

Microplastic - Microbiome Analysis

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Experimental Setup

Arabidopsis thaliana plants were grown on sand or soil. The Plants were treated either with no plastic (CTRL), microplastic, macroplastic or starch. The plastic were added in 3% V/V concentration or 10% V/V concentrations. As additional controls, all the treatments were also performed without a plant.

Figure 1.1 | Rarefaction

In a first step we conduct a rarefaction plot to make sure that we have sequenced deep enough.

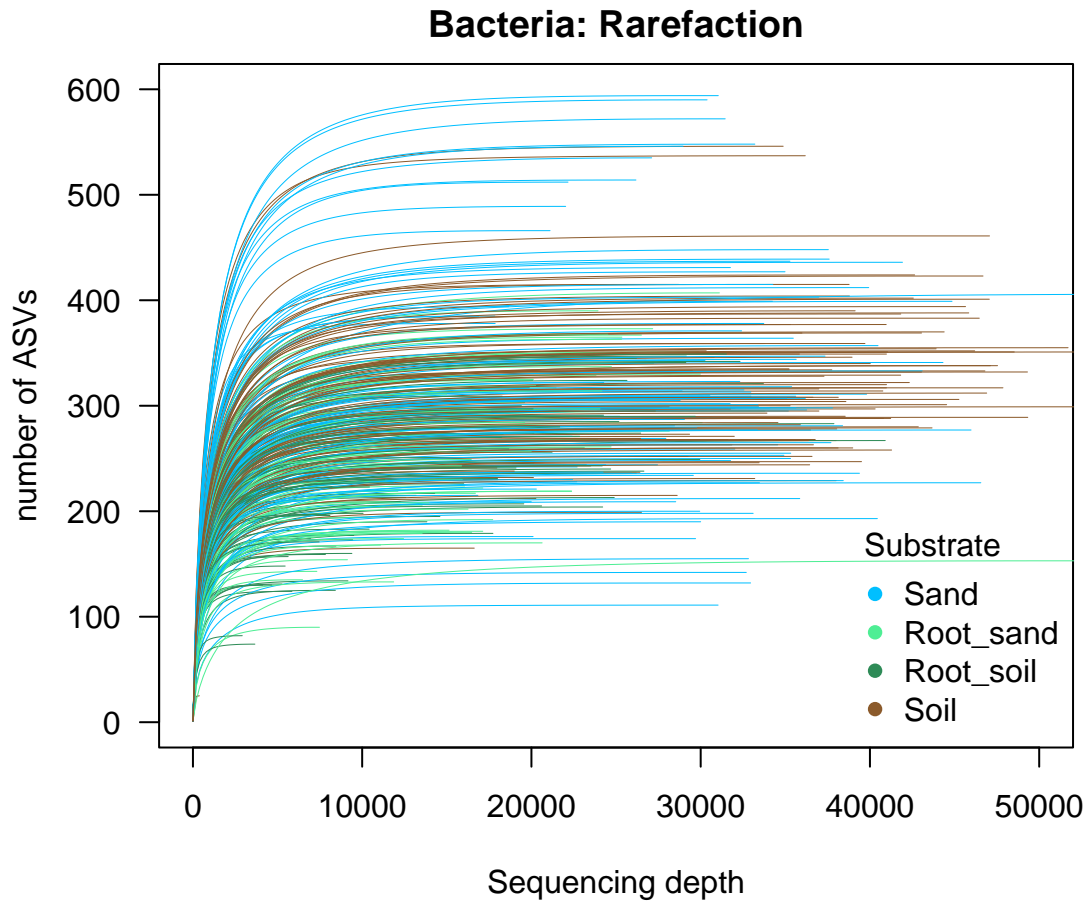
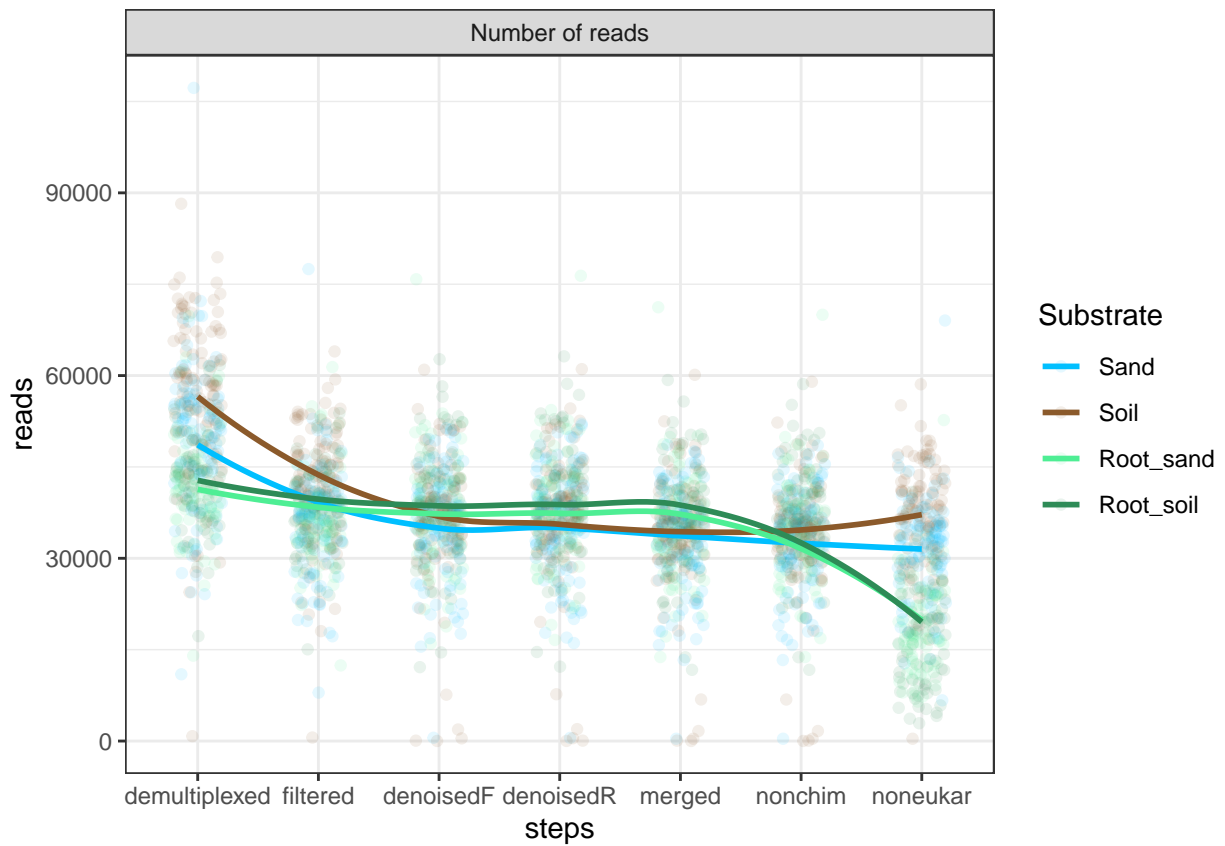


Figure 1.2 | Number of reads

Before we normalize, we do another control plot. We plot the amount of reads during each pipeline step. This allows us to see where we lose how much reads.



Conclusion: Samples from sand, roots and soil seems to be sequenced similar deep. We loose more roots samples at the end by filtering out plant and other eukaryotic reads. Nevertheless, the samples are similar deep sequenced so that we can set a common threshold.

Normalization

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 treatments in each substrate by using the non-parametric Kruskal-Wallis Test.

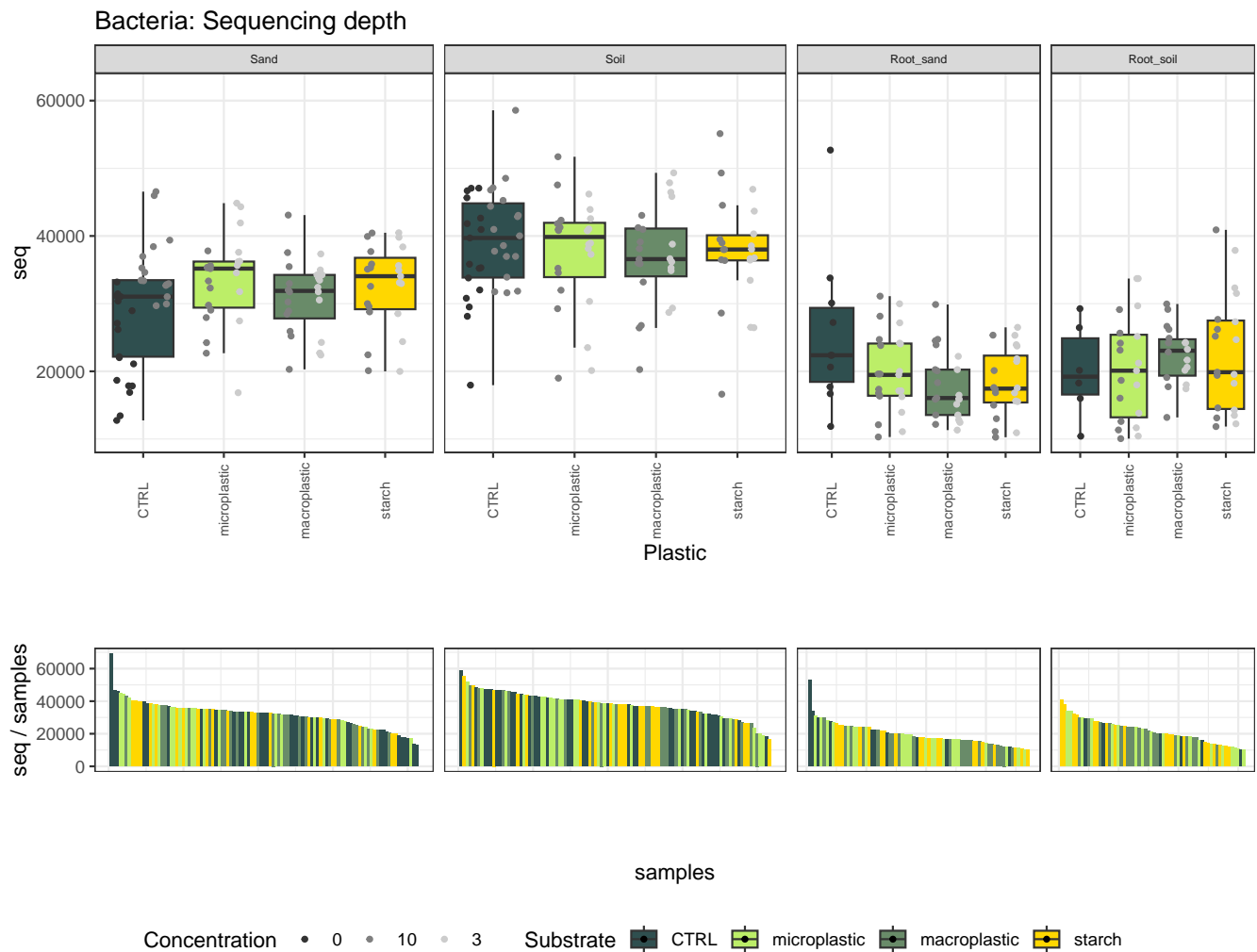
Sequencing depth

We show the sum, range and median over all samples. Eight bacterial samples were previous removed due to very low sequencing depth.

Bacteria

Substrate	removed_samples	sum	min	max	median
Sand	1	3266638	12746	69039	32824
Soil	1	3937323	16622	58567	38126
Root_sand	7	1453295	10264	52690	17661
Root_soil	19	1342540	10053	40904	20912

Figure 2 | Sequencing depth



Asymptotic Kruskal-Wallis Test

We test for different sequencing depths between the treatments

```
## [1] "Sand"

##
## Asymptotic Kruskal-Wallis Test
##
## data: sample_depth by
## group (CTRL_plant, CTRL_substrate, CTRL_substrate_microplastic_10, CTRL_substrate_macroplastic_10, CTRL_substrate_starch_3, CTRL_substrate_starch_10)
## chi-squared = 32.674, df = 10, p-value = 0.000309

## [1] "Root_sand"

##
## Asymptotic Kruskal-Wallis Test
##
## data: sample_depth by
## group (CTRL_plant, microplastic_3, microplastic_10, macroplastic_3, macroplastic_10, starch_3, starch_10)
## chi-squared = 11.593, df = 6, p-value = 0.07169

## [1] "Root_soil"

##
## Asymptotic Kruskal-Wallis Test
##
## data: sample_depth by
## group (CTRL_plant, microplastic_3, microplastic_10, macroplastic_3, macroplastic_10, starch_3, starch_10)
## chi-squared = 2.7782, df = 6, p-value = 0.8361

## [1] "Soil"

##
## Asymptotic Kruskal-Wallis Test
##
## data: sample_depth by
## group (CTRL_plant, CTRL_substrate, CTRL_substrate_microplastic_10, CTRL_substrate_macroplastic_10, CTRL_substrate_starch_3, CTRL_substrate_starch_10)
## chi-squared = 5.5373, df = 10, p-value = 0.8525
```

Conclusion: Because there are significant differences between the treatments in sand and marginal differences in roots from sand, we normalize the data by rarefaction for diversity comparisons (see Weiss et al. (2017), Microbiome Journal). For consistency we do it for sand, root and soil. We define the rarefaction threshold per sample to 10^4 .

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 rarefied 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 2: Number of outliers

Plastic	Sand	Root_sand	Root_soil	Soil
CTRL	0	1	1	0
microplastic	2	1	1	0
macroplastic	2	2	1	2
starch	0	0	1	3

Sample Size

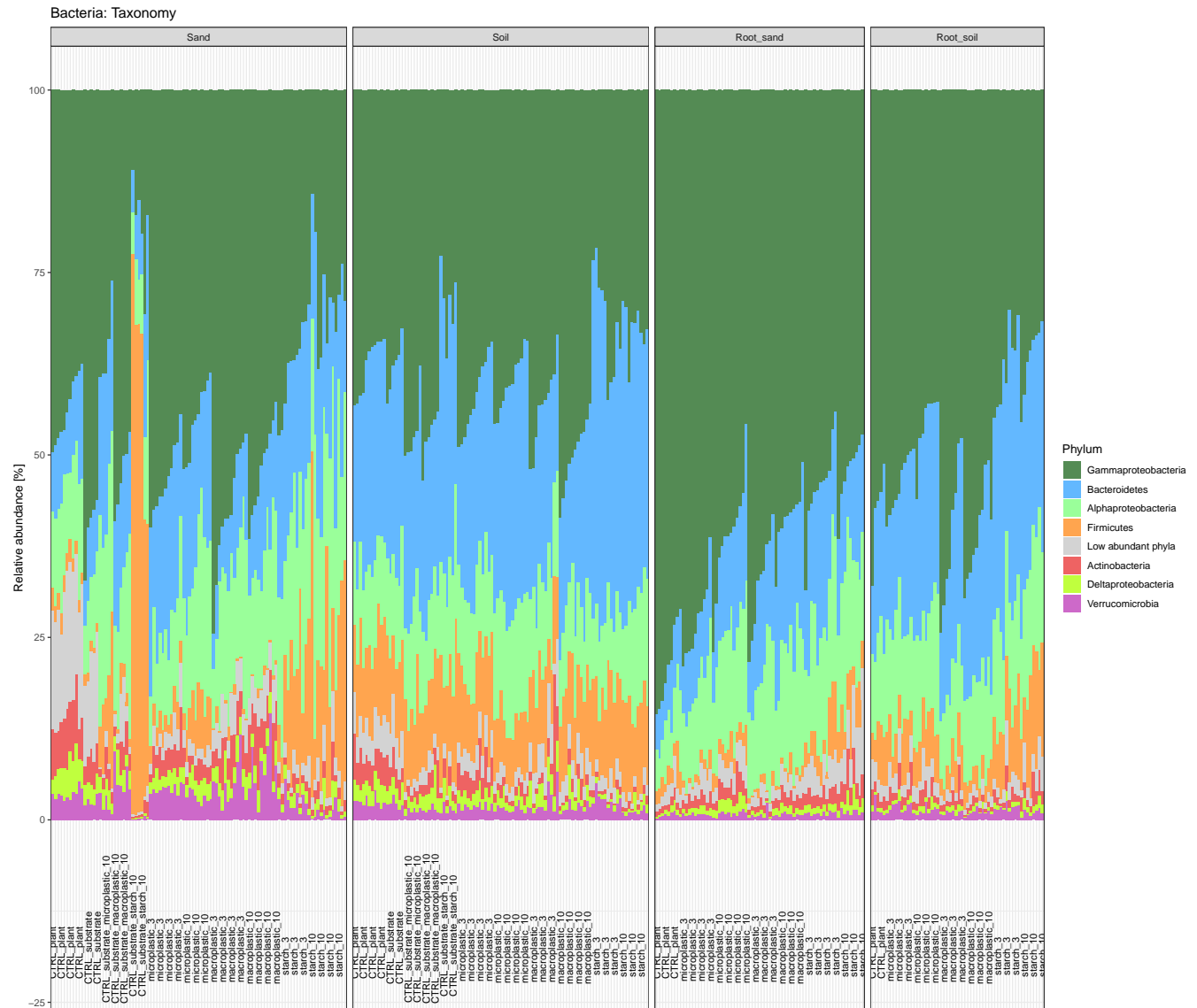
We end up with the following number of samples per treatment for the analysis.

