

# 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

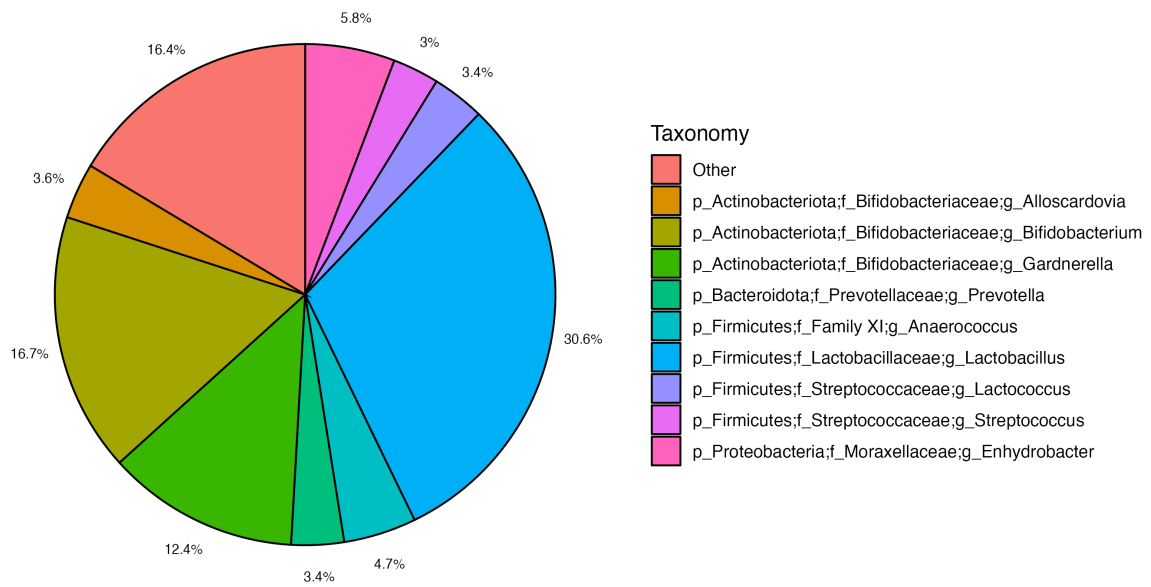


Figure 1: Taxonomic composition of PBS sample

```
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.

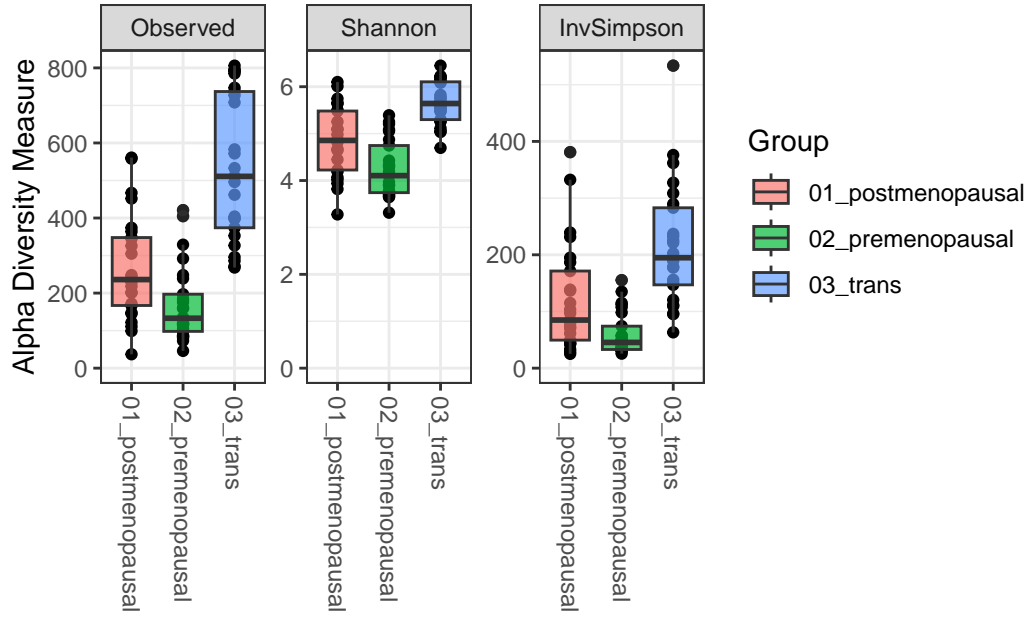
In this dataset, alpha diversity is markedly different between the group of interest (i.e. *03\_trans*) and the two control groups. However, the intervention group is more similar to the first control group (i.e. *postmenopausal women*).

```
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")+
  expand_limits(y = 0)
```



```
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")
```

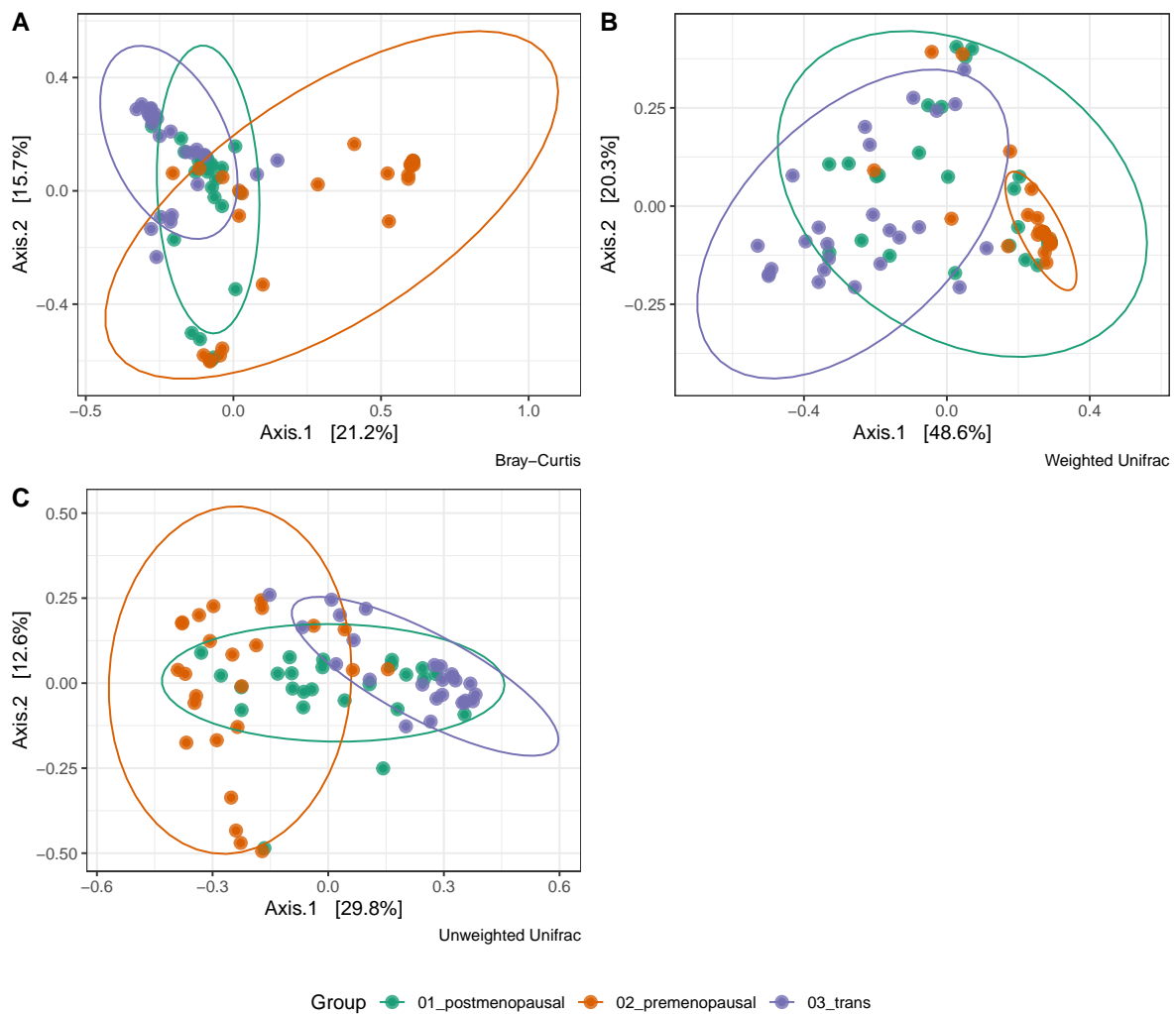


)

```
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	FoldChange	negLog10Pval	adjPval	Kingdom	Phylum	Class	Order	Family	Genus	
ASV55	8.9674	5.588	1.502	3.0550	0.0020	0.009	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV62	1.674	4.442	1.443	3.0690	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV75	8.4584	1.132	1.432	2.8830	0.0040	0.013	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV85	8.5634	1.669	1.500	3.1120	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV95	6.4434	1.629	1.483	3.1160	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV156	1.4324	1.277	1.501	2.8500	0.0040	0.014	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV156	1.1334	1.037	1.432	2.8100	0.0050	0.016	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV156	1.2984	1.465	1.510	2.9580	0.0030	0.011	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV153	1.1694	1.740	1.443	3.2890	0.0010	0.005	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV151	1.2413	1.860	1.451	2.6600	0.0080	0.022	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV147	1.3954	1.291	1.482	2.8950	0.0040	0.013	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV148	1.6634	1.439	1.440	3.0830	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV147	1.2534	1.649	1.473	3.1480	0.0020	0.007	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV246	1.1774	1.292	1.393	3.0770	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV243	1.1014	1.616	1.453	3.1720	0.0020	0.007	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV241	1.9824	1.235	1.520	2.7860	0.0050	0.017	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV242	1.9054	1.446	1.382	3.2170	0.0010	0.006	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV340	1.8994	1.442	1.414	3.1410	0.0020	0.007	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV339	1.2904	1.235	1.492	2.8320	0.0050	0.015	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV339	1.1614	1.514	1.461	3.0890	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV337	1.8594	1.511	1.497	3.0120	0.0030	0.009	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV338	1.1474	1.606	1.479	3.1150	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV430	1.3044	1.236	1.472	2.8720	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
	3.329		2.459									
ASV537	3.625	-	1.333	-	0.0160	0.039	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.196		2.398									
ASV537	3.608	-	1.336	-	0.0130	0.033	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.317		2.483									
ASV534	1.361	-	1.466	-	0.0230	0.050	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.341		2.279									
ASV532	1.825	-	1.483	-	0.0060	0.018	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	4.053		2.733									
ASV632	1.056	-	1.415	-	0.0050	0.016	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.957		2.796									
ASV632	1.648	-	1.379	-	0.0050	0.017	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.842		2.787									
ASV631	1.023	-	1.384	-	0.0120	0.031	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.482		2.517									
ASV627	1.851	-	1.404	-	0.0030	0.012	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	4.106		2.924									

ASV	baseMean	log2FoldChange	negLog10Padj	significance	Kingdom	Phylum	Class	Order	Family	Genus		
ASV627	0.069	-	1.465	-	0.009	0.026	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.805		2.597									
ASV626	0.421	-	1.399	-	0.009	0.024	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.679		2.629									
ASV726	0.234	-	1.342	-	0.015	0.036	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.269		2.437									
ASV725	0.051	-	1.505	-	0.020	0.043	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.510		2.333									
ASV722	0.871	-	1.379	-	0.015	0.037	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.350		2.429									
ASV722	0.816	-	1.468	-	0.010	0.028	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.757		2.560									
ASV822	0.716	-	1.432	-	0.022	0.049	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.270		2.283									
ASV822	0.808	-	1.500	-	0.005	0.017	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	4.167		2.778									
ASV822	0.674	-	1.453	-	0.014	0.034	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.577		2.462									
ASV920	0.463	-	1.440	-	0.018	0.043	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.392		2.356									
ASV100	0.793	-	1.414	-	0.003	0.009	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	4.268		3.018									
ASV122	0.144	-	1.444	-	0.012	0.032	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.610		2.500									
ASV120	0.759	-	1.455	-	0.022	0.049	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Finegoldia
	3.325		2.285					Tissierellales	XI			
ASV120	0.523	-	1.743	-	0.002	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	5.367		3.079									
ASV130	0.781	-	1.443	-	0.019	0.043	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.399		2.355									
ASV130	0.041	-	1.606	-	0.004	0.014	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	4.593		2.860									
ASV140	0.964	-	1.656	-	0.000	0.000	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	7.262		4.385									
ASV152	0.289	-	1.779	-	0.000	0.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	7.158		4.024									
ASV155	0.947	-	1.469	-	0.001	0.004	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	4.982		3.391									
ASV163	0.189	-	1.455	-	0.000	0.002	Bacteria	Bacteroidia	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	5.168		3.551									

