Arsenic - Microbiome Analysis

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Experimental Setup

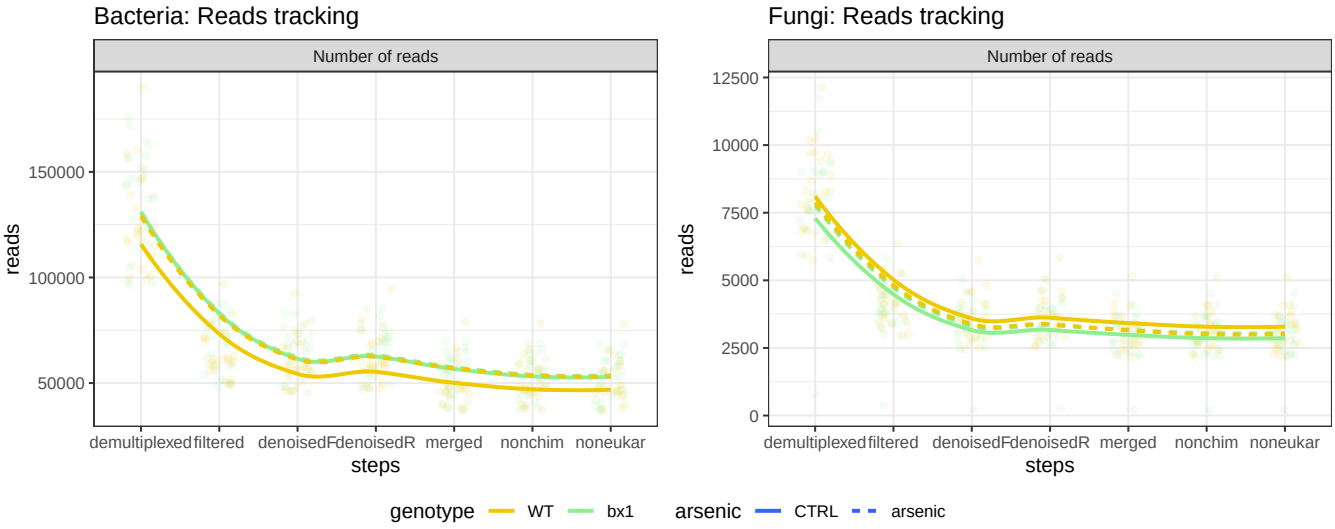
WT maize plants and bx1 mutants with a W22 background were grown. Half of them were treated with 0 mg/kg As (CTRL) and the other half with 100 mg/kg As (arsenic). We analyse the shift of the bacterial and fungal communities in the plant rhizosphere.

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.

