

Herbicide Effects - Microbiome-Analysis

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Experimental Setup

Two experiments were performed. In experiment 1 (exp1) we kept a constant 60% water holding capacity (WHC) while in experiment 2 (exp2) all pots were flushed (100% WHC) before keeping a constant 60% WHC. In both experiments, we had three Herbicide (Hc) treatments: water control (ctr), glyphosate (gly) and terbuthylazine (tb) which were applied on two different targets: Bare soil (S) or weeds (Ca; *Chenopodium album*). Two weeks after applying herbicides, maize was planted in the soil. Soil samples were collected at: 2W (2 weeks after herbicide application, maize planting, 7 reps. per treatment) and 5W (5 weeks after herbicide application, 3 weeks old maize, 7 reps. per treatment).

Description all data

Sequencing depth

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

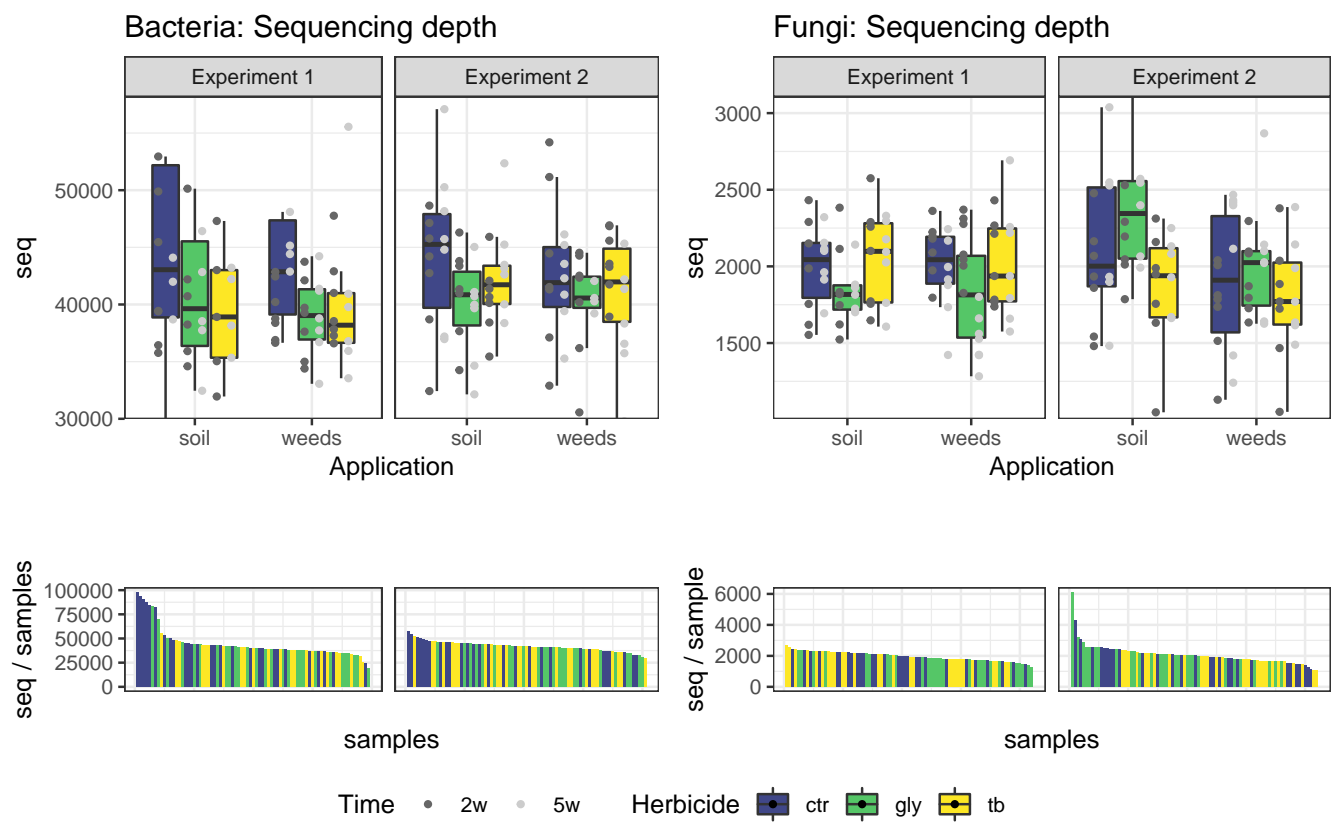
Bacteria

```
## [1] "8 samples removed"
## [1] "sum: 6888025"
## [1] "min: 19462" "max: 97860"
## [1] "median: 41264"
```

Fungi

```
## [1] "No samples removed"
## [1] "sum: 338223"
## [1] "min: 1048" "max: 6108"
## [1] "median: 1989"
```

Figure 1 | Sequencing depth



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 by using the non-parametric Kruskal-Wallis Test.

Bacteria

```
##  
## Kruskal-Wallis rank sum test  
##  
## data: sample_depth by group  
## Kruskal-Wallis chi-squared = 15.778, df = 11, p-value = 0.1496
```

Fungi

```
##  
## Kruskal-Wallis rank sum test  
##  
## data: sample_depth by group  
## Kruskal-Wallis chi-squared = 22.451, df = 11, p-value = 0.0211
```

Conclusion: Because there are significant differences for fungi between the sample groups, we normalized the data by rarefaction for diversity comparisons (see Weiss et al. (2017), Microbiome Journal). For consistency, we rarefied for bacteria and fungi. We defined the rarefaction threshold per sample to 1.9×10^4 for bacteria and 1000 for fungi .

Sample Size

We discarded eight bacterial samples with too few reads per sample while we kept all fungal samples. We ended up with the following number of samples per treatment for the analysis. The samples were labeled as “Herbicide_Application_Experiment_Time”:

Table 1: Sample profile

Sample	Bacteria	Fungi
ctr_soil_1_2	7	7
ctr_soil_1_5	7	7
ctr_soil_2_2	7	7
ctr_soil_2_5	7	7
ctr_weeds_1_2	7	7
ctr_weeds_1_5	7	7
ctr_weeds_2_2	7	7
ctr_weeds_2_5	7	7
gly_soil_1_2	7	7
gly_soil_1_5	7	7
gly_soil_2_2	7	7
gly_soil_2_5	7	7
gly_weeds_1_2	7	7
gly_weeds_1_5	7	7
gly_weeds_2_2	7	7
gly_weeds_2_5	4	7
tb_soil_1_2	5	7
tb_soil_1_5	4	7
tb_soil_2_2	7	7
tb_soil_2_5	7	7
tb_weeds_1_2	7	7
tb_weeds_1_5	7	7
tb_weeds_2_2	7	7
tb_weeds_2_5	7	7