Table 3: Sample profile

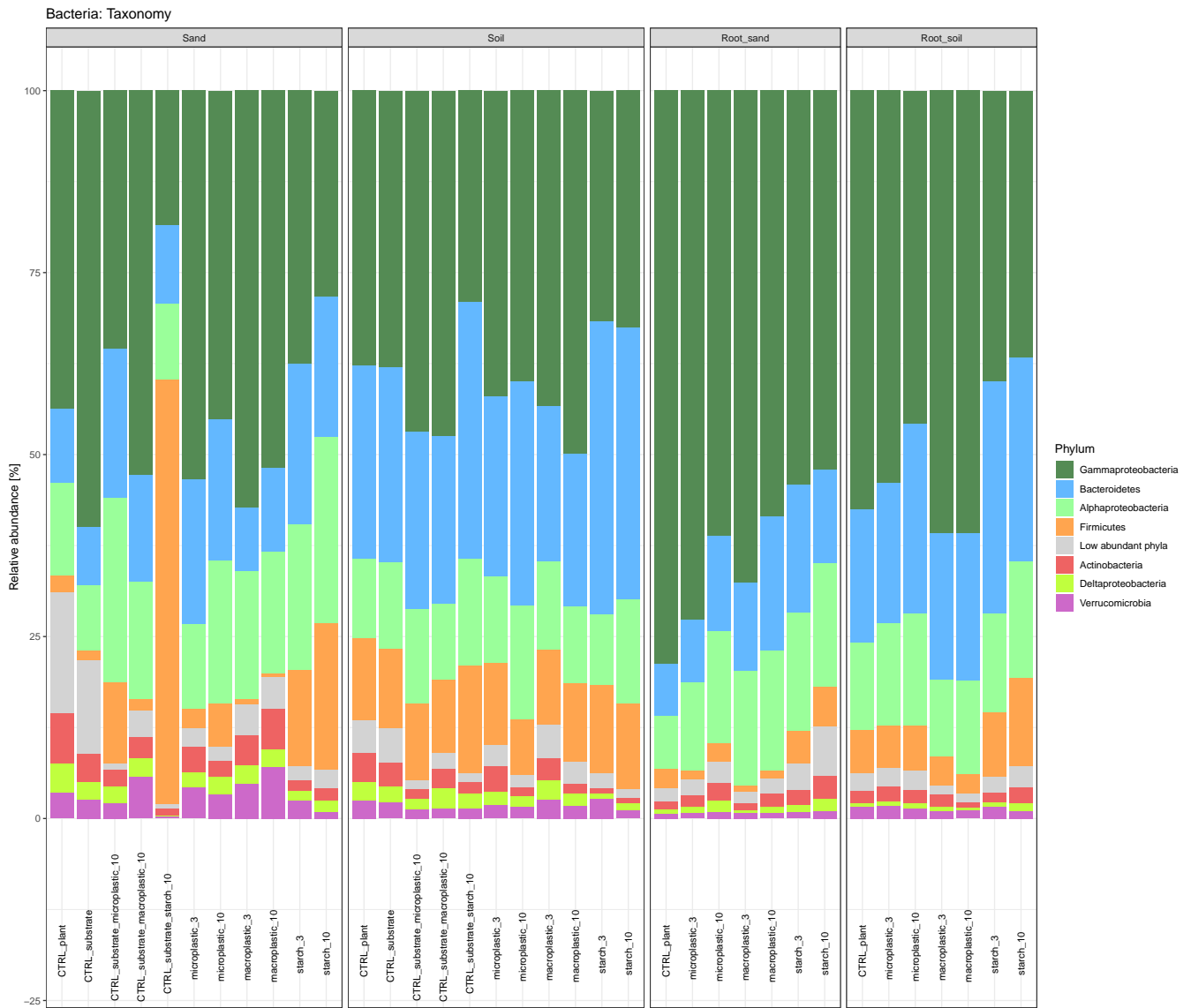
Sample	Sand	Root_sand	Root_soil	Soil
CTRL_plant	11	9	5	11
CTRL_substrate	5	0	0	6
CTRL_substrate_microplastic_10	5	0	0	6
CTRL_substrate_macroplastic_10	6	0	0	6
CTRL_substrate_starch_10	6	0	0	6
microplastic_3	11	10	10	12
microplastic_10	10	12	8	12
macroplastic_3	12	9	8	10
macroplastic_10	10	10	9	11
starch_3	11	11	10	10
starch_10	12	9	8	9

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

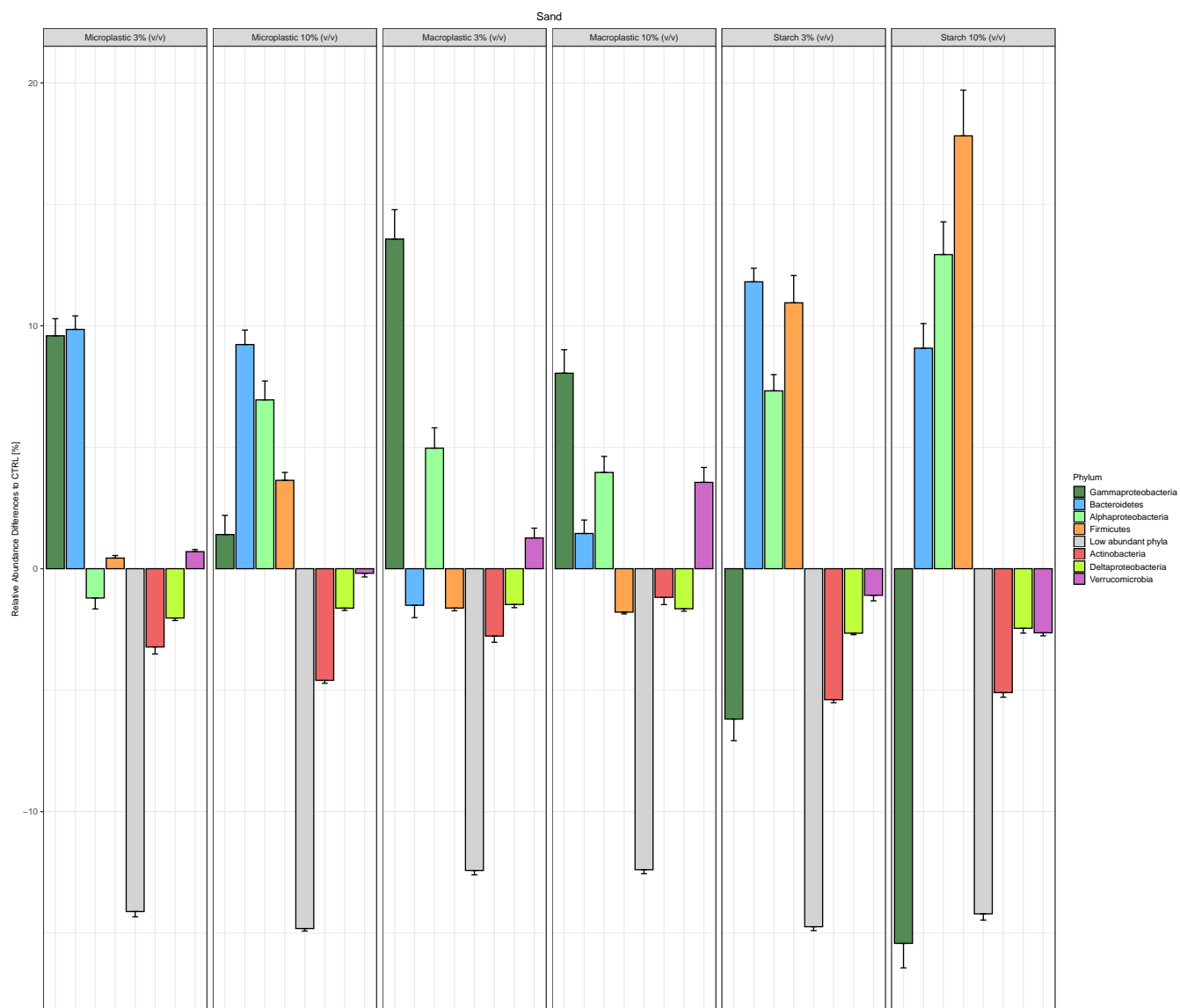
Figure 3 | Phylum level taxonomy

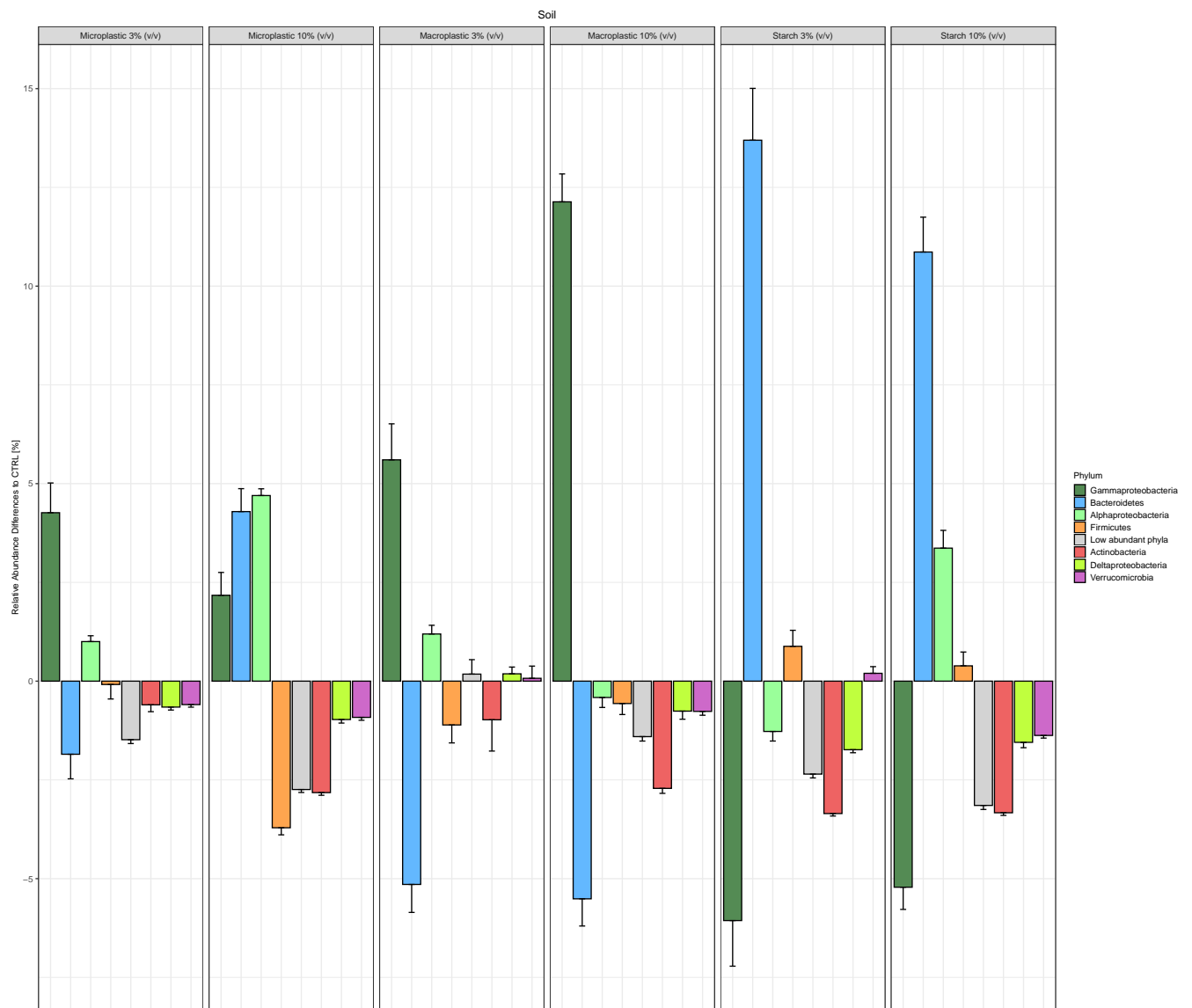


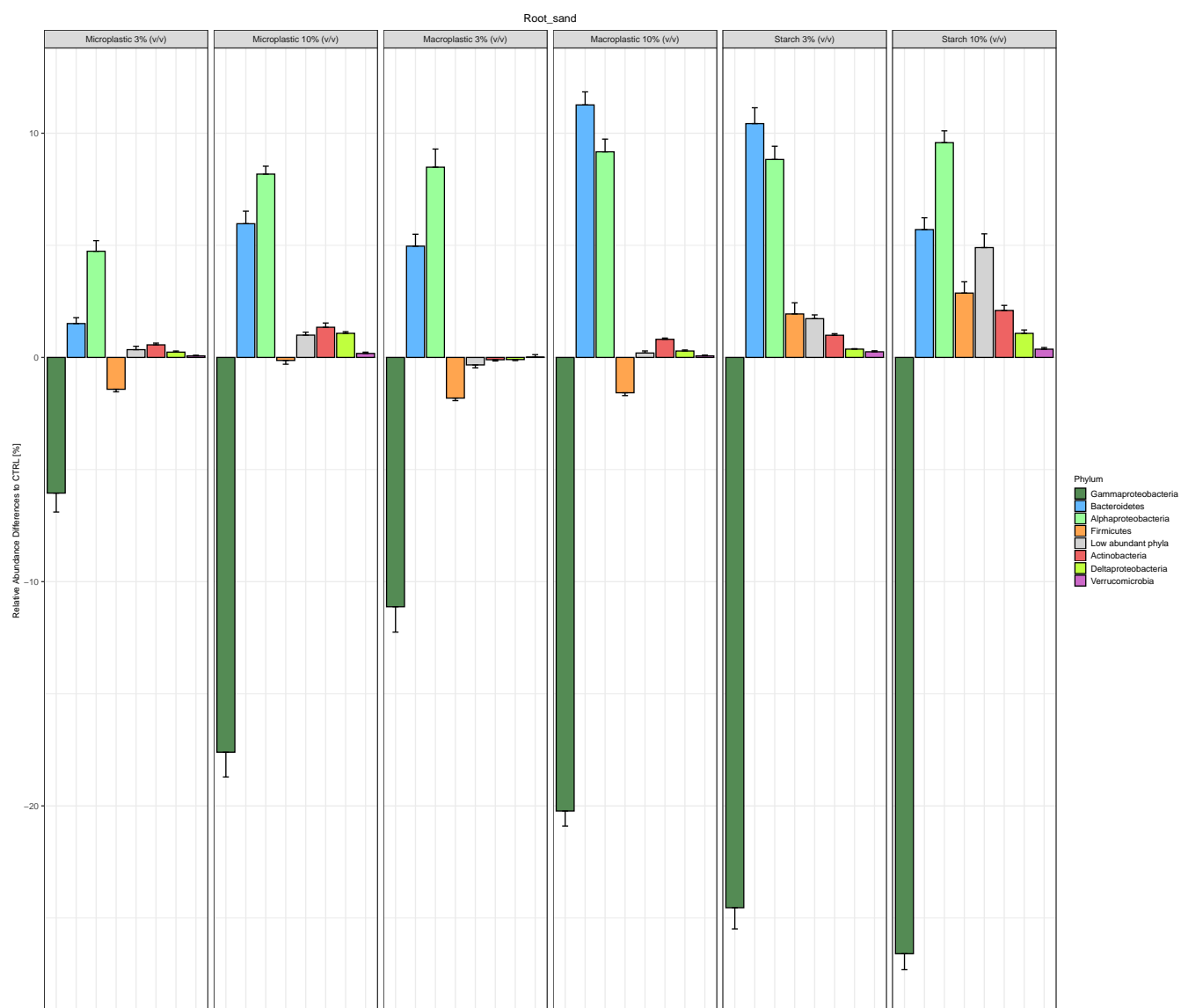
We repeat the plot by plotting the abundances means for each treatment in each substrate.

