

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 sprayed on two different targets: Bare soil (S) or weeds (Ca; *Chenopodium album*). Two weeks after spraying, maize was planted in the soil. Soil samples were collected at: 2W (2 weeks after spraying, maize planting, 7 reps. per treatment) and 5W (5 weeks after spraying, 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.

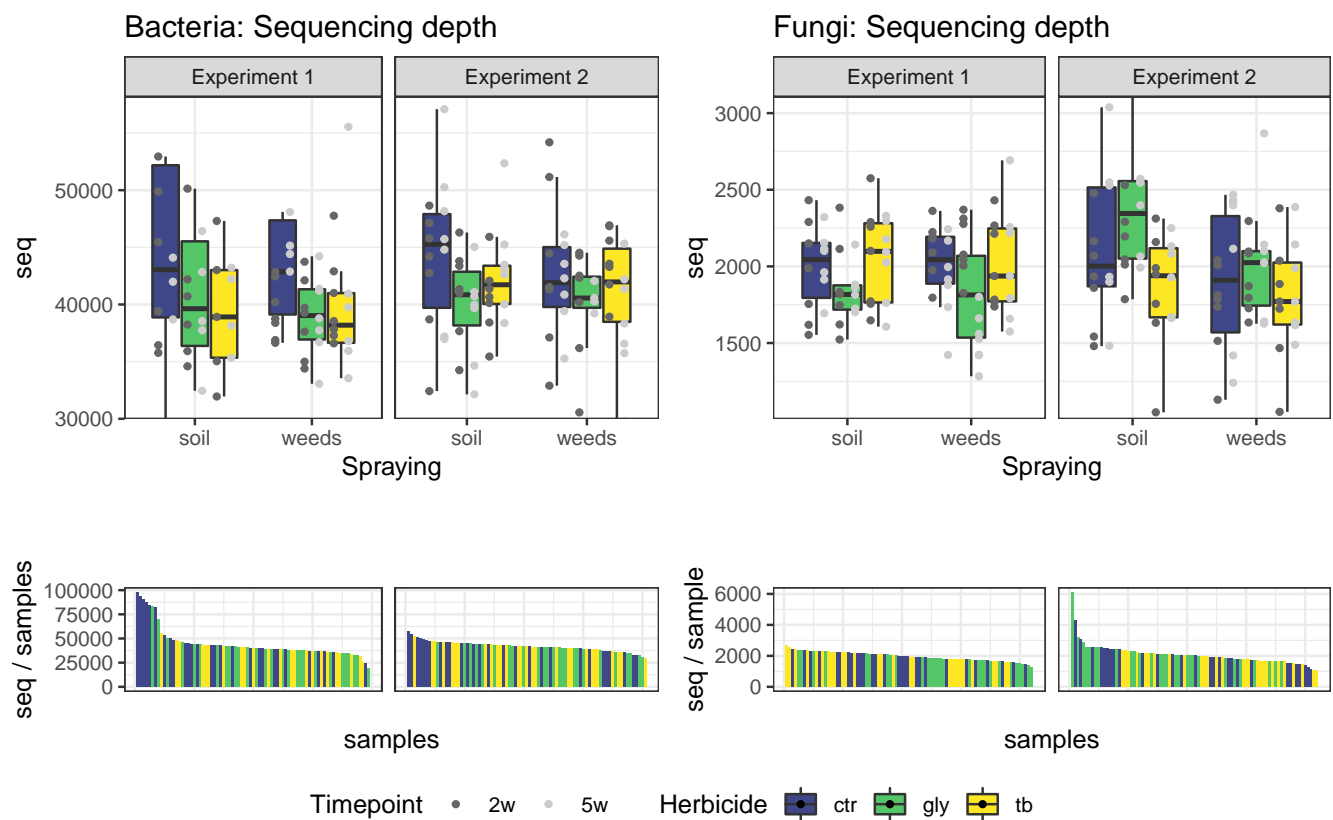
Bacteria

```
## [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_Spraying_Experiment_Timepoint”:

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

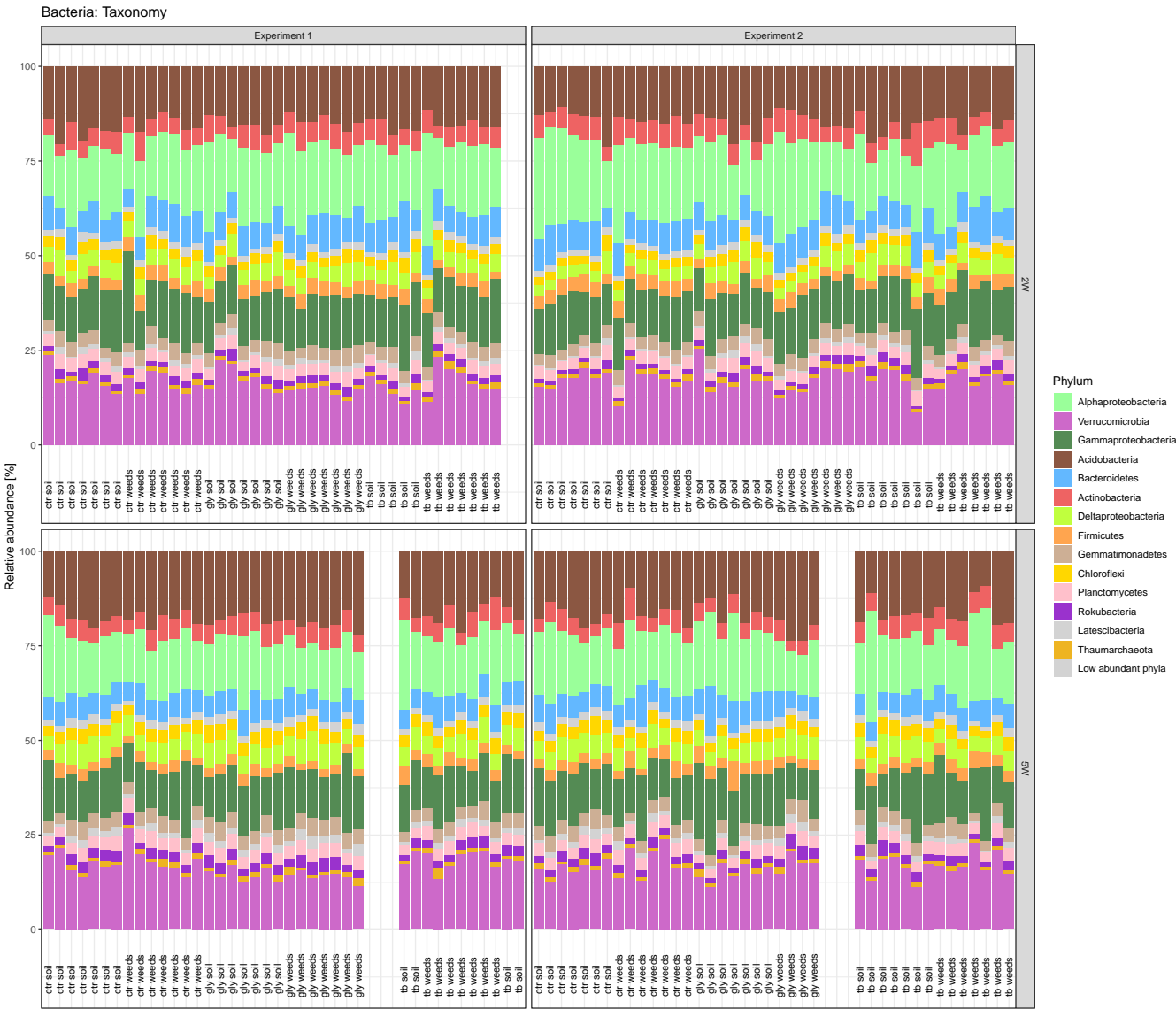
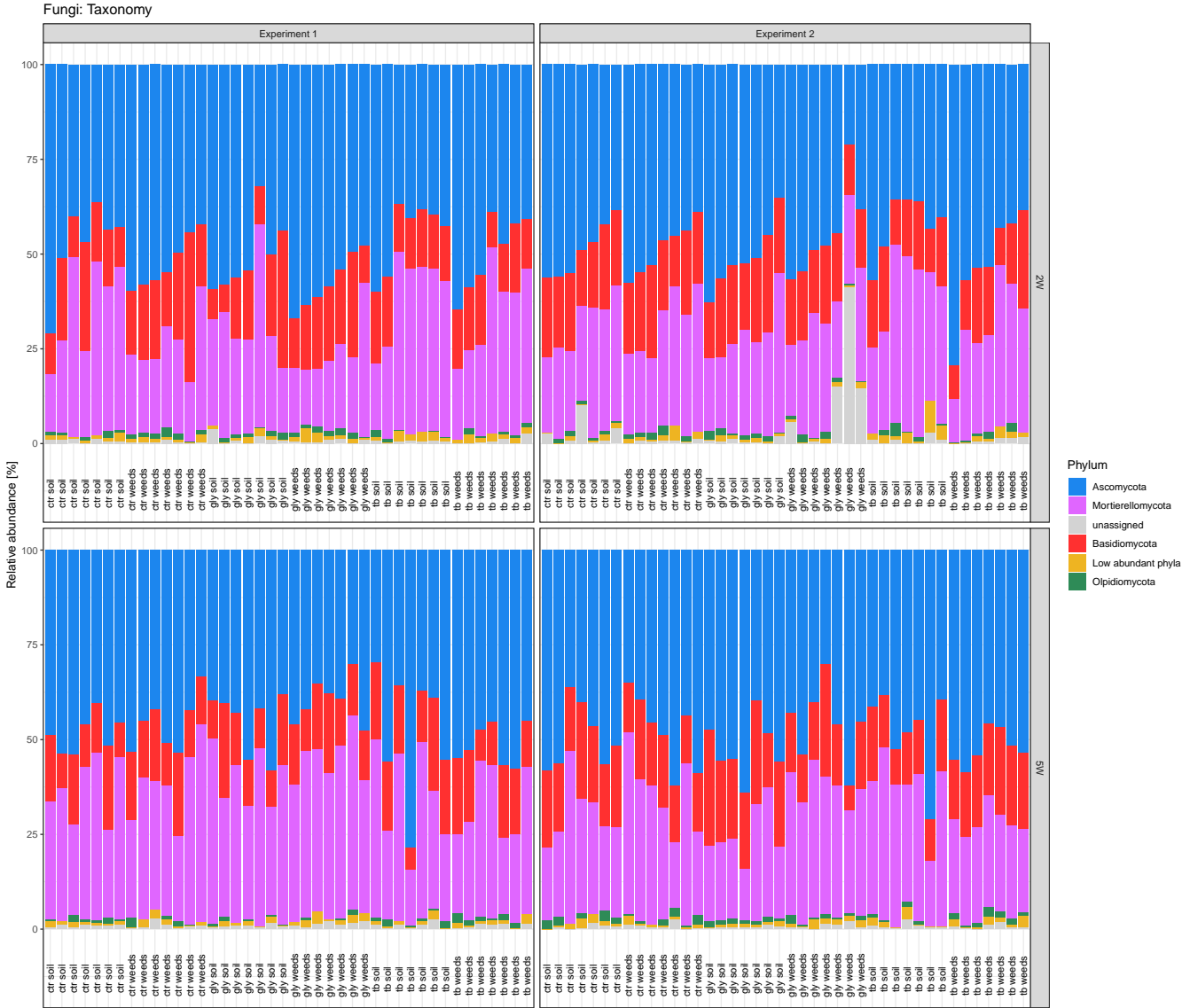


Figure 2.2 | Fungi: Phylum level taxonomy



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 |
| Spraying | 1 | 0.00241 | 0.004702 | 0.4463 | 0.796 |
| Timepoint | 1 | 0.07635 | 0.149 | 14.14 | 0.001 |
| Plate | 1 | 0.01166 | 0.02275 | 2.16 | 0.078 |
| Herbicide:Spraying | 2 | 0.00836 | 0.01631 | 0.7741 | 0.583 |
| Herbicide:Timepoint | 2 | 0.02097 | 0.04091 | 1.942 | 0.082 |
| Spraying:Timepoint | 1 | 0.00866 | 0.0169 | 1.604 | 0.158 |
| Herbicide:Spraying:Timepoint | 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 |
| Spraying | 1 | 0.003506 | 0.004953 | 0.4699 | 0.706 |
| Timepoint | 1 | 0.07476 | 0.1056 | 10.02 | 0.002 |
| Plate | 1 | 0.01548 | 0.02188 | 2.076 | 0.101 |
| Herbicide:Spraying | 2 | 0.01575 | 0.02225 | 1.055 | 0.375 |
| Herbicide:Timepoint | 2 | 0.0275 | 0.03885 | 1.843 | 0.12 |
| Spraying:Timepoint | 1 | 0.02114 | 0.02986 | 2.833 | 0.048 |
| Herbicide:Spraying:Timepoint | 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 (continued below)

| | Df | SumOfSqs | R2 | F |
|-------------------------------------|----|------------|------------|----------|
| Herbicide | 2 | 0.004059 | 0.002904 | 0.1921 |
| Spraying | 1 | 0.02543 | 0.01819 | 2.407 |
| Timepoint | 1 | 0.074 | 0.05294 | 7.004 |
| Plate | 1 | 0.4487 | 0.321 | 42.47 |
| Herbicide:Spraying | 2 | 0.01659 | 0.01187 | 0.7849 |
| Herbicide:Timepoint | 2 | 0.03144 | 0.02249 | 1.488 |
| Spraying:Timepoint | 1 | 0.0483 | 0.03455 | 4.571 |
| Herbicide:Spraying:Timepoint | 2 | -0.0008099 | -0.0005794 | -0.03833 |
| Residual | 71 | 0.7501 | 0.5366 | NA |
