SI Tables and Figures

Root exuded specialized metabolites reduce arsenic toxicity in maize

Veronica Caggìa; Jan Wälchli; Gabriel Deslandes-Hérold; Pierre Mateo; Christelle AM Robert; Hang Guan; Moritz Bigalke; Sandra Spielvogel; Adrien Mestrot; Klaus Schläppi; Matthias Erb

Supplementary figures and tables

Table S1. Soil characterization for Q-Matte

pH	6.61 ± 0.04
Clay (%)	10.2 ± 0.8
Silt (%)	53 ± 2
Sand (%)	37 ± 3
Plant available P (mg/kg)	2.41 ± 0.02
Total carbon (g/kg)	26.49 ± 0.07
Nitrogen (g/kg)	2.91 ± 0.01
Sulfur (g/kg)	0.35 ± 0.03
Total organic carbon (g/kg)	25.41 ± 0.09
Arsenic (mg/kg)	2.9 ± 0.5
Magnesium (g/kg)	4.1 ± 0.5
Potassium (g/kg)	1.5 ± 0.1
Iron (g/kg)	17.7 ± 0.7
Manganese (g/kg)	0.74 ± 0.02

Table S2. Characteristics of Changins and Posieux soils

Parameters	Changins	Posieux
clay %	27.5	16
sand %	60.635	72.2
pH	7.043	6.775
phosphorus-CO2 (mg/kg)	5.96	12.675
potassium-CO2 (mg/kg)	1.395	1.6
magnesium-CO2 (mg/kg)	11.64	6.3625
nitrate-H2O (mg/kg)	11.1565	33.35625
phosphorus-H2O (mg/kg)	4.301	7.265
potassium-H2O (mg/kg)	17.327	16.6125
calcium-H2O (mg/kg)	77.146	68.7575
magnesium-H2O (mg/kg)	9.189	7.67125
iron-H2O (mg/kg)	15.43455	7.197875
phosphorus-AAE (mg/kg)	34.5015	41.56125
potassium-AAE (mg/kg)	159.79	77.78625
magnesium-AAE (mg/kg)	189.16	85.34375
manganese-AAE (mg/kg)	260.9	217.625
boron-AAE (mg/kg)	0.7	0.1375
copper-AAE (mg/kg)	5.57	3.9625
iron-AAE (mg/kg)	270.45	200

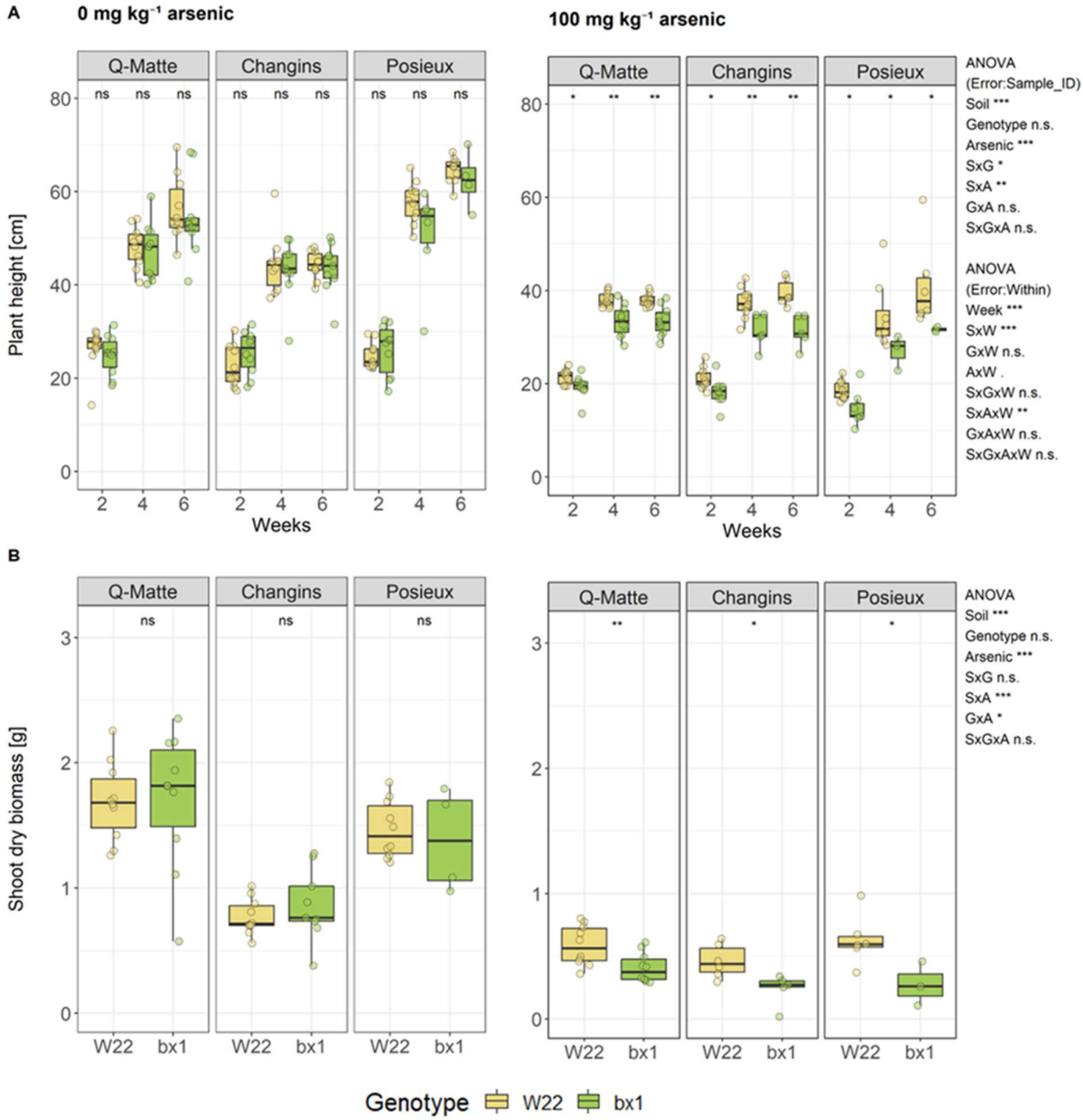


Figure S1. The protective effect of benzoxazinoids is conserved across different soils. (A) Plant height and (B) shoot dry biomass of wild type (W22) and benzoxazinoid-deficient *bx1* mutant plants growing in three different soils with different characteristics without (0 mg kg^{-1}) or with arsenic addition (100 mg kg^{-1}). Levels of significance (between genotypes): n.s. non-significant, . = marginally significant, *p <.05, **p <.01, ***p <.001.

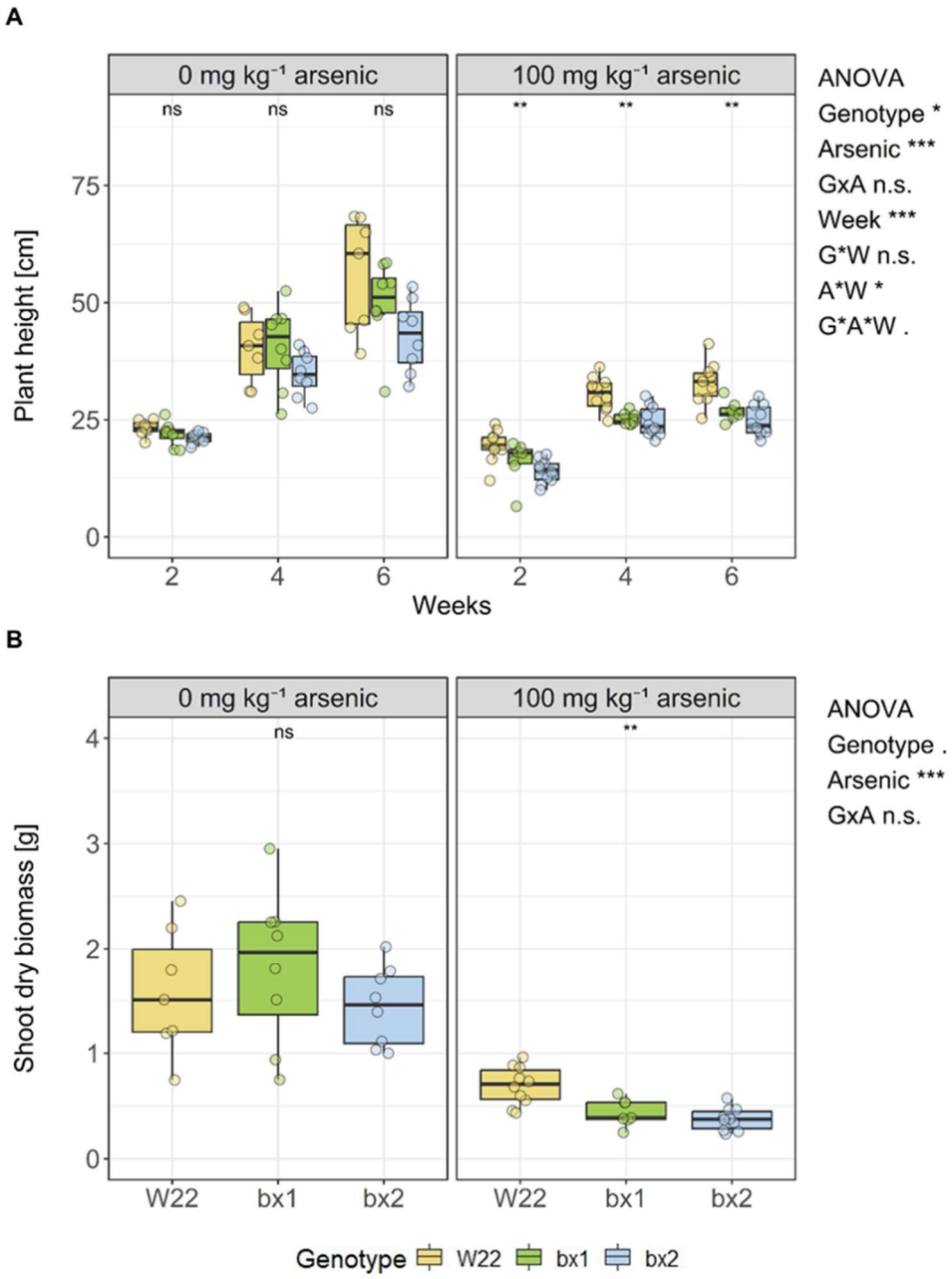


Figure S2. The protective effect of benzoxazinoids is conserved across different benzoxazinoid mutants. (A) Plant height and (B) shoot dry biomass of wild type (W22) and benzoxazinoid-deficient *bx1* and *bx2* mutant plants growing in soil without (0 mg kg^{-1}) or with arsenic addition (100 mg kg^{-1}). Levels of significance (among genotypes): n.s. non-significant, . = marginally significant, *p <.05, **p <.01, ***p <.001. See **Additional file 2** for detailed results of Tukey HSD tests.

