

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

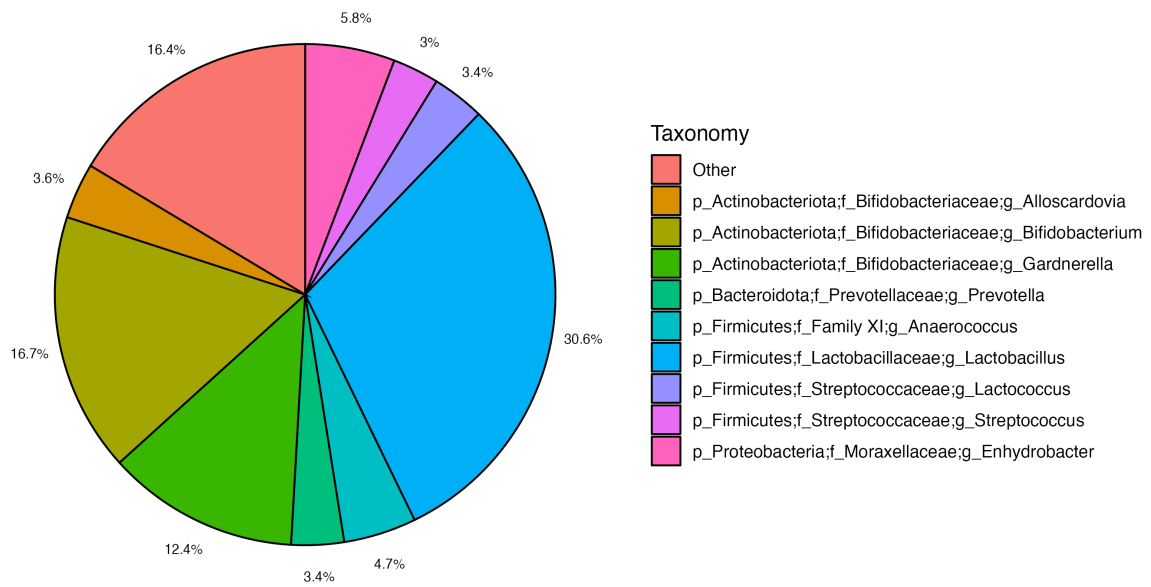


Figure 1: Taxonomic composition of PBS sample

```
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.

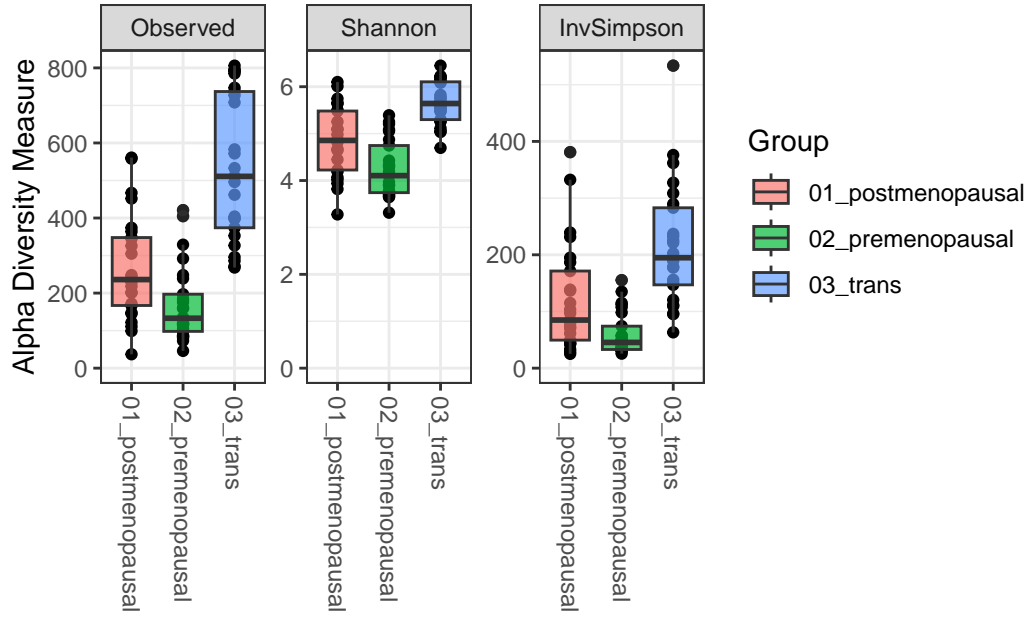
In this dataset, alpha diversity is markedly different between the group of interest (i.e. *03_trans*) and the two control groups. However, the intervention group is more similar to the first control group (i.e. *postmenopausal women*).

```
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")
```

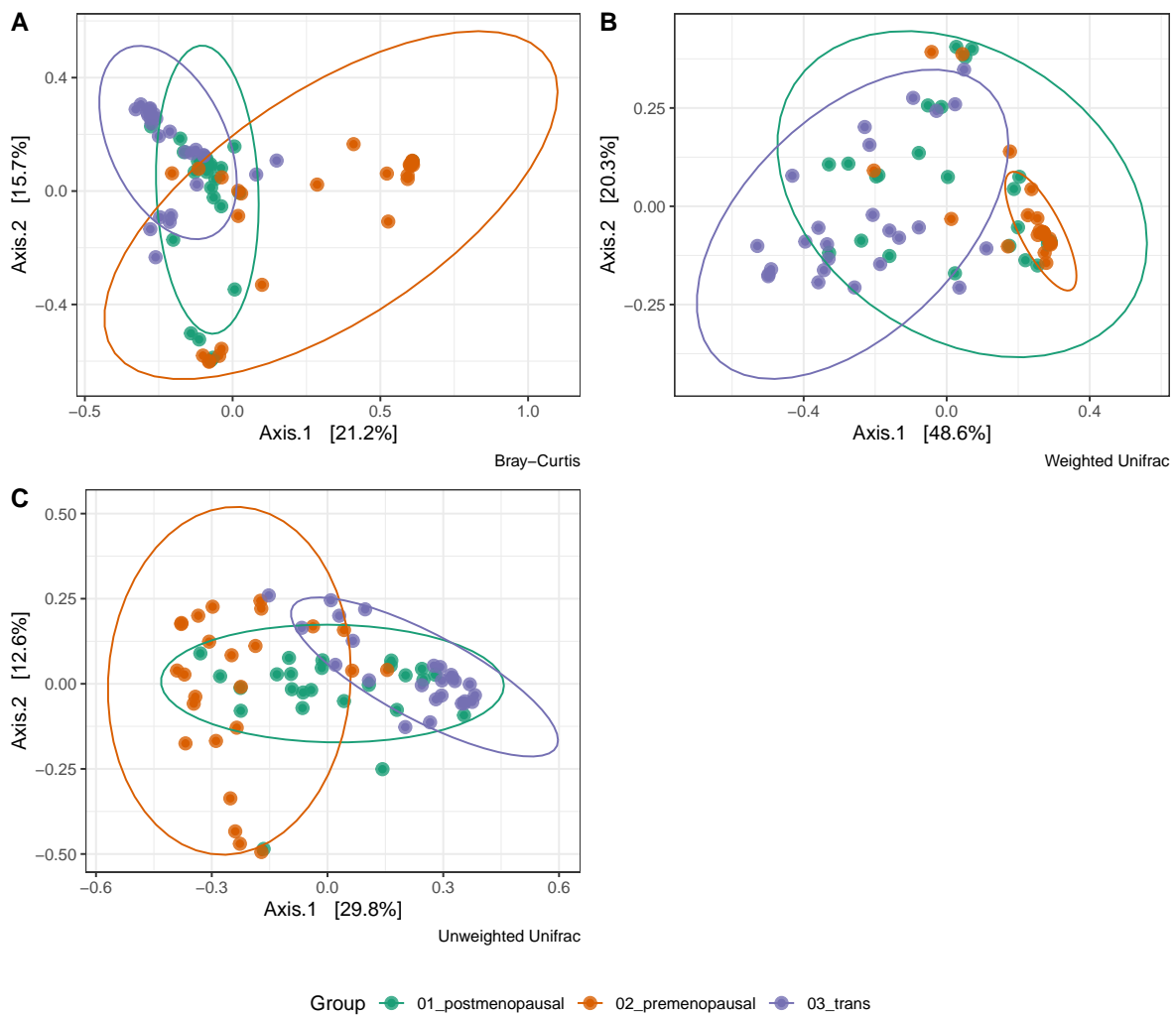


```
)
```

```
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	1.5023	0.0550	0.0020	0.0090	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV62	1.674	1.4483	0.0690	0.0020	0.0080	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV75	8.4584	1.4342	0.8830	0.0040	0.0130	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV85	8.5634	1.5003	0.1120	0.0020	0.0080	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV95	6.4434	1.4853	0.1160	0.0020	0.0080	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV156	6.4324	1.5012	0.8500	0.0040	0.0140	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV156	1.1334	1.4302	0.8100	0.0050	0.0160	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV156	2.2984	1.5102	0.9580	0.0030	0.0110	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV153	1.694	1.4413	0.2890	0.0010	0.0050	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV151	1.2413	1.4512	0.6600	0.0080	0.0220	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV147	3.3954	1.4822	0.8950	0.0040	0.0130	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV148	6.634	1.4403	0.0830	0.0020	0.0080	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV147	2.534	1.4773	0.1480	0.0020	0.0070	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV246	1.774	1.3953	0.0770	0.0020	0.0080	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV243	1.1014	1.4553	0.1720	0.0020	0.0070	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV241	2.9824	1.5202	0.7860	0.0050	0.0170	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV242	2.9054	1.3823	0.2170	0.0010	0.0060	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV340	0.8994	1.4143	0.1410	0.0020	0.0070	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV339	2.904	1.4952	0.8320	0.0050	0.0150	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV339	1.614	1.4613	0.0890	0.0020	0.0080	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV337	3.8594	1.4973	0.0120	0.0030	0.0090	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV338	1.474	1.4793	0.1150	0.0020	0.0080	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV430	3.044	1.4752	0.8720	0.0040	0.0140	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV538	0.312	-	1.354	-	0.0140	0.0340	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae
ASV537	3.329	-	2.459	-	-	-	-	-	-	-	-
ASV537	0.625	-	1.333	-	0.0160	0.0390	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae
ASV537	3.196	-	2.398	-	-	-	-	-	-	-	-
ASV537	3.608	-	1.336	-	0.0130	0.0330	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae
ASV537	3.317	-	2.483	-	-	-	-	-	-	-	-
ASV534	0.361	-	1.466	-	0.0230	0.0500	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae
ASV534	3.341	-	2.279	-	-	-	-	-	-	-	-
ASV532	0.825	-	1.483	-	0.0060	0.0180	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae
ASV532	4.053	-	2.733	-	-	-	-	-	-	-	-
ASV632	0.056	-	1.415	-	0.0050	0.0160	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae
ASV632	3.957	-	2.796	-	-	-	-	-	-	-	-
ASV632	1.648	-	1.379	-	0.0050	0.0170	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae
ASV632	3.842	-	2.787	-	-	-	-	-	-	-	-
ASV631	0.023	-	1.384	-	0.0120	0.0310	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae
ASV631	3.482	-	2.517	-	-	-	-	-	-	-	-
ASV627	0.851	-	1.404	-	0.0030	0.0120	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae
ASV627	4.106	-	2.924	-	-	-	-	-	-	-	-

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

ASV	baseMean	log2FoldChange	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	Faleis	Finegoldia
	3.295		2.286					Tissierellales	XI	
ASV250.985	-	1.770	-	0.0110.029	Bacteria	Firmicutes	Negativibacteres	Veillonellales	Veillonellaceae	Dialister
	4.498		2.542					Selenomonadales		
ASV257.002	6.402	2.096	0.0550.0020.009	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	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	Lactobacillaceae	Lactobacillus	Lactobacillus
ASV303.355	-	2.077	-	0.0170.040	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	4.951		2.384							
ASV304.550	-	1.770	-	0.0020.007	Bacteria	Firmicutes	Negativibacteres	Veillonellales	Veillonellaceae	Dialister
	5.598		3.162					Selenomonadales		
ASV305.581	-	1.646	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.538		3.973							
ASV307.649	-	1.439	-	0.0130.033	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faleis	Finegoldia
	3.570		2.481					Tissierellales	XI	
ASV308.117	-	1.502	-	0.0110.029	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.819		2.543							
ASV309.167	-	1.821	-	0.0000.003	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.434		3.534							

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

ASV	baseMean	log2FoldChange	negLog10Pvalue	negLog10PadjPvalue	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	Negativicutes	Veillonellales	Veillonellaceae	Dialister
	5.572		3.060					Selenomonadales		
ASV48615	-	1.743	-	0.0020.007	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
	5.458		3.131					Selenomonadales		
ASV49264	-	1.947	-	0.0000.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	7.143		3.668					Tissierellales	XI	
ASV49702	-	1.548	-	0.0000.000	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	6.716		4.339					Tissierellales	XI	
ASV50856	-	1.418	-	0.0000.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	5.438		3.835					Tissierellales	XI	
ASV52334	-	2.397	-	0.0170.041	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Akkermansia
	5.696		2.376							
ASV53965	-	2.186	-	0.0130.032	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Akkermansia
	5.449		2.493							
ASV56299	-	1.815	-	0.0000.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	6.993		3.852					Tissierellales	XI	
ASV57606	-	1.939	-	0.0000.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	7.154		3.690					Tissierellales	XI	
ASV58261	-	1.881	-	0.0000.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	7.004		3.724					Tissierellales	XI	
ASV52795	-	1.630	-	0.0030.010	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	4.893		3.002					Tissierellales	XI	
ASV57280	-	1.699	-	0.0000.002	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
	6.016		3.542					Selenomonadales		
ASV58111	-	1.511	-	0.0000.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	6.394		4.231					Tissierellales	XI	
ASV58609	-	1.770	-	0.0000.002	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	6.358		3.592							
ASV55891	-	1.447	-	0.0000.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	6.114		4.225					Tissierellales	XI	
ASV56332	-	1.345	-	0.0000.000	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	6.632		4.929					Tissierellales	XI	
ASV55997	-	1.708	-	0.0000.002	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
	6.177		3.617					Selenomonadales		
ASV56039	-	1.779	-	0.0010.003	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	6.099		3.428					Tissierellales	XI	

