

Arsenic Plant - Microbiome-Analysis

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Description all data

To decide on how to normalize the data we followed the recommendation of Weiss et al. (2017, Microbiome Journal) and we inspected whether there are differences in sequencing depths between the different sample groups utilizing the non-parametric Kruskal-Wallis Test.

Sequencing depth

We show the sum, range and median over all samples we include in the analysis.

Bacteria

```
## [1] "samples removed (low sequencing depth)"
```

Table 1: Bacteria: seq depth of removed samples

	Sequences
F70_R52	4619
F74_R43	4493
F71_R52	4484
F62_R55	4250
F73_R55	3794
F60_R52	3726
F73_R52	3708
F60_R53	3564
F66_R52	3430
F73_R45	3254
F62_R54	3108
F62_R53	2736
F74_R42	2353
F75_R45	2290
F74_R41	2261
F74_R45	2148
F62_R52	2123
F61_R52	2057
F75_R42	1644
F75_R41	1630
F73_R41	1585
F66_R55	1493
F66_R54	1479
F73_R42	1421
F73_R54	1143
F66_R53	733
F74_R40	447
F64_R52	227
F73_R40	176
F75_R40	92

```
## [1] "sum: 1099987"
```

```
## [1] "range: 5304" "range: 26983"
```

```
## [1] "median: 16086.5"
```

Fungi

```
## [1] "samples removed (low sequencing depth)"
```

Table 2: Fungi: seq depth of removed samples

	Sequences
F9_R6	177
F8_R6	66
F7_R6	29
F6_R6	26

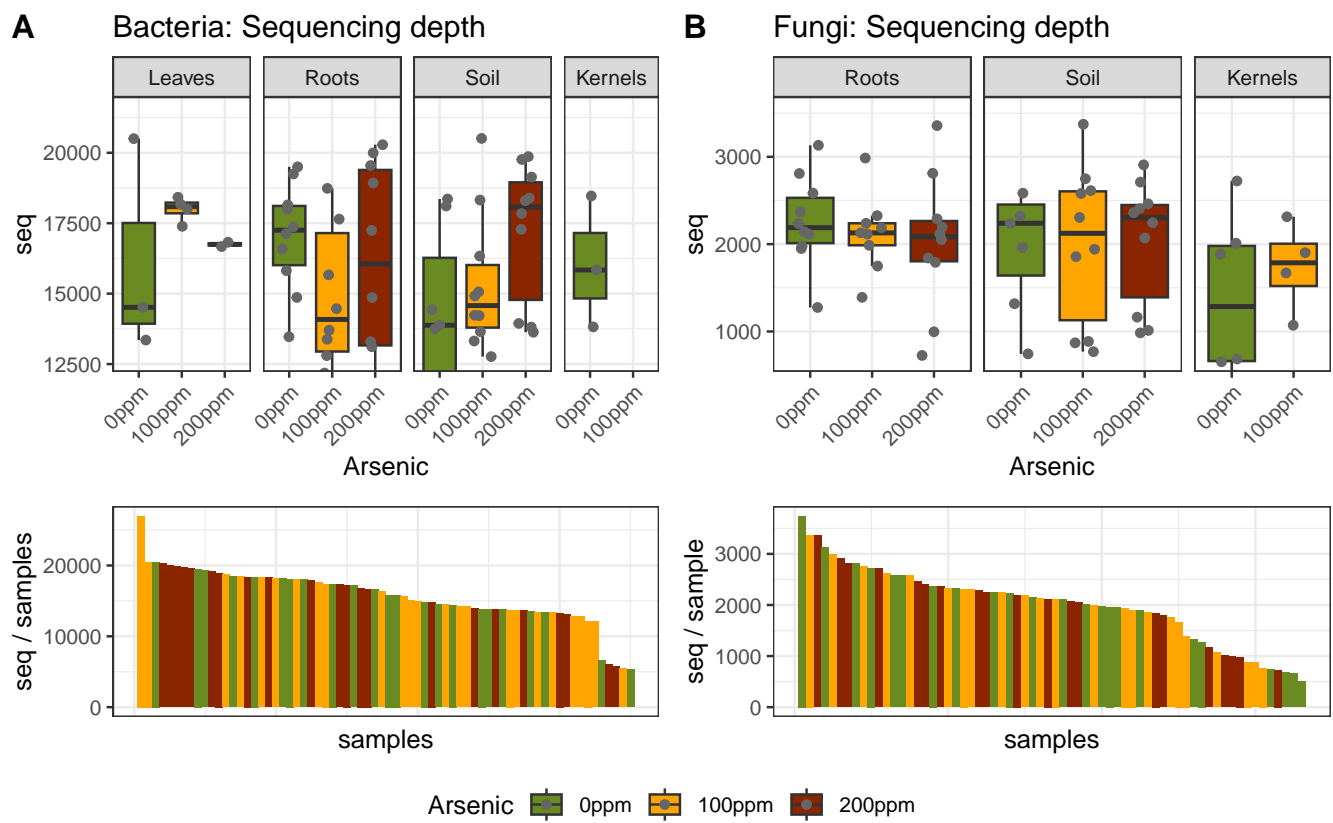
```
## [1] "sum: 132467"
```

```
## [1] "range: 500" "range: 3742"
```

```
## [1] "median: 2116.5"
```

We excluded all bacterial samples with less than 5000 sequences and fungal samples with 500 sequences. respectively.

Figure 1 | Sequencing depth



Data normalization

Asymptotic Kruskal-Wallis Test

We test for different sequencing depths between the groups.

