treatments (three levels: As_0, As_{100}, As_{200}), and tissue types (roots, stem, leaves, grains) as well as their interactions were analyzed on individual As species (Table S3.3). The interactions stand for the combined effects of the experimental factors on the response variable, e.g. totAs concentration in maize. For multiple As species (multiple response variables), the multivariate analysis of

variance (MANOVA) was applied to the comparison of multivariate sample means in maize tissues, studying the interaction effects and individual effects of the four experimental factors on individual As species (Table S3.5). Moreover, the estimated marginal means (in the *emmeans* package) were calculated for the post-hoc analysis. 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. The original data, emmeans, are listed in the supplementary document (Table S3.2, S3.6, and S3.8-S3.11). Besides, conventional (Pearson) correlation and partial correlation were analyzed to investigate the impact of an individual As species in maize on its dry biomass. Partial correlation was applied for the pairwise correlation of each individual As species (inAs, MMA^V, DMA^V, and TMAO) and the dry biomass, aiming to find the responsible As species for the reduction in dry biomass.

3 Results

3.1 Total As and As Translocation in Maize

In most cases, we found a significant interaction among the three experimental factors (microbial disturbance, As treatment, and tissue types) due to our intersectional experimental design. Total As concentration was determined in both the entire maize and in its four tissues (roots, stem, leaves, grains). The effects of microbial disturbance and As treatment on totAs concentration in the entire maize are described first. Total As concentration in the entire maize was increased by microbial disturbance ($F_{2, 66}$ = 12.157, p < 0.001) and As treatment ($F_{2, 66}$ = 226.431, p < 0.001) as well as by their interactions ($F_{4, 66}$ = 2.182, p = 0.0081) (Table S3.3). The interactions meant the combined effects of the two experimental factors (microbial disturbance and As treatment) on the response variable (totAs in the entire maize), which suggested that the effect of microbial disturbance on totAs concentration depended on the levels of As treatment and vice versa.

Total As concentration in maize tissues was significantly affected by the interactions between microbial disturbance, As treatment and tissue types ($F_{7, 195}$ = 2.727, p = 0.010) (Table S3.3). Significant interactions were also found between microbial disturbance and tissue types ($F_{7, 195}$ = 3.545, p = 0.002) and between As treatment and tissue types ($F_{5, 195}$ = 20.974, p < 0.001). Specifically, the sterilization effect (difference NS-RS) increased totAs concentrations in maize tissues in RS, i.e., in stem of the As_0 and As_{100} group, and in leaves of the As_{200} group (Figure III-2b). Moreover, there was a difference in totAs concentrations between un- and contaminated soils in different maize tissues. In contaminated soils (As_{100} and As_{200} groups for NS, RS and DS), totAs concentration decreased in the order roots > stem ≥ leaves > grains, while in uncontaminated soils (As_0 group for NS, RS and DS), totAs had a higher concentration in the leaves than the stem.

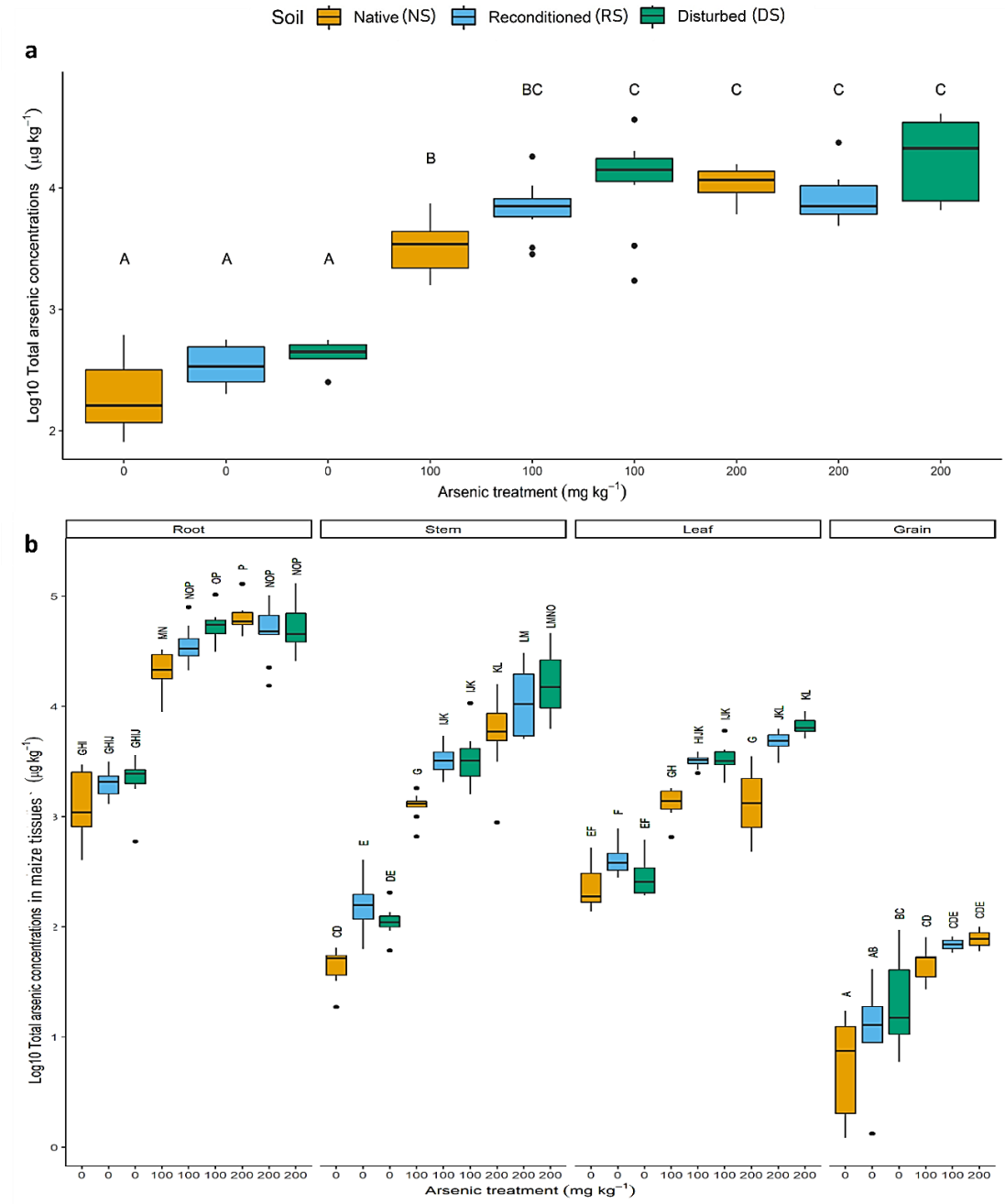


Figure III-2. Total As concentrations (totAs) (a) in the entire maize; and (b) in maize tissues. Data are mean values ± standard error. Pairwise comparisons were explored and reported using CLD letters. Different CLD letters indicated a statistically significant difference between emmeans ($p < 0.05$).

The values of BAC and BCF represent the relative translocation of As in maize and are shown in Table S3.4. Interestingly, the relative translocation of As in leaves and grains was highest in the As_0 group. The BAC values decreased as follows: leaves > stem ≥ grains in the As_0 group, stem ≥ leaves > grains in the As_{100} group, and stem > leaves ≈ grains in the As_{200} group (">" means significance at $\alpha = 0.05$). The higher the As levels in soils, the less As was

translocated into maize leaves, suggesting less As input into the essential upper tissues. Root BCF values were one order of magnitude greater than stem BAC. Arsenic relative translocation in the stem was considerably higher in the As_{200} group than in the As_0 and As_{100} groups, and the highest As accumulation in the stem reached even 31% of As levels in the roots (TF = 0.31 in DS of the As_{200} group). Leaf BAC was in the same range as stem BAC and grain BAC was one order of magnitude lower. When comparing the three soils, the relative As translocation in leaves and stem was always lower in NS (lower BAC values) than in RS and DS, regardless of the As levels in soils.

3.2 Arsenic Speciation in Maize Tissues

The inAs and three orgAs (MMA^V, DMA^V, and TMAO) were detected in all four maize tissues (roots, stem, leaves and grains) (Figure III-3, III-4 and III-5). The effects of microbial disturbance, As treatment, and tissue types on these four species are elucidated here, and their interactions had a significant effect on As speciation in all maize tissues ($p < 0.001$) (Table S3.5). The microbial disturbance effect (difference RS-DS) led to higher inAs concentrations in the stem of DS than RS in the As_{200} group (Figure III-3). Because of the sterilization effect (difference NS-RS) shown in Figure III-3, higher inAs levels were detected in the tissues of maize grown in RS than in NS: in stems of the As_{200} group, in leaves of the As0 and As_{100} groups, and in grains of the As_0 group. Noticeably, the sterilization effects were observed in the leaves of all three As groups. Overall, inAs concentrations in different maize tissues followed the same orders as totAs concentrations shown above.

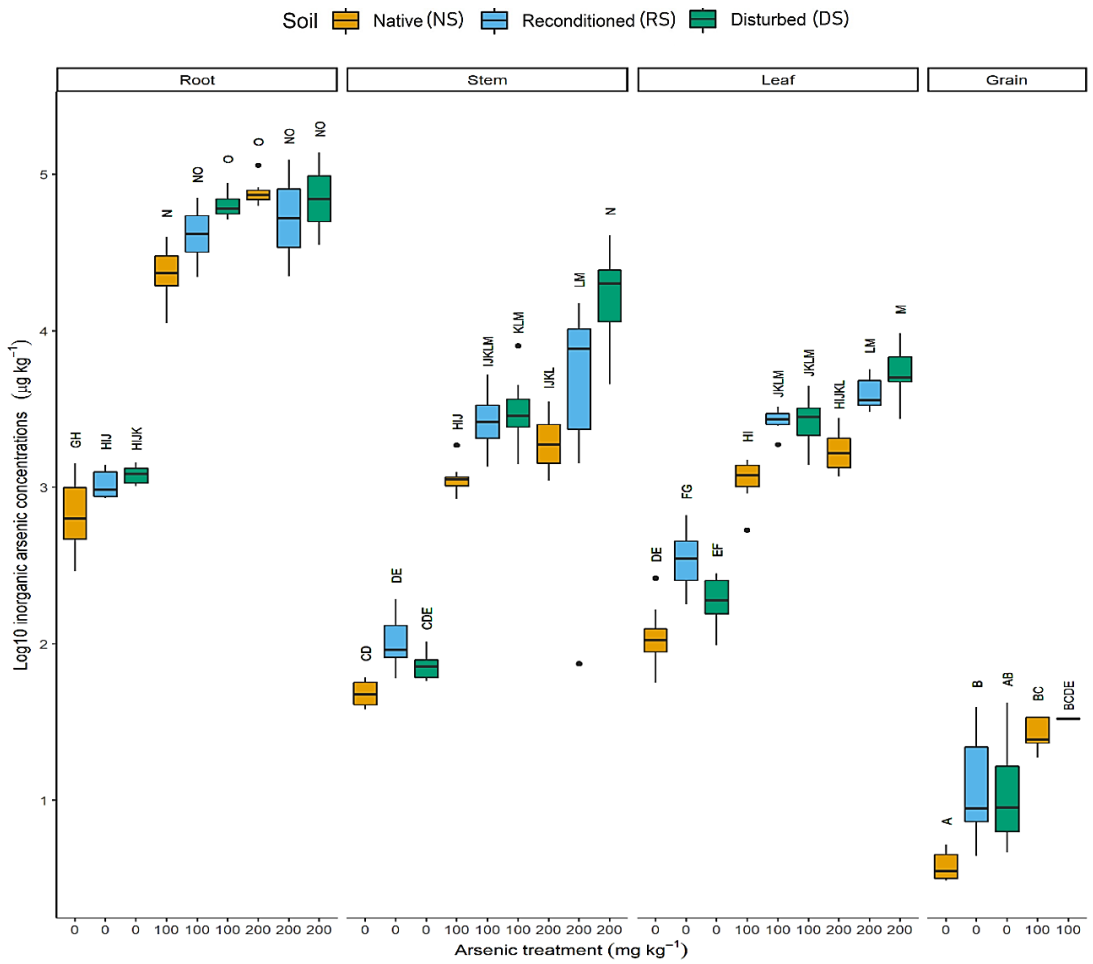


Figure III-3. The concentrations of inorganic As species (inAs) in maize tissues. Data are mean values ± standard error. Data were statistically analyzed by three-way ANOVA followed by Tukey posthoc test for comparing the means. Different letters indicate a statistically significant difference between means ($p < 0.05$).

The sum of orgAs was the sum concentrations of the three identified orgAs, i.e., MMA^V, DMA^V and TMAO. The orgAs levels in maize tissues were significantly affected by microbial disturbance ($F_{2, 195} = 36.833$, $p < 0.001$). The microbial disturbance effect resulted in higher orgAs levels in the stem of maize grown in DS than in RS at 200 mg kg^{-1}, while the sterilization effects increased orgAs levels in maize grown in RS, which was observed in stem and leaves of the As_{200} group (Figure III-4a). Moreover, orgAs levels in maize tissues showed no significant difference among the three uncontaminated soils (As_0 group for NS, RS, and DS). Whereas in contaminated soils (As_{100} and As_{200} groups), orgAs levels in maize tissues increased with increasing soil microbial disturbance (NS < RS ≤ DS; Figure III-4b). This trend was more pronounced at higher soil As concentrations, suggesting that more orgAs were taken up by maize as soil As levels increased. In addition,

orgAs concentrations in maize tissues was affected by tissue types ($F_{3, 195}$ = 310.927, $p < 0.001$), and it decreased from roots ≥ leaves > stem > grains, in contrast to what we observed for totAs and inAs. Maize in NS had higher orgAs% than in RS and DS (Figure III-4b), implying that the toxicity level of As was lowest in NS, as orgAs generally have a lower toxicity level than inAs. Regarding individual orgAs, the microbial disturbance effect was only identified for MMA^V in the stem of the As_{200} group with higher concentrations in DS than in RS (Figure S3.1). The sterilization effect caused higher concentrations in RS than in NS for all three orgAs in the leaves of the As_{200} group and for TMAO in the stems of As_{200} group.

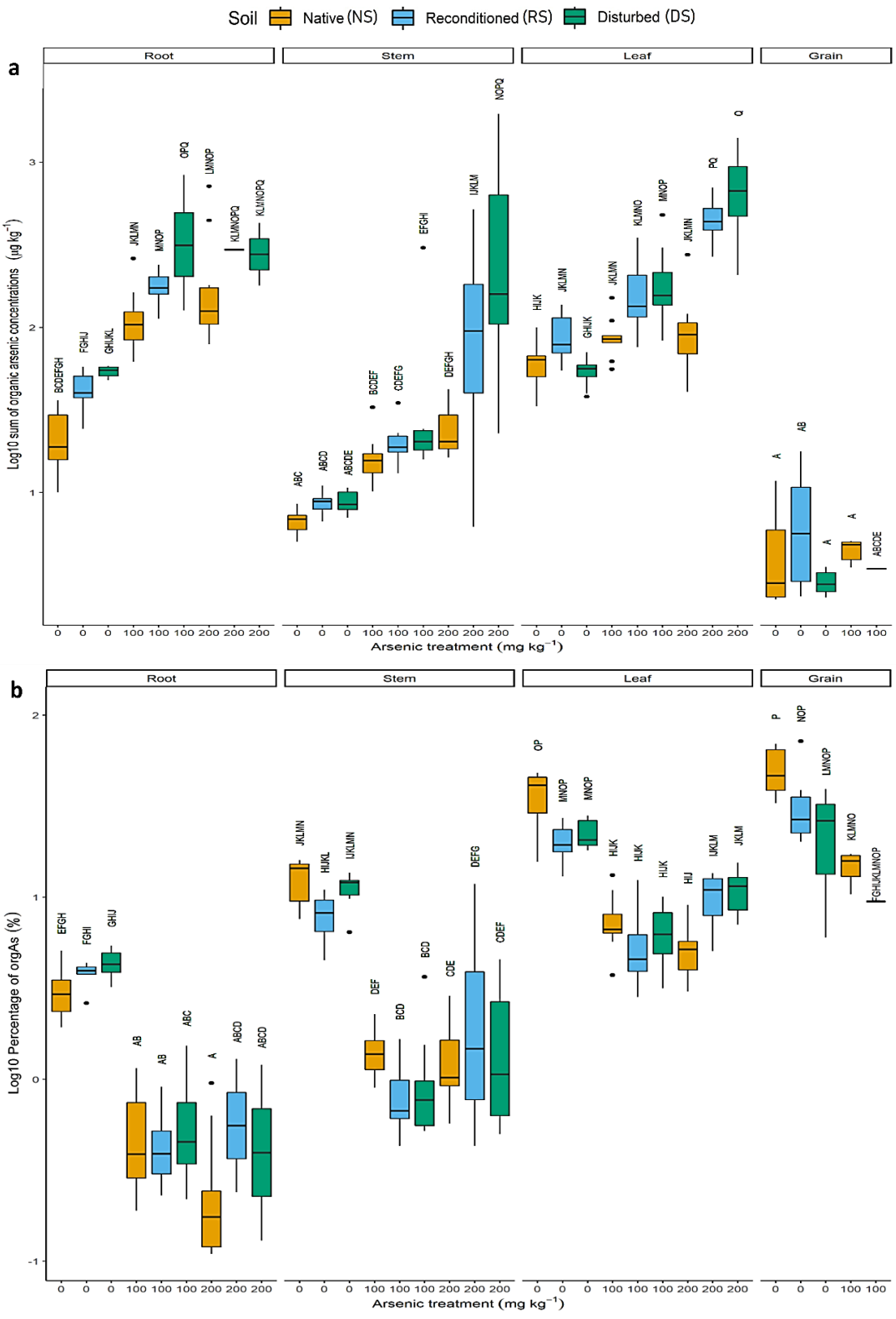


Figure III-4. The values of (a) sum of organic As species (orgAs) and (b) orgAs% in maize tissues. Data are mean values ± standard error. Pairwise comparisons were explored and reported using CLD letters. Different CLD letters indicated a statistically significant difference between emmeans ($p < 0.05$). Missing data in grains were due to the inability of maize to bear grains under high As stress and the absence of grains in cobs.

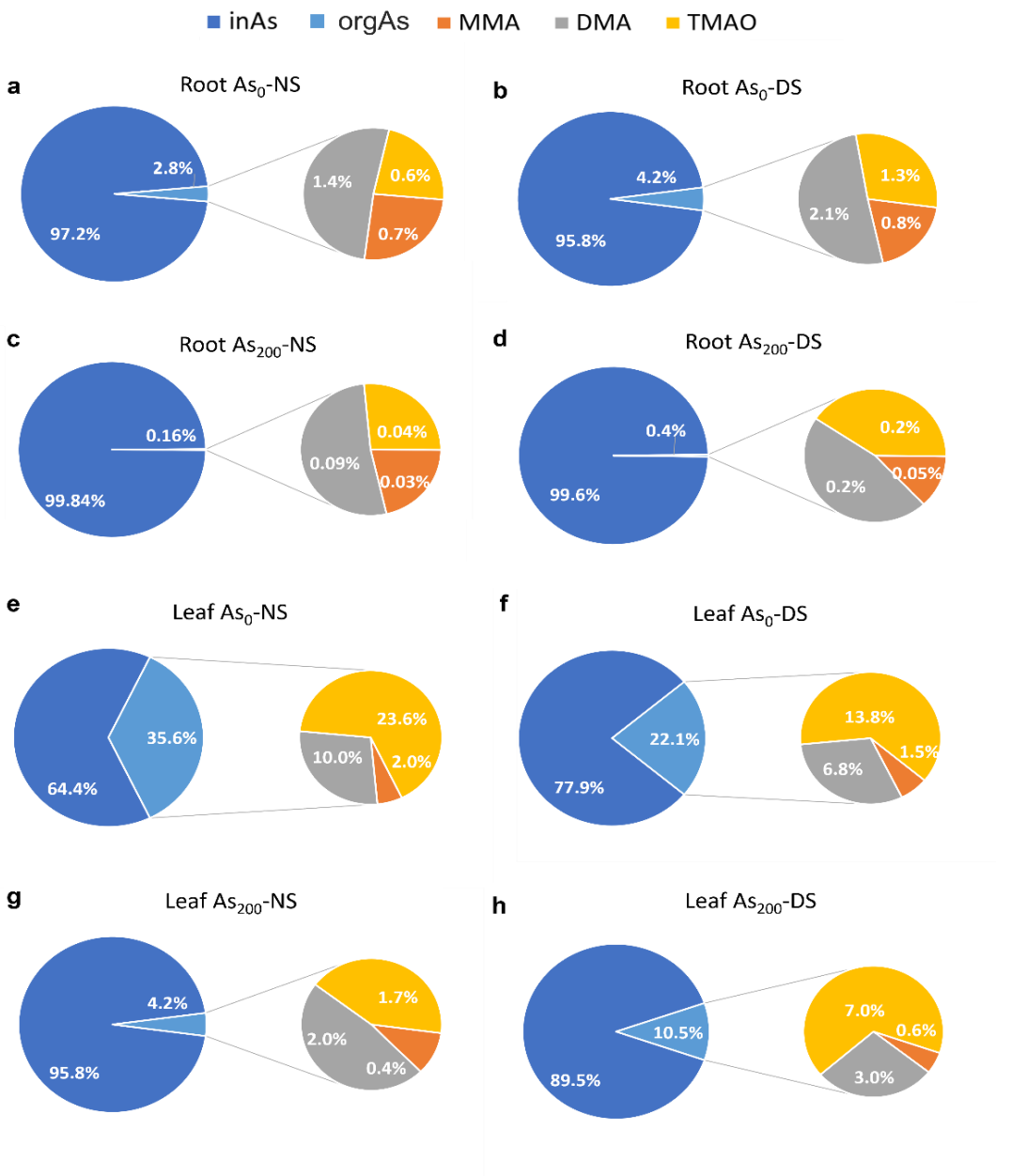


Figure III-5. The changes in As species in maize tissues with the increasing soil As levels (from the As_0 group to As_{200} group) and the increasing soil microbial disturbance (from NS to DS), presenting the percentages of inorganic As species (inAs%) and organic As species (orgAs%, i.e., MMA^V, DMA^V, and TMAO) in (a) root in NS of the As_0 group; (b) root in DS of the As_0 group; (c) root in NS of the As_{200} group; (d) root in DS of the As_{200} group; (e) leaves in NS of the As_0 group; (f) leaves in DS of the As_0 group; (g) leaves in NS of the As_{200} group; and (h) leaves in DS of the As_{200} group.

The orgAs% was exceedingly low in maize roots, being only 0.16% in the As_{200} group (Figure III-5c). As soil microbial disturbance increased, orgAs% in maize roots increased, while inAs% decreased (Figure III-5a and III-5b or Figure III-5c and III-5d). However, in

uncontaminated soils, orgAs% in maize leaves decreased with increasing soil microbial disturbance (Figure III-5e and III-5f). Furthermore, in both roots and leaves, orgAs% decreased sharply because of the increasing As levels in soils. For instance, orgAs% in maize leaves decreased from 35.6% to 4.2% in NS (Figure III-5e and III-5g). In addition, orgAs% showed an ascending trend of roots < stem < leaves < grains (Figure III-4b). Their proportions in the maize grown in NS increased from 2.8% in roots (Figure III-5a) to 35.6% in leaves (Figure III-5e) due to the rapid translocation of orgAs in plants (Raab et al. 2005a; Carey et al. 2011; Ye et al. 2010; Awasthi et al. 2017). As in the study of (CI et al. 2012), we discovered the highest orgAs% in maize grains, reaching up to 48% (Table S3.11). Besides, the primary orgAs differed in different maize tissues. In maize roots, DMA^V levels were generally higher than that of MMA^V and TMAO. On the other hand, TMAO was the primary As species in maize stem and leaves, although having the lowest level in grains.

3.3 Arsenic Correlations in Soil Water and Maize and Other Chemical Parameters

Total As concentration in soil water can be found in our previous paper (Chapter II). Total As concentration in soil water strongly positively correlated with the totAs concentration in roots ($r = 0.94$, $p < 0.001$), stem ($r = 0.97$, $p < 0.001$), leaves ($r = 0.91$, $p < 0.001$), but not in grains ($r = 0.48$, $p > 0.05$). There were also strong positive correlations between inAs in soil water and inAs in roots ($r = 0.94$, $p < 0.001$), leaves ($r = 0.93$, $p < 0.001$), and stem ($r = 0.82$, $p < 0.001$), but not in grains ($r = 0.59$, $p > 0.05$) (Figure S3.2). TMAO concentration in soil water was positively correlated with that in the leaves ($r = 0.57$, $p = 0.008$), which was not the case for MMA^V and DMA^V. This signified that only TMAO was significantly translocated from soil water to maize leaves, which might be explained by the higher TMAO levels than MMA^V and DMA^V in soil water (Chapter II). This could also be explained by the possibly different transporters of TMAO from MMA and DMA, which remained unknown.

To have a better understanding of the plant chemistry as a big picture, the redundancy analysis (RDA) was applied to explore the effects of microbial disturbance

and As treatment (experimental factors) on multielement concentrations in maize plants (response variables). This RDA model was able to explain 5.54% of the variations in the multielement concentrations. Microbial disturbance and As treatment had a significant impact on the multiple response variables ($F_{4, 222}$ = 4.316, p < 0.001) with adjusted R^2 values of 1.20% and 4.52%, respectively. This means that e.g., microbial disturbance explained 1.20% of the variations in multielement concentrations in maize plants. Parameters that point in the same direction in an RDA plot indicates their positive correlations and vice versa. The As_{200} group showed the same direction as the concentrations of As, K, and Na in maize plants (Figure III-6), indicating positive correlations between them. Contrarily, low concentrations of nutrients P, Mg, Ca, Fe, Al, and Mn were observed in the sterilized soils spiked at 200 mg kg^{-1} As, as indicated by their opposite directions in Figure III-6.

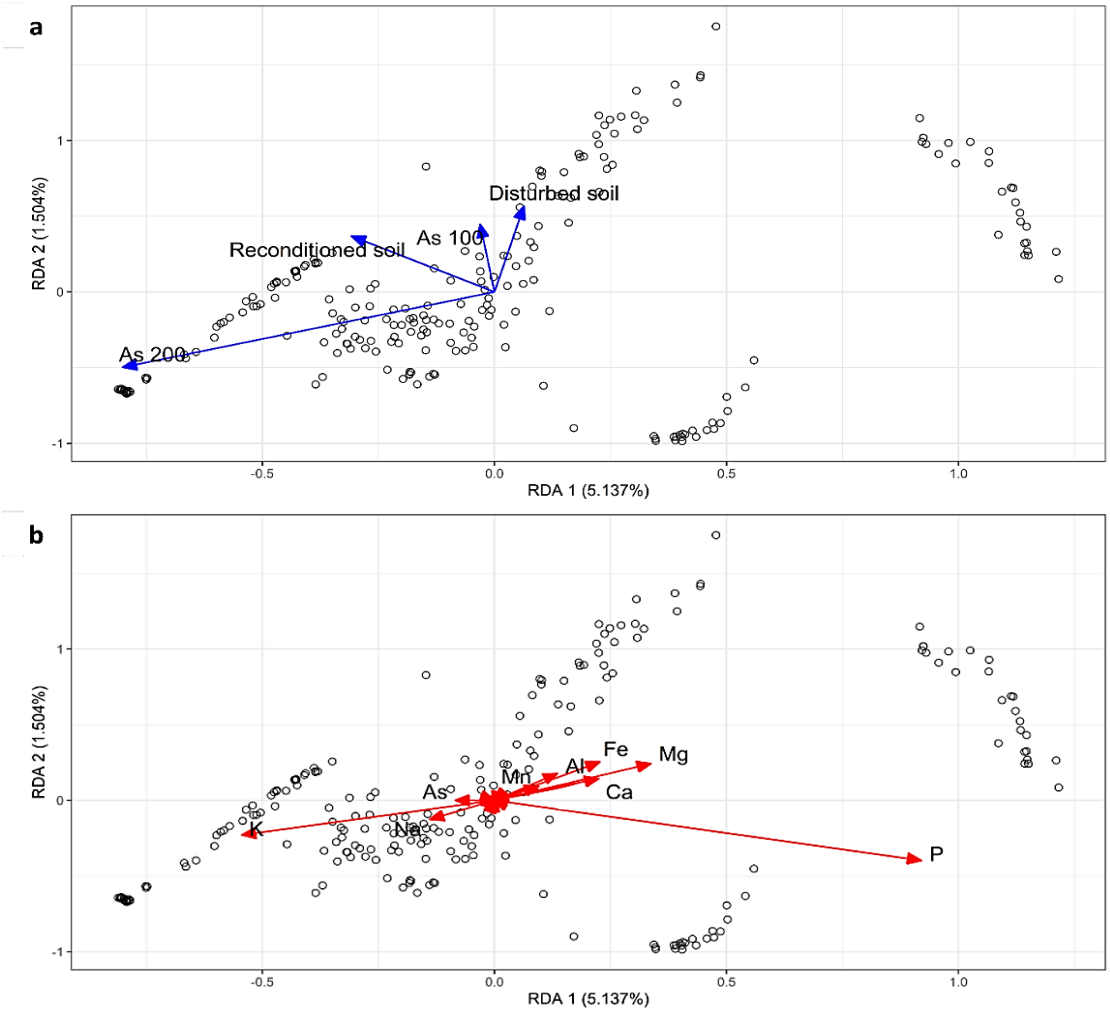


Figure III-6. Redundancy analysis (RDA) triplot showing samples as dots with a) experimental factors (microbial disturbance and As treatments), b) corresponding changes in response variables (plant chemistry parameters) in the system. The percentage of explained variance was indicated on each axis.

3.4 Dry Biomass and Its Correlation with As Species in Maize

The dry biomass in both the entire maize and maize tissues were significantly influenced by microbial disturbance, As treatment and their interaction effects ($p < 0.001$) (Table S3.3). The microbial disturbance effect (difference RS-DS) was not observed, while the sterilization effect (difference NS-RS) significantly reduced the total dry biomass of maize in the As_{100} and As_{200} groups (Figure III-7a and Table S3.3). Maize in uncontaminated soils could buffer the sterilization effect and showed no changes in the total dry biomass between un- and sterilized soils. At a soil As level of 100 mg kg^{-1}, the total dry biomass of maize grown in NS was at the same level as those in uncontaminated soils. More specifically, the dry biomass of maize grown in RS and DS was however reduced by the sterilization effect in stem, leaves and cob of the As_{100} group and only in stem of the As_{200} group (Figure III-7b). In comparison to the other tissues, stem biomass was the most reduced by the adverse effect of As. The stem biomass of maize in RS and DS decreased sharply due to the sterilization effect, which was more evident at higher As concentrations in soils.

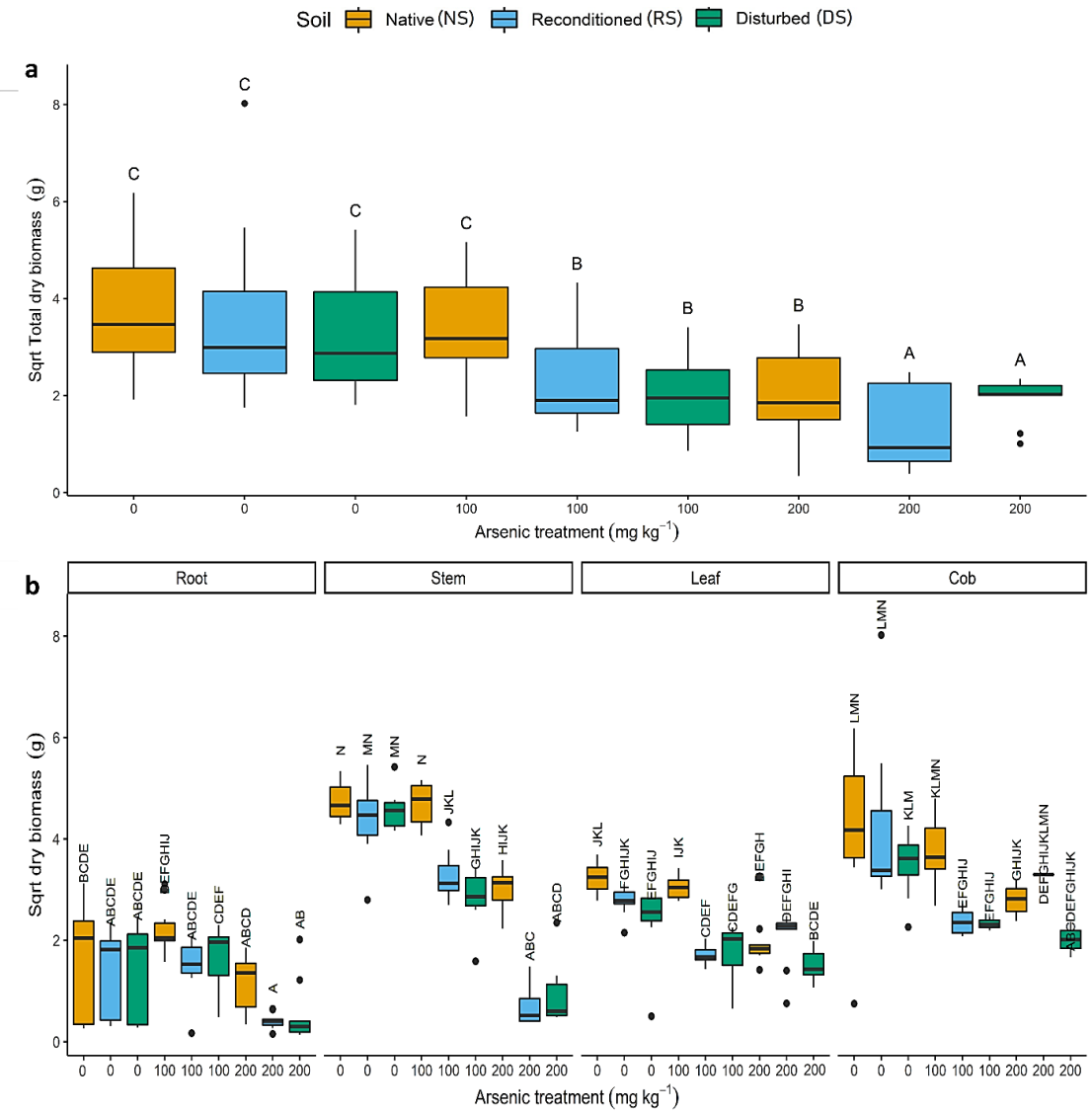


Figure III-7. The dry biomass (a) in the entire maize; and (b) in maize tissues under microbial disturbance effect and As treatments. Pairwise comparisons were explored and reported using CLD letters. Different letters indicated a statistically significant difference between emmeans ($p < 0.05$).