ASV	baseMean	log2FoldChange	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							
ASV56659	-	1.831	- 0.0000.002	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Fenollaria
	6.749	3.687					Tissierellales	XI	
ASV56374	-	2.082	- 0.0100.027	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacter	Alloscardovia
	5.372	2.581							
ASV56581	-	2.021	- 0.0020.007	Bacteria	Bacteroid	Bacteroid	Bacteroidales	Prevotella	Prevotella
	6.373	3.153							
ASV56837	-	1.817	- 0.0000.002	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Fenollaria
	6.718	3.698					Tissierellales	XI	
ASV56713	-	2.022	- 0.0010.004	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Fenollaria
	6.738	3.332					Tissierellales	XI	
ASV56754	-	1.984	- 0.0020.009	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Anaerococcus
	6.003	3.026					Tissierellales	XI	
ASV60373	-	1.497	- 0.0000.001	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Peptoniphilus
	6.334	4.231					Tissierellales	XI	
ASV60265	-	1.833	- 0.0010.004	Bacteria	Bacteroid	Bacteroid	Bacteroidales	Prevotella	Prevotella
	6.142	3.350							
ASV61423	-	2.005	- 0.0020.007	Bacteria	Bacteroid	Bacteroid	Bacteroidales	Prevotella	Prevotella
	6.314	3.150							
ASV61674	-	1.962	- 0.0010.004	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Anaerococcus
	6.636	3.382					Tissierellales	XI	
ASV61806	-	1.792	- 0.0000.001	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Fenollaria
	6.887	3.843					Tissierellales	XI	
ASV62350	-	1.499	- 0.0000.000	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Peptoniphilus
	6.543	4.365					Tissierellales	XI	
ASV62583	-	2.027	- 0.0020.008	Bacteria	Bacteroid	Bacteroid	Bacteroidales	Prevotella	Prevotella
	6.315	3.116							
ASV63387	-	2.019	- 0.0020.007	Bacteria	Bacteroid	Bacteroid	Bacteroidales	Prevotella	Prevotella
	6.332	3.136							

ASV	baseMean	log2FoldChange	negLog10Pval	negLog10Padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV63766	-	2.645	-	0.0170.040	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces
	6.323		2.391							
ASV63794	-	1.947	-	0.0010.003	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	6.717		3.450					Tissierellales	XI	
ASV63866	-	1.646	-	0.0000.002	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
	6.007		3.649					Selenomonadales		
ASV65184	-	1.900	-	0.0010.003	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Anaerococcus
	6.585		3.466					Tissierellales	XI	
ASV65130	-	1.880	-	0.0000.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	6.689		3.558					Tissierellales	XI	
ASV65746	-	1.530	-	0.0000.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	5.438		3.554					Tissierellales	XI	
ASV65900	-	1.948	-	0.0010.004	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Anaerococcus
	6.577		3.376					Tissierellales	XI	
ASV65224	-	1.908	-	0.0000.003	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	6.721		3.523					Tissierellales	XI	
ASV65364	-	2.197	-	0.0070.020	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces
	5.939		2.702							
ASV65709	-	1.601	-	0.0010.003	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	5.480		3.422					Tissierellales	XI	
ASV66046	-	1.937	-	0.0010.005	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Anaerococcus
	6.415		3.312					Tissierellales	XI	
ASV65334	-	1.494	-	0.0000.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	6.197		4.147					Tissierellales	XI	
ASV65747	-	2.281	-	0.0060.017	Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Howarthella
	6.315		2.768							
ASV63792	-	2.283	-	0.0140.034	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Anaerococcus
	5.607		2.457					Tissierellales	XI	
ASV65841	-	1.929	-	0.0010.005	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.274		3.253							
ASV64359	-	1.873	-	0.0000.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	6.653		3.552					Tissierellales	XI	
ASV65363	-	1.513	-	0.0000.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	5.828		3.852					Tissierellales	XI	
ASV70250	-	1.741	-	0.0010.006	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
	5.609		3.221					Selenomonadales		
ASV70036	-	1.976	-	0.0020.008	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	6.176		3.125							
ASV70884	-	1.766	-	0.0000.001	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							
ASV84564	-	2.401	- 0.0160.039	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecoalcaliphilaceae	Anaerococcus
	5.775	2.405					Tissierellales	XI	
ASV84930	-	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	
ASV84117	-	1.919	- 0.0010.005	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecoalcaliphilaceae	Fenollaria
	6.309	3.288					Tissierellales	XI	
ASV84351	-	2.276	- 0.0090.025	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecoalcaliphilaceae	Parvimonas
	5.944	2.612					Tissierellales	XI	
ASV84420	-	1.975	- 0.0020.008	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.175	3.127							
ASV84473	-	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		
ASV84710	-	1.589	- 0.0010.004	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecoalcaliphilaceae	Peptoniphilus
	5.344	3.363					Tissierellales	XI	
ASV84406	-	2.265	- 0.0100.026	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecoalcaliphilaceae	Anaerococcus
	5.865	2.589					Tissierellales	XI	
ASV84988	-	1.734	- 0.0000.001	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.692	3.860							
ASV84985	-	2.000	- 0.0040.014	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	5.745	2.873							
ASV84710	-	2.056	- 0.0040.013	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecoalcaliphilaceae	Anaerococcus
	5.918	2.879					Tissierellales	XI	

ASV	baseMean	log2FoldChange	negLog10Pvalue	negLog10Padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV83970	-	2.171	-	0.0080.022	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Faecalibacterium	Anaerococcus
	5.789		2.667					Tissierellales	XI	
ASV84604	-	2.177	-	0.0060.018	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Faecalibacterium	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							
ASV91021	-	1.735	-	0.0020.009	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Faecalibacterium	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	Faecalibacterium	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	Faecalibacterium	Anaerococcus
	5.817		2.811					Tissierellales	XI	
ASV97514	-	2.298	-	0.0200.045	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Faecalibacterium	Parvimonas
	5.353		2.329					Tissierellales	XI	
ASV98847	-	2.364	-	0.0140.034	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Faecalibacterium	Anaerococcus
	5.816		2.461					Tissierellales	XI	
ASV10229	-	2.287	-	0.0120.031	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Faecalibacterium	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	Faecalibacterium	Parvimonas
	5.942		2.509					Tissierellales	XI	
ASV10861	-	2.037	-	0.0030.012	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Faecalibacterium	Ezakiella
	5.963		2.927					Tissierellales	XI	
ASV11280	-	2.097	-	0.0050.016	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Faecalibacterium	Anaerococcus
	5.882		2.805					Tissierellales	XI	
ASV11324	-	2.306	-	0.0190.044	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Faecalibacterium	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	negLog10PValue	negLog10AdjPValue	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

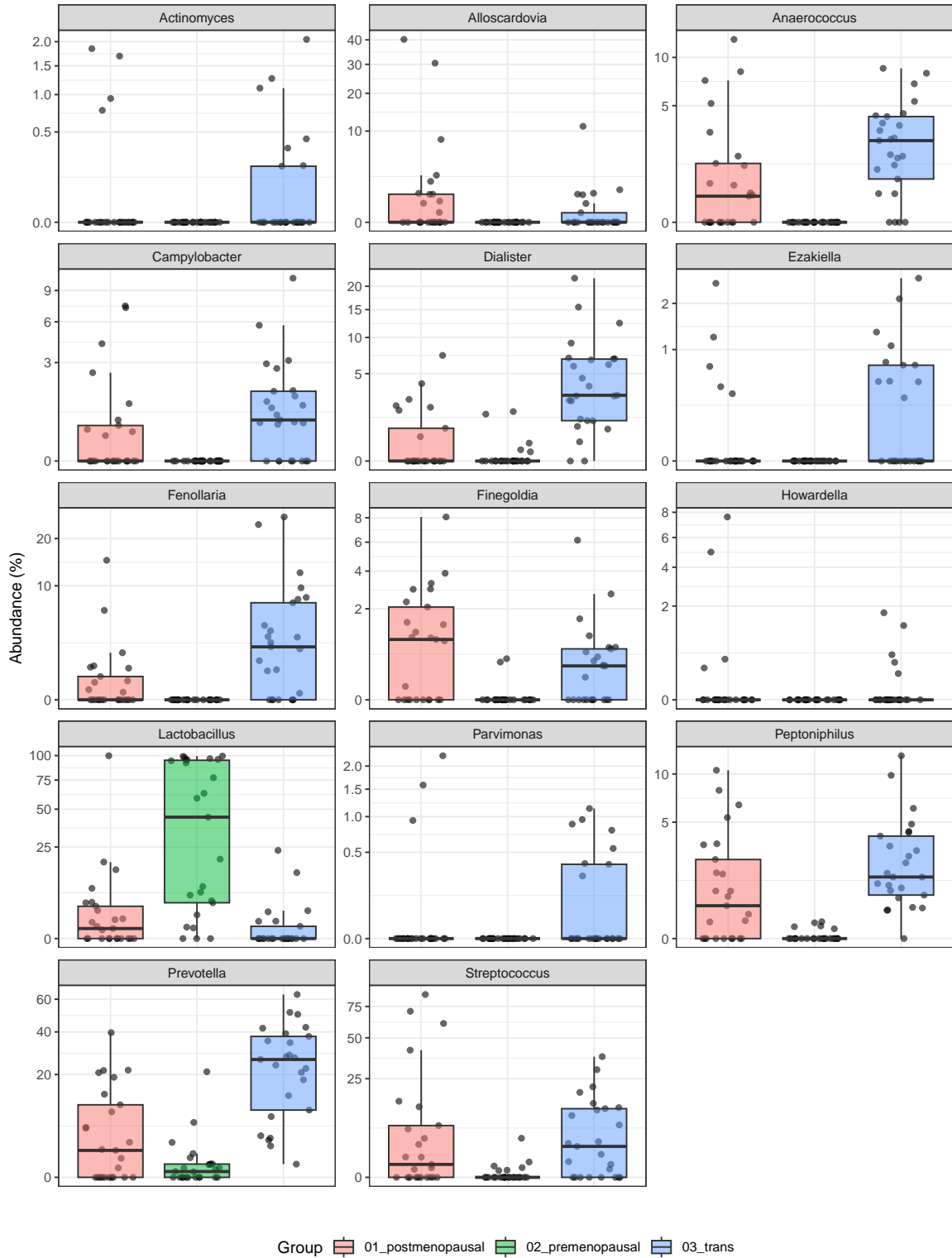
This analysis reveals, that the *Trans* group is characterized by a loss of *Lactobacillus* and an

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:Estradiole, Abundance)
genus$Taxonomy[genus$Abundance < 10] <- "< 10 % abund."

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

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

phyloseq-class experiment-level object

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

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

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

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

phyloseq-class experiment-level object

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

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

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

# Class Level
```

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

phyloseq-class experiment-level object

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

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

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

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

phyloseq-class experiment-level object

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

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

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

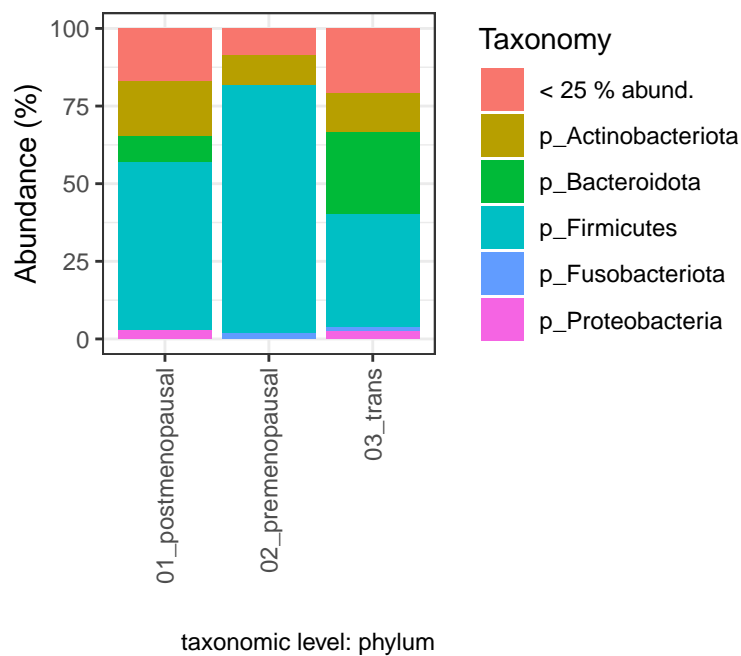