Bacteria

```
##
## Asymptotic Kruskal-Wallis Test
##
## data: colSums(bDAT) by
## bDESIGN$group (Oppm_Leaves, Oppm_Roots, Oppm_Soil, Oppm_Kernels, 100ppm_Leaves, 100ppm_Roots, 100ppm_Soil, 100ppm_Kernels, 200ppm_Leaves, 200ppm_Roots, 200ppm_Soil, 200ppm_Kernels)
## chi-squared = 11.099, df = 10, p-value = 0.3499
```

Fungi

```
##
## Asymptotic Kruskal-Wallis Test
##
## data: colSums(fDAT) by
## fDESIGN$group (Oppm_Roots, Oppm_Soil, Oppm_Kernels, 100ppm_Roots, 100ppm_Soil, 100ppm_Kernels, 200ppm_Roots, 200ppm_Soil, 200ppm_Kernels)
## chi-squared = 6.0333, df = 7, p-value = 0.5359
```

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. We set the number of nearest neighbors to 60% of the samples size and chose an empirical outlier percentile of 5%. We remove all outliers from our data.

Bacteria

```
## [1] "samples removed (outliers)"
## [1] "F75_R43" "F75_R44"
```

Fungi

```
## [1] "samples removed (outliers)"
## [1] "F7_R1" "F9_R12"
```

Sample Control

Sample Size

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

Table 3: Bacteria: Sample profiles

	0ppm	100ppm	200ppm
Leaves	3	4	1
Roots	10	10	10
Soil	7	10	10
Kernels	2	1	0

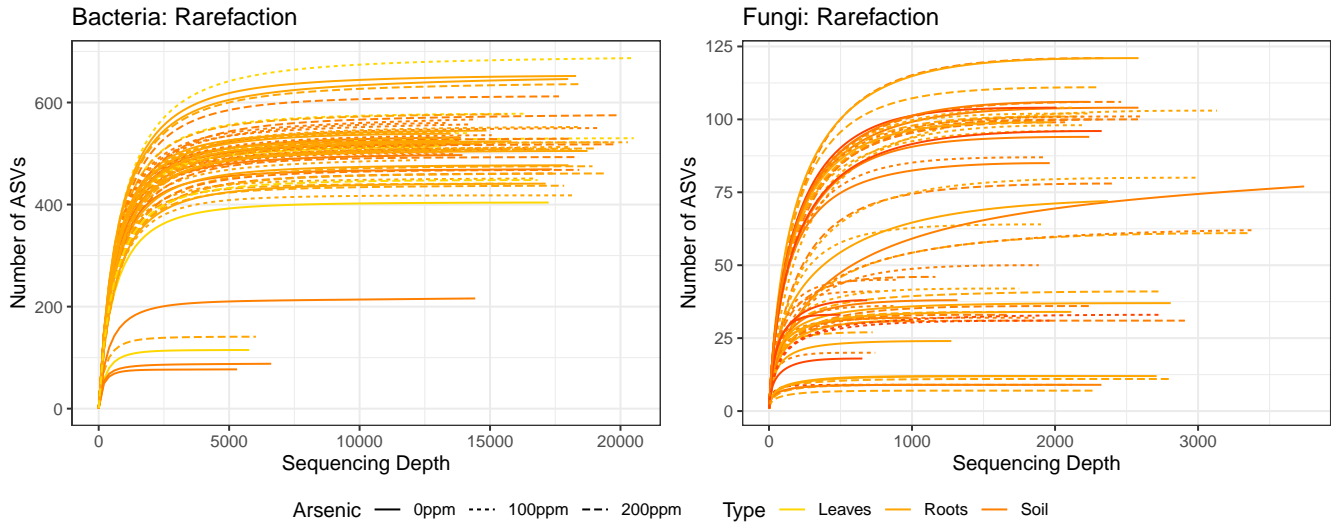
Table 4: Fungi: Sample profiles

	0ppm	100ppm	200ppm
Roots	10	9	10
Soil	7	10	10
Kernels	5	3	0

Conclusion: Because there are not enough replicates for bacterial kernel samples, we exclude them for all further analyses.

