

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

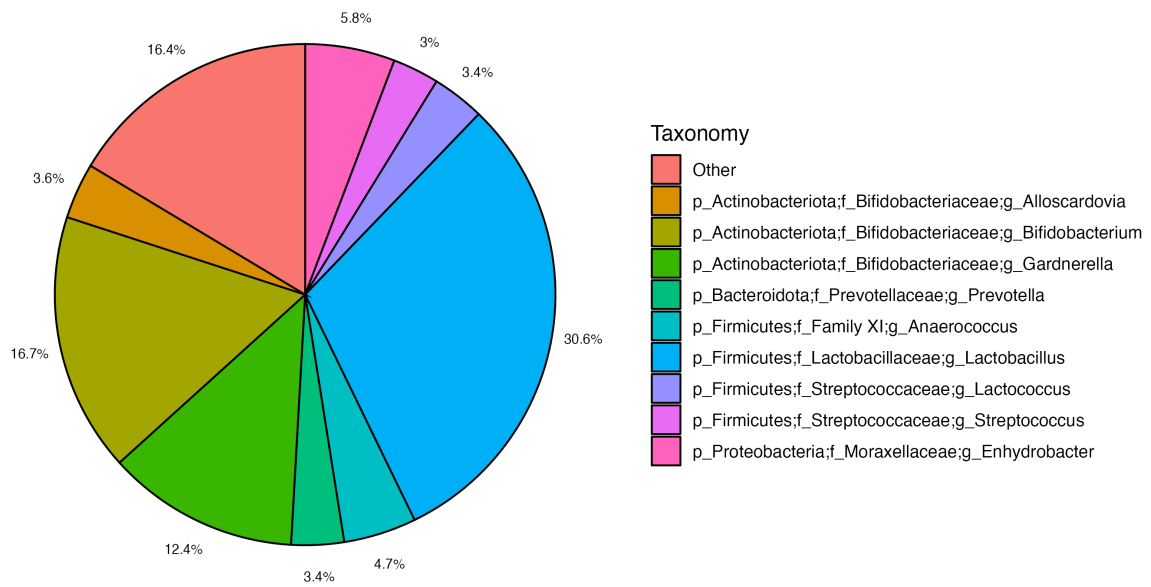


Figure 1: Taxonomic composition of PBS sample

```
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.

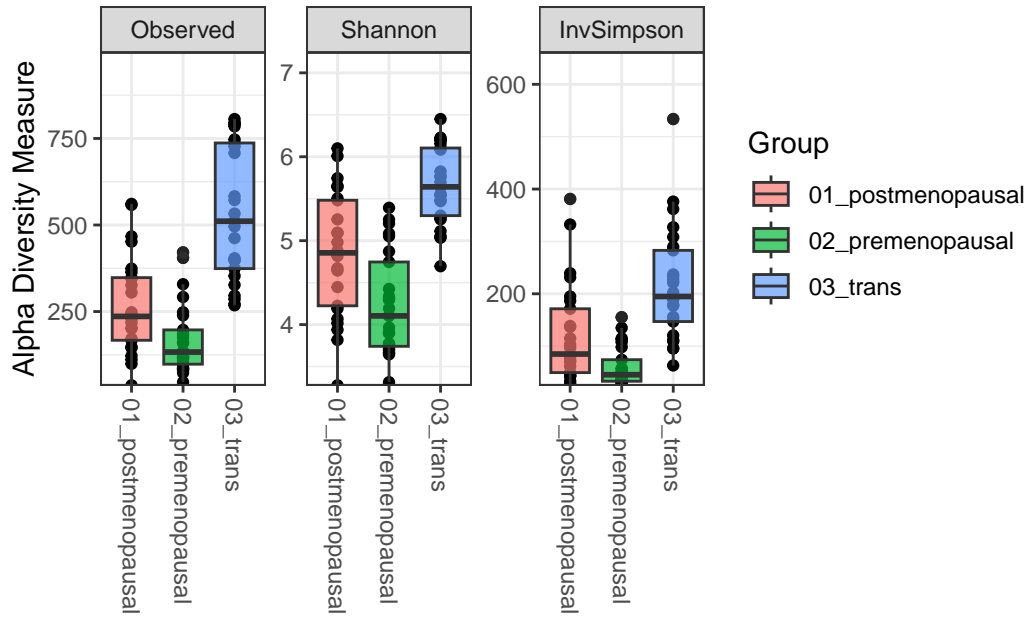
In this dataset, alpha diversity is markedly different between the group of interest (i.e. *03_trans*) and the two control groups. However, the intervention group is more similar to the first control group (i.e. *postmenopausal women*).

```
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)))
```



```
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

```
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*.

```
# 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 ]
```

```
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")
```

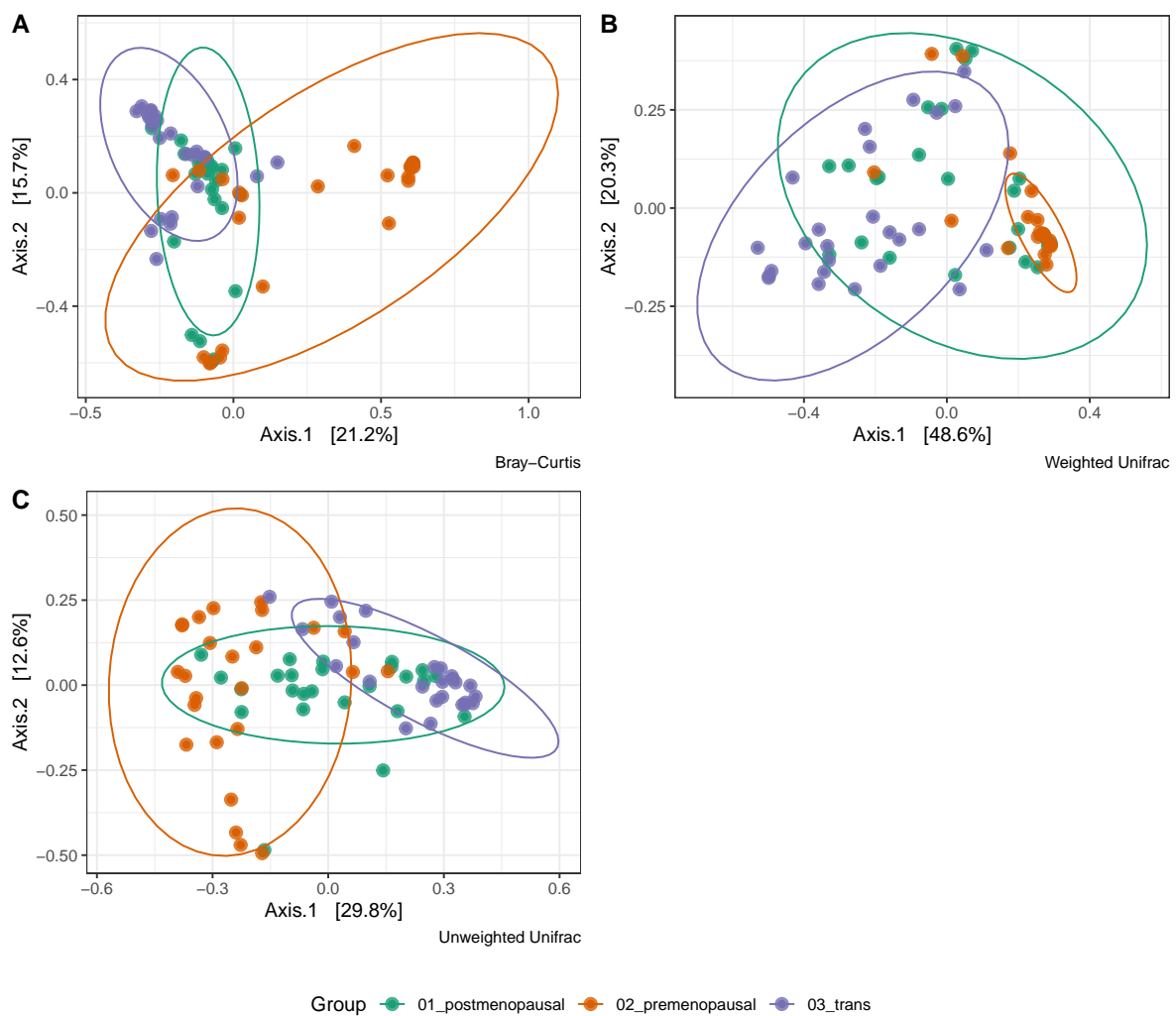
```
# extract the legend from one of the plots
legend_b <- get_legend(
  p1 +
  guides(color = guide_legend(nrow = 1)) +
  theme(legend.position = "bottom")
```


)

```
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))
```



```

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	*

```

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	*