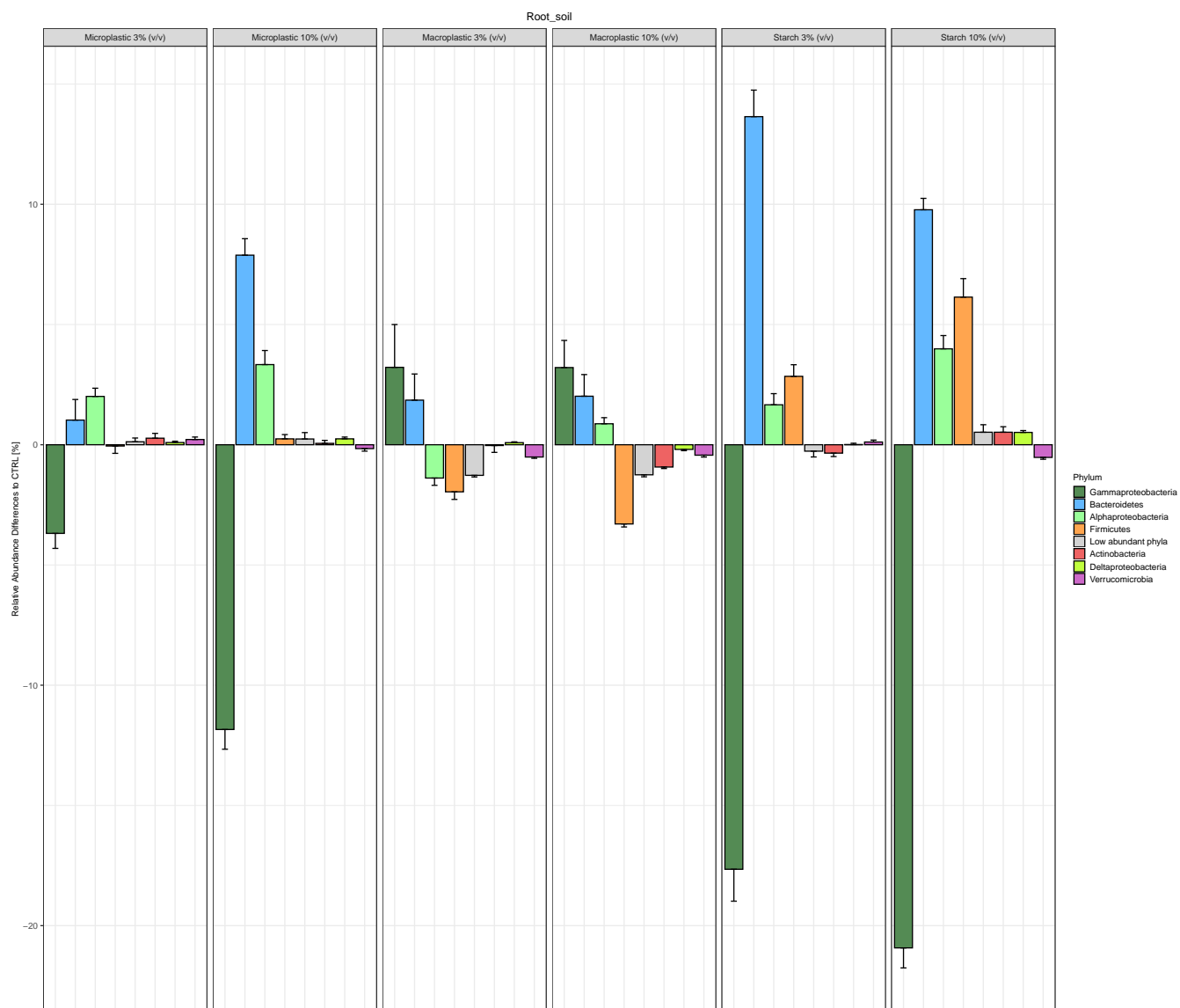


We repeat the plot showing in a different way for each phyla if it is increasing or decreasing compared to the plant control. For simplicity, we exclude substrate controls in this plot.









Alpha diversity

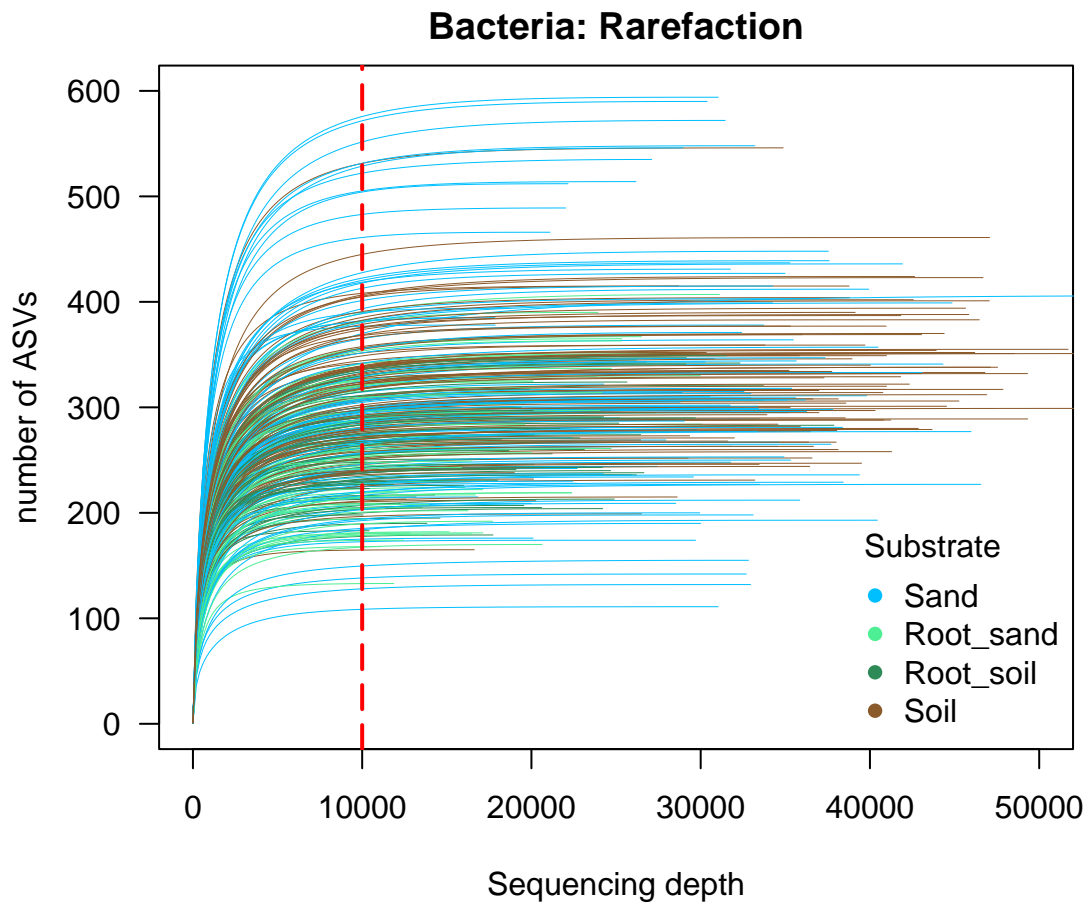
We answer the following question for the alpha diversity in each substrate:

- Q1: Are there differences in alpha diversity between the different treatments?
- Q2: Are there differences in alpha diversity between the different concentrations?

Rarefaction

Figure 3 | Rarefaction

Before analyzing the alpha diversity, we conduct the rarefaction plot to make sure that no diversity is lost due to a too low rarefaction threshold.



Conclusion: We chose a rarefaction threshold that do cover the majority of diversity in each sample.

Alpha Diversity

Method: We rarefy the dataset by the sequencing depth of 10^4 and calculate the Shannon diversity for each sample. This is repeated 100 times. Then, the mean value from the 100 iterations is taken for statistical analysis between the different samples.

Overview

We get an overview for each substrate by investigating the effect on alpha diversity by the factors of plastic, concentration and the interaction between them. We model the alpha diversity against these factors in an aov-model and perform a F-Test.

Table 4: Sand: F test

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Plastic	3	26447	8816	12.73	5.216e-07
Concentration	2	65634	32817	47.39	8.726e-15
Plant	1	5115	5115	7.387	0.00788
Plastic:Concentration	2	429.2	214.6	0.3099	0.7343
Residuals	90	62320	692.4	NA	NA

Table 5: Root_sand: F test

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Plastic	3	31314	10438	31.33	1.622e-12
Concentration	1	8102	8102	24.32	6.269e-06
Plastic:Concentration	2	2893	1447	4.342	0.01712
Residuals	63	20992	333.2	NA	NA

Table 6: Root_soil: F test

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Plastic	3	3918	1306	4.749	0.005375
Concentration	1	185.2	185.2	0.6736	0.4156
Plastic:Concentration	2	1220	610.1	2.219	0.1192
Residuals	51	14026	275	NA	NA

Table 7: Soil: F test

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Plastic	3	17882	5961	15.79	2.481e-08
Concentration	2	12144	6072	16.09	1.063e-06
Plant	1	115.9	115.9	0.3071	0.5809
Plastic:Concentration	2	1912	956.2	2.533	0.08503
Residuals	90	33968	377.4	NA	NA

Conclusion: We find an effect on the alpha diversity between the different plastics and for the plastic concentration in sand.

Plastic effect

Q1: Are there differences in alpha diversity between the different treatments (samples with plant)?

For this analysis we only conduct samples with plants. Plants were either treated with no plastic (control), microplastic, macroplastic or starch. We saw above that the treatment is shaping the alpha diversity. We investigate now in which direction is the alpha diversity shifted by the single treatments by looking at Shannon diversity, richness and evenness.

Shannon Diversity

Table 8: Plastic effect in sand

Plastic	Shannon	letters
CTRL	161.9	c
microplastic	80.04	a
macroplastic	100.9	b
starch	64.04	a

Table 9: Plastic effect in root_sand

Plastic	Shannon	letters
CTRL	35.36	a
microplastic	74.41	b
macroplastic	76.06	b
starch	105	c

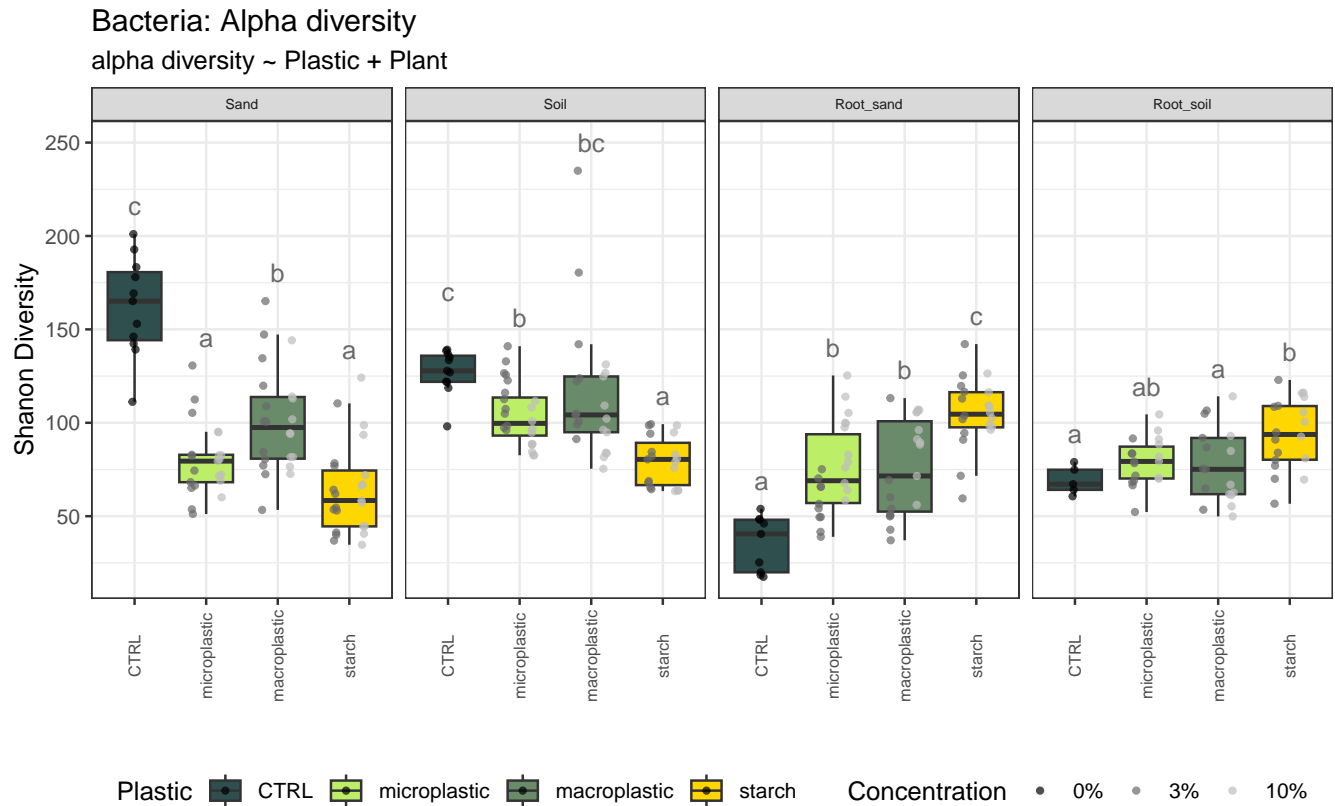
Table 10: Plastic effect in root_soil

Plastic	Shannon	letters
CTRL	69.15	a
microplastic	79.21	ab
macroplastic	76.98	a
starch	93.85	b

Table 11: Plastic effect in soil

Plastic	Shannon	letters
CTRL	127.1	c
microplastic	104.4	b
macroplastic	114.9	bc
starch	79.4	a

Figure 4.1 | Plastic effect on alpha diversity



Conclusion: In sand and, the alpha diversity is in overall reduced by treating with plastics. In roots from sand and soil, the opposite effect can be observed.

Richness

Table 12: Plastic effect in sand

Plastic	Shannon	letters
CTRL	161.9	c
microplastic	80.04	ab
macroplastic	100.9	b
starch	64.04	a

Table 13: Plastic effect in root_sand

Plastic	Shannon	letters
CTRL	35.36	a
microplastic	74.41	a
macroplastic	76.06	a
starch	105	b

Table 14: Plastic effect in root_soil

Plastic	Shannon	letters
CTRL	69.15	a
microplastic	79.21	a
macroplastic	76.98	a
starch	93.85	a

Table 15: Plastic effect in soil

Plastic	Shannon	letters
CTRL	127.1	b
microplastic	104.4	b
macroplastic	114.9	b
starch	79.4	a

Bacteria: Alpha diversity alpha diversity ~ Plastic + Plant

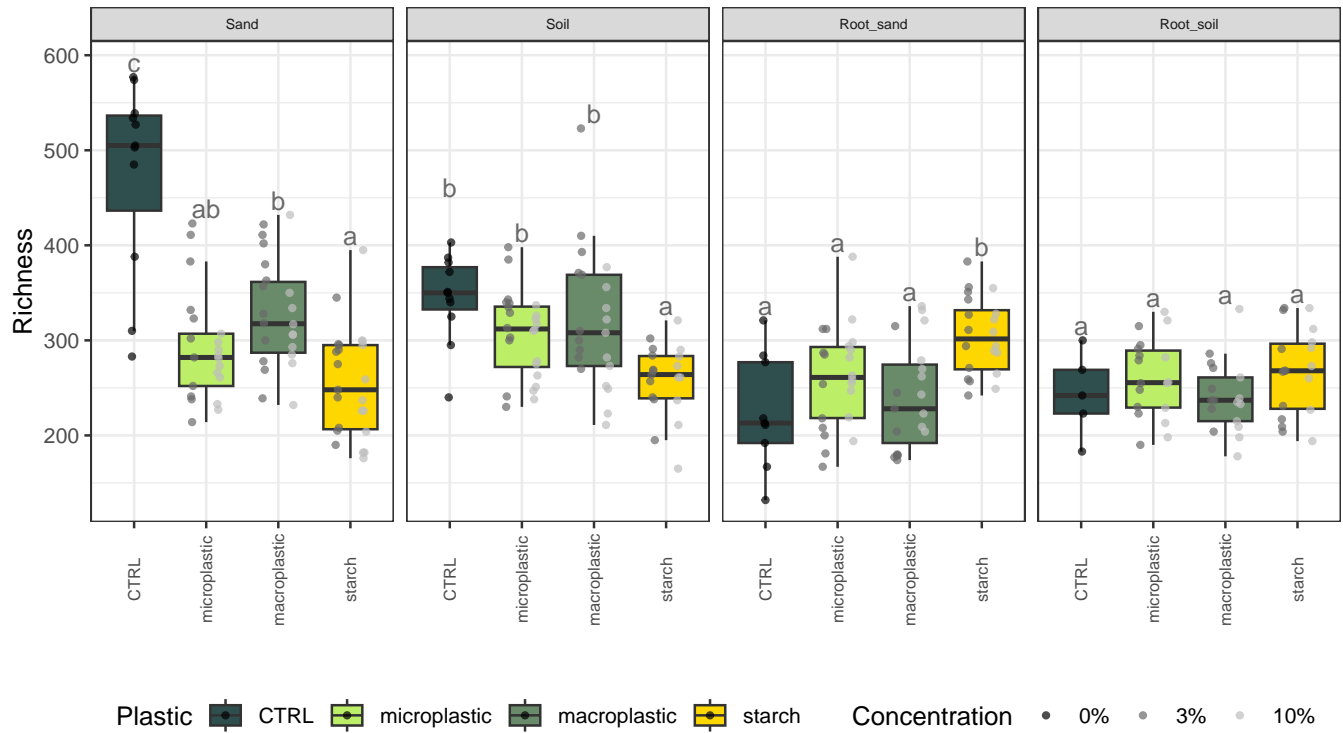


Table 16: Plastic effect in sand

Plastic	Shannon	letters
CTRL	161.9	c
microplastic	80.04	b
macroplastic	100.9	b
starch	64.04	a

Table 17: Plastic effect in root_sand

Plastic	Shannon	letters
CTRL	35.36	a
microplastic	74.41	b
macroplastic	76.06	bc
starch	105	c