| Total | 83 | 1.398 | 1 | NA |

| | Pr(>F) |
|-------------------------------------|--------|
| Herbicide | 0.951 |
| Spraying | 0.108 |
| Timepoint | 0.003 |
| Plate | 0.001 |
| Herbicide:Spraying | 0.503 |
| Herbicide:Timepoint | 0.21 |
| Spraying:Timepoint | 0.017 |
| Herbicide:Spraying:Timepoint | 1 |
| Residual | NA |
| Total | NA |

Table 6: Fungi PERMANOVA: Experiment 1

| | Df | SumOfSqs | R2 | F | Pr(>F) |
|-------------------------------------|----|----------|----------|--------|--------|
| Herbicide | 2 | 0.03628 | 0.02971 | 1.656 | 0.146 |
| Spraying | 1 | 0.007012 | 0.005744 | 0.6402 | 0.569 |
| Timepoint | 1 | 0.0104 | 0.008521 | 0.9499 | 0.39 |
| Plate | 1 | 0.2516 | 0.2061 | 22.97 | 0.001 |
| Herbicide:Spraying | 2 | 0.05161 | 0.04228 | 2.356 | 0.046 |
| Herbicide:Timepoint | 2 | 0.02951 | 0.02417 | 1.347 | 0.218 |
| Spraying:Timepoint | 1 | 0.01951 | 0.01598 | 1.781 | 0.124 |
| Herbicide:Spraying:Timepoint | 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

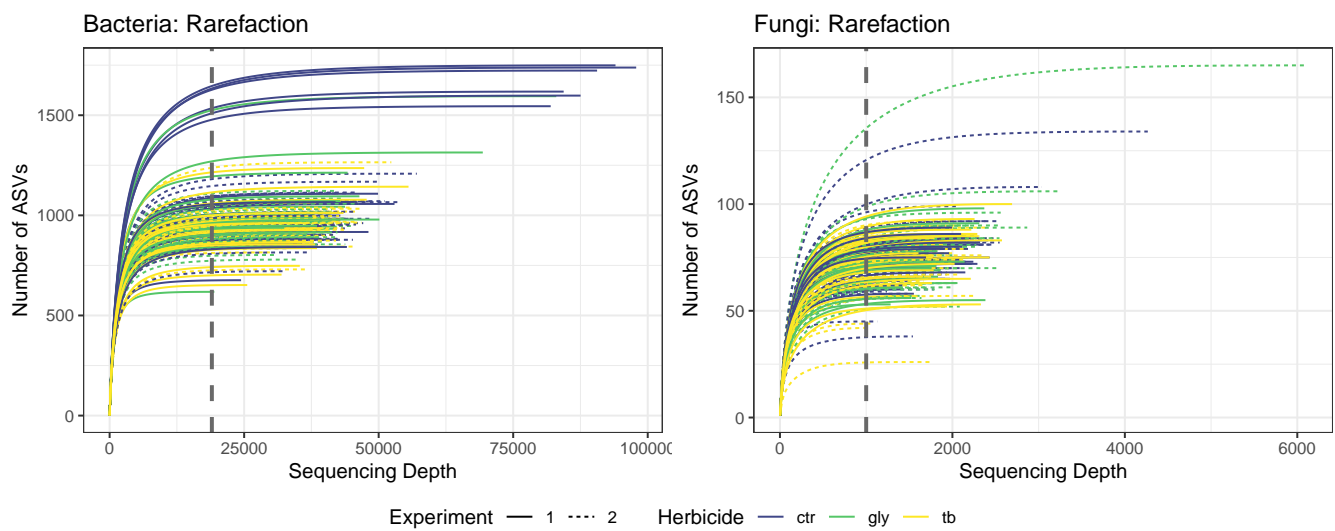
We answered the following questions for the bacterial and fungal alpha diversity:

- Q1: Are there differences in alpha diversity between the spraying 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 spraying targets, 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 7: Bacteria, experiment 1: F test

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

Table 8: Bacteria, experiment 2: F test

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

Fungi

Table 9: Fungi, experiment 1: F test

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

Table 10: Fungi, experiment 2: F test

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|-------------------------------------|----|--------|---------|---------|-----------|
| Herbicide | 2 | 257.8 | 128.9 | 1.927 | 0.1531 |
| Spraying | 1 | 220.4 | 220.4 | 3.295 | 0.07371 |
| Timepoint | 1 | 171.5 | 171.5 | 2.564 | 0.1137 |
| Plate | 1 | 1431 | 1431 | 21.39 | 1.639e-05 |
| Herbicide:Spraying | 2 | 31.9 | 15.95 | 0.2385 | 0.7884 |
| Herbicide:Timepoint | 2 | 174 | 86.99 | 1.301 | 0.2787 |
| Spraying:Timepoint | 1 | 19.81 | 19.81 | 0.2961 | 0.588 |
