

Arsenic Soil - 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] "No samples with low sequencing depth removed"
## [1] "samples removed (not sequenced)"
## [1] "FLD0112" "FLD0373"
## [1] "sum: 3913520"
## [1] "range: 26812" "range: 88499"
## [1] "median: 43546"
```

Fungi

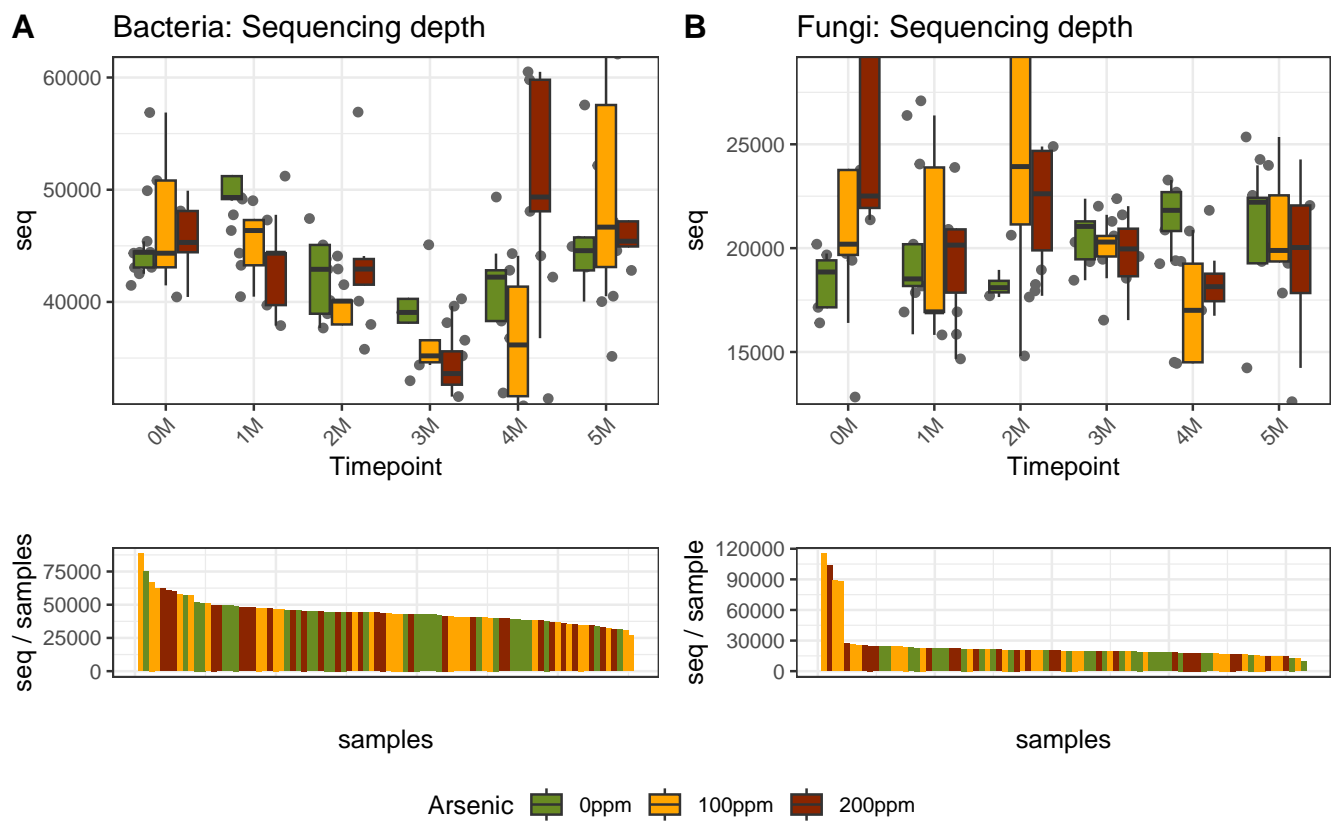
```
## [1] "samples removed (low sequencing depth)"
```

FLD0310	FLD0324	FLD0299	FLD0269
8978	4246	4188	1255

```
## [1] "samples removed (not sequenced)"
## [1] "FLD0268" "FLD0196" "FLD0284"
## [1] "sum: 1951222"
## [1] "range: 9346" "range: 115292"
## [1] "median: 19889"
```

We define the rarefaction threshold per sample to 2.5×10^4 for bacteria and 9000 for fungi .

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_0M, Oppm_1M, Oppm_2M, Oppm_3M, Oppm_4M, Oppm_5M, 100ppm_0M, 100ppm_1M, 100ppm_2M,  
## chi-squared = 37.963, df = 17, p-value = 0.002486
```

Fungi

```
##  
## Asymptotic Kruskal-Wallis Test  
##  
## data: colSums(fDAT) by  
## fDESIGN$group (Oppm_0M, Oppm_1M, Oppm_2M, Oppm_3M, Oppm_4M, Oppm_5M, 100ppm_0M, 100ppm_1M, 100ppm_2M,  
## chi-squared = 20.278, df = 17, p-value = 0.2603
```

Conclusion: Because we find significant differences in sequencing depths between the sample groups for bacteria, we will normalize the data by rarefaction for diversity comparisons (see Weiss et al. (2017), Microbiome Journal). For consistency we rarefy for bacteria and fungi.