```
theme(legend.position = "right") +
labs(y = "Abundance (%)", x = "", caption = "taxonomic level: phylum") +
rotate_x_text()
```

5.1 Phylum level

On the phylum level, the taxonomic distribution is dominated by *Firmicutes* (these correspond to *Lactobacilli*, see below). The *Trans* group also shows an increase in *Bacteroidota* compared to the other 2 groups.

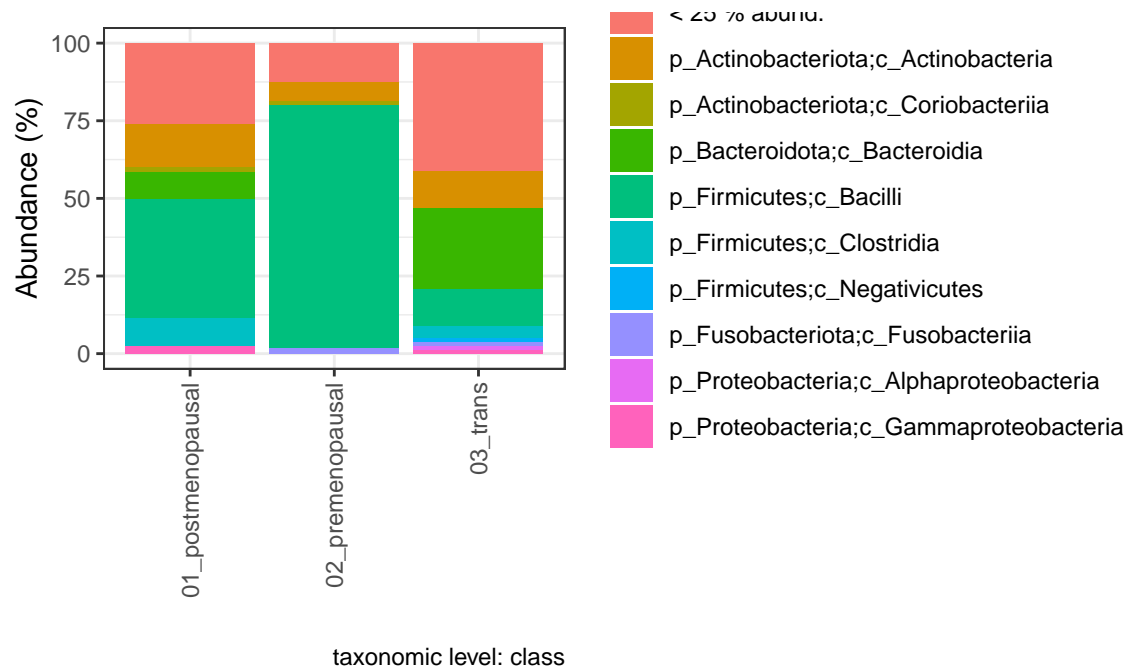
```
phylumplot
```



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

5.2 Class level

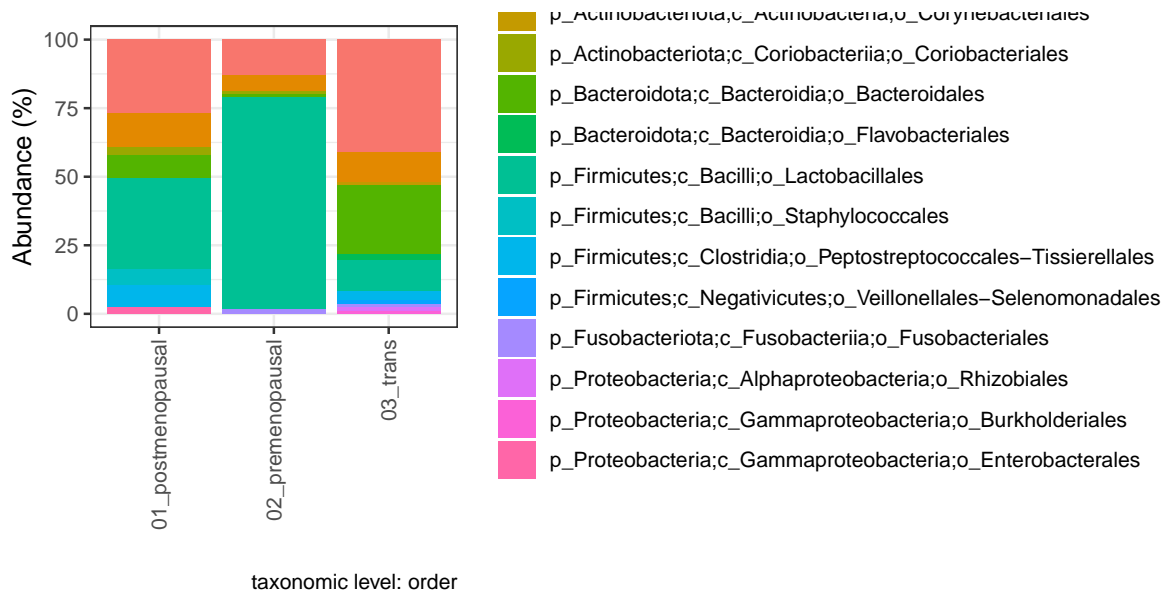
```
classplot
```



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

5.3 Order level

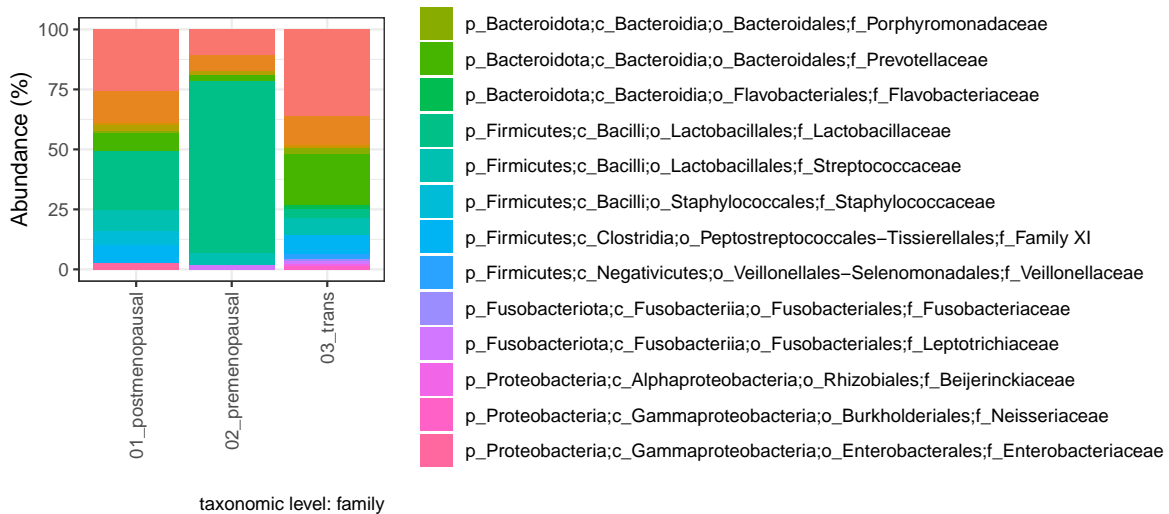
```
orderplot
```



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

5.4 Family level

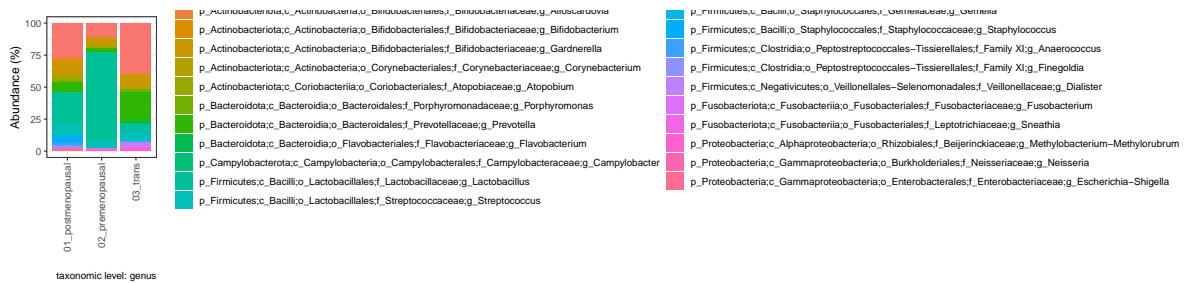
familyplot



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

5.5 Genus level

genusplot



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

6 Secondary analyses

6.0.1 Duration of menopause

This analysis is only performed in the postmenopausal group

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

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

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

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

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

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

```
2023-12-08 20:21:34.921341 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/asv_menopause-duration/maaslin2.1
2023-12-08 20:21:41.124612 INFO::Writing function arguments to log file
2023-12-08 20:21:41.130971 INFO::Verifying options selected are valid
2023-12-08 20:21:41.157955 INFO::Determining format of input files
2023-12-08 20:21:41.158437 INFO::Input format is data samples as rows and metadata samples as
2023-12-08 20:21:41.200029 INFO::Formula for fixed effects: expr ~ DurationMenopause
2023-12-08 20:21:41.200578 INFO::Filter data based on min abundance and min prevalence
2023-12-08 20:21:41.200874 INFO::Total samples in data: 25
2023-12-08 20:21:41.201143 INFO::Min samples required with min abundance for a feature not t
2023-12-08 20:21:41.247273 INFO::Total filtered features: 8831
2023-12-08 20:21:41.249217 INFO::Filtered feature names from abundance and prevalence filter
2023-12-08 20:21:41.251746 INFO::Total filtered features with variance filtering: 0
2023-12-08 20:21:41.252082 INFO::Filtered feature names from variance filtering:
2023-12-08 20:21:41.252672 INFO::Running selected normalization method: TSS
```

```

2023-12-08 20:21:41.254277 INFO::Applying z-score to standardize continuous metadata
2023-12-08 20:21:41.258862 INFO::Running selected transform method: LOG
2023-12-08 20:21:41.259851 INFO::Running selected analysis method: LM
2023-12-08 20:21:41.27833 INFO::Fitting model to feature number 1, ASV14
2023-12-08 20:21:41.281713 INFO::Fitting model to feature number 2, ASV16
2023-12-08 20:21:41.286726 INFO::Counting total values for each feature
2023-12-08 20:21:41.288139 INFO::Writing filtered data to file results/maaslin2/asv_menopause-
2023-12-08 20:21:41.289714 INFO::Writing filtered, normalized data to file results/maaslin2/
2023-12-08 20:21:41.29113 INFO::Writing filtered, normalized, transformed data to file resul
2023-12-08 20:21:41.292601 WARNING::Deleting existing residuals file: results/maaslin2/asv_m
2023-12-08 20:21:41.293281 INFO::Writing residuals to file results/maaslin2/asv_menopause-du
2023-12-08 20:21:41.29405 WARNING::Deleting existing fitted file: results/maaslin2/asv_menopau
2023-12-08 20:21:41.294526 INFO::Writing fitted values to file results/maaslin2/asv_menopaus
2023-12-08 20:21:41.29514 INFO::Writing all results to file (ordered by increasing q-values)
2023-12-08 20:21:41.296438 INFO::Writing the significant results (those which are less than c
2023-12-08 20:21:41.297098 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2023-12-08 20:21:41.298964 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")

```

```

2023-12-08 20:21:48.65669 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_menopause-duration/maaslin2
2023-12-08 20:21:48.873873 INFO::Writing function arguments to log file
2023-12-08 20:21:48.877985 INFO::Verifying options selected are valid
2023-12-08 20:21:48.878302 INFO::Determining format of input files
2023-12-08 20:21:48.878601 INFO::Input format is data samples as rows and metadata samples as
2023-12-08 20:21:48.880852 INFO::Formula for fixed effects: expr ~ DurationMenopause
2023-12-08 20:21:48.881188 INFO::Filter data based on min abundance and min prevalence
2023-12-08 20:21:48.881455 INFO::Total samples in data: 25
2023-12-08 20:21:48.881713 INFO::Min samples required with min abundance for a feature not t

```

```

2023-12-08 20:21:48.883462 INFO::Total filtered features: 294
2023-12-08 20:21:48.8838 INFO::Filtered feature names from abundance and prevalence filtering
2023-12-08 20:21:48.884315 INFO::Total filtered features with variance filtering: 0
2023-12-08 20:21:48.884603 INFO::Filtered feature names from variance filtering:
2023-12-08 20:21:48.884865 INFO::Running selected normalization method: TSS
2023-12-08 20:21:48.885313 INFO::Applying z-score to standardize continuous metadata
2023-12-08 20:21:48.888203 INFO::Running selected transform method: LOG
2023-12-08 20:21:48.888649 INFO::Running selected analysis method: LM
2023-12-08 20:21:48.888973 INFO::Fitting model to feature number 1, ASV16
2023-12-08 20:21:48.890224 INFO::Fitting model to feature number 2, ASV82
2023-12-08 20:21:48.891318 INFO::Fitting model to feature number 3, ASV271
2023-12-08 20:21:48.892395 INFO::Fitting model to feature number 4, ASV526
2023-12-08 20:21:48.895481 INFO::Counting total values for each feature
2023-12-08 20:21:48.896228 INFO::Writing filtered data to file results/maaslin2/genus_menopause-
2023-12-08 20:21:48.897139 INFO::Writing filtered, normalized data to file results/maaslin2/g
2023-12-08 20:21:48.897842 INFO::Writing filtered, normalized, transformed data to file resu
2023-12-08 20:21:48.898514 WARNING::Deleting existing residuals file: results/maaslin2/genus
2023-12-08 20:21:48.899007 INFO::Writing residuals to file results/maaslin2/genus_menopause-c
2023-12-08 20:21:48.899505 WARNING::Deleting existing fitted file: results/maaslin2/genus_mer
2023-12-08 20:21:48.899937 INFO::Writing fitted values to file results/maaslin2/genus_menopa
2023-12-08 20:21:48.900374 INFO::Writing all results to file (ordered by increasing q-values)
2023-12-08 20:21:48.90105 INFO::Writing the significant results (those which are less than o
2023-12-08 20:21:48.901462 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2023-12-08 20:21:48.902008 INFO::Writing association plots (one for each significant associat
[1] "There are no associations to plot!"

```

```

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

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

```

```

2023-12-08 20:21:52.961373 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/family_menopause-duration/maaslin
2023-12-08 20:21:53.046169 INFO::Writing function arguments to log file

```

```

2023-12-08 20:21:53.049227 INFO::Verifying options selected are valid
2023-12-08 20:21:53.049535 INFO::Determining format of input files
2023-12-08 20:21:53.049821 INFO::Input format is data samples as rows and metadata samples as columns
2023-12-08 20:21:53.051175 INFO::Formula for fixed effects: expr ~ DurationMenopause
2023-12-08 20:21:53.051501 INFO::Filter data based on min abundance and min prevalence
2023-12-08 20:21:53.051771 INFO::Total samples in data: 25
2023-12-08 20:21:53.052029 INFO::Min samples required with min abundance for a feature not to be filtered: 3
2023-12-08 20:21:53.05293 INFO::Total filtered features: 111
2023-12-08 20:21:53.053237 INFO::Filtered feature names from abundance and prevalence filtering:
2023-12-08 20:21:53.053663 INFO::Total filtered features with variance filtering: 0
2023-12-08 20:21:53.053943 INFO::Filtered feature names from variance filtering:
2023-12-08 20:21:53.0542 INFO::Running selected normalization method: TSS
2023-12-08 20:21:53.054636 INFO::Applying z-score to standardize continuous metadata
2023-12-08 20:21:53.057624 INFO::Running selected transform method: LOG
2023-12-08 20:21:53.058321 INFO::Running selected analysis method: LM
2023-12-08 20:21:53.058712 INFO::Fitting model to feature number 1, ASV16
2023-12-08 20:21:53.060166 INFO::Fitting model to feature number 2, ASV82
2023-12-08 20:21:53.061272 INFO::Fitting model to feature number 3, ASV120
2023-12-08 20:21:53.062352 INFO::Fitting model to feature number 4, ASV271
2023-12-08 20:21:53.065564 INFO::Counting total values for each feature
2023-12-08 20:21:53.066468 INFO::Writing filtered data to file results/maaslin2/family_menopause/
2023-12-08 20:21:53.067346 INFO::Writing filtered, normalized data to file results/maaslin2/family_menopause/
2023-12-08 20:21:53.068162 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/family_menopause/
2023-12-08 20:21:53.068847 WARNING::Deleting existing residuals file: results/maaslin2/family_menopause/
2023-12-08 20:21:53.069573 INFO::Writing residuals to file results/maaslin2/family_menopause/
2023-12-08 20:21:53.070107 WARNING::Deleting existing fitted file: results/maaslin2/family_menopause/
2023-12-08 20:21:53.070513 INFO::Writing fitted values to file results/maaslin2/family_menopause/
2023-12-08 20:21:53.070953 INFO::Writing all results to file (ordered by increasing q-values)
2023-12-08 20:21:53.071641 INFO::Writing the significant results (those which are less than 0.05)
2023-12-08 20:21:53.072081 INFO::Writing heatmap of significant results to file: results/maaslin2/family_menopause/
[1] "There are no associations to plot!"
2023-12-08 20:21:53.072655 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) %>%