ASV	baseMean	log2FoldChange	negLog10Pval	negLog10Padj	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	Negativicutes	Mitronellales- 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	negLog10Pval	adjPval	Kingdom	Phylum	Class	Order	Family	Genus
ASV247.258	-	1.441	-	0.0220.049	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	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		
ASV257.602	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	Familia	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	Reptostreptococcales	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	Reptostreptococcales	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	Reptostreptococcales	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	Reptostreptococcales	Familia	Fenollaria
	7.522		4.078					Tissierellales	XI	
ASV48228	-	1.778	-	0.0000.000	Bacteria	Firmicutes	Glostridia	Reptostreptococcales	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	Reptostreptococcales	Familia	Fenollaria
	7.384		3.951					Tissierellales	XI	
ASV49397	-	1.545	-	0.0000.000	Bacteria	Firmicutes	Glostridia	Reptostreptococcales	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	Reptostreptococcales	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	Reptostreptococcales	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	Negativibacteriia	Veillonellales	Veillonellaceae	Dialister
	5.572		3.060					Selenomonadales		
ASV48615	-	1.743	-	0.0020.007	Bacteria	Firmicutes	Negativibacteriia	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	Negativibacteriia	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	Campylobacteriia	Campylobacteriia	Campylobacterales	Campylobacteraceae	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	Negativibacteriia	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	negLog10Padj	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							
ASV56559	-	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	
ASV65924	-	1.908	-	0.000	0.003	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	6.721		3.523						Tissierellales	XI	
ASV67364	-	2.197	-	0.007	0.020	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces
	5.939		2.702								
ASV67509	-	1.601	-	0.001	0.003	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	5.480		3.422						Tissierellales	XI	
ASV68046	-	1.937	-	0.001	0.005	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Anaerococcus
	6.415		3.312						Tissierellales	XI	
ASV68334	-	1.494	-	0.000	0.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	6.197		4.147						Tissierellales	XI	
ASV68747	-	2.281	-	0.006	0.017	Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Howarthella
	6.315		2.768								
ASV68792	-	2.283	-	0.014	0.034	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Anaerococcus
	5.607		2.457						Tissierellales	XI	
ASV69841	-	1.929	-	0.001	0.005	Bacteria	Bacteroidetes	Bacteroidetes	Bacteroidales	Prevotellaceae	Prevotella
	6.274		3.253								
ASV69959	-	1.873	-	0.000	0.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	6.653		3.552						Tissierellales	XI	
ASV69963	-	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	negLog10Padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV70337	-	2.053	- 0.0030.010	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecoalcaliphilaceae	Anaerococcus
	6.118	2.980					Tissierellales	XI	
ASV74980	-	2.066	- 0.0020.009	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	6.300	3.049							
ASV74576	-	1.662	- 0.0000.002	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
	6.110	3.675					Selenomonadales		
ASV84402	-	2.285	- 0.0080.022	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecoalcaliphilaceae	Ezakiella
	6.101	2.670					Tissierellales	XI	
ASV84365	-	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	Faecoalcaliphilaceae	Anaerococcus
	5.775	2.405					Tissierellales	XI	
ASV80930	-	2.044	- 0.0030.010	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecoalcaliphilaceae	Anaerococcus
	6.075	2.973					Tissierellales	XI	
ASV84568	-	1.684	- 0.0010.004	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecoalcaliphilaceae	Peptoniphilus
	5.638	3.348					Tissierellales	XI	
ASV82017	-	1.919	- 0.0010.005	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecoalcaliphilaceae	Fenollaria
	6.309	3.288					Tissierellales	XI	
ASV82351	-	2.276	- 0.0090.025	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecoalcaliphilaceae	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	Negativicutes	Veillonellales	Veillonellaceae	Dialister
	6.128	3.686					Selenomonadales		
ASV84485	-	1.742	- 0.0010.005	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecoalcaliphilaceae	Peptoniphilus
	5.719	3.283					Tissierellales	XI	
ASV83900	-	2.198	- 0.0060.017	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecoalcaliphilaceae	Anaerococcus
	6.097	2.775					Tissierellales	XI	
ASV84592	-	1.689	- 0.0010.003	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
	5.777	3.420					Selenomonadales		
ASV83270	-	1.589	- 0.0010.004	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecoalcaliphilaceae	Peptoniphilus
	5.344	3.363					Tissierellales	XI	
ASV83406	-	2.265	- 0.0100.026	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecoalcaliphilaceae	Anaerococcus
	5.865	2.589					Tissierellales	XI	
ASV83488	-	1.734	- 0.0000.001	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.692	3.860							
ASV84585	-	2.000	- 0.0040.014	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	5.745	2.873							
ASV83470	-	2.056	- 0.0040.013	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecoalcaliphilaceae	Anaerococcus
	5.918	2.879					Tissierellales	XI	

ASV	baseMean	log2FoldChange	negLog10Pval	negLog10Padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV83970	-	2.171	-	0.0080.022	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Anaerococcus
	5.789		2.667					Tissierellales	XI	
ASV84604	-	2.177	-	0.0060.018	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	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	Peptostreptococcales	Familia	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	Peptostreptococcales	Familia	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	Peptostreptococcales	Familia	Anaerococcus
	5.817		2.811					Tissierellales	XI	
ASV97514	-	2.298	-	0.0200.045	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Parvimonas
	5.353		2.329					Tissierellales	XI	
ASV98847	-	2.364	-	0.0140.034	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Anaerococcus
	5.816		2.461					Tissierellales	XI	
ASV10229	-	2.287	-	0.0120.031	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	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	Peptostreptococcales	Familia	Parvimonas
	5.942		2.509					Tissierellales	XI	
ASV10861	-	2.037	-	0.0030.012	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Ezakiella
	5.963		2.927					Tissierellales	XI	
ASV12280	-	2.097	-	0.0050.016	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Anaerococcus
	5.882		2.805					Tissierellales	XI	
ASV12324	-	2.306	-	0.0190.044	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	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") +
  expand_limits(y = 0)

# 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
Finegoldia	02_premenopausal	0.0300835	0.0000000	0.1045203	0.000000	0.4076266

Genus	grp	mean	median	sd	min	max
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

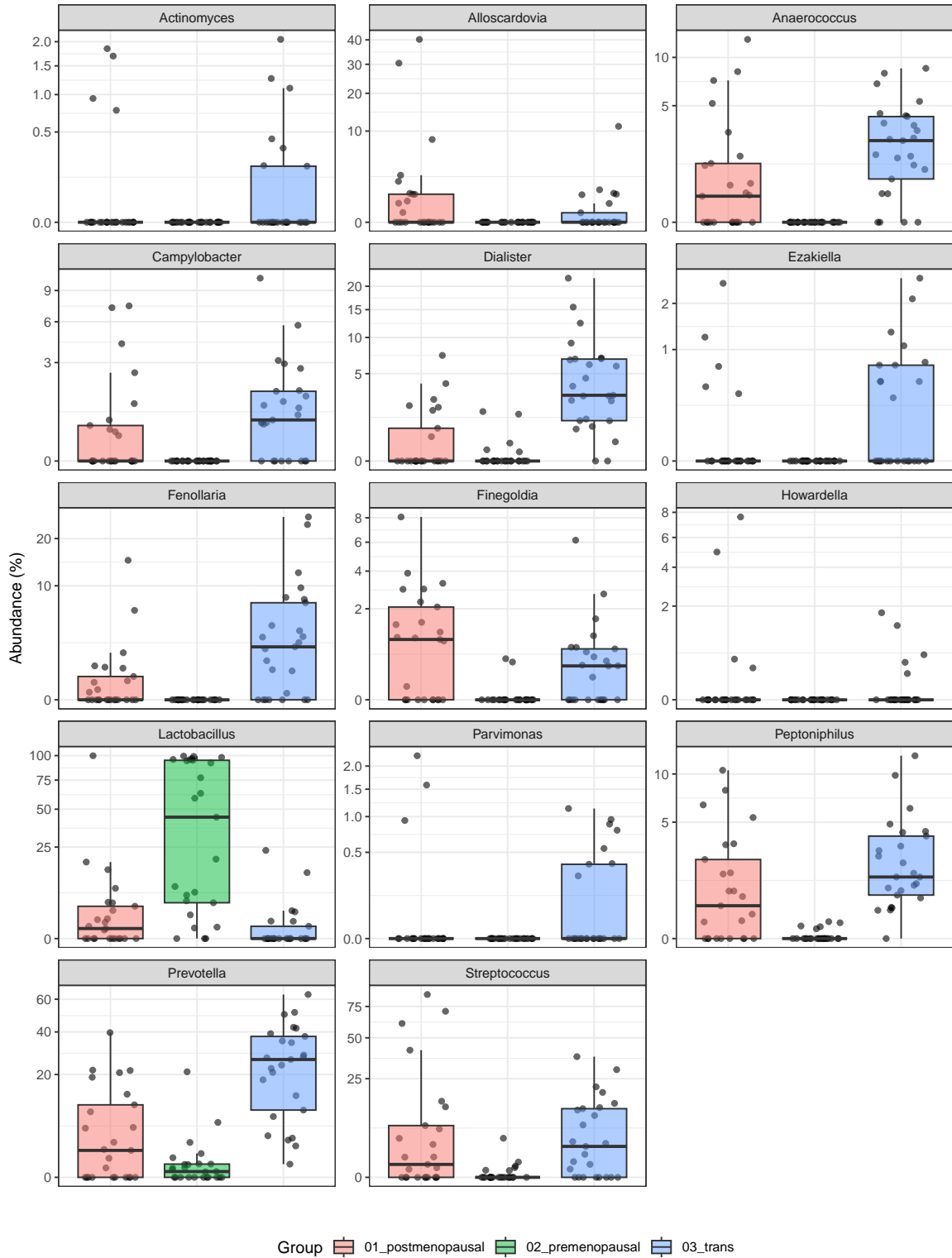
This analysis reveals, that the *Trans* group is characterized by a loss of *Lactobacillus* and an

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:Estradiol, 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") +
```

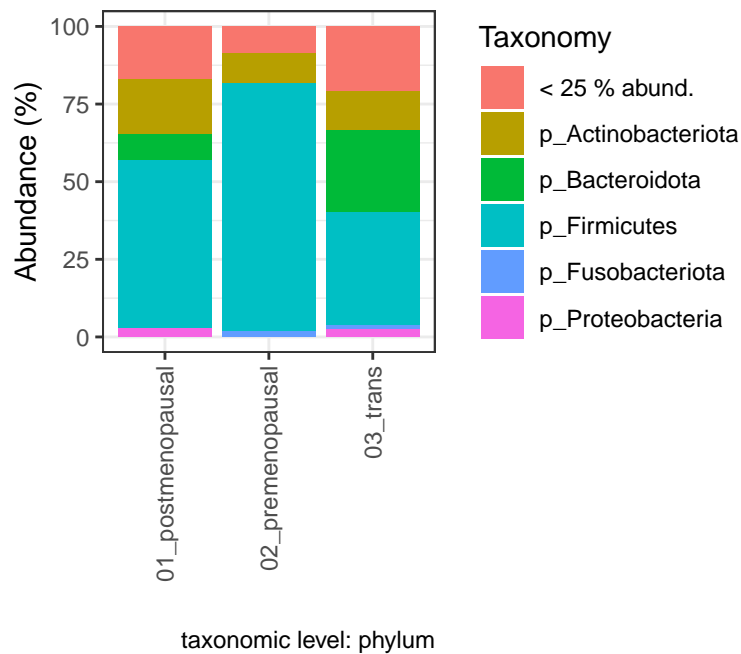


```
theme(legend.position = "right") +
labs(y = "Abundance (%)", x = "", caption = "taxonomic level: phylum") +
rotate_x_text()
```

## 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.

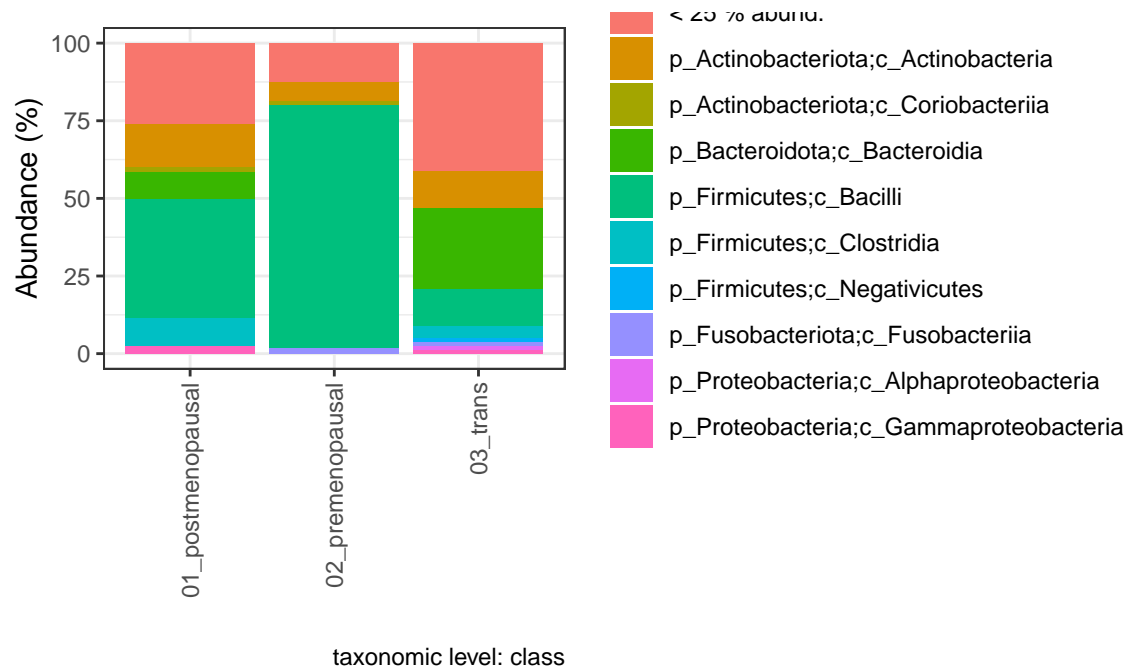
```
phylumplot
```



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

## 5.2 Class level

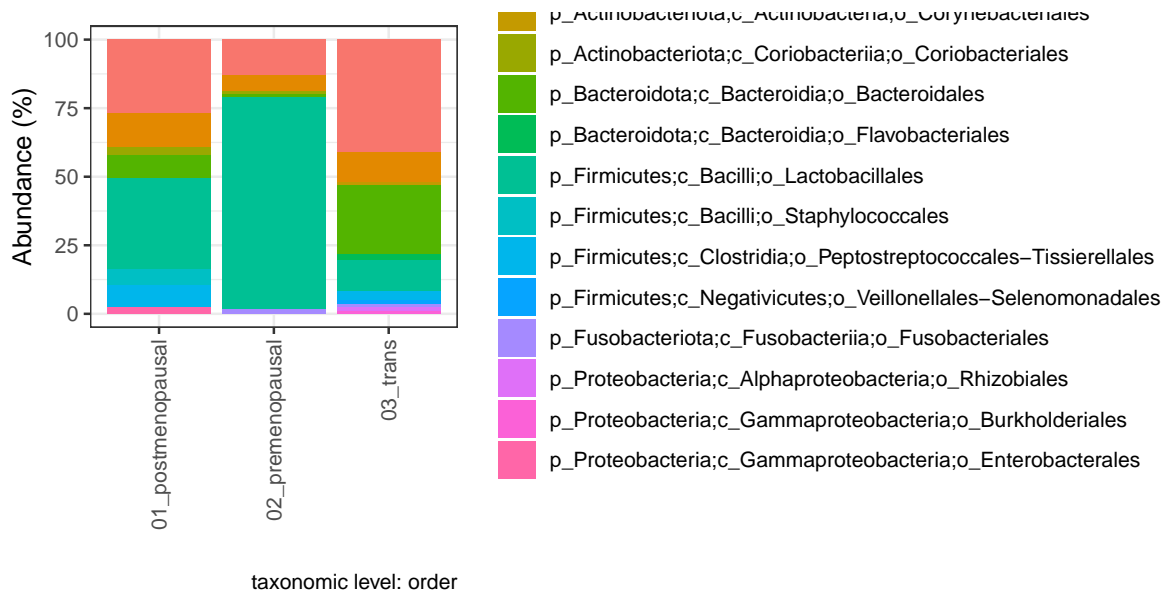
```
classplot
```