Figure 2 | Rarefaction plot

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

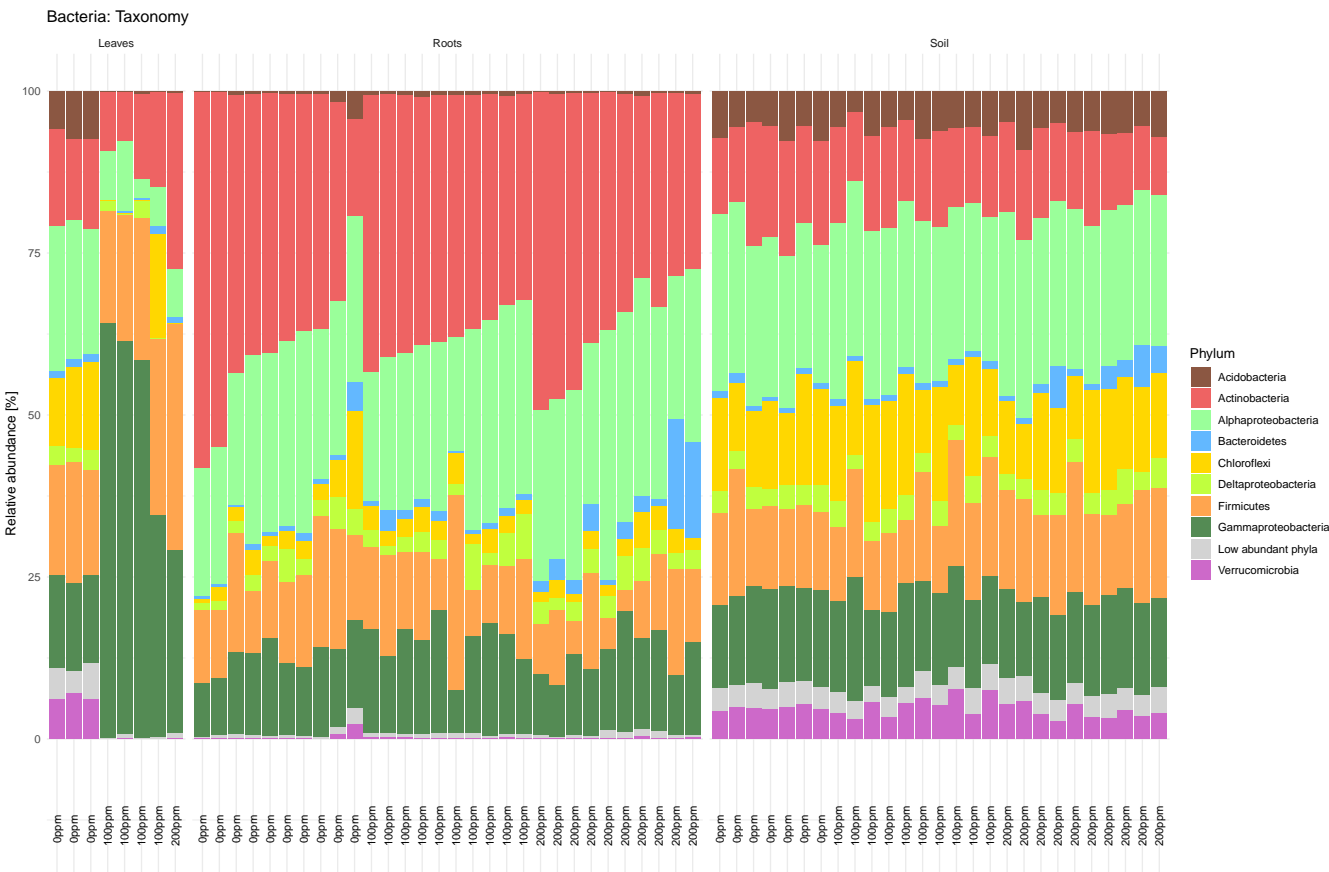


Conclusion: The remaining samples are sequenced deep enough to cover the majority of microbial diversity.