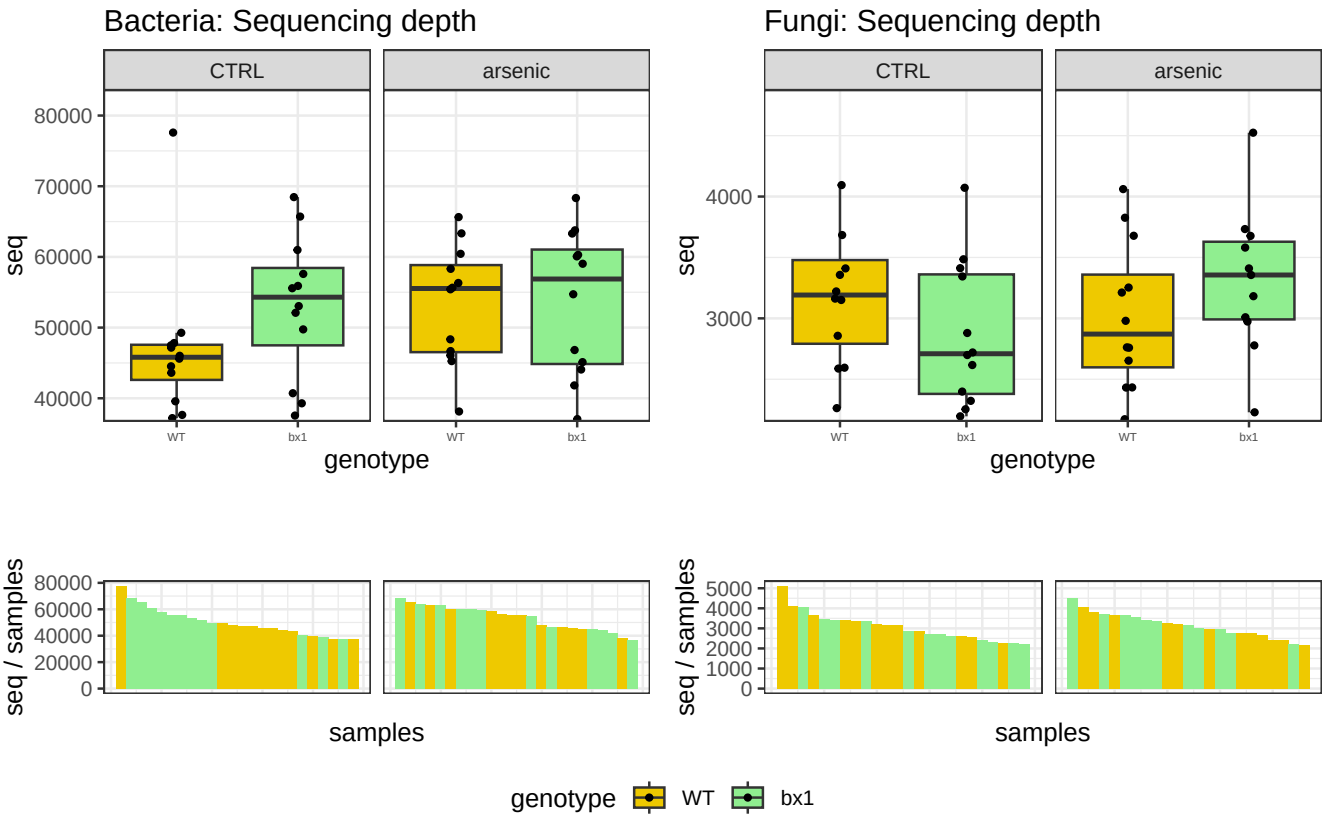
Number of sequences

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

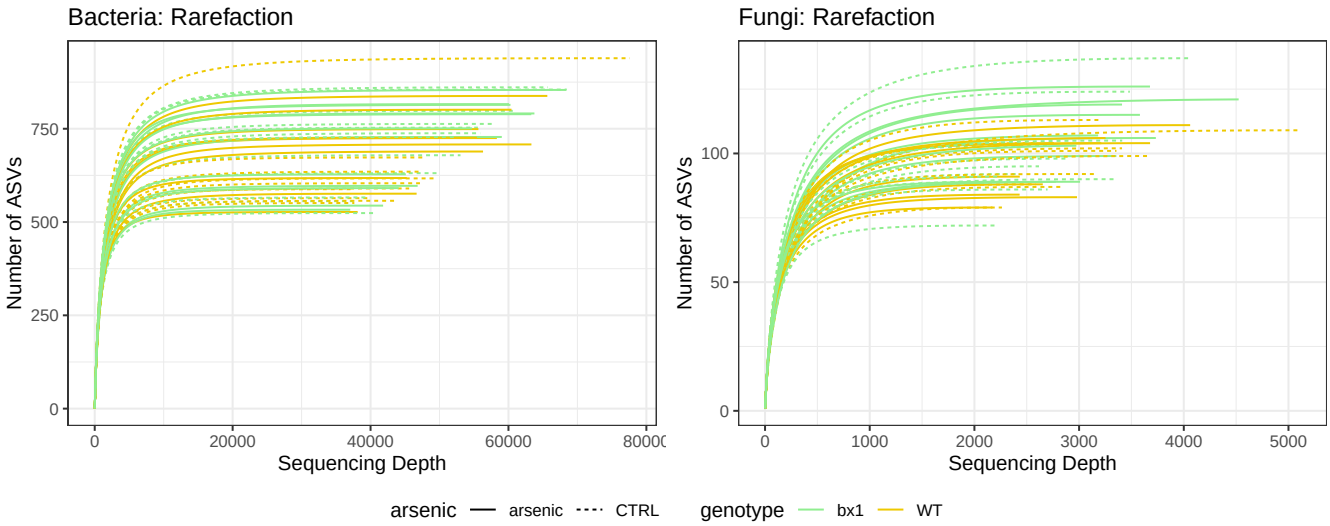