```
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

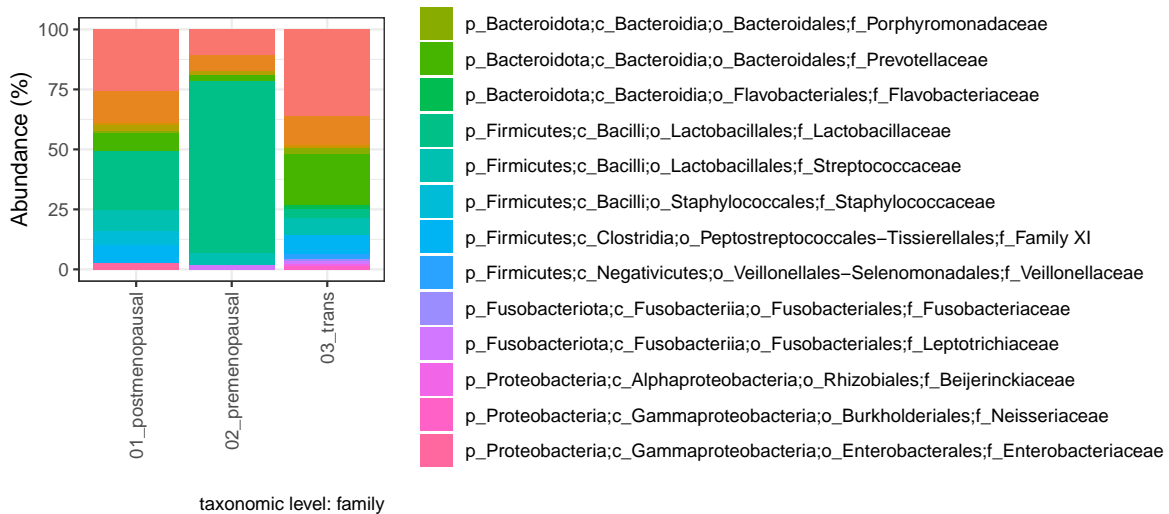
```
orderplot
```



```
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

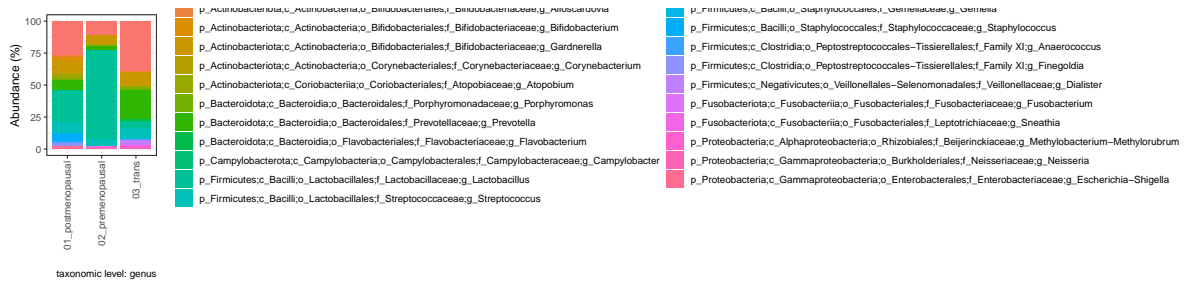
familyplot



```
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")
```

```
2023-11-18 17:25:16.857848 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/asv_menopause-duration/maaslin2.1
2023-11-18 17:25:23.056202 INFO::Writing function arguments to log file
2023-11-18 17:25:23.062647 INFO::Verifying options selected are valid
2023-11-18 17:25:23.087287 INFO::Determining format of input files
2023-11-18 17:25:23.087746 INFO::Input format is data samples as rows and metadata samples as
2023-11-18 17:25:23.128715 INFO::Formula for fixed effects: expr ~ DurationMenopause
2023-11-18 17:25:23.129223 INFO::Filter data based on min abundance and min prevalence
2023-11-18 17:25:23.129508 INFO::Total samples in data: 25
2023-11-18 17:25:23.129771 INFO::Min samples required with min abundance for a feature not t
2023-11-18 17:25:23.174388 INFO::Total filtered features: 8831
2023-11-18 17:25:23.176191 INFO::Filtered feature names from abundance and prevalence filter
2023-11-18 17:25:23.178637 INFO::Total filtered features with variance filtering: 0
2023-11-18 17:25:23.17893 INFO::Filtered feature names from variance filtering:
2023-11-18 17:25:23.179191 INFO::Running selected normalization method: TSS
```

```

2023-11-18 17:25:23.180203 INFO::Applying z-score to standardize continuous metadata
2023-11-18 17:25:23.183175 INFO::Running selected transform method: LOG
2023-11-18 17:25:23.183692 INFO::Running selected analysis method: LM
2023-11-18 17:25:23.187355 INFO::Fitting model to feature number 1, ASV14
2023-11-18 17:25:23.189595 INFO::Fitting model to feature number 2, ASV16
2023-11-18 17:25:23.193053 INFO::Counting total values for each feature
2023-11-18 17:25:23.193872 INFO::Writing filtered data to file results/maaslin2/asv_menopause-
2023-11-18 17:25:23.195206 INFO::Writing filtered, normalized data to file results/maaslin2/
2023-11-18 17:25:23.19584 INFO::Writing filtered, normalized, transformed data to file resul
2023-11-18 17:25:23.196486 WARNING::Deleting existing residuals file: results/maaslin2/asv_m
2023-11-18 17:25:23.197008 INFO::Writing residuals to file results/maaslin2/asv_menopause-dur
2023-11-18 17:25:23.19751 WARNING::Deleting existing fitted file: results/maaslin2/asv_menopau
2023-11-18 17:25:23.198221 INFO::Writing fitted values to file results/maaslin2/asv_menopaus
2023-11-18 17:25:23.198684 INFO::Writing all results to file (ordered by increasing q-values)
2023-11-18 17:25:23.19936 INFO::Writing the significant results (those which are less than o
2023-11-18 17:25:23.199794 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2023-11-18 17:25:23.20085 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")

```

```

2023-11-18 17:25:30.925497 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_menopause-duration/maaslin2
2023-11-18 17:25:31.142897 INFO::Writing function arguments to log file
2023-11-18 17:25:31.145477 INFO::Verifying options selected are valid
2023-11-18 17:25:31.145783 INFO::Determining format of input files
2023-11-18 17:25:31.146062 INFO::Input format is data samples as rows and metadata samples as
2023-11-18 17:25:31.148276 INFO::Formula for fixed effects: expr ~ DurationMenopause
2023-11-18 17:25:31.148604 INFO::Filter data based on min abundance and min prevalence
2023-11-18 17:25:31.14887 INFO::Total samples in data: 25
2023-11-18 17:25:31.149124 INFO::Min samples required with min abundance for a feature not t

```

```

2023-11-18 17:25:31.15089 INFO::Total filtered features: 294
2023-11-18 17:25:31.151225 INFO::Filtered feature names from abundance and prevalence filter
2023-11-18 17:25:31.151725 INFO::Total filtered features with variance filtering: 0
2023-11-18 17:25:31.152 INFO::Filtered feature names from variance filtering:
2023-11-18 17:25:31.152254 INFO::Running selected normalization method: TSS
2023-11-18 17:25:31.152687 INFO::Applying z-score to standardize continuous metadata
2023-11-18 17:25:31.155516 INFO::Running selected transform method: LOG
2023-11-18 17:25:31.155978 INFO::Running selected analysis method: LM
2023-11-18 17:25:31.156302 INFO::Fitting model to feature number 1, ASV16
2023-11-18 17:25:31.157575 INFO::Fitting model to feature number 2, ASV82
2023-11-18 17:25:31.158651 INFO::Fitting model to feature number 3, ASV271
2023-11-18 17:25:31.159706 INFO::Fitting model to feature number 4, ASV526
2023-11-18 17:25:31.162714 INFO::Counting total values for each feature
2023-11-18 17:25:31.163446 INFO::Writing filtered data to file results/maaslin2/genus_menopau
2023-11-18 17:25:31.164353 INFO::Writing filtered, normalized data to file results/maaslin2/g
2023-11-18 17:25:31.164979 INFO::Writing filtered, normalized, transformed data to file resu
2023-11-18 17:25:31.16562 WARNING::Deleting existing residuals file: results/maaslin2/genus_r
2023-11-18 17:25:31.16612 INFO::Writing residuals to file results/maaslin2/genus_menopause-d
2023-11-18 17:25:31.166621 WARNING::Deleting existing fitted file: results/maaslin2/genus_mer
2023-11-18 17:25:31.167026 INFO::Writing fitted values to file results/maaslin2/genus_menopau
2023-11-18 17:25:31.167462 INFO::Writing all results to file (ordered by increasing q-values)
2023-11-18 17:25:31.168122 INFO::Writing the significant results (those which are less than c
2023-11-18 17:25:31.168548 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2023-11-18 17:25:31.16909 INFO::Writing association plots (one for each significant associati
[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")

```

```

2023-11-18 17:25:34.879676 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/family_menopause-duration/maaslin
2023-11-18 17:25:34.960218 INFO::Writing function arguments to log file

```

```

2023-11-18 17:25:34.962794 INFO::Verifying options selected are valid
2023-11-18 17:25:34.963099 INFO::Determining format of input files
2023-11-18 17:25:34.963378 INFO::Input format is data samples as rows and metadata samples as columns
2023-11-18 17:25:34.964726 INFO::Formula for fixed effects: expr ~ DurationMenopause
2023-11-18 17:25:34.965048 INFO::Filter data based on min abundance and min prevalence
2023-11-18 17:25:34.965321 INFO::Total samples in data: 25
2023-11-18 17:25:34.965575 INFO::Min samples required with min abundance for a feature not met
2023-11-18 17:25:34.966498 INFO::Total filtered features: 111
2023-11-18 17:25:34.966799 INFO::Filtered feature names from abundance and prevalence filtering: 111
2023-11-18 17:25:34.96722 INFO::Total filtered features with variance filtering: 0
2023-11-18 17:25:34.967499 INFO::Filtered feature names from variance filtering: 0
2023-11-18 17:25:34.967753 INFO::Running selected normalization method: TSS
2023-11-18 17:25:34.968197 INFO::Applying z-score to standardize continuous metadata
2023-11-18 17:25:34.971055 INFO::Running selected transform method: LOG
2023-11-18 17:25:34.971528 INFO::Running selected analysis method: LM
2023-11-18 17:25:34.971849 INFO::Fitting model to feature number 1, ASV16
2023-11-18 17:25:34.973094 INFO::Fitting model to feature number 2, ASV82
2023-11-18 17:25:34.974195 INFO::Fitting model to feature number 3, ASV120
2023-11-18 17:25:34.975256 INFO::Fitting model to feature number 4, ASV271
2023-11-18 17:25:34.978265 INFO::Counting total values for each feature
2023-11-18 17:25:34.978993 INFO::Writing filtered data to file results/maaslin2/family_menopause/
2023-11-18 17:25:34.979707 INFO::Writing filtered, normalized data to file results/maaslin2/family_menopause/
2023-11-18 17:25:34.980343 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/family_menopause/
2023-11-18 17:25:34.980989 WARNING::Deleting existing residuals file: results/maaslin2/family_menopause/
2023-11-18 17:25:34.981508 INFO::Writing residuals to file results/maaslin2/family_menopause/
2023-11-18 17:25:34.982018 WARNING::Deleting existing fitted file: results/maaslin2/family_menopause/
2023-11-18 17:25:34.982432 INFO::Writing fitted values to file results/maaslin2/family_menopause/
2023-11-18 17:25:34.982959 INFO::Writing all results to file (ordered by increasing q-values)
2023-11-18 17:25:34.983624 INFO::Writing the significant results (those which are less than 0.05)
2023-11-18 17:25:34.984031 INFO::Writing heatmap of significant results to file: results/maaslin2/family_menopause/
[1] "There are no associations to plot!"
2023-11-18 17:25:34.984581 INFO::Writing association plots (one for each significant association)
[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", "InvSimpson"))

