

# Changins - Microbiome analysis

Jan Waelchli

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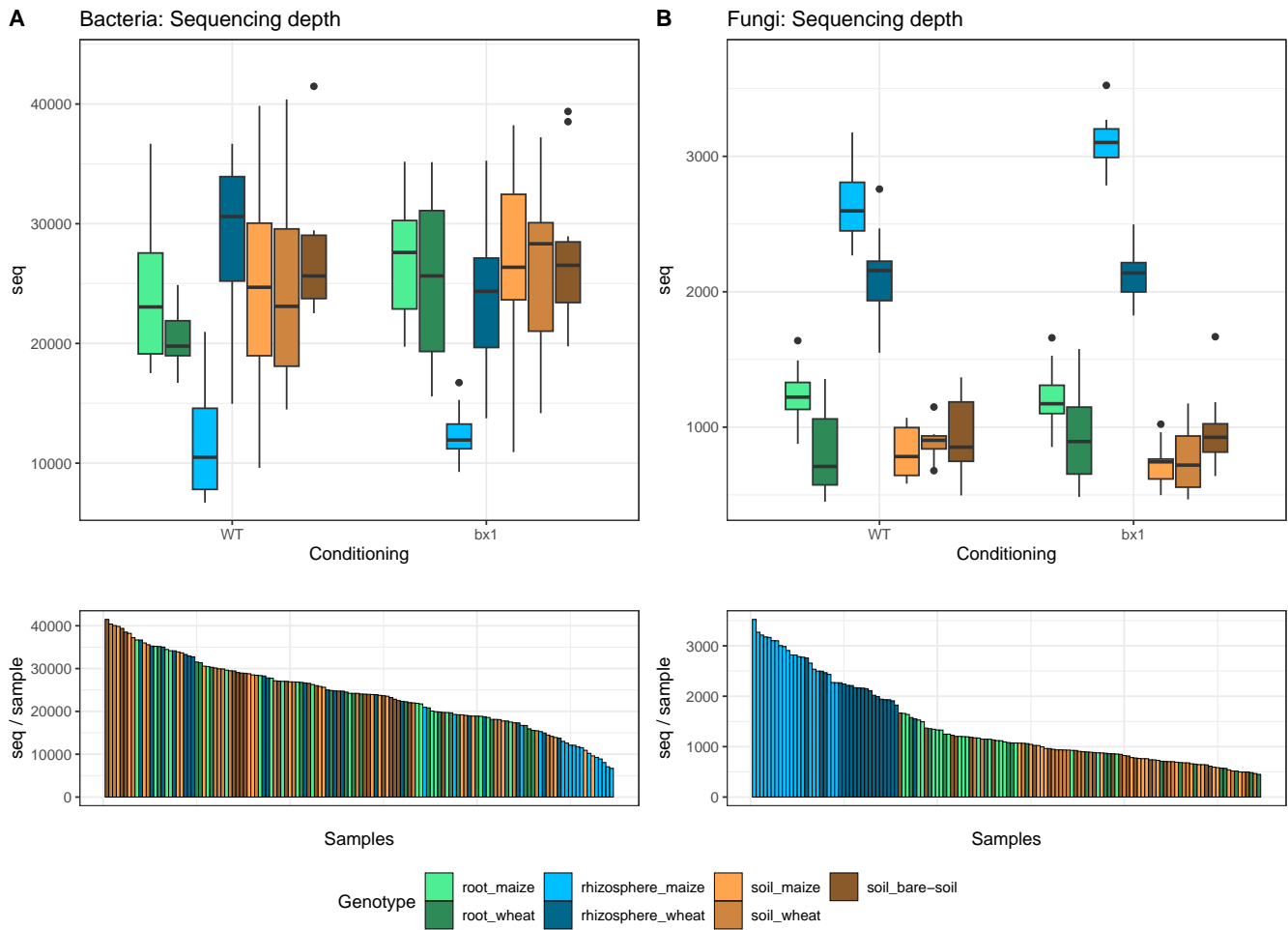
# Data description

## Sequencing depth

We removed 4 bacterial samples with less than 6700 sequences and 4 fungal samples with less than 450 sequences. We show the sum, range, median and total number of ASVs over all remaining samples.

taxa	sum	min	max	median	ASVs
Bacteria	3276744	6702	41478	24131	12069
Fungi	189461	450	3524	1088	1924

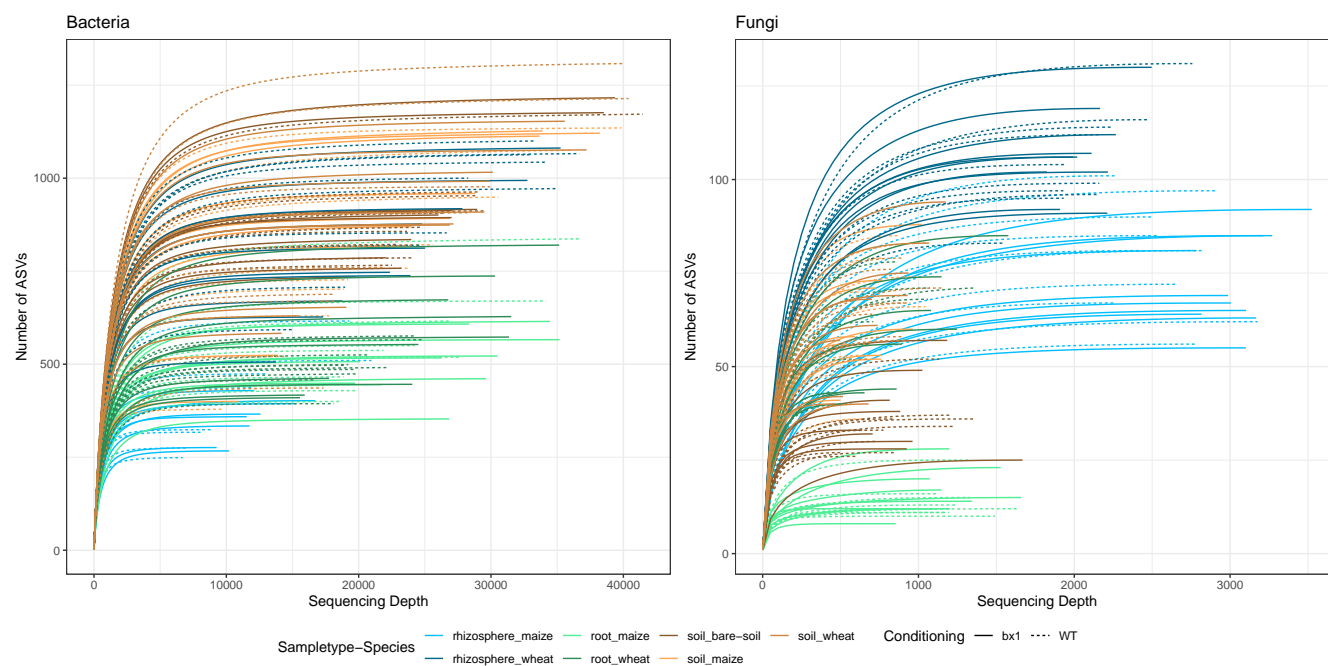
Figure 1 | Sequencing depth



## Rarefaction curve

We checked if the sequence depth is enough to capture the microbial diversity by plotting the rarefaction curve for each sample.

**Figure 2 | Rarefaction curve**



**Conclusion:** We have sequenced deep enough.

## Data 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 sample groups utilizing the non-parametric Kruskal-Wallis Test.

### Asymptotic Kruskal-Wallis test

We tested different sequencing depths for bacteria and fungi.

```
##
```

```
## Asymptotic Kruskal-Wallis Test
```

```
##
```

```
## data: colSums(bDAT) by
```

```
## bDESIGN$Sample_type_Species_Conditioning (rhizosphere_maize_bx1, rhizosphere_maize_WT, rhizosphere_maize_WT)
```

```
## chi-squared = 43.503, df = 13, p-value = 3.709e-05
```

```
##
```

```
## Asymptotic Kruskal-Wallis Test
```

```
##
```

```
## data: colSums(fDAT) by
```

```
## fDESIGN$Sample_type_Species_Conditioning (rhizosphere_maize_bx1, rhizosphere_maize_WT, rhizosphere_maize_WT)
```

```
## chi-squared = 101.53, df = 13, p-value = 8.882e-16
```

**Conclusion:** We found significant differences in sequencing depths between the different sample groups for bacteria and fungi. Therefore we rarefied the data for bacteria and fungi to equalize sequence differences! We defined the rarefaction threshold for bacteria to 6700 sequences per sample and for fungi to 450 sequences.

## Final number of samples

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

Table 2: Sample profile

Sample	Bacteria	Fungi
rhizosphere_maize_bx1	8	10
rhizosphere_maize_WT	8	10
rhizosphere_wheat_bx1	10	10
rhizosphere_wheat_WT	10	10
root_maize_bx1	10	10
root_maize_WT	10	10
root_wheat_bx1	10	9
root_wheat_WT	10	9
soil_bare-soil_bx1	10	9
soil_bare-soil_WT	10	10
soil_maize_bx1	10	10
soil_maize_WT	10	9
soil_wheat_bx1	10	10
soil_wheat_WT	10	10

# Taxonomy Overview

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

Figure 3.1 | Bacteria: Taxonomy overview

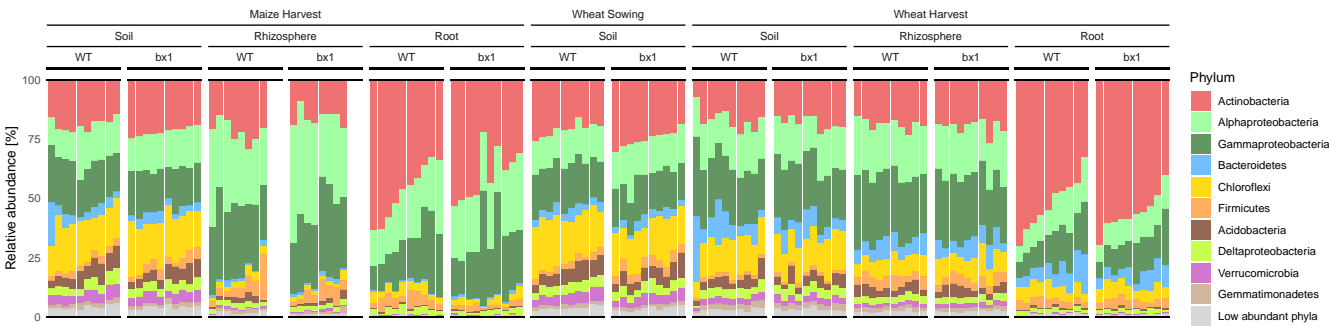
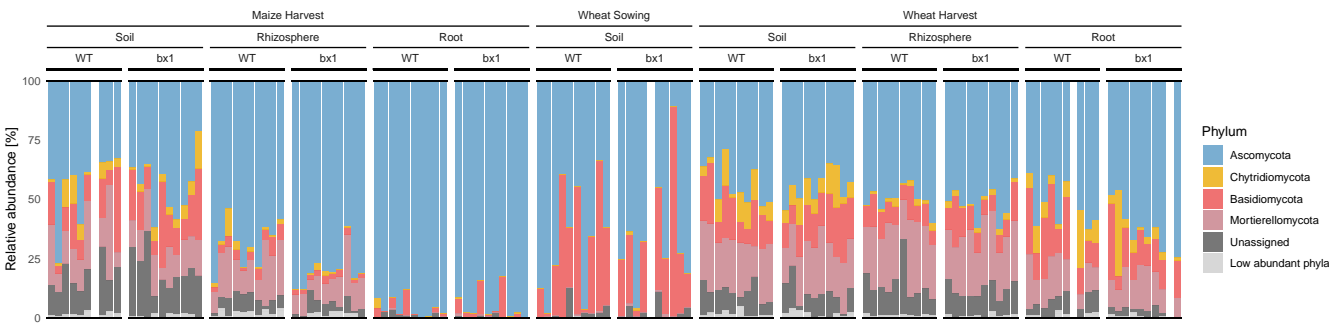


Figure 3.2 | Fungi: Taxonomy overview



## Data analysis - Maize harvest

Here we analysed the microbiome data which has been collected during maize harvesting.