	CTRL	arsenic
WT	10	10
bx1	12	12

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

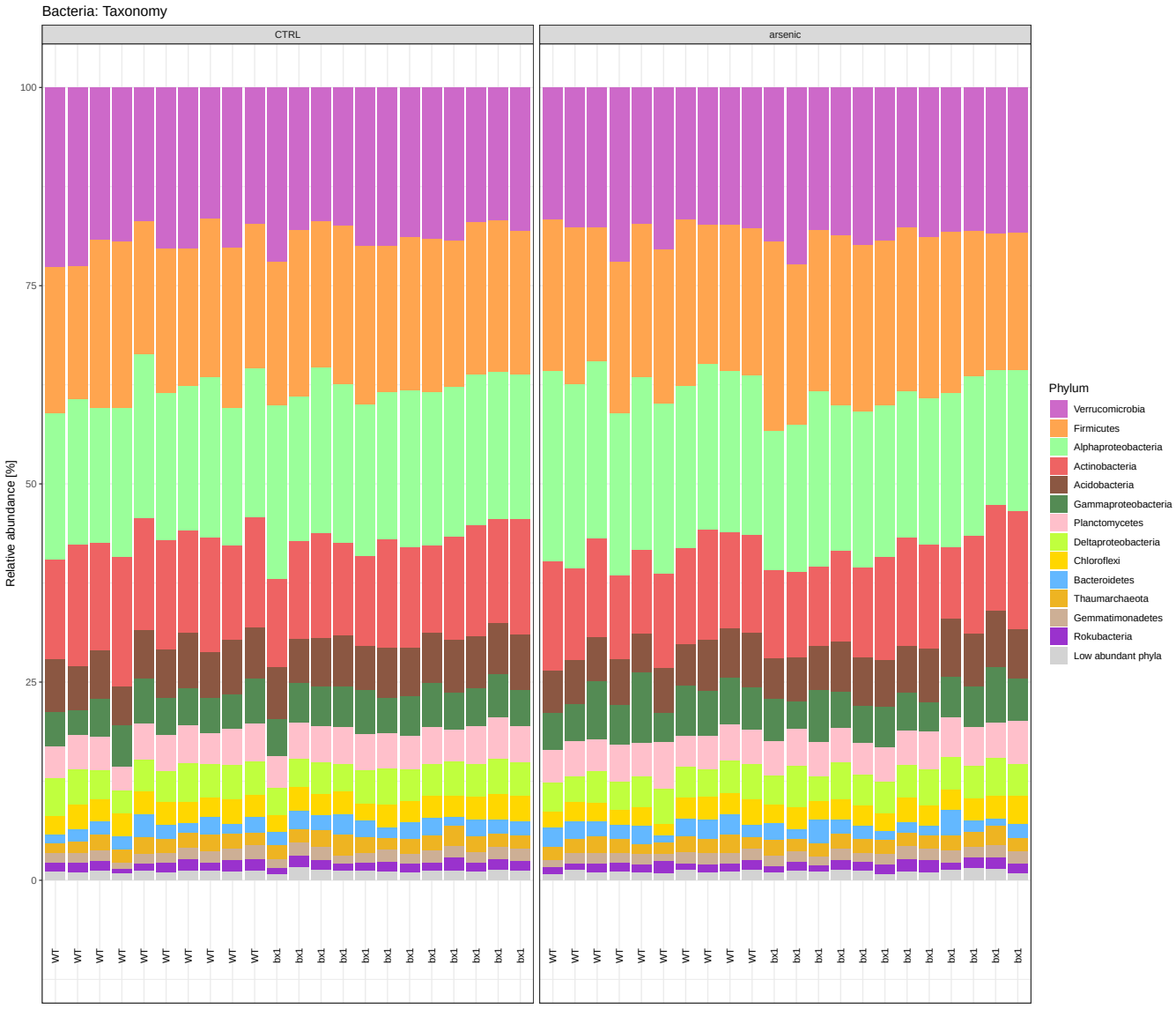
