MBVagTrans Results

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1 Introduction

Include here metadata summaries and summary of phyloseq object

2 PBS empty control

The PBS empty control seems to be contaminated with normal vaginal flora.

```
taxdistri = read_csv2( "results/microbiome/pbs_taxonomic_composition.csv")
kable(mutate(taxdistri, Abundance = round(Abundance, digits = 2)))
```

Taxonomy	Abundance
p__Firmicutes;f__Lactobacillaceae;g__Lactobacillus	30.57
p__Actinobacteriota;f__Bifidobacteriaceae;g__Bifidobacterium	16.69
p__Actinobacteriota;f__Bifidobacteriaceae;g__Gardnerella	12.40
p__Proteobacteria;f__Moraxellaceae;g__Enhydrobacter	5.80
p__Firmicutes;f__Family XI;g__Anaerococcus	4.72
p__Actinobacteriota;f__Bifidobacteriaceae;g__Alloscardovia	3.61
p__Firmicutes;f__Streptococcaceae;g__Lactococcus	3.41
p__Bacteroidota;f__Prevotellaceae;g__Prevotella	3.37
p__Firmicutes;f__Streptococcaceae;g__Streptococcus	3.05
p__Proteobacteria;f__Moraxellaceae;g__Acinetobacter	2.45
p__Actinobacteriota;f__Atopobiaceae;g__Atopobium	2.16
p__Firmicutes;f__Family XI;g__Finegoldia	1.55
p__Firmicutes;f__Staphylococcaceae;g__Staphylococcus	1.31
p__Actinobacteriota;f__Propionibacteriaceae;g__Cutibacterium	1.17
p__Firmicutes;f__Lachnospiraceae;g__Anaerostipes	1.17
p__Firmicutes;f__Ruminococcaceae;g__Faecalibacterium	1.08
p__Actinobacteriota;f__Corynebacteriaceae;g__Corynebacterium	0.92
p__Actinobacteriota;f__Actinomycetaceae;g__NA	0.69
p__Proteobacteria;f__Xanthobacteraceae;g__Bradyrhizobium	0.66
p__Actinobacteriota;f__Actinomycetaceae;g__Varibaculum	0.59
p__Firmicutes;f__Family XI;g__Ezakiella	0.59
p__Firmicutes;f__Enterococcaceae;g__Enterococcus	0.58
p__Actinobacteriota;f__Corynebacteriaceae;g__NA	0.54
p__Actinobacteriota;f__Pseudonocardiaceae;g__Prauserella	0.54
p__Firmicutes;f__Marinococcaceae;g__Alteribacillus	0.39

5.8%

16.4%

3%

3.4%

3.6%

30.6%

16.7%

12.4%

3.4%

4.7%

Taxonomy

- Other
- p_Actinobacteriota;f_Bifidobacteriaceae;g_Alloscardovia
- p_Actinobacteriota;f_Bifidobacteriaceae;g_Bifidobacterium
- p_Actinobacteriota;f_Bifidobacteriaceae;g_Gardnerella
- p_Bacteroidota;f_Prevotellaceae;g_Prevotella
- p_Firmicutes;f_Family XI;g_Anaerococcus
- p_Firmicutes;f_Lactobacillaceae;g_Lactobacillus
- p_Firmicutes;f_Streptococcaceae;g_Lactococcus
- p_Firmicutes;f_Streptococcaceae;g_Streptococcus
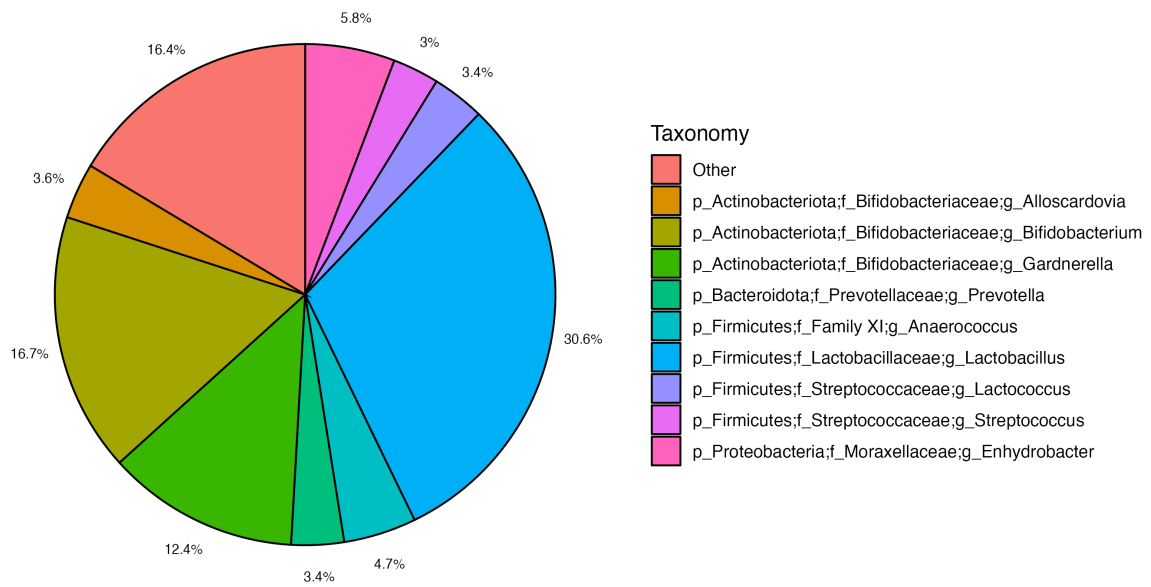- p_Proteobacteria;f_Moraxellaceae;g_Enhydrobacter

Figure 1: Taxonomic composition of PBS sample

3

```
ps_samples = readRDS("intermediate/ps_samples.rds")
```

3 Diversity Analysis

3.1 Alpha-diversity

Alpha diversity describes the number and distribution of taxa *within* a sample or group of samples. Different indices exist that capture and describe different aspects of this ecologic measure.

In this dataset, alpha diversity is markedly different between the group of interest (i.e. *03_trans*) and the two control groups. However, the intervention group is more similar to the first control group (i.e. *postmenopausal women*).

```
adiv = plot_richness(ps_samples, x = "grp", measures = c("Observed", "InvSimpson", "Shanno

aov = adiv$data %>%
  group_by(variable) %>%
  rstatix::anova_test(value~grp)

tukey = adiv$data %>%
  group_by(variable) %>%
  tukey_hsd(value~grp)

adiv +
  geom_boxplot(aes(fill = grp),alpha = 0.7) +
  labs(x = "", fill = "Group")+
  scale_y_continuous(expand = expansion(mult = c(0, 0.25)))  +
  scale_fill_manual(values = custom_pal)
```
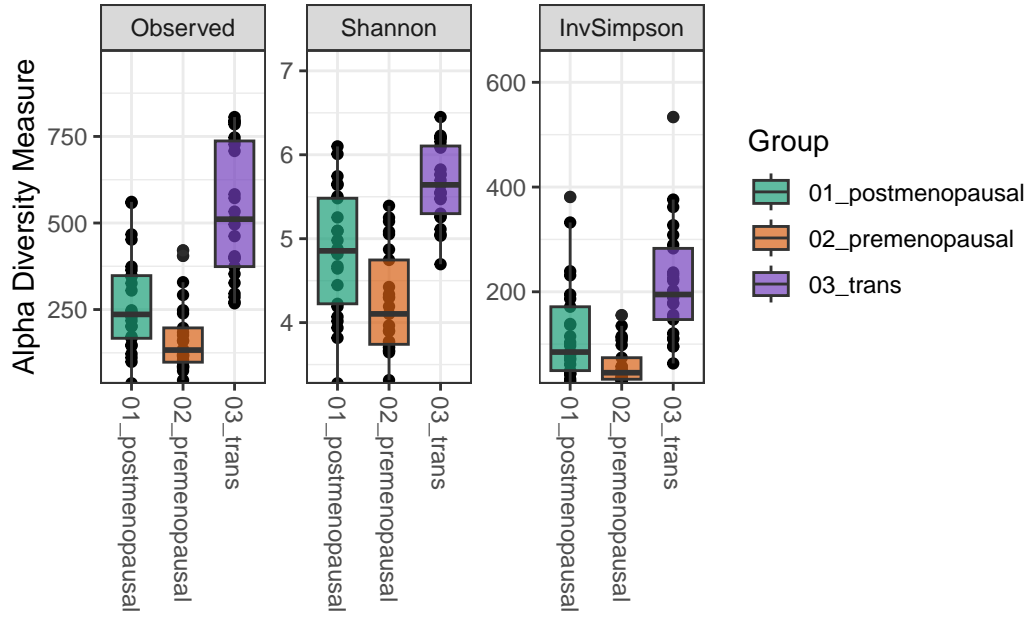
```
kable(select(tukey, variable, group1,group2,p.adj, p.adj.signif))
```

variable	group1	group2	p.adj	p.adj.signif
Observed	01_postmenopausal	02_premenopausal	5.99e-02	ns
Observed	01_postmenopausal	03_trans	1.00e-07	****
Observed	02_premenopausal	03_trans	0.00e+00	****
Shannon	01_postmenopausal	02_premenopausal	2.03e-03	**
Shannon	01_postmenopausal	03_trans	3.20e-05	****
Shannon	02_premenopausal	03_trans	0.00e+00	****
InvSimpson	01_postmenopausal	02_premenopausal	3.55e-02	*
InvSimpson	01_postmenopausal	03_trans	6.88e-04	***
InvSimpson	02_premenopausal	03_trans	0.00e+00	****

3.1.1 Effect of covariates

To rule out a possible effect of age on alpha diversity, pearson correlation of alpha diversity measures with age are performed within each of the 3 groups separately.

```
adiv$data %>%
  group_by(variable, grp) %>%
  cor_test(value, Age) %>%
  select(grp, variable, cor, statistic, p, conf.low, conf.high) %>%
  kable()
```

grp	variable	cor	statistic	p	conf.low	conf.high
01_postmenopausal	Observed	0.092	0.4418424	0.663	-0.3148005	0.4698411
02_premenopausal	Observed	-0.067	-0.3210136	0.751	-0.4500411	0.3372440
03_trans	Observed	-0.042	-0.1997907	0.843	-0.4296871	0.3594190
01_postmenopausal	Shannon	0.082	0.3922556	0.698	-0.3240500	0.4617756
02_premenopausal	Shannon	-0.150	-0.7415709	0.466	-0.5167418	0.2578904
03_trans	Shannon	-0.190	-0.9438783	0.355	-0.5465364	0.2187115
01_postmenopausal	InvSimpson	0.130	0.6133610	0.546	-0.2824268	0.4970754
02_premenopausal	InvSimpson	-0.110	-0.5345112	0.598	-0.4846848	0.2973790
03_trans	InvSimpson	-0.094	-0.4540420	0.654	-0.4718124	0.3125169

No comparison is significant.

3.1.1.1 Length of GAHT within patient group

```
adiv$data %>%
  filter(grp =="03_trans") %>%
  group_by(variable) %>%
  cor_test(value, GHAT_Length) %>%
  select(variable, cor, statistic, p, conf.low, conf.high) %>%
  kable(digits = 3, caption = "Pearson Correlation of a-diversity measures with length of
```

Table 4: Pearson Correlation of a-diversity measures with length of GAHT in the Trans patient group

variable	cor	statistic	p	conf.low	conf.high
Observed	-0.29	-1.450	0.161	-0.614	0.119
Shannon	-0.50	-2.760	0.011	-0.747	-0.129
InvSimpson	-0.35	-1.817	0.082	-0.657	0.047

In this analysis, a significant effect on Shannon index with length of hormone replacement therapy could be observed.

3.1.1.2 Influence of Nugent score within patient group

```r
adiv$data %>%
  group_by(variable) %>%
  tukey_hsd(value~NugentScore) %>%
  select(variable, group1, group2, estimate, p.adj, p.adj.signif, conf.low, conf.high) %>%
  kable(digits = 3, caption = "Tukey Test on alpha diversity measures by Nugent Score")
```

Table 5: Tukey Test on alpha diversity measures by Nugent Score

variable	group1	group2	estimate	p.adj	p.adj.signif	conf.low	conf.high
Observed	0-3	4-6	212.029	0.006	**	47.224	376.833
Observed	0-3	7-10	-31.233	0.993	ns	-339.555	277.088
Observed	0-3	no bacteria	200.600	0.001	**	66.804	334.396
Observed	4-6	7-10	-243.262	0.207	ns	-567.204	80.681
Observed	4-6	no bacteria	-11.429	0.998	ns	-178.096	155.238
Observed	7-10	no bacteria	231.833	0.208	ns	-77.488	541.154
Shannon	0-3	4-6	0.973	0.001	***	0.354	1.592
Shannon	0-3	7-10	0.028	1.000	ns	-1.130	1.186
Shannon	0-3	no bacteria	0.851	0.000	***	0.349	1.353
Shannon	4-6	7-10	-0.945	0.182	ns	-2.161	0.271
Shannon	4-6	no bacteria	-0.122	0.956	ns	-0.748	0.504
Shannon	7-10	no bacteria	0.823	0.253	ns	-0.338	1.984
InvSimpson	0-3	4-6	93.802	0.022	*	10.066	177.538
InvSimpson	0-3	7-10	-9.942	0.998	ns	-166.598	146.713
InvSimpson	0-3	no bacteria	84.009	0.009	**	16.028	151.989
InvSimpson	4-6	7-10	-103.744	0.353	ns	-268.336	60.848
InvSimpson	4-6	no bacteria	-9.793	0.990	ns	-94.475	74.889
InvSimpson	7-10	no bacteria	93.951	0.401	ns	-63.212	251.114

Notably, if analysis is split by patient group, none of these differences remain significant. This might be due to a lower sample size.

3.2 Beta-diversity

This diversity metric compares different samples or groups of samples regarding their similarity or difference in community composition. The distance matrix can be visualized using dimension reduction techniques (e.g. PCA) or pairwise distances can be calculated. For this analysis, the main hypothesis is, that overall community composition differs significantly between the patient group (i.e. *03_trans*) and the two control groups. This will be tested using *adonis.*

```r
# first, filter out low abundance taxa
lowabund_filter = genefilter_sample(ps_samples, filterfun_sample(function(x) x > 10), A=0.
ps_filtered = prune_taxa(lowabund_filter, ps_samples)
ps_filtered
```

```
phyloseq-class experiment-level object
otu_table()   OTU Table:          [ 567 taxa and 75 samples ]
sample_data() Sample Data:        [ 75 samples by 15 sample variables ]
tax_table()   Taxonomy Table:     [ 567 taxa by 7 taxonomic ranks ]
phy_tree()    Phylogenetic Tree:  [ 567 tips and 566 internal nodes ]
refseq()      DNAStringSet:       [ 567 reference sequences ]
```

```r
ps_filtered_rel = transform_sample_counts(ps_filtered, function(x) 100 * x/sum(x))

ord.bray<- ordinate(ps_filtered_rel, "PCoA", "bray")
ord.wunifrac <- ordinate(ps_filtered_rel, "PCoA", "unifrac", weighted = T)
ord.unifrac <- ordinate(ps_filtered_rel, "PCoA", "unifrac", weighted = F)

p1 = plot_ordination(ps_filtered_rel, ord.bray, type="samples", color="grp") +
  geom_point(size = 3, alpha = 0.8) +
  scale_color_brewer(type = "qual",palette = "Dark2") +
  stat_ellipse() +
  labs(color = "Group", caption = "Bray-Curtis")

p2 = plot_ordination(ps_filtered_rel, ord.wunifrac, type="samples", color="grp") +
  geom_point(size = 3, alpha = 0.8) +
  scale_color_brewer(type = "qual",palette = "Dark2") +
  stat_ellipse() +
  labs(color = "Group", caption = "Weighted Unifrac")

p3 = plot_ordination(ps_filtered_rel, ord.unifrac, type="samples", color="grp") +
  geom_point(size = 3, alpha = 0.8) +
  scale_color_brewer(type = "qual",palette = "Dark2")+
  stat_ellipse() +
  labs(color = "Group", caption = "Unweighted Unifrac")

# extract the legend from one of the plots
legend_b <- get_legend(
  p1 +
    guides(color = guide_legend(nrow = 1)) +
    theme(legend.position = "bottom")
```

```
)

plot_ordinations = cowplot::plot_grid(p1 + theme(legend.position="none"),
                         p2 + theme(legend.position="none"),
                         p3 + theme(legend.position="none"),
                         labels =  "AUTO", ncol = 2)
```

Warning in MASS::cov.trob(data[, vars]): Probable convergence failure

```
cowplot::plot_grid(plot_ordinations, legend_b, ncol = 1, rel_heights = c(1, .1))
```
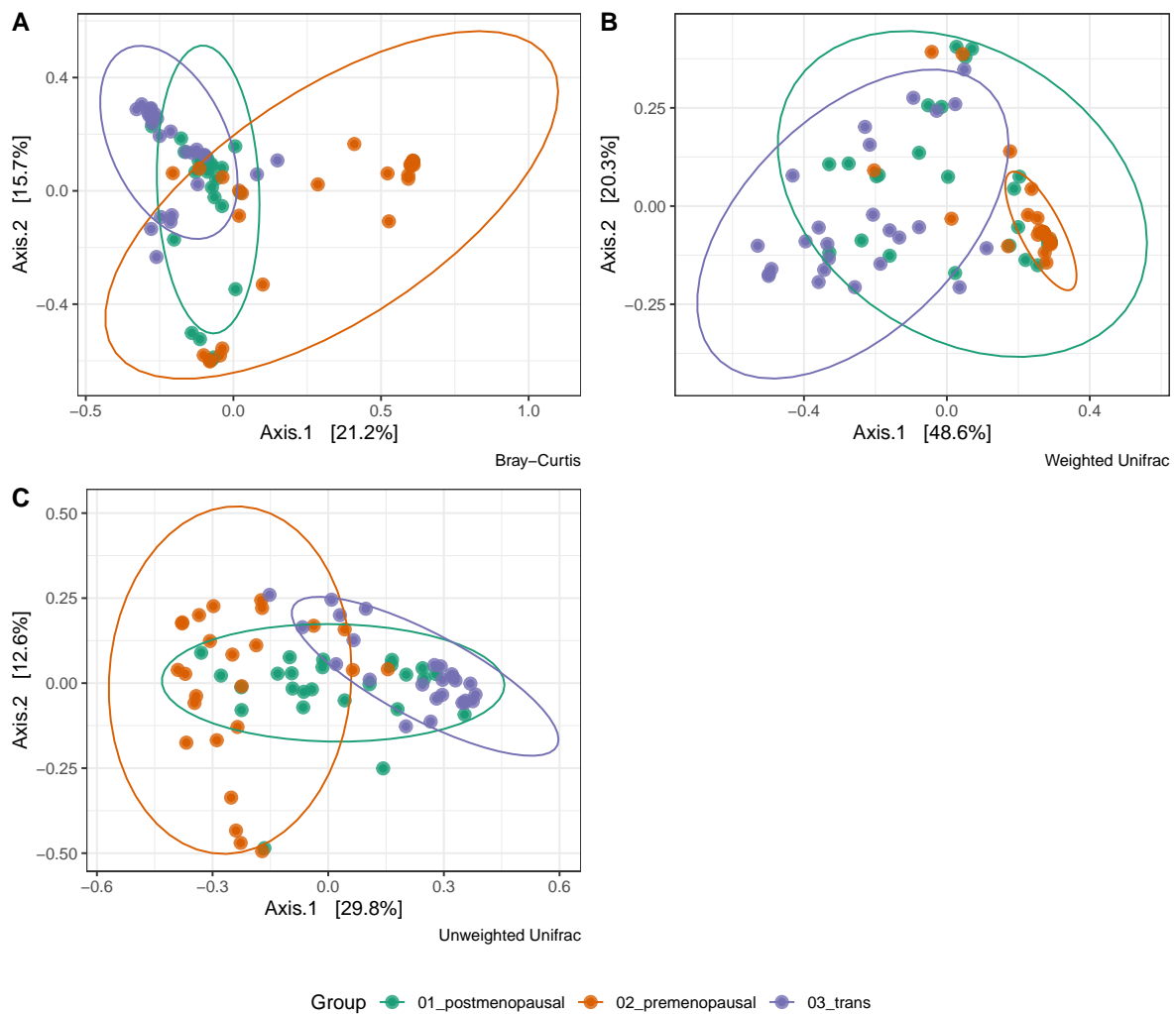
```r
D_BC <- phyloseq::distance(ps_filtered_rel, "bray")
D_UF <- phyloseq::distance(ps_filtered_rel,  "unifrac")
D_wUF <- phyloseq::distance(ps_filtered_rel, "wunifrac")

adonis_bc = vegan::adonis2(D_BC ~ phyloseq::sample_data(ps_filtered_rel)$grp)
adonis_pw_bc = pairwise.adonis(D_BC, phyloseq::sample_data(ps_filtered_rel)$grp)

adonis_uf = vegan::adonis2(D_UF ~ phyloseq::sample_data(ps_filtered_rel)$grp)
adonis_pw_uf = pairwise.adonis(D_UF, phyloseq::sample_data(ps_filtered_rel)$grp)

adonis_wUF = vegan::adonis2(D_wUF ~ phyloseq::sample_data(ps_filtered_rel)$grp)
adonis_pw_wUF = pairwise.adonis(D_wUF, phyloseq::sample_data(ps_filtered_rel)$grp)

kable(adonis_pw_bc, digits = 3, caption = "Bray-Curtis")
```

Table 6: Bray-Curtis

pairs	Df	SumsOfSqsF.Model	R2	p.value	p.adjusted	sig
01_postmenopausal vs 02_premenopausal	1	1.803	4.965	0.094	0.001	0.003	*
01_postmenopausal vs 03_trans	1	1.411	3.997	0.077	0.001	0.003	*
02_premenopausal vs 03_trans	1	3.513	11.173	0.189	0.001	0.003	*

```r
kable(adonis_pw_uf, digits = 3, caption ="Unweigthed Unifrac")
```

Table 7: Unweigthed Unifrac

pairs	Df	SumsOfSqsF.Model	R2	p.value	p.adjusted	sig
01_postmenopausal vs 02_premenopausal	1	1.096	5.754	0.107	0.001	0.003	*
01_postmenopausal vs 03_trans	1	0.888	5.901	0.109	0.001	0.003	*
02_premenopausal vs 03_trans	1	2.559	16.713	0.258	0.001	0.003	*

```r
kable(adonis_pw_wUF, digits = 3, caption = "Weighted Unifrac")
```

Table 8: Weighted Unifrac

pairs	Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted	sig
01_postmenopausal vs 02_premenopausal	1	0.583	6.608	0.121	0.001	0.003	*
01_postmenopausal vs 03_trans	1	0.995	8.073	0.144	0.001	0.003	*
02_premenopausal vs 03_trans	1	2.563	30.133	0.386	0.001	0.003	*

4 Differential abundance

```
library(DESeq2)

dds = phyloseq_to_deseq2(ps_filtered, ~ grp)
dds$grp<-relevel(dds$grp,ref="03_trans")
ds <- estimateSizeFactors(dds, type="poscounts")
ds = DESeq(ds, test="Wald", fitType="parametric")
alpha = 0.05
res_premenopausal = results(ds, contrast = c("grp", "02_premenopausal", "03_trans"))
taxa_sig_premenopausal = dplyr::filter(as.data.frame(res_premenopausal), padj < 0.05) %>%
  rownames_to_column("ASV")

taxa_table = tax_table(ps_filtered) %>%
  as.data.frame() %>%
  rownames_to_column("ASV")

diffabund_pre = left_join(taxa_sig_premenopausal, taxa_table, by = "ASV") %>%
  select(-Species) %>%
  write_csv2("results/microbiome/deseq_diff_trans-premenopausal.csv")

kable(diffabund_pre, digits = 3, row.names = F, caption = "Differential abundance between
```

Table 9: Differential abundance between premenopausal women and trans patients (pre vs trans)

ASV	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV1	74.357	4.694	1.528	3.072	0.002	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV2	67.716	4.210	1.454	2.895	0.004	0.013	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV3	67.172	3.754	1.422	2.639	0.008	0.023	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV4	64.609	4.388	1.475	2.975	0.003	0.010	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus

ASV	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV5	58.967	4.588	1.502	3.055	0.002	0.009	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV6	62.167	4.442	1.448	3.069	0.002	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV7	58.458	4.132	1.434	2.883	0.004	0.013	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV8	58.563	4.669	1.500	3.112	0.002	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV9	56.443	4.629	1.485	3.116	0.002	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV10	56.432	4.277	1.501	2.850	0.004	0.014	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV11	56.133	4.037	1.436	2.810	0.005	0.016	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV12	56.298	4.465	1.510	2.958	0.003	0.011	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV13	53.169	4.740	1.441	3.289	0.001	0.005	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV15	51.241	3.860	1.451	2.660	0.008	0.022	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV14	47.395	4.291	1.482	2.895	0.004	0.013	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV18	48.663	4.439	1.440	3.083	0.002	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV19	47.253	4.649	1.477	3.148	0.002	0.007	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV20	46.177	4.292	1.395	3.077	0.002	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV24	43.101	4.616	1.455	3.172	0.002	0.007	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV25	41.982	4.235	1.520	2.786	0.005	0.017	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV26	42.905	4.446	1.382	3.217	0.001	0.006	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV30	40.899	4.442	1.414	3.141	0.002	0.007	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV31	39.290	4.235	1.495	2.832	0.005	0.015	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV33	39.161	4.514	1.461	3.089	0.002	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV35	37.859	4.511	1.497	3.012	0.003	0.009	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV37	38.147	4.606	1.479	3.115	0.002	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV43	30.304	4.236	1.475	2.872	0.004	0.014	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV54	8.312	-3.329	1.354	-2.459	0.014	0.034	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV53	7.625	-3.196	1.333	-2.398	0.016	0.039	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV57	3.608	-3.317	1.336	-2.483	0.013	0.033	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV58	4.361	-3.341	1.466	-2.279	0.023	0.050	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV59	2.825	-4.053	1.483	-2.733	0.006	0.018	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV61	2.056	-3.957	1.415	-2.796	0.005	0.016	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV62	1.648	-3.842	1.379	-2.787	0.005	0.017	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV63	1.023	-3.482	1.384	-2.517	0.012	0.031	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV67	6.851	-4.106	1.404	-2.924	0.003	0.012	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella

ASV	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV63	7.069	-3.805	1.465	-2.597	0.009	0.026	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV62	6.421	-3.679	1.399	-2.629	0.009	0.024	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV72	6.234	-3.269	1.342	-2.437	0.015	0.036	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV72	5.051	-3.510	1.505	-2.333	0.020	0.045	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV72	2.871	-3.350	1.379	-2.429	0.015	0.037	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV72	2.816	-3.757	1.468	-2.560	0.010	0.028	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV81	2.716	-3.270	1.432	-2.283	0.022	0.049	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV82	2.808	-4.167	1.500	-2.778	0.005	0.017	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV84	2.674	-3.577	1.453	-2.462	0.014	0.034	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV92	0.463	-3.392	1.440	-2.356	0.018	0.043	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV100	0.793	-4.268	1.414	-3.018	0.003	0.009	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV120	2.144	-3.610	1.444	-2.500	0.012	0.032	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV12	7.759	-3.325	1.455	-2.285	0.022	0.049	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Finegoldia
ASV120	1.523	-5.367	1.743	-3.079	0.002	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV13	7.781	-3.399	1.443	-2.355	0.019	0.043	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV13	7.041	-4.593	1.606	-2.860	0.004	0.014	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV14	8.964	-7.262	1.656	-4.385	0.000	0.000	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV15	2.289	-7.158	1.779	-4.024	0.000	0.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV15	5.947	-4.982	1.469	-3.391	0.001	0.004	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV16	3.189	-5.168	1.455	-3.551	0.000	0.002	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella

ASV	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV155	4.436	-7.124	1.351	-5.272	0.000	0.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV156	8.300	-6.944	1.721	-4.035	0.000	0.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV177	5.624	-4.849	1.457	-3.329	0.001	0.004	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV178	8.120	-6.800	1.770	-3.842	0.000	0.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV184	7.069	-5.346	1.452	-3.683	0.000	0.002	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV194	2.983	-6.993	1.651	-4.237	0.000	0.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV196	6.878	-5.670	1.413	-4.011	0.000	0.001	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV197	7.760	4.556	1.883	2.420	0.016	0.037	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV199	9.687	-6.759	1.711	-3.949	0.000	0.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV205	5.564	-4.616	1.871	-2.467	0.014	0.034	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
ASV209	9.992	-6.638	1.838	-3.612	0.000	0.002	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV215	5.669	-5.944	2.409	-2.467	0.014	0.034	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV214	4.706	-6.698	1.850	-3.620	0.000	0.002	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV220	0.907	-6.788	1.648	-4.120	0.000	0.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV222	2.862	-5.804	1.518	-3.824	0.000	0.001	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV223	3.076	-6.698	1.431	-4.679	0.000	0.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV228	8.003	-5.895	1.422	-4.146	0.000	0.001	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV230	0.649	-6.779	1.644	-4.124	0.000	0.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV235	5.725	-6.607	1.440	-4.588	0.000	0.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV232	2.549	-6.767	1.699	-3.983	0.000	0.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV242	2.202	-6.970	1.437	-4.851	0.000	0.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella

ASV	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV247	7.258	-3.295	1.441	-2.286	0.022	0.049	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Finegoldia
ASV25	5.985	-4.498	1.770	-2.542	0.011	0.029	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
ASV257	7.602	6.402	2.096	3.055	0.002	0.009	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV29	9.211	-4.327	1.749	-2.474	0.013	0.034	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
ASV261	1.064	-5.407	2.078	-2.602	0.009	0.025	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV263	3.753	-5.113	1.410	-3.626	0.000	0.002	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV265	5.502	-6.663	1.836	-3.629	0.000	0.002	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV266	6.957	-7.244	1.387	-5.222	0.000	0.000	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV270	0.388	-5.665	1.495	-3.789	0.000	0.001	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV272	2.277	-6.448	1.705	-3.781	0.000	0.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV274	4.888	-5.302	2.078	-2.551	0.011	0.029	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV276	6.402	-6.603	1.761	-3.749	0.000	0.002	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV277	7.702	-4.607	1.666	-2.765	0.006	0.017	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
ASV301	1.231	-6.591	1.454	-4.532	0.000	0.000	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV302	2.878	4.772	1.962	2.433	0.015	0.036	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV303	3.355	-4.951	2.077	-2.384	0.017	0.040	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV304	4.550	-5.598	1.770	-3.162	0.002	0.007	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
ASV305	5.581	-6.538	1.646	-3.973	0.000	0.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV307	7.649	-3.570	1.439	-2.481	0.013	0.033	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Finegoldia
ASV308	8.117	-3.819	1.502	-2.543	0.011	0.029	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV309	9.167	-6.434	1.821	-3.534	0.000	0.003	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus

ASV	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV311	1.472	-6.621	1.334	-4.963	0.000	0.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV315	5.049	-6.474	1.901	-3.406	0.001	0.004	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV32	2.322	-4.198	1.732	-2.424	0.015	0.037	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
ASV322	2.599	-24.683	2.756	-8.956	0.000	0.000	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV326	6.260	-7.621	1.709	-4.459	0.000	0.000	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Fenollaria
ASV328	8.403	-6.609	1.697	-3.896	0.000	0.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV330	0.768	-6.550	1.759	-3.724	0.000	0.002	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV333	3.823	-6.456	1.420	-4.545	0.000	0.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV335	5.261	-6.415	1.689	-3.797	0.000	0.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV330	0.987	4.962	1.951	2.544	0.011	0.029	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV335	5.530	-6.688	1.518	-4.405	0.000	0.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV335	5.892	-6.879	1.426	-4.824	0.000	0.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV352	2.595	-4.410	1.863	-2.368	0.018	0.042	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV353	3.607	-6.464	1.826	-3.540	0.000	0.002	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV36	6.413	-6.310	1.399	-4.512	0.000	0.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV36	6.705	-6.673	1.652	-4.038	0.000	0.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus
ASV37	7.138	-5.341	1.757	-3.040	0.002	0.009	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
ASV375	5.926	6.086	2.014	3.022	0.003	0.009	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV37	7.472	-5.068	1.865	-2.717	0.007	0.019	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
ASV378	8.028	-5.967	1.439	-4.148	0.000	0.001	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV384	4.733	-7.241	1.749	-4.141	0.000	0.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Fenollaria

ASV	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV3	8.926	-5.573	2.147	-2.596	0.009	0.026	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV4	8.266	-4.984	2.067	-2.412	0.016	0.038	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV4	6.730	-5.167	2.259	-2.288	0.022	0.049	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV4	8.586	-7.156	1.462	-4.896	0.000	0.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV4	1.246	-6.049	1.879	-3.220	0.001	0.006	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV4	1.926	-5.876	2.168	-2.710	0.007	0.019	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Alloscardovia
ASV4	2.365	-6.250	1.467	-4.261	0.000	0.001	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV4	2.561	-5.611	2.022	-2.775	0.006	0.017	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Alloscardovia
ASV4	3.018	-7.522	1.845	-4.078	0.000	0.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Fenollaria
ASV4	3.228	-7.325	1.778	-4.119	0.000	0.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Fenollaria
ASV4	3.438	-4.107	1.714	-2.395	0.017	0.039	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
ASV4	3.744	-5.359	1.932	-2.774	0.006	0.017	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Alloscardovia
ASV4	4.370	-7.384	1.869	-3.951	0.000	0.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Fenollaria
ASV4	4.897	-6.882	1.545	-4.456	0.000	0.000	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Peptoniphilus
ASV4	4.795	-6.066	1.735	-3.496	0.000	0.003	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV4	5.072	-7.278	1.957	-3.719	0.000	0.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Fenollaria
ASV4	5.430	-5.133	2.046	-2.508	0.012	0.031	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV4	6.956	-7.223	1.965	-3.676	0.000	0.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Fenollaria
ASV4	6.787	-5.002	2.131	-2.347	0.019	0.043	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
ASV4	6.952	-5.177	2.223	-2.329	0.020	0.045	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus

ASV	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV47.141		-7.008	1.427	-4.910	0.000	0.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV48.741	4.968	1.998	2.486	0.013	0.033		Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV46.544		-5.413	1.918	-2.823	0.005	0.015	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Alloscardovia
ASV43.254		-5.572	1.821	-3.060	0.002	0.009	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
ASV43.615		-5.458	1.743	-3.131	0.002	0.007	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
ASV49.364		-7.143	1.947	-3.668	0.000	0.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Fenollaria
ASV49.602		-6.716	1.548	-4.339	0.000	0.000	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Peptoniphilus
ASV50.856		-5.438	1.418	-3.835	0.000	0.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Peptoniphilus
ASV52.334		-5.696	2.397	-2.376	0.017	0.041	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Alloscardovia
ASV50.165		-5.449	2.186	-2.493	0.013	0.032	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Alloscardovia
ASV51.399		-6.993	1.815	-3.852	0.000	0.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Fenollaria
ASV51.606		-7.154	1.939	-3.690	0.000	0.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Fenollaria
ASV51.261		-7.004	1.881	-3.724	0.000	0.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Fenollaria
ASV52.795		-4.893	1.630	-3.002	0.003	0.010	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Peptoniphilus
ASV53.280		-6.016	1.699	-3.542	0.000	0.002	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
ASV54.511		-6.394	1.511	-4.231	0.000	0.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Peptoniphilus
ASV54.809		-6.358	1.770	-3.592	0.000	0.002	Bacteria	Campylobacterota	Campylobacteria	Campylobacterales	Campylobacteraceae	Campylobacter
ASV55.891		-6.114	1.447	-4.225	0.000	0.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Peptoniphilus
ASV55.332		-6.632	1.345	-4.929	0.000	0.000	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Peptoniphilus
ASV55.997		-6.177	1.708	-3.617	0.000	0.002	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
ASV56.039		-6.099	1.779	-3.428	0.001	0.003	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Peptoniphilus

ASV	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV55	5.422	-6.426	1.739	-3.695	0.000	0.002	Bacteria	Campylobacterota	Campylobacteria	Campylobacterales	Campylobacteraceae	Campylobacter
ASV55	5.359	-6.548	1.602	-4.088	0.000	0.001	Bacteria	Campylobacterota	Campylobacteria	Campylobacterales	Campylobacteraceae	Campylobacter
ASV56	6.796	-6.705	1.518	-4.416	0.000	0.000	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Peptoniphilus
ASV56	6.153	-5.335	1.792	-2.977	0.003	0.010	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV56	6.831	-6.801	1.795	-3.790	0.000	0.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus
ASV57	7.303	-6.260	1.929	-3.245	0.001	0.006	Bacteria	Campylobacterota	Campylobacteria	Campylobacterales	Campylobacteraceae	Campylobacter
ASV58	8.659	-6.749	1.831	-3.687	0.000	0.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Fenollaria
ASV58	8.174	-5.372	2.082	-2.581	0.010	0.027	Bacteria	Actinobacteriota	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Alloscardovia
ASV58	8.781	-6.373	2.021	-3.153	0.002	0.007	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV58	8.837	-6.718	1.817	-3.698	0.000	0.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Fenollaria
ASV59	9.713	-6.738	2.022	-3.332	0.001	0.004	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Fenollaria
ASV59	9.454	-6.003	1.984	-3.026	0.002	0.009	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus
ASV60	0.773	-6.334	1.497	-4.231	0.000	0.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Peptoniphilus
ASV60	0.265	-6.142	1.833	-3.350	0.001	0.004	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV61	1.423	-6.314	2.005	-3.150	0.002	0.007	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV61	1.874	-6.636	1.962	-3.382	0.001	0.004	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus
ASV61	1.806	-6.887	1.792	-3.843	0.000	0.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Fenollaria
ASV62	2.350	-6.543	1.499	-4.365	0.000	0.000	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Peptoniphilus
ASV62	2.583	-6.315	2.027	-3.116	0.002	0.008	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV63	3.387	-6.332	2.019	-3.136	0.002	0.007	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella

ASV	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV	63.766	-6.323	2.645	-2.391	0.017	0.040	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces
ASV	63.794	-6.717	1.947	-3.450	0.001	0.003	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Fenollaria
ASV	64.466	-6.007	1.646	-3.649	0.000	0.002	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
ASV	65.184	-6.585	1.900	-3.466	0.001	0.003	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus
ASV	65.730	-6.689	1.880	-3.558	0.000	0.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Fenollaria
ASV	66.646	-5.438	1.530	-3.554	0.000	0.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Peptoniphilus
ASV	66.900	-6.577	1.948	-3.376	0.001	0.004	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus
ASV	67.424	-6.721	1.908	-3.523	0.000	0.003	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Fenollaria
ASV	67.364	-5.939	2.197	-2.702	0.007	0.020	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces
ASV	67.709	-5.480	1.601	-3.422	0.001	0.003	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Peptoniphilus
ASV	68.746	-6.415	1.937	-3.312	0.001	0.005	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus
ASV	68.534	-6.197	1.494	-4.147	0.000	0.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Peptoniphilus
ASV	68.347	-6.315	2.281	-2.768	0.006	0.017	Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Howardella
ASV	68.792	-5.607	2.283	-2.457	0.014	0.034	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus
ASV	69.841	-6.274	1.929	-3.253	0.001	0.005	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV	69.959	-6.653	1.873	-3.552	0.000	0.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Fenollaria
ASV	69.363	-5.828	1.513	-3.852	0.000	0.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Peptoniphilus
ASV	70.350	-5.609	1.741	-3.221	0.001	0.006	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
ASV	70.036	-6.176	1.976	-3.125	0.002	0.008	Bacteria	Campylobacterota	Campylobacteria	Campylobacterales	Campylobacteraceae	Campylobacter
ASV	70.884	-6.665	1.766	-3.774	0.000	0.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Fenollaria

ASV	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV7	40.937	-6.680	1.848	-3.615	0.000	0.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Fenollaria
ASV7	31.962	-5.838	2.162	-2.700	0.007	0.020	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus
ASV7	61.448	-7.086	1.705	-4.156	0.000	0.001	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV7	51.620	-6.228	1.917	-3.249	0.001	0.006	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV7	22.423	-6.512	2.056	-3.168	0.002	0.007	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus
ASV7	62.854	-6.890	1.773	-3.885	0.000	0.001	Bacteria	Campylobacterota	Campylobacteria	Campylobacterales	Campylobacteraceae	Campylobacter
ASV7	43.591	-6.612	1.882	-3.513	0.000	0.003	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Fenollaria
ASV7	43.815	-6.597	1.880	-3.509	0.000	0.003	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Fenollaria
ASV7	33.710	-5.506	1.816	-3.032	0.002	0.009	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
ASV7	33.357	-5.022	1.893	-2.653	0.008	0.022	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV7	52.256	-5.046	2.189	-2.305	0.021	0.047	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus
ASV7	53.890	-6.033	1.613	-3.741	0.000	0.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Peptoniphilus
ASV7	45.071	-6.300	1.890	-3.334	0.001	0.004	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV7	55.839	-6.922	1.565	-4.422	0.000	0.000	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV7	66.894	-6.007	2.160	-2.781	0.005	0.017	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus
ASV7	46.959	-6.144	1.601	-3.837	0.000	0.001	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
ASV7	73.485	-6.092	2.223	-2.740	0.006	0.018	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus
ASV7	47.951	-5.833	1.502	-3.884	0.000	0.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Peptoniphilus
ASV7	83.529	-6.056	2.228	-2.718	0.007	0.019	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus
ASV7	84.209	-5.862	2.093	-2.800	0.005	0.016	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella

ASV	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV7	9.337	-6.118	2.053	-2.980	0.003	0.010	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus
ASV7	9.980	-6.300	2.066	-3.049	0.002	0.009	Bacteria	Campylobacterota	Campylobacteria	Campylobacterales	Campylobacteraceae	Campylobacter
ASV7	9.576	-6.110	1.662	-3.675	0.000	0.002	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
ASV8	0.802	-6.101	2.285	-2.670	0.008	0.022	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Ezakiella
ASV8	0.365	-6.153	1.794	-3.430	0.001	0.003	Bacteria	Campylobacterota	Campylobacteria	Campylobacterales	Campylobacteraceae	Campylobacter
ASV8	0.564	-5.775	2.401	-2.405	0.016	0.039	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus
ASV8	0.930	-6.075	2.044	-2.973	0.003	0.010	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus
ASV8	1.568	-5.638	1.684	-3.348	0.001	0.004	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Peptoniphilus
ASV8	2.017	-6.309	1.919	-3.288	0.001	0.005	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Fenollaria
ASV8	2.951	-5.944	2.276	-2.612	0.009	0.025	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Parvimonas
ASV8	2.620	-6.175	1.975	-3.127	0.002	0.008	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV8	2.473	-6.128	1.662	-3.686	0.000	0.002	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
ASV8	3.485	-5.719	1.742	-3.283	0.001	0.005	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Peptoniphilus
ASV8	3.400	-6.097	2.198	-2.775	0.006	0.017	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus
ASV8	4.592	-5.777	1.689	-3.420	0.001	0.003	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
ASV8	4.710	-5.344	1.589	-3.363	0.001	0.004	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Peptoniphilus
ASV8	4.406	-5.865	2.265	-2.589	0.010	0.026	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus
ASV8	5.988	-6.692	1.734	-3.860	0.000	0.001	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV8	4.585	-5.745	2.000	-2.873	0.004	0.014	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV8	8.710	-5.918	2.056	-2.879	0.004	0.013	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus

ASV	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV88	3.970	-5.789	2.171	-2.667	0.008	0.022	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus
ASV89	9.604	-5.980	2.177	-2.747	0.006	0.018	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Ezakiella
ASV90	0.242	-5.982	2.173	-2.753	0.006	0.018	Bacteria	Campylobacterota	Campylobacteria	Campylobacterales	Campylobacteraceae	Campylobacter
ASV90	0.653	-6.616	1.739	-3.804	0.000	0.001	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV93	1.221	-5.250	1.735	-3.026	0.002	0.009	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Peptoniphilus
ASV94	2.347	-6.074	1.746	-3.478	0.001	0.003	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
ASV92	4.492	-6.428	1.759	-3.655	0.000	0.002	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV93	3.552	-6.020	2.030	-2.966	0.003	0.011	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Ezakiella
ASV94	1.892	4.227	1.821	2.322	0.020	0.046	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV95	3.877	-5.817	2.069	-2.811	0.005	0.016	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus
ASV97	5.514	-5.353	2.298	-2.329	0.020	0.045	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Parvimonas
ASV98	3.847	-5.816	2.364	-2.461	0.014	0.034	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus
ASV103	2.439	-5.743	2.287	-2.511	0.012	0.031	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Ezakiella
ASV103	3.484	-5.855	2.057	-2.846	0.004	0.015	Bacteria	Campylobacterota	Campylobacteria	Campylobacterales	Campylobacteraceae	Campylobacter
ASV103	2.458	-5.942	2.368	-2.509	0.012	0.031	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Parvimonas
ASV103	3.881	-5.963	2.037	-2.927	0.003	0.012	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Ezakiella
ASV112	2.880	-5.882	2.097	-2.805	0.005	0.016	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus
ASV112	3.224	-5.388	2.306	-2.337	0.019	0.044	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Family XI	Anaerococcus

```r
# repeat analysis for postmenopausal group
res_postmenopausal = results(ds, contrast = c("grp", "01_postmenopausal", "03_trans"))
taxa_sig_postmenopausal = dplyr::filter(as.data.frame(res_postmenopausal), padj < 0.05) %>
```

```r
    rownames_to_column("ASV")

  diffabund_post = left_join(taxa_sig_postmenopausal, taxa_table, by = "ASV") %>%
    write_csv2("results/microbiome/deseq_diff_trans-postmenopausal.csv")

  kable(diffabund_post, digits = 3, row.names = F, caption = "Differential abundance between
```

Table 10: Differential abundance between postmenopausal women and trans patients (post vs trans)

ASV	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Kingdom	Phylum	Class	Order	Family	Genus	Species
ASV7	26.234	-4.408	1.350	-3.264	0.001	0.035	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	NA
ASV7	22.871	-5.000	1.395	-3.584	0.000	0.026	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	NA
ASV8	22.716	-4.845	1.446	-3.350	0.001	0.034	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	NA
ASV110	0.793	-5.282	1.432	-3.689	0.000	0.026	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	NA
ASV25	811	-6.944	1.776	-3.910	0.000	0.026	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister	NA
ASV30	450	-6.873	1.785	-3.851	0.000	0.026	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister	NA
ASV32	022	-5.740	1.754	-3.272	0.001	0.035	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister	NA
ASV37	138	-6.614	1.771	-3.734	0.000	0.026	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister	NA
ASV39	045	-6.536	1.858	-3.517	0.000	0.027	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister	NA
ASV43	012	-6.279	1.856	-3.383	0.001	0.034	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister	NA
ASV43	838	-6.417	1.739	-3.691	0.000	0.026	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister	NA
ASV45	659	-6.421	1.784	-3.599	0.000	0.026	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister	NA
ASV48	341	-6.084	1.843	-3.301	0.001	0.034	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister	NA
ASV48	854	-6.153	1.831	-3.361	0.001	0.034	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister	NA
ASV48	015	-6.246	1.754	-3.560	0.000	0.026	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister	NA

ASV	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Kingdom	Phylum	Class	Order	Family	Genus	Species
ASV50	291	-6.125	1.896	-3.231	0.001	0.037	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister	NA
ASV51	638	-6.040	1.828	-3.304	0.001	0.034	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister	NA
ASV56	753	-6.083	1.804	-3.373	0.001	0.034	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	NA
ASV75	639	-5.221	1.562	-3.342	0.001	0.034	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	NA

```r
asvs = unique(c(diffabund_pre$ASV, diffabund_post$ASV)) # get asvs identified by Deseq2

# next, melt the phyloseq object into a data.frame and filter OTUs for significant ASVs as
ps_sig_melt = psmelt(ps_filtered_rel) %>%
  filter(OTU %in% asvs)

# Summarize Abundance at the genus level for plotting, almost no species are assigned in t
ps_sig_melt2 = ps_sig_melt %>%
  group_by(Sample, Genus, grp) %>%
  summarize(Abundance = sum(Abundance))

# Plot the final figure panel
deseq_res_fig = ggplot(ps_sig_melt2, aes(x = grp, y=Abundance)) +
  geom_jitter(width = 0.3, alpha = 0.6) +
  geom_boxplot(aes(fill = grp), alpha = 0.5, outlier.shape = NA) +
  facet_wrap(~Genus, scales = "free", ncol = 3) +
  scale_y_sqrt() +
  theme(axis.text.x=element_blank(),
      axis.ticks.x=element_blank()) +
  labs(x = "", fill = "Group", y= "Abundance (%)") +
  theme(legend.position = "bottom") +
  scale_y_continuous(expand = expansion(mult = c(0, 0.2))) +
  scale_fill_manual(values = custom_pal)

deseq_res_fig
```

| | Actinomyces | Alloscardovia | Anaerococcus |
| | Campylobacter | Dialister | Ezakiella |
Abundance (%) | Fenollaria | Finegoldia | Howardella |
| | Lactobacillus | Parvimonas | Peptoniphilus |
| | Prevotella | Streptococcus | |

Group 🟩 01_postmenopausal 🟧 02_premenopausal 🟪 03_trans
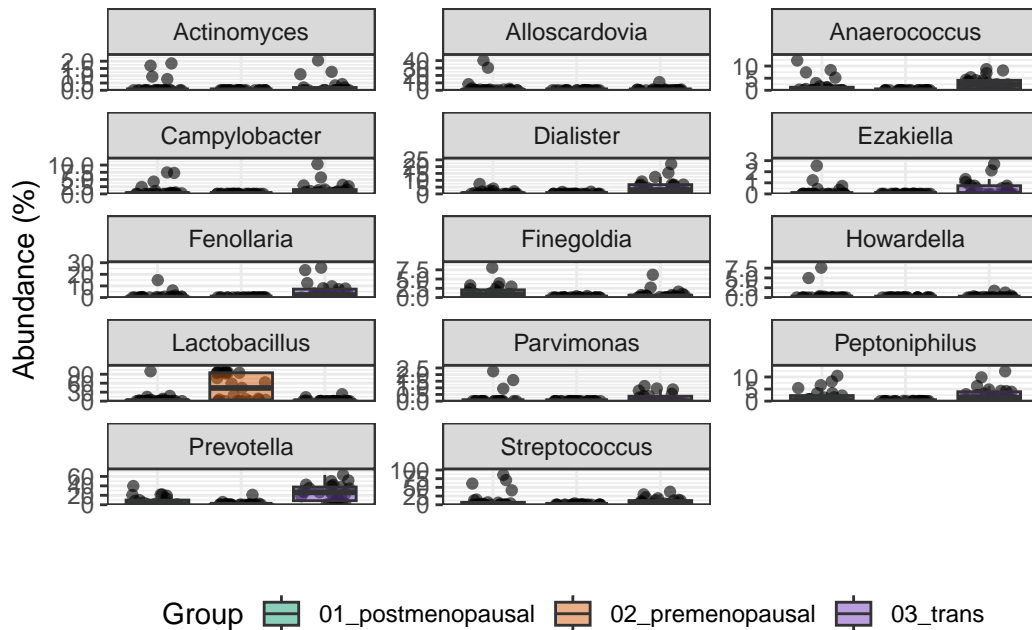
```r
ggsave(filename = "MbVagTrans_Results_files/figure-html/deseq_taxa_final.pdf", plot = dese
       device = "pdf", width = 8, height = 8)

# summary statistics
summarystat1 = ps_sig_melt2 %>%
  group_by(Genus, grp) %>%
  summarize(mean = mean(Abundance, na.rm=T),
            median = median(Abundance, na.rm =T),
            sd = sd(Abundance, na.rm = T),
            min = min(Abundance, na.rm =T),
            max = max(Abundance, na.rm =T))

write_csv2(x = summarystat1, "results/microbiome/deseq_summarystat_complete.csv")

summarystat2 = ps_sig_melt2 %>%
  group_by(Genus, grp) %>%
  summarize(mean = mean(Abundance, na.rm=T) %>% round(digits = 2),
            sd = sd(Abundance, na.rm = T) %>% round(digits = 2)) %>%
  mutate(stat = paste(mean, "±", sd)) %>%
  select(Genus, grp, stat) %>%
  pivot_wider(id_cols = "Genus", names_from = grp, values_from = stat)
```

```r
write_csv2(summarystat2, "results/microbiome/deseq_summarystat_complete.csv")

kable(summarystat1)
```