Table S3. Composition and purity of BXs used for complementation.

Compound	Conc_ug_ml	
MBOA-Glc	0	
HMBOA-Glc	0.514	
HM2BOA-Glc	0	
DIMBOA-Glc	**24.019**	
DIM2BOA-Glc	0.409	
HMBOA	0	
DIMBOA	0.244	
HDMBOA-Glc	**2.229**	
HDM2BOA-Glc	0	
MBOA	0	
DIMBOA-2xHexose	0	
DIMBOA-3xHexose	0.031	
HMBOA-2xHexose	0.297	
Total:	**27.743**	**ug/ml**
Prepared for analysis:	50	ug/ml
Final BXs purity:	56%	

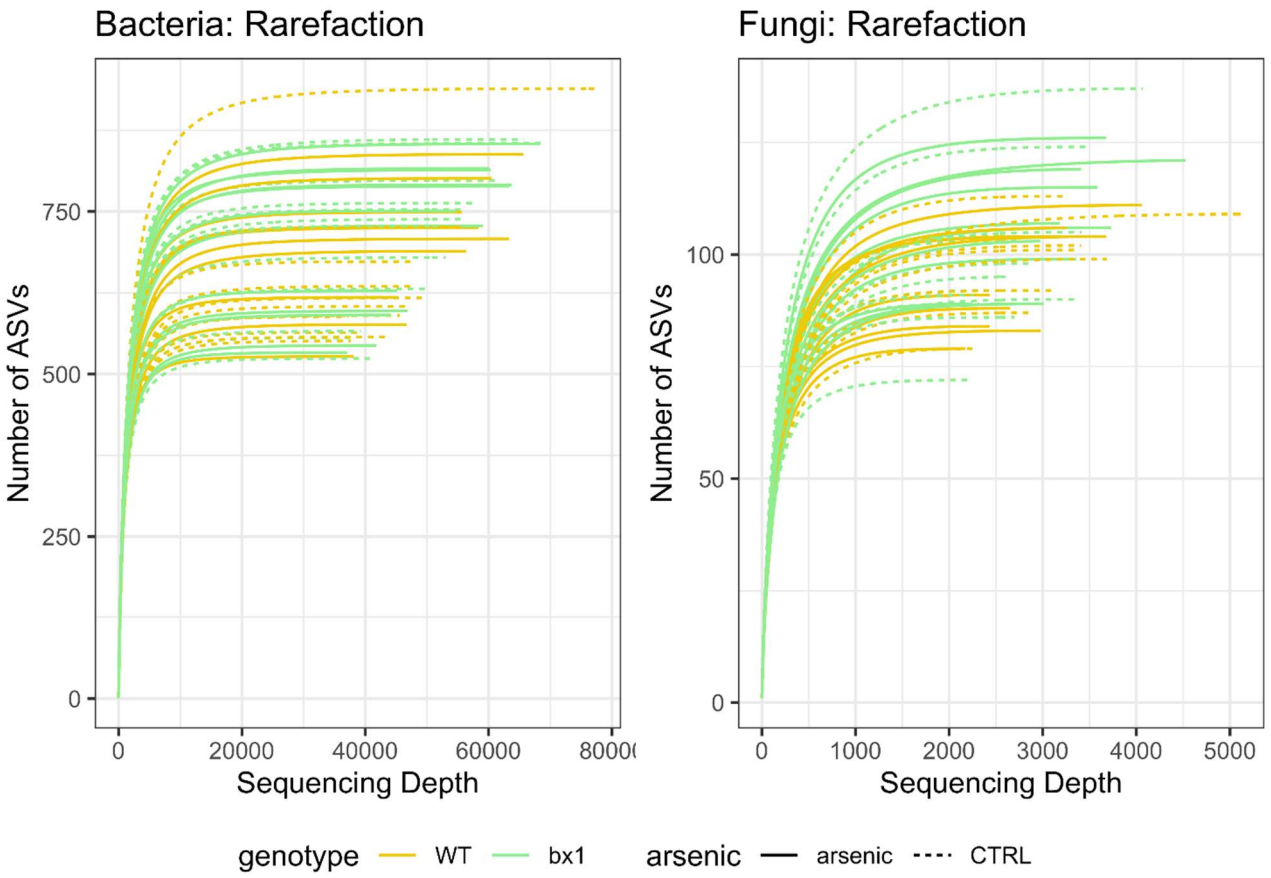


Figure S3. Rarefaction plot with the rarefaction threshold labelled as red line for bacteria (A) and fungi (B). Rhizosphere samples of wild type (W22) and benzoxazinoid-deficient *bx1* mutant plants growing without (0 mg kg^{-1}, CTRL) or with arsenic addition (100 mg kg^{-1}, arsenic) were analyzed.

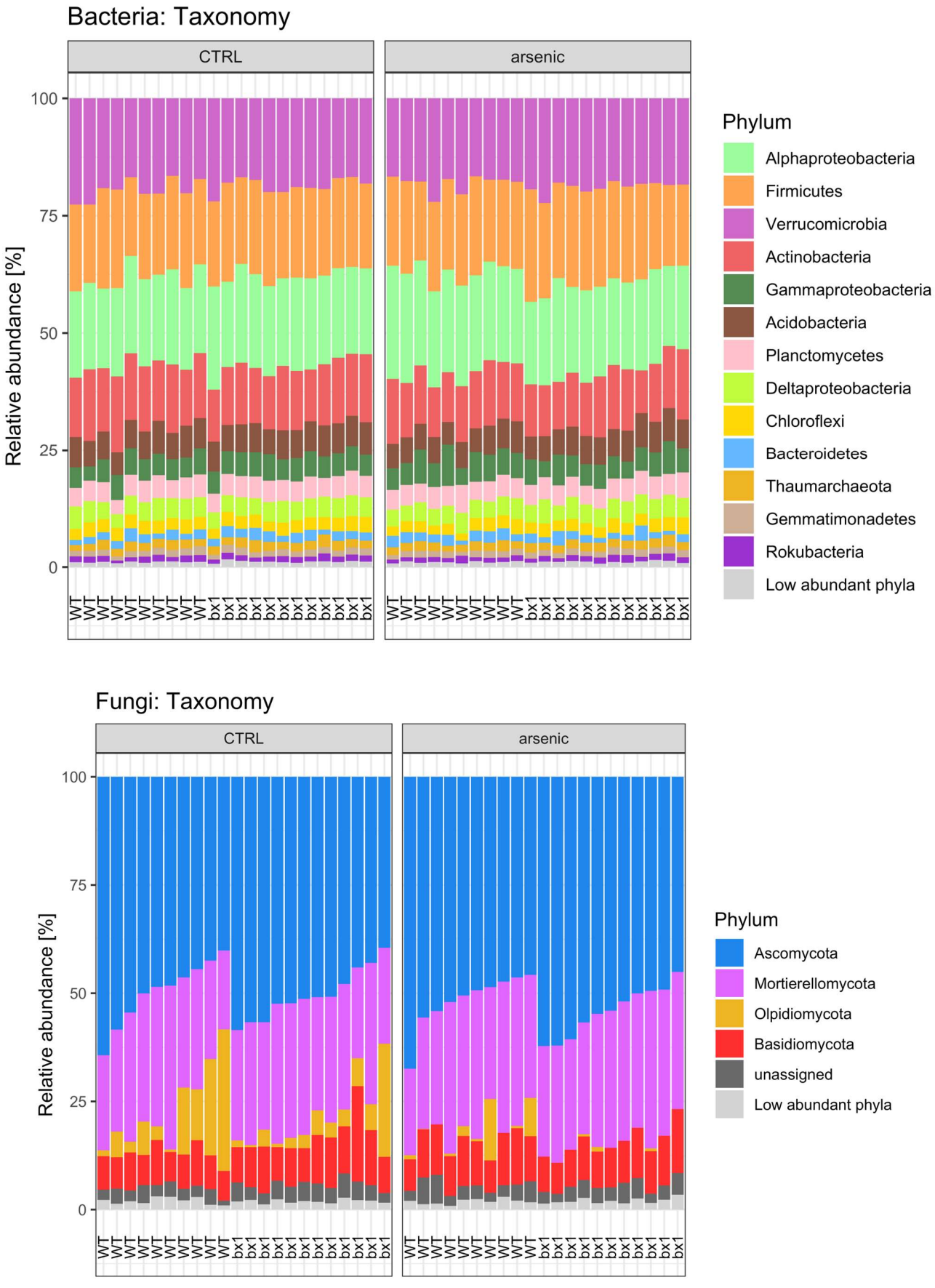


Figure S4. Relative abundance of different phyla for bacteria (A) and fungi (B).