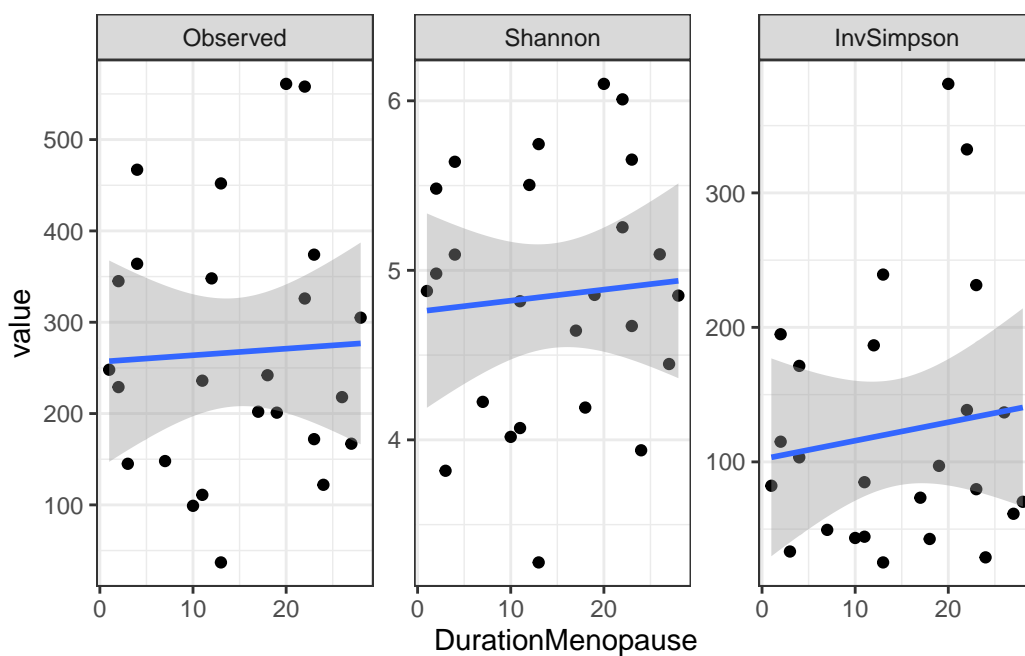
```



```
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")
```

```
2023-12-08 20:21:53.481882 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/asv_GHAT_Length/maaslin2.log"
2023-12-08 20:22:00.193383 INFO::Writing function arguments to log file
2023-12-08 20:22:00.19599 INFO::Verifying options selected are valid
2023-12-08 20:22:00.196295 INFO::Determining format of input files
2023-12-08 20:22:00.196576 INFO::Input format is data samples as rows and metadata samples as
2023-12-08 20:22:00.237106 INFO::Formula for fixed effects: expr ~ GHAT_Length
2023-12-08 20:22:00.237656 INFO::Filter data based on min abundance and min prevalence
2023-12-08 20:22:00.237936 INFO::Total samples in data: 25
2023-12-08 20:22:00.238201 INFO::Min samples required with min abundance for a feature not t
2023-12-08 20:22:00.280161 INFO::Total filtered features: 8824
```

```

2023-12-08 20:22:00.282 INFO::Filtered feature names from abundance and prevalence filtering
2023-12-08 20:22:00.284672 INFO::Total filtered features with variance filtering: 0
2023-12-08 20:22:00.285031 INFO::Filtered feature names from variance filtering:
2023-12-08 20:22:00.285324 INFO::Running selected normalization method: TSS
2023-12-08 20:22:00.285852 INFO::Applying z-score to standardize continuous metadata
2023-12-08 20:22:00.288891 INFO::Running selected transform method: LOG
2023-12-08 20:22:00.289412 INFO::Running selected analysis method: LM
2023-12-08 20:22:00.289757 INFO::Fitting model to feature number 1, ASV54
2023-12-08 20:22:00.29111 INFO::Fitting model to feature number 2, ASV55
2023-12-08 20:22:00.292236 INFO::Fitting model to feature number 3, ASV57
2023-12-08 20:22:00.29363 INFO::Fitting model to feature number 4, ASV58
2023-12-08 20:22:00.29493 INFO::Fitting model to feature number 5, ASV59
2023-12-08 20:22:00.296112 INFO::Fitting model to feature number 6, ASV60
2023-12-08 20:22:00.297249 INFO::Fitting model to feature number 7, ASV61
2023-12-08 20:22:00.298378 INFO::Fitting model to feature number 8, ASV62
2023-12-08 20:22:00.299463 INFO::Fitting model to feature number 9, ASV64
2023-12-08 20:22:00.303113 INFO::Counting total values for each feature
2023-12-08 20:22:00.303984 INFO::Writing filtered data to file results/maaslin2/asv_GHAT_Length
2023-12-08 20:22:00.304845 INFO::Writing filtered, normalized data to file results/maaslin2/asv_GHAT_Length
2023-12-08 20:22:00.305572 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/asv_GHAT_Length
2023-12-08 20:22:00.306375 WARNING::Deleting existing residuals file: results/maaslin2/asv_GHAT_Length
2023-12-08 20:22:00.306886 INFO::Writing residuals to file results/maaslin2/asv_GHAT_Length
2023-12-08 20:22:00.315223 WARNING::Deleting existing fitted file: results/maaslin2/asv_GHAT_Length
2023-12-08 20:22:00.315791 INFO::Writing fitted values to file results/maaslin2/asv_GHAT_Length
2023-12-08 20:22:00.316322 INFO::Writing all results to file (ordered by increasing q-values)
2023-12-08 20:22:00.317258 INFO::Writing the significant results (those which are less than 0.05)
2023-12-08 20:22:00.318034 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."
2023-12-08 20:22:00.3189 INFO::Writing association plots (one for each significant association)
2023-12-08 20:22:00.319862 INFO::Plotting associations from most to least significant, grouped by metadata number
2023-12-08 20:22:00.320319 INFO::Plotting data for metadata number 1, GHAT_Length
2023-12-08 20:22:00.321635 INFO::Creating scatter plot for continuous data, GHAT_Length vs ASV54

```

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

```

2023-12-08 20:22:00.413798 INFO::Creating scatter plot for continuous data, GHAT_Length vs ASV55

```

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

```

2023-12-08 20:22:00.504695 INFO::Creating scatter plot for continuous data, GHAT_Length vs ASV57

```

```

2023-12-08 20:22:00.585125 INFO::Creating scatter plot for continuous data, GHAT_Length vs ASV58

```

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

2023-12-08 20:22:00.670821 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")

```

```

2023-12-08 20:22:08.335083 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_GHAT_Length/maaslin2.log"
2023-12-08 20:22:08.548896 INFO::Writing function arguments to log file
2023-12-08 20:22:08.55153 INFO::Verifying options selected are valid
2023-12-08 20:22:08.551842 INFO::Determining format of input files
2023-12-08 20:22:08.552128 INFO::Input format is data samples as rows and metadata samples as columns
2023-12-08 20:22:08.554371 INFO::Formula for fixed effects: expr ~ GHAT_Length
2023-12-08 20:22:08.554754 INFO::Filter data based on min abundance and min prevalence
2023-12-08 20:22:08.555028 INFO::Total samples in data: 25
2023-12-08 20:22:08.555283 INFO::Min samples required with min abundance for a feature not to be filtered: 5
2023-12-08 20:22:08.557051 INFO::Total filtered features: 288
2023-12-08 20:22:08.557427 INFO::Filtered feature names from abundance and prevalence filtering:
2023-12-08 20:22:08.558005 INFO::Total filtered features with variance filtering: 0
2023-12-08 20:22:08.558297 INFO::Filtered feature names from variance filtering:
2023-12-08 20:22:08.558581 INFO::Running selected normalization method: TSS
2023-12-08 20:22:08.559087 INFO::Applying z-score to standardize continuous metadata
2023-12-08 20:22:08.561908 INFO::Running selected transform method: LOG
2023-12-08 20:22:08.562441 INFO::Running selected analysis method: LM
2023-12-08 20:22:08.562878 INFO::Fitting model to feature number 1, ASV14
2023-12-08 20:22:08.56423 INFO::Fitting model to feature number 2, ASV55
2023-12-08 20:22:08.56542 INFO::Fitting model to feature number 3, ASV66
2023-12-08 20:22:08.566664 INFO::Fitting model to feature number 4, ASV87
2023-12-08 20:22:08.568058 INFO::Fitting model to feature number 5, ASV120
2023-12-08 20:22:08.569414 INFO::Fitting model to feature number 6, ASV205
2023-12-08 20:22:08.570646 INFO::Fitting model to feature number 7, ASV241
2023-12-08 20:22:08.571794 INFO::Fitting model to feature number 8, ASV443
2023-12-08 20:22:08.572962 INFO::Fitting model to feature number 9, ASV568
2023-12-08 20:22:08.574083 INFO::Fitting model to feature number 10, ASV940
2023-12-08 20:22:08.577524 INFO::Counting total values for each feature
2023-12-08 20:22:08.578387 INFO::Writing filtered data to file results/maaslin2/genus_GHAT_Length/maaslin2_filtered_data.csv
2023-12-08 20:22:08.579304 INFO::Writing filtered, normalized data to file results/maaslin2/genus_GHAT_Length/maaslin2_normalized_data.csv
2023-12-08 20:22:08.580091 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/genus_GHAT_Length/maaslin2_transformed_data.csv
2023-12-08 20:22:08.580914 WARNING::Deleting existing residuals file: results/maaslin2/genus_GHAT_Length/maaslin2_residuals.csv
2023-12-08 20:22:08.581399 INFO::Writing residuals to file results/maaslin2/genus_GHAT_Length/maaslin2_residuals.csv
2023-12-08 20:22:08.581924 WARNING::Deleting existing fitted file: results/maaslin2/genus_GHAT_Length/maaslin2_fitted_values.csv
2023-12-08 20:22:08.58233 INFO::Writing fitted values to file results/maaslin2/genus_GHAT_Length/maaslin2_fitted_values.csv

```

```

2023-12-08 20:22:08.582802 INFO::Writing all results to file (ordered by increasing q-values)
2023-12-08 20:22:08.583531 INFO::Writing the significant results (those which are less than 0.2)
2023-12-08 20:22:08.584022 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."
2023-12-08 20:22:08.584705 INFO::Writing association plots (one for each significant association)
2023-12-08 20:22:08.585429 INFO::Plotting associations from most to least significant, grouped by metadata number
2023-12-08 20:22:08.585783 INFO::Plotting data for metadata number 1, GHAT_Length
2023-12-08 20:22:08.586621 INFO::Creating scatter plot for continuous data, GHAT_Length vs Abundance

2023-12-08 20:22:08.669696 INFO::Creating scatter plot for continuous data, GHAT_Length vs Abundance

2023-12-08 20:22:08.758801 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>

```

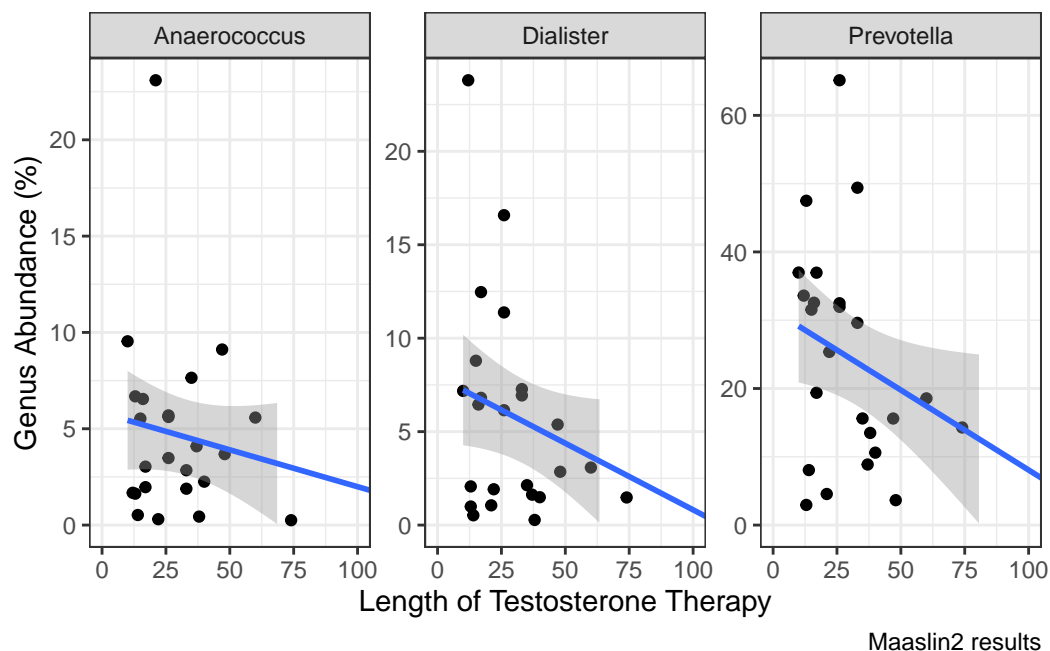
# plot correlation for these 3 ASVs
psmelt_trans_genus = psmelt(ps_trans_genus)
maaslin_res_genus_ghat = ggplot(filter(psmelt_trans_genus, OTU %in% filter(genus_GHAT$results, qval < 0.2)$feature)) +
  aes(x = GHAT_Length, y = Abundance) +
  geom_point() +
  geom_smooth(method = "lm") +
  facet_wrap(~Genus,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