Genus	grp	mean	median	sd	min	max
Actinomyces	01_postmenopausal	0.2100274	0.0000000	0.5266452	0.000000	1.8481086
Actinomyces	02_premenopausal	0.0000000	0.0000000	0.0000000	0.000000	0.0000000
Actinomyces	03_trans	0.2229707	0.0000000	0.5065297	0.000000	2.0506634
Alloscardovia	01_postmenopausal	3.5007549	0.0000000	9.8283602	0.000000	40.2019002
Alloscardovia	02_premenopausal	0.0000000	0.0000000	0.0000000	0.000000	0.0000000
Alloscardovia	03_trans	0.6295872	0.0000000	2.2086704	0.000000	11.0647182
Anaerococcus	01_postmenopausal	1.6874306	0.2502002	3.2065695	0.000000	12.3004140
Anaerococcus	02_premenopausal	0.0000000	0.0000000	0.0000000	0.000000	0.0000000
Anaerococcus	03_trans	2.7214480	2.4524715	2.5349074	0.000000	8.7163233
Campylobacter	01_postmenopausal	0.9645998	0.0000000	2.1495525	0.000000	7.4512123
Campylobacter	02_premenopausal	0.0000000	0.0000000	0.0000000	0.000000	0.0000000
Campylobacter	03_trans	1.4005780	0.5219207	2.2975664	0.000000	10.3474131
Dialister	01_postmenopausal	0.8158066	0.0000000	1.7006613	0.000000	7.3039742
Dialister	02_premenopausal	0.1356403	0.0000000	0.4200930	0.000000	1.6030737
Dialister	03_trans	4.9814930	2.8316665	5.2787486	0.000000	21.8879224
Ezakiella	01_postmenopausal	0.2122119	0.0000000	0.5666188	0.000000	2.5409836
Ezakiella	02_premenopausal	0.0000000	0.0000000	0.0000000	0.000000	0.0000000
Ezakiella	03_trans	0.4530339	0.0000000	0.7102635	0.000000	2.6899612
Fenollaria	01_postmenopausal	1.0540922	0.0000000	3.1589236	0.000000	14.9726776
Fenollaria	02_premenopausal	0.0000000	0.0000000	0.0000000	0.000000	0.0000000
Fenollaria	03_trans	4.7089272	2.1564210	6.9444378	0.000000	25.7131537
Finegoldia	01_postmenopausal	1.3231500	0.8786381	1.8566051	0.000000	8.0745342
Finegoldia	02_premenopausal	0.0300835	0.0000000	0.1045203	0.000000	0.4076266
Finegoldia	03_trans	0.6289195	0.2773376	1.3062089	0.000000	6.1532654
Howardella	01_postmenopausal	0.5273837	0.0000000	1.7774515	0.000000	7.6086957
Howardella	02_premenopausal	0.0000000	0.0000000	0.0000000	0.000000	0.0000000
Howardella	03_trans	0.1568124	0.0000000	0.4222741	0.000000	1.7256011
Lactobacillus	01_postmenopausal	6.2509499	0.3005464	20.0031717	0.000000	99.8372992
Lactobacillus	02_premenopausal	46.3527996	44.0280598	43.5730941	0.000000	99.4823570
Lactobacillus	03_trans	1.7154789	0.0000000	5.1853138	0.000000	23.2060113
Parvimonas	01_postmenopausal	0.1907625	0.0000000	0.5601033	0.000000	2.2471910
Parvimonas	02_premenopausal	0.0000000	0.0000000	0.0000000	0.000000	0.0000000
Parvimonas	03_trans	0.2127434	0.0000000	0.3603008	0.000000	1.1369939
Peptoniphilus	01_postmenopausal	1.8298909	0.3984946	2.8734076	0.000000	10.4592997
Peptoniphilus	02_premenopausal	0.0137179	0.0000000	0.0304223	0.000000	0.1051940
Peptoniphilus	03_trans	2.6848862	1.3996501	3.0425141	0.000000	12.3415046

Genus	grp	mean	median	sd	min	max
Prevotella	01_postmenopausal	6.7637229	1.3728720	10.2919027	0.000000	39.6079484
Prevotella	02_premenopausal	1.3104803	0.0634317	4.2906424	0.000000	21.0881295
Prevotella	03_trans	25.1653767	26.2678288	17.5531345	0.329713	63.2318501
Streptococcus	01_postmenopausal	12.3690232	0.4296455	24.5652088	0.000000	85.8695652
Streptococcus	02_premenopausal	0.2141191	0.0000000	0.7868471	0.000000	3.9297356
Streptococcus	03_trans	7.4102552	2.4581275	10.2297815	0.000000	37.4280546

```
kable(summarystat2)
```

Genus	01_postmenopausal	02_premenopausal	03_trans
Actinomyces	0.21 ± 0.53	0 ± 0	0.22 ± 0.51
Alloscardovia	3.5 ± 9.83	0 ± 0	0.63 ± 2.21
Anaerococcus	1.69 ± 3.21	0 ± 0	2.72 ± 2.53
Campylobacter	0.96 ± 2.15	0 ± 0	1.4 ± 2.3
Dialister	0.82 ± 1.7	0.14 ± 0.42	4.98 ± 5.28
Ezakiella	0.21 ± 0.57	0 ± 0	0.45 ± 0.71
Fenollaria	1.05 ± 3.16	0 ± 0	4.71 ± 6.94
Finegoldia	1.32 ± 1.86	0.03 ± 0.1	0.63 ± 1.31
Howardella	0.53 ± 1.78	0 ± 0	0.16 ± 0.42
Lactobacillus	6.25 ± 20	46.35 ± 43.57	1.72 ± 5.19
Parvimonas	0.19 ± 0.56	0 ± 0	0.21 ± 0.36
Peptoniphilus	1.83 ± 2.87	0.01 ± 0.03	2.68 ± 3.04
Prevotella	6.76 ± 10.29	1.31 ± 4.29	25.17 ± 17.55
Streptococcus	12.37 ± 24.57	0.21 ± 0.79	7.41 ± 10.23

This analysis reveals, that the *Trans* group is characterized by a loss of *Lactobacillus* and an increase in bacteria more commonly associated with the intestinal flora (e.g. *Campylobacter, Anaerococcus, Dialister, Prevotella)* compared to the premenopausal group.

Comparing the *Trans* to the *postmenopausal* group only revealed an even larger reduction in *Lactobacillus.*

Thus, overall the *trans* group is strinkingly similar in its taxonomic composition compared to postmenopausal women.

```
deseq_res_fig
```
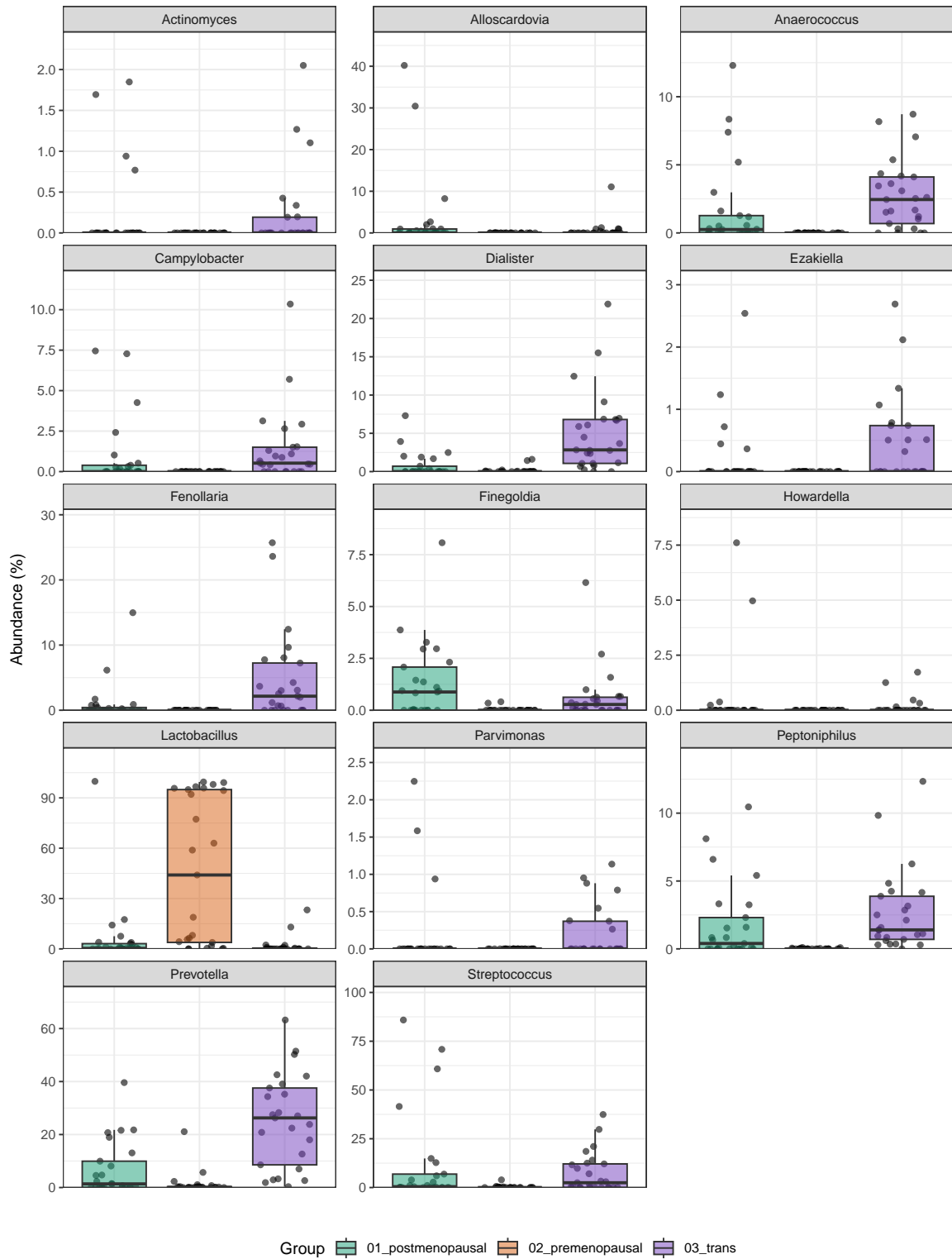
```
ggsave(deseq_res_fig, width = 7, height = 10, filename = "results/microbiome/deseq_asv_fig
ggsave(deseq_res_fig, width = 7, height = 8, filename = "results/microbiome/deseq_asv_figu
```

5 Taxonomic composition

The following plots illustrate taxonomic composition at all levels of the taxonomy, The taxon
table is filtered to only differentiate the most abundant taxa at each level (depending on the
level).

```
ps.ns = prune_taxa(taxa_sums(ps_samples) >1, ps_samples) # remove singleton ASVs
ps_rel = transform_sample_counts(ps.ns, function(x) x/sum(x) *100) # transform to rel coun

# Generate agglomerated phyloseq objects
genus_rel = tax_glom(ps_rel, taxrank="Genus", NArm = F)
genus_rel # 383 genera
```

```
phyloseq-class experiment-level object
otu_table()   OTU Table:          [ 383 taxa and 75 samples ]
sample_data() Sample Data:        [ 75 samples by 15 sample variables ]
tax_table()   Taxonomy Table:     [ 383 taxa by 7 taxonomic ranks ]
phy_tree()    Phylogenetic Tree: [ 383 tips and 382 internal nodes ]
refseq()      DNAStringSet:       [ 383 reference sequences ]
```

```
genus = psmelt(genus_rel) %>%
  mutate(Taxonomy = paste0("p_", Phylum, ";c_", Class, ";o_", Order, ";f_", Family, ";g_",
  select(Sample, Taxonomy, grp, Age:Estradiole, Abundance)
genus$Taxonomy[genus$Abundance < 10] <- "< 10 % abund."

genusplot = ggplot(genus, aes(x = grp, y = Abundance/25, fill = Taxonomy)) +
  geom_col(position = "stack") +
  theme(legend.position = "right") +
  labs(y = "Abundance (%)", x= "", caption = "taxonomic level: genus") +
  rotate_x_text()

# Family Level
family_rel = tax_glom(ps_rel, taxrank="Family", NArm = F)
family_rel # 383 genera
```

30

```
phyloseq-class experiment-level object
otu_table()   OTU Table:         [ 151 taxa and 75 samples ]
sample_data() Sample Data:       [ 75 samples by 15 sample variables ]
tax_table()   Taxonomy Table:    [ 151 taxa by 7 taxonomic ranks ]
phy_tree()    Phylogenetic Tree: [ 151 tips and 150 internal nodes ]
refseq()      DNAStringSet:      [ 151 reference sequences ]
```

```r
  family = psmelt(family_rel) %>%
    mutate(Taxonomy = paste0("p_", Phylum, ";c_", Class, ";o_", Order, ";f_", Family)) %>%
    select(Sample, Taxonomy, grp, Age:Estradiole, Abundance)
  family$Taxonomy[family$Abundance < 15] <- "< 15 % abund."

  familyplot = ggplot(family, aes(x = grp, y = Abundance/25, fill = Taxonomy)) +
    geom_col(position = "stack") +
    theme(legend.position = "right") +
    labs(y = "Abundance (%)", x= "", caption = "taxonomic level: family") +
    rotate_x_text()

  # Order Level
  order_rel = tax_glom(ps_rel, taxrank="Order", NArm = F)
  order_rel # 75 taxa
```

```
phyloseq-class experiment-level object
otu_table()   OTU Table:         [ 76 taxa and 75 samples ]
sample_data() Sample Data:       [ 75 samples by 15 sample variables ]
tax_table()   Taxonomy Table:    [ 76 taxa by 7 taxonomic ranks ]
phy_tree()    Phylogenetic Tree: [ 76 tips and 75 internal nodes ]
refseq()      DNAStringSet:      [ 76 reference sequences ]
```

```r
  order = psmelt(order_rel) %>%
    mutate(Taxonomy = paste0("p_", Phylum, ";c_", Class, ";o_", Order)) %>%
    select(Sample, Taxonomy, grp, Age:Estradiole, Abundance)
  order$Taxonomy[order$Abundance < 20] <- "< 20 % abund."

  orderplot = ggplot(order, aes(x = grp, y = Abundance/25, fill = Taxonomy)) +
    geom_col(position = "stack") +
    theme(legend.position = "right") +
    labs(y = "Abundance (%)", x= "", caption = "taxonomic level: order") +
    rotate_x_text()

  # Class Level
```

```
  class_rel = tax_glom(ps_rel, taxrank="Class", NArm = F)
  class_rel # 30 taxa
```

```
phyloseq-class experiment-level object
otu_table()   OTU Table:          [ 30 taxa and 75 samples ]
sample_data() Sample Data:        [ 75 samples by 15 sample variables ]
tax_table()   Taxonomy Table:     [ 30 taxa by 7 taxonomic ranks ]
phy_tree()    Phylogenetic Tree: [ 30 tips and 29 internal nodes ]
refseq()      DNAStringSet:       [ 30 reference sequences ]
```

```
  class = psmelt(class_rel) %>%
    mutate(Taxonomy = paste0("p_", Phylum, ";c_", Class)) %>%
    select(Sample, Taxonomy, grp, Age:Estradiole, Abundance)
  class$Taxonomy[class$Abundance < 25] <- "< 25 % abund."

  classplot = ggplot(class, aes(x = grp, y = Abundance/25, fill = Taxonomy)) +
    geom_col(position = "stack") +
    theme(legend.position = "right") +
    labs(y = "Abundance (%)", x= "", caption = "taxonomic level: class") +
    rotate_x_text()

  # Phylum Level
  phylum_rel = tax_glom(ps_rel, taxrank="Phylum", NArm = F)
  phylum_rel # 18 taxa
```

```
phyloseq-class experiment-level object
otu_table()   OTU Table:          [ 18 taxa and 75 samples ]
sample_data() Sample Data:        [ 75 samples by 15 sample variables ]
tax_table()   Taxonomy Table:     [ 18 taxa by 7 taxonomic ranks ]
phy_tree()    Phylogenetic Tree: [ 18 tips and 17 internal nodes ]
refseq()      DNAStringSet:       [ 18 reference sequences ]
```

```
  phylum = psmelt(phylum_rel) %>%
    mutate(Taxonomy = paste0("p_", Phylum)) %>%
    select(Sample, Taxonomy, grp, Age:Estradiole, Abundance)
  phylum$Taxonomy[phylum$Abundance < 25] <- "< 25 % abund."

  phylumplot = ggplot(phylum, aes(x = grp, y = Abundance/25, fill = Taxonomy)) +
    geom_col(position = "stack") +
```

```
theme(legend.position = "right") +
labs(y = "Abundance (%)", x= "", caption = "taxonomic level: phylum") +
rotate_x_text()
```

5.1 Phylum level

On the phylum level, the taxonomic distribution is dominated by *Firmicutes* (these correspond to *Lactobacilli*, see below). The *Trans* group also shows an increase in *Bacteroidota* compared to the other 2 groups.
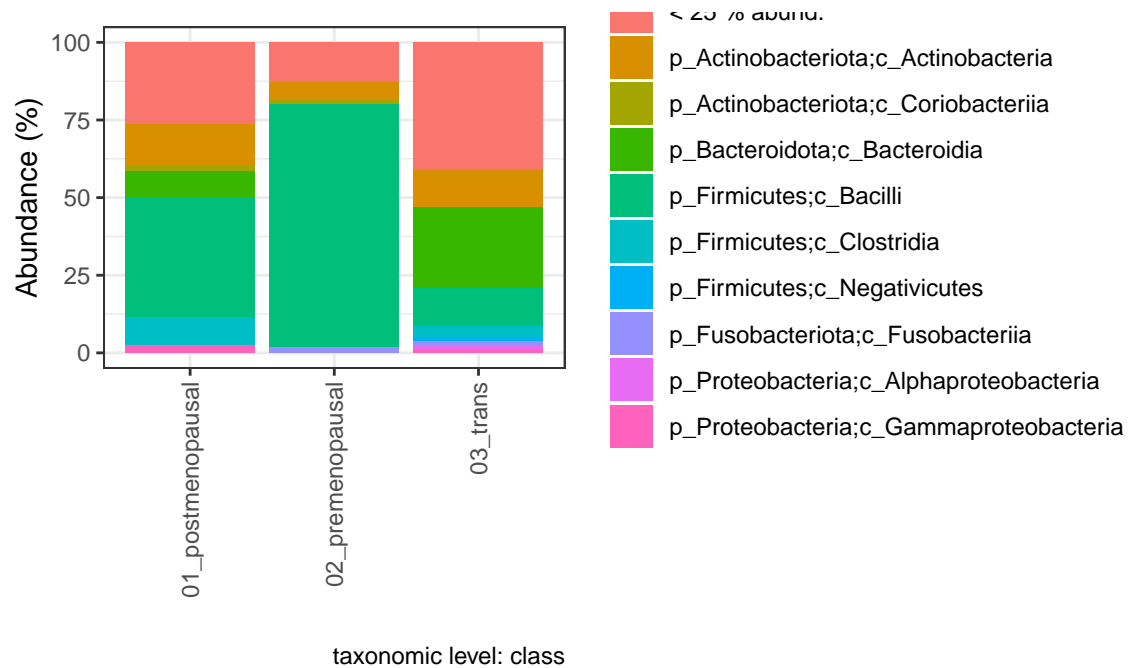
```
phylumplot = phylumplot + scale_x_discrete(limits = rev(levels(phylum$grp))) + coord_flip(
phylumplot
```


taxonomic level: phylum
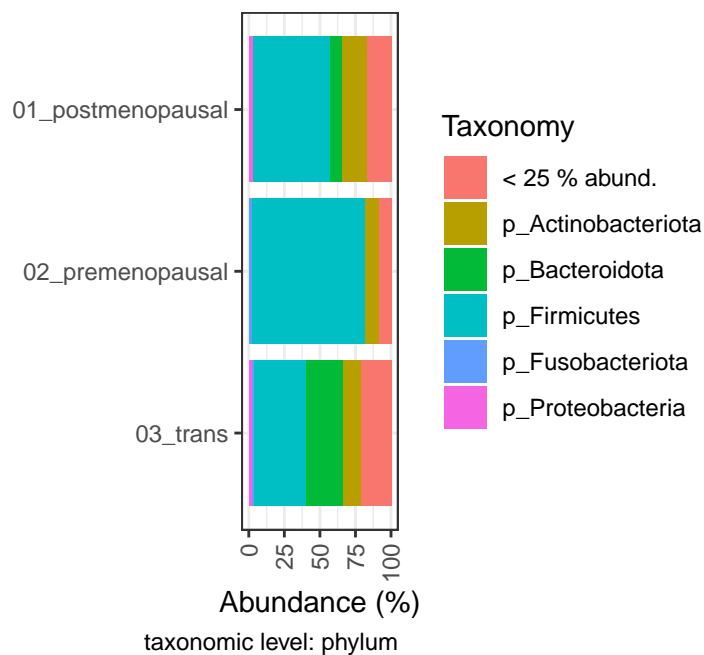
```
ggsave(plot = phylumplot, filename = "results/microbiome/taxplots_phylum.pdf", width = 7,
```

5.2 Class level

```
classplot
```


taxonomic level: class

```
ggsave(plot = classplot, filename = "results/microbiome/taxplots_class.pdf", width = 6, he
ggsave(plot = classplot, filename = "results/microbiome/taxplots_class.png", width = 6, he
```

5.3 Order level

```
orderplot
```

p_Actinobacteriota;c_Actinobacteria;o_Corynebacteriales
p_Actinobacteriota;c_Coriobacteriia;o_Coriobacteriales
p_Bacteroidota;c_Bacteroidia;o_Bacteroidales
p_Bacteroidota;c_Bacteroidia;o_Flavobacteriales
p_Firmicutes;c_Bacilli;o_Lactobacillales
p_Firmicutes;c_Bacilli;o_Staphylococcales
p_Firmicutes;c_Clostridia;o_Peptostreptococcales–Tissierellales
p_Firmicutes;c_Negativicutes;o_Veillonellales–Selenomonadales
p_Fusobacteriota;c_Fusobacteriia;o_Fusobacteriales
p_Proteobacteria;c_Alphaproteobacteria;o_Rhizobiales
p_Proteobacteria;c_Gammaproteobacteria;o_Burkholderiales
p_Proteobacteria;c_Gammaproteobacteria;o_Enterobacterales

taxonomic level: order

```r
ggsave(plot = orderplot, filename = "results/microbiome/taxplots_order.pdf", width = 7, he
ggsave(plot = orderplot, filename = "results/microbiome/taxplots_order.png", width = 7, he
```

5.4 Family level

```r
familyplot
```


p_Bacteroidota;c_Bacteroidia;o_Bacteroidales;f_Porphyromonadaceae
p_Bacteroidota;c_Bacteroidia;o_Bacteroidales;f_Prevotellaceae
p_Bacteroidota;c_Bacteroidia;o_Flavobacteriales;f_Flavobacteriaceae
p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae
p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Streptococcaceae
p_Firmicutes;c_Bacilli;o_Staphylococcales;f_Staphylococcaceae
p_Firmicutes;c_Clostridia;o_Peptostreptococcales–Tissierellales;f_Family XI
p_Firmicutes;c_Negativicutes;o_Veillonellales–Selenomonadales;f_Veillonellaceae
p_Fusobacteriota;c_Fusobacteriia;o_Fusobacteriales;f_Fusobacteriaceae
p_Fusobacteriota;c_Fusobacteriia;o_Fusobacteriales;f_Leptotrichiaceae
p_Proteobacteria;c_Alphaproteobacteria;o_Rhizobiales;f_Beijerinckiaceae
p_Proteobacteria;c_Gammaproteobacteria;o_Burkholderiales;f_Neisseriaceae
p_Proteobacteria;c_Gammaproteobacteria;o_Enterobacterales;f_Enterobacteriaceae

taxonomic level: family

```
ggsave(plot = familyplot, filename = "results/microbiome/taxplots_family.pdf", width = 8,
ggsave(plot = familyplot, filename = "results/microbiome/taxplots_family.png", width = 8,
```

5.5 Genus level

```
genusplot
```


taxonomic level: genus

```
ggsave(plot = genusplot, filename = "results/microbiome/taxplots_genus.pdf", width = 15, h
ggsave(plot = genusplot, filename = "results/microbiome/taxplots_family.png", width = 15,
```