```

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

```

Table 8: 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	*

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
ASV174	3574.694	1.528	3.072	0.002	0.008	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV267	7164.210	1.454	2.895	0.004	0.013	0.013	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV367	1723.754	1.422	2.639	0.008	0.023	0.023	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV464	6094.388	1.475	2.975	0.003	0.010	0.010	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus

ASV	baseMean	log2FoldChange	negLog10Pval	negLog10Padj	Kingdom	Phylum	Class	Order	Family	Genus		
ASV55	8.9674	1.5023	0.0550	0.0020	0.009	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV62	1.674	1.4483	0.0690	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV75	8.4584	1.4342	0.8830	0.0040	0.013	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV85	8.5634	1.5003	0.1120	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV95	6.4434	1.4853	0.1160	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV156	6.4324	1.5012	0.8500	0.0040	0.014	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV156	1.334	1.4302	0.8100	0.0050	0.016	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV156	2.2984	1.5102	0.9580	0.0030	0.011	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV153	1.694	1.4413	0.2890	0.0010	0.005	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV151	1.2413	1.4512	0.6600	0.0080	0.022	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV147	3.3954	1.4822	0.8950	0.0040	0.013	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV148	6.634	1.4403	0.0830	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV147	2.534	1.4773	0.1480	0.0020	0.007	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV246	1.774	1.3953	0.0770	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV243	1.1014	1.4553	0.1720	0.0020	0.007	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV241	2.9824	1.5202	0.7860	0.0050	0.017	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV242	2.9054	1.3823	0.2170	0.0010	0.006	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV340	0.8994	1.4143	0.1410	0.0020	0.007	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV339	2.904	1.4952	0.8320	0.0050	0.015	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV339	1.614	1.4613	0.0890	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV337	3.8594	1.4973	0.0120	0.0030	0.009	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV338	1.474	1.4793	0.1150	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV430	3.044	1.4752	0.8720	0.0040	0.014	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV538	3.312	-	1.354	-	0.0140	0.034	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV537	3.625	-	1.333	-	0.0160	0.039	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV537	3.608	-	1.336	-	0.0130	0.033	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV534	3.361	-	1.466	-	0.0230	0.050	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV532	3.825	-	1.483	-	0.0060	0.018	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV632	3.056	-	1.415	-	0.0050	0.016	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV632	3.648	-	1.379	-	0.0050	0.017	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV631	3.023	-	1.384	-	0.0120	0.031	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV627	3.851	-	1.404	-	0.0030	0.012	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella

ASV	baseMean	log2FoldChange	negLog10Padj	Kingdom	Phylum	Class	Order	Family	Genus		
ASV627	0.069	-	1.465	-	0.0090.026	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.805		2.597								
ASV626	0.421	-	1.399	-	0.0090.024	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.679		2.629								
ASV726	0.234	-	1.342	-	0.0150.036	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.269		2.437								
ASV725	0.051	-	1.505	-	0.0200.043	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.510		2.333								
ASV722	0.871	-	1.379	-	0.0150.037	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.350		2.429								
ASV722	0.816	-	1.468	-	0.0100.028	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.757		2.560								
ASV822	0.716	-	1.432	-	0.0220.049	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.270		2.283								
ASV822	0.808	-	1.500	-	0.0050.017	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	4.167		2.778								
ASV822	0.674	-	1.453	-	0.0140.034	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.577		2.462								
ASV920	0.463	-	1.440	-	0.0180.043	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.392		2.356								
ASV100	0.793	-	1.414	-	0.0030.009	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	4.268		3.018								
ASV122	0.144	-	1.444	-	0.0120.032	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.610		2.500								
ASV120	0.759	-	1.455	-	0.0220.049	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Finegoldia
	3.325		2.285					Tissierellales	XI		
ASV120	0.523	-	1.743	-	0.0020.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	5.367		3.079								
ASV130	0.781	-	1.443	-	0.0190.043	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.399		2.355								
ASV130	0.041	-	1.606	-	0.0040.014	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	4.593		2.860								
ASV140	0.964	-	1.656	-	0.0000.000	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	7.262		4.385								
ASV152	0.289	-	1.779	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	7.158		4.024								
ASV155	0.947	-	1.469	-	0.0010.004	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	4.982		3.391								
ASV163	0.189	-	1.455	-	0.0000.002	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	5.168		3.551								

ASV	baseMean	log2FoldChange	negLog10Pvalue	negLog10PadjPvalue	Kingdom	Phylum	Class	Order	Family	Genus
ASV151436	-	1.351	-	0.0000.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	7.124		5.272							
ASV158300	-	1.721	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.944		4.035							
ASV175624	-	1.457	-	0.0010.004	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	4.849		3.329							
ASV175120	-	1.770	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.800		3.842							
ASV184069	-	1.452	-	0.0000.002	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	5.346		3.683							
ASV192983	-	1.651	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.993		4.237							
ASV196878	-	1.413	-	0.0000.001	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	5.670		4.011							
ASV197360	4.556	1.882	2.420	0.0160.037	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV199687	-	1.711	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.759		3.949							
ASV203564	-	1.871	-	0.0140.034	Bacteria	Firmicutes	Negativibacilli	Veillonellales- Selenomonadales	Veillonellaceae	Dialister
	4.616		2.467							
ASV203992	-	1.838	-	0.0000.002	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.638		3.612							
ASV210669	-	2.409	-	0.0140.034	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	5.944		2.467							
ASV214706	-	1.850	-	0.0000.002	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.698		3.620							
ASV220907	-	1.648	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.788		4.120							
ASV222862	-	1.518	-	0.0000.001	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	5.804		3.824							
ASV223076	-	1.431	-	0.0000.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.698		4.679							
ASV223003	-	1.422	-	0.0000.001	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	5.895		4.146							
ASV230649	-	1.644	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.779		4.124							
ASV232725	-	1.440	-	0.0000.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.607		4.588							
ASV232549	-	1.699	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.767		3.983							
ASV242202	-	1.437	-	0.0000.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.970		4.851							

ASV	baseMean	log2FoldChange	negLog10Pvalue	negLog10Pvalueadj	Kingdom	Phylum	Class	Order	Family	Genus
ASV247.258	-	1.441	-	0.0220.049	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faleis	Finegoldia
	3.295		2.286					Tissierellales	XI	
ASV250.985	-	1.770	-	0.0110.029	Bacteria	Firmicutes	Negativibacteres	Veillonellales	Veillonellaceae	Dialister
	4.498		2.542					Selenomonadales		
ASV257.002	6.402	2.096	0.0550.0020.009	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV258.211	-	1.749	-	0.0130.034	Bacteria	Firmicutes	Negativibacteres	Veillonellales	Veillonellaceae	Dialister
	4.327		2.474					Selenomonadales		
ASV261.064	-	2.078	-	0.0090.025	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	5.407		2.602							
ASV262.753	-	1.410	-	0.0000.002	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	5.113		3.626							
ASV263.502	-	1.836	-	0.0000.002	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.663		3.629							
ASV266.957	-	1.387	-	0.0000.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	7.244		5.222							
ASV270.388	-	1.495	-	0.0000.001	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	5.665		3.789							
ASV272.277	-	1.705	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.448		3.781							
ASV274.888	-	2.078	-	0.0110.029	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	5.302		2.551							
ASV275.402	-	1.761	-	0.0000.002	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.603		3.749							
ASV277.702	-	1.666	-	0.0060.017	Bacteria	Firmicutes	Negativibacteres	Veillonellales	Veillonellaceae	Dialister
	4.607		2.765					Selenomonadales		
ASV301.231	-	1.454	-	0.0000.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.591		4.532							
ASV302.778	4.772	1.962	0.4330.0150.036	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV303.355	-	2.077	-	0.0170.040	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	4.951		2.384							
ASV304.550	-	1.770	-	0.0020.007	Bacteria	Firmicutes	Negativibacteres	Veillonellales	Veillonellaceae	Dialister
	5.598		3.162					Selenomonadales		
ASV305.581	-	1.646	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.538		3.973							
ASV307.649	-	1.439	-	0.0130.033	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faleis	Finegoldia
	3.570		2.481					Tissierellales	XI	
ASV308.117	-	1.502	-	0.0110.029	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.819		2.543							
ASV309.167	-	1.821	-	0.0000.003	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.434		3.534							

ASV	baseMean	log2FoldChange	negLog10Pval	negLog10Padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV311472	-	1.334	-	0.0000.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.621		4.963							
ASV315049	-	1.901	-	0.0010.004	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.474		3.406							
ASV32322	-	1.732	-	0.0150.037	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
	4.198		2.424							
ASV32399	-	2.756	-	0.0000.000	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	24.683		8.956							
ASV326260	-	1.709	-	0.0000.000	Bacteria	Firmicutes	Clostridia	Reptostreptococcaceae	Familia	Fenollaria
	7.621		4.459					Tissierellales	XI	
ASV328403	-	1.697	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.609		3.896							
ASV330768	-	1.759	-	0.0000.002	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.550		3.724							
ASV333823	-	1.420	-	0.0000.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.456		4.545							
ASV335261	-	1.689	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.415		3.797							
ASV335987	4.962	1.951	2.544	0.0110.029	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV33530	-	1.518	-	0.0000.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.688		4.405							
ASV335892	-	1.426	-	0.0000.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.879		4.824							
ASV335595	-	1.863	-	0.0180.042	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	4.410		2.368							
ASV335607	-	1.826	-	0.0000.002	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.464		3.540							
ASV336113	-	1.399	-	0.0000.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.310		4.512							
ASV336705	-	1.652	-	0.0000.001	Bacteria	Firmicutes	Clostridia	Reptostreptococcaceae	Familia	Anaerococcus
	6.673		4.038					Tissierellales	XI	
ASV337138	-	1.757	-	0.0020.009	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
	5.341		3.040							
ASV337926	6.086	2.014	3.022	0.0030.009	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV337472	-	1.865	-	0.0070.019	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
	5.068		2.717							
ASV3378028	-	1.439	-	0.0000.001	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	5.967		4.148							
ASV3381733	-	1.749	-	0.0000.001	Bacteria	Firmicutes	Clostridia	Reptostreptococcaceae	Familia	Fenollaria
	7.241		4.141					Tissierellales	XI	

ASV	baseMean	log2FoldChange	negLog10Pval	negLog10Padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV38926	-	2.147	-	0.0090.026	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	5.573		2.596							
ASV48266	-	2.067	-	0.0160.038	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	4.984		2.412							
ASV46730	-	2.259	-	0.0220.049	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	5.167		2.288							
ASV48586	-	1.462	-	0.0000.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	7.156		4.896							
ASV41546	-	1.879	-	0.0010.006	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.049		3.220							
ASV43926	-	2.168	-	0.0070.019	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Alloscardovia
	5.876		2.710							
ASV42365	-	1.467	-	0.0000.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.250		4.261							
ASV42561	-	2.022	-	0.0060.017	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Alloscardovia
	5.611		2.775							
ASV43018	-	1.845	-	0.0000.000	Bacteria	Firmicutes	Glostridia	Peptostreptococcales	Familia	Fenollaria
	7.522		4.078					Tissierellales	XI	
ASV48228	-	1.778	-	0.0000.000	Bacteria	Firmicutes	Glostridia	Peptostreptococcales	Familia	Fenollaria
	7.325		4.119					Tissierellales	XI	
ASV46438	-	1.714	-	0.0170.039	Bacteria	Firmicutes	Negativibacteria	Veillonellales	Veillonellaceae	Dialister
	4.107		2.395					Selenomonadales		
ASV48744	-	1.932	-	0.0060.017	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Alloscardovia
	5.359		2.774							
ASV48270	-	1.869	-	0.0000.000	Bacteria	Firmicutes	Glostridia	Peptostreptococcales	Familia	Fenollaria
	7.384		3.951					Tissierellales	XI	
ASV49397	-	1.545	-	0.0000.000	Bacteria	Firmicutes	Glostridia	Peptostreptococcales	Familia	Peptoniphilus
	6.882		4.456					Tissierellales	XI	
ASV48795	-	1.735	-	0.0000.003	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.066		3.496							
ASV48072	-	1.957	-	0.0000.002	Bacteria	Firmicutes	Glostridia	Peptostreptococcales	Familia	Fenollaria
	7.278		3.719					Tissierellales	XI	
ASV45430	-	2.046	-	0.0120.031	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	5.133		2.508							
ASV46956	-	1.965	-	0.0000.002	Bacteria	Firmicutes	Glostridia	Peptostreptococcales	Familia	Fenollaria
	7.223		3.676					Tissierellales	XI	
ASV46287	-	2.131	-	0.0190.043	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	5.002		2.347							
ASV46352	-	2.223	-	0.0200.045	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	5.177		2.329							

ASV	baseMean	log2FoldChange	negLog10Pval	negLog10Padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV46141	-	1.427	-	0.0000.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	7.008		4.910							
ASV48741	4.968	1.998	2.486	0.0130.033	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV48544	-	1.918	-	0.0050.015	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Akkermansia
	5.413		2.823							
ASV48254	-	1.821	-	0.0020.009	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
	5.572		3.060					Selenomonadales		
ASV48615	-	1.743	-	0.0020.007	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
	5.458		3.131					Selenomonadales		
ASV49264	-	1.947	-	0.0000.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	7.143		3.668					Tissierellales	XI	
ASV49702	-	1.548	-	0.0000.000	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	6.716		4.339					Tissierellales	XI	
ASV50856	-	1.418	-	0.0000.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	5.438		3.835					Tissierellales	XI	
ASV52334	-	2.397	-	0.0170.041	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Akkermansia
	5.696		2.376							
ASV53965	-	2.186	-	0.0130.032	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Akkermansia
	5.449		2.493							
ASV56299	-	1.815	-	0.0000.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	6.993		3.852					Tissierellales	XI	
ASV57606	-	1.939	-	0.0000.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	7.154		3.690					Tissierellales	XI	
ASV58261	-	1.881	-	0.0000.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	7.004		3.724					Tissierellales	XI	
ASV52795	-	1.630	-	0.0030.010	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	4.893		3.002					Tissierellales	XI	
ASV57280	-	1.699	-	0.0000.002	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
	6.016		3.542					Selenomonadales		
ASV58111	-	1.511	-	0.0000.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	6.394		4.231					Tissierellales	XI	
ASV58609	-	1.770	-	0.0000.002	Bacteria	Campylobacter	Campylobacter	Campylobacteriales	Campylobacteriaceae	Campylobacter
	6.358		3.592							
ASV55891	-	1.447	-	0.0000.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	6.114		4.225					Tissierellales	XI	
ASV56332	-	1.345	-	0.0000.000	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	6.632		4.929					Tissierellales	XI	
ASV55997	-	1.708	-	0.0000.002	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
	6.177		3.617					Selenomonadales		
ASV56039	-	1.779	-	0.0010.003	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	6.099		3.428					Tissierellales	XI	

ASV	baseMean	log2FoldChange	negLog10Pval	adjPval	Kingdom	Phylum	Class	Order	Family	Genus
ASV56422	-	1.739	-	0.0000.002	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	6.426		3.695							
ASV56359	-	1.602	-	0.0000.001	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	6.548		4.088							
ASV56796	-	1.518	-	0.0000.000	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Peptoniphilus
	6.705		4.416					Tissierellales	XI	
ASV56753	-	1.792	-	0.0030.010	Bacteria	Bacteroid	Bacteroid	Bacteroidales	Prevotella	Prevotella
	5.335		2.977							
ASV56531	-	1.795	-	0.0000.001	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Anaerococcus
	6.801		3.790					Tissierellales	XI	
ASV56303	-	1.929	-	0.0010.006	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	6.260		3.245							
ASV56659	-	1.831	-	0.0000.002	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Fenollaria
	6.749		3.687					Tissierellales	XI	
ASV56374	-	2.082	-	0.0100.027	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacter	Alloscardovia
	5.372		2.581							
ASV56581	-	2.021	-	0.0020.007	Bacteria	Bacteroid	Bacteroid	Bacteroidales	Prevotella	Prevotella
	6.373		3.153							
ASV56837	-	1.817	-	0.0000.002	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Fenollaria
	6.718		3.698					Tissierellales	XI	
ASV56713	-	2.022	-	0.0010.004	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Fenollaria
	6.738		3.332					Tissierellales	XI	
ASV56754	-	1.984	-	0.0020.009	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Anaerococcus
	6.003		3.026					Tissierellales	XI	
ASV60373	-	1.497	-	0.0000.001	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Peptoniphilus
	6.334		4.231					Tissierellales	XI	
ASV60265	-	1.833	-	0.0010.004	Bacteria	Bacteroid	Bacteroid	Bacteroidales	Prevotella	Prevotella
	6.142		3.350							
ASV61423	-	2.005	-	0.0020.007	Bacteria	Bacteroid	Bacteroid	Bacteroidales	Prevotella	Prevotella
	6.314		3.150							
ASV61674	-	1.962	-	0.0010.004	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Anaerococcus
	6.636		3.382					Tissierellales	XI	
ASV61806	-	1.792	-	0.0000.001	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Fenollaria
	6.887		3.843					Tissierellales	XI	
ASV62350	-	1.499	-	0.0000.000	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Peptoniphilus
	6.543		4.365					Tissierellales	XI	
ASV62583	-	2.027	-	0.0020.008	Bacteria	Bacteroid	Bacteroid	Bacteroidales	Prevotella	Prevotella
	6.315		3.116							
ASV63387	-	2.019	-	0.0020.007	Bacteria	Bacteroid	Bacteroid	Bacteroidales	Prevotella	Prevotella
	6.332		3.136							

ASV base	log2 Fold	FC	Signif	pval	padj	Kingdm	Phylum	Class	Order	Family	Genus
ASV63766	-	2.645	-	0.017	0.040	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces
	6.323		2.391								
ASV63794	-	1.947	-	0.001	0.003	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	6.717		3.450						Tissierellales	XI	
ASV63866	-	1.646	-	0.000	0.002	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
	6.007		3.649						Selenomonadales		
ASV65184	-	1.900	-	0.001	0.003	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Anaerococcus
	6.585		3.466						Tissierellales	XI	
ASV65130	-	1.880	-	0.000	0.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	6.689		3.558						Tissierellales	XI	
ASV65746	-	1.530	-	0.000	0.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	5.438		3.554						Tissierellales	XI	
ASV65900	-	1.948	-	0.001	0.004	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Anaerococcus
	6.577		3.376						Tissierellales	XI	
ASV65224	-	1.908	-	0.000	0.003	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	6.721		3.523						Tissierellales	XI	
ASV65364	-	2.197	-	0.007	0.020	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces
	5.939		2.702								
ASV65709	-	1.601	-	0.001	0.003	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	5.480		3.422						Tissierellales	XI	
ASV66046	-	1.937	-	0.001	0.005	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Anaerococcus
	6.415		3.312						Tissierellales	XI	
ASV66334	-	1.494	-	0.000	0.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	6.197		4.147						Tissierellales	XI	
ASV66747	-	2.281	-	0.006	0.017	Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Howarthella
	6.315		2.768								
ASV66792	-	2.283	-	0.014	0.034	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Anaerococcus
	5.607		2.457						Tissierellales	XI	
ASV66841	-	1.929	-	0.001	0.005	Bacteria	Bacteroidetes	Bacteroidetes	Bacteroidales	Prevotellaceae	Prevotella
	6.274		3.253								
ASV66959	-	1.873	-	0.000	0.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	6.653		3.552						Tissierellales	XI	
ASV69363	-	1.513	-	0.000	0.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	5.828		3.852						Tissierellales	XI	
ASV70250	-	1.741	-	0.001	0.006	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
	5.609		3.221						Selenomonadales		
ASV70736	-	1.976	-	0.002	0.008	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	6.176		3.125								
ASV70884	-	1.766	-	0.000	0.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	6.665		3.774						Tissierellales	XI	

ASV	baseMean	log2FoldChange	negLog10Pval	negLog10Padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV74937	-	1.848	-	0.0000.002	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecalibacterium	Fenollaria
	6.680		3.615					Tissierellales	XI	
ASV73962	-	2.162	-	0.0070.020	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecalibacterium	Anaerococcus
	5.838		2.700					Tissierellales	XI	
ASV70448	-	1.705	-	0.0000.001	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	7.086		4.156							
ASV71620	-	1.917	-	0.0010.006	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.228		3.249							
ASV72423	-	2.056	-	0.0020.007	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecalibacterium	Anaerococcus
	6.512		3.168					Tissierellales	XI	
ASV72854	-	1.773	-	0.0000.001	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	6.890		3.885							
ASV74591	-	1.882	-	0.0000.003	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecalibacterium	Fenollaria
	6.612		3.513					Tissierellales	XI	
ASV74815	-	1.880	-	0.0000.003	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecalibacterium	Fenollaria
	6.597		3.509					Tissierellales	XI	
ASV73710	-	1.816	-	0.0020.009	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
	5.506		3.032					Selenomonadales		
ASV74657	-	1.893	-	0.0080.022	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	5.022		2.653							
ASV73956	-	2.189	-	0.0210.047	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecalibacterium	Anaerococcus
	5.046		2.305					Tissierellales	XI	
ASV75390	-	1.613	-	0.0000.002	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecalibacterium	Peptoniphilus
	6.033		3.741					Tissierellales	XI	
ASV74971	-	1.890	-	0.0010.004	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.300		3.334							
ASV75639	-	1.565	-	0.0000.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.922		4.422							
ASV76894	-	2.160	-	0.0050.017	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecalibacterium	Anaerococcus
	6.007		2.781					Tissierellales	XI	
ASV76659	-	1.601	-	0.0000.001	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
	6.144		3.837					Selenomonadales		
ASV73485	-	2.223	-	0.0060.018	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecalibacterium	Anaerococcus
	6.092		2.740					Tissierellales	XI	
ASV74951	-	1.502	-	0.0000.001	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecalibacterium	Peptoniphilus
	5.833		3.884					Tissierellales	XI	
ASV73329	-	2.228	-	0.0070.019	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecalibacterium	Anaerococcus
	6.056		2.718					Tissierellales	XI	
ASV73409	-	2.093	-	0.0050.016	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	5.862		2.800							

ASV	baseMean	log2FoldChange	negLog10Pvalue	negLog10FDR	padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV70337	-	2.053	-	0.0030.010		Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Anaerococcus
	6.118		2.980						Tissierellales	XI	
ASV70980	-	2.066	-	0.0020.009		Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	6.300		3.049								
ASV70576	-	1.662	-	0.0000.002		Bacteria	Firmicutes	Negativicoccus	Veillonellales	Veillonella	Dialister
	6.110		3.675						Selenomonadales		
ASV80402	-	2.285	-	0.0080.022		Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Ezakiella
	6.101		2.670						Tissierellales	XI	
ASV80365	-	1.794	-	0.0010.003		Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	6.153		3.430								
ASV80564	-	2.401	-	0.0160.039		Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Anaerococcus
	5.775		2.405						Tissierellales	XI	
ASV80930	-	2.044	-	0.0030.010		Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Anaerococcus
	6.075		2.973						Tissierellales	XI	
ASV81068	-	1.684	-	0.0010.004		Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Peptoniphilus
	5.638		3.348						Tissierellales	XI	
ASV82017	-	1.919	-	0.0010.005		Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Fenollaria
	6.309		3.288						Tissierellales	XI	
ASV82051	-	2.276	-	0.0090.025		Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Parvimonas
	5.944		2.612						Tissierellales	XI	
ASV82420	-	1.975	-	0.0020.008		Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.175		3.127								
ASV82473	-	1.662	-	0.0000.002		Bacteria	Firmicutes	Negativicoccus	Veillonellales	Veillonella	Dialister
	6.128		3.686						Selenomonadales		
ASV83085	-	1.742	-	0.0010.005		Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Peptoniphilus
	5.719		3.283						Tissierellales	XI	
ASV83100	-	2.198	-	0.0060.017		Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Anaerococcus
	6.097		2.775						Tissierellales	XI	
ASV84192	-	1.689	-	0.0010.003		Bacteria	Firmicutes	Negativicoccus	Veillonellales	Veillonella	Dialister
	5.777		3.420						Selenomonadales		
ASV84210	-	1.589	-	0.0010.004		Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Peptoniphilus
	5.344		3.363						Tissierellales	XI	
ASV84106	-	2.265	-	0.0100.026		Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Anaerococcus
	5.865		2.589						Tissierellales	XI	
ASV84088	-	1.734	-	0.0000.001		Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.692		3.860								
ASV84085	-	2.000	-	0.0040.014		Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	5.745		2.873								
ASV84110	-	2.056	-	0.0040.013		Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Anaerococcus
	5.918		2.879						Tissierellales	XI	

ASV	baseMean	log2FoldChange	negLog10Pvalue	negLog10Pvalueadj	Kingdom	Phylum	Class	Order	Family	Genus
ASV83970	-	2.171	-	0.0080.022	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Anaerococcus
	5.789		2.667					Tissierellales	XI	
ASV84604	-	2.177	-	0.0060.018	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Ezakiella
	5.980		2.747					Tissierellales	XI	
ASV90242	-	2.173	-	0.0060.018	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	5.982		2.753							
ASV90653	-	1.739	-	0.0000.001	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.616		3.804							
ASV91221	-	1.735	-	0.0020.009	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Peptoniphilus
	5.250		3.026					Tissierellales	XI	
ASV92347	-	1.746	-	0.0010.003	Bacteria	Firmicutes	Negativibacter	Veillonellales	Veillonellaceae	Dialister
	6.074		3.478					Selenomonadales		
ASV92492	-	1.759	-	0.0000.002	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.428		3.655							
ASV93552	-	2.030	-	0.0030.011	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Ezakiella
	6.020		2.966					Tissierellales	XI	
ASV941892	4.227	1.821	2.322	0.0200.046	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV95877	-	2.069	-	0.0050.016	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Anaerococcus
	5.817		2.811					Tissierellales	XI	
ASV97514	-	2.298	-	0.0200.045	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Parvimonas
	5.353		2.329					Tissierellales	XI	
ASV98347	-	2.364	-	0.0140.034	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Anaerococcus
	5.816		2.461					Tissierellales	XI	
ASV10229	-	2.287	-	0.0120.031	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Ezakiella
	5.743		2.511					Tissierellales	XI	
ASV10364	-	2.057	-	0.0040.015	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	5.855		2.846							
ASV10458	-	2.368	-	0.0120.031	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Parvimonas
	5.942		2.509					Tissierellales	XI	
ASV10361	-	2.037	-	0.0030.012	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Ezakiella
	5.963		2.927					Tissierellales	XI	
ASV12280	-	2.097	-	0.0050.016	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Anaerococcus
	5.882		2.805					Tissierellales	XI	
ASV12294	-	2.306	-	0.0190.044	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Anaerococcus
	5.388		2.337					Tissierellales	XI	