### Soil chemistry PC1

We know from previous analyses that we have a soil-chemical gradient (soil chemistry PC1) along the field. We tested if the microbiome changes along soil chemistry PC1. We tested this effect with a PERMANOVA and illustrated it with a PCoA.

### PERMANOVA

*Model: ~ Sample\_type + Soil Chemistry PC1*

Table 3: Bacteria

	R2	Pr(>F)
<b>Sampletype</b>	0.3213	0.001
<b>Soil chemistry PC1</b>	0.163	0.001
<b>Residual</b>	0.5156	NA

Table 4: Fungi

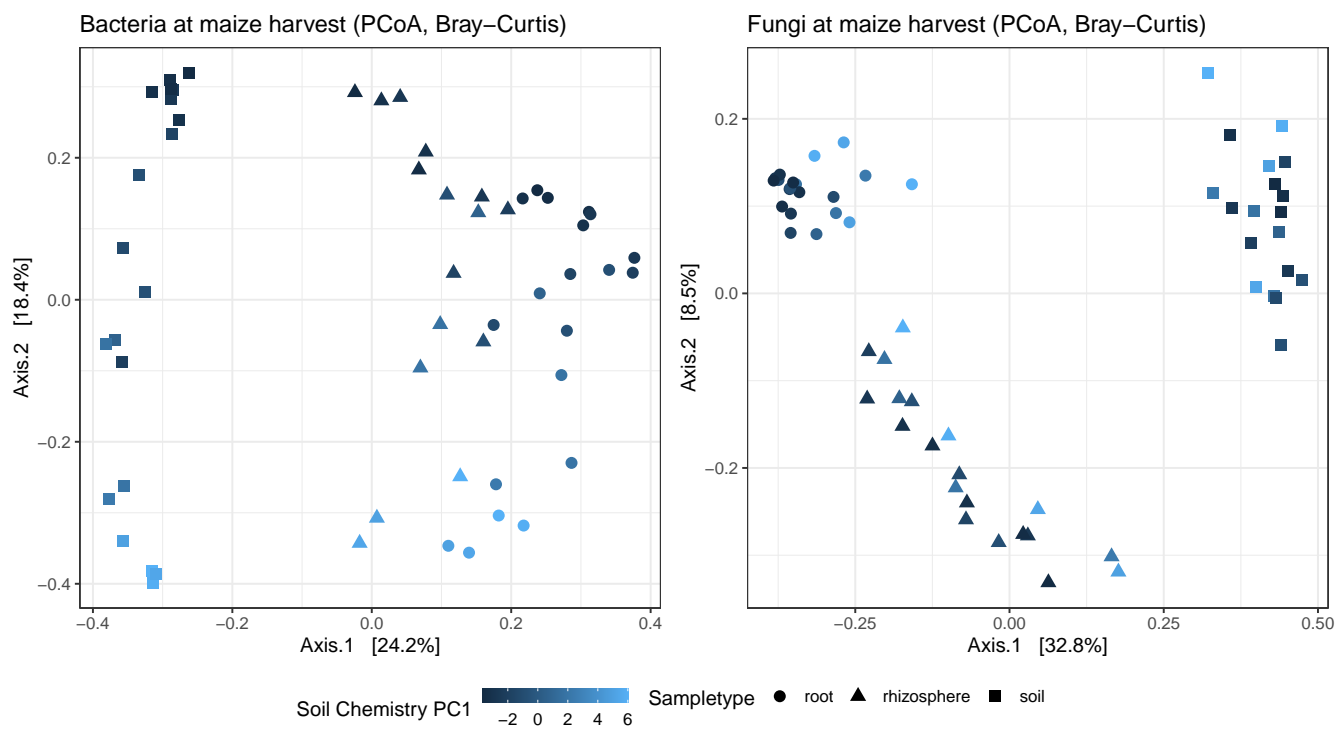
	R2	Pr(>F)
<b>Sampletype</b>	0.3803	0.001
<b>Soil chemistry PC1</b>	0.03033	0.012
<b>Residual</b>	0.5894	NA



## PCoA

We performed an unconstrained ordination with Bray-Curtis distances.

Figure 4 | PCoA with Bray-Curtis



## Correlation analysis: PCoA (microbiota) ~ Soil chemistry PC1

Do soil chemistry PC1 correlate with the beta diversity? We tested for the first two axes.

Figure 5.1 | Bacteria: Correlation (microbiota) ~ Soil chemistry PC1

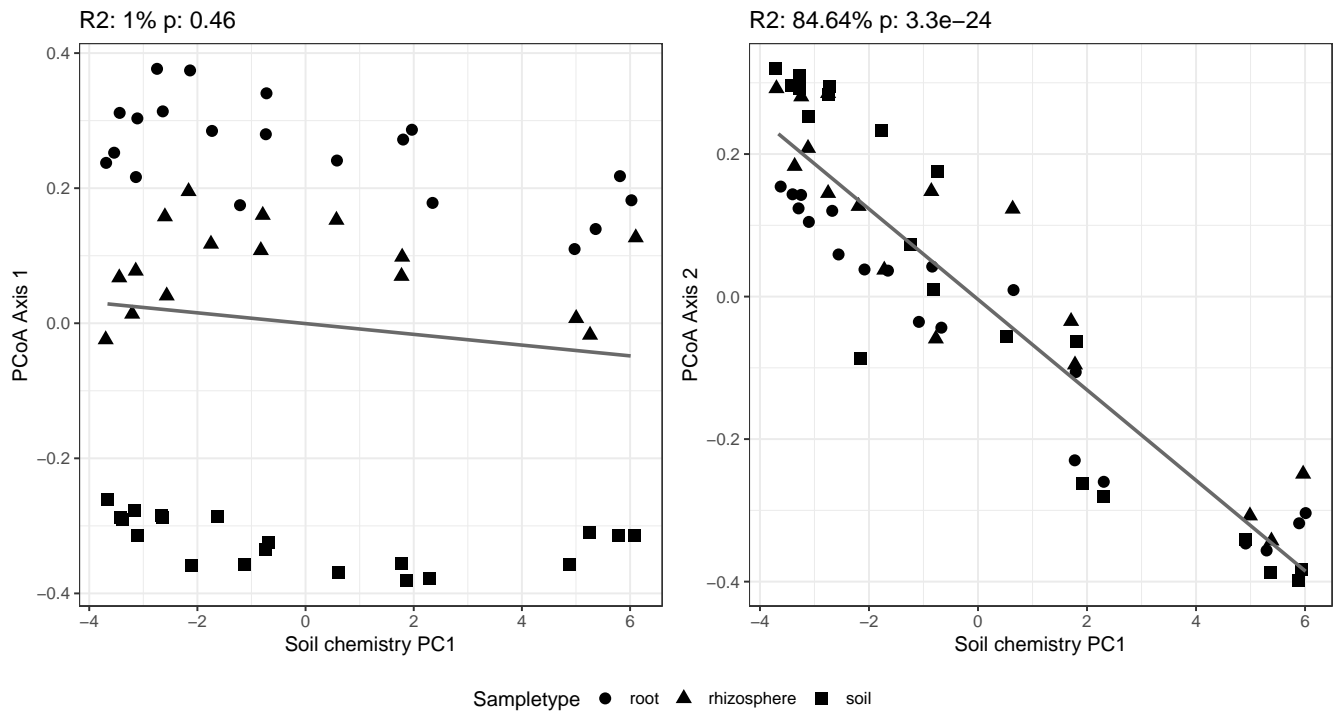
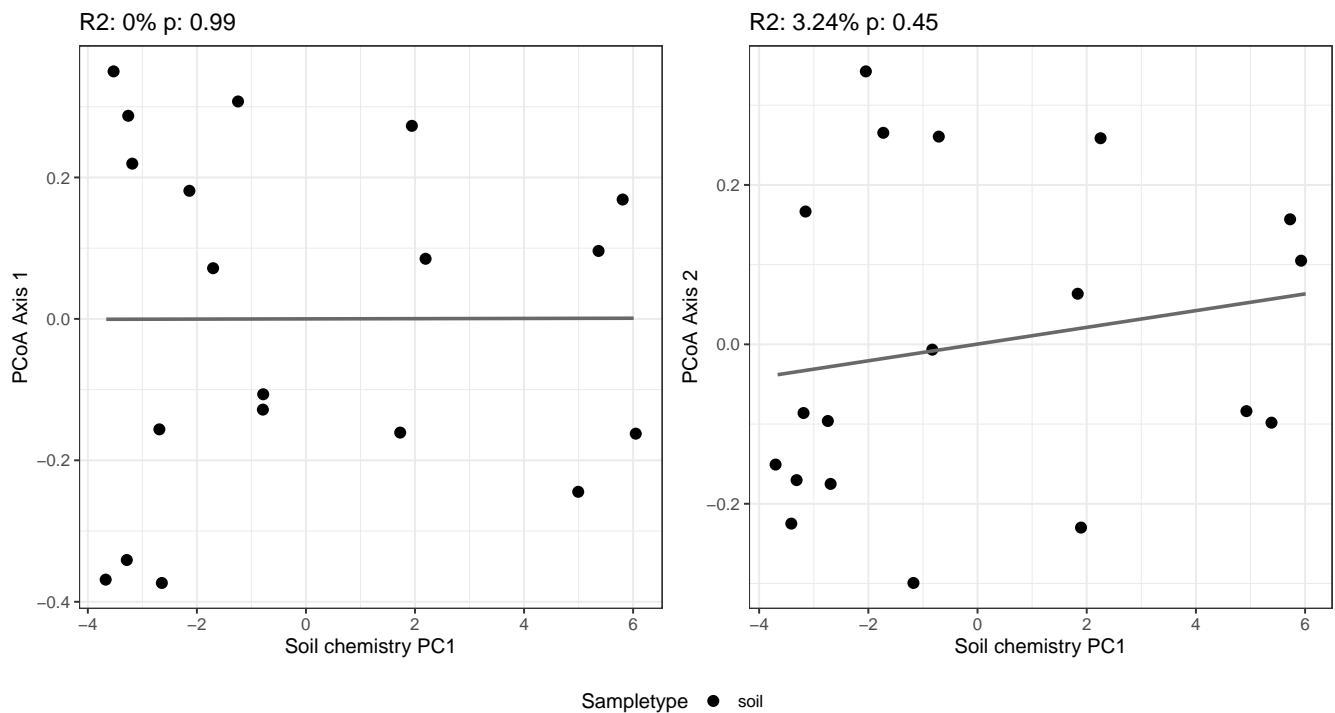


Figure 5.2 | Fungi: Correlation (microbiota) ~ Soil chemistry PC1



**Conclusion:** The microbiome is influenced by the soil chemistry PC1. To take soil chemistry PC1 into account, we included it as independent variable in all following models.