6 Secondary analyses

6.0.1 Duration of menopause

This analysis is only performed in the postmenopausal group

```
head(sample_data(ps_samples))
```

```
           CID          grp Age GHAT_Length GNRHA Virgo SexuallyActive
22010-0001 101 01_postmenopausal  64           0  <NA>    no            yes
22010-0002 102 01_postmenopausal  49           0  <NA>    no            yes
22010-0003 103 01_postmenopausal  61           0  <NA>    no            yes
22010-0004 104 01_postmenopausal  70           0  <NA>    no            yes
22010-0005 105 01_postmenopausal  66           0  <NA>    no            yes
22010-0006 106 01_postmenopausal  71           0  <NA>    no             no
           SexOfPartner NugentScore Testosterone Estradiole DurationMenopause
22010-0001            M        7-10           NA         NA                11
22010-0002            M         0-3           NA         NA                 2
```

```
22010-0003              M         7-10        NA         NA               10
22010-0004              M         4-6         NA         NA               22
22010-0005         M no bacteria             NA         NA               12
22010-0006              M         0-3         NA         NA               22
         DurationAmenorrhea CycleDaySampling Duration_GNRH
22010-0001                NA               NA           NA
22010-0002                NA               NA           NA
22010-0003                NA               NA           NA
22010-0004                NA               NA           NA
22010-0005                NA               NA           NA
22010-0006                NA               NA           NA
```

```r
# for menopause duration, use maaslin2
library(Maaslin2)

# only 01_postmenopausal samples
ps_menopause = subset_samples(ps_samples, grp =="01_postmenopausal") %>%
  transform_sample_counts(function(x) x/sum(x) * 100) # relative abundances are necessary

asv_durationMenopause = Maaslin2(input_data = otu_table(ps_menopause),
        input_metadata = as(sample_data(ps_menopause), "data.frame"),
        output = "results/maaslin2/asv_menopause-duration",
        min_abundance = 2.5,
        min_prevalence = 0.1,
        max_significance = 0.2,
        fixed_effects = "DurationMenopause")
```

```
2024-01-19 19:51:27.63807 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/asv_menopause-duration/maaslin2.lo
2024-01-19 19:51:33.989674 INFO::Writing function arguments to log file
2024-01-19 19:51:33.996557 INFO::Verifying options selected are valid
2024-01-19 19:51:34.022036 INFO::Determining format of input files
2024-01-19 19:51:34.022545 INFO::Input format is data samples as rows and metadata samples as
2024-01-19 19:51:34.063714 INFO::Formula for fixed effects: expr ~  DurationMenopause
2024-01-19 19:51:34.064265 INFO::Filter data based on min abundance and min prevalence
2024-01-19 19:51:34.064559 INFO::Total samples in data: 25
2024-01-19 19:51:34.064834 INFO::Min samples required with min abundance for a feature not to
2024-01-19 19:51:34.109829 INFO::Total filtered features: 8831
2024-01-19 19:51:34.112505 INFO::Filtered feature names from abundance and prevalence filteri
2024-01-19 19:51:34.115022 INFO::Total filtered features with variance filtering: 0
2024-01-19 19:51:34.115336 INFO::Filtered feature names from variance filtering:
2024-01-19 19:51:34.115617 INFO::Running selected normalization method: TSS
```

```
2024-01-19 19:51:34.116684 INFO::Applying z-score to standardize continuous metadata
2024-01-19 19:51:34.12034 INFO::Running selected transform method: LOG
2024-01-19 19:51:34.121061 INFO::Running selected analysis method: LM
2024-01-19 19:51:34.12528 INFO::Fitting model to feature number 1, ASV14
2024-01-19 19:51:34.127564 INFO::Fitting model to feature number 2, ASV16
2024-01-19 19:51:34.130743 INFO::Counting total values for each feature
2024-01-19 19:51:34.131523 INFO::Writing filtered data to file results/maaslin2/asv_menopause
2024-01-19 19:51:34.132653 INFO::Writing filtered, normalized data to file results/maaslin2/a
2024-01-19 19:51:34.133276 INFO::Writing filtered, normalized, transformed data to file resul
2024-01-19 19:51:34.133888 WARNING::Deleting existing residuals file: results/maaslin2/asv_me
2024-01-19 19:51:34.134389 INFO::Writing residuals to file results/maaslin2/asv_menopause-dur
2024-01-19 19:51:34.135694 WARNING::Deleting existing fitted file: results/maaslin2/asv_menop
2024-01-19 19:51:34.136142 INFO::Writing fitted values to file results/maaslin2/asv_menopause
2024-01-19 19:51:34.136784 INFO::Writing all results to file (ordered by increasing q-values)
2024-01-19 19:51:34.137471 INFO::Writing the significant results (those which are less than
2024-01-19 19:51:34.137927 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2024-01-19 19:51:34.138992 INFO::Writing association plots (one for each significant associat
[1] "There are no associations to plot!"
```

```r
# no significant effect on ASV level, try glom at genuslevel
ps_menopause_genus = tax_glom(ps_menopause, taxrank = "Genus")

genus_durationMenopause = Maaslin2(input_data = otu_table(ps_menopause_genus),
        input_metadata = as(sample_data(ps_menopause_genus), "data.frame"),
        output = "results/maaslin2/genus_menopause-duration",
        min_abundance = 5,
        min_prevalence = 0.2,
        max_significance = 0.2,
        fixed_effects = "DurationMenopause")
```

```
2024-01-19 19:51:42.017116 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_menopause-duration/maaslin2
2024-01-19 19:51:42.244783 INFO::Writing function arguments to log file
2024-01-19 19:51:42.248205 INFO::Verifying options selected are valid
2024-01-19 19:51:42.24854 INFO::Determining format of input files
2024-01-19 19:51:42.248826 INFO::Input format is data samples as rows and metadata samples as
2024-01-19 19:51:42.250987 INFO::Formula for fixed effects: expr ~  DurationMenopause
2024-01-19 19:51:42.251331 INFO::Filter data based on min abundance and min prevalence
2024-01-19 19:51:42.251606 INFO::Total samples in data: 25
2024-01-19 19:51:42.251865 INFO::Min samples required with min abundance for a feature not to
```

```
2024-01-19 19:51:42.253633 INFO::Total filtered features: 294
2024-01-19 19:51:42.253973 INFO::Filtered feature names from abundance and prevalence filter:
2024-01-19 19:51:42.254484 INFO::Total filtered features with variance filtering: 0
2024-01-19 19:51:42.254774 INFO::Filtered feature names from variance filtering:
2024-01-19 19:51:42.255033 INFO::Running selected normalization method: TSS
2024-01-19 19:51:42.255478 INFO::Applying z-score to standardize continuous metadata
2024-01-19 19:51:42.258914 INFO::Running selected transform method: LOG
2024-01-19 19:51:42.259469 INFO::Running selected analysis method: LM
2024-01-19 19:51:42.259838 INFO::Fitting model to feature number 1, ASV16
2024-01-19 19:51:42.261216 INFO::Fitting model to feature number 2, ASV82
2024-01-19 19:51:42.262522 INFO::Fitting model to feature number 3, ASV271
2024-01-19 19:51:42.263927 INFO::Fitting model to feature number 4, ASV526
2024-01-19 19:51:42.267637 INFO::Counting total values for each feature
2024-01-19 19:51:42.268598 INFO::Writing filtered data to file results/maaslin2/genus_menopau
2024-01-19 19:51:42.269536 INFO::Writing filtered, normalized data to file results/maaslin2/g
2024-01-19 19:51:42.270204 INFO::Writing filtered, normalized, transformed data to file resul
2024-01-19 19:51:42.2709 WARNING::Deleting existing residuals file: results/maaslin2/genus_me
2024-01-19 19:51:42.271421 INFO::Writing residuals to file results/maaslin2/genus_menopause-o
2024-01-19 19:51:42.271962 WARNING::Deleting existing fitted file: results/maaslin2/genus_men
2024-01-19 19:51:42.272382 INFO::Writing fitted values to file results/maaslin2/genus_menopau
2024-01-19 19:51:42.272856 INFO::Writing all results to file (ordered by increasing q-values)
2024-01-19 19:51:42.273563 INFO::Writing the significant results (those which are less than 
2024-01-19 19:51:42.273987 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2024-01-19 19:51:42.274551 INFO::Writing association plots (one for each significant associat
[1] "There are no associations to plot!"
```

```r
# no significant effect on genus level, try on family level
ps_menopause_family = tax_glom(ps_menopause, taxrank = "Family")

family_durationMenopause = Maaslin2(input_data = otu_table(ps_menopause_family),
        input_metadata = as(sample_data(ps_menopause_family), "data.frame"),
        output = "results/maaslin2/family_menopause-duration",
        min_abundance = 10,
        min_prevalence = 0.3,
        max_significance = 0.2,
        fixed_effects = "DurationMenopause")
```

```
2024-01-19 19:51:45.759722 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/family_menopause-duration/maaslin2
2024-01-19 19:51:45.84358 INFO::Writing function arguments to log file
```

```
2024-01-19 19:51:45.84669 INFO::Verifying options selected are valid
2024-01-19 19:51:45.847004 INFO::Determining format of input files
2024-01-19 19:51:45.84729 INFO::Input format is data samples as rows and metadata samples as
2024-01-19 19:51:45.848639 INFO::Formula for fixed effects: expr ~  DurationMenopause
2024-01-19 19:51:45.848966 INFO::Filter data based on min abundance and min prevalence
2024-01-19 19:51:45.849233 INFO::Total samples in data: 25
2024-01-19 19:51:45.84949 INFO::Min samples required with min abundance for a feature not to
2024-01-19 19:51:45.850382 INFO::Total filtered features: 111
2024-01-19 19:51:45.850706 INFO::Filtered feature names from abundance and prevalence filter:
2024-01-19 19:51:45.851151 INFO::Total filtered features with variance filtering: 0
2024-01-19 19:51:45.851438 INFO::Filtered feature names from variance filtering:
2024-01-19 19:51:45.851698 INFO::Running selected normalization method: TSS
2024-01-19 19:51:45.852159 INFO::Applying z-score to standardize continuous metadata
2024-01-19 19:51:45.85533 INFO::Running selected transform method: LOG
2024-01-19 19:51:45.856133 INFO::Running selected analysis method: LM
2024-01-19 19:51:45.856565 INFO::Fitting model to feature number 1, ASV16
2024-01-19 19:51:45.857983 INFO::Fitting model to feature number 2, ASV82
2024-01-19 19:51:45.859119 INFO::Fitting model to feature number 3, ASV120
2024-01-19 19:51:45.860218 INFO::Fitting model to feature number 4, ASV271
2024-01-19 19:51:45.86356 INFO::Counting total values for each feature
2024-01-19 19:51:45.864419 INFO::Writing filtered data to file results/maaslin2/family_menopa
2024-01-19 19:51:45.865257 INFO::Writing filtered, normalized data to file results/maaslin2/
2024-01-19 19:51:45.865961 INFO::Writing filtered, normalized, transformed data to file resu
2024-01-19 19:51:45.866658 WARNING::Deleting existing residuals file: results/maaslin2/family
2024-01-19 19:51:45.867207 INFO::Writing residuals to file results/maaslin2/family_menopause-
2024-01-19 19:51:45.867773 WARNING::Deleting existing fitted file: results/maaslin2/family_me
2024-01-19 19:51:45.868309 INFO::Writing fitted values to file results/maaslin2/family_menopa
2024-01-19 19:51:45.868804 INFO::Writing all results to file (ordered by increasing q-values)
2024-01-19 19:51:45.869802 INFO::Writing the significant results (those which are less than
2024-01-19 19:51:45.870412 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2024-01-19 19:51:45.871011 INFO::Writing association plots (one for each significant associat
[1] "There are no associations to plot!"
```

```r
## Alpha diversity by duration of menopause
ps_menopause_abs = subset_samples(ps_samples, grp =="01_postmenopausal")

adiv_menopause = plot_richness(ps_menopause_abs, measures = c("Observed", "Shannon", "InvS

adiv_menopause$data %>%
  select(DurationMenopause, variable, value) %>%
```

```
group_by(variable) %>%
cor_test(value, DurationMenopause) %>%
kable(digits = 3)
```

variable	var1	var2	cor	statistic	p	conf.low	conf.high	method
Observed	value	DurationMenopause	0.045	0.216	0.831	-0.357	0.432	Pearson
Shannon	value	DurationMenopause	0.078	0.375	0.711	-0.327	0.459	Pearson
InvSimpson	value	DurationMenopause	0.130	0.617	0.543	-0.282	0.498	Pearson

```
adiv_menopause$data %>%
  ggplot(aes(x = DurationMenopause, y = value)) +
  geom_point()+
  geom_smooth(method ="lm") +
  facet_wrap(~variable, scales = "free_y")
```
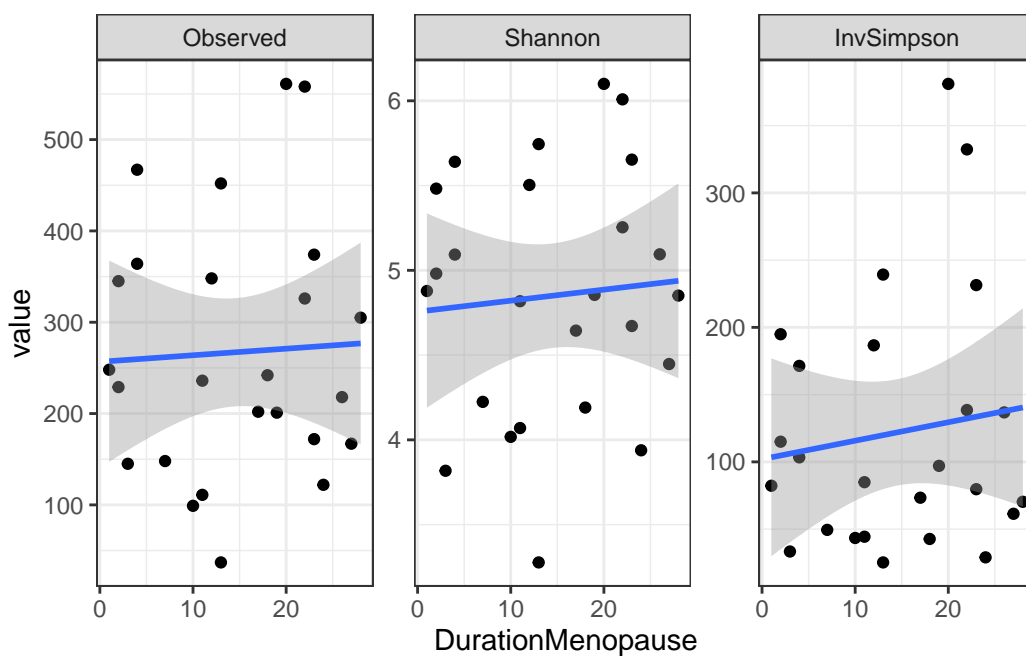


```
BC_menopause_genus = vegan::vegdist(otu_table(ps_menopause_genus))

menopauseduration_adonis_bc = vegan::adonis2(BC_menopause_genus ~ phyloseq::sample_data(ps
```

```
# no significant effect on the beta diversity level as well

# Summary statistics of duration menopause
sample_data(ps_menopause_genus)$DurationMenopause %>%
  summary()
```

```
  Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
  1.00    7.00   13.00   14.48   22.00   28.00
```

There is no significant correlation of duration of menopause (years) with alpha diversity of vaginal microbiomes and overall community composition (beta-diversity). Duration of menopause in this sample is rather varied ranging from 1 to 28 years (mean 14.5 years).

6.0.2 Duration of gender-affirming hormone therapy (GAHT)

This analysis is only performed in the TRANS group.

```
# only 03_trans samples
ps_trans = subset_samples(ps_samples, grp =="03_trans") %>%
  transform_sample_counts(function(x) x/sum(x) * 100) # relative abundances are necessary

asv_GHAT = Maaslin2(input_data = otu_table(ps_trans),
         input_metadata = as(sample_data(ps_trans), "data.frame"),
         output = "results/maaslin2/asv_GHAT_Length",
         min_abundance = 1,
         min_prevalence = 0.1,
         max_significance = 0.2,
         fixed_effects = "GHAT_Length")
```

```
2024-01-19 19:51:46.302029 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/asv_GHAT_Length/maaslin2.log"
2024-01-19 19:51:52.545116 INFO::Writing function arguments to log file
2024-01-19 19:51:52.547719 INFO::Verifying options selected are valid
2024-01-19 19:51:52.548028 INFO::Determining format of input files
2024-01-19 19:51:52.548313 INFO::Input format is data samples as rows and metadata samples as
2024-01-19 19:51:52.595862 INFO::Formula for fixed effects: expr ~  GHAT_Length
2024-01-19 19:51:52.596384 INFO::Filter data based on min abundance and min prevalence
2024-01-19 19:51:52.59667 INFO::Total samples in data: 25
2024-01-19 19:51:52.596933 INFO::Min samples required with min abundance for a feature not to
2024-01-19 19:51:52.637645 INFO::Total filtered features: 8824
```

```
2024-01-19 19:51:52.639332 INFO::Filtered feature names from abundance and prevalence filter:
2024-01-19 19:51:52.64182 INFO::Total filtered features with variance filtering: 0
2024-01-19 19:51:52.64212 INFO::Filtered feature names from variance filtering:
2024-01-19 19:51:52.64238 INFO::Running selected normalization method: TSS
2024-01-19 19:51:52.642843 INFO::Applying z-score to standardize continuous metadata
2024-01-19 19:51:52.645635 INFO::Running selected transform method: LOG
2024-01-19 19:51:52.646108 INFO::Running selected analysis method: LM
2024-01-19 19:51:52.64643 INFO::Fitting model to feature number 1, ASV54
2024-01-19 19:51:52.647681 INFO::Fitting model to feature number 2, ASV55
2024-01-19 19:51:52.648771 INFO::Fitting model to feature number 3, ASV57
2024-01-19 19:51:52.649827 INFO::Fitting model to feature number 4, ASV58
2024-01-19 19:51:52.650866 INFO::Fitting model to feature number 5, ASV59
2024-01-19 19:51:52.651898 INFO::Fitting model to feature number 6, ASV60
2024-01-19 19:51:52.652937 INFO::Fitting model to feature number 7, ASV61
2024-01-19 19:51:52.65398 INFO::Fitting model to feature number 8, ASV62
2024-01-19 19:51:52.655021 INFO::Fitting model to feature number 9, ASV64
2024-01-19 19:51:52.658244 INFO::Counting total values for each feature
2024-01-19 19:51:52.659055 INFO::Writing filtered data to file results/maaslin2/asv_GHAT_Leng
2024-01-19 19:51:52.659865 INFO::Writing filtered, normalized data to file results/maaslin2/a
2024-01-19 19:51:52.660573 INFO::Writing filtered, normalized, transformed data to file resul
2024-01-19 19:51:52.661372 WARNING::Deleting existing residuals file: results/maaslin2/asv_GH
2024-01-19 19:51:52.661851 INFO::Writing residuals to file results/maaslin2/asv_GHAT_Length/:
2024-01-19 19:51:52.662369 WARNING::Deleting existing fitted file: results/maaslin2/asv_GHAT_
2024-01-19 19:51:52.662819 INFO::Writing fitted values to file results/maaslin2/asv_GHAT_Leng
2024-01-19 19:51:52.663271 INFO::Writing all results to file (ordered by increasing q-values)
2024-01-19 19:51:52.663956 INFO::Writing the significant results (those which are less than c
2024-01-19 19:51:52.66443 INFO::Writing heatmap of significant results to file: results/maasl
[1] "There is not enough metadata in the associations to create a heatmap plot. Please review
2024-01-19 19:51:52.665079 INFO::Writing association plots (one for each significant associat
2024-01-19 19:51:52.665777 INFO::Plotting associations from most to least significant, groupe
2024-01-19 19:51:52.666116 INFO::Plotting data for metadata number 1, GHAT_Length
2024-01-19 19:51:52.667143 INFO::Creating scatter plot for continuous data, GHAT_Length vs AS

Warning: Removed 1 rows containing missing values (`geom_point()`).

2024-01-19 19:51:52.75044 INFO::Creating scatter plot for continuous data, GHAT_Length vs ASV

Warning: Removed 1 rows containing missing values (`geom_point()`).

2024-01-19 19:51:52.833286 INFO::Creating scatter plot for continuous data, GHAT_Length vs AS

2024-01-19 19:51:52.913005 INFO::Creating scatter plot for continuous data, GHAT_Length vs AS
```

Warning: Removed 1 rows containing missing values (`geom_point()`).

2024-01-19 19:51:53.006507 INFO::Creating scatter plot for continuous data, GHAT_Length vs AS

Warning: Removed 1 rows containing missing values (`geom_point()`).
Removed 1 rows containing missing values (`geom_point()`).
Removed 1 rows containing missing values (`geom_point()`).
Removed 1 rows containing missing values (`geom_point()`).
Removed 1 rows containing missing values (`geom_point()`).

```r
tax_table(ps_trans) %>%
  as.data.frame() %>%
  rownames_to_column("ASV") %>%
  filter(ASV %in% asv_GHAT$results$feature)
```

```
    ASV  Kingdom        Phylum       Class          Order        Family
1 ASV54 Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae
2 ASV55 Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae
3 ASV57 Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae
4 ASV58 Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae
5 ASV59 Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae
6 ASV60 Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae
7 ASV61 Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae
8 ASV62 Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae
9 ASV64 Bacteria Bacteroidota Bacteroidia Bacteroidales Prevotellaceae
       Genus Species
1 Prevotella    <NA>
2 Prevotella    <NA>
3 Prevotella    <NA>
4 Prevotella    <NA>
5 Prevotella    <NA>
6 Prevotella    <NA>
7 Prevotella    <NA>
8 Prevotella    <NA>
9 Prevotella    <NA>
```

```r
# all ASVs map to Genus Prevotella
ps_trans_genus = tax_glom(ps_trans, taxrank = "Genus")
genus_GHAT = Maaslin2(input_data = otu_table(ps_trans_genus),
        input_metadata = as(sample_data(ps_trans_genus), "data.frame"),
```

```
            output = "results/maaslin2/genus_GHAT_Length",
            min_abundance = 5,
            min_prevalence = 0.1,
            max_significance = 0.2,
            fixed_effects = "GHAT_Length")
```

2024-01-19 19:52:00.518009 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_GHAT_Length/maaslin2.log"
2024-01-19 19:52:00.727061 INFO::Writing function arguments to log file
2024-01-19 19:52:00.72987 INFO::Verifying options selected are valid
2024-01-19 19:52:00.730174 INFO::Determining format of input files
2024-01-19 19:52:00.730454 INFO::Input format is data samples as rows and metadata samples as
2024-01-19 19:52:00.732631 INFO::Formula for fixed effects: expr ~ GHAT_Length
2024-01-19 19:52:00.732952 INFO::Filter data based on min abundance and min prevalence
2024-01-19 19:52:00.733221 INFO::Total samples in data: 25
2024-01-19 19:52:00.733476 INFO::Min samples required with min abundance for a feature not to
2024-01-19 19:52:00.735255 INFO::Total filtered features: 288
2024-01-19 19:52:00.735587 INFO::Filtered feature names from abundance and prevalence filter
2024-01-19 19:52:00.73613 INFO::Total filtered features with variance filtering: 0
2024-01-19 19:52:00.736417 INFO::Filtered feature names from variance filtering:
2024-01-19 19:52:00.736677 INFO::Running selected normalization method: TSS
2024-01-19 19:52:00.737144 INFO::Applying z-score to standardize continuous metadata
2024-01-19 19:52:00.740007 INFO::Running selected transform method: LOG
2024-01-19 19:52:00.74056 INFO::Running selected analysis method: LM
2024-01-19 19:52:00.740912 INFO::Fitting model to feature number 1, ASV14
2024-01-19 19:52:00.742205 INFO::Fitting model to feature number 2, ASV55
2024-01-19 19:52:00.743376 INFO::Fitting model to feature number 3, ASV66
2024-01-19 19:52:00.744515 INFO::Fitting model to feature number 4, ASV87
2024-01-19 19:52:00.745636 INFO::Fitting model to feature number 5, ASV120
2024-01-19 19:52:00.74673 INFO::Fitting model to feature number 6, ASV205
2024-01-19 19:52:00.747936 INFO::Fitting model to feature number 7, ASV241
2024-01-19 19:52:00.749135 INFO::Fitting model to feature number 8, ASV443
2024-01-19 19:52:00.750277 INFO::Fitting model to feature number 9, ASV568
2024-01-19 19:52:00.751388 INFO::Fitting model to feature number 10, ASV940
2024-01-19 19:52:00.754938 INFO::Counting total values for each feature
2024-01-19 19:52:00.755816 INFO::Writing filtered data to file results/maaslin2/genus_GHAT_Le
2024-01-19 19:52:00.75698 INFO::Writing filtered, normalized data to file results/maaslin2/ge
2024-01-19 19:52:00.757804 INFO::Writing filtered, normalized, transformed data to file resul
2024-01-19 19:52:00.75865 WARNING::Deleting existing residuals file: results/maaslin2/genus_G
2024-01-19 19:52:00.759177 INFO::Writing residuals to file results/maaslin2/genus_GHAT_Length
2024-01-19 19:52:00.759756 WARNING::Deleting existing fitted file: results/maaslin2/genus_GHA
2024-01-19 19:52:00.760197 INFO::Writing fitted values to file results/maaslin2/genus_GHAT_Le

```
2024-01-19 19:52:00.760676 INFO::Writing all results to file (ordered by increasing q-values
2024-01-19 19:52:00.761435 INFO::Writing the significant results (those which are less than
2024-01-19 19:52:00.761948 INFO::Writing heatmap of significant results to file: results/maas
[1] "There is not enough metadata in the associations to create a heatmap plot. Please revie
2024-01-19 19:52:00.762627 INFO::Writing association plots (one for each significant associat
2024-01-19 19:52:00.76333 INFO::Plotting associations from most to least significant, groupe
2024-01-19 19:52:00.763681 INFO::Plotting data for metadata number 1, GHAT_Length
2024-01-19 19:52:00.764816 INFO::Creating scatter plot for continuous data, GHAT_Length vs AS


2024-01-19 19:52:00.84514 INFO::Creating scatter plot for continuous data, GHAT_Length vs ASV


2024-01-19 19:52:00.922032 INFO::Creating scatter plot for continuous data, GHAT_Length vs AS


Warning: Removed 1 rows containing missing values (`geom_point()`).
Removed 1 rows containing missing values (`geom_point()`).
```

```r
tax_table(ps_trans_genus) %>%
  as.data.frame() %>%
  rownames_to_column("ASV") %>%
  filter(ASV %in% filter(genus_GHAT$results, qval < 0.2)$feature)
```

```
    ASV  Kingdom       Phylum        Class
1  ASV55 Bacteria Bacteroidota    Bacteroidia
2 ASV205 Bacteria   Firmicutes Negativicutes
3 ASV568 Bacteria   Firmicutes    Clostridia
                            Order         Family        Genus Species
1                    Bacteroidales  Prevotellaceae    Prevotella    <NA>
2      Veillonellales-Selenomonadales Veillonellaceae     Dialister    <NA>
3 Peptostreptococcales-Tissierellales       Family XI Anaerococcus    <NA>
```

```r
# plot correlation for these 3 ASVs
psmelt_trans_genus = psmelt(ps_trans_genus)
maaslin_res_genus_ghat = ggplot(filter(psmelt_trans_genus, OTU %in% filter(genus_GHAT$resu
                                aes(x = GHAT_Length, y = Abundance)) +
  geom_point() +
  geom_smooth(method = "lm") +
  facet_wrap(~Genus, scales = "free") +
  scale_y_continuous(limits = function(x){c(0, max(0.1, x))}) +
  coord_cartesian(xlim = c(0,100)) +
```

```
    labs(x = "Length of Testosterone Therapy",
         y = "Genus Abundance (%)",
         caption = "Maaslin2 results")

maaslin_res_genus_ghat
```