Taxonomy

Phyla abundance plot

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

Figure 2.1 | Bacteria: Phylum level taxonomy

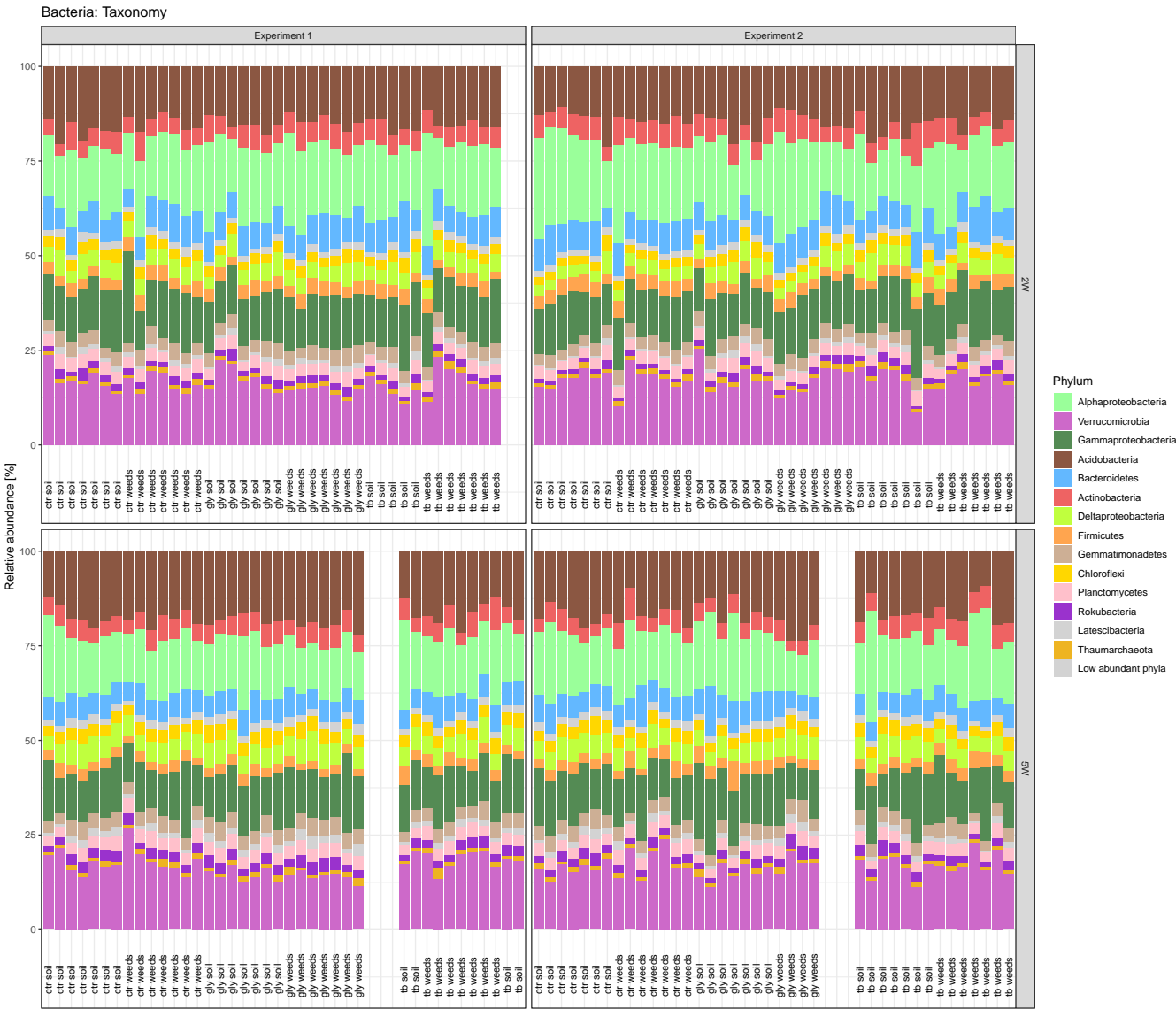
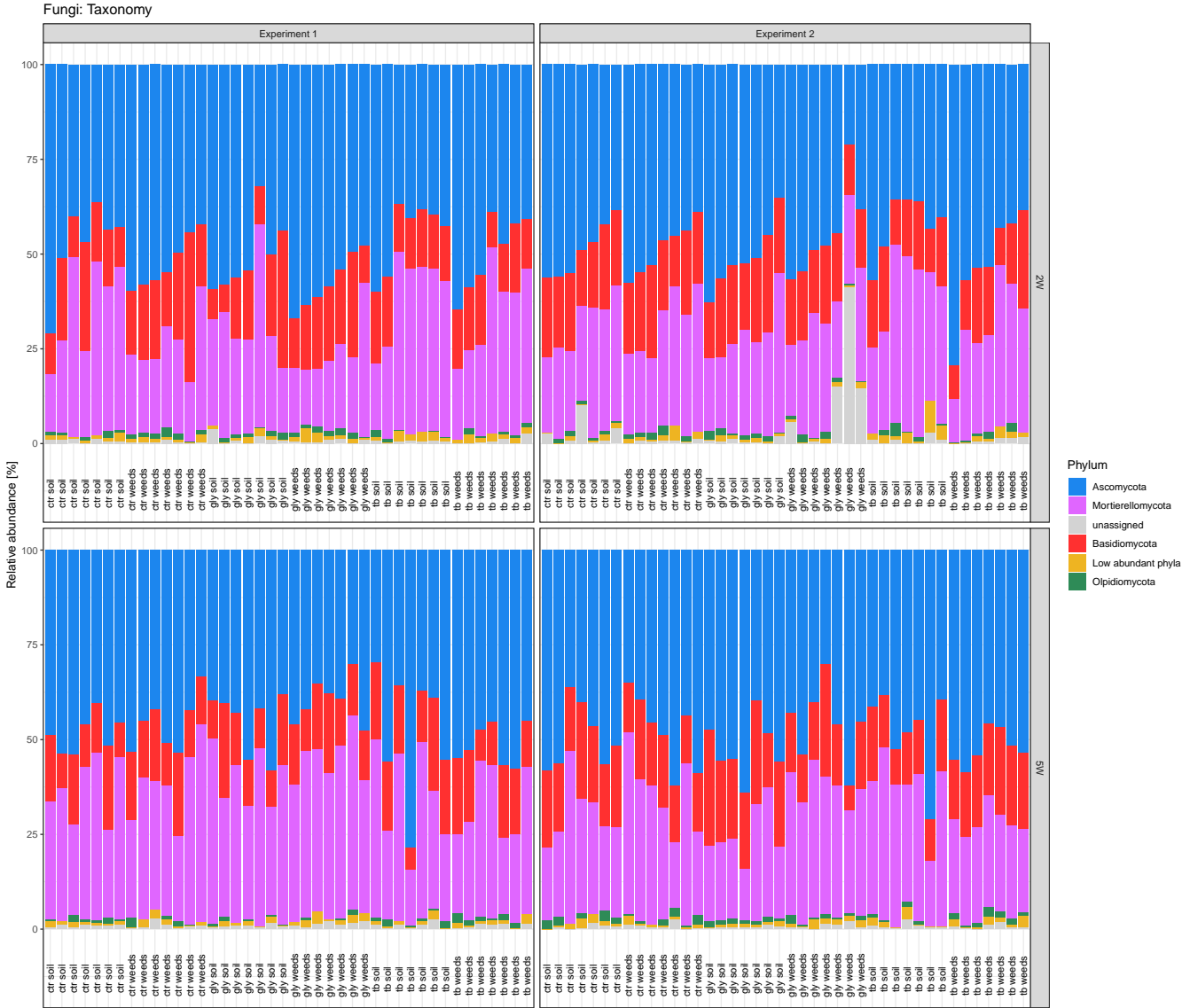


Figure 2.2 | Fungi: Phylum level taxonomy



Effect of all factors on phyla abundances

We test if there is any difference between the phyla abundances between the experiments, the two timepoints, the modes of application, herbicide treatments, any of their interactions or technical lab artefacts (the DNA extraction and PCR amplification was done on two plates) by performing a PERMANOVA (permutations = 999).

Bacteria

Table 2: Bacteria PERMANOVA: Experiment 1

	Df	SumOfSqs	R2	F	Pr(>F)
Herbicide	2	0.02272	0.04433	2.104	0.039
Application	1	0.00241	0.004702	0.4463	0.796
Time	1	0.07635	0.149	14.14	0.001
Plate	1	0.01166	0.02275	2.16	0.078
Herbicide:Application	2	0.00836	0.01631	0.7741	0.583
Herbicide:Time	2	0.02097	0.04091	1.942	0.082
Application:Time	1	0.00866	0.0169	1.604	0.158
Herbicide:Application:Time	2	0.00503	0.009814	0.4658	0.878
Residual	66	0.3564	0.6953	NA	NA
Total	78	0.5125	1	NA	NA

Table 3: Bacteria PERMANOVA: Experiment 1

	Df	SumOfSqs	R2	F	Pr(>F)
Herbicide	2	0.009393	0.01327	0.6295	0.683
Application	1	0.003506	0.004953	0.4699	0.706
Time	1	0.07476	0.1056	10.02	0.002
Plate	1	0.01548	0.02188	2.076	0.101
Herbicide:Application	2	0.01575	0.02225	1.055	0.375
Herbicide:Time	2	0.0275	0.03885	1.843	0.12
Application:Time	1	0.02114	0.02986	2.833	0.048
Herbicide:Application:Time	2	0.03294	0.04654	2.207	0.054
Residual	68	0.5073	0.7168	NA	NA
Total	80	0.7078	1	NA	NA

Fungi

Table 4: Fungi PERMANOVA: Experiment 1

	Df	SumOfSqs	R2	F	Pr(>F)
Herbicide	2	0.004059	0.002904	0.1921	0.951
Application	1	0.02543	0.01819	2.407	0.108
Time	1	0.074	0.05294	7.004	0.003
Plate	1	0.4487	0.321	42.47	0.001
Herbicide:Application	2	0.01659	0.01187	0.7849	0.503
Herbicide:Time	2	0.03144	0.02249	1.488	0.21
Application:Time	1	0.0483	0.03455	4.571	0.017
Herbicide:Application:Time	2	-0.0008099	-0.0005794	-0.03833	1
Residual	71	0.7501	0.5366	NA	NA
Total	83	1.398	1	NA	NA

Table 5: Fungi PERMANOVA: Experiment 1

	Df	SumOfSqs	R2	F	Pr(>F)
Herbicide	2	0.03628	0.02971	1.656	0.146
Application	1	0.007012	0.005744	0.6402	0.569
Time	1	0.0104	0.008521	0.9499	0.39
Plate	1	0.2516	0.2061	22.97	0.001
Herbicide:Application	2	0.05161	0.04228	2.356	0.046
Herbicide:Time	2	0.02951	0.02417	1.347	0.218
Application:Time	1	0.01951	0.01598	1.781	0.124
Herbicide:Application:Time	2	0.03729	0.03055	1.703	0.134
Residual	71	0.7776	0.637	NA	NA
Total	83	1.221	1	NA	NA

Conclusion: The relative phyla abundances change over time for bacteria and fungi.