```

# 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) %>

```

```

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	negLog10Padj	Kingdom	Phylum	Class	Order	Family	Genus	Species
ASV26.234	-	1.350	- 0.0010.035	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	NA
	4.408		3.264							
ASV22.871	-	1.395	- 0.0000.026	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	NA
	5.000		3.584							
ASV22.716	-	1.446	- 0.0010.034	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	NA
	4.845		3.350							
ASV110.793	-	1.432	- 0.0000.026	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	NA
	5.282		3.689							
ASV25.811	-	1.776	- 0.0000.026	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister	NA
	6.944		3.910				Selenomonadales			
ASV30.450	-	1.785	- 0.0000.026	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister	NA
	6.873		3.851				Selenomonadales			
ASV32.022	-	1.754	- 0.0010.035	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister	NA
	5.740		3.272				Selenomonadales			
ASV37.138	-	1.771	- 0.0000.026	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister	NA
	6.614		3.734				Selenomonadales			
ASV39.045	-	1.858	- 0.0000.027	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister	NA
	6.536		3.517				Selenomonadales			
ASV43.012	-	1.856	- 0.0010.034	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister	NA
	6.279		3.383				Selenomonadales			
ASV43.838	-	1.739	- 0.0000.026	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister	NA
	6.417		3.691				Selenomonadales			
ASV45.659	-	1.784	- 0.0000.026	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister	NA
	6.421		3.599				Selenomonadales			
ASV45.241	-	1.843	- 0.0010.034	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister	NA
	6.084		3.301				Selenomonadales			
ASV45.854	-	1.831	- 0.0010.034	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister	NA
	6.153		3.361				Selenomonadales			
ASV45.015	-	1.754	- 0.0000.026	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister	NA
	6.246		3.560				Selenomonadales			

ASV baseMean	log2FoldChange	negLog10Padj	Kingdom	Phylum	Class	Order	Family	Genus	Species
ASV50091	- 1.896	- 0.0010.037	Bacteria	Firmicutes	Negative	Veillonellales	Veillonellaceae	Dialister	NA
	6.125	3.231				Selenomonadales			
ASV51638	- 1.828	- 0.0010.034	Bacteria	Firmicutes	Negative	Veillonellales	Veillonellaceae	Dialister	NA
	6.040	3.304				Selenomonadales			
ASV56753	- 1.804	- 0.0010.034	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	NA
	6.083	3.373							
ASV75639	- 1.562	- 0.0010.034	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	NA
	5.221	3.342							

```

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, 0.15)))

# 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)

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

kable(summarystat1)

```

Genus	grp	mean	median	sd	min	max
Actinomyces	01_postmenopausal	D.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	B.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	l.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	D.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	D.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	D.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	l.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	l.3231500	0.8786381	1.8566051	0.000000	8.0745342

Genus	grp	mean	median	sd	min	max
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
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)`

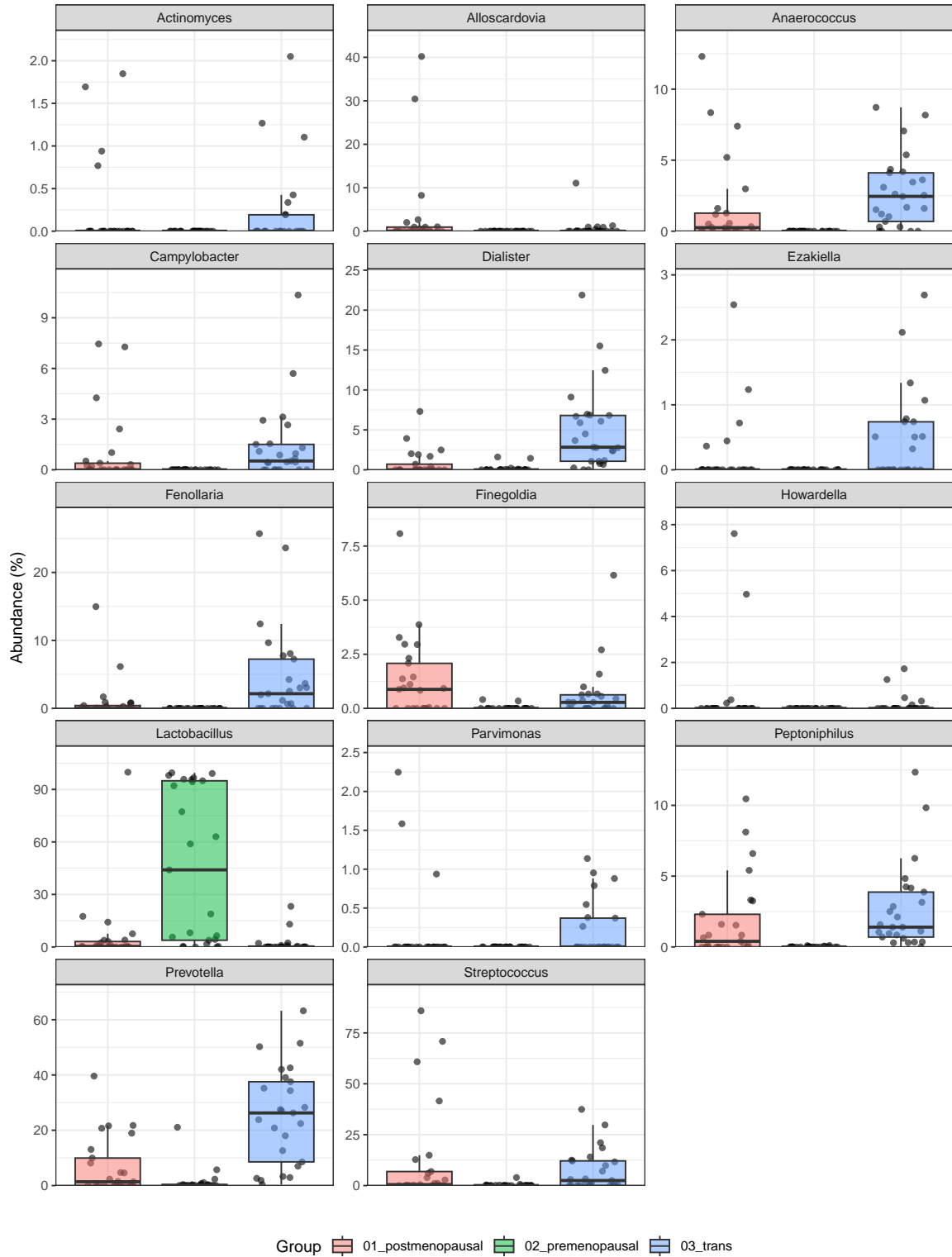
Genus	01_postmenopausal	02_premenopausal	03_trans
Actinomyces	0.21 ± 0.53	0 ± 0	0.22 ± 0.51
Alloscardovia	3.5 ± 9.83	0 ± 0	0.63 ± 2.21
Anaerococcus	1.69 ± 3.21	0 ± 0	2.72 ± 2.53
Campylobacter	0.96 ± 2.15	0 ± 0	1.4 ± 2.3
Dialister	0.82 ± 1.7	0.14 ± 0.42	4.98 ± 5.28
Ezakiella	0.21 ± 0.57	0 ± 0	0.45 ± 0.71
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 strikingly 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_fig")
```

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 counts

# 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_", Genus))
  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
```

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 ]
```

```
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 ]
```

```
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") +
```

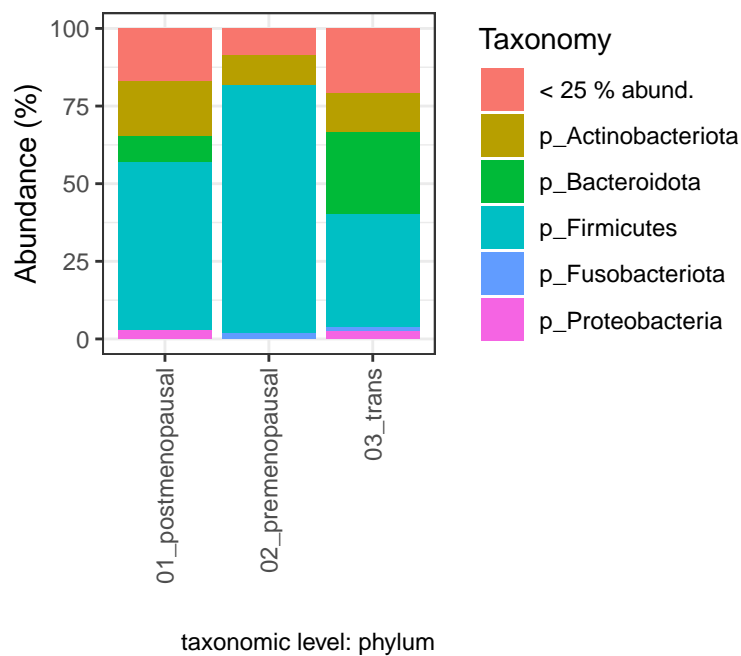


```
theme(legend.position = "right") +
labs(y = "Abundance (%)", x = "", caption = "taxonomic level: phylum") +
rotate_x_text()
```

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.

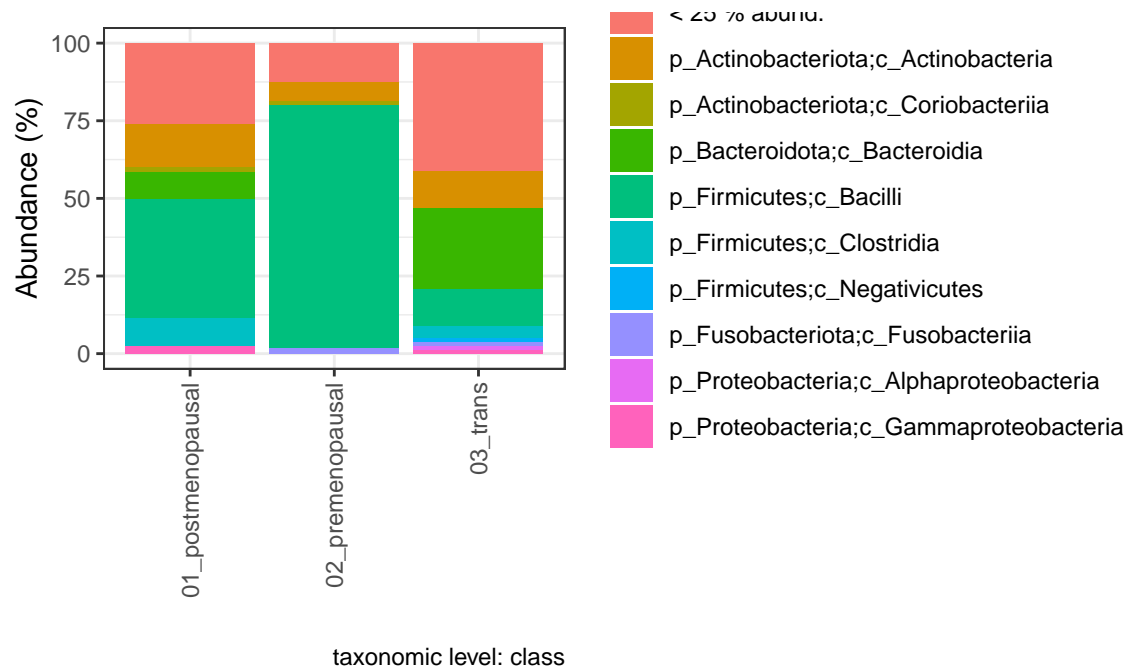
```
phylumplot
```



```
ggsave(plot = phylumplot, filename = "results/microbiome/taxplots_phylum.pdf", width = 4,
```

5.2 Class level

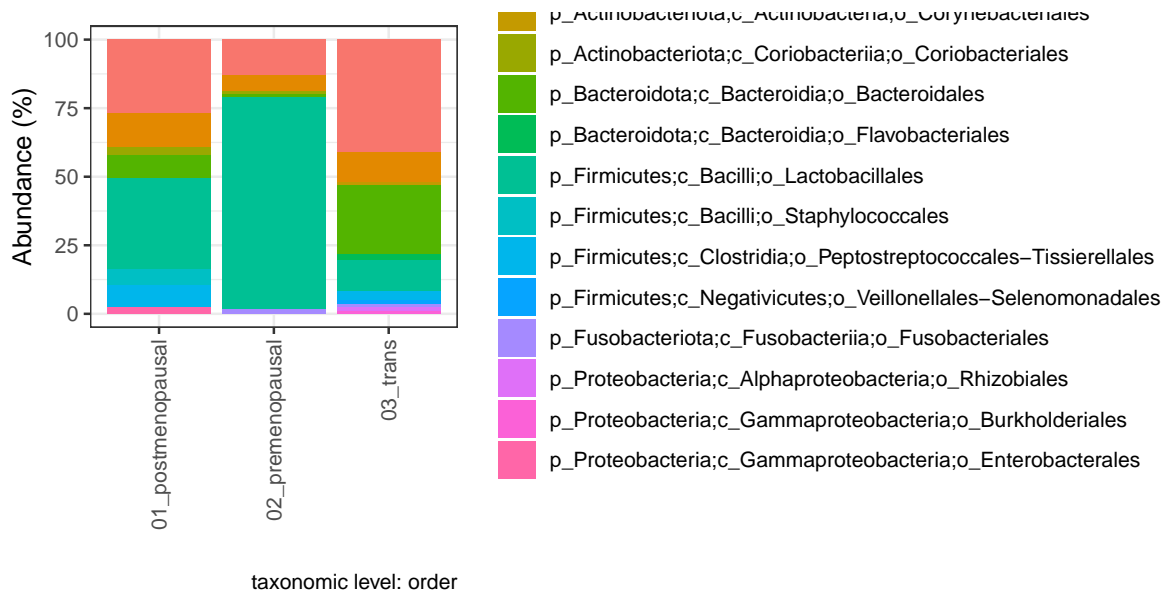
```
classplot
```



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

5.3 Order level

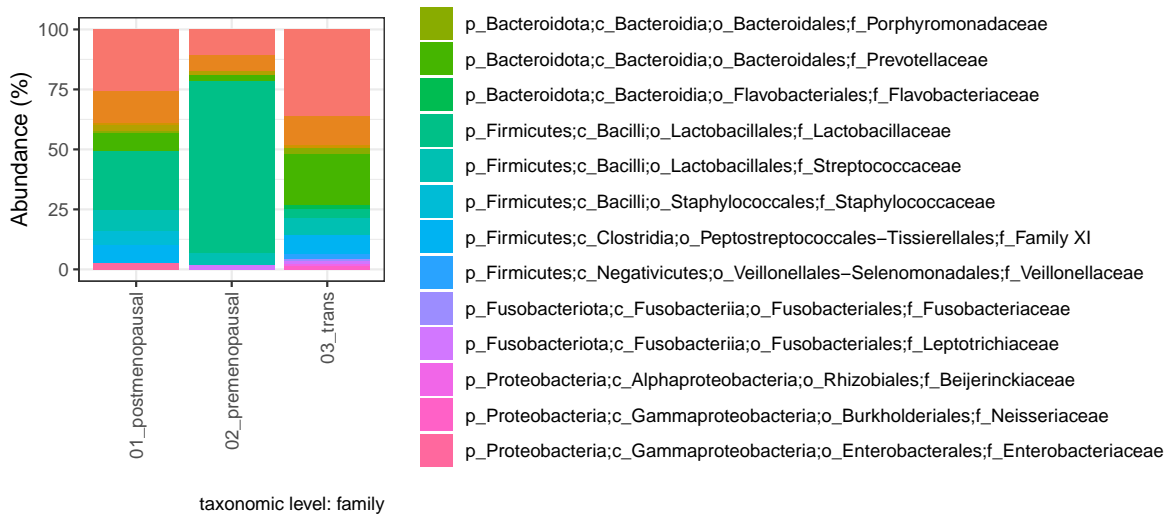
```
orderplot
```



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

5.4 Family level

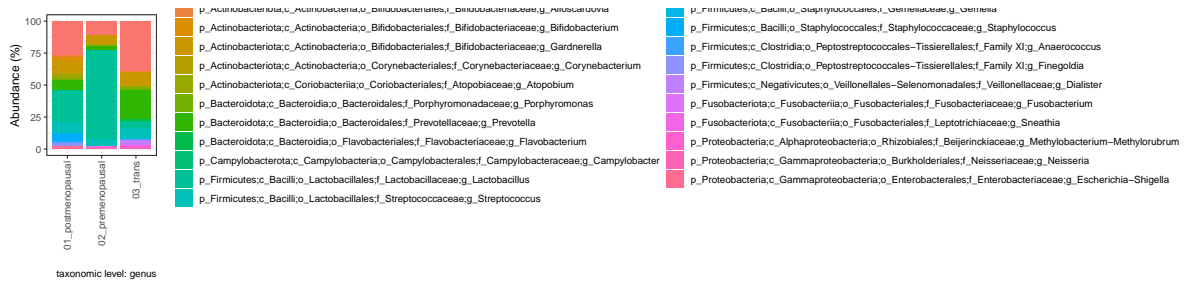
familyplot