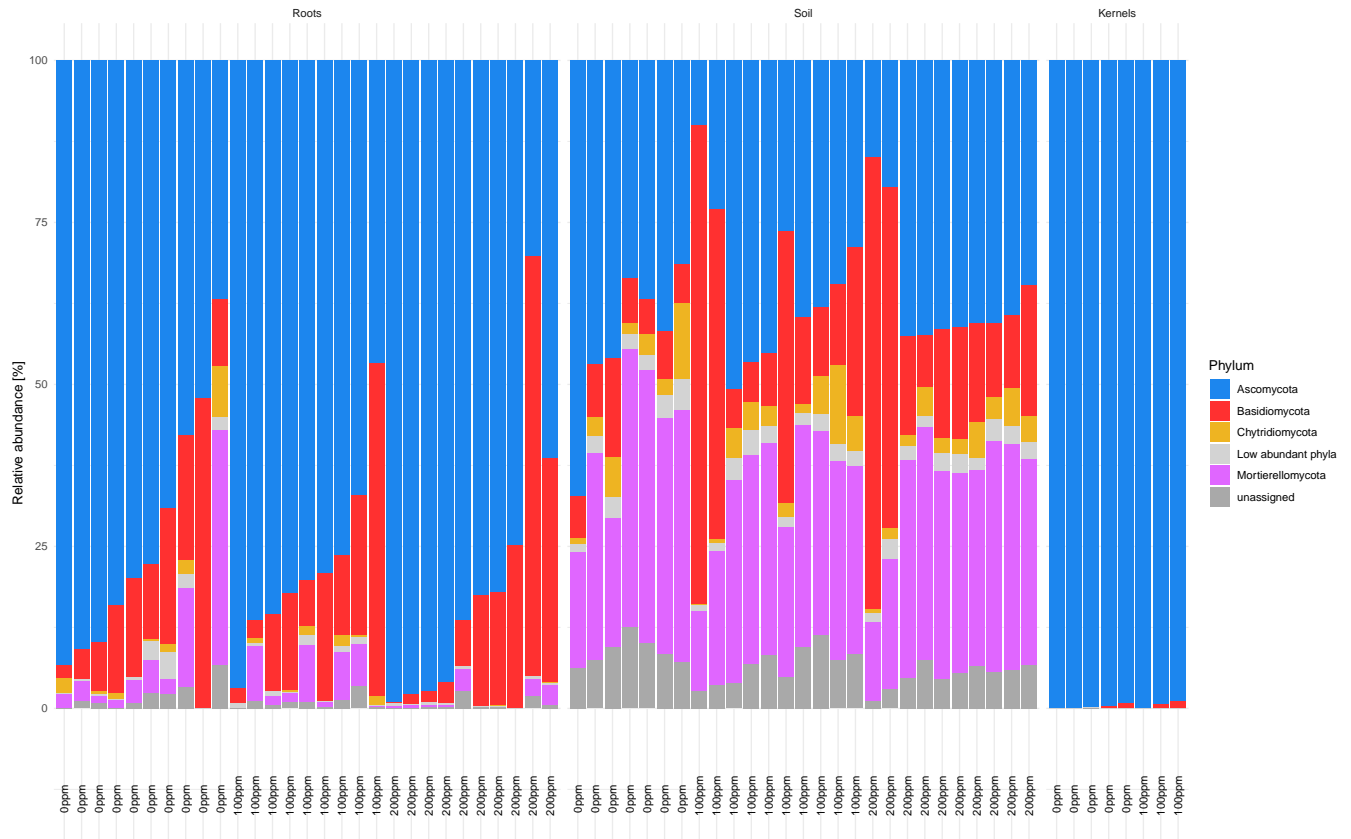
Taxonomy

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

Figure 3.1 | phylum level taxonomy

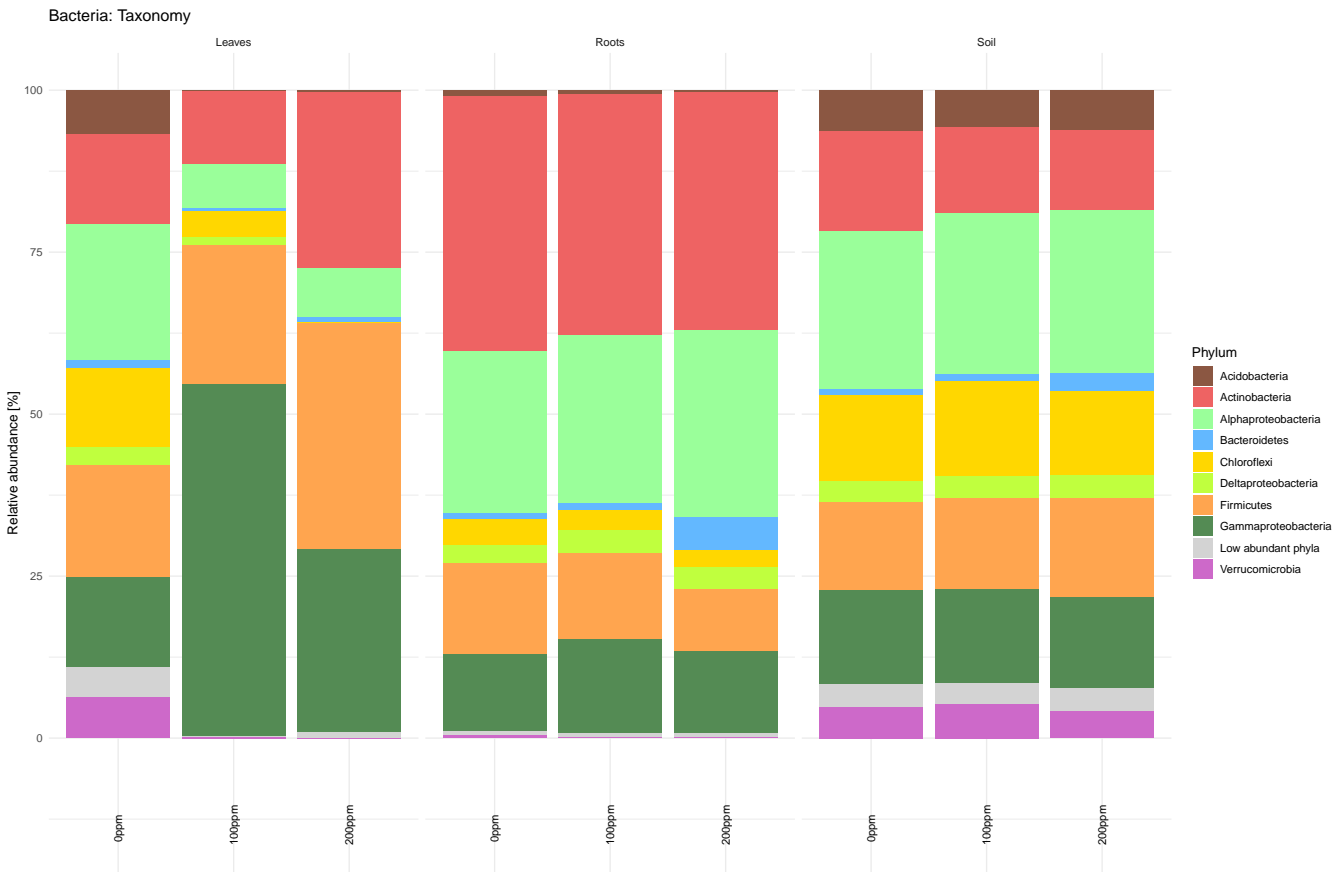


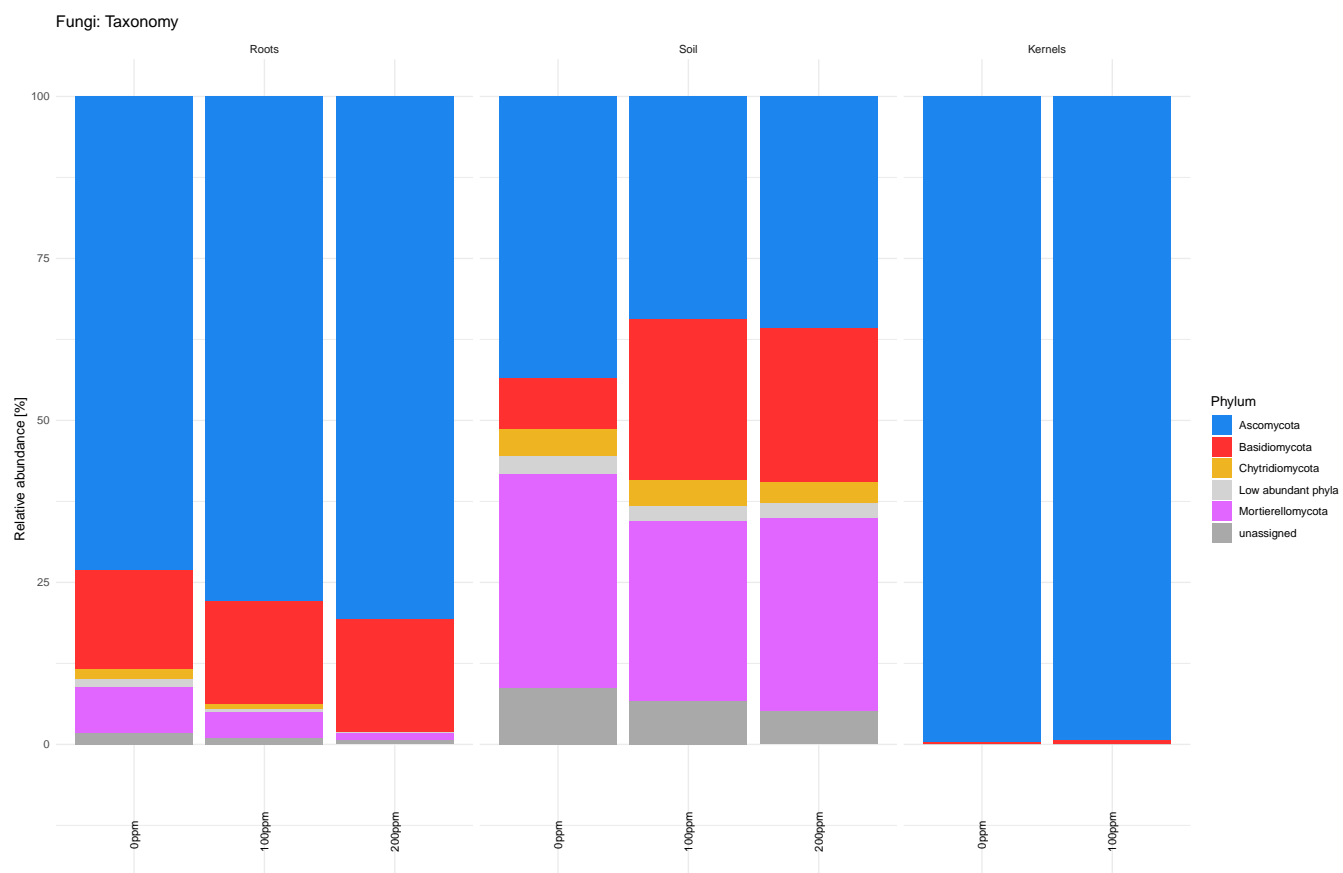
Fungi: Taxonomy



We repeat the plot by plotting the abundances means for each arsenic concentration.

Figure 3.2 | mean abundances on phylum level





Alpha diversity

Shannon diversity

Method: We calculated Shannon diversity for each sample.

Bacteria - ANOVA

Table 5: Bacteria: Arsenic effect

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Arsenic	2	2.728	1.364	4.126	0.0213
Type	2	0.6156	0.3078	0.9313	0.4001
Arsenic:Type	4	4.648	1.162	3.516	0.01249
Residuals	56	18.51	0.3305	NA	NA

Fungi - ANOVA

Table 6: Fungi: Arsenic effect

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Arsenic	2	0.04729	0.02365	0.02883	0.9716
Type	2	2.477	1.238	1.51	0.2298
Arsenic:Type	3	5.105	1.702	2.075	0.1139
Residuals	56	45.93	0.8202	NA	NA