Both the conventional and partial correlations were performed to investigate the correlations between the total dry biomass and As species in the entire maize (Table S3.7). From the conventional correlation, we found that the total dry biomass was negatively correlated with the concentrations of inAs, MMA^V, DMA^V, and TMAO in the entire maize ($r = -0.69, -0.64, -0.67$, and -0.70, respectively; $p < 0.001$). DMA^V and TMAO had higher coefficient values in the conventional correlation than in the partial correlation (Table S3.7). This is because the conventional correlation was inflated by the other coexisting As species (MMA^V and inAs), whereas the partial correlation can separate the confounding effects of other As species in each of the pairwise correlations

with a individual As species (Sharma et al. 2019). In the partial analysis results, the total dry biomass was negatively correlated with MMA^V ($r' = -0.20$, $p = 0.003$) and inAs ($r' = -0.18$, $p = 0.008$) concentrations in maize. In summary, the total dry biomass was reduced primarily in stem and as a result of the negative effects of MMA^V and inAs in maize.

4 Discussion

4.1 Differential As Translocation in Maize at Different Soil As Concentrations

Maize plants can accumulate high levels of As in their roots and have a very slow acropetal transport of As from their roots to stems (Requejo and Tena 2006; Gulz et al. 2005). In the present study, As was accumulated greatest in the roots of maize grown in both un- and contaminated soils. Root BCF values were an order of magnitude higher than stem BAC, implying that there was a significant As uptake by maize roots. In our high-As soils, maize stem had higher BAC and TF values than leaves and grains, showing that inAs translocation to leaves and grains was restricted, probably due to an increase in chelating agents and antioxidants in maize roots and stem. The translocation efficiency of inAs can be affected by the presence of chelating agents such as glutathione (GSH) and phytochelatins (PCs) in plant roots and stem, i.e., more intracellular chelation with inAs, lower inAs translocation and accumulation in plant upper tissues (Yadav 2010; Rosas-Castor et al. 2014; Srivastava et al. 2016). Plants have evolved to produce both antioxidant enzymes (e.g., catalase and peroxidase) and non-enzymatic antioxidants (e.g., GSH and proline) (Sharma and Dietz 2009), which act as the first defense line against the radicals generated during As oxidative stress (Herrera-Estrella and Guevara-García 2009; Mittler 2002). Proline and some peroxidase can decompose radicals such as H_2O_2 and OH· (Sharma and Dietz 2009). Arsenite has a high affinity for the sulfhydryl groups (-SH) of thiol-rich peptides such as PCs and GSH and can form metal(loid)-GSH and metal(loid)-PCs complexes, which are sequestered in vacuoles to protect cellular components against As exposure (Garbinski et al. 2019; Schmöger et al. 2000). Because of their storage in vacuoles, these complexes in plant roots reduce As mobility for efflux and long-distance transport (Liu et al. 2010). Overall, we hypothesize that the translocation of inAs in our maize plants was limited to the upper tissues due to the potential presence of chelating agents in maize roots and stems.

In soils with low As levels, plants probably did not produce antioxidants as they do in high-As soils, so that As could be translocated from stem to leaves and to grains more efficiently. In line with our results, As translocation from soils or rice roots to grains is lower when rice is exposed to increased soil As levels due to the production of PCs. The PCs and As-PCs

complexes produced in rice roots reduce inAs translocation into stem and grains (Batista et al. 2014). Therefore, the translocation efficiency of As can be affected by the concentration of soluble As in soils (Rosas-Castor et al. 2014). In our maize plants, As translocation into grains tended to be lower in high-As soils than in moderate-As soils, resulting in less As accumulation in the grains of the former. Similar to this result, a previous study also found that as the concentration of soluble As in soils rises, the translocation efficiency of As from maize roots to leaves decreases, as evidenced by their declining translocation values (Mallick et al. 2011). Taken together, we assume that maize plants in uncontaminated soils or contaminated soils with As levels to which maize can adapt, did not tend to, or only to a lesser extent, control As translocation to the essential upper tissues, as they did in high-As soils. This resulted in increased As accumulation in maize leaves and grains. Maize plants in high-As soils, on the other hand, could limit acropetal translocation of inAs by increasing chelating agents and antioxidants in roots and stem, leading to low BAC and TF of leaves and grains, and thus low accumulation of inAs in maize leaves and grains.

In our study, orgAs showed the same distribution amongst maize tissues in both un- and contaminated soils, partly because they are directly transported through plant stem and formed fewer complexes than As^{III} (Carey et al. 2011). Although rice roots absorb inAs much faster than orgAs (Li et al. 2009b; Carey et al. 2011; Awasthi et al. 2017), orgAs are more mobile than inAs in the phloem and can be translocated in both phloem and xylem (Ye et al. 2010). MMA^{V} and DMA^{V}, for instance, can be readily re-mobilized from rice leaves to grains *via* the phloem. Same in our study, orgAs% increased greatly from 2.8% in roots to 35.6% in leaves in NS due to their rapid translocation and the far less efficient translocation of inAs in maize plants (Raab et al. 2005a; Carey et al. 2011; Ye et al. 2010; Awasthi et al. 2017). In addition, the increase in soil As levels has no influence on DMA^{III} translocation in rice, as their complexes with chelating agents such as DMA^{III}-peptide complexes (Kala et al. 2000; Raab et al. 2007a) are instable or less likely to be formed (Batista et al. 2014). While MMA^{III}-PC complexes have been detected in sun flower (Raab et al. 2005b) and in rice root vacuoles (Kerl et al. 2019), which might explain the lower concentration of MMA^{V} compared to DMA^{V} in our maize tissues. Pentavalent MMA and DMA are less likely to bind to PCs (Mishra et al. 2017) and only the DMA^{V}-GSH complex has been observed in Brassicaceae plants with a high sulfur content (Raab et al. 2007b). The production of tervalent As-peptide complexes begins with the reduction of pentavalent As, which can be a limiting step for thiol complexation (Mishra et al. 2017). Taken together, our results are consistent with previous reports on rice

plant. More research on maize plants is needed, because rice and maize plants may differ in some ways. On the one hand, orgAs can pass directly through the stem and be readily translocated in plants. While on the other hand, their complexes appear less likely to be formed, and some of them are very unstable, which might have resulted in the same distribution of orgAs in different maize tissues.

Since higher plants appear to lack the ability to methylate As (Zheng et al. 2013; Jia et al. 2013), orgAs in plants are known to originate from As methylation by soil microbes and following uptake by plants from soil water (Lomax et al. 2012). Thus, the reduction in orgAs% in maize grown in our contaminated soils compared to uncontaminated soils resulted from the low orgAs% in contaminated soil water. Because inAs are the most common As species in the soil environment, the fraction of orgAs in contaminated soil water can be reduced due to a higher background level of inAs in contaminated soils (Chapter II). Moreover, the proportion of orgAs also increased in the roots of maize plants grown in sterilized soils, because that microbial disturbance mobilized As into soil water and more orgAs were available in soil water to plant roots. However, the situation was different in maize leaves. In uncontaminated soils, orgAs% in maize leaves was lower in sterilized than in unsterilized soils, whereas it was higher in sterilized soils in the presence of As. This was probably because inAs translocation into the leaves of maize grown in contaminated soils was prohibited, leading to a lower inAs% and a corresponding higher orgAs% in maize leaves. Meanwhile, when contaminated soils were sterilized, a higher percentage of orgAs were mobilized into soil water and taken up by maize plants, which then efficiently translocated into maize leaves. Organic As species in soil water has been found correlated negatively with the copy number of Streptomyces As methylating gene (arsM), which is responsible for the methylation and volatilization of As from bacteria (Zhao et al. 2013a). We hypothesized that arsM was less available in our sterilized soil water due to soil sterilization, resulting in higher concentrations of orgAs in sterilised soil water and subsequent uptake by maize plants as well as higher levels of orgAs in their tissues (Chapter III). On the other hand, soil microbes can also demethylate As (Lehr et al. 2003; Yoshinaga et al. 2011). The As-demethlyting microbes might have been eleminated by soil sterilization, which prevented the conversion of orgAs into inAs, resulting in higher levels of orgAs in the sterilized soil water.

4.2 Microbial Disturbance Effects on As in Plants

In our study, the microbial disturbance effect was observed only in the stem, where maize grown in DS had higher concentrations of inAs, orgAs, and MMA^V than maize in RS. Contrary results have been reported, indicating that rice cultivated in sterilized soils accumulates less As than rice in unsterilized soils, which is ascribed to the differences in soil bacterial communities (Huang et al. 2021). Certain bacterial taxa such as PGPMs are found in higher abundance in sterilized soils, and their relative abundance is negatively correlated with As concentrations in grains (Huang et al. 2021). Despite that this finding contradicts ours, the role of PGPMs in As mitigation in rice plants is in line with our findings. Soil indigenous microbes such as PGPMs can promote the bioleaching and volatilization of As from contaminated soils (Roychowdhury et al. 2018; Tran et al. 2020; Turpeinen et al. 2002). By enhancing root activity and reducing radial oxygen loss, PGPMs stimulate rice growth and increase As sequestration within roots (Huang et al. 2021). They can also secrete antioxidant enzymes or stimulate plants to synthesize antioxidant enzymes in order to prevent or minimize As oxidative damage to plants and themselves (Kavita et al. 2008).

Since the foremost consequence of soil sterilization is the elimination of soil indigenous microbes, it is possible that more beneficial indigenous microbes were present in our unsterilized soils than in sterilized soils. Although soil sterilization eliminated some pathogens, it also killed beneficial microbes in soils like PGPMs, and thus disrupted their possible beneficial effects on the host plant (Ochieno 2022). In contaminated soils, soil sterilization may be unfavorable as it reduced microbial efficiency in As control and mitigation by eliminating soil indigenous microbes. Soil sterilization alters microbial composition and inhibits microbial activity significantly (Mahmood et al. 2014; Marschner and Rumberger 2004). Microbial activity has found to be negatively correlated with As mobility, i.e., As release from soils would be high at a low microbial activity (Kumpiene et al. 2007). ϒ-sterilization can cause damage to proteins by ionizing radiation, disrupting enzyme activity, and halting microbial exoenzyme production (Blankinship et al. 2014). Therefore, it is suspected in our study that the presence or higher relative abundance of soil indigenous microbes in unsterilized soils might have contributed to lower As accumulation in maize plants.

In our uncontaminated soils, the concentrations of totAs and inAs followed a pattern of roots > leaves > stem > grains, but a distinct pattern of roots > stem ≥ leaves > grains in contaminated soils. We hypothesize that the increased As accumulation in roots and the decreased As translocation to upper tissues may be a mutual attempt by maize plants and soil microbes under As stress to mitigate the deleterious effects of As through their interactions. A similar hypothesis has been put forward, claiming that plants susceptible to As toxicity tend to limit As translocation from roots to stem, possibly as a survival mechanism (Caporale et al. 2013). Plant-microbe interactions have reciprocal effects on both partners and play an important role in their adaptation and survival in stressed environments (Berg 2009). Plants and microbes are both capable to produce phytohormones such as auxin to stimulate plant growth and nutrition. Auxin-producing bacteria can not only increase plant nutrient uptake by proliferating plant roots, also loosen plant cell walls to increase the amount of root exudates and thereby additional nutrients to sustain microbial growth (Vacheron et al. 2013). Auxin has been shown to be reduced by half after soil sterilization (Lu et al. 2022). In our experiment, soil sterilization might have also killed beneficial soil microbes such as PGPMs and auxin-producing bacteria and disrupted their interactions with the host plant, losing their beneficial functions in promoting plant growth and potential resistance to As stress. Taken together, eliminating soil indigenous microbes through soil sterilization could disrupt plant-microbe interactions and negate the beneficial effects of soil microbes that promoted plant health and assist plants to cope with As stress.

4.3 Sterilization Effects on As in Plants

In the current study, P deficiency was observed in sterilized soils of all three As groups (Figure S3.3), lower than the required amount in plant stem (2000 mg kg^{-1}) for adequate growth (Kirkby 2012). Previous research has reported P deficiency after soil sterilization due to the elimination of symbiotic mycorrhizae involved in P absorption (Wallace et al. 1973). Phosphorus deficiency may have a negative impact on plant growth and health due to either a decrease in photosynthesis or an increase in energy investment (Malhotra et al. 2018). Plants can undergo various morphological, physiological, and biochemical adaptations under P deficiency conditions (Malhotra et al. 2018). Nonetheless, in our study, P efficiency resulting from soil sterilization had no effect on the dry biomass of maize grown in uncontaminated soils, suggesting that maize plants were resilient to P deficiency in the absence of As stress, with no modification of the morphological trait, i.e.,

dry biomass. In the presence of As in the environment, however, P deficiency caused by soil sterilization might have significantly reduced the dry biomass of our maize. Due to similar chemical structures and shared phosphate transporters, low P can result in more transporters being available for As and more As being taken up by plants (Wu et al. 2022). Due to the sterilization effect, we observed higher As accumulation, including totAs and all measured As species in our maize cultivated in sterilized soils than unsterilized soils (NS < RS). Therefore, in our maize grown in contaminated soils, the induced P deficiency by soil sterilization exacerbated the reduction in dry biomass and potentially increased As accumulation in maize plants. Further, the unfavorable impact of microbial disturbance and As treatments on maize dry biomass was enhanced by their interactions.

When compared to other tissues, the stem was most affected by the negative effect of As, probably because the concentrations of inAs and orgAs in the stem were increased by both the microbial disturbance and sterilization effects. Same findings have been reported that crops cultivated in sterilized soils grow much worse than those on unsterilized soils, and the dry biomass production of stem in different crops is significantly depressed at a low P supply, which is particularly noticeable in sterilized soils (Ortas 2003). Moreover, As translocation to stem in this study was higher than in previous studies at comparable soil As concentrations, which reported lower BAC and TF values in the stem (Drličková et al. 2013; Gulz et al. 2005). While the available As in soil water (4.70 mg kg^{-1}) was higher than in our experiment (2.16 mg kg^{-1}) as well as root BCF value (2.0) was higher than ours (0.3, As_{200} group), their stem TF value (0.005) was significantly lower than ours (0.191, As_{200} group) (Gulz et al. 2005). The high As translocation into the stem might explain the observed highest reduction in the dry biomass of stem. Furthermore, although As accumulated more in our maize roots than in stem, the stem was substantially more susceptible to As than the roots. This is because that antioxidant defense mechanisms do not appear to be formed in the stem, making the stem less effective than the roots in defending against As (Requejo and Tena 2006). In total, we observed that both the microbial disturbance and sterilization effects increased As levels in the stem as well as a high As translocation into the stem, which might explain why the dry biomass of stem was reduced more than the other tissues.

4.4 Correlation Between As in Soil Water and in Maize

Arsenic methylation process is known to follow the sequence from As^{III} to orgAs, e.g. MMA, DMA and TMAO, and we found the same concentration sequence in our maize plants (MMA^V < DMA^V ≤ TMAO). It can be speculated that As methylation took place actively in our soils, resulting in a higher accumulation of TMAO and DMA^V in maize than MMA^V. Moreover, our partial correlation analysis revealed that the total dry biomass of maize was mainly influenced by inAs and MMA^V concentrations in maize. It is not surprising that inAs was one of the responsible species, given that they have the highest concentration in maize and are more toxic than orgAs (Khairul et al. 2017; Di et al. 2019). The uptake of MMA^V and DMA^V is mediated by the influx transporter OsLsi1 with a much lower efficiency than As (Du et al. 2019; Li et al. 2019). MMA^V and DMA^V are stated to possess higher toxicity levels than TMAO (Khairul et al. 2017; Di et al. 2019), which might explain why the presence of TMAO in maize was not associated with the reduction in total dry biomass of our maize. In addition, the uptake rate of MMA^V by rice is reported higher than that of DMA^V (Abedin et al. 2002; Meharg and Hartley-Whitaker 2002). More research is needed to determine whether the rapid translocation of MMA^V in plants led to the positive correlation with the reduction in maize biomass.

4.5 Environmental Relevance and Implications

The lowest limit set by the European Union (EU) for As in complete feed and feed materials is 2 mg kg^{-1} (Adamse et al. 2017). Arsenic concentrations in our maize roots exceeded the 2 mg kg^{-1} limit, whereas As levels in the stem and leaves of maize grown in uncontaminated soils were below the limit. As a result, maize grown in soils with As levels higher than 100 mg kg^{-1} may produce fodders that exceed the safety limit for animals, posing a health risk to humans if the animal flesh is consumed. Moreover, some countries and authorities, such as China, World Health Organization (WHO), and the EU have established limits for arsenic in human food (0.2 mg kg^{-1} of inAs). The EU advised an even lower concentration of inAs for infants and young children (0.1 mg kg^{-1}) (WHO 2018). The concentrations of inAs in our maize grains were lower than the limit for infants and young children, indicating a low environmental exposure risk to humans when maize plants are grown in soils containing 100 mg As kg^{-1} soils (data in soils at 200 mg kg^{-1} remain unknown due to fruitless grains). Due to low acropetal transport to upper tissues and low As levels in grains, maize is considered a suitable replacement crop for As-contaminated farmlands (Guo et al. 2019), which was also demonstrated in our study. In comparison to maize as an As excluder (Abbas and Abdelhafez

2013), rice is regarded as an exceedingly high As accumulator (Nath et al. 2014). More than half of the 20 rice genotypes can accumulate As in their grains above the limit of 0.3 mg kg^{-1} permitted by the Codex Alimentarius, even up to 2.2 mg kg^{-1} (Tuli et al. 2010). Therefore, the cultivation of maize plants instead of rice species in high-As soils is recommended.

5 Conclusion

In contaminated soils, plant-microbe interactions tended to limit the translocation of inAs to maize essential upper tissues, probably due to an increased peptide formation and corresponding complexation and sequestration of inAs in maize roots and stem. Conversely, the translocation of orgAs was not reduced at high soil As levels, as they have a higher translocation efficiency in plants than inAs due to their direct transport through the stem and less likely formation and instability of As-complexes. Furthermore, in high-As soils, both the microbial disturbance and sterilization effects increased As concentrations in the stem of maize grown in sterilized soils, with even higher relative translocation of As (higher BAC and TF values) than the leaves. Both results may explain why the dry biomass of the stem was reduced by As more than the other tissues. Moreover, in the absence of As, soil sterilization had no effect on the dry biomass. When As was present in the environment, due to P deficiency caused by the sterilization effect, the reduction in dry biomass was larger in maize grown in sterilized than unsterilized soils. This study completed the study of As in the soil environment in our previous research (Chapter II) and highlighted the significance of plant-microbe interactions in reducing the translocation and accumulation of inAs in maize upper tissues, which may allow for a better understanding of As uptake, translocation, speciation, and detoxification in the systematic soil-plant system. To complete the systemic research of As in the soil-plant system, research on the morphological and physiological response of maize plants is still awaited.

Associated Content

Supporting Information

Additional information: *I Materials and Methods*, including the chemical characterizations of Q-matte soil (Table S1.1); the preparation of microbial extracts (Figure S1.1); the maize biomass estimating model (Figure S1.2); an example of pore water sampler in pot (Figure S1.3); and the operating parameters of HPLC (Table S1.2). *II Results*, the CRMs data on totAs and As species in maize tissues (Table S3.1); the emmeans values for totAs concentration and dry biomass in the entire maize (Table S3.2); the *p* values of ANOVA on totAs concentration, As species concentrations, and dry biomass in maize tissues (Table S3.3); The bioconcentration factor (BCF), bioaccumulation coefficient (BAC), and translocation factor (TF) in maize tissues under different soil and As treatments (Table S3.4); the *p* values of MANOVA statistical analysis on As species in maize tissues (Table S3.5); the concentrations of individual orgAs (a) MMA^V; (b) DMA^V; and (c) TMAO in maize tissues (Figure S3.1); Pearson correlations of totAs and inAs concentrations in soil water (Figure S3.2); the emmeans of dry biomass in maize tissues (Table S3.6); Conventional Pearson correlations r and partial correlation coefficients r′ between As species concentrations in the entire maize and dry biomass (Table S3.7); the emmeans of totAs and As species concentrations in maize roots, stem, leaves, and grains (Table S3.8-S3.11); and the concentrations of total P in maize stem (Figure S3.3).

Acknowledgements

This research was financially supported by the University of Bern through the Interfaculty Research Cooperation One Health (https://www.onehealth.unibe.ch/). We especially thank Dr. Daniela Fischer and Patrick Neuhaus for their support in the laboratory, Valentin Gfeller for his guidance in plant cultivation, as well as Xiaowen Liu, Pia Bergamaschi, Andrea Weber, Florian Enz, and Christina Désirée Widmer for their help with plant incubation and data collection. We would also like to thank Dr. Suyi Hu for his help with data organisation.

Author Contributions

Hang Guan: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - Original draft, Visualization, Project administration. Veronica Caggìa: Investigation, Writing - Review and Editing. Andrea Gómez-Chamorro: Formal analysis, Writing - Review and Editing. Miquel Coll Crespi: Investigation. Teresa Chávez-Capilla: Methodology, Writing - Review and Editing. Alban Ramette: Conceptualization, Formal analysis, Writing - Review and Editing. Klaus Schlaeppi: Conceptualization, Resources, Writing - Review and Editing. Adrien Mestrot: Methodology, Conceptualization, Supervision, Funding acquisition, Writing - Review and Editing. Moritz Bigalke: Conceptualization, Supervision, Writing - Review and Editing, Funding acquisition, Project administration.

Reference

Abbas, Mohamed H. H.; Abdelhafez, Ahmed A. (2013): Role of EDTA in arsenic mobilization and its uptake by maize grown on an As-polluted soil. In *Chemosphere* 90 (2), pp. 588–594. DOI: 10.1016/j.chemosphere.2012.08.042.

Abedin, Mohammed Joinal; Feldmann, Jörg; Meharg, Andy A. (2002): Uptake kinetics of arsenic species in rice plants. In *Plant physiology* 128 (3), pp. 1120–1128. DOI: 10.1104/pp.010733.

Adamse, Paulien; van der Fels-Klerx, H. J. Ine; Jong, Jacob de (2017): Cadmium, lead, mercury and arsenic in animal feed and feed materials - trend analysis of monitoring results. In *Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment* 34 (8), pp. 1298–1311. DOI: 10.1080/19440049.2017.1300686.

Afroz, Hasina; Su, Shiming; Carey, Manus; Meharg, Andy A.; Meharg, Caroline (2019): Inhibition of Microbial Methylation via arsM in the Rhizosphere: Arsenic Speciation in the Soil to Plant Continuum. In *Environmental science & technology* 53 (7), pp. 3451–3463. DOI: 10.1021/acs.est.8b07008.

Anjum, Naser A.; Gill, Sarvajeet Singh; Tuteja, Narendra (2017): Enhancing Cleanup of Environmental Pollutants. Cham: Springer International Publishing.

Armienta, María Aurora; Beltrán, Margarita; Martínez, Sarayth; Labastida, Israel (2020): Heavy metal assimilation in maize (Zea mays L.) plants growing near mine tailings. In *Environmental geochemistry and health* 42 (8), pp. 2361–2375. DOI: 10.1007/s10653-019-00424-1.

Arora; Jha (2019): Microbial interventions in agriculture and environment: Volume 2: Rhizosphere, microbiome and agro-ecology. Impact of Plant-Associated Microbial Communities on Host Plants Under Abiotic Stresses. 2 volumes: Springer Nature. Available online at https://link.springer.com/content/pdf/10.1007/978-981-13-8383-0.pdf.

Awasthi, Surabhi; Chauhan, Reshu; Srivastava, Sudhakar; Tripathi, Rudra D. (2017): The Journey of Arsenic from Soil to Grain in Rice. In *Frontiers in plant science* 8, p. 1007. DOI: 10.3389/fpls.2017.01007.

Batista, Bruno Lemos; Nigar, Meher; Mestrot, Adrien; Rocha, Bruno Alves; Barbosa Júnior, Fernando; Price, Adam H. et al. (2014): Identification and quantification of phytochelatins in roots of rice to long-term exposure: evidence of individual role on arsenic accumulation and translocation. In *J Exp Bot* 65 (6), pp. 1467–1479. DOI: 10.1093/jxb/eru018.

Berg, Gabriele (2009): Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. In *Applied microbiology and biotechnology* 84 (1), pp. 11–18. DOI: 10.1007/s00253-009-2092-7.

Blankinship, Joseph C.; Becerra, Caryl A.; Schaeffer, Sean M.; Schimel, Joshua P. (2014): Separating cellular metabolism from exoenzyme activity in soil organic matter decomposition. In *Soil Biology and Biochemistry* 71, pp. 68–75. DOI: 10.1016/j.soilbio.2014.01.010.

Borch, Thomas; Kretzschmar, Ruben; Kappler, Andreas; van Cappellen, Philippe; Ginder-Vogel, Matthew; Voegelin, Andreas; Campbell, Kate (2010): Biogeochemical redox processes and their impact on contaminant dynamics. In *Environmental science & technology* 44 (1), pp. 15–23. DOI: 10.1021/es9026248.

Boyd, H. W. (1971): Manganese toxicity to peanuts in autoclaved soil. In *Plant Soil* 35 (1-3), pp. 133–144. DOI: 10.1007/BF01372638.

Cao, Xiaoxia; Bai, Lingyu; Zeng, Xibai; Zhang, Junzheng; Wang, Yanan; Wu, Cuixia; Su, Shiming (2019): Is maize suitable for substitution planting in arsenic-contaminated farmlands? In *Plant Soil Environ.* 65 (No. 9), pp. 425–434. DOI: 10.17221/155/2019-PSE.

Caporale, Antonio G.; Pigna, Massimo; Sommella, Alessia; Dynes, James J.; Cozzolino, Vincenza; Violante, Antonio (2013): Influence of compost on the mobility of arsenic in soil and its uptake by bean plants (Phaseolus vulgaris L.) irrigated with arsenite-contaminated water. In *Journal of environmental management* 128, pp. 837–843. DOI: 10.1016/j.jenvman.2013.06.041.

Carey, Anne-Marie; Norton, Gareth J.; Deacon, Claire; Scheckel, Kirk G.; Lombi, Enzo; Punshon, Tracy et al. (2011): Phloem transport of arsenic species from flag leaf to grain during grain filling. In *New Phytologist* 192 (1), pp. 87–98. DOI: 10.1111/j.1469-8137.2011.03789.x.

Cavalca, Lucia; Zecchin, Sarah; Zaccheo, Patrizia; Abbas, Ben; Rotiroti, Marco; Bonomi, Tullia; Muyzer, Gerard (2019): Exploring Biodiversity and Arsenic Metabolism of Microbiota Inhabiting Arsenic-Rich Groundwaters in Northern Italy. In *Frontiers in microbiology* 10, p. 1480. DOI: 10.3389/fmicb.2019.01480.

CI, Xiao-ke; LIU, Hua-lin; HAO, Yu-bo; ZHANG, Ji-wang; LIU, Peng; DONG, Shu-ting (2012): Arsenic Distribution, Species, and Its Effect on Maize Growth Treated with Arsenate. In *Journal of Integrative Agriculture* 11 (3), pp. 416–423. DOI: 10.1016/S2095-3119(12)60026-4.

Dao, T. H.; Marx, D. B.; Lavy, T. L.; Dragun, J. (1982): Effect, and Statistical Evaluation, of Soil Sterilization on Aniline and Diuron Adsorption Isotherms. In *Soil Science Society of America Journal* 46 (5), pp. 963–969. DOI: 10.2136/sssaj1982.03615995004600050016x.

Di, Xuerong; Beesley, Luke; Zhang, Zulin; Zhi, Suli; Jia, Yan; Ding, Yongzhen (2019): Microbial Arsenic Methylation in Soil and Uptake and Metabolism of Methylated Arsenic in Plants: A Review. In *International journal of environmental research and public health* 16 (24). DOI: 10.3390/ijerph16245012.

Drličková, Gabriela; Vaculík, Marek; Matejkovič, Peter; Lux, Alexander (2013): Bioavailability and toxicity of arsenic in maize (Zea mays L.) grown in contaminated soils. In *Bulletin of environmental contamination and toxicology* 91 (2), pp. 235–239. DOI: 10.1007/s00128-013-1035-2.

Du, Fan; LIU, Peng; Wang, Kai; Yang, Zhaoguang; Wang, Lin (2019): Influence of different arsenic species on uptake, speciation and efflux of arsenic in hydroponic rice plants. In *Ecotoxicology and environmental safety* 186, p. 109791. DOI: 10.1016/ j.ecoenv.2019.109791.

Etesami, Hassan (2018): Bacterial mediated alleviation of heavy metal stress and decreased accumulation of metals in plant tissues: Mechanisms and future prospects. In *Ecotoxicology and environmental safety* 147, pp. 175–191. DOI: 10.1016/j.ecoenv.2017.08.032.

Fellet, G.; Marchiol, L.; Perosa, D.; Zerbi, G. (2007): The application of phytoremediation technology in a soil contaminated by pyrite cinders. In *Ecological Engineering* 31 (3), pp. 207–214. DOI: 10.1016/j.ecoleng.2007.06.011.

Finnegan, Patrick M.; Chen, Weihua (2012): Arsenic toxicity: the effects on plant metabolism. In *Frontiers in physiology* 3, p. 182. DOI: 10.3389/fphys.2012.00182.

Garbinski, Luis D.; Rosen, Barry P.; Chen, Jian (2019): Pathways of arsenic uptake and efflux. In *Environment International* 126, pp. 585–597. DOI: 10.1016/j.envint.2019.02.058.

Gulz, Petra Angela; Gupta, Satish-Kumar; Schulin, Rainer (2005): Arsenic accumulation of common plants from contaminated soils. In *Plant Soil* 272 (1-2), pp. 337–347. DOI: 10.1007/s11104-004-5960-z.

Hasanuzzaman, Mirza (Ed.) (op. 2018): Mechanisms of arsenic toxicity and tolerance in plants. Singapore: Springer.

Herrera-Estrella, Luis Rafael; Guevara-García, Angel Arturo (2009): Heavy Metal Adaptation. 1st ed.: Wiley.

Huang, Lu; Wang, Xun; Chi, Yihan; Huang, Linan; Li, Wai Chin; Ye, Zhihong (2021): Rhizosphere bacterial community composition affects cadmium and arsenic accumulation in rice (Oryza sativa L.). In *Ecotoxicology and environmental safety* 222, p. 112474. DOI: 10.1016/j.ecoenv.2021.112474.

Jackson, Brian (2015): Fast ion chromatography-ICP-QQQ for arsenic speciation. In *Journal of analytical atomic spectrometry* 30 (6), pp. 1405–1407. DOI: 10.1039/C5JA00049A.

Jia, Yan; Huang, Hai; Zhong, Min; Wang, Feng-Hua; Zhang, Li-Mei; Zhu, Yong-Guan (2013): Microbial arsenic methylation in soil and rice rhizosphere. In *Environmental science & technology* 47 (7), pp. 3141–3148. DOI: 10.1021/es303649v.

Kabir, M. S.; Salam, M. A.; Paul, D. N. R.; Hossain, M. I.; Rahman, N. M. F.; Aziz, Abdullah; Latif, M. A. (2016): Spatial Variation of Arsenic in Soil, Irrigation Water, and Plant Parts: A Microlevel Study. In *TheScientificWorldJournal* 2016, p. 2186069. DOI: 10.1155/2016/2186069.

Kala, S. V.; Neely, M. W.; Kala, G.; Prater, C. I.; Atwood, D. W.; Rice, J. S.; Lieberman, M. W. (2000): The MRP2/cMOAT transporter and arsenic-glutathione complex formation are required for biliary excretion of arsenic. In *The Journal of biological chemistry* 275 (43), pp. 33404–33408. DOI: 10.1074/jbc.M007030200.

Kavita, B.; Shukla, Sapna; Naresh Kumar, G.; Archana, G. (2008): Amelioration of phytotoxic effects of Cd on mung bean seedlings by gluconic acid secreting rhizobacterium Enterobacter asburiae PSI3 and implication of role of organic acid. In *World J Microbiol Biotechnol* 24 (12), pp. 2965–2972. DOI: 10.1007/s11274-008-9838-8.

Kerl, Carolin F.; Schindele, Ruth Alina; Brüggenwirth, Lena; Colina Blanco, Andrea E.; Rafferty, Colleen; Clemens, Stephan; Planer-Friedrich, Britta (2019): Methylated Thioarsenates and Monothioarsenate Differ in Uptake, Transformation, and Contribution to Total Arsenic Translocation in Rice Plants. In *Environmental science & technology* 53 (10), pp. 5787–5796. DOI: 10.1021/acs.est.9b00592.

Khairul, Islam; Wang, Qianqian; Jiang, Yuhan; Chao, Wang; Naranmandura, Hua (2017): Metabolism, toxicity and anticancer activities of arsenic compounds 8, Article 14, pp. 23905–23926.

Kirkby, Ernest (2012): Introduction, Definition and Classification of Nutrients. In : Marschner's Mineral Nutrition of Higher Plants: Elsevier, pp. 3–5.

Kumpiene, Jurate; Castillo Montesinos, Isaac; Lagerkvist, Anders; Maurice, Christian (2007): Evaluation of the critical factors controlling stability of chromium, copper, arsenic and zinc in iron-treated soil. In *Chemosphere* 67 (2), pp. 410–417. DOI: 10.1016/j.chemosphere.2006.08.031.

Lehr, Corinne R.; Polishchuk, Elena; Radoja, Una; Cullen, William R. (2003): Demethylation of methylarsenic species byMycobacterium neoaurum. In *Appl. Organometal. Chem.* 17 (11), pp. 831–834. DOI: 10.1002/aoc.544.

Li, Feng; Zheng, Yuan-Ming; He, Ji-Zheng (2009a): Microbes influence the fractionation of arsenic in paddy soils with different fertilization regimes. In *The Science of the total environment* 407 (8), pp. 2631–2640. DOI: 10.1016/j.scitotenv.2008.12.021.

Li, Jinglong; Sun, Yuqing; Jiang, Xuelian; Chen, Baodong; Zhang, Xin (2018): Arbuscular mycorrhizal fungi alleviate arsenic toxicity to Medicago sativa by influencing arsenic speciation and partitioning. In *Ecotoxicology and environmental safety* 157, pp. 235–243. DOI: 10.1016/j.ecoenv.2018.03.073.

Li, Kun; DiLegge, Michael J.; Minas, Ioannis S.; Hamm, Alison; Manter, Daniel; Vivanco, Jorge M. (2019): Soil sterilization leads to re-colonization of a healthier rhizosphere microbiome. In *Rhizosphere* 12, p. 100176. DOI: 10.1016/j.rhisph.2019.100176.

Li, Ren-Ying; Ago, Yukiko; Liu, Wen-Ju; Mitani, Namiki; Feldmann, Jörg; McGrath, Steve P. et al. (2009b): The rice aquaporin Lsi1 mediates uptake of methylated arsenic species. In *Plant Physiol* 150 (4), pp. 2071–2080. DOI: 10.1104/pp.109.140350.