## Analysis of genotype effects - Alpha diversity

How did the genotypes affect the microbiome? We compared the root, rhizosphere and soil microbiomes of maize whether the microbial communities differ between the genotypes (WT, *bx1*). To measure the alpha diversity, we rarefied the data to the sequencing depth of 6700 (bacteria) and 450 (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.

### Anova statistics

*Model: ~ Genotype \* Soil chemistry PC1*

Table 5: Bacteria all compartments

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
<b>Genotype</b>	1	19051	19051	0.5718	0.453
<b>Soil chemistry PC1</b>	1	24558	24558	0.7371	0.3945
<b>Genotype : Soil chemistry PC1</b>	1	4544	4544	0.1364	0.7134
<b>Residuals</b>	52	1732525	33318	NA	NA

Table 6: Bacteria roots

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
<b>Genotype</b>	1	25391	25391	5.466	0.0327
<b>Soil chemistry PC1</b>	1	8470	8470	1.823	0.1957
<b>Genotype : Soil chemistry PC1</b>	1	1009	1009	0.2173	0.6474
<b>Residuals</b>	16	74324	4645	NA	NA

Table 7: Bacteria rhizosphere

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
<b>Genotype</b>	1	27461	27461	6.092	0.02959
<b>Soil chemistry PC1</b>	1	8027	8027	1.781	0.2068
<b>Genotype : Soil chemistry PC1</b>	1	1584	1584	0.3515	0.5643
<b>Residuals</b>	12	54092	4508	NA	NA

Table 8: Bacteria soil

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
<b>Genotype</b>	1	5868	5868	0.3755	0.5486
<b>Soil chemistry PC1</b>	1	15387	15387	0.9847	0.3358
<b>Genotype : Soil chemistry PC1</b>	1	1643	1643	0.1051	0.7499
<b>Residuals</b>	16	250015	15626	NA	NA

Table 9: Fungi all compartments

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
<b>Conditioning</b>	1	294.6	294.6	1.173	0.2835
<b>Soil chemistry PC1</b>	1	30.84	30.84	0.1228	0.7274
<b>Genotype : Soil chemistry PC1</b>	1	220.3	220.3	0.8773	0.353

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
<b>Residuals</b>	55	13812	251.1	NA	NA

Table 10: Fungi roots

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
<b>Genotype</b>	1	1.237	1.237	0.8502	0.3702
<b>Soil chemistry PC1</b>	1	1.225	1.225	0.8418	0.3725
<b>Genotype : Soil chemistry PC1</b>	1	3.196	3.196	2.196	0.1578
<b>Residuals</b>	16	23.29	1.455	NA	NA

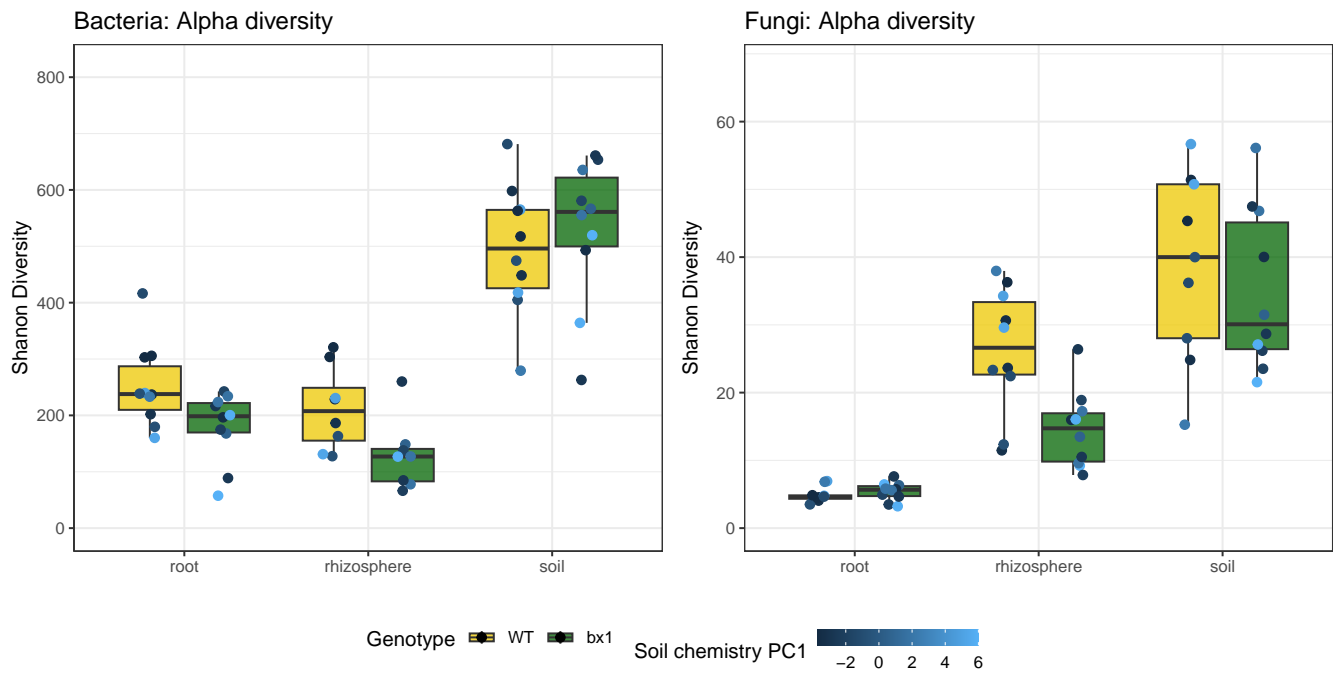
Table 11: Fungi rhizosphere

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
<b>Genotype</b>	1	682.2	682.2	11.62	0.003591
<b>Soil chemistry PC1</b>	1	15.01	15.01	0.2557	0.62
<b>Genotype : Soil chemistry PC1</b>	1	105.8	105.8	1.803	0.1981
<b>Residuals</b>	16	939.2	58.7	NA	NA

Table 12: Fungi soil

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
<b>Genotype</b>	1	69.29	69.29	0.3858	0.5438
<b>Soil chemistry PC1</b>	1	2.535	2.535	0.01412	0.907
<b>Genotype : Soil chemistry PC1</b>	1	111.6	111.6	0.6214	0.4428
<b>Residuals</b>	15	2694	179.6	NA	NA

**Figure 6 | Shannon diversity by sampletype and genotype**

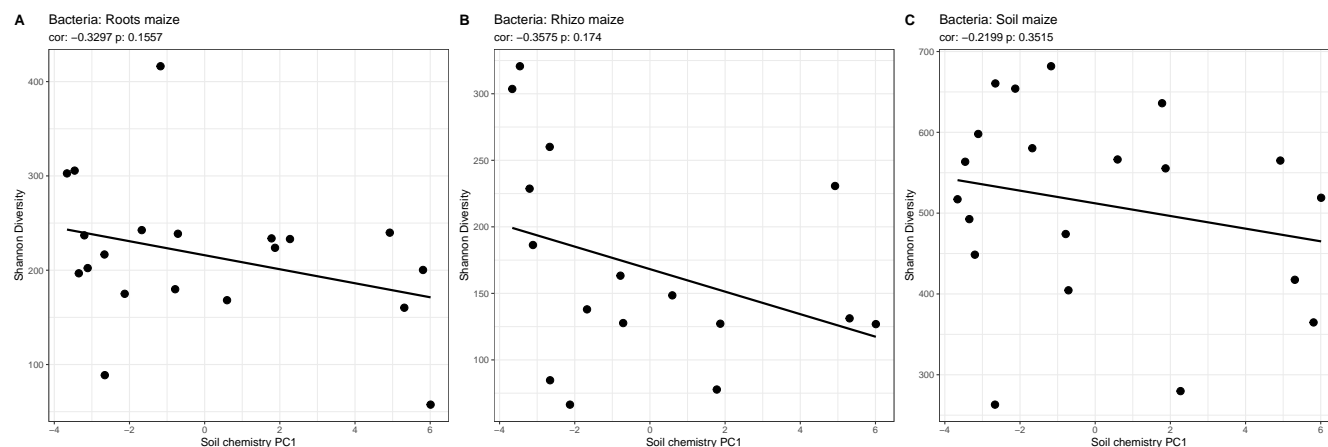


**Conclusion:** Soil chemistry PC1 influenced the alpha diversity of the microbial communities. Root and rhizosphere bacterial communities have higher Shannon diversity in WT genotypes compared to *bx1* genotypes (weakly significant). Rhizosphere fungal communities have higher Shannon diversity in WT compared to *bx1* (significant).

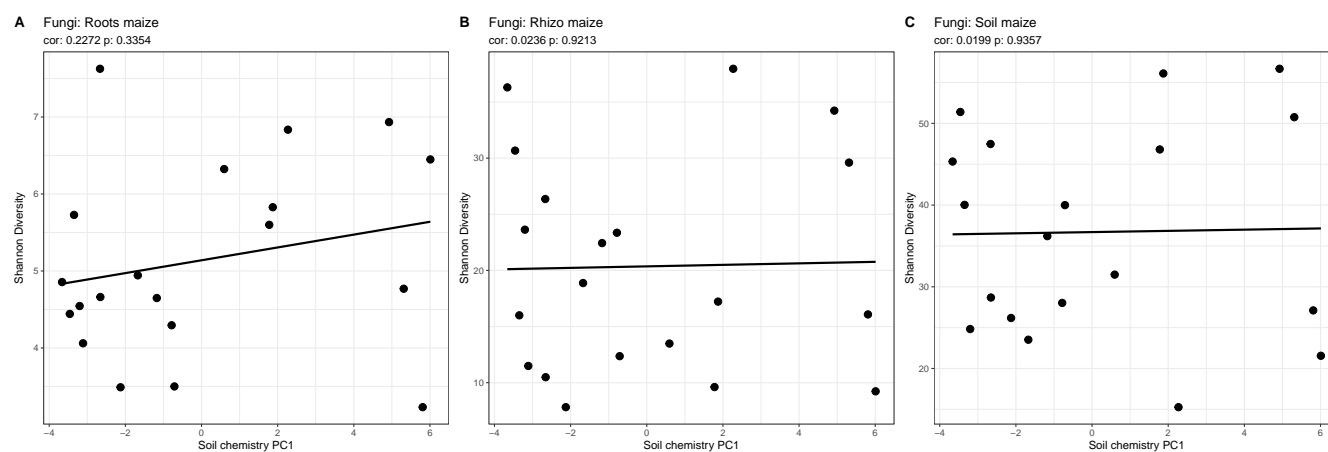
## Correlation analysis: Alpha diversity ~ soil chemistry PC1

Is there a correlation between the alpha diversity and soil chemistry PC1? We tested for each sampletype.

**Figure 7.1 | Bacteria: Correlation alpha diversity ~ soil chemistry PC1**



**Figure 7.2 | Fungi: Correlation alpha diversity ~ soil chemistry PC1**



**Conclusion:** We found no correlation.

## Analysis of genotype effects - Beta diversity

We measured the beta diversity using Bray-Curtis distances. We asked for genotype effects in each compartment by using PERMANOVA (function ‘adonis2()’ of the package vegan) and CAP ordination.