| Herbicide:Spraying:Timepoint | 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. Spraying on different targets and using different herbicides did not significantly influence the alpha diversity. For fungi, spraying either on weeds or direct on soil affected the alpha diversity while the time nor the herbicide had a significant influence.

Spraying effect

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

The herbicide was either sprayed 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 spraying targets did shape the communities. For consistency we investigated the bacterial and fungal communities.

Bacteria

Table 11: Bacteria: Spraying effect in experiment 1

| | Shannon | .group |
|--------------|---------|--------|
| soil | 474.1 | a |
| weeds | 467.4 | a |

Table 12: Bacteria: Spraying effect in experiment 2

| | Shannon | .group |
|--------------|---------|--------|
| soil | 450.3 | a |
| weeds | 430.2 | a |

Fungi

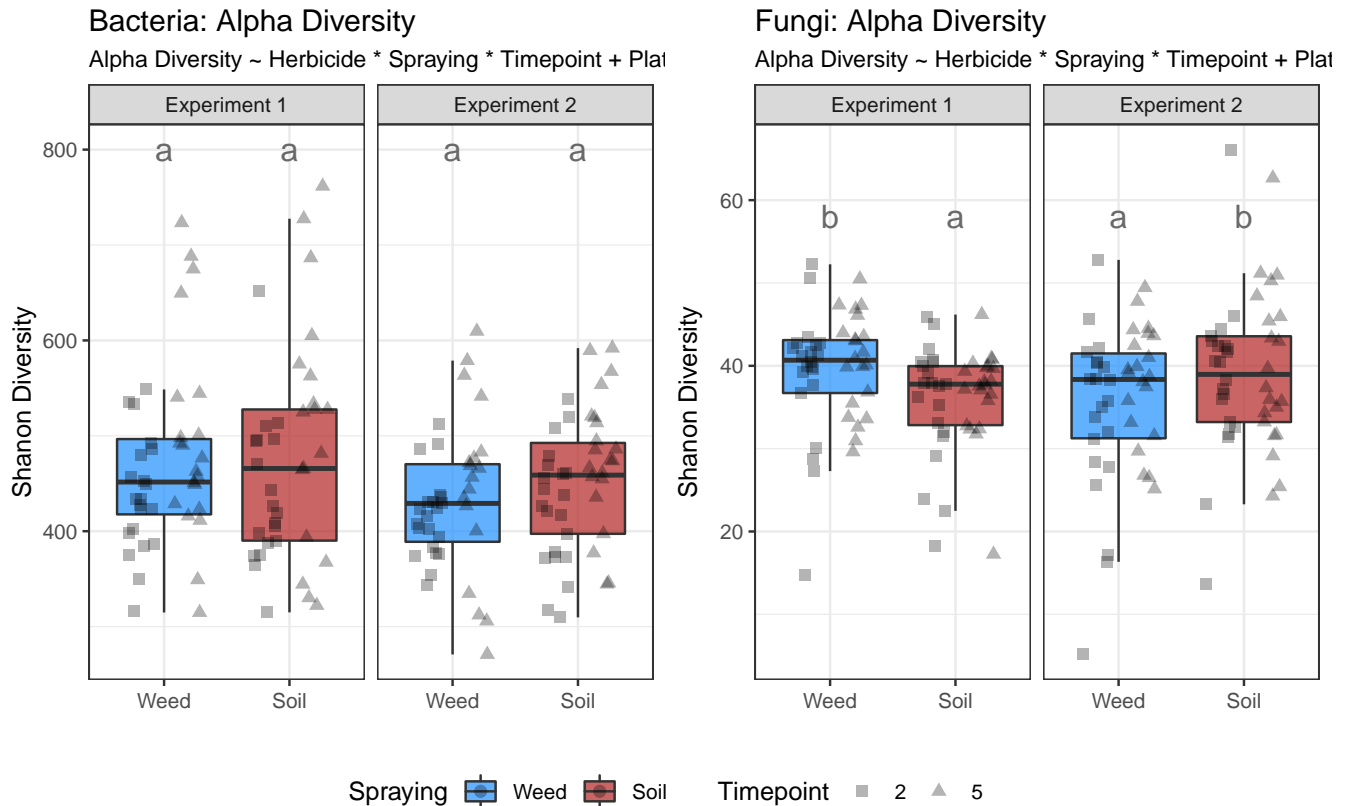
Table 13: Fungi: Spraying effect in experiment 1

| | Shannon | .group |
|--------------|---------|--------|
| soil | 36.15 | a |
| weeds | 39.57 | b |

Table 14: Fungi: Spraying effect in experiment 2

| | Shannon | .group |
|--------------|---------|--------|
| soil | 39.07 | b |
| weeds | 35.83 | a |

Figure 4.1 | Shannon diversity - Spraying



Conclusion: As already seen, bacterial alpha diversity remains constant between the two spraying targets. In experiment 1 led spraying 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 spraying targets. 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 spraying 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 15: Bacteria PERMANOVA: Experiment 1

| | Df | SumOfSqs | R2 | F | Pr(>F) |
|-------------------------------------|----|----------|---------|-------|--------|
| Herbicide | 2 | 0.2347 | 0.03485 | 1.479 | 0.005 |
| Spraying | 1 | 0.1001 | 0.01486 | 1.261 | 0.11 |
| Timepoint | 1 | 0.4064 | 0.06034 | 5.123 | 0.001 |
| Plate | 1 | 0.1078 | 0.016 | 1.358 | 0.065 |
| Herbicide:Spraying | 2 | 0.1603 | 0.0238 | 1.01 | 0.402 |
| Herbicide:Timepoint | 2 | 0.2196 | 0.03261 | 1.384 | 0.026 |
| Spraying:Timepoint | 1 | 0.1056 | 0.01568 | 1.331 | 0.059 |
| Herbicide:Spraying:Timepoint | 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 16: Bacteria PERMANOVA: Experiment 2