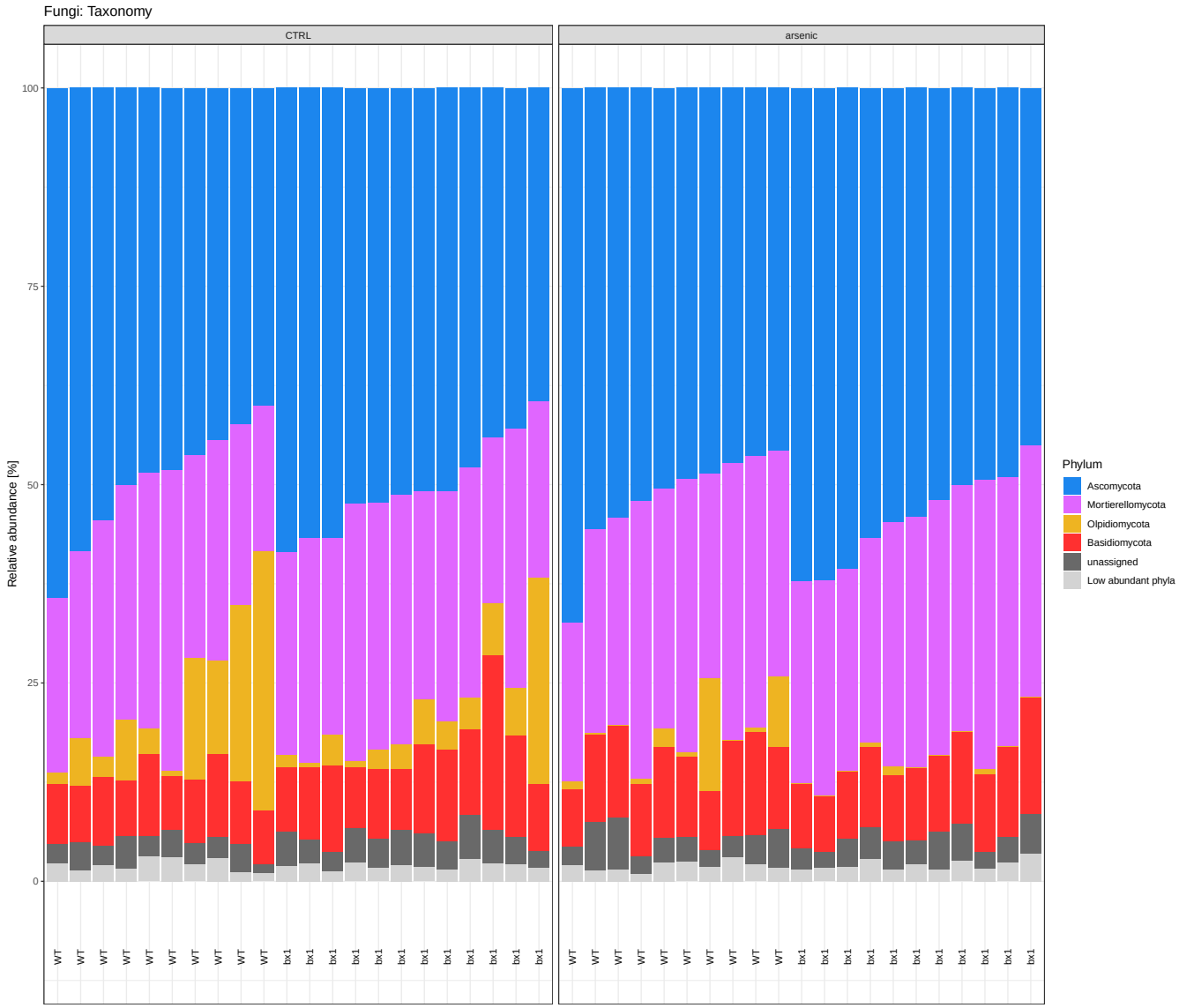


Figure S14.2 | Fungi: Phylum level taxonomy



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.