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

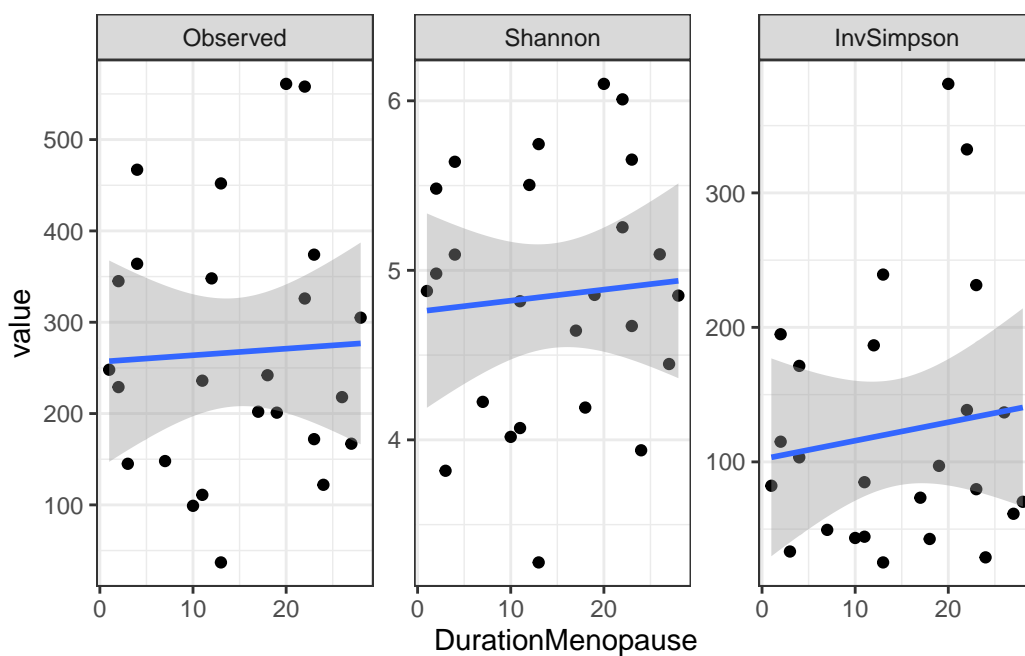
```



```
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")
```

```
2023-11-18 17:25:35.397143 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/asv_GHAT_Length/maaslin2.log"
2023-11-18 17:25:41.497534 INFO::Writing function arguments to log file
2023-11-18 17:25:41.500091 INFO::Verifying options selected are valid
2023-11-18 17:25:41.500392 INFO::Determining format of input files
2023-11-18 17:25:41.500669 INFO::Input format is data samples as rows and metadata samples as columns
2023-11-18 17:25:41.548481 INFO::Formula for fixed effects: expr ~ GHAT_Length
2023-11-18 17:25:41.549016 INFO::Filter data based on min abundance and min prevalence
2023-11-18 17:25:41.549312 INFO::Total samples in data: 25
2023-11-18 17:25:41.549583 INFO::Min samples required with min abundance for a feature not to be filtered: 3
2023-11-18 17:25:41.591398 INFO::Total filtered features: 8824
```

```

2023-11-18 17:25:41.593207 INFO::Filtered feature names from abundance and prevalence filter
2023-11-18 17:25:41.595711 INFO::Total filtered features with variance filtering: 0
2023-11-18 17:25:41.595993 INFO::Filtered feature names from variance filtering:
2023-11-18 17:25:41.596247 INFO::Running selected normalization method: TSS
2023-11-18 17:25:41.596719 INFO::Applying z-score to standardize continuous metadata
2023-11-18 17:25:41.599501 INFO::Running selected transform method: LOG
2023-11-18 17:25:41.599969 INFO::Running selected analysis method: LM
2023-11-18 17:25:41.600296 INFO::Fitting model to feature number 1, ASV54
2023-11-18 17:25:41.601525 INFO::Fitting model to feature number 2, ASV55
2023-11-18 17:25:41.602611 INFO::Fitting model to feature number 3, ASV57
2023-11-18 17:25:41.60369 INFO::Fitting model to feature number 4, ASV58
2023-11-18 17:25:41.604747 INFO::Fitting model to feature number 5, ASV59
2023-11-18 17:25:41.605779 INFO::Fitting model to feature number 6, ASV60
2023-11-18 17:25:41.606804 INFO::Fitting model to feature number 7, ASV61
2023-11-18 17:25:41.607827 INFO::Fitting model to feature number 8, ASV62
2023-11-18 17:25:41.608836 INFO::Fitting model to feature number 9, ASV64
2023-11-18 17:25:41.612076 INFO::Counting total values for each feature
2023-11-18 17:25:41.612887 INFO::Writing filtered data to file results/maaslin2/asv_GHAT_Length
2023-11-18 17:25:41.613673 INFO::Writing filtered, normalized data to file results/maaslin2/asv_GHAT_Length
2023-11-18 17:25:41.614373 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/asv_GHAT_Length
2023-11-18 17:25:41.615158 WARNING::Deleting existing residuals file: results/maaslin2/asv_GHAT_Length
2023-11-18 17:25:41.615646 INFO::Writing residuals to file results/maaslin2/asv_GHAT_Length
2023-11-18 17:25:41.616176 WARNING::Deleting existing fitted file: results/maaslin2/asv_GHAT_Length
2023-11-18 17:25:41.616606 INFO::Writing fitted values to file results/maaslin2/asv_GHAT_Length
2023-11-18 17:25:41.618275 INFO::Writing all results to file (ordered by increasing q-values)
2023-11-18 17:25:41.618975 INFO::Writing the significant results (those which are less than 0.05)
2023-11-18 17:25:41.619451 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."
2023-11-18 17:25:41.620103 INFO::Writing association plots (one for each significant association)
2023-11-18 17:25:41.620789 INFO::Plotting associations from most to least significant, grouped by metadata number
2023-11-18 17:25:41.62112 INFO::Plotting data for metadata number 1, GHAT_Length
2023-11-18 17:25:41.621701 INFO::Creating scatter plot for continuous data, GHAT_Length vs ASV54

```

Warning: Removed 1 rows containing missing values (`geom\_point()`).

```

2023-11-18 17:25:41.704812 INFO::Creating scatter plot for continuous data, GHAT_Length vs ASV55

```

Warning: Removed 1 rows containing missing values (`geom\_point()`).

```

2023-11-18 17:25:41.789122 INFO::Creating scatter plot for continuous data, GHAT_Length vs ASV57

```

```

2023-11-18 17:25:41.865695 INFO::Creating scatter plot for continuous data, GHAT_Length vs ASV58

```

Warning: Removed 1 rows containing missing values (`geom\_point()`).

2023-11-18 17:25:41.957435 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")

```

```

2023-11-18 17:25:49.755784 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_GHAT_Length/maaslin2.log"
2023-11-18 17:25:49.978966 INFO::Writing function arguments to log file
2023-11-18 17:25:49.981519 INFO::Verifying options selected are valid
2023-11-18 17:25:49.981824 INFO::Determining format of input files
2023-11-18 17:25:49.982098 INFO::Input format is data samples as rows and metadata samples as columns
2023-11-18 17:25:49.984224 INFO::Formula for fixed effects: expr ~ GHAT_Length
2023-11-18 17:25:49.984544 INFO::Filter data based on min abundance and min prevalence
2023-11-18 17:25:49.984816 INFO::Total samples in data: 25
2023-11-18 17:25:49.985072 INFO::Min samples required with min abundance for a feature not to be filtered: 5
2023-11-18 17:25:49.986837 INFO::Total filtered features: 288
2023-11-18 17:25:49.987196 INFO::Filtered feature names from abundance and prevalence filtering:
2023-11-18 17:25:49.987763 INFO::Total filtered features with variance filtering: 0
2023-11-18 17:25:49.988056 INFO::Filtered feature names from variance filtering:
2023-11-18 17:25:49.988316 INFO::Running selected normalization method: TSS
2023-11-18 17:25:49.988809 INFO::Applying z-score to standardize continuous metadata
2023-11-18 17:25:49.991685 INFO::Running selected transform method: LOG
2023-11-18 17:25:49.992208 INFO::Running selected analysis method: LM
2023-11-18 17:25:49.992541 INFO::Fitting model to feature number 1, ASV14
2023-11-18 17:25:49.993814 INFO::Fitting model to feature number 2, ASV55
2023-11-18 17:25:49.994968 INFO::Fitting model to feature number 3, ASV66
2023-11-18 17:25:49.99606 INFO::Fitting model to feature number 4, ASV87
2023-11-18 17:25:49.997106 INFO::Fitting model to feature number 5, ASV120
2023-11-18 17:25:49.998163 INFO::Fitting model to feature number 6, ASV205
2023-11-18 17:25:49.999207 INFO::Fitting model to feature number 7, ASV241
2023-11-18 17:25:50.000266 INFO::Fitting model to feature number 8, ASV443
2023-11-18 17:25:50.001314 INFO::Fitting model to feature number 9, ASV568
2023-11-18 17:25:50.002353 INFO::Fitting model to feature number 10, ASV940
2023-11-18 17:25:50.005804 INFO::Counting total values for each feature
2023-11-18 17:25:50.006697 INFO::Writing filtered data to file results/maaslin2/genus_GHAT_Length/maaslin2_filtered_data.csv
2023-11-18 17:25:50.007587 INFO::Writing filtered, normalized data to file results/maaslin2/genus_GHAT_Length/maaslin2_normalized_data.csv
2023-11-18 17:25:50.008385 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/genus_GHAT_Length/maaslin2_transformed_data.csv
2023-11-18 17:25:50.009255 WARNING::Deleting existing residuals file: results/maaslin2/genus_GHAT_Length/maaslin2_residuals.csv
2023-11-18 17:25:50.00975 INFO::Writing residuals to file results/maaslin2/genus_GHAT_Length/maaslin2_residuals.csv
2023-11-18 17:25:50.010281 WARNING::Deleting existing fitted file: results/maaslin2/genus_GHAT_Length/maaslin2_fitted_values.csv
2023-11-18 17:25:50.010703 INFO::Writing fitted values to file results/maaslin2/genus_GHAT_Length/maaslin2_fitted_values.csv

```

```

2023-11-18 17:25:50.011179 INFO::Writing all results to file (ordered by increasing q-values)
2023-11-18 17:25:50.011927 INFO::Writing the significant results (those which are less than 0.2)
2023-11-18 17:25:50.01242 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"
2023-11-18 17:25:50.013122 INFO::Writing association plots (one for each significant association)
2023-11-18 17:25:50.013846 INFO::Plotting associations from most to least significant, grouped by metadata number
2023-11-18 17:25:50.014207 INFO::Plotting data for metadata number 1, GHAT_Length
2023-11-18 17:25:50.014869 INFO::Creating scatter plot for continuous data, GHAT_Length vs Abundance

```

```

2023-11-18 17:25:50.090791 INFO::Creating scatter plot for continuous data, GHAT_Length vs Abundance

```

```

2023-11-18 17:25:50.166648 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>

```

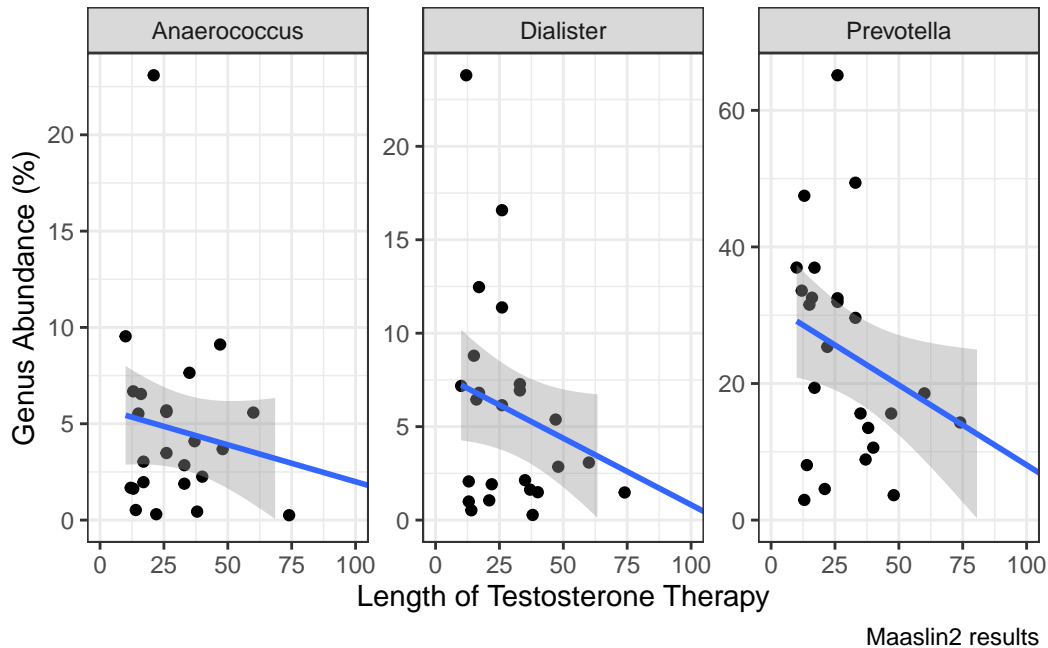
# plot correlation for these 3 ASVs
psmelt_trans_genus = psmelt(ps_trans_genus)
maaslin_res_genus_ghat = ggplot(filter(psmelt_trans_genus, OTU %in% filter(genus_GHAT$results, qval < 0.2)$feature)) +
  aes(x = GHAT_Length, y = Abundance) +
  geom_point() +
  geom_smooth(method = "lm") +
  facet_wrap(~Genus,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