Outlier Detection

We use the method CLOUD developped by Montassier et al. 2018, which is a non.parametric detection test for outliers. We perform the test with Bray-Curtis distances from the rarefied 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] "FLD0100" "FLD0118" "FLD0120" "FLD0132"
```

Fungi

```
## [1] "samples removed (outliers)"  
## [1] "FLD0254" "FLD0292" "FLD0297" "FLD0298"
```

Sample Size

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

Table 2: Bacteria: Sample profiles

	0ppm	100ppm	200ppm
0M	5	5	5
1M	5	5	5
2M	5	5	5
3M	5	4	4
4M	5	4	3
5M	5	5	4

Table 3: Fungi: Sample profiles

	0ppm	100ppm	200ppm
0M	4	5	3
1M	5	5	5
2M	4	5	4
3M	5	4	2
4M	5	5	3
5M	5	5	5

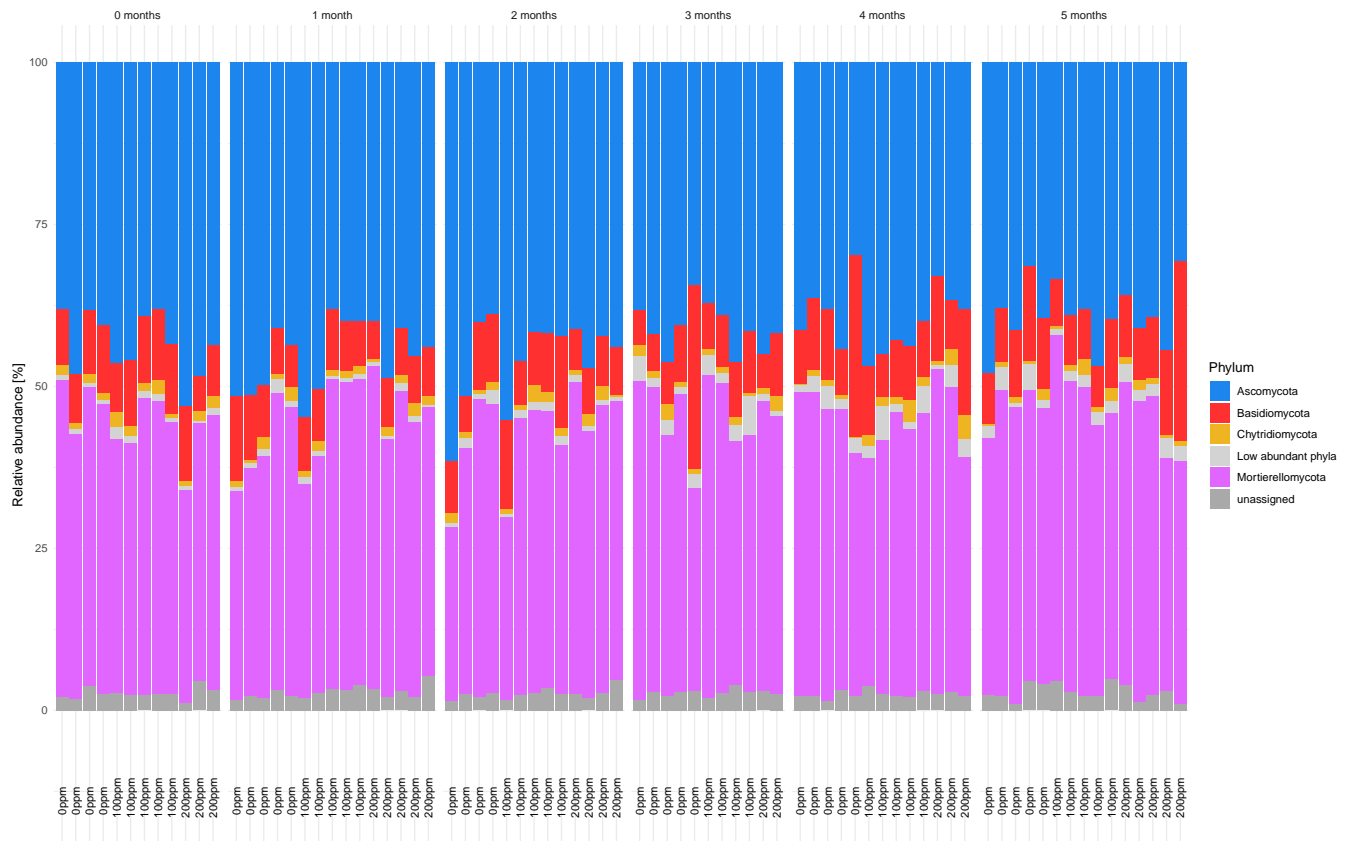
Taxonomy

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

Figure 2.1 | abundances on phylum level

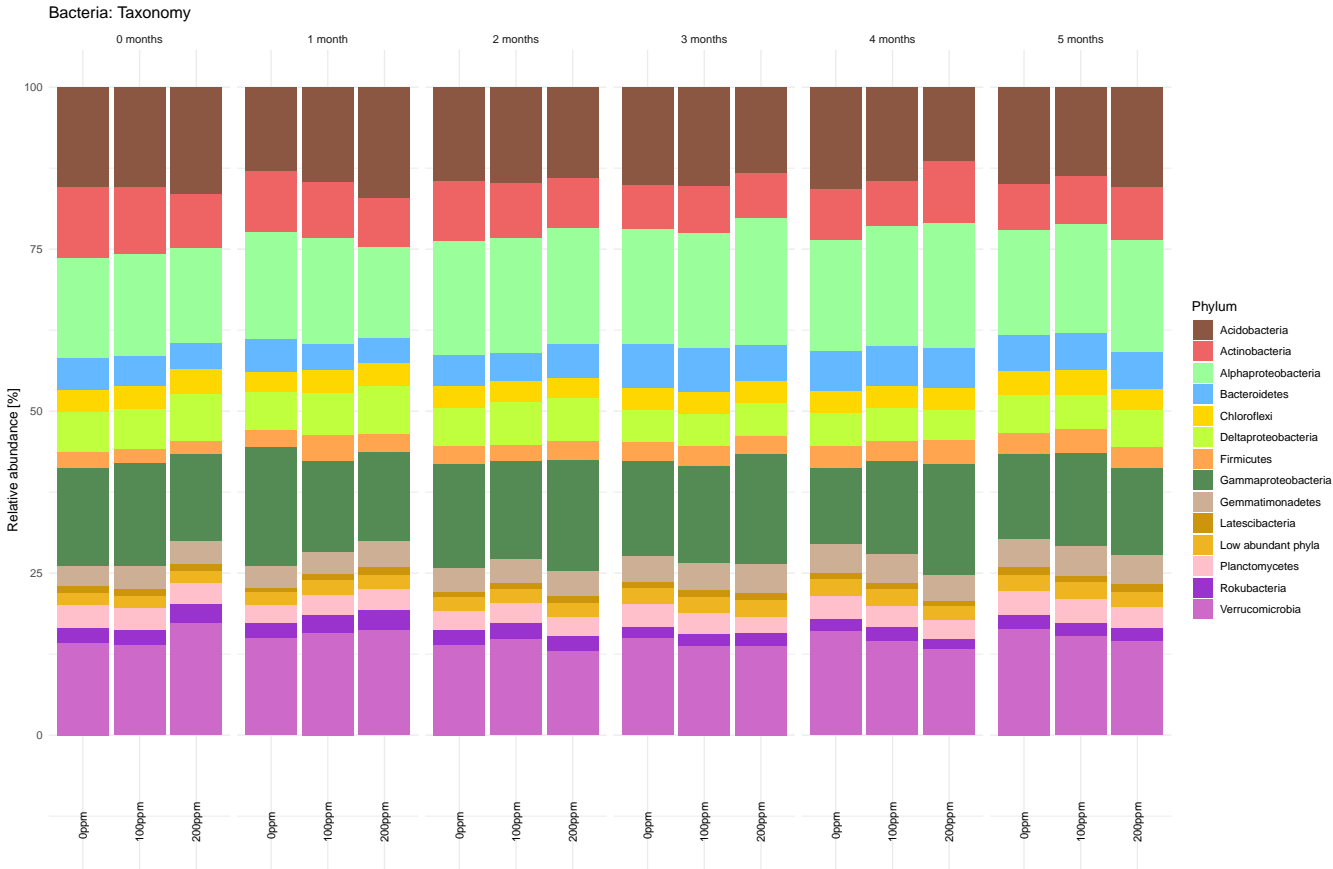


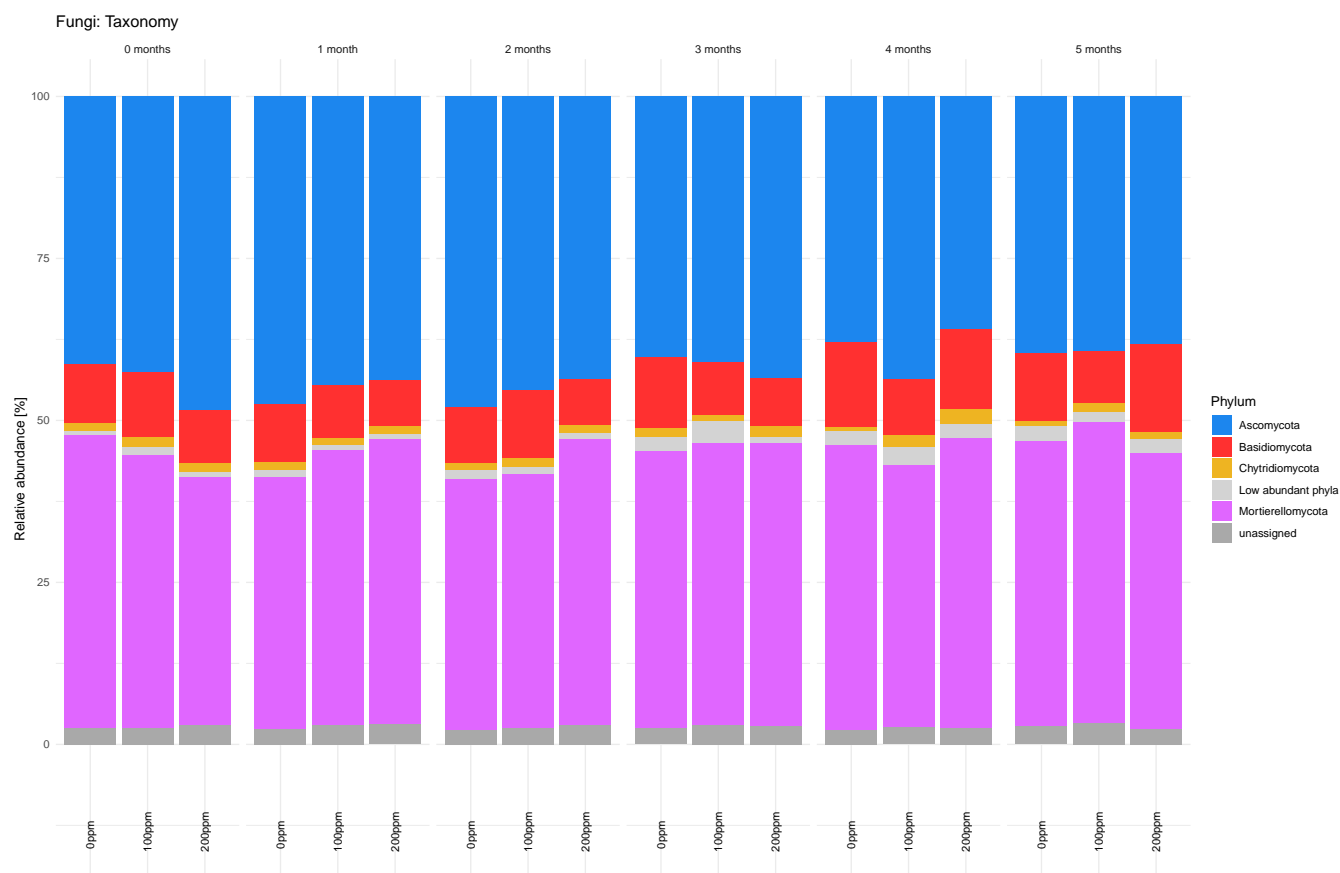
Fungi: Taxonomy



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

Figure 2.2 | mean abundances on phylum level

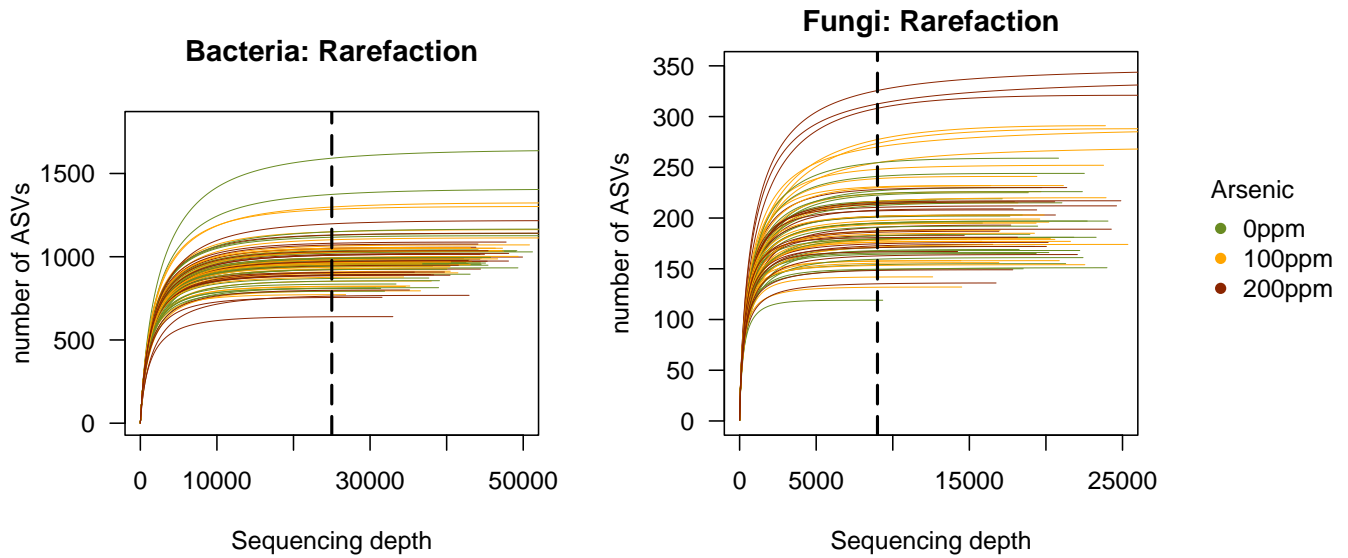




Alpha diversity

Figure 3 | rarefaction

Before analysing the alpha diversity, we conduct the rarefaction plot to make sure that we don't lose diversity due to a too low rarefaction threshold.



Conclusion: We chose rarefaction thresholds that do not affect the alpha diversity.

Shannon diversity

Method: we rarefy the dataset to the sequencing depth of 2.5×10^4 for bacteria and 9000 for fungi. Then we calculated Shannon diversity in each sample. This was repeated 100 times and the mean value from the 100 iterations was taken for statistical analysis between the different samples.

Bacteria

We check how the Arsenic shifts the alpha diversity over 5 months.

Table 4: Bacteria: Arsenic effect

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Arsenic	2	7259	3630	0.6323	0.5345
Timepoint	5	57248	11450	1.995	0.09088
Arsenic:Timepoint	10	52326	5233	0.9116	0.528
Residuals	66	378842	5740	NA	NA

Conclusion: We find no statistical support that Arsenic alters bacterial alpha diversity.