```
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



```
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	

```
# 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")
```

```
2024-01-03 15:37:42.512517 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/asv_menopause-duration/maaslin2.1
2024-01-03 15:37:47.277044 INFO::Writing function arguments to log file
2024-01-03 15:37:47.281812 INFO::Verifying options selected are valid
2024-01-03 15:37:47.301691 INFO::Determining format of input files
2024-01-03 15:37:47.30209 INFO::Input format is data samples as rows and metadata samples as
2024-01-03 15:37:47.334609 INFO::Formula for fixed effects: expr ~ DurationMenopause
2024-01-03 15:37:47.335055 INFO::Filter data based on min abundance and min prevalence
2024-01-03 15:37:47.335277 INFO::Total samples in data: 25
2024-01-03 15:37:47.335483 INFO::Min samples required with min abundance for a feature not t
2024-01-03 15:37:47.369608 INFO::Total filtered features: 8831
2024-01-03 15:37:47.371083 INFO::Filtered feature names from abundance and prevalence filter
2024-01-03 15:37:47.372948 INFO::Total filtered features with variance filtering: 0
2024-01-03 15:37:47.373181 INFO::Filtered feature names from variance filtering:
2024-01-03 15:37:47.373389 INFO::Running selected normalization method: TSS
```

```

2024-01-03 15:37:47.374245 INFO::Applying z-score to standardize continuous metadata
2024-01-03 15:37:47.376724 INFO::Running selected transform method: LOG
2024-01-03 15:37:47.377141 INFO::Running selected analysis method: LM
2024-01-03 15:37:47.380159 INFO::Fitting model to feature number 1, ASV14
2024-01-03 15:37:47.381845 INFO::Fitting model to feature number 2, ASV16
2024-01-03 15:37:47.384286 INFO::Counting total values for each feature
2024-01-03 15:37:47.384851 INFO::Writing filtered data to file results/maaslin2/asv_menopause
2024-01-03 15:37:47.3857 INFO::Writing filtered, normalized data to file results/maaslin2/asv
2024-01-03 15:37:47.386171 INFO::Writing filtered, normalized, transformed data to file resu
2024-01-03 15:37:47.386659 WARNING::Deleting existing residuals file: results/maaslin2/asv_m
2024-01-03 15:37:47.387087 INFO::Writing residuals to file results/maaslin2/asv_menopause-du
2024-01-03 15:37:47.38747 WARNING::Deleting existing fitted file: results/maaslin2/asv_menopau
2024-01-03 15:37:47.387803 INFO::Writing fitted values to file results/maaslin2/asv_menopaus
2024-01-03 15:37:47.388139 INFO::Writing all results to file (ordered by increasing q-values)
2024-01-03 15:37:47.388657 INFO::Writing the significant results (those which are less than c
2024-01-03 15:37:47.389005 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2024-01-03 15:37:47.389813 INFO::Writing association plots (one for each significant associat
[1] "There are no associations to plot!"

```

```

# 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")

```

```

2024-01-03 15:37:53.145047 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_menopause-duration/maaslin2
2024-01-03 15:37:53.302844 INFO::Writing function arguments to log file
2024-01-03 15:37:53.304919 INFO::Verifying options selected are valid
2024-01-03 15:37:53.30516 INFO::Determining format of input files
2024-01-03 15:37:53.305378 INFO::Input format is data samples as rows and metadata samples as
2024-01-03 15:37:53.307103 INFO::Formula for fixed effects: expr ~ DurationMenopause
2024-01-03 15:37:53.307362 INFO::Filter data based on min abundance and min prevalence
2024-01-03 15:37:53.30758 INFO::Total samples in data: 25
2024-01-03 15:37:53.307789 INFO::Min samples required with min abundance for a feature not t

```

```

2024-01-03 15:37:53.309191 INFO::Total filtered features: 294
2024-01-03 15:37:53.309456 INFO::Filtered feature names from abundance and prevalence filter
2024-01-03 15:37:53.309859 INFO::Total filtered features with variance filtering: 0
2024-01-03 15:37:53.310086 INFO::Filtered feature names from variance filtering:
2024-01-03 15:37:53.310288 INFO::Running selected normalization method: TSS
2024-01-03 15:37:53.310641 INFO::Applying z-score to standardize continuous metadata
2024-01-03 15:37:53.31292 INFO::Running selected transform method: LOG
2024-01-03 15:37:53.313271 INFO::Running selected analysis method: LM
2024-01-03 15:37:53.313533 INFO::Fitting model to feature number 1, ASV16
2024-01-03 15:37:53.314551 INFO::Fitting model to feature number 2, ASV82
2024-01-03 15:37:53.315407 INFO::Fitting model to feature number 3, ASV271
2024-01-03 15:37:53.316247 INFO::Fitting model to feature number 4, ASV526
2024-01-03 15:37:53.318657 INFO::Counting total values for each feature
2024-01-03 15:37:53.319242 INFO::Writing filtered data to file results/maaslin2/genus_menopau
2024-01-03 15:37:53.320309 INFO::Writing filtered, normalized data to file results/maaslin2/g
2024-01-03 15:37:53.32105 INFO::Writing filtered, normalized, transformed data to file resul
2024-01-03 15:37:53.321631 WARNING::Deleting existing residuals file: results/maaslin2/genus
2024-01-03 15:37:53.322071 INFO::Writing residuals to file results/maaslin2/genus_menopause-c
2024-01-03 15:37:53.322498 WARNING::Deleting existing fitted file: results/maaslin2/genus_mer
2024-01-03 15:37:53.322842 INFO::Writing fitted values to file results/maaslin2/genus_menopau
2024-01-03 15:37:53.323203 INFO::Writing all results to file (ordered by increasing q-values)
2024-01-03 15:37:53.323771 INFO::Writing the significant results (those which are less than c
2024-01-03 15:37:53.324112 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2024-01-03 15:37:53.32457 INFO::Writing association plots (one for each significant associat
[1] "There are no associations to plot!"

```

```

# 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")

```

```

2024-01-03 15:37:56.104929 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/family_menopause-duration/maaslin
2024-01-03 15:37:56.166436 INFO::Writing function arguments to log file

```

```

2024-01-03 15:37:56.168543 INFO::Verifying options selected are valid
2024-01-03 15:37:56.168792 INFO::Determining format of input files
2024-01-03 15:37:56.16902 INFO::Input format is data samples as rows and metadata samples as
2024-01-03 15:37:56.170138 INFO::Formula for fixed effects: expr ~ DurationMenopause
2024-01-03 15:37:56.170402 INFO::Filter data based on min abundance and min prevalence
2024-01-03 15:37:56.170608 INFO::Total samples in data: 25
2024-01-03 15:37:56.170813 INFO::Min samples required with min abundance for a feature not to
2024-01-03 15:37:56.171543 INFO::Total filtered features: 111
2024-01-03 15:37:56.171783 INFO::Filtered feature names from abundance and prevalence filter
2024-01-03 15:37:56.172138 INFO::Total filtered features with variance filtering: 0
2024-01-03 15:37:56.172359 INFO::Filtered feature names from variance filtering:
2024-01-03 15:37:56.172568 INFO::Running selected normalization method: TSS
2024-01-03 15:37:56.172944 INFO::Applying z-score to standardize continuous metadata
2024-01-03 15:37:56.175366 INFO::Running selected transform method: LOG
2024-01-03 15:37:56.175719 INFO::Running selected analysis method: LM
2024-01-03 15:37:56.175977 INFO::Fitting model to feature number 1, ASV16
2024-01-03 15:37:56.177012 INFO::Fitting model to feature number 2, ASV82
2024-01-03 15:37:56.177885 INFO::Fitting model to feature number 3, ASV120
2024-01-03 15:37:56.178708 INFO::Fitting model to feature number 4, ASV271
2024-01-03 15:37:56.181094 INFO::Counting total values for each feature
2024-01-03 15:37:56.181683 INFO::Writing filtered data to file results/maaslin2/family_menopau
2024-01-03 15:37:56.182285 INFO::Writing filtered, normalized data to file results/maaslin2/f
2024-01-03 15:37:56.182821 INFO::Writing filtered, normalized, transformed data to file resu
2024-01-03 15:37:56.183348 WARNING::Deleting existing residuals file: results/maaslin2/famil
2024-01-03 15:37:56.183779 INFO::Writing residuals to file results/maaslin2/family_menopau
2024-01-03 15:37:56.184846 WARNING::Deleting existing fitted file: results/maaslin2/family_m
2024-01-03 15:37:56.185179 INFO::Writing fitted values to file results/maaslin2/family_menop
2024-01-03 15:37:56.185536 INFO::Writing all results to file (ordered by increasing q-values)
2024-01-03 15:37:56.186082 INFO::Writing the significant results (those which are less than 0
2024-01-03 15:37:56.186408 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2024-01-03 15:37:56.186857 INFO::Writing association plots (one for each significant associat
[1] "There are no associations to plot!"

```

```

## 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) %>%

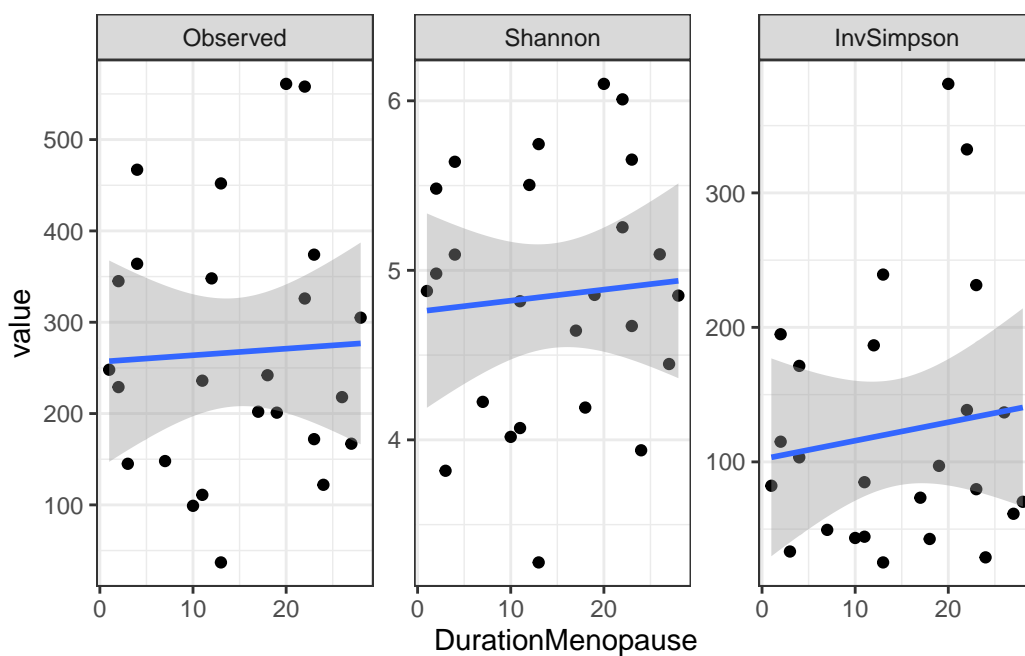
```



```
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")
```

```
2024-01-03 15:37:56.513168 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/asv_GHAT_Length/maaslin2.log"
2024-01-03 15:38:01.183655 INFO::Writing function arguments to log file
2024-01-03 15:38:01.185808 INFO::Verifying options selected are valid
2024-01-03 15:38:01.186061 INFO::Determining format of input files
2024-01-03 15:38:01.186291 INFO::Input format is data samples as rows and metadata samples as columns
2024-01-03 15:38:01.21829 INFO::Formula for fixed effects: expr ~ GHAT_Length
2024-01-03 15:38:01.218885 INFO::Filter data based on min abundance and min prevalence
2024-01-03 15:38:01.219146 INFO::Total samples in data: 25
2024-01-03 15:38:01.219375 INFO::Min samples required with min abundance for a feature not to be filtered: 3
2024-01-03 15:38:01.258518 INFO::Total filtered features: 8824
```

```

2024-01-03 15:38:01.260066 INFO::Filtered feature names from abundance and prevalence filter
2024-01-03 15:38:01.262137 INFO::Total filtered features with variance filtering: 0
2024-01-03 15:38:01.26238 INFO::Filtered feature names from variance filtering:
2024-01-03 15:38:01.262596 INFO::Running selected normalization method: TSS
2024-01-03 15:38:01.262987 INFO::Applying z-score to standardize continuous metadata
2024-01-03 15:38:01.265328 INFO::Running selected transform method: LOG
2024-01-03 15:38:01.265714 INFO::Running selected analysis method: LM
2024-01-03 15:38:01.265975 INFO::Fitting model to feature number 1, ASV54
2024-01-03 15:38:01.266991 INFO::Fitting model to feature number 2, ASV55
2024-01-03 15:38:01.267847 INFO::Fitting model to feature number 3, ASV57
2024-01-03 15:38:01.268714 INFO::Fitting model to feature number 4, ASV58
2024-01-03 15:38:01.269572 INFO::Fitting model to feature number 5, ASV59
2024-01-03 15:38:01.270398 INFO::Fitting model to feature number 6, ASV60
2024-01-03 15:38:01.27123 INFO::Fitting model to feature number 7, ASV61
2024-01-03 15:38:01.272095 INFO::Fitting model to feature number 8, ASV62
2024-01-03 15:38:01.272926 INFO::Fitting model to feature number 9, ASV64
2024-01-03 15:38:01.275487 INFO::Counting total values for each feature
2024-01-03 15:38:01.276147 INFO::Writing filtered data to file results/maaslin2/asv_GHAT_Length
2024-01-03 15:38:01.27682 INFO::Writing filtered, normalized data to file results/maaslin2/asv_GHAT_Length
2024-01-03 15:38:01.277385 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/asv_GHAT_Length
2024-01-03 15:38:01.278024 WARNING::Deleting existing residuals file: results/maaslin2/asv_GHAT_Length
2024-01-03 15:38:01.278578 INFO::Writing residuals to file results/maaslin2/asv_GHAT_Length
2024-01-03 15:38:01.279069 WARNING::Deleting existing fitted file: results/maaslin2/asv_GHAT_Length
2024-01-03 15:38:01.279456 INFO::Writing fitted values to file results/maaslin2/asv_GHAT_Length
2024-01-03 15:38:01.279839 INFO::Writing all results to file (ordered by increasing q-values)
2024-01-03 15:38:01.280408 INFO::Writing the significant results (those which are less than 0.05)
2024-01-03 15:38:01.280812 INFO::Writing heatmap of significant results to file: results/maaslin2/asv_GHAT_Length
[1] "There is not enough metadata in the associations to create a heatmap plot. Please review the metadata."
2024-01-03 15:38:01.281354 INFO::Writing association plots (one for each significant association)
2024-01-03 15:38:01.281916 INFO::Plotting associations from most to least significant, grouped by metadata number
2024-01-03 15:38:01.282185 INFO::Plotting data for metadata number 1, GHAT_Length
2024-01-03 15:38:01.282686 INFO::Creating scatter plot for continuous data, GHAT_Length vs ASV54

```

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

```

2024-01-03 15:38:01.346385 INFO::Creating scatter plot for continuous data, GHAT_Length vs ASV55

```

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

```

2024-01-03 15:38:01.411253 INFO::Creating scatter plot for continuous data, GHAT_Length vs ASV57

```

```

2024-01-03 15:38:01.470233 INFO::Creating scatter plot for continuous data, GHAT_Length vs ASV58

```

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

2024-01-03 15:38:01.540302 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()`).