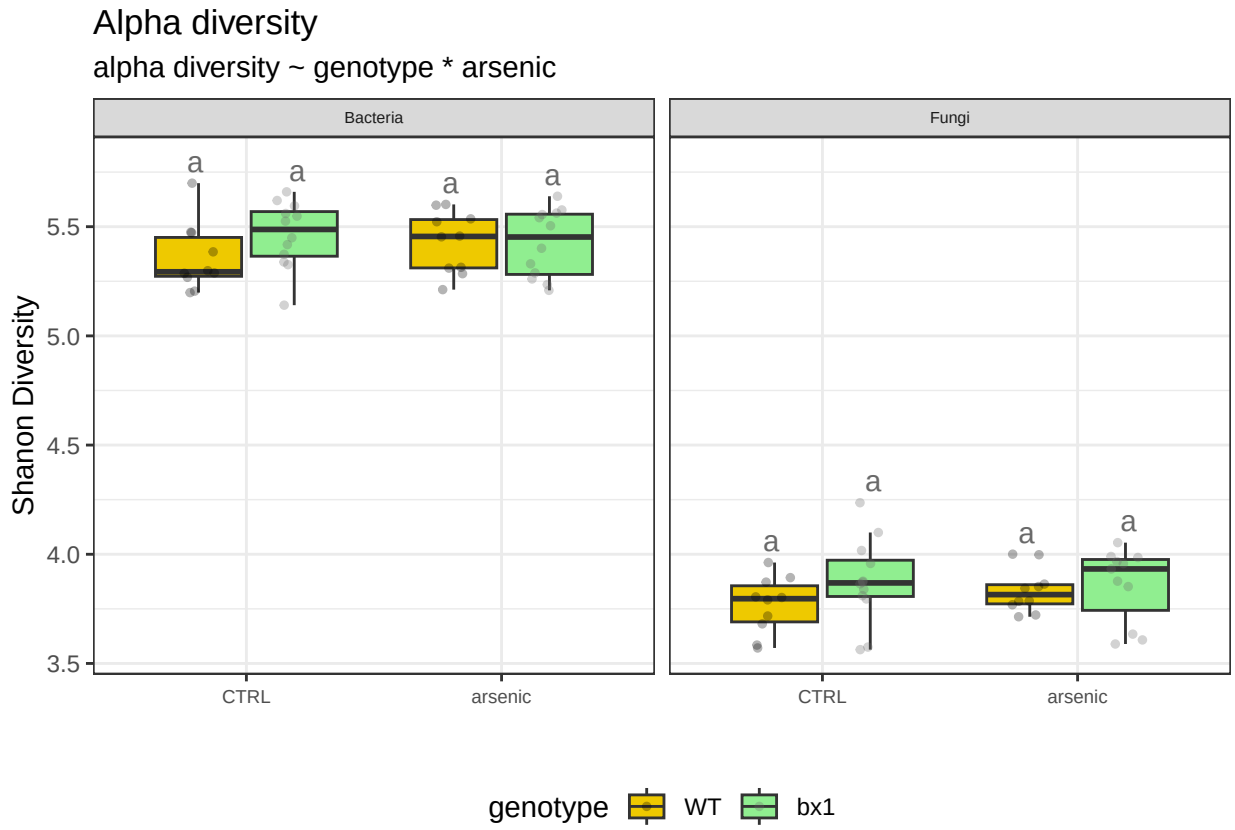
Table S17: Bacteria: F test

	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