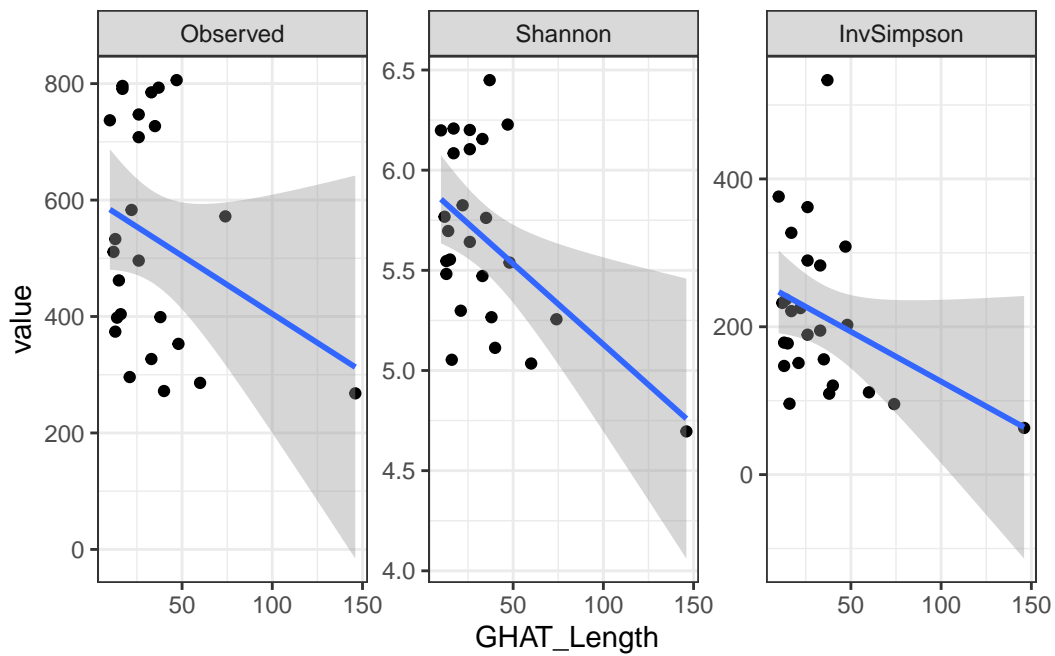
```

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	0.003
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()

```

```

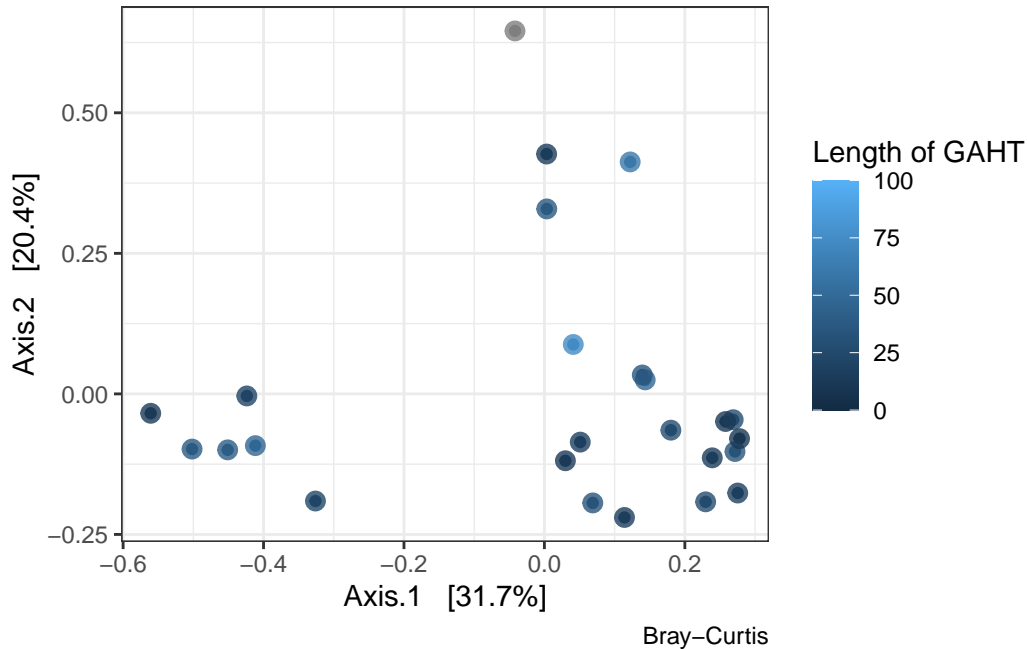
Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
10.00  16.00   26.00   33.56  38.00  146.00

```

```

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
```

```
2023-11-18 17:25:57.76908 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_Testosterone/maaslin2.log"
2023-11-18 17:25:57.987135 INFO::Writing function arguments to log file
2023-11-18 17:25:57.989984 INFO::Verifying options selected are valid
```

```

2023-11-18 17:25:57.990288 INFO::Determining format of input files
2023-11-18 17:25:57.990567 INFO::Input format is data samples as rows and metadata samples as
2023-11-18 17:25:57.992731 INFO::Formula for fixed effects: expr ~ Testosterone
2023-11-18 17:25:57.99307 INFO::Filter data based on min abundance and min prevalence
2023-11-18 17:25:57.993345 INFO::Total samples in data: 25
2023-11-18 17:25:57.993598 INFO::Min samples required with min abundance for a feature not to
2023-11-18 17:25:57.995372 INFO::Total filtered features: 292
2023-11-18 17:25:57.995713 INFO::Filtered feature names from abundance and prevalence filter:
2023-11-18 17:25:57.996225 INFO::Total filtered features with variance filtering: 0
2023-11-18 17:25:57.996504 INFO::Filtered feature names from variance filtering:
2023-11-18 17:25:57.996754 INFO::Running selected normalization method: TSS
2023-11-18 17:25:57.997201 INFO::Applying z-score to standardize continuous metadata
2023-11-18 17:25:57.999951 INFO::Running selected transform method: LOG
2023-11-18 17:25:58.000411 INFO::Running selected analysis method: LM
2023-11-18 17:25:58.000731 INFO::Fitting model to feature number 1, ASV14
2023-11-18 17:25:58.002137 INFO::Fitting model to feature number 2, ASV54
2023-11-18 17:25:58.003258 INFO::Fitting model to feature number 3, ASV66
2023-11-18 17:25:58.004351 INFO::Fitting model to feature number 4, ASV87
2023-11-18 17:25:58.005426 INFO::Fitting model to feature number 5, ASV205
2023-11-18 17:25:58.006502 INFO::Fitting model to feature number 6, ASV568
2023-11-18 17:25:58.009605 INFO::Counting total values for each feature
2023-11-18 17:25:58.010366 INFO::Writing filtered data to file results/maaslin2/genus_Testos
2023-11-18 17:25:58.011117 INFO::Writing filtered, normalized data to file results/maaslin2/g
2023-11-18 17:25:58.011771 INFO::Writing filtered, normalized, transformed data to file resu
2023-11-18 17:25:58.012511 WARNING::Deleting existing residuals file: results/maaslin2/genus
2023-11-18 17:25:58.01306 INFO::Writing residuals to file results/maaslin2/genus_Testosterone
2023-11-18 17:25:58.013566 WARNING::Deleting existing fitted file: results/maaslin2/genus_Tes
2023-11-18 17:25:58.014026 INFO::Writing fitted values to file results/maaslin2/genus_Testos
2023-11-18 17:25:58.014476 INFO::Writing all results to file (ordered by increasing q-values)
2023-11-18 17:25:58.015173 INFO::Writing the significant results (those which are less than
2023-11-18 17:25:58.01558 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2023-11-18 17:25:58.01616 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
```

```
2023-11-18 17:25:58.023913 WARNING::Input is a matrix, passing through as.data.frame() .  
[1] "Warning: Deleting existing log file: results/maaslin2/genus_DurationGNRH/maaslin2.log"  
2023-11-18 17:25:58.230568 INFO::Writing function arguments to log file  
2023-11-18 17:25:58.233421 INFO::Verifying options selected are valid  
2023-11-18 17:25:58.233755 INFO::Determining format of input files  
2023-11-18 17:25:58.234059 INFO::Input format is data samples as rows and metadata samples as columns  
2023-11-18 17:25:58.236395 INFO::Formula for fixed effects: expr ~ Duration_GNRH  
2023-11-18 17:25:58.236769 INFO::Filter data based on min abundance and min prevalence  
2023-11-18 17:25:58.237041 INFO::Total samples in data: 25  
2023-11-18 17:25:58.237299 INFO::Min samples required with min abundance for a feature not to be filtered: 5  
2023-11-18 17:25:58.239107 INFO::Total filtered features: 292  
2023-11-18 17:25:58.239456 INFO::Filtered feature names from abundance and prevalence filtering: 292  
2023-11-18 17:25:58.239988 INFO::Total filtered features with variance filtering: 0  
2023-11-18 17:25:58.240271 INFO::Filtered feature names from variance filtering:  
2023-11-18 17:25:58.240532 INFO::Running selected normalization method: TSS  
2023-11-18 17:25:58.240985 INFO::Applying z-score to standardize continuous metadata  
2023-11-18 17:25:58.243774 INFO::Running selected transform method: LOG  
2023-11-18 17:25:58.244234 INFO::Running selected analysis method: LM  
2023-11-18 17:25:58.244561 INFO::Fitting model to feature number 1, ASV14  
2023-11-18 17:25:58.245845 INFO::Fitting model to feature number 2, ASV54  
2023-11-18 17:25:58.246958 INFO::Fitting model to feature number 3, ASV66  
2023-11-18 17:25:58.248039 INFO::Fitting model to feature number 4, ASV87  
2023-11-18 17:25:58.249119 INFO::Fitting model to feature number 5, ASV205  
2023-11-18 17:25:58.250203 INFO::Fitting model to feature number 6, ASV568  
2023-11-18 17:25:58.253334 INFO::Counting total values for each feature
```

```

2023-11-18 17:25:58.254129 INFO::Writing filtered data to file results/maaslin2/genus_DurationGN
2023-11-18 17:25:58.255084 INFO::Writing filtered, normalized data to file results/maaslin2/g
2023-11-18 17:25:58.255778 INFO::Writing filtered, normalized, transformed data to file resu
2023-11-18 17:25:58.256478 WARNING::Deleting existing residuals file: results/maaslin2/genus_
2023-11-18 17:25:58.256994 INFO::Writing residuals to file results/maaslin2/genus_DurationGN
2023-11-18 17:25:58.25772 WARNING::Deleting existing fitted file: results/maaslin2/genus_Dura
2023-11-18 17:25:58.258403 INFO::Writing fitted values to file results/maaslin2/genus_Durati
2023-11-18 17:25:58.258923 INFO::Writing all results to file (ordered by increasing q-values)
2023-11-18 17:25:58.259684 INFO::Writing the significant results (those which are less than c
2023-11-18 17:25:58.260128 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2023-11-18 17:25:58.260706 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 premenopausal 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

```

```

2023-11-18 17:25:58.327019 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/asv_cycle_premeno/maaslin2.log"
2023-11-18 17:26:04.603272 INFO::Writing function arguments to log file
2023-11-18 17:26:04.606179 INFO::Verifying options selected are valid
2023-11-18 17:26:04.606499 INFO::Determining format of input files
2023-11-18 17:26:04.606789 INFO::Input format is data samples as rows and metadata samples as
2023-11-18 17:26:04.649203 INFO::Formula for fixed effects: expr ~ CycleDaySampling
2023-11-18 17:26:04.649851 INFO::Filter data based on min abundance and min prevalence
2023-11-18 17:26:04.650243 INFO::Total samples in data: 25

```

```

2023-11-18 17:26:04.650547 INFO::Min samples required with min abundance for a feature not t
2023-11-18 17:26:04.703994 INFO::Total filtered features: 8808
2023-11-18 17:26:04.706034 INFO::Filtered feature names from abundance and prevalence filter
2023-11-18 17:26:04.708924 INFO::Total filtered features with variance filtering: 0
2023-11-18 17:26:04.709366 INFO::Filtered feature names from variance filtering:
2023-11-18 17:26:04.709661 INFO::Running selected normalization method: TSS
2023-11-18 17:26:04.710237 INFO::Applying z-score to standardize continuous metadata
2023-11-18 17:26:04.713123 INFO::Running selected transform method: LOG
2023-11-18 17:26:04.713732 INFO::Running selected analysis method: LM
2023-11-18 17:26:04.714065 INFO::Fitting model to feature number 1, ASV1
2023-11-18 17:26:04.715355 INFO::Fitting model to feature number 2, ASV2
2023-11-18 17:26:04.716451 INFO::Fitting model to feature number 3, ASV3
2023-11-18 17:26:04.717518 INFO::Fitting model to feature number 4, ASV4
2023-11-18 17:26:04.718892 INFO::Fitting model to feature number 5, ASV5
2023-11-18 17:26:04.720429 INFO::Fitting model to feature number 6, ASV6
2023-11-18 17:26:04.721509 INFO::Fitting model to feature number 7, ASV7
2023-11-18 17:26:04.722559 INFO::Fitting model to feature number 8, ASV8
2023-11-18 17:26:04.723614 INFO::Fitting model to feature number 9, ASV9
2023-11-18 17:26:04.724679 INFO::Fitting model to feature number 10, ASV10
2023-11-18 17:26:04.725714 INFO::Fitting model to feature number 11, ASV11
2023-11-18 17:26:04.726748 INFO::Fitting model to feature number 12, ASV12
2023-11-18 17:26:04.727782 INFO::Fitting model to feature number 13, ASV13
2023-11-18 17:26:04.729315 INFO::Fitting model to feature number 14, ASV14
2023-11-18 17:26:04.730883 INFO::Fitting model to feature number 15, ASV15
2023-11-18 17:26:04.732091 INFO::Fitting model to feature number 16, ASV16
2023-11-18 17:26:04.733312 INFO::Fitting model to feature number 17, ASV17
2023-11-18 17:26:04.734607 INFO::Fitting model to feature number 18, ASV18
2023-11-18 17:26:04.735875 INFO::Fitting model to feature number 19, ASV19
2023-11-18 17:26:04.737158 INFO::Fitting model to feature number 20, ASV20
2023-11-18 17:26:04.73865 INFO::Fitting model to feature number 21, ASV21
2023-11-18 17:26:04.740034 INFO::Fitting model to feature number 22, ASV22
2023-11-18 17:26:04.741248 INFO::Fitting model to feature number 23, ASV23
2023-11-18 17:26:04.742455 INFO::Fitting model to feature number 24, ASV27
2023-11-18 17:26:04.743645 INFO::Fitting model to feature number 25, ASV32
2023-11-18 17:26:04.747896 INFO::Counting total values for each feature
2023-11-18 17:26:04.749035 INFO::Writing filtered data to file results/maaslin2/asv_cycle_pr
2023-11-18 17:26:04.750387 INFO::Writing filtered, normalized data to file results/maaslin2/a
2023-11-18 17:26:04.751368 INFO::Writing filtered, normalized, transformed data to file resu
2023-11-18 17:26:04.752501 WARNING::Deleting existing residuals file: results/maaslin2/asv_cy
2023-11-18 17:26:04.75317 INFO::Writing residuals to file results/maaslin2/asv_cycle_premeno
2023-11-18 17:26:04.753809 WARNING::Deleting existing fitted file: results/maaslin2/asv_cycl
2023-11-18 17:26:04.754259 INFO::Writing fitted values to file results/maaslin2/asv_cycle_pr
2023-11-18 17:26:04.754761 INFO::Writing all results to file (ordered by increasing q-values)