```
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>

```
# 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")

```

```

2024-01-03 15:38:07.50059 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_GHAT_Length/maaslin2.log"
2024-01-03 15:38:07.656874 INFO::Writing function arguments to log file
2024-01-03 15:38:07.658957 INFO::Verifying options selected are valid
2024-01-03 15:38:07.659197 INFO::Determining format of input files
2024-01-03 15:38:07.659417 INFO::Input format is data samples as rows and metadata samples as columns
2024-01-03 15:38:07.66114 INFO::Formula for fixed effects: expr ~ GHAT_Length
2024-01-03 15:38:07.661414 INFO::Filter data based on min abundance and min prevalence
2024-01-03 15:38:07.661637 INFO::Total samples in data: 25
2024-01-03 15:38:07.661847 INFO::Min samples required with min abundance for a feature not to be filtered: 5
2024-01-03 15:38:07.669728 INFO::Total filtered features: 288
2024-01-03 15:38:07.670167 INFO::Filtered feature names from abundance and prevalence filtering:
2024-01-03 15:38:07.670643 INFO::Total filtered features with variance filtering: 0
2024-01-03 15:38:07.670886 INFO::Filtered feature names from variance filtering:
2024-01-03 15:38:07.671096 INFO::Running selected normalization method: TSS
2024-01-03 15:38:07.671477 INFO::Applying z-score to standardize continuous metadata
2024-01-03 15:38:07.673685 INFO::Running selected transform method: LOG
2024-01-03 15:38:07.674063 INFO::Running selected analysis method: LM
2024-01-03 15:38:07.674331 INFO::Fitting model to feature number 1, ASV14
2024-01-03 15:38:07.675349 INFO::Fitting model to feature number 2, ASV55
2024-01-03 15:38:07.67621 INFO::Fitting model to feature number 3, ASV66
2024-01-03 15:38:07.677034 INFO::Fitting model to feature number 4, ASV87
2024-01-03 15:38:07.677847 INFO::Fitting model to feature number 5, ASV120
2024-01-03 15:38:07.678655 INFO::Fitting model to feature number 6, ASV205
2024-01-03 15:38:07.679449 INFO::Fitting model to feature number 7, ASV241
2024-01-03 15:38:07.680254 INFO::Fitting model to feature number 8, ASV443
2024-01-03 15:38:07.681063 INFO::Fitting model to feature number 9, ASV568
2024-01-03 15:38:07.68187 INFO::Fitting model to feature number 10, ASV940
2024-01-03 15:38:07.684401 INFO::Counting total values for each feature
2024-01-03 15:38:07.685051 INFO::Writing filtered data to file results/maaslin2/genus_GHAT_Length/maaslin2_filtered_data.csv
2024-01-03 15:38:07.685737 INFO::Writing filtered, normalized data to file results/maaslin2/genus_GHAT_Length/maaslin2_normalized_data.csv
2024-01-03 15:38:07.686365 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/genus_GHAT_Length/maaslin2_transformed_data.csv
2024-01-03 15:38:07.68699 WARNING::Deleting existing residuals file: results/maaslin2/genus_GHAT_Length/maaslin2_residuals.csv
2024-01-03 15:38:07.687398 INFO::Writing residuals to file results/maaslin2/genus_GHAT_Length/maaslin2_residuals.csv
2024-01-03 15:38:07.687806 WARNING::Deleting existing fitted file: results/maaslin2/genus_GHAT_Length/maaslin2_fitted_values.csv
2024-01-03 15:38:07.688156 INFO::Writing fitted values to file results/maaslin2/genus_GHAT_Length/maaslin2_fitted_values.csv

```

```

2024-01-03 15:38:07.688532 INFO::Writing all results to file (ordered by increasing q-values)
2024-01-03 15:38:07.689074 INFO::Writing the significant results (those which are less than 0.2)
2024-01-03 15:38:07.689461 INFO::Writing heatmap of significant results to file: results/maaslin_res_genus_ghat
[1] "There is not enough metadata in the associations to create a heatmap plot. Please review the metadata"
2024-01-03 15:38:07.690004 INFO::Writing association plots (one for each significant association)
2024-01-03 15:38:07.690541 INFO::Plotting associations from most to least significant, grouped by metadata number
2024-01-03 15:38:07.690818 INFO::Plotting data for metadata number 1, GHAT_Length
2024-01-03 15:38:07.691318 INFO::Creating scatter plot for continuous data, GHAT_Length vs Abundance

```

```

2024-01-03 15:38:07.748778 INFO::Creating scatter plot for continuous data, GHAT_Length vs Abundance

```

```

2024-01-03 15:38:07.808434 INFO::Creating scatter plot for continuous data, GHAT_Length vs Abundance

```

```

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

```

```

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>

```

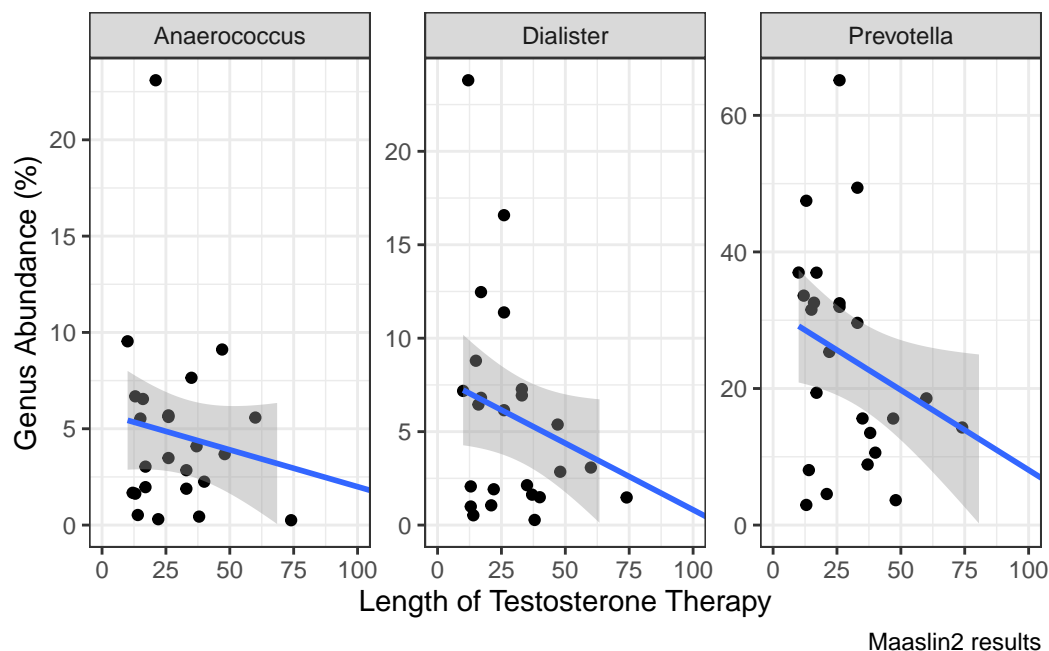
# plot correlation for these 3 ASVs
psmelt_trans_genus = psmelt(ps_trans_genus)
maaslin_res_genus_ghat = ggplot(filter(psmelt_trans_genus, OTU %in% filter(genus_GHAT$results, qval < 0.2)$feature),
  aes(x = GHAT_Length, y = Abundance)) +
  geom_point() +
  geom_smooth(method = "lm") +
  facet_wrap(~Genus, 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()``).



```
ggsave(maaslin_res_genus_ghat, filename = "results/maaslin2/genus_GHAT_Length_panelplot.png",
       height = 5, 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)

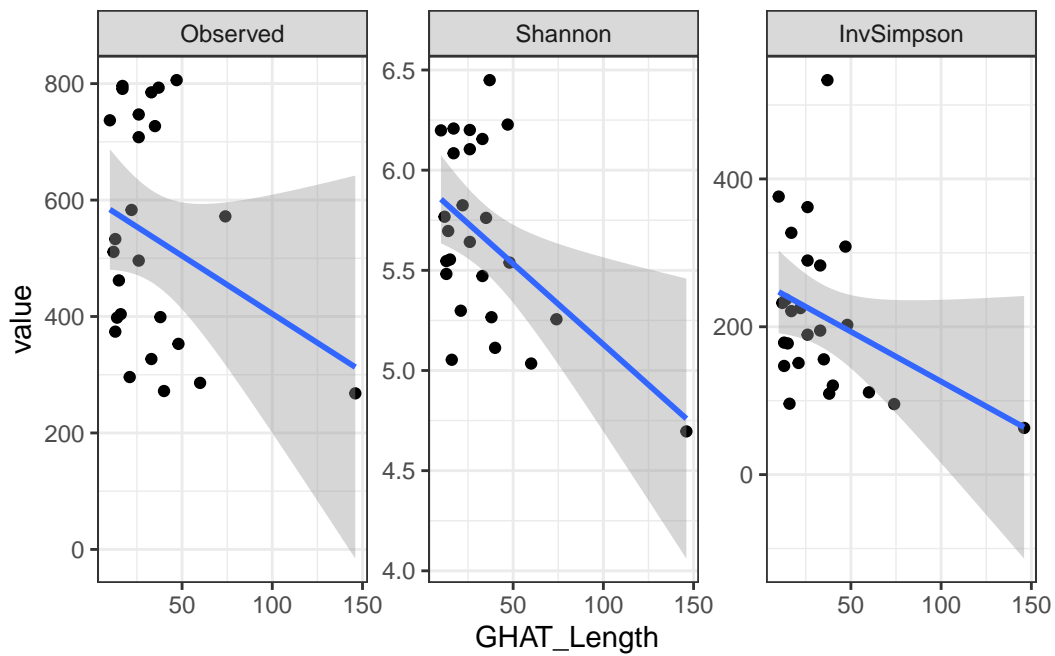
```

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)$GHAT_Length)

#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	24	5.648191	0.1133731	2.941013	0.002
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()

```

```

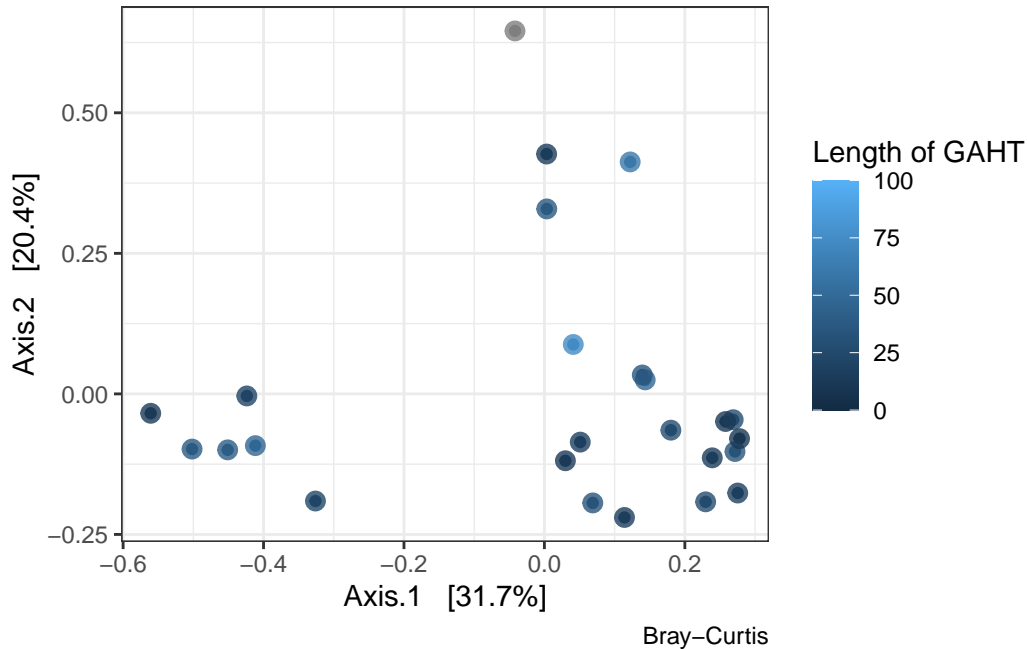
Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
10.00  16.00   26.00   33.56  38.00  146.00

```

```

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

```



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
```

```
2024-01-03 15:38:14.763433 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_Testosterone/maaslin2.log"
2024-01-03 15:38:14.930925 INFO::Writing function arguments to log file
2024-01-03 15:38:14.93308 INFO::Verifying options selected are valid
```

```

2024-01-03 15:38:14.933339 INFO::Determining format of input files
2024-01-03 15:38:14.933579 INFO::Input format is data samples as rows and metadata samples as
2024-01-03 15:38:14.935417 INFO::Formula for fixed effects: expr ~ Testosterone
2024-01-03 15:38:14.93574 INFO::Filter data based on min abundance and min prevalence
2024-01-03 15:38:14.935963 INFO::Total samples in data: 25
2024-01-03 15:38:14.936175 INFO::Min samples required with min abundance for a feature not to
2024-01-03 15:38:14.937621 INFO::Total filtered features: 292
2024-01-03 15:38:14.937939 INFO::Filtered feature names from abundance and prevalence filter:
2024-01-03 15:38:14.938391 INFO::Total filtered features with variance filtering: 0
2024-01-03 15:38:14.938625 INFO::Filtered feature names from variance filtering:
2024-01-03 15:38:14.938835 INFO::Running selected normalization method: TSS
2024-01-03 15:38:14.939226 INFO::Applying z-score to standardize continuous metadata
2024-01-03 15:38:14.941697 INFO::Running selected transform method: LOG
2024-01-03 15:38:14.942109 INFO::Running selected analysis method: LM
2024-01-03 15:38:14.942381 INFO::Fitting model to feature number 1, ASV14
2024-01-03 15:38:14.94361 INFO::Fitting model to feature number 2, ASV54
2024-01-03 15:38:14.944592 INFO::Fitting model to feature number 3, ASV66
2024-01-03 15:38:14.945519 INFO::Fitting model to feature number 4, ASV87
2024-01-03 15:38:14.946621 INFO::Fitting model to feature number 5, ASV205
2024-01-03 15:38:14.947709 INFO::Fitting model to feature number 6, ASV568
2024-01-03 15:38:14.950311 INFO::Counting total values for each feature
2024-01-03 15:38:14.950933 INFO::Writing filtered data to file results/maaslin2/genus_Testos
2024-01-03 15:38:14.951578 INFO::Writing filtered, normalized data to file results/maaslin2/g
2024-01-03 15:38:14.952123 INFO::Writing filtered, normalized, transformed data to file resu
2024-01-03 15:38:14.952678 WARNING::Deleting existing residuals file: results/maaslin2/genus
2024-01-03 15:38:14.95309 INFO::Writing residuals to file results/maaslin2/genus_Testosterone
2024-01-03 15:38:14.953518 WARNING::Deleting existing fitted file: results/maaslin2/genus_Tes
2024-01-03 15:38:14.953862 INFO::Writing fitted values to file results/maaslin2/genus_Testos
2024-01-03 15:38:14.954219 INFO::Writing all results to file (ordered by increasing q-values)
2024-01-03 15:38:14.954754 INFO::Writing the significant results (those which are less than
2024-01-03 15:38:14.955086 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2024-01-03 15:38:14.955529 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

