

Changins - Microbiome analysis

Jan Waelchli

2023-03-20

Contents

Data description	2
Data normalization	4
Final number of samples	5
Data analysis - Maize harvest	6
Soil-chemical gradient	6
Analysis of BX conditioning effects - Alpha diversity	9
Analysis of BX conditioning effects - Beta diversity	13
Data analysis - Wheat sowing	16
Soil-chemical gradient	16
Analysis of BX conditioning effects - Alpha diversity	19
Analysis of BX conditioning effects - Beta diversity	20
Data analysis - Wheat growth	22
Soil-chemical gradient	22
Analysis of BX conditioning effects - Alpha diversity	25
Analysis of BX conditioning effects - Beta diversity	29

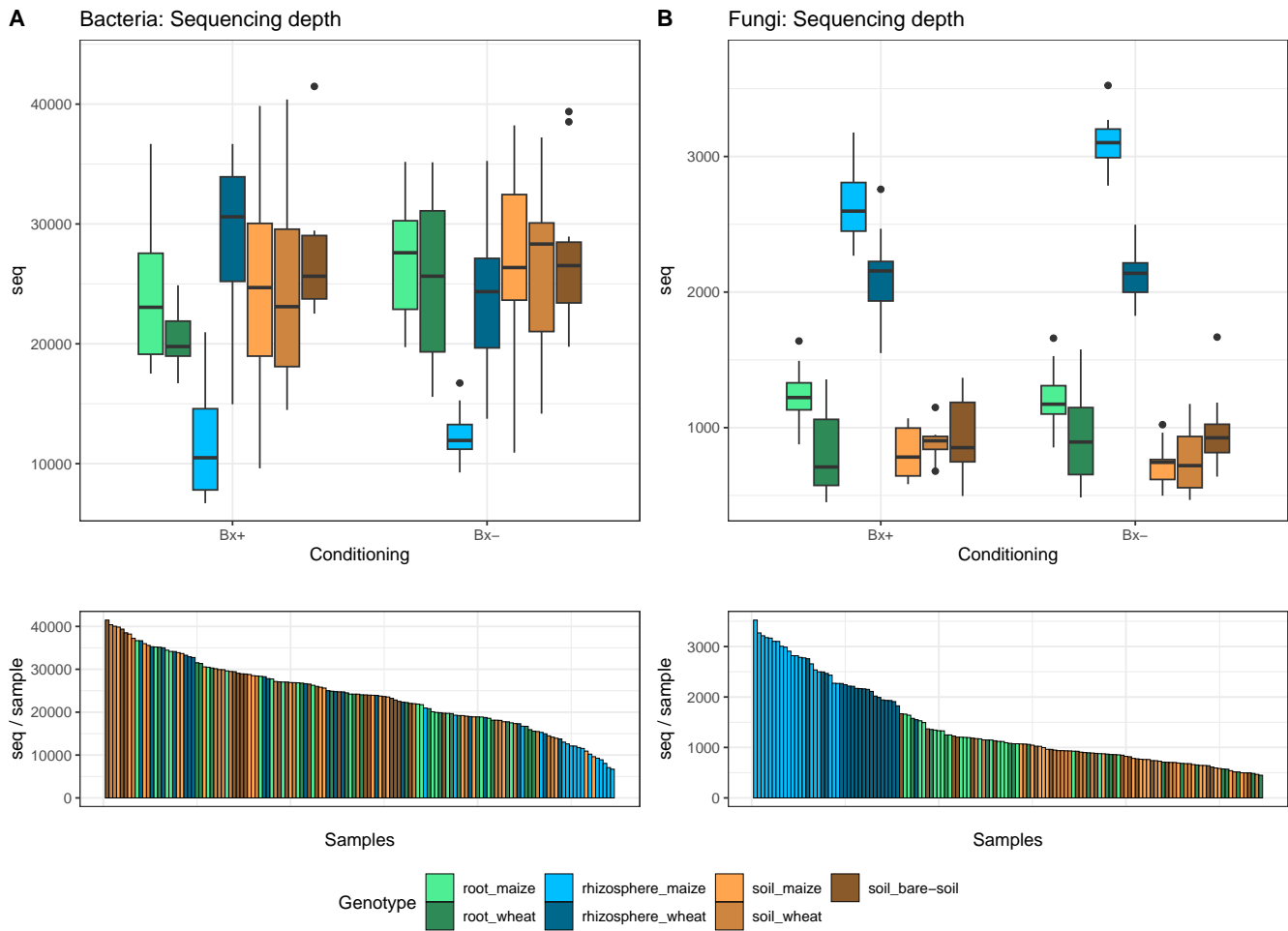
Data description

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

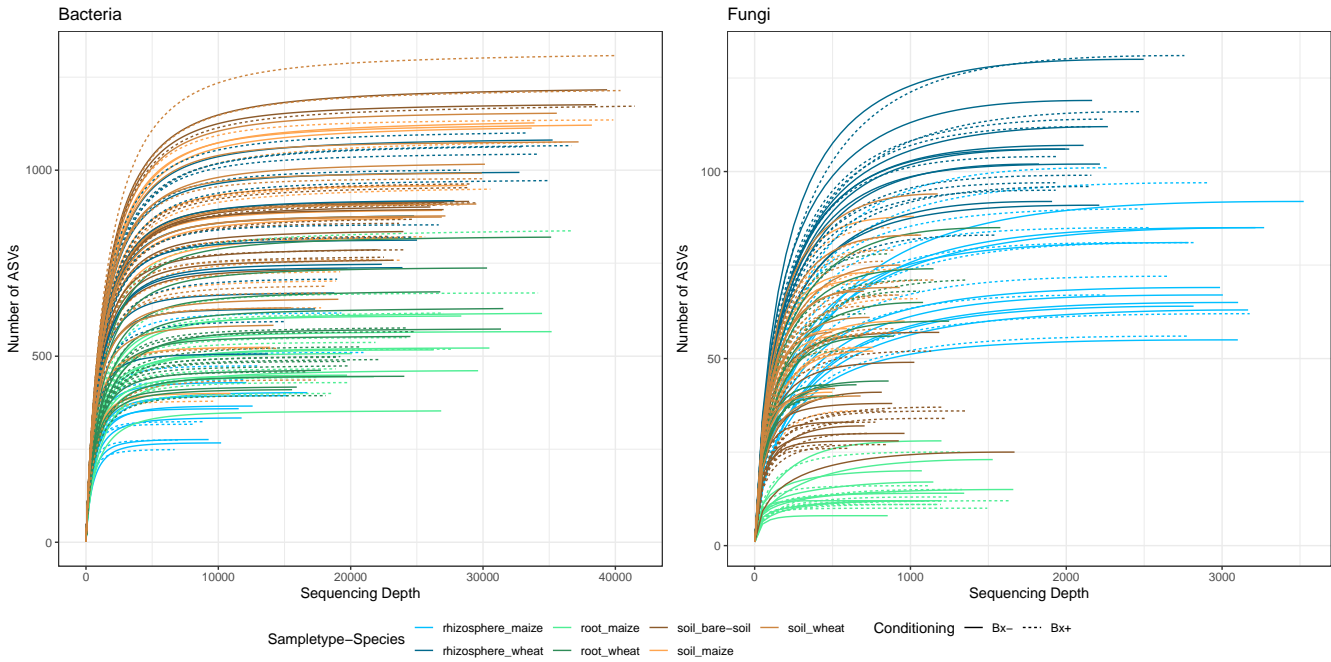
Figure 1 | Sequencing depth



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



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_Bx-, rhizosphere_maize_Bx+, rhizosphere_w
## 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_Bx-, rhizosphere_maize_Bx+, rhizosphere_w
## 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_Bx-	8	10
rhizosphere_maize_Bx+	8	10
rhizosphere_wheat_Bx-	10	10
rhizosphere_wheat_Bx+	10	10
root_maize_Bx-	10	10
root_maize_Bx+	10	10
root_wheat_Bx-	10	9
root_wheat_Bx+	10	9
soil_bare-soil_Bx-	10	9
soil_bare-soil_Bx+	10	10
soil_maize_Bx-	10	10
soil_maize_Bx+	10	9
soil_wheat_Bx-	10	10
soil_wheat_Bx+	10	10

Data analysis - Maize harvest

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

Soil-chemical gradient

We know from previous analyses that we have a soil-chemical gradient along the field. We tested if the microbiome changes along this gradient. 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)
Sampletype	0.3213	0.001
Soil chemistry PC1	0.163	0.001
Residual	0.5156	NA