### PERMANOVA by sampletype

*Model: ~ Genotype \* Soil chemistry PC1*

Table 13: Bacteria: roots maize

	Df	SumOfSqs	R2	F	Pr(>F)
<b>Genotype</b>	1	0.2589	0.07545	2.295	0.04
<b>Soil chemistry PC1</b>	1	1.25	0.3642	11.08	0.001
<b>Genotype : Soil chemistry PC1</b>	1	0.1177	0.03431	1.044	0.327
<b>Residual</b>	16	1.805	0.526	NA	NA
<b>Total</b>	19	3.431	1	NA	NA

Table 14: Bacteria: rhizo maize

	Df	SumOfSqs	R2	F	Pr(>F)
<b>Genotype</b>	1	0.2207	0.07626	1.605	0.108
<b>Soil chemistry PC1</b>	1	0.8099	0.2799	5.892	0.001
<b>Genotype : Soil chemistry PC1</b>	1	0.2136	0.07381	1.554	0.104
<b>Residual</b>	12	1.649	0.57	NA	NA
<b>Total</b>	15	2.893	1	NA	NA

Table 15: Bacteria: soil maize

	Df	SumOfSqs	R2	F	Pr(>F)
<b>Genotype</b>	1	0.1209	0.03026	0.8875	0.424
<b>Soil chemistry PC1</b>	1	1.609	0.4028	11.82	0.001
<b>Genotype : Soil chemistry PC1</b>	1	0.0859	0.0215	0.6308	0.688
<b>Residual</b>	16	2.179	0.5454	NA	NA
<b>Total</b>	19	3.995	1	NA	NA

Table 16: Fungi: roots maize

	Df	SumOfSqs	R2	F	Pr(>F)
<b>Genotype</b>	1	0.3267	0.1333	3.259	0.001
<b>Soil chemistry PC1</b>	1	0.4101	0.1673	4.091	0.001
<b>Genotype : Soil chemistry PC1</b>	1	0.1099	0.04483	1.096	0.379
<b>Residual</b>	16	1.604	0.6545	NA	NA
<b>Total</b>	19	2.451	1	NA	NA

Table 17: Fungi: rhizo maize

	Df	SumOfSqs	R2	F	Pr(>F)
<b>Genotype</b>	1	0.3649	0.1376	3.295	0.004
<b>Soil chemistry PC1</b>	1	0.3597	0.1356	3.248	0.003

	Df	SumOfSqs	R2	F	Pr(>F)
<b>Genotype : Soil chemistry PC1</b>	1	0.1562	0.05887	1.41	0.157
<b>Residual</b>	16	1.772	0.668	NA	NA
<b>Total</b>	19	2.653	1	NA	NA

Table 18: Fungi: soil maize

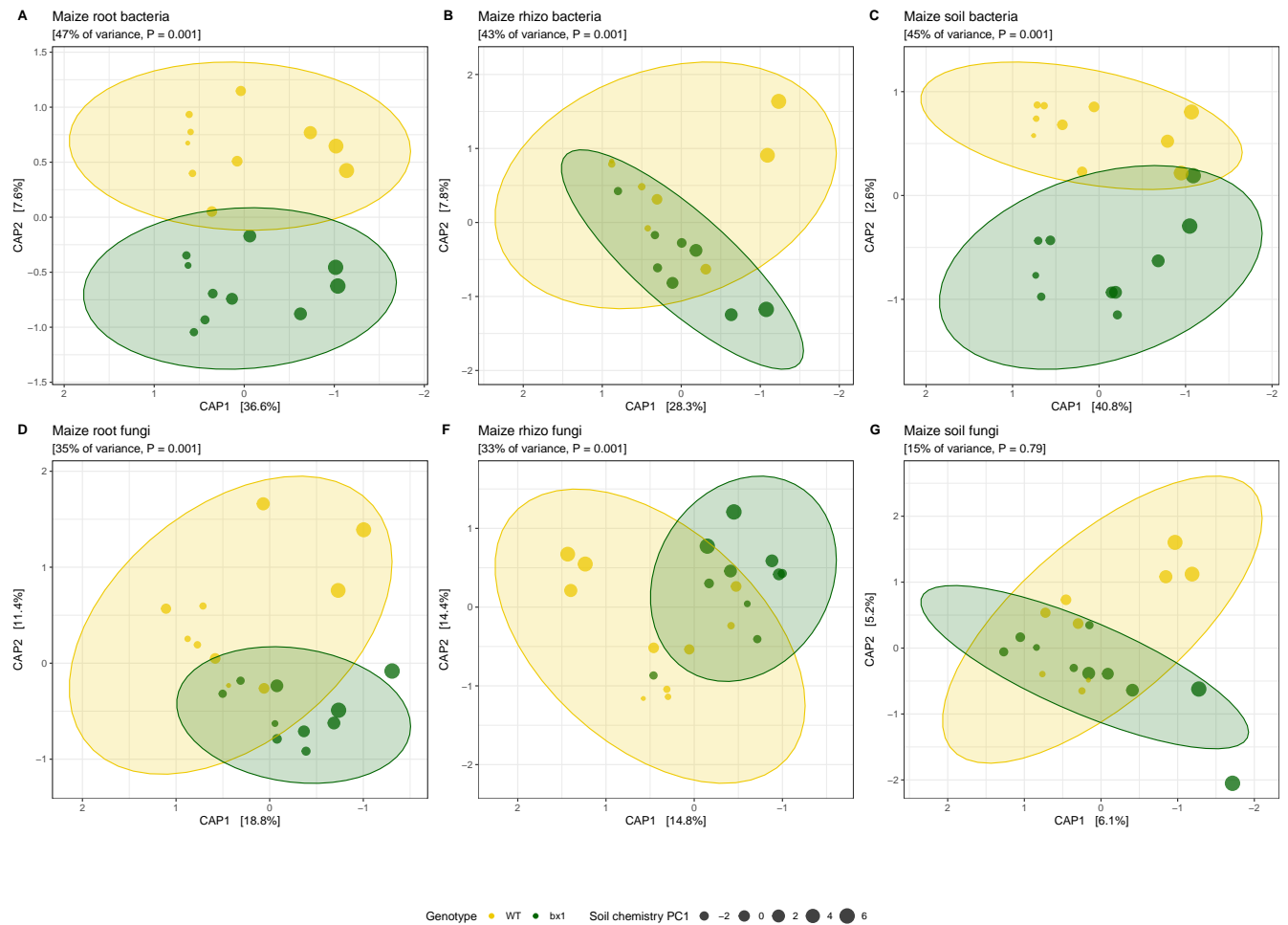
	Df	SumOfSqs	R2	F	Pr(>F)
<b>Genotype</b>	1	0.2556	0.0437	0.774	0.913
<b>Soil chemistry PC1</b>	1	0.3557	0.06082	1.077	0.295
<b>Genotype : Soil chemistry PC1</b>	1	0.284	0.04855	0.8599	0.775
<b>Residual</b>	15	4.954	0.8469	NA	NA
<b>Total</b>	18	5.849	1	NA	NA



## CAP by samplotype

Model:  $\sim \text{Genotype} * \text{PCA\_soil\_chem\_1}$

Figure 8 | CAP of maize (by samplotype)



**Conclusion:** Soil chemistry PC1 affected the bacterial communities more strongly (sig. in all compartments) compared to genotypes. A significant genotype effect was found in maize roots for bacteria. Rhizosphere and soil are not affected. soil chemistry PC1 affected the fungal communities less than the bacteria (lower R2 and only in root and rhizosphere). The genotype effect however was more pronounced, being significant in roots and rhizosphere, but not soil samples.

## Data analysis - Wheat sowing

Here we analysed the microbiome data which has been collected during wheat sowing. All samples are bare-soil samples.

### Soil chemistry PC1

We know from previous analyses that we have a soil-chemical gradient (soil chemistry PC1) along the field. We tested if the microbiome changes along soil chemistry PC1. We tested this effect with a PERMANOVA and illustrated it with a PCoA.

### PERMANOVA

*Model: ~ Conditioning + Soil Chemistry PC1*

Table 19: Bacteria

	R2	Pr(>F)
<b>Conditioning</b>	0.02803	0.379
<b>Soil chemistry PC1</b>	0.4649	0.001
<b>Conditioning : Soil chemistry PC1</b>	0.01959	0.651

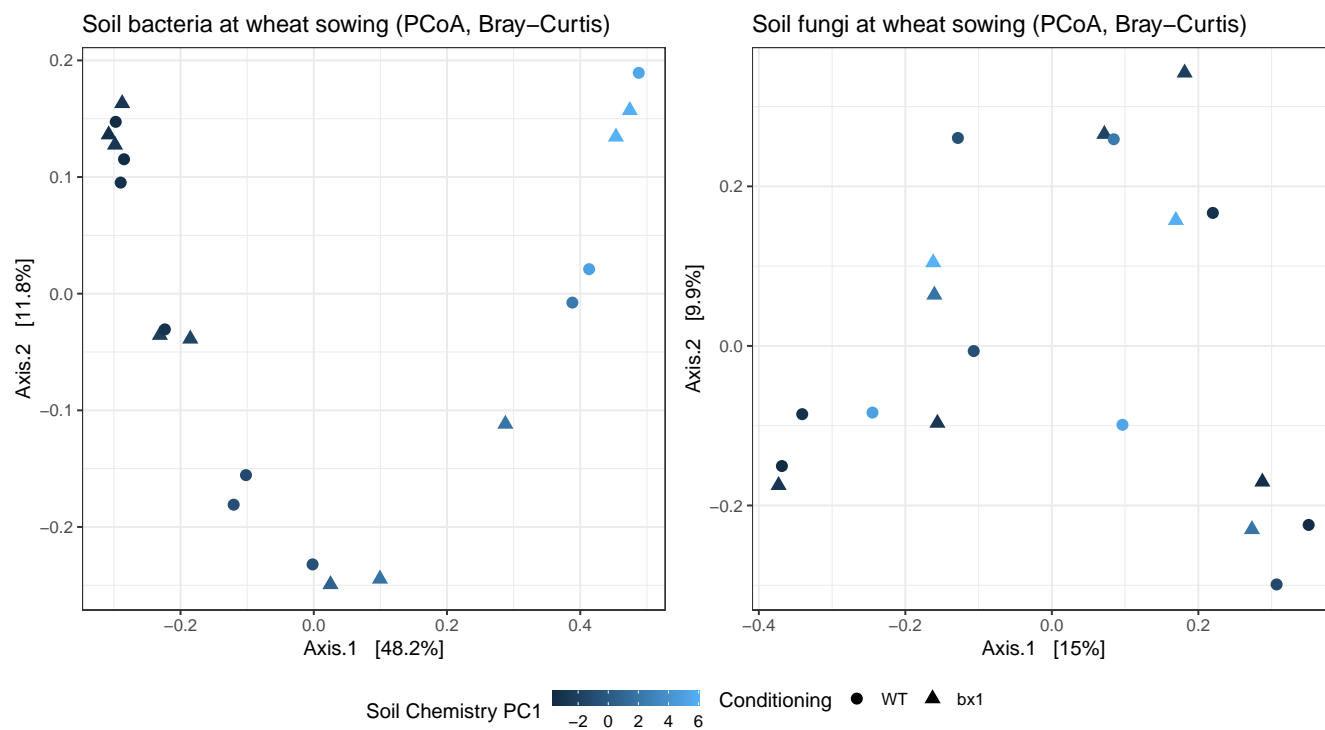
Table 20: Fungi

	R2	Pr(>F)
<b>Conditioning</b>	0.04453	0.888
<b>Soil chemistry PC1</b>	0.06556	0.157
<b>Conditioning : Soil chemistry PC1</b>	0.05155	0.62

## PCoA

We performed an unconstrained ordination with Bray-Curtis distances.

