

MBVagTrans Results

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Table of contents

1	Introduction	2
2	PBS empty control	2
3	Diversity Analysis	4
3.1	Alpha-diversity	4
3.1.1	Effect of covariates	5
3.2	Beta-diversity	7
4	Differential abundance	11
5	Taxonomic composition	30
5.1	Phylum level	33
5.2	Class level	33
5.3	Order level	34
5.4	Family level	35
5.5	Genus level	36
6	Secondary analyses	36
6.0.1	Duration of menopause	36
6.0.2	Duration of gender-affirming hormone therapy (GAHT)	42
6.0.3	Absolute Height of Testosterone in TRANS group	50
6.0.4	Duration of GnRH therapy in TRANS group	52
6.0.5	Cycle dependency in premenopausal group	53
6.0.6	Influence of sexual activity on microbiome composition	57
6.0.7	Influence of the duration of amenorrhea on microbiome composition	60

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

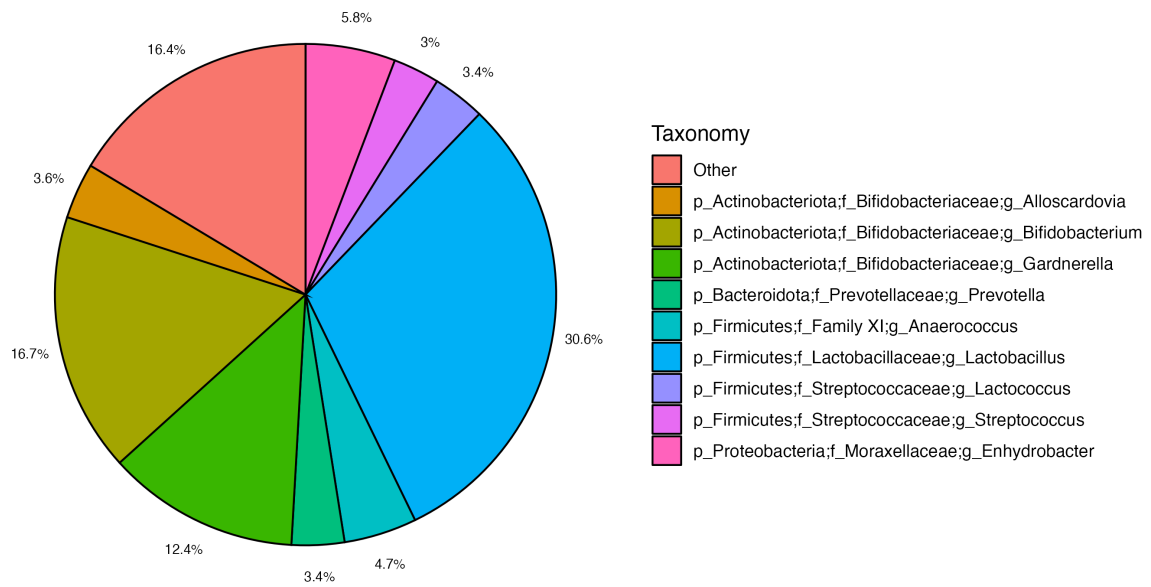


Figure 1: Taxonomic composition of PBS sample

```
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.

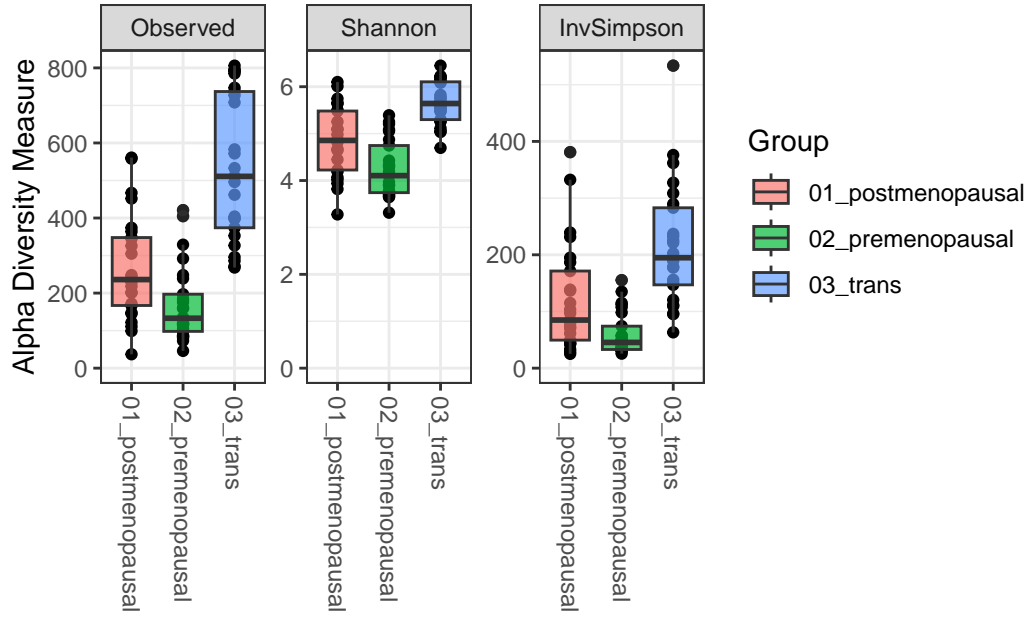
In this dataset, alpha diversity is markedly different between the group of interest (i.e. *03_trans*) and the two control groups. However, the intervention group is more similar to the first control group (i.e. *postmenopausal women*).