Alpha diversity

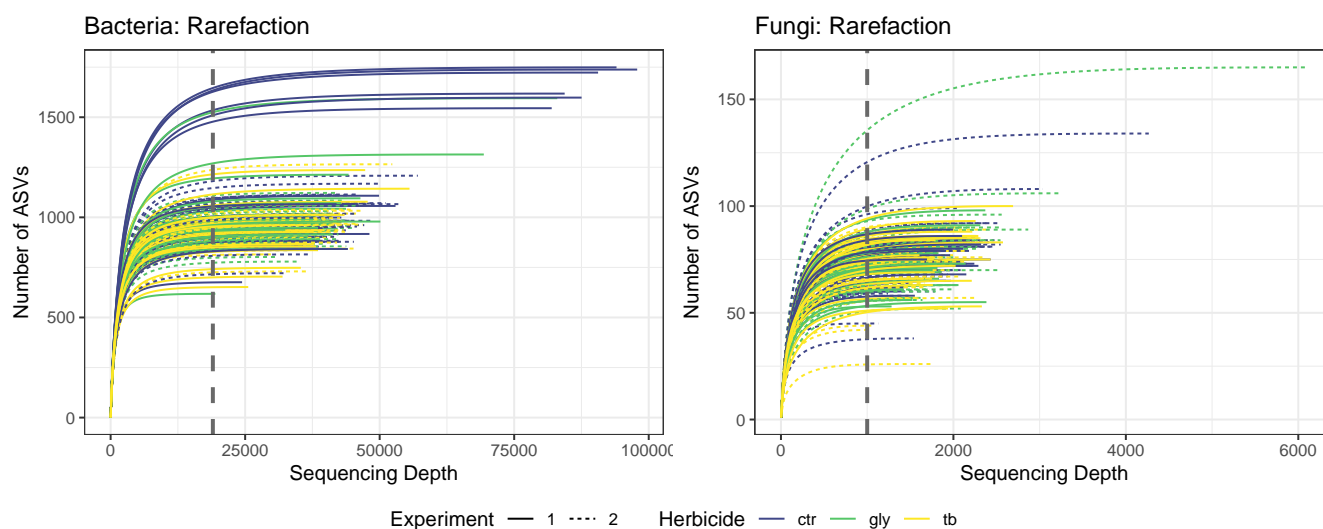
We answered the following questions for the bacterial and fungal alpha diversity:

- **Q1:** Are there differences in alpha diversity between the herbicide applications?
- **Q2:** Are there differences in alpha diversity between the herbicide treatments?

Rarefaction

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

Figure 3 | Rarefaction



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

Method

We rarefied the dataset by the sequencing depth of 1.9×10^4 for bacteria and 1000 for fungi and calculated the Shannon diversity as a index for alpha diversity for each sample. This was repeated 100 times. Then, the mean value from the 100 iterations was taken for statistical analysis between the different samples.

Effect of all factors on alpha diversity

Before answering the questions, we got an overview for both experiments by investigating the effect on alpha diversity by the factors of different herbicides, different herbicide applications, time, any of their interactions and technical lab artefacts (the DNA extraction and PCR amplification was done on two plates). We modeled the alpha diversity against these factors in an aov-model and performed a F-Test.

Bacteria

Table 6: Bacteria, experiment 1: F test

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Herbicide	2	28546	14273	1.779	0.1768
Application	1	167.6	167.6	0.02089	0.8855
Time	1	82941	82941	10.34	0.002019
Plate	1	92269	92269	11.5	0.001179
Herbicide:Application	2	5080	2540	0.3166	0.7297
Herbicide:Time	2	22518	11259	1.403	0.253
Application:Time	1	7.181	7.181	0.000895	0.9762
Herbicide:Application:Time	2	30952	15476	1.929	0.1534
Residuals	66	529533	8023	NA	NA

Table 7: Bacteria, experiment 2: F test

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Herbicide	2	24.55	12.28	0.002736	0.9973
Application	1	8165	8165	1.819	0.1819
Time	1	36301	36301	8.089	0.005878
Plate	1	8069	8069	1.798	0.1844
Herbicide:Application	2	2063	1032	0.2299	0.7953
Herbicide:Time	2	38443	19221	4.283	0.0177
Application:Time	1	1827	1827	0.4071	0.5256
Herbicide:Application:Time	2	19276	9638	2.148	0.1246
Residuals	68	305171	4488	NA	NA

Fungi

Table 8: Fungi, experiment 1: F test

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Herbicide	2	39.26	19.63	0.4862	0.617
Application	1	245.8	245.8	6.088	0.01602
Time	1	38.67	38.67	0.9578	0.3311
Plate	1	398.2	398.2	9.863	0.002459
Herbicide:Application	2	11.26	5.63	0.1394	0.8701
Herbicide:Time	2	146.3	73.16	1.812	0.1708
Application:Time	1	0.001223	0.001223	3.029e-05	0.9956
Herbicide:Application:Time	2	243.2	121.6	3.012	0.05552
Residuals	71	2867	40.38	NA	NA

Table 9: Fungi, experiment 2: F test

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Herbicide	2	257.8	128.9	1.927	0.1531
Application	1	220.4	220.4	3.295	0.07371
Time	1	171.5	171.5	2.564	0.1137
Plate	1	1431	1431	21.39	1.639e-05
Herbicide:Application	2	31.9	15.95	0.2385	0.7884
Herbicide:Time	2	174	86.99	1.301	0.2787
Application:Time	1	19.81	19.81	0.2961	0.588
Herbicide:Application:Time	2	590.3	295.2	4.414	0.01561
Residuals	71	4748	66.88	NA	NA

Conclusion: We see a strong effect on the alpha diversity between the two plates for bacteria and fungi. Samples were randomized on the two plates. We are not interested in differences between the two plates, that's why we kept the "plate-treatment" in the following models as a factor so that effects of the other factors were quantified while accounting for the plate variance. In bacteria, the alpha diversity changed over time. Herbicide application on different targets and using different herbicides did not significantly influence the alpha diversity. For fungi, applying herbicides either on weeds or direct on soil affected the alpha diversity while the time nor the herbicide had a significant influence.

Application effect

Q1: Are there differences in alpha diversity between the herbicides applications?

The herbicide was either applied directly on soil or on weeds. While we couldn't find any effect on the alpha diversity in bacterial communities, we found differences for fungi. We further investigated for both experiments how the different herbicides applications did shape the communities. For consistency we investigated the bacterial and fungal communities.

Bacteria

Table 10: Bacteria: Application effect in experiment 1

	Shannon	.group
soil	474.1	a
weeds	467.4	a

Table 11: Bacteria: Application effect in experiment 2

	Shannon	.group
soil	450.3	a
weeds	430.2	a

Fungi

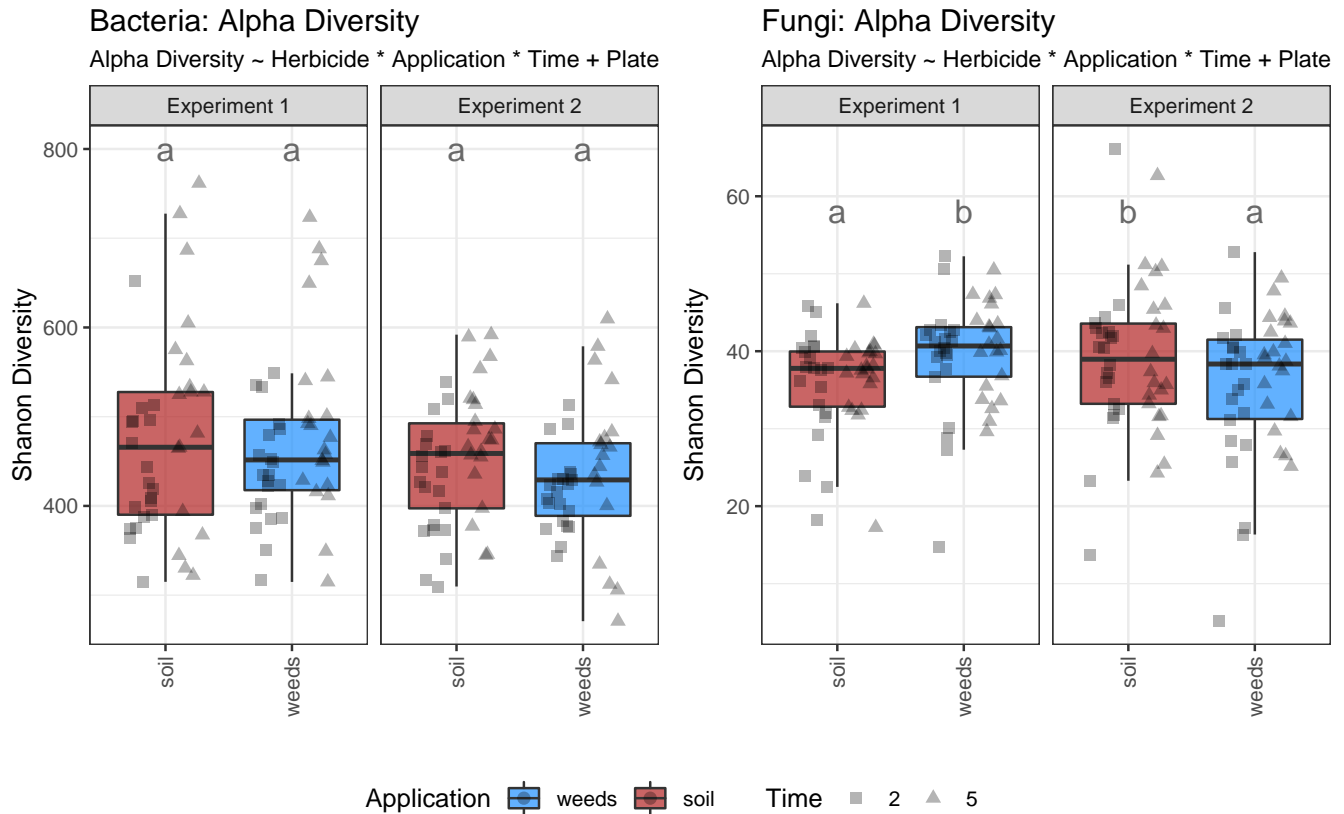
Table 12: Fungi: Application effect in experiment 1