Warning: Removed 28 rows containing missing values (`geom_smooth()`).
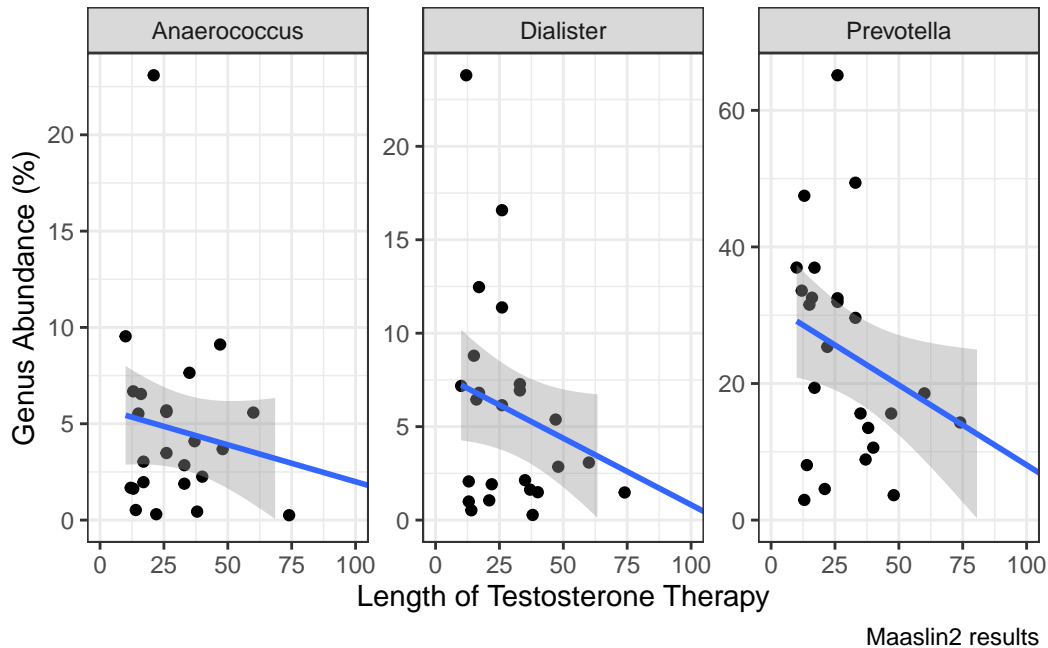
Maaslin2 results

```
ggsave(maaslin_res_genus_ghat, filename = "results/maaslin2/genus_GHAT_Length_panelplot.pd
       height = 4, width =6)
```

Warning: Removed 28 rows containing missing values (`geom_smooth()`).

```
## Alpha diversity by duration of gaht
ps_trans_abs = subset_samples(ps_samples, grp =="03_trans")
ps_trans_genus = tax_glom(ps_trans_abs, taxrank ="Genus") %>%
  transform_sample_counts(function(x) x/sum(x) * 100)

adiv_trans = plot_richness(ps_trans_abs, measures = c("Observed", "Shannon", "InvSimpson")
```

```
adiv_trans$data %>%
    select(GHAT_Length, variable, value) %>%
    group_by(variable) %>%
    cor_test(value, GHAT_Length) %>%
    kable(digits = 3)
```
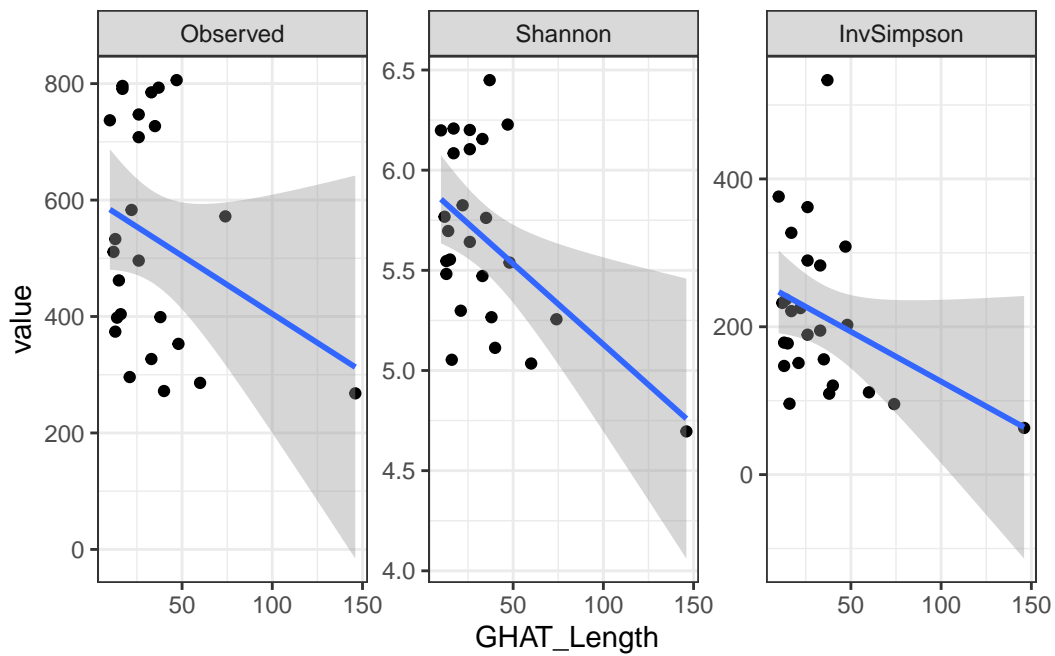
variable	var1	var2	cor	statistic	p	conf.low	conf.high	method
Observed	value	GHAT_Length	-0.29	-1.450	0.161	-0.614	0.119	Pearson
Shannon	value	GHAT_Length	-0.50	-2.760	0.011	-0.747	-0.129	Pearson
InvSimpson	value	GHAT_Length	-0.35	-1.817	0.082	-0.657	0.047	Pearson

```
adiv_trans$data %>%
    ggplot(aes(x = GHAT_Length, y = value)) +
    geom_point()+
    geom_smooth(method ="lm") +
    facet_wrap(~variable, scales = "free_y")
```

```
BC_trans_genus = vegan::vegdist(otu_table(ps_trans_genus))
ord.bc = ordinate(ps_trans_genus,method = "PCoA")

gaht_adonis_bc = vegan::adonis2(BC_trans_genus ~ phyloseq::sample_data(ps_trans_genus)$GHA

#This is highly significant on the genus level!
kable(gaht_adonis_bc)
```

	Df	SumOfSqs	R2	F	Pr(>F)
phyloseq::sample_data(ps_trans_genus)$GHAT_Length	1	0.648191	0.1133731	2.941013	0.005
Residual	23	5.069135	0.8866269	NA	NA
Total	24	5.717326	1.0000000	NA	NA

```
# Summary statistics of duration menopause
sample_data(ps_trans_genus)$GHAT_Length %>%
  summary()
```

```
  Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
 10.00   16.00   26.00   33.56   38.00  146.00
```

```
p_gaht_beta = plot_ordination(ps_trans_genus, ord.bc, type="samples", color="GHAT_Length")
  geom_point(size = 3, alpha = 0.8) +
  labs(color = "Length of GAHT", caption = "Bray-Curtis") +
  scale_color_continuous(limits = c(0,100))
p_gaht_beta
```

Bray–Curtis
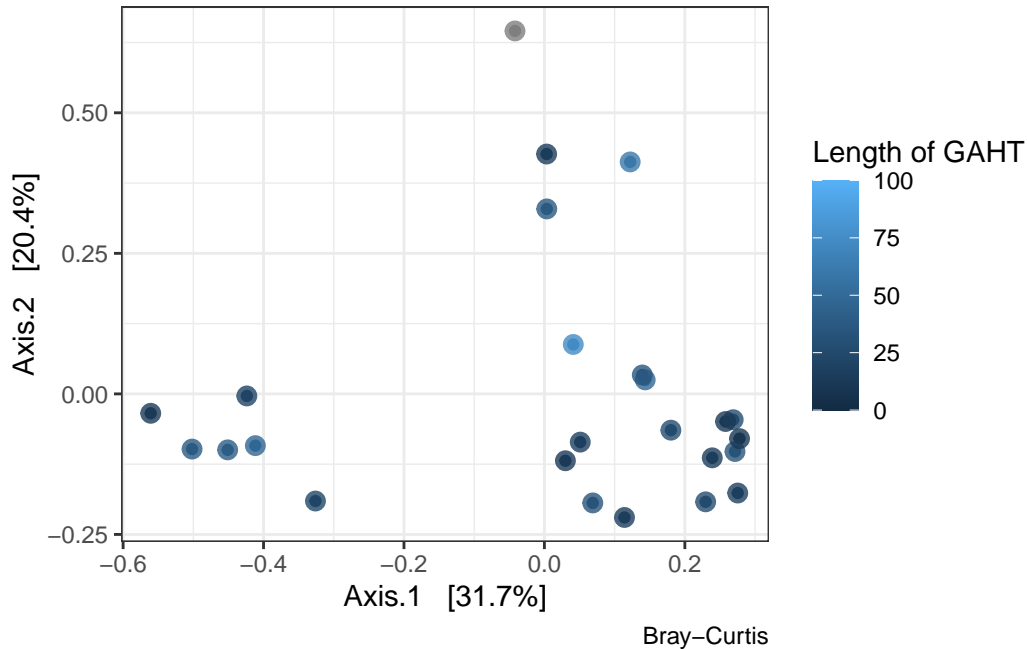
There is a significant effect of duration of GAHT on the shannon index value in the trans group and on overall community composition although this seems to depend on a single outlier with a duration of 150 of hormone therapy. On the genus level, there are 3 taxa (Anaerococcus, Dialister and Prevotella) who are inversely correlated to duration of testosterone supplementation.

6.0.3 Absolute Height of Testosterone in TRANS group

```
# Height of testosterone
genus_testosterone = Maaslin2(input_data = otu_table(ps_trans_genus),
        input_metadata = as(sample_data(ps_trans_genus), "data.frame"),
        output = "results/maaslin2/genus_Testosterone",
        min_abundance = 5,
        min_prevalence = 0.3,
        max_significance = 0.2,
        fixed_effects = "Testosterone") # no significant effect
```

2024-01-19 19:52:08.971151 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_Testosterone/maaslin2.log"
2024-01-19 19:52:09.196832 INFO::Writing function arguments to log file
2024-01-19 19:52:09.199577 INFO::Verifying options selected are valid

```
2024-01-19 19:52:09.199891 INFO::Determining format of input files
2024-01-19 19:52:09.200181 INFO::Input format is data samples as rows and metadata samples as
2024-01-19 19:52:09.202819 INFO::Formula for fixed effects: expr ~  Testosterone
2024-01-19 19:52:09.203256 INFO::Filter data based on min abundance and min prevalence
2024-01-19 19:52:09.203554 INFO::Total samples in data: 25
2024-01-19 19:52:09.203839 INFO::Min samples required with min abundance for a feature not to
2024-01-19 19:52:09.205654 INFO::Total filtered features: 292
2024-01-19 19:52:09.206037 INFO::Filtered feature names from abundance and prevalence filteri
2024-01-19 19:52:09.206606 INFO::Total filtered features with variance filtering: 0
2024-01-19 19:52:09.206896 INFO::Filtered feature names from variance filtering:
2024-01-19 19:52:09.207166 INFO::Running selected normalization method: TSS
2024-01-19 19:52:09.207634 INFO::Applying z-score to standardize continuous metadata
2024-01-19 19:52:09.210553 INFO::Running selected transform method: LOG
2024-01-19 19:52:09.211053 INFO::Running selected analysis method: LM
2024-01-19 19:52:09.211388 INFO::Fitting model to feature number 1, ASV14
2024-01-19 19:52:09.212866 INFO::Fitting model to feature number 2, ASV54
2024-01-19 19:52:09.214033 INFO::Fitting model to feature number 3, ASV66
2024-01-19 19:52:09.215162 INFO::Fitting model to feature number 4, ASV87
2024-01-19 19:52:09.216259 INFO::Fitting model to feature number 5, ASV205
2024-01-19 19:52:09.217357 INFO::Fitting model to feature number 6, ASV568
2024-01-19 19:52:09.220561 INFO::Counting total values for each feature
2024-01-19 19:52:09.221361 INFO::Writing filtered data to file results/maaslin2/genus_Testost
2024-01-19 19:52:09.222171 INFO::Writing filtered, normalized data to file results/maaslin2/g
2024-01-19 19:52:09.22286 INFO::Writing filtered, normalized, transformed data to file resul
2024-01-19 19:52:09.223557 WARNING::Deleting existing residuals file: results/maaslin2/genus_
2024-01-19 19:52:09.224089 INFO::Writing residuals to file results/maaslin2/genus_Testosteron
2024-01-19 19:52:09.224625 WARNING::Deleting existing fitted file: results/maaslin2/genus_Tes
2024-01-19 19:52:09.225094 INFO::Writing fitted values to file results/maaslin2/genus_Testost
2024-01-19 19:52:09.225561 INFO::Writing all results to file (ordered by increasing q-values)
2024-01-19 19:52:09.226251 INFO::Writing the significant results (those which are less than
2024-01-19 19:52:09.226686 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2024-01-19 19:52:09.227251 INFO::Writing association plots (one for each significant associat
[1] "There are no associations to plot!"
```

No significant associations.

6.0.4 Duration of GnRH therapy in TRANS group

```r
summary(sample_data(ps_trans_genus)$Duration_GNRH) # only 10 values!
```

```
  Min. 1st Qu.  Median    Mean 3rd Qu.    Max.    NA's
  12.0    18.0    36.0    37.6    56.0    74.0      15
```

```r
genus_gnrh= Maaslin2(input_data = otu_table(ps_trans_genus),
          input_metadata = as(sample_data(ps_trans_genus), "data.frame"),
          output = "results/maaslin2/genus_DurationGNRH",
          min_abundance = 5,
          min_prevalence = 0.3,
          max_significance = 0.2,
          fixed_effects = "Duration_GNRH") # no significant effect
```

```
2024-01-19 19:52:09.234463 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_DurationGNRH/maaslin2.log"
2024-01-19 19:52:09.44517 INFO::Writing function arguments to log file
2024-01-19 19:52:09.448206 INFO::Verifying options selected are valid
2024-01-19 19:52:09.448517 INFO::Determining format of input files
2024-01-19 19:52:09.448808 INFO::Input format is data samples as rows and metadata samples as
2024-01-19 19:52:09.450961 INFO::Formula for fixed effects: expr ~  Duration_GNRH
2024-01-19 19:52:09.451294 INFO::Filter data based on min abundance and min prevalence
2024-01-19 19:52:09.451567 INFO::Total samples in data: 25
2024-01-19 19:52:09.451824 INFO::Min samples required with min abundance for a feature not to
2024-01-19 19:52:09.453568 INFO::Total filtered features: 292
2024-01-19 19:52:09.45391 INFO::Filtered feature names from abundance and prevalence filterin
2024-01-19 19:52:09.454435 INFO::Total filtered features with variance filtering: 0
2024-01-19 19:52:09.454711 INFO::Filtered feature names from variance filtering:
2024-01-19 19:52:09.454965 INFO::Running selected normalization method: TSS
2024-01-19 19:52:09.455413 INFO::Applying z-score to standardize continuous metadata
2024-01-19 19:52:09.458148 INFO::Running selected transform method: LOG
2024-01-19 19:52:09.458594 INFO::Running selected analysis method: LM
2024-01-19 19:52:09.458937 INFO::Fitting model to feature number 1, ASV14
2024-01-19 19:52:09.460205 INFO::Fitting model to feature number 2, ASV54
2024-01-19 19:52:09.46131 INFO::Fitting model to feature number 3, ASV66
2024-01-19 19:52:09.462404 INFO::Fitting model to feature number 4, ASV87
2024-01-19 19:52:09.463495 INFO::Fitting model to feature number 5, ASV205
2024-01-19 19:52:09.464577 INFO::Fitting model to feature number 6, ASV568
2024-01-19 19:52:09.46771 INFO::Counting total values for each feature
```

```
2024-01-19 19:52:09.468481 INFO::Writing filtered data to file results/maaslin2/genus_Duratio
2024-01-19 19:52:09.469477 INFO::Writing filtered, normalized data to file results/maaslin2/g
2024-01-19 19:52:09.470144 INFO::Writing filtered, normalized, transformed data to file resul
2024-01-19 19:52:09.470839 WARNING::Deleting existing residuals file: results/maaslin2/genus_
2024-01-19 19:52:09.471529 INFO::Writing residuals to file results/maaslin2/genus_DurationGNI
2024-01-19 19:52:09.47217 WARNING::Deleting existing fitted file: results/maaslin2/genus_Dura
2024-01-19 19:52:09.472646 INFO::Writing fitted values to file results/maaslin2/genus_Duratio
2024-01-19 19:52:09.473122 INFO::Writing all results to file (ordered by increasing q-values)
2024-01-19 19:52:09.473843 INFO::Writing the significant results (those which are less than
2024-01-19 19:52:09.474283 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2024-01-19 19:52:09.474843 INFO::Writing association plots (one for each significant associat
[1] "There are no associations to plot!"
```

```
# no significant associations
```

No significant associations.

6.0.5 Cycle dependency in pre-menopausal group

```
ps_pre = subset_samples(ps_samples, grp =="02_premenopausal") %>%
  transform_sample_counts(function(x) x/sum(x) * 100) # relative abundances are necessary

asv_cycleday = Maaslin2(input_data = otu_table(ps_pre),
        input_metadata = as(sample_data(ps_pre), "data.frame"),
        output = "results/maaslin2/asv_cycle_premeno",
        min_abundance = 3,
        min_prevalence = 0.1,
        max_significance = 0.2,
        fixed_effects = "CycleDaySampling") # no significant effect
```

```
2024-01-19 19:52:09.537941 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/asv_cycle_premeno/maaslin2.log"
2024-01-19 19:52:15.972216 INFO::Writing function arguments to log file
2024-01-19 19:52:15.975258 INFO::Verifying options selected are valid
2024-01-19 19:52:15.975575 INFO::Determining format of input files
2024-01-19 19:52:15.975865 INFO::Input format is data samples as rows and metadata samples as
2024-01-19 19:52:16.017141 INFO::Formula for fixed effects: expr ~  CycleDaySampling
2024-01-19 19:52:16.017759 INFO::Filter data based on min abundance and min prevalence
2024-01-19 19:52:16.018086 INFO::Total samples in data: 25
```

```
2024-01-19 19:52:16.018375 INFO::Min samples required with min abundance for a feature not t
2024-01-19 19:52:16.060347 INFO::Total filtered features: 8808
2024-01-19 19:52:16.062211 INFO::Filtered feature names from abundance and prevalence filter:
2024-01-19 19:52:16.06491 INFO::Total filtered features with variance filtering: 0
2024-01-19 19:52:16.065234 INFO::Filtered feature names from variance filtering:
2024-01-19 19:52:16.065513 INFO::Running selected normalization method: TSS
2024-01-19 19:52:16.066067 INFO::Applying z-score to standardize continuous metadata
2024-01-19 19:52:16.068871 INFO::Running selected transform method: LOG
2024-01-19 19:52:16.069572 INFO::Running selected analysis method: LM
2024-01-19 19:52:16.069931 INFO::Fitting model to feature number 1, ASV1
2024-01-19 19:52:16.071197 INFO::Fitting model to feature number 2, ASV2
2024-01-19 19:52:16.07228 INFO::Fitting model to feature number 3, ASV3
2024-01-19 19:52:16.073429 INFO::Fitting model to feature number 4, ASV4
2024-01-19 19:52:16.074506 INFO::Fitting model to feature number 5, ASV5
2024-01-19 19:52:16.075555 INFO::Fitting model to feature number 6, ASV6
2024-01-19 19:52:16.076598 INFO::Fitting model to feature number 7, ASV7
2024-01-19 19:52:16.077642 INFO::Fitting model to feature number 8, ASV8
2024-01-19 19:52:16.078682 INFO::Fitting model to feature number 9, ASV9
2024-01-19 19:52:16.080112 INFO::Fitting model to feature number 10, ASV10
2024-01-19 19:52:16.081493 INFO::Fitting model to feature number 11, ASV11
2024-01-19 19:52:16.082625 INFO::Fitting model to feature number 12, ASV12
2024-01-19 19:52:16.08372 INFO::Fitting model to feature number 13, ASV13
2024-01-19 19:52:16.084806 INFO::Fitting model to feature number 14, ASV14
2024-01-19 19:52:16.085862 INFO::Fitting model to feature number 15, ASV15
2024-01-19 19:52:16.086909 INFO::Fitting model to feature number 16, ASV16
2024-01-19 19:52:16.087954 INFO::Fitting model to feature number 17, ASV17
2024-01-19 19:52:16.089001 INFO::Fitting model to feature number 18, ASV18
2024-01-19 19:52:16.090049 INFO::Fitting model to feature number 19, ASV19
2024-01-19 19:52:16.091249 INFO::Fitting model to feature number 20, ASV20
2024-01-19 19:52:16.092579 INFO::Fitting model to feature number 21, ASV21
2024-01-19 19:52:16.093942 INFO::Fitting model to feature number 22, ASV22
2024-01-19 19:52:16.095174 INFO::Fitting model to feature number 23, ASV23
2024-01-19 19:52:16.096316 INFO::Fitting model to feature number 24, ASV27
2024-01-19 19:52:16.097402 INFO::Fitting model to feature number 25, ASV32
2024-01-19 19:52:16.101837 INFO::Counting total values for each feature
2024-01-19 19:52:16.10306 INFO::Writing filtered data to file results/maaslin2/asv_cycle_prem
2024-01-19 19:52:16.104226 INFO::Writing filtered, normalized data to file results/maaslin2/a
2024-01-19 19:52:16.105216 INFO::Writing filtered, normalized, transformed data to file resu
2024-01-19 19:52:16.106325 WARNING::Deleting existing residuals file: results/maaslin2/asv_cy
2024-01-19 19:52:16.106811 INFO::Writing residuals to file results/maaslin2/asv_cycle_premend
2024-01-19 19:52:16.107466 WARNING::Deleting existing fitted file: results/maaslin2/asv_cycle
2024-01-19 19:52:16.107912 INFO::Writing fitted values to file results/maaslin2/asv_cycle_pre
2024-01-19 19:52:16.108416 INFO::Writing all results to file (ordered by increasing q-values)
```

2024-01-19 19:52:16.109221 INFO::Writing the significant results (those which are less than
2024-01-19 19:52:16.109666 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2024-01-19 19:52:16.110223 INFO::Writing association plots (one for each significant associat
[1] "There are no associations to plot!"

```r
ps_pre_genus = tax_glom(ps_pre, "Genus")

genus_cycleday = Maaslin2(input_data = otu_table(ps_pre_genus),
        input_metadata = as(sample_data(ps_pre_genus), "data.frame"),
        output = "results/maaslin2/genus_cycle_premeno",
        min_abundance = 5,
        min_prevalence = 0.3,
        max_significance = 0.2,
        fixed_effects = "CycleDaySampling")
```