```
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 14 sample variables ]
tax_table() Taxonomy Table: [ 567 taxa by 7 taxonomic ranks ]
phy_tree() Phylogenetic Tree: [ 567 tips and 566 internal nodes ]
refseq() DNASTringSet: [ 567 reference sequences ]
```

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

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

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

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

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

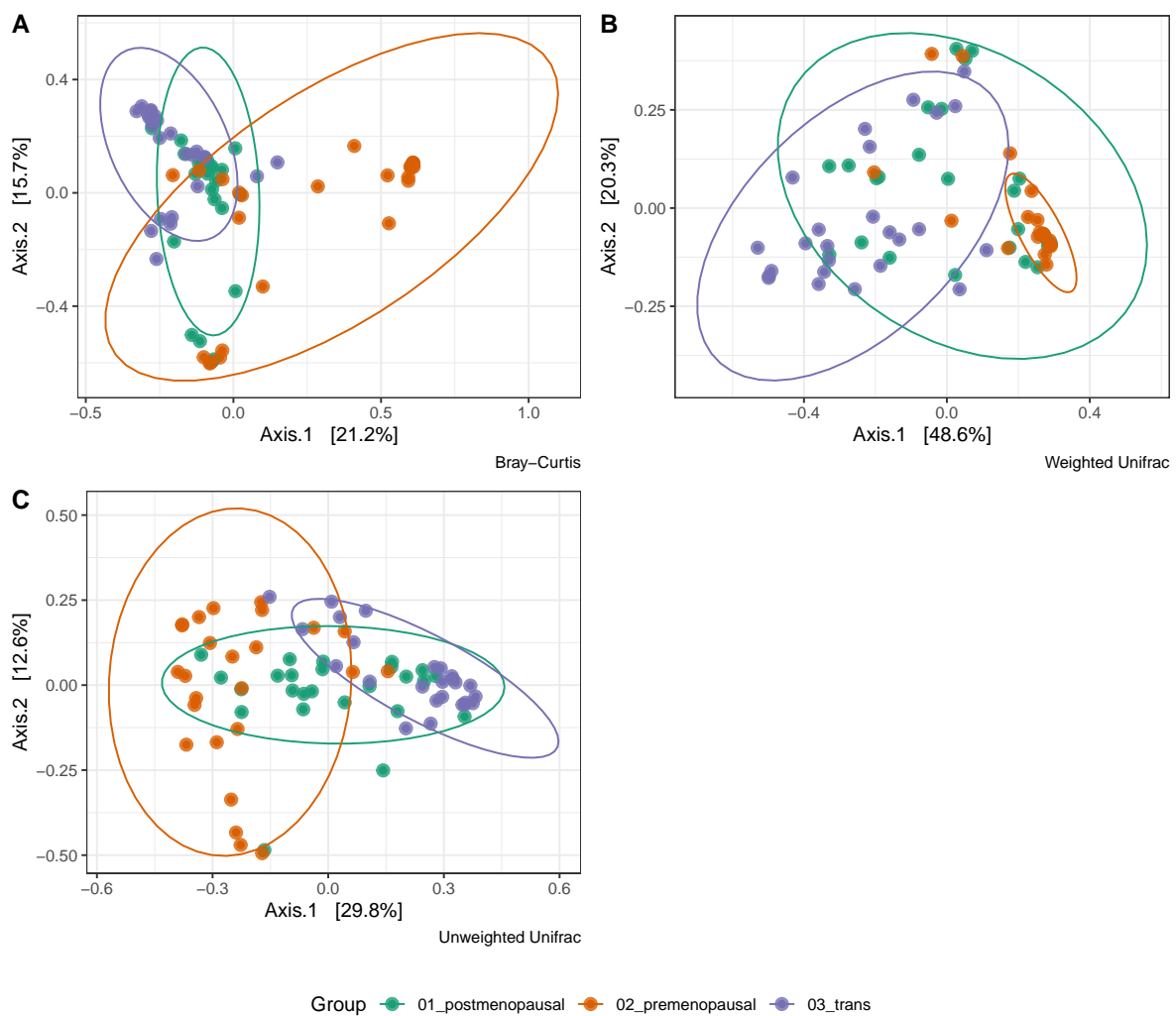
```
# extract the legend from one of the plots
legend_b <- get_legend(
  p1 +
  guides(color = guide_legend(nrow = 1)) +
  theme(legend.position = "bottom")
```


)

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

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

```
cowplot::plot_grid(plot_ordinations, legend_b, ncol = 1, rel_heights = c(1, .1))
```



```

D_BC <- phyloseq::distance(ps_filtered_rel, "bray")
D_UF <- phyloseq::distance(ps_filtered_rel, "unifrac")
D_wUF <- phyloseq::distance(ps_filtered_rel, "wunifrac")

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

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

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

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

```

Table 6: Bray-Curtis

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

```

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

```

Table 7: Unweigthed Unifrac

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

```

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

```

Table 8: Weighted Unifrac

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

4 Differential abundance

```
library(DESeq2)