Because we found significant differences in bacterial alpha diversity between the different arsenic treatments and within the arsenic:type interaction, we check how arsenic shifts the alpha diversity in the different plant compartments (type).

Bacteria - Tukey post-hoc

Table 7: Bacteria: Arsenic effect

	Shannon	.group
0ppm_Leaves	4.75	ab
100ppm_Leaves	5.861	b
200ppm_Leaves	5.451	ab
0ppm_Roots	5.751	b
100ppm_Roots	5.66	b
200ppm_Roots	5.424	ab
0ppm_Soil	4.765	a
100ppm_Soil	5.81	b
200ppm_Soil	5.619	b

Conclusion: There is no constant evidence that arsenic alters the alpha diversity in plant compartments. In soil we find statistical support that samples with no arsenic have lower alpha diversity. But considering those samples, we find a few samples with a much lower diversity than the rest of the samples.

Fungi - Tukey post-hoc

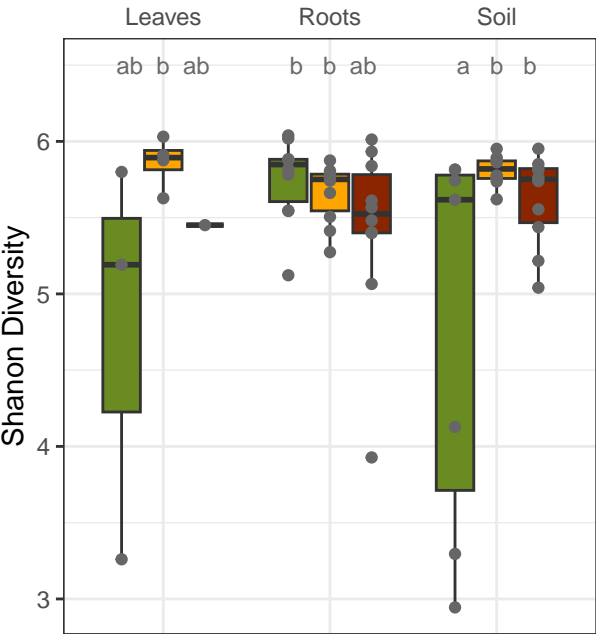
Table 8: Fungi: Arsenic effect

	Shannon	.group
0ppm_Roots	2.685	a
100ppm_Roots	3.204	a
200ppm_Roots	2.489	a
0ppm_Soil	3.137	a
100ppm_Soil	3.03	a
200ppm_Soil	3.318	a
0ppm_Kernels	3.174	a
100ppm_Kernels	2.072	a