Table S4. PERMANOVA of phyla abundances (abundance ~ genotype * arsenic, 999 repetitions).

Taxa	Factor	Df	SumOfSqs	R2	F	Pr(>F)
Bacteria	arsenic	1	0.0038882	0.04372751	1.90381578	0.097
Bacteria	genotype	1	0.00297084	0.03341072	1.45464156	0.188
Bacteria	arsenic:genotype	1	0.00036702	0.00412761	0.17970875	0.957
Bacteria	Residual	40	0.08169277	0.91873415	NA	NA
Bacteria	Total	43	0.08891884	1	NA	NA
Fungi	arsenic	1	0.00397966	0.01001834	0.41468005	0.729
Fungi	genotype	1	0.0089264	0.02247121	0.93013019	0.427
Fungi	arsenic:genotype	1	0.01005068	0.02530145	1.04728	0.368
Fungi	Residual	39	0.37428052	0.942209	NA	NA
Fungi	Total	42	0.39723726	1	NA	NA

Table S5. ANOVA of alpha diversity (shannon diversity ~ genotype * arsenic).

Taxa	Factor	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Bacteria	genotype	1	0.02808331	0.02808331	1.24414974	0.27132934
Bacteria	arsenic	1	0.00156474	0.00156474	0.06932106	0.79367946
Bacteria	genotype:arsenic	1	0.03245025	0.03245025	1.43761428	0.23758074
Bacteria	Residuals	40	0.9028917	0.02257229	NA	NA
Fungi	genotype	1	0.0478357	0.0478357	1.99925905	0.16530961
Fungi	arsenic	1	0.00489366	0.00489366	0.20452691	0.65359694
Fungi	genotype:arsenic	1	0.01818744	0.01818744	0.76013099	0.38862342
Fungi	Residuals	39	0.93314185	0.02392671	NA	NA

Table S6. The number of sensitive ASVs which have an altered relative abundance between the genotypes in arsenic-contaminated soil. The last column represents the relative abundance sum of all sensitive ASVs in the control treatment.

taxa	lower in CTRL	unchanged	higher in CTRL	rel. abu. of sens. ASVs
bacteria	0	1284	0	0%
fungi	0	176	0	0%

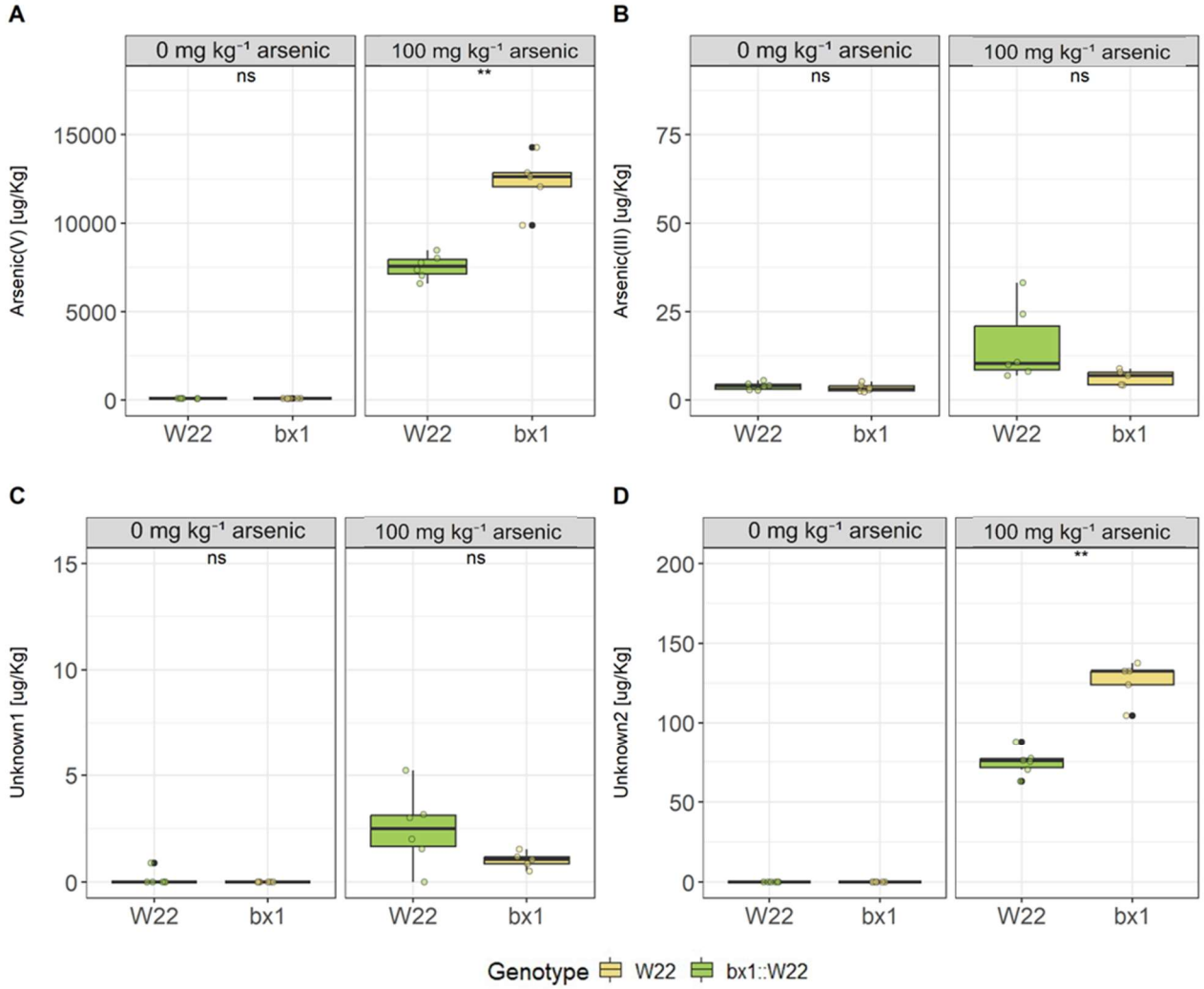


Figure S5. Arsenic speciation data in the maize rhizosphere. Concentrations of As^{V} and As^{III} and two unknown arsenic species in the rhizosphere of wild type (W22) and benzoxazinoid-deficient *bx1* mutant plants growing in soil without (0 mg kg^{-1}) or with arsenic addition (100 mg kg^{-1}) are shown. Levels of significance (among genotypes): n.s. non-significant, . = marginally significant, *p <.05, **p <.01, ***p <.001. See **Additional file 2** for detailed results of Tukey HSD tests.

Table S7. Soil parameter of in the respective As- and As+ fields.

Parameters	As- field	As+ field
clay %	16	21
silt %	21	21
pH	7.3	7.4
phosphorus-CO2 (mg/kg)	24.3	18.5
potassium-CO2 (mg/kg)	14.5	8.1
magnesium-CCMg (mg/kg)	9.2	8.3
phosphorus-H2O (mg/kg)	70.8	62.8
potassium-H2O (mg/kg)	723.5	493.5
magnesium-H2O (mg/kg)	266.9	217.6
iron (mg/kg)	222	213
manganese (mg/kg)	233	285
boron (mg/kg)	1.7	1.5
copper (mg/kg)	6.7	7.2

A

B

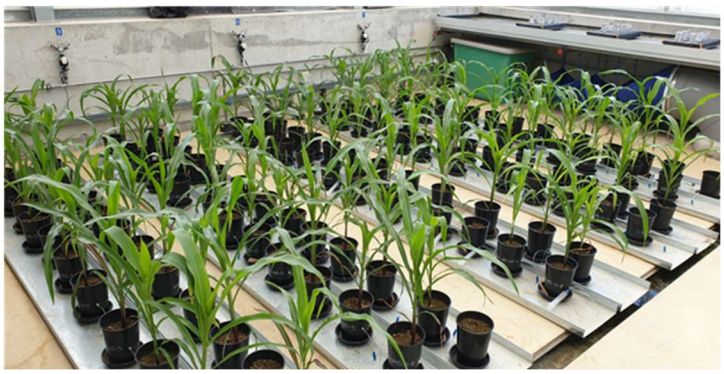


Figure S6. (A) Soil incubation setup in plastic boxes with plastic lid in the corridor of the greenhouse. (B) Greenhouse setting of experiments with randomized design and weekly randomization.

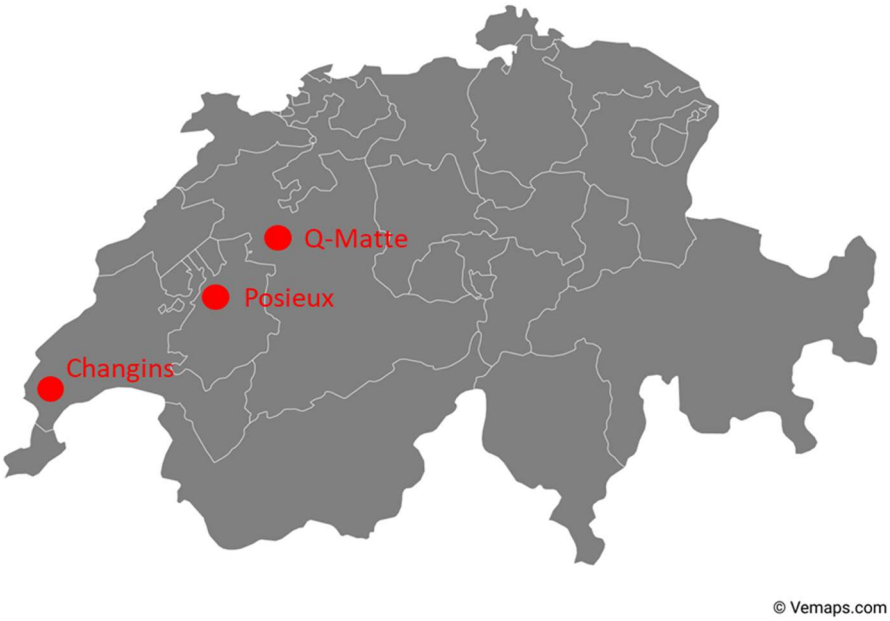


Figure S7. Switzerland map with the origin of the three used agricultural soils: Frauenkappelen (Canton Bern), Posieux (Canton Fribourg) and Changings (Canton Vaud).