	Shannon	.group
soil	36.15	a
weeds	39.57	b

Table 13: Fungi: Application effect in experiment 2

	Shannon	.group
soil	39.07	b
weeds	35.83	a

Figure 4.1 | Shannon diversity - Application



Conclusion: As already seen, bacterial alpha diversity remains constant between the two herbicide applications. In experiment 1 led applying herbicides on weeds to a higher fungal alpha diversity while we observed the opposite effect in experiment 2.

Herbicide effect

Q2: Are there differences in alpha diversity between the herbicide treatments?

We treated samples either with water (control), glyphosate or terbuthylazine. We saw above that the different herbicides did not influence the bacterial nor fungal alpha diversity.

Conclusion alpha diversity

A inconsistent effect on the fungal alpha diversity was found between the different herbicide applications. The alpha diversity of bacteria and fungi did not changed between water control, glyphosate and terbuthylazine.

Beta diversity

We answered the following questions for the bacterial and fungal beta diversity:

- **Q1: Are there differences in beta diversity between the herbicide applications?**
- **Q2: Are there differences in beta diversity between the herbicide treatments?**

Method

For the two questions we followed the same logic. First we used the function ‘adonis()’ (package vegan) to analyze the beta diversity with a PERMANOVA (permutations = 999). Then, we graphically represent the beta diversity with a CAP plot (constrained ordination).

Effect of all factors on beta diversity

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

Bacteria

Table 14: Bacteria PERMANOVA: Experiment 1

	Df	SumOfSqs	R2	F	Pr(>F)
Herbicide	2	0.2347	0.03485	1.479	0.005
Application	1	0.1001	0.01486	1.261	0.11
Time	1	0.4064	0.06034	5.123	0.001
Plate	1	0.1078	0.016	1.358	0.065
Herbicide:Application	2	0.1603	0.0238	1.01	0.402
Herbicide:Time	2	0.2196	0.03261	1.384	0.026
Application:Time	1	0.1056	0.01568	1.331	0.059
Herbicide:Application:Time	2	0.1647	0.02445	1.038	0.338
Residual	66	5.236	0.7774	NA	NA
Total	78	6.735	1	NA	NA

Table 15: Bacteria PERMANOVA: Experiment 2

	Df	SumOfSqs	R2	F	Pr(>F)
Herbicide	2	0.2397	0.03335	1.505	0.034
Application	1	0.09258	0.01288	1.163	0.188
Time	1	0.6086	0.08467	7.644	0.001
Plate	1	0.08531	0.01187	1.071	0.291
Herbicide:Application	2	0.1798	0.02502	1.129	0.218
Herbicide:Time	2	0.2713	0.03774	1.704	0.013
Application:Time	1	0.09744	0.01356	1.224	0.142
Herbicide:Application:Time	2	0.1989	0.02767	1.249	0.121
Residual	68	5.415	0.7533	NA	NA
Total	80	7.188	1	NA	NA

Fungi

Table 16: Fungi PERMANOVA: Experiment 1

	Df	SumOfSqs	R2	F	Pr(>F)
Herbicide	2	0.3079	0.02258	1.151	0.207
Application	1	0.2561	0.01878	1.915	0.023
Time	1	0.5082	0.03726	3.8	0.001
Plate	1	1.994	0.1462	14.91	0.001
Herbicide:Application	2	0.251	0.01841	0.9386	0.562
Herbicide:Time	2	0.295	0.02163	1.103	0.263
Application:Time	1	0.2239	0.01642	1.674	0.041
Herbicide:Application:Time	2	0.3071	0.02252	1.148	0.222
Residual	71	9.494	0.6962	NA	NA
Total	83	13.64	1	NA	NA

Table 17: Fungi PERMANOVA: Experiment 2

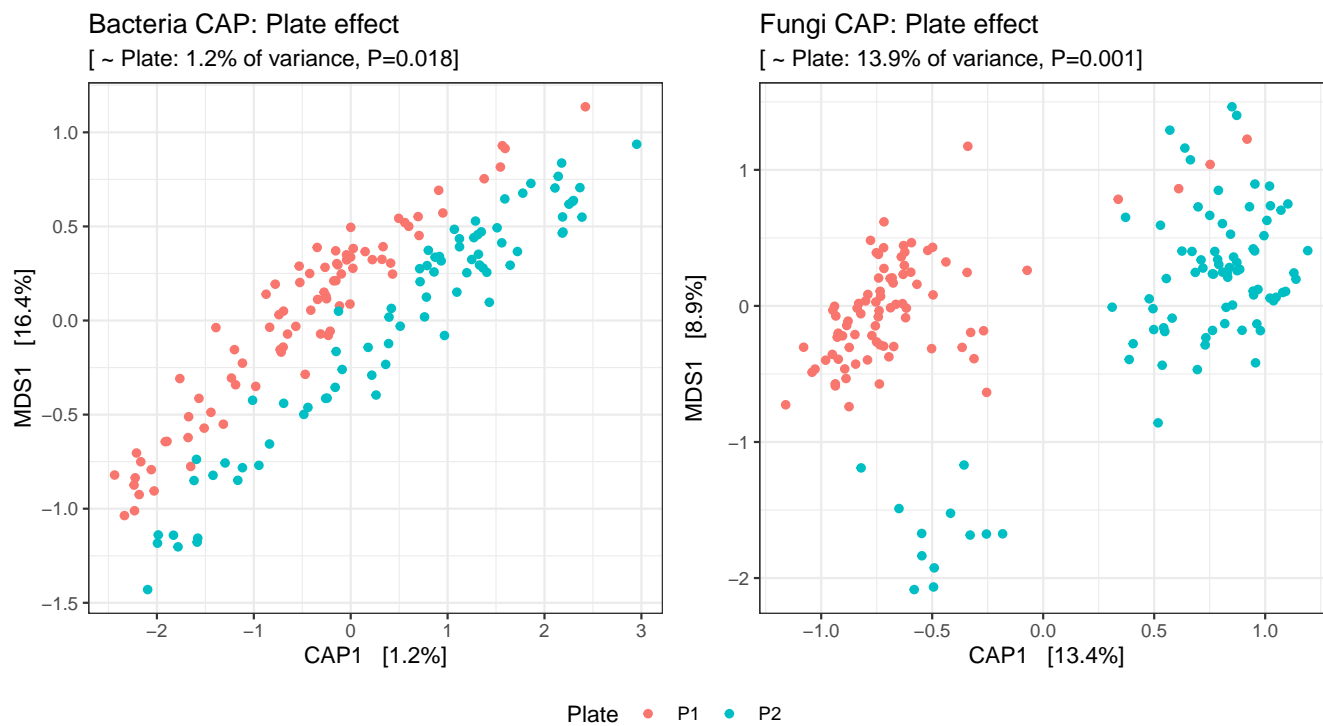
	Df	SumOfSqs	R2	F	Pr(>F)
Herbicide	2	0.4031	0.02927	1.414	0.056
Application	1	0.1593	0.01156	1.117	0.273
Time	1	0.214	0.01553	1.501	0.062
Plate	1	1.646	0.1195	11.55	0.001
Herbicide:Application	2	0.3757	0.02727	1.318	0.093
Herbicide:Time	2	0.3267	0.02372	1.146	0.221
Application:Time	1	0.1702	0.01236	1.194	0.185
Herbicide:Application:Time	2	0.3603	0.02616	1.264	0.109
Residual	71	10.12	0.7346	NA	NA
Total	83	13.77	1	NA	NA

Conclusion: We found an effect on the beta diversity between the two plates for fungi. Samples were randomized on the two plates. We are not interested in differences between the two plates, that's why we kept the "plate-treatment" in the following models as factor so that effects of the other factors were quantified while accounting for the plate variance. For constancy we kept plate as a factor in the model for bacteria and fungi. In bacteria, the beta diversity changed over time and between the different herbicides. Herbicide application on different targets did not significantly influence the beta diversity. Fungi were more constant, only the fungal microbiome shifted over time in experiment 1.

Technical artefacts

We visualized the technical artefacts with a CAP (constrained ordination).

Figure 5.1 | CAP - Plate Effect



Application effect

Q1: Are there differences in beta diversity between the herbicide applications?