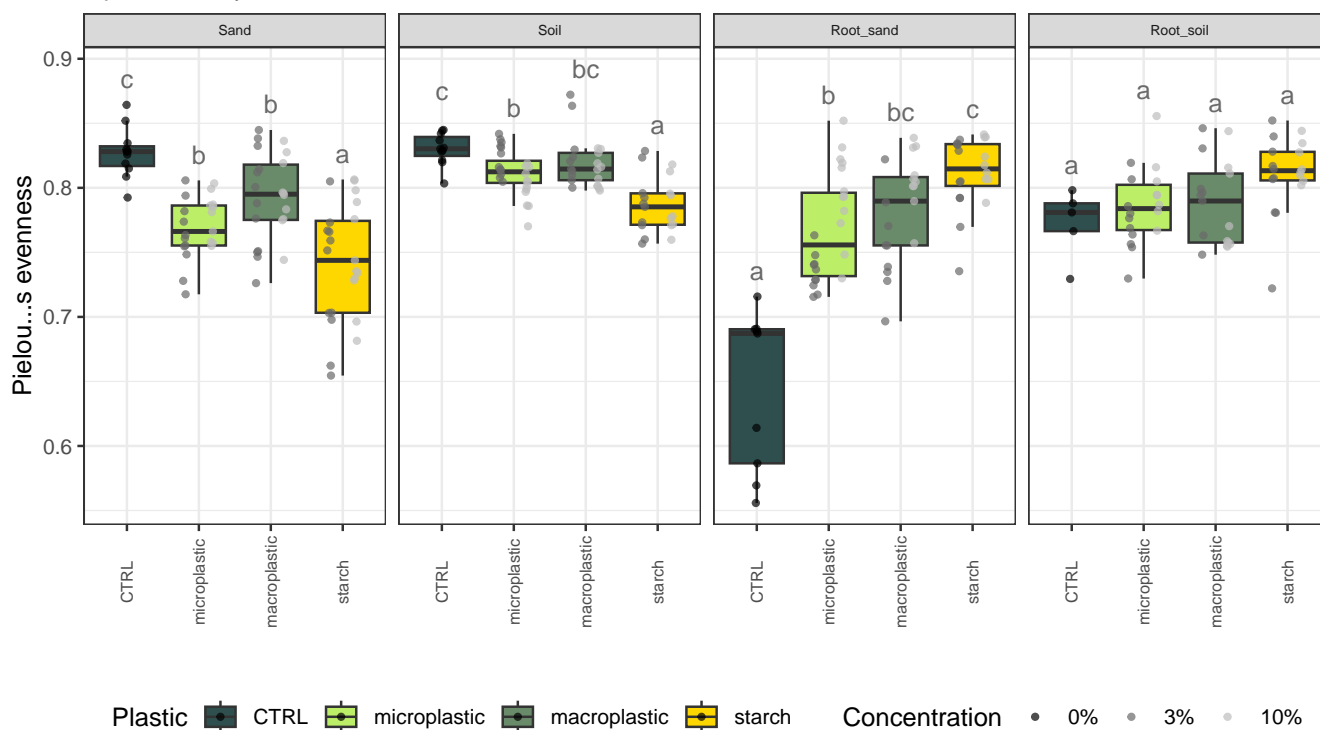
Table 18: Plastic effect in root_soil

Plastic	Shannon	letters
CTRL	69.15	a
microplastic	79.21	a
macroplastic	76.98	a
starch	93.85	a

Table 19: Plastic effect in soil

Plastic	Shannon	letters
CTRL	127.1	c
microplastic	104.4	b
macroplastic	114.9	bc
starch	79.4	a

Bacteria: Alpha diversity alpha diversity ~ Plastic + Plant



Concentrations effect

Q2: Are there differences in alpha diversity between the different concentrations?

Plants were either treated with no plastic (control), microplastic, macroplastic or starch. Plastic was added in a 3% or 10% concentration. From the stats above we could see that the concentration has an effect on the alpha diversity in sand. We investigate now in which direction is the alpha diversity shifted by the different concentrations.

Table 20: Concentration effect in sand

	Concentration	Shannon	letters
1	0%	161.9	b
3	3%	82.61	a
2	10%	80.12	a

Table 21: Concentration effect in root_sand

	Concentration	Shannon	letters
1	0%	35.36	a
3	3%	74.46	b
2	10%	95.13	c

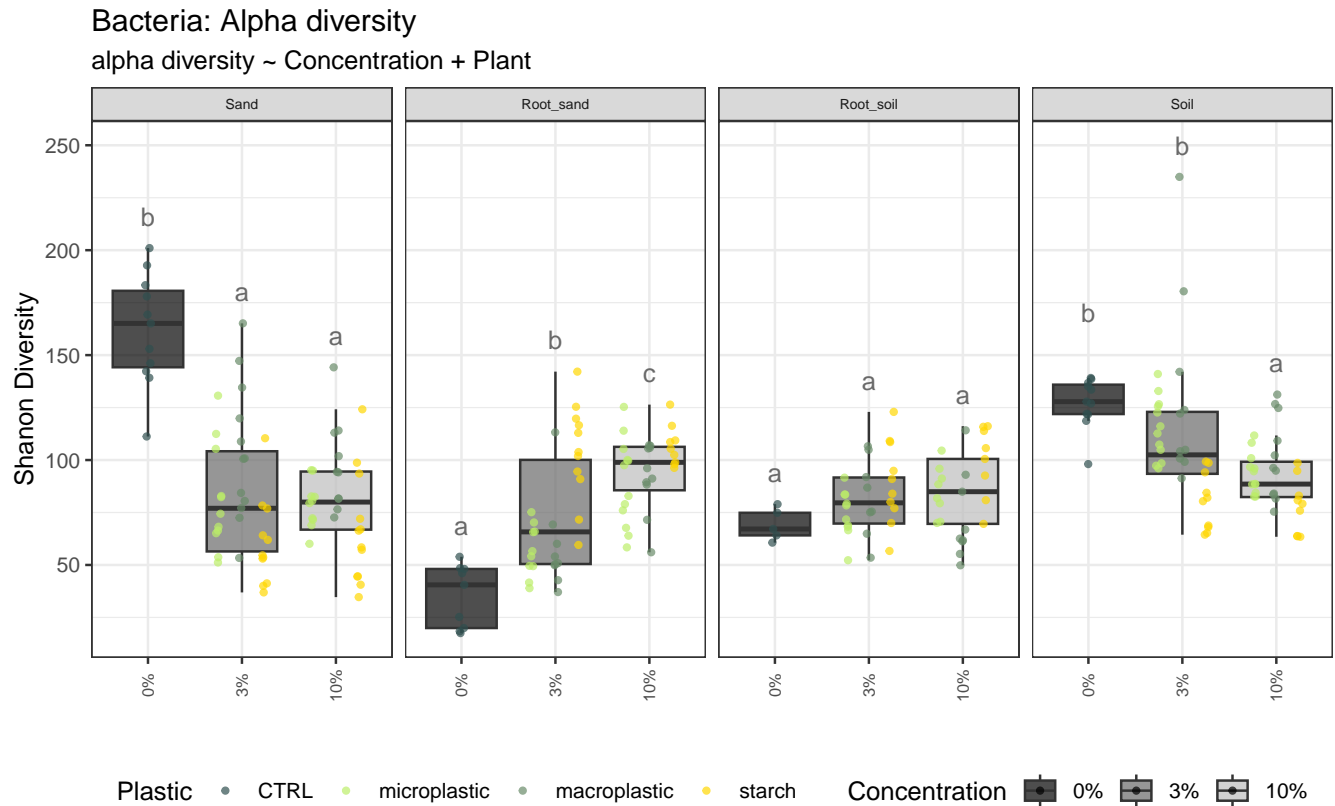
Table 22: Concentration effect in root_soil

	Concentration	Shannon	letters
1	0%	69.15	a
3	3%	82.04	a
2	10%	85.06	a

Table 23: Concentration effect in soil

	Concentration	Shannon	letters
1	0%	127.1	b
3	3%	109.1	b
2	10%	91.77	a

Figure 4.1 | Concentration effect on alpha diversity



Conclusion: A sand and soil is the alpha diversity is in overall reduced by treating with plastics. In roots from sand, the opposite effect can be observed. Roots from soil don't show effects. There is no clear effect that a higher concentration of plastic has a stronger effect than a lower concentration.

Conclusion alpha diversity: In sand and soil, the alpha diversity was reduced by treating samples with microplastic, macroplastic or starch. In roots from sand or soil, the opposite effect could be observed. There is no clear effect, that a higher plastic concentration shows a stronger response on the alpha diversity.

Beta diversity

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

- **Q1: Are there differences in beta diversity between the different treatments?**
- **Q2: Are there differences in beta diversity between the different concentrations?**

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).

Overview

Before answering the questions, we get an overview by investigating the full model to see which factors alters the beta diversity.

Table 24: PERMANOVA: sand

	Df	SumOfSqs	R2	F	Pr(>F)
Plastic	3	6.387	0.2676	16.73	0.001
Concentration	2	4.196	0.1758	16.48	0.001
Plant	1	0.6976	0.02923	5.482	0.001
Plastic:Concentration	2	1.135	0.04753	4.457	0.001
Residual	90	11.45	0.4799	NA	NA
Total	98	23.87	1	NA	NA

Table 25: PERMANOVA: root_sand

	Df	SumOfSqs	R2	F	Pr(>F)
Plastic	3	3.674	0.3214	12.85	0.001
Concentration	1	1.053	0.09209	11.04	0.001
Plastic:Concentration	2	0.6989	0.06114	3.665	0.001
Residual	63	6.006	0.5254	NA	NA
Total	69	11.43	1	NA	NA

Table 26: PERMANOVA: root_soil

	Df	SumOfSqs	R2	F	Pr(>F)
Plastic	3	2.313	0.2887	8.15	0.001
Concentration	1	0.3404	0.0425	3.599	0.001
Plastic:Concentration	2	0.5328	0.0665	2.816	0.001
Residual	51	4.825	0.6023	NA	NA
Total	57	8.011	1	NA	NA

Table 27: PERMANOVA: soil

	Df	SumOfSqs	R2	F	Pr(>F)
Plastic	3	3.14	0.235	12.44	0.001
Concentration	2	1.841	0.1377	10.94	0.001
Plant	1	0.0507	0.003794	0.6027	0.891
Plastic:Concentration	2	0.7601	0.05688	4.518	0.001
Residual	90	7.571	0.5666	NA	NA
Total	98	13.36	1	NA	NA

Conclusion: The bacterial communities are different between the different plastic treatments, the plastic concentration, the plastic:concentration interaction and between samples with plant and without plant. We further investigate the single factors in the following analyses.

Plastic effect

Q1: Are there differences in beta diversity between the different treatments (samples with plant)?
 For this analysis we only conduct samples with plants. Plants were either treated with no plastic (control), microplastic, macroplastic or starch. We saw above that the treatment is shaping the beta diversity. We investigate now in which direction is the beta diversity shifted by the single treatments.

Table 28: PERMANOVA: sand

	Df	SumOfSqs	R2	F	Pr(>F)
Plastic	3	7.757	0.4345	23.5	0.001
Concentration	1	1.259	0.0705	11.44	0.001
Plastic:Concentration	2	1.135	0.06355	5.155	0.001
Residual	70	7.703	0.4315	NA	NA
Total	76	17.85	1	NA	NA

Table 29: PERMANOVA: root_sand

	Df	SumOfSqs	R2	F	Pr(>F)
Plastic	3	3.674	0.3214	12.85	0.001
Concentration	1	1.053	0.09209	11.04	0.001
Plastic:Concentration	2	0.6989	0.06114	3.665	0.001
Residual	63	6.006	0.5254	NA	NA
Total	69	11.43	1	NA	NA

Table 30: PERMANOVA: root_soil

	Df	SumOfSqs	R2	F	Pr(>F)
Plastic	3	2.313	0.2887	8.15	0.001
Concentration	1	0.3404	0.0425	3.599	0.001
Plastic:Concentration	2	0.5328	0.0665	2.816	0.001
Residual	51	4.825	0.6023	NA	NA
Total	57	8.011	1	NA	NA

Table 31: PERMANOVA: soil

	Df	SumOfSqs	R2	F	Pr(>F)
Plastic	3	3.408	0.3474	15.22	0.001
Concentration	1	0.5646	0.05756	7.562	0.001
Plastic:Concentration	2	0.7601	0.07749	5.091	0.001
Residual	68	5.077	0.5175	NA	NA
Total	74	9.809	1	NA	NA

Figure 5.1.1 | PCoA - Plastic effect on beta diversity

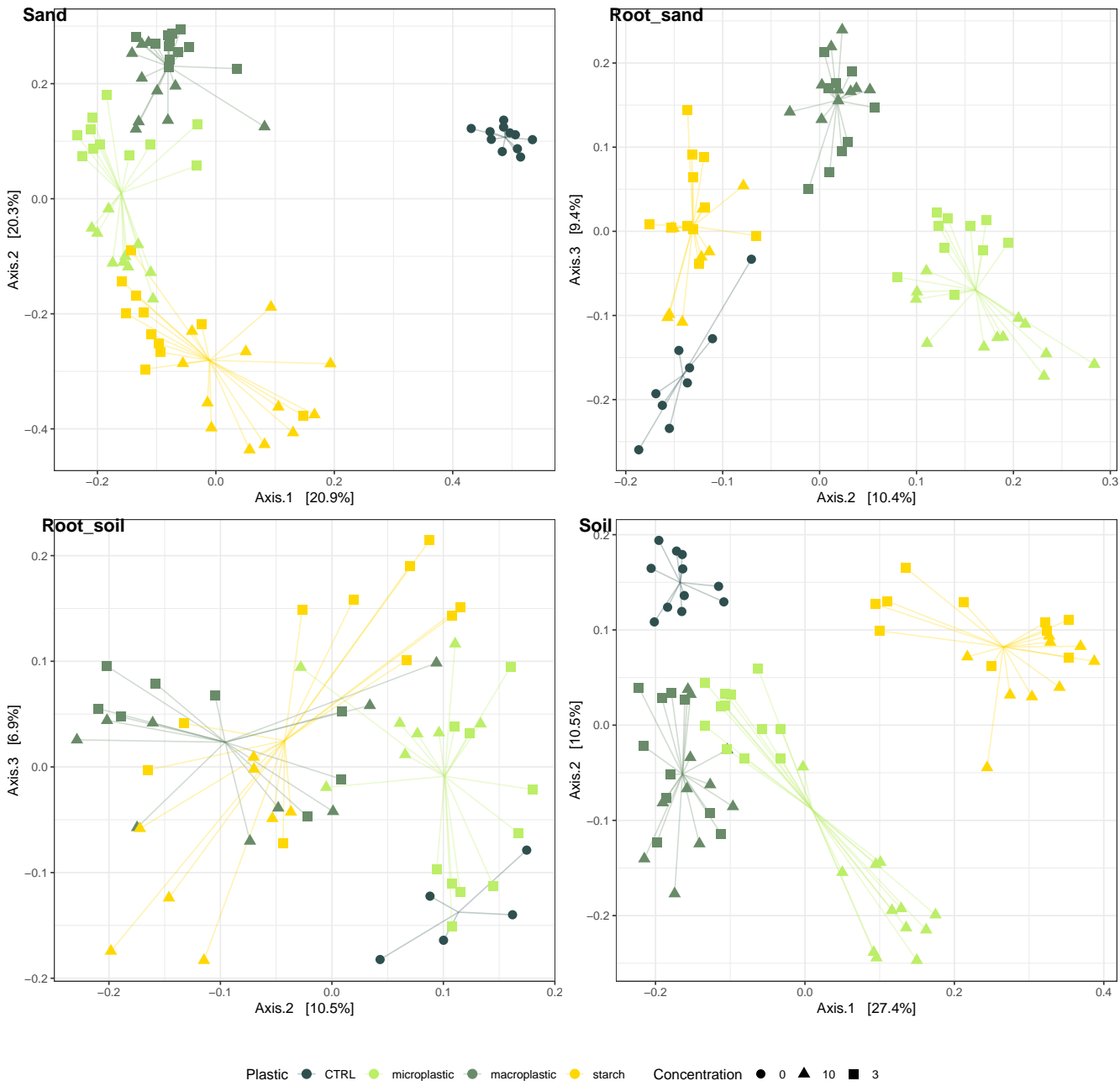
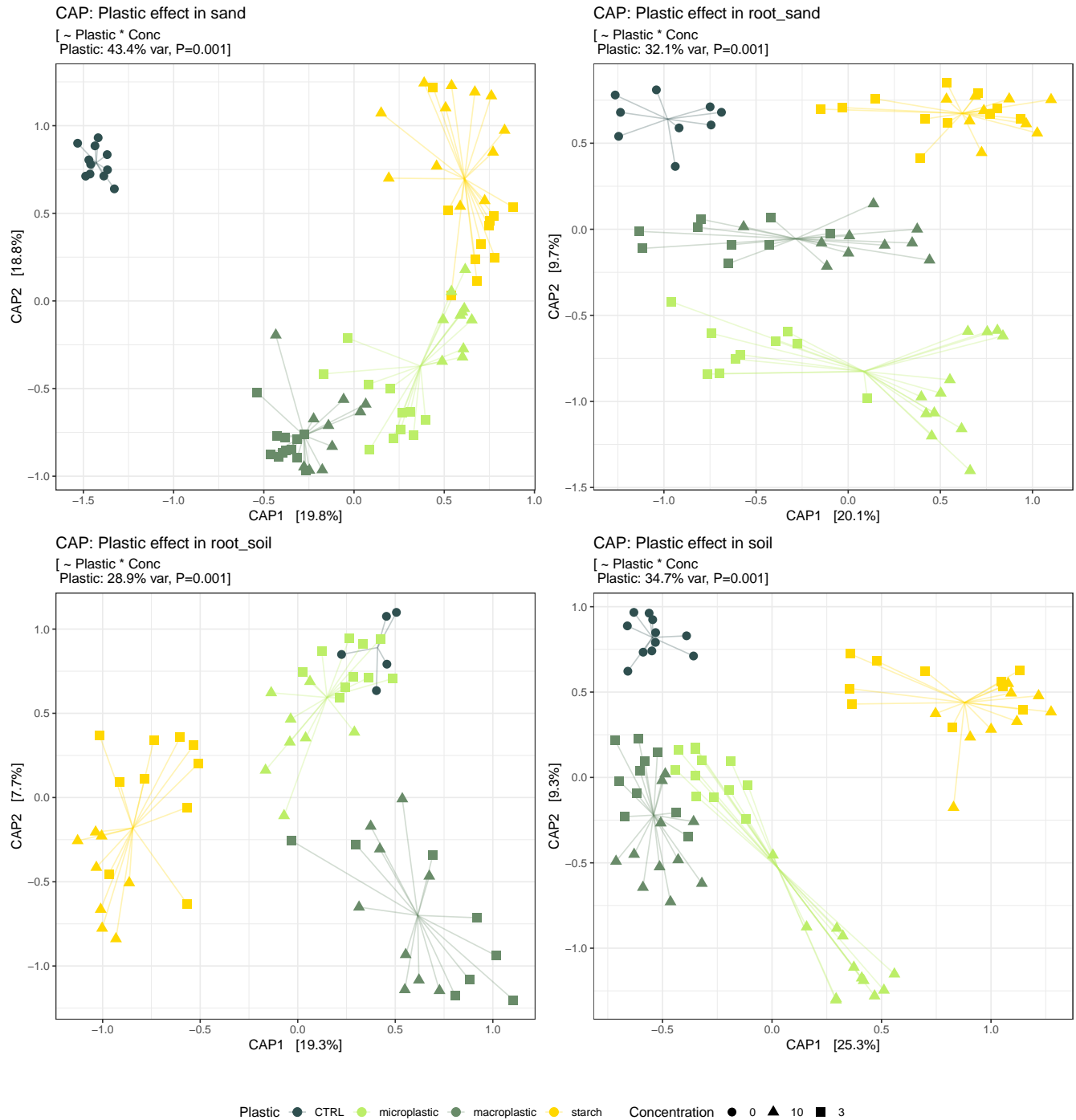