```

```

2023-11-18 17:26:04.755581 INFO::Writing the significant results (those which are less than 0.05) to file: results/maaslin2/genus_cycle_premeno/maaslin2.sig
2023-11-18 17:26:04.756016 INFO::Writing heatmap of significant results to file: results/maaslin2/genus_cycle_premeno/maaslin2.heatmap
[1] "There are no associations to plot!"
2023-11-18 17:26:04.756578 INFO::Writing association plots (one for each significant association) to file: results/maaslin2/genus_cycle_premeno/maaslin2.assoc
[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")

```

```

2023-11-18 17:26:12.952231 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_cycle_premeno/maaslin2.log"
2023-11-18 17:26:13.160032 INFO::Writing function arguments to log file
2023-11-18 17:26:13.162807 INFO::Verifying options selected are valid
2023-11-18 17:26:13.163111 INFO::Determining format of input files
2023-11-18 17:26:13.163403 INFO::Input format is data samples as rows and metadata samples as columns
2023-11-18 17:26:13.165596 INFO::Formula for fixed effects: expr ~ CycleDaySampling
2023-11-18 17:26:13.165918 INFO::Filter data based on min abundance and min prevalence
2023-11-18 17:26:13.166183 INFO::Total samples in data: 25
2023-11-18 17:26:13.166437 INFO::Min samples required with min abundance for a feature not to be filtered: 5
2023-11-18 17:26:13.168205 INFO::Total filtered features: 296
2023-11-18 17:26:13.168544 INFO::Filtered feature names from abundance and prevalence filtering: 296
2023-11-18 17:26:13.169022 INFO::Total filtered features with variance filtering: 0
2023-11-18 17:26:13.169298 INFO::Filtered feature names from variance filtering: 0
2023-11-18 17:26:13.169551 INFO::Running selected normalization method: TSS
2023-11-18 17:26:13.169977 INFO::Applying z-score to standardize continuous metadata
2023-11-18 17:26:13.172838 INFO::Running selected transform method: LOG
2023-11-18 17:26:13.173286 INFO::Running selected analysis method: LM
2023-11-18 17:26:13.173607 INFO::Fitting model to feature number 1, ASV1
2023-11-18 17:26:13.174843 INFO::Fitting model to feature number 2, ASV66
2023-11-18 17:26:13.177867 INFO::Counting total values for each feature
2023-11-18 17:26:13.17861 INFO::Writing filtered data to file results/maaslin2/genus_cycle_premeno/maaslin2.filtered
2023-11-18 17:26:13.179344 INFO::Writing filtered, normalized data to file results/maaslin2/genus_cycle_premeno/maaslin2.filtered_norm
2023-11-18 17:26:13.179966 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/genus_cycle_premeno/maaslin2.filtered_norm_trans
2023-11-18 17:26:13.180589 WARNING::Deleting existing residuals file: results/maaslin2/genus_cycle_premeno/maaslin2.residuals

```

```

2023-11-18 17:26:13.181079 INFO::Writing residuals to file results/maaslin2/genus_cycle_prema
2023-11-18 17:26:13.181548 WARNING::Deleting existing fitted file: results/maaslin2/genus_cy
2023-11-18 17:26:13.181953 INFO::Writing fitted values to file results/maaslin2/genus_cycle_
2023-11-18 17:26:13.182363 INFO::Writing all results to file (ordered by increasing q-values)
2023-11-18 17:26:13.183019 INFO::Writing the significant results (those which are less than 0
2023-11-18 17:26:13.183475 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
2023-11-18 17:26:13.184115 INFO::Writing association plots (one for each significant associat
2023-11-18 17:26:13.184771 INFO::Plotting associations from most to least significant, group
2023-11-18 17:26:13.185115 INFO::Plotting data for metadata number 1, CycleDaySampling
2023-11-18 17:26:13.185726 INFO::Creating scatter plot for continuous data, CycleDaySampling

2023-11-18 17:26:13.269959 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

```

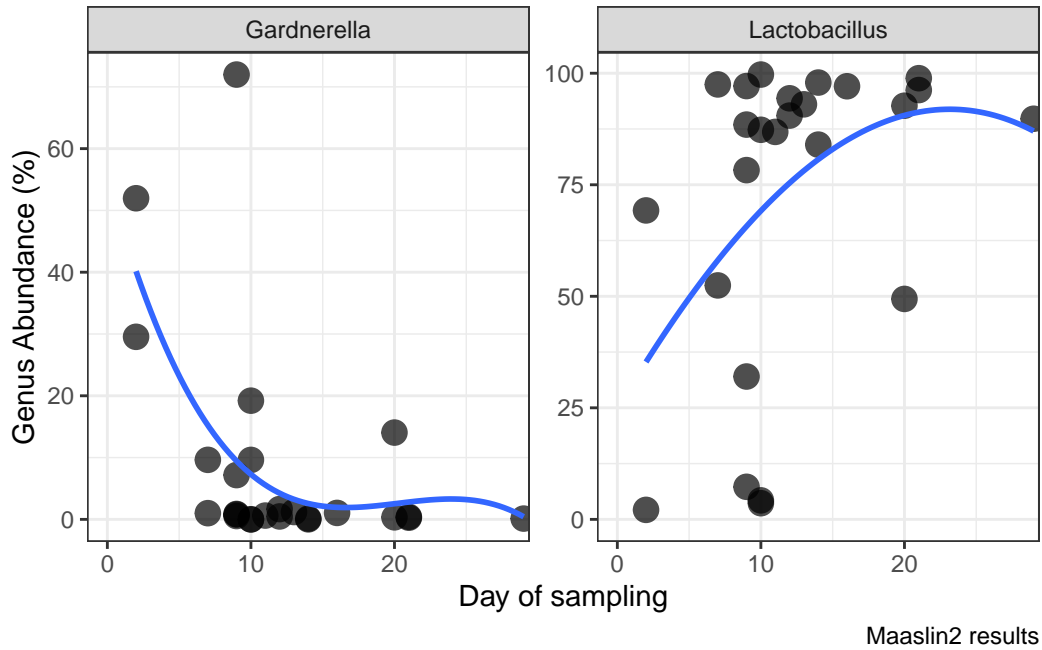
psmelt_pre_genus = psmelt(ps_pre_genus)

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.p",
       height = 5, width = 6)
```

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

## 6.0.6 Influence of sexual activity on microbiome composition

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

```

```

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")

```

```

2023-11-18 17:26:23.100606 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_SexuallyActive/maaslin2.log"
2023-11-18 17:26:23.304504 INFO::Writing function arguments to log file
2023-11-18 17:26:23.307093 INFO::Verifying options selected are valid
2023-11-18 17:26:23.307407 INFO::Determining format of input files
2023-11-18 17:26:23.30769 INFO::Input format is data samples as rows and metadata samples as columns
2023-11-18 17:26:23.309999 INFO::Formula for fixed effects: expr ~ SexuallyActive
2023-11-18 17:26:23.310349 INFO::Factor detected for categorical metadata 'SexuallyActive'. Processing as factor
2023-11-18 17:26:23.310613 INFO::Filter data based on min abundance and min prevalence
2023-11-18 17:26:23.31087 INFO::Total samples in data: 72
2023-11-18 17:26:23.31112 INFO::Min samples required with min abundance for a feature not to be filtered: 5
2023-11-18 17:26:23.313011 INFO::Total filtered features: 294
2023-11-18 17:26:23.313348 INFO::Filtered feature names from abundance and prevalence filtering:
2023-11-18 17:26:23.313856 INFO::Total filtered features with variance filtering: 0
2023-11-18 17:26:23.314143 INFO::Filtered feature names from variance filtering:
2023-11-18 17:26:23.314401 INFO::Running selected normalization method: TSS
2023-11-18 17:26:23.314885 INFO::Applying z-score to standardize continuous metadata
2023-11-18 17:26:23.317419 INFO::Running selected transform method: LOG
2023-11-18 17:26:23.317879 INFO::Running selected analysis method: LM
2023-11-18 17:26:23.318206 INFO::Fitting model to feature number 1, ASV1
2023-11-18 17:26:23.31964 INFO::Fitting model to feature number 2, ASV54
2023-11-18 17:26:23.32094 INFO::Fitting model to feature number 3, ASV66
2023-11-18 17:26:23.330511 INFO::Fitting model to feature number 4, ASV87
2023-11-18 17:26:23.334145 INFO::Counting total values for each feature
2023-11-18 17:26:23.334937 INFO::Writing filtered data to file results/maaslin2/genus_SexuallyActive/maaslin2_filtered_data.csv
2023-11-18 17:26:23.335878 INFO::Writing filtered, normalized data to file results/maaslin2/genus_SexuallyActive/maaslin2_normalized_data.csv
2023-11-18 17:26:23.336671 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/genus_SexuallyActive/maaslin2_transformed_data.csv

```

```

2023-11-18 17:26:23.337485 WARNING::Deleting existing residuals file: results/maaslin2/genus_SexuallyActive
2023-11-18 17:26:23.337972 INFO::Writing residuals to file results/maaslin2/genus_SexuallyActive
2023-11-18 17:26:23.338493 WARNING::Deleting existing fitted file: results/maaslin2/genus_SexuallyActive
2023-11-18 17:26:23.3389 INFO::Writing fitted values to file results/maaslin2/genus_SexuallyActive
2023-11-18 17:26:23.339337 INFO::Writing all results to file (ordered by increasing q-values)
2023-11-18 17:26:23.34003 INFO::Writing the significant results (those which are less than or equal to 0.05)
2023-11-18 17:26:23.340502 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"
2023-11-18 17:26:23.341152 INFO::Writing association plots (one for each significant association)
2023-11-18 17:26:23.341866 INFO::Plotting associations from most to least significant, grouped by metadata number
2023-11-18 17:26:23.342224 INFO::Plotting data for metadata number 1, SexuallyActive
2023-11-18 17:26:23.342895 INFO::Creating boxplot for categorical data, SexuallyActive vs ASV

```

2023-11-18 17:26:23.410805 INFO::Creating boxplot for categorical data, SexuallyActive vs ASV

```

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>			

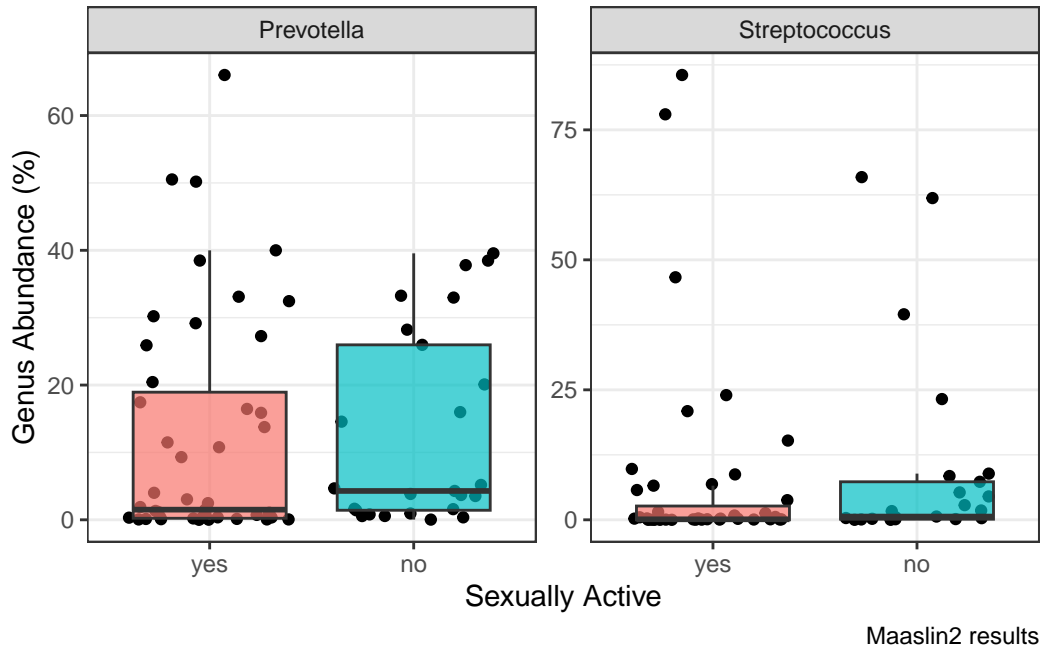
```

# 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")

```

```
maaslin_res_genus_sexactive
```

Warning: Removed 16 rows containing missing values (`geom\_point()`).



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

Warning: Removed 10 rows containing missing values (`geom\_point()`).

Sexual activity is associated with lower levels of *Prevotella* and *Streptococcus*.

### 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.

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

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

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

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

```
[1] NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA
[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
sample_data(ps_menopause)$DurationCombined = ifelse(is.na(sample_data(ps_menopause)$DurationMenopauseMonths),
  sample_data(ps_menopause)$DurationAmenorrhea, sample_data(ps_menopause)$DurationMenopauseMonths)