We could see that there was no effect for bacteria nor for fungi. Herbicide application direct on soil or on weeds was not relevant for the microbial communities. That's why we do not further investigate the mode of application.

Herbicide effect

Q2: Are there differences in beta diversity between the herbicide treatments?

We treated samples either with water (control), glyphosate or terbuthylazine. We saw that herbicides influenced the beta diversity of bacteria (both experiments) and fungi (only experiment 2). We checked for both experiments for differences between the herbicides.

Bacteria

Table 18: Bacteria: Glyphosate effect in experiment 1 (tested against control)

	Df	SumOfSqs	R2	F	Pr(>F)
Herbicide	1	0.1309	0.02842	1.715	0.014

Table 19: Bacteria: Terbuthylazine effect in experiment 1 (tested against control)

	Df	SumOfSqs	R2	F	Pr(>F)
Herbicide	1	0.1191	0.02771	1.455	0.03

Table 20: Bacteria: Glyphosate effect in experiment 2 (tested against control)

	Df	SumOfSqs	R2	F	Pr(>F)
Herbicide	1	0.1363	0.02906	1.767	0.033

Table 21: Bacteria: Terbuthylazine effect in experiment 2 (tested against control)

	Df	SumOfSqs	R2	F	Pr(>F)
Herbicide	1	0.1498	0.03029	1.908	0.023

Fungi

Table 22: Fungi: Glyphosate effect in experiment 1 (tested against control)

	Df	SumOfSqs	R2	F	Pr(>F)
Herbicide	1	0.1733	0.01973	1.324	0.142

Table 23: Fungi: Terbutylazine effect in experiment 1 (tested against control)

	Df	SumOfSqs	R2	F	Pr(>F)
Herbicide	1	0.157	0.01758	1.185	0.211

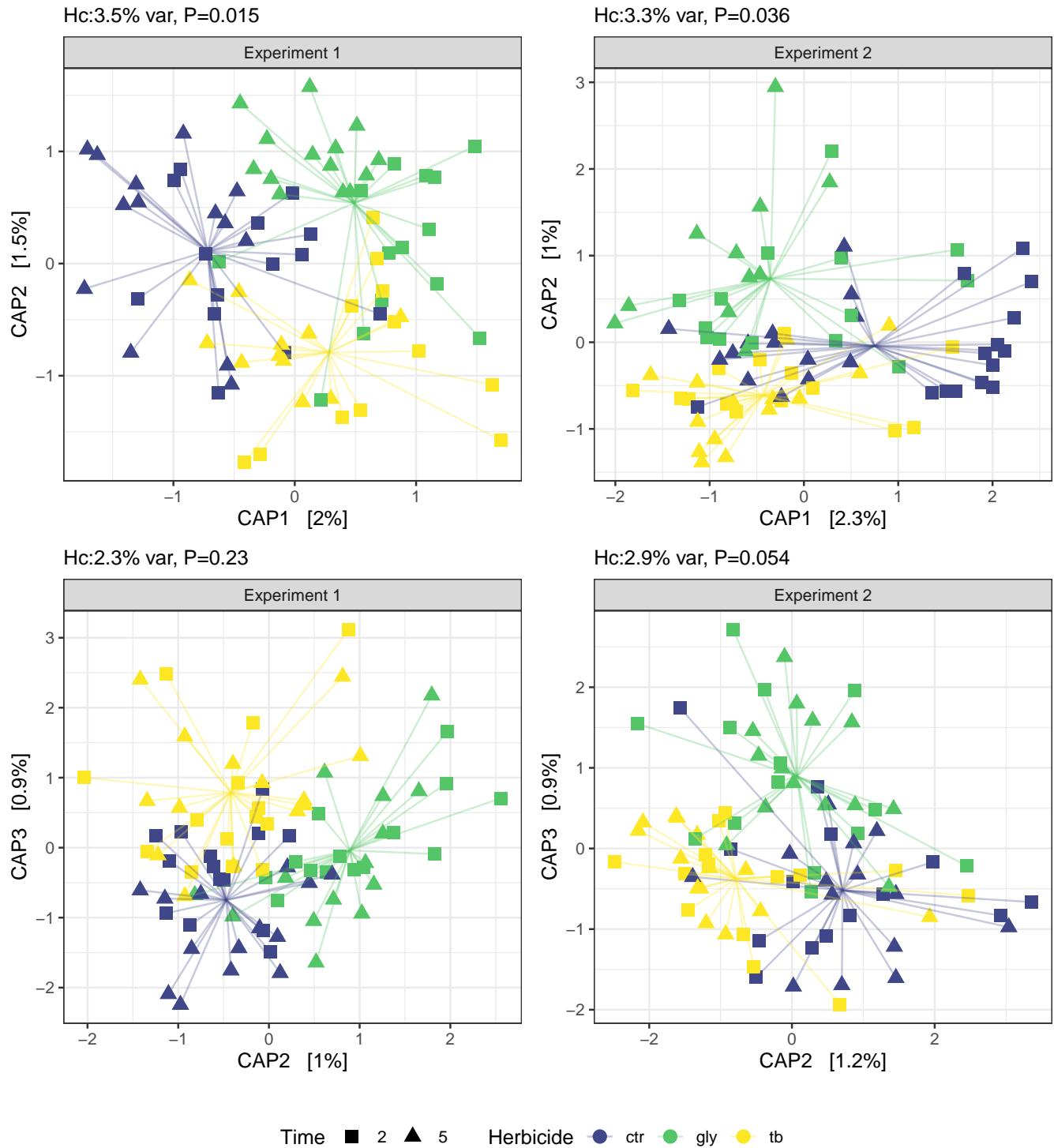
Table 24: Fungi: Glyphosate effect in experiment 2 (tested against control)

	Df	SumOfSqs	R2	F	Pr(>F)
Herbicide	1	0.1472	0.01697	1.068	0.309

Table 25: Fungi: Terbutylazine effect in experiment 2 (tested against control)

	Df	SumOfSqs	R2	F	Pr(>F)
Herbicide	1	0.2682	0.02902	1.87	0.023

Figure 5.2 | CAP - Herbicides



Conclusion: Applying glyphosate and terbuthylazine altered the microbial beta diversity of bacteria and fungi. But conduction the CAP plots showed that herbicides had a small effect which only explained up to 3% of variation between communities.

Conclusion beta diversity

Different applications of herbicide did not alter microbial communities. Herbicides had an effect on bacterial and fungal microbial community compositions. They could explain up to 3% of the microbiome community variation.

Taxa response

Did a core of microbial taxa respond to herbicides? We searched sensitive ASVs – ASVs being differential abundant between two treatments. We followed the same structure as before and answered the 2 questions:

- **Q1: Are there sensitive ASVs between the herbicide applications?**
- **Q2: Are there sensitive ASVs between the herbicide treatments?**

Method

To answer the questions we used the package edgeR (Robinson et al., 2010). We fitted a negative binomial generalized log-linear model to the number of reads for each ASV, conducted a likelihood ratio test for the given coefficient contrasts and controlled the family-wise error rate with a Bonferroni-Holms correction.

Application effect

Q1: Are there sensitive ASVs between the herbicide applications?

The herbicide was applied directly on soil or on weeds. We checked for different abundant ASVs after application herbicide.

Bacteria

higher in weeds	unchanged	higher in soil
81	1847	86

[1] "1.57% of the bacterial abundance changed between the different application targets."

Table 27: The top abundant ASVs

	phylum	class	logFC weeds:soil	rAbu_weeds	rAbu_soil
ASV16	Proteobacteria	Alphaproteobacteria	0.268	0.558%	0.496%
ASV503	Bacteroidetes	Bacteroidia	-7.301	0.049%	0.043%
ASV863	Proteobacteria	Gammaproteobacteria	6.004	0.028%	0.002%
ASV889	Planctomycetes	Planctomycetacia	5.046	0.025%	0.012%
ASV718	Proteobacteria	Gammaproteobacteria	4.402	0.024%	0.015%
ASV874	Bacteroidetes	Bacteroidia	-5.834	0.02%	0.022%
ASV870	Proteobacteria	Gammaproteobacteria	5.924	0.018%	0.012%
ASV1018	Proteobacteria	Gammaproteobacteria	5.359	0.018%	0.012%
ASV1226	Bacteroidetes	Bacteroidia	2.798	0.015%	0.008%
ASV1094	Bacteroidetes	Bacteroidia	4.545	0.014%	0.006%