Figure 5.1.2 | CAP - Plastic effect on beta diversity



Conclusion: The bacterial communities differ between the different plastic treatments. Plastic can describe between 30% and 45% of the microbial variety. Most of the variety within the plastic treatments comes from the different plastic concentrations which have been used.

Concentration effect

Q2: Are there differences in beta diversity between the different concentrations (samples with plant)?

For this analysis we only conduct samples with plants. Plants were either treated with no plastic (control) or microplastic, macroplastic or starch in a concentration of 3% or 10%. We saw above that the concentration has an effect on the beta diversity. We investigate now in which direction is the beta diversity shifted by the different concentrations.

Table 32: PERMANOVA: sand microplastic

	Df	SumOfSqs	R2	F	Pr(>F)
Concentration	2	4.021	0.636	25.34	0.001
Residual	29	2.301	0.364	NA	NA
Total	31	6.322	1	NA	NA

Table 33: PERMANOVA: sand macroplastic

	Df	SumOfSqs	R2	F	Pr(>F)
Concentration	2	3.444	0.5112	15.69	0.001
Residual	30	3.293	0.4888	NA	NA
Total	32	6.737	1	NA	NA

Table 34: PERMANOVA: sand starch

	Df	SumOfSqs	R2	F	Pr(>F)
Concentration	2	4.199	0.5189	16.72	0.001
Residual	31	3.893	0.4811	NA	NA
Total	33	8.092	1	NA	NA

Table 35: PERMANOVA: root_sand microplastic

	Df	SumOfSqs	R2	F	Pr(>F)
Concentration	2	2.17	0.4704	12.44	0.001
Residual	28	2.443	0.5296	NA	NA
Total	30	4.613	1	NA	NA

Table 36: PERMANOVA: root_sand macroplastic

	Df	SumOfSqs	R2	F	Pr(>F)
Concentration	2	1.629	0.4349	9.619	0.001
Residual	25	2.117	0.5651	NA	NA
Total	27	3.746	1	NA	NA

Table 37: PERMANOVA: root_sand starch

	Df	SumOfSqs	R2	F	Pr(>F)
Concentration	2	1.955	0.4244	9.586	0.001
Residual	26	2.651	0.5756	NA	NA
Total	28	4.606	1	NA	NA

	Df	SumOfSqs	R2	F	Pr(>F)
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Table 38: PERMANOVA: root_soil microplastic

	Df	SumOfSqs	R2	F	Pr(>F)
Concentration	2	0.4787	0.2417	3.187	0.001
Residual	20	1.502	0.7583	NA	NA
Total	22	1.981	1	NA	NA

Table 39: PERMANOVA: root_soil macroplastic

	Df	SumOfSqs	R2	F	Pr(>F)
Concentration	2	0.6609	0.251	3.184	0.001
Residual	19	1.972	0.749	NA	NA
Total	21	2.633	1	NA	NA

Table 40: PERMANOVA: root_soil starch

	Df	SumOfSqs	R2	F	Pr(>F)
Concentration	2	1.068	0.3458	5.285	0.001
Residual	20	2.021	0.6542	NA	NA
Total	22	3.09	1	NA	NA

Table 41: PERMANOVA: soil microplastic

	Df	SumOfSqs	R2	F	Pr(>F)
Concentration	2	1.287	0.3992	10.63	0.001
Residual	32	1.938	0.6008	NA	NA
Total	34	3.225	1	NA	NA

Table 42: PERMANOVA: soil macroplastic

	Df	SumOfSqs	R2	F	Pr(>F)
Concentration	2	0.8573	0.269	5.337	0.001
Residual	29	2.329	0.731	NA	NA
Total	31	3.187	1	NA	NA

Table 43: PERMANOVA: soil starch

	Df	SumOfSqs	R2	F	Pr(>F)
Concentration	2	1.94	0.4854	12.74	0.001
Residual	27	2.057	0.5146	NA	NA
Total	29	3.997	1	NA	NA

Figure 5.2.1 | PCoA - Concentration effect on beta diversity

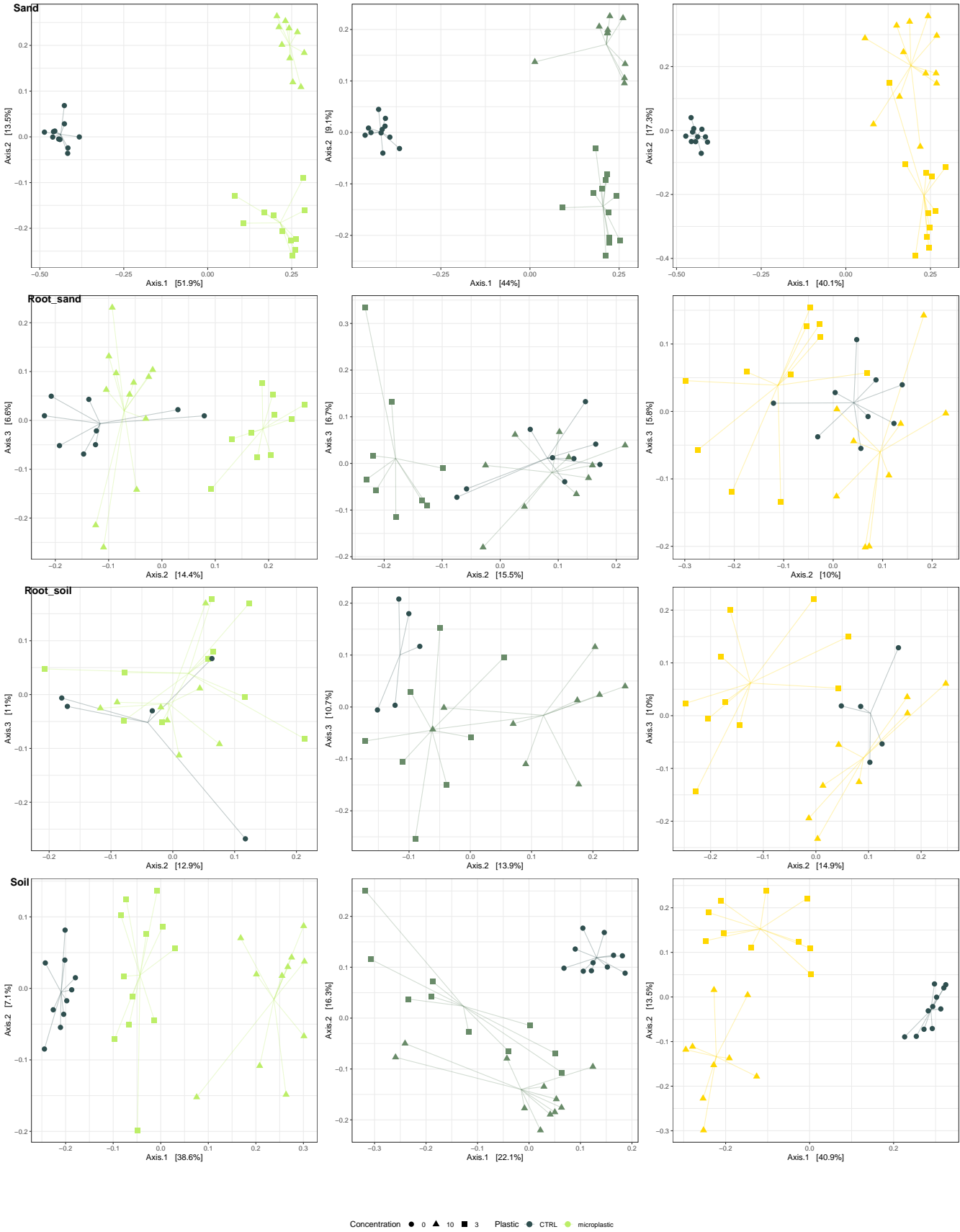
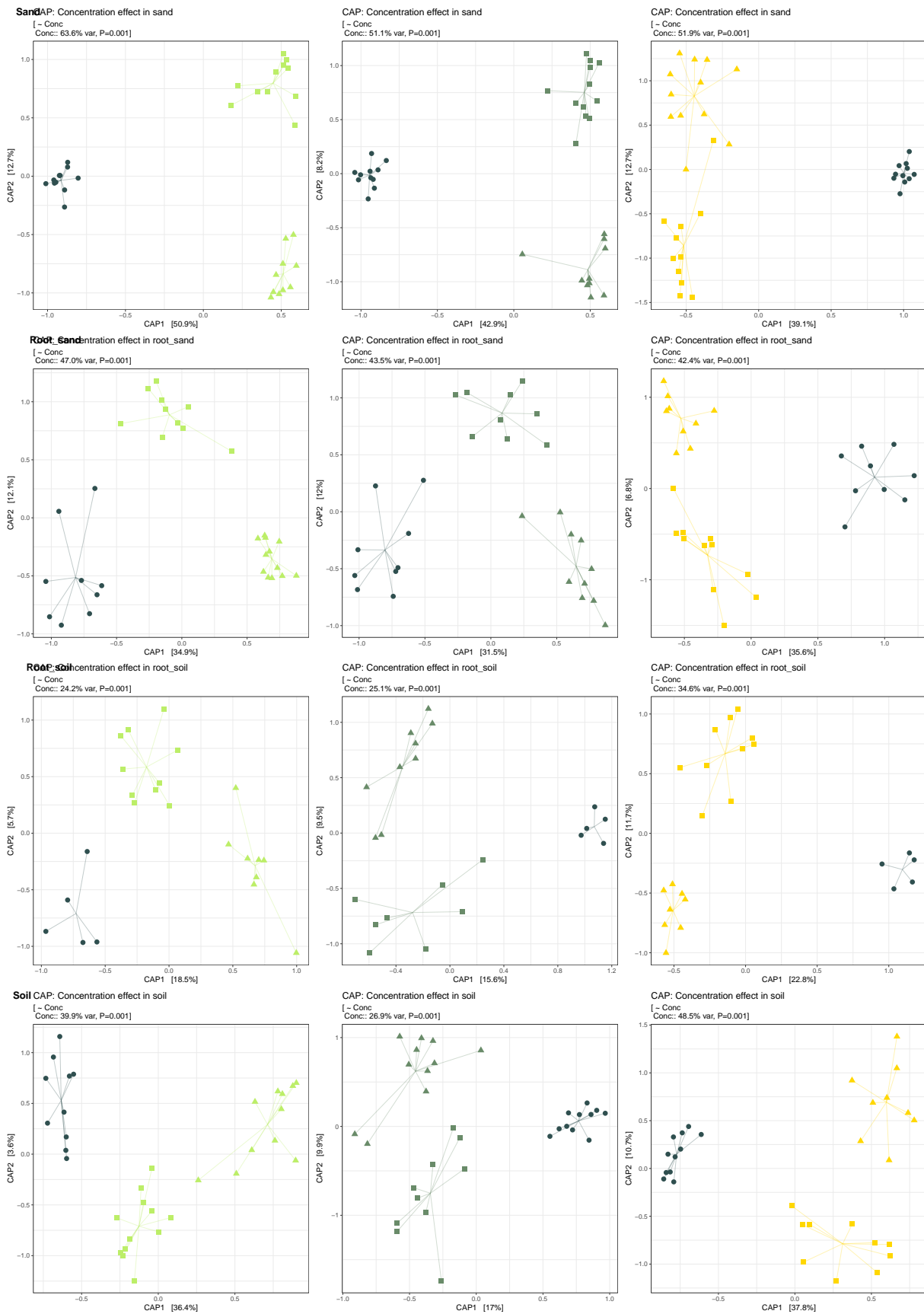


Figure 5.2.2 | CAP - Concentration effect on beta diversity



Conclusion: The bacterial communities differs between the different concentrations in all substrate. The strongest response could be detected in the sand where the concentration explains around 50% of variety within the different plastic treatments.

Conclusion beta diversity: *The microbiome between the different plastic treatments are very different in all four substrates. The plastic concentration has a big impact on the microbial community.*

Taxa Response

Is there a core of microbial taxa respond to the different plastic treatments? We searched sensitive ASVs – ASVs being differential abundant between CTRL samples and samples containing plastic. We followed the same structure as before and answer the question:

Q1: Are there sensitive ASVs between control and plastic-treated samples?

We answer the question by using for different tools to measure differential abundances - aldex2, acomb, maaslin2 and metagenomeSeq - and predict ASVs to be different if they were detected by 3 or more tools.

Relative abundance in sand

We check for each ASV if it is sensitive or not to the different plastics. We show how many sensitive ASVs we have found for each plastic-treatment. Then, we plot the top 50 most abundant ASVs and label significant differences in red. Down below we show the phyla and class of those top abundant sensitive ASVs.

Different abundant ASVs

We show how many ASVs has been changed due to the plastic treatments how much of the relative abundance belongs to those sensitive ASVs.

Table 44: microplastic effect in sand

lower in CTRL	unchanged	higher in CTRL
159	315	336

76.16% of the bacterial community in sand was changed in abundance due to microplastic.

Table 45: macroplastic effect in sand

lower in CTRL	unchanged	higher in CTRL
152	427	290

67.6% of the bacterial community in sand was changed in abundance due to macroplastic.

Table 46: starch effect in sand

lower in CTRL	unchanged	higher in CTRL
97	411	283

61.8% of the bacterial community in sand was changed in abundance due to starch.

We plot the 50 top abundant ASVs (in controls) and label plastic sensitive ASVs in red.



Taxonomy of abundant ASVs

We show the taxonomy of top abundant ASVs which are plastic sensitive.