Fungi

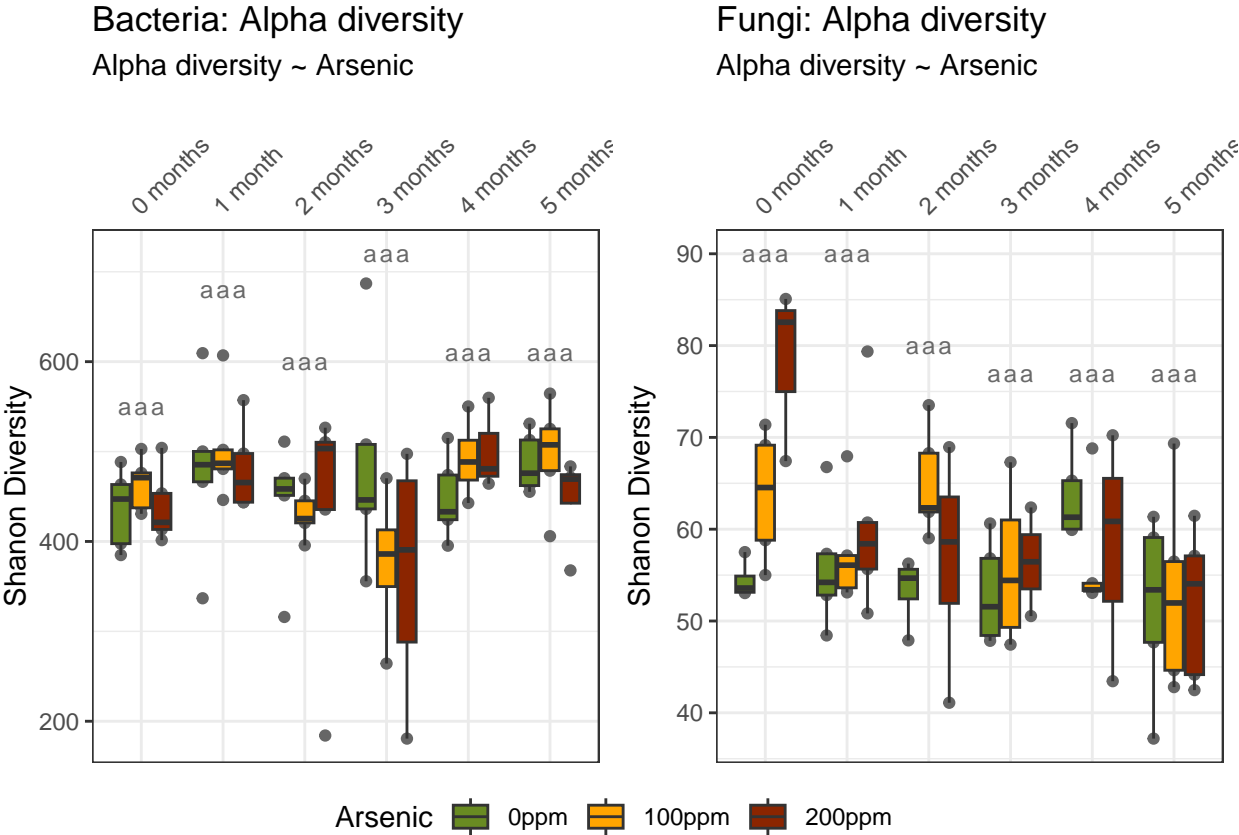
We check how the Arsenic shifts the alpha diversity over 5 months.

Table 5: Fungi: Arsenic effect

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Arsenic	2	260.1	130.1	1.989	0.1457
Timepoint	5	1136	227.1	3.473	0.007939
Arsenic:Timepoint	10	1288	128.8	1.969	0.05269
Residuals	61	3990	65.4	NA	NA

Conclusion: We find no statistical support that Arsenic alters fungal alpha diversity.

Figure 4 | shannon diversity



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 6: Bacteria: all

	Df	SumOfSqs	R2	F	Pr(>F)
Arsenic	2	0.1044	0.01752	0.8303	0.791
Timepoint	5	0.8838	0.1484	2.812	0.001
Arsenic:Timepoint	10	0.8198	0.1376	1.304	0.006
Residual	66	4.149	0.6965	NA	NA
Total	83	5.957	1	NA	NA

Conclusion: We find differences in the beta diversity over time. There is trend for an interaction effect of arsenic and time. Therefore we investigate in a next step the arsenic effect for each timepoint.

We test if the bacterial compositions differ between 0ppm and 100ppm arsenic concentration over 5 months.

Table 7: Arsenic effect (100ppm) over 5 months

Timepoint	Factors	SumOfSqs	R2	F	p	p_adj
0 months	Arsenic	0.05507	0.1216	1.108	0.215	0.9108
0 months	Residual	0.3976	0.8784	NA	NA	NA
0 months	Total	0.4527	1	NA	NA	NA
1 month	Arsenic	0.04597	0.07656	0.6633	1	1
1 month	Residual	0.5544	0.9234	NA	NA	NA
1 month	Total	0.6004	1	NA	NA	NA
2 months	Arsenic	0.04382	0.1055	0.9434	0.54	0.9108
2 months	Residual	0.3716	0.8945	NA	NA	NA
2 months	Total	0.4154	1	NA	NA	NA
3 months	Arsenic	0.05751	0.119	0.9457	0.551	0.9108
3 months	Residual	0.4257	0.881	NA	NA	NA
3 months	Total	0.4832	1	NA	NA	NA
4 months	Arsenic	0.05004	0.1151	0.9101	0.759	0.9108
4 months	Residual	0.3849	0.8849	NA	NA	NA
4 months	Total	0.435	1	NA	NA	NA
5 months	Arsenic	0.04546	0.09813	0.8705	0.723	0.9108
5 months	Residual	0.4178	0.9019	NA	NA	NA
5 months	Total	0.4632	1	NA	NA	NA

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

We test if the bacterial compositions differ between 0ppm and 200ppm arsenic concentration over 5 months.