2024-01-19 19:52:23.325947 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_cycle_premeno/maaslin2.log"
2024-01-19 19:52:23.535141 INFO::Writing function arguments to log file
2024-01-19 19:52:23.537903 INFO::Verifying options selected are valid
2024-01-19 19:52:23.538228 INFO::Determining format of input files
2024-01-19 19:52:23.538526 INFO::Input format is data samples as rows and metadata samples as
2024-01-19 19:52:23.540734 INFO::Formula for fixed effects: expr ~ CycleDaySampling
2024-01-19 19:52:23.541063 INFO::Filter data based on min abundance and min prevalence
2024-01-19 19:52:23.54133 INFO::Total samples in data: 25
2024-01-19 19:52:23.541588 INFO::Min samples required with min abundance for a feature not to
2024-01-19 19:52:23.543367 INFO::Total filtered features: 296
2024-01-19 19:52:23.543712 INFO::Filtered feature names from abundance and prevalence filteri
2024-01-19 19:52:23.544203 INFO::Total filtered features with variance filtering: 0
2024-01-19 19:52:23.544488 INFO::Filtered feature names from variance filtering:
2024-01-19 19:52:23.544758 INFO::Running selected normalization method: TSS
2024-01-19 19:52:23.545196 INFO::Applying z-score to standardize continuous metadata
2024-01-19 19:52:23.547985 INFO::Running selected transform method: LOG
2024-01-19 19:52:23.548426 INFO::Running selected analysis method: LM
2024-01-19 19:52:23.54875 INFO::Fitting model to feature number 1, ASV1
2024-01-19 19:52:23.549993 INFO::Fitting model to feature number 2, ASV66
2024-01-19 19:52:23.552948 INFO::Counting total values for each feature
2024-01-19 19:52:23.553659 INFO::Writing filtered data to file results/maaslin2/genus_cycle_p
2024-01-19 19:52:23.554358 INFO::Writing filtered, normalized data to file results/maaslin2/g
2024-01-19 19:52:23.554967 INFO::Writing filtered, normalized, transformed data to file resul
2024-01-19 19:52:23.555588 WARNING::Deleting existing residuals file: results/maaslin2/genus_

```
2024-01-19 19:52:23.556258 INFO::Writing residuals to file results/maaslin2/genus_cycle_preme
2024-01-19 19:52:23.556888 WARNING::Deleting existing fitted file: results/maaslin2/genus_cyc
2024-01-19 19:52:23.557354 INFO::Writing fitted values to file results/maaslin2/genus_cycle_p
2024-01-19 19:52:23.557842 INFO::Writing all results to file (ordered by increasing q-values)
2024-01-19 19:52:23.559824 INFO::Writing the significant results (those which are less than o
2024-01-19 19:52:23.560344 INFO::Writing heatmap of significant results to file: results/maas
[1] "There is not enough metadata in the associations to create a heatmap plot. Please review
2024-01-19 19:52:23.56099 INFO::Writing association plots (one for each significant associati
2024-01-19 19:52:23.561634 INFO::Plotting associations from most to least significant, groupe
2024-01-19 19:52:23.56197 INFO::Plotting data for metadata number 1, CycleDaySampling
2024-01-19 19:52:23.562576 INFO::Creating scatter plot for continuous data, CycleDaySampling


2024-01-19 19:52:23.63731 INFO::Creating scatter plot for continuous data, CycleDaySampling w


Warning: Removed 1 rows containing missing values (`geom_point()`).
Removed 1 rows containing missing values (`geom_point()`).
```

```r
tax_table(ps_pre_genus) %>%
  as.data.frame() %>%
  rownames_to_column("ASV") %>%
  filter(ASV %in% filter(genus_cycleday$results, qval < 0.2)$feature) %>%
  kable()
```

ASV	Kingdom	Phylum	Class	Order	Family	Genus	Species
ASV1	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	NA
ASV66	Bacteria	Actinobacteriota	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Gardnerella	NA

```r
psmelt_pre_genus = psmelt(ps_pre_genus)

maaslin_res_genus_cycleday = ggplot(filter(psmelt_pre_genus, OTU %in% filter(genus_cycleda
                                  aes(x = CycleDaySampling, y = Abundance)) +
  geom_point(size = 4, alpha = 0.7) +
  facet_wrap(~Genus, scales = "free") +
  geom_smooth(method = "lm", se = F, formula = y ~poly(x, 3, raw =T)) +
  scale_y_continuous(limits = function(x){c(0, max(0.1, x))}) +
  coord_cartesian(xlim = c(0,28)) +
  labs(x = "Day of sampling",
       y = "Genus Abundance (%)",
       caption = "Maaslin2 results")
```

```
maaslin_res_genus_cycleday
```
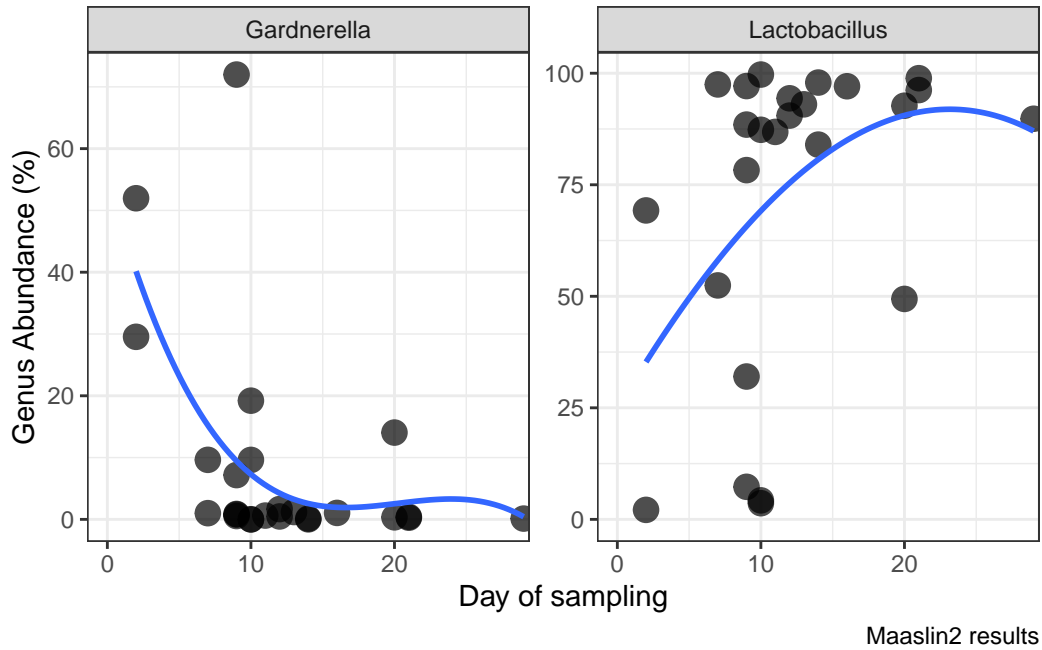

Maaslin2 results

```
ggsave(maaslin_res_genus_cycleday, filename = "results/maaslin2/genus_CycleDay_panelplot.p
        height = 5, width = 6)
```

Gardnerella is highest in the early days of the menstrual cycle while *Lactobacillus* during the middle of the cycle. This seems to correspond to *Vomstein et al 2022*.

6.0.6 Influence of sexual activity on microbiome composition

```
# Sexual activity
ps_rel_genus = tax_glom(ps_samples, taxrank = "Genus") %>%
  transform_sample_counts(function(x) x/sum(x) * 100)
ps_rel_genus_sexuallyactive = subset_samples(ps_rel_genus, sample_data(ps_rel_genus)$Sexua

ps_abs_genus = tax_glom(ps_samples, taxrank = "Genus")
ps_abs_genus_sexuallyactive = subset_samples(ps_abs_genus, sample_data(ps_abs_genus)$Sexua
```

```
ps_rel_genus_sexuallyactive
```

```
phyloseq-class experiment-level object
otu_table()   OTU Table:          [ 298 taxa and 72 samples ]
sample_data() Sample Data:        [ 72 samples by 15 sample variables ]
tax_table()   Taxonomy Table:     [ 298 taxa by 7 taxonomic ranks ]
phy_tree()    Phylogenetic Tree:  [ 298 tips and 297 internal nodes ]
refseq()      DNAStringSet:       [ 298 reference sequences ]
```

```
# Via Maaslin2
genus_SexuallyActive = Maaslin2(input_data = otu_table(ps_rel_genus_sexuallyactive),
        input_metadata = as(sample_data(ps_rel_genus_sexuallyactive), "data.frame"),
        output = "results/maaslin2/genus_SexuallyActive",
        min_abundance = 5,
        min_prevalence = 0.2,
        max_significance = 0.2,
        fixed_effects = "SexuallyActive")
```

```
2024-01-19 19:52:41.966095 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_SexuallyActive/maaslin2.log"
2024-01-19 19:52:42.202703 INFO::Writing function arguments to log file
2024-01-19 19:52:42.205789 INFO::Verifying options selected are valid
2024-01-19 19:52:42.206096 INFO::Determining format of input files
2024-01-19 19:52:42.20638 INFO::Input format is data samples as rows and metadata samples as
2024-01-19 19:52:42.208751 INFO::Formula for fixed effects: expr ~  SexuallyActive
2024-01-19 19:52:42.209125 INFO::Factor detected for categorial metadata 'SexuallyActive'. P
2024-01-19 19:52:42.209397 INFO::Filter data based on min abundance and min prevalence
2024-01-19 19:52:42.209654 INFO::Total samples in data: 72
2024-01-19 19:52:42.209912 INFO::Min samples required with min abundance for a feature not to
2024-01-19 19:52:42.211814 INFO::Total filtered features: 294
2024-01-19 19:52:42.212159 INFO::Filtered feature names from abundance and prevalence filter
2024-01-19 19:52:42.212685 INFO::Total filtered features with variance filtering: 0
2024-01-19 19:52:42.212976 INFO::Filtered feature names from variance filtering:
2024-01-19 19:52:42.213239 INFO::Running selected normalization method: TSS
2024-01-19 19:52:42.213738 INFO::Applying z-score to standardize continuous metadata
2024-01-19 19:52:42.216786 INFO::Running selected transform method: LOG
2024-01-19 19:52:42.217287 INFO::Running selected analysis method: LM
2024-01-19 19:52:42.217617 INFO::Fitting model to feature number 1, ASV1
2024-01-19 19:52:42.219012 INFO::Fitting model to feature number 2, ASV54
2024-01-19 19:52:42.220232 INFO::Fitting model to feature number 3, ASV66
```

```
2024-01-19 19:52:42.221409 INFO::Fitting model to feature number 4, ASV87
2024-01-19 19:52:42.224712 INFO::Counting total values for each feature
2024-01-19 19:52:42.225519 INFO::Writing filtered data to file results/maaslin2/genus_Sexuall
2024-01-19 19:52:42.226603 INFO::Writing filtered, normalized data to file results/maaslin2/g
2024-01-19 19:52:42.227492 INFO::Writing filtered, normalized, transformed data to file resul
2024-01-19 19:52:42.228352 WARNING::Deleting existing residuals file: results/maaslin2/genus_
2024-01-19 19:52:42.228848 INFO::Writing residuals to file results/maaslin2/genus_SexuallyAct
2024-01-19 19:52:42.229382 WARNING::Deleting existing fitted file: results/maaslin2/genus_Se
2024-01-19 19:52:42.229797 INFO::Writing fitted values to file results/maaslin2/genus_Sexuall
2024-01-19 19:52:42.230233 INFO::Writing all results to file (ordered by increasing q-values
2024-01-19 19:52:42.230903 INFO::Writing the significant results (those which are less than
2024-01-19 19:52:42.231385 INFO::Writing heatmap of significant results to file: results/maa
[1] "There is not enough metadata in the associations to create a heatmap plot. Please revie
2024-01-19 19:52:42.232025 INFO::Writing association plots (one for each significant associa
2024-01-19 19:52:42.232736 INFO::Plotting associations from most to least significant, group
2024-01-19 19:52:42.233085 INFO::Plotting data for metadata number 1, SexuallyActive
2024-01-19 19:52:42.234258 INFO::Creating boxplot for categorical data, SexuallyActive vs AS


2024-01-19 19:52:42.309856 INFO::Creating boxplot for categorical data, SexuallyActive vs AS
```

```r
tax_table(ps_rel_genus_sexuallyactive) %>%
  as.data.frame() %>%
  rownames_to_column("ASV") %>%
  filter(ASV %in% filter(genus_SexuallyActive$results, qval < 0.2)$feature)
```

```
    ASV  Kingdom      Phylum      Class         Order          Family
1 ASV54 Bacteria Bacteroidota Bacteroidia   Bacteroidales   Prevotellaceae
2 ASV87 Bacteria   Firmicutes     Bacilli Lactobacillales Streptococcaceae
        Genus Species
1   Prevotella    <NA>
2 Streptococcus    <NA>
```

```r
# plot sexuallyActive vs these 2 genera
psmelt_rel_genus_sexuallyactive = psmelt(ps_rel_genus_sexuallyactive)
maaslin_res_genus_sexactive = ggplot(filter(psmelt_rel_genus_sexuallyactive, OTU %in% filt
                              aes(x = SexuallyActive, y = Abundance,fill = SexuallyActiv
  geom_jitter() +
  geom_boxplot(alpha = 0.7, outlier.shape = NA) +
  facet_wrap(~Genus, scales = "free") +
  scale_y_continuous(limits = function(x){c(0, max(0.1, x))}) +
```

```
      theme(legend.position = "none")+
      labs(x = "Sexually Active",
           y = "Genus Abundance (%)",
           caption = "Maaslin2 results")+
        scale_y_continuous(expand = expansion(mult = c(0, 0.2))) +
      scale_fill_manual(values = c("black", "lightgrey"))
```

```
maaslin_res_genus_sexactive
```
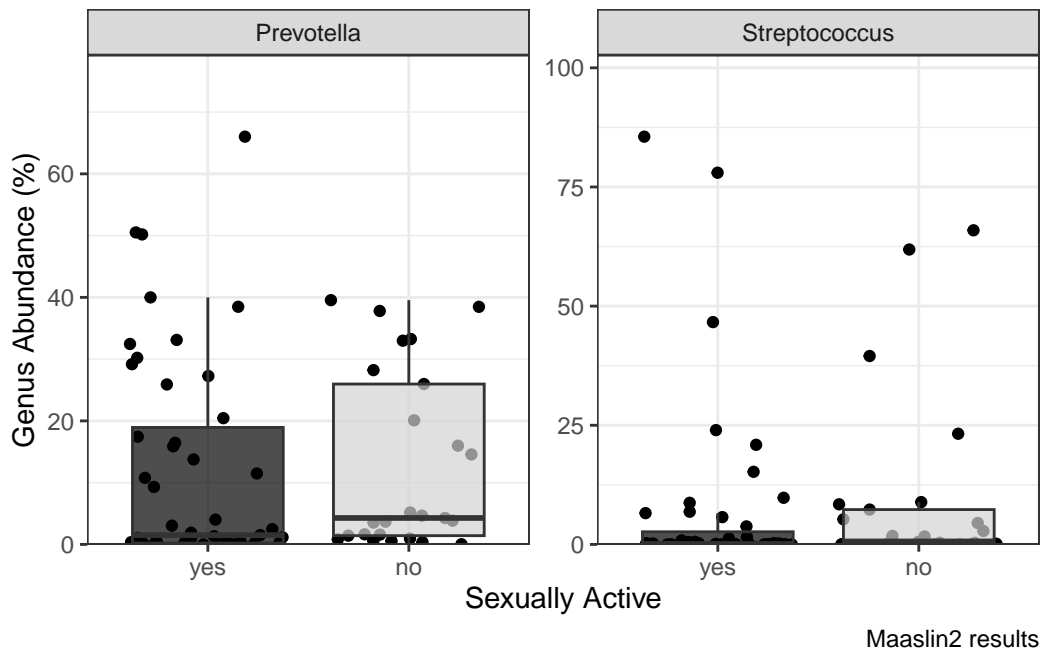

Maaslin2 results

```
ggsave(maaslin_res_genus_sexactive, filename = "results/maaslin2/genus_SexuallyActive_pane
        height = 3, width = 4)

# Via Deseq2
sexactive_ds = phyloseq_to_deseq2(ps_abs_genus_sexuallyactive, ~SexuallyActive)
cts = counts(sexactive_ds)
geoMeans = apply(cts, 1, function(row) if (all(row == 0)) 0 else exp(mean(log(row[row != 0
sexactive_ds = estimateSizeFactors(sexactive_ds, geoMeans=geoMeans)
sexactive_ds = DESeq(sexactive_ds, test = "Wald", fitType = "parametric")
res = results(sexactive_ds, cooksCutoff = FALSE)
alpha = 0.5
```

```
sigtab = res[which(res$padj < alpha), ]
sigtab = cbind(as(sigtab, "data.frame"), as(tax_table(ps_abs_genus_sexuallyactive)[rowname
sigtab = as.data.frame(sigtab) %>%
  rownames_to_column("feature")

sigtab
```

```
  feature  baseMean log2FoldChange    lfcSE      stat      pvalue      padj
1  ASV556 12.793256       5.241888 1.912155  2.741351 0.006118719 0.4332856
2  ASV898 29.633042      -5.829075 1.818941 -3.204653 0.001352252 0.2992866
3 ASV1006  7.381661      -6.291604 2.093133 -3.005832 0.002648554 0.2992866
4 ASV4650  2.993678      -4.442670 1.666221 -2.666315 0.007668771 0.4332856
   Kingdom          Phylum          Class                              Order
1 Bacteria Actinobacteriota Actinobacteria              Bifidobacteriales
2 Bacteria       Firmicutes        Bacilli                 Lactobacillales
3 Bacteria       Firmicutes  Negativicutes Veillonellales-Selenomonadales
4 Bacteria Actinobacteriota Actinobacteria              Bifidobacteriales
             Family          Genus Species
1 Bifidobacteriaceae Bifidobacterium    <NA>
2   Lactobacillaceae          HT002    <NA>
3     Veillonellaceae    Megasphaera    <NA>
4 Bifidobacteriaceae    Neoscardovia    <NA>
```

```
# this does not detect any significant difference



# Wilcoxon
psmelt_rel_genus_sexuallyactive %>%
  group_by(OTU) %>%
  rstatix::t_test(data = ., Abundance~SexuallyActive) %>%
  rstatix::adjust_pvalue(method = "fdr") %>%
  filter(p.adj < 0.1)
```

```
# A tibble: 0 x 10
# i 10 variables: OTU <chr>, .y. <chr>, group1 <chr>, group2 <chr>, n1 <int>,
#   n2 <int>, statistic <dbl>, df <dbl>, p <dbl>, p.adj <dbl>
```

```
# using simple parametric statistical test (t-test) no significant diff.
```

Although Maaslin2 detects significant changes in some taxa, this might not be statistically relevant due to the extremely skewed distribution of data and non-suitability of a linear model for comparison of 2 groups. Likewise, using a simple parametric statistical test such as the t-test is sub-optimal. This approach fails to detect any significantly altered taxa.

Next, we analyse and describe the taxonomic composition within the *trans* and control groups by sexual activity. There are no significant statistical differences and the sample size is small. Therefore, the top 10 abundant taxa are listed for sexually active and non-active subgroups:

```
# Trans Group taxa selection
ps_rel_genus_sexuallyactive_trans = subset_samples(ps_rel_genus_sexuallyactive, grp =="03_
top <- names(sort(taxa_sums(ps_rel_genus_sexuallyactive_trans), decreasing = TRUE))[1:20]
psmelt_rel_genus_sexuallyactive_trans.top <- prune_taxa(top, ps_rel_genus_sexuallyactive_t
  psmelt() %>%
  mutate(tax = paste(Phylum, Class, Order, Family, Genus,sep = "|")) %>%
  group_by(tax, SexuallyActive)

# Plot
genus_sexactive_trans_top20 = ggplot(psmelt_rel_genus_sexuallyactive_trans.top, aes(x = ta
  geom_boxplot(position="dodge", alpha = 0.5) +
  geom_point(aes(color = SexuallyActive), position = position_dodge(width = 0.7), alpha =
  scale_fill_manual(values = c("black", "lightgrey")) +
  scale_color_manual(values = c("black", "lightgrey")) +
  coord_flip() +
  theme_bw() +
  lims(y = c(0,100)) +
  labs(title = "Trans-men group", y = "Abundance (%)", x = "", color = "Sexually Active",

# Summarize
Genus_Sexuallyactive_Trans_top20 = psmelt_rel_genus_sexuallyactive_trans.top %>%
  summarize(mean = mean(Abundance, na.rm=T),
            median = median(Abundance, na.rm =T),
            sd = sd(Abundance, na.rm=T),
            IQR = IQR(Abundance ,na.rm=T))

knitr::kable(Genus_Sexuallyactive_Trans_top20, digits = 2)
```

tax	SexuallyActive	mean	median	sd	IQR
Actinobacteriota|Actinobacteria|Actinomycetales|Actinomycetaceae|Actino	yes	0.89 0.27		1.56	1.03
Actinobacteriota|Actinobacteria|Actinomycetales|Actinomycetaceae|Actino	no	0.74 0.30		1.19	0.85
Actinobacteriota|Actinobacteria|Bifidobacteriales|Bifidobacteriaceae|Allos	yes	3.00 0.00		6.57	0.18
Actinobacteriota|Actinobacteria|Bifidobacteriales|Bifidobacteriaceae|Allos	no	0.61 0.00		0.94	0.98

tax	Sexually Active	mean	median	sd	IQR
Actinobacteriota|Actinobacteria|Bifidobacteriales|Bifidobacteriaceae|Gardnerella	yes	17.08	11.56	22.03	35.43
Actinobacteriota|Actinobacteria|Bifidobacteriales|Bifidobacteriaceae|Gardnerella	no	8.30	0.11	15.58	5.60
Actinobacteriota|Actinobacteria|Corynebacteriales|Corynebacteriaceae|Corynebacterium	yes	1.52	0.34	2.56	1.39
Actinobacteriota|Actinobacteria|Corynebacteriales|Corynebacteriaceae|Corynebacterium	no	2.32	0.63	2.86	3.54
Bacteroidota|Bacteroidia|Bacteroidales|Porphyromonadaceae|Porphyromonas	yes	3.54	2.21	6.50	3.19
Bacteroidota|Bacteroidia|Bacteroidales|Porphyromonadaceae|Porphyromonas	no	5.52	1.90	11.55	2.30
Bacteroidota|Bacteroidia|Bacteroidales|Prevotellaceae|Prevotella	yes	26.39	25.90	19.93	22.34
Bacteroidota|Bacteroidia|Bacteroidales|Prevotellaceae|Prevotella	no	24.10	26.54	13.99	21.74
Bacteroidota|Bacteroidia|Flavobacteriales|Flavobacteriaceae|Flavobacterium	yes	0.03	0.00	0.07	0.00
Bacteroidota|Bacteroidia|Flavobacteriales|Flavobacteriaceae|Flavobacterium	no	5.16	0.00	16.31	0.00
Campylobacterota|Campylobacteria|Campylobacterales|Campylobacteraceae|Campylobacter	yes	0.73	0.35	1.04	0.74
Campylobacterota|Campylobacteria|Campylobacterales|Campylobacteraceae|Campylobacter	no	2.14	0.85	3.18	0.78
Firmicutes|Bacilli|Lactobacillales|Lactobacillaceae|Lactobacillus	yes	5.23	0.35	7.78	8.42
Firmicutes|Bacilli|Lactobacillales|Lactobacillaceae|Lactobacillus	no	7.85	0.28	14.70	8.71
Firmicutes|Bacilli|Lactobacillales|Streptococcaceae|Streptococcus	yes	9.79	1.52	21.33	6.38
Firmicutes|Bacilli|Lactobacillales|Streptococcaceae|Streptococcus	no	2.86	0.99	3.77	5.88
Firmicutes|Clostridia|Peptostreptococcales-Tissierellales|Family XI|Anaerococcus	yes	3.50	2.89	2.80	4.01
Firmicutes|Clostridia|Peptostreptococcales-Tissierellales|Family XI|Anaerococcus	no	6.52	4.78	6.75	5.21
Firmicutes|Clostridia|Peptostreptococcales-Tissierellales|Family XI|Ezakiella	yes	0.79	0.06	1.27	1.02
Firmicutes|Clostridia|Peptostreptococcales-Tissierellales|Family XI|Ezakiella	no	0.87	0.62	0.79	0.55
Firmicutes|Clostridia|Peptostreptococcales-Tissierellales|Family XI|Fenollaria	yes	1.81	0.55	2.60	2.35
Firmicutes|Clostridia|Peptostreptococcales-Tissierellales|Family XI|Fenollaria	no	2.89	2.96	2.30	3.80
Firmicutes|Clostridia|Peptostreptococcales-Tissierellales|Family XI|Finegoldia	yes	1.13	0.56	1.47	0.87
Firmicutes|Clostridia|Peptostreptococcales-Tissierellales|Family XI|Finegoldia	no	1.34	0.82	1.81	0.90
Firmicutes|Clostridia|Peptostreptococcales-Tissierellales|Family XI|Peptoniphilus	yes	2.49	1.87	2.47	3.17
Firmicutes|Clostridia|Peptostreptococcales-Tissierellales|Family XI|Peptoniphilus	no	3.07	2.71	2.07	3.08
Firmicutes|Negativicutes|Veillonellales-Selenomonadales|Veillonellaceae|Dialister	yes	4.68	2.20	4.76	5.56
Firmicutes|Negativicutes|Veillonellales-Selenomonadales|Veillonellaceae|Dialister	no	8.05	6.80	7.78	8.44
Fusobacteriota|Fusobacteriia|Fusobacteriales|Fusobacteriaceae|Fusobacterium	yes	1.01	0.22	1.28	1.71

tax	SexuallyActive	mean	median	sd	IQR
Fusobacteriota|Fusobacteriia|Fusobacteriales|Fusobacteriaceae|Fusobacterium	no	2.31	0.12	5.90	1.12
Fusobacteriota|Fusobacteriia|Fusobacteriales|Leptotrichiaceae|Sneathia	yes	3.36	0.15	7.59	3.35
Fusobacteriota|Fusobacteriia|Fusobacteriales|Leptotrichiaceae|Sneathia	no	0.56	0.00	1.27	0.00
Proteobacteria|Alphaproteobacteria|Rhizobiales|Beijerinckiaceae|Methylobacterium-Methylorubrum	yes	0.01	0.00	0.02	0.00
Proteobacteria|Alphaproteobacteria|Rhizobiales|Beijerinckiaceae|Methylobacterium-Methylorubrum	no	3.25	0.00	9.73	0.02
Proteobacteria|Gammaproteobacteria|Burkholderiales|Neisseriaceae|Neisseria	yes	2.51	0.00	8.87	0.09
Proteobacteria|Gammaproteobacteria|Burkholderiales|Neisseriaceae|Neisseria	no	0.04	0.00	0.12	0.00

```r
# Postmenopausal group
ps_rel_genus_sexuallyactive_post = subset_samples(ps_rel_genus_sexuallyactive, grp =="01_p
top2 <- names(sort(taxa_sums(ps_rel_genus_sexuallyactive_post), decreasing = TRUE))[1:20]
psmelt_rel_genus_sexuallyactive_post.top <- prune_taxa(top2, ps_rel_genus_sexuallyactive_p
  psmelt() %>%
  mutate(tax = paste(Phylum, Class, Order, Family, Genus,sep = "|")) %>%
  group_by(tax, SexuallyActive)

# Plot
genus_sexactive_post_top20 = ggplot(psmelt_rel_genus_sexuallyactive_post.top, aes(x = tax,
  geom_boxplot(position="dodge", alpha = 0.5) +
  geom_point(aes(color = SexuallyActive), position = position_dodge(width = 0.7), alpha =
  scale_fill_manual(values = c("black", "lightgrey")) +
  scale_color_manual(values = c("black", "lightgrey")) +
  coord_flip() +
  theme_bw() +
  lims(y = c(0,100)) +
  labs(title = "Postmenopausal group", y = "Abundance (%)", x = "", color = "Sexually Acti

ggsave(filename = "results/Genus_SexuallyActive_Descriptive_Postmenopausal.pdf", plot = ge
       height = 8, width = 11)
ggsave(filename = "results/Genus_SexuallyActive_Descriptive_Trans.pdf", plot = genus_sexac
       height = 8, width = 11)

# Summarize
Genus_Sexuallyactive_Post_top20 = psmelt_rel_genus_sexuallyactive_post.top %>%
  summarize(mean = mean(Abundance, na.rm=T),
            median = median(Abundance, na.rm =T),
            sd = sd(Abundance, na.rm=T),
```

```
            IQR = IQR(Abundance ,na.rm=T))

knitr::kable(Genus_Sexuallyactive_Post_top20, digits = 2)
```