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

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

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

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

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

ASV	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV174	3574.694	1.528	3.072	0.002	0.008	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV267	7164.210	1.454	2.895	0.004	0.013	0.013	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV367	1723.754	1.422	2.639	0.008	0.023	0.023	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV464	6094.388	1.475	2.975	0.003	0.010	0.010	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus

ASV	baseMean	log2FoldChange	negLog10Pval	negLog10AdjPval	Kingdom	Phylum	Class	Order	Family	Genus		
ASV55	8.9674	1.5023	0.0550	0.0020	0.009	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV62	1.674	1.4483	0.0690	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV75	8.4584	1.4342	0.8830	0.0040	0.013	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV85	8.5634	1.5003	0.1120	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV95	6.4434	1.4853	0.1160	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV156	6.4324	1.5012	0.8500	0.0040	0.014	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV156	1.1334	1.4302	0.8100	0.0050	0.016	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV156	2.2984	1.5102	0.9580	0.0030	0.011	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV153	1.694	1.4413	0.2890	0.0010	0.005	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV151	1.2413	1.4512	0.6600	0.0080	0.022	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV147	1.3954	1.4822	0.8950	0.0040	0.013	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV148	1.6634	1.4403	0.0830	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV147	1.2534	1.4773	0.1480	0.0020	0.007	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV246	1.1774	1.3953	0.0770	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV243	1.1014	1.4553	0.1720	0.0020	0.007	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV241	1.9824	1.5202	0.7860	0.0050	0.017	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV242	1.9054	1.3823	0.2170	0.0010	0.006	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV340	1.8994	1.4113	0.1410	0.0020	0.007	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV339	1.2904	1.4952	0.8320	0.0050	0.015	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV339	1.1614	1.4613	0.0890	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV337	1.8594	1.4973	0.0120	0.0030	0.009	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV338	1.1474	1.4793	0.1150	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV430	1.3044	1.4752	0.8720	0.0040	0.014	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
ASV538	0.312	-	1.354	-	0.0140	0.034	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV537	0.625	-	1.333	-	0.0160	0.039	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV537	1.608	-	1.336	-	0.0130	0.033	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV534	1.361	-	1.466	-	0.0230	0.050	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV532	1.825	-	1.483	-	0.0060	0.018	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV632	1.056	-	1.415	-	0.0050	0.016	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV632	1.648	-	1.379	-	0.0050	0.017	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV631	1.023	-	1.384	-	0.0120	0.031	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV627	1.851	-	1.404	-	0.0030	0.012	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella

ASV	baseMean	log2FoldChange	negLog10Padj	negLog10P	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 XI	Finegoldia
	3.325		2.285					Tissierellales			
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	negLog10Pvalue	padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV247.258	-	1.441	-	0.0220.049	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Finegoldia
	3.295		2.286					Tissierellales	XI	
ASV250.985	-	1.770	-	0.0110.029	Bacteria	Firmicutes	Negativibacteres	Veillonellales	Veillonellaceae	Dialister
	4.498		2.542					Selenomonadales		
ASV257.602	6.402	2.096	0.0550.0020.009		Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV258.211	-	1.749	-	0.0130.034	Bacteria	Firmicutes	Negativibacteres	Veillonellales	Veillonellaceae	Dialister
	4.327		2.474					Selenomonadales		
ASV261.064	-	2.078	-	0.0090.025	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	5.407		2.602							
ASV262.753	-	1.410	-	0.0000.002	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	5.113		3.626							
ASV263.502	-	1.836	-	0.0000.002	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.663		3.629							
ASV266.957	-	1.387	-	0.0000.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	7.244		5.222							
ASV270.388	-	1.495	-	0.0000.001	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	5.665		3.789							
ASV272.277	-	1.705	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.448		3.781							
ASV274.888	-	2.078	-	0.0110.029	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	5.302		2.551							
ASV275.402	-	1.761	-	0.0000.002	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.603		3.749							
ASV277.702	-	1.666	-	0.0060.017	Bacteria	Firmicutes	Negativibacteres	Veillonellales	Veillonellaceae	Dialister
	4.607		2.765					Selenomonadales		
ASV301.231	-	1.454	-	0.0000.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.591		4.532							
ASV302.778	4.772	1.962	2.4330.0150.036		Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV303.355	-	2.077	-	0.0170.040	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	4.951		2.384							
ASV304.550	-	1.770	-	0.0020.007	Bacteria	Firmicutes	Negativibacteres	Veillonellales	Veillonellaceae	Dialister
	5.598		3.162					Selenomonadales		
ASV305.581	-	1.646	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.538		3.973							
ASV307.649	-	1.439	-	0.0130.033	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Finegoldia
	3.570		2.481					Tissierellales	XI	
ASV308.117	-	1.502	-	0.0110.029	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.819		2.543							
ASV309.167	-	1.821	-	0.0000.003	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.434		3.534							