Table 8: Arsenic effect (200ppm) over 5 months

Timepoint	Factors	SumOfSqs	R2	F	p	p_adj
0 months	Arsenic	0.05742	0.1184	1.074	0.281	0.562
0 months	Residual	0.4276	0.8816	NA	NA	NA
0 months	Total	0.485	1	NA	NA	NA
1 month	Arsenic	0.113	0.2013	2.017	0.009	0.054
1 month	Residual	0.4482	0.7987	NA	NA	NA
1 month	Total	0.5612	1	NA	NA	NA
2 months	Arsenic	0.05558	0.09033	0.7944	0.843	0.843
2 months	Residual	0.5597	0.9097	NA	NA	NA
2 months	Total	0.6153	1	NA	NA	NA
3 months	Arsenic	0.09847	0.1785	1.521	0.025	0.075
3 months	Residual	0.4532	0.8215	NA	NA	NA
3 months	Total	0.5517	1	NA	NA	NA
4 months	Arsenic	0.06558	0.1315	0.9083	0.531	0.6372
4 months	Residual	0.4332	0.8685	NA	NA	NA
4 months	Total	0.4987	1	NA	NA	NA
5 months	Arsenic	0.05069	0.1244	0.9943	0.459	0.6372
5 months	Residual	0.3568	0.8756	NA	NA	NA
5 months	Total	0.4075	1	NA	NA	NA

Conclusion: The bacterial beta diversity is not affected by the arsenic concentration of 200ppm.

Figure 5 | Bacteria: PCoA ordination

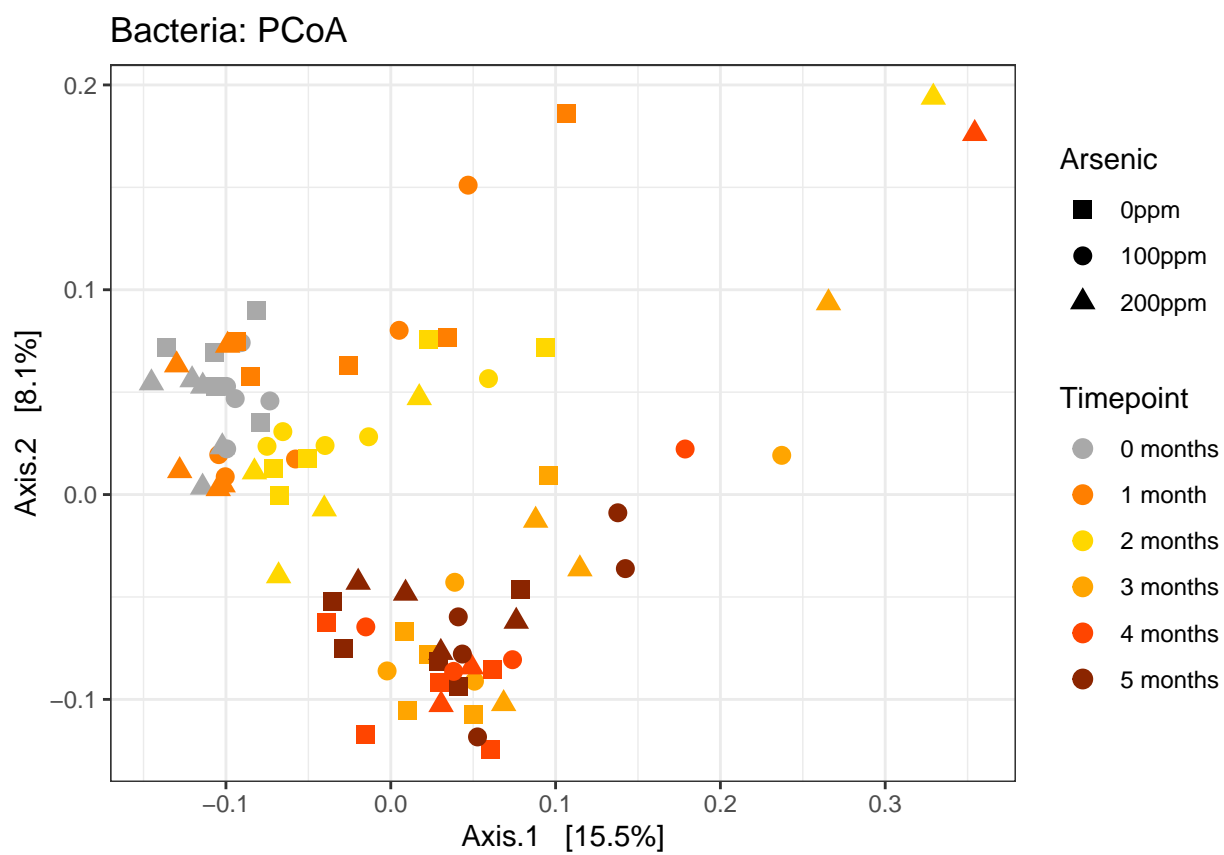
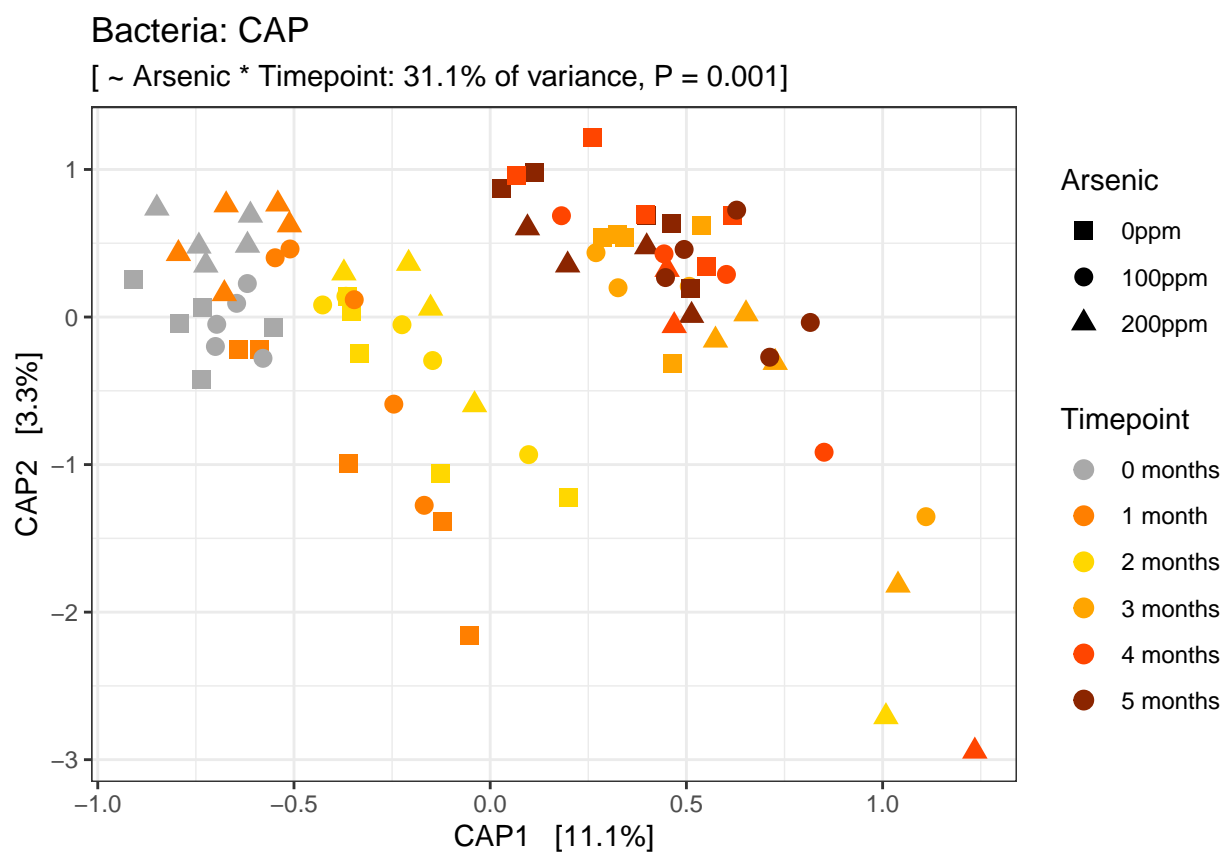


Figure 6 | Bacteria: CAP ordination



Fungi

Table 9: Fungi: all

	Df	SumOfSqs	R2	F	Pr(>F)
Arsenic	2	0.2432	0.02219	0.9151	0.666
Timepoint	5	0.8665	0.07907	1.304	0.012
Arsenic:Timepoint	10	1.744	0.1591	1.312	0.006