tax	SexuallyActive	mean	median	sd	IQR
Actinobacteriota|Actinobacteria|Actinomycetales|Actinomycetaceae|Actinomyces	yes	0.45	0.00	0.83	0.17
Actinobacteriota|Actinobacteria|Actinomycetales|Actinomycetaceae|Actinomyces	no	0.63	0.00	1.84	0.15
Actinobacteriota|Actinobacteria|Bifidobacteriales|Bifidobacteriaceae|Alloscardovia	yes	1.85	0.26	3.54	2.07
Actinobacteriota|Actinobacteria|Bifidobacteriales|Bifidobacteriaceae|Alloscardovia	no	2.40	0.07	6.66	0.39
Actinobacteriota|Actinobacteria|Bifidobacteriales|Bifidobacteriaceae|Bifidobacterium	yes	6.50	0.10	16.70	0.00
Actinobacteriota|Actinobacteria|Bifidobacteriales|Bifidobacteriaceae|Bifidobacterium	no	2.67	0.14	6.90	0.33
Actinobacteriota|Actinobacteria|Bifidobacteriales|Bifidobacteriaceae|Gardnerella	yes	17.54	1.30	28.06	15.26
Actinobacteriota|Actinobacteria|Bifidobacteriales|Bifidobacteriaceae|Gardnerella	no	2.99	0.32	5.54	2.13
Actinobacteriota|Actinobacteria|Corynebacteriales|Corynebacteriaceae|Corynebacterium	yes	0.71	0.46	0.75	1.14
Actinobacteriota|Actinobacteria|Corynebacteriales|Corynebacteriaceae|Corynebacterium	no	3.07	0.82	4.78	3.61
Actinobacteriota|Coriobacteriia|Coriobacteriales|Atopobiaceae|Atopobium	yes	4.30	1.48	8.04	4.18
Actinobacteriota|Coriobacteriia|Coriobacteriales|Atopobiaceae|Atopobium	no	3.70	0.20	11.57	1.82
Bacteroidota|Bacteroidia|Bacteroidales|Porphyromonadaceae|Porphyromonas	yes	2.96	0.07	5.61	2.98
Bacteroidota|Bacteroidia|Bacteroidales|Porphyromonadaceae|Porphyromonas	no	0.51	0.22	0.83	0.54
Bacteroidota|Bacteroidia|Bacteroidales|Prevotellaceae|Prevotella	yes	17.05	11.49	16.62	27.86
Bacteroidota|Bacteroidia|Bacteroidales|Prevotellaceae|Prevotella	no	5.26	1.58	9.01	3.37
Bacteroidota|Bacteroidia|Bacteroidales|Prevotellaceae|Prevotella_9	yes	0.00	0.00	0.00	0.00
Bacteroidota|Bacteroidia|Bacteroidales|Prevotellaceae|Prevotella_9	no	0.86	0.00	2.59	0.00
Campylobacterota|Campylobacteria|Campylobacterales|Campylobacteraceae|Campylobacter	yes	0.74	0.00	2.57	2.54
Campylobacterota|Campylobacteria|Campylobacterales|Campylobacteraceae|Campylobacter	no	0.34	0.16	0.44	0.37
Firmicutes|Bacilli|Lactobacillales|Lactobacillaceae|Lactobacillus	yes	21.64	7.48	33.40	19.36
Firmicutes|Bacilli|Lactobacillales|Lactobacillaceae|Lactobacillus	no	31.00	10.93	35.78	51.90
Firmicutes|Bacilli|Lactobacillales|Streptococcaceae|Streptococcus	yes	5.42	0.83	8.61	6.77
Firmicutes|Bacilli|Lactobacillales|Streptococcaceae|Streptococcus	no	13.66	0.63	23.19	14.12
Firmicutes|Bacilli|Staphylococcales|Gemellaceae|Gemella	yes	0.08	0.00	0.21	0.00
Firmicutes|Bacilli|Staphylococcales|Gemellaceae|Gemella	no	1.19	0.00	2.92	0.30
Firmicutes|Bacilli|Staphylococcales|Staphylococcaceae|Staphylococcus	yes	0.07	0.00	0.09	0.14
Firmicutes|Bacilli|Staphylococcales|Staphylococcaceae|Staphylococcus	no	9.65	0.06	25.44	0.29
Firmicutes|Clostridia|Peptostreptococcales-Tissierellales|Family XI|Anaerococcus	yes	3.72	1.73	5.84	3.35
Firmicutes|Clostridia|Peptostreptococcales-Tissierellales|Family XI|Anaerococcus	no	2.48	1.18	4.08	2.20
Firmicutes|Clostridia|Peptostreptococcales-Tissierellales|Family XI|Ezakiella	yes	1.21	0.13	2.01	1.79
Firmicutes|Clostridia|Peptostreptococcales-Tissierellales|Family XI|Ezakiella	no	0.34	0.05	0.63	0.43

tax	Sexually Active	mean	median	sd	IQR
Firmicutes|Clostridia|Peptostreptococcales-Tissierellales|Family XI|Finegoldia	yes	3.02	0.88	4.61	2.57
Firmicutes|Clostridia|Peptostreptococcales-Tissierellales|Family XI|Finegoldia	no	4.13	0.54	6.38	4.05
Firmicutes|Clostridia|Peptostreptococcales-Tissierellales|Family XI|Peptoniphilus	yes	2.75	1.66	3.43	3.93
Firmicutes|Clostridia|Peptostreptococcales-Tissierellales|Family XI|Peptoniphilus	no	1.61	0.51	2.29	1.38
Firmicutes|Negativicutes|Veillonellales-Selenomonadales|Veillonellaceae|Dialister	yes	1.98	0.00	3.25	3.03
Firmicutes|Negativicutes|Veillonellales-Selenomonadales|Veillonellaceae|Dialister	no	0.60	0.54	0.63	0.82
Proteobacteria|Gammaproteobacteria|Enterobacterales|Enterobacteriaceae|Escherichia-Shigella	yes	0.35	0.00	0.87	0.06
Proteobacteria|Gammaproteobacteria|Enterobacterales|Enterobacteriaceae|Escherichia-Shigella	no	5.09	0.00	16.84	2.16

```
genus_sexactive_trans_top20
```

Trans–

Proteobacteria|Gammaproteobacteria|Burkholderiales|Neisseriaceae|Neisseria
...eria|Alphaproteobacteria|Rhizobiales|Beijerinckiaceae|Methylobacterium–Methylorubrum
Fusobacteriota|Fusobacteriia|Fusobacteriales|Leptotrichiaceae|Sneathia
Fusobacteriota|Fusobacteriia|Fusobacteriales|Fusobacteriaceae|Fusobacterium
Firmicutes|Negativicutes|Veillonellales–Selenomonadales|Veillonellaceae|Dialister
Firmicutes|Clostridia|Peptostreptococcales–Tissierellales|Family XI|Peptoniphilus
Firmicutes|Clostridia|Peptostreptococcales–Tissierellales|Family XI|Finegoldia
Firmicutes|Clostridia|Peptostreptococcales–Tissierellales|Family XI|Fenollaria
Firmicutes|Clostridia|Peptostreptococcales–Tissierellales|Family XI|Ezakiella
Firmicutes|Clostridia|Peptostreptococcales–Tissierellales|Family XI|Anaerococcus
Firmicutes|Bacilli|Lactobacillales|Streptococcaceae|Streptococcus
Firmicutes|Bacilli|Lactobacillales|Lactobacillaceae|Lactobacillus
...pylobacterota|Campylobacteria|Campylobacterales|Campylobacteraceae|Campylobacter
Bacteroidota|Bacteroidia|Flavobacteriales|Flavobacteriaceae|Flavobacterium
Bacteroidota|Bacteroidia|Bacteroidales|Prevotellaceae|Prevotella
Bacteroidota|Bacteroidia|Bacteroidales|Porphyromonadaceae|Porphyromonas
...Actinobacteriota|Actinobacteria|Corynebacteriales|Corynebacteriaceae|Corynebacterium
Actinobacteriota|Actinobacteria|Bifidobacteriales|Bifidobacteriaceae|Gardnerella
Actinobacteriota|Actinobacteria|Bifidobacteriales|Bifidobacteriaceae|Alloscardovia
Actinobacteriota|Actinobacteria|Actinomycetales|Actinomycetaceae|Actinomyces

Sexu

Abundance (%)

```
genus_sexactive_post_top20
```

Postme

ꞓteria|Gammaproteobacteria|Enterobacterales|Enterobacteriaceae|Escherichia−Shigella
Firmicutes|Negativicutes|Veillonellales−Selenomonadales|Veillonellaceae|Dialister
Firmicutes|Clostridia|Peptostreptococcales−Tissierellales|Family XI|Peptoniphilus
Firmicutes|Clostridia|Peptostreptococcales−Tissierellales|Family XI|Finegoldia
Firmicutes|Clostridia|Peptostreptococcales−Tissierellales|Family XI|Ezakiella
Firmicutes|Clostridia|Peptostreptococcales−Tissierellales|Family XI|Anaerococcus
Firmicutes|Bacilli|Staphylococcales|Staphylococcaceae|Staphylococcus
Firmicutes|Bacilli|Staphylococcales|Gemellaceae|Gemella
Firmicutes|Bacilli|Lactobacillales|Streptococcaceae|Streptococcus
Firmicutes|Bacilli|Lactobacillales|Lactobacillaceae|Lactobacillus
ylobacterota|Campylobacteria|Campylobacterales|Campylobacteraceae|Campylobacter
Bacteroidota|Bacteroidia|Bacteroidales|Prevotellaceae|Prevotella_9
Bacteroidota|Bacteroidia|Bacteroidales|Prevotellaceae|Prevotella
Bacteroidota|Bacteroidia|Bacteroidales|Porphyromonadaceae|Porphyromonas
Actinobacteriota|Coriobacteriia|Coriobacteriales|Atopobiaceae|Atopobium
ꞓtinobacteriota|Actinobacteria|Corynebacteriales|Corynebacteriaceae|Corynebacterium
Actinobacteriota|Actinobacteria|Bifidobacteriales|Bifidobacteriaceae|Gardnerella
Actinobacteriota|Actinobacteria|Bifidobacteriales|Bifidobacteriaceae|Bifidobacterium
Actinobacteriota|Actinobacteria|Bifidobacteriales|Bifidobacteriaceae|Alloscardovia
Actinobacteriota|Actinobacteria|Actinomycetales|Actinomycetaceae|Actinomyces

Sexuᴀ

☒ y

⊟ n

100

Abundance (%)

```
write_csv(Genus_Sexuallyactive_Post_top20, file = "results/Genus_SexuallyActive_Descriptiv
write_csv(Genus_Sexuallyactive_Trans_top20, file = "results/Genus_SexuallyActive_Descripti

# Analysis in Trans group with male sexual partner only
genus_Sexofpartner = psmelt_rel_genus_sexuallyactive_trans.top %>%
  filter(!is.na(SexOfPartner)) %>%
  mutate(SexOfPartner2 = ifelse(SexOfPartner %in% c("M", "M+F"), "M", "F only"))
genus_sexactive_trans_malepartner_top20 = ggplot(genus_Sexofpartner,
                                                  aes(x = tax, y = Abundance, fill = Sexual
  geom_boxplot(position="dodge", alpha = 0.5) +
  geom_point(aes(color = SexuallyActive), position = position_dodge(width = 0.7), alpha =
  scale_fill_manual(values = c("black", "lightgrey")) +
  scale_color_manual(values = c("black", "lightgrey")) +
  coord_flip() +
  theme_bw() +
  facet_wrap(~SexOfPartner2) +
  lims(y = c(0,100)) +
  labs(title = "Trans-men group", y = "Abundance (%)", x = "", color = "Sexually Active",
```

```
ggsave(filename = "results/Genus_SexuallyActive_Descriptive_Trans_SexOfPartner.pdf",
       genus_sexactive_trans_malepartner_top20,
       height = 8, width = 11)
```

6.0.7 Influence of the duration of amenorrhea on microbiome composition

Both postmenopausal women as well as trans men undergoing GAHT experience amenorrhea.
This section explores a potential interaction between time since last menstruation and compo-
sition of the vaginal microbiome.

**This analysis is performed within each group separately and results are com-
pared.**

```
# generate relevant phyloseq object
ps_menopause = subset_samples(ps_samples, grp !="02_premenopausal")

sample_data(ps_menopause)$DurationMenopause # this is the duration of menopause in postmen
```

```
 [1] 11  2 10 22 12 22  4 28  7 26 11  2 18 20 17 24 23 19 23 27 13  1 13  4  3
[26] NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA
```

```
sample_data(ps_menopause)$DurationAmenorrhea # duration of amenorrhea in trans group (mont
```

```
 [1] NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA
[26] 19 42  2 33 17 35  6  2 37 14  0 60 46  4 26  9 16 27  0 74  9  1 35 16 18
```

```
sample_data(ps_menopause)$DurationMenopauseMonths = sample_data(ps_menopause)$DurationMeno
sample_data(ps_menopause)$DurationCombined = ifelse(is.na(sample_data(ps_menopause)$Durati

ps_menopause_rel =  transform_sample_counts(ps_menopause,function(x) x/sum(x) * 100)
# Diversity analysis
adiv_menopause =  plot_richness(ps_menopause, measures = c("Observed", "Shannon", "InvSimp

adiv_menopause = adiv_menopause$data %>%
  select(samples, grp, DurationMenopause, DurationAmenorrhea, DurationCombined, variable,

corr_trans = filter(adiv_menopause, grp =="03_trans") %>%
  group_by(variable) %>%
  rstatix::cor_test(value,DurationAmenorrhea)
```

```
kable(corr_trans)
```

variable	var1	var2	cor	statistic	p	conf.low	conf.high	method
Observed	value	DurationAmenorrhea	0.078	0.3754983	0.711	-0.3271637	0.4590309	Pearson
Shannon	value	DurationAmenorrhea	-0.100	-0.4841405	0.633	-0.4766538	0.3068698	Pearson
InvSimpson	value	DurationAmenorrhea	-0.140	-0.6598812	0.516	-0.5042806	0.2735542	Pearson

```
ggplot(filter(adiv_menopause, grp =="03_trans"), aes(x = DurationAmenorrhea, y = value)) +
  geom_point() +
  geom_smooth(method = "lm", color = "darkred") +
  facet_wrap(~variable, scales = "free") +
  labs(x = "Duration of Amenorrhea in postmenopausal group", y = "Alpha diversity value")
```
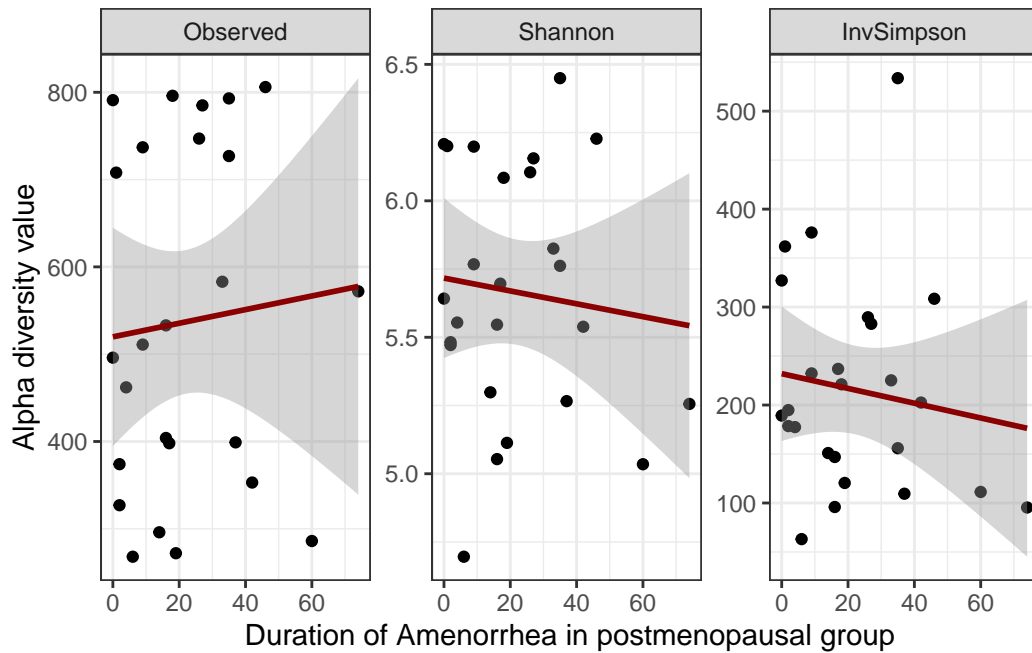


```
ggplot(filter(adiv_menopause, grp =="01_postmenopausal"), aes(x = DurationMenopause, y = v
  geom_point() +
  geom_smooth(method = "lm") +
```

```
facet_wrap(~variable, scales = "free") +
labs(x = "Duration of Amenorrhea in Trans group", y = "Alpha diversity value")
```
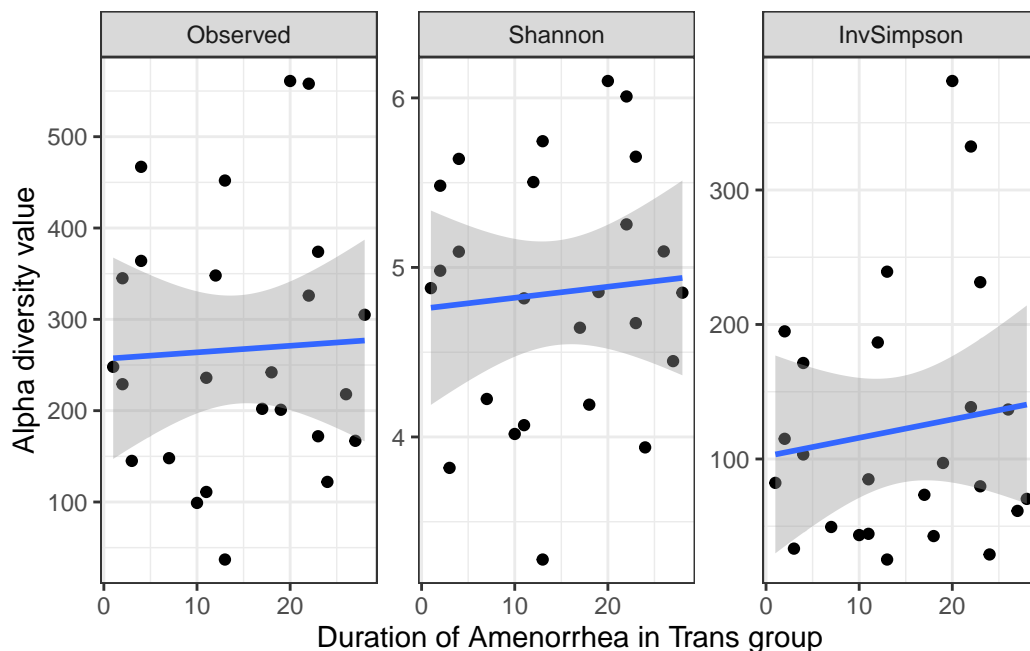


```
corr_postmeno = filter(adiv_menopause, grp =="01_postmenopausal") %>%
  group_by(variable) %>%
  rstatix::cor_test(value,DurationMenopause,method = "pearson")
kable(corr_postmeno)
```

variable	var1	var2	cor	statistic	p	conf.low	conf.high	method
Observed	value	DurationMenopause	0.045	0.2158134	0.831	-0.3565088	0.4324048	Pearson
Shannon	value	DurationMenopause	0.078	0.3754553	0.711	-0.3271717	0.4590238	Pearson
InvSimpson	value	DurationMenopause	0.130	0.6169177	0.543	-0.2817497	0.4976290	Pearson

```
# correlations are not signficiant for trans and postmenopausal group separately

# Next, both groups are combined: in months
```

```r
corr_menopause = adiv_menopause %>%
  group_by(variable) %>%
  rstatix::cor_test(value, DurationCombined, method = "pearson")
kable(corr_menopause)
```

variable	var1	var2	cor	statistic	p	conf.low	conf.high	method
Observed	value	DurationCombined	0.43	-3.298352	0.00184	-0.6325179	-0.1720921	Pearson
Shannon	value	DurationCombined	0.37	-2.766912	0.00801	-0.5884815	-0.1031910	Pearson
InvSimpson	value	DurationCombined	0.26	-1.900510	0.06340	-0.5056562	0.0149028	Pearson

```r
comb_menopause_plot = ggplot(adiv_menopause, aes(x = DurationCombined, y = value)) +
  geom_point(aes(color = grp)) +
  geom_smooth(method = "lm", color = "darkgrey") +
  facet_wrap(~variable, scales = "free") +
  labs(x = "Duration of Amenorrhea in postmenopausal and trans group (months)", y = "Alpha
  scale_color_manual(values = custom_pal[c(1,3)])


ggsave(plot = comb_menopause_plot,device = "pdf",
       filename = "MbVagTrans_Results_files/figure-html/duration-menopause_comb.pdf", heig

## Maaslin2 analysis of abundance with duration of amenorrhea
ps_menopause_rel_genus = tax_glom(ps_menopause_rel, taxrank = "Genus")

genus_MenoPauseDurationMonths = Maaslin2(input_data = otu_table(ps_menopause_rel_genus),
          input_metadata = as(sample_data(ps_menopause_rel_genus), "data.frame"),
          output = "results/maaslin2/genus_MenopausedurationMonths",
          min_abundance = 5,
          min_prevalence = 0.2,
          max_significance = 0.2,
          fixed_effects = "DurationCombined")
```

```
2024-01-19 19:52:59.598506 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_MenopausedurationMonths/maas
2024-01-19 19:52:59.808319 INFO::Writing function arguments to log file
2024-01-19 19:52:59.810921 INFO::Verifying options selected are valid
2024-01-19 19:52:59.811227 INFO::Determining format of input files
```

```
2024-01-19 19:52:59.811508 INFO::Input format is data samples as rows and metadata samples as
2024-01-19 19:52:59.813767 INFO::Formula for fixed effects: expr ~  DurationCombined
2024-01-19 19:52:59.814124 INFO::Filter data based on min abundance and min prevalence
2024-01-19 19:52:59.8144 INFO::Total samples in data: 50
2024-01-19 19:52:59.81466 INFO::Min samples required with min abundance for a feature not to
2024-01-19 19:52:59.816559 INFO::Total filtered features: 292
2024-01-19 19:52:59.816954 INFO::Filtered feature names from abundance and prevalence filter:
2024-01-19 19:52:59.817489 INFO::Total filtered features with variance filtering: 0
2024-01-19 19:52:59.817774 INFO::Filtered feature names from variance filtering:
2024-01-19 19:52:59.818036 INFO::Running selected normalization method: TSS
2024-01-19 19:52:59.818515 INFO::Applying z-score to standardize continuous metadata
2024-01-19 19:52:59.821326 INFO::Running selected transform method: LOG
2024-01-19 19:52:59.821828 INFO::Running selected analysis method: LM
2024-01-19 19:52:59.822153 INFO::Fitting model to feature number 1, ASV14
2024-01-19 19:52:59.823439 INFO::Fitting model to feature number 2, ASV54
2024-01-19 19:52:59.824586 INFO::Fitting model to feature number 3, ASV82
2024-01-19 19:52:59.825699 INFO::Fitting model to feature number 4, ASV205
2024-01-19 19:52:59.826801 INFO::Fitting model to feature number 5, ASV210
2024-01-19 19:52:59.827869 INFO::Fitting model to feature number 6, ASV369
2024-01-19 19:52:59.831383 INFO::Counting total values for each feature
2024-01-19 19:52:59.832293 INFO::Writing filtered data to file results/maaslin2/genus_Menopau
2024-01-19 19:52:59.833223 INFO::Writing filtered, normalized data to file results/maaslin2/g
2024-01-19 19:52:59.834037 INFO::Writing filtered, normalized, transformed data to file resul
2024-01-19 19:52:59.834884 WARNING::Deleting existing residuals file: results/maaslin2/genus_
2024-01-19 19:52:59.835369 INFO::Writing residuals to file results/maaslin2/genus_Menopausedu
2024-01-19 19:52:59.835902 WARNING::Deleting existing fitted file: results/maaslin2/genus_Men
2024-01-19 19:52:59.836321 INFO::Writing fitted values to file results/maaslin2/genus_Menopau
2024-01-19 19:52:59.8368 INFO::Writing all results to file (ordered by increasing q-values):
2024-01-19 19:52:59.837489 INFO::Writing the significant results (those which are less than
2024-01-19 19:52:59.837978 INFO::Writing heatmap of significant results to file: results/maas
[1] "There is not enough metadata in the associations to create a heatmap plot. Please revie
2024-01-19 19:52:59.838642 INFO::Writing association plots (one for each significant associat
2024-01-19 19:52:59.839342 INFO::Plotting associations from most to least significant, groupe
2024-01-19 19:52:59.839902 INFO::Plotting data for metadata number 1, DurationCombined
2024-01-19 19:52:59.840808 INFO::Creating scatter plot for continuous data, DurationCombined

Warning: Removed 1 rows containing missing values (`geom_point()`).

2024-01-19 19:52:59.925146 INFO::Creating scatter plot for continuous data, DurationCombined

Warning: Removed 1 rows containing missing values (`geom_point()`).
```

```
2024-01-19 19:53:00.007827 INFO::Creating scatter plot for continuous data, DurationCombined

2024-01-19 19:53:00.087426 INFO::Creating scatter plot for continuous data, DurationCombined
```

```
Warning: Removed 1 rows containing missing values (`geom_point()`).
Removed 1 rows containing missing values (`geom_point()`).
Removed 1 rows containing missing values (`geom_point()`).
Removed 1 rows containing missing values (`geom_point()`).
```

```r
# 4 ASVs

tax_table(ps_menopause_rel_genus) %>%
  as.data.frame() %>%
  rownames_to_column("ASV") %>%
  filter(ASV %in% filter(genus_MenoPauseDurationMonths$results, qval < 0.2)$feature)
```

```
    ASV  Kingdom       Phylum          Class
1  ASV14 Bacteria    Firmicutes        Bacilli
2  ASV54 Bacteria Bacteroidota    Bacteroidia
3 ASV205 Bacteria    Firmicutes Negativicutes
4 ASV369 Bacteria    Firmicutes     Clostridia
                                Order          Family        Genus Species
1                     Lactobacillales Lactobacillaceae Lactobacillus    <NA>
2                       Bacteroidales   Prevotellaceae    Prevotella    <NA>
3       Veillonellales-Selenomonadales  Veillonellaceae     Dialister    <NA>
4 Peptostreptococcales-Tissierellales        Family XI   Anaerococcus    <NA>
```

```r
# Lactobacillus, dialister, Anaerococcus, Prevotella

# plot DurationCombined vs these 4 genera
psmelt_rel_genus_durationComb = psmelt(ps_menopause_rel_genus)
maaslin_res_genus_durationComb = ggplot(filter(psmelt_rel_genus_durationComb, OTU %in% fil
                             aes(x = DurationCombined, y = Abundance,color = grp)) +
  geom_jitter(size = 2) +
  facet_wrap(~Genus, scales = "free") +
  scale_y_continuous(limits = function(x){c(0, max(0.1, x))}) +
  theme(legend.position = "none")+
  labs(x = "Sexually Active",
       y = "Genus Abundance (%)",
       caption = "Maaslin2 results")
```

```
maaslin_res_genus_durationComb
```