| | Df | SumOfSqs | R2 | F | Pr(>F) |
|-------------------------------------|----|----------|---------|-------|--------|
| Herbicide | 2 | 0.2397 | 0.03335 | 1.505 | 0.034 |
| Spraying | 1 | 0.09258 | 0.01288 | 1.163 | 0.188 |
| Timepoint | 1 | 0.6086 | 0.08467 | 7.644 | 0.001 |
| Plate | 1 | 0.08531 | 0.01187 | 1.071 | 0.291 |
| Herbicide:Spraying | 2 | 0.1798 | 0.02502 | 1.129 | 0.218 |
| Herbicide:Timepoint | 2 | 0.2713 | 0.03774 | 1.704 | 0.013 |
| Spraying:Timepoint | 1 | 0.09744 | 0.01356 | 1.224 | 0.142 |
| Herbicide:Spraying:Timepoint | 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 17: Fungi PERMANOVA: Experiment 1

| | Df | SumOfSqs | R2 | F | Pr(>F) |
|-------------------------------------|----|----------|---------|--------|--------|
| Herbicide | 2 | 0.3079 | 0.02258 | 1.151 | 0.207 |
| Spraying | 1 | 0.2561 | 0.01878 | 1.915 | 0.023 |
| Timepoint | 1 | 0.5082 | 0.03726 | 3.8 | 0.001 |
| Plate | 1 | 1.994 | 0.1462 | 14.91 | 0.001 |
| Herbicide:Spraying | 2 | 0.251 | 0.01841 | 0.9386 | 0.562 |
| Herbicide:Timepoint | 2 | 0.295 | 0.02163 | 1.103 | 0.263 |
| Spraying:Timepoint | 1 | 0.2239 | 0.01642 | 1.674 | 0.041 |
| Herbicide:Spraying:Timepoint | 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 18: Fungi PERMANOVA: Experiment 2

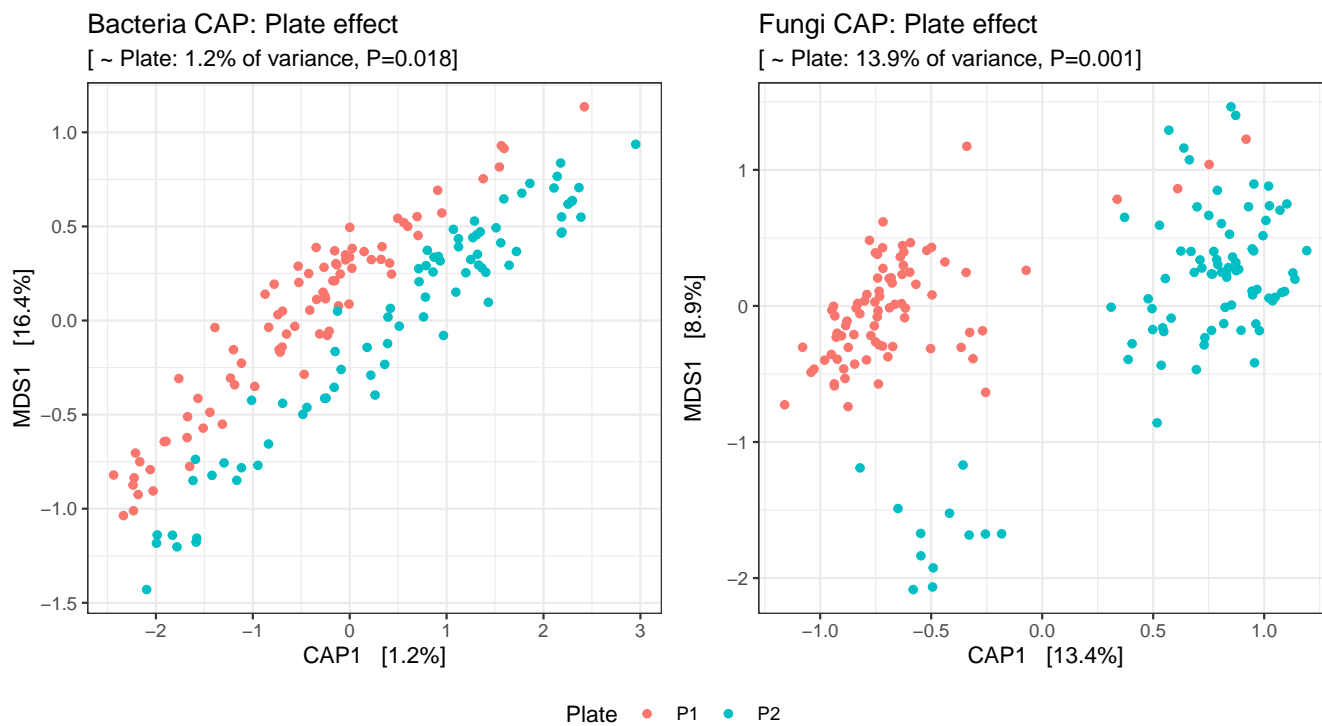
| | Df | SumOfSqs | R2 | F | Pr(>F) |
|-------------------------------------|----|----------|---------|-------|--------|
| Herbicide | 2 | 0.4031 | 0.02927 | 1.414 | 0.056 |
| Spraying | 1 | 0.1593 | 0.01156 | 1.117 | 0.273 |
| Timepoint | 1 | 0.214 | 0.01553 | 1.501 | 0.062 |
| Plate | 1 | 1.646 | 0.1195 | 11.55 | 0.001 |
| Herbicide:Spraying | 2 | 0.3757 | 0.02727 | 1.318 | 0.093 |
| Herbicide:Timepoint | 2 | 0.3267 | 0.02372 | 1.146 | 0.221 |
| Spraying:Timepoint | 1 | 0.1702 | 0.01236 | 1.194 | 0.185 |
| Herbicide:Spraying:Timepoint | 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 a 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. Spraying 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



Spraying effect

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

We could see that there was no effect for bacteria nor for fungi. Spraying 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 effect.