Liu, Wen-Ju; Wood, B. Alan; Raab, Andrea; McGrath, Steve P.; Zhao, Fang-Jie; Feldmann, Jörg (2010): Complexation of arsenite with phytochelatins reduces arsenite efflux and translocation from roots to shoots in Arabidopsis. In *Plant Physiol* 152 (4), pp. 2211–2221. DOI: 10.1104/pp.109.150862.

Lomax, Charlotte; Liu, Wen-Ju; Wu, Liyou; Xue, Kai; Xiong, Jinbo; Zhou, Jizhong et al. (2012): Methylated arsenic species in plants originate from soil microorganisms. In *New Phytologist* 193 (3), pp. 665–672. DOI: 10.1111/j.1469-8137.2011.03956.x.

López-Bucio, José; Nieto-Jacobo, María Fernanda; Ramírez-Rodríguez, Verenice; Herrera-Estrella, Luis (2000): Organic acid metabolism in plants: from adaptive physiology to transgenic varieties for cultivation in extreme soils. In *Plant Science* 160 (1), pp. 1–13. DOI: 10.1016/S0168-9452(00)00347-2.

Lu, Yuqiu; Wang, Enzhao; Tang, Zhiyu; Rui, Junpeng; Li, Yanling; Tang, Zhixi et al. (2022): Roots and microbiome jointly drive the distributions of 17 phytohormones in the plant soil continuum in a phytohormone‐specific manner. In *Plant Soil* 470 (1-2), pp. 153–165. DOI: 10.1007/s11104-021-04898-w.

Mahmood, T.; Mehnaz, S.; Fleischmann, F.; Ali, R.; Hashmi, Z. H.; Iqbal, Z. (2014): Soil sterilization effects on root growth and formation of rhizosheaths in wheat seedlings. In *Pedobiologia* 57 (3), pp. 123–130. DOI: 10.1016/j.pedobi.2013.12.005.

Malhotra, Hina; Vandana; Sharma, Sandeep; Pandey, Renu (2018): Phosphorus Nutrition: Plant Growth in Response to Deficiency and Excess. In : Plant Nutrients and Abiotic Stress Tolerance: Springer, Singapore, pp. 171–190. Available online at https://link.springer.com/chapter/10.1007/978-981-10-9044-8_7.

Mallick, Shekhar; Sinam, Geetgovind; Sinha, Sarita (2011): Study on arsenate tolerant and sensitive cultivars of Zea mays L.: differential detoxification mechanism and effect on nutrients status. In *Ecotoxicology and environmental safety* 74 (5), pp. 1316–1324. DOI: 10.1016/j.ecoenv.2011.02.012.

Marschner, Petra; Rumberger, Angelika (2004): Rapid changes in the rhizosphere bacterial community structure during re-colonization of sterilized soil. In *Biol Fertil Soils* 40 (1), pp. 1–6. DOI: 10.1007/s00374-004-0736-4.

Meharg, Andrew A.; Hartley‐Whitaker, Jeanette (2002): Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. In *New Phytologist* 154 (1), pp. 29–43. DOI: 10.1046/j.1469-8137.2002.00363.x.

Meharg, Andrew A.; Zhao, Fang-Jie (2012): Arsenic in Rice Grain. In Andrew A. Meharg, Fang-Jie Zjao (Eds.): Arsenic & rice. Springer: Dordrecht [u.a.] (Life sciences), pp. 11–30.

Mishra, Seema; Mattusch, Jürgen; Wennrich, Rainer (2017): Accumulation and transformation of inorganic and organic arsenic in rice and role of thiol-complexation to restrict their translocation to shoot. In *Sci Rep* 7 (1), p. 40522. DOI: 10.1038/srep40522.

Mittler, Ron (2002): Oxidative stress, antioxidants and stress tolerance. In *Trends in Plant Science* 7 (9), pp. 405–410. DOI: 10.1016/S1360-1385(02)02312-9.

Nath, Shwetosmita; Panda, Piyalee; Mishra, Sagarika; Dey, Mohitosh; Choudhury, Shuvasish; Sahoo, Lingaraj; Panda, Sanjib Kumar (2014): Arsenic stress in rice: redox consequences and regulation by iron. In *Plant physiology and biochemistry : PPB* 80, pp. 203–210. DOI: 10.1016/j.plaphy.2014.04.013.

Neidhardt, H.; Norra, S.; Tang, X.; Guo, H.; Stüben, D. (2012): Impact of irrigation with high arsenic burdened groundwater on the soil-plant system: Results from a case study in the Inner Mongolia, China. In *Environmental pollution (Barking, Essex : 1987)* 163, pp. 8–13. DOI: 10.1016/j.envpol.2011.12.033.

Neumann, Kerstin; Klukas, Christian; Friedel, Swetlana; Rischbeck, Pablo; Chen, Dijun; Entzian, Alexander et al. (2015): Dissecting spatiotemporal biomass accumulation in barley under different water regimes using high-throughput image analysis. In *Plant, cell & environment* 38 (10), pp. 1980–1996. DOI: 10.1111/pce.12516.

Norton, Gareth; Deacon, Claire; Mestrot, Adrien; Feldmann, Joerg; Jenkins, Paul; Baskaran, Christina; Meharg, Andrew A. (2013): Arsenic speciation and localization in horticultural produce grown in a historically impacted mining region. In *Environmental science & technology* 47 (12), pp. 6164–6172. DOI: 10.1021/es400720r.

Ochieno, Dennis M. W. (2022): Soil Sterilization Eliminates Beneficial Microbes That Provide Natural Pest Suppression Ecosystem Services Against Radopholus similis and Fusarium Oxysporum V5w2 in the Endosphere and Rhizosphere of Tissue Culture Banana Plants. In *Front. Sustain. Food Syst.* 6, Article 688194. DOI: 10.3389/fsufs.2022.688194.

Ortas, Ibrahim (2003): Effect of Selected Mycorrhizal Inoculation on Phosphorus Sustainability in Sterile and Non‐sterile Soils in the Harran Plain in South Anatolia. In *Journal of Plant Nutrition* 26 (1), pp. 1–17. DOI: 10.1081/PLN-120016494.

Pandey, Neha; Chandrakar, Vibhuti; Keshavkant, Sahu (op. 2018): Mitigating Arsenic Toxicity in Plants: Role of Microbiota. In Mirza Hasanuzzaman (Ed.): Mechanisms of arsenic toxicity and tolerance in plants. Singapore: Springer, pp. 191–218.

Punshon, Tracy; Jackson, Brian P.; Meharg, Andrew A.; Warczack, Todd; Scheckel, Kirk; Guerinot, Mary Lou (2017): Understanding arsenic dynamics in agronomic systems to predict and prevent uptake by crop plants. In *The Science of the total environment* 581-582, pp. 209–220. DOI: 10.1016/j.scitotenv.2016.12.111.

Raab; Schat, Henk; Meharg, Andrew A.; Feldmann, Jörg (2005a): Uptake, translocation and transformation of arsenate and arsenite in sunflower (Helianthus annuus): formation of arsenic-phytochelatin complexes during exposure to high arsenic concentrations. In *New Phytologist* 168 (3), pp. 551–558. DOI: 10.1111/j.1469-8137.2005.01519.x.

Raab; Williams, Paul N.; Meharg, Andrew; Feldmann, Jörg (2007a): Uptake and translocation of inorganic and methylated arsenic species by plants. In *Environ. Chem.* 4 (3), p. 197. DOI: 10.1071/EN06079.

Raab, Andrea; Schat, Henk; Meharg, Andrew A.; Feldmann, Jörg (2005b): Uptake, translocation and transformation of arsenate and arsenite in sunflower (Helianthus annuus): formation of arsenic-phytochelatin complexes during exposure to high arsenic concentrations. In *New Phytologist* 168 (3), pp. 551–558. DOI: 10.1111/j.1469-8137.2005.01519.x.

Raab, Andrea; Wright, Stephen H.; Jaspars, Marcel; Meharg, Andrew A.; Feldmann, Jörg (2007b): Pentavalent Arsenic Can Bind to Biomolecules. In *Angewandte Chemie* 119 (15), pp. 2648–2651. DOI: 10.1002/ange.200604805.

Rajkumar, M.; Sandhya, S.; Prasad, M. N. V.; Freitas, H. (2012): Perspectives of plant-associated microbes in heavy metal phytoremediation. In *Biotechnology Advances* 30 (6), pp. 1562–1574. DOI: 10.1016/j.biotechadv.2012.04.011.

Razavi; Lakzian (2007): Evaluation of chemical and biological consequences of soil sterilization methods 5, pp. 87–91.

Requejo, Raquel; Tena, Manuel (2006): Maize response to acute arsenic toxicity as revealed by proteome analysis of plant shoots. In *PROTEOMICS* 6 Suppl 1 (S1), S156-62. DOI: 10.1002/pmic.200500381.

Rios-Valenciana, Erika E.; Briones-Gallardo, Roberto; Cházaro-Ruiz, Luis F.; Martínez-Villegas, Nadia; Celis, Lourdes B. (2017): Role of indigenous microbiota from heavily contaminated sediments in the bioprecipitation of arsenic. In *Journal of Hazardous Materials* 339, pp. 114–121. DOI: 10.1016/j.jhazmat.2017.06.019.

Rosas-Castor, J. M.; Guzmán-Mar, J. L.; Hernández-Ramírez, A.; Garza-González, M. T.; Hinojosa-Reyes, L. (2014): Arsenic accumulation in maize crop (Zea mays): a review. In *The Science of the total environment* 488-489, pp. 176–187. DOI: 10.1016/j.scitotenv.2014.04.075.

Roychowdhury, Roopali; Roy, Madhumita; Rakshit, Annanya; Sarkar, Sangita; Mukherjee, Pritam (2018): Arsenic Bioremediation by Indigenous Heavy Metal Resistant Bacteria of Fly

Ash Pond. In *Bulletin of environmental contamination and toxicology* 101 (4), pp. 527–535. DOI: 10.1007/s00128-018-2428-z.

Ruiz-Chancho, Maria José; López-Sánchez, José Fermín; Schmeisser, Ernst; Goessler, Walter; Francesconi, Kevin A.; Rubio, R. (2008): Arsenic speciation in plants growing in arsenic-contaminated sites. In *Chemosphere* 71 (8), pp. 1522–1530. DOI: 10.1016/j.chemosphere.2007.11.054.

Schmöger, M. E.; Oven, M.; Grill, E. (2000): Detoxification of arsenic by phytochelatins in plants. In *Plant Physiol* 122 (3), pp. 793–801. DOI: 10.1104/pp.122.3.793.

Sharma, Aakriti; Muyskens, Amanda; Guinness, Joseph; Polizzotto, Matthew L.; Fuentes, Montserrat; Tappero, Ryan V. et al. (2019): Multi-element effects on arsenate accumulation in a geochemical matrix determined using μ-XRF, μ-XANES and spatial statistics. In *Journal of synchrotron radiation* 26 (Pt 6), pp. 1967–1979. DOI: 10.1107/S1600577519012785.

Sharma, Shanti S.; Dietz, Karl-Josef (2009): The relationship between metal toxicity and cellular redox imbalance. In *Trends in Plant Science* 14 (1), pp. 43–50. DOI: 10.1016/j.tplants.2008.10.007.

Skipper, Horace D.; Westermann, Dale T. (1973): Comparative effects of propylene oxide, sodium azide, and autoclaving on selected soil properties. In *Soil Biology and Biochemistry* 5 (4), pp. 409–414. DOI: 10.1016/0038-0717(73)90067-9.

Song, Jing; Zhao, Fang-Jie; McGrath, Steve P.; Luo, Yong-Ming (2006): Influence of soil properties and aging on arsenic phytotoxicity. In *Environmental toxicology and chemistry* 25 (6), pp. 1663–1670. DOI: 10.1897/05-480r2.1.

Srivastava, Sudhakar (2020): Arsenic in Drinking Water and Food. Singapore: Springer Singapore.

Srivastava, Sudhakar; Akkarakaran, Jincy Joseph; Sounderajan, Suvarna; Shrivastava, Manoj; Suprasanna, Penna (2016): Arsenic toxicity in rice (Oryza sativa L.) is influenced by sulfur supply: Impact on the expression of transporters and thiol metabolism. In *Geoderma* 270, pp. 33–42. DOI: 10.1016/j.geoderma.2015.11.006.

Thirunavukkarasu, O.S; Viraraghavan, T.; Subramanian, K.S; Tanjore, S. (2002): Organic arsenic removal from drinking water. In *Urban Water* 4 (4), pp. 415–421. DOI: 10.1016/S1462-0758(02)00029-8.

Tran, Thi Minh; Han, Hyeop-Jo; Ko, Ju-In; Lee, Jong-Un (2020): Effect of Indigenous Microbial Consortium on Bioleaching of Arsenic from Contaminated Soil by Shewanella putrefaciens. In *Sustainability* 12 (8), p. 3286. DOI: 10.3390/su12083286.

Tuli, Rakesh; Chakrabarty, Debasis; Trivedi, Prabodh Kumar; Tripathi, Rudra Deo (2010): Recent advances in arsenic accumulation and metabolism in rice. In *Mol Breeding* 26 (2), pp. 307–323. DOI: 10.1007/s11032-010-9412-6.

Turpeinen, Riina; Pantsar-Kallio, Mari; Kairesalo, Timo (2002): Role of microbes in controlling the speciation of arsenic and production of arsines in contaminated soils 285, pp. 133–145.

Vacheron, Jordan; Desbrosses, Guilhem; Bouffaud, Marie-Lara; Touraine, Bruno; Moënne-Loccoz, Yvan; Muller, Daniel et al. (2013): Plant growth-promoting rhizobacteria and root system functioning. In *Frontiers in plant science* 4, p. 356. DOI: 10.3389/fpls.2013.00356.

Valasek, John; Thomasson, J. Alex (Eds.) (2016): Autonomous Air and Ground Sensing Systems for Agricultural Optimization and Phenotyping. SPIE Commercial + Scientific Sensing and Imaging. Baltimore, Maryland, United States, Sunday 17 April 2016: SPIE.

Wagner, Stefan; Hoefer, Christoph; Puschenreiter, Markus; Wenzel, Walter W.; Oburger, Eva; Hann, Stephan et al. (2020): Arsenic redox transformations and cycling in the rhizosphere of Pteris vittata and Pteris quadriaurita. In *Environmental and Experimental Botany* 177, p. 104122. DOI: 10.1016/j.envexpbot.2020.104122.

Wallace, A.; Frolich, E. F.; Alexander, G. V. (1973): Effect of steam sterilization of soil on two desert plant species. In *Plant Soil* 39 (2), pp. 453–456. DOI: 10.1007/BF00014815.

Wang, Jia; Xie, Zuoming; Wei, Xiaofan; Chen, Mengna; Luo, Yan; Wang, Yanxin (2020): An indigenous bacterium Bacillus XZM for phosphate enhanced transformation and migration of arsenate. In *The Science of the total environment* 719, p. 137183. DOI: 10.1016/j.scitotenv.2020.137183.

WHO (2018): Arsenic Primer: guidance on the investigations & mitigation of arsenic contamination.

Wu, Jingwen; Liang, Jieliang; Björn, Lars Olof; Li, Jintian; Shu, Wensheng; Wang, Yutao (2022): Phosphorus-arsenic interaction in the 'soil-plant-microbe' system and its influence on arsenic pollution. In *The Science of the total environment* 802, p. 149796. DOI: 10.1016/j.scitotenv.2021.149796.

Xiao, Enzong; Cui, Jinli; Sun, Weimin; Jiang, Shiming; Huang, Mengyan; Kong, Deguan et al. (2020): Root microbiome assembly of As-hyperaccumulator Pteris vittata and its efficacy in arsenic requisition. In *Environmental microbiology*. DOI: 10.1111/1462-2920.15299.

Yadav, S. K. (2010): Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. In *South African Journal of Botany* 76 (2), pp. 167–179. DOI: 10.1016/j.sajb.2009.10.007.

Yang, Ningke; Wang, Hongbin; Wang, Haijuan; Wang, Zhongzhen; Ran, Jiakang; Guo, Siyu; Peng, Yu (2021): Screening maize (Zea mays L.) varieties with low accumulation of cadmium, arsenic, and lead in edible parts but high accumulation in other parts: a field plot experiment. In *Environ Sci Pollut Res* 28 (25), pp. 33583–33598. DOI: 10.1007/s11356-021-12958-y.

Ye, Wen-Ling; Wood, B. Alan; Stroud, Jacqueline L.; Andralojc, P. John; Raab, Andrea; McGrath, Steve P. et al. (2010): Arsenic speciation in phloem and xylem exudates of castor bean. In *Plant Physiol* 154 (3), pp. 1505–1513. DOI: 10.1104/pp.110.163261.

Yoon, Joonki; Cao, Xinde; Zhou, Qixing; Ma, Lena Q. (2006): Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. In *The Science of the total environment* 368 (2-3), pp. 456–464. DOI: 10.1016/j.scitotenv.2006.01.016.

Yoshinaga, Masafumi; Cai, Yong; Rosen, Barry P. (2011): Demethylation of methylarsonic acid by a microbial community. In *Environmental microbiology* 13 (5), pp. 1205–1215. DOI: 10.1111/j.1462-2920.2010.02420.x.

Yu, Yang; Zhang, Shuzhen; Huang, Honglin; Luo, Lei; Wen, Bei (2009): Arsenic accumulation and speciation in maize as affected by inoculation with arbuscular mycorrhizal fungus Glomus mosseae. In *Journal of agricultural and food chemistry* 57 (9), pp. 3695–3701. DOI: 10.1021/jf900107y.

Zhao, Fang-Jie; Harris, Eleanor; Yan, Jia; Ma, Jincai; Wu, Liyou; Liu, Wenju et al. (2013a): Arsenic methylation in soils and its relationship with microbial arsM abundance and diversity, and as speciation in rice. In *Environmental science & technology* 47 (13), pp. 7147–7154. DOI: 10.1021/es304977m.

Zhao, Fang-Jie; Zhu, Yong-Guan; Meharg, Andrew A. (2013b): Methylated arsenic species in rice: geographical variation, origin, and uptake mechanisms. In *Environmental science & technology* 47 (9), pp. 3957–3966. DOI: 10.1021/es304295n.

Zhao, Zhan; Zhang, Huaisheng; Fu, Zhongjun; Chen, Hao; Lin, Yanan; Yan, Pengshuai et al. (2018): Genetic-based dissection of arsenic accumulation in maize using a genome-wide association analysis method. In *Plant biotechnology journal* 16 (5), pp. 1085–1093. DOI: 10.1111/pbi.12853.

Zheng, RuiLun; Sun, GuoXin; Zhu, YongGuan (2013): Effects of microbial processes on the fate of arsenic in paddy soil. In *Chin. Sci. Bull.* 58 (2), pp. 186–193. DOI: 10.1007/s11434-012-5489-0.

Chapter IV Soil indigenous microbes protect maize plants cultivated in soils with varying arsenic levels

Hang Guan[†], Veronica Caggìa[‡], Andrea Gómez-Chamorro[¶], Xiaowen Liu[¤], Klaus Schlaeppi[‡§], Alban Ramette[¶], Adrien Mestrot[†] and Moritz Bigalke[†*]

[†]Institute of Geography, University of Bern, Hallerstrasse 12, CH-3012 Bern

[‡]Institute of Plant Sciences, University of Bern, Altenbergrain 21, CH-3013 Bern

[¶]Institute for Infectious Diseases, University of Bern, Friedbühlstrasse 51, CH-3001 Bern

¤Center of Deep Sea Research, Institute of Oceanology, Center for Ocean Mega-Science, Chinese Academy of Sciences, CN-266071 Qingdao, China

§Department of Environmental Sciences, University of Basel, CH-4056 Basel, Switzerland

*Institute of Applied Geoscience, Technical University of Darmstadt, Schnittspahnstrasse 9, D-64287 Darmstadt, Germany

This is a preprint version of the manuscript to be submitted for publication.

Abstract

Arsenic (As) contamination of agricultural soils causes adverse effects on crop development and yield loss. But little is known about how soil microbes influence plant health in soils with different As levels. Therefore, we performed a greenhouse pot experiment with maize (*Zea mays* L.) plants grown on different conditioned soil and As levels, studying the role of soil indigenous microbes on plant health. Pots with ten replicates were prepared at three soil treatments: native soil (NS), reconditioned soil (RS, sterilized soils and reconditioned with native soil microbes), and disturbed soil (DS, sterilized soils before planting). Because soil sterilization may casue both biotic and abiotic changes, DS and RS treatments were introduced to differentiate between the biotic (microbial disturbance) and abiotic (soil sterilization) effects. The soil treatments were intersected with three As treatments: uncontaminated soils (As_0), moderate-As soils (As_{100}, addition of 100 mg As kg^{-1} soil), and high-As soils (As_{200}, addition of 200 mg As kg^{-1} soil). Various plant health parameters were recorded regularly, including plant height, fresh biomass, BBCH-scale, leaf numbers, leaf chlorophyll content and damage scale of leaf spot. Plant height and BBCH-scale of maize on uncontaminated soils were not reduced by both microbial disturbance and sterilization effects, implying that plants were capable to buffer the microbial disturbance effect. In the presence of As stressor, the microbial disturbance effect reduced plant height, leaf numbers, and chlorophyll content, while enhancing damage scale. However, these affected health parameters were not or only slightly retarded in maize grown on NS even at a high As level, thanks to the undisturbed soil indigenous microbes. The sterilization effect induced P and Mn deficiencies in maize grown on high-As soils, which was in line with enhanced As accumulation in plants and its adverse effects on plant health. The strongest correlation was found between the As concentrations in leaves and plant health parameters. This might be due to the fact that high As reduced leaf chlorophyll content and photosynthesis rate, thus delaying the phenological development and the biomass production of maize plants.

1 Introduction

Arsenic (As) accumulation in crop plants has implications for agricultural sustainability, because As exposure has an adverse effect on morphological (e.g., chlorosis), physiological (e.g., growth processes inhibition), and biochemical (e.g., oxidative stress) responses of plants (Zemanová et al. 2021). Plant growth can be rigorously constrained by As that slows or arrests cell expansion and biomass production. Arsenic also reduces plant reproductivity and yield by decreasing its fertility and inhibiting the development of reproductive organs (Alam et al. 2019). Reactive oxygen species can be generated during the conversion of As valence forms or indirectly by inactivating antioxidant molecules through binding with their sulfhydryl groups (-SH). The formation of reactive oxygen species induced by As can lead to plant physiological disorders (Flora 2011), as they cause oxidative damage to biomolecules such as lipids and proteins and can eventually lead to cell death (Garg and Singla 2011). By impairing metabolic processes, sufficiently high As levels may result in plant death (Finnegan and Chen 2012).

Arsenic speciation is of great importance because of differences in phytotoxicity and phytoavailability among species. Arsenic occurs predominantly in inorganic As species (inAs) as arsenate (As^{V}) and arsenite (As^{III}) in soil and aquatic environments. The most common organic As species (orgAs) are methylarsonic acid (MMA^{V}), dimethylarsinic acid (DMA^{V}), and trimethylarsine oxide (TMAO). In general, the toxicity of As species decreases as follows: As^{III} > As^{V} > MMA^{V}, DMA^{V} > TMAO (Khairul et al. 2017; Di et al. 2019). The inAs is found to be most abundant in numerous terrestrial plant samples, including maize roots, stem and leaves (Ruiz-Chancho et al. 2008). Whereas orgAs represent only small amounts (0.17 - 0.23 % of the As level in soils) (Rosas-Castor et al. 2014b). Nonetheless, orgAs can be found in maize grains, accounting for up to 61% of the total As concentrations (CI et al. 2012). DMA^{V} and MMA^{V} are the most frequently identified orgAs in maize (Abbas and Meharg 2008; CI et al. 2012; Yu et al. 2009).

Soil microbes perform essential ecosystem functions, e.g., promoting plant growth and health and aiding with nutrient availability and uptake. They supply both macronutrients and micronutrients (Berg 2009). Bacteria may contribute to plant nutrition by liberating P from organic compounds such as phytates (Unno et al. 2005). More importantly, soil microbes can

enhance stress tolerance and disease resistance by activating the defense systems of host plant and inducing their systemic resistance (Maksimov et al. 2015). Soil indigenous microbes can promote As bioleaching and volatilisation to arsines that are removed from contaminated soils (Mestrot et al. 2013; Roychowdhury et al. 2018; Tran et al. 2020; Turpeinen et al. 2002; Majumder et al. 2013; Wang et al. 2014). Soil indigenous microbes can also impart plant tolerance through the secretion of organic acids and siderophores, which can form stable complexes with As to reduce its bioavailability and toxicity in the soil-plant-microbe system (Drewniak and Sklodowska 2013; Singh et al. 2021).

The plant-microbe interactions have reciprocal effects on both partners. For example, plants commonly respond to root colonization by microbes with an increased release of root exudates (Phillips et al. 2004), or they produce several compounds that mimic quorum sensing signals to recruit bacterial communities (Bauer and Mathesius 2004). Root exudates contain organic components that play a key role in nutrient solubilization as carbon sources for microbial nutrition, thereby attracting beneficial microbes (Schaechter 2009). In response to environmental stressors, the excretion of these organic compounds increases frequently (López-Bucio et al. 2000). In the presence of toxic levels of aluminum, maize roots emit more organic acids such as oxalic acid, malic acid and citric acid, which may act as signals to attract microbes (Piñeros et al. 2002) and form internal complexes with aluminum to detoxify it (Ma et al. 2001). Concurrently, soil microbes produce volatile organic compounds that can be sensed by plants to alter morphogenesis or trigger plant defense and stress responses (Ortíz-Castro et al. 2009). Bacteria and fungi in the roots have an intimate interaction with their host plants to promote plant growth and suppress plant pathogens (Whipps 2001; Berg 2009).

Some studies have reported that soil sterilization increases significantly the growth of different crop species by removing pathogens, promoting rapid changes in microbial communities to reach more beneficial and healthier soil microbes. Plant-beneficial microbial functions are promoted after soil sterilization, including nitrogen fixation, P solubilization, biological control or root growth promotion (Li et al. 2019). Maize height, total dry biomass and plant P concentrations are significantly higher in sterilized than unsterilized soils, because of increased nutrient availability from the decomposition of soil biota, elimination of plant pathogens, and decreased microbial competitors for inorganic nutrients following

soil sterilization (Zhang et al. 2011). However, other studies have claimed the negative responses of plants to soil sterilization. Soil sterilization can have adverse effects on plant growth because of the elimination of soil indigenous microbes, which decreases plant growth, photosynthesis, glycyrrhizin, and liquiritin accumulation as well as downregulates the expression of related biosynthesis genes (Yu et al. 2019). While soil sterilization eliminates some pathogens, it could also disrupt the available beneficial effects of soil indigenous microbes on the host plant (Ochieno 2022). Particularly in As-contaminated soils, soil sterilization may be unfavorable, as the elimination of soil indigenous microbes may lose microbial efficacy in As control and mitigation (Majumder et al. 2013). Meanwhile, soil sterilization also causes abiotic changes by altering the sorptive behavior of As, due to ion competition such as P for sorption sites on soils (Tiberg et al. 2020; Hongshao and Stanforth 2001) and altered As reactivity to organic matter owing to organic carbon or pH changes (Dao et al. 1982; Razavi and Lakzian 2007).

In previously chapters about this experiment (Chapter II & III), we found that in both soil water and maize tissues, the concentrations of total As (totAs) and inAs followed a general pattern of NS < RS ≤ DS, as both the microbial disturbance and sterilization effects mobilized As into soil water. In this experiment, we noticed that the presence of maize plants mitigated both the microbial disturbance and sterilization effects, assisting soil microbes to recover from soil sterilization. The plant-microbe interactions played a role in minimizing As concentrations in soil water for the survival of both plants and soil microbes (Chapter II). In terms of how microbial disturbance and sterilization effects affected the total dry biomass of maize grown on soils with various As levels, we found that in uncontaminated soils, both both effects had no influence on maize dry biomass. Due to the presence of soil indigenous microbes, the decrease in dry biomass was mitigated in maize on contaminated soils (Chapter III). Moreover, the simultaneous presence of microbial disturbance and sterilization effects exacerbated the adverse effects of As on maize dry biomass. Both the microbial disturbance and sterilization effects in the stem led to a greater reduction in its dry biomass than in the other tissues (Chapter IV). In this study, we aim to investigate the interaction effects of microbial disturbance and As treatment on the plant health as well as to disentangle the microbial-based effect on plant health. We intend to answer the following research questions with this greenhouse pot experiment: 1) What is the microbial disturbance effect on the plant health in soils with varying As levels? 2) What is the

sterilization effect on the plant health in soils with varying As levels? 3) Are the As concentrations in maize tissue linked to the plant health parameters?

2 Materials and Methods

The soil (silty loam) was sampled by a soil recycling company (Kästli Bau AG) from the uppermost 20 cm of an agricultural site in Frauenkappelen, Switzerland. The soil pile was then stored outside the greenhouse of the Institute of Plant Sciences at the University of Bern (Ostermundigen, Switzerland). For this pot greenhouse experiment, about 800 kg of soils were sampled from both sides of the soil pile for homogeneity and sieved to 1 cm. This experiment had nine treatment groups: three soil treatments (native soil (NS), reconditioned soil (RS), and disturbed soil (DS)) × three As treatments (As_0, As_{100}, and As_{200} mg As kg^{-1} soil), with ten replicates in each group (Figure IV-1). The soils in the As_0 group have a naturally occurring concentration of 2.91 ± 0.54 mg kg^{-1} without the addition of As. For As_{100} and As_{200} groups, around 400 kg of soils were spiked with sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$, ≥ 98.0%; Sigma-Aldrich®, CH) to enrich an additional 100 and 200 mg kg^{-1} As in soils. The soils were incubated at room temperature for two months at 50% of water holding capacity (WHC), allowing for As equilibration between soil water and soil phases (data not shown) and simulating aging (Song et al. 2006).

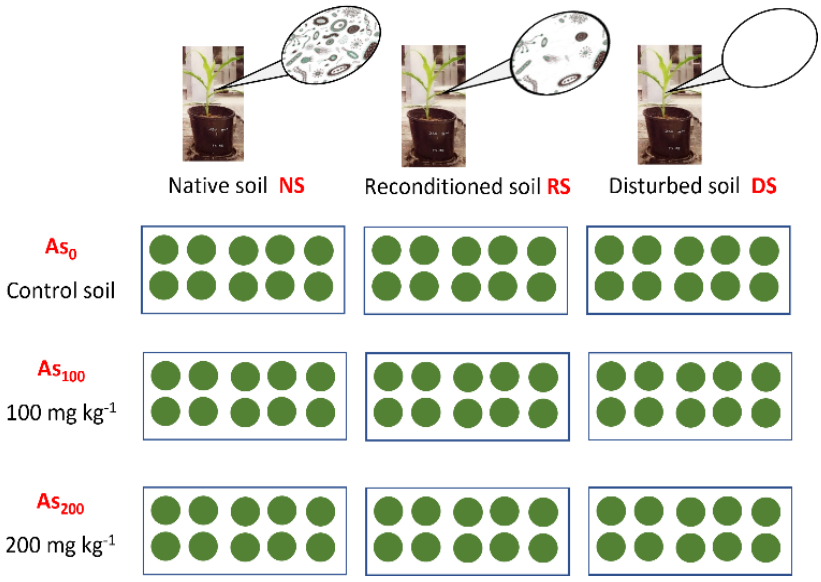


Figure IV-1. Overview of the experimental design

Afterward, soils in the three As treatments were further subdivided into three subgroups for the three soil treatments (NS, RS, and DS). The first treatment was kept untreated and named as NS. The second and third segments were sterilized by X-ray (25 kGy minimum to 60 kGy maximum at Synergy Health Däniken AG, Switzerland). The second segment was reconditioned with microbial extracts from NS after sterilization and designated as RS. The

third segment was referred to as DS without microbial reconditioning. The microbial extracts for the RS treatment were obtained by entirely mixing 70 kg native soils with 70 L of Milli-Q water (> 18.2 MΩ·cm at 25°C) in a pre-sterilized concrete mixer (sterilized with ethanol and a gas burner) (Figure S1.1). The solutions were left to stand for 2 h and filtered through a 250 µm stainless sieve and 25 µm 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) and allowed 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). Due to the presence of microbes in the greenhouse, DS was not assumed to be free of microbes but rather to have a disturbed microbial composition.

The sterilization effect was the same between DS and RS, while the microbial disturbance was partly eliminated in the RS treatment due to the reconditioning of microbial extracts. Therefore, it is assumed that the difference between RS and DS showed the microbial disturbance effects, and the difference between NS and RS reflected the sterilization effects. The detailed characterizations of NS and DS can be found in Table S1.1. All soils were adequately homogenized and decanted into 117 pots. Each pot (7 L) was filled with 6.5 kg of soils and reached the same height to ensure uniform bulk density of soils. In the end, 90 pots with maize plants were cultivated from April to September 2019.

Maize seeds (*Zea mays* L., W22 genotype) were soaked for 6 minutes in a commercial bleach solution followed by 6 washes and an 8-hour soak in autoclaved MilliQ-water (> 18.2 MΩ·cm at 25°C). Before sowing, one week after soil sterilization, seeds were placed overnight in plastic Petri plates with moist filter papers. Each pot was initially sown with three pre-sterilized maize kernels and only the best performing seedling was kept per pot for further growth. 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 randomly placed in the greenhouse. In the beginning, plants were watered weekly by weighing pots and adjusting the WHC to 50%. From the third month of growth, they were watered more frequently. The weekly fertilization of maize plants 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 contains 52% phosphate (P_2O_5), 10% total nitrogen (8.4% NH_3-N and 1.4% NO_3-N), and 10% potassium oxide (K_2O). The maize plants were cultivated in the greenhouse with 14 h of light each day and a temperature of 18 - 26°C during the day and 16 - 24°C at night. The greenhouse cabin is heated in case of temperatures below 18°C during the day and below 16°C at night. The cooling system automatically turns on if the temperature exceeds 26°C. The ventilation system turns on once the temperature is over 22°C in the daytime or over 20°C at night. The humidity ranged between 30 and 60%.

Plant height, fresh biomass, and leaf chlorophyll content were recorded biweekly, while BBCH-scale and leaf numbers per plant were measured monthly. Plant height was measured from the base to the tip of the plant with a carpenter's ruler. Leaf chlorophyll content was assessed by averaging three readings from three positions between the base and the apex of a leaf with the Soil Plant Analysis Development chlorophyll meter (SPAD-502, Minolta Camera CO., LTD., Japan). BBCH-scale is a universal scale using a decimal code for the description of the growth stages of most agricultural crops and weeds (Lancashire et al. 1991), where similar growth stages of maize plants were given the same code. The lower the code, the slower the development of the plant. Leaf numbers on the entire plant were counted visually. Damage scale of the leaf spot was also visually inspected once in the middle of the plant growth.