higher in weeds	unchanged	higher in soil
77	1748	110

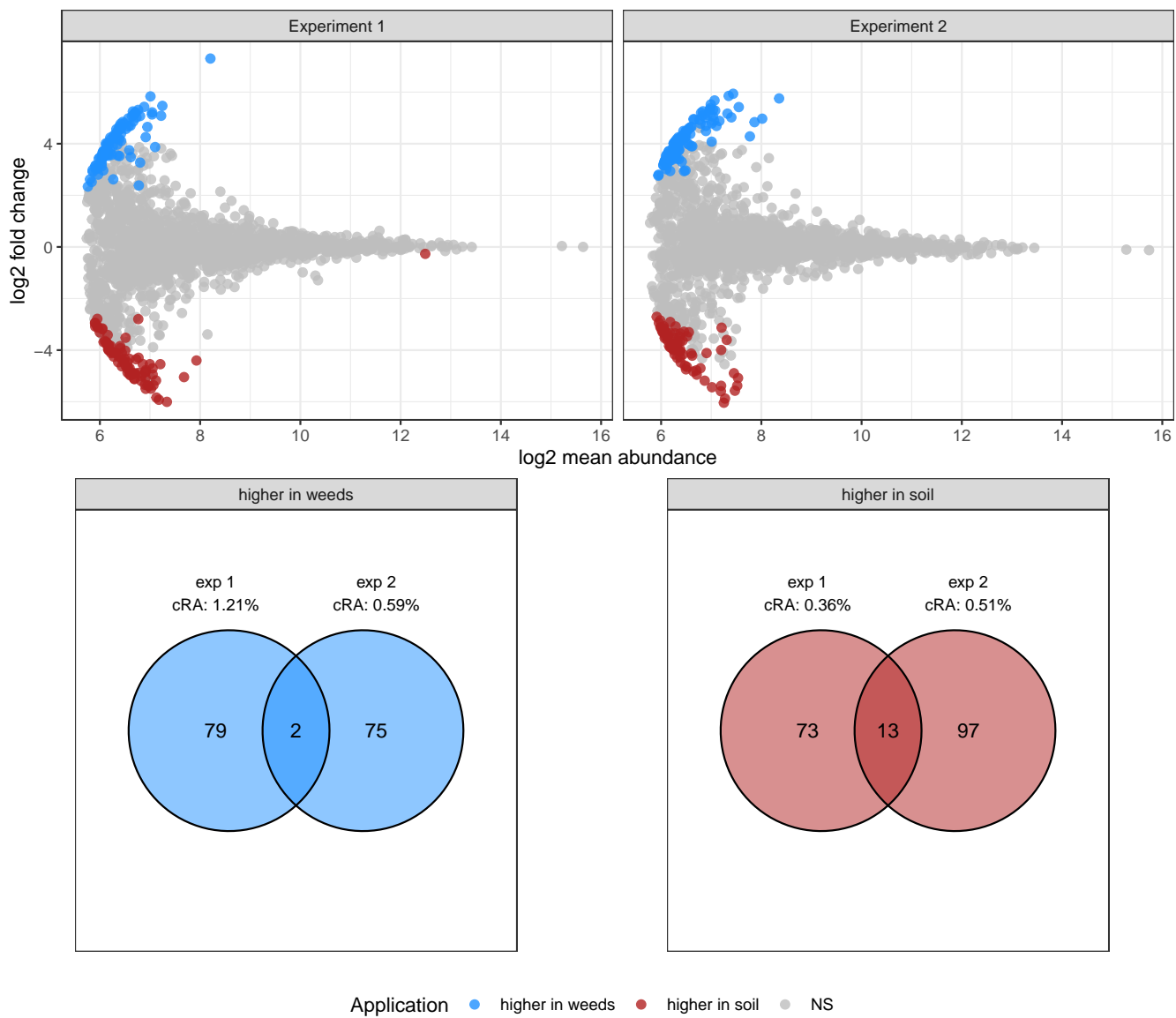
[1] "1.09% of the bacterial abundance changed between the different application targets."

Table 29: The top abundant ASVs

	phylum	class	logFC weeds:soil	rAbu_weeds	rAbu_soil
ASV497	Bacteroidetes	Bacteroidia	5.876	0.049%	0.01%
ASV719	Acidobacteria	Blastocatellia_(Subgroup_4)	-4.284	0.025%	0.022%
ASV602	Proteobacteria	Gammaproteobacteria	-5.755	0.022%	0.04%
ASV930	Actinobacteria	Thermoleophilia	4.895	0.02%	0.01%
ASV874	Bacteroidetes	Bacteroidia	-4.691	0.02%	0.022%
ASV677	Acidobacteria	Acidobacteriia	-4.970	0.02%	0.032%
ASV1100	Proteobacteria	Gammaproteobacteria	3.851	0.02%	0.006%
ASV873	Bacteroidetes	Bacteroidia	-5.284	0.018%	0.015%
ASV1011	Actinobacteria	Actinobacteria	5.377	0.017%	0.006%
ASV794	Verrucomicrobia	Verrucomicrobiae	5.593	0.017%	0.017%

In experiment 1 were 167 bacterial ASVs sensitive to different application targets while we found in experiment 2 187 sensitive bacterial ASVs.

Figure 6.1 | Bacteria: MA & venn



Fungi

higher in weeds	unchanged	higher in soil
10	107	4

[1] "1.99% of the fungal abundance changed between the different application targets."

Table 31: The top abundant ASVs

	phylum	class	logFC weeds:soil	rAbu_weeds	rAbu_soil
ASV33	Basidiomycota	Agaricomycetes	-4.642	0.263%	0.821%
ASV63	Ascomycota	Sordariomycetes	4.283	0.225%	0.176%
ASV76	Ascomycota	Sordariomycetes	5.316	0.204%	0.076%
ASV60	Ascomycota	unassigned	-2.813	0.18%	0.323%
ASV86	Ascomycota	Sordariomycetes	3.901	0.173%	0.049%
ASV95	Ascomycota	Leotiomycetes	4.554	0.165%	0.081%
ASV88	Ascomycota	Leotiomycetes	3.751	0.148%	0.068%
ASV80	Ascomycota	Sordariomycetes	3.621	0.146%	0.121%
ASV92	Ascomycota	Eurotiomycetes	4.742	0.123%	0.067%
ASV91	Ascomycota	Pezizomycetes	-2.564	0.106%	0.157%

higher in weeds	unchanged	higher in soil
4	111	11

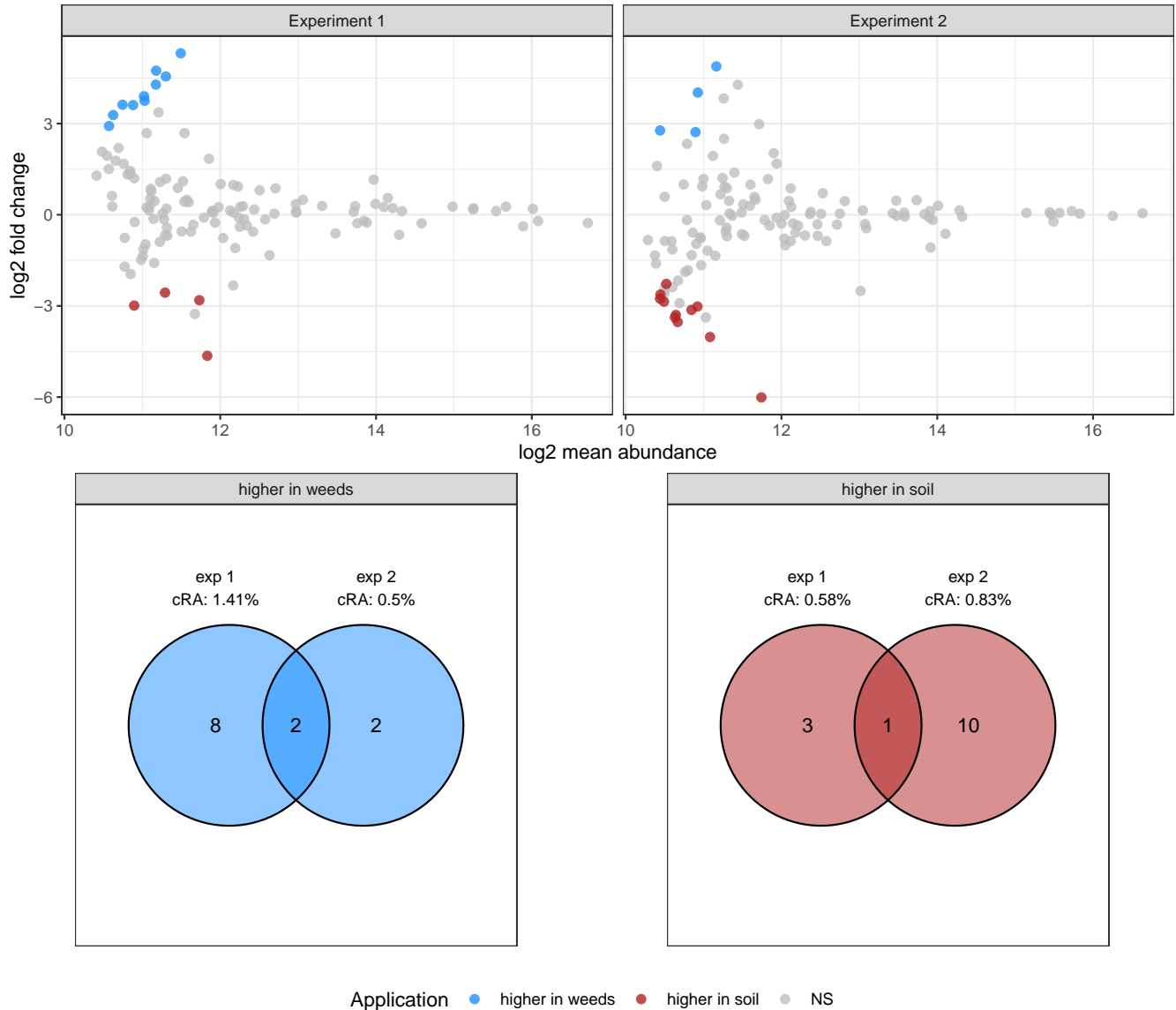
[1] "1.33% of the fungal abundance changed between the different application targets."