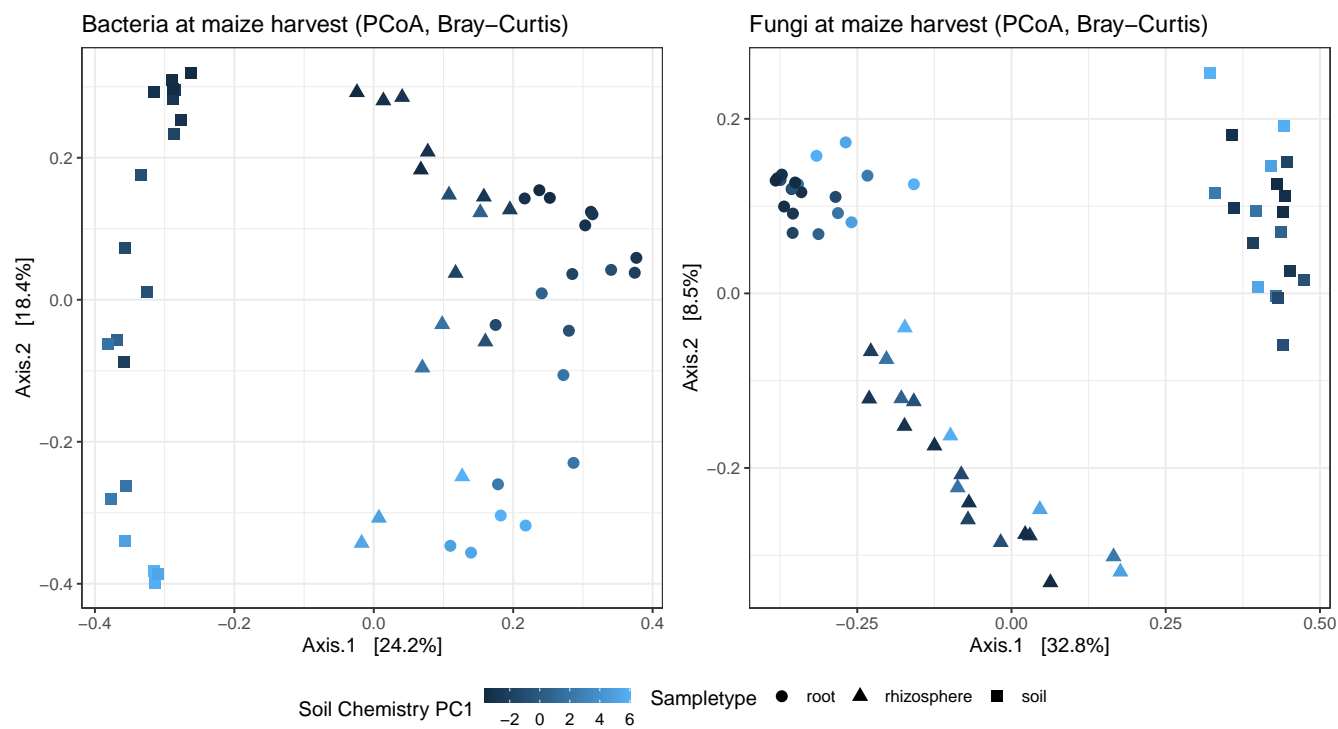
Table 4: Fungi

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

PCoA

We performed an unconstrained ordination with Bray-Curtis distances.

Figure 3 | PCoA with Bray-Curtis



Correlation analysis: PCoA ~ position

Do the field positions correlate with the beta diversity? We tested for the first two axes.

Figure 4.1 | Bacteria: Correlation PCoA ~ position

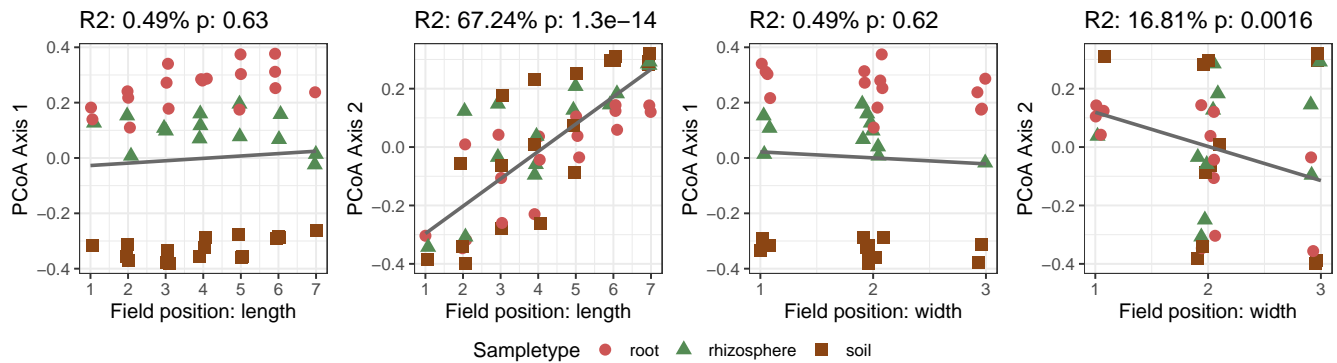
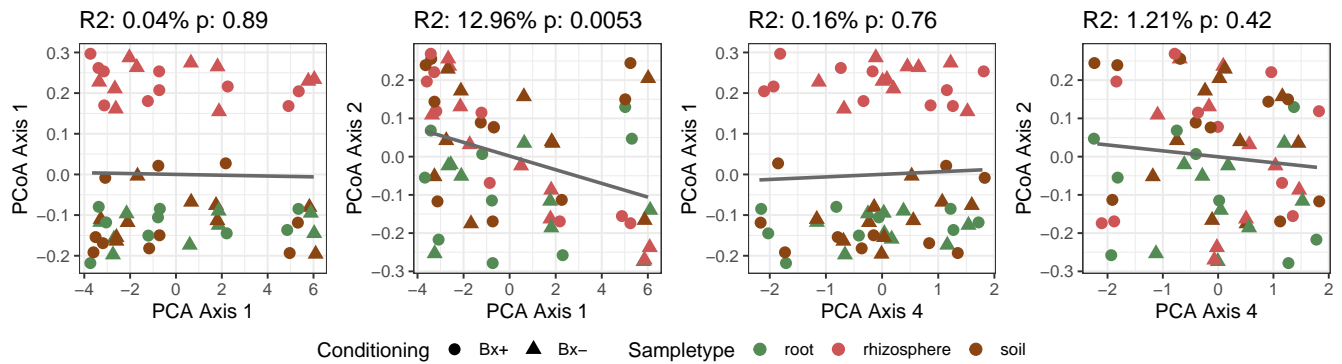


Figure 4.2 | Fungi: Correlation PCoA ~ position



Conclusion: The microbiome is influenced by the chemical soil gradient. To take this chemical soil gradient into account, we included the axis 1 of the PCA of the soil chemistry data 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 maize whether the microbial communities differ between the conditioning treatments (Bx+, Bx-). 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 * PCA_soil_chem_1*

Table 5: Bacteria all compartments

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

Table 6: Bacteria roots

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

Table 7: Bacteria rhizosphere

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Conditioning	1	27462	27462	6.092	0.02959
Soil chemistry PC1	1	8027	8027	1.781	0.2068
Conditioning : Soil chemistry PC1	1	1584	1584	0.3515	0.5643
Residuals	12	54092	4508	NA	NA

Table 8: Bacteria soil

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

Table 9: Fungi all compartments

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Conditioning	1	294.6	294.6	1.173	0.2835
Soil chemistry PC1	1	30.84	30.84	0.1228	0.7274
Conditioning : Soil chemistry PC1	1	220.3	220.3	0.8773	0.353
Residuals	55	13812	251.1	NA	NA