Table 47: Top abundant ASVs sensitive to microplastic in sand

ASV	phylum	class	abu CTRL	abu trt
ASV5	Proteobacteria	Gammaproteobacteria	6.9%	8.96%
ASV3	Proteobacteria	Gammaproteobacteria	4.67%	1.18%
ASV6	Bacteroidetes	Bacteroidia	3.44%	11.3%
ASV15	Proteobacteria	Alphaproteobacteria	2.06%	2.34%
ASV51	Chloroflexi	Anaerolineae	1.94%	0.34%
ASV36	Proteobacteria	Gammaproteobacteria	1.74%	2.52%
ASV8	Bacteroidetes	Bacteroidia	1.18%	1%
ASV58	Firmicutes	Clostridia	1.11%	0.11%
ASV17	Proteobacteria	Gammaproteobacteria	1.07%	0.17%
ASV32	Proteobacteria	Gammaproteobacteria	0.85%	1.19%
ASV4	Proteobacteria	Gammaproteobacteria	0.83%	2.27%
ASV71	Firmicutes	Bacilli	0.72%	0.87%
ASV28	Proteobacteria	Alphaproteobacteria	0.68%	1.26%
ASV244	Proteobacteria	Gammaproteobacteria	0.66%	0.08%
ASV88	Proteobacteria	Gammaproteobacteria	0.65%	0.97%
ASV123	Actinobacteria	Actinobacteria	0.63%	0.18%
ASV100	Actinobacteria	Actinobacteria	0.61%	0.1%
ASV22	Proteobacteria	Alphaproteobacteria	0.61%	1.08%
ASV136	Proteobacteria	Alphaproteobacteria	0.55%	0.33%
ASV62	Proteobacteria	Gammaproteobacteria	0.5%	0.06%
ASV54	Proteobacteria	Gammaproteobacteria	0.49%	1.28%
ASV322	Proteobacteria	Gammaproteobacteria	0.46%	0%
ASV39	Proteobacteria	Gammaproteobacteria	0.4%	3.03%
ASV23	Proteobacteria	Gammaproteobacteria	0.4%	0.06%
ASV61	Proteobacteria	Alphaproteobacteria	0.4%	0.37%
ASV151	Bacteroidetes	Bacteroidia	0.39%	0.08%
ASV134	Firmicutes	Bacilli	0.36%	0.58%
ASV187	Proteobacteria	Alphaproteobacteria	0.35%	0.04%
ASV116	Firmicutes	Bacilli	0.35%	0.17%
ASV98	Proteobacteria	Alphaproteobacteria	0.33%	0.12%
ASV238	Bacteroidetes	Bacteroidia	0.32%	0.07%
ASV70	Verrucomicrobia	Verrucomicrobiae	0.32%	1.04%
ASV73	Proteobacteria	Gammaproteobacteria	0.32%	0.83%
ASV188	Proteobacteria	Alphaproteobacteria	0.29%	0.04%

Table 48: Top abundant ASVs sensitive to macroplastic in sand

ASV	phylum	class	abu CTRL	abu trt
ASV5	Proteobacteria	Gammaproteobacteria	6.9%	6.48%
ASV3	Proteobacteria	Gammaproteobacteria	4.67%	0.84%
ASV6	Bacteroidetes	Bacteroidia	3.44%	2.79%
ASV51	Chloroflexi	Anaerolineae	1.94%	0.18%
ASV40	Proteobacteria	Gammaproteobacteria	1.52%	1.9%
ASV37	Actinobacteria	Actinobacteria	1.35%	3.4%
ASV17	Proteobacteria	Gammaproteobacteria	1.07%	0.06%
ASV32	Proteobacteria	Gammaproteobacteria	0.85%	2.16%
ASV4	Proteobacteria	Gammaproteobacteria	0.83%	1.31%
ASV28	Proteobacteria	Alphaproteobacteria	0.68%	3.95%
ASV244	Proteobacteria	Gammaproteobacteria	0.66%	0.22%
ASV88	Proteobacteria	Gammaproteobacteria	0.65%	1.02%
ASV123	Actinobacteria	Actinobacteria	0.63%	0.23%
ASV100	Actinobacteria	Actinobacteria	0.61%	0.1%
ASV22	Proteobacteria	Alphaproteobacteria	0.61%	0.71%
ASV62	Proteobacteria	Gammaproteobacteria	0.5%	0.04%
ASV108	Proteobacteria	Alphaproteobacteria	0.44%	1.58%
ASV23	Proteobacteria	Gammaproteobacteria	0.4%	0.19%
ASV61	Proteobacteria	Alphaproteobacteria	0.4%	0.41%
ASV151	Bacteroidetes	Bacteroidia	0.39%	0.03%
ASV187	Proteobacteria	Alphaproteobacteria	0.35%	0.03%
ASV35	Bacteroidetes	Bacteroidia	0.35%	0.05%
ASV238	Bacteroidetes	Bacteroidia	0.32%	0.03%
ASV70	Verrucomicrobia	Verrucomicrobiae	0.32%	1.69%
ASV73	Proteobacteria	Gammaproteobacteria	0.32%	0.86%
ASV188	Proteobacteria	Alphaproteobacteria	0.29%	0.06%
ASV24	Proteobacteria	Gammaproteobacteria	0.28%	0.63%
ASV128	Proteobacteria	Gammaproteobacteria	0.28%	1.35%
ASV97	Proteobacteria	Gammaproteobacteria	0.28%	0.8%

Table 49: Top abundant ASVs sensitive to starch in sand

ASV	phylum	class	abu CTRL	abu trt
ASV3	Proteobacteria	Gammaproteobacteria	4.67%	1.29%
ASV6	Bacteroidetes	Bacteroidia	3.44%	7.55%
ASV31	Firmicutes	Clostridia	3.39%	3.38%
ASV55	Firmicutes	Clostridia	2.76%	1.26%
ASV15	Proteobacteria	Alphaproteobacteria	2.06%	3.54%
ASV51	Chloroflexi	Anaerolineae	1.94%	0.59%
ASV40	Proteobacteria	Gammaproteobacteria	1.52%	0.07%
ASV8	Bacteroidetes	Bacteroidia	1.18%	4.9%
ASV58	Firmicutes	Clostridia	1.11%	2.92%
ASV17	Proteobacteria	Gammaproteobacteria	1.07%	0.22%
ASV32	Proteobacteria	Gammaproteobacteria	0.85%	0.72%
ASV4	Proteobacteria	Gammaproteobacteria	0.83%	0.49%
ASV65	Proteobacteria	Gammaproteobacteria	0.8%	0.04%
ASV71	Firmicutes	Bacilli	0.72%	1.79%
ASV28	Proteobacteria	Alphaproteobacteria	0.68%	0.24%
ASV244	Proteobacteria	Gammaproteobacteria	0.66%	0%
ASV88	Proteobacteria	Gammaproteobacteria	0.65%	0.01%
ASV123	Actinobacteria	Actinobacteria	0.63%	0.11%
ASV100	Actinobacteria	Actinobacteria	0.61%	0.12%
ASV22	Proteobacteria	Alphaproteobacteria	0.61%	3.19%
ASV62	Proteobacteria	Gammaproteobacteria	0.5%	0.1%
ASV322	Proteobacteria	Gammaproteobacteria	0.46%	0%
ASV23	Proteobacteria	Gammaproteobacteria	0.4%	0.11%
ASV61	Proteobacteria	Alphaproteobacteria	0.4%	0.73%
ASV151	Bacteroidetes	Bacteroidia	0.39%	0.17%
ASV77	Proteobacteria	Alphaproteobacteria	0.37%	0.39%
ASV134	Firmicutes	Bacilli	0.36%	0.64%
ASV187	Proteobacteria	Alphaproteobacteria	0.35%	0.07%
ASV116	Firmicutes	Bacilli	0.35%	0.78%
ASV98	Proteobacteria	Alphaproteobacteria	0.33%	1.1%
ASV238	Bacteroidetes	Bacteroidia	0.32%	0.12%
ASV147	Verrucomicrobia	Verrucomicrobiae	0.32%	0.06%
ASV73	Proteobacteria	Gammaproteobacteria	0.32%	0.64%
ASV150	Verrucomicrobia	Verrucomicrobiae	0.32%	0.1%
ASV149	Bacteroidetes	Bacteroidia	0.3%	0.07%

Sensitive Taxonomies

We plot how many ASVs are sensitive to multiple plastic treatments and show frequent phyla, classes and families which are sensitive to all three plastic treatments.

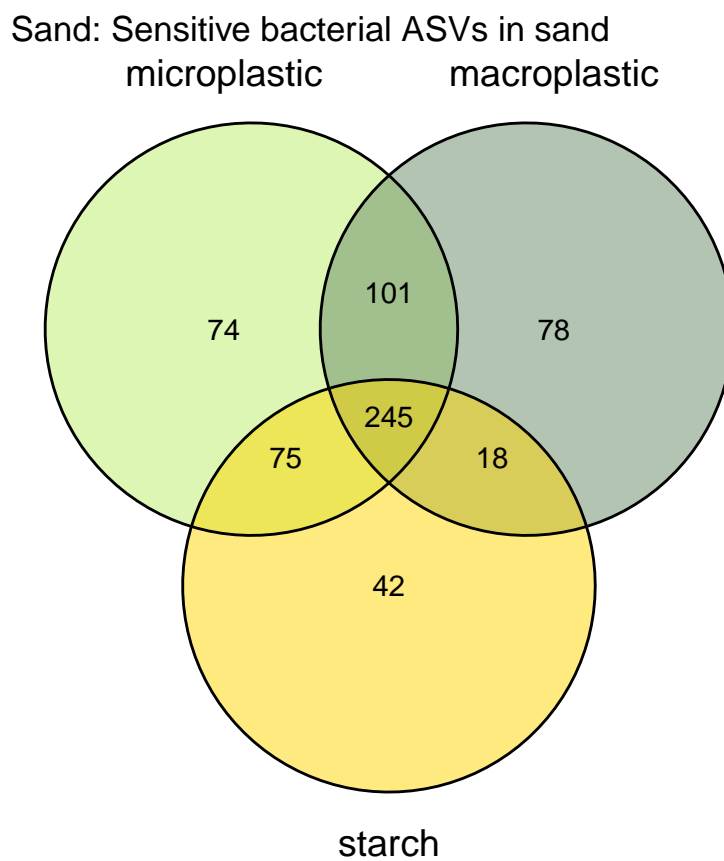


Table 50: Frequent phyla sensitive to all plastic treatments in sand

phylum	frequency
Proteobacteria	128
Bacteroidetes	18
Firmicutes	16
Actinobacteria	12
Chloroflexi	12
Planctomycetes	12
Euryarchaeota	9
Gemmatimonadetes	8
Verrucomicrobia	7
Acidobacteria	5

Table 51: Frequent classes sensitive to all plastic treatments in sand

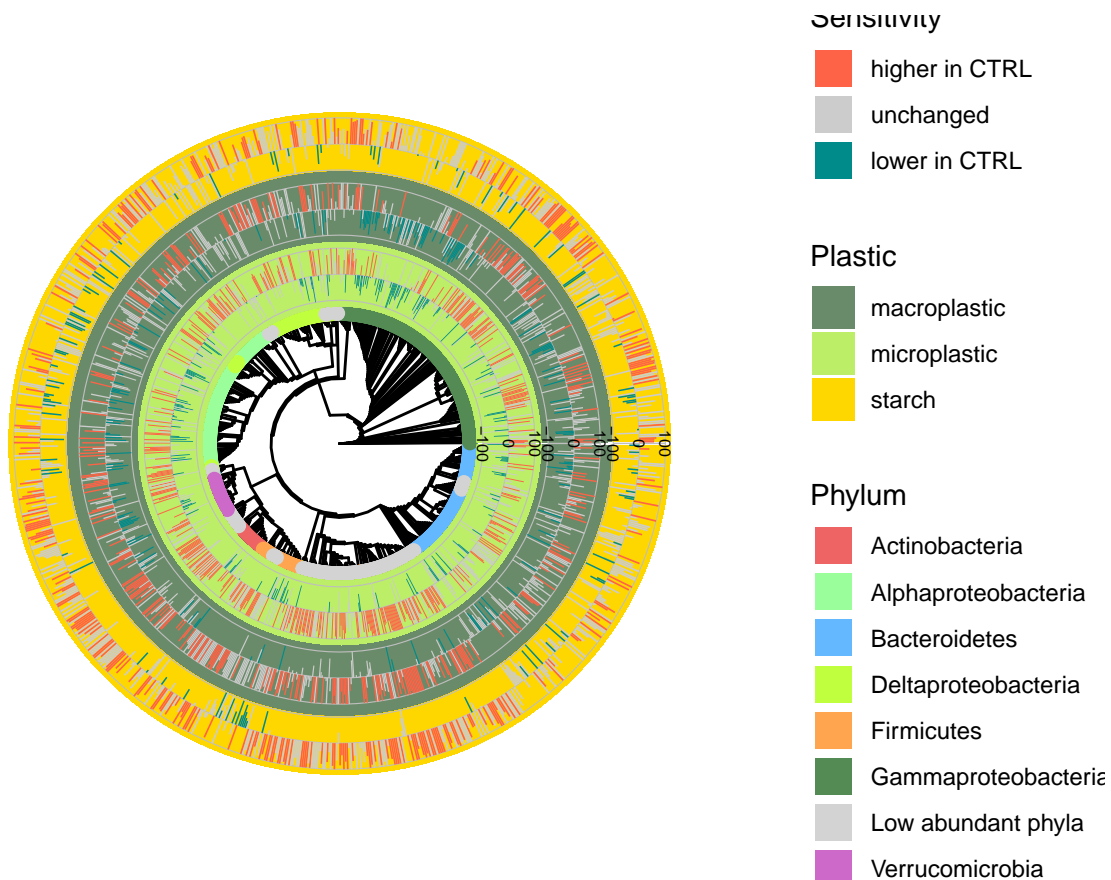
class	frequency
Gammaproteobacteria	63
Alphaproteobacteria	45
Deltaproteobacteria	20
Bacteroidia	17
Anaerolineae	10
Planctomycetacia	9
Actinobacteria	8
Clostridia	8
Bacilli	7
Thermoplasmata	7
Verrucomicrobiae	7
Gemmatimonadetes	6
unassigned	5

Table 52: Frequent families sensitive to all plastic treatments in sand

family	frequency
unassigned	38
Burkholderiaceae	15
Solimonadaceae	10
Rhizobiaceae	9
Sphingomonadaceae	8
Nitrosomonadaceae	7
Pirellulaceae	7
Caulobacteraceae	6
Chitinophagaceae	6
Gemmatimonadaceae	6
Polyangiaceae	5

Phylogenetic tree

We plot a phylogenetic tree with all ASVs tested for sensitivity and label with the rings around the tree if they are sensitive to microplastic, macroplastic and starch.



Conclusion differential abundance analysis: *A huge part of the microbiome is shaped by adding plastic to the soil. Microplastic, macroplastic and starch has a similar effect. Bacteria reacting to the plastic are phylogenetic diverse.*

Relative abundance in root sand

We check for each ASV if it is sensitive or not to the different plastics. We show how many sensitive ASVs we have found for each plastic-treatment. Then, we plot the top 50 most abundant ASVs and label significant differences in red. Down below we show the phyla and class of those top abundant sensitive ASVs.

Different abundant ASVs

We show how many ASVs has been changed due to the plastic treatments how much of the relative abundance belongs to those sensitive ASVs.

Table 53: microplastic effect in root_sand

lower in CTRL	unchanged	higher in CTRL
20	435	18

27.07% of the bacterial community in root was changed in abundance due to microplastic.

Table 54: macroplastic effect in root_sand

lower in CTRL	unchanged	higher in CTRL
22	479	22

27.12% of the bacterial community in root was changed in abundance due to macroplastic.

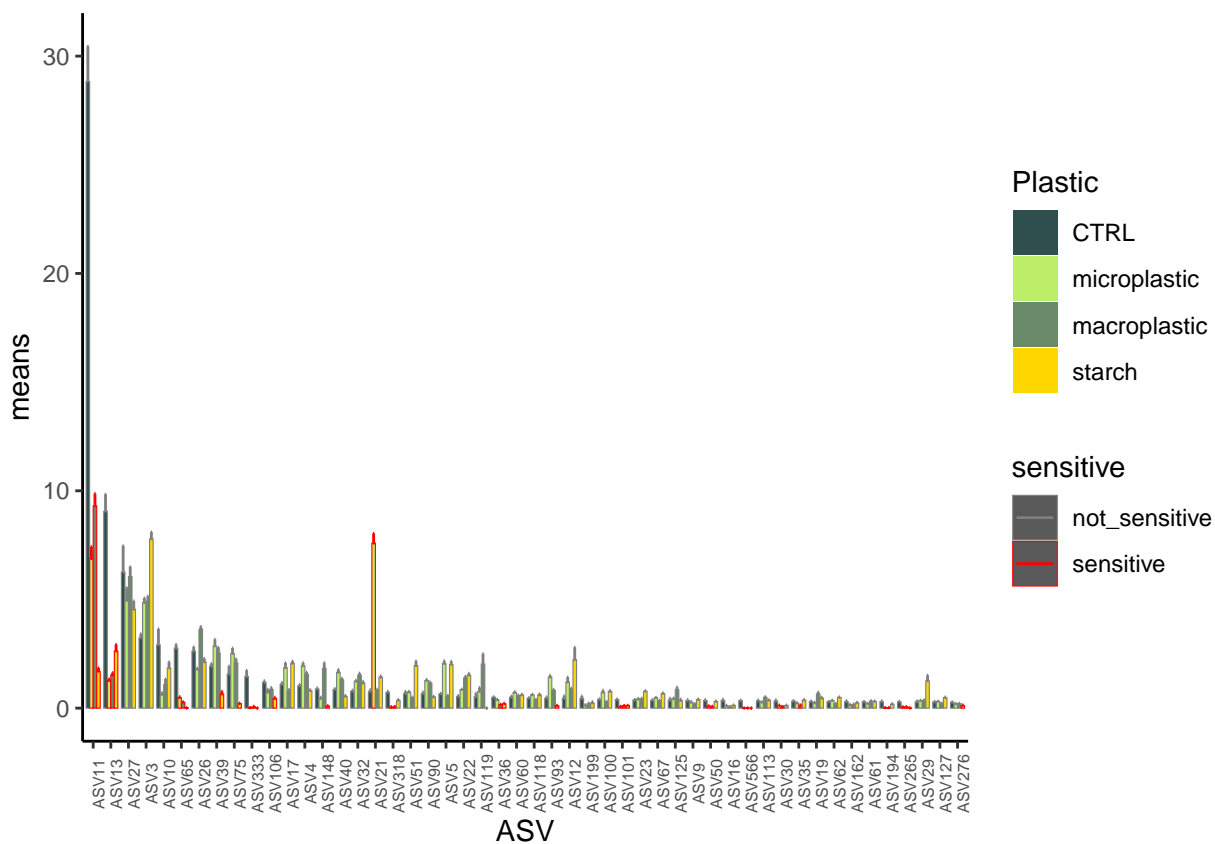
Table 55: starch effect in root_sand

lower in CTRL	unchanged	higher in CTRL
16	596	27

16.18% of the bacterial community in root_sand was changed in abundance due to starch.