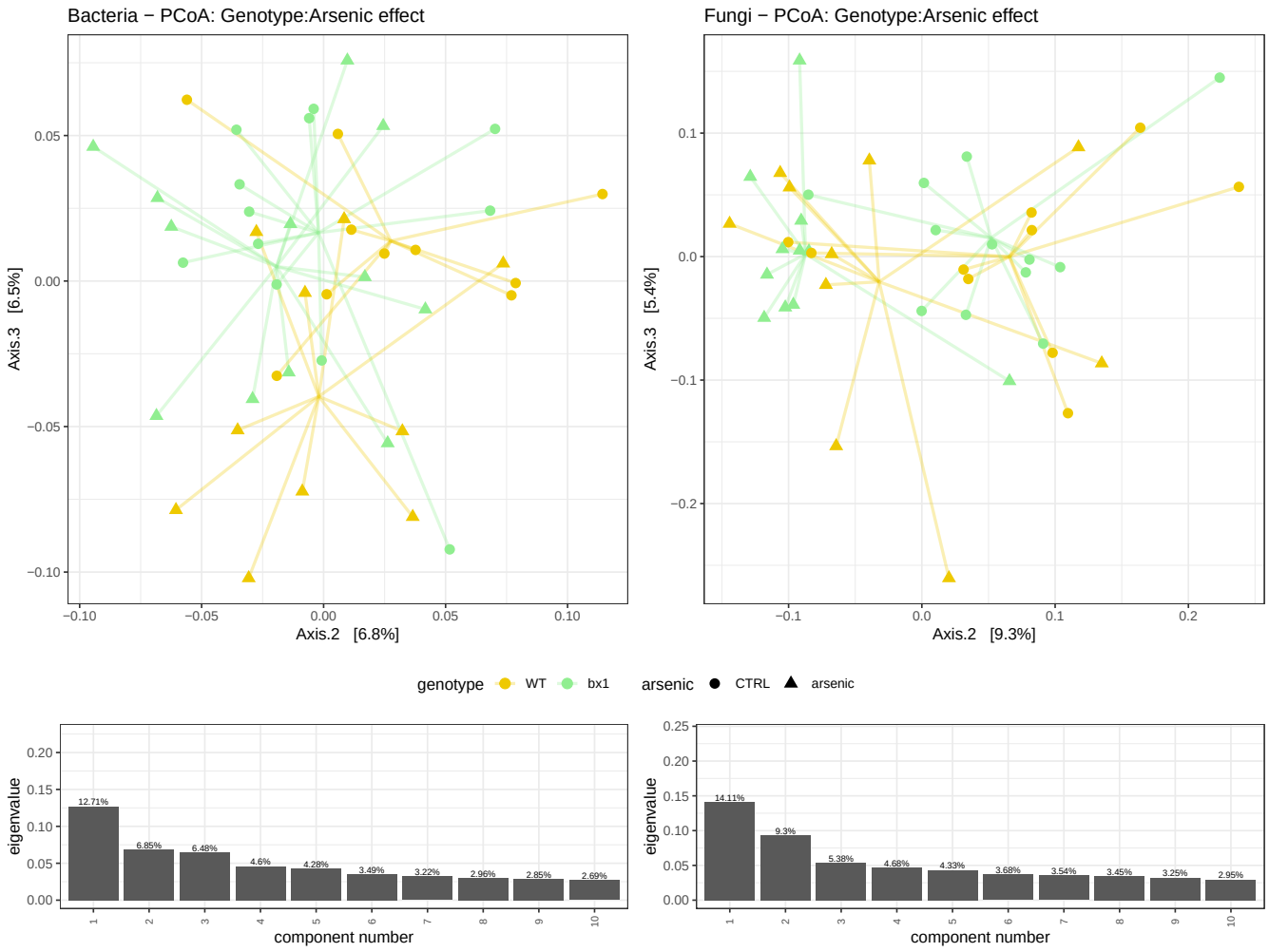
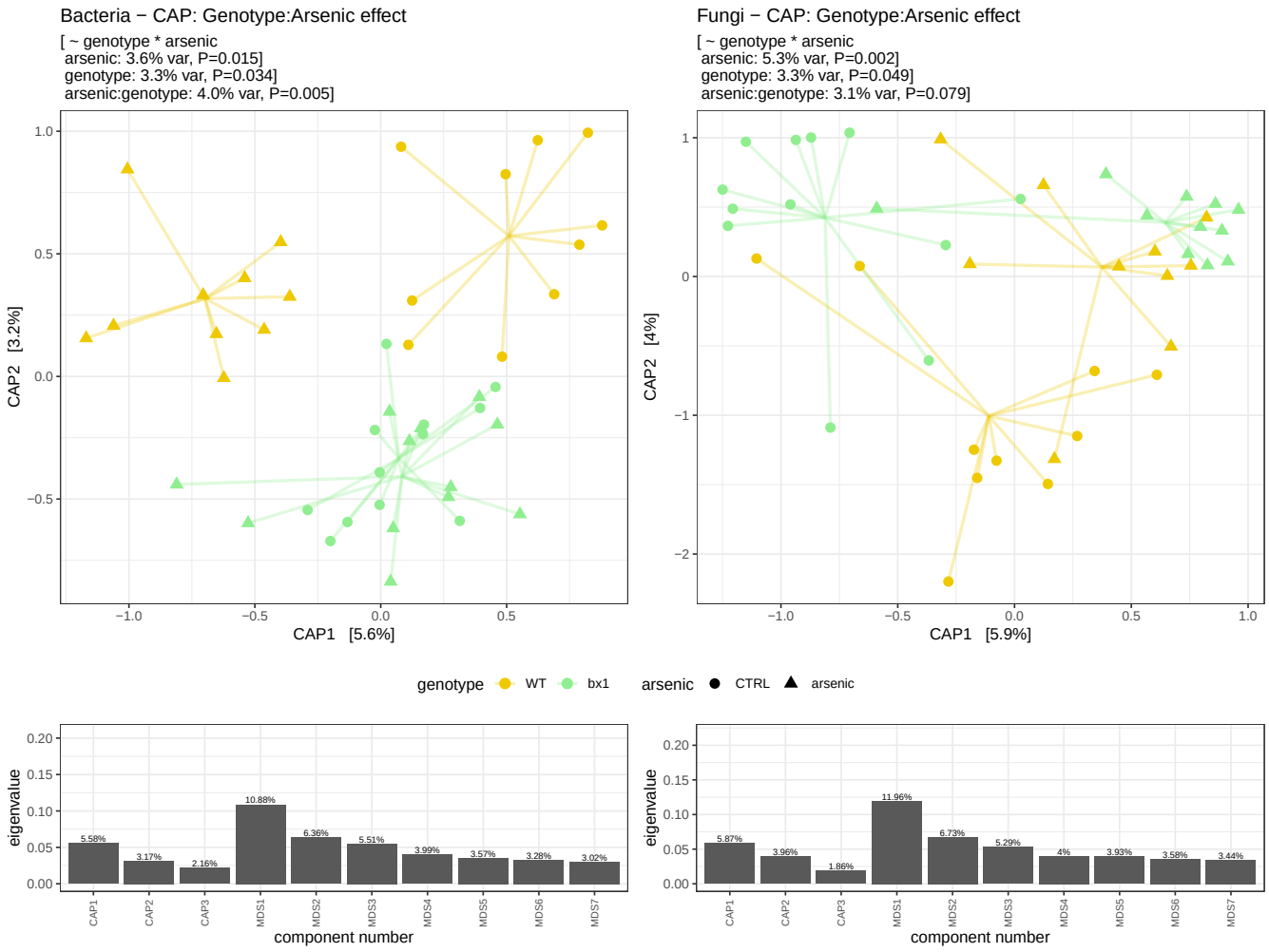


Figure S16.2 | CAP - genotype:arsenic effect on beta diversity



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.