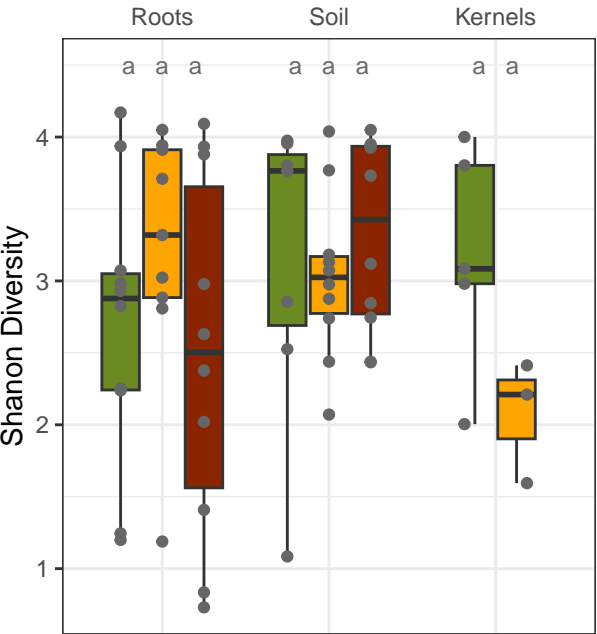
Conclusion: We find no statistical support that Arsenic alters fungal alpha diversity in the different compartments.

Figure 4 | shannon diversity

A Bacteria: Alpha diversity
alpha diversity ~ Arsenic



B Fungi: Alpha diversity
alpha diversity ~ Arsenic



Arsenic 0ppm 100ppm 200ppm

Beta diversity

PERMANOVA

We use the function ‘adonis2()’ (package vegan) to analyse the beta diversity with a PERMANOVA (permutations = 999). First we investigate the full model to see which factors alters the beta diversity.

Bacteria

Table 9: Bacteria: all

	Df	SumOfSqs	R2	F	Pr(>F)
Arsenic	2	0.9543	0.06402	2.161	0.026
Type	2	0.3842	0.02577	0.8701	0.528
Arsenic:Type	4	1.205	0.0808	1.364	0.129
Residual	56	12.36	0.8294	NA	NA
Total	64	14.91	1	NA	NA

Conclusion: There is an arsenic effect on the bacterial composition.

We test if the bacterial compositions differ between 0ppm and 200ppm arsenic concentration in different compartments.

Table 10: Arsenic effect (100ppm) in different compartments

Type	Factors	SumOfSqs	R2	F	p	p_adj
Leaves	Arsenic	0.2748	0.1363	0.7892	0.455	0.455
Leaves	Residual	1.741	0.8637	NA	NA	NA
Leaves	Total	2.016	1	NA	NA	NA
Roots	Arsenic	0.1285	0.06186	1.187	0.23	0.455
Roots	Residual	1.949	0.9381	NA	NA	NA
Roots	Total	2.078	1	NA	NA	NA
Soil	Arsenic	0.07076	0.06397	1.025	0.382	0.455
Soil	Residual	1.035	0.936	NA	NA	NA
Soil	Total	1.106	1	NA	NA	NA

Table 11: Arsenic effect (200ppm) in different compartments

Type	Factors	SumOfSqs	R2	F	p	p_adj
Leaves	Arsenic	0.1357	0.171	0.4127	0.5	0.75
Leaves	Residual	0.6575	0.829	NA	NA	NA
Leaves	Total	0.7931	1	NA	NA	NA
Roots	Arsenic	0.07874	0.03102	0.5763	0.985	0.985
Roots	Residual	2.459	0.969	NA	NA	NA
Roots	Total	2.538	1	NA	NA	NA
Soil	Arsenic	0.07818	0.06806	1.096	0.228	0.684
Soil	Residual	1.07	0.9319	NA	NA	NA
Soil	Total	1.149	1	NA	NA	NA

Conclusion: The bacterial beta diversity is not altered by treating the soil with Arsenic.