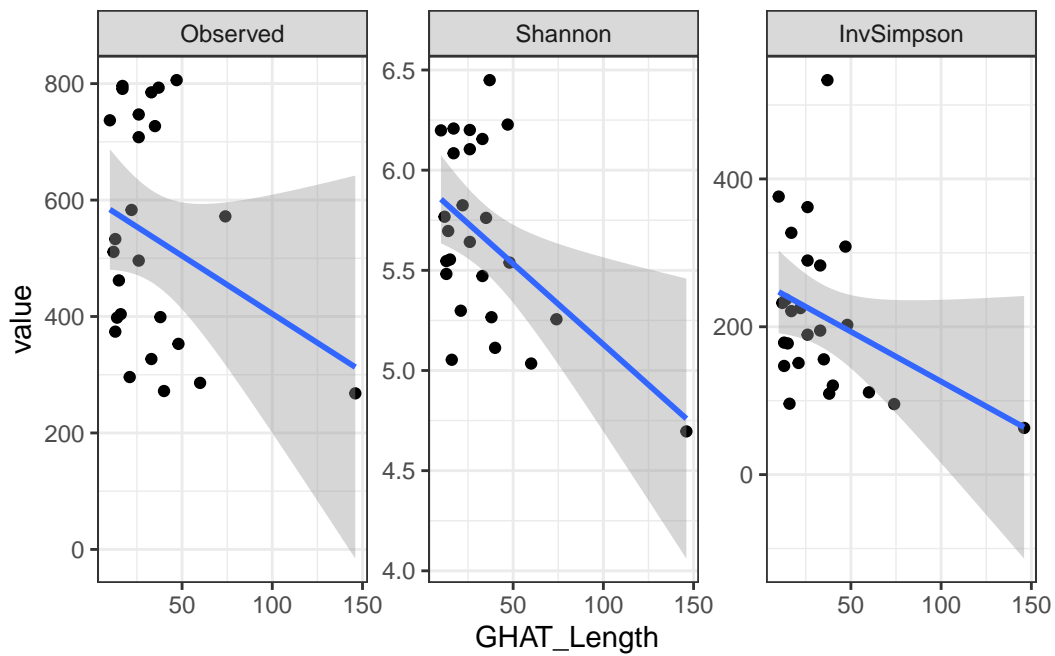
```

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	0.009
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()

```

```

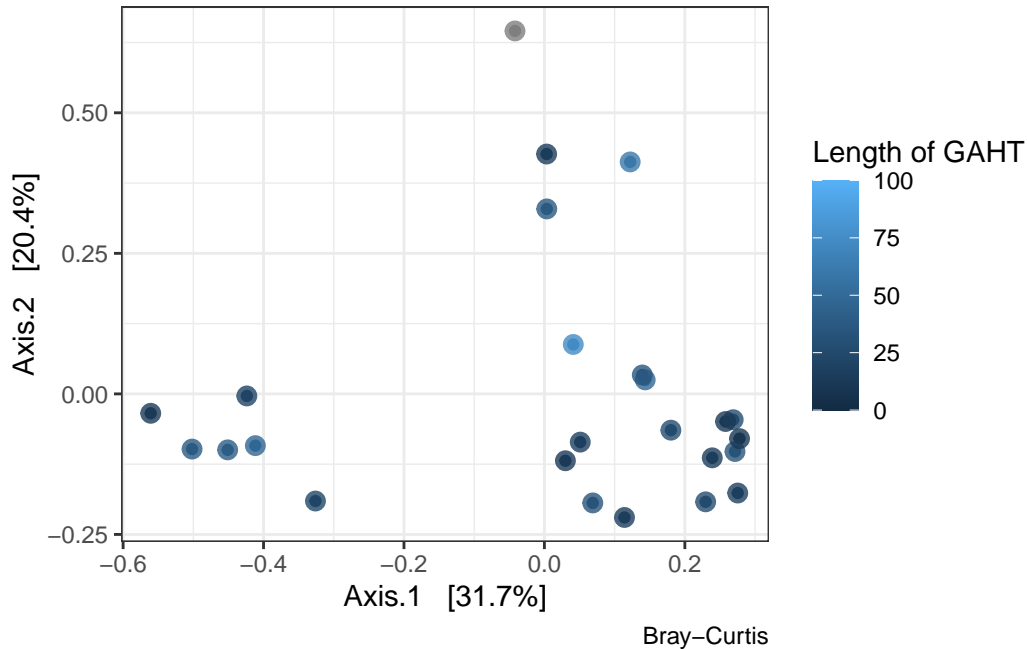
Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
10.00  16.00   26.00   33.56  38.00  146.00

```

```

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
```

```
2023-12-08 20:22:17.051641 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_Testosterone/maaslin2.log"
2023-12-08 20:22:17.279667 INFO::Writing function arguments to log file
2023-12-08 20:22:17.282374 INFO::Verifying options selected are valid
```

```

2023-12-08 20:22:17.282914 INFO::Determining format of input files
2023-12-08 20:22:17.283304 INFO::Input format is data samples as rows and metadata samples as
2023-12-08 20:22:17.285553 INFO::Formula for fixed effects: expr ~ Testosterone
2023-12-08 20:22:17.285898 INFO::Filter data based on min abundance and min prevalence
2023-12-08 20:22:17.286173 INFO::Total samples in data: 25
2023-12-08 20:22:17.286457 INFO::Min samples required with min abundance for a feature not to
2023-12-08 20:22:17.288258 INFO::Total filtered features: 292
2023-12-08 20:22:17.288607 INFO::Filtered feature names from abundance and prevalence filter:
2023-12-08 20:22:17.289148 INFO::Total filtered features with variance filtering: 0
2023-12-08 20:22:17.289438 INFO::Filtered feature names from variance filtering:
2023-12-08 20:22:17.28971 INFO::Running selected normalization method: TSS
2023-12-08 20:22:17.290181 INFO::Applying z-score to standardize continuous metadata
2023-12-08 20:22:17.293234 INFO::Running selected transform method: LOG
2023-12-08 20:22:17.293784 INFO::Running selected analysis method: LM
2023-12-08 20:22:17.29414 INFO::Fitting model to feature number 1, ASV14
2023-12-08 20:22:17.295637 INFO::Fitting model to feature number 2, ASV54
2023-12-08 20:22:17.296807 INFO::Fitting model to feature number 3, ASV66
2023-12-08 20:22:17.297931 INFO::Fitting model to feature number 4, ASV87
2023-12-08 20:22:17.29903 INFO::Fitting model to feature number 5, ASV205
2023-12-08 20:22:17.300127 INFO::Fitting model to feature number 6, ASV568
2023-12-08 20:22:17.303679 INFO::Counting total values for each feature
2023-12-08 20:22:17.304526 INFO::Writing filtered data to file results/maaslin2/genus_Testos
2023-12-08 20:22:17.305306 INFO::Writing filtered, normalized data to file results/maaslin2/g
2023-12-08 20:22:17.305993 INFO::Writing filtered, normalized, transformed data to file resu
2023-12-08 20:22:17.306698 WARNING::Deleting existing residuals file: results/maaslin2/genus
2023-12-08 20:22:17.307203 INFO::Writing residuals to file results/maaslin2/genus_Testosteron
2023-12-08 20:22:17.307715 WARNING::Deleting existing fitted file: results/maaslin2/genus_Tes
2023-12-08 20:22:17.308145 INFO::Writing fitted values to file results/maaslin2/genus_Testos
2023-12-08 20:22:17.308612 INFO::Writing all results to file (ordered by increasing q-values)
2023-12-08 20:22:17.309336 INFO::Writing the significant results (those which are less than
2023-12-08 20:22:17.309769 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2023-12-08 20:22:17.310327 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
```

```
2023-12-08 20:22:17.317813 WARNING::Input is a matrix, passing through as.data.frame() .  
[1] "Warning: Deleting existing log file: results/maaslin2/genus_DurationGNRH/maaslin2.log"  
2023-12-08 20:22:17.546622 INFO::Writing function arguments to log file  
2023-12-08 20:22:17.549376 INFO::Verifying options selected are valid  
2023-12-08 20:22:17.549683 INFO::Determining format of input files  
2023-12-08 20:22:17.549962 INFO::Input format is data samples as rows and metadata samples as columns  
2023-12-08 20:22:17.552113 INFO::Formula for fixed effects: expr ~ Duration_GNRH  
2023-12-08 20:22:17.55244 INFO::Filter data based on min abundance and min prevalence  
2023-12-08 20:22:17.552711 INFO::Total samples in data: 25  
2023-12-08 20:22:17.552967 INFO::Min samples required with min abundance for a feature not to be filtered: 5  
2023-12-08 20:22:17.554738 INFO::Total filtered features: 292  
2023-12-08 20:22:17.555084 INFO::Filtered feature names from abundance and prevalence filtering: 292  
2023-12-08 20:22:17.555611 INFO::Total filtered features with variance filtering: 0  
2023-12-08 20:22:17.555891 INFO::Filtered feature names from variance filtering:  
2023-12-08 20:22:17.556158 INFO::Running selected normalization method: TSS  
2023-12-08 20:22:17.556605 INFO::Applying z-score to standardize continuous metadata  
2023-12-08 20:22:17.559625 INFO::Running selected transform method: LOG  
2023-12-08 20:22:17.560341 INFO::Running selected analysis method: LM  
2023-12-08 20:22:17.560732 INFO::Fitting model to feature number 1, ASV14  
2023-12-08 20:22:17.562285 INFO::Fitting model to feature number 2, ASV54  
2023-12-08 20:22:17.563601 INFO::Fitting model to feature number 3, ASV66  
2023-12-08 20:22:17.564816 INFO::Fitting model to feature number 4, ASV87  
2023-12-08 20:22:17.565989 INFO::Fitting model to feature number 5, ASV205  
2023-12-08 20:22:17.567101 INFO::Fitting model to feature number 6, ASV568  
2023-12-08 20:22:17.57079 INFO::Counting total values for each feature
```

```

2023-12-08 20:22:17.571815 INFO::Writing filtered data to file results/maaslin2/genus_DurationGN
2023-12-08 20:22:17.573074 INFO::Writing filtered, normalized data to file results/maaslin2/g
2023-12-08 20:22:17.574057 INFO::Writing filtered, normalized, transformed data to file resu
2023-12-08 20:22:17.575024 WARNING::Deleting existing residuals file: results/maaslin2/genus_
2023-12-08 20:22:17.575889 INFO::Writing residuals to file results/maaslin2/genus_DurationGN
2023-12-08 20:22:17.576658 WARNING::Deleting existing fitted file: results/maaslin2/genus_Dur
2023-12-08 20:22:17.577187 INFO::Writing fitted values to file results/maaslin2/genus_Durati
2023-12-08 20:22:17.57772 INFO::Writing all results to file (ordered by increasing q-values)
2023-12-08 20:22:17.578536 INFO::Writing the significant results (those which are less than c
2023-12-08 20:22:17.579016 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2023-12-08 20:22:17.57961 INFO::Writing association plots (one for each significant associat
[1] "There are no associations to plot!"

```

```
# no significant associations
```

No significant associations.

6.0.5 Cycle dependency in pre-menopausal group

```

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

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

```

```

2023-12-08 20:22:17.659022 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/asv_cycle_premeno/maaslin2.log"
2023-12-08 20:22:23.776262 INFO::Writing function arguments to log file
2023-12-08 20:22:23.779249 INFO::Verifying options selected are valid
2023-12-08 20:22:23.779612 INFO::Determining format of input files
2023-12-08 20:22:23.779918 INFO::Input format is data samples as rows and metadata samples as
2023-12-08 20:22:23.820784 INFO::Formula for fixed effects: expr ~ CycleDaySampling
2023-12-08 20:22:23.82141 INFO::Filter data based on min abundance and min prevalence
2023-12-08 20:22:23.821722 INFO::Total samples in data: 25

```

```

2023-12-08 20:22:23.822003 INFO::Min samples required with min abundance for a feature not t
2023-12-08 20:22:23.863895 INFO::Total filtered features: 8808
2023-12-08 20:22:23.865663 INFO::Filtered feature names from abundance and prevalence filter
2023-12-08 20:22:23.868329 INFO::Total filtered features with variance filtering: 0
2023-12-08 20:22:23.868641 INFO::Filtered feature names from variance filtering:
2023-12-08 20:22:23.868911 INFO::Running selected normalization method: TSS
2023-12-08 20:22:23.869466 INFO::Applying z-score to standardize continuous metadata
2023-12-08 20:22:23.872332 INFO::Running selected transform method: LOG
2023-12-08 20:22:23.872952 INFO::Running selected analysis method: LM
2023-12-08 20:22:23.873282 INFO::Fitting model to feature number 1, ASV1
2023-12-08 20:22:23.874553 INFO::Fitting model to feature number 2, ASV2
2023-12-08 20:22:23.875645 INFO::Fitting model to feature number 3, ASV3
2023-12-08 20:22:23.876843 INFO::Fitting model to feature number 4, ASV4
2023-12-08 20:22:23.877947 INFO::Fitting model to feature number 5, ASV5
2023-12-08 20:22:23.879013 INFO::Fitting model to feature number 6, ASV6
2023-12-08 20:22:23.880069 INFO::Fitting model to feature number 7, ASV7
2023-12-08 20:22:23.881111 INFO::Fitting model to feature number 8, ASV8
2023-12-08 20:22:23.88216 INFO::Fitting model to feature number 9, ASV9
2023-12-08 20:22:23.883206 INFO::Fitting model to feature number 10, ASV10
2023-12-08 20:22:23.88427 INFO::Fitting model to feature number 11, ASV11
2023-12-08 20:22:23.885318 INFO::Fitting model to feature number 12, ASV12
2023-12-08 20:22:23.886367 INFO::Fitting model to feature number 13, ASV13
2023-12-08 20:22:23.887412 INFO::Fitting model to feature number 14, ASV14
2023-12-08 20:22:23.888496 INFO::Fitting model to feature number 15, ASV15
2023-12-08 20:22:23.889538 INFO::Fitting model to feature number 16, ASV16
2023-12-08 20:22:23.890566 INFO::Fitting model to feature number 17, ASV17
2023-12-08 20:22:23.891596 INFO::Fitting model to feature number 18, ASV18
2023-12-08 20:22:23.89268 INFO::Fitting model to feature number 19, ASV19
2023-12-08 20:22:23.893714 INFO::Fitting model to feature number 20, ASV20
2023-12-08 20:22:23.894746 INFO::Fitting model to feature number 21, ASV21
2023-12-08 20:22:23.895851 INFO::Fitting model to feature number 22, ASV22
2023-12-08 20:22:23.896875 INFO::Fitting model to feature number 23, ASV23
2023-12-08 20:22:23.897899 INFO::Fitting model to feature number 24, ASV27
2023-12-08 20:22:23.898918 INFO::Fitting model to feature number 25, ASV32
2023-12-08 20:22:23.902954 INFO::Counting total values for each feature
2023-12-08 20:22:23.904042 INFO::Writing filtered data to file results/maaslin2/asv_cycle_pr
2023-12-08 20:22:23.905443 INFO::Writing filtered, normalized data to file results/maaslin2/a
2023-12-08 20:22:23.906536 INFO::Writing filtered, normalized, transformed data to file resu
2023-12-08 20:22:23.907678 WARNING::Deleting existing residuals file: results/maaslin2/asv_cy
2023-12-08 20:22:23.908187 INFO::Writing residuals to file results/maaslin2/asv_cycle_premen
2023-12-08 20:22:23.908803 WARNING::Deleting existing fitted file: results/maaslin2/asv_cycl
2023-12-08 20:22:23.909247 INFO::Writing fitted values to file results/maaslin2/asv_cycle_pr
2023-12-08 20:22:23.909744 INFO::Writing all results to file (ordered by increasing q-values)