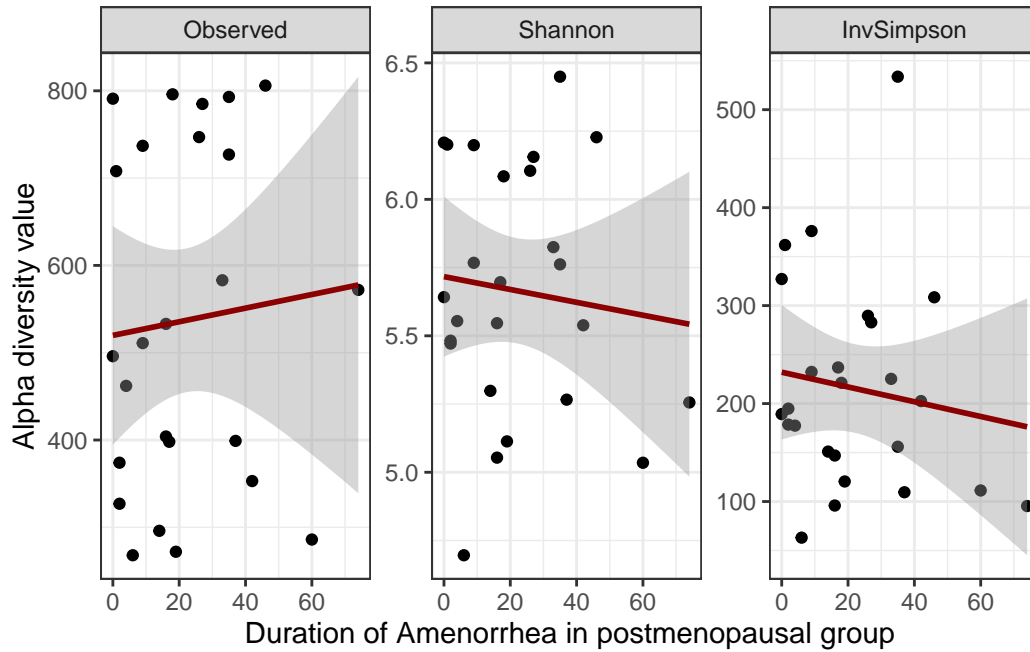
ps_menopause_rel = transform_sample_counts(ps_menopause, function(x) x/sum(x) * 100)
# Diversity analysis
adiv_menopause = plot_richness(ps_menopause, measures = c("Observed", "Shannon", "InvSimpson"))

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

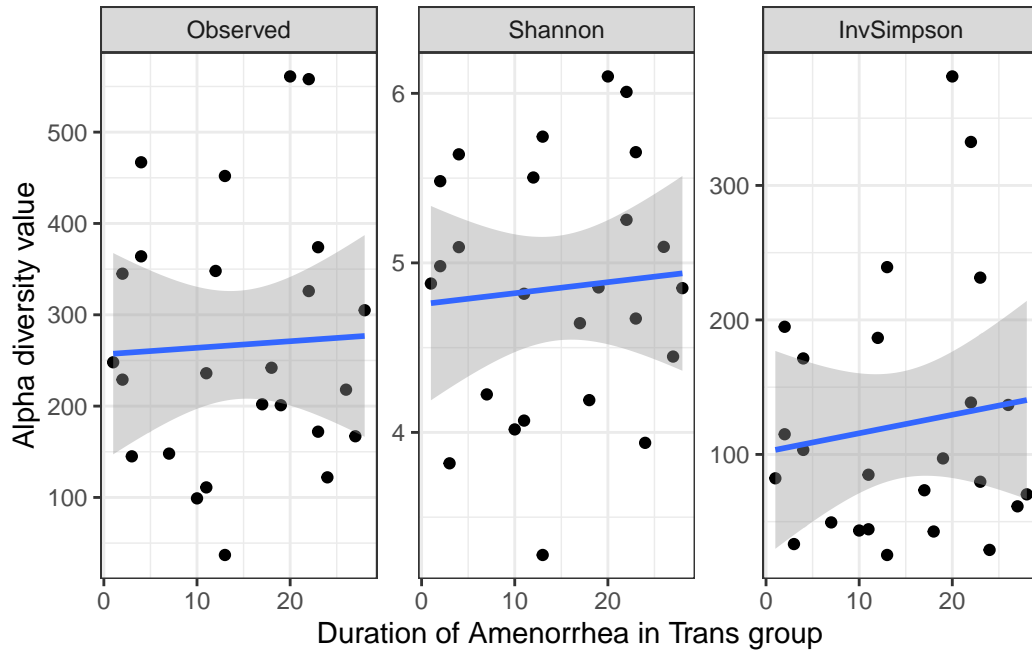
corr_trans = filter(adiv_menopause, grp == "03_trans") %>%
  group_by(variable) %>%
  rstatix::cor_test(value, DurationAmenorrhea)
kable(corr_trans)
```

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)
```

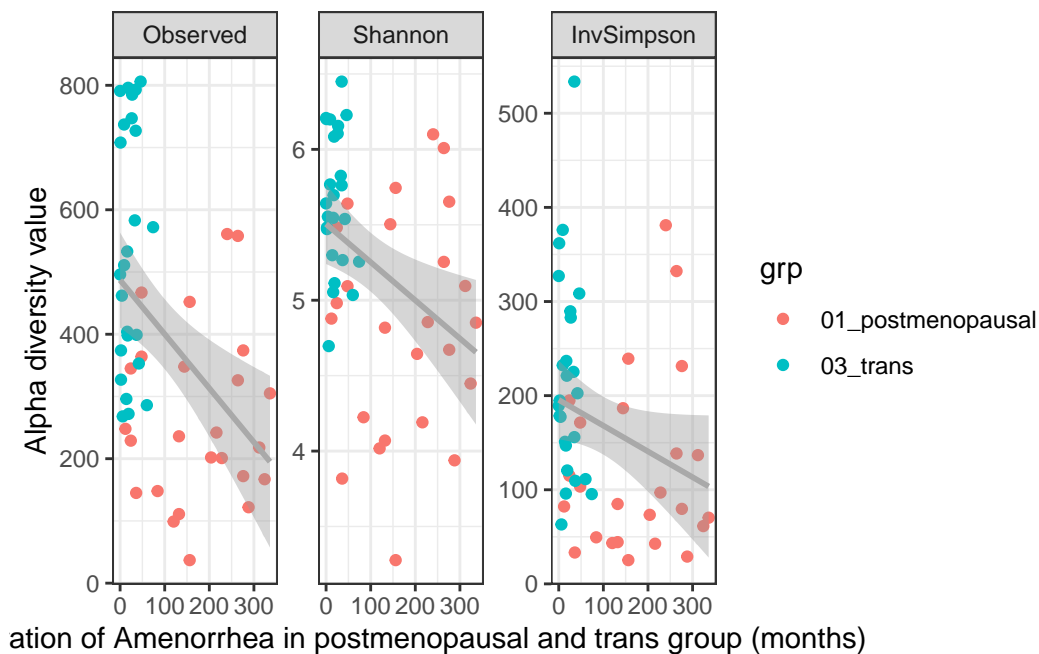
variable	var1	var2	cor	statistic	p	conf.low	conf.high	method
Observed	value	DurationMenopause	0.045	0.2158134	0.831	-0.3565088	0.4324048	Pearson
Shannon	value	DurationMenopause	0.078	0.3754553	0.711	-0.3271717	0.4590238	Pearson
InvSimpson	value	DurationMenopause	0.130	0.6169177	0.543	-0.2817497	0.4976290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

```

```

2023-11-18 17:26:34.02076 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_MenopausedurationMonths/maaslin2.log"
2023-11-18 17:26:34.559655 INFO::Writing function arguments to log file
2023-11-18 17:26:34.562631 INFO::Verifying options selected are valid
2023-11-18 17:26:34.562949 INFO::Determining format of input files
2023-11-18 17:26:34.563231 INFO::Input format is data samples as rows and metadata samples as columns
2023-11-18 17:26:34.565428 INFO::Formula for fixed effects: expr ~ DurationCombined
2023-11-18 17:26:34.565756 INFO::Filter data based on min abundance and min prevalence
2023-11-18 17:26:34.566017 INFO::Total samples in data: 50
2023-11-18 17:26:34.566271 INFO::Min samples required with min abundance for a feature not to be filtered: 5
2023-11-18 17:26:34.568118 INFO::Total filtered features: 292
2023-11-18 17:26:34.56845 INFO::Filtered feature names from abundance and prevalence filtering: ASV14, ASV54, ASV82, ASV205, ASV210, ASV369
2023-11-18 17:26:34.568964 INFO::Total filtered features with variance filtering: 0
2023-11-18 17:26:34.569247 INFO::Filtered feature names from variance filtering:
2023-11-18 17:26:34.569501 INFO::Running selected normalization method: TSS
2023-11-18 17:26:34.569969 INFO::Applying z-score to standardize continuous metadata
2023-11-18 17:26:34.572698 INFO::Running selected transform method: LOG
2023-11-18 17:26:34.573162 INFO::Running selected analysis method: LM
2023-11-18 17:26:34.573485 INFO::Fitting model to feature number 1, ASV14
2023-11-18 17:26:34.574748 INFO::Fitting model to feature number 2, ASV54
2023-11-18 17:26:34.575845 INFO::Fitting model to feature number 3, ASV82
2023-11-18 17:26:34.57691 INFO::Fitting model to feature number 4, ASV205
2023-11-18 17:26:34.577965 INFO::Fitting model to feature number 5, ASV210
2023-11-18 17:26:34.579012 INFO::Fitting model to feature number 6, ASV369
2023-11-18 17:26:34.582115 INFO::Counting total values for each feature
2023-11-18 17:26:34.582885 INFO::Writing filtered data to file results/maaslin2/genus_MenopausedurationMonths/maaslin2.filtered.csv
2023-11-18 17:26:34.583769 INFO::Writing filtered, normalized data to file results/maaslin2/genus_MenopausedurationMonths/maaslin2.filtered.normalized.csv
2023-11-18 17:26:34.584558 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/genus_MenopausedurationMonths/maaslin2.filtered.normalized.transformed.csv
2023-11-18 17:26:34.585371 WARNING::Deleting existing residuals file: results/maaslin2/genus_MenopausedurationMonths/maaslin2.residuals.csv
2023-11-18 17:26:34.586078 INFO::Writing residuals to file results/maaslin2/genus_MenopausedurationMonths/maaslin2.residuals.csv
2023-11-18 17:26:34.586642 WARNING::Deleting existing fitted file: results/maaslin2/genus_MenopausedurationMonths/maaslin2.fitted.csv
2023-11-18 17:26:34.587069 INFO::Writing fitted values to file results/maaslin2/genus_MenopausedurationMonths/maaslin2.fitted.csv
2023-11-18 17:26:34.58755 INFO::Writing all results to file (ordered by increasing q-values)
2023-11-18 17:26:34.588249 INFO::Writing the significant results (those which are less than 0.05) to file results/maaslin2/genus_MenopausedurationMonths/maaslin2.significant.csv
2023-11-18 17:26:34.588733 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 and associations."
2023-11-18 17:26:34.589404 INFO::Writing association plots (one for each significant association) to file: results/maaslin2/genus_MenopausedurationMonths/maaslin2.significant.association.plots

```

```
2023-11-18 17:26:34.590108 INFO::Plotting associations from most to least significant, group
2023-11-18 17:26:34.590454 INFO::Plotting data for metadata number 1, DurationCombined
2023-11-18 17:26:34.59105 INFO::Creating scatter plot for continuous data, DurationCombined
```

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

```
2023-11-18 17:26:34.671491 INFO::Creating scatter plot for continuous data, DurationCombined
```

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

```
2023-11-18 17:26:34.753253 INFO::Creating scatter plot for continuous data, DurationCombined
```