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 19: 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 20: 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 21: 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 22: 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 23: 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 24: 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 |

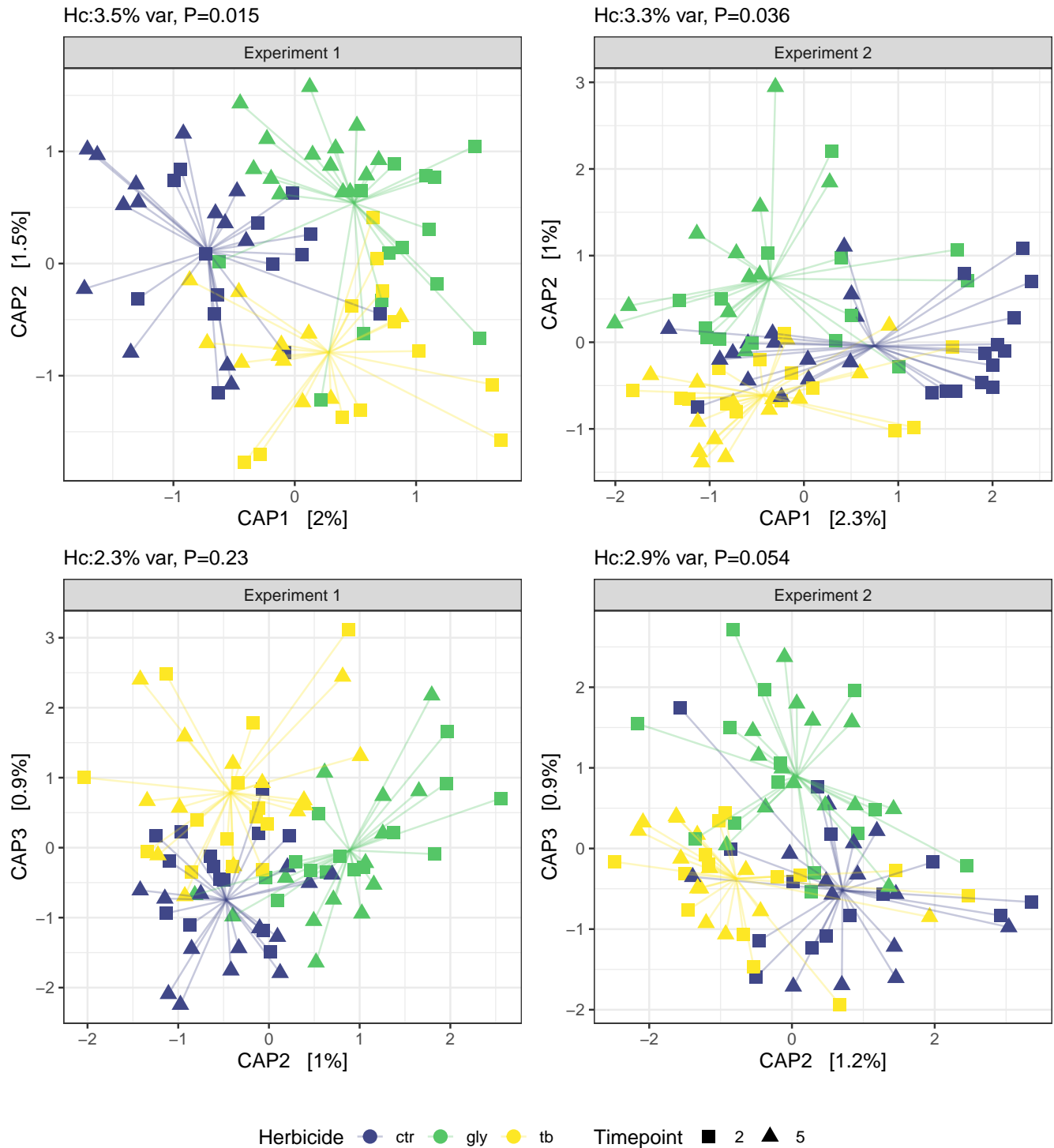
Table 25: Fungi: Glyphosate effect in experiment 2 (tested against control)

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

Table 26: 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: Spraying glyphosate and terbuthylazine altered the microbial beta diversity of bacteria and fungi. But conducting the CAP plots showed that herbicides had a small effect which only explained up to 3% of variation between communities.

Conclusion beta diversity

Different spraying targets 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 spraying 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.

Spraying effect

Q1: Are there sensitive ASVs between the spraying applications?

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

Bacteria

Table 27: Bacteria: Spraying effect in experiment 1

| higher in soil | unchanged | higher in weeds |
|----------------|-----------|-----------------|
| 86 | 1847 | 81 |

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

Table 28: The top abundant ASVs

| | phylum | class | logFC | rAbu |
|----------------|----------------|---------------------|--------|-------|
| ASV16 | Proteobacteria | Alphaproteobacteria | 0.2684 | 0.56% |
| ASV503 | Bacteroidetes | Bacteroidia | -7.301 | 0.05% |
| ASV863 | Proteobacteria | Gammaproteobacteria | 6.004 | 0.03% |
| ASV889 | Planctomycetes | Planctomycetacia | 5.046 | 0.02% |
| ASV718 | Proteobacteria | Gammaproteobacteria | 4.402 | 0.02% |
| ASV874 | Bacteroidetes | Bacteroidia | -5.834 | 0.02% |
| ASV870 | Proteobacteria | Gammaproteobacteria | 5.924 | 0.02% |
| ASV1018 | Proteobacteria | Gammaproteobacteria | 5.359 | 0.02% |
| ASV1226 | Bacteroidetes | Bacteroidia | 2.798 | 0.02% |
| ASV1094 | Bacteroidetes | Bacteroidia | 4.545 | 0.01% |

Table 29: Bacteria: Spraying effect in experiment 2

| higher in soil | unchanged | higher in weeds |
|----------------|-----------|-----------------|
| 110 | 1748 | 77 |

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

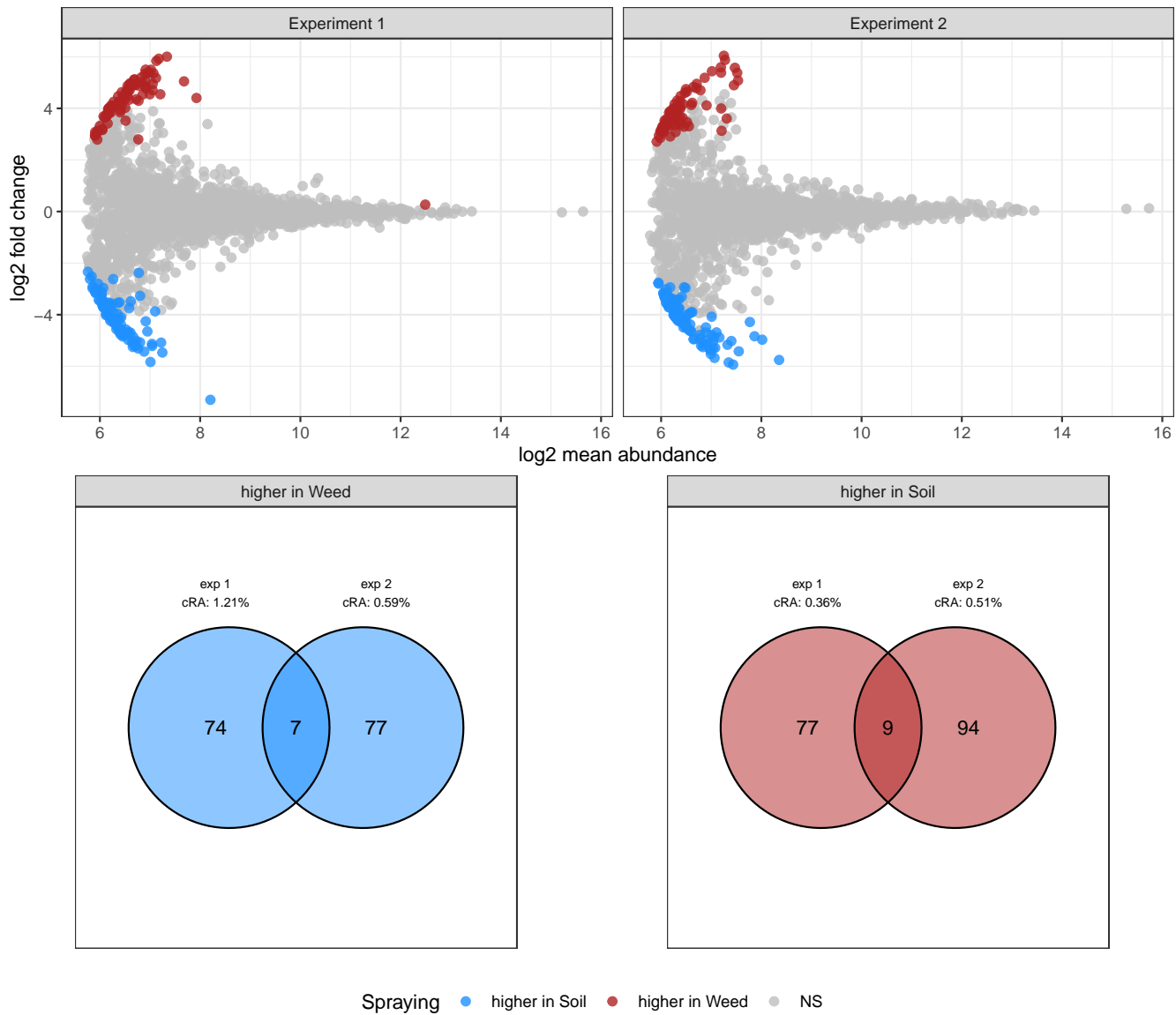
Table 30: The top abundant ASVs

| | phylum | class | logFC | rAbu |
|----------------|-----------------|-----------------------------|--------|-------|
| ASV497 | Bacteroidetes | Bacteroidia | 5.876 | 0.05% |
| ASV719 | Acidobacteria | Blastocatellia_(Subgroup_4) | -4.284 | 0.02% |
| ASV602 | Proteobacteria | Gammaproteobacteria | -5.755 | 0.02% |
| ASV930 | Actinobacteria | Thermoleophilia | 4.895 | 0.02% |
| ASV874 | Bacteroidetes | Bacteroidia | -4.691 | 0.02% |
| ASV677 | Acidobacteria | Acidobacteriia | -4.97 | 0.02% |
| ASV1100 | Proteobacteria | Gammaproteobacteria | 3.851 | 0.02% |
| ASV873 | Bacteroidetes | Bacteroidia | -5.284 | 0.02% |
| ASV1011 | Actinobacteria | Actinobacteria | 5.377 | 0.02% |
| ASV794 | Verrucomicrobia | Verrucomicrobiae | 5.593 | 0.02% |

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

sensitive bacterial ASVs.