Additionally, a side experiment was conducted to estimate the fresh biomass of maize during growth while maintaining the same WHC in soils by controlling the weight of the pots. In this experiment, 60 maize plants were grown for five months and three of them were harvested weekly to determine their fresh biomass. Plant images were simultaneously recorded to derive the green pixels area of plant leaves. Therefore, a linear model could be built between the calculated biomass and the leaf area to estimate the plant's actual fresh biomass (Figure S1.2) (Neumann et al. 2015; Valasek and Thomasson 2016). The estimated fresh biomass was then applied to calculate the amount of irrigation water and correct the weight of pots to retain 50% of WHC.

All the statistical analysis were performed in R software (version 1.2.5033) including the following packages: car, multcomp, emmeans and vegan. The univariate analysis of variance (ANOVA) was applied to study the influence of the three experimental factors, i.e., microbial disturbance (three levels: NS, DS, RS), As treatments (three levels: As_0, As_{100}, As_{200}) and temporal effect as well as their interactions on the plant health parameters. The temporal

effect was considered due to the changes in plant health parameters over time. The interactions stand for the combined effects of the experimental factors on the response variable, e.g. plant height. The estimated marginal means (in the *emmeans* package) were calculated for the post-hoc analysis. 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. Besides, the conventional correlation was analyzed to investigate the relation between the concentrations of totAs and As species in maize (inAs, MMA^V, DMA^V, and TMAO) and plant health parameters. These As data have been shown in Chapter III. Only the damage scale was performed using Spearman rank correlation as it was a categorical variable, while all other plant health parameters were conducted with Pearson correlation analysis. Among the plant health parameters, the percentages of BBCH-scale loss (BBCH-scale/the highest BBCH-scale * 100) and leaf loss (leaf numbers/the highest leaf numbers * 100) were calculated for their correlation analyses.

3 Results

According to the univariate ANOVA results, interactions between microbial disturbance and As treatments significantly influenced plant height, chlorophyll content, leaf numbers, BBCH-scale, damage scale ($p < 0.001$), and fresh biomass ($p = 0.023$) (Table S4.1). Microbial disturbance and As treatments individually decreased all the measured plant health parameters, including plant height, fresh biomass, chlorophyll content, leaf numbers, BBCH-scale and damage scale ($p < 0.001$).

In terms of plant height, maize plants grown on DS were smaller than those on RS in the As_{100} group due to the microbial disturbance effect (difference between DS and RS) (Figure S4.1). Plant height was lower in RS than in NS due to the sterilization effect (difference between NS and RS). The sterilization effect was evident in both As_{100} and As_{200} groups but not in the As_0 group, indicating that plant height was not reduced solely due to the disturbance of soil indigenous microbes in the absence of As. As a result, the significant decrease in plant height observed in RS and DS was attributed to the interaction effects between microbial disturbance and As treatments. Unlike maize on RS and DS, the maize height on NS of the As_{200} group was not greatly retarded thanks to the undisturbed soil indigenous microbes (Figure IV-2a). Concerning fresh biomass, the microbial disturbance effect had no effect on fresh biomass, it was the sterilization effect that reduced the fresh biomass of maize plants in all three As groups (Figure S4.2). In As_{100} and As_{200} groups, the reduction in fresh biomass in NS was not as severe as in DS and RS (Figure IV-2b). The fresh biomass of maize in RS and DS was severely reduced by As effect, especially in the As_{200} group.

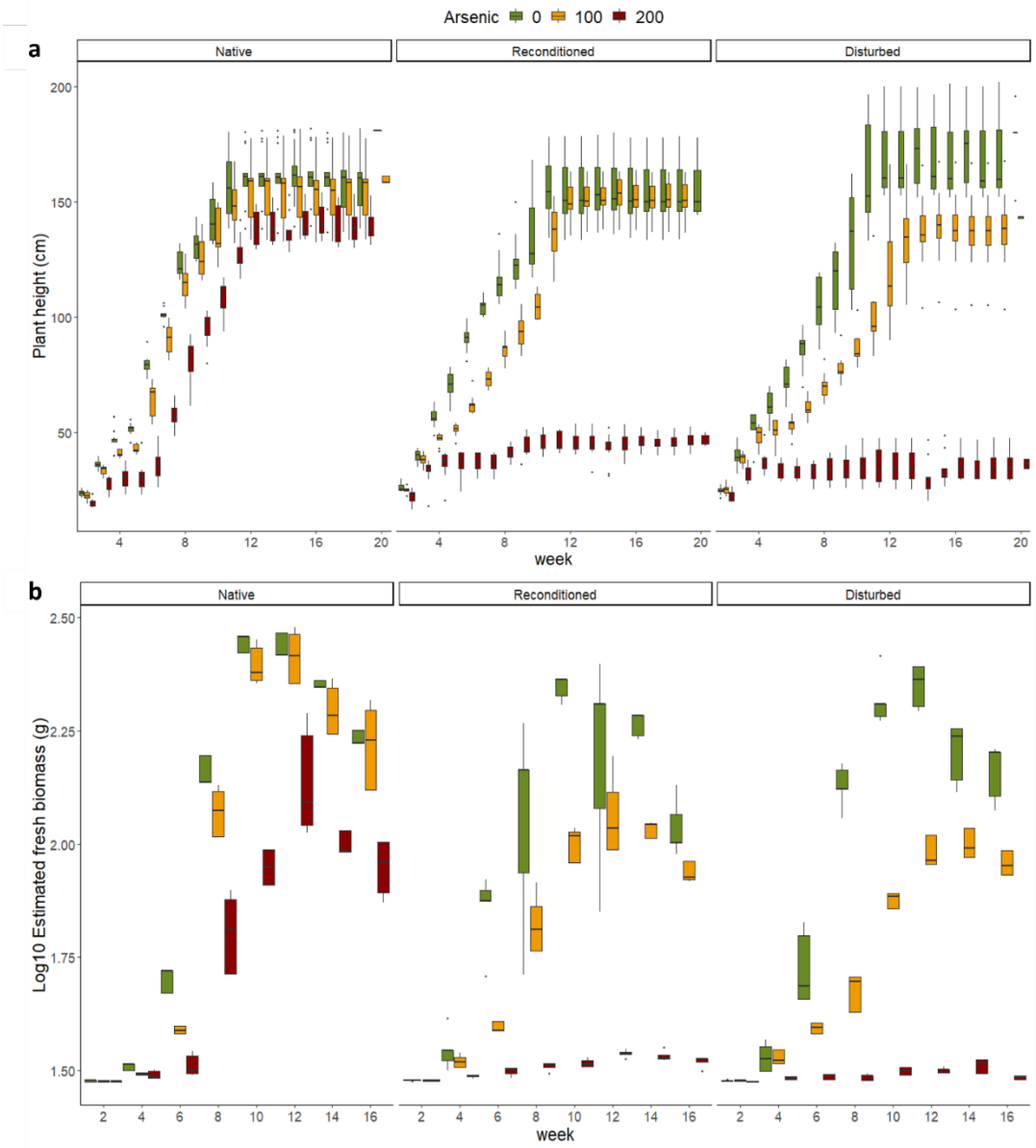


Figure IV-2. Changes in (a) plant height; (b) fresh biomass over time under soil sterilization and As treatments

The microbial disturbance effect had no influence on BBCH-scale, while the sterilization effect reduced BBCH-scale only in the As_{200} group, resulting in a lower scale in RS and DS than in NS (Figure IV-3a and S4.3). Regarding leaf numbers, maize in the As_{200} group had more leaves on RS than on DS due to the microbial disturbance effect (Figure IV-3b and S4.4). The sterilization effect in the As_0 group promoted maize growth with greater leaf numbers in RS and DS than in NS. This effect was also seen in maize on DS of the As_{100} group, which had more leaves than maize on NS and RS. Collectively, the sterilization effect promoted leaf development in soils with low As levels. Whereas at high As concentration in soils, leaf numbers were not influenced by the sterilization

effect but increased significantly due to microbial reconditioning.

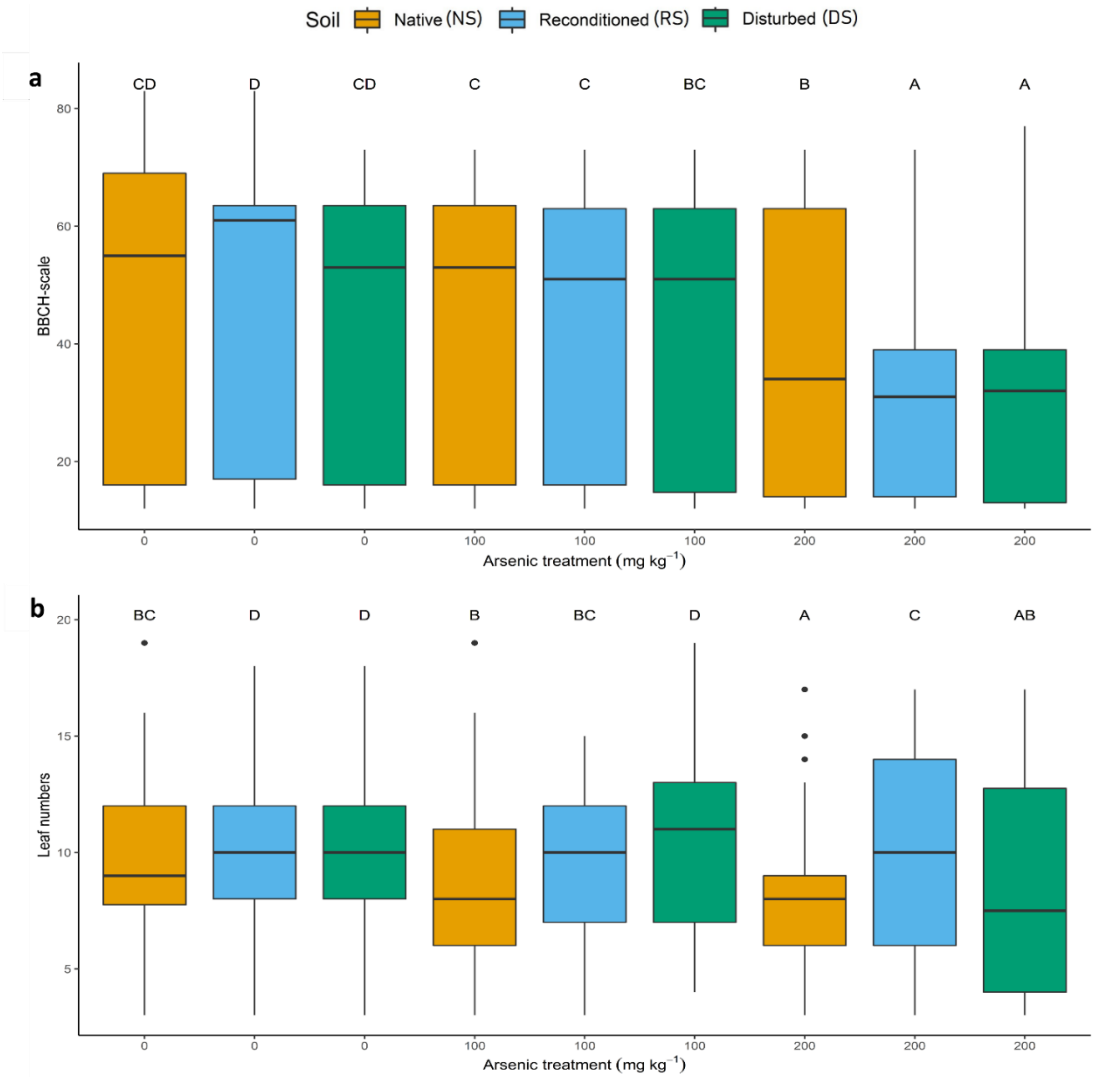


Figure IV-3. Changes in (a) BBCH-scale and (b) leaf numbers under soil sterilization and As treatments. Data are mean values ± standard error. Pairwise comparisons were explored and reported using CLD letters. Different letters indicated a statistically significant difference between emmeans ($p < 0.05$).

The microbial disturbance effect decreased significantly the chlorophyll content in maize leaves on DS compared to RS in the As_{200} group (Figure IV-4a and S4.5). In all three As groups, the sterilization effect was observed, with maize leaves on RS containing less chlorophyll content than those on NS. Despite As addition, maize grown on NS had similar chlorophyll content in leaves. However, chlorophyll content in maize grown on RS and

DS decreased significantly as As levels in soils increased. Leaf spot is a foliar disease caused by nutrient deficiency (Ochieno 2022) or fungal and bacterial plant diseases (Kaur et al. 2019). Only maize cultivated on RS and DS was found to have it. These discolored spots or lesions frequently have a center of necrosis or cell death, resulting in loss of chlorophyll content only observed in maize on RS and DS (Figure IV-4b). However, due to the presence of undisturbed soil indigenous microbes, maize on NS was mostly resistant to this disease. In addition, four levels of damage scale for the leaf spot were established: 0 (0%), 1 (1 - 33%), 2 (34 - 66%), and 3 (67 - 100%). The significant increase in damage scale of spot disease was attributed to both the microbial disturbance and sterilization effects, with a trend of NS < RS < DS in all three As treatments (Figure S4.6). The scale ranges were around 0 for NS, 1-2 for RS, and 2-3 for DS. The leaves in the As_{200} group were highly stressed by As and were substantially smaller than those in the As_0 and As_{100} groups, displaying infection of large portions of the foliage, i.e., leaf blight. Consequently, only a few leaves survived in the As_{200} group.

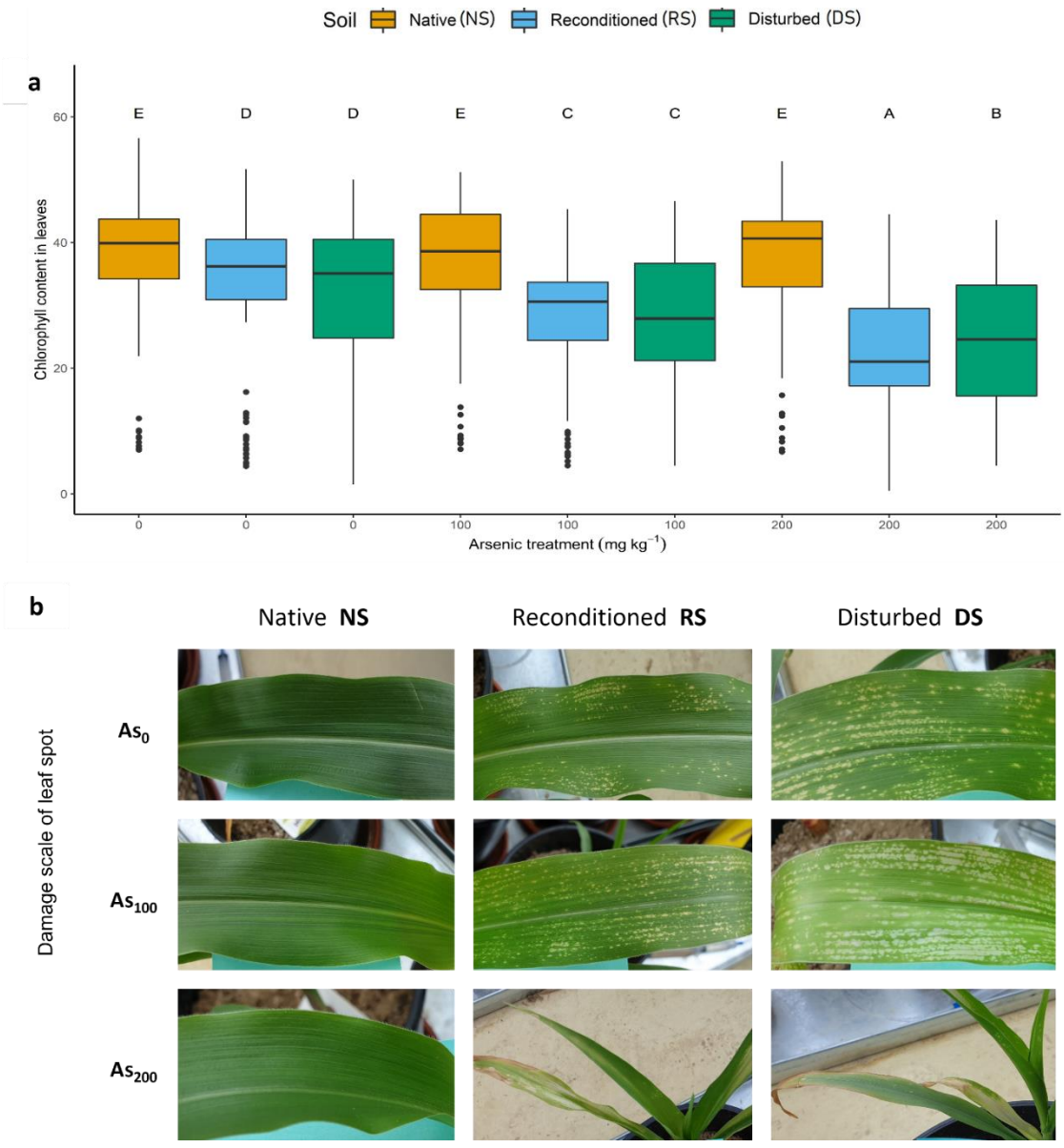


Figure IV-4. The effects of soil sterilization and As treatments on (a) chlorophyll content; (b) leaf spot (foliar diseases). Data are mean values ± standard error. Pairwise comparisons were explored and reported using CLD letters. Different letters indicated a statistically significant difference between emmeans ($p < 0.05$).

As for the correlation analysis between As in maize tissues and the plant health, the three plant health parameters, i.e. plant height, fresh biomass, and chlorophyll were significantly negatively correlated with the concentrations of totAs and As species in maize roots, stem, and leaves ($p < 0.05$) (Table S4.2-S4.5). While the damage scale was significantly positively correlated with totAs and As species in maize leaves ($p < 0.01$) (Table S4.4). The leaves showed the strongest correlations between the concentrations of totAs and As species and

plant height and chlorophyll (negative correlation), as well as BBCH-scale loss (positive correlation). Surprisingly, no significant correlation was detected between As concentration in maize tissues and the loss in leaf numbers.

4 Discussion

4.1 Plant Health Responses to Microbial Disturbance

In uncontaminated soils, some studies have reported that plants respond positively to soil sterilization (Moreira et al. 2019; Mahmood et al. 2014; Miransari et al. 2009; CI et al. 2012). Peanut plants grown in sterilized soils have greater leaf numbers, stem height, and stem and root biomass than those in unsterilized soils, probably due to more available nutrients and less microbial competition in sterilized soils (Al-Khaliel 2010). Competition may be greater in unsterilized soils, as mycoparasites, for instance, can attack the arbuscular mycorrhization that promotes plant growth and production. Other studies, however, have found a negative impact of soil sterilization on plant growth and health (Lu et al. 2022; Ochieno 2022; Yu et al. 2019). On ϒ-sterilized soils, maize plants have significantly lower plant growth (e.g., chlorophyll, stem thickness, plant height, and dry biomass), photosynthesis efficiency (e.g., leaf net photosynthetic rate and stomatal conductance), P in roots and stem (Lu et al. 2022). Plants grown in sterilized soils are less healthy than those in unsterilized soils, with more root damage and lower fresh biomass, plant height, and leaf size. This is likely because soil sterilization eliminates beneficial microbes that provide natural pest suppression ecosystem services in the endosphere and rhizosphere of banana plants. In unsterilized soils, the pest inhibitive biota might be present or there can be competition amongst plant-parasitic nematodes (Ochieno 2022). Microbial competition in soils, according to studies on both sterilized and unsterilized soils, was the reason given to explain the observed worse plant health.

Therefore, we believe that plant responses to microbial disturbance depend on the existing microbial communities and whether microbial competition increased or decreased after soil sterilization. Plants could react negatively to microbial disturbance if there are fewer beneficial microbes, fewer nutrients, or more microbial competition following sterilization. In our uncontaminated soils we discovered inconsistent results. Soil sterilization increased significantly leaf numbers and damage scalem, while significantly reducing fresh biomass and chlorophyll, but had no effect on plant height and BBCH-scale. The increase in leaf numbers could be due to nutrient release from soil sterilization (Al-Khaliel 2010), while the decrease in fresh biomass and chlorophyll could be due to the elimination of beneficial microbes by soil sterilization (Ochieno 2022). When the soil was contaminated by As, our plants usually

presented poorer health in sterilized than in unsterilized soils. Arsenic exposure to plants is known to induce the production of reactive oxygen species, which may cause irreversible DNA damage and cell death (Huang et al. 2019) and impact oxidative carbon metabolism, amino acid and protein connections, and nitrogen and sulfur assimilation pathways (Finnegan and Chen 2012). This study discovered that the interaction effects between microbial disturbance or sterilization effects and As treatments exacerbated the adverse effects of As on plant health, which is detailed in the following chapters.

4.2 Microbial disturbance Effects in Different As-containing Soils

The difference between DS and RS in our experiment showed the microbial disturbance effect, indicating that the disturbance of soil indigenous microbes resulted in an significant increase in As accumulation in plants (Chapter III) and the associated adverse effects on plant health. The microbial disturbance effect was only observed in our uncontaminated soils for the damage scale, implying that plants can cope with the disturbance of soil indigenous microbes in uncontaminated soils. In contaminated soils, on the other hand, the microbial disturbance effect significantly reduced plant height, leaf numbers and chlorophyll, and significantly increased damage scale, demonstrating the role of soil indigenous microbes in plant growth exposed to As. The reduction in plant height and leaf damage scale by As was greatly alleviated, and leaf numbers were even increased significantly due to the reconditioning of soil indigenous microorganisms in RS. Previous research has revealed the same phenomenon. A sterilization treatment plus 1% unsterilized arable or grassland soils increases significantly maize height and total biomass (Lu et al. 2022). Leaf numbers, lateral branch numbers, and plant growth rate are higher in sterilized soils with the addition of microbes, as it contributes to the success of mycorrhizal sporulation that promotes the growth and production of peanut plants (Al-Khaliel 2010).

Due to the undisturbed soil indigenous microbes in NS, even in high-As soils, all plant health parameters were not or only slightly affected by As in the present study. Our findings support previous research showing that soil indigenous microbes and their potential interactions with the host plant can promote plant growth and impart As tolerance (Rai et al. 2019; Huang

et al. 2021). Soil indigenous microbes can promote plant growth by regulating plant hormones, improving nutrition acquisition, siderophore production and antioxidant system (Kumar and Verma 2018). The reciprocal plant-microbe interactions are critical for both partners' survival in the face of environmental stressors. In response to the exposure to aboveground pathogens, plants can recruit beneficial rhizosphere communities *via* a change in root exudations (Yuan et al. 2018; Broeckling et al. 2008; Phillips et al. 2004; Bauer and Mathesius 2004). Extracellular organic chelators and hormones generated by soil microbes can stimulate plant growth and reduce the accumulation of heavy metal(loid)s in plants (Etesami 2018; Yu et al. 2019). Through an intimate interaction with the host plants, the recruited soil microbes can promote plant growth and suppress plant pathogens (Berg 2009), improve plant stress tolerance and disease resistance (Whipps 2001) and enhance the host plant's capability to adapt to As stressor (Xiao et al. 2020).

The foremost consequence of soil sterilization is the elimination of soil indigenous microbes, although they rapidly recolonize and recruit a new disturbed microbial community that is less diverse (Mahmood et al. 2014; Marschner and Rumberger 2004). ϒ-sterilization causes physical damage to proteins by ionizing radiation, which disrupts enzyme activity and halts microbial exoenzyme production (Blankinship et al. 2014). Enzyme activities of sterilized and recovered soils are lower than those of unsterilized soil, including the activities of catalase, invertase, urease, protease, acid phosphatase, and phytase (Xun et al. 2015). Changes in their activities can modify microbial composition and activities, as these enzymes are involved in the hydrolysis of carbon-substrates and organic nutrients, which alters nutrient availability in the rhizosphere (Gianfreda 2015). Taken together, these findings broadly support the fact that the elimination and disturbance of soil indigenous microbes by soil sterilization may cause negative effects on soil microbial and enzyme activity, hampering their ability to support plant growth and imparting plant resistance to As through interactions with their host plants. This highlights the essential role of soil indigenous microbes in promoting plant growth and resilience to the environmental stressors such as As.

4.3 Sterilization Effects in Different As-containing Soils

In uncontaminated soils, the sterilization effect resulted in a significant decrease in fresh biomass and chlorophyll content ($p < 0.05$). The well-documented correlations between chlorophyll and biomass may explain their similar responses to As stress in the environment. Rice stem biomass was reported to correlate strongly with photosynthetic pigments in the leaves (Rahman et al. 2007). Because the chlorophyll-a content is directly related to the carbohydrate production that can be stored by rice in the grains, the decline of chlorophyll content in plant leaves can lead to reduced plant growth and yield (Rahman et al. 2007). In the presence of As, however, all the plant health parameters were significantly retarded in our high-As soils (NS < RS ≤ DS), due to the interaction between the sterilization effect and As treatments. For example, plant height and BBCH-scale were decreased significantly by the sterilization effect only in contaminated soils, but not in the absence of As. Similarly, leaf numbers were increased in uncontaminated and moderate-As soils, while they decreased in high-As soils.

The sterilization effect can lead to nutrient releases from the death and lysis of cells (Berns et al. 2008). In our uncontaminated soils, the sterilization effect resulted in a significant increase in leaf numbers ($p < 0.05$), which was likely attributable to the higher concentration of potassium (K) in maize leaves in sterilized than in unsterilized soils (Figure S4.7). The increase in plant available K has been reported to be a sterilization effect (Dietrich et al. 2020) and the addition of K-salt to rice can enhance leaf area and stem biomass (Zain and Ismail 2016; Lin and Yeh 2008). However, the sterilization effect may lead to nutrient imbalances, which affect plant uptake and utilization of these elements. In the current study, the sterilization effect caused significant nutrient deficiencies of P and Mn, contributing to the poorer plant growth in sterilized soils (Figure S4.8). Nutrient deficiencies in plants caused by the sterilization effect have been reported in previous research. Banana plants in steam-sterilized soils grow poorly due to the modification of nutrient compounds to less available forms (Ochieno 2022). Although the sterilization effect increases the amount of nutrients released by dead microbial cells (McNamara et al. 2003), these amounts may still be insufficient for plants such as liquorice, which has been demonstrated to require a large supply of nutrients to thrive (Öztürk et al. 2017). Simultaneously, while the sterilization effect increases the availability of nutrients from dead microbial cells, their availability to plants

may be limited in the absence of decomposers after soil sterilization (Noreika et al. 2021). Since poorly soluble inorganic nutrients can be made available through the solubilization of bacterial siderophores and the secretion of organic acids, the elimination of soil microbes would result in limited plant availability of soluble inorganic nutrients (Berg 2009).

In terms of P, its concentrations decreased significantly in our maize in sterilized soils, regardless of As treatments (Figure S4.8). The concentrations were significantly lower than the amount required for adequate growth (2000 mg kg^{-1} stem) (Kirkby 2012). Crops cultivated in sterilized soils grow much worse than those in unsterilized soils, and dry biomass production of the stem in various crops is significantly depressed at a low P supply, which is particularly noticeable in sterilized soils (Ortas 2003). Soil sterilization can induce abiotic changes associated with P deficiency by eliminating symbiotic myccorrhizae involved in P absorption (Wallace et al. 1973). Meanwhile, P is known to usually reduce As uptake, mainly As^{V}, in plant and microbial systems and to interfere with As biotransformation by competing for As transporters (Wu et al. 2022). High inorganic P treatment can upregulate glutathione biosynthesis in plants by increasing glutathione reductase activity (Souri et al. 2018) as well as upregulating the expression of arsenate reductase in wheat to reduce As^{V} to As^{III} (Pigna et al. 2010), thereby promoting the binding to As^{III} for detoxification. These findings broadly support the fact that P deficiency induced by the sterilization effect not only is detrimental to plant growth and production, also plays a role in high As uptake and accumulation in plants in sterilized soils.

Furthermore, nutrient deficiency was observed for Mn in our maize in sterilized soils, but only when soil As concentration was high (200 mg kg^{-1}) (Figure S4.8). Soil sterilization has been reported to enhance the release of extractable Mn in soils (Mahmood et al. 2014). Similar to this result, a significant increase in Mn concentration was also observed in our maize in sterilized soils with low As levels (Figure S4.8). Conversely, Mn concentration in maize was lower in sterilized than in unsterilized soils with high As content. This could be ascribed to a possible complex in plant roots between amorphous hydroxides of Mn and As^{V} (Rosas-Castor et al. 2014a). As a result of the complexation, there is a strong negative correlation between Mn concentrations in agricultural soil water and As translocation into plant stem. The low concentration of Mn in soils can raise As concentration in plant aerial tissues and increase As transference across the food chain (Rosas-Castor et al. 2014a).

Altogether, our findings indicated that the sterilization effect caused a significant increase in K concentration (Figure S4.7) and a significant decrease in P concentration (Figure S4.8) in maize grown in sterilized soils. An adequate supply of K may have resulted in a large increase in maize leaf numbers in sterilized soils with low As content. Phosphorus deficiencies might have influenced As translocation and accumulation in maize plants grown on both un- and contaminated soils. Further, Mn deficiency in maize in sterilized soils was observed only when As content in the soils was high, which could have contributed to poor plant health and increased As accumulation in plants, aggravating the harmful effects of As on plant health.

4.4 Correlation Between As in Maize Tissues and Plant Health

When maize plants are exposed to high As, either in soils or in hydroculture, they exhibit toxicity symptoms such as reduction in plant height, root and stem growth, chlorophyll content, leaf area, and photosynthesis (Stoeva et al. 2003; Hakeem et al. 2015; Duquesnoy et al. 2010). In the present study, the strongest correlation was found in the leaves between the concentrations of totAs and As species and plant height, fresh biomass, BBCH-scale loss, chlorophyll, and damage scale. Chlorophyll, as the primary pigment in photosynthesis, requires N to form its core molecular component. High As concentrations in soils can decrease leaf N content (Peng 2000) and inhibit the activity and synthesis of aminolaevulinic acid and protochlorophyll reductase (Anjum et al. 2017), thereby lowering chlorophyll synthesis and the photosynthesis rate. The induced low photosynthetic rate can lead to a reduction in plant height and BBCH-scale (Spagnoletti and Lavado 2015) as well as in plant biomass and yield (Sandil et al. 2021), as the addition of As delays plant phenological progress. Moreover, the positive correlation between As concentrations in the leaves and the leaf damage scale might be an indication of As-stressed leaves. Brown spots on maize leaves indicate the accumulation of As-induced hydrogen peroxide in plant cells (Ghosh et al. 2017). In addition, no significant correlations were found between As concentration and leaf numbers in our study, possibly because that K, rather than As, was the driving factor for leaf development.

5 Conclusion

Maize plants grown in unsterilized soils generally presented better health than those in sterilized soils. The microbial disturbance effect was not observed in uncontaminated soils (except damage scale), implying that maize plants were capable of buffering the effect of microbial disturbance in the absence of As. However, when the As stressor was present in soils, plant height, leaf numbers, chlorophyll content, and damage scale were retarded by the interaction effects of microbial disturbance and As treatments. Less damage was observed in maize grown on soil with undisturbed soil indigenous microbes, demonstrating the importance of soil indigenous microbes in protecting maize plants from As poisoning. In sterilized soils with high As levels, the sterilization effect resulted in higher K concentrations in the leaves as well as P and Mn deficiencies in maize. Nutrient deficiencies hindered plant growth and production, which corresponded to increased uptake and accumulation of As in plants, exacerbating the detrimental effects of As on plant health. The As concentrations in the leaves correlated strongly negatively with all the plant health parameters except leaf numbers, which were likely controlled primarily by K rather than As. This study highlighted the role of soil indigenous microbes in promoting plant growth and imparting plant resistance to As, which could provide reference value for the role of soil indigenous microbes in plants grown in As-contaminated soils and ensure the sustainability of agricultural production.

Associated Content

Supporting Information

Additional information: *I Materials and Methods*, including the chemical characterizations of soils (Table S1.1); the preparation of microbial extracts (Figure S1.1); maize biomass estimation model (Figure S1.2). *II Results*, including the *p* values of univariate ANOVA statistical analysis on plant health parameters (Table S4.1); changes in plant height and fresh biomass under soil sterilization and As treatments (Figure S4.1-S4.2); changes in BBCH-scale, leaf numbers, and chlorophyll of maize over time under soil sterilization and As treatments (Figure S4.3-S4.5); changes in the damage scale of maize under soil sterilization and As treatments (Figure S4.6); the correlation coefficients between phenotyping parameters and As species in maize roots, stem, leaves, and grains (Table S4.2-S4.5); the concentrations of total K in maize leaves under soil sterilization and As treatments (Figure S4.7); and the concentrations of total P and total Mn under soil sterilization and As treatments (Figure S4.8); the estimated marginal means (emmeans, R Package) of totAs concentration and As species concentrations in maize roots, stem, leaves, and grains (Table S4.6-S4.9).

Acknowledgements

This research was financially supported by the University of Bern through the Interfaculty Research Cooperation One Health (https://www.onehealth.unibe.ch/). We especially thank Dr. Daniela Fischer and Patrick Neuhaus for their support in the laboratory, Valentin Gfeller for his guidance in plant cultivation, as well as Pia Bergamaschi, Andrea Weber, Florian Enz and Christina Désirée Widmer for their help with plant incubation and data collection. We would also like to thank Dr. Suyi Hu for his help with data organisation.

Author Contributions

Hang Guan: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - Original draft, Visualization, Project administration. Veronica Caggìa: Investigation, Writing - Review and Editing. Andrea Gómez-Chamorro: Formal analysis, Writing - Review and Editing. Xiaowen Liu: Investigation. Alban Ramette: Conceptualization, Formal analysis, Writing - Review and Editing. Klaus Schlaeppi: Conceptualization, Resources, Writing - Review and Editing. Adrien Mestrot: Conceptualization, Supervision, Funding acquisition, Writing - Review and Editing. Moritz Bigalke: Conceptualization, Supervision, Writing - Review and Editing, Funding acquisition, Project administration.

Reference

Abbas, Mohamed H. H.; Meharg, Andrew A. (2008): Arsenate, arsenite and dimethyl arsinic acid (DMA) uptake and tolerance in maize (Zea mays L.). In *Plant Soil* 304 (1-2), pp. 277–289. DOI: 10.1007/s11104-008-9549-9.

Alam, Mohammad Zahangeer; Hoque, Md Anamul; Ahammed, Golam Jalal; McGee, Rebecca; Carpenter-Boggs, Lynne (2019): Arsenic accumulation in lentil (Lens culinaris) genotypes and risk associated with the consumption of grains. In *Sci Rep* 9 (1), p. 9431. DOI: 10.1038/s41598-019-45855-z.