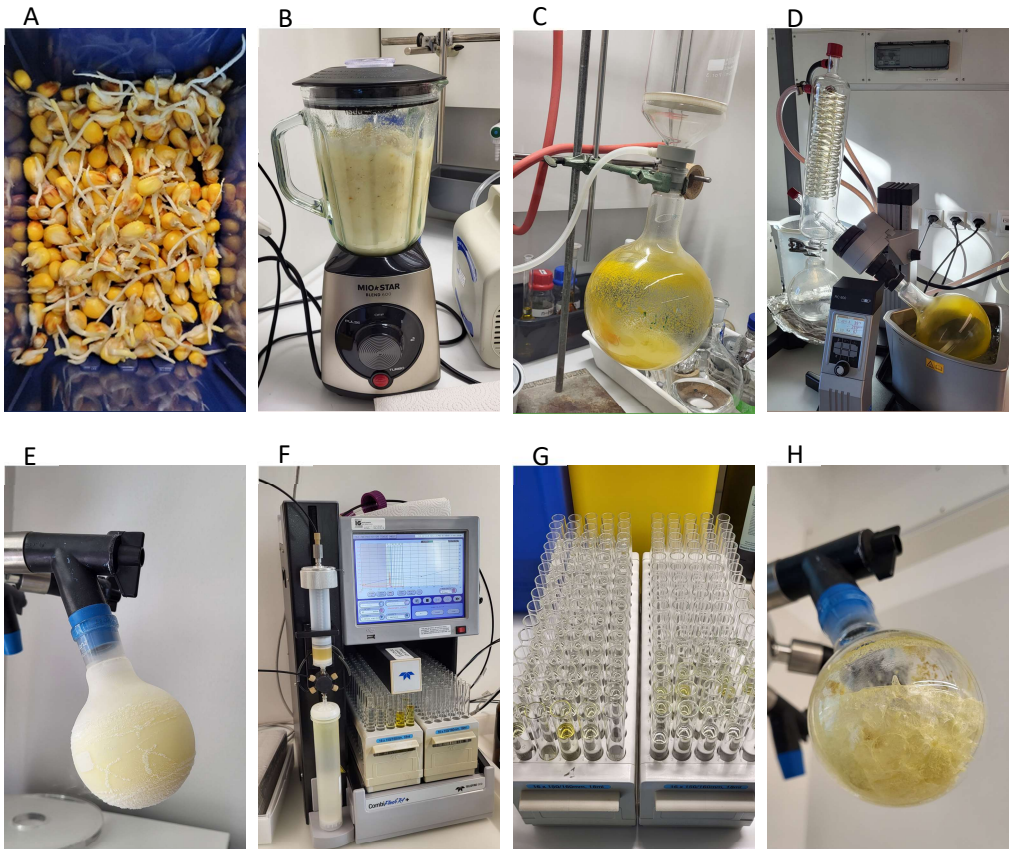


Figure S8. Purification BXs from wild-type germinated seeds

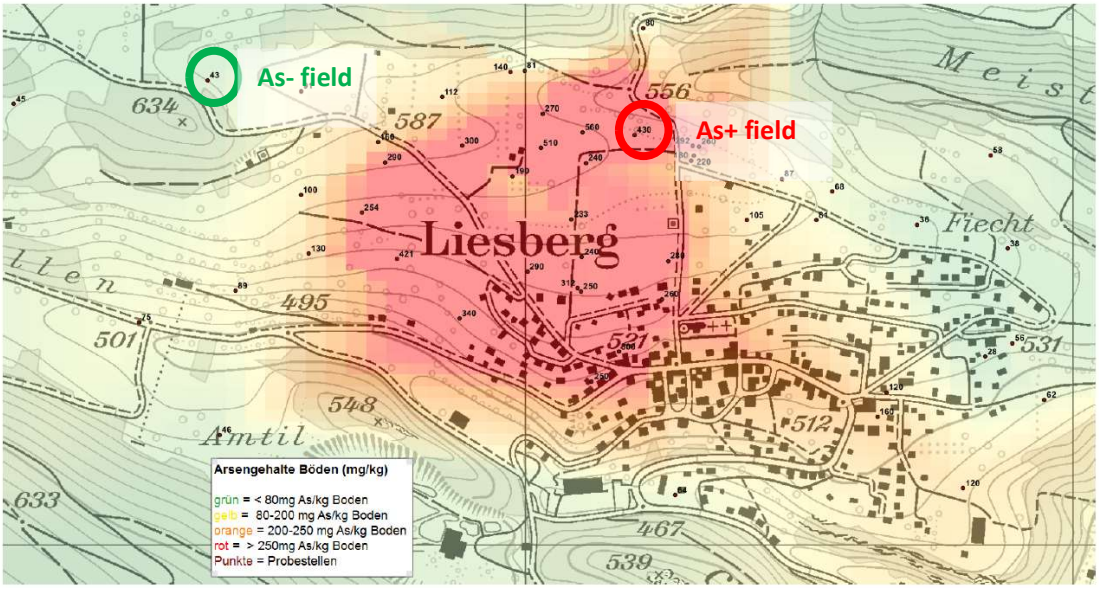


Figure S9. Map of the arsenic-contaminated area in Liesberg, Basel-Landschaft, Switzerland. In green, the least contaminated field and in red the heavily contaminated field. Both fields are owned and managed by the same farmer.

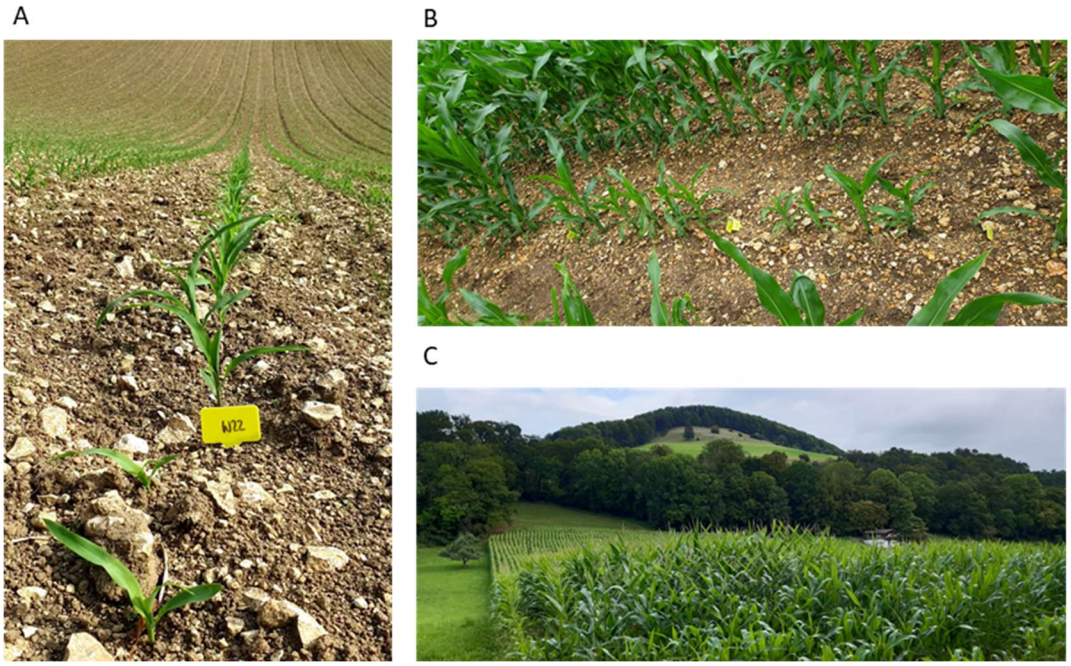


Figure S10. (A) Our seeds were planted, after removal of the farmer's seeds, in the field lines. (B) Subplot of 6 W22 plants and 6 *bx1* mutant plants. (C) Field just before harvesting.

Table S8. Dates and treatments that the farmer applied in its fields. Therefore, also our plants were treated in the same way during the growth period.

Type of management	Date	Comments
Fertilization	before plowing	30 m3 liquid manure
Pre-crop (plow)	24.05.2021	
Pre-crop (arrow)	26.05.2021	
Sowing maize	01.06.2021	Amarock (As+), Robertino (As-)
Fertilization	16.06.2021	NPK 3x15% approx. 200 Kg/ha
Herbicide	26.06.2021	1.5 L/ha EquipPower + 0.5L Bavel
Fertilization	28.06.2021	Urea 150 Kg/ha

Table S9. ANOVA tables of plant height and chlorophyll content measured over time. Variables were untransformed (§), log transformed (¥), sqrt transformed (∞) or ranked transformed (†) to meet the requirements to perform the analysis. F-values and significance levels for a three-way analysis of variance with herbicide (Hc), application (ApGxFX) and time (Ti) as separate factors and their interaction term are shown. P-values: ns not significant, . 0.1 < P < 0.05, * 0.05 < P < 0.01, ** 0.01 < P < 0.001, *** 0 < P < 0.001.