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

ASV	baseMean	log2FoldChange	negLog10Pval	adjPval	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	Glostridii	Reptostreptococcales	Familia	Fenollaria
	7.522		4.078					Tissierellales	XI	
ASV48228	-	1.778	-	0.0000.000	Bacteria	Firmicutes	Glostridii	Reptostreptococcales	Familia	Fenollaria
	7.325		4.119					Tissierellales	XI	
ASV46438	-	1.714	-	0.0170.039	Bacteria	Firmicutes	Negativicutes	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	Glostridii	Reptostreptococcales	Familia	Fenollaria
	7.384		3.951					Tissierellales	XI	
ASV49397	-	1.545	-	0.0000.000	Bacteria	Firmicutes	Glostridii	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	Glostridii	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	Glostridii	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	FDR	negLog10Pval	padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV46141	-	1.427	-	0.0000.000		Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	7.008		4.910								
ASV48741	4.968	1.998	2.486	0.0130.033		Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV48544	-	1.918	-	0.0050.015		Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Akkermansia
	5.413		2.823								
ASV48254	-	1.821	-	0.0020.009		Bacteria	Firmicutes	Negativibacteriia	Veillonellales	Veillonellaceae	Dialister
	5.572		3.060						Selenomonadales		
ASV48615	-	1.743	-	0.0020.007		Bacteria	Firmicutes	Negativibacteriia	Veillonellales	Veillonellaceae	Dialister
	5.458		3.131						Selenomonadales		
ASV49264	-	1.947	-	0.0000.002		Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	7.143		3.668						Tissierellales	XI	
ASV49702	-	1.548	-	0.0000.000		Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	6.716		4.339						Tissierellales	XI	
ASV50856	-	1.418	-	0.0000.001		Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	5.438		3.835						Tissierellales	XI	
ASV52334	-	2.397	-	0.0170.041		Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Akkermansia
	5.696		2.376								
ASV53965	-	2.186	-	0.0130.032		Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Akkermansia
	5.449		2.493								
ASV56299	-	1.815	-	0.0000.001		Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	6.993		3.852						Tissierellales	XI	
ASV57606	-	1.939	-	0.0000.002		Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	7.154		3.690						Tissierellales	XI	
ASV58261	-	1.881	-	0.0000.002		Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	7.004		3.724						Tissierellales	XI	
ASV52795	-	1.630	-	0.0030.010		Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	4.893		3.002						Tissierellales	XI	
ASV57280	-	1.699	-	0.0000.002		Bacteria	Firmicutes	Negativibacteriia	Veillonellales	Veillonellaceae	Dialister
	6.016		3.542						Selenomonadales		
ASV58111	-	1.511	-	0.0000.001		Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	6.394		4.231						Tissierellales	XI	
ASV58609	-	1.770	-	0.0000.002		Bacteria	Campylobacteriia	Campylobacteriia	Campylobacterales	Campylobacteriaceae	Campylobacter
	6.358		3.592								
ASV55891	-	1.447	-	0.0000.001		Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	6.114		4.225						Tissierellales	XI	
ASV56332	-	1.345	-	0.0000.000		Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	6.632		4.929						Tissierellales	XI	
ASV55997	-	1.708	-	0.0000.002		Bacteria	Firmicutes	Negativibacteriia	Veillonellales	Veillonellaceae	Dialister
	6.177		3.617						Selenomonadales		
ASV56039	-	1.779	-	0.0010.003		Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	6.099		3.428						Tissierellales	XI	

ASV	baseMean	log2FoldChange	negLog10Padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV56422	-	1.739	- 0.0000.002	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	6.426	3.695							
ASV56359	-	1.602	- 0.0000.001	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	6.548	4.088							
ASV56796	-	1.518	- 0.0000.000	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Peptoniphilus
	6.705	4.416					Tissierellales	XI	
ASV56753	-	1.792	- 0.0030.010	Bacteria	Bacteroid	Bacteroid	Bacteroidales	Prevotella	Prevotella
	5.335	2.977							
ASV56531	-	1.795	- 0.0000.001	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Anaerococcus
	6.801	3.790					Tissierellales	XI	
ASV56303	-	1.929	- 0.0010.006	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	6.260	3.245							
ASV56659	-	1.831	- 0.0000.002	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Fenollaria
	6.749	3.687					Tissierellales	XI	
ASV56374	-	2.082	- 0.0100.027	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacter	Alloscardovia
	5.372	2.581							
ASV56581	-	2.021	- 0.0020.007	Bacteria	Bacteroid	Bacteroid	Bacteroidales	Prevotella	Prevotella
	6.373	3.153							
ASV56837	-	1.817	- 0.0000.002	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Fenollaria
	6.718	3.698					Tissierellales	XI	
ASV56713	-	2.022	- 0.0010.004	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Fenollaria
	6.738	3.332					Tissierellales	XI	
ASV56754	-	1.984	- 0.0020.009	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Anaerococcus
	6.003	3.026					Tissierellales	XI	
ASV60373	-	1.497	- 0.0000.001	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Peptoniphilus
	6.334	4.231					Tissierellales	XI	
ASV60265	-	1.833	- 0.0010.004	Bacteria	Bacteroid	Bacteroid	Bacteroidales	Prevotella	Prevotella
	6.142	3.350							
ASV61423	-	2.005	- 0.0020.007	Bacteria	Bacteroid	Bacteroid	Bacteroidales	Prevotella	Prevotella
	6.314	3.150							
ASV61674	-	1.962	- 0.0010.004	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Anaerococcus
	6.636	3.382					Tissierellales	XI	
ASV61806	-	1.792	- 0.0000.001	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Fenollaria
	6.887	3.843					Tissierellales	XI	
ASV62350	-	1.499	- 0.0000.000	Bacteria	Firmicutes	Clostridi	Peptostreptococ	Famil	Peptoniphilus
	6.543	4.365					Tissierellales	XI	
ASV62583	-	2.027	- 0.0020.008	Bacteria	Bacteroid	Bacteroid	Bacteroidales	Prevotella	Prevotella
	6.315	3.116							
ASV63387	-	2.019	- 0.0020.007	Bacteria	Bacteroid	Bacteroid	Bacteroidales	Prevotella	Prevotella
	6.332	3.136							