Table 33: The top abundant ASVs

	phylum	class	logFC weeds:soil	rAbu_weeds	rAbu_soil
ASV33	Basidiomycota	Agaricomycetes	-6.012	0.263%	0.821%
ASV86	Ascomycota	Sordariomycetes	4.885	0.173%	0.049%
ASV95	Ascomycota	Leotiomycetes	4.023	0.165%	0.081%
ASV79	Ascomycota	Sordariomycetes	-4.024	0.157%	0.11%
ASV94	Ascomycota	Leotiomycetes	2.720	0.132%	0.086%
ASV116	Ascomycota	Dothideomycetes	-3.385	0.086%	0.098%
ASV141	Ascomycota	Eurotiomycetes	-3.531	0.086%	0.064%
ASV119	Ascomycota	Dothideomycetes	-3.020	0.069%	0.099%
ASV122	Basidiomycota	Microbotryomycetes	-3.134	0.054%	0.094%
ASV215	Ascomycota	unassigned	2.775	0.033%	0.033%

In experiment 1 were 14 fungal ASVs sensitive to different application targets while we found in experiment 2 15 sensitive fungal ASVs.

Figure 6.2 | Fungi: MA & venn



Conclusion: Different application targets altered bacterial and fungal community members in their relative abundance. The majority of sensitive ASVs are not abundant in the rhizosphere, that's why only small_exp1 percentages were changed due to application herbicides. Most of the changed ASVs were only sensitive either in experiment 1 or experiment 2.

Herbicide effect

Q2: Are there sensitive ASVs between the herbicide treatments?

We treated samples either with water (control), glyphosate or terbuthylazine. We checked for both experiments for differences in the ASV abundance by comparing the herbicides against the water control.

Bacteria

lower in gly	unchanged	higher in gly
85	1605	33

[1] "6.12% of the bacterial abundance changed after applying glyphosate"

Table 35: The top abundant ASVs

	phylum	class	logFC ctr:hc	rAbu_ctr	rAbu_hc
ASV2	Verrucomicrobia	Verrucomicrobiae	0.252	3.855%	3.549%
ASV7	Verrucomicrobia	Verrucomicrobiae	0.451	0.817%	0.724%
ASV18	Proteobacteria	Deltaproteobacteria	0.276	0.553%	0.51%
ASV32	Actinobacteria	Actinobacteria	-0.733	0.25%	0.332%
ASV651	Verrucomicrobia	Verrucomicrobiae	6.508	0.061%	0.036%
ASV711	Acidobacteria	Subgroup_5	4.571	0.051%	0.035%
ASV794	Verrucomicrobia	Verrucomicrobiae	6.769	0.042%	0.026%
ASV724	Bacteroidetes	Bacteroidia	6.367	0.041%	0.025%
ASV781	Bacteroidetes	Bacteroidia	6.747	0.039%	0.026%
ASV860	Acidobacteria	Holophagae	6.533	0.031%	0.018%

lower in tb	unchanged	higher in tb
109	1492	32

[1] "1.49% of the bacterial abundance changed after applying terbuthylazine"

Table 37: The top abundant ASVs

	phylum	class	logFC ctr:hc	rAbu_ctr	rAbu_hc
ASV477	Proteobacteria	Gammaproteobacteria	3.770	0.076%	0.049%
ASV569	Acidobacteria	Subgroup_17	3.857	0.063%	0.042%
ASV651	Verrucomicrobia	Verrucomicrobiae	5.444	0.061%	0.04%
ASV794	Verrucomicrobia	Verrucomicrobiae	7.110	0.042%	0.026%
ASV763	Acidobacteria	Holophagae	6.536	0.038%	0.025%
ASV654	Proteobacteria	Deltaproteobacteria	4.627	0.038%	0.028%
ASV649	Acidobacteria	Subgroup_6	3.858	0.037%	0.029%
ASV803	Latescibacteria	unassigned	5.380	0.035%	0.023%
ASV790	Acidobacteria	Subgroup_5	5.896	0.033%	0.02%
ASV860	Acidobacteria	Holophagae	6.230	0.031%	0.019%

lower in gly	unchanged	higher in gly
60	1464	31

[1] "2.05% of the bacterial abundance changed after applying glyphosate"

Table 39: The top abundant ASVs

	phylum	class	logFC ctr:hc	rAbu_ctr	rAbu_hc
ASV19	Proteobacteria	Alphaproteobacteria	0.549	0.622%	0.527%
ASV39	Proteobacteria	Alphaproteobacteria	0.681	0.476%	0.393%
ASV32	Actinobacteria	Actinobacteria	-0.645	0.223%	0.33%
ASV842	Proteobacteria	Alphaproteobacteria	7.292	0.06%	0.034%
ASV683	Bacteroidetes	Bacteroidia	6.734	0.039%	0.024%
ASV816	Proteobacteria	Gammaproteobacteria	6.542	0.034%	0.019%
ASV654	Proteobacteria	Deltaproteobacteria	6.403	0.033%	0.021%
ASV946	Acidobacteria	Subgroup_6	4.695	0.029%	0.021%
ASV1071	Bacteroidetes	Bacteroidia	5.948	0.026%	0.016%
ASV929	Proteobacteria	Gammaproteobacteria	5.678	0.026%	0.016%

lower in tb	unchanged	higher in tb
67	1467	41

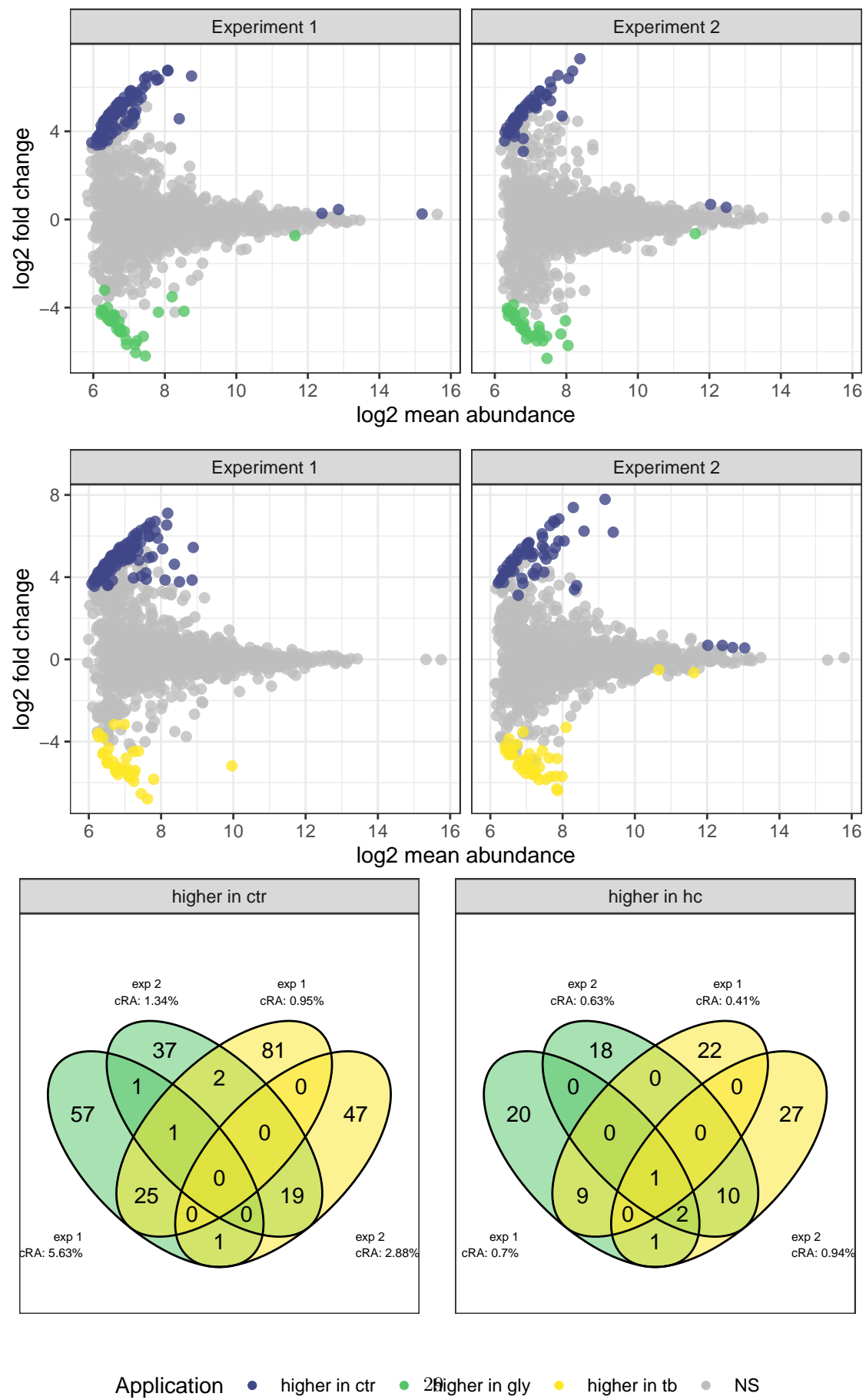
[1] "4.15% of the bacterial abundance changed after applying terbuthylazine"