Figure 5 | Bacteria: PCoA ordination

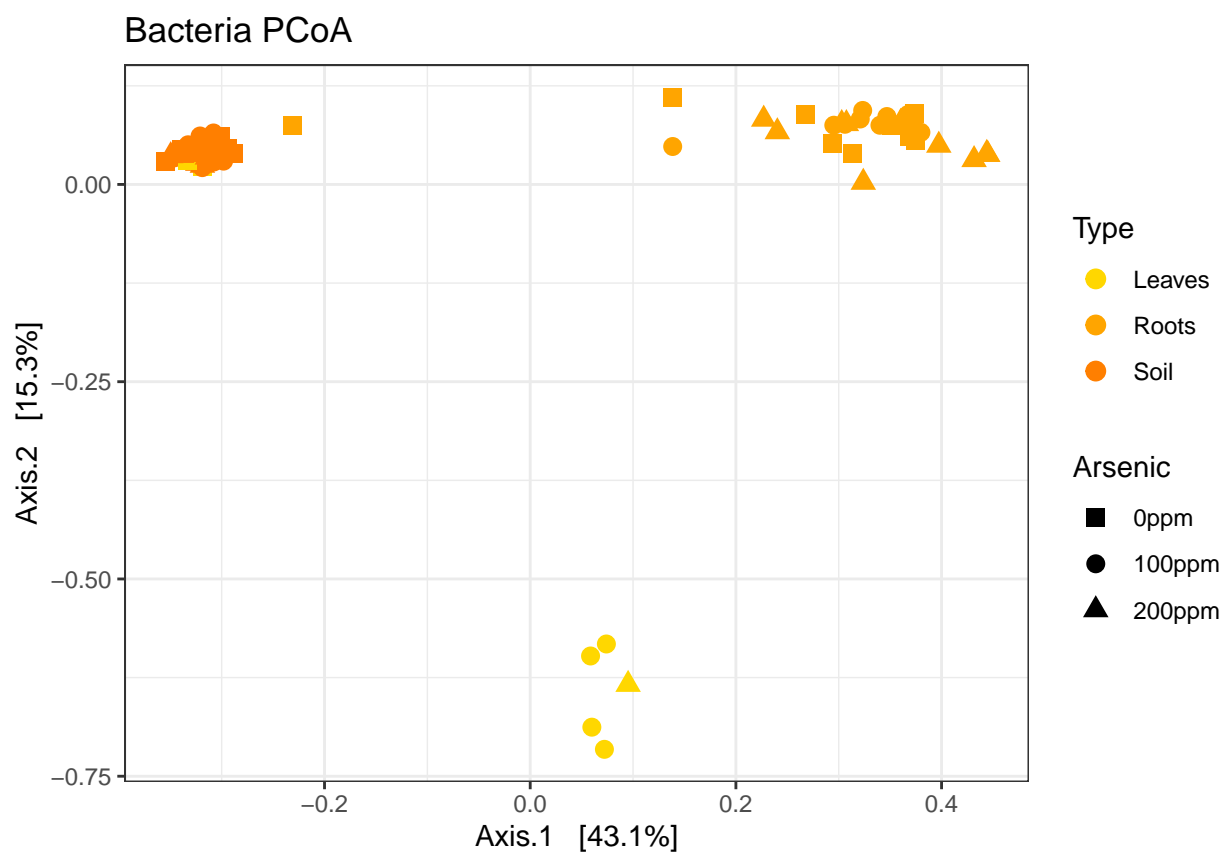
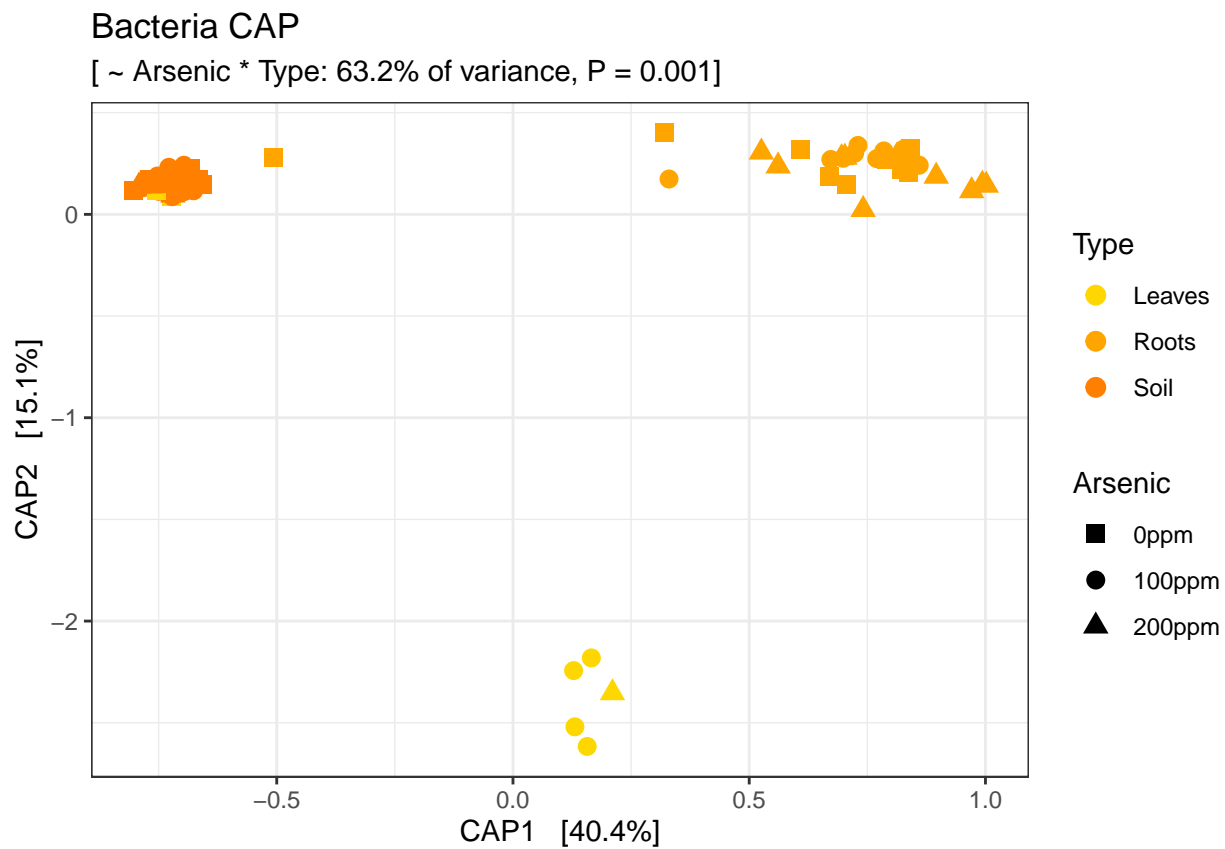


Figure 6 | Bacteria: CAP ordination



Fungi

Table 12: Fungi: all

	Df	SumOfSqs	R2	F	Pr(>F)
Arsenic	2	0.5097	0.02091	0.6618	0.94
Type	2	0.6605	0.0271	0.8576	0.691
Arsenic:Type	3	1.641	0.06732	1.42	0.054
Residual	56	21.57	0.8847	NA	NA
Total	63	24.38	1	NA	NA

Conclusion: No effect was found on the beta diversity.