ASV base	log2 Fold	FC	Signif	pval	padj	Kingdm	Phylum	Class	Order	Family	Genus
ASV63766	-	2.645	-	0.017	0.040	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces
	6.323		2.391								
ASV63794	-	1.947	-	0.001	0.003	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	6.717		3.450						Tissierellales	XI	
ASV63866	-	1.646	-	0.000	0.002	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
	6.007		3.649						Selenomonadales		
ASV65184	-	1.900	-	0.001	0.003	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Anaerococcus
	6.585		3.466						Tissierellales	XI	
ASV65130	-	1.880	-	0.000	0.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	6.689		3.558						Tissierellales	XI	
ASV65746	-	1.530	-	0.000	0.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	5.438		3.554						Tissierellales	XI	
ASV65900	-	1.948	-	0.001	0.004	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Anaerococcus
	6.577		3.376						Tissierellales	XI	
ASV65924	-	1.908	-	0.000	0.003	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	6.721		3.523						Tissierellales	XI	
ASV67364	-	2.197	-	0.007	0.020	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces
	5.939		2.702								
ASV67509	-	1.601	-	0.001	0.003	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	5.480		3.422						Tissierellales	XI	
ASV68046	-	1.937	-	0.001	0.005	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Anaerococcus
	6.415		3.312						Tissierellales	XI	
ASV68334	-	1.494	-	0.000	0.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	6.197		4.147						Tissierellales	XI	
ASV68747	-	2.281	-	0.006	0.017	Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Howarthella
	6.315		2.768								
ASV68792	-	2.283	-	0.014	0.034	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Anaerococcus
	5.607		2.457						Tissierellales	XI	
ASV69841	-	1.929	-	0.001	0.005	Bacteria	Bacteroidetes	Bacteroidetes	Bacteroidales	Prevotellaceae	Prevotella
	6.274		3.253								
ASV69959	-	1.873	-	0.000	0.002	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	6.653		3.552						Tissierellales	XI	
ASV69963	-	1.513	-	0.000	0.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Peptoniphilus
	5.828		3.852						Tissierellales	XI	
ASV70250	-	1.741	-	0.001	0.006	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
	5.609		3.221						Selenomonadales		
ASV70736	-	1.976	-	0.002	0.008	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	6.176		3.125								
ASV70884	-	1.766	-	0.000	0.001	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Familia	Fenollaria
	6.665		3.774						Tissierellales	XI	

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

ASV	baseMean	log2FoldChange	negLog10Padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV70337	-	2.053	- 0.0030.010	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	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	Veillonella	Dialister
	6.110	3.675					Selenomonadales		
ASV84402	-	2.285	- 0.0080.022	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Ezakiella
	6.101	2.670					Tissierellales	XI	
ASV84365	-	1.794	- 0.0010.003	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	6.153	3.430							
ASV80564	-	2.401	- 0.0160.039	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Anaerococcus
	5.775	2.405					Tissierellales	XI	
ASV80930	-	2.044	- 0.0030.010	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Anaerococcus
	6.075	2.973					Tissierellales	XI	
ASV84568	-	1.684	- 0.0010.004	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Peptoniphilus
	5.638	3.348					Tissierellales	XI	
ASV82017	-	1.919	- 0.0010.005	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Fenollaria
	6.309	3.288					Tissierellales	XI	
ASV82351	-	2.276	- 0.0090.025	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Parvimonas
	5.944	2.612					Tissierellales	XI	
ASV82420	-	1.975	- 0.0020.008	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.175	3.127							
ASV82473	-	1.662	- 0.0000.002	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonella	Dialister
	6.128	3.686					Selenomonadales		
ASV84485	-	1.742	- 0.0010.005	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Peptoniphilus
	5.719	3.283					Tissierellales	XI	
ASV83900	-	2.198	- 0.0060.017	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Anaerococcus
	6.097	2.775					Tissierellales	XI	
ASV84592	-	1.689	- 0.0010.003	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonella	Dialister
	5.777	3.420					Selenomonadales		
ASV832710	-	1.589	- 0.0010.004	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Peptoniphilus
	5.344	3.363					Tissierellales	XI	
ASV832406	-	2.265	- 0.0100.026	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Anaerococcus
	5.865	2.589					Tissierellales	XI	
ASV83288	-	1.734	- 0.0000.001	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.692	3.860							
ASV84585	-	2.000	- 0.0040.014	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	5.745	2.873							
ASV832710	-	2.056	- 0.0040.013	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faeco	Anaerococcus
	5.918	2.879					Tissierellales	XI	