Warning: Removed 12 rows containing missing values (`geom_point()`).
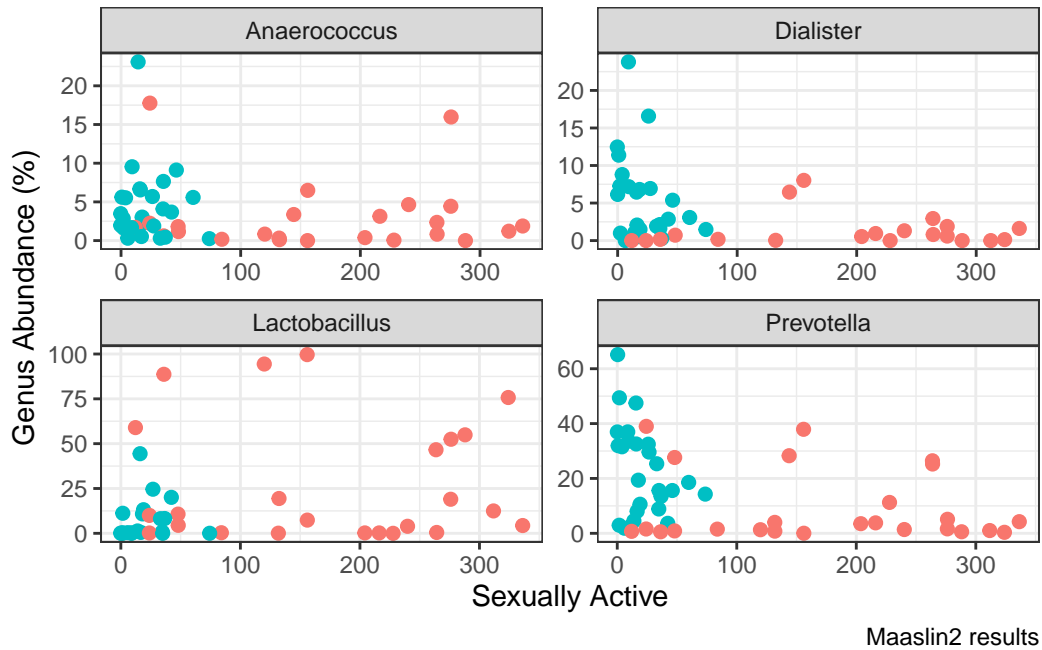
Maaslin2 results

```
ggsave(maaslin_res_genus_durationComb, filename = "results/maaslin2/genus_DurationMenopaus
       height = 5, width = 6)
```

Warning: Removed 13 rows containing missing values (`geom_point()`).

6.1 Influence of sex of partner

```
## Analysis in complete cohort
### alpha diversity
rstatix::shapiro_test(filter(adiv$data, variable=="Observed"), value)
```

```
# A tibble: 1 x 3
  variable statistic          p
```

```
  <chr>         <dbl>     <dbl>
1 value         0.903 0.0000290
```

```r
rstatix::shapiro_test(filter(adiv$data, variable=="Shannon"), value)
```

```
# A tibble: 1 x 3
  variable statistic      p
  <chr>        <dbl>  <dbl>
1 value        0.960 0.0184
```

```r
rstatix::shapiro_test(filter(adiv$data, variable=="InvSimpson"), value)
```

```
# A tibble: 1 x 3
  variable statistic           p
  <chr>        <dbl>       <dbl>
1 value        0.859 0.000000647
```

```r
adiv$data %>%
  filter(variable %in% c("Observed", "Shannon", "InvSimpson")) %>%
  group_by(variable) %>%
  rstatix::kruskal_test(value~SexOfPartner)
```

```
# A tibble: 3 x 7
  variable   .y.     n statistic    df     p method
* <fct>      <chr> <int>     <dbl> <int> <dbl> <chr>
1 Observed   value    75     -27.4     2     1 Kruskal-Wallis
2 Shannon    value    75     -26.4     2     1 Kruskal-Wallis
3 InvSimpson value    75     -27.5     2     1 Kruskal-Wallis
```

```r
adiv$data %>%
  filter(variable %in% c("Observed", "Shannon", "InvSimpson")) %>%
  group_by(variable) %>%
  rstatix::anova_test(value~SexOfPartner)
```

```
# A tibble: 3 x 8
  variable   Effect          DFn   DFd     F           p `p<.05`   ges
* <fct>      <chr>         <dbl> <dbl> <dbl>       <dbl> <chr>    <dbl>
```

```
1 Observed    SexOfPartner    3    71 13.3  0.000000566 *       0.359
2 Shannon     SexOfPartner    3    71  8.50 0.0000674   *       0.264
3 InvSimpson SexOfPartner     3    71  4.21 0.008        *       0.151
```

Observed is not distributed normally, and no significant effect of sex of partner is detected using Kruskal Wallis test. However, Shannon and InvSimpson indices show a significant effect.

```r
adonis_bc_partnersex = vegan::adonis2(D_BC ~ phyloseq::sample_data(ps_filtered_rel)$SexOfP
adonis_pw_bc_partnersex = pairwise.adonis(D_BC, phyloseq::sample_data(ps_filtered_rel)$Sex

adonis_uf_partnersex = vegan::adonis2(D_UF ~ phyloseq::sample_data(ps_filtered_rel)$SexOfP
adonis_pw_uf_partnersex = pairwise.adonis(D_UF, phyloseq::sample_data(ps_filtered_rel)$Sex

adonis_wUF_partnersex = vegan::adonis2(D_wUF ~ phyloseq::sample_data(ps_filtered_rel)$SexO
adonis_pw_wUF_partnersex = pairwise.adonis(D_wUF, phyloseq::sample_data(ps_filtered_rel)$S

kable(adonis_pw_bc, digits = 3, caption = "Bray-Curtis")
```

Table 22: Bray-Curtis

pairs	Df	SumsOfSqsF.Model	R2	p.value	p.adjusted	sig
01_postmenopausal vs 02_premenopausal	1	1.803 4.965	0.094	0.001	0.003	*
01_postmenopausal vs 03_trans	1	1.411 3.997	0.077	0.001	0.003	*
02_premenopausal vs 03_trans	1	3.513 11.173	0.189	0.001	0.003	*

```r
kable(adonis_pw_uf, digits = 3, caption ="Unweigthed Unifrac")
```

Table 23: Unweigthed Unifrac

pairs	Df	SumsOfSqsF.Model	R2	p.value	p.adjusted	sig
01_postmenopausal vs 02_premenopausal	1	1.096 5.754	0.107	0.001	0.003	*
01_postmenopausal vs 03_trans	1	0.888 5.901	0.109	0.001	0.003	*
02_premenopausal vs 03_trans	1	2.559 16.713	0.258	0.001	0.003	*

```r
kable(adonis_pw_wUF, digits = 3, caption = "Weighted Unifrac")
```

Table 24: Weighted Unifrac

pairs	Df	SumsOfSqsF.Model	R2	p.value	p.adjusted	sig
01_postmenopausal vs 02_premenopausal	1	0.583	6.608	0.121	0.001	0.003	*
01_postmenopausal vs 03_trans	1	0.995	8.073	0.144	0.001	0.003	*
02_premenopausal vs 03_trans	1	2.563	30.133	0.386	0.001	0.003	*

Adonis identifies significant influence of sex of partner on bray curtis dissimilarities overall. Pairwise uncorrected p value is only significant for comparison of M vs M+F and borderline sig for comaprison of M vs F.

Using unweighted Unicfrac, there is a uncorrected singificant p value of 0.021 for the comparison of male vs female.

```
genus_SexOfPartner = Maaslin2(input_data = otu_table(ps_rel_genus_sexuallyactive),
         input_metadata = as(sample_data(ps_rel_genus_sexuallyactive), "data.frame"),
         output = "results/maaslin2/genus_SexOfPartner",
         min_abundance = 5,
         min_prevalence = 0.2,
         max_significance = 0.2,
         fixed_effects = "SexOfPartner")
```

```
2024-01-19 19:53:01.986982 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_SexOfPartner/maaslin2.log"
2024-01-19 19:53:02.21887 INFO::Writing function arguments to log file
2024-01-19 19:53:02.221494 INFO::Verifying options selected are valid
2024-01-19 19:53:02.223644 INFO::Determining format of input files
2024-01-19 19:53:02.224042 INFO::Input format is data samples as rows and metadata samples a
2024-01-19 19:53:02.231397 INFO::Formula for fixed effects: expr ~  SexOfPartner
2024-01-19 19:53:02.231785 INFO::Factor detected for categorial metadata 'SexOfPartner'. Pro
2024-01-19 19:53:02.232059 INFO::Filter data based on min abundance and min prevalence
2024-01-19 19:53:02.232328 INFO::Total samples in data: 72
2024-01-19 19:53:02.232595 INFO::Min samples required with min abundance for a feature not to
2024-01-19 19:53:02.234547 INFO::Total filtered features: 294
2024-01-19 19:53:02.234916 INFO::Filtered feature names from abundance and prevalence filter
2024-01-19 19:53:02.235443 INFO::Total filtered features with variance filtering: 0
2024-01-19 19:53:02.235745 INFO::Filtered feature names from variance filtering:
2024-01-19 19:53:02.236011 INFO::Running selected normalization method: TSS
2024-01-19 19:53:02.2365 INFO::Applying z-score to standardize continuous metadata
2024-01-19 19:53:02.239057 INFO::Running selected transform method: LOG
```

```
2024-01-19 19:53:02.239511 INFO::Running selected analysis method: LM
2024-01-19 19:53:02.239829 INFO::Fitting model to feature number 1, ASV1
2024-01-19 19:53:02.241259 INFO::Fitting model to feature number 2, ASV54
2024-01-19 19:53:02.242515 INFO::Fitting model to feature number 3, ASV66
2024-01-19 19:53:02.243721 INFO::Fitting model to feature number 4, ASV87
2024-01-19 19:53:02.247111 INFO::Counting total values for each feature
2024-01-19 19:53:02.247985 INFO::Writing filtered data to file results/maaslin2/genus_SexOfPa
2024-01-19 19:53:02.249217 INFO::Writing filtered, normalized data to file results/maaslin2/g
2024-01-19 19:53:02.250133 INFO::Writing filtered, normalized, transformed data to file resul
2024-01-19 19:53:02.251008 WARNING::Deleting existing residuals file: results/maaslin2/genus_
2024-01-19 19:53:02.251538 INFO::Writing residuals to file results/maaslin2/genus_SexOfPartne
2024-01-19 19:53:02.25209 WARNING::Deleting existing fitted file: results/maaslin2/genus_SexO
2024-01-19 19:53:02.252516 INFO::Writing fitted values to file results/maaslin2/genus_SexOfPa
2024-01-19 19:53:02.25296 INFO::Writing all results to file (ordered by increasing q-values)
2024-01-19 19:53:02.253686 INFO::Writing the significant results (those which are less than
2024-01-19 19:53:02.254158 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2024-01-19 19:53:02.254738 INFO::Writing association plots (one for each significant associat
2024-01-19 19:53:02.255423 INFO::Plotting associations from most to least significant, groupe
2024-01-19 19:53:02.255767 INFO::Plotting data for metadata number 1, SexOfPartner
2024-01-19 19:53:02.256537 INFO::Creating boxplot for categorical data, SexOfPartner vs ASV54
```

```
  genus_SexOfPartner2  = Maaslin2(input_data = otu_table(ps_rel_genus_sexuallyactive),
            input_metadata = as(sample_data(ps_rel_genus_sexuallyactive), "data.frame"),
            output = "results/maaslin2/genus_SexOfPartnergrp",
            min_abundance = 5,
            min_prevalence = 0.2,
            max_significance = 0.2,
            fixed_effects = c("SexOfPartner", "grp", "SexOfPartner*grp"))
```

```
2024-01-19 19:53:02.419353 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_SexOfPartnergrp/maaslin2.log
2024-01-19 19:53:02.63361 INFO::Writing function arguments to log file
2024-01-19 19:53:02.636282 INFO::Verifying options selected are valid
2024-01-19 19:53:02.636595 INFO::Determining format of input files
2024-01-19 19:53:02.636883 INFO::Input format is data samples as rows and metadata samples as
2024-01-19 19:53:02.639273 WARNING::Feature name not found in metadata so not applied to form
2024-01-19 19:53:02.639729 INFO::Formula for fixed effects: expr ~  SexOfPartner + grp
2024-01-19 19:53:02.640068 INFO::Factor detected for categorial metadata 'SexOfPartner'. Prov
2024-01-19 19:53:02.640339 INFO::Factor detected for categorial metadata 'grp'. Provide a ref
2024-01-19 19:53:02.640588 INFO::Filter data based on min abundance and min prevalence
```

```
2024-01-19 19:53:02.640844 INFO::Total samples in data: 72
2024-01-19 19:53:02.641099 INFO::Min samples required with min abundance for a feature not t
2024-01-19 19:53:02.642994 INFO::Total filtered features: 294
2024-01-19 19:53:02.643363 INFO::Filtered feature names from abundance and prevalence filter:
2024-01-19 19:53:02.643877 INFO::Total filtered features with variance filtering: 0
2024-01-19 19:53:02.644164 INFO::Filtered feature names from variance filtering:
2024-01-19 19:53:02.644428 INFO::Running selected normalization method: TSS
2024-01-19 19:53:02.644913 INFO::Applying z-score to standardize continuous metadata
2024-01-19 19:53:02.64768 INFO::Running selected transform method: LOG
2024-01-19 19:53:02.648133 INFO::Running selected analysis method: LM
2024-01-19 19:53:02.648464 INFO::Fitting model to feature number 1, ASV1
2024-01-19 19:53:02.649993 INFO::Fitting model to feature number 2, ASV54
2024-01-19 19:53:02.651319 INFO::Fitting model to feature number 3, ASV66
2024-01-19 19:53:02.65264 INFO::Fitting model to feature number 4, ASV87
2024-01-19 19:53:02.656293 INFO::Counting total values for each feature
2024-01-19 19:53:02.657309 INFO::Writing filtered data to file results/maaslin2/genus_SexOfPa
2024-01-19 19:53:02.658226 INFO::Writing filtered, normalized data to file results/maaslin2/g
2024-01-19 19:53:02.659033 INFO::Writing filtered, normalized, transformed data to file resu
2024-01-19 19:53:02.659865 WARNING::Deleting existing residuals file: results/maaslin2/genus_
2024-01-19 19:53:02.66036 INFO::Writing residuals to file results/maaslin2/genus_SexOfPartner
2024-01-19 19:53:02.660888 WARNING::Deleting existing fitted file: results/maaslin2/genus_Sex
2024-01-19 19:53:02.661307 INFO::Writing fitted values to file results/maaslin2/genus_SexOfPa
2024-01-19 19:53:02.661759 INFO::Writing all results to file (ordered by increasing q-values)
2024-01-19 19:53:02.662515 INFO::Writing the significant results (those which are less than
2024-01-19 19:53:02.66301 INFO::Writing heatmap of significant results to file: results/maas
2024-01-19 19:53:02.696136 INFO::Writing association plots (one for each significant associat
2024-01-19 19:53:02.69717 INFO::Plotting associations from most to least significant, groupe
2024-01-19 19:53:02.697558 INFO::Plotting data for metadata number 1, grp
2024-01-19 19:53:02.698216 INFO::Creating boxplot for categorical data, grp vs ASV54

2024-01-19 19:53:02.771064 INFO::Creating boxplot for categorical data, grp vs ASV87

2024-01-19 19:53:02.853809 INFO::Creating boxplot for categorical data, grp vs ASV1

2024-01-19 19:53:02.930657 INFO::Creating boxplot for categorical data, grp vs ASV1

2024-01-19 19:53:03.007428 INFO::Creating boxplot for categorical data, grp vs ASV54

2024-01-19 19:53:03.474507 INFO::Plotting data for metadata number 2, SexOfPartner
2024-01-19 19:53:03.475424 INFO::Creating boxplot for categorical data, SexOfPartner vs ASV87
```

```
tax_table(ps_rel_genus_sexuallyactive) %>%
  as.data.frame() %>%
  rownames_to_column("ASV") %>%
  filter(ASV %in% filter(genus_SexOfPartner2$results, qval < 0.2)$feature)
```

```
    ASV  Kingdom        Phylum        Class           Order          Family
1  ASV1 Bacteria    Firmicutes      Bacilli Lactobacillales Lactobacillaceae
2 ASV54 Bacteria Bacteroidota Bacteroidia    Bacteroidales    Prevotellaceae
3 ASV87 Bacteria    Firmicutes      Bacilli Lactobacillales Streptococcaceae
          Genus Species
1 Lactobacillus    <NA>
2    Prevotella    <NA>
3 Streptococcus    <NA>
```

```
maaslin_res_genus_sexpartner = ggplot(filter(psmelt_rel_genus_sexuallyactive, OTU %in% fil
                            aes(x = SexOfPartner, y = Abundance,fill = SexOfPartner))
  geom_jitter() +
  geom_boxplot(alpha = 0.7, outlier.shape = NA) +
  facet_grid(grp~Genus, scales = "free") +
  scale_y_continuous(limits = function(x){c(0, max(0.1, x))}) +
  theme(legend.position = "none")+
  labs(x = "Sex of Partner",
       y = "Genus Abundance (%)",
       caption = "Maaslin2 results") +
      scale_y_continuous(expand = expansion(mult = c(0, 0.2)))
```

```
maaslin_res_genus_sexpartner
```
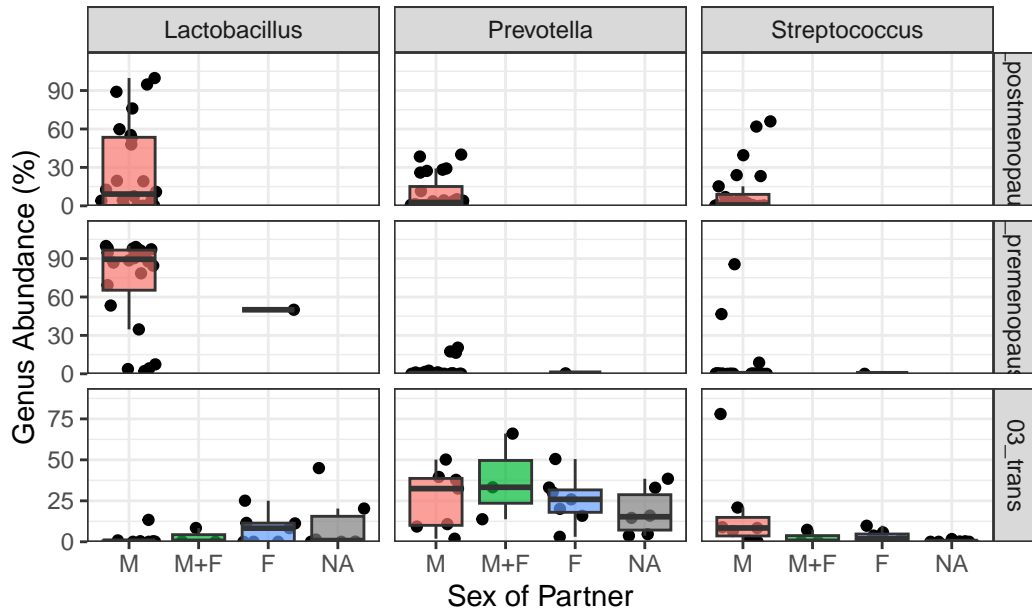
Maaslin2 results

```r
ggsave(maaslin_res_genus_sexpartner, filename = "results/maaslin2/genus_SexOfPartner_panel
        height = 5, width = 6)



# M/F distinction only in trans - filter for this group
ps_rel_genus_sexuallyactive_trans = subset_samples(ps_rel_genus_sexuallyactive, grp =="03_
genus_SexOfPartnerTrans = Maaslin2(input_data = otu_table(ps_rel_genus_sexuallyactive_tran
        input_metadata = as(sample_data(ps_rel_genus_sexuallyactive_trans), "data.frame")
        output = "results/maaslin2/genus_SexOfPartnerTrans",
        min_abundance = 5,
        min_prevalence = 0.2,
        max_significance = 0.2,
        fixed_effects = "SexOfPartner")
```

```
2024-01-19 19:53:04.197763 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_SexOfPartnerTrans/maaslin2.l
2024-01-19 19:53:04.409465 INFO::Writing function arguments to log file
2024-01-19 19:53:04.412178 INFO::Verifying options selected are valid
2024-01-19 19:53:04.412486 INFO::Determining format of input files
2024-01-19 19:53:04.412765 INFO::Input format is data samples as rows and metadata samples as
2024-01-19 19:53:04.414923 INFO::Formula for fixed effects: expr ~  SexOfPartner
2024-01-19 19:53:04.415266 INFO::Factor detected for categorial metadata 'SexOfPartner'. Pro
```

```
2024-01-19 19:53:04.415527 INFO::Filter data based on min abundance and min prevalence
2024-01-19 19:53:04.415786 INFO::Total samples in data: 23
2024-01-19 19:53:04.416039 INFO::Min samples required with min abundance for a feature not to
2024-01-19 19:53:04.417806 INFO::Total filtered features: 291
2024-01-19 19:53:04.418164 INFO::Filtered feature names from abundance and prevalence filter:
2024-01-19 19:53:04.418706 INFO::Total filtered features with variance filtering: 0
2024-01-19 19:53:04.418987 INFO::Filtered feature names from variance filtering:
2024-01-19 19:53:04.419246 INFO::Running selected normalization method: TSS
2024-01-19 19:53:04.419698 INFO::Applying z-score to standardize continuous metadata
2024-01-19 19:53:04.422279 INFO::Running selected transform method: LOG
2024-01-19 19:53:04.422753 INFO::Running selected analysis method: LM
2024-01-19 19:53:04.423079 INFO::Fitting model to feature number 1, ASV1
2024-01-19 19:53:04.42444 INFO::Fitting model to feature number 2, ASV54
2024-01-19 19:53:04.425639 INFO::Fitting model to feature number 3, ASV66
2024-01-19 19:53:04.426807 INFO::Fitting model to feature number 4, ASV87
2024-01-19 19:53:04.427963 INFO::Fitting model to feature number 5, ASV205
2024-01-19 19:53:04.429115 INFO::Fitting model to feature number 6, ASV369
2024-01-19 19:53:04.430261 INFO::Fitting model to feature number 7, ASV397
2024-01-19 19:53:04.433915 INFO::Counting total values for each feature
2024-01-19 19:53:04.434978 INFO::Writing filtered data to file results/maaslin2/genus_SexOfPa
2024-01-19 19:53:04.435963 INFO::Writing filtered, normalized data to file results/maaslin2/g
2024-01-19 19:53:04.436657 INFO::Writing filtered, normalized, transformed data to file resu
2024-01-19 19:53:04.437392 WARNING::Deleting existing residuals file: results/maaslin2/genus_
2024-01-19 19:53:04.4379 INFO::Writing residuals to file results/maaslin2/genus_SexOfPartner7
2024-01-19 19:53:04.438428 WARNING::Deleting existing fitted file: results/maaslin2/genus_Sex
2024-01-19 19:53:04.438847 INFO::Writing fitted values to file results/maaslin2/genus_SexOfPa
2024-01-19 19:53:04.439279 INFO::Writing all results to file (ordered by increasing q-values)
2024-01-19 19:53:04.440025 INFO::Writing the significant results (those which are less than d
2024-01-19 19:53:04.440442 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2024-01-19 19:53:04.441002 INFO::Writing association plots (one for each significant associat
[1] "There are no associations to plot!"

  # no significant results in trans group only
```