**Figure 9 | PCoA with Bray-Curtis**



## Correlation analysis: PCoA (microbiota) ~ Soil chemistry PC1

Do soil chemistry PC1 correlate with the beta diversity? We tested for the first two axes.

Figure 10.1 | Bacteria: Correlation (microbiota) ~ Soil chemistry PC1

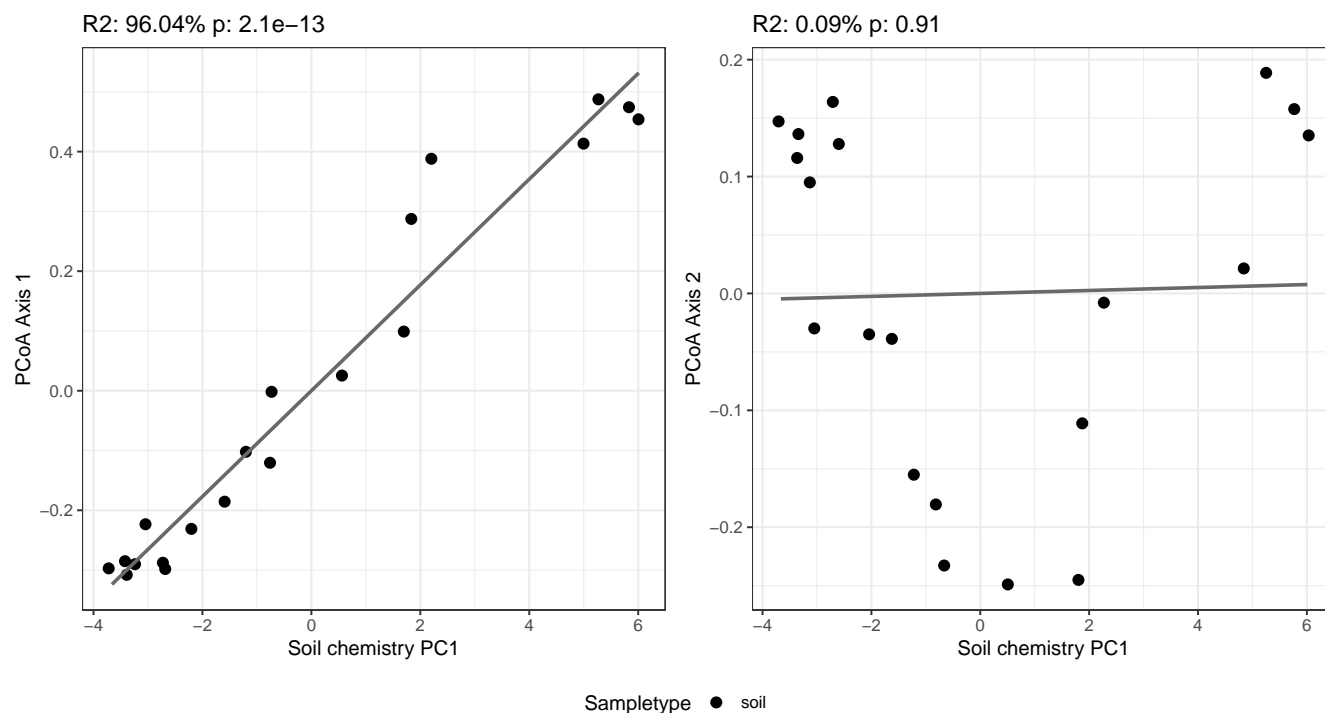
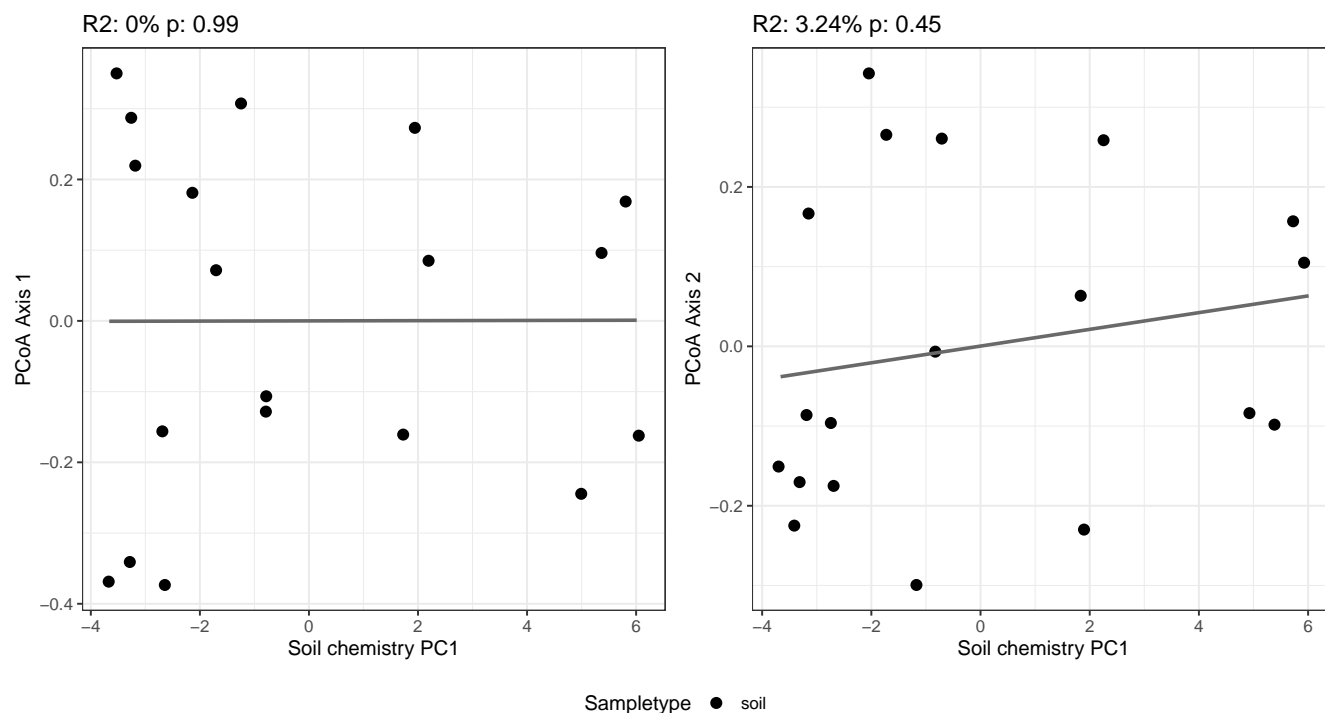


Figure 10.2 | Fungi: Correlation (microbiota) ~ Soil chemistry PC1



**Conclusion:** The microbiome is influenced by soil chemistry PC1. To take soil chemistry PC1 into account, we included it as independent variable in all following models.

## Analysis of BX conditioning effects - Alpha diversity

How did BX conditioning affect the microbiome? We searched for differences between the conditioning treatments (WT, *bx1*). To measure the alpha diversity, we rarefied the data to the sequencing depth of 6700 (bacteria) and 450 (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.

### Anova statistics

*Model: ~ Conditioning \* Soil chemistry PC1*

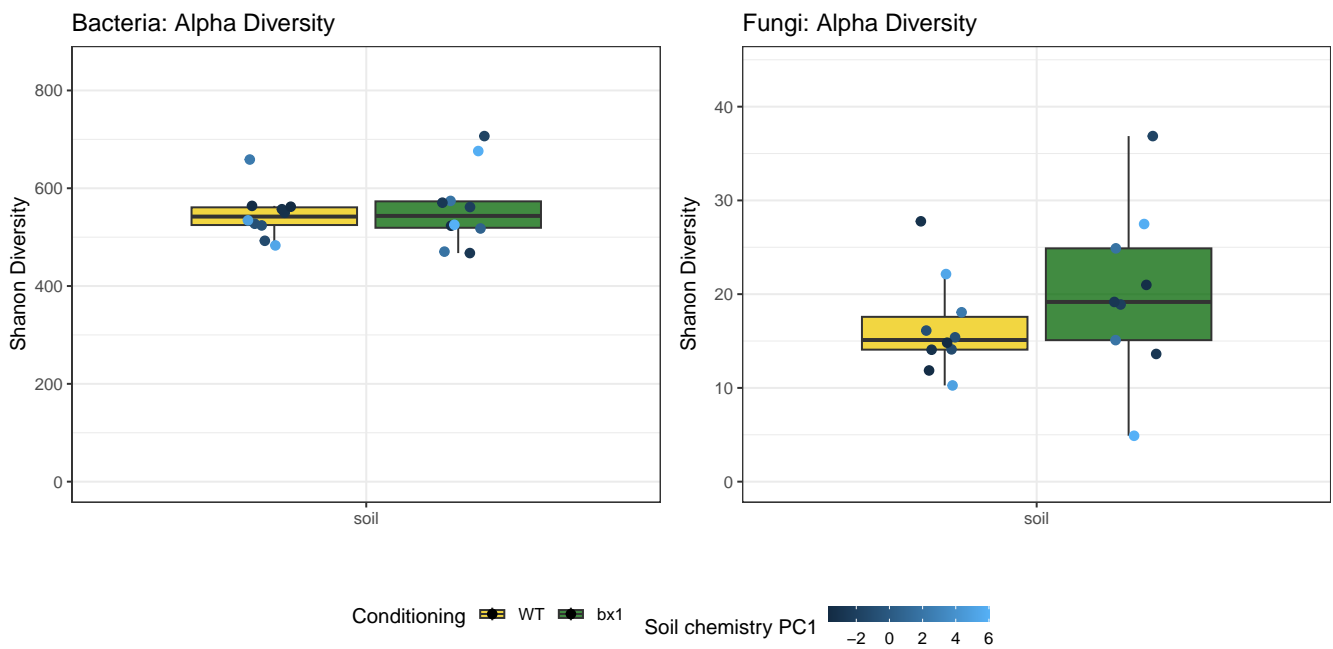
Table 21: Bacteria bare-soil

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
<b>Conditioning</b>	1	979.1	979.1	0.2075	0.6549
<b>Soil chemistry PC1</b>	1	167.8	167.8	0.03556	0.8528
<b>Conditioning : Soil chemistry PC1</b>	1	1769	1769	0.3749	0.549
<b>Residuals</b>	16	75509	4719	NA	NA

Table 22: Fungi bare-soil

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
<b>Conditioning</b>	1	66.56	66.56	1.153	0.2998
<b>Soil chemistry PC1</b>	1	15.28	15.28	0.2648	0.6143
<b>Conditioning : Soil chemistry PC1</b>	1	16.28	16.28	0.2821	0.6031
<b>Residuals</b>	15	865.6	57.71	NA	NA

Figure 11 | Shannon diversity by conditioning



**Conclusion:** soil chemistry PC1 did not influence the alpha diversity of the microbial communities in bare soil.

## Analysis of BX conditioning effects - Beta diversity

We measured the beta diversity using Bray-Curtis distances. We asked for conditioning effects by using PERMANOVA (function ‘adonis2()’ of the package vegan) and CAP ordination.