Figure 6.1 | Bacteria: MA & venn



Fungi

Table 31: Fungi: Spraying effect in experiment 1

| higher in soil | unchanged | higher in weeds |
|----------------|-----------|-----------------|
| 4 | 107 | 10 |

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

Table 32: The top abundant ASVs

| | phylum | class | logFC | rAbu |
|--------------|---------------|-----------------|--------|-------|
| ASV33 | Basidiomycota | Agaricomycetes | -4.642 | 0.26% |
| ASV63 | Ascomycota | Sordariomycetes | 4.283 | 0.22% |
| ASV76 | Ascomycota | Sordariomycetes | 5.316 | 0.2% |
| ASV60 | Ascomycota | unassigned | -2.813 | 0.18% |
| ASV86 | Ascomycota | Sordariomycetes | 3.901 | 0.17% |
| ASV95 | Ascomycota | Leotiomycetes | 4.554 | 0.17% |
| ASV88 | Ascomycota | Leotiomycetes | 3.751 | 0.15% |
| ASV80 | Ascomycota | Sordariomycetes | 3.621 | 0.15% |
| ASV92 | Ascomycota | Eurotiomycetes | 4.742 | 0.12% |
| ASV91 | Ascomycota | Pezizomycetes | -2.564 | 0.11% |

Table 33: Fungi: Spraying effect in experiment 2

| higher in soil | unchanged | higher in weeds |
|----------------|-----------|-----------------|
| 11 | 111 | 4 |

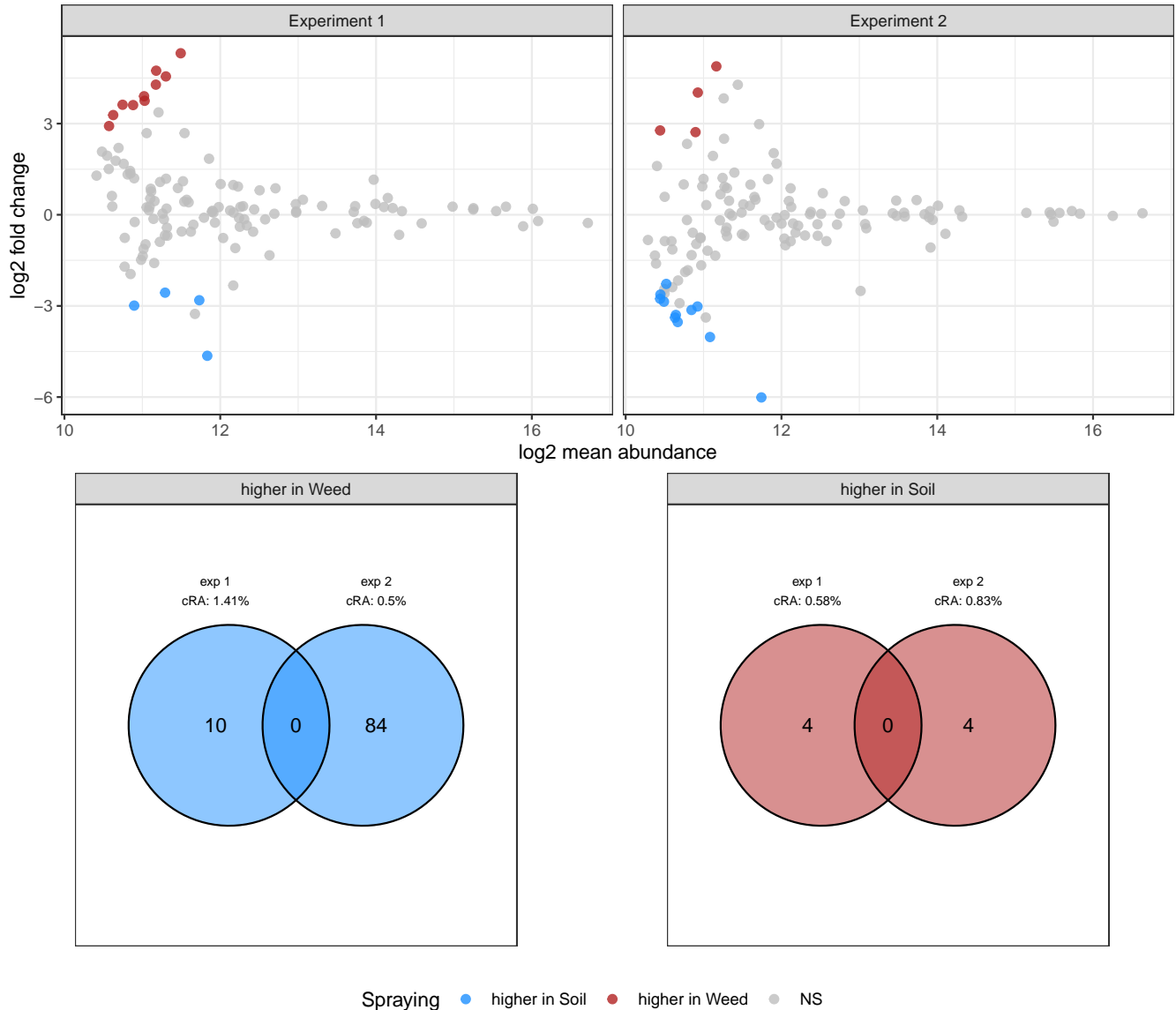
[1] "1.33% of the fungal abundance changed between the different spraying targets."