Residual	61	8.106	0.7396	NA	NA
Total	78	10.96	1	NA	NA

Conclusion: We find differences in the beta diversity over time. There is a trend for an interaction effect of arsenic and time. Therefore we investigate in a next step the arsenic effect for each timepoint.

We test if the fungal compositions differ between 0ppm and 100ppm arsenic concentration over 5 months.

Table 10: Arsenic effect (100ppm) over 5 months

Timepoint	Factors	SumOfSqs	R2	F	p	p_adj
0 months	Arsenic	0.1052	0.1323	1.068	0.35	0.573
0 months	Residual	0.6896	0.8677	NA	NA	NA
0 months	Total	0.7948	1	NA	NA	NA
1 month	Arsenic	0.1097	0.121	1.101	0.211	0.573
1 month	Residual	0.7972	0.879	NA	NA	NA
1 month	Total	0.9069	1	NA	NA	NA
2 months	Arsenic	0.1141	0.1245	0.9951	0.382	0.573
2 months	Residual	0.8027	0.8755	NA	NA	NA
2 months	Total	0.9168	1	NA	NA	NA
3 months	Arsenic	0.1112	0.1107	0.8709	0.737	0.8844
3 months	Residual	0.8935	0.8893	NA	NA	NA
3 months	Total	1.005	1	NA	NA	NA
4 months	Arsenic	0.1133	0.09329	0.8231	0.965	0.965
4 months	Residual	1.102	0.9067	NA	NA	NA
4 months	Total	1.215	1	NA	NA	NA
5 months	Arsenic	0.1499	0.135	1.249	0.041	0.246
5 months	Residual	0.9602	0.865	NA	NA	NA
5 months	Total	1.11	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 over 5 months.