Table 10: Fungi roots

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

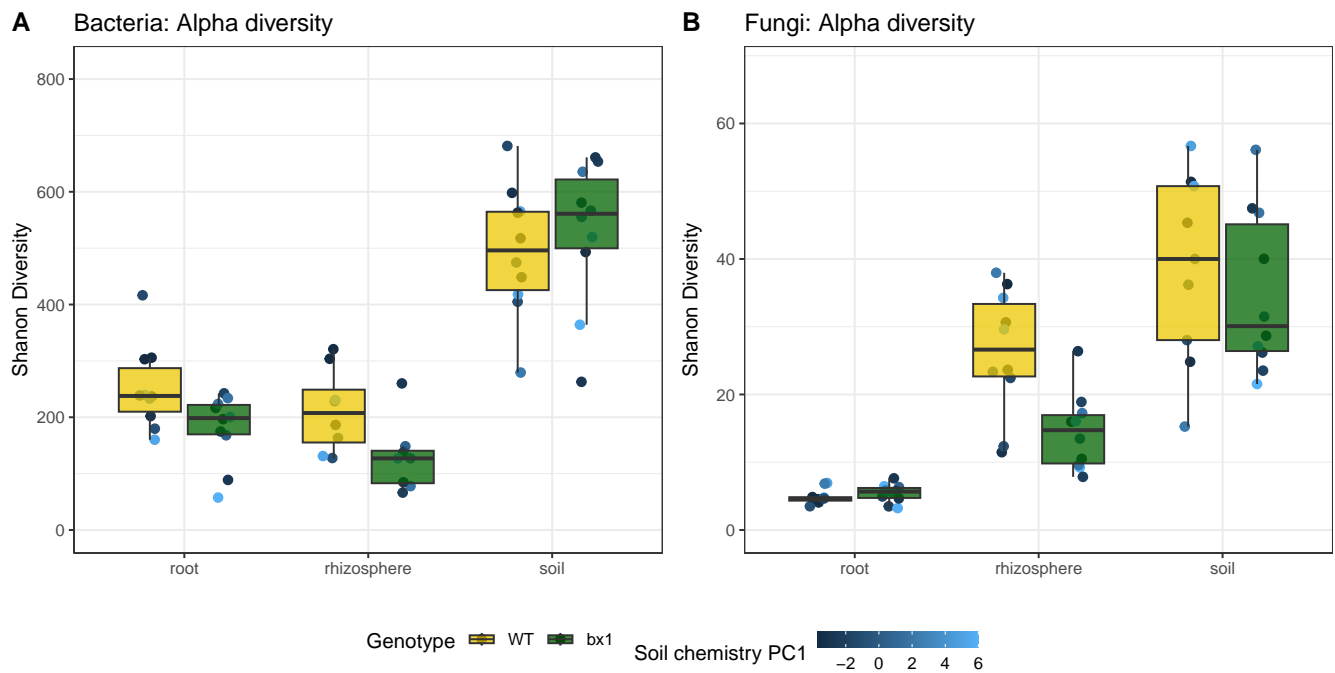
Table 11: Fungi rhizosphere

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

Table 12: Fungi soil

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

Figure 5 | Shannon diversity by samplotype and conditioning



Conclusion: The chemical field gradient influenced the alpha diversity of the microbial communities. Root and rhizosphere bacterial communities have higher Shannon diversity in BX+ compared to BX- conditions (weakly significant). Rhizosphere fungal communities have higher Shannon diversity in BX+ compared to BX- conditions (significant).

Correlation analysis: Alpha diversity ~ soil chemical gradient

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

Figure 6.1 | Bacteria: Correlation alpha diversity ~ soil chemical gradient

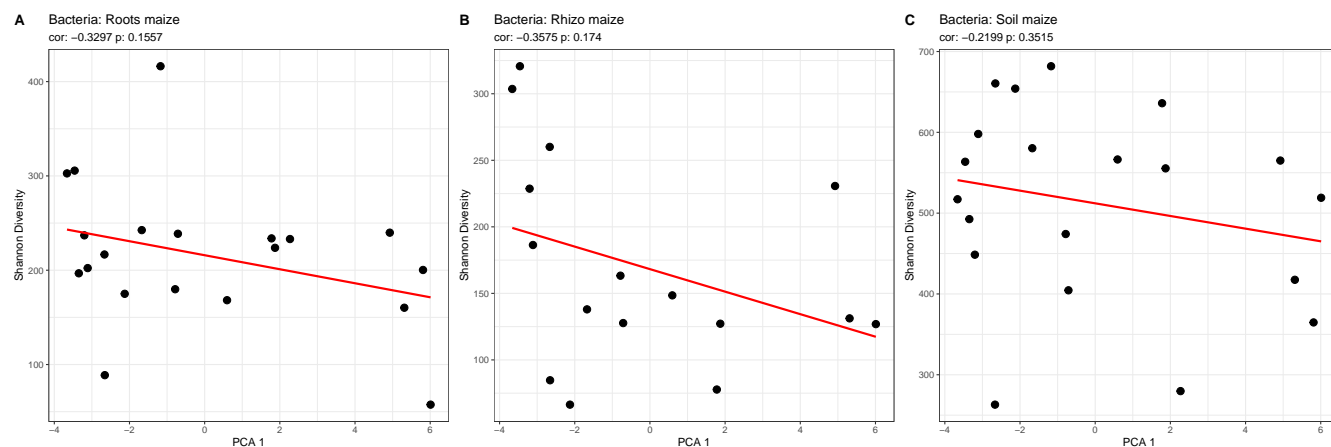
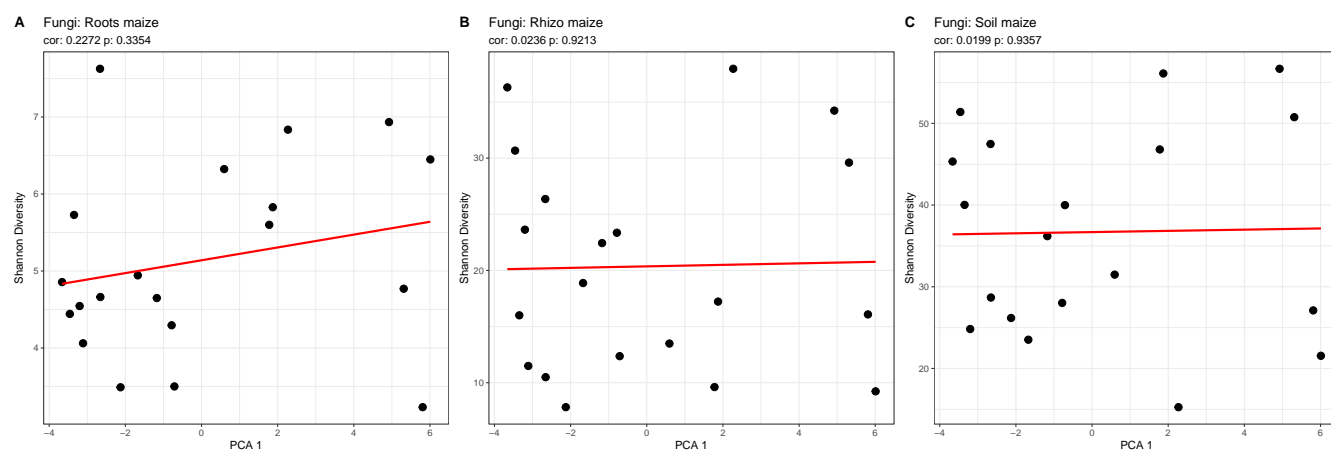


Figure 6.2 | Fungi: Correlation alpha diversity ~ soil chemical gradient



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 samplotype

Model: $\sim \text{Conditioning} * \text{PCA_soil_chem_1}$

Table 13: Bacteria: roots maize

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

Table 14: Bacteria: rhizo maize

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

Table 15: Bacteria: soil maize

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

Table 16: Fungi: roots maize

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

Table 17: Fungi: rhizo maize

	Df	SumOfSqs	R2	F	Pr(>F)
Conditioning	1	0.3649	0.1376	3.295	0.004
Soil chemistry PC1	1	0.3597	0.1356	3.248	0.003
Conditioning : Soil chemistry PC1	1	0.1562	0.05887	1.41	0.157
Residual	16	1.772	0.668	NA	NA
Total	19	2.653	1	NA	NA