ASV	baseMean	log2FoldChange	negLog10Pval	negLog10Padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV83970	-	2.171	-	0.0080.022	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Faecalibacteriaceae	Anaerococcus
	5.789		2.667					Tissierellales	XI	
ASV84604	-	2.177	-	0.0060.018	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Faecalibacteriaceae	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	Faecalibacteriaceae	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	Faecalibacteriaceae	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	Faecalibacteriaceae	Anaerococcus
	5.817		2.811					Tissierellales	XI	
ASV97514	-	2.298	-	0.0200.045	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Faecalibacteriaceae	Parvimonas
	5.353		2.329					Tissierellales	XI	
ASV98347	-	2.364	-	0.0140.034	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Faecalibacteriaceae	Anaerococcus
	5.816		2.461					Tissierellales	XI	
ASV10229	-	2.287	-	0.0120.031	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Faecalibacteriaceae	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	Faecalibacteriaceae	Parvimonas
	5.942		2.509					Tissierellales	XI	
ASV10361	-	2.037	-	0.0030.012	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Faecalibacteriaceae	Ezakiella
	5.963		2.927					Tissierellales	XI	
ASV12280	-	2.097	-	0.0050.016	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Faecalibacteriaceae	Anaerococcus
	5.882		2.805					Tissierellales	XI	
ASV12294	-	2.306	-	0.0190.044	Bacteria	Firmicutes	Clostridia	Peptostreptococcales	Faecalibacteriaceae	Anaerococcus
	5.388		2.337					Tissierellales	XI	

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

```

rownames_to_column("ASV")

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

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

```

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

ASV baseMean	log2FoldChange	negLog10Padj	Kingdom	Phylum	Class	Order	Family	Genus	Species
ASV26.234	- 4.408	1.350 - 3.264	0.0010.035	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV22.871	- 5.000	1.395 - 3.584	0.0000.026	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV22.716	- 4.845	1.446 - 3.350	0.0010.034	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV110.793	- 5.282	1.432 - 3.689	0.0000.026	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
ASV25.811	- 6.944	1.776 - 3.910	0.0000.026	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
ASV30.450	- 6.873	1.785 - 3.851	0.0000.026	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
ASV32.022	- 5.740	1.754 - 3.272	0.0010.035	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
ASV37.138	- 6.614	1.771 - 3.734	0.0000.026	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
ASV39.045	- 6.536	1.858 - 3.517	0.0000.027	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
ASV43.012	- 6.279	1.856 - 3.383	0.0010.034	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
ASV43.838	- 6.417	1.739 - 3.691	0.0000.026	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
ASV45.659	- 6.421	1.784 - 3.599	0.0000.026	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
ASV45.241	- 6.084	1.843 - 3.301	0.0010.034	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
ASV45.854	- 6.153	1.831 - 3.361	0.0010.034	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
ASV45.015	- 6.246	1.754 - 3.560	0.0000.026	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister

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

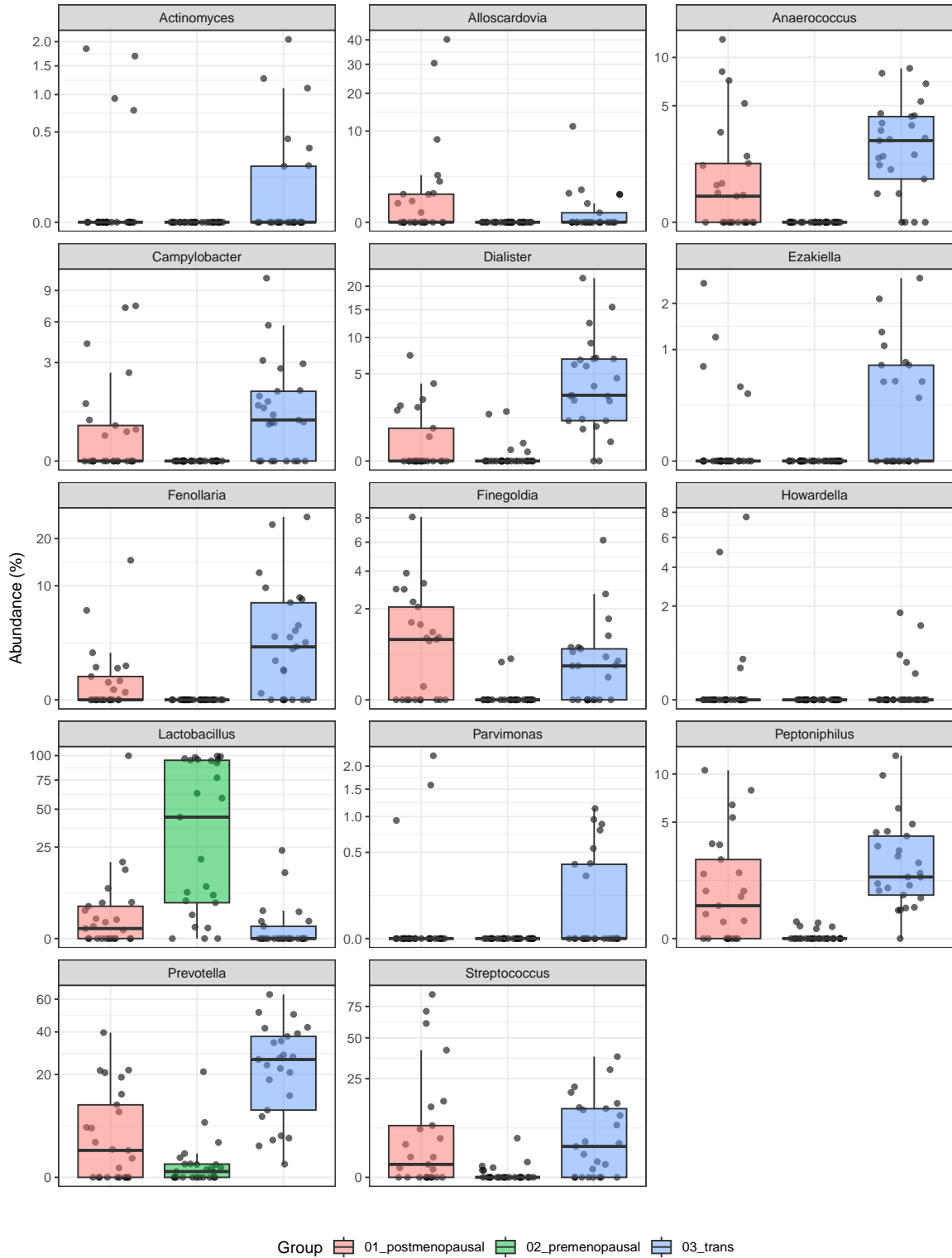
This analysis reveals, that the *Trans* group is characterized by a loss of *Lactobacillus* and an