```

```

2023-12-08 20:22:23.910521 INFO::Writing the significant results (those which are less than 0.05) to file: results/maaslin2/genus_cycle_premeno/maaslin2.sig
2023-12-08 20:22:23.910946 INFO::Writing heatmap of significant results to file: results/maaslin2/genus_cycle_premeno/maaslin2.sig
[1] "There are no associations to plot!"
2023-12-08 20:22:23.9115 INFO::Writing association plots (one for each significant association) to file: results/maaslin2/genus_cycle_premeno/maaslin2.sig
[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")

```

```

2023-12-08 20:22:31.45246 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_cycle_premeno/maaslin2.log"
2023-12-08 20:22:31.662842 INFO::Writing function arguments to log file
2023-12-08 20:22:31.665603 INFO::Verifying options selected are valid
2023-12-08 20:22:31.665928 INFO::Determining format of input files
2023-12-08 20:22:31.666225 INFO::Input format is data samples as rows and metadata samples as columns
2023-12-08 20:22:31.668469 INFO::Formula for fixed effects: expr ~ CycleDaySampling
2023-12-08 20:22:31.668808 INFO::Filter data based on min abundance and min prevalence
2023-12-08 20:22:31.669104 INFO::Total samples in data: 25
2023-12-08 20:22:31.669384 INFO::Min samples required with min abundance for a feature not to be filtered: 5
2023-12-08 20:22:31.671312 INFO::Total filtered features: 296
2023-12-08 20:22:31.671815 INFO::Filtered feature names from abundance and prevalence filtering: 296
2023-12-08 20:22:31.672354 INFO::Total filtered features with variance filtering: 0
2023-12-08 20:22:31.672676 INFO::Filtered feature names from variance filtering: 0
2023-12-08 20:22:31.672955 INFO::Running selected normalization method: TSS
2023-12-08 20:22:31.6734 INFO::Applying z-score to standardize continuous metadata
2023-12-08 20:22:31.676745 INFO::Running selected transform method: LOG
2023-12-08 20:22:31.677388 INFO::Running selected analysis method: LM
2023-12-08 20:22:31.677797 INFO::Fitting model to feature number 1, ASV1
2023-12-08 20:22:31.679147 INFO::Fitting model to feature number 2, ASV66
2023-12-08 20:22:31.682387 INFO::Counting total values for each feature
2023-12-08 20:22:31.683214 INFO::Writing filtered data to file results/maaslin2/genus_cycle_premeno/maaslin2.sig
2023-12-08 20:22:31.683979 INFO::Writing filtered, normalized data to file results/maaslin2/genus_cycle_premeno/maaslin2.sig
2023-12-08 20:22:31.684608 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/genus_cycle_premeno/maaslin2.sig
2023-12-08 20:22:31.685245 WARNING::Deleting existing residuals file: results/maaslin2/genus_cycle_premeno/maaslin2.sig

```

```

2023-12-08 20:22:31.685737 INFO::Writing residuals to file results/maaslin2/genus_cycle_prema
2023-12-08 20:22:31.686222 WARNING::Deleting existing fitted file: results/maaslin2/genus_cy
2023-12-08 20:22:31.686633 INFO::Writing fitted values to file results/maaslin2/genus_cycle_
2023-12-08 20:22:31.687054 INFO::Writing all results to file (ordered by increasing q-values)
2023-12-08 20:22:31.687765 INFO::Writing the significant results (those which are less than 0
2023-12-08 20:22:31.688279 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
2023-12-08 20:22:31.688937 INFO::Writing association plots (one for each significant associat
2023-12-08 20:22:31.689604 INFO::Plotting associations from most to least significant, group
2023-12-08 20:22:31.689953 INFO::Plotting data for metadata number 1, CycleDaySampling
2023-12-08 20:22:31.690582 INFO::Creating scatter plot for continuous data, CycleDaySampling

2023-12-08 20:22:31.774781 INFO::Creating scatter plot for continuous data, CycleDaySampling

```

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

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

```

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

```

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

```

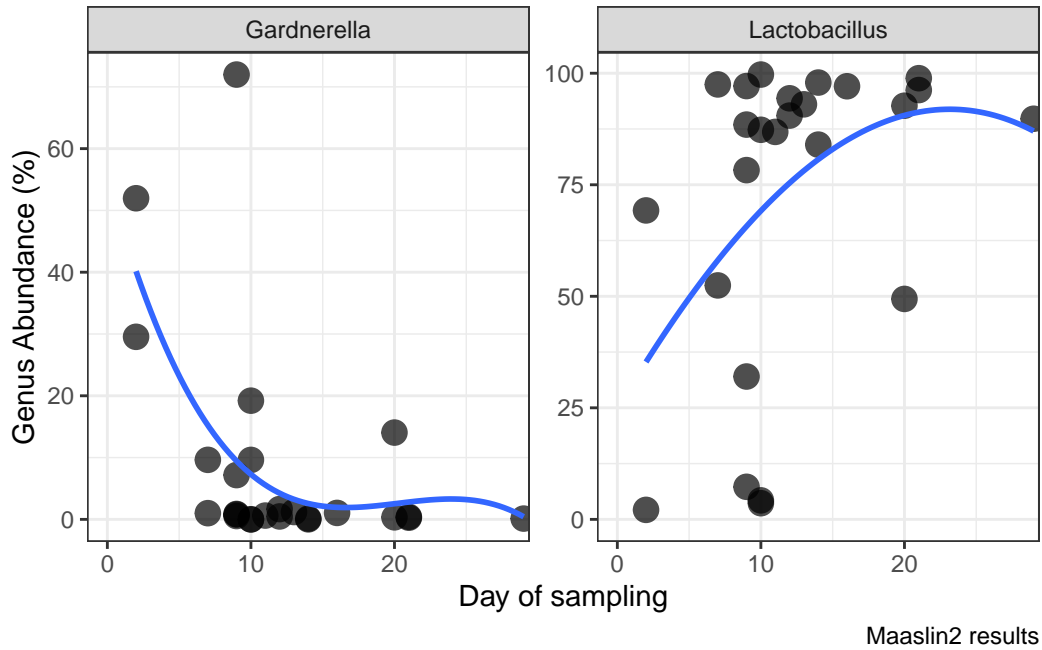
psmelt_pre_genus = psmelt(ps_pre_genus)

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

```



```
maaslin_res_genus_cycleday
```



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

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

6.0.6 Influence of sexual activity on microbiome composition

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

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

```
ps_rel_genus_sexuallyactive
```

```
phyloseq-class experiment-level object
```

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

```
# Via Maaslin2
```

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

```
2023-12-08 20:22:51.244178 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_SexuallyActive/maaslin2.log"
2023-12-08 20:22:51.457018 INFO::Writing function arguments to log file
2023-12-08 20:22:51.459817 INFO::Verifying options selected are valid
2023-12-08 20:22:51.460142 INFO::Determining format of input files
2023-12-08 20:22:51.460432 INFO::Input format is data samples as rows and metadata samples as columns
2023-12-08 20:22:51.462781 INFO::Formula for fixed effects: expr ~ SexuallyActive
2023-12-08 20:22:51.463321 INFO::Factor detected for categorical metadata 'SexuallyActive'. Proceeding with factor
2023-12-08 20:22:51.463651 INFO::Filter data based on min abundance and min prevalence
2023-12-08 20:22:51.463945 INFO::Total samples in data: 72
2023-12-08 20:22:51.464223 INFO::Min samples required with min abundance for a feature not to be filtered: 5
2023-12-08 20:22:51.466152 INFO::Total filtered features: 294
2023-12-08 20:22:51.466527 INFO::Filtered feature names from abundance and prevalence filtering: 294
2023-12-08 20:22:51.467145 INFO::Total filtered features with variance filtering: 0
2023-12-08 20:22:51.467529 INFO::Filtered feature names from variance filtering: 0
2023-12-08 20:22:51.467831 INFO::Running selected normalization method: TSS
2023-12-08 20:22:51.468352 INFO::Applying z-score to standardize continuous metadata
2023-12-08 20:22:51.471389 INFO::Running selected transform method: LOG
2023-12-08 20:22:51.471888 INFO::Running selected analysis method: LM
2023-12-08 20:22:51.472232 INFO::Fitting model to feature number 1, ASV1
2023-12-08 20:22:51.47367 INFO::Fitting model to feature number 2, ASV54
2023-12-08 20:22:51.474916 INFO::Fitting model to feature number 3, ASV66
```

```

2023-12-08 20:22:51.476154 INFO::Fitting model to feature number 4, ASV87
2023-12-08 20:22:51.479583 INFO::Counting total values for each feature
2023-12-08 20:22:51.480357 INFO::Writing filtered data to file results/maaslin2/genus_SexuallyActive
2023-12-08 20:22:51.482058 INFO::Writing filtered, normalized data to file results/maaslin2/genus_SexuallyActive
2023-12-08 20:22:51.483029 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/genus_SexuallyActive
2023-12-08 20:22:51.483984 WARNING::Deleting existing residuals file: results/maaslin2/genus_SexuallyActive
2023-12-08 20:22:51.484742 INFO::Writing residuals to file results/maaslin2/genus_SexuallyActive
2023-12-08 20:22:51.485329 WARNING::Deleting existing fitted file: results/maaslin2/genus_SexuallyActive
2023-12-08 20:22:51.486042 INFO::Writing fitted values to file results/maaslin2/genus_SexuallyActive
2023-12-08 20:22:51.486582 INFO::Writing all results to file (ordered by increasing q-values)
2023-12-08 20:22:51.487319 INFO::Writing the significant results (those which are less than 0.05)
2023-12-08 20:22:51.487817 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."
2023-12-08 20:22:51.488483 INFO::Writing association plots (one for each significant association)
2023-12-08 20:22:51.489197 INFO::Plotting associations from most to least significant, grouped by metadata number
2023-12-08 20:22:51.489543 INFO::Plotting data for metadata number 1, SexuallyActive
2023-12-08 20:22:51.490968 INFO::Creating boxplot for categorical data, SexuallyActive vs ASV
2023-12-08 20:22:51.563603 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(
  aes(x = SexuallyActive, y = Abundance, fill = SexuallyActive)

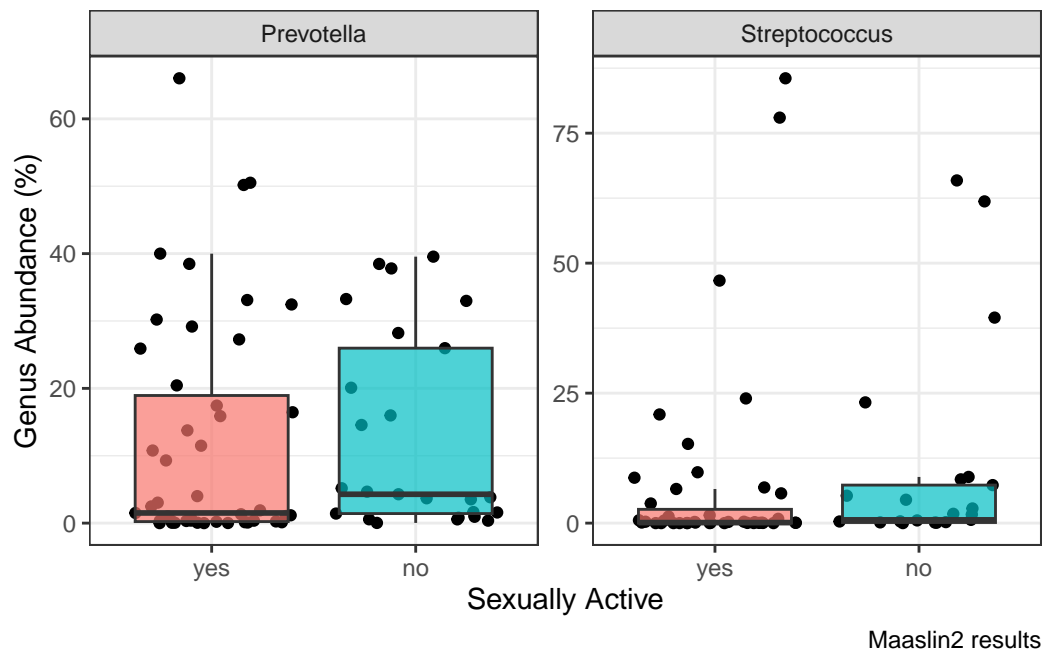
geom_jitter() +
geom_boxplot(alpha = 0.7, outlier.shape = NA) +
facet_wrap(~Genus, scales = "free") +
scale_y_continuous(limits = function(x){c(0, max(0.1, x))}) +

```

```
theme(legend.position = "none")+
labs(x = "Sexually Active",
     y = "Genus Abundance (%)",
     caption = "Maaslin2 results")
```

```
maaslin_res_genus_sexactive
```

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



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

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

```
# Via Deseq2
sexactive_ds = phyloseq_to_deseq2(ps_abs_genus_sexuallyactive, ~SexuallyActive)
cts = counts(sexactive_ds)
geoMeans = apply(cts, 1, function(row) if (all(row == 0)) 0 else exp(mean(log(row[row != 0])))
sexactive_ds = estimateSizeFactors(sexactive_ds, geoMeans=geoMeans)
```

```
sexactive_ds = DESeq(sexactive_ds, test = "Wald", fitType = "parametric")
res = results(sexactive_ds, cooksCutoff = FALSE)
alpha = 0.5
sigtab = res[which(res$padj < alpha), ]
sigtab = cbind(as(sigtab, "data.frame"), as(tax_table(ps_abs_genus_sexuallyactive)[rownames(sigtab), ], "data.frame"))
sigtab = as.data.frame(sigtab) %>%
  rownames_to_column("feature")
```

```
sigtab
```

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

```
# this does not detect any significant difference
```