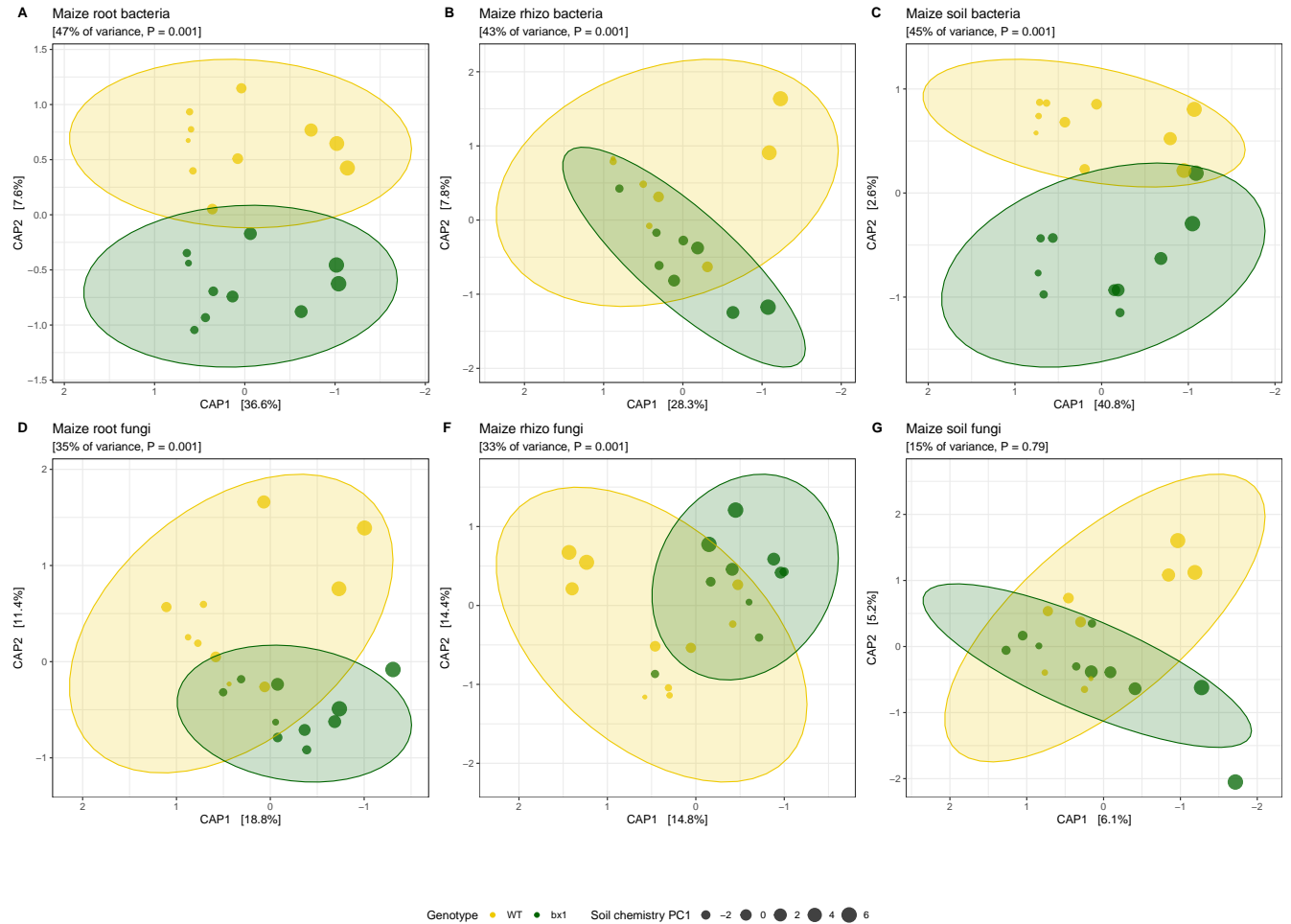
Table 18: Fungi: soil maize

	Df	SumOfSqs	R2	F	Pr(>F)
Conditioning	1	0.2556	0.0437	0.774	0.913
PCA_soil_chem_1	1	0.3557	0.06082	1.077	0.295
Conditioning:PCA_soil_chem_1	1	0.284	0.04855	0.8599	0.775
Residual	15	4.954	0.8469	NA	NA
Total	18	5.849	1	NA	NA

CAP by sampletype

Model: $\sim \text{Conditioning} * \text{PCA_soil_chem_1}$

Figure 7 | CAP of maize (by sampletype)



Conclusion: The chemical gradient affected the bacterial communities more strongly (sig. in all compartments) compared to BX conditioning. A significant BX effect was found in maize roots for bacteria. Rhizosphere and soil are not affected. The chemical gradient affected the fungal communities less than the bacteria (lower R^2 and only in root and rhizosphere). The BX conditioning 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-chemical gradient

We know from previous analyses that we have a soil-chemical gradient along the field. We tested if the microbiome changes along this gradient. 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)
Conditioning	0.02803	0.379
Soil chemistry PC1	0.4649	0.001
Conditioning : Soil chemistry PC1	0.01959	0.651

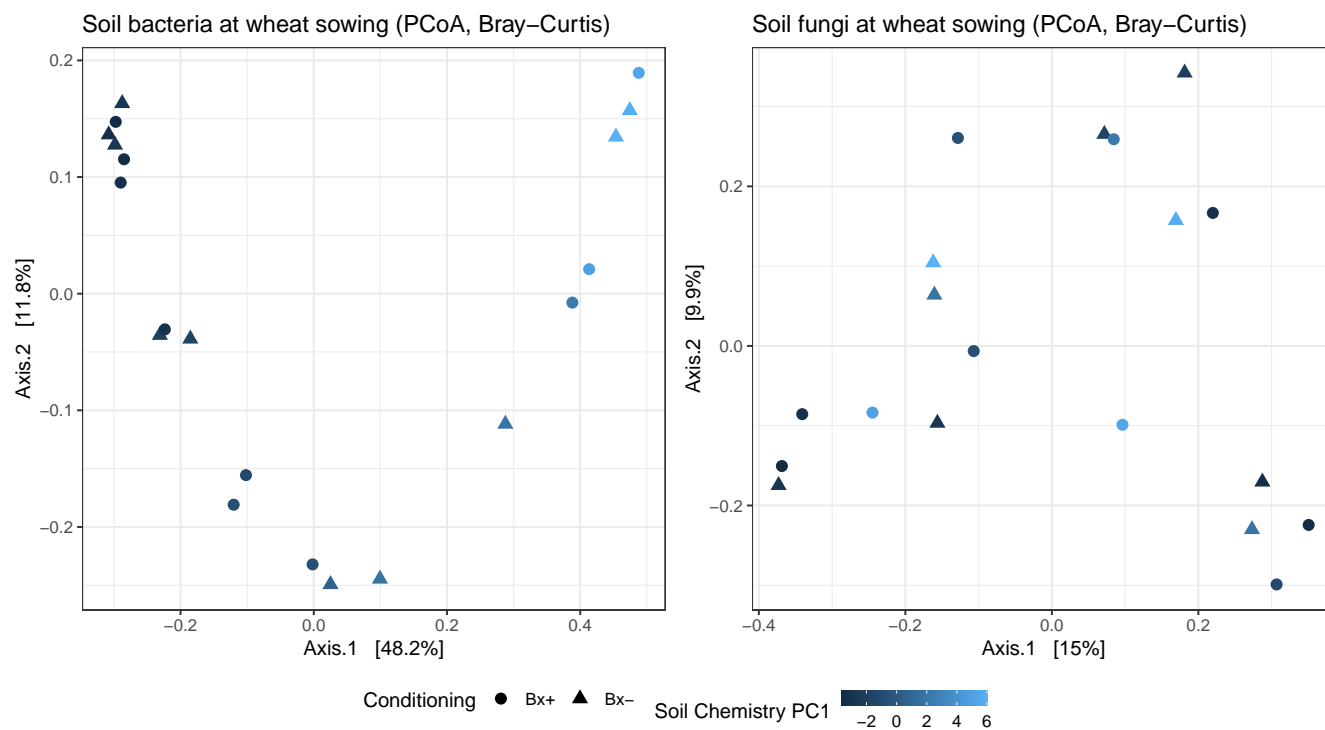
Table 20: Fungi

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

PCoA

We performed an unconstrained ordination with Bray-Curtis distances.