	Error:Sample_ID						Error:Within							
Variable	Genotype		Arsenic		G*A		Week		G*W		A*W		G*A*W	
	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)
Figure 1														
Plant height†	24.78	1.04e-05 ***	20.851	3.98e-05 ***	2.103	0.154	819.931	< 2e-16***	8.713	0.000000148 ***	5.355	0.000115***	0.323	0.898617
Chlorophyll†	0.187	0.668	0.001	0.977	1.276	0.265	3.92E+01	<2e-16 ***	0.56	0.7309	2.176	0.0578 .	0.056	0.998
Figure 2														
Plant height†	3.754	0.0298*	27.107	3.07e-06 ***	1.62	0.2073	273.403	<2e-16 ***	0.4	8.08E-01	3.337	0.0392*	2.225	0.0710.
Figure 4														
Plant height†	5.424	0.00714 **	114.877	5.55e-15 ***	3.112	0.05258.	669.28	< 2e-16 ***	1.27	2.86E-01	16.28	6.61e-07 ***	0.03	0.998

Variable	Soil		Genotype		Arsenic		Week		S*G		S*A		G*A	
	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)
Figure 2S														
Plant height†	12.364	1.67e-05 ***	27.855	8.11e-07 ***	328.438	< 2e-16 ***	33.092	1.17e-11 ***	1.094	0.339	20.321	4.37e-08 ***	5.905	0.0170*

S*W		G*W		A*W		S*G*A		S*A*W	
F	Pr(>F)	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)
2.464	0.0904.	0.66	0.5193	1.181	0.28	0.765	0.4681	0.014	0.9075

	Error:Sample_ID						Error:Within							
Variable	Genotype		Field		G*F		Week		G*W		F*W		G*F*W	
	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)
Figure 5														
Plant height†	9.724	0.002208 **	14.35	0.000225 ***	0.123	0.725803	476.655	< 2e-16 ***	7.697	5.12e-05 ***	1.907	0.128	0.762	0.516

	Error:Sample_ID						Error:Within							
Variable	Conditioning		Arsenic		C*A		Week		C*W		A*W		C*A*W	
	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)
Figure 6														
Plant height§	8.332	0.00663 **	69.38	8.04e-10 ***	3.249	0.08010.	485.729	< 2e-16 ***	6.001	0.00393 **	66.129	< 2e-16 ***	0.975	0.38233

Table S10. ANOVA tables of leaves biomass and arsenic uptake in roots and leaves. Variables were untransformed (§), log transformed (¥), sqrt transformed (∞) or ranked transformed (†) to meet the requirements to perform the analysis. F-values and significance levels for a three-way analysis of variance with herbicide (Hc), application (Ap) and time (Ti) as separate factors and their interaction term are shown. P-values: ns not significant, . 0.1 < P < 0.05, * 0.05 < P < 0.01, ** 0.01 < P < 0.001, *** 0 < P < 0.001.

Variable	Genotype		Arsenic		G*A	
	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)
Figure 1						
Leaves dry biomass †	9.953	0.00289 **	56.023	2.24e-09 ***	4.583	0.03787 *
Figure S3						
Leaves dry biomass §	2.684	0.0794 .	89.078	3.85e-12 ***	1.431	0.25
Figure 2						
Leaves dry biomass §	5.165	0.00899 **	312.543	< 2e-16 ***	4.194	0.02049 *
Figure 4						
As uptake roots †	9.353	0.00363 **	199.33	< 2e-16 ***	10.573	0.00210 **
As uptake leaves ¥	4.418	0.0406 *	3087.608	<2e-16 ***	1.381	0.2455

Variable	Genotype		Field		G*F	
	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)
Figure 5						
Leaves dry biomass †	33.88	1.81e-07 ***	7.138	0.00946 **	0.682	0.41199

Variable	Conditioning		Arsenic		C*A	
	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)
Figure 6						
Leaves dry biomass §	7.756	0.00858 **	74.195	3.61e-10 ***	2.491	0.1235

Variable	Soil		Genotype		Arsenic	
	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)
Figure S2						
Leaves dry biomass §	26.526	1.38e-09 ***	2.65	0.1074	241.717	< 2e-16 ***

	S*G		S*A		G*A		S*G*A	
	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)	F	Pr(>F)
	0.548	0.5803	15.25	2.39e-06 ***	4.491	0.0371 *	0.099	0.9061

Arsenic - Microbiome Analysis

Jan Waelchli

2024-02-05

Contents

Experimental Setup

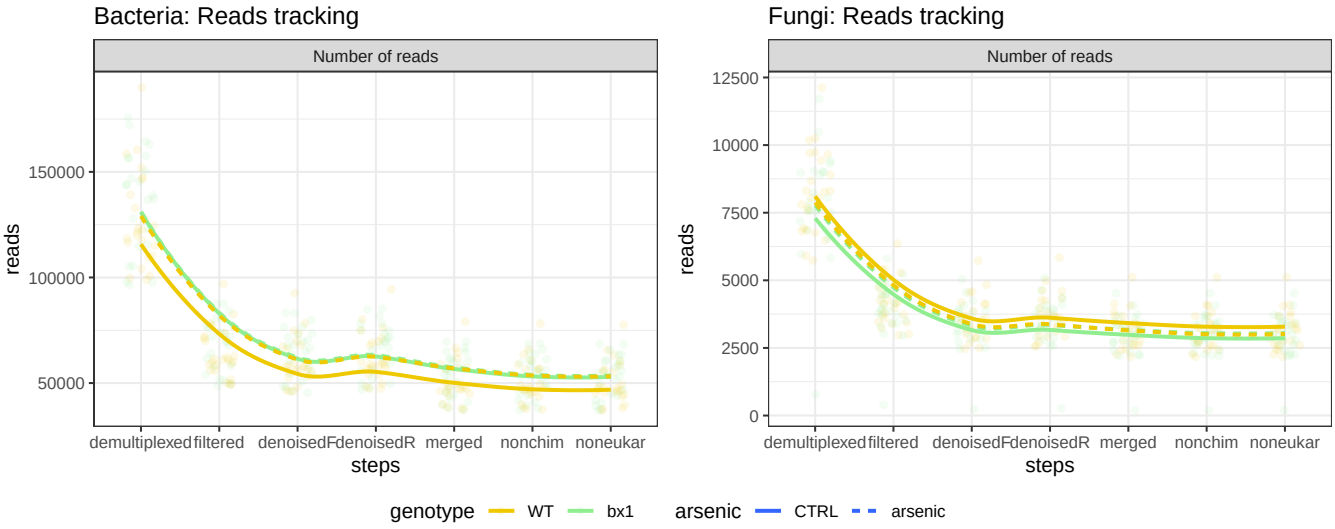
WT maize plants and bx1 mutants with a W22 background were grown. Half of them were treated with 0 mg/kg As (CTRL) and the other half with 100 mg/kg As (arsenic). We analyse the shift of the bacterial and fungal communities in the plant rhizosphere.

Description all data

Sequencing Depth

Figure S11 | Reads tracking

We plot the amount of reads during each pipeline step. This allows us to see where we loses reads and if the samples from the different groups behave similar.



Conclusion: We lose the expected amount of reads. Samples from different group behave very similar.

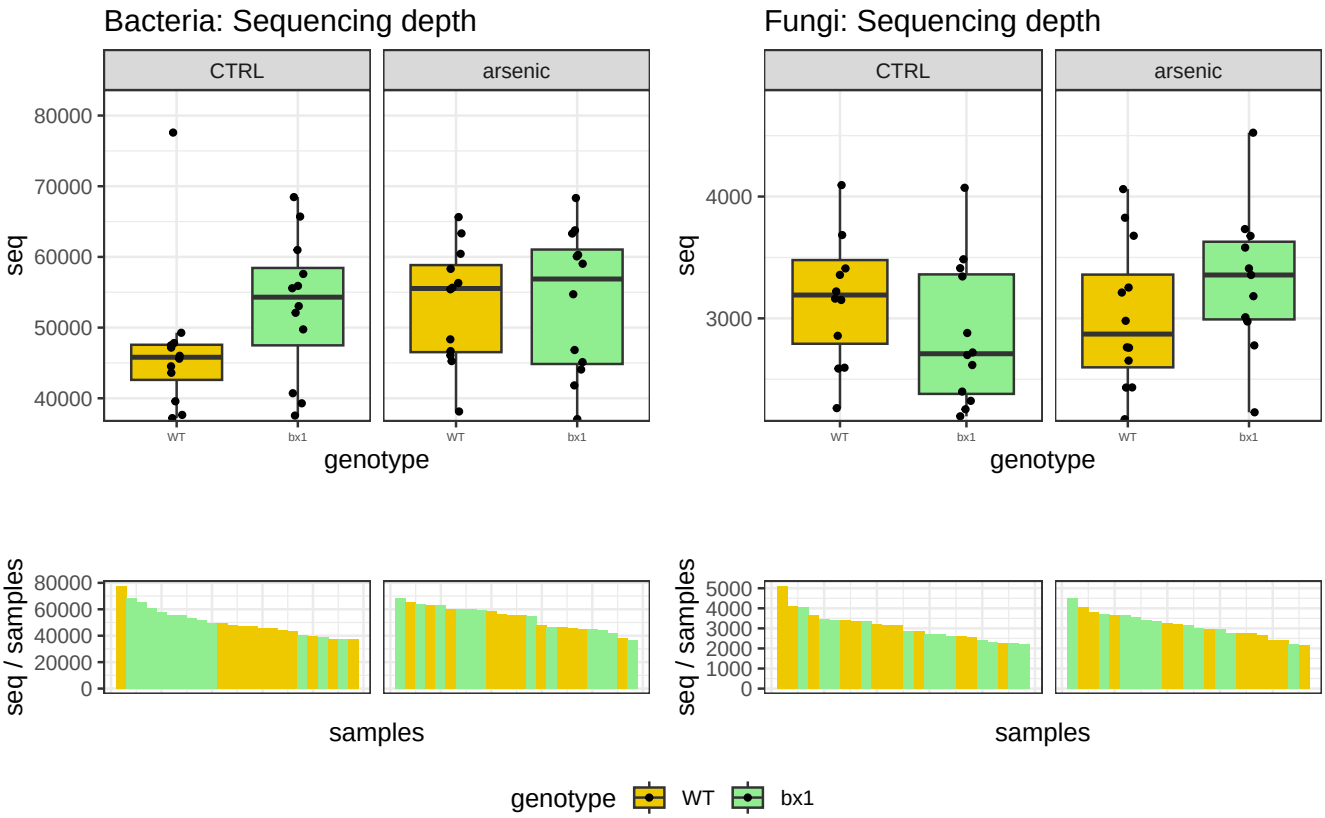
Number of sequences

We show the sum, range and median of sequecnes over all samples.