Al-Khaliel, Abdulla Saleh (2010): Effects of Arbuscular Mycorrhization in Sterile and Non-sterile Soils. In *Tropical Life Sciences Research* 21 (1), pp. 55–70.

Anjum, Shakeel Ahmad; Tanveer, Mohsin; Hussain, Saddam; Ashraf, Umair; Khan, Imran; Wang, Longchang (2017): Alteration in Growth, Leaf Gas Exchange, and Photosynthetic Pigments of Maize Plants Under Combined Cadmium and Arsenic Stress. In *Water Air Soil Pollut* 228 (1), pp. 1–12. DOI: 10.1007/s11270-016-3187-2.

Bauer, Wolfgang D.; Mathesius, Ulrike (2004): Plant responses to bacterial quorum sensing signals. In *Current opinion in plant biology* 7 (4), pp. 429–433. DOI: 10.1016/j.pbi.2004.05.008.

Berg, Gabriele (2009): Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. In *Applied microbiology and biotechnology* 84 (1), pp. 11–18. DOI: 10.1007/s00253-009-2092-7.

Berns; Philipp, H.; Narres, H.-D.; Burauel, P.; Vereecken, H.; Tappe, W. (2008): Effect of gamma-sterilization and autoclaving on soil organic matter structure as studied by solid state NMR, UV and fluorescence spectroscopy. In *Eur J Soil Science* 59 (3), pp. 540–550. DOI: 10.1111/j.1365-2389.2008.01016.x.

Blankinship, Joseph C.; Becerra, Caryl A.; Schaeffer, Sean M.; Schimel, Joshua P. (2014): Separating cellular metabolism from exoenzyme activity in soil organic matter decomposition. In *Soil Biology and Biochemistry* 71, pp. 68–75. DOI: 10.1016/j.soilbio.2014.01.010.

Broeckling, Corey D.; Broz, Amanda K.; Bergelson, Joy; Manter, Daniel K.; Vivanco, Jorge M. (2008): Root exudates regulate soil fungal community composition and diversity. In *Applied and environmental microbiology* 74 (3), pp. 738–744. DOI: 10.1128/AEM.02188-07.

CI, Xiao-ke; LIU, Hua-lin; HAO, Yu-bo; ZHANG, Ji-wang; LIU, Peng; DONG, Shu-ting (2012): Arsenic Distribution, Species, and Its Effect on Maize Growth Treated with Arsenate. In *Journal of Integrative Agriculture* 11 (3), pp. 416–423. DOI: 10.1016/S2095-3119(12)60026-4.

Dao, T. H.; Marx, D. B.; Lavy, T. L.; Dragun, J. (1982): Effect, and Statistical Evaluation, of Soil Sterilization on Aniline and Diuron Adsorption Isotherms. In *Soil Science Society of America Journal* 46 (5), pp. 963–969. DOI: 10.2136/sssaj1982.03615995004600050016x.

Di, Xuerong; Beesley, Luke; Zhang, Zulin; Zhi, Suli; Jia, Yan; Ding, Yongzhen (2019): Microbial Arsenic Methylation in Soil and Uptake and Metabolism of Methylated Arsenic in Plants: A

Review. In *International journal of environmental research and public health* 16 (24). DOI: 10.3390/ijerph16245012.

Dietrich, Peter; Cesarz, Simone; Eisenhauer, Nico; Roscher, Christiane (2020): Effects of steam sterilization on soil abiotic and biotic properties. In *SOIL ORG* 92 (2), 99–108-99–108. DOI: 10.25674/so92iss2pp99.

Drewniak, L.; Sklodowska, A. (2013): Arsenic-transforming microbes and their role in biomining processes. In *Environ Sci Pollut Res* 20 (11), pp. 7728–7739. DOI: 10.1007/s11356-012-1449-0.

Duquesnoy, Isabelle; Champeau, Gabrielle Marie; Evray, Germaine; Ledoigt, Gérard; Piquet-Pissaloux, Agnès (2010): Enzymatic adaptations to arsenic-induced oxidative stress in Zea mays and genotoxic effect of arsenic in root tips of Vicia faba and Zea mays. In *Comptes rendus biologies* 333 (11-12), pp. 814–824. DOI: 10.1016/j.crvi.2010.07.004.

Etesami, Hassan (2018): Bacterial mediated alleviation of heavy metal stress and decreased accumulation of metals in plant tissues: Mechanisms and future prospects. In *Ecotoxicology and environmental safety* 147, pp. 175–191. DOI: 10.1016/j.ecoenv.2017.08.032.

Finnegan, Patrick M.; Chen, Weihua (2012): Arsenic toxicity: the effects on plant metabolism. In *Front. Physiol.* 3, p. 182. DOI: 10.3389/fphys.2012.00182.

Flora, Swaran J. S. (2011): Arsenic-induced oxidative stress and its reversibility. In *Free radical biology & medicine* 51 (2), pp. 257–281. DOI: 10.1016/j.freeradbiomed.2011.04.008.

Garg, Neera; Singla, Priyanka (2011): Arsenic toxicity in crop plants: physiological effects and tolerance mechanisms. In *Environ Chem Lett* 9 (3), pp. 303–321. DOI: 10.1007/s10311-011-0313-7.

Ghosh, Supriya; Singh, Kashmir; Shaw, Arun K.; Azahar, Ikbal; Adhikari, Sinchan; Ghosh, Ujjal et al. (2017): Insights into the miRNA-mediated response of maize leaf to arsenate stress. In *Environmental and Experimental Botany* 137, pp. 96–109. DOI: 10.1016/j.envexpbot.2017.01.015.

Gianfreda, L. (2015): Enzymes of importance to rhizosphere processes. In *J. Soil Sci. Plant Nutr.* (ahead), p. 0. DOI: 10.4067/S0718-95162015005000022.

Hakeem, Khalid; Sabir, Muhammad; Ozturk, Munir; Mermut, Ahmet (2015): Soil Remediation and Plants. Chapter 16. Arsenic Toxicity in Plants and Possible Remediation. Amsterdam, Boston: Academic Press, an imprint of Elsevier.

Hongshao, Z.; Stanforth, R. (2001): Competitive adsorption of phosphate and arsenate on goethite. In *Environmental science & technology* 35 (24), pp. 4753–4757. DOI: 10.1021/es010890y.

Hu, Lingfei; Robert, Christelle A. M.; Cadot, Selma; Zhang, Xi; Ye, Meng; Li, Beibei et al. (2018): Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. In *Nature communications* 9 (1), p. 2738. DOI: 10.1038/s41467-018-05122-7.

Huang, Honglin; Ullah, Farhan; Zhou, Dao-Xiu; Yi, Ming; Zhao, Yu (2019): Mechanisms of ROS Regulation of Plant Development and Stress Responses. In *Frontiers in plant science* 10, p. 800. DOI: 10.3389/fpls.2019.00800.

Huang, Lu; Wang, Xun; Chi, Yihan; Huang, Linan; Li, Wai Chin; Ye, Zhihong (2021): Rhizosphere bacterial community composition affects cadmium and arsenic accumulation in rice (Oryza sativa L.). In *Ecotoxicology and environmental safety* 222, p. 112474. DOI: 10.1016/j.ecoenv.2021.112474.

Kaur, Sukhvir; Pandey, Shreelekha; Goel, Shivani (2019): Plants Disease Identification and Classification Through Leaf Images: A Survey. In *Arch Computat Methods Eng* 26 (2), pp. 507–530. DOI: 10.1007/s11831-018-9255-6.

Khairul, Islam; Wang, Qianqian; Jiang, Yuhan; Chao, Wang; Naranmandura, Hua (2017): Metabolism, toxicity and anticancer activities of arsenic compounds 8, Article 14, pp. 23905–23926.

Kirkby, Ernest (2012): Introduction, Definition and Classification of Nutrients. In : Marschner's Mineral Nutrition of Higher Plants: Elsevier, pp. 3–5.

Kumar, Akhilesh; Verma, Jay Prakash (2018): Does plant-Microbe interaction confer stress tolerance in plants: A review? In *Microbiological research* 207, pp. 41–52. DOI: 10.1016/j.micres.2017.11.004.

Lancashire, Peter D.; H. Bleiholder; T. van den Boom; P. Langelüddeke; R. Stauss; Elfriede Weber; and A. Witzenberger (1991): A uniform decimal code for growth stages of crops and weeds. In *Ann Applied Biology* 119 (3), pp. 561–601. DOI: 10.1111/j.1744-7348.1991.tb04895.x.

Li, Kun; DiLegge, Michael J.; Minas, Ioannis S.; Hamm, Alison; Manter, Daniel; Vivanco, Jorge M. (2019): Soil sterilization leads to re-colonization of a healthier rhizosphere microbiome. In *Rhizosphere* 12, p. 100176. DOI: 10.1016/j.rhisph.2019.100176.

Lin, Chao-Yi; Yeh, Der-Ming (2008): Potassium Nutrition Affects Leaf Growth, Anatomy, and Macroelements of Guzmania. In *horts* 43 (1), pp. 146–148. DOI: 10.21273/HORTSCI.43.1.146.

López-Bucio, José; Nieto-Jacobo, María Fernanda; Ramírez-Rodríguez, Verenice; Herrera-Estrella, Luis (2000): Organic acid metabolism in plants: from adaptive physiology to transgenic varieties for cultivation in extreme soils. In *Plant Science* 160 (1), pp. 1–13. DOI: 10.1016/S0168-9452(00)00347-2.

Lu, Yuqiu; Wang, Enzhao; Tang, Zhiyu; Rui, Junpeng; Li, Yanling; Tang, Zhixi et al. (2022): Roots and microbiome jointly drive the distributions of 17 phytohormones in the plant soil continuum in a phytohormone‐specific manner. In *Plant Soil* 470 (1-2), pp. 153–165. DOI: 10.1007/s11104-021-04898-w.

Ma, Jian Feng; Ryan, Peter R.; Delhaize, Emmanuel (2001): Aluminium tolerance in plants and the complexing role of organic acids. In *Trends in Plant Science* 6 (6), pp. 273–278. DOI: 10.1016/S1360-1385(01)01961-6.

Mahmood, T.; Mehnaz, S.; Fleischmann, F.; Ali, R.; Hashmi, Z. H.; Iqbal, Z. (2014): Soil sterilization effects on root growth and formation of rhizosheaths in wheat seedlings. In *Pedobiologia* 57 (3), pp. 123–130. DOI: 10.1016/j.pedobi.2013.12.005.

Majumder, Aparajita; Bhattacharyya, Kallol; Kole, S. C.; Ghosh, Sagarmoy (2013): Efficacy of indigenous soil microbes in arsenic mitigation from contaminated alluvial soil of India. In *Environ Sci Pollut Res* 20 (8), pp. 5645–5653. DOI: 10.1007/s11356-013-1560-x.

Maksimov, I. V.; Veselova, S. V.; Nuzhnaya, T. V.; Sarvarova, E. R.; Khairullin, R. M. (2015): Plant growth-promoting bacteria in regulation of plant resistance to stress factors. In *Russ J Plant Physiol* 62 (6), pp. 715–726. DOI: 10.1134/S1021443715060114.

Marschner, Petra; Rumberger, Angelika (2004): Rapid changes in the rhizosphere bacterial community structure during re-colonization of sterilized soil. In *Biol Fertil Soils* 40 (1), pp. 1–6. DOI: 10.1007/s00374-004-0736-4.

McNamara, N. P.; Black, H.I.J.; Beresford, N. A.; Parekh, N. R. (2003): Effects of acute gamma irradiation on chemical, physical and biological properties of soils. In *Applied Soil Ecology* 24 (2), pp. 117–132. DOI: 10.1016/S0929-1393(03)00073-8.

Mestrot, Adrien; Planer-Friedrich, Britta; Feldmann, Jörg (2013): Biovolatilisation: a poorly studied pathway of the arsenic biogeochemical cycle. In *Environmental science. Processes & impacts* 15 (9), pp. 1639–1651. DOI: 10.1039/c3em00105a.

Miransari, M.; Bahrami, H. A.; Rejali, F.; Malakouti, M. J. (2009): Effects of soil compaction and arbuscular mycorrhiza on corn (Zea mays L.) nutrient uptake. In *Soil and Tillage Research* 103 (2), pp. 282–290. DOI: 10.1016/j.still.2008.10.015.

Moreira, Helena; Pereira, Sofia I.A.; Marques, Ana P.G.C.; Rangel, António O.S.S.; Castro, Paula M.L. (2019): Effects of soil sterilization and metal spiking in plant growth promoting rhizobacteria selection for phytotechnology purposes. In *Geoderma* 334, pp. 72–81. DOI: 10.1016/j.geoderma.2018.07.025.

Neumann, Kerstin; Klukas, Christian; Friedel, Swetlana; Rischbeck, Pablo; Chen, Dijun; Entzian, Alexander et al. (2015): Dissecting spatiotemporal biomass accumulation in barley under different water regimes using high-throughput image analysis. In *Plant, cell & environment* 38 (10), pp. 1980–1996. DOI: 10.1111/pce.12516.

Noreika, Norbertas; Pärtel, Meelis; Öpik, Maarja (2021): Effects of mutualistic and pathogenic soil mycobiota on forest ecosystem functioning: herbaceous phytometer growth on natural and sterilised soils. In *Ecological Indicators* 127, p. 107792. DOI: 10.1016/j.ecolind.2021.107792.

Ochieno, Dennis M. W. (2022): Soil Sterilization Eliminates Beneficial Microbes That Provide Natural Pest Suppression Ecosystem Services Against Radopholus similis and Fusarium Oxysporum V5w2 in the Endosphere and Rhizosphere of Tissue Culture Banana Plants. In *Front. Sustain. Food Syst.* 6, Article 688194. DOI: 10.3389/fsufs.2022.688194.

Ortas, Ibrahim (2003): Effect of Selected Mycorrhizal Inoculation on Phosphorus Sustainability in Sterile and Non‐sterile Soils in the Harran Plain in South Anatolia. In *Journal of Plant Nutrition* 26 (1), pp. 1–17. DOI: 10.1081/PLN-120016494.

Ortíz-Castro, Randy; Contreras-Cornejo, Hexon Angel; Macías-Rodríguez, Lourdes; López-Bucio, José (2009): The role of microbial signals in plant growth and development. In *Plant signaling & behavior* 4 (8), pp. 701–712. DOI: 10.4161/psb.4.8.9047.

Peng, S. (2000): Single-leaf and canopy photosynthesis of rice11Citation: Sheehy JE, Mitchell PL, Hardy B, editors. 2000. Redesigning rice photosynthesis to increase yield. Proceedings of the Workshop on The Quest to Reduce Hunger: Redesigning Rice Photosynthesis, 30 Nov.-3 Dec. 1999, Los Baños. Philippines. Makati City (Philippines): International Rice Research Institute and Amsterdam (The Netherlands): Elsevier Science B.V. 293 p. In *Studies in Plant Science* 7, pp. 213–228. DOI: 10.1016/S0928-3420(00)80017-8.

Phillips, Donald A.; Fox, Tama C.; King, Maria D.; Bhuvaneswari, T. V.; Teuber, Larry R. (2004): Microbial products trigger amino acid exudation from plant roots. In *Plant Physiol* 136 (1), pp. 2887–2894. DOI: 10.1104/pp.104.044222.

Pigna, M.; Cozzolino, V.; Giandonato Caporale, A.; Mora, M.L; Di Meo, V.; Jara, A.A; Violante, A. (2010): EFFECTS OF PHOSPHORUS FERTILIZATION ON ARSENIC UPTAKE BY WHEAT GROWN IN POLLUTED SOILS. In *J. Soil Sci. Plant Nutr.* 10 (4), pp. 428–442. DOI: 10.4067/S0718-95162010000200004.

Piñeros, Miguel A.; Magalhaes, Jurandir V.; Carvalho Alves, Vera M.; Kochian, Leon V. (2002): The physiology and biophysics of an aluminum tolerance mechanism based on root citrate exudation in maize. In *Plant physiology* 129 (3), pp. 1194–1206. DOI: 10.1104/pp.002295.

Rahman, M.; Hasegawa, H.; Mahfuzur Rahman, M.; Nazrul Islam, M.; Majid Miah, M. A.; Tasmen, A. (2007): Effect of arsenic on photosynthesis, growth and yield of five widely cultivated rice (Oryza sativa L.) varieties in Bangladesh. In *Chemosphere* 67 (6), pp. 1072–1079. DOI: 10.1016/j.chemosphere.2006.11.061.

Rai, Prabhat Kumar; Lee, Sang Soo; Zhang, Ming; Tsang, Yiu Fai; Kim, Ki-Hyun (2019): Heavy metals in food crops: Health risks, fate, mechanisms, and management. In *Environment International* 125, pp. 365–385. DOI: 10.1016/j.envint.2019.01.067.

Razavi; Lakzian (2007): Evaluation of chemical and biological consequences of soil sterilization methods 5, pp. 87–91.

Rosas-Castor, J. M.; Guzmán-Mar, J. L.; Alfaro-Barbosa, J. M.; Hernández-Ramírez, A.; Pérez-Maldonado, I. N.; Caballero-Quintero, A.; Hinojosa-Reyes, L. (2014a): Evaluation of the transfer of soil arsenic to maize crops in suburban areas of San Luis Potosi, Mexico. In *The Science of the total environment* 497-498, pp. 153–162. DOI: 10.1016/j.scitotenv.2014.07.072.

Rosas-Castor, J. M.; Guzmán-Mar, J. L.; Hernández-Ramírez, A.; Garza-González, M. T.; Hinojosa-Reyes, L. (2014b): Arsenic accumulation in maize crop (Zea mays): a review. In *The Science of the total environment* 488-489, pp. 176–187. DOI: 10.1016/j.scitotenv.2014.04.075.

Roychowdhury, Roopali; Roy, Madhumita; Rakshit, Annanya; Sarkar, Sangita; Mukherjee, Pritam (2018): Arsenic Bioremediation by Indigenous Heavy Metal Resistant Bacteria of Fly Ash Pond. In *Bulletin of environmental contamination and toxicology* 101 (4), pp. 527–535. DOI: 10.1007/s00128-018-2428-z.

Ruiz-Chancho, Maria José; López-Sánchez, José Fermín; Schmeisser, Ernst; Goessler, Walter; Francesconi, Kevin A.; Rubio, R. (2008): Arsenic speciation in plants growing in arsenic-contaminated sites. In *Chemosphere* 71 (8), pp. 1522–1530. DOI: 10.1016/j.chemosphere.2007.11.054.

Sandil, Sirat; Óvári, Mihály; Dobosy, Péter; Vetési, Viktória; Endrédi, Anett; Takács, Anita et al. (2021): Effect of arsenic-contaminated irrigation water on growth and elemental composition of tomato and cabbage cultivated in three different soils, and related health risk assessment. In *Environmental research* 197, p. 111098. DOI: 10.1016/j.envres.2021.111098.

Schaechter, Moselio (2009): Encyclopedia of microbiology. 3rd ed. / Moselio Schaechter. London: Academic.

Singh, Pratika; Khan, Azmi; Srivastava, Amrita (2021): Biological Means of Arsenic Minimization with Special Reference to Siderophore. In : Arsenic Toxicity: Challenges and Solutions: Springer, Singapore, pp. 253–278. Available online at https://link.springer.com/chapter/10.1007/978-981-33-6068-6_10.

Song, Jing; Zhao, Fang-Jie; McGrath, Steve P.; Luo, Yong-Ming (2006): Influence of soil properties and aging on arsenic phytotoxicity. In *Environmental toxicology and chemistry* 25 (6), pp. 1663–1670. DOI: 10.1897/05-480r2.1.

Souri, Zahra; Karimi, Naser; Oliveira, Letúzia M. de (2018): Antioxidant enzymes responses in shoots of arsenic hyperaccumulator, Isatis cappadocica Desv., under interaction of arsenate and phosphate. In *Environmental technology* 39 (10), pp. 1316–1327. DOI: 10.1080/09593330.2017.1329349.

Spagnoletti, Federico; Lavado, Raúl (2015): The Arbuscular Mycorrhiza Rhizophagus intraradices Reduces the Negative Effects of Arsenic on Soybean Plants. In *Agronomy* 5 (2), pp. 188–199. DOI: 10.3390/agronomy5020188.

Stoeva, N.; Berova, M.; Zlatev, Z. (2003): Physiological Response of Maize to Arsenic Contamination. In *Biologia Plantarum* 46 (3), pp. 449–452. DOI: 10.1023/B:BIOP.0000023893.12939.48.

Tiberg, Charlotta; Sjöstedt, Carin; Eriksson, Ann Kristin; Klysubun, Wantana; Gustafsson, Jon Petter (2020): Phosphate competition with arsenate on poorly crystalline iron and aluminum (hydr)oxide mixtures. In *Chemosphere* 255, p. 126937. DOI: 10.1016/j.chemosphere.2020.126937.

Tran, Thi Minh; Han, Hyeop-Jo; Ko, Ju-In; Lee, Jong-Un (2020): Effect of Indigenous Microbial Consortium on Bioleaching of Arsenic from Contaminated Soil by Shewanella putrefaciens. In *Sustainability* 12 (8), p. 3286. DOI: 10.3390/su12083286.

Turpeinen, Riina; Pantsar-Kallio, Mari; Kairesalo, Timo (2002): Role of microbes in controlling the speciation of arsenic and production of arsines in contaminated soils 285, pp. 133–145.

Valasek, John; Thomasson, J. Alex (Eds.) (2016): Autonomous Air and Ground Sensing Systems for Agricultural Optimization and Phenotyping. SPIE Commercial + Scientific Sensing and Imaging. Baltimore, Maryland, United States, Sunday 17 April 2016: SPIE (SPIE Proceedings).

Wallace, A.; Frolich, E. F.; Alexander, G. V. (1973): Effect of steam sterilization of soil on two desert plant species. In *Plant Soil* 39 (2), pp. 453–456. DOI: 10.1007/BF00014815.

Wang, Peipei; Sun, GuoXin; Jia, Yan; Meharg, Andrew A.; Zhu, YongGuan (2014): A review on completing arsenic biogeochemical cycle: Microbial volatilization of arsines in environment. In *Journal of Environmental Sciences* 26 (2), pp. 371–381. DOI: 10.1016/S1001-0742(13)60432-5.

Whipps, J. M. (2001): Microbial interactions and biocontrol in the rhizosphere. In *Journal of experimental botany* 52 (Spec Issue), pp. 487–511. DOI: 10.1093/jexbot/52.suppl_1.487.

Wu, Jingwen; Liang, Jieliang; Björn, Lars Olof; Li, Jintian; Shu, Wensheng; Wang, Yutao (2022): Phosphorus-arsenic interaction in the 'soil-plant-microbe' system and its influence on arsenic pollution. In *The Science of the total environment* 802, p. 149796. DOI: 10.1016/j.scitotenv.2021.149796.

Xiao, Enzong; Cui, Jinli; Sun, Weimin; Jiang, Shiming; Huang, Mengyan; Kong, Deguan et al. (2020): Root microbiome assembly of As-hyperaccumulator Pteris vittata and its efficacy in arsenic requisition. In *Environmental microbiology* 23 (4), pp. 1959–1971. DOI: 10.1111/1462-2920.15299.

Xun, Weibing; Huang, Ting; Zhao, Jun; Ran, Wei; Wang, Boren; Shen, Qirong; Zhang, Ruifu (2015): Environmental conditions rather than microbial inoculum composition determine the bacterial composition, microbial biomass and enzymatic activity of reconstructed soil microbial communities. In *Soil Biology and Biochemistry* 90, pp. 10–18. DOI: 10.1016/j.soilbio.2015.07.018.

Yu, Meng; Xie, Wei; Zhang, Xin; Zhang, Shubin; Wang, Youshan; Hao, Zhipeng; Chen, Baodong (2019): Arbuscular Mycorrhizal Fungi Can Compensate for the Loss of Indigenous Microbial Communities to Support the Growth of Liquorice (Glycyrrhiza uralensis Fisch.). In *Plants* 9 (1), p. 7. DOI: 10.3390/plants9010007.

Yu, Yang; Zhang, Shuzhen; Huang, Honglin; Luo, Lei; Wen, Bei (2009): Arsenic accumulation and speciation in maize as affected by inoculation with arbuscular mycorrhizal fungus Glomus mosseae. In *Journal of agricultural and food chemistry* 57 (9), pp. 3695–3701. DOI: 10.1021/jf900107y.

Yuan, Jun; Zhao, Jun; Wen, Tao; Zhao, Mengli; Li, Rong; Goossens, Pim et al. (2018): Root exudates drive the soil-borne legacy of aboveground pathogen infection. In *Microbiome* 6 (1), p. 156. DOI: 10.1186/s40168-018-0537-x.

Zain, Nurul Amalina; Ismail, Mohd Razi (2016): Effects of potassium rates and types on growth, leaf gas exchange and biochemical changes in rice (Oryza sativa) planted under cyclic water stress. In *Agricultural Water Management* 164, pp. 83–90. DOI: 10.1016/j.agwat.2015.09.022.

Zemanová, Veronika; Pavlíková, Daniela; Hnilička, František; Pavlík, Milan (2021): Arsenic Toxicity-Induced Physiological and Metabolic Changes in the Shoots of Pteris cretica and Spinacia oleracea. In *Plants (Basel, Switzerland)* 10 (10). DOI: 10.3390/plants10102009.

Zhang, Gui-Yun; Zhang, Li-Ping; Wei, Ming-Feng; Liu, Zhen; Fan, Qiao-Lan; Shen, Qi-Rong; Xu, Guo-Hua (2011): Effect of arbuscular mycorrhizal fungi, organic fertilizer and soil sterilization on maize growth. In *Acta Ecologica Sinica* 31 (4), pp. 192–196. DOI: 10.1016/j.chnaes.2011.04.005.

Chapter I Appendix

Summarizing Overview

Content includes two tables and two figures.

1 Materials and Methods (detailed information)

1.1 Soil Incubation

After sampling soils from the field, the maximum water holding capacity (WHC) of the soil was measured following the standard of ISO DIS 11268-2 (Appendix A). And ISO 11465 was applied to the quantification of soil water content on the same day of soil sampling (Appendix B). During soil incubation time, the water content in soils was hold at 50% of WHC. The soils were incubated for two months in the greenhouse building Nr. 24 in Ostermundigen, Switzerland, until reaching the equilibrium of As concentrations between soil and soil water phases (data not shown) and simulate aging (Song et al. 2006).

1.2 Preparation of Microbial Extracts

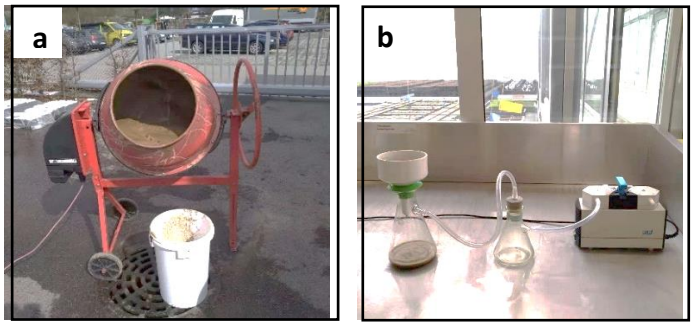


Figure S1.1. (a) Preparation of microbial extracts in a concrete mixer and (b) sieving of their supernatant

1.3 Chemical Characterizations of Soils

Table S1.1. Characterizations of native soil (NS) and disturbed soil (DS).

	pH	**Clay** [%]	**Silt** [%]	**Sand** [%]	**C_{org}** [g kg^{-1}]	**N** [g kg^{-1}]	**CEC** [$mmol_c$ kg^{-1}]
Native Soil (**NS**)	7.03 ± 0.06	17.27 ± 0.76	66.04 ± 1.04	16.69 ± 0.30	21.92 ± 0.01	2.70 ± 0.01	268.41 ± 3.40
Disturbed Soil (**DS**)	7.14 ± 0.07	17.96 ± 0.80	70.95 ± 2.48	11.09 ± 3.21	22.79 ± 0.01	2.76 ± 0.01	282.83 ± 1.12
	Fe [g kg^{-1}]	**Na** [g kg^{-1}]	**Mg** [g kg^{-1}]	**Al** [mg kg^{-1}]	**K** [g kg^{-1}]	**Mn** [g kg^{-1}]	**S** [g kg^{-1}]
Native Soil (**NS**)	21.71 ± 0.20	0.33 ± 0.01	2.21 ± 0.02	11.25 ± 7.30	2.40 ± 0.06	0.03 ± 0.00	0.37 ± 0.003
Disturbed Soil (**DS**)	22.21 ± 1.43	0.32 ± 0.05	2.23 ± 0.03	10.72 ± 0.54	2.64 ± 0.04 g	0.21 ± 0.00	0.42 ± 0.01

± standard deviations

1.4 Greenhosue Conditions

The maize plants were cultivated in the greenhouse with 14 h of light each day and a temperature of 18 - 26°C during the day and 16 - 24°C at night. The greenhouse cabin is heated in case of temperatures below 18°C during the day and below 16°C at night. The cooling system automatically turns on if the temperature exceeds 26°C. The ventilation system turns on once temperature is over 22°C in the daytime or over 20°C at night. The humidity ranged from 30% to 60%.

1.5 A Model to Estimate Maize Biomass

To control WHC at 50% in the main experiment, we would need to know the growing biomass of maize over time. Maize biomass needs to be known to control soil WHC, but maize cannot be harvested during growth. The idea was to build a model between the real maize biomass and the estimated digital biomass. The digital biomass was derived from transformed leaf area data of maize images. By developing a model that correlates maize fresh biomass with green pixel areas, we can estimate their fresh biomass over time. We stood on a fixed position and used a conventional camera to take images of maize weekly as shown on Figure S1.2a. Afterwards, we collected data of green pixel area of images by Adobe Photoshop and calculated the fresh biomass following the equation below. Through recording plant images and calculating their green pixel areas, we could estimate the digital biomass of maize. We grew 72 maize plants and recorded their images biweekly for four months using a conventional camera and harvested them to determine their actual weight. Every time we recorded maize photos, we harvested and weighed the fresh biomass of maize. Three plants were harvested each week for five months. The resulting linear model is shown as Figure S1.2b. In our experiment, we applied this estimation model for growing maize plants and controlling WHC at 50% in soils.

$$\text{Digital Biomass } [cm^3] = \sqrt{\mathrm{A_0} \times \mathrm{A_{90}} \times \mathrm{A_{TV}}}$$

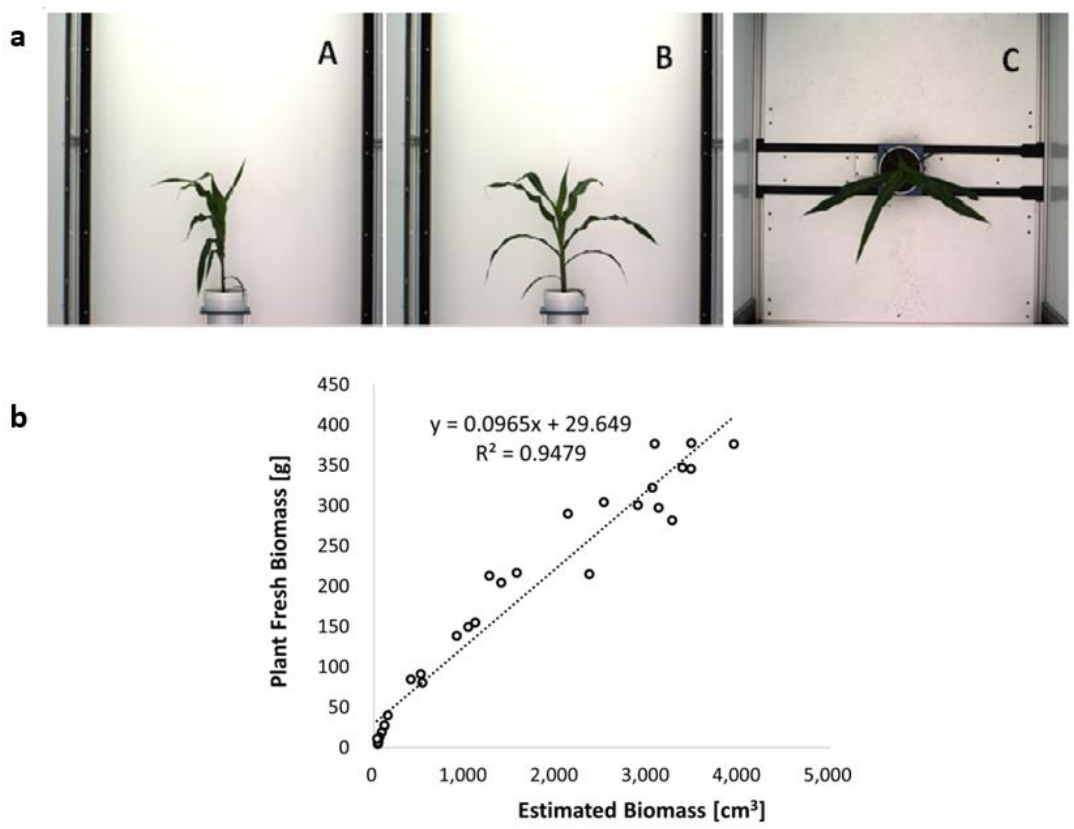


Figure S1.2 (a) An example of maize photos at A: 0 degree, B: 90 degrees, C: top view (Ge et al. 2016); (b) the derived maize biomass estimation model

1.6 Soil Water Sampling

From the side of a pot, a hole was established 2 cm above the water level of the saucer with a wood stick. To avoid space between soils and the soil water sampler, a soil-water slurry was made to fill the hole. The soil water sampler was then installed for 10 cm in a pot (Figure S1.3). The sampler was well fixed in the hole by sealing with a hot melt glue gun. The sampler is composed of three parts: the front tip is a porous part with a bulb diameter of 2.8 mm and a porous tubing of 10 cm with an average pore diameter of 0.15 µm; the middle is an extension tube of 12 cm (made of PE/PVC tubing); and the end is a joint with a female luer lock used to connect the syringe. The sampler was then connected with a 30 mL syringe and extended by a yellow wood stick to suck up soil water. The pressure of the syringe allowed soil water to be sampled overnight.

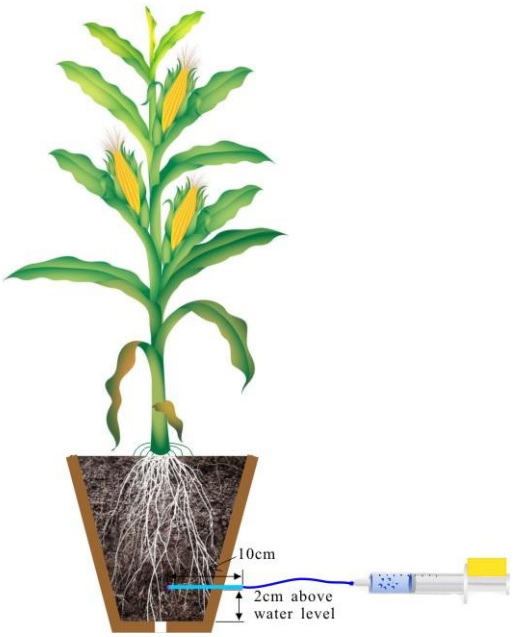


Figure S1.3. An example of a soil water sampler in a 7 L pot

1.7 HPLC Operating Parameters

Table S2 gives information about the details of HPLC operating parameters. The column recovery for the column was 90.60 ± 14.67 % based on the calculations of 28 soil samples.