```
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

```
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
```

```
2024-01-03 15:38:14.961273 WARNING::Input is a matrix, passing through as.data.frame() .  
[1] "Warning: Deleting existing log file: results/maaslin2/genus_DurationGNRH/maaslin2.log"  
2024-01-03 15:38:15.124547 INFO::Writing function arguments to log file  
2024-01-03 15:38:15.12706 INFO::Verifying options selected are valid  
2024-01-03 15:38:15.127323 INFO::Determining format of input files  
2024-01-03 15:38:15.127553 INFO::Input format is data samples as rows and metadata samples as columns  
2024-01-03 15:38:15.129278 INFO::Formula for fixed effects: expr ~ Duration_GNRH  
2024-01-03 15:38:15.129533 INFO::Filter data based on min abundance and min prevalence  
2024-01-03 15:38:15.129744 INFO::Total samples in data: 25  
2024-01-03 15:38:15.129945 INFO::Min samples required with min abundance for a feature not to be filtered: 5  
2024-01-03 15:38:15.131345 INFO::Total filtered features: 292  
2024-01-03 15:38:15.131611 INFO::Filtered feature names from abundance and prevalence filtering: 292  
2024-01-03 15:38:15.132041 INFO::Total filtered features with variance filtering: 0  
2024-01-03 15:38:15.132265 INFO::Filtered feature names from variance filtering:  
2024-01-03 15:38:15.132473 INFO::Running selected normalization method: TSS  
2024-01-03 15:38:15.132839 INFO::Applying z-score to standardize continuous metadata  
2024-01-03 15:38:15.135147 INFO::Running selected transform method: LOG  
2024-01-03 15:38:15.135524 INFO::Running selected analysis method: LM  
2024-01-03 15:38:15.135791 INFO::Fitting model to feature number 1, ASV14  
2024-01-03 15:38:15.136816 INFO::Fitting model to feature number 2, ASV54  
2024-01-03 15:38:15.137687 INFO::Fitting model to feature number 3, ASV66  
2024-01-03 15:38:15.138533 INFO::Fitting model to feature number 4, ASV87  
2024-01-03 15:38:15.139378 INFO::Fitting model to feature number 5, ASV205  
2024-01-03 15:38:15.140224 INFO::Fitting model to feature number 6, ASV568  
2024-01-03 15:38:15.142707 INFO::Counting total values for each feature
```

```

2024-01-03 15:38:15.143324 INFO::Writing filtered data to file results/maaslin2/genus_DurationGNI
2024-01-03 15:38:15.144134 INFO::Writing filtered, normalized data to file results/maaslin2/g
2024-01-03 15:38:15.144697 INFO::Writing filtered, normalized, transformed data to file resu
2024-01-03 15:38:15.145253 WARNING::Deleting existing residuals file: results/maaslin2/genus
2024-01-03 15:38:15.145651 INFO::Writing residuals to file results/maaslin2/genus_DurationGNI
2024-01-03 15:38:15.146073 WARNING::Deleting existing fitted file: results/maaslin2/genus_Dur
2024-01-03 15:38:15.146401 INFO::Writing fitted values to file results/maaslin2/genus_Durati
2024-01-03 15:38:15.146748 INFO::Writing all results to file (ordered by increasing q-values:
2024-01-03 15:38:15.147286 INFO::Writing the significant results (those which are less than c
2024-01-03 15:38:15.147634 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2024-01-03 15:38:15.148078 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

```

```

2024-01-03 15:38:15.206338 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/asv_cycle_premeno/maaslin2.log"
2024-01-03 15:38:19.885841 INFO::Writing function arguments to log file
2024-01-03 15:38:19.887902 INFO::Verifying options selected are valid
2024-01-03 15:38:19.88814 INFO::Determining format of input files
2024-01-03 15:38:19.888359 INFO::Input format is data samples as rows and metadata samples as
2024-01-03 15:38:19.919321 INFO::Formula for fixed effects: expr ~ CycleDaySampling
2024-01-03 15:38:19.919767 INFO::Filter data based on min abundance and min prevalence
2024-01-03 15:38:19.919994 INFO::Total samples in data: 25

```

```

2024-01-03 15:38:19.920203 INFO::Min samples required with min abundance for a feature not t
2024-01-03 15:38:19.952395 INFO::Total filtered features: 8808
2024-01-03 15:38:19.953843 INFO::Filtered feature names from abundance and prevalence filter
2024-01-03 15:38:19.955894 INFO::Total filtered features with variance filtering: 0
2024-01-03 15:38:19.956139 INFO::Filtered feature names from variance filtering:
2024-01-03 15:38:19.956356 INFO::Running selected normalization method: TSS
2024-01-03 15:38:19.956801 INFO::Applying z-score to standardize continuous metadata
2024-01-03 15:38:19.959111 INFO::Running selected transform method: LOG
2024-01-03 15:38:19.95958 INFO::Running selected analysis method: LM
2024-01-03 15:38:19.959844 INFO::Fitting model to feature number 1, ASV1
2024-01-03 15:38:19.960857 INFO::Fitting model to feature number 2, ASV2
2024-01-03 15:38:19.96171 INFO::Fitting model to feature number 3, ASV3
2024-01-03 15:38:19.962531 INFO::Fitting model to feature number 4, ASV4
2024-01-03 15:38:19.963345 INFO::Fitting model to feature number 5, ASV5
2024-01-03 15:38:19.964224 INFO::Fitting model to feature number 6, ASV6
2024-01-03 15:38:19.965043 INFO::Fitting model to feature number 7, ASV7
2024-01-03 15:38:19.965866 INFO::Fitting model to feature number 8, ASV8
2024-01-03 15:38:19.966678 INFO::Fitting model to feature number 9, ASV9
2024-01-03 15:38:19.967498 INFO::Fitting model to feature number 10, ASV10
2024-01-03 15:38:19.96832 INFO::Fitting model to feature number 11, ASV11
2024-01-03 15:38:19.969138 INFO::Fitting model to feature number 12, ASV12
2024-01-03 15:38:19.969952 INFO::Fitting model to feature number 13, ASV13
2024-01-03 15:38:19.970778 INFO::Fitting model to feature number 14, ASV14
2024-01-03 15:38:19.971582 INFO::Fitting model to feature number 15, ASV15
2024-01-03 15:38:19.972388 INFO::Fitting model to feature number 16, ASV16
2024-01-03 15:38:19.973211 INFO::Fitting model to feature number 17, ASV17
2024-01-03 15:38:19.974022 INFO::Fitting model to feature number 18, ASV18
2024-01-03 15:38:19.974829 INFO::Fitting model to feature number 19, ASV19
2024-01-03 15:38:19.975651 INFO::Fitting model to feature number 20, ASV20
2024-01-03 15:38:19.976482 INFO::Fitting model to feature number 21, ASV21
2024-01-03 15:38:19.977353 INFO::Fitting model to feature number 22, ASV22
2024-01-03 15:38:19.978166 INFO::Fitting model to feature number 23, ASV23
2024-01-03 15:38:19.978971 INFO::Fitting model to feature number 24, ASV27
2024-01-03 15:38:19.979773 INFO::Fitting model to feature number 25, ASV32
2024-01-03 15:38:19.982889 INFO::Counting total values for each feature
2024-01-03 15:38:19.983745 INFO::Writing filtered data to file results/maaslin2/asv_cycle_pr
2024-01-03 15:38:19.984617 INFO::Writing filtered, normalized data to file results/maaslin2/a
2024-01-03 15:38:19.985381 INFO::Writing filtered, normalized, transformed data to file resu
2024-01-03 15:38:19.986274 WARNING::Deleting existing residuals file: results/maaslin2/asv_cy
2024-01-03 15:38:19.98668 INFO::Writing residuals to file results/maaslin2/asv_cycle_premeno
2024-01-03 15:38:19.987237 WARNING::Deleting existing fitted file: results/maaslin2/asv_cycl
2024-01-03 15:38:19.987571 INFO::Writing fitted values to file results/maaslin2/asv_cycle_pr
2024-01-03 15:38:19.987984 INFO::Writing all results to file (ordered by increasing q-values)

```

```

2024-01-03 15:38:19.988598 INFO::Writing the significant results (those which are less than 0.05) to file: results/maaslin2/genus_cycle_premeno/maaslin2.log
2024-01-03 15:38:19.988955 INFO::Writing heatmap of significant results to file: results/maaslin2/genus_cycle_premeno/maaslin2_heatmap.png
[1] "There are no associations to plot!"
2024-01-03 15:38:19.989399 INFO::Writing association plots (one for each significant association) to file: results/maaslin2/genus_cycle_premeno/maaslin2_association_plots/
[1] "There are no associations to plot!"

```

```

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")

```

```

2024-01-03 15:38:26.269373 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_cycle_premeno/maaslin2.log"
2024-01-03 15:38:26.427398 INFO::Writing function arguments to log file
2024-01-03 15:38:26.429401 INFO::Verifying options selected are valid
2024-01-03 15:38:26.429647 INFO::Determining format of input files
2024-01-03 15:38:26.429866 INFO::Input format is data samples as rows and metadata samples as columns
2024-01-03 15:38:26.431567 INFO::Formula for fixed effects: expr ~ CycleDaySampling
2024-01-03 15:38:26.431818 INFO::Filter data based on min abundance and min prevalence
2024-01-03 15:38:26.432031 INFO::Total samples in data: 25
2024-01-03 15:38:26.432231 INFO::Min samples required with min abundance for a feature not to be filtered: 5
2024-01-03 15:38:26.433632 INFO::Total filtered features: 296
2024-01-03 15:38:26.433913 INFO::Filtered feature names from abundance and prevalence filtering: 296
2024-01-03 15:38:26.434325 INFO::Total filtered features with variance filtering: 0
2024-01-03 15:38:26.434559 INFO::Filtered feature names from variance filtering: 0
2024-01-03 15:38:26.4348 INFO::Running selected normalization method: TSS
2024-01-03 15:38:26.435145 INFO::Applying z-score to standardize continuous metadata
2024-01-03 15:38:26.437398 INFO::Running selected transform method: LOG
2024-01-03 15:38:26.437727 INFO::Running selected analysis method: LM
2024-01-03 15:38:26.437982 INFO::Fitting model to feature number 1, ASV1
2024-01-03 15:38:26.438977 INFO::Fitting model to feature number 2, ASV66
2024-01-03 15:38:26.44126 INFO::Counting total values for each feature
2024-01-03 15:38:26.441809 INFO::Writing filtered data to file results/maaslin2/genus_cycle_premeno/maaslin2_filtered_data.csv
2024-01-03 15:38:26.442355 INFO::Writing filtered, normalized data to file results/maaslin2/genus_cycle_premeno/maaslin2_normalized_data.csv
2024-01-03 15:38:26.442842 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/genus_cycle_premeno/maaslin2_transformed_data.csv
2024-01-03 15:38:26.443313 WARNING::Deleting existing residuals file: results/maaslin2/genus_cycle_premeno/maaslin2_residuals.csv

```

```

2024-01-03 15:38:26.443715 INFO::Writing residuals to file results/maaslin2/genus_cycle_prema
2024-01-03 15:38:26.444116 WARNING::Deleting existing fitted file: results/maaslin2/genus_cy
2024-01-03 15:38:26.444445 INFO::Writing fitted values to file results/maaslin2/genus_cycle_p
2024-01-03 15:38:26.444783 INFO::Writing all results to file (ordered by increasing q-values)
2024-01-03 15:38:26.445308 INFO::Writing the significant results (those which are less than 0
2024-01-03 15:38:26.445677 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
2024-01-03 15:38:26.446187 INFO::Writing association plots (one for each significant associat
2024-01-03 15:38:26.446682 INFO::Plotting associations from most to least significant, group
2024-01-03 15:38:26.446945 INFO::Plotting data for metadata number 1, CycleDaySampling
2024-01-03 15:38:26.447422 INFO::Creating scatter plot for continuous data, CycleDaySampling

2024-01-03 15:38:26.511803 INFO::Creating scatter plot for continuous data, CycleDaySampling

```

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

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

```

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

```

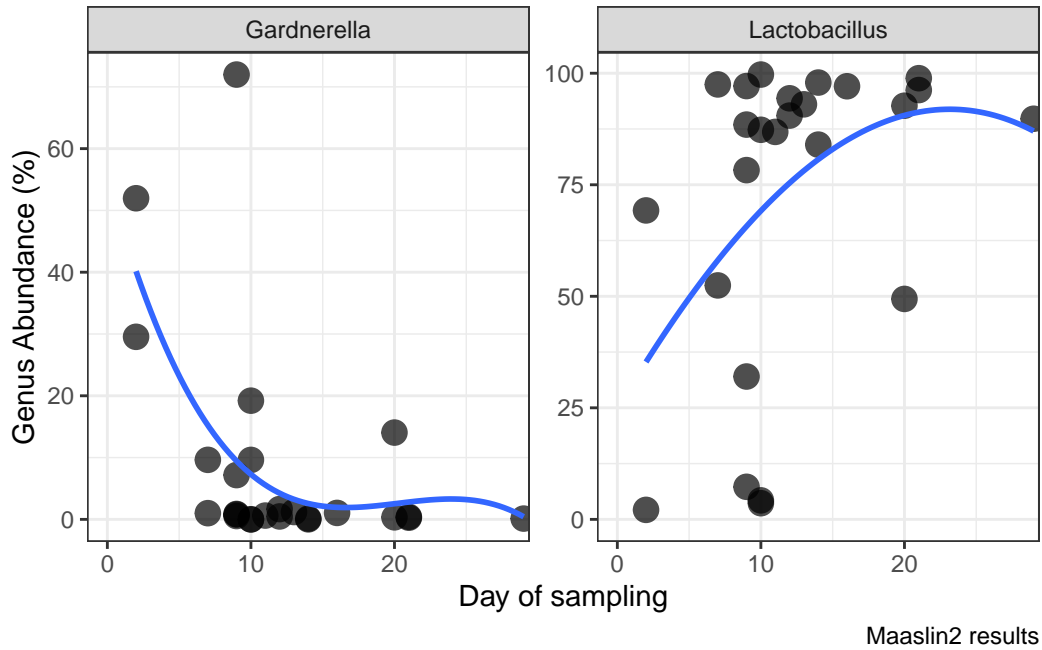
psmelt_pre_genus = psmelt(ps_pre_genus)

maaslin_res_genus_cycleday = ggplot(filter(psmelt_pre_genus, OTU %in% filter(genus_cycleday
  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
```



```
ggsave(maaslin_res_genus_cycleday, filename = "results/maaslin2/genus_CycleDay_panelplot.pdf",
       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)$SexualActivity == "Active")

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

```
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")
```

```
2024-01-03 15:38:42.09834 WARNING::Input is a matrix, passing through as.data.frame() .
```

```
[1] "Warning: Deleting existing log file: results/maaslin2/genus_SexuallyActive/maaslin2.log"
```

```
2024-01-03 15:38:42.26398 INFO::Writing function arguments to log file
```

```
2024-01-03 15:38:42.266298 INFO::Verifying options selected are valid
```

```
2024-01-03 15:38:42.266547 INFO::Determining format of input files
```

```
2024-01-03 15:38:42.266772 INFO::Input format is data samples as rows and metadata samples as columns
```

```
2024-01-03 15:38:42.268672 INFO::Formula for fixed effects: expr ~ SexuallyActive
```

```
2024-01-03 15:38:42.268949 INFO::Factor detected for categorical metadata 'SexuallyActive'. Proceeding with factor
```

```
2024-01-03 15:38:42.269167 INFO::Filter data based on min abundance and min prevalence
```

```
2024-01-03 15:38:42.269371 INFO::Total samples in data: 72
```

```
2024-01-03 15:38:42.26957 INFO::Min samples required with min abundance for a feature not to be filtered: 5
```

```
2024-01-03 15:38:42.271178 INFO::Total filtered features: 294
```

```
2024-01-03 15:38:42.271453 INFO::Filtered feature names from abundance and prevalence filtering: 294
```

```
2024-01-03 15:38:42.271871 INFO::Total filtered features with variance filtering: 0
```

```
2024-01-03 15:38:42.272108 INFO::Filtered feature names from variance filtering: 0
```

```
2024-01-03 15:38:42.272321 INFO::Running selected normalization method: TSS
```

```
2024-01-03 15:38:42.272725 INFO::Applying z-score to standardize continuous metadata
```

```
2024-01-03 15:38:42.275082 INFO::Running selected transform method: LOG
```

```
2024-01-03 15:38:42.275448 INFO::Running selected analysis method: LM
```

```
2024-01-03 15:38:42.275711 INFO::Fitting model to feature number 1, ASV1
```

```
2024-01-03 15:38:42.276848 INFO::Fitting model to feature number 2, ASV54
```

```
2024-01-03 15:38:42.277805 INFO::Fitting model to feature number 3, ASV66
```

```

2024-01-03 15:38:42.278756 INFO::Fitting model to feature number 4, ASV87
2024-01-03 15:38:42.281277 INFO::Counting total values for each feature
2024-01-03 15:38:42.28187 INFO::Writing filtered data to file results/maaslin2/genus_SexuallyActive
2024-01-03 15:38:42.282594 INFO::Writing filtered, normalized data to file results/maaslin2/genus_SexuallyActive
2024-01-03 15:38:42.283217 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/genus_SexuallyActive
2024-01-03 15:38:42.283873 WARNING::Deleting existing residuals file: results/maaslin2/genus_SexuallyActive
2024-01-03 15:38:42.284475 INFO::Writing residuals to file results/maaslin2/genus_SexuallyActive
2024-01-03 15:38:42.284998 WARNING::Deleting existing fitted file: results/maaslin2/genus_SexuallyActive
2024-01-03 15:38:42.285382 INFO::Writing fitted values to file results/maaslin2/genus_SexuallyActive
2024-01-03 15:38:42.285753 INFO::Writing all results to file (ordered by increasing q-values)
2024-01-03 15:38:42.286332 INFO::Writing the significant results (those which are less than 0.05)
2024-01-03 15:38:42.286721 INFO::Writing heatmap of significant results to file: results/maaslin2/genus_SexuallyActive
[1] "There is not enough metadata in the associations to create a heatmap plot. Please review the metadata."
2024-01-03 15:38:42.287247 INFO::Writing association plots (one for each significant association)
2024-01-03 15:38:42.287805 INFO::Plotting associations from most to least significant, grouped by metadata number
2024-01-03 15:38:42.288084 INFO::Plotting data for metadata number 1, SexuallyActive
2024-01-03 15:38:42.28861 INFO::Creating boxplot for categorical data, SexuallyActive vs ASV54
2024-01-03 15:38:42.341264 INFO::Creating boxplot for categorical data, SexuallyActive vs ASV87