Table S11: Number of sequences

Taxa	removed_samples	sum	min	max	median
Bacteria	0	2484130	37055	77593	49508
Fungi	1	146569	2172	5112	3151

Figure S12 | Sequencing depth



Normalization

Asymptotic Kruskal-Wallis Test & Normalization

To decide on how to normalize the data we follow the recommendation of Weiss et al. (2017, Microbiome Journal) and inspect whether there are differences in sequencing depths between the different arsenic-treatments and genotypes by using the non-parametric Kruskal-Wallis Test.

```
## [1] "Bacteria"
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  sample_depth by group
## Kruskal-Wallis chi-squared = 4.9192, df = 3, p-value = 0.1778
```

```
## [1] "Fungi"
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  sample_depth by group
## Kruskal-Wallis chi-squared = 3.798, df = 3, p-value = 0.2841
```

Conclusion: We don't find significant differences between the groups in bacteria or fungi. We follow the recommendation of Weiss et al. (2017) to use TSS normalization for samples with small sequencing-depth differences.

Outlier Detection

We use the method CLOUD developed by Montassier et al. 2018, which is a non-parametric detection test for outliers. We perform the test with Bray-Curtis distances from the normalized data for each substrate and each plastic treatment individually. We set the number of nearest neighbors to 60% of the samples size and chose an empirical outlier percentile of 0.1. We remove all outliers from our data.

Table S12: Number of outliers

Species	Arsenic.CTRL	Arsenic.arsenic
Bacteria	2	2
Fungi	2	2

Sample Control

Sample Size

We end up with the following number of samples per treatment for the analysis.

Table S13: Bacteria: Sample profile

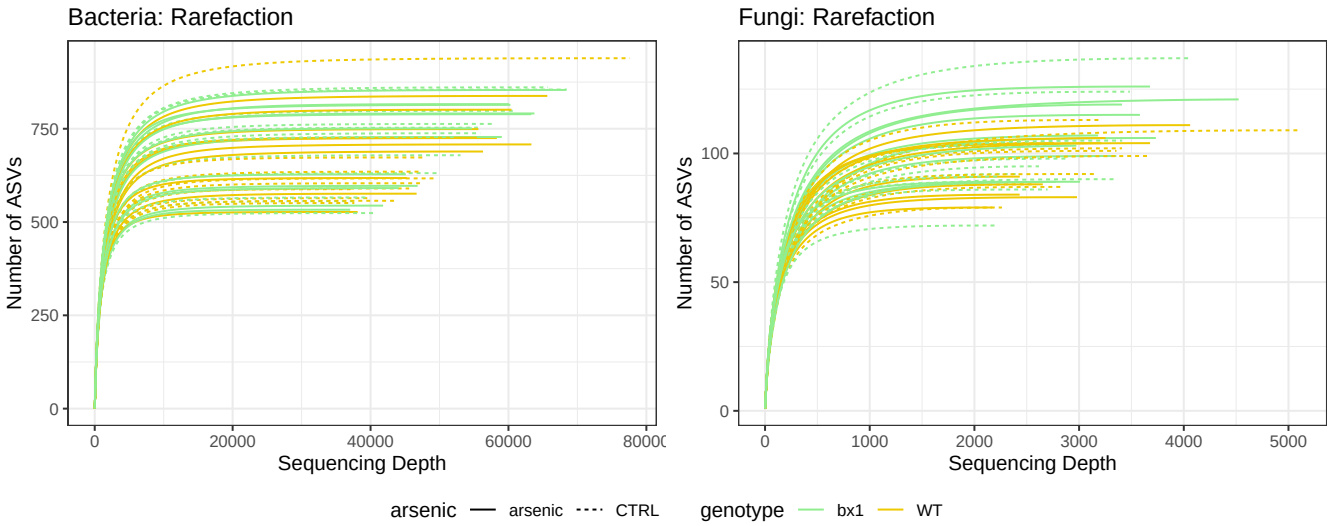
	CTRL	arsenic
WT	10	10
bx1	12	12

Table S14: Fungi: Sample profile

	CTRL	arsenic
WT	10	10
bx1	12	11

Figure S13 | Rarefaction plot

We plot a rarefaction plot with the remaining samples to check if the sequence depth is enough to capture the microbial diversity.



Conclusion: All samples were sequenced deep enough.

Taxonomy

Phyla abundance plot

We get an overview over the abundance of bacterial taxonomy by showing the most abundant phyla for each sample.

Figure S14.1 | Bacteria: Phylum level taxonomy

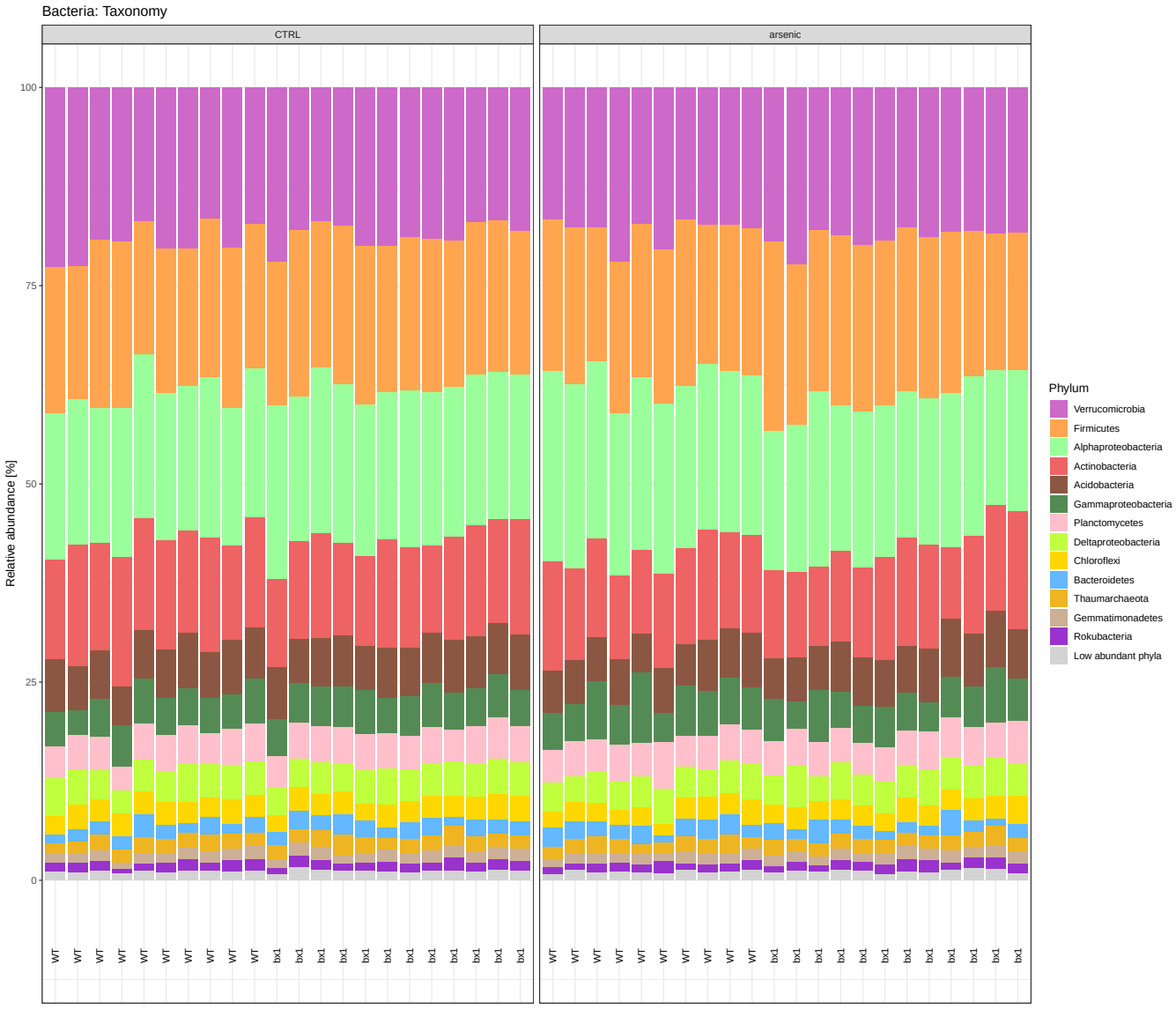
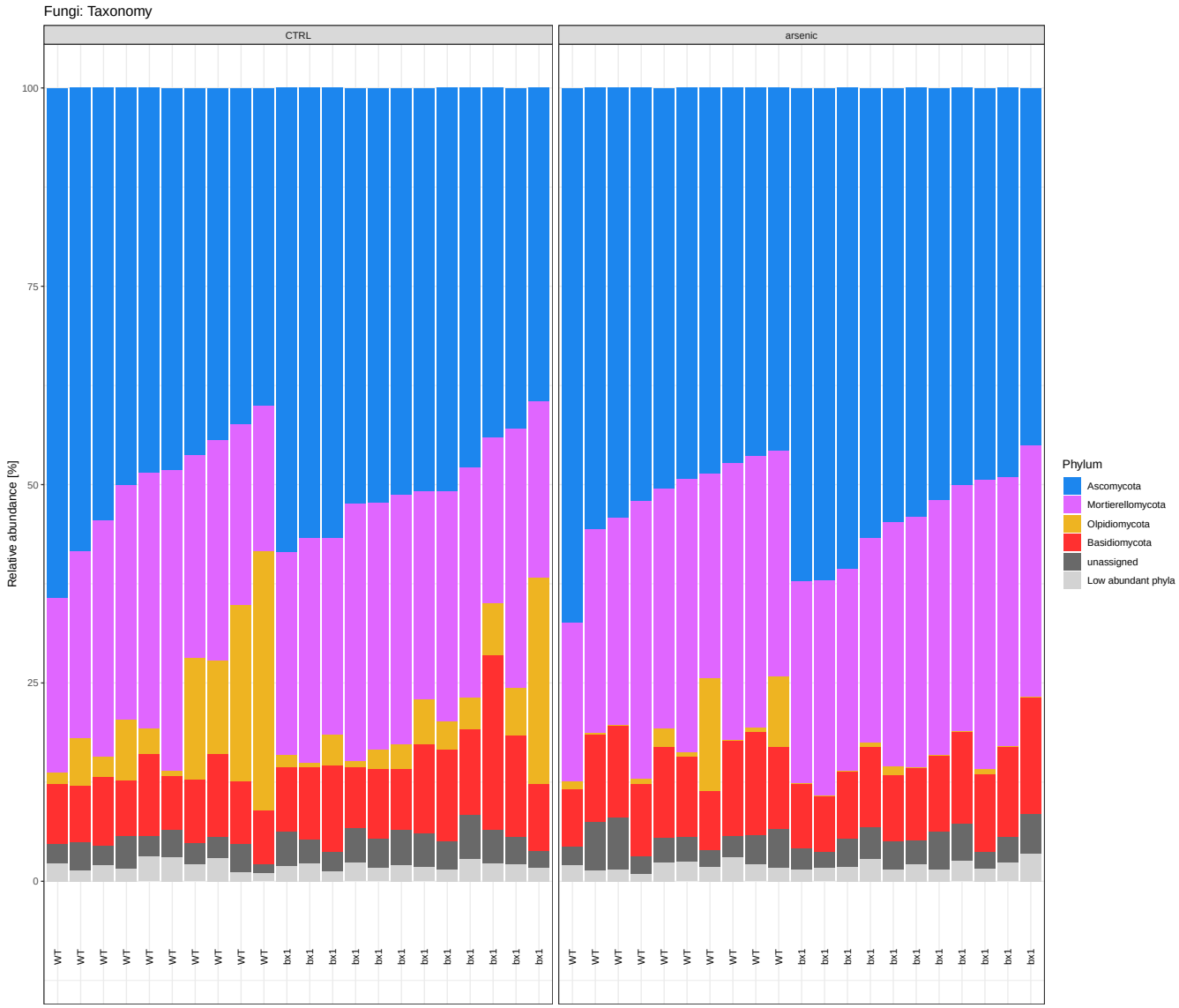