Table S1.2. The operating parameters for HPLC

HPLC conditions	
Injection volume	5 µl
Column temperature	20 °C
Mobile phase	50 mM ammonium carbonate $(NH_4)_2CO_3$ (pH 8.9) in 3% methanol
Flow rate	1 mL min^{-1}
Calibration standards	DMA^V (0 - 150 ppb)
Quality controls	DMA^V (0.4, 4, and 8 ppb)

1.8 Determination of the Maximum WHC of the Soil

The following method for determining the maximum water holding capacity (WHC) of the soil has been found to be appropriate. It is described in Annex C of the ISO DIS 11268-2 (Soil Quality - Effects of pollutants on earthworms (Eisenia fetida). Part 2: Determination of effects on reproduction (3)).

Collect a defined quantity (e.g. 5 g) of the test soil substrate using a suitable sampling device (auger tube etc.). Cover the bottom of the tube with a wet piece of filter paper and then place it on a rack in a water bath. The tube should be gradually submerged until the water level is above the top of the soil. It should then be left in the water for about three hours. Since not all water absorbed by the soil capillaries can be retained, the soil sample should be allowed to drain for a period of two hours by placing the tube onto a bed of very wet finely ground quartz sand contained within a covered vessel (to prevent drying). The sample should then be weighed and dried to a constant mass at 105 °C. The water holding capacity (WHC) must be calculated as follows:

$$WHC\ (\text{in\% of dry mass}) = (S - T - D)/D \times 100$$

Where:

S = water-saturated substrate + mass of tube + mass of filter paper

T = tare (mass of tube + mass of filter paper)

D = dry mass of substrate

1.9 Determination of Dry Matter and Water Content on a Mass Basis

1 Scope

This International Standard ISO 11465:1993 (en) specifies a method for the determination of the dry matter content and water content of soil samples on a mass basis.

This method can be applied to all types of soil samples. Different procedures are specified for air-dried soil samples, for example, samples pretreated according to ISO 11464, and for field-moist soil samples.

For the determination of soil water content on a volume basis, refer to ISO 11461.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

- ISO 11461:— 1), Soil quality — Determination of soil water content on a volume basis — Gravimetric method.
- ISO 11464:— 1), Soil quality — Pretreatment of samples for physico-chemical analyses.

3 Definitions

For the purposes of this International Standard, the following definitions apply.

3.1 Dry matter content on a mass basis w_{dm}

Dry residue of soil, expressed as a percentage by mass, after drying according to this International Standard.

3.2 Water content on a dry mass basis w_{H2O}

Mass of water evaporating from the soil when dried to constant mass at 105 °C, divided by the dry mass of the soil and multiplied by 100.

3.3 Constant mass

Mass is reached when, during the drying process, the difference between two successive weightings of the cooled sample, with an interval of 4 h between them, does not exceed 0,1% (m/m) of the last determined mass.

Note 1 to entry: Usually, 16 h to 24 h is sufficient for drying most soils to constant mass, but certain soil types and large samples will require longer.

Chapter II Appendix

Maize plants and soil microbes interact to reduce arsenic concentrations and influence arsenic speciation in the soil water

Content includes five tables and eight figures.

1 Results

1.1 Three-way Interactions on Soil Water and Soils

The univariate analysis of variance (ANOVA) results showed that the interactions among the three experimental factors of microbial disturbance, plants, and As treatments were significant for totAs concentrations in soil water ($p < 0.05$), but not with the temporal effect (Table S3). It was also significant between microbial disturbance and As treatments ($F_{4, 397} = 21.428$, $p < 0.001$) as well as between plants and As treatments ($F_{2, 397} = 61.668$, $p < 0.001$). This could explain the intersection of lines in the As_{100} and As_{200} groups (Figure S2.1). More interaction effects showed in the As_{100} and As_{200} groups than in As_0 group, which suggested enhanced interaction effects among the experimental factors under As stress.

The multivariate analysis of variance (MANOVA) statistical results showed that the three-way and two-way interactions among the microbial disturbance, plants, and As treatments significantly affected As species in soil water ($p < 0.001$) (Table S5). In contrast, MANOVA analysis in soils showed insignificant three-way interactions ($F_{4, 294} = 0.854$, $p = 0.647$), which was also true for the ANOVA statistical results (Table S6). The three-way interactions were insignificant for inAs, orgAs, orgAs%, and individual orgAs (MMA^V, DMA^V and TMAO). The microbial disturbance made no difference in As speciation in soils. All the three soils had similar levels of inAs, but not of orgAs. Due to the few proportions of orgAs in soils, the difference of orgAs played a negligible role in the statistical result.

Table S2.1 The *p* values of univariate ANOVA statistical analysis on totAs and As species in soil water

Experimental factors	**totAs**	**inAs**	**orgAs**	**orgAs%**	**MMA^V**	**DMA^V**	**TMAO**
microbial disturbance	***	***	***	***	***	***	***
plants	0.047*	***	0.007**	0.364	0.231	0.010**	0.007**
As treatments	***	***	***	***	***	***	***
time	***	***	***	***	***	***	***
microbial disturbance × plants × As treatments	***	0.676	0.002**	***	0.202	0.055	***
microbial disturbance × plants	***	0.710	0.301	0.223	***	0.385	0.483
microbial disturbance × As treatments	***	***	***	***	0.117	***	***
plants × As treatments	***	***	***	0.003**	0.006**	***	0.032*

× interaction terms

***: $p < 0.001$; **: significant at $\alpha = 0.01$; *: significant at $\alpha = 0.05$

Table S2.2. The estimated marginal means (emmeans) of totAs and As species concentrations in soil water

Treatments	totAs µg/kg	inAs µg/kg	orgAs µg/kg	orgAs % %	MMA^V µg/kg	DMA^V µg/kg	TMAO µg/kg
As_0-NS-NP	2.82 ± 0.20	1.73 ± 0.15	1.64 ± 0.19	47.67 ± 4.31	1.06 ± 0.04	1.07 ± 0.10	1.09 ± 0.14
As_0-RS-NP	5.59 ± 0.36	1.75 ± 0.15	2.62 ± 0.29	68.33 ± 5.90	1.07 ± 0.04	1.19 ± 0.11	1.36 ± 0.17
As_0-DS-NP	7.76 ± 0.55	1.89 ± 0.17	3.43 ± 0.39	71.87 ± 6.50	1.15 ± 0.04	1.32 ± 0.13	1.78 ± 0.24
As_{100}-NS-NP	217.95 ± 26.4	132.72 ± 13.7	2.88 ± 0.38	2.44 ± 0.26	1.08 ± 0.05	1.37 ± 0.15	1.93 ± 0.3
As_{100}-RS-NP	239.56 ± 16.0	181.72 ± 15.5	6.57 ± 0.72	3.98 ± 0.35	1.09 ± 0.04	1.81 ± 0.17	3.83 ± 0.49
As_{100}-DS-NP	255.22 ± 16.2	190.58 ± 16.0	10.10 ± 1.10	5.51 ± 0.48	1.39 ± 0.05	2.99 ± 0.27	5.42 ± 0.68
As_{200}-NS-NP	872.16 ± 82.1	682.75 ± 79.9	2.76 ± 0.42	1.32 ± 0.16	1.11 ± 0.06	1.62 ± 0.21	1.57 ± 0.28
As_{200}-RS-NP	962.33 ± 67.7	689.96 ± 64.1	8.47 ± 1.02	2.19 ± 0.21	1.11 ± 0.04	1.81 ± 0.18	4.77 ± 0.67
As_{200}-DS-NP	941.42 ± 62.9	665.62 ± 60.7	4.66 ± 0.55	1.58 ± 0.15	1.28 ± 0.05	1.50 ± 0.15	2.32 ± 0.32
As_0-NS-P	5.71 ± 0.49	1.76 ± 0.18	2.09 ± 0.27	59.84 ± 6.18	1.07 ± 0.05	1.20 ± 0.13	1.12 ± 0.17
As_0-RS-P	7.02 ± 0.4	1.87 ± 0.15	2.87 ± 0.30	68.06 ± 5.70	1.17 ± 0.04	1.23 ± 0.11	1.37 ± 0.17
As_0-DS-P	8.00 ± 0.48	1.97 ± 0.15	3.15 ± 0.32	68.45 ± 5.50	1.12 ± 0.04	1.34 ± 0.11	1.45 ± 0.17
As_{100}-NS-P	133.23 ± 13.0	126.29 ± 12.7	2.25 ± 0.29	2.13 ± 0.22	1.10 ± 0.05	1.19 ± 0.13	1.34 ± 0.2
As_{100}-RS-P	162.98 ± 8.3	117.33 ± 7.9	5.94 ± 0.52	5.09 ± 0.35	1.14 ± 0.03	1.55 ± 0.11	3.65 ± 0.37
As_{100}-DS-P	221.00 ± 12.8	162.68 ± 12.0	5.38 ± 0.51	3.63 ± 0.27	1.17 ± 0.04	1.81 ± 0.15	3.20 ± 0.35
As_{200}-NS-P	733.26 ± 77.9	467.02 ± 53.1	3.34 ± 0.49	1.57 ± 0.18	1.18 ± 0.06	1.40 ± 0.17	2.31 ± 0.39
As_{200}-RS-P	791.16 ± 42.4	579.18 ± 40.8	6.76 ± 0.62	2.09 ± 0.15	1.23 ± 0.04	2.18 ± 0.17	3.44 ± 0.36
As_{200}-DS-P	690.30 ± 38.0	476.86 ± 34.7	5.72 ± 0.54	2.05 ± 0.15	1.28 ± 0.04	1.80 ± 0.14	2.89 ± 0.32

± standard errors; NP: No-plant pots and P: Plant pots

Table S2.3. The *p* values of MANOVA statistical analysis on As species in soil water and in soils

Experimental factors	As species in soil water	As species in soil
microbial disturbance	***	0.337
plants	***	0.022*
As treatments	***	***
time	***	***
microbial disturbance × plants × As treatments	***	0.647
microbial disturbance × plants	0.008**	0.941
microbial disturbance × As treatments	***	0.236
plants × As treatments	***	0.126

× interaction terms

***: $p < 0.001$; **: significant at $\alpha = 0.01$; *: significant at $\alpha = 0.05$

Table S2.4. The *p* values of univariate ANOVA statistical analysis on totAs and As species in soils

Experimental factors	inAs	orgAs	orgAs%	MMA^V	DMA^V	TMAO
Microbial disturbance	0.459	0.639	0.996	0.021*	0.737	0.419
Plants	0.610	0.046*	0.016*	0.208	0.075	0.003**
As treatments	***	***	***	***	***	***
Time	***	***	0.607	***	***	***
Microbial disturbance × plants × As treatments	0.727	0.676	0.981	0.225	0.940	0.219
Microbial disturbance × plants	0.447	0.710	0.977	0.565	0.752	0.374
Microbial disturbance × As treatments	0.478	0.620	0.242	0.021*	0.764	0.482
plants × As treatments	0.186	0.835	0.008**	0.681	0.670	0.525

× interaction terms

***: $p < 0.001$; **: significant at $\alpha = 0.01$; *: significant at $\alpha = 0.05$

Table S2.5. The emmeans of totAs and As species concentrations in soils

Treatment	inAs µg/kg	orgAs µg/kg	orgAs % %	MMA^V µg/kg	DMA^V µg/kg	TMAO µg/kg
As_0-NS-NP	727 ± 109.7	37.6 ± 13.48	5.84 ± 0.55	3.56 ± 1.80	31.2 ± 8.52	1.83 ± 0.68
As_0-RS-NP	778 ± 124.5	38.3 ± 14.29	5.62 ± 0.56	3.16 ± 1.70	27.4 ± 7.93	2.49 ± 0.98
As_0-DS-NP	987 ± 148.8	44.7 ± 13.48	5.14 ± 0.48	6.88 ± 3.49	33.6 ± 9.16	1.26 ± 0.47
As_{100}-NS-NP	44421 ± 6700	69.0 ± 13.48	1.15 ± 0.11	6.51 ± 3.29	49.3 ± 13.45	2.46 ± 0.92
As_{100}-RS-NP	43391 ± 6545	88.7 ± 13.48	1.19 ± 0.11	13.41 ± 6.79	40.1 ± 10.94	4.68 ± 1.74
As_{100}-DS-NP	41443 ± 6251	115.2 ± 13.48	1.26 ± 0.12	20.40 ± 10.33	42.8 ± 11.68	8.17 ± 3.04
As_{200}-NS-NP	107111 ± 16157	93.9 ± 13.48	1.09 ± 0.11	7.73 ± 3.91	64.4 ± 17.57	3.27 ± 1.22
As_{200}-RS-NP	102585 ± 15474	145.7 ± 13.48	1.14 ± 0.11	23.86 ± 12.08	88.3 ± 24.10	2.50 ± 0.93
As_{200}-DS-NP	108431 ± 16356	169.8 ± 13.48	1.15 ± 0.11	14.13 ± 7.15	103.9 ± 28.37	6.70 ± 2.49
As_0-NS-P	895 ± 76.5	38.4 ± 7.64	4.59 ± 0.24	3.63 ± 1.04	24.2 ± 3.74	1.49 ± 0.31
As_0-RS-P	1154 ± 109	39.9 ± 8.43	4.23 ± 0.25	5.69 ± 1.80	26.6 ± 4.54	1.31 ± 0.31
As_0-DS-P	1007 ± 95	37.8 ± 8.43	4.02 ± 0.24	4.01 ± 1.27	24.0 ± 4.10	1.32 ± 0.31
As_{100}-NS-P	41774 ± 3451	66.5 ± 7.38	1.15 ± 0.06	9.14 ± 2.53	40.6 ± 6.07	2.20 ± 0.45
As_{100}-RS-P	44979 ± 3992	74.7 ± 7.93	1.16 ± 0.06	7.38 ± 2.20	43.3 ± 6.96	2.94 ± 0.64
As_{100}-DS-P	45206 ± 4092	73.3 ± 8.09	1.16 ± 0.07	8.41 ± 2.56	43.5 ± 7.13	2.39 ± 0.53
As_{200}-NS-P	104216 ± 8610	85.3 ± 7.38	1.08 ± 0.06	4.90 ± 1.36	57.1 ± 8.53	2.16 ± 0.44
As_{200}-RS-P	107456 ± 10139	139.3 ± 8.43	1.14 ± 0.07	25.73 ± 8.15	74.7 ± 12.76	2.11 ± 0.49
As_{200}-DS-P	92074 ± 8171	130.6 ± 7.93	1.18 ± 0.07	20.94 ± 6.24	72.2 ± 11.59	2.1 ± 0.47

± standard errors; NP: No-plant pots and P: Plant pots

1.2 Time-series Plots

According to ANOVA results on Table S5, significant difference was observed for MMA^V, DMA^V, and TMAO concentrations in soil water for all four experimental factors (microbial disturbance, plants, As treatments, and time) ($p < 0.05$) (Figure S2.5). The concentrations of

MMA^{V}, DMA^{V}, and TMAO in soils were affected by the time effect ($p < 0.001$), which explained the large variations in the boxplot (Figure S2.6).

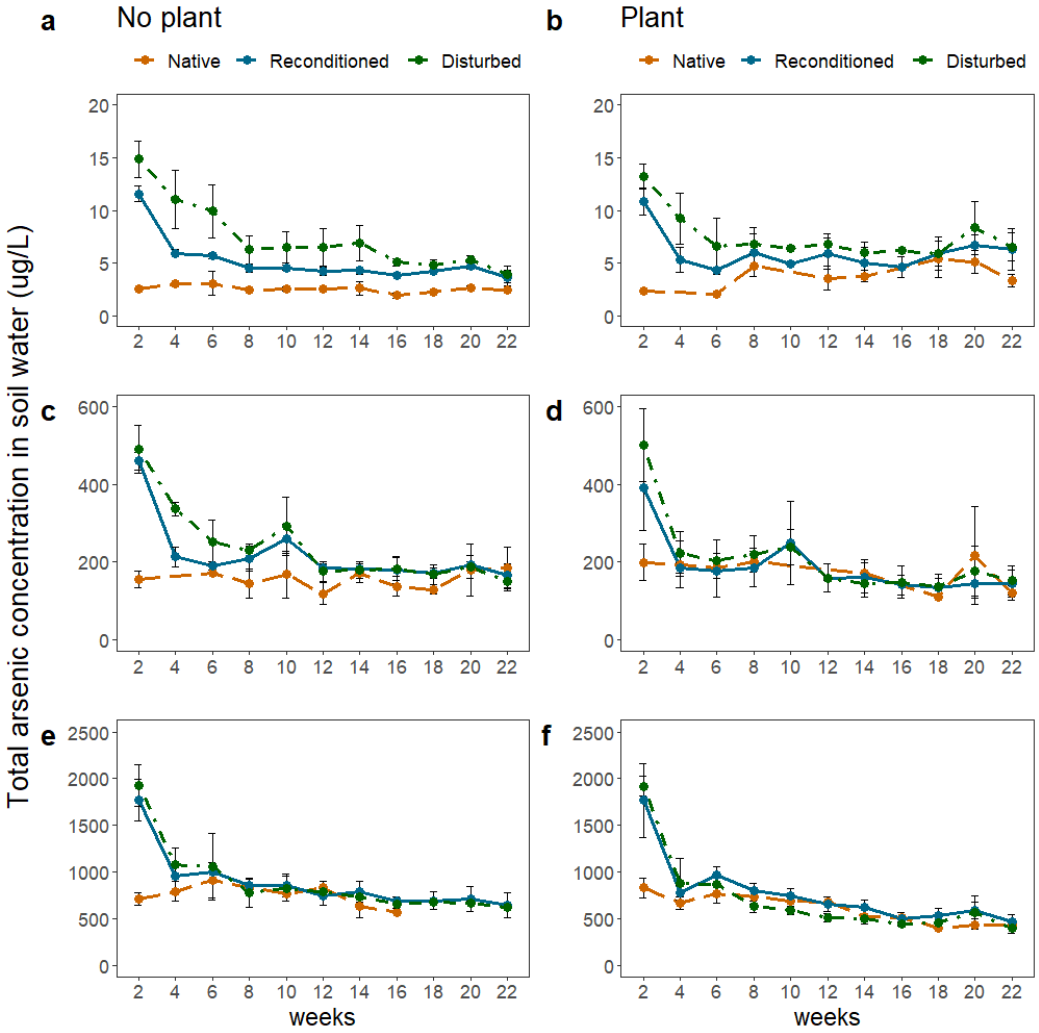


Figure S2.1. The totAs concentration in soil water of (a) NP_ As_0 group; (b) P_ As_0 group; (c) NP_ As_{100} group; (d) P_ As_{100} group; (e) NP_ As_{200} group; and (f) P_ As_{200} group. NP: No-plant pots and P: Plant pots

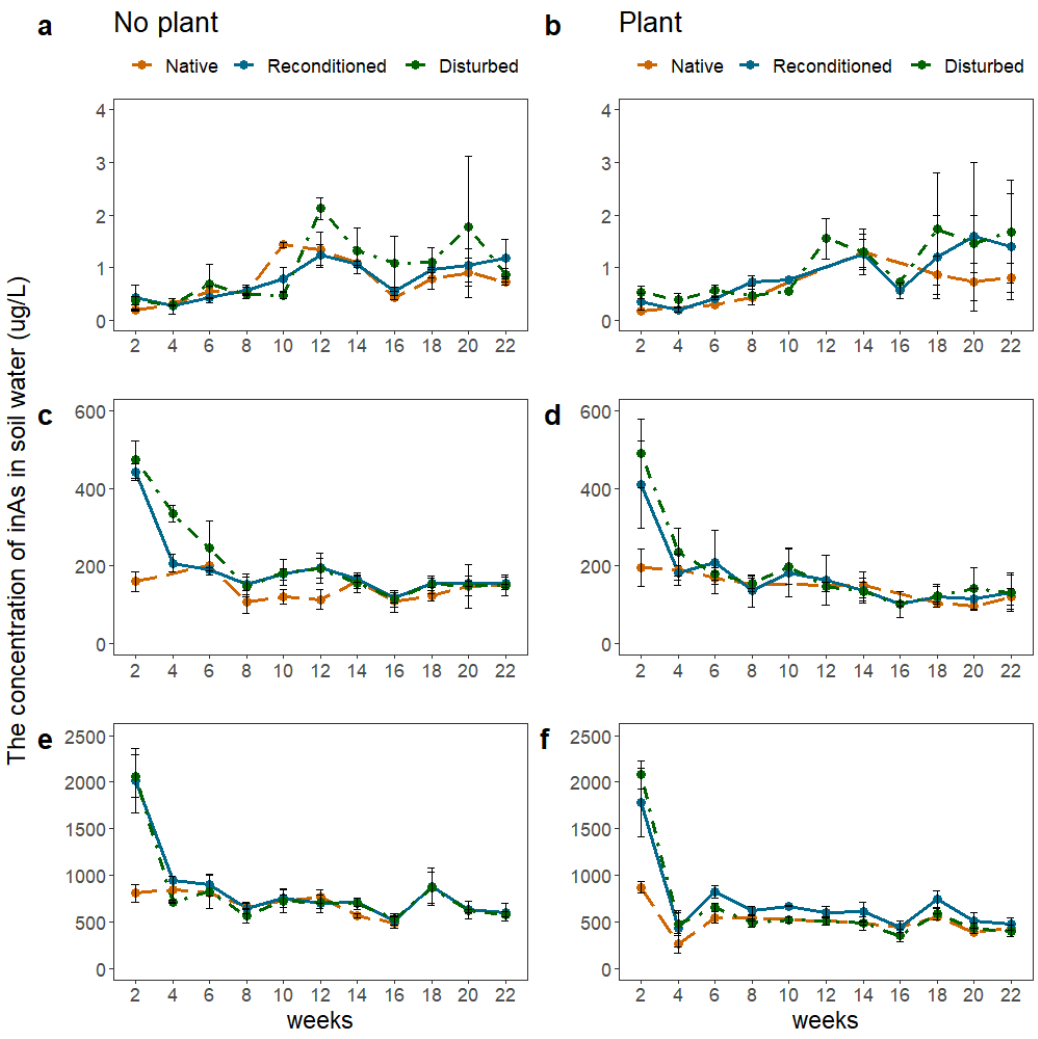


Figure S2.2. The inAs concentration in soil water of (a) NP_ As_0 group; (b) P_ As_0 group; (c) NP_ As_{100} group; (d) P_ As_{100} group; (e) NP_ As_{200} group; and (f) P_ As_{200} group. NP: No-plant pots and P: Plant pots

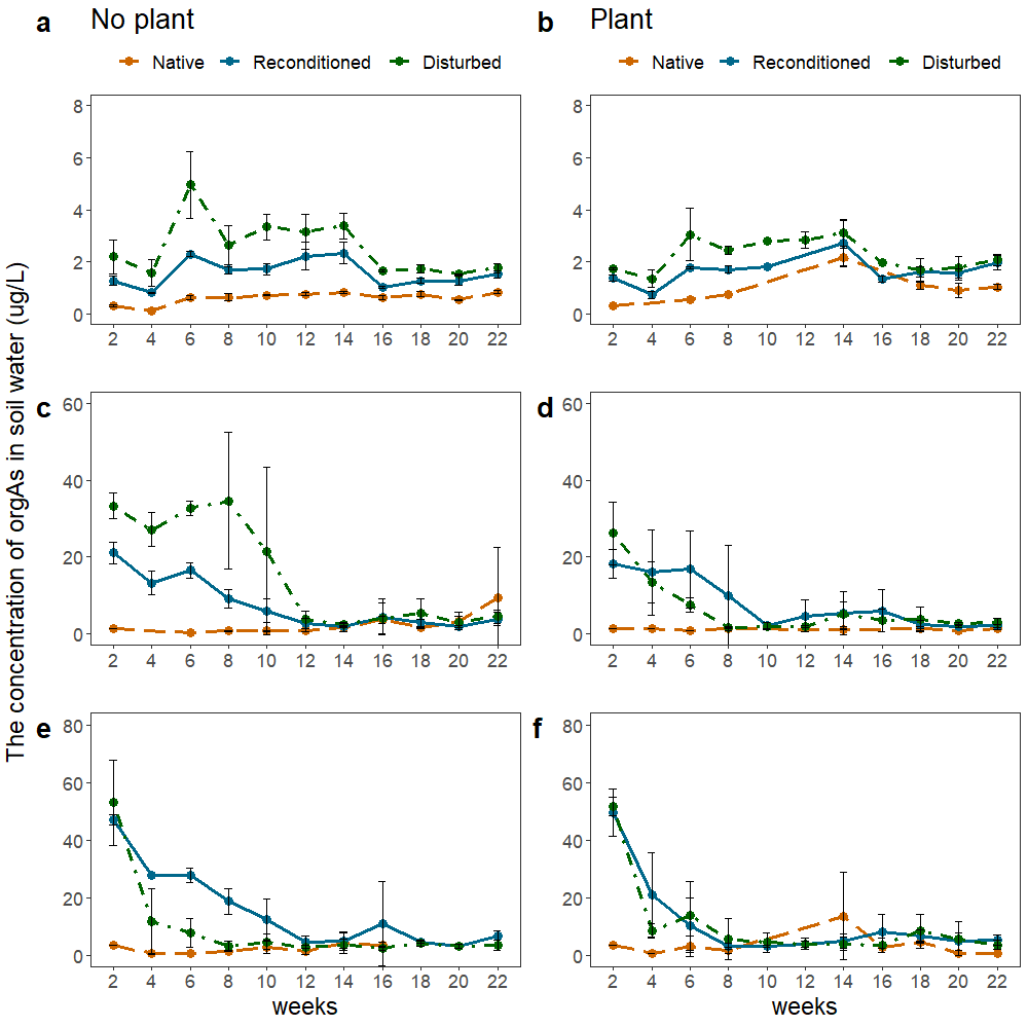


Figure S2.3. The orgAs concentration in soil water of (a) NP_ As_0 group; (b) P_ As_0 group; (c) NP_ As_{100} group; (d) P_ As_{100} group; (e) NP_ As_{200} group; and (f) P_ As_{200} group. NP: No-plant pots and P: Plant pots

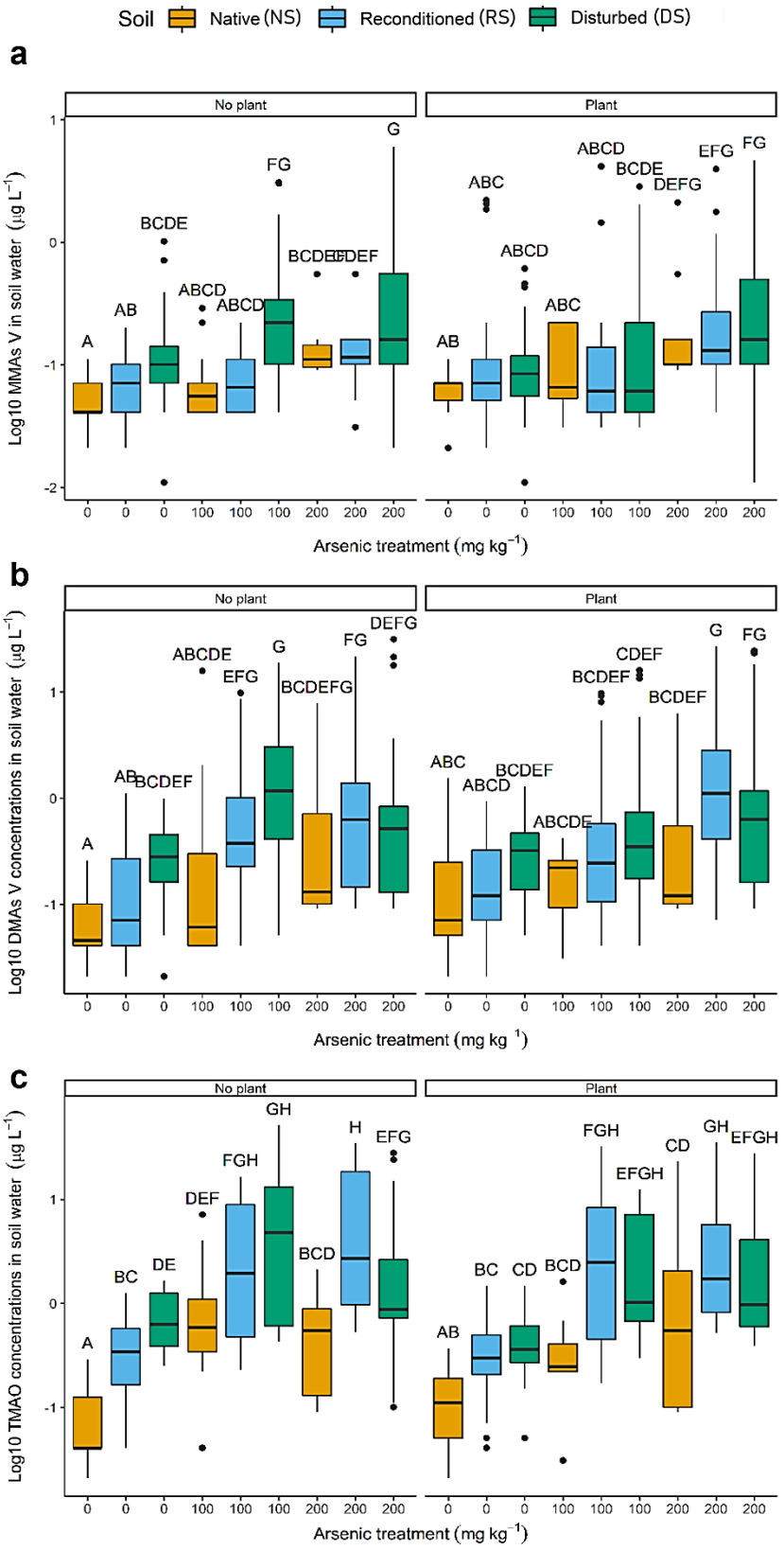


Figure S2.4. The concentration of the individual orgAs: (a) MMA^V; (b) DMA^V; and (c) TMAO in soil water

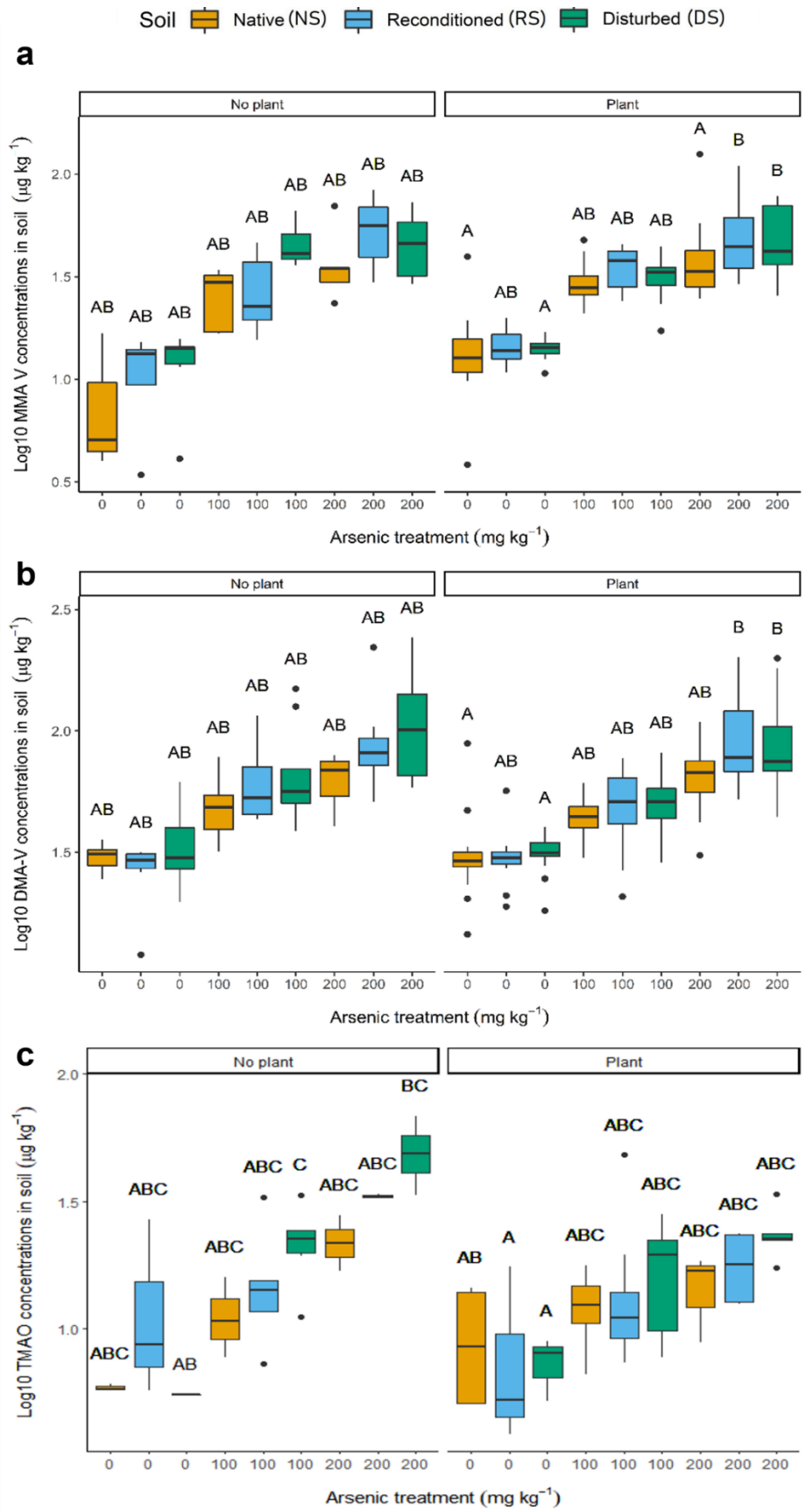


Figure S2.5. The concentration of the individual orgAs: (a) MMA^V; (b) DMA^V; and (c) TMAO in soils