Figure 8 | PCoA with Bray-Curtis



Correlation analysis: PCoA (microbiota) ~ PCA (soil chemistry)

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

Figure 9.1 | Bacteria: Correlation (microbiota) ~ PCA (soil chemistry)

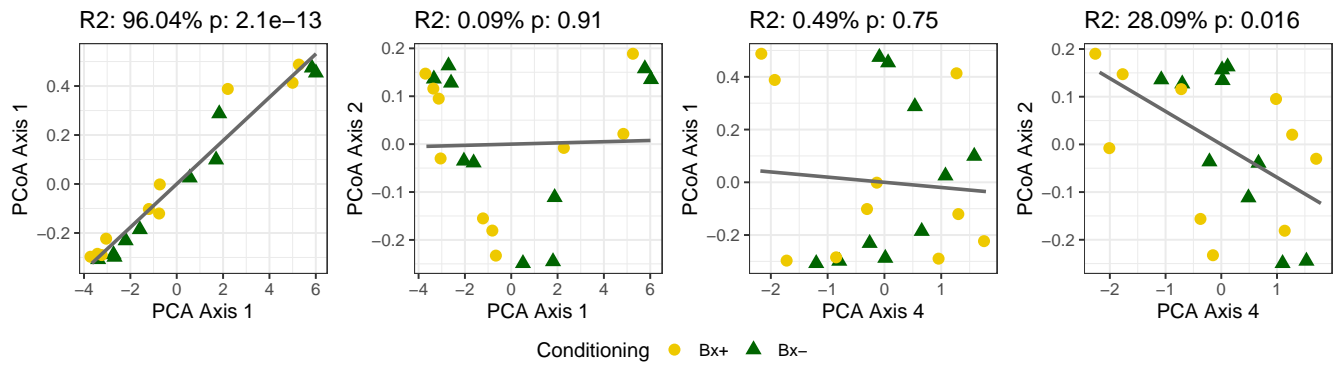
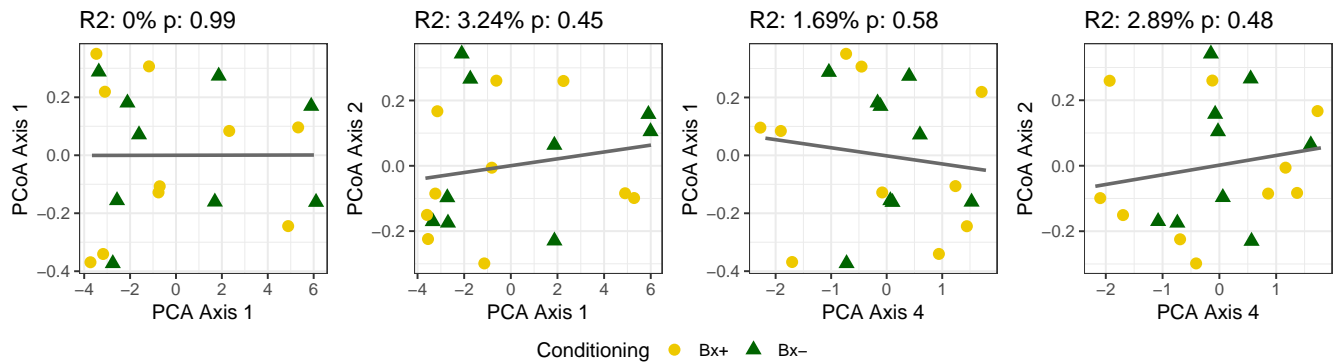


Figure 9.2 | Fungi: Correlation (microbiota) ~ PCA (soil chemistry)



Conclusion: The microbiome is influenced by the chemical soil gradient. To take this chemical soil gradient into account, we included the axis 1 of the PCA of the soil chemistry data 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 (Bx+, Bx-). 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: $\sim \text{Conditioning} * \text{PCA_soil_chem_1}$

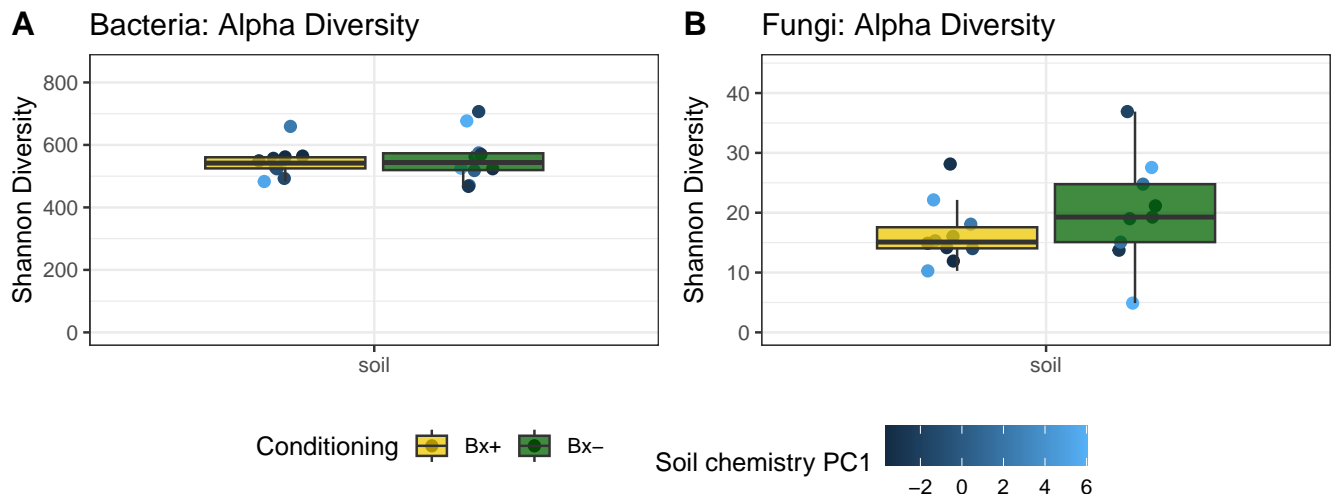
Table 21: Bacteria bare-soil

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Conditioning	1	993.9	993.9	0.21	0.6529
Soil chemistry PC1	1	184.7	184.7	0.03903	0.8459
Conditioning : Soil chemistry PC1	1	1784	1784	0.3771	0.5478
Residuals	16	75719	4732	NA	NA

Table 22: Fungi bare-soil

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Conditioning	1	67.18	67.18	1.156	0.2992
Soil chemistry PC1	1	16.8	16.8	0.2891	0.5987
Conditioning : Soil chemistry PC1	1	16.2	16.2	0.2787	0.6052
Residuals	15	871.5	58.1	NA	NA

Figure 10 | Shannon diversity by conditioning



Conclusion: The chemical field gradient 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 * PCA_soil_chem_1*

Table 23: Bacteria: soil at sowing

	Df	SumOfSqs	R2	F	Pr(>F)
Conditioning	1	0.1027	0.02803	0.9201	0.384
Soil chemistry PC1	1	1.704	0.4649	15.26	0.001
Conditioning : Soil chemistry PC1	1	0.07178	0.01959	0.6429	0.654
Residual	16	1.786	0.4875	NA	NA
Total	19	3.664	1	NA	NA