### PERMANOVA

*Model: ~ Conditioning \* Soil chemistry PC1*

Table 23: Bacteria: soil at sowing

	Df	SumOfSqs	R2	F	Pr(>F)
<b>Conditioning</b>	1	0.1027	0.02803	0.9201	0.379
<b>Soil chemistry PC1</b>	1	1.704	0.4649	15.26	0.001
<b>Conditioning : Soil chemistry PC1</b>	1	0.07178	0.01959	0.6429	0.652
<b>Residual</b>	16	1.786	0.4875	NA	NA
<b>Total</b>	19	3.664	1	NA	NA

### Fungi

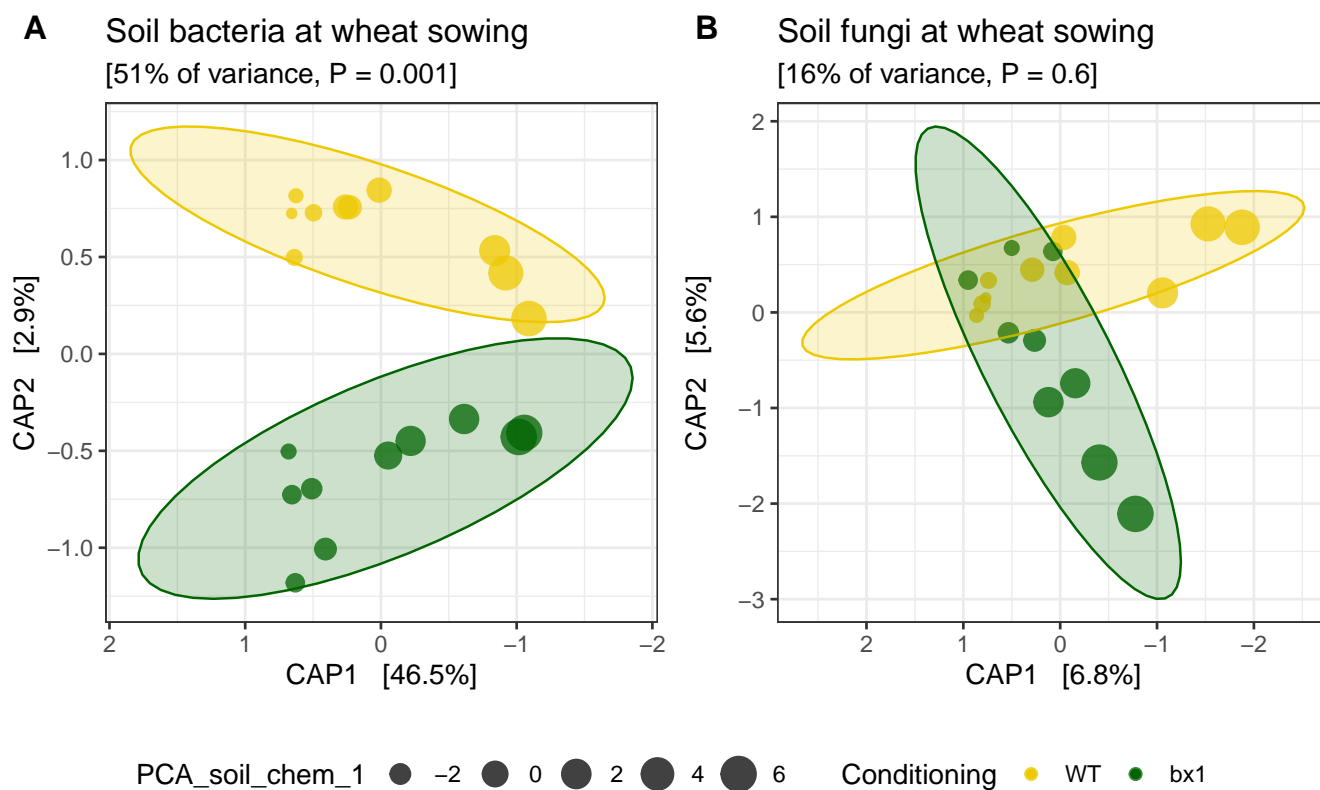
Table 24: Fungi: soil at sowing

	Df	SumOfSqs	R2	F	Pr(>F)
<b>Conditioning</b>	1	0.3156	0.04453	0.7968	0.89
<b>Soil chemistry PC1</b>	1	0.4645	0.06556	1.173	0.163
<b>Conditioning : Soil chemistry PC1</b>	1	0.3653	0.05155	0.9223	0.645
<b>Residual</b>	15	5.941	0.8384	NA	NA
<b>Total</b>	18	7.086	1	NA	NA

## CAP

Model:  $\sim$  Conditioning \* Soil chemistry PC1

Figure 12 | CAP



**Conclusion:** soil chemistry PC1 affected bacterial but not fungal communities in bare-soil. No difference in beta diversity were found between communities from different conditioned soils.



## Data analysis - Wheat growth

Here we analysed the microbiome data which has been collected after wheat has grown.

### Soil chemistry PC1

We know from previous analyses that we have a soil-chemical gradient (soil chemistry PC1) along the field. We tested if the microbiome changes along soil chemistry PC1. We tested this effect with a PERMANOVA and illustrated it with a PCoA.

### PERMANOVA

*Model: ~ Sample\_type + Soil Chemistry PC1*

Table 25: Bacteria

	R2	Pr(>F)
<b>Sampletype</b>	0.2427	0.001
<b>Soil chemistry PC1</b>	0.2423	0.001
<b>Residual</b>	0.5149	NA

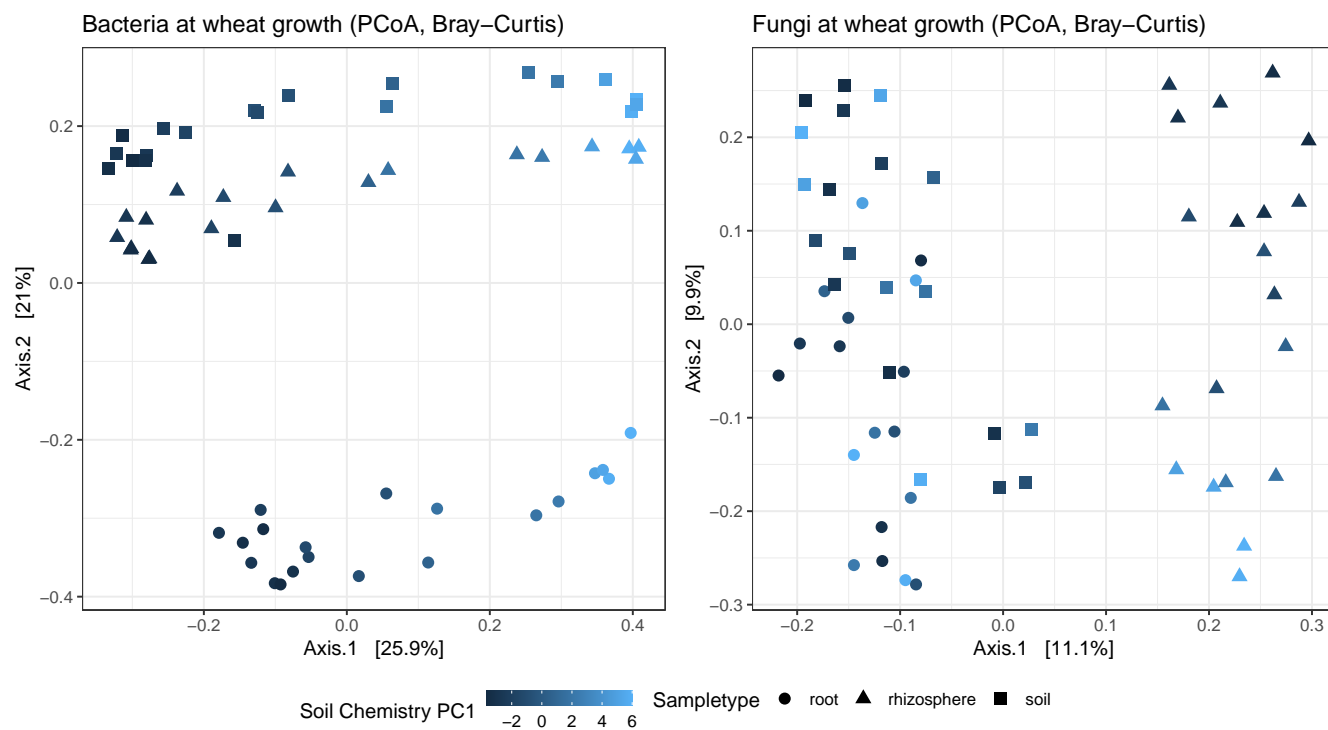
Table 26: Fungi

	R2	Pr(>F)
<b>Sampletype</b>	0.1773	0.001
<b>Soil chemistry PC1</b>	0.02767	0.006
<b>Residual</b>	0.7951	NA

## PCoA

We performed an unconstrained ordination with Bray-Curtis distances.

**Figure 13 | PCoA with Bray-Curtis**



## Correlation analysis: PCoA (microbiota) ~ Soil chemistry PC1

Do soil chemistry PC1 correlate with the beta diversity? We tested for the first two axes.