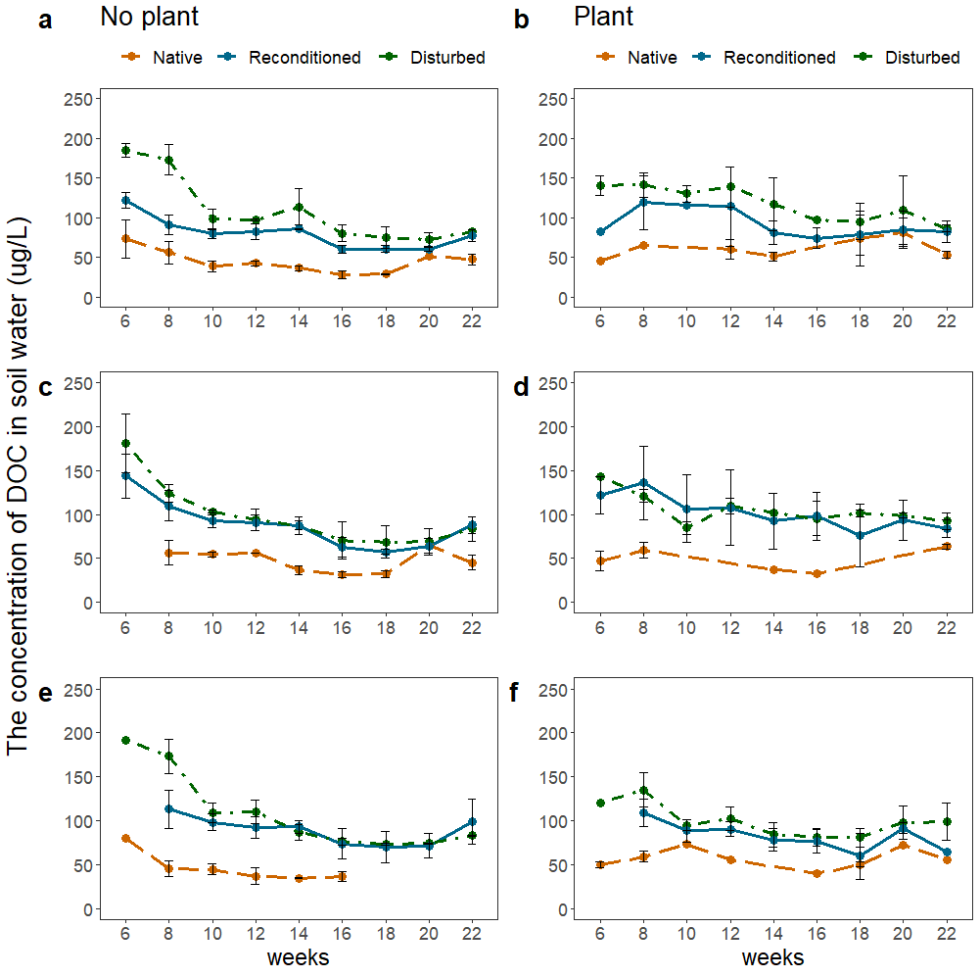


Figure S2.6. The concentration of DOC in soil water of (a) NP_ As_0 group; (b) P_ As_0 group; (c) NP_ As_{100} group; (d) P_As_{100} group; (e) NP_ As_{200} group; and (f) P_ As_{200} group. Missing data were due to insufficient quantity of samples. NP: No-plant pots and P: Plant pots

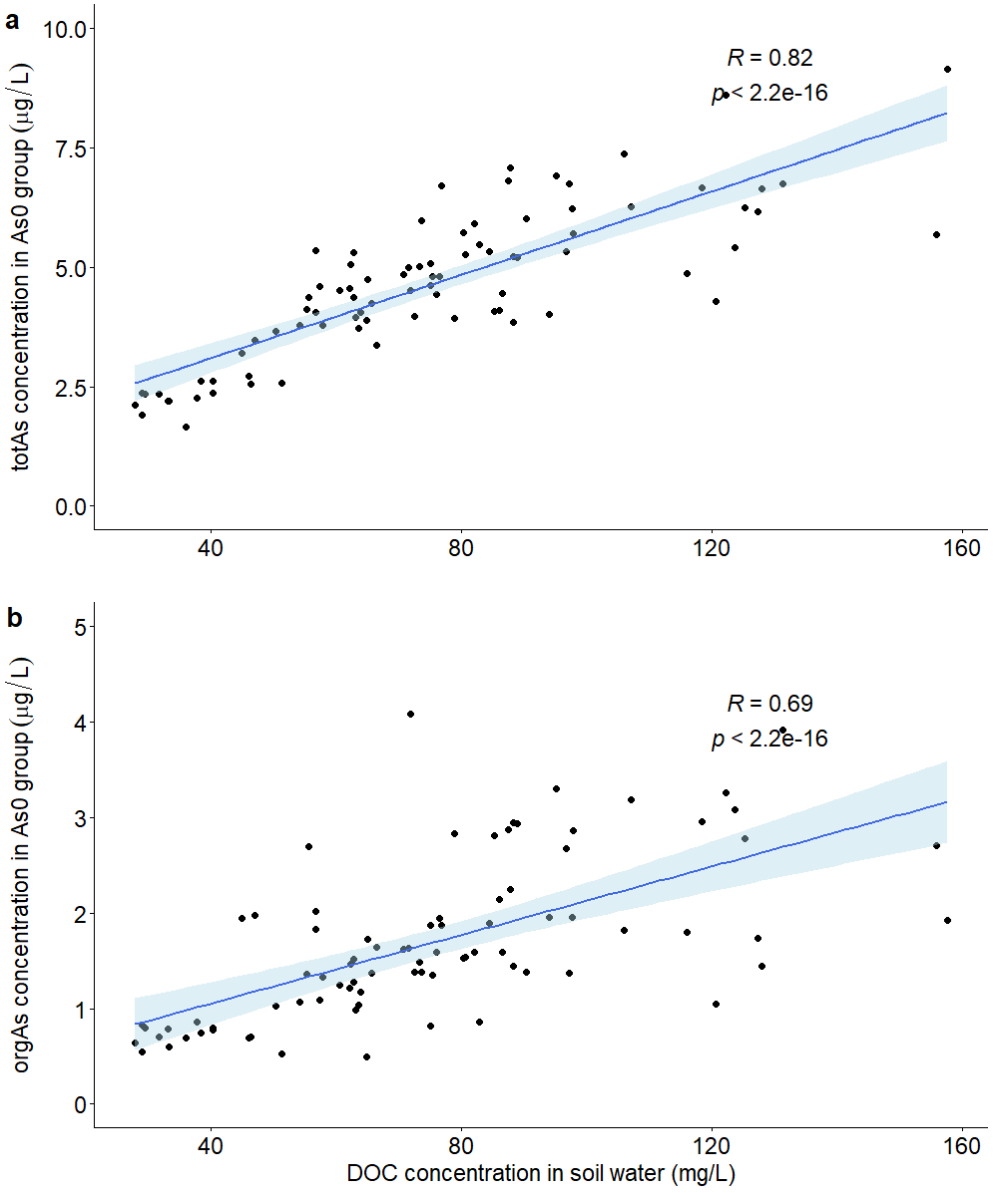


Figure S2.7. The positive correlations of DOC concentration with (a) totAs concentration and (b) orgAs concentration in soil water of As_0 group

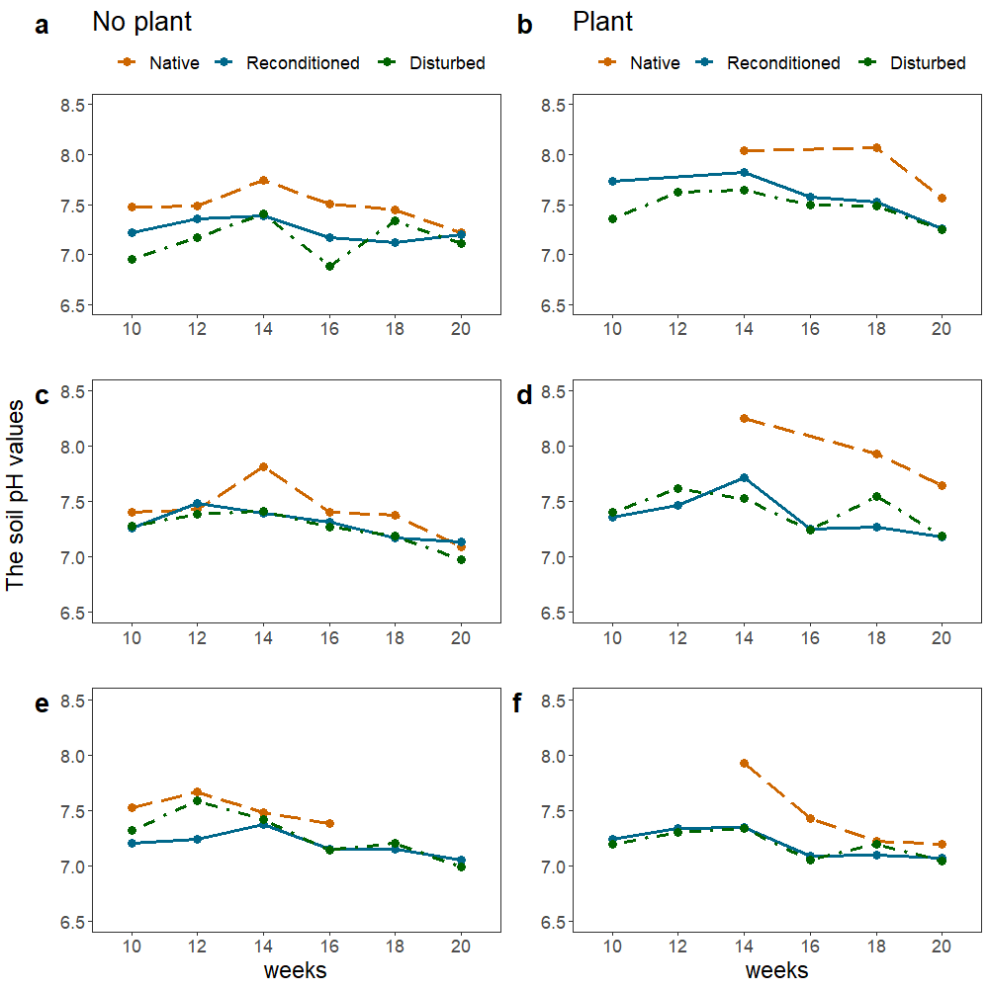


Figure S2.8. The soil pH in (a) NP_ As_0 group; (b) P_ As_0 group; (c) NP_ As_{100} group; (d) P_ As_{100}; (e) NP_As_{200} group; and (f) P_ As_{200} group. Each sampling point contains 2-6 soil water samples with standard deviation calculated based on the ion concentration, which was too small to be visible. Missing data were due to insufficient quantity of maize grain samples. NP: No-plant pots and P: Plant pots

Chapter III Appendix

Maize plants in high-arsenic soils interact with soil microbes to limit the translocation of inorganic arsenic species to aerial plant tissues

Content includes 11 tables and three figures.

1 Materials and Methods (detailed information)

1.1 Plant Cultivation

In each pot, three maize kernels were put on the soil surface in the middle of the pot with the embryo facing upwards. The kernels were then pressed with one finger into 15-20 mm deep and covered by loose soils. During 1.5 days of the sowing, pots were watered from the saucers. During this period, maize required more water so that water content was adjusted to 80% of WHC. After three to four days of germination, pots were watered regularly from the top, and the weights of pots were recorded weekly to keep at 50% of WHC. The needed watering mass was equal to the differences between the target of requested WHC amount and the estimated growing biomass of maize. The fresh biomass of maize could be estimated based on our model derived from a side experiment above.

1.2 Plant Harvest

Maize was harvested after four months of growing. Aerial plant tissues were cut approximately 1 cm above the soil surface and separated into roots, stem, leaves, and cob (grains). Roots were first cleaned with distilled water to remove most soil particles from the root surface and then washed with Milli-Q water (> 18.2 MΩ·cm at 25°C). Each part of the plants was drying in the oven at 70°C until constant weight before the determination of dry biomass (Gulz et al. 2005). After the measurements of dry biomass, further trace elemental analyses were applied.

2 Results

The Certified Reference Material (CRM) of Rye grass and Tomato leaves were applied for total As (totAs) analysis in maize tissues. Rice CRM was used in the analysis of As speciation in soils. Table S3.1 shows the percentage recoveries of acid digestion/extraction for certified

total As, inAs and DMA concentrations in the CRM. Rice represented a great percentage recovery for DMA and a lower percentage for inAs.

Table S3.1. The CRMs data on the total As and As speciation analysis in maize tissues

Parameter	CRM	Average extraction recovery (%)		n
Total As (totAs)	ERM®- CD281 Rye grass	81.80 ± 13.08		20
	SRM® 1573a Tomato leaves	124.36 ± 14.88		23
	ERM®- BC211 Rice	106.36 ± 16.63		14
As species	ERM®- BC211 Rice	inAs	DMA	26
		101.15 ± 17.21	107.93 ± 8.50	

± standard errors

Table S3.2. The emmeans of total As concentration and dry biomass in the entire maize

Treatment	Total As (µg/L)	Dry biomass (g)
As_0-NS	196 ± 44.6	14.01 ± 0.98
As_0-RS	346 ± 78.7	10.97 ± 0.99
As_0-DS	421 ± 104	8.79 ± 1.06
As_{100}-NS	3266 ± 621	10.62 ± 0.98
As_{100}-RS	6789 ± 1292	5.57 ± 1.00
As_{100}-DS	11248 ± 2140	4.85 ± 1.11
As_{200}-NS	10859 ± 2178	5.68 ± 0.98
As_{200}-RS	8156 ± 1636	2.02 ± 1.15
As_{200}-DS	17013 ± 3869	1.87 ± 1.24

± standard errors

Table S3.3. The *p* values of ANOVA on total As and As species concentrations, and dry biomass in maize tissues

Treatment	totAs	inAs	orgAs	orgAs%	MMA^V	DMA^V	TMAO	Dry biomass
Microbial disturbance	***	***	***	0.574	***	***	***	***
As treatments	***	***	***	***	***	***	***	***
Tissue types	***	***	***	***	***	***	***	***
Microbial disturbance × As treatments	0.151	0.018*	***	***	0.008***	0.011*	***	***
Microbial disturbance × tissue types	0.002***	0.010**	0.102	0.010**	0.269	0.198	0.246	0.002***
As treatments × tissue types	***	***	***	***	***	0.109	***	***
Microbial disturbance × As treatments × tissue types	0.010**	0.366	0.002***	0.387	0.140	0.033*	0.008***	***

***: $p < 0.001$; **: significant at $\alpha = 0.01$; *: significant at $\alpha = 0.05$

× interaction terms

Table S3.4. The bioconcentration factor (BCF), bioaccumulation coefficient (BAC), and translocation factor (TF) in maize tissues under microbial disturbance and As treatments

Treatment		BCF	BAC			TF		
			Stem	Leaf	Grain	Stem	Leaf	Grain
As_0	NS	0.44	0.01	0.08	0.002	0.03	0.18	0.004
	RS	0.68	0.05	0.14	0.004	0.08	0.21	0.005
	DS	0.70	0.04	0.10	0.007	0.05	0.14	0.010
As_{100}	NS	0.21	0.01	0.01	0.0005	0.06	0.06	0.002
	RS	0.35	0.03	0.03	0.0007	0.09	0.09	0.002
	DS	0.53	0.03	0.03	n.d.	0.06	0.06	n.d.
As_{200}	NS	0.31	0.03	0.01	0.0004	0.09	0.02	0.001
	RS	0.23	0.05	0.02	n.d.	0.23	0.10	n.d.
	DS	0.26	0.08	0.03	n.d.	0.31	0.13	n.d.

n.d.: no data available due to the fruitless grains

Table S3.5. The *p* values of the multivariate analysis of variance (MANOVA) on As species in maize tissues

Treatment	Arsenic species
Microbial disturbance	***
As treatments	***
Tissue types	***
Microbial disturbance × As treatments	***
Microbial disturbance × tissue types	***
As treatments × tissue types	***
Microbial disturbance × As treatments × tissue types	0.075

***: $p < 0.001$

× interaction terms

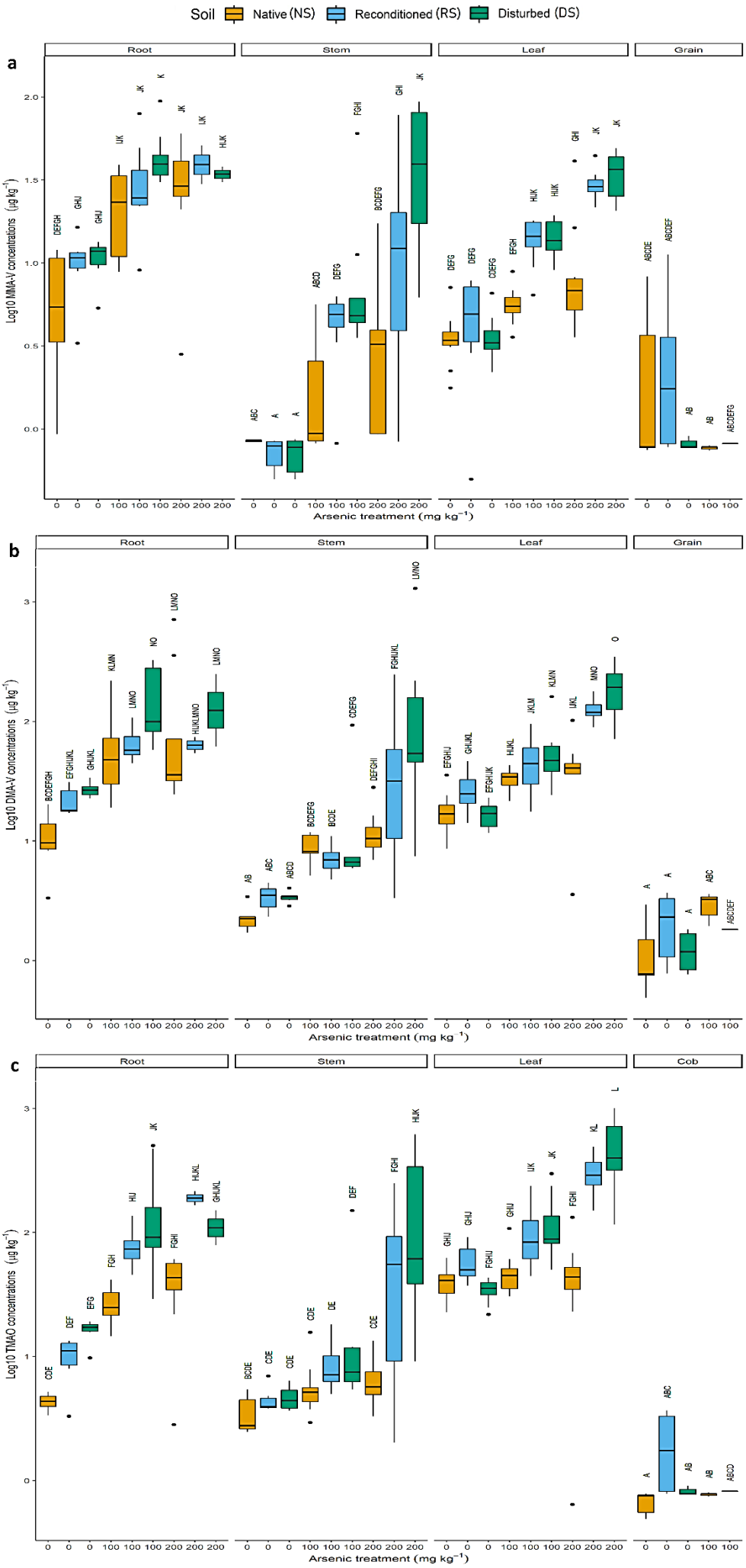

Soil
Native (NS)
Reconditioned (RS)
Disturbed (DS)
a
Root
Stem
Leaf
Grain
Log10 MMA-V concentrations (µg kg⁻¹)
Arsenic treatment (mg kg⁻¹)
b
Log10 DMA-V concentrations (µg kg⁻¹)
c
Cob
Log10 TMAO concentrations (µg kg⁻¹)

Figure S3.1. The concentrations of individual orgAs (a) MMA^V; (b) DMA^V; and (c) TMAO in maize tissues. Data were emmeans ± standard error. Pairwise comparisons were explored and reported using CLD letters. Different CLD letters indicated a statistically significant difference between emmeans ($p < 0.05$).

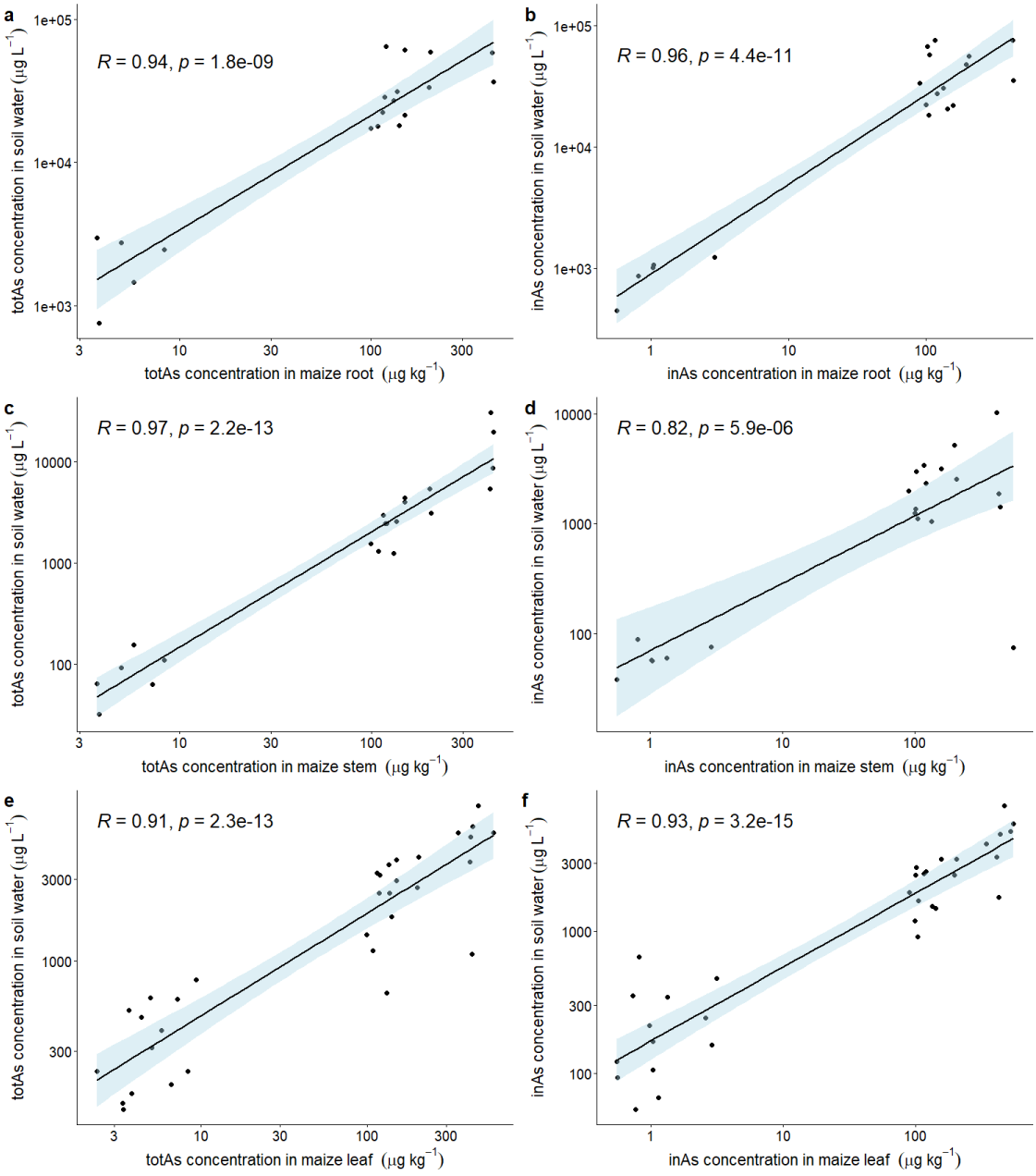


Figure S3.2. Pearson correlations of (a) totAs in maize roots; (b) inAs in maize roots; (c) totAs in maize stem; (d) inAs in maize stem; (e) totAs in maize leaves; and (f) inAs in maize leaves with the respective concentrations in soil water

Table S3.6. The estimated marginal means (emmeans, R Package) of dry biomass in maize tissues

Treatment	Root	Stem	Leaf	Cob
As_0-NS	2.48 ± 0.65	22.42 ± 1.97	10.35 ± 1.34	17.37 ± 1.73
As_{100}-NS	4.84 ± 0.91	22.10 ± 1.95	9.21 ± 1.26	14.44 ± 1.58
As_{200}-NS	1.37 ± 0.49	9.03 ± 1.25	3.83 ± 0.81	7.76 ± 1.16
As_0-RS	1.88 ± 0.57	19.09 ± 1.81	7.65 ± 1.15	17.15 ± 1.72
As_{100}-RS	2.25 ± 0.66	10.71 ± 1.36	2.90 ± 0.70	5.36 ± 1.03
As_{200}-RS	0.17 ± 0.17	0.48 ± 0.30	4.19 ± 0.90	10.89 ± 4.33
As_0-DS	1.93 ± 0.58	20.81 ± 1.89	5.79 ± 1.00	12.27 ± 1.45
As_{100}-DS	2.82 ± 0.74	8.14 ± 1.18	2.28 ± 0.63	5.40 ± 1.08
As_{200}-DS	0.29 ± 0.22	0.95 ± 0.52	3.06 ± 0.87	4.07 ± 1.87

± standard errors

Table S3.7. Conventional Pearson correlation coefficients r and partial correlation coefficients r′ between the concentrations of As species in the entire maize and its dry biomass

As species in maize	**Conventional correlation** n = 230		**Partial correlation** n = 230	
	r	*p*-value	r′	*p*-value
MMA^V	-0.70	***	-0.20	0.003**
DMA^V	-0.69		-0.10	0.122
TMAO	-0.64		-0.10	0.642
inAs	-0.67		-0.18	0.008**

***: $p < 0.001$; **: significant at $\alpha = 0.01$

Table S3.8. The emmeans of total As concentration and As species concentrations in maize roots

Treatments	totAs µg/kg	inAs µg/kg	orgAs µg/kg	orgAs %	MMA^V µg/kg	DMA^V µg/kg	TMAO µg/kg
As_0-NS	1277.29 ± 259	655.92 ± 140	20.09 ± 4.84	2.97 ± 0.63	4.82 ± 1.34	9.67 ± 2.61	4.28 ± 1.24
As_0-RS	1986.79 ± 402	1043.37 ± 223	40.67 ± 9.79	3.75 ± 0.79	9.31 ± 2.59	21.21 ± 5.72	9.10 ± 2.64
As_0-DS	2045.62 ± 414	1202.37 ± 257	53.86 ± 12.96	4.29 ± 0.90	10.26 ± 2.86	26.89 ± 7.25	15.82 ± 4.57
As_{100}-NS	21402.44± 3627	23725.32 ± 3920	108.11 ± 20.15	0.45 ± 0.07	20.34 ± 4.39	50.61 ± 10.57	25.92 ± 5.79
As_{100}-RS	35563.44 ± 6027	41399.20 ± 7650	173.79 ± 36.21	0.42 ± 0.08	27.75 ± 6.70	62.81 ± 14.67	74.87 ± 18.71
As_{100}-DS	54131.32 ± 9173	63427.99 ± 11700	322.62 ± 67.23	0.50 ± 0.09	43.08 ± 10.39	132.55 ± 30.95	115.03 ± 28.75
As_{200}-NS	63521.76 ± 10765	75844.48 ± 12500	161.61 ± 30.12	0.21 ± 0.03	26.37 ± 5.69	64.63 ± 13.50	32.97 ± 7.37
As_{200}-RS	46344.70 ± 8279	52526.19 ± 19400	295.31 ± 123	0.56 ± 0.20	39.18 ± 18.90	63.34 ± 29.58	188.98 ± 94.46
As_{200}-DS	52669.58 ± 10668	69804.82 ± 25800	276.89 ± 115	0.40 ± 0.14	34.23 ± 16.51	123.65 ± 57.75	109.02 ± 54.49

± standard errors

Table S3.9. The emmeans of total As concentration and As species concentrations in maize stem

Treatments	totAs µg/kg	inAs µg/kg	orgAs µg/kg	orgAs %	MMA^V µg/kg	DMA^V µg/kg	TMAO µg/kg
As_0-NS	42.47 ± 9.29	48.12 ± 11.30	6.63 ± 1.75	12.04 ± 2.78	0.85 ± 0.26	2.27 ± 0.67	3.37 ± 1.07
As_0-RS	155.25 ± 31.45	102.86 ± 20.30	8.58 ± 1.91	7.67 ± 1.50	0.70 ± 0.18	3.34 ± 0.83	4.43 ± 1.18
As_0-DS	111.61 ± 22.61	72.29 ± 15.40	8.73 ± 2.10	10.73 ± 2.27	0.69 ± 0.19	3.39 ± 0.92	4.61 ± 1.33
As_{100}-NS	1243.77 ± 211	1129.21 ± 197	15.86 ± 3.12	1.38 ± 0.24	1.54 ± 0.35	8.40 ± 1.85	5.40 ± 1.27
As_{100}-RS	3235.71 ± 549	2612.71 ± 432	19.65 ± 3.66	0.75 ± 0.12	4.08 ± 0.88	6.99 ± 1.46	7.92 ± 1.77
As_{100}-DS	3221.61 ± 546	3058.78 ± 565	27.91 ± 5.82	0.90 ± 0.16	6.92 ± 1.67	9.15 ± 2.14	11.13 ± 2.78
As_{200}-NS	5551.31 ± 941	2061.47 ± 867	22.95 ± 4.51	1.19 ± 0.20	2.77 ± 0.63	11.62 ± 2.56	6.48 ± 1.53
As_{200}-RS	10787.09 ± 1927	7665.46 ± 5115	66.99 ± 13.16	1.70 ± 0.29	7.95 ± 1.81	23.42 ± 5.16	30.50 ± 7.19
As_{200}-DS	16358.46 ± 5062	16270.66 ± 3470	216.36 ± 52.06	1.31 ± 0.28	32.70 ± 9.11	80.63 ± 21.74	86.57 ± 24.98

± standard errors

Table S3.10. The emmeans of total As concentration and As species concentrations in maize leaves

Treatments	totAs µg/kg	inAs µg/kg	orgAs µg/kg	orgAs %	MMA^V µg/kg	DMA^V µg/kg	TMAO µg/kg
As_0-NS	224.00 ± 37.96	108.61 ± 18.00	60.60 ± 11.23	34.80 ± 5.69	3.42 ± 0.74	16.83 ± 3.52	39.86 ± 8.91
As_0-RS	410.83 ± 69.63	342.60 ± 56.60	85.72 ± 15.98	19.74 ± 3.23	4.04 ± 0.87	25.39 ± 5.30	55.16 ± 12.33
As_0-DS	279.51 ± 52.96	188.51 ± 34.80	53.74 ± 11.20	22.09 ± 4.04	3.54 ± 0.85	16.47 ± 3.85	33.44 ± 8.36
As_{100}-NS	1330.18 ± 225	1115.32 ± 184	85.71 ± 15.98	7.10 ± 1.16	5.55 ± 1.20	32.94 ± 6.88	46.12 ± 10.31
As_{100}-RS	3196.80 ± 542	2700.84 ± 447	147.07 ± 27.41	5.14 ± 0.84	13.52 ± 2.92	41.35 ± 8.64	89.51 ± 20.07
As_{100}-DS	3307.89 ± 561	2661.00 ± 440	173.75 ± 32.38	6.11 ± 1.00	13.97 ± 3.01	50.13 ± 10.47	107.41 ± 24.01
As_{200}-NS	1313.29 ± 223	1696.67 ± 281	89.59 ± 16.70	5.00 ± 0.82	7.83 ± 1.69	35.33 ± 7.38	30.95 ± 6.92
As_{200}-RS	4759.75 ± 807	3956.35 ± 689	435.85 ± 85.63	9.88 ± 1.70	29.38 ± 6.68	120.94 ± 26.63	280.37 ± 66.06
As_{200}-DS	6637.53 ± 1258	5370.38 ± 993	642.52 ± 134	10.64 ± 1.94	33.55 ± 8.09	177.81 ± 41.52	421.76 ± 105

± standard errors

Table S3.11. The emmeans of total As concentration and As species concentrations in maize grain

Treatments	totAs µg/kg	inAs µg/kg	orgAs µg/kg	orgAs %	MMA^V µg/kg	DMA^V µg/kg	TMAO µg/kg
As_0-NS	5.32 ± 1.16	3.79 ± 0.81	3.89 ± 0.94	48.42 ± 10.22	1.62 ± 0.45	1.03 ± 0.28	0.66 ± 0.19
As_0-RS	10.38 ± 2.49	11.85 ± 2.53	5.87 ± 1.41	30.80 ± 6.50	2.06 ± 0.58	1.90 ± 0.51	1.68 ± 0.49
As_0-DS	20.52 ± 4.16	10.96 ± 2.16	2.85 ± 0.64	19.74 ± 3.86	0.81 ± 0.21	1.19 ± 0.30	0.81 ± 0.22
As_{100}-NS	46.44 ± 11.13	26.16 ± 6.12	4.42 ± 1.17	14.42 ± 3.33	0.77 ± 0.24	2.85 ± 0.84	0.77 ± 0.24
As_{100}-RS	69.05 ± 26.17	33.14 ± 17.3	3.46 ± 2.04	9.45 ± 4.88	0.82 ± 0.56	1.82 ± 1.20	0.82 ± 0.58
As_{100}-DS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
As_{200}-NS	77.58 ± 29.40	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
As_{200}-RS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
As_{200}-DS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

± standard errors; n.d.: no data available due to the fruitless grains

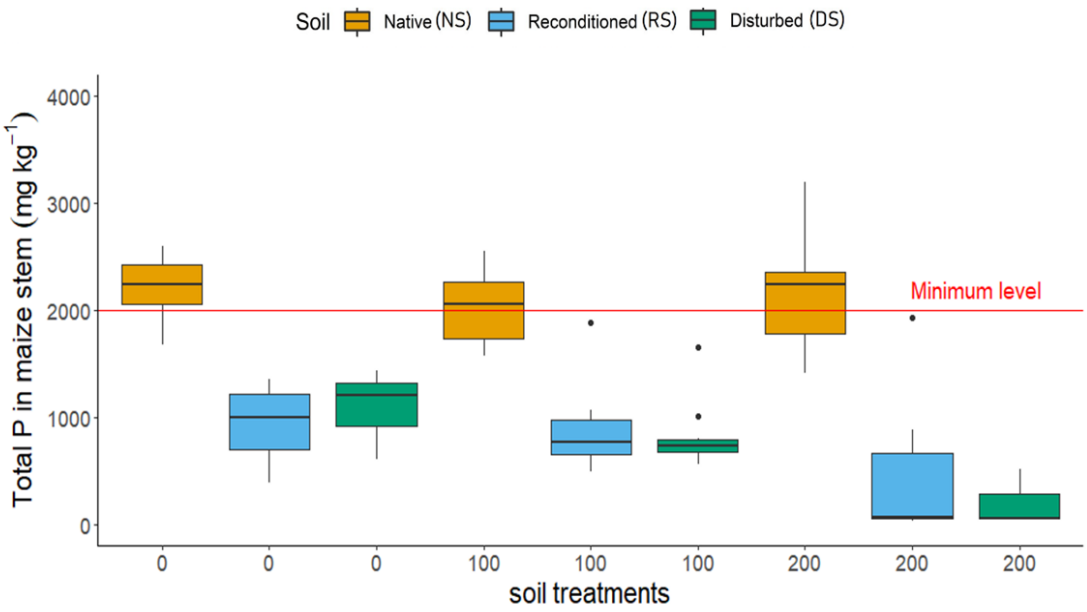


Figure S3.3. The concentrations of total P in maize leaves under microbial disturbance and As treatments

Chapter IV Appendix

Soil indigenous microbes protect maize plants cultivated on soils with varying arsenic levels

Content includes nine tables and eight figures.