Table 34: The top abundant ASVs

| | phylum | class | logFC | rAbu |
|---------------|---------------|--------------------|--------|-------|
| ASV33 | Basidiomycota | Agaricomycetes | -6.012 | 0.26% |
| ASV86 | Ascomycota | Sordariomycetes | 4.885 | 0.17% |
| ASV95 | Ascomycota | Leotiomycetes | 4.023 | 0.17% |
| ASV79 | Ascomycota | Sordariomycetes | -4.024 | 0.16% |
| ASV94 | Ascomycota | Leotiomycetes | 2.72 | 0.13% |
| ASV116 | Ascomycota | Dothideomycetes | -3.385 | 0.09% |
| ASV141 | Ascomycota | Eurotiomycetes | -3.531 | 0.09% |
| ASV119 | Ascomycota | Dothideomycetes | -3.02 | 0.07% |
| ASV122 | Basidiomycota | Microbotryomycetes | -3.134 | 0.05% |
| ASV215 | Ascomycota | unassigned | 2.775 | 0.03% |

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

Figure 6.2 | Fungi: MA & venn



Conclusion: Different spraying 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 spraying 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

Table 35: Bacteria: Glyphosate effect in experiment 1

| lower in gly | unchanged | higher in gly |
|--------------|-----------|---------------|
| 85 | 1605 | 33 |

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

Table 36: The top abundant ASVs

| | phylum | class | logFC | rAbu |
|---------------|------------------|---------------------|---------|-------|
| ASV2 | Verrucomicrobia | Verrucomicrobiae | -0.2518 | 3.55% |
| ASV7 | Verrucomicrobia | Verrucomicrobiae | -0.4515 | 0.72% |
| ASV18 | Proteobacteria | Deltaproteobacteria | -0.2761 | 0.51% |
| ASV32 | Actinobacteria | Actinobacteria | 0.733 | 0.33% |
| ASV651 | Verrucomicrobia | Verrucomicrobiae | -6.508 | 0.04% |
| ASV711 | Acidobacteria | Subgroup_5 | -4.571 | 0.04% |
| ASV461 | Actinobacteria | Actinobacteria | 4.167 | 0.04% |
| ASV470 | Gemmatimonadetes | Gemmatimonadetes | 3.509 | 0.03% |
| ASV781 | Bacteroidetes | Bacteroidia | -6.747 | 0.03% |
| ASV794 | Verrucomicrobia | Verrucomicrobiae | -6.769 | 0.03% |

Table 37: Bacteria: Terbuthylazine effect in experiment 1

| lower in tb | unchanged | higher in tb |
|-------------|-----------|--------------|
| 109 | 1492 | 32 |

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

Table 38: The top abundant ASVs

| | phylum | class | logFC | rAbu |
|---------------|-----------------|---------------------|--------|-------|
| ASV170 | Proteobacteria | Alphaproteobacteria | 5.184 | 0.12% |
| ASV477 | Proteobacteria | Gammaproteobacteria | -3.77 | 0.05% |
| ASV569 | Acidobacteria | Subgroup_17 | -3.857 | 0.04% |
| ASV651 | Verrucomicrobia | Verrucomicrobiae | -5.444 | 0.04% |
| ASV649 | Acidobacteria | Subgroup_6 | -3.858 | 0.03% |
| ASV654 | Proteobacteria | Deltaproteobacteria | -4.627 | 0.03% |
| ASV794 | Verrucomicrobia | Verrucomicrobiae | -7.11 | 0.03% |
| ASV763 | Acidobacteria | Holophagae | -6.536 | 0.03% |
| ASV803 | Latescibacteria | unassigned | -5.38 | 0.02% |
| ASV869 | Acidobacteria | Subgroup_6 | 6.792 | 0.02% |

Table 39: Bacteria: Glyphosate effect in experiment 2

| lower in gly | unchanged | higher in gly |
|--------------|-----------|---------------|
| 60 | 1464 | 31 |

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

Table 40: The top abundant ASVs

| | phylum | class | logFC | rAbu |
|---------------|----------------|---------------------|---------|-------|
| ASV19 | Proteobacteria | Alphaproteobacteria | -0.5489 | 0.53% |
| ASV39 | Proteobacteria | Alphaproteobacteria | -0.681 | 0.39% |
| ASV32 | Actinobacteria | Actinobacteria | 0.6449 | 0.33% |
| ASV464 | Firmicutes | Bacilli | 4.601 | 0.04% |
| ASV842 | Proteobacteria | Alphaproteobacteria | -7.292 | 0.03% |
| ASV683 | Bacteroidetes | Bacteroidia | -6.734 | 0.02% |
| ASV629 | Proteobacteria | Gammaproteobacteria | 5.72 | 0.02% |
| ASV654 | Proteobacteria | Deltaproteobacteria | -6.403 | 0.02% |
| ASV946 | Acidobacteria | Subgroup_6 | -4.695 | 0.02% |
| ASV956 | Actinobacteria | Actinobacteria | 5.195 | 0.02% |

Table 41: Bacteria: Terbutylazine effect in experiment 2