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 14 sample variables ]
tax_table() Taxonomy Table: [ 383 taxa by 7 taxonomic ranks ]
phy_tree() Phylogenetic Tree: [ 383 tips and 382 internal nodes ]
refseq() DNASTringSet: [ 383 reference sequences ]
```

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

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

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

phyloseq-class experiment-level object

```
otu_table() OTU Table: [ 151 taxa and 75 samples ]
sample_data() Sample Data: [ 75 samples by 14 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 14 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 14 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 14 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") +
```

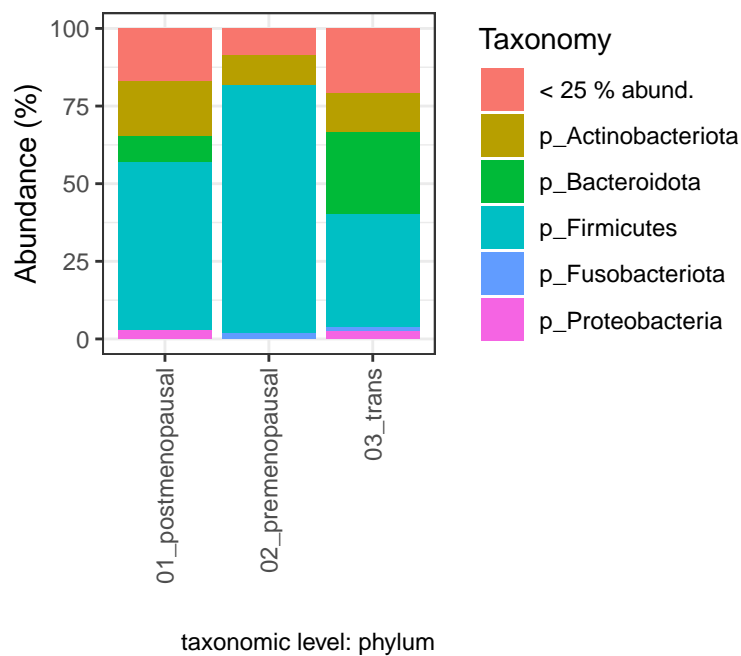