Table S4.1. The *p* values of univariate ANOVA statistical analysis on plant health parameters

Experimental factors	**Plant height**	**Fresh biomass**	**BBCH-scale**	**Leaf numbers**	**Chlorophyll**	**Damage scale**
Microbial disturbance	***	***	***	***	***	***
As treatments	***	***	***	***	***	***
time	***	***	***	***	***	***
Microbial disturbance × As treatments	***	0.024*	***	***	***	***

× interaction terms

***: p < 0.001; *: significant at α = 0.05

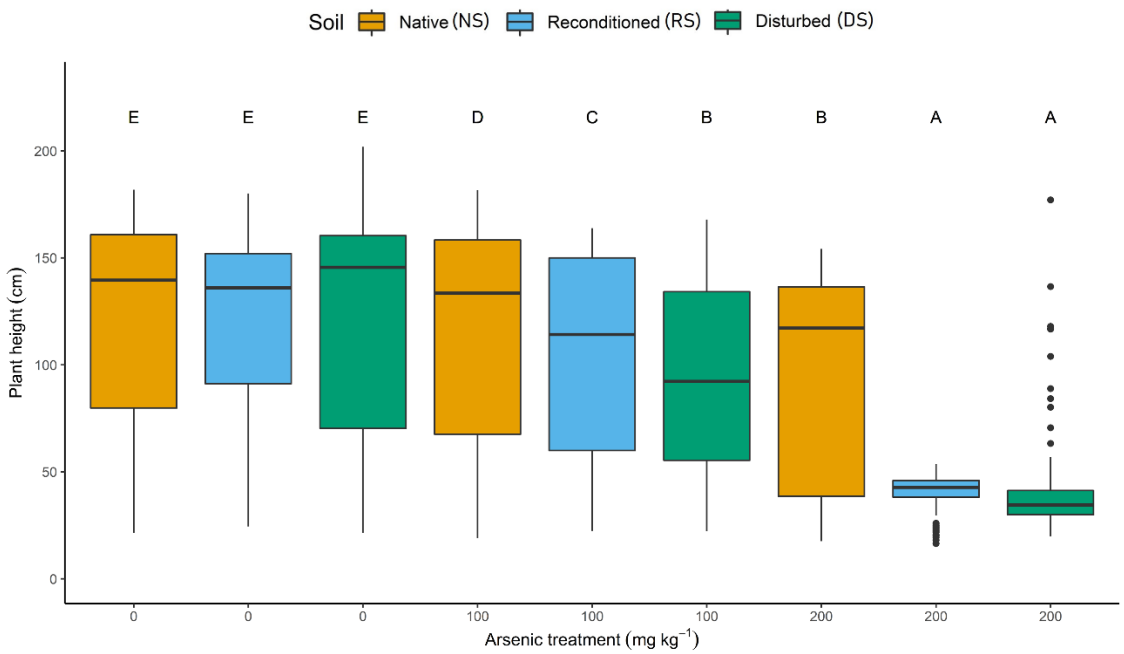


Figure S4.1. Changes in plant height under microbial disturbance and As treatments

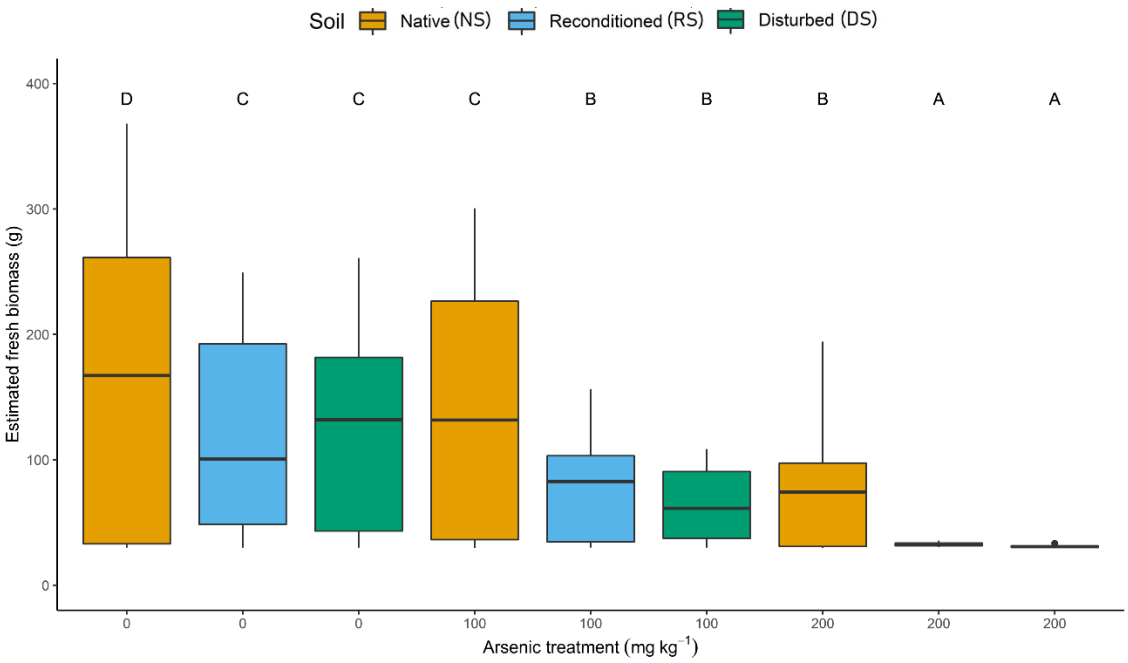


Figure S4.2. Changes in fresh biomass under microbial disturbance and As treatments

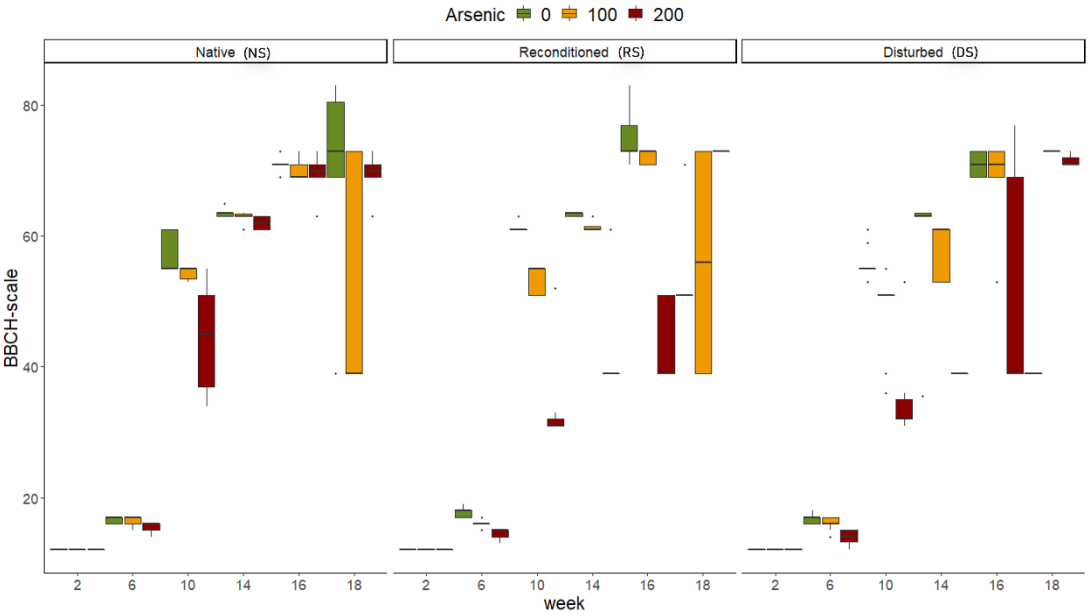


Figure S4.3. Changes in BBCH-scale of maize over time under microbial disturbance and As treatments. Native: NS, reconditioned: RS, disturbed: DS.

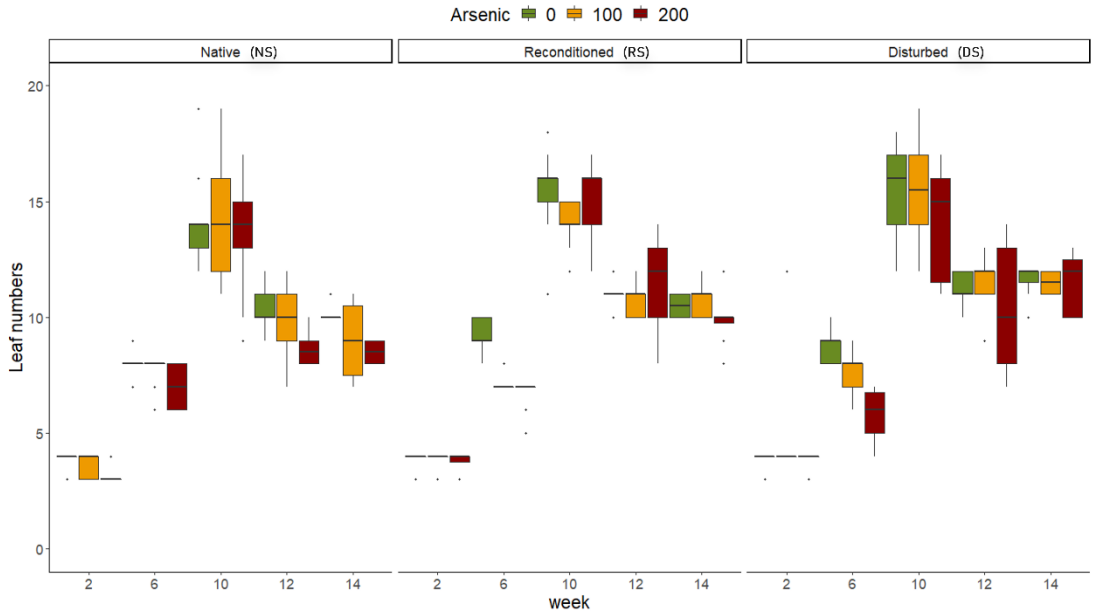


Figure S4.4. Changes in the leaf numbers over time under microbial disturbance and As treatments

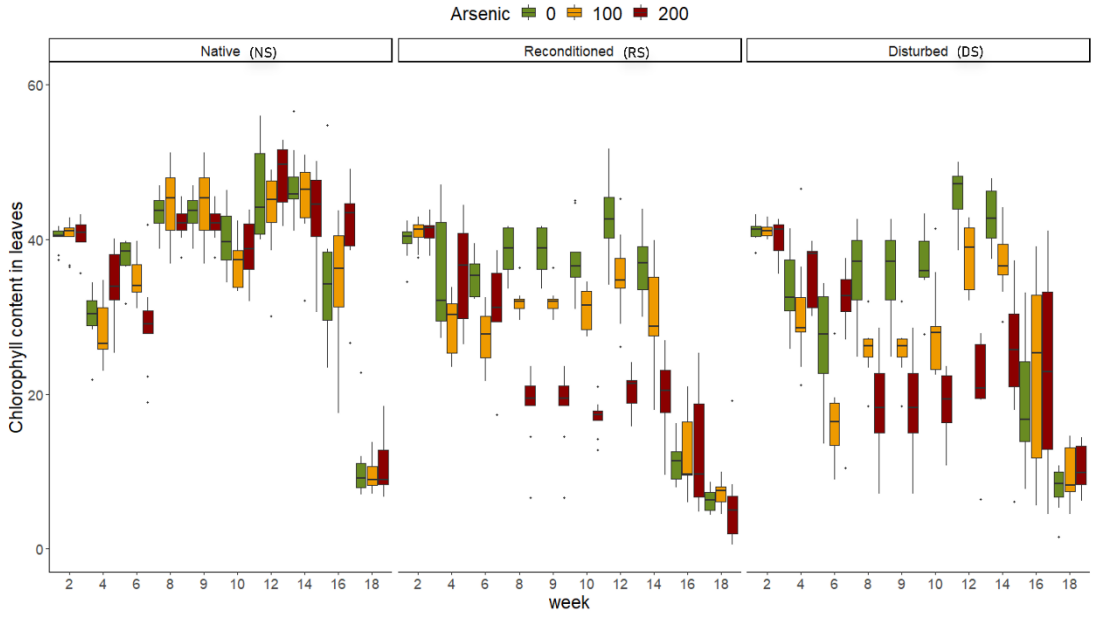


Figure S4.5. Changes in the chlorophyll content in maize leaves over time under microbial disturbance and As treatments

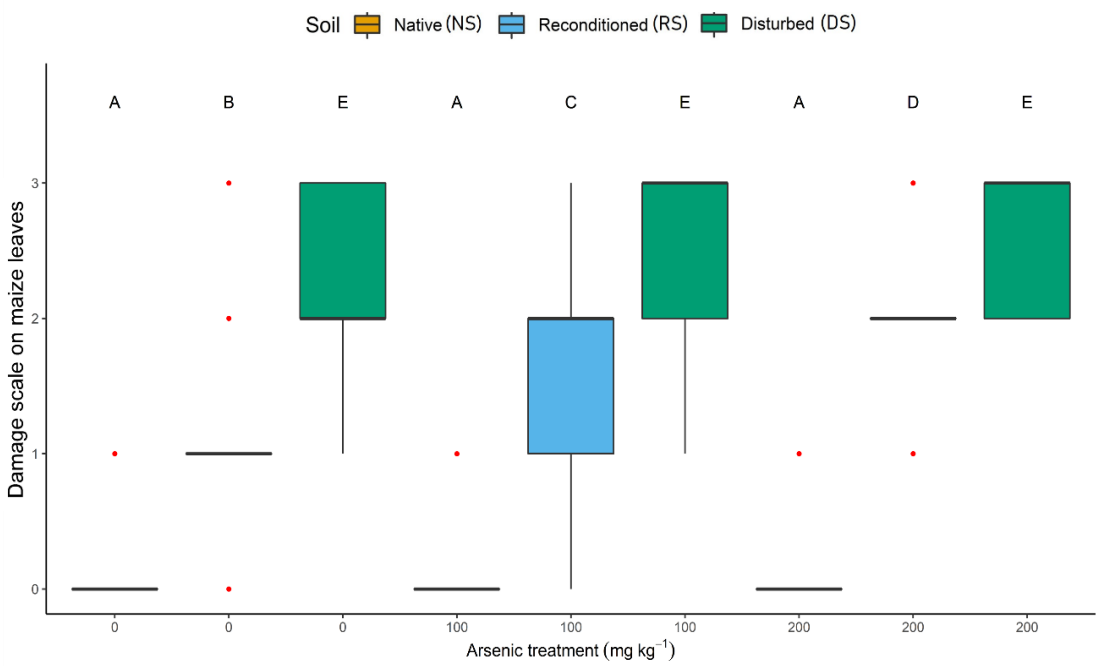


Figure S4.6. Damage scale of leaf spot under microbial disturbance and As treatments. Red dots represent the outliers. A line instead of a box is due to the data concentration.

Table S4.2. The correlation coefficient between As species in maize root and plant health parameters

	Plant height	Fresh biomass	BBCH-scale loss	Leaf loss	Chlorophyll
n	57	16	50	58	58
inAs	-0.38**	-0.73**	0.30	0.18	-0.34*
MMA^V	-0.41**	-0.79**	0.31	-0.0001	-0.42**
DMA^V	-0.41**	-0.54*	0.29	0.24	-0.38*
TMAO	-0.47**	-0.90***	0.47**	0.10	-0.53***
totAs	-0.39**	-0.77**	0.33	0.15	-0.38*

n: observation numbers

***: significant at α = 0.001; **: significant at α = 0.01; *: significant at α = 0.05

Table S4.3. The correlation coefficient between As species in maize stem and plant health parameters

	Plant height	Fresh biomass	BBCH-scale loss	Leaf loss	Chlorophyll
n	67	20	68	65	67
inAs	-0.43***	-0.83***	0.15	0.26	-0.39**
MMA^V	-0.44***	-0.74**	0.22	0.18	-0.40**
DMA^V	-0.55***	-0.74**	0.21	0.23	-0.26*
TMAO	-0.58***	-0.67*	0.23	0.09	-0.42**
totAs	-0.56***	-0.85***	0.43**	0.27	-0.43**

n: observation numbers

***: significant at α = 0.001; **: significant at α = 0.01; *: significant at α = 0.05

Table S4.4. The correlation coefficient between As species in maize leaf and plant health parameters

	Plant height	Fresh biomass	BBCH-scale loss	Leaf loss	Chlorophyll	Damage scale
n	82	23	71	84	84	58
inAs	-0.62***	-0.85***	0.51***	0.15	-0.64***	0.51***
MMA^V	-0.72***	-0.79***	0.65***	0.05	-0.73***	0.50***
DMA^V	-0.78***	-0.70***	0.71***	0.05	-0.69***	0.41**
TMAO	-0.71***	-0.60**	0.65***	-0.04	-0.66***	0.60***
totAs	-0.66***	-0.84***	0.55***	0.09	-0.71***	0.48***

n: observation numbers

***: significant at α = 0.001; **: significant at α = 0.01; *: significant at α = 0.05

Table S4.5. The correlation coefficient between As species in maize grain and plant health parameters

	Plant height	Fresh biomass	BBCH-scale loss	Leaf loss	Chlorophyll
n	25	8	22	25	25
inAs	-0.04	-0.75	0.13	0.22	-0.42
MMA^V	-0.02	0.48	-0.50	0.04	0.44
DMA^V	-0.44	-0.66	-0.20	0.11	-0.11
TMAO	-0.23	-0.58	-0.16	-0.34	0.11
totAs	0.03	-0.73	0.15	0.28	-0.28

n: observation numbers

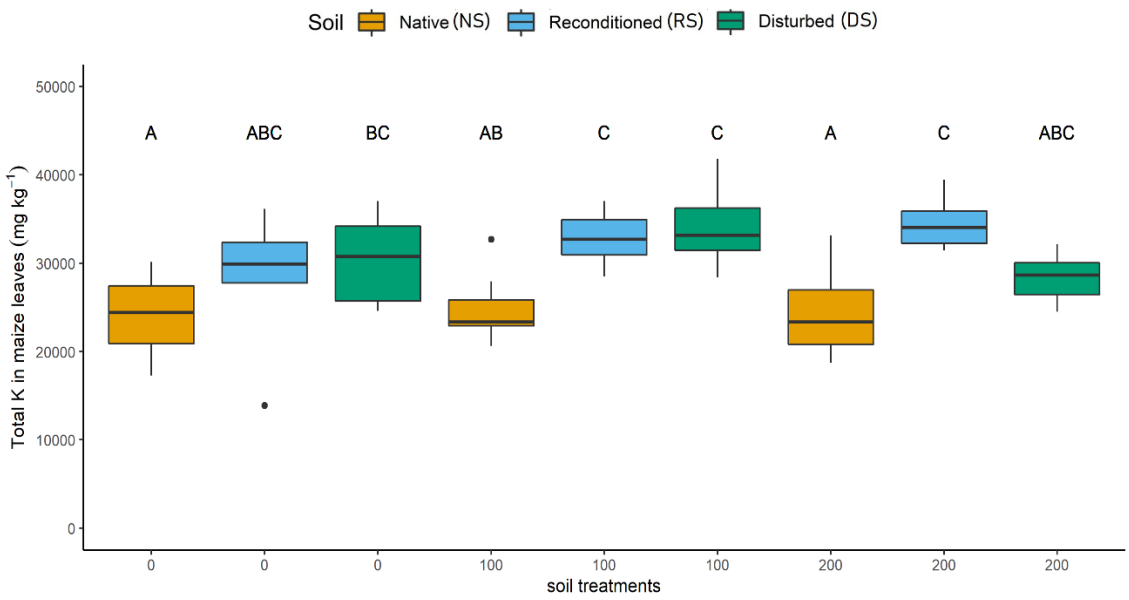


Figure S4.7. The concentrations of total K in maize leaves under microbial disturbance and As treatments

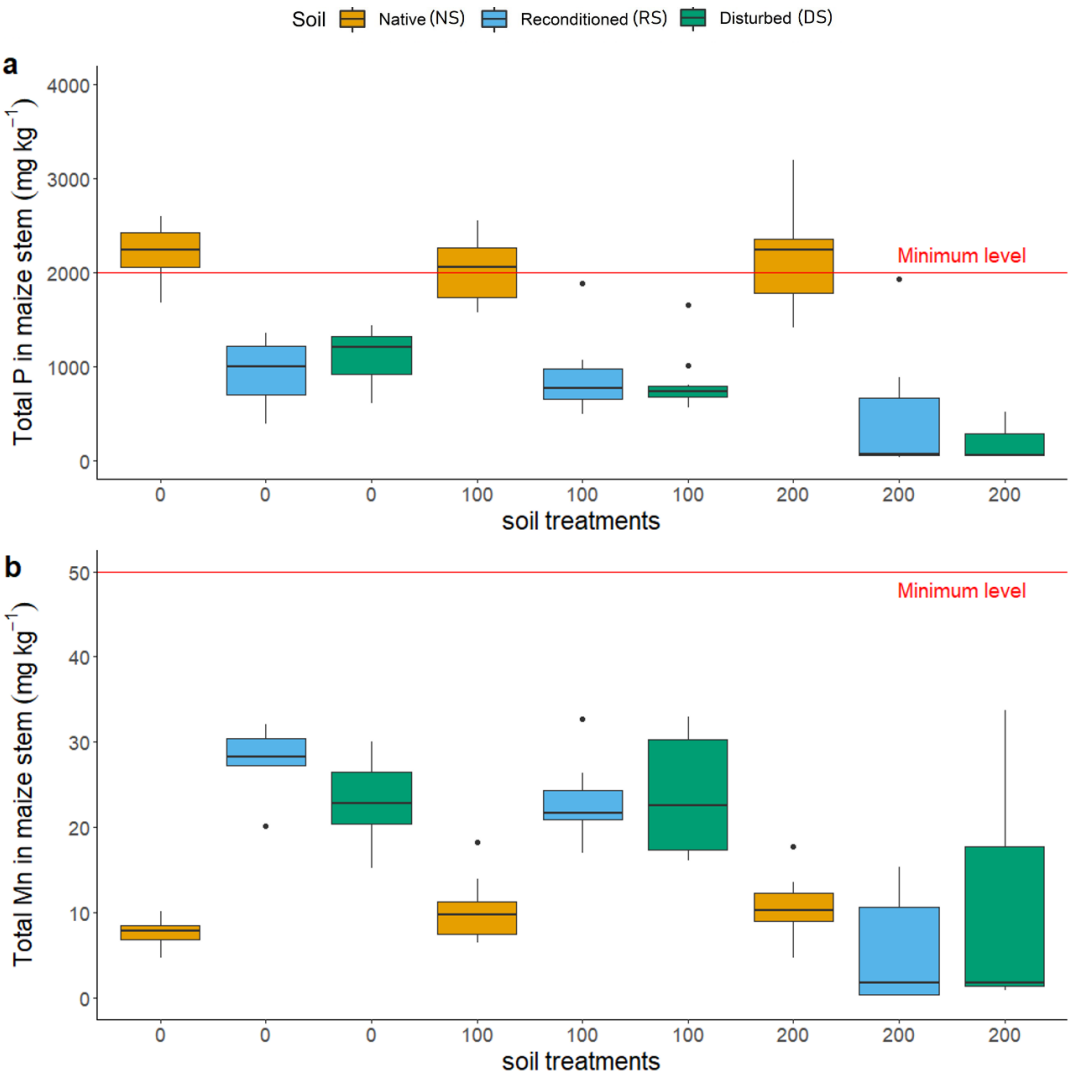


Figure S4.8. The concentrations of essential nutrients (a) P; (b) Mn in maize stem under microbial disturbance and As treatments. Minimum level stands for the average concentrations of these mineral elements in the dry stem required for adequate plant growth (Kirkby 2012).

Table S4.6. The estimated marginal means (emmeans, R Package) of totAs concentration and As species concentrations in maize roots

Treatments	totAs µg/kg	inAs µg/kg	orgAs µg/kg	orgAs %	MMA^V µg/kg	DMA^V µg/kg	TMAO µg/kg
As_0-NS	1277.29 ± 259	655.92 ± 140	20.09 ± 4.84	2.97 ± 0.63	4.82 ± 1.34	9.67 ± 2.61	4.28 ± 1.24
As_0-RS	1986.79 ± 402	1043.37 ± 223	40.67 ± 9.79	3.75 ± 0.79	9.31 ± 2.59	21.21 ± 5.72	9.10 ± 2.64
As_0-DS	2045.62 ± 414	1202.37 ± 257	53.86 ± 12.96	4.29 ± 0.90	10.26 ± 2.86	26.89 ± 7.25	15.82 ± 4.57
As_{100}-NS	21402.44± 3627	23725.32 ± 3920	108.11 ± 20.15	0.45 ± 0.07	20.34 ± 4.39	50.61 ± 10.57	25.92 ± 5.79
As_{100}-RS	35563.44 ± 6027	41399.20 ± 7650	173.79 ± 36.21	0.42 ± 0.08	27.75 ± 6.70	62.81 ± 14.67	74.87 ± 18.71
As_{100}-DS	54131.32 ± 9173	63427.99 ± 11700	322.62 ± 67.23	0.50 ± 0.09	43.08 ± 10.39	132.55 ± 30.95	115.03 ± 28.75
As_{200}-NS	63521.76 ± 10765	75844.48 ± 12500	161.61 ± 30.12	0.21 ± 0.03	26.37 ± 5.69	64.63 ± 13.50	32.97 ± 7.37
As_{200}-RS	46344.70 ± 8279	52526.19 ± 19400	295.31 ± 123	0.56 ± 0.20	39.18 ± 18.90	63.34 ± 29.58	188.98 ± 94.46
As_{200}-DS	52669.58 ± 10668	69804.82 ± 25800	276.89 ± 115	0.40 ± 0.14	34.23 ± 16.51	123.65 ± 57.75	109.02 ± 54.49

± standard errors

Table S4.7. The emmeans of totAs concentration and As species concentrations in maize stem

Treatments	totAs µg/kg	inAs µg/kg	orgAs µg/kg	orgAs %	MMA^V µg/kg	DMA^V µg/kg	TMAO µg/kg
As_0-NS	42.47 ± 9.29	48.12 ± 11.30	6.63 ± 1.75	12.04 ± 2.78	0.85 ± 0.26	2.27 ± 0.67	3.37 ± 1.07
As_0-RS	155.25 ± 31.45	102.86 ± 20.30	8.58 ± 1.91	7.67 ± 1.50	0.70 ± 0.18	3.34 ± 0.83	4.43 ± 1.18
As_0-DS	111.61 ± 22.61	72.29 ± 15.40	8.73 ± 2.10	10.73 ± 2.27	0.69 ± 0.19	3.39 ± 0.92	4.61 ± 1.33
As_{100}-NS	1243.77 ± 211	1129.21 ± 197	15.86 ± 3.12	1.38 ± 0.24	1.54 ± 0.35	8.40 ± 1.85	5.40 ± 1.27
As_{100}-RS	3235.71 ± 549	2612.71 ± 432	19.65 ± 3.66	0.75 ± 0.12	4.08 ± 0.88	6.99 ± 1.46	7.92 ± 1.77
As_{100}-DS	3221.61 ± 546	3058.78 ± 565	27.91 ± 5.82	0.90 ± 0.16	6.92 ± 1.67	9.15 ± 2.14	11.13 ± 2.78
As_{200}-NS	5551.31 ± 941	2061.47 ± 867	22.95 ± 4.51	1.19 ± 0.20	2.77 ± 0.63	11.62 ± 2.56	6.48 ± 1.53
As_{200}-RS	10787.09 ± 1927	7665.46 ± 5115	66.99 ± 13.16	1.70 ± 0.29	7.95 ± 1.81	23.42 ± 5.16	30.50 ± 7.19
As_{200}-DS	16358.46 ± 5062	16270.66 ± 3470	216.36 ± 52.06	1.31 ± 0.28	32.70 ± 9.11	80.63 ± 21.74	86.57 ± 24.98

± standard errors

Table S4.8. The emmeans of totAs concentration and As species concentrations in maize leaves

Treatments	totAs µg/kg	inAs µg/kg	orgAs µg/kg	orgAs %	MMA^V µg/kg	DMA^V µg/kg	TMAO µg/kg
As_0-NS	224.00 ± 37.96	108.61 ± 18.00	60.60 ± 11.23	34.80 ± 5.69	3.42 ± 0.74	16.83 ± 3.52	39.86 ± 8.91
As_0-RS	410.83 ± 69.63	342.60 ± 56.60	85.72 ± 15.98	19.74 ± 3.23	4.04 ± 0.87	25.39 ± 5.30	55.16 ± 12.33
As_0-DS	279.51 ± 52.96	188.51 ± 34.80	53.74 ± 11.20	22.09 ± 4.04	3.54 ± 0.85	16.47 ± 3.85	33.44 ± 8.36
As_{100}-NS	1330.18 ± 225	1115.32 ± 184	85.71 ± 15.98	7.10 ± 1.16	5.55 ± 1.20	32.94 ± 6.88	46.12 ± 10.31
As_{100}-RS	3196.80 ± 542	2700.84 ± 447	147.07 ± 27.41	5.14 ± 0.84	13.52 ± 2.92	41.35 ± 8.64	89.51 ± 20.07
As_{100}-DS	3307.89 ± 561	2661.00 ± 440	173.75 ± 32.38	6.11 ± 1.00	13.97 ± 3.01	50.13 ± 10.47	107.41 ± 24.01
As_{200}-NS	1313.29 ± 223	1696.67 ± 281	89.59 ± 16.70	5.00 ± 0.82	7.83 ± 1.69	35.33 ± 7.38	30.95 ± 6.92
As_{200}-RS	4759.75 ± 807	3956.35 ± 689	435.85 ± 85.63	9.88 ± 1.70	29.38 ± 6.68	120.94 ± 26.63	280.37 ± 66.06
As_{200}-DS	6637.53 ± 1258	5370.38 ± 993	642.52 ± 134	10.64 ± 1.94	33.55 ± 8.09	177.81 ± 41.52	421.76 ± 105

± standard errors

Table S4.9. The emmeans of totAs concentration and As species concentrations in maize grains

Treatments	totAs µg/kg	inAs µg/kg	orgAs µg/kg	orgAs %	MMA^V µg/kg	DMA^V µg/kg	TMAO µg/kg
As_0-NS	5.32 ± 1.16	3.79 ± 0.81	3.89 ± 0.94	48.42 ± 10.22	1.62 ± 0.45	1.03 ± 0.28	0.66 ± 0.19
As_0-RS	10.38 ± 2.49	11.85 ± 2.53	5.87 ± 1.41	30.80 ± 6.50	2.06 ± 0.58	1.90 ± 0.51	1.68 ± 0.49
As_0-DS	20.52 ± 4.16	10.96 ± 2.16	2.85 ± 0.64	19.74 ± 3.86	0.81 ± 0.21	1.19 ± 0.30	0.81 ± 0.22
As_{100}-NS	46.44 ± 11.13	26.16 ± 6.12	4.42 ± 1.17	14.42 ± 3.33	0.77 ± 0.24	2.85 ± 0.84	0.77 ± 0.24
As_{100}-RS	69.05 ± 26.17	33.14 ± 17.3	3.46 ± 2.04	9.45 ± 4.88	0.82 ± 0.56	1.82 ± 1.20	0.82 ± 0.58
As_{100}-DS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
As_{200}-NS	77.58 ± 29.40	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
As_{200}-RS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
As_{200}-DS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

± standard errors; n.d.: no data available due to the fruitless grains

Appendix Reference

Ge, Yufeng; Pandey, Piyush; Bai, Geng (2016): Estimating fresh biomass of maize plants from their RGB images in greenhouse phenotyping. In John Valasek, J. Alex Thomasson (Eds.): Autonomous Air and Ground Sensing Systems for Agricultural Optimization and Phenotyping. SPIE Commercial + Scientific Sensing and Imaging. Baltimore, Maryland, United States, Sunday 17 April 2016: SPIE (SPIE Proceedings), p. 986605.

Gulz, Petra Angela; Gupta, Satish-Kumar; Schulin, Rainer (2005): Arsenic accumulation of common plants from contaminated soils. In *Plant Soil* 272 (1-2), pp. 337–347. DOI: 10.1007/s11104-004-5960-z.

Kirkby, Ernest (2012): Introduction, Definition and Classification of Nutrients. In : Marschner's Mineral Nutrition of Higher Plants: Elsevier, pp. 3–5.

Song, Jing; Zhao, Fang-Jie; McGrath, Steve P.; Luo, Yong-Ming (2006): Influence of soil properties and aging on arsenic phytotoxicity. In *Environmental toxicology and chemistry* 25 (6), pp. 1663–1670. DOI: 10.1897/05-480r2.1.

Declaration of Consent

on the basis of Article 18 of the PromR Phil.-nat. 19

Name/First Name: Guan/Hang

Registration Number: 18-120-295

Study program: PhD in Geography

Bachelor ☐ Master ☐ Dissertation ☑

Title of the thesis: The role of soil indigenous microbes and their interactions with maize (*Zea mays* L.) plants in arsenic uptake, translocation, speciation, and detoxification in the soil-plant system

Supervisors: Prof. Dr. Moritz Bigalke & Prof. Dr. Adrien Mestrot

I declare here with that this thesis is my own work and that I have not used any sources other than those stated. I have indicated the adoption of quotations as well as thoughts taken from other authors as such in the thesis. I am aware that the Senate pursuant to Article 36 paragraph 1 litera r of the University Act of September 5th, 1996 and Article 69 of the University Statute of June 7th, 2011 is authorized to revoke the doctoral degree awarded on the basis of this thesis.

For the purposes of evaluation and verification of compliance with the declaration of originality and theregulations governing plagiarism, I hereby grant the University of Bern the right to process my personal data and to perform the acts of use this requires, in particular, to reproduce the written thesis and to store it permanently in a database, and to use said database, or to make said database available, to enable comparison with theses submitted by others.

Bern, 22.07.2022 *Hang Guan*

Place/Date Signature