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

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

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

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

6.0.7 Influence of the duration of amenorrhea on microbiome composition

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

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

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

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

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

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

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

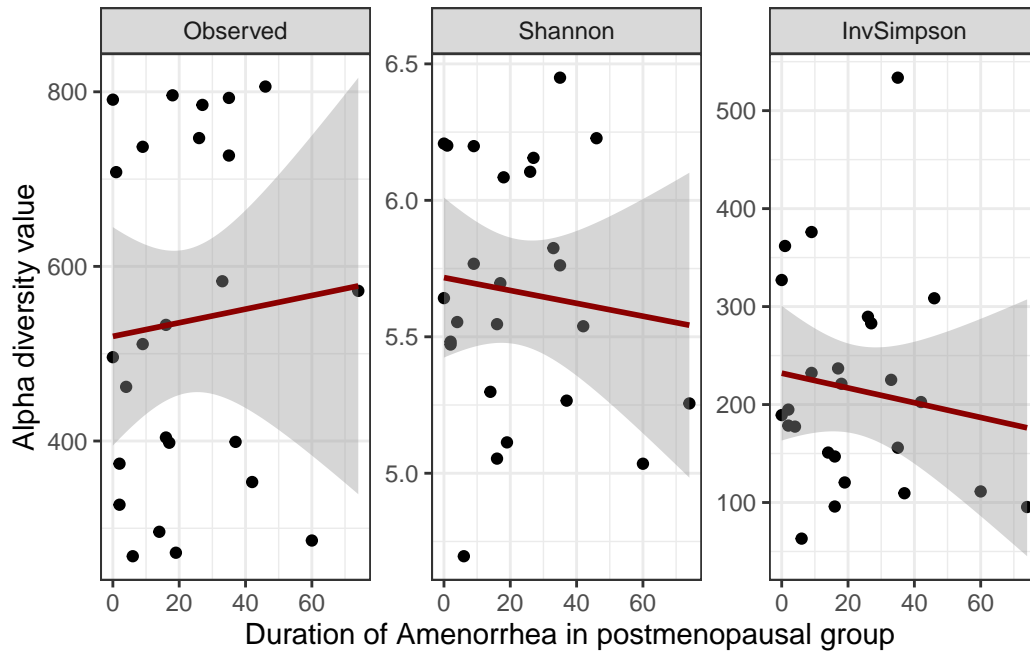
ps_menopause_rel = transform_sample_counts(ps_menopause, function(x) x/sum(x) * 100)
# Diversity analysis
adiv_menopause = plot_richness(ps_menopause, measures = c("Observed", "Shannon", "InvSimpson"))

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

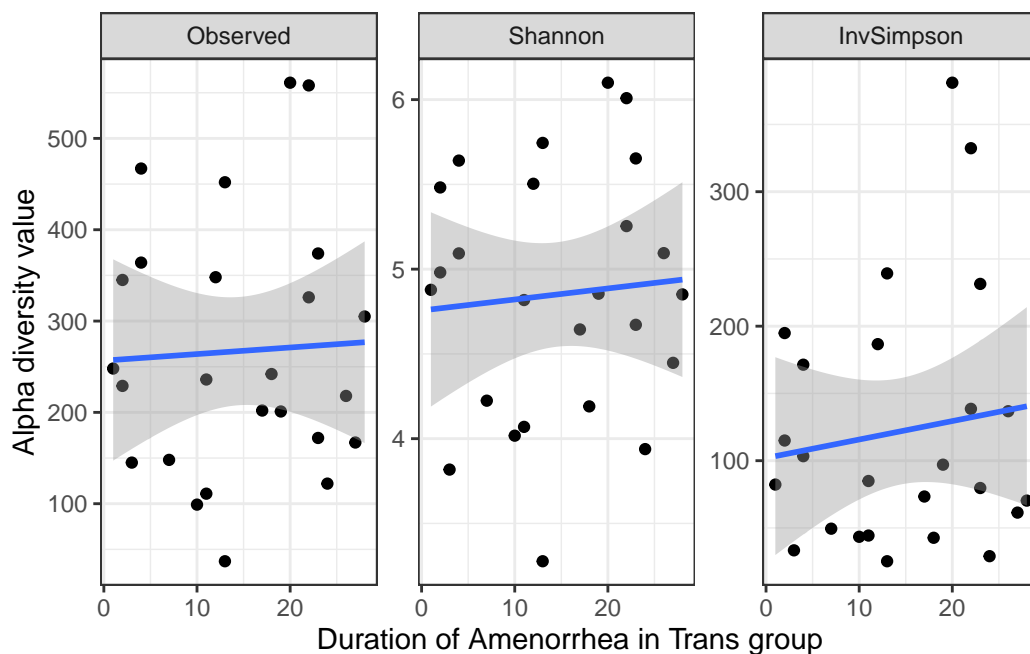
```
corr_trans = filter(adiv_menopause, grp == "03_trans") %>%
  group_by(variable) %>%
  rstatix::cor_test(value, DurationAmenorrhea)
kable(corr_trans)
```

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

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



```
ggplot(filter(adv_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)
```

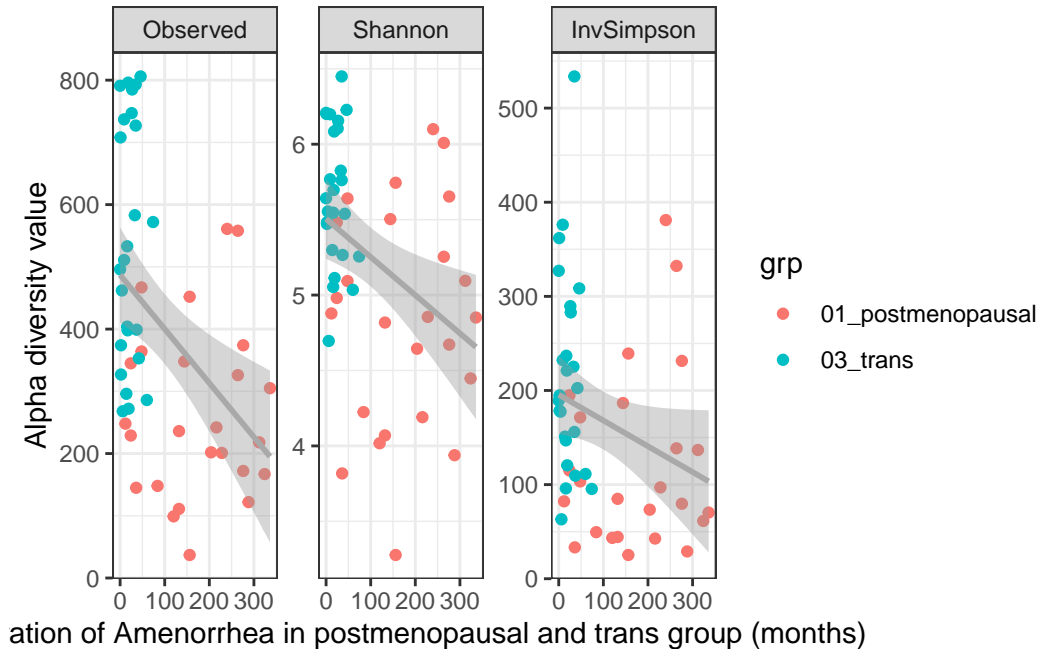
variable	var1	var2	cor	statistic	p	conf.low	conf.high	method
Observed	value	DurationMenopause	0.045	0.2158134	0.831	-0.3565088	0.4324048	Pearson
Shannon	value	DurationMenopause	0.078	0.3754553	0.711	-0.3271717	0.4590238	Pearson
InvSimpson	value	DurationMenopause	0.130	0.6169177	0.543	-0.2817497	0.4976290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


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

# Next, both groups are combined: in months
corr_menopause = adiv_menopause %>%
  group_by(variable) %>%
  rstatix::cor_test(value, DurationCombined, method = "pearson")
kable(corr_menopause)
```

variable	var1	var2	cor	statistic	p	conf.low	conf.high	method
Observed	value	DurationCombined	0.43	-	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(adiv_menopause, aes(x = DurationCombined, y = value)) +
  geom_point(aes(color = grp)) +
  geom_smooth(method = "lm", color = "darkgrey") +
  facet_wrap(~variable, scales = "free") +
  labs(x = "Duration of Amenorrhea in postmenopausal and trans group (months)", y = "Alpha")
```



```
## 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")
```

```
2023-12-08 20:23:05.714449 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_MenopausedurationMonths/maaslin2_log.txt"
2023-12-08 20:23:05.951459 INFO::Writing function arguments to log file
2023-12-08 20:23:05.954551 INFO::Verifying options selected are valid
2023-12-08 20:23:05.954896 INFO::Determining format of input files
2023-12-08 20:23:05.955188 INFO::Input format is data samples as rows and metadata samples as columns
2023-12-08 20:23:05.9576 INFO::Formula for fixed effects: expr ~ DurationCombined
2023-12-08 20:23:05.95818 INFO::Filter data based on min abundance and min prevalence
2023-12-08 20:23:05.958508 INFO::Total samples in data: 50
2023-12-08 20:23:05.958793 INFO::Min samples required with min abundance for a feature not to be filtered: 5
2023-12-08 20:23:05.960833 INFO::Total filtered features: 292
```

```

2023-12-08 20:23:05.96128 INFO::Filtered feature names from abundance and prevalence filtering
2023-12-08 20:23:05.96185 INFO::Total filtered features with variance filtering: 0
2023-12-08 20:23:05.962149 INFO::Filtered feature names from variance filtering:
2023-12-08 20:23:05.962419 INFO::Running selected normalization method: TSS
2023-12-08 20:23:05.962917 INFO::Applying z-score to standardize continuous metadata
2023-12-08 20:23:05.965817 INFO::Running selected transform method: LOG
2023-12-08 20:23:05.966296 INFO::Running selected analysis method: LM
2023-12-08 20:23:05.96664 INFO::Fitting model to feature number 1, ASV14
2023-12-08 20:23:05.968169 INFO::Fitting model to feature number 2, ASV54
2023-12-08 20:23:05.969849 INFO::Fitting model to feature number 3, ASV82
2023-12-08 20:23:05.971231 INFO::Fitting model to feature number 4, ASV205
2023-12-08 20:23:05.972382 INFO::Fitting model to feature number 5, ASV210
2023-12-08 20:23:05.973488 INFO::Fitting model to feature number 6, ASV369
2023-12-08 20:23:05.976807 INFO::Counting total values for each feature
2023-12-08 20:23:05.977852 INFO::Writing filtered data to file results/maaslin2/genus_Menopar
2023-12-08 20:23:05.979185 INFO::Writing filtered, normalized data to file results/maaslin2/
2023-12-08 20:23:05.980316 INFO::Writing filtered, normalized, transformed data to file resu
2023-12-08 20:23:05.981433 WARNING::Deleting existing residuals file: results/maaslin2/genus
2023-12-08 20:23:05.982007 INFO::Writing residuals to file results/maaslin2/genus_Menopaused
2023-12-08 20:23:05.982823 WARNING::Deleting existing fitted file: results/maaslin2/genus_Mer
2023-12-08 20:23:05.983272 INFO::Writing fitted values to file results/maaslin2/genus_Menopar
2023-12-08 20:23:05.983772 INFO::Writing all results to file (ordered by increasing q-values)
2023-12-08 20:23:05.984579 INFO::Writing the significant results (those which are less than
2023-12-08 20:23:05.985073 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
2023-12-08 20:23:05.985761 INFO::Writing association plots (one for each significant associat
2023-12-08 20:23:05.986485 INFO::Plotting associations from most to least significant, group
2023-12-08 20:23:05.986853 INFO::Plotting data for metadata number 1, DurationCombined
2023-12-08 20:23:05.98748 INFO::Creating scatter plot for continuous data, DurationCombined v

```

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

```

2023-12-08 20:23:06.07133 INFO::Creating scatter plot for continuous data, DurationCombined v

```

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

```

2023-12-08 20:23:06.154134 INFO::Creating scatter plot for continuous data, DurationCombined

```

```

2023-12-08 20:23:06.570413 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)
```

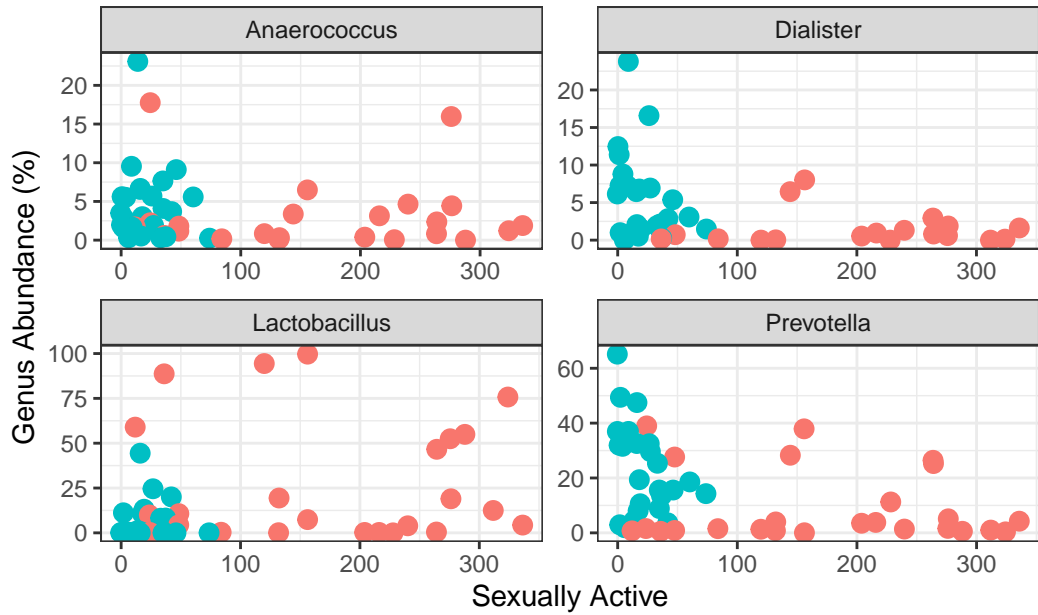
	ASV	Kingdom	Phylum	Class					
1	ASV14	Bacteria	Firmicutes	Bacilli					
2	ASV54	Bacteria	Bacteroidota	Bacteroidia					
3	ASV205	Bacteria	Firmicutes	Negativicutes					
4	ASV369	Bacteria	Firmicutes	Clostridia					
					Order	Family	Genus	Species	
1					Lactobacillales	Lactobacillaceae	Lactobacillus	<NA>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



Maaslin2 results

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

Warning: Removed 7 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(adv$data, variable=="InvSimpson"), value)
```

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

```
adv$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
```

```
adv$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)$SexOfP
adonis_pw_bc_partnersex = pairwise.adonis(D_BC, phyloseq::sample_data(ps_filtered_rel)$Sex

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

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

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: 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 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 *

pairs	Df	SumsOfSqsF.Model	R2	p.value	p.adjusted	sig
-------	----	------------------	----	---------	------------	-----