```

```

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>			

```

# 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% filter(genus_SexuallyActive$results, qval < 0.2)$feature)) +
  aes(x = SexuallyActive, y = Abundance, fill = SexuallyActive) +
  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))}) +

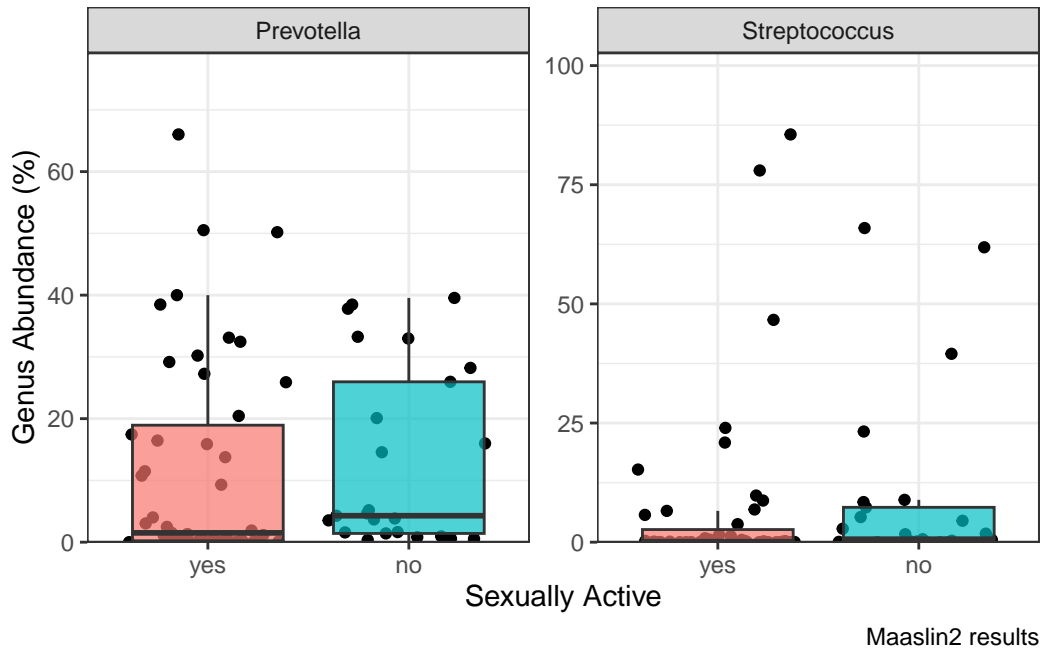
```

```

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

```

```
maaslin_res_genus_sexactive
```



```

ggsave(maaslin_res_genus_sexactive, filename = "results/maaslin2/genus_SexuallyActive_panel1.png",
       height = 5, width = 6)

```

```

# 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.

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 composition of the vaginal microbiome.

This analysis is performed within each group separately and results are compared.

[illegible]

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

[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
[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)$DurationMenopauseMonths = sample_data(ps_menopause)$DurationMenopauseMonths / 12
sample_data(ps_menopause)$DurationCombined = ifelse(is.na(sample_data(ps_menopause)$DurationMenopauseMonths), 0, sample_data(ps_menopause)$DurationMenopauseMonths + sample_data(ps_menopause)$DurationAmenorrhea)

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"))

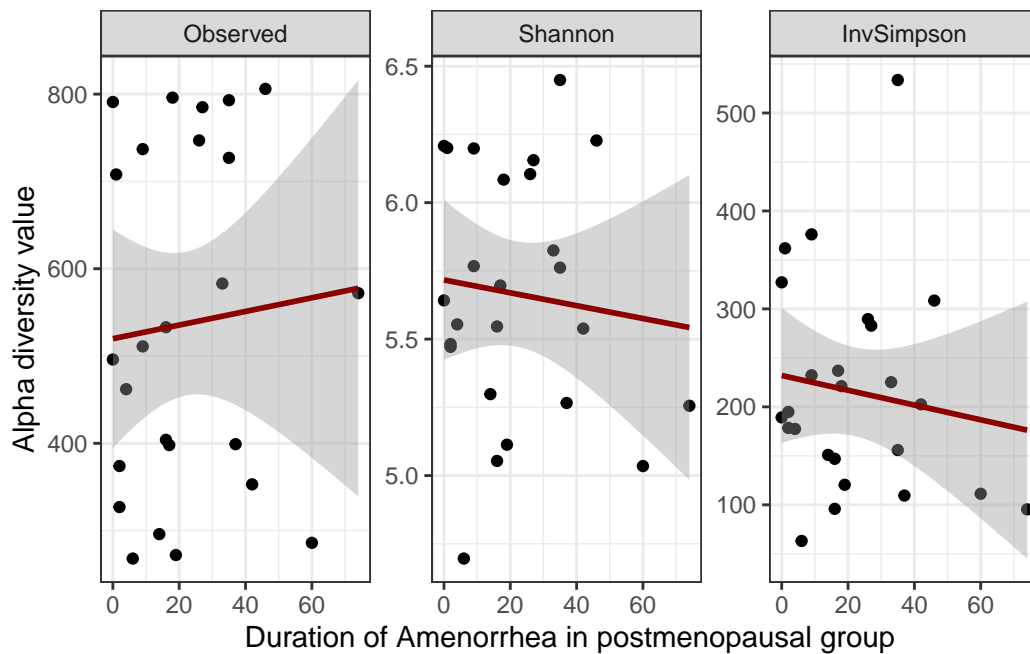
adiv_menopause = adiv_menopause$data %>%
  select(samples, grp, DurationMenopause, DurationAmenorrhea, DurationCombined, variable,
         count)

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

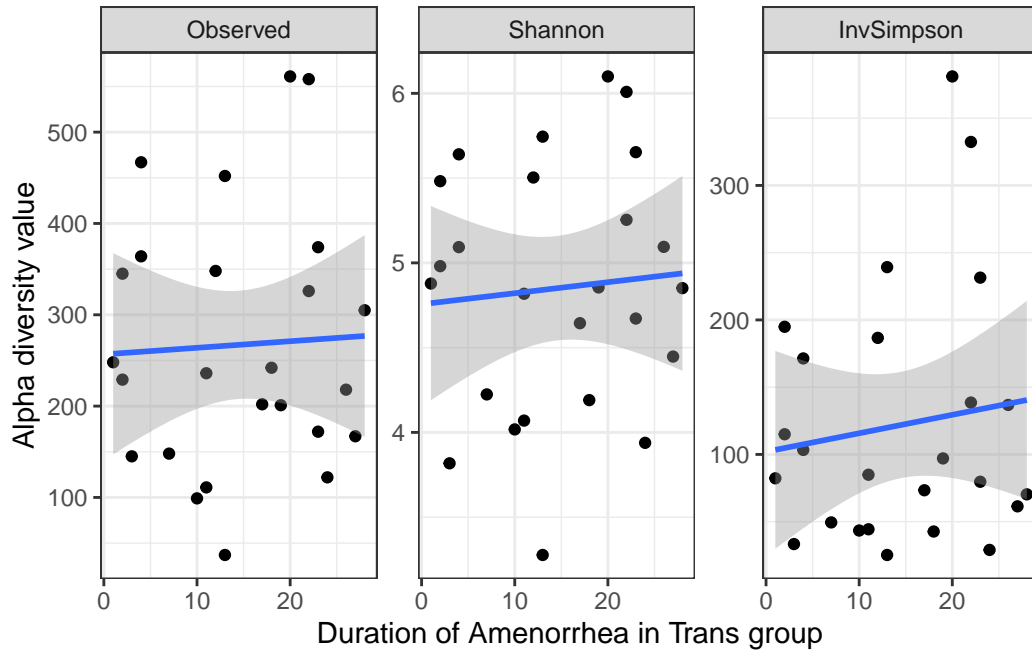
kable(corr_trans)
```

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

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

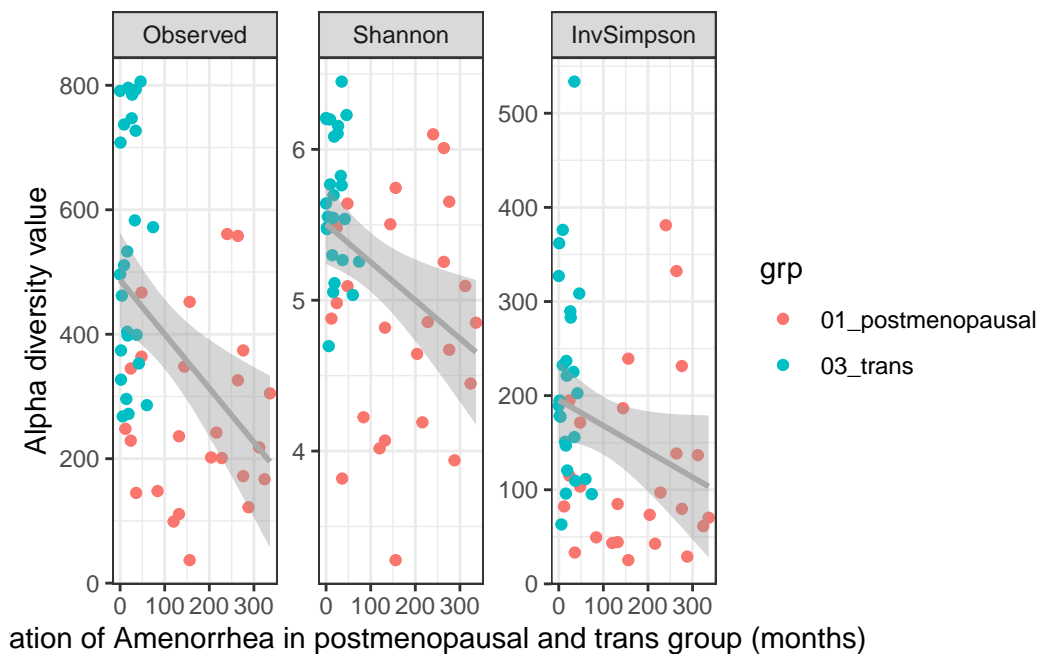
variable	var1	var2	cor	statistic	p	conf.low	conf.high	method
Observed	value	DurationMenopause	0.045	0.2158134	0.831	-0.3565088	0.4324048	Pearson
Shannon	value	DurationMenopause	0.078	0.3754553	0.711	-0.3271717	0.4590238	Pearson
InvSimpson	value	DurationMenopause	0.130	0.6169177	0.543	-0.2817497	0.4976290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

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

# Next, both groups are combined: in months
corr_menopause = adv_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	-	0.00184	-	-	Pearson
				3.298352		0.6325179	0.1720921	
Shannon	value	DurationCombined	0.37	-	0.00801	-	-	Pearson
				2.766912		0.5884815	0.1031910	
InvSimpson	value	DurationCombined	0.26	-	0.06340	-	0.0149028	Pearson
				1.900510		0.5056562		

```
ggplot(activemenopause, 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
```



```
## 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")

```

```

2024-01-03 15:38:55.549841 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_MenopausedurationMonths/maaslin2.log"
2024-01-03 15:38:55.719607 INFO::Writing function arguments to log file
2024-01-03 15:38:55.721651 INFO::Verifying options selected are valid
2024-01-03 15:38:55.721897 INFO::Determining format of input files
2024-01-03 15:38:55.722116 INFO::Input format is data samples as rows and metadata samples as columns
2024-01-03 15:38:55.723874 INFO::Formula for fixed effects: expr ~ DurationCombined
2024-01-03 15:38:55.724136 INFO::Filter data based on min abundance and min prevalence
2024-01-03 15:38:55.724347 INFO::Total samples in data: 50
2024-01-03 15:38:55.724549 INFO::Min samples required with min abundance for a feature not to be filtered: 5
2024-01-03 15:38:55.726018 INFO::Total filtered features: 292
2024-01-03 15:38:55.726289 INFO::Filtered feature names from abundance and prevalence filtering: ASV14, ASV54, ASV82, ASV205, ASV210, ASV369
2024-01-03 15:38:55.726733 INFO::Total filtered features with variance filtering: 0
2024-01-03 15:38:55.726954 INFO::Filtered feature names from variance filtering:
2024-01-03 15:38:55.727156 INFO::Running selected normalization method: TSS
2024-01-03 15:38:55.72753 INFO::Applying z-score to standardize continuous metadata
2024-01-03 15:38:55.729857 INFO::Running selected transform method: LOG
2024-01-03 15:38:55.730236 INFO::Running selected analysis method: LM
2024-01-03 15:38:55.730494 INFO::Fitting model to feature number 1, ASV14
2024-01-03 15:38:55.731542 INFO::Fitting model to feature number 2, ASV54
2024-01-03 15:38:55.732398 INFO::Fitting model to feature number 3, ASV82
2024-01-03 15:38:55.733238 INFO::Fitting model to feature number 4, ASV205
2024-01-03 15:38:55.734067 INFO::Fitting model to feature number 5, ASV210
2024-01-03 15:38:55.734896 INFO::Fitting model to feature number 6, ASV369
2024-01-03 15:38:55.737405 INFO::Counting total values for each feature
2024-01-03 15:38:55.738014 INFO::Writing filtered data to file results/maaslin2/genus_MenopausedurationMonths/maaslin2.filtered.csv
2024-01-03 15:38:55.738943 INFO::Writing filtered, normalized data to file results/maaslin2/genus_MenopausedurationMonths/maaslin2.filtered.normalized.csv
2024-01-03 15:38:55.739572 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/genus_MenopausedurationMonths/maaslin2.filtered.normalized.transformed.csv
2024-01-03 15:38:55.740215 WARNING::Deleting existing residuals file: results/maaslin2/genus_MenopausedurationMonths/maaslin2.residuals.csv
2024-01-03 15:38:55.740616 INFO::Writing residuals to file results/maaslin2/genus_MenopausedurationMonths/maaslin2.residuals.csv
2024-01-03 15:38:55.741044 WARNING::Deleting existing fitted file: results/maaslin2/genus_MenopausedurationMonths/maaslin2.fitted.csv
2024-01-03 15:38:55.741137 INFO::Writing fitted values to file results/maaslin2/genus_MenopausedurationMonths/maaslin2.fitted.csv
2024-01-03 15:38:55.741743 INFO::Writing all results to file (ordered by increasing q-values): results/maaslin2/genus_MenopausedurationMonths/maaslin2.results.csv
2024-01-03 15:38:55.742292 INFO::Writing the significant results (those which are less than 0.05) to file: results/maaslin2/genus_MenopausedurationMonths/maaslin2.significant.csv
2024-01-03 15:38:55.742677 INFO::Writing heatmap of significant results to file: results/maaslin2/genus_MenopausedurationMonths/maaslin2.significant.heatmap.png
[1] "There is not enough metadata in the associations to create a heatmap plot. Please review the metadata."
2024-01-03 15:38:55.743211 INFO::Writing association plots (one for each significant association) to file: results/maaslin2/genus_MenopausedurationMonths/maaslin2.association.plots

```

```
2024-01-03 15:38:55.743763 INFO::Plotting associations from most to least significant, group
2024-01-03 15:38:55.744037 INFO::Plotting data for metadata number 1, DurationCombined
2024-01-03 15:38:55.744549 INFO::Creating scatter plot for continuous data, DurationCombined
```

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

```
2024-01-03 15:38:55.807905 INFO::Creating scatter plot for continuous data, DurationCombined
```

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

```
2024-01-03 15:38:55.873022 INFO::Creating scatter plot for continuous data, DurationCombined
```

```
2024-01-03 15:38:55.93341 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()`).
```

```
# 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>			

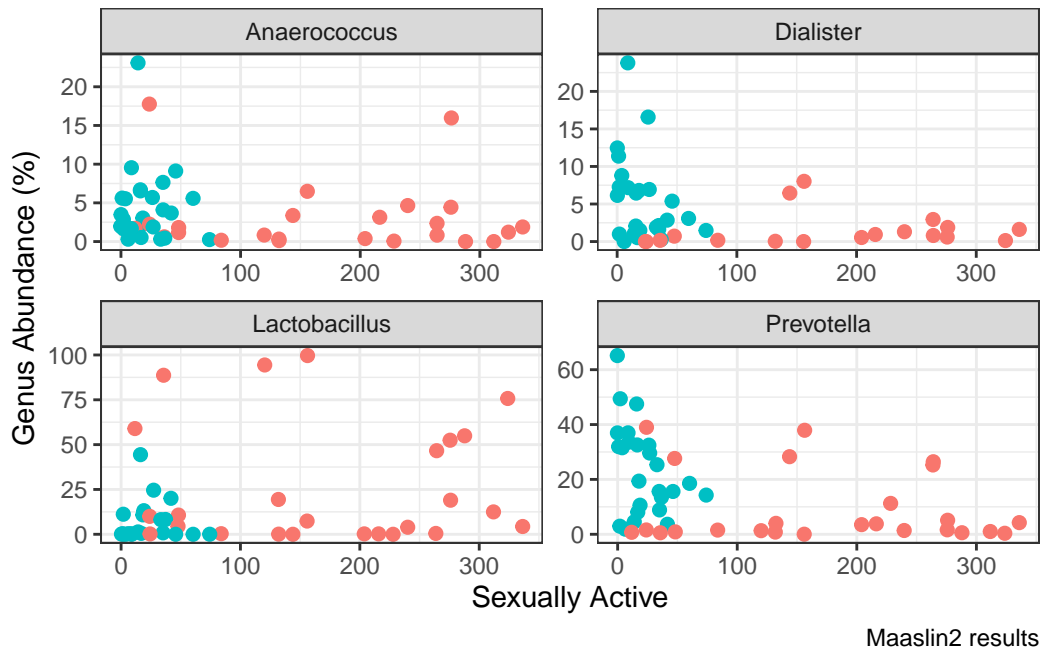
```
# Lactobacillus, dialister, Anaerococcus, Prevotella

# 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()`).



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

Warning: Removed 12 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

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

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

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
```

```

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.