Figure S14.2 | Fungi: Phylum level taxonomy



Effect of all factors on phyla abundances

We test if there are any difference between the phyla abundances between genotypes, arsenic-treatments or their interaction by performing a PERMNOVA (permutations = 999).

Bacteria

Table S15: Bacteria: PERMANOVA

	Df	SumOfSqs	R2	F	Pr(>F)
arsenic	1	0.003888	0.04373	1.904	0.097
genotype	1	0.002971	0.03341	1.455	0.188
arsenic:genotype	1	0.000367	0.004128	0.1797	0.957
Residual	40	0.08169	0.9187	NA	NA
Total	43	0.08892	1	NA	NA

Fungi

Table S16: Fungi: PERMANOVA

	Df	SumOfSqs	R2	F	Pr(>F)
arsenic	1	0.00398	0.01002	0.4147	0.729
genotype	1	0.008926	0.02247	0.9301	0.427
arsenic:genotype	1	0.01005	0.0253	1.047	0.368
Residual	39	0.3743	0.9422	NA	NA
Total	42	0.3972	1	NA	NA

Conclusion: No differences found.

Alpha diversity

We answer the following questions for the alpha diversity in each substrate:

- **Q1: Has arsenic changed the beta diversity?**
- **Q2: Is beta diversity different between the genotypes?**
- **Q3: Are there differences in beta diversity between the different genotypes after treating plants with arsenic?**

Method

We calculate the Shannon diversity for each sample with the normalized data.

Genotype*Arsenic Effect

We investigate the effect on alpha diversity by the factors of genotype, arsenic and the interaction between them. We model the alpha diversity against these factors in an aov-model and perform a F-Test.

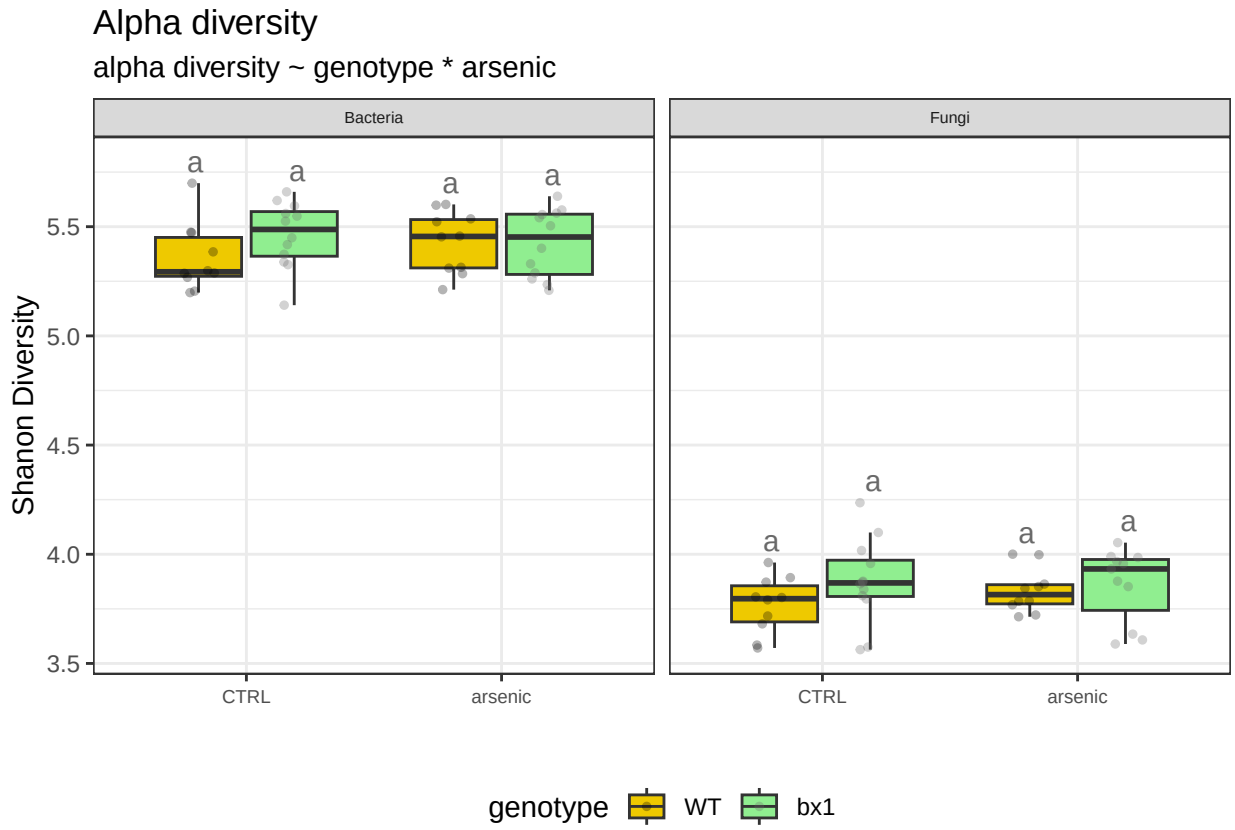
Table S17: Bacteria: F test

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
genotype	1	0.02808	0.02808	1.244	0.2713
arsenic	1	0.001565	0.001565	0.06932	0.7937
genotype:arsenic	1	0.03245	0.03245	1.438	0.2376
Residuals	40	0.9029	0.02257	NA	NA

Table S18: Fungi: F test

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
genotype	1	0.04784	0.04784	1.999	0.1653
arsenic	1	0.004894	0.004894	0.2045	0.6536
genotype:arsenic	1	0.01819	0.01819	0.7601	0.3886
Residuals	39	0.9331	0.02393	NA	NA

Figure S15 | Genotype*Arsenic effect on alpha diversity



Conclusion: No effect has been found.

Beta diversity

We answered the following question for the bacterial and fungal beta diversity in each compartment:

- **Q1: Has arsenic changed the beta diversity?**
- **Q2: Is beta diversity different between the genotypes?**
- **Q3: Are there differences in beta diversity between the different genotypes after treating plants with arsenic?**

Method

First we use the function 'adonis()' (package vegan) to analyze the beta diversity with a PERMANOVA (permutations = 999). Then, we graphically represent the beta diversity with a PCoA (unconstrained ordination) and a CAP plot (constrained ordination).

Genotype*Arsenic Effect

We investigate the full model to see which factors alters the beta diversity.