Fungi

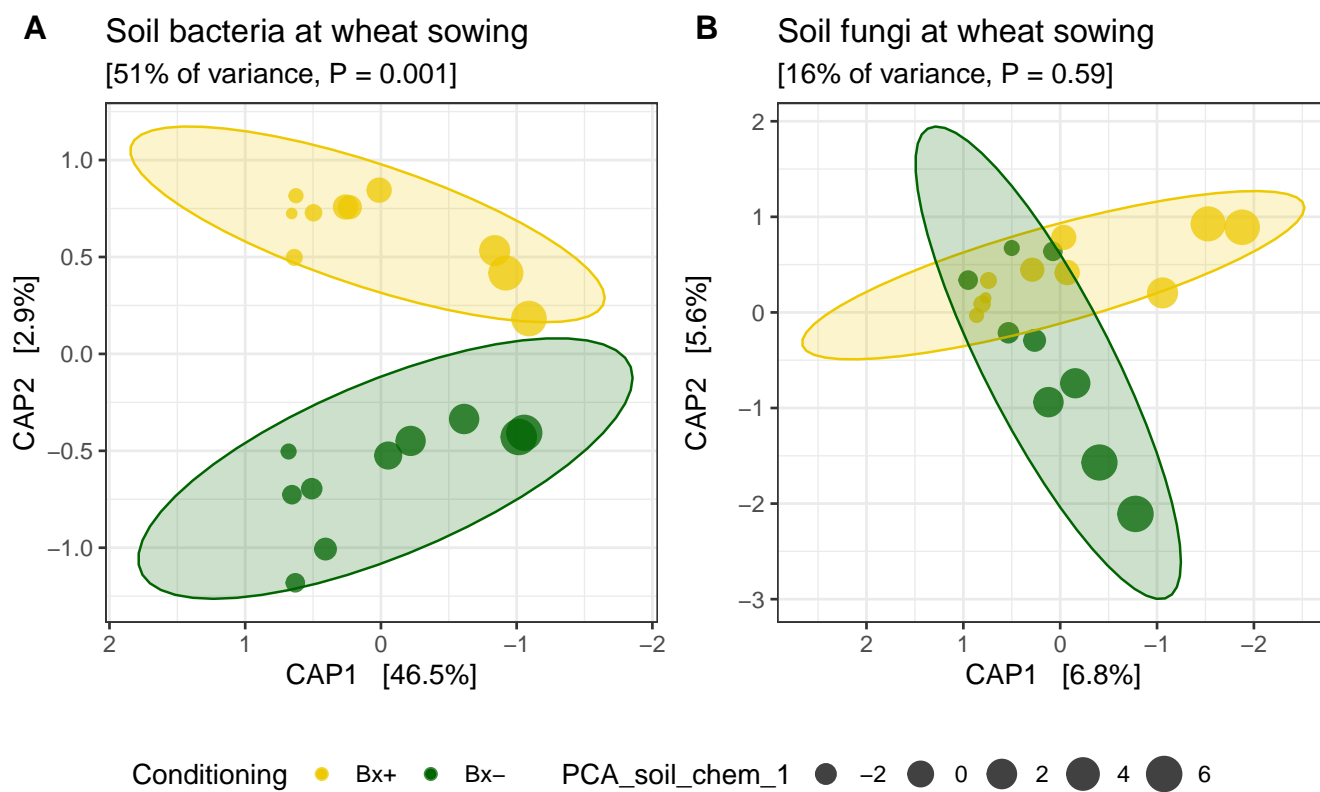
Table 24: Fungi: soil at sowing

	Df	SumOfSqs	R2	F	Pr(>F)
Conditioning	1	0.3156	0.04453	0.7968	0.89
Soil chemistry PC1	1	0.4645	0.06556	1.173	0.173
Conditioning : Soil chemistry PC1	1	0.3653	0.05155	0.9223	0.636
Residual	15	5.941	0.8384	NA	NA
Total	18	7.086	1	NA	NA

CAP

Model: $\sim \text{Conditioning} * \text{PCA_soil_chem_1}$

Figure 11 | CAP



Conclusion: The chemical gradient 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-chemical gradient

We know from previous analyses that we have a soil-chemical gradient along the field. We tested if the microbiome changes along this gradient. 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)
Sampletype	0.2427	0.001
Soil chemistry PC1	0.2423	0.001
Residual	0.5149	NA

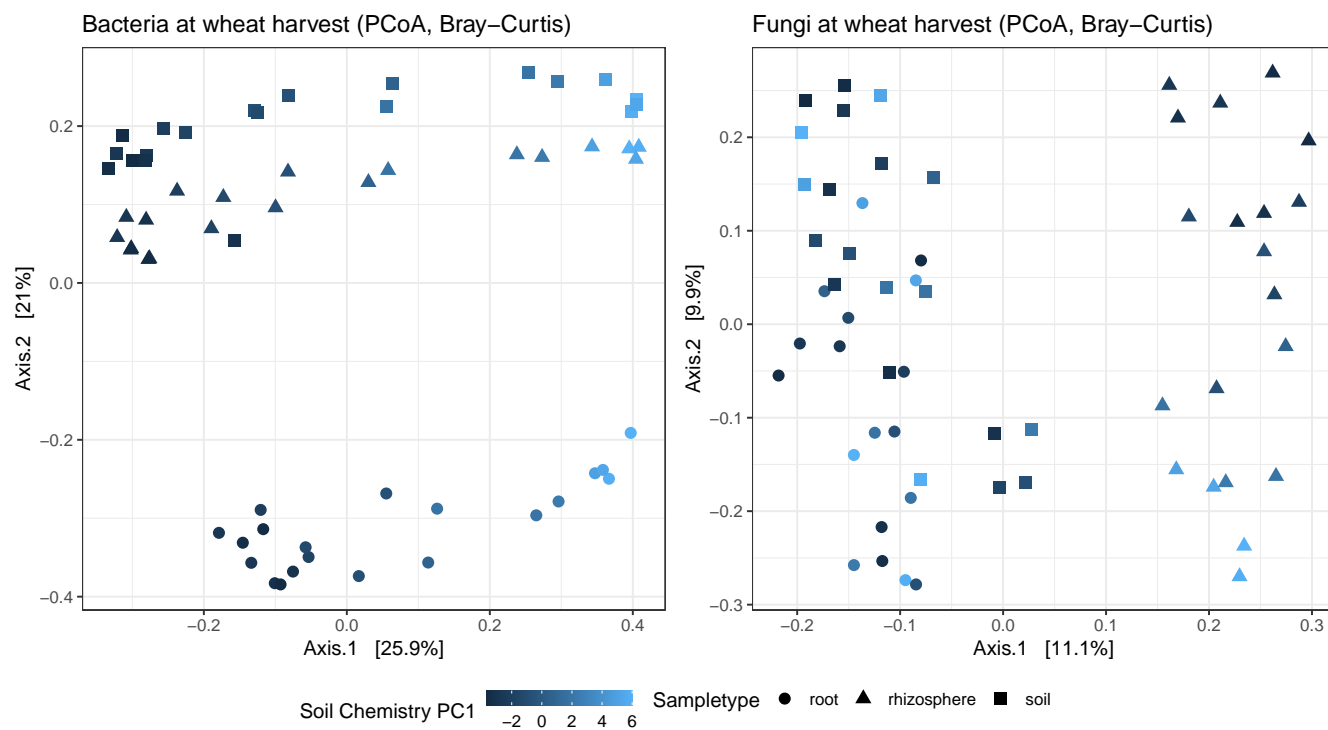
Table 26: Fungi

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

PCoA

We performed an unconstrained ordination with Bray-Curtis distances.

Figure 12 | PCoA with Bray-Curtis



Correlation analysis: PCoA (microbiota) ~ PCA (soil chemistry)

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

Figure 13.1 | Bacteria: Correlation (microbiota) ~ PCA (soil chemistry)

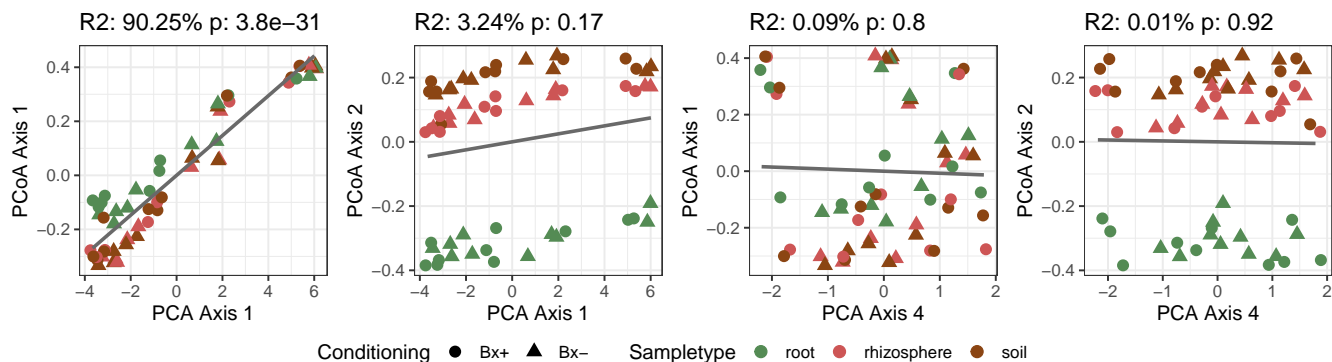
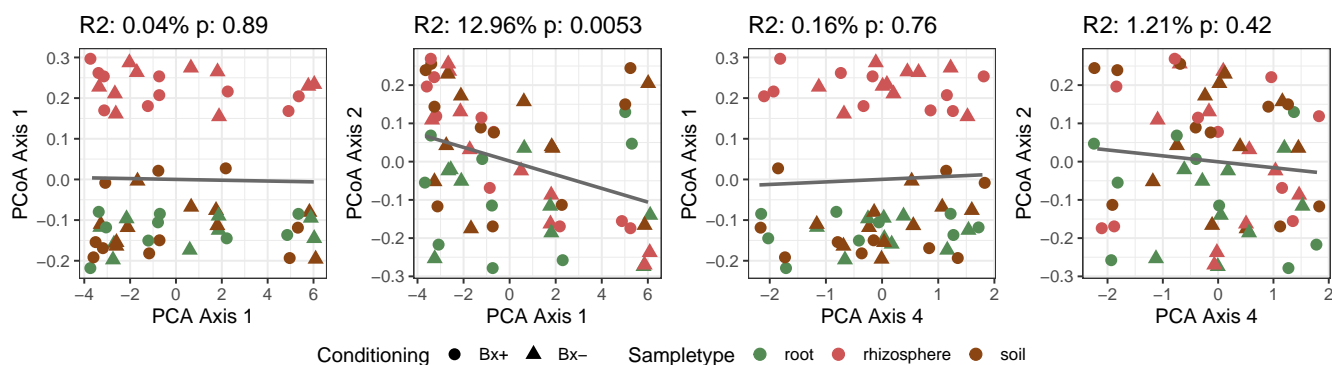