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

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

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

```
2023-12-08 20:23:08.547088 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_SexOfPartner/maaslin2.log"
2023-12-08 20:23:08.749568 INFO::Writing function arguments to log file
2023-12-08 20:23:08.752165 INFO::Verifying options selected are valid
2023-12-08 20:23:08.752477 INFO::Determining format of input files
2023-12-08 20:23:08.752764 INFO::Input format is data samples as rows and metadata samples as columns
2023-12-08 20:23:08.755128 INFO::Formula for fixed effects: expr ~ SexOfPartner
2023-12-08 20:23:08.755482 INFO::Factor detected for categorical metadata 'SexOfPartner'. Proceeding with factor
2023-12-08 20:23:08.755748 INFO::Filter data based on min abundance and min prevalence
2023-12-08 20:23:08.75602 INFO::Total samples in data: 72
2023-12-08 20:23:08.756282 INFO::Min samples required with min abundance for a feature not to be filtered: 5
2023-12-08 20:23:08.75834 INFO::Total filtered features: 294
2023-12-08 20:23:08.758694 INFO::Filtered feature names from abundance and prevalence filtering: 294
2023-12-08 20:23:08.759225 INFO::Total filtered features with variance filtering: 0
2023-12-08 20:23:08.759525 INFO::Filtered feature names from variance filtering: 0
2023-12-08 20:23:08.759807 INFO::Running selected normalization method: TSS
2023-12-08 20:23:08.760315 INFO::Applying z-score to standardize continuous metadata
2023-12-08 20:23:08.763004 INFO::Running selected transform method: LOG
2023-12-08 20:23:08.763521 INFO::Running selected analysis method: LM
2023-12-08 20:23:08.763891 INFO::Fitting model to feature number 1, ASV1
2023-12-08 20:23:08.765321 INFO::Fitting model to feature number 2, ASV54
2023-12-08 20:23:08.766579 INFO::Fitting model to feature number 3, ASV66
2023-12-08 20:23:08.767794 INFO::Fitting model to feature number 4, ASV87
2023-12-08 20:23:08.771186 INFO::Counting total values for each feature
```



```

2023-12-08 20:23:08.772069 INFO::Writing filtered data to file results/maaslin2/genus_SexOfPartner2
2023-12-08 20:23:08.773005 INFO::Writing filtered, normalized data to file results/maaslin2/genus_SexOfPartner2
2023-12-08 20:23:08.773808 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/genus_SexOfPartner2
2023-12-08 20:23:08.774633 WARNING::Deleting existing residuals file: results/maaslin2/genus_SexOfPartner2
2023-12-08 20:23:08.77512 INFO::Writing residuals to file results/maaslin2/genus_SexOfPartner2
2023-12-08 20:23:08.775654 WARNING::Deleting existing fitted file: results/maaslin2/genus_SexOfPartner2
2023-12-08 20:23:08.776067 INFO::Writing fitted values to file results/maaslin2/genus_SexOfPartner2
2023-12-08 20:23:08.776505 INFO::Writing all results to file (ordered by increasing q-values)
2023-12-08 20:23:08.777214 INFO::Writing the significant results (those which are less than 0.05)
2023-12-08 20:23:08.777712 INFO::Writing heatmap of significant results to file: results/maaslin2/genus_SexOfPartner2
[1] "There are no associations to plot!"
2023-12-08 20:23:08.778322 INFO::Writing association plots (one for each significant association)
2023-12-08 20:23:08.779049 INFO::Plotting associations from most to least significant, grouped by metadata number
2023-12-08 20:23:08.779398 INFO::Plotting data for metadata number 1, SexOfPartner
2023-12-08 20:23:08.780068 INFO::Creating boxplot for categorical data, SexOfPartner vs ASV54

```

```

genus_SexOfPartner2 = Maaslin2(input_data = otu_table(ps_rel_genus_sexuallyactive),
                                input_metadata = as(sample_data(ps_rel_genus_sexuallyactive), "data.frame"),
                                output = "results/maaslin2/genus_SexOfPartner2grp",
                                min_abundance = 5,
                                min_prevalence = 0.2,
                                max_significance = 0.2,
                                fixed_effects = c("SexOfPartner", "grp"))

```

```

2023-12-08 20:23:08.948469 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_SexOfPartner2grp/maaslin2.log"
2023-12-08 20:23:09.149292 INFO::Writing function arguments to log file
2023-12-08 20:23:09.151796 INFO::Verifying options selected are valid
2023-12-08 20:23:09.152091 INFO::Determining format of input files
2023-12-08 20:23:09.152365 INFO::Input format is data samples as rows and metadata samples as columns
2023-12-08 20:23:09.154654 INFO::Formula for fixed effects: expr ~ SexOfPartner + grp
2023-12-08 20:23:09.154999 INFO::Factor detected for categorical metadata 'SexOfPartner'. Provide a reference level.
2023-12-08 20:23:09.155277 INFO::Factor detected for categorical metadata 'grp'. Provide a reference level.
2023-12-08 20:23:09.155529 INFO::Filter data based on min abundance and min prevalence
2023-12-08 20:23:09.155783 INFO::Total samples in data: 72
2023-12-08 20:23:09.156036 INFO::Min samples required with min abundance for a feature not to be filtered: 5
2023-12-08 20:23:09.157933 INFO::Total filtered features: 294
2023-12-08 20:23:09.15827 INFO::Filtered feature names from abundance and prevalence filtering: 294
2023-12-08 20:23:09.158765 INFO::Total filtered features with variance filtering: 0
2023-12-08 20:23:09.159039 INFO::Filtered feature names from variance filtering: 0
2023-12-08 20:23:09.159289 INFO::Running selected normalization method: TSS

```

```

2023-12-08 20:23:09.159768 INFO::Applying z-score to standardize continuous metadata
2023-12-08 20:23:09.162466 INFO::Running selected transform method: LOG
2023-12-08 20:23:09.162938 INFO::Running selected analysis method: LM
2023-12-08 20:23:09.163281 INFO::Fitting model to feature number 1, ASV1
2023-12-08 20:23:09.164804 INFO::Fitting model to feature number 2, ASV54
2023-12-08 20:23:09.166151 INFO::Fitting model to feature number 3, ASV66
2023-12-08 20:23:09.167484 INFO::Fitting model to feature number 4, ASV87
2023-12-08 20:23:09.171141 INFO::Counting total values for each feature
2023-12-08 20:23:09.172148 INFO::Writing filtered data to file results/maaslin2/genus_SexOfPartner2/
2023-12-08 20:23:09.173036 INFO::Writing filtered, normalized data to file results/maaslin2/genus_SexOfPartner2/
2023-12-08 20:23:09.173829 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/genus_SexOfPartner2/
2023-12-08 20:23:09.174649 WARNING::Deleting existing residuals file: results/maaslin2/genus_SexOfPartner2/
2023-12-08 20:23:09.17513 INFO::Writing residuals to file results/maaslin2/genus_SexOfPartner2/
2023-12-08 20:23:09.175647 WARNING::Deleting existing fitted file: results/maaslin2/genus_SexOfPartner2/
2023-12-08 20:23:09.17624 INFO::Writing fitted values to file results/maaslin2/genus_SexOfPartner2/
2023-12-08 20:23:09.176818 INFO::Writing all results to file (ordered by increasing q-values)
2023-12-08 20:23:09.177623 INFO::Writing the significant results (those which are less than 0.2)
2023-12-08 20:23:09.178132 INFO::Writing heatmap of significant results to file: results/maaslin2/genus_SexOfPartner2/
2023-12-08 20:23:09.218119 INFO::Writing association plots (one for each significant association)
2023-12-08 20:23:09.219176 INFO::Plotting associations from most to least significant, grouped by metadata number
2023-12-08 20:23:09.219591 INFO::Plotting data for metadata number 1, grp
2023-12-08 20:23:09.220285 INFO::Creating boxplot for categorical data, grp vs ASV54

2023-12-08 20:23:09.291088 INFO::Creating boxplot for categorical data, grp vs ASV87

2023-12-08 20:23:09.365612 INFO::Creating boxplot for categorical data, grp vs ASV1

2023-12-08 20:23:09.436924 INFO::Creating boxplot for categorical data, grp vs ASV1

2023-12-08 20:23:09.509076 INFO::Creating boxplot for categorical data, grp vs ASV54

2023-12-08 20:23:09.976137 INFO::Plotting data for metadata number 2, SexOfPartner
2023-12-08 20:23:09.977073 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$results, qual < 0.2)$feature)

```

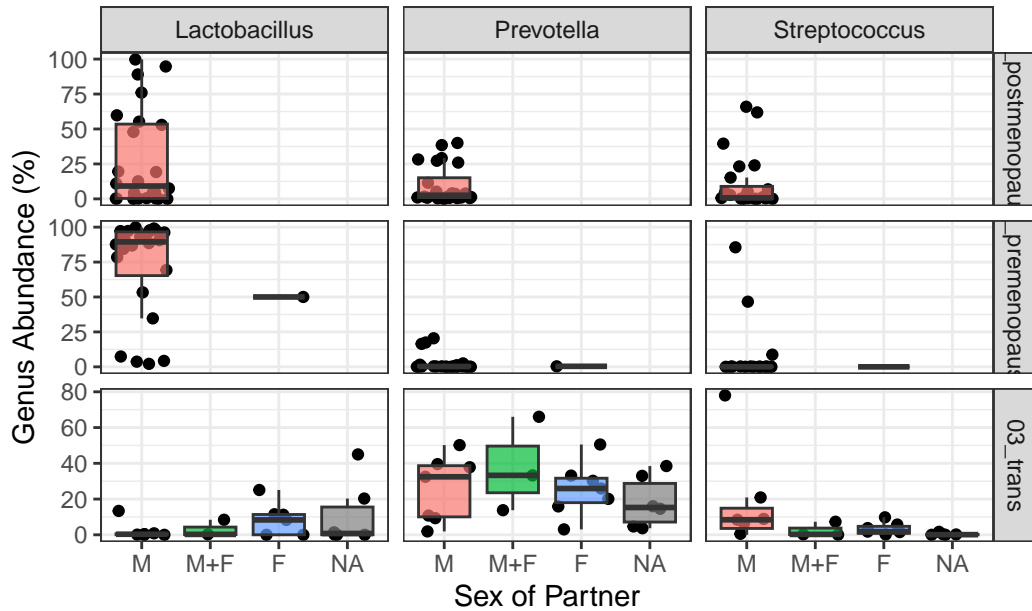
	ASV	Kingdom	Phylum	Class	Order	Family
1	ASV1	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae
2	ASV54	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae
3	ASV87	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae
		Genus	Species			
1	Lactobacillus	<NA>				
2	Prevotella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

```
# plot abundance of Provetella
maaslin_res_genus_sexpartner = ggplot(filter(psmelt_rel_genus_sexuallyactive, OTU %in% fil
aes(x = SexOfPartner, y = Abundance, fill = SexOfPartner))

geom_jitter() +
geom_boxplot(alpha = 0.7, outlier.shape = NA) +
facet_grid(grp~Genus, scales = "free") +
scale_y_continuous(limits = function(x){c(0, max(0.1, x))}) +
theme(legend.position = "none")+
labs(x = "Sex of Partner",
y = "Genus Abundance (%)",
caption = "Maaslin2 results")

maaslin_res_genus_sexpartner
```

Warning: Removed 14 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 17 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")
```

```
2023-12-08 20:23:10.806382 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_SexOfPartnerTrans/maaslin2.
2023-12-08 20:23:11.024479 INFO::Writing function arguments to log file
2023-12-08 20:23:11.027016 INFO::Verifying options selected are valid
2023-12-08 20:23:11.027322 INFO::Determining format of input files
```

```

2023-12-08 20:23:11.027639 INFO::Input format is data samples as rows and metadata samples as
2023-12-08 20:23:11.030063 INFO::Formula for fixed effects: expr ~ SexOfPartner
2023-12-08 20:23:11.030536 INFO::Factor detected for categorical metadata 'SexOfPartner'. Pro
2023-12-08 20:23:11.030819 INFO::Filter data based on min abundance and min prevalence
2023-12-08 20:23:11.031091 INFO::Total samples in data: 23
2023-12-08 20:23:11.031358 INFO::Min samples required with min abundance for a feature not to
2023-12-08 20:23:11.03313 INFO::Total filtered features: 291
2023-12-08 20:23:11.033504 INFO::Filtered feature names from abundance and prevalence filter
2023-12-08 20:23:11.034044 INFO::Total filtered features with variance filtering: 0
2023-12-08 20:23:11.034337 INFO::Filtered feature names from variance filtering:
2023-12-08 20:23:11.034594 INFO::Running selected normalization method: TSS
2023-12-08 20:23:11.035056 INFO::Applying z-score to standardize continuous metadata
2023-12-08 20:23:11.037615 INFO::Running selected transform method: LOG
2023-12-08 20:23:11.038094 INFO::Running selected analysis method: LM
2023-12-08 20:23:11.038413 INFO::Fitting model to feature number 1, ASV1
2023-12-08 20:23:11.039796 INFO::Fitting model to feature number 2, ASV54
2023-12-08 20:23:11.040983 INFO::Fitting model to feature number 3, ASV66
2023-12-08 20:23:11.042153 INFO::Fitting model to feature number 4, ASV87
2023-12-08 20:23:11.043309 INFO::Fitting model to feature number 5, ASV205
2023-12-08 20:23:11.044458 INFO::Fitting model to feature number 6, ASV369
2023-12-08 20:23:11.045597 INFO::Fitting model to feature number 7, ASV397
2023-12-08 20:23:11.049198 INFO::Counting total values for each feature
2023-12-08 20:23:11.050234 INFO::Writing filtered data to file results/maaslin2/genus_SexOfPa
2023-12-08 20:23:11.05119 INFO::Writing filtered, normalized data to file results/maaslin2/g
2023-12-08 20:23:11.051897 INFO::Writing filtered, normalized, transformed data to file resu
2023-12-08 20:23:11.052745 WARNING::Deleting existing residuals file: results/maaslin2/genus
2023-12-08 20:23:11.053246 INFO::Writing residuals to file results/maaslin2/genus_SexOfPartn
2023-12-08 20:23:11.053757 WARNING::Deleting existing fitted file: results/maaslin2/genus_Se
2023-12-08 20:23:11.054208 INFO::Writing fitted values to file results/maaslin2/genus_SexOfPa
2023-12-08 20:23:11.054646 INFO::Writing all results to file (ordered by increasing q-values
2023-12-08 20:23:11.055403 INFO::Writing the significant results (those which are less than
2023-12-08 20:23:11.055833 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2023-12-08 20:23:11.056394 INFO::Writing association plots (one for each significant associat
[1] "There are no associations to plot!"

```

```

# no significant results in trans group only

```