Figure 14.1 | Bacteria: Correlation (microbiota) ~ Soil chemistry PC1

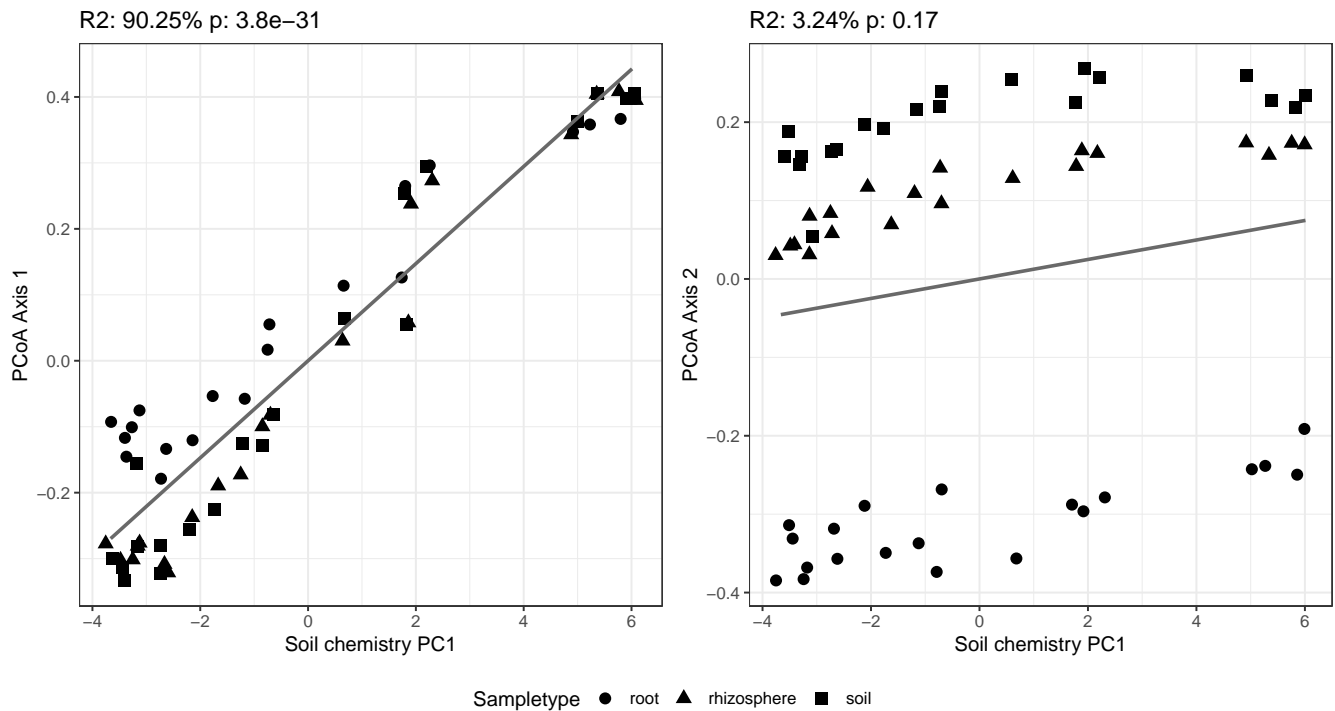
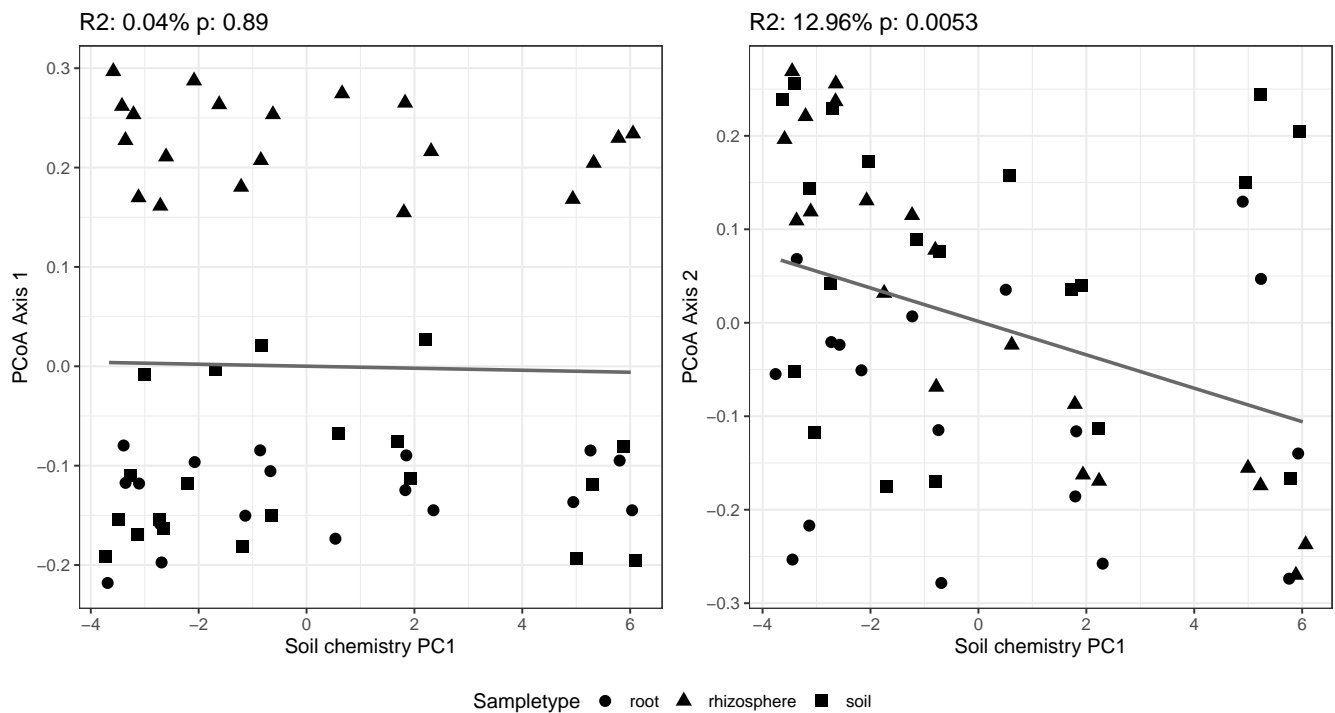


Figure 14.2 | Fungi: Correlation (microbiota) ~ Soil chemistry PC1



**Conclusion:** The microbiome is influenced by soil chemistry PC1. To take soil chemistry PC1 into account, we included it as independent variable in all following models.

## Analysis of BX conditioning effects - Alpha diversity

How did BX conditioning affect the microbiome? We compared the root, rhizosphere and soil microbiomes of wheat whether the microbial communities would differ between the conditioning treatments (WT, *bx1*). To measure the alpha diversity, we rarefied the data to the sequencing depth of 6700 (bacteria) and 450 (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.

### Anova statistics

*Model: ~ Conditioning \* Soil chemistry PC1*

Table 27: Bacteria all compartments

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
<b>Conditioning</b>	1	2.349	2.349	8.088e-05	0.9929
<b>Soil chemistry PC1</b>	1	7243	7243	0.2494	0.6195
<b>Conditioning : Soil chemistry PC1</b>	1	3004	3004	0.1034	0.7489
<b>Residuals</b>	56	1626403	29043	NA	NA

Table 28: Bacteria roots

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
<b>Conditioning</b>	1	1695	1695	0.5301	0.4771
<b>Soil chemistry PC1</b>	1	9639	9639	3.015	0.1017
<b>Conditioning : Soil chemistry PC1</b>	1	988.4	988.4	0.3092	0.5859
<b>Residuals</b>	16	51153	3197	NA	NA

Table 29: Bacteria rhizosphere

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
<b>Conditioning</b>	1	13527	13527	1.835	0.1944
<b>Soil chemistry PC1</b>	1	1808	1808	0.2452	0.6272
<b>Conditioning : Soil chemistry PC1</b>	1	1955	1955	0.2651	0.6137
<b>Residuals</b>	16	117949	7372	NA	NA

Table 30: Bacteria soil

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
<b>Conditioning</b>	1	5254	5254	0.3258	0.576
<b>Soil chemistry PC1</b>	1	41238	41238	2.558	0.1293
<b>Conditioning : Soil chemistry PC1</b>	1	29100	29100	1.805	0.1979
<b>Residuals</b>	16	257973	16123	NA	NA

Table 31: Fungi all compartments

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
<b>Conditioning</b>	1	25.66	25.66	0.2008	0.6559
<b>Soil chemistry PC1</b>	1	0.4902	0.4902	0.003836	0.9508
<b>Conditioning : Soil chemistry PC1</b>	1	0.001797	0.001797	1.406e-05	0.997
<b>Residuals</b>	54	6901	127.8	NA	NA

Table 32: Fungi roots

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
<b>Conditioning</b>	1	255.4	255.4	4.687	0.04816
<b>Soil chemistry PC1</b>	1	3.561	3.561	0.06533	0.802
<b>Conditioning : Soil chemistry PC1</b>	1	99.2	99.2	1.82	0.1987
<b>Residuals</b>	14	763	54.5	NA	NA

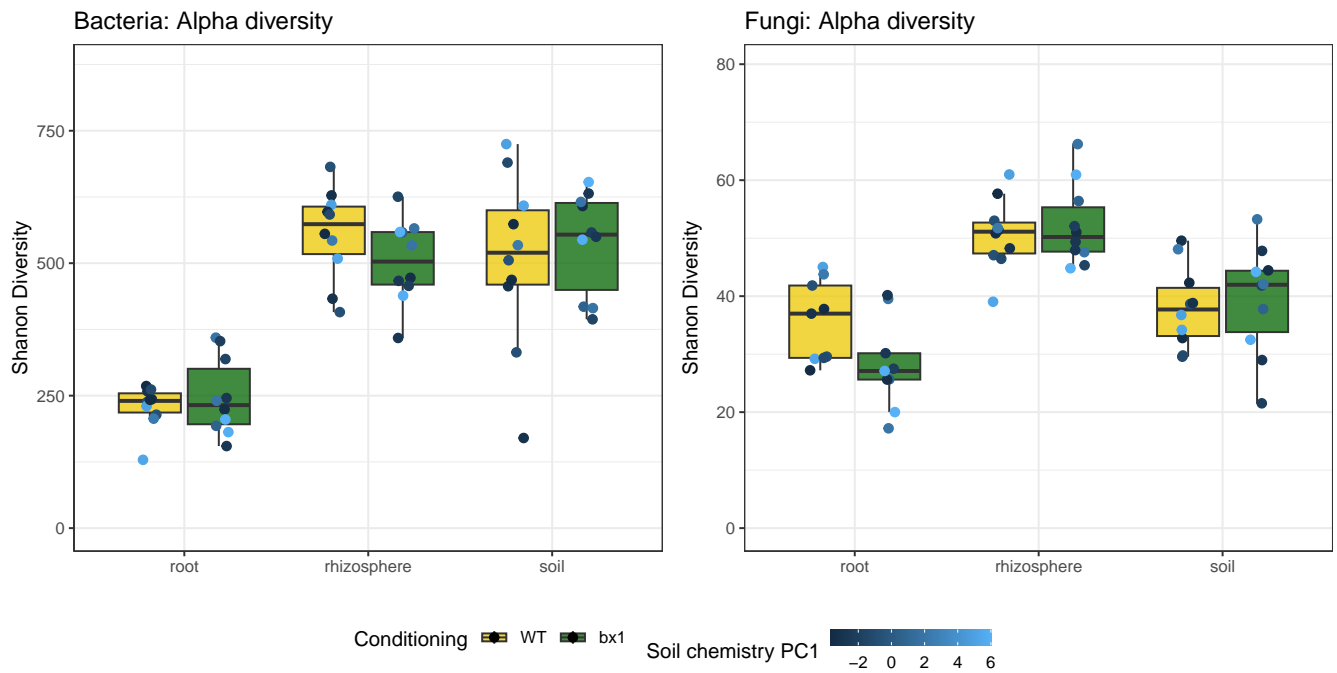
Table 33: Fungi rhizosphere

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
<b>Conditioning</b>	1	11.73	11.73	0.2544	0.6209
<b>Soil chemistry PC1</b>	1	6.058	6.058	0.1315	0.7217
<b>Conditioning : Soil chemistry PC1</b>	1	32.53	32.53	0.7058	0.4132
<b>Residuals</b>	16	737.4	46.09	NA	NA

Table 34: Fungi soil

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
<b>Conditioning</b>	1	9.683	9.683	0.127	0.7263
<b>Soil chemistry PC1</b>	1	1.076	1.076	0.01411	0.9069
<b>Conditioning : Soil chemistry PC1</b>	1	19.04	19.04	0.2497	0.6241
<b>Residuals</b>	16	1220	76.27	NA	NA

Figure 15 | Shannon diversity by sampletype and conditioning



**Conclusion:** Soil chemistry PC1 influenced the alpha diversity of root fungal communities. They have higher Shannon diversity in WT compared to *bx1* conditions (significant).

## Correlation analysis: alpha diversity ~ soil chemistry PC1

Is there a correlation between the alpha diversity and soil chemistry PC1? We tested for each sampletype.