Figure 13.2 | Fungi: Correlation (microbiota) ~ PCA (soil chemistry)



Conclusion: The microbiome is influenced by the chemical soil gradient. To take this chemical soil gradient into account, we included the axis 1 of the PCA of the soil chemistry data 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 (Bx+, Bx-). 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 * PCA_soil_chem_1*

Table 27: Bacteria all compartments

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Conditioning	1	2.32	2.32	7.989e-05	0.9929
Soil chemistry PC1	1	7243	7243	0.2494	0.6195
Conditioning : Soil chemistry PC1	1	3004	3004	0.1034	0.7489
Residuals	56	1626419	29043	NA	NA

Table 28: Bacteria roots

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Conditioning	1	1695	1695	0.5302	0.4771
Soil chemistry PC1	1	9639	9639	3.015	0.1017
Conditioning : Soil chemistry PC1	1	988.3	988.3	0.3091	0.5859
Residuals	16	51152	3197	NA	NA

Table 29: Bacteria rhizosphere

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Conditioning	1	13526	13526	1.835	0.1944
Soil chemistry PC1	1	1808	1808	0.2452	0.6272
Conditioning : Soil chemistry PC1	1	1955	1955	0.2651	0.6136
Residuals	16	117950	7372	NA	NA

Table 30: Bacteria soil

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Conditioning	1	5255	5255	0.3259	0.576
Soil chemistry PC1	1	41239	41239	2.558	0.1293
Conditioning : Soil chemistry PC1	1	29099	29099	1.805	0.1979
Residuals	16	257978	16124	NA	NA

Table 31: Fungi all compartments

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

Table 32: Fungi roots

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

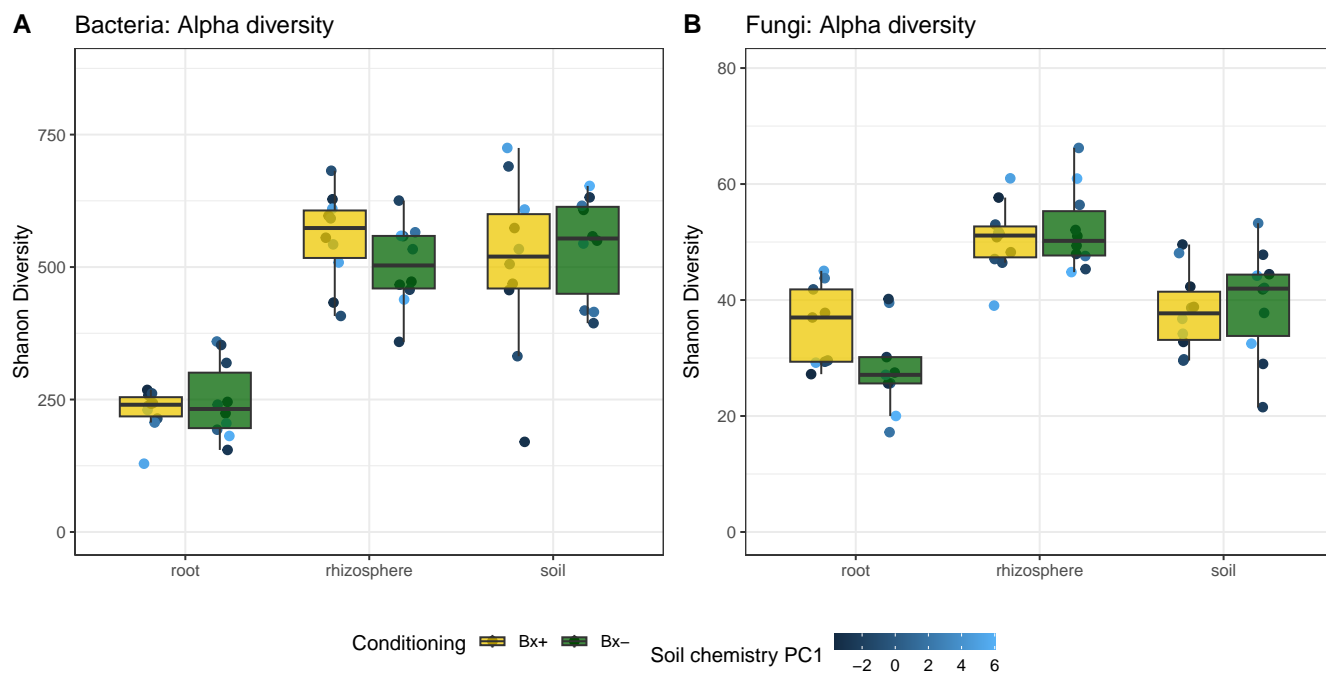
Table 33: Fungi rhizosphere

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

Table 34: Fungi soil

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

Figure 14 | Shannon diversity by samplotype and conditioning



Conclusion: The chemical field gradient influenced the alpha diversity of root fungal communities. They have higher Shannon diversity in BX+ compared to BX- conditions (significant).

Correlation analysis: alpha diversity ~ soil chemical gradient

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

Figure 15.1 | Bacteria: Correlation alpha diversity ~ soil chemical gradient

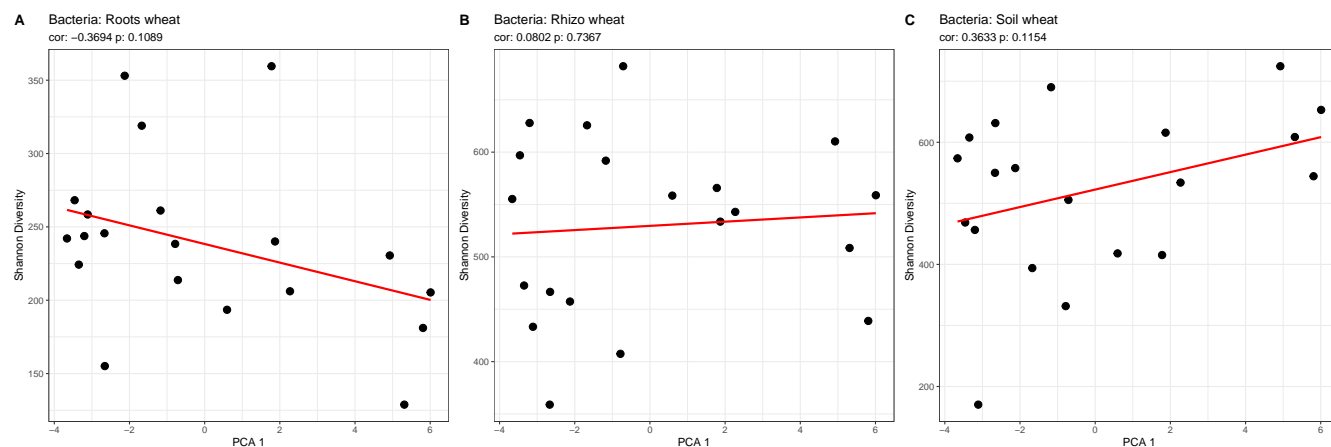
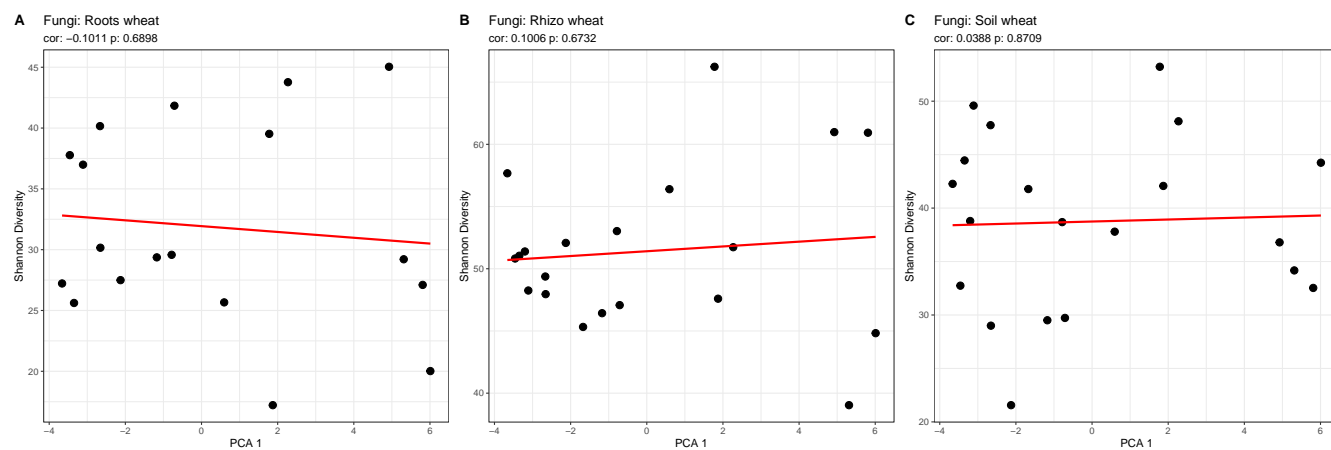