```

adonis_bc_partnersex = vegan::adonis2(D_BC ~ phyloseq::sample_data(ps_filtered_rel)$SexOfPartner)
adonis_pw_bc_partnersex = pairwise.adonis(D_BC, phyloseq::sample_data(ps_filtered_rel)$SexOfPartner)

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

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

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

```

Table 20: 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	*

```

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

```

Table 21: Unweighted 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 *

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

Table 22: 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 comparison of M vs F.

Using unweighted Unifrac, there is a uncorrected significant 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")
```

```
2024-01-03 15:38:57.520458 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_SexOfPartner/maaslin2.log"
2024-01-03 15:38:57.680692 INFO::Writing function arguments to log file
2024-01-03 15:38:57.682784 INFO::Verifying options selected are valid
2024-01-03 15:38:57.683031 INFO::Determining format of input files
2024-01-03 15:38:57.683254 INFO::Input format is data samples as rows and metadata samples as
```

```

2024-01-03 15:38:57.685129 INFO::Formula for fixed effects: expr ~ SexOfPartner
2024-01-03 15:38:57.685413 INFO::Factor detected for categorical metadata 'SexOfPartner'. Pro
2024-01-03 15:38:57.685629 INFO::Filter data based on min abundance and min prevalence
2024-01-03 15:38:57.68584 INFO::Total samples in data: 72
2024-01-03 15:38:57.686045 INFO::Min samples required with min abundance for a feature not t
2024-01-03 15:38:57.68758 INFO::Total filtered features: 294
2024-01-03 15:38:57.687869 INFO::Filtered feature names from abundance and prevalence filter
2024-01-03 15:38:57.688327 INFO::Total filtered features with variance filtering: 0
2024-01-03 15:38:57.688574 INFO::Filtered feature names from variance filtering:
2024-01-03 15:38:57.688789 INFO::Running selected normalization method: TSS
2024-01-03 15:38:57.68919 INFO::Applying z-score to standardize continuous metadata
2024-01-03 15:38:57.691356 INFO::Running selected transform method: LOG
2024-01-03 15:38:57.691738 INFO::Running selected analysis method: LM
2024-01-03 15:38:57.691997 INFO::Fitting model to feature number 1, ASV1
2024-01-03 15:38:57.693121 INFO::Fitting model to feature number 2, ASV54
2024-01-03 15:38:57.694087 INFO::Fitting model to feature number 3, ASV66
2024-01-03 15:38:57.695065 INFO::Fitting model to feature number 4, ASV87
2024-01-03 15:38:57.697783 INFO::Counting total values for each feature
2024-01-03 15:38:57.698481 INFO::Writing filtered data to file results/maaslin2/genus_SexOfPa
2024-01-03 15:38:57.699203 INFO::Writing filtered, normalized data to file results/maaslin2/
2024-01-03 15:38:57.699843 INFO::Writing filtered, normalized, transformed data to file resu
2024-01-03 15:38:57.700515 WARNING::Deleting existing residuals file: results/maaslin2/genus
2024-01-03 15:38:57.70095 INFO::Writing residuals to file results/maaslin2/genus_SexOfPartne
2024-01-03 15:38:57.701455 WARNING::Deleting existing fitted file: results/maaslin2/genus_Se
2024-01-03 15:38:57.701792 INFO::Writing fitted values to file results/maaslin2/genus_SexOfPa
2024-01-03 15:38:57.702151 INFO::Writing all results to file (ordered by increasing q-values
2024-01-03 15:38:57.702726 INFO::Writing the significant results (those which are less than
2024-01-03 15:38:57.703106 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2024-01-03 15:38:57.703576 INFO::Writing association plots (one for each significant associat
2024-01-03 15:38:57.704121 INFO::Plotting associations from most to least significant, group
2024-01-03 15:38:57.704392 INFO::Plotting data for metadata number 1, SexOfPartner
2024-01-03 15:38:57.70491 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"))

```



```

2024-01-03 15:38:57.844129 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_SexOfPartnergrp/maaslin2.log"
2024-01-03 15:38:58.002822 INFO::Writing function arguments to log file
2024-01-03 15:38:58.004873 INFO::Verifying options selected are valid
2024-01-03 15:38:58.005119 INFO::Determining format of input files
2024-01-03 15:38:58.005349 INFO::Input format is data samples as rows and metadata samples as columns
2024-01-03 15:38:58.007208 INFO::Formula for fixed effects: expr ~ SexOfPartner + grp
2024-01-03 15:38:58.007483 INFO::Factor detected for categorical metadata 'SexOfPartner'. Provide a reference level
2024-01-03 15:38:58.007705 INFO::Factor detected for categorical metadata 'grp'. Provide a reference level
2024-01-03 15:38:58.007905 INFO::Filter data based on min abundance and min prevalence
2024-01-03 15:38:58.008108 INFO::Total samples in data: 72
2024-01-03 15:38:58.008309 INFO::Min samples required with min abundance for a feature not to be filtered: 5
2024-01-03 15:38:58.009839 INFO::Total filtered features: 294
2024-01-03 15:38:58.010111 INFO::Filtered feature names from abundance and prevalence filtering:
2024-01-03 15:38:58.010509 INFO::Total filtered features with variance filtering: 0
2024-01-03 15:38:58.01073 INFO::Filtered feature names from variance filtering:
2024-01-03 15:38:58.010934 INFO::Running selected normalization method: TSS
2024-01-03 15:38:58.011315 INFO::Applying z-score to standardize continuous metadata
2024-01-03 15:38:58.013571 INFO::Running selected transform method: LOG
2024-01-03 15:38:58.013937 INFO::Running selected analysis method: LM
2024-01-03 15:38:58.0142 INFO::Fitting model to feature number 1, ASV1
2024-01-03 15:38:58.015419 INFO::Fitting model to feature number 2, ASV54
2024-01-03 15:38:58.016469 INFO::Fitting model to feature number 3, ASV66
2024-01-03 15:38:58.017494 INFO::Fitting model to feature number 4, ASV87
2024-01-03 15:38:58.020555 INFO::Counting total values for each feature
2024-01-03 15:38:58.021428 INFO::Writing filtered data to file results/maaslin2/genus_SexOfPartnergrp/maaslin2.filtered.data
2024-01-03 15:38:58.022167 INFO::Writing filtered, normalized data to file results/maaslin2/genus_SexOfPartnergrp/maaslin2.filtered.normalized.data
2024-01-03 15:38:58.022801 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/genus_SexOfPartnergrp/maaslin2.filtered.normalized.transformed.data
2024-01-03 15:38:58.023458 WARNING::Deleting existing residuals file: results/maaslin2/genus_SexOfPartnergrp/maaslin2.residuals
2024-01-03 15:38:58.023875 INFO::Writing residuals to file results/maaslin2/genus_SexOfPartnergrp/maaslin2.residuals
2024-01-03 15:38:58.024331 WARNING::Deleting existing fitted file: results/maaslin2/genus_SexOfPartnergrp/maaslin2.fitted
2024-01-03 15:38:58.02466 INFO::Writing fitted values to file results/maaslin2/genus_SexOfPartnergrp/maaslin2.fitted
2024-01-03 15:38:58.025015 INFO::Writing all results to file (ordered by increasing q-values)
2024-01-03 15:38:58.025614 INFO::Writing the significant results (those which are less than 0.05) to file results/maaslin2/genus_SexOfPartnergrp/maaslin2.significant
2024-01-03 15:38:58.026013 INFO::Writing heatmap of significant results to file: results/maaslin2/genus_SexOfPartnergrp/maaslin2.significant.heatmap
2024-01-03 15:38:58.054175 INFO::Writing association plots (one for each significant association) to file results/maaslin2/genus_SexOfPartnergrp/maaslin2.significant.association.plots
2024-01-03 15:38:58.055065 INFO::Plotting associations from most to least significant, grouped by metadata
2024-01-03 15:38:58.055397 INFO::Plotting data for metadata number 1, grp
2024-01-03 15:38:58.055977 INFO::Creating boxplot for categorical data, grp vs ASV54

2024-01-03 15:38:58.124797 INFO::Creating boxplot for categorical data, grp vs ASV87

2024-01-03 15:38:58.181969 INFO::Creating boxplot for categorical data, grp vs ASV1

```

2024-01-03 15:38:58.238614 INFO::Creating boxplot for categorical data, grp vs ASV1

2024-01-03 15:38:58.296178 INFO::Creating boxplot for categorical data, grp vs ASV54

2024-01-03 15:38:58.6583 INFO::Plotting data for metadata number 2, SexOfPartner

2024-01-03 15:38:58.659027 INFO::Creating boxplot for categorical data, SexOfPartner vs ASV87

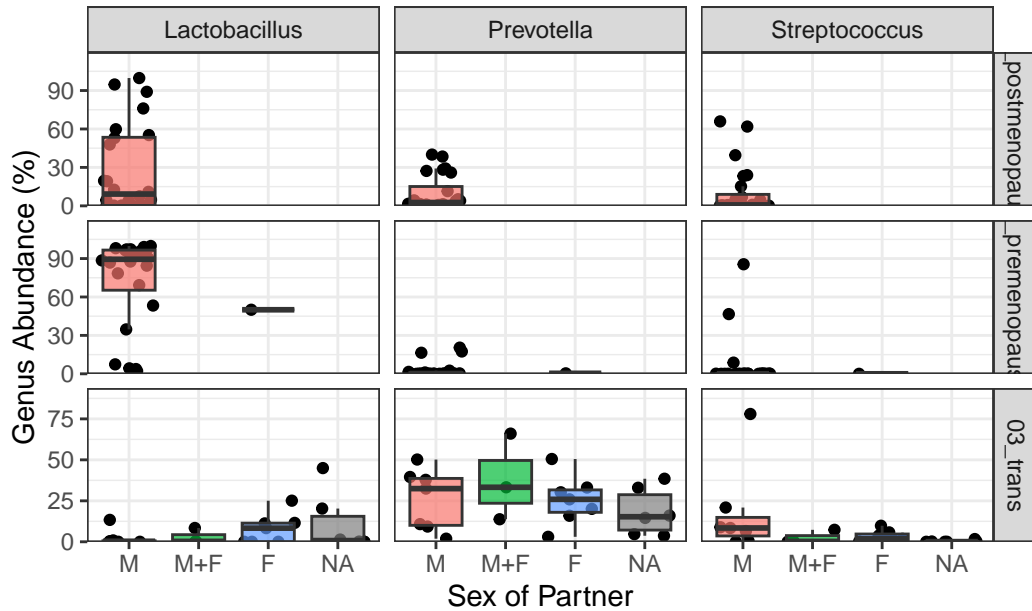
```
tax_table(ps_rel_genus_sexuallyactive) %>%
  as.data.frame() %>%
  rownames_to_column("ASV") %>%
  filter(ASV %in% filter(genus_SexOfPartner2$results, qval < 0.2)$feature)
```

	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>				

```
# plot abundance of Prevotella
maaslin_res_genus_sexpartner = ggplot(filter(psmelt_rel_genus_sexuallyactive, OTU %in% filter(
  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

```
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")
```

```
2024-01-03 15:38:59.300612 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_SexOfPartnerTrans/maaslin2.
2024-01-03 15:38:59.467214 INFO::Writing function arguments to log file
2024-01-03 15:38:59.46934 INFO::Verifying options selected are valid
2024-01-03 15:38:59.469601 INFO::Determining format of input files
2024-01-03 15:38:59.469839 INFO::Input format is data samples as rows and metadata samples as
2024-01-03 15:38:59.471682 INFO::Formula for fixed effects: expr ~ SexOfPartner
2024-01-03 15:38:59.471949 INFO::Factor detected for categorical metadata 'SexOfPartner'. Pro
```

```

2024-01-03 15:38:59.472154 INFO::Filter data based on min abundance and min prevalence
2024-01-03 15:38:59.472359 INFO::Total samples in data: 23
2024-01-03 15:38:59.472563 INFO::Min samples required with min abundance for a feature not to
2024-01-03 15:38:59.473963 INFO::Total filtered features: 291
2024-01-03 15:38:59.474229 INFO::Filtered feature names from abundance and prevalence filter
2024-01-03 15:38:59.474652 INFO::Total filtered features with variance filtering: 0
2024-01-03 15:38:59.474875 INFO::Filtered feature names from variance filtering:
2024-01-03 15:38:59.475077 INFO::Running selected normalization method: TSS
2024-01-03 15:38:59.475457 INFO::Applying z-score to standardize continuous metadata
2024-01-03 15:38:59.477648 INFO::Running selected transform method: LOG
2024-01-03 15:38:59.478023 INFO::Running selected analysis method: LM
2024-01-03 15:38:59.478282 INFO::Fitting model to feature number 1, ASV1
2024-01-03 15:38:59.479427 INFO::Fitting model to feature number 2, ASV54
2024-01-03 15:38:59.480365 INFO::Fitting model to feature number 3, ASV66
2024-01-03 15:38:59.481277 INFO::Fitting model to feature number 4, ASV87
2024-01-03 15:38:59.482186 INFO::Fitting model to feature number 5, ASV205
2024-01-03 15:38:59.483089 INFO::Fitting model to feature number 6, ASV369
2024-01-03 15:38:59.484011 INFO::Fitting model to feature number 7, ASV397
2024-01-03 15:38:59.486896 INFO::Counting total values for each feature
2024-01-03 15:38:59.487692 INFO::Writing filtered data to file results/maaslin2/genus_SexOfPa
2024-01-03 15:38:59.488532 INFO::Writing filtered, normalized data to file results/maaslin2/g
2024-01-03 15:38:59.489174 INFO::Writing filtered, normalized, transformed data to file resu
2024-01-03 15:38:59.489786 WARNING::Deleting existing residuals file: results/maaslin2/genus
2024-01-03 15:38:59.49038 INFO::Writing residuals to file results/maaslin2/genus_SexOfPartne
2024-01-03 15:38:59.490795 WARNING::Deleting existing fitted file: results/maaslin2/genus_Se
2024-01-03 15:38:59.491124 INFO::Writing fitted values to file results/maaslin2/genus_SexOfPa
2024-01-03 15:38:59.49146 INFO::Writing all results to file (ordered by increasing q-values)
2024-01-03 15:38:59.492051 INFO::Writing the significant results (those which are less than c
2024-01-03 15:38:59.492387 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2024-01-03 15:38:59.49285 INFO::Writing association plots (one for each significant associat
[1] "There are no associations to plot!"

```

```

# no significant results in trans group only

```