Table 41: The top abundant ASVs

	phylum	class	logFC ctr:hc	rAbu_ctr	rAbu_hc
ASV9	Proteobacteria	Alphaproteobacteria	0.553	0.926%	0.812%
ASV14	Proteobacteria	Alphaproteobacteria	0.574	0.762%	0.638%
ASV19	Proteobacteria	Alphaproteobacteria	0.679	0.622%	0.554%
ASV39	Proteobacteria	Alphaproteobacteria	0.685	0.476%	0.388%
ASV32	Actinobacteria	Actinobacteria	-0.648	0.223%	0.36%
ASV125	Proteobacteria	Gammaproteobacteria	-0.515	0.125%	0.149%
ASV318	Bacteroidetes	Bacteroidia	7.785	0.116%	0.061%
ASV435	Proteobacteria	Alphaproteobacteria	6.186	0.106%	0.064%
ASV842	Proteobacteria	Alphaproteobacteria	7.389	0.06%	0.03%
ASV707	Bacteroidetes	Bacteroidia	6.240	0.055%	0.035%

Glyphosate changed the abundance of 118 bacterial ASVs in experiment 1 and 91 bacterial ASVs in experiment 2. Application terbuthylazine led to changes in abundance for 141 bacterial ASVs in experiment 1 and 108 bacterial ASVs in experiment 2.

Figure 7.1 | Bacteria: MA & venn



Fungi

lower in gly	unchanged	higher in gly
3	87	2

[1] "1.24% of the fungal abundance changed after applying glyphosate"

Table 43: The top abundant ASVs

	phylum	class	logFC ctr:hc	rAbu_ctr	rAbu_hc
ASV19	Mortierellomycota	Mortierellomycetes	6.537	0.814%	0.493%
ASV62	Ascomycota	Sordariomycetes	5.655	0.421%	0.248%
ASV76	Ascomycota	Sordariomycetes	5.018	0.321%	0.191%
ASV75	Mortierellomycota	Mortierellomycetes	-4.801	0.05%	0.184%
ASV95	Ascomycota	Leotiomycetes	-5.319	NA%	0.125%

lower in tb	unchanged	higher in tb
1	95	3

[1] "1.58% of the fungal abundance changed after applying terbuthylazine"

Table 45: The top abundant ASVs

	phylum	class	logFC ctr:hc	rAbu_ctr	rAbu_hc
ASV33	Basidiomycota	Agaricomycetes	-6.339	0.614%	0.836%
ASV55	Ascomycota	Sordariomycetes	3.097	0.336%	0.243%
ASV83	Ascomycota	Leotiomycetes	-5.134	0.189%	0.268%
ASV54	Mortierellomycota	Mortierellomycetes	-3.316	0.154%	0.234%

lower in gly	unchanged	higher in gly
2	100	1

[1] "0.36% of the fungal abundance changed after applying glyphosate"

Table 47: The top abundant ASVs

	phylum	class	logFC ctr:hc	rAbu_ctr	rAbu_hc
ASV69	Ascomycota	Sordariomycetes	4.580	0.246%	0.161%
ASV116	Ascomycota	Dothideomycetes	4.294	0.132%	0.071%
ASV95	Ascomycota	Leotiomycetes	-4.928	0.054%	0.129%

lower in tb	unchanged	higher in tb
2	92	0

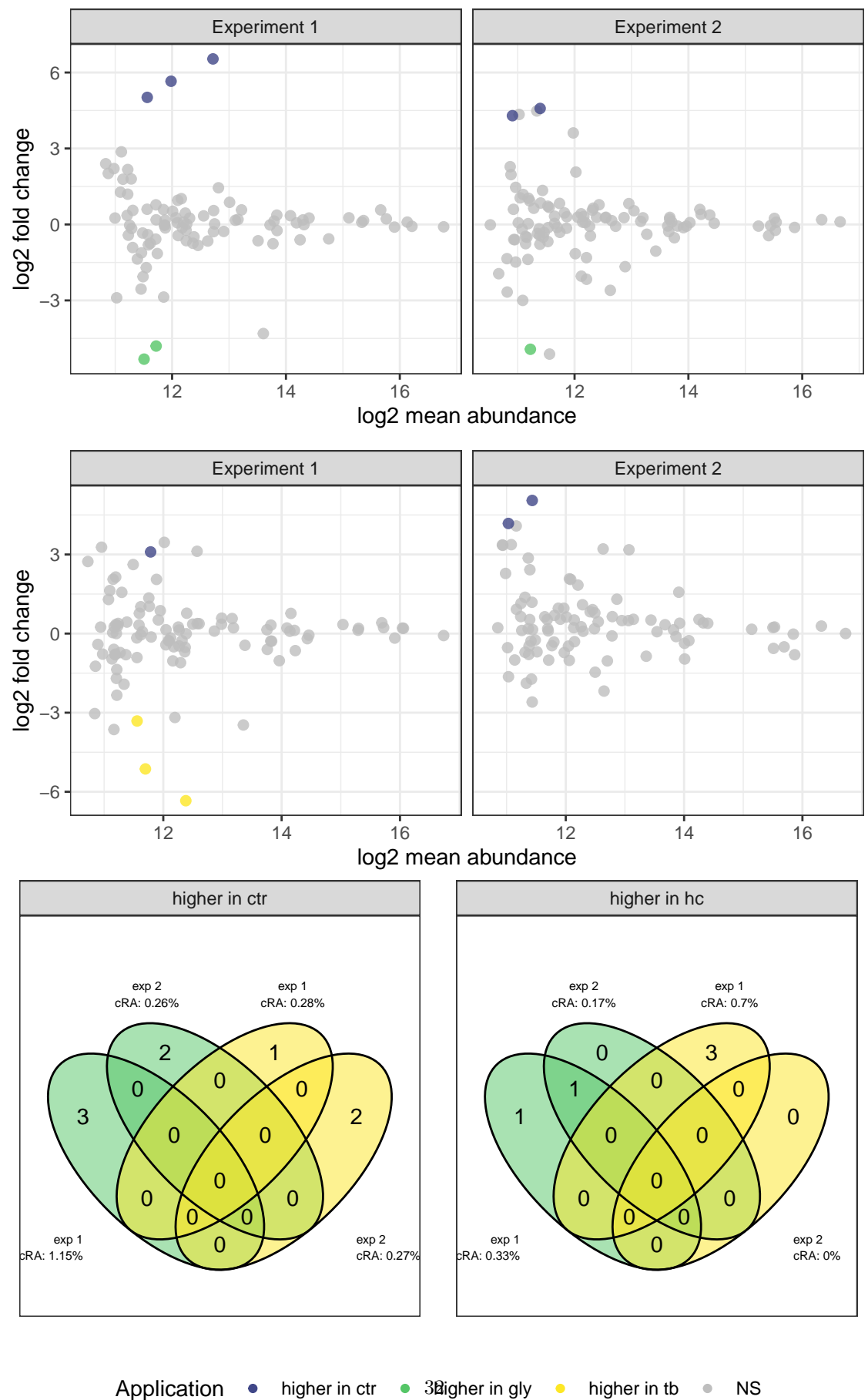
[1] "0.23% of the fungal abundance changed after applying terbuthylazine"

Table 49: The top abundant ASVs

	phylum	class	logFC ctr:hc	rAbu_ctr	rAbu_hc
ASV73	Ascomycota	Sordariomycetes	5.050	0.236%	0.138%
ASV108	Ascomycota	Dothideomycetes	4.177	0.15%	0.089%

Glyphosate changed the abundance of 5 bacterial ASVs in experiment 1 and 3 bacterial ASVs in experiment 2. Applying terbuthylazine led to changes in abundance for 4 bacterial ASVs in experiment 1 and 2 bacterial ASVs in experiment 2.

Figure 7.2 | Fungi: MA & venn



Conclusion: Applying glyphosate and terbuthylazine led to similar effect. In both experiments were around 30 ASVs sensitive in their abundance to glyphosate and terbuthylazine but non of those ASVs was sensitive in both experiments. We found high abundant bacterial ASVs reacting sensitive to herbicides which was up to 12 % of the bacterial community. Only a few fungal ASVs were changed in their abundance and non of them was sensitive for both herbicides or in both experiments.

Conclusion taxa response

Applying herbicides to different targets did not influence high abundant ASVs. Applying glyphosate and terbuthylazine caused similar effects. Both altered, multiple bacterial ASVs. Almost no effect was found on fungi. Sensitive ASVs were not consistent between the two experiments.