Table S19: Bacteria: PERMANOVA

	Df	SumOfSqs	R2	F	Pr(>F)
genotype	1	0.04415	0.03271	1.469	0.034
arsenic	1	0.0492	0.03646	1.637	0.015
genotype:arsenic	1	0.05384	0.0399	1.791	0.005
Residual	40	1.202	0.8909	NA	NA
Total	43	1.35	1	NA	NA

Table S20: Fungi: PERMANOVA

	Df	SumOfSqs	R2	F	Pr(>F)
genotype	1	0.1526	0.0328	1.448	0.049
arsenic	1	0.2483	0.05335	2.356	0.002
genotype:arsenic	1	0.1431	0.03076	1.358	0.079
Residual	39	4.109	0.8831	NA	NA
Total	42	4.653	1	NA	NA

Figure S16.1 | PCoA - genotype:arsenic effect on beta diversity

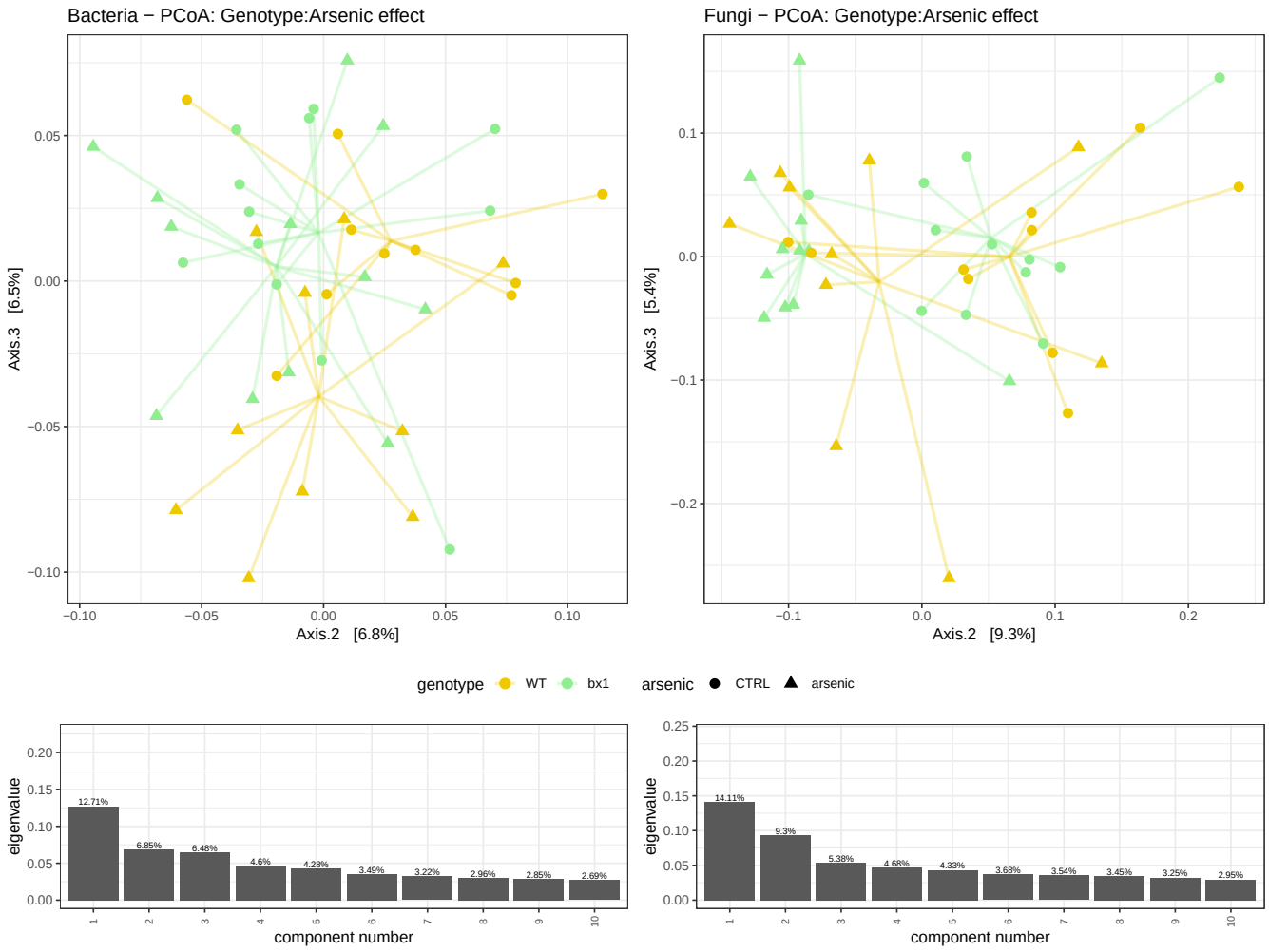
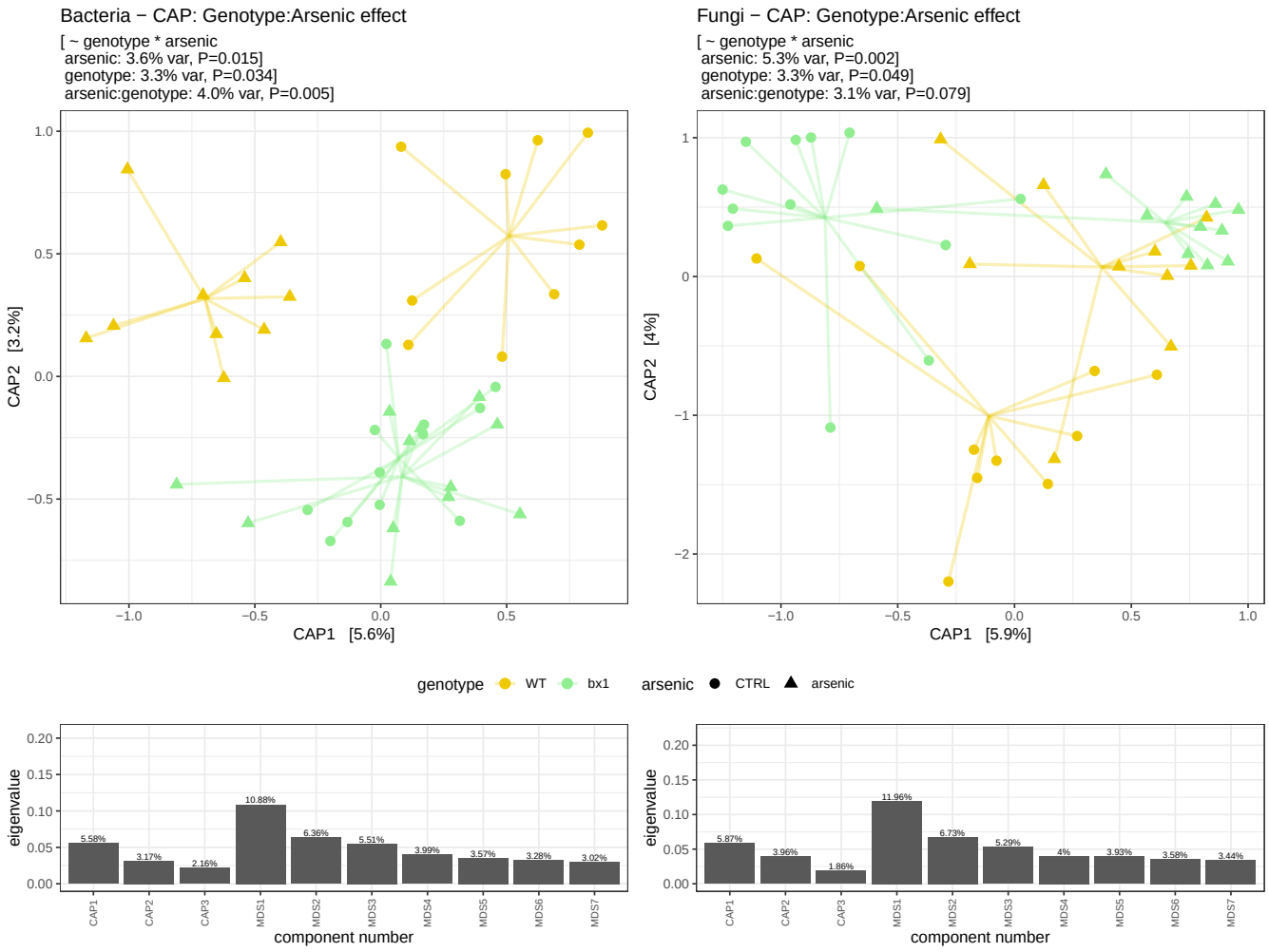


Figure S16.2 | CAP - genotype:arsenic effect on beta diversity



Conclusion: There are differences in the bacterial and fungal communities due to the arsenic treatment, the genotypes and their interactions. We can explain about 4% of bacterial and 3% of fungal variety due to the arsenic:genotype interaction effect.

Taxa Response

Is there a core of sensitive microbial taxa? We searched sensitive ASVs – ASVs being differential abundant between WT and bx1. We answer the following question in non-arsenic and arsenic conditions:

Q1: Are there sensitive ASVs between control and WT and bx1 samples in non-arsenic and arsenic soil?

Method

We answered the question by using four different tools to measure differential abundances - aldex2, acombc, maaslin2 and metagenomeSeq - and predict ASVs to be different if they were detected by 2 or more tools.

Genotype*Arsenic Effect

We check for each ASV if it is sensitive or not. Then, we show how many ASVs has been changed between the genotypes and how much of the relative abundance belongs to those sensitive ASVs.

Table S21: Bacteria: genotype effect

taxa	arsenic	lower in WT	unchanged	higher in WT	rel. abu. of sens. ASVs
bac	CTRL	0	1236	0	0%
bac	arsenic	0	1284	0	0%

0% in non-arsenic and 0% in arsenic conditions of the bacterial community was changed in abundance due to genotype.

Table S22: Fungi: gneotype effect

taxa	arsenic	lower in WT	unchanged	higher in WT	rel. abu. of sens. ASVs
fungi	CTRL	0	167	1	0.056%
fungi	arsenic	0	176	0	0%

0.32% in non-arsenic and 0% in arsenic conditions of the fungal community was changed in abundance due to genotype.

Conclusion: Most ASVs are insensitive.