Table 11: Arsenic effect (200ppm) over 5 months

Timepoint	Factors	SumOfSqs	R2	F	p	p_adj
0 months	Arsenic	0.0885	0.1373	0.7957	0.913	0.913
0 months	Residual	0.5561	0.8627	NA	NA	NA
0 months	Total	0.6446	1	NA	NA	NA
1 month	Arsenic	0.1286	0.1319	1.215	0.049	0.147
1 month	Residual	0.8461	0.8681	NA	NA	NA
1 month	Total	0.9747	1	NA	NA	NA
2 months	Arsenic	0.1331	0.1662	1.196	0.075	0.15
2 months	Residual	0.668	0.8338	NA	NA	NA
2 months	Total	0.8011	1	NA	NA	NA
3 months	Arsenic	0.1226	0.1611	0.9603	0.497	0.5964
3 months	Residual	0.6382	0.8389	NA	NA	NA
3 months	Total	0.7608	1	NA	NA	NA
4 months	Arsenic	0.1417	0.1518	1.074	0.238	0.357
4 months	Residual	0.7915	0.8482	NA	NA	NA
4 months	Total	0.9331	1	NA	NA	NA
5 months	Arsenic	0.204	0.1703	1.642	0.006	0.036
5 months	Residual	0.9939	0.8297	NA	NA	NA
5 months	Total	1.198	1	NA	NA	NA

Conclusion: The fungal beta diversity is not shaped by 200ppm arsenic.

Figure 7 | Fungi: PCoA ordination

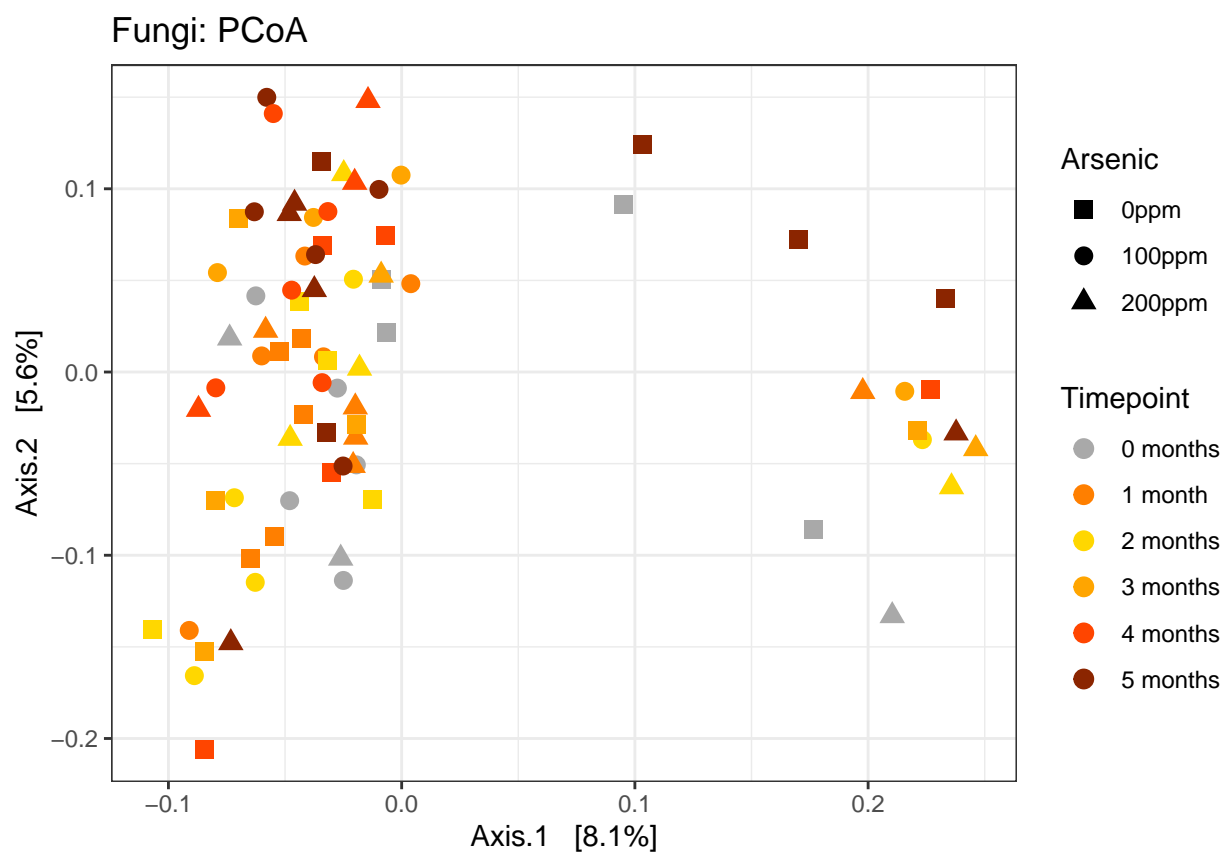


Figure 8 | Fungi: CAP ordination