We test if the arsenic treatments alter the bacterial compositions in different compartments.

Table 13: Arsenic effect (100ppm) in different compartments

Type	Factors	SumOfSqs	R2	F	p	p_adj
Roots	Arsenic	0.2326	0.03812	0.6738	0.866	0.866
Roots	Residual	5.868	0.9619	NA	NA	NA
Roots	Total	6.101	1	NA	NA	NA
Soil	Arsenic	0.326	0.09676	1.607	0.094	0.282
Soil	Residual	3.043	0.9032	NA	NA	NA
Soil	Total	3.369	1	NA	NA	NA
Kernels	Arsenic	0.1258	0.07484	0.4854	0.686	0.866
Kernels	Residual	1.555	0.9252	NA	NA	NA
Kernels	Total	1.681	1	NA	NA	NA

Conclusion: The fungal beta diversity is not altered by treating the soil with 100ppm Arsenic.

We test if the fungal compositions differ between 0ppm and 200ppm arsenic concentration.

Table 14: Arsenic effect (200ppm) in different compartments

Type	Factors	SumOfSqs	R2	F	p	p_adj
Roots	Arsenic	0.1262	0.01974	0.3624	1	1
Roots	Residual	6.269	0.9803	NA	NA	NA
Roots	Total	6.396	1	NA	NA	NA
Soil	Arsenic	0.281	0.0864	1.418	0.138	0.276
Soil	Residual	2.971	0.9136	NA	NA	NA
Soil	Total	3.252	1	NA	NA	NA

Conclusion: The fungal beta diversity is not altered by treating the soil with Arsenic.

Figure 7 | Fungi: PCoA ordination

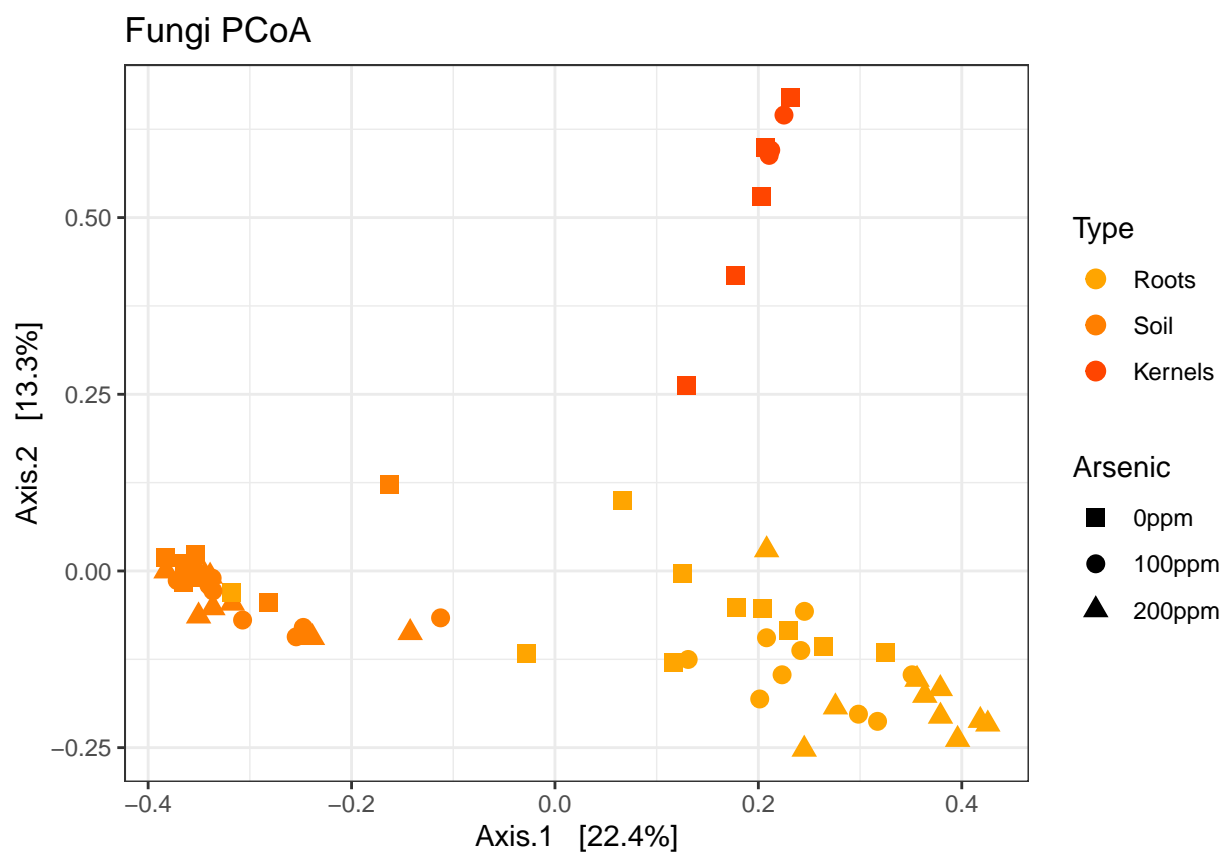


Figure 8 | Fungi: CAP ordination