Relative abundance plot

We plot the 50 top abundant ASVs (in controls) and label plastic sensitive ASVs in red.



Taxonomy of abundant ASVs

We show the taxonomy of top abundant ASVs which are plastic sensitive.

Table 56: Top abundant ASVs sensitive to microplastic in root_sand

ASV	phylum	class	abu CTRL	abu trt
ASV11	Proteobacteria	Gammaproteobacteria	28.83%	6.88%
ASV13	Proteobacteria	Gammaproteobacteria	9.06%	1.26%
ASV65	Proteobacteria	Gammaproteobacteria	2.76%	0.48%
ASV333	Firmicutes	Bacilli	1.46%	0.02%
ASV21	Proteobacteria	Gammaproteobacteria	0.75%	7.59%
ASV318	Proteobacteria	Gammaproteobacteria	0.72%	0.05%
ASV101	Proteobacteria	Gammaproteobacteria	0.39%	0.08%
ASV50	Proteobacteria	Gammaproteobacteria	0.35%	0.1%
ASV566	Proteobacteria	Alphaproteobacteria	0.34%	0%
ASV30	Proteobacteria	Gammaproteobacteria	0.33%	0.12%
ASV194	Proteobacteria	Gammaproteobacteria	0.29%	0.02%
ASV265	Proteobacteria	Gammaproteobacteria	0.29%	0.05%

Table 57: Top abundant ASVs sensitive to macroplastic in
root_sand

ASV	phylum	class	abu CTRL	abu trt
ASV11	Proteobacteria	Gammaproteobacteria	28.83%	9.31%
ASV13	Proteobacteria	Gammaproteobacteria	9.06%	1.53%
ASV65	Proteobacteria	Gammaproteobacteria	2.76%	0.27%
ASV333	Firmicutes	Bacilli	1.46%	0.06%
ASV318	Proteobacteria	Gammaproteobacteria	0.72%	0.06%
ASV36	Proteobacteria	Gammaproteobacteria	0.5%	0.16%
ASV101	Proteobacteria	Gammaproteobacteria	0.39%	0.12%
ASV50	Proteobacteria	Gammaproteobacteria	0.35%	0.08%
ASV566	Proteobacteria	Alphaproteobacteria	0.34%	0%
ASV30	Proteobacteria	Gammaproteobacteria	0.33%	0.07%
ASV35	Bacteroidetes	Bacteroidia	0.32%	0.13%
ASV194	Proteobacteria	Gammaproteobacteria	0.29%	0.02%
ASV265	Proteobacteria	Gammaproteobacteria	0.29%	0.06%

Table 58: Top abundant ASVs sensitive to starch in root_sand

ASV	phylum	class	abu CTRL	abu trt
ASV11	Proteobacteria	Gammaproteobacteria	28.83%	1.69%
ASV13	Proteobacteria	Gammaproteobacteria	9.06%	2.62%
ASV65	Proteobacteria	Gammaproteobacteria	2.76%	0.01%
ASV39	Proteobacteria	Gammaproteobacteria	1.93%	0.64%
ASV75	Proteobacteria	Gammaproteobacteria	1.57%	0.21%
ASV333	Firmicutes	Bacilli	1.46%	0%
ASV106	Bacteroidetes	Bacteroidia	1.18%	0.44%
ASV148	Proteobacteria	Gammaproteobacteria	0.87%	0.09%
ASV36	Proteobacteria	Gammaproteobacteria	0.5%	0.2%
ASV93	Proteobacteria	Gammaproteobacteria	0.45%	0.11%
ASV101	Proteobacteria	Gammaproteobacteria	0.39%	0.12%
ASV566	Proteobacteria	Alphaproteobacteria	0.34%	0%
ASV265	Proteobacteria	Gammaproteobacteria	0.29%	0.01%
ASV276	Proteobacteria	Gammaproteobacteria	0.27%	0.12%

Sensitive Taxonomies

We plot how many ASVs are sensitive to multiple plastic treatments and show frequent phyla, classes and families which are sensitive to all three plastic treatments.

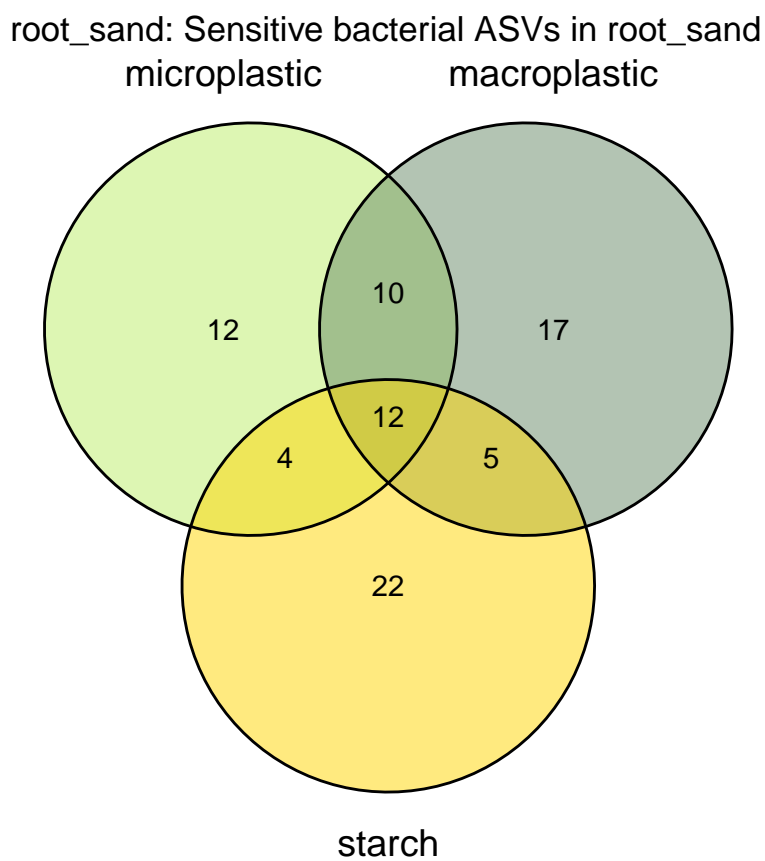


Table 59: Frequent phyla sensitive to all plastic treatments in root_sand

phylum	frequency
Proteobacteria	10

Table 60: Frequent classes sensitive to all plastic treatments in root_sand

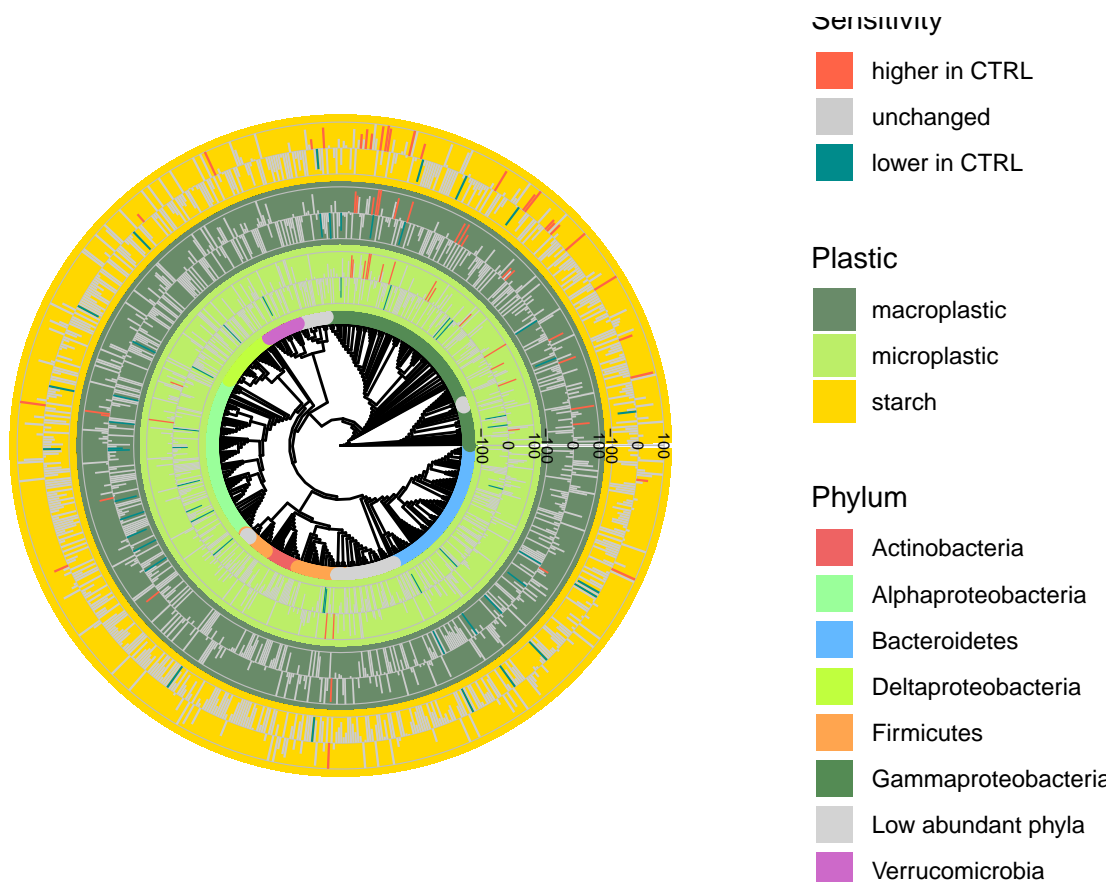
class	frequency
Gammaproteobacteria	8

Table 61: Frequent families sensitive to all plastic treatments in root_sand

family	frequency
Burkholderiaceae	6

Phylogenetic tree

We plot a phylogenetic tree with all ASVs tested for sensitivity and label with the rings around the tree if they are sensitive to microplastic, macroplastic and starch.



Relative abundance in root soil

We check for each ASV if it is sensitive or not to the different plastics. We show how many sensitive ASVs we have found for each plastic-treatment. Then, we plot the top 50 most abundant ASVs and label significant differences in red. Down below we show the phyla and class of those top abundant sensitive ASVs.

Different abundant ASVs

We show how many ASVs has been changed due to the plastic treatments how much of the relative abundance belongs to those sensitive ASVs.

Table 62: microplastic effect in root_soil

lower in CTRL	unchanged	higher in CTRL
6	475	3

1.95% of the bacterial community in root was changed in abundance due to microplastic.

Table 63: macroplastic effect in root_soil

lower in CTRL	unchanged	higher in CTRL
7	436	29

7.54% of the bacterial community in root was changed in abundance due to macroplastic.

Table 64: starch effect in root_soil

lower in CTRL	unchanged	higher in CTRL
17	462	27

25.87% of the bacterial community in root_soil was changed in abundance due to starch.

We plot the 50 top abundant ASVs (in controls) and label plastic sensitive ASVs in red.



Taxonomy of abundant ASVs

We show the taxonomy of top abundant ASVs which are plastic sensitive.

Table 65: Top abundant ASVs sensitive to microplastic in root_soil

ASV	phylum	class	abu CTRL	abu trt
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Table 66: Top abundant ASVs sensitive to macroplastic in root_soil

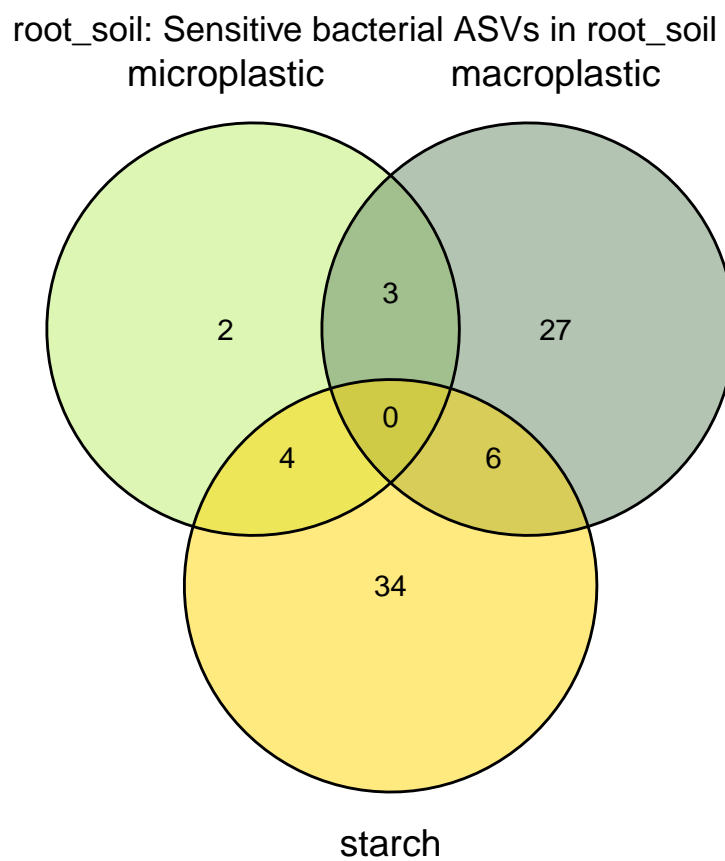
ASV	phylum	class	abu CTRL	abu trt
ASV101	Proteobacteria	Gammaproteobacteria	1.7%	0.3%
ASV67	Bacteroidetes	Bacteroidia	0.98%	0.42%
ASV127	Bacteroidetes	Bacteroidia	0.8%	0.29%
ASV62	Proteobacteria	Gammaproteobacteria	0.7%	0.23%
ASV18	Bacteroidetes	Bacteroidia	0.57%	0.11%
ASV35	Bacteroidetes	Bacteroidia	0.46%	0.17%

Table 67: Top abundant ASVs sensitive to starch in root_soil

ASV	phylum	class	abu CTRL	abu trt
ASV10	Proteobacteria	Gammaproteobacteria	9.94%	2.2%
ASV44	Proteobacteria	Gammaproteobacteria	9%	1.03%
ASV4	Proteobacteria	Gammaproteobacteria	3.19%	1.5%
ASV111	Bacteroidetes	Bacteroidia	2.08%	0.44%
ASV161	Proteobacteria	Gammaproteobacteria	1.76%	0.07%
ASV173	Proteobacteria	Gammaproteobacteria	1.6%	0%
ASV172	Firmicutes	Bacilli	1.12%	0.31%
ASV182	Bacteroidetes	Bacteroidia	0.87%	0.2%
ASV127	Bacteroidetes	Bacteroidia	0.8%	0.24%
ASV38	Proteobacteria	Alphaproteobacteria	0.7%	0.48%
ASV14	Bacteroidetes	Bacteroidia	0.44%	3.46%

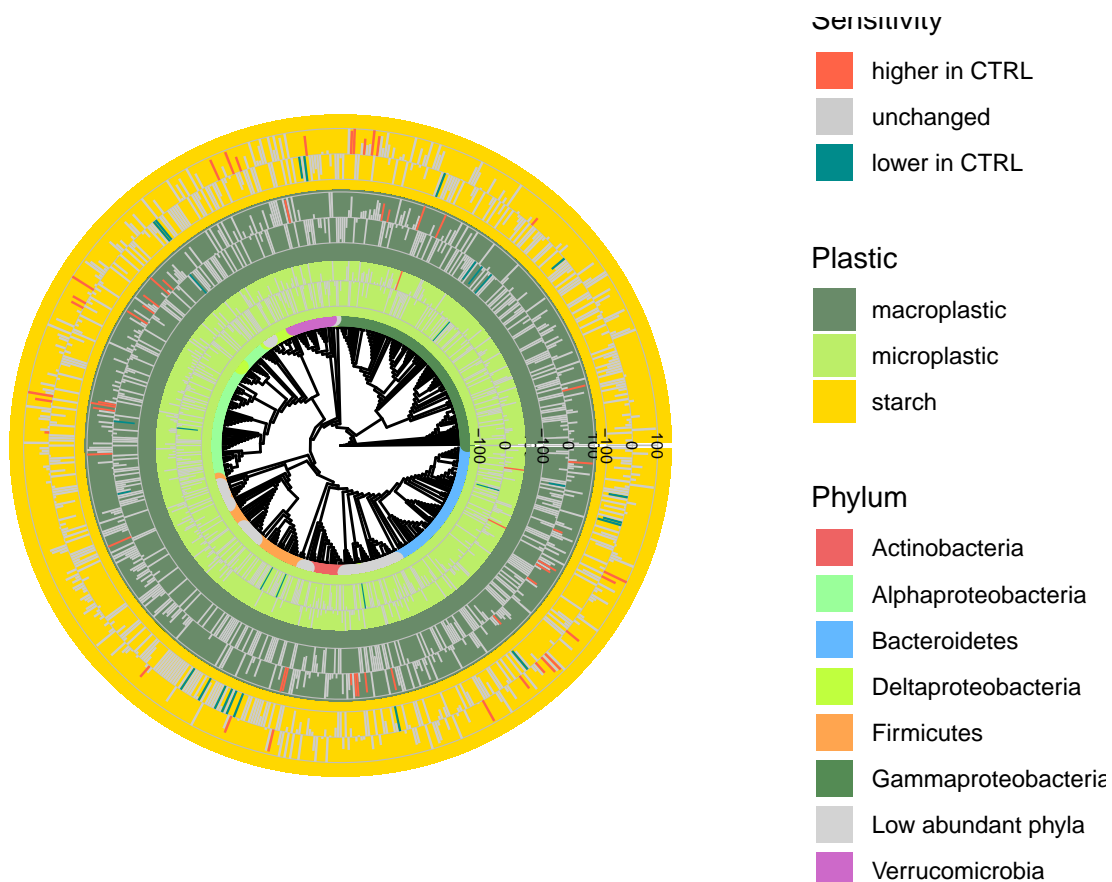
Sensitive Taxonomies

We plot how many ASVs are sensitive to multiple plastic treatments and show frequent phyla, classes and families which are sensitive to all three plastic treatments.