```
2023-11-18 17:26:34.828971 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)
```

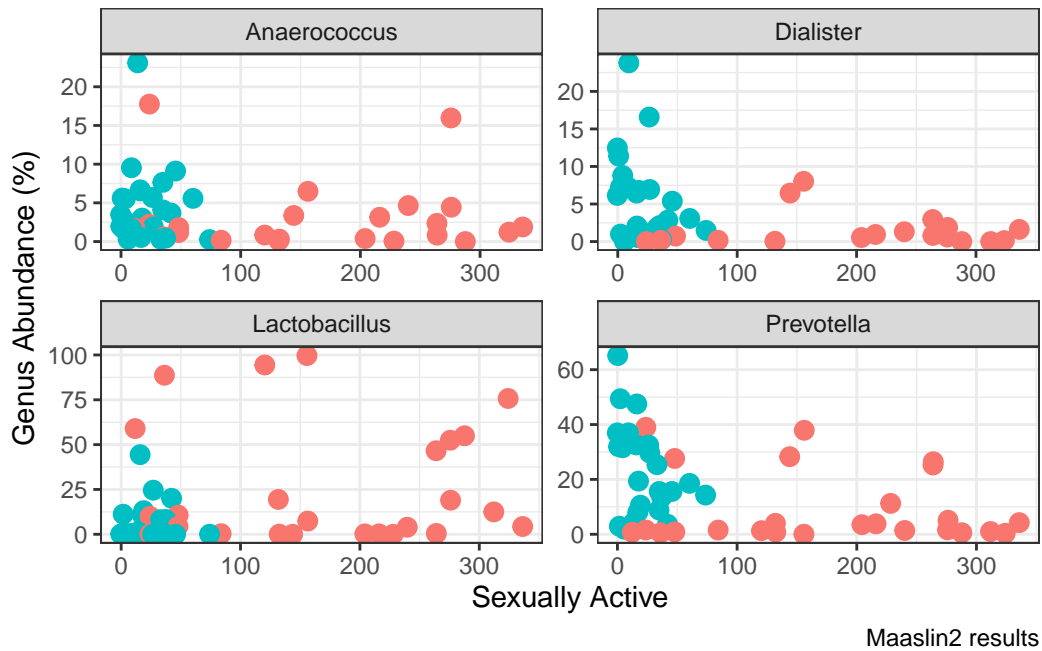
	ASV	Kingdom	Phylum	Class					
1	ASV14	Bacteria	Firmicutes	Bacilli					
2	ASV54	Bacteria	Bacteroidota	Bacteroidia					
3	ASV205	Bacteria	Firmicutes	Negativicutes					
4	ASV369	Bacteria	Firmicutes	Clostridia					
			Order	Family	Genus	Species			
1			Lactobacillales	Lactobacillaceae	Lactobacillus	<NA>			
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 = 3) +
  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 13 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")
```

```
2023-11-18 17:26:36.795587 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_SexOfPartner/maaslin2.log"
2023-11-18 17:26:36.998334 INFO::Writing function arguments to log file
2023-11-18 17:26:37.00082 INFO::Verifying options selected are valid
2023-11-18 17:26:37.001123 INFO::Determining format of input files
2023-11-18 17:26:37.001404 INFO::Input format is data samples as rows and metadata samples as
```

```

2023-11-18 17:26:37.003843 INFO::Formula for fixed effects: expr ~ SexOfPartner
2023-11-18 17:26:37.004213 INFO::Factor detected for categorical metadata 'SexOfPartner'. Pro
2023-11-18 17:26:37.004484 INFO::Filter data based on min abundance and min prevalence
2023-11-18 17:26:37.004751 INFO::Total samples in data: 72
2023-11-18 17:26:37.005018 INFO::Min samples required with min abundance for a feature not t
2023-11-18 17:26:37.00701 INFO::Total filtered features: 294
2023-11-18 17:26:37.007374 INFO::Filtered feature names from abundance and prevalence filter
2023-11-18 17:26:37.007916 INFO::Total filtered features with variance filtering: 0
2023-11-18 17:26:37.008215 INFO::Filtered feature names from variance filtering:
2023-11-18 17:26:37.008482 INFO::Running selected normalization method: TSS
2023-11-18 17:26:37.008974 INFO::Applying z-score to standardize continuous metadata
2023-11-18 17:26:37.011527 INFO::Running selected transform method: LOG
2023-11-18 17:26:37.011993 INFO::Running selected analysis method: LM
2023-11-18 17:26:37.012316 INFO::Fitting model to feature number 1, ASV1
2023-11-18 17:26:37.013711 INFO::Fitting model to feature number 2, ASV54
2023-11-18 17:26:37.01494 INFO::Fitting model to feature number 3, ASV66
2023-11-18 17:26:37.016128 INFO::Fitting model to feature number 4, ASV87
2023-11-18 17:26:37.019459 INFO::Counting total values for each feature
2023-11-18 17:26:37.020321 INFO::Writing filtered data to file results/maaslin2/genus_SexOfPa
2023-11-18 17:26:37.021211 INFO::Writing filtered, normalized data to file results/maaslin2/
2023-11-18 17:26:37.021999 INFO::Writing filtered, normalized, transformed data to file resu
2023-11-18 17:26:37.022789 WARNING::Deleting existing residuals file: results/maaslin2/genus
2023-11-18 17:26:37.023284 INFO::Writing residuals to file results/maaslin2/genus_SexOfPartn
2023-11-18 17:26:37.023801 WARNING::Deleting existing fitted file: results/maaslin2/genus_Se
2023-11-18 17:26:37.024217 INFO::Writing fitted values to file results/maaslin2/genus_SexOfP
2023-11-18 17:26:37.024665 INFO::Writing all results to file (ordered by increasing q-values
2023-11-18 17:26:37.025376 INFO::Writing the significant results (those which are less than
2023-11-18 17:26:37.025839 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2023-11-18 17:26:37.026415 INFO::Writing association plots (one for each significant associat
2023-11-18 17:26:37.027109 INFO::Plotting associations from most to least significant, group
2023-11-18 17:26:37.027448 INFO::Plotting data for metadata number 1, SexOfPartner
2023-11-18 17:26:37.028094 INFO::Creating boxplot for categorical data, SexOfPartner vs ASV5

```

```

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"))

```

```

2023-11-18 17:26:37.195717 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_SexOfPartnergrp/maaslin2.log
2023-11-18 17:26:37.409166 INFO::Writing function arguments to log file
2023-11-18 17:26:37.412196 INFO::Verifying options selected are valid
2023-11-18 17:26:37.412524 INFO::Determining format of input files
2023-11-18 17:26:37.412818 INFO::Input format is data samples as rows and metadata samples as
2023-11-18 17:26:37.415193 INFO::Formula for fixed effects: expr ~ SexOfPartner + grp
2023-11-18 17:26:37.415567 INFO::Factor detected for categorical metadata 'SexOfPartner'. Prov
2023-11-18 17:26:37.415849 INFO::Factor detected for categorical metadata 'grp'. Provide a re
2023-11-18 17:26:37.416101 INFO::Filter data based on min abundance and min prevalence
2023-11-18 17:26:37.416354 INFO::Total samples in data: 72
2023-11-18 17:26:37.416605 INFO::Min samples required with min abundance for a feature not t
2023-11-18 17:26:37.41853 INFO::Total filtered features: 294
2023-11-18 17:26:37.418921 INFO::Filtered feature names from abundance and prevalence filter
2023-11-18 17:26:37.41945 INFO::Total filtered features with variance filtering: 0
2023-11-18 17:26:37.419734 INFO::Filtered feature names from variance filtering:
2023-11-18 17:26:37.419992 INFO::Running selected normalization method: TSS
2023-11-18 17:26:37.420487 INFO::Applying z-score to standardize continuous metadata
2023-11-18 17:26:37.423346 INFO::Running selected transform method: LOG
2023-11-18 17:26:37.423874 INFO::Running selected analysis method: LM
2023-11-18 17:26:37.424211 INFO::Fitting model to feature number 1, ASV1
2023-11-18 17:26:37.425855 INFO::Fitting model to feature number 2, ASV54
2023-11-18 17:26:37.427262 INFO::Fitting model to feature number 3, ASV66
2023-11-18 17:26:37.428629 INFO::Fitting model to feature number 4, ASV87
2023-11-18 17:26:37.432334 INFO::Counting total values for each feature
2023-11-18 17:26:37.433386 INFO::Writing filtered data to file results/maaslin2/genus_SexOfPa
2023-11-18 17:26:37.434539 INFO::Writing filtered, normalized data to file results/maaslin2/
2023-11-18 17:26:37.43547 INFO::Writing filtered, normalized, transformed data to file resul
2023-11-18 17:26:37.436361 WARNING::Deleting existing residuals file: results/maaslin2/genus
2023-11-18 17:26:37.436923 INFO::Writing residuals to file results/maaslin2/genus_SexOfPartn
2023-11-18 17:26:37.437481 WARNING::Deleting existing fitted file: results/maaslin2/genus_Se
2023-11-18 17:26:37.438085 INFO::Writing fitted values to file results/maaslin2/genus_SexOfPa
2023-11-18 17:26:37.438715 INFO::Writing all results to file (ordered by increasing q-values)
2023-11-18 17:26:37.439621 INFO::Writing the significant results (those which are less than
2023-11-18 17:26:37.440505 INFO::Writing heatmap of significant results to file: results/maas
2023-11-18 17:26:37.473932 INFO::Writing association plots (one for each significant associat
2023-11-18 17:26:37.475038 INFO::Plotting associations from most to least significant, group
2023-11-18 17:26:37.475446 INFO::Plotting data for metadata number 1, grp
2023-11-18 17:26:37.476133 INFO::Creating boxplot for categorical data, grp vs ASV54

2023-11-18 17:26:37.55169 INFO::Creating boxplot for categorical data, grp vs ASV87

2023-11-18 17:26:37.634359 INFO::Creating boxplot for categorical data, grp vs ASV1

```



2023-11-18 17:26:37.70737 INFO::Creating boxplot for categorical data, grp vs ASV1

2023-11-18 17:26:37.779533 INFO::Creating boxplot for categorical data, grp vs ASV54

2023-11-18 17:26:38.23964 INFO::Plotting data for metadata number 2, SexOfPartner

2023-11-18 17:26:38.240594 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)
```

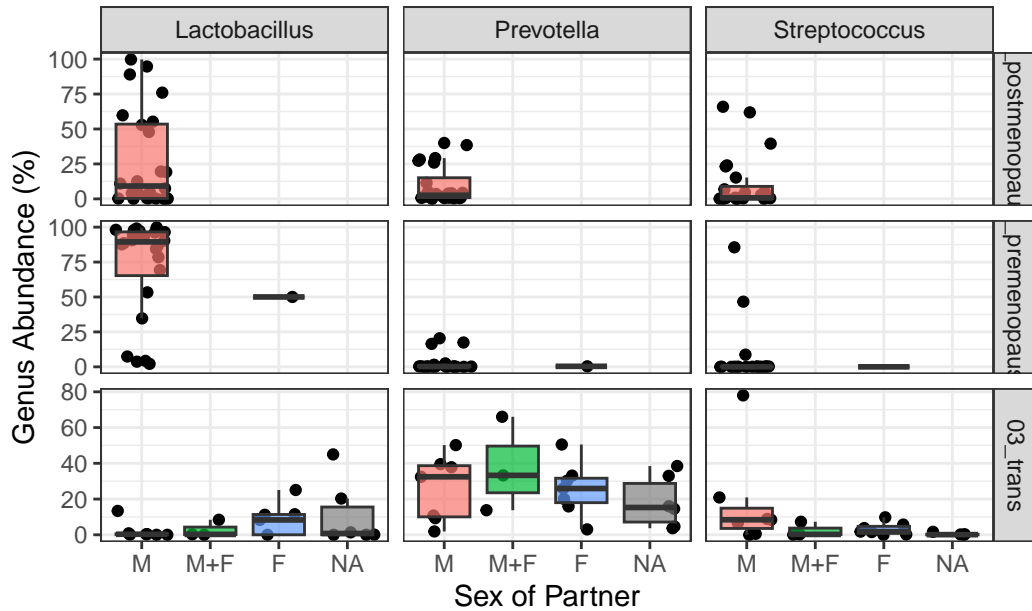
	ASV	Kingdom	Phylum	Class	Order	Family
1	ASV1	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae
2	ASV54	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae
3	ASV87	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae

	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(genus_SexOfPartner2$results, qval < 0.2)$feature))  
  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")  
  
maaslin_res_genus_sexpartner
```

Warning: Removed 13 rows containing missing values (`geom\_point()`).



Maaslin2 results

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

Warning: Removed 13 rows containing missing values (`geom\_point()`).

```
# 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")
```

```
2023-11-18 17:26:39.093295 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_SexOfPartnerTrans/maaslin2.
2023-11-18 17:26:39.310083 INFO::Writing function arguments to log file
2023-11-18 17:26:39.312889 INFO::Verifying options selected are valid
2023-11-18 17:26:39.313208 INFO::Determining format of input files
```

```

2023-11-18 17:26:39.313495 INFO::Input format is data samples as rows and metadata samples as
2023-11-18 17:26:39.315729 INFO::Formula for fixed effects: expr ~ SexOfPartner
2023-11-18 17:26:39.316104 INFO::Factor detected for categorical metadata 'SexOfPartner'. Pro
2023-11-18 17:26:39.316382 INFO::Filter data based on min abundance and min prevalence
2023-11-18 17:26:39.316649 INFO::Total samples in data: 23
2023-11-18 17:26:39.316904 INFO::Min samples required with min abundance for a feature not to
2023-11-18 17:26:39.318707 INFO::Total filtered features: 291
2023-11-18 17:26:39.319104 INFO::Filtered feature names from abundance and prevalence filter
2023-11-18 17:26:39.319648 INFO::Total filtered features with variance filtering: 0
2023-11-18 17:26:39.319935 INFO::Filtered feature names from variance filtering:
2023-11-18 17:26:39.320203 INFO::Running selected normalization method: TSS
2023-11-18 17:26:39.320659 INFO::Applying z-score to standardize continuous metadata
2023-11-18 17:26:39.323281 INFO::Running selected transform method: LOG
2023-11-18 17:26:39.32377 INFO::Running selected analysis method: LM
2023-11-18 17:26:39.324218 INFO::Fitting model to feature number 1, ASV1
2023-11-18 17:26:39.325708 INFO::Fitting model to feature number 2, ASV54
2023-11-18 17:26:39.326958 INFO::Fitting model to feature number 3, ASV66
2023-11-18 17:26:39.328213 INFO::Fitting model to feature number 4, ASV87
2023-11-18 17:26:39.32943 INFO::Fitting model to feature number 5, ASV205
2023-11-18 17:26:39.330639 INFO::Fitting model to feature number 6, ASV369
2023-11-18 17:26:39.331862 INFO::Fitting model to feature number 7, ASV397
2023-11-18 17:26:39.335696 INFO::Counting total values for each feature
2023-11-18 17:26:39.33675 INFO::Writing filtered data to file results/maaslin2/genus_SexOfPart
2023-11-18 17:26:39.337782 INFO::Writing filtered, normalized data to file results/maaslin2/g
2023-11-18 17:26:39.338588 INFO::Writing filtered, normalized, transformed data to file resu
2023-11-18 17:26:39.339344 WARNING::Deleting existing residuals file: results/maaslin2/genus
2023-11-18 17:26:39.339864 INFO::Writing residuals to file results/maaslin2/genus_SexOfPartn
2023-11-18 17:26:39.340392 WARNING::Deleting existing fitted file: results/maaslin2/genus_Se
2023-11-18 17:26:39.340825 INFO::Writing fitted values to file results/maaslin2/genus_SexOfP
2023-11-18 17:26:39.341261 INFO::Writing all results to file (ordered by increasing q-values
2023-11-18 17:26:39.341999 INFO::Writing the significant results (those which are less than
2023-11-18 17:26:39.342412 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2023-11-18 17:26:39.342972 INFO::Writing association plots (one for each significant associat
[1] "There are no associations to plot!"

```

```

# no significant results in trans group only

```