Figure 16.1 | Bacteria: Correlation alpha diversity ~ soil chemistry PC1

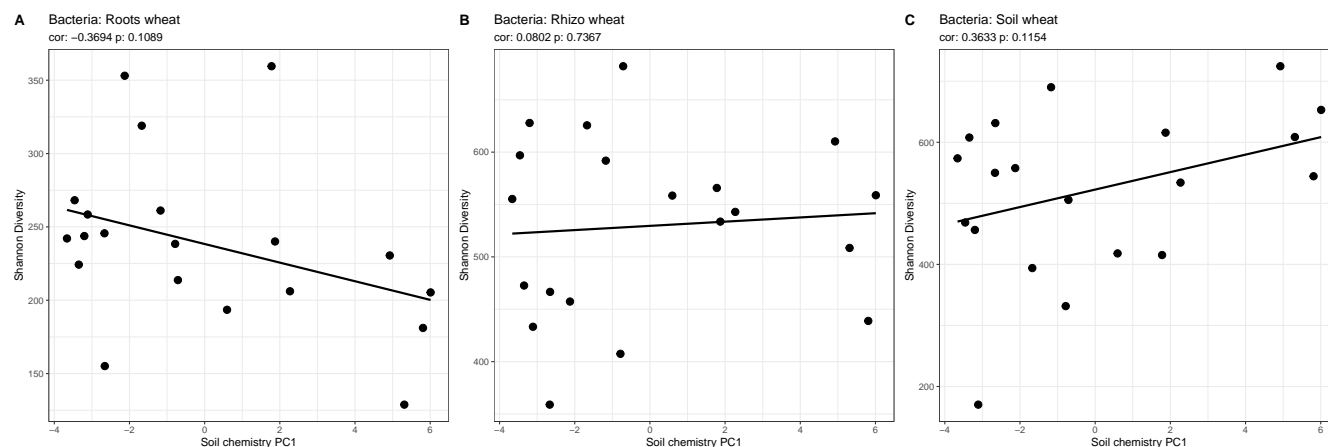
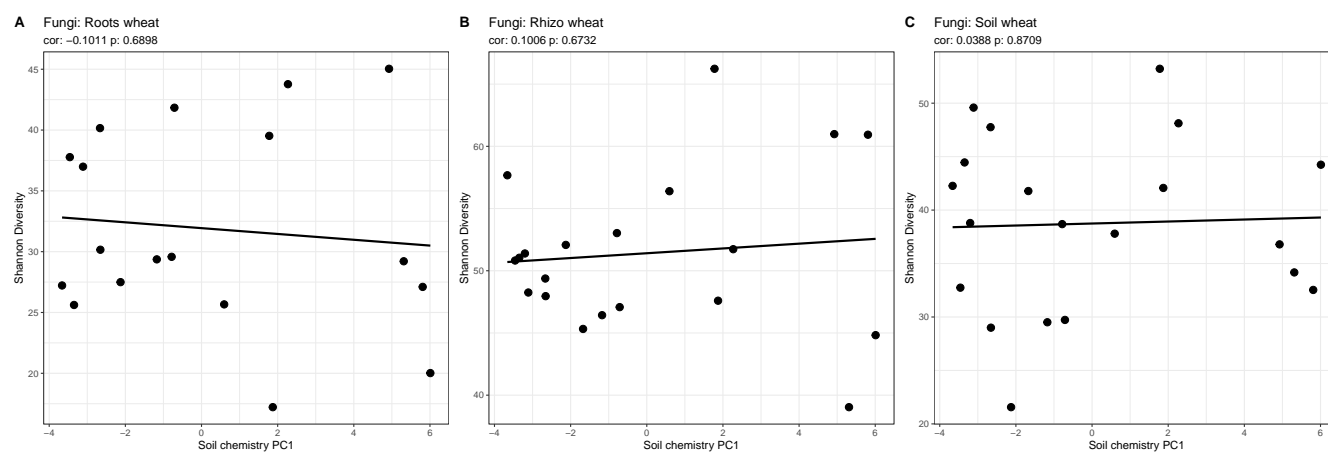


Figure 16.2 | Fungi: Correlation alpha diversity ~ soil chemistry PC1



## Analysis of BX conditioning effects - Beta diversity

We measured the beta diversity using Bray-Curtis distances. We asked for conditioning effects in each compartment by using PERMANOVA (function ‘adonis2()’ of the package vegan) and CAP ordination.

### PERMANOVA by sampletype

*Model: ~ Conditioning \* Soil chemistry PC1*

#### Bacteria

Table 35: Bacteria: roots wheat

	Df	SumOfSqs	R2	F	Pr(>F)
<b>Conditioning</b>	1	0.07777	0.02221	0.6698	0.683
<b>Soil chemistry PC1</b>	1	1.474	0.4208	12.69	0.001
<b>Genotype : Soil chemistry PC1</b>	1	0.09307	0.02658	0.8016	0.513
<b>Residual</b>	16	1.858	0.5305	NA	NA
<b>Total</b>	19	3.502	1	NA	NA

Table 36: Bacteria: rhizo wheat

	Df	SumOfSqs	R2	F	Pr(>F)
<b>Conditioning</b>	1	0.09221	0.02583	0.8217	0.486
<b>Soil chemistry PC1</b>	1	1.595	0.4467	14.21	0.001
<b>Genotype : Soil chemistry PC1</b>	1	0.08748	0.02451	0.7795	0.49
<b>Residual</b>	16	1.796	0.503	NA	NA
<b>Total</b>	19	3.57	1	NA	NA

Table 37: Bacteria: soil wheat

	Df	SumOfSqs	R2	F	Pr(>F)
<b>Conditioning</b>	1	0.1082	0.02549	0.7411	0.597
<b>Soil chemistry PC1</b>	1	1.698	0.3998	11.62	0.001
<b>Genotype : Soil chemistry PC1</b>	1	0.1042	0.02453	0.7132	0.648
<b>Residual</b>	16	2.337	0.5502	NA	NA
<b>Total</b>	19	4.247	1	NA	NA

**Conclusion:** Soil chemistry PC1 affected the bacterial communities more strongly (sig. in all compartments) compared to BX conditioning. A significant BX effect was found in wheat roots for bacteria. Rhizosphere and soil are not affected.



## Fungi

Table 38: Fungi: roots wheat

	Df	SumOfSqs	R2	F	Pr(>F)
<b>Conditioning</b>	1	0.3169	0.08461	1.492	0.026
<b>Soil chemistry PC1</b>	1	0.2146	0.0573	1.01	0.46
<b>Genotype : Soil chemistry PC1</b>	1	0.2402	0.06412	1.131	0.236
<b>Residual</b>	14	2.974	0.794	NA	NA
<b>Total</b>	17	3.746	1	NA	NA

Table 39: Fungi: rhizo wheat

	Df	SumOfSqs	R2	F	Pr(>F)
<b>Conditioning</b>	1	0.1354	0.03239	0.7218	0.815
<b>Soil chemistry PC1</b>	1	0.9027	0.216	4.812	0.001
<b>Genotype : Soil chemistry PC1</b>	1	0.1406	0.03364	0.7497	0.779
<b>Residual</b>	16	3.001	0.718	NA	NA
<b>Total</b>	19	4.18	1	NA	NA

Table 40: Fungi: soil wheat

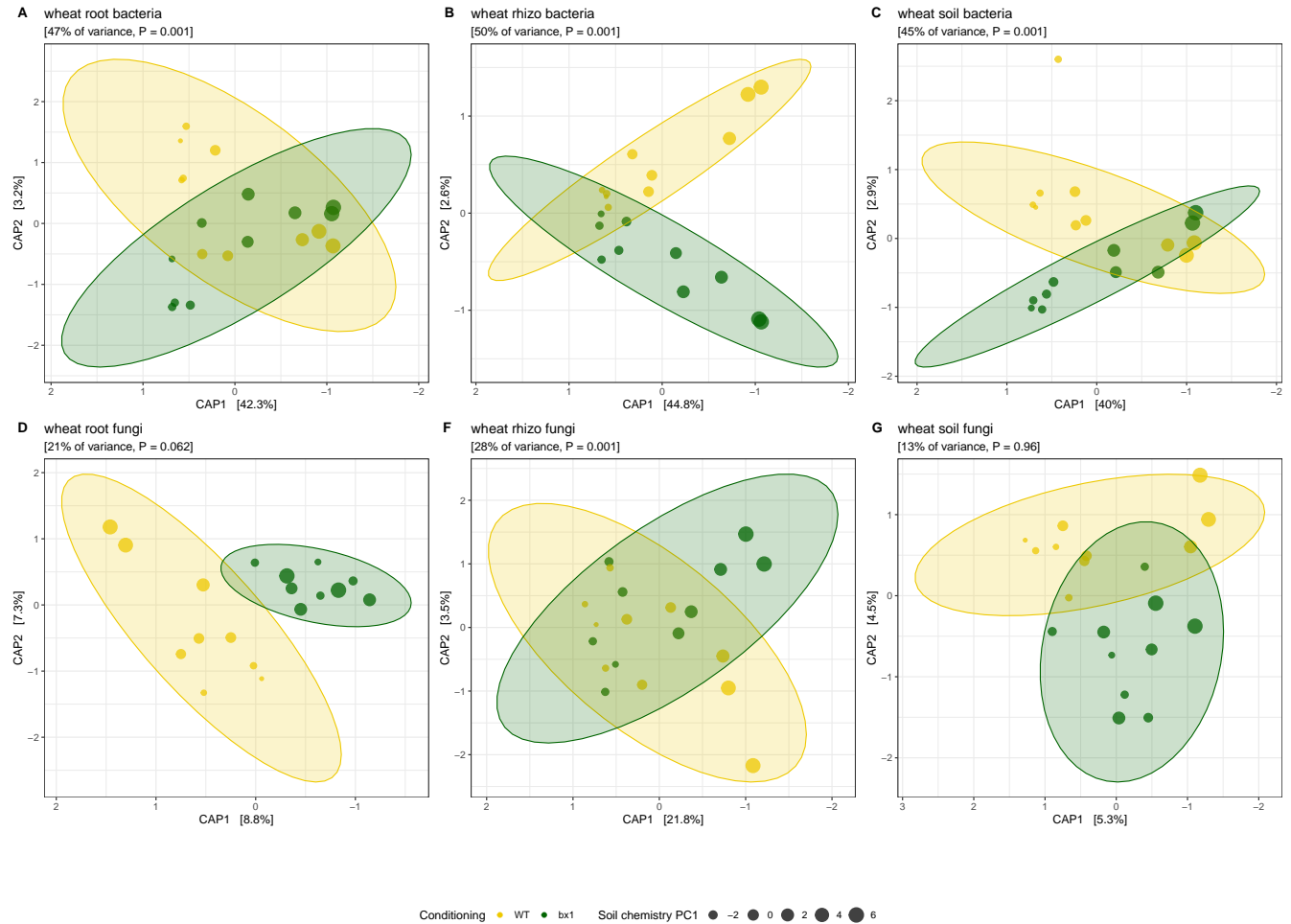
	Df	SumOfSqs	R2	F	Pr(>F)
<b>Conditioning</b>	1	0.2239	0.0459	0.8427	0.766
<b>Soil chemistry PC1</b>	1	0.232	0.04757	0.8733	0.685
<b>Genotype : Soil chemistry PC1</b>	1	0.1706	0.03498	0.6421	0.989
<b>Residual</b>	16	4.251	0.8715	NA	NA
<b>Total</b>	19	4.877	1	NA	NA

**Conclusion:** Soil chemistry PC1 affected the fungal communities less than the bacteria (lower R2 and only in root and rhizo). The BX conditioning however was more pronounced, being significant in roots and rhizosphere, but not soil samples.

## CAP by samplotype

Model:  $\sim \text{Conditioning} * \text{Soil chemistry PC1}$

Figure 17 | CAP of wheat (by samplotype)



**Conclusion:** Soil chemistry PC1 affected the bacterial communities more strongly (sig. in all compartments) compared to BX conditioning. No significant BX effect was found for bacteria. Soil chemistry PC1 affected the fungal communities less than the bacteria (lower R2 and only in and rhizosphere). A BX conditioning effect was found for fungal communities in roots.