Phylogenetic tree

We plot a phylogenetic tree with all ASVs tested for sensitivity and label with the rings around the tree if they are sensitive to microplastic, macroplastic and starch.



Relative abundance in soil

We check for each ASV if it is sensitive or not to the different plastics. We show how many sensitive ASVs we have found for each plastic-treatment. Then, we plot the top 50 most abundant ASVs and label significant differences in red. Down below we show the phyla and class of those top abundant sensitive ASVs.

Different abundant ASVs

We show how many ASVs has been changed due to the plastic treatments how much of the relative abundance belongs to those sensitive ASVs.

Table 68: microplastic effect in soil

lower in CTRL	unchanged	higher in CTRL
39	558	42

38.35% of the bacterial community in soil was changed in abundance due to microplastic.

Table 69: macroplastic effect in soil

lower in CTRL	unchanged	higher in CTRL
16	603	33

18.03% of the bacterial community in soil was changed in abundance due to macroplastic.

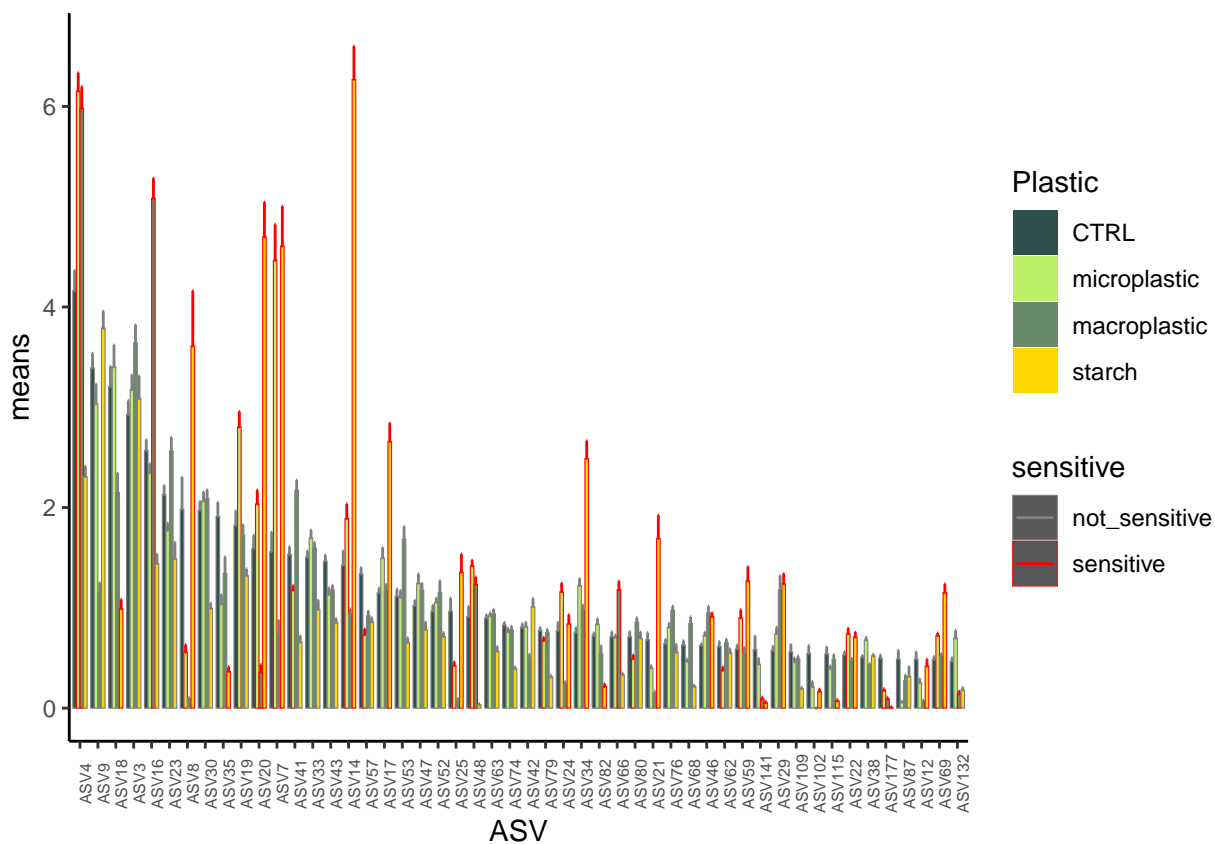
Table 70: starch effect in soil

lower in CTRL	unchanged	higher in CTRL
63	373	125

54.69% of the bacterial community in soil was changed in abundance due to starch.

Relative abundance plot

We plot the 50 top abundant ASVs (in controls) and label plastic sensitive ASVs in red.



Taxonomy of abundant ASVs

We show the taxonomy of top abundant ASVs which are plastic sensitive.

Table 71: Top abundant ASVs sensitive to microplastic in soil

ASV	phylum	class	abu CTRL	abu trt
ASV4	Proteobacteria	Gammaproteobacteria	4.16%	6.15%
ASV8	Bacteroidetes	Bacteroidia	1.98%	0.56%
ASV19	Proteobacteria	Gammaproteobacteria	1.82%	2.8%
ASV20	Bacteroidetes	Bacteroidia	1.59%	2.04%
ASV7	Bacteroidetes	Bacteroidia	1.56%	4.46%
ASV41	Proteobacteria	Gammaproteobacteria	1.53%	1.18%
ASV14	Bacteroidetes	Bacteroidia	1.42%	1.89%
ASV57	Proteobacteria	Gammaproteobacteria	1.34%	0.73%
ASV25	Bacteroidetes	Bacteroidia	0.97%	0.42%
ASV48	Proteobacteria	Gammaproteobacteria	0.91%	1.42%
ASV79	Firmicutes	Bacilli	0.77%	0.67%
ASV24	Proteobacteria	Gammaproteobacteria	0.77%	1.16%
ASV80	Bacteroidetes	Bacteroidia	0.71%	0.49%
ASV62	Proteobacteria	Gammaproteobacteria	0.62%	0.38%
ASV59	Bacteroidetes	Bacteroidia	0.59%	0.9%
ASV22	Proteobacteria	Alphaproteobacteria	0.53%	0.74%
ASV177	Bacteroidetes	Bacteroidia	0.5%	0.18%
ASV69	Firmicutes	Bacilli	0.48%	0.72%

Table 72: Top abundant ASVs sensitive to macroplastic in soil

ASV	phylum	class	abu CTRL	abu trt
ASV4	Proteobacteria	Gammaproteobacteria	4.16%	5.98%
ASV16	Proteobacteria	Gammaproteobacteria	2.57%	5.08%
ASV20	Bacteroidetes	Bacteroidia	1.59%	0.36%
ASV48	Proteobacteria	Gammaproteobacteria	0.91%	1.23%
ASV66	Proteobacteria	Gammaproteobacteria	0.71%	1.18%
ASV141	Bacteroidetes	Bacteroidia	0.58%	0.09%
ASV177	Bacteroidetes	Bacteroidia	0.5%	0.09%
ASV132	Bacteroidetes	Bacteroidia	0.47%	0.14%

Table 73: Top abundant ASVs sensitive to starch in soil

ASV	phylum	class	abu CTRL	abu trt
ASV18	Bacteroidetes	Bacteroidia	3.21%	0.99%
ASV8	Bacteroidetes	Bacteroidia	1.98%	3.61%
ASV35	Bacteroidetes	Bacteroidia	1.91%	0.36%
ASV20	Bacteroidetes	Bacteroidia	1.59%	4.7%
ASV7	Bacteroidetes	Bacteroidia	1.56%	4.61%
ASV14	Bacteroidetes	Bacteroidia	1.42%	6.27%
ASV17	Proteobacteria	Gammaproteobacteria	1.15%	2.66%
ASV25	Bacteroidetes	Bacteroidia	0.97%	1.36%
ASV24	Proteobacteria	Gammaproteobacteria	0.77%	0.84%
ASV34	Bacteroidetes	Bacteroidia	0.76%	2.49%
ASV82	Actinobacteria	Actinobacteria	0.72%	0.21%
ASV21	Proteobacteria	Gammaproteobacteria	0.68%	1.69%
ASV46	Bacteroidetes	Bacteroidia	0.62%	0.91%
ASV59	Bacteroidetes	Bacteroidia	0.59%	1.27%
ASV141	Bacteroidetes	Bacteroidia	0.58%	0.05%
ASV29	Proteobacteria	Gammaproteobacteria	0.58%	1.24%
ASV102	Bacteroidetes	Bacteroidia	0.55%	0.16%
ASV115	Proteobacteria	Deltaproteobacteria	0.54%	0.07%
ASV22	Proteobacteria	Alphaproteobacteria	0.53%	0.71%
ASV177	Bacteroidetes	Bacteroidia	0.5%	0.01%
ASV12	Proteobacteria	Gammaproteobacteria	0.49%	0.42%
ASV69	Firmicutes	Bacilli	0.48%	1.15%

Sensitive Taxonomies

We plot how many ASVs are sensitive to multiple plastic treatments and show frequent phyla, classes and families which are sensitive to all three plastic treatments.

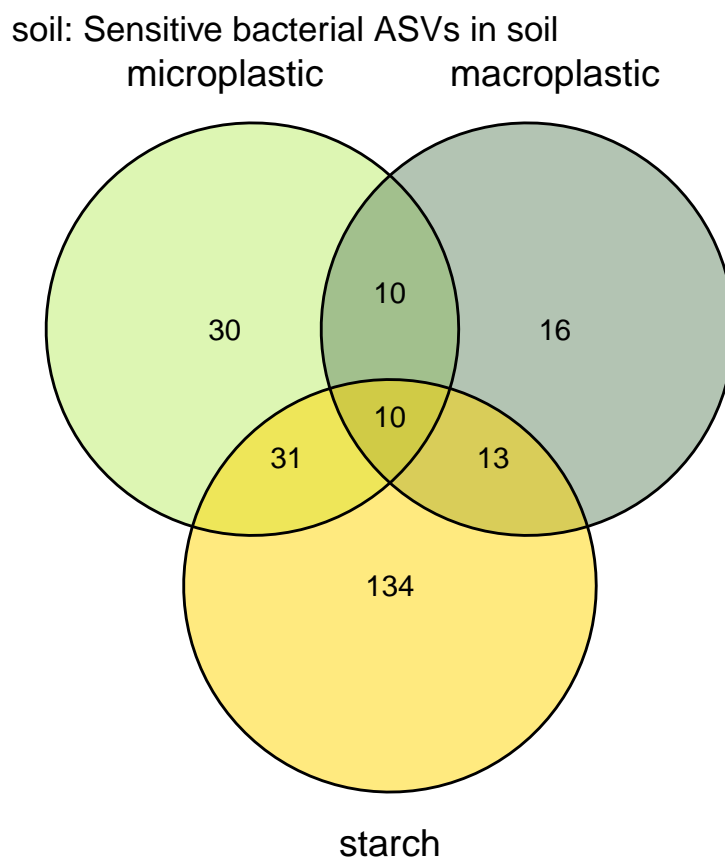


Table 74: Phyla sensitive to all plastic treatments in soil

phylum	frequency
Proteobacteria	5
Bacteroidetes	4
Verrucomicrobia	1

Table 75: Classes sensitive to all plastic treatments in soil

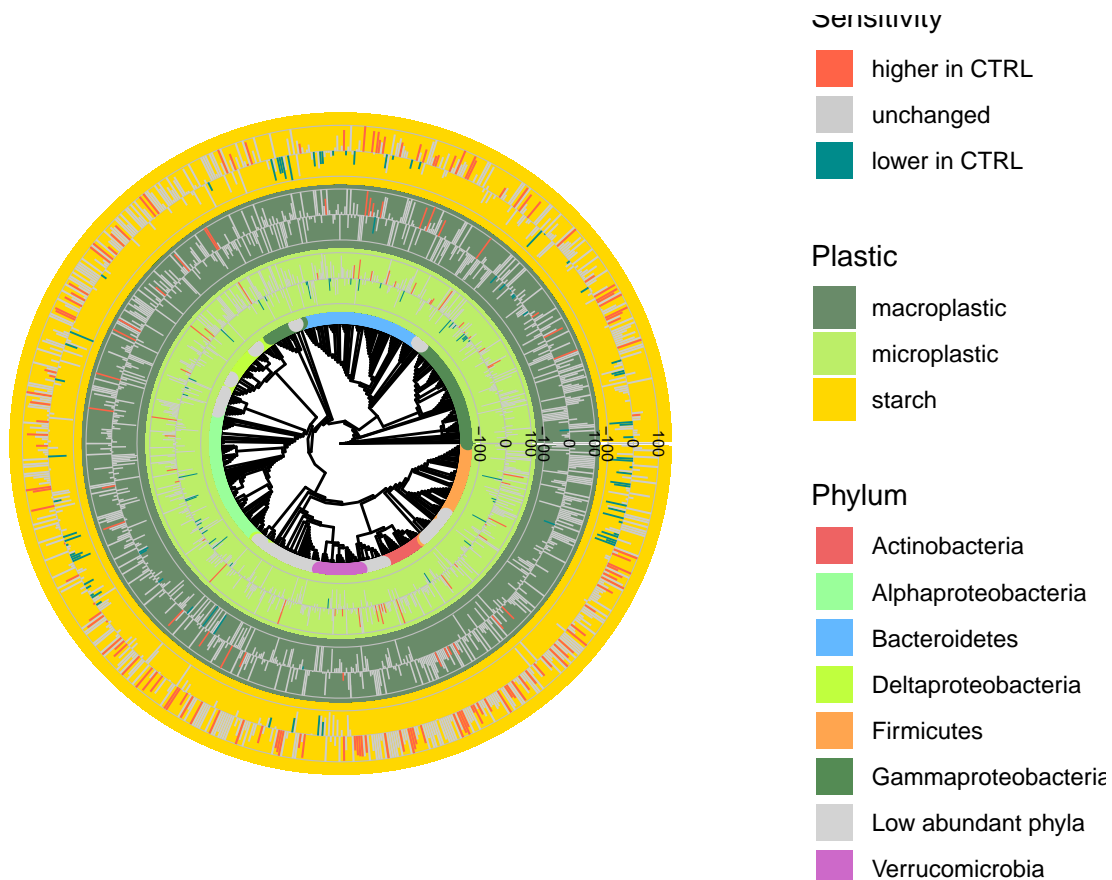
class	frequency
Bacteroidia	4
Alphaproteobacteria	3
Deltaproteobacteria	1
Gammaproteobacteria	1
Verrucomicrobiae	1

Table 76: Families sensitive to all plastic treatments in soil

family	frequency
Caulobacteraceae	1
Chitinophagaceae	1
Chthoniobacteraceae	1
Crocinitomicaceae	1
Devosiaceae	1
env.OPS_17	1
Hymenobacteraceae	1
Oligoflexaceae	1
TRA3-20	1
Xanthobacteraceae	1

Phylogenetic tree

We plot a phylogenetic tree with all ASVs tested for sensitivity and label with the rings around the tree if they are sensitive to microplastic, macroplastic and starch.



Conclusion differential abundance analysis: *A huge part of the microbiome is shaped by adding plastic to the soil. Microplastic, macroplastic and starch has a similar effect. Bacteria reacting to the plastic are phylogenetic diverse.*