Figure 15.2 | Fungi: Correlation alpha diversity ~ soil chemical gradient



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: $\sim \text{Conditioning} * \text{PCA_soil_chem_1}$

Bacteria

Table 35: Bacteria: roots wheat

	Df	SumOfSqs	R2	F	Pr(>F)
Conditioning	1	0.07777	0.02221	0.6698	0.683
PCA_soil_chem_1	1	1.474	0.4208	12.69	0.001
Conditioning:PCA_soil_chem_1	1	0.09307	0.02658	0.8016	0.513
Residual	16	1.858	0.5305	NA	NA
Total	19	3.502	1	NA	NA

Table 36: Bacteria: rhizo wheat

	Df	SumOfSqs	R2	F	Pr(>F)
Conditioning	1	0.09221	0.02583	0.8217	0.486
PCA_soil_chem_1	1	1.595	0.4467	14.21	0.001
Conditioning:PCA_soil_chem_1	1	0.08748	0.02451	0.7795	0.49
Residual	16	1.796	0.503	NA	NA
Total	19	3.57	1	NA	NA

Table 37: Bacteria: soil wheat

	Df	SumOfSqs	R2	F	Pr(>F)
Conditioning	1	0.1082	0.02549	0.7411	0.597
PCA_soil_chem_1	1	1.698	0.3998	11.62	0.001
Conditioning:PCA_soil_chem_1	1	0.1042	0.02453	0.7132	0.648
Residual	16	2.337	0.5502	NA	NA
Total	19	4.247	1	NA	NA

Conclusion: The chemical gradient 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)
Conditioning	1	0.3169	0.08461	1.492	0.026
PCA_soil_chem_1	1	0.2146	0.0573	1.01	0.46
Conditioning:PCA_soil_chem_1	1	0.2402	0.06412	1.131	0.236
Residual	14	2.974	0.794	NA	NA
Total	17	3.746	1	NA	NA

Table 39: Fungi: rhizo wheat

	Df	SumOfSqs	R2	F	Pr(>F)
Conditioning	1	0.1354	0.03239	0.7218	0.815
PCA_soil_chem_1	1	0.9027	0.216	4.812	0.001
Conditioning:PCA_soil_chem_1	1	0.1406	0.03364	0.7497	0.779
Residual	16	3.001	0.718	NA	NA
Total	19	4.18	1	NA	NA

Table 40: Fungi: soil wheat

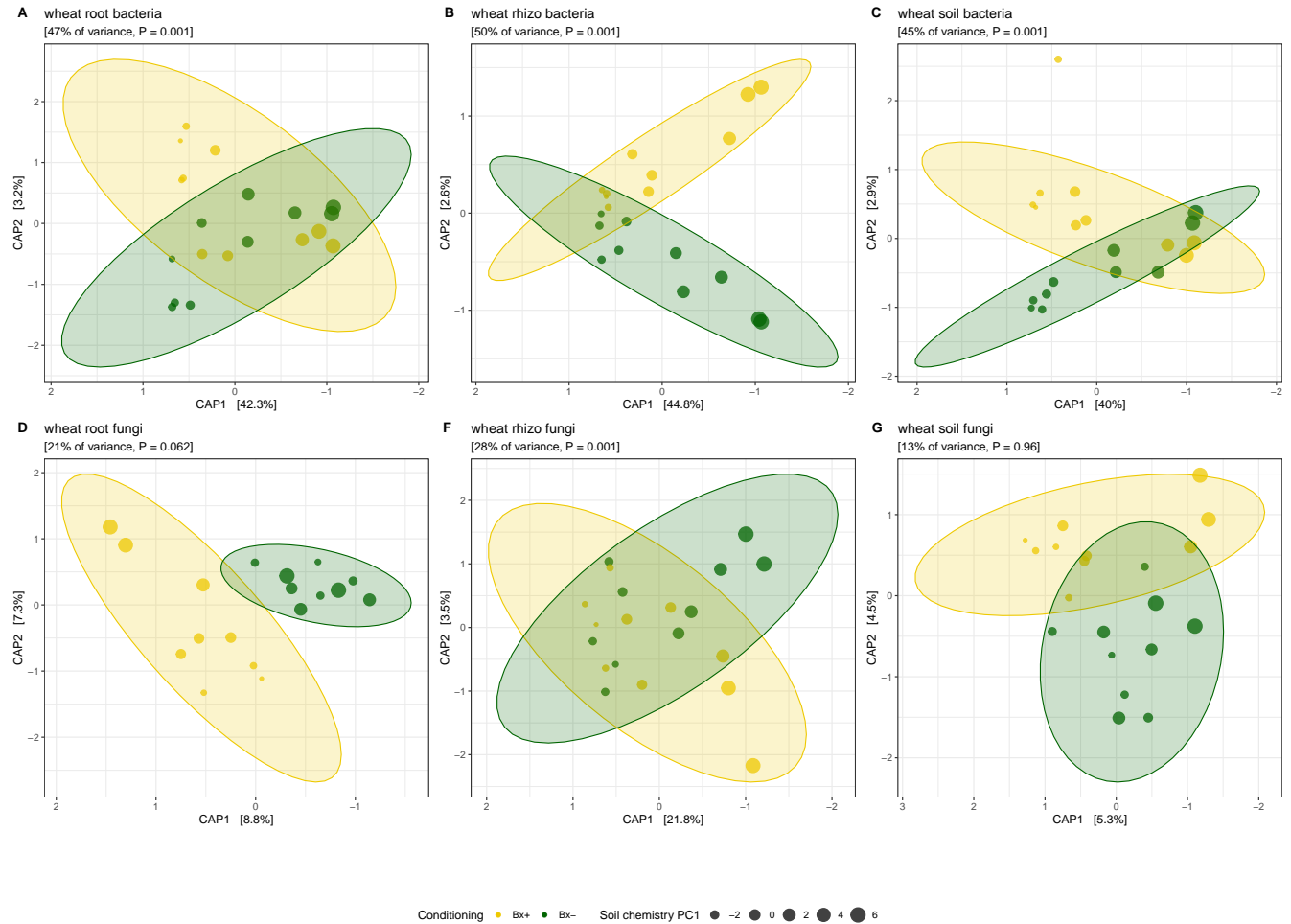
	Df	SumOfSqs	R2	F	Pr(>F)
Conditioning	1	0.2239	0.0459	0.8427	0.766
PCA_soil_chem_1	1	0.232	0.04757	0.8733	0.685
Conditioning:PCA_soil_chem_1	1	0.1706	0.03498	0.6421	0.989
Residual	16	4.251	0.8715	NA	NA
Total	19	4.877	1	NA	NA

Conclusion: The chemical gradient 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: $\sim \text{Conditioning} * \text{PCA_soil_chem_1}$

Figure 16 | CAP of wheat (by samplotype)



Conclusion: The chemical gradient affected the bacterial communities more strongly (sig. in all compartments) compared to BX conditioning. No significant BX effect was found for bacteria. The chemical gradient 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 in roots.