| lower in tb | unchanged | higher in tb |
|-------------|-----------|--------------|
| 67 | 1467 | 41 |

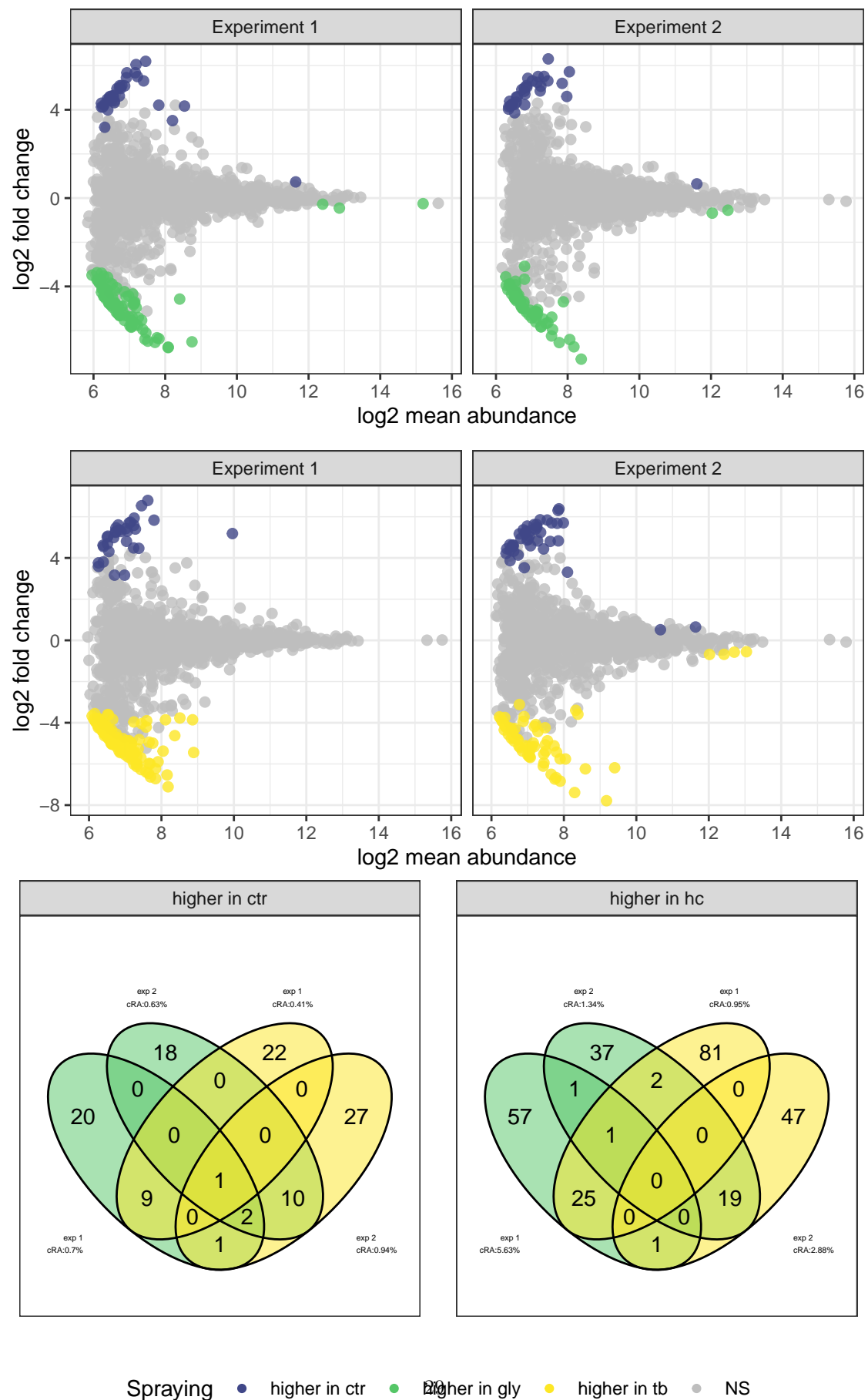
[1] "4.15% of the bacterial abundance changed after spraying terbutylazine"

Table 42: The top abundant ASVs

| | phylum | class | logFC | rAbu |
|---------------|----------------|---------------------|---------|-------|
| ASV9 | Proteobacteria | Alphaproteobacteria | -0.5531 | 0.81% |
| ASV14 | Proteobacteria | Alphaproteobacteria | -0.5741 | 0.64% |
| ASV19 | Proteobacteria | Alphaproteobacteria | -0.6788 | 0.55% |
| ASV39 | Proteobacteria | Alphaproteobacteria | -0.6846 | 0.39% |
| ASV32 | Actinobacteria | Actinobacteria | 0.6485 | 0.36% |
| ASV125 | Proteobacteria | Gammaproteobacteria | 0.5151 | 0.15% |
| ASV435 | Proteobacteria | Alphaproteobacteria | -6.186 | 0.06% |
| ASV318 | Bacteroidetes | Bacteroidia | -7.785 | 0.06% |
| ASV707 | Bacteroidetes | Bacteroidia | -6.24 | 0.03% |
| ASV457 | Proteobacteria | Gammaproteobacteria | -5.755 | 0.03% |

Glyphosate changed the abundance of 118 bacterial ASVs in experiment 1 and 91 bacterial ASVs in experiment 2. Spraying terbutylazine 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

Table 43: Fungi: Glyphosate effect in experiment 1

| lower in gly | unchanged | higher in gly |
|--------------|-----------|---------------|
| 3 | 87 | 2 |

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

Table 44: The top abundant ASVs

| | phylum | class | cols_phyla | rank |
|--------------|-------------------|--------------------|---------------|------|
| ASV19 | Mortierellomycota | Mortierellomycetes | mediumorchid1 | 19 |
| ASV62 | Ascomycota | Sordariomycetes | dodgerblue2 | 62 |
| ASV76 | Ascomycota | Sordariomycetes | dodgerblue2 | 76 |
| ASV75 | Mortierellomycota | Mortierellomycetes | mediumorchid1 | 75 |
| ASV95 | Ascomycota | Leotiomycetes | dodgerblue2 | 95 |

Table 45: Fungi: Terbutylazine effect in experiment 1

| lower in tb | unchanged | higher in tb |
|-------------|-----------|--------------|
| 1 | 95 | 3 |

[1] "1.58% of the fungal abundance changed after spraying terbutylazine"

Table 46: The top abundant ASVs

| | phylum | class | cols_phyla | rank |
|--------------|-------------------|--------------------|---------------|------|
| ASV33 | Basidiomycota | Agaricomycetes | firebrick1 | 33 |
| ASV83 | Ascomycota | Leotiomycetes | dodgerblue2 | 83 |
| ASV55 | Ascomycota | Sordariomycetes | dodgerblue2 | 55 |
| ASV54 | Mortierellomycota | Mortierellomycetes | mediumorchid1 | 54 |

Table 47: Fungi: Glyphosate effect in experiment 2

| lower in gly | unchanged | higher in gly |
|--------------|-----------|---------------|
| 2 | 100 | 1 |

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

Table 48: The top abundant ASVs

| | phylum | class | logFC | rAbu |
|---------------|------------|-----------------|--------|-------|
| ASV69 | Ascomycota | Sordariomycetes | -4.58 | 0.16% |
| ASV95 | Ascomycota | Leotiomycetes | 4.928 | 0.13% |
| ASV116 | Ascomycota | Dothideomycetes | -4.294 | 0.07% |

Table 49: Fungi: Terbutylazine effect in experiment 2

| lower in tb | unchanged | higher in tb |
|-------------|-----------|--------------|
| 2 | 92 | 0 |

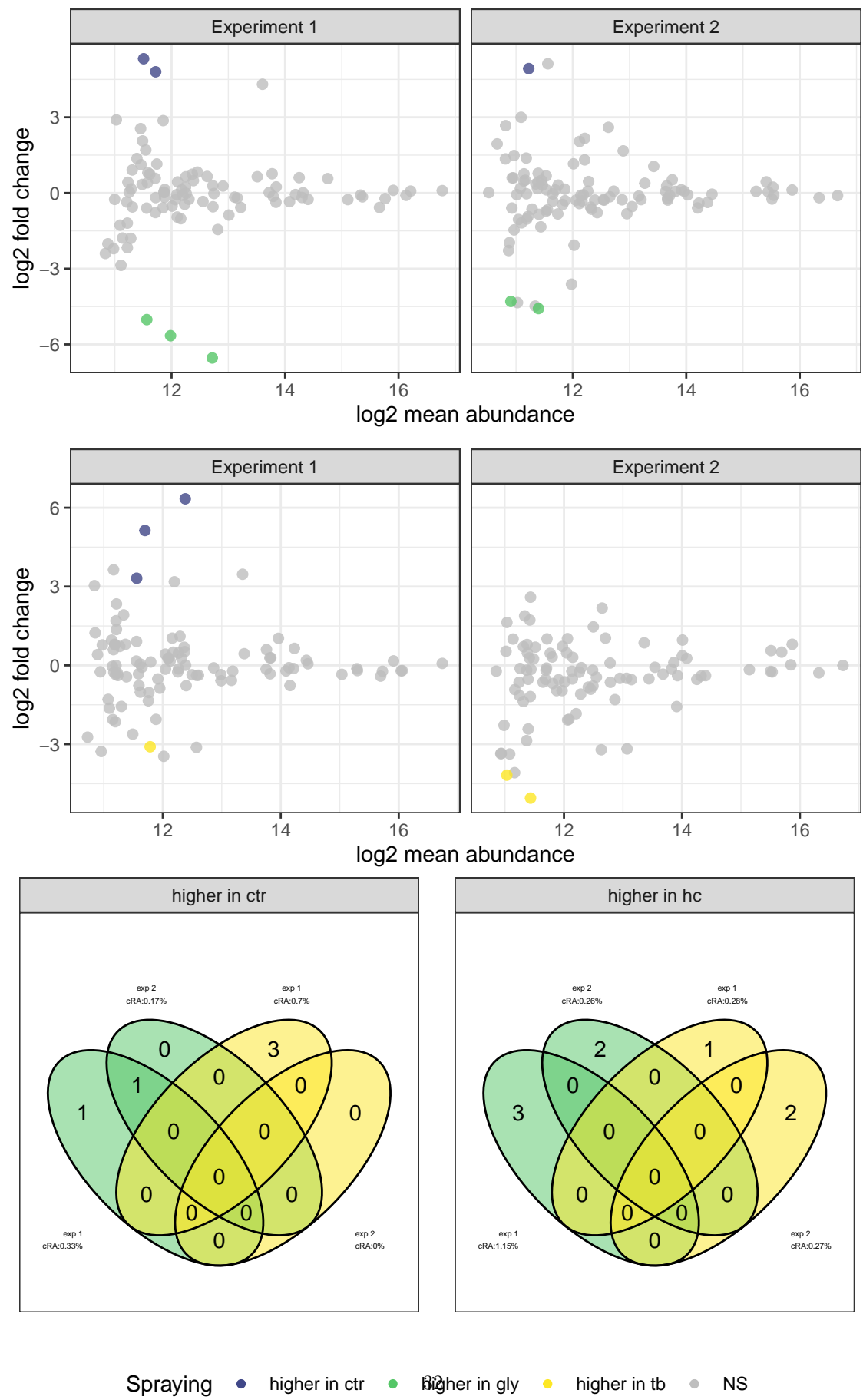
[1] "0.23% of the fungal abundance changed after spraying terbutylazine"

Table 50: The top abundant ASVs

| | phylum | class | logFC | rAbu |
|---------------|------------|-----------------|--------|-------|
| ASV73 | Ascomycota | Sordariomycetes | -5.05 | 0.14% |
| ASV108 | Ascomycota | Dothideomycetes | -4.177 | 0.09% |

Glyphosate changed the abundance of 5 bacterial ASVs in experiment 1 and 3 bacterial ASVs in experiment 2. Spraying terbutylazine 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: Spraying 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

Spraying to different targets did not influence high abundant ASVs. Spraying 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.