```
theme(legend.position = "right") +
labs(y = "Abundance (%)", x = "", caption = "taxonomic level: phylum") +
rotate_x_text()
```

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.

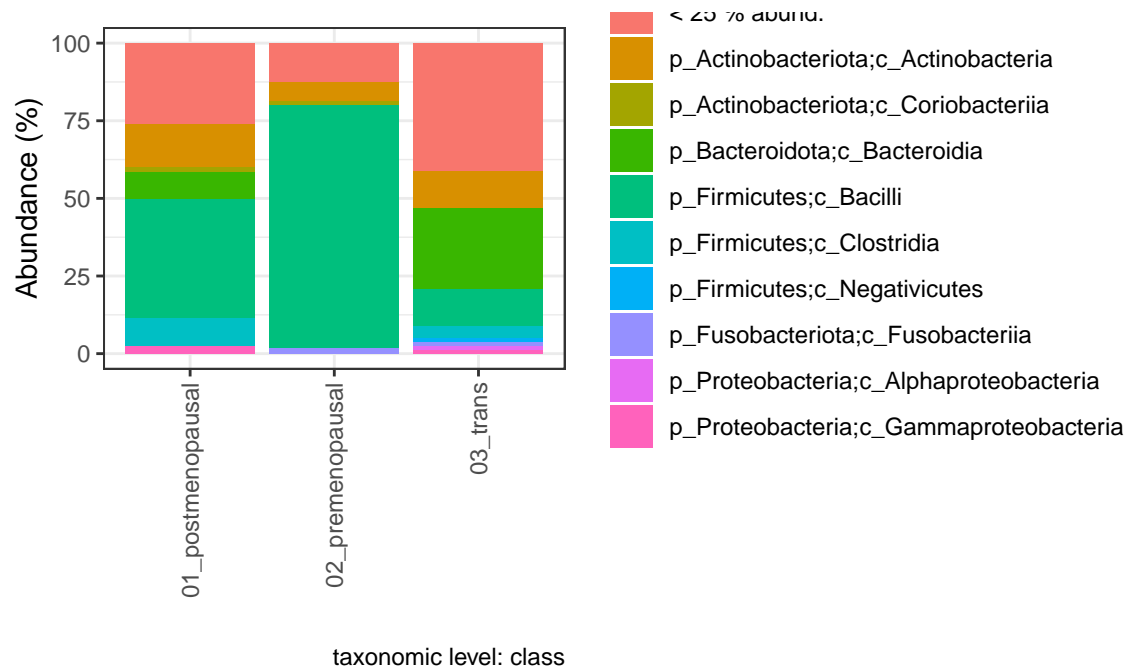
```
phylumplot
```



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

5.2 Class level

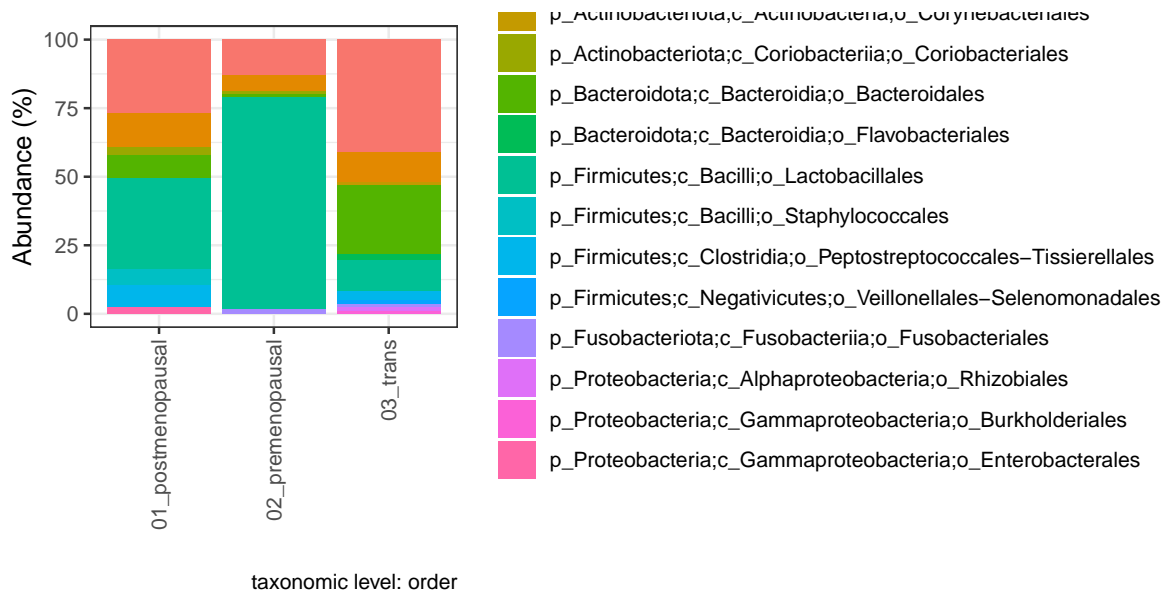
```
classplot
```



```
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

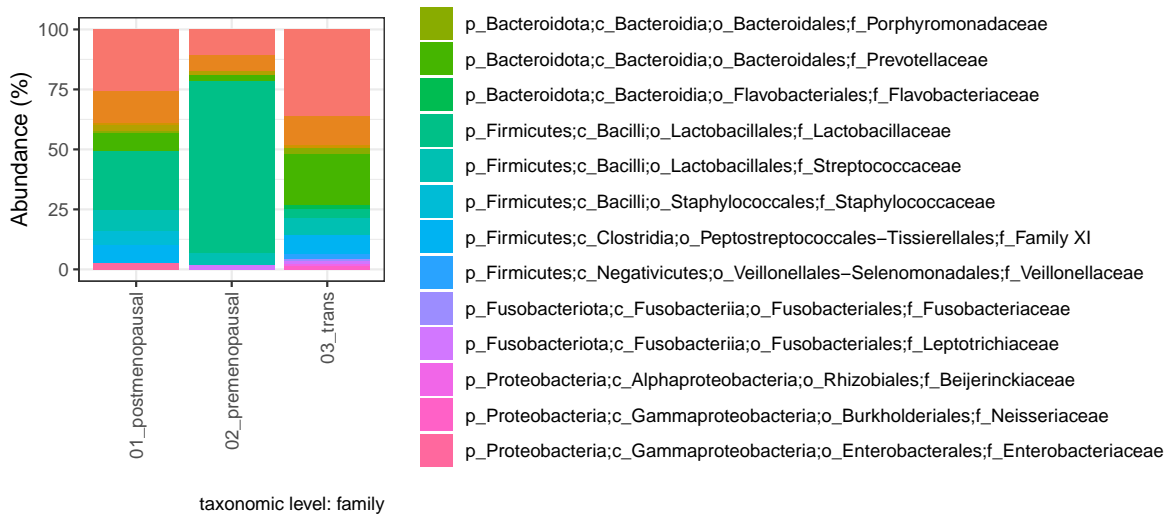
```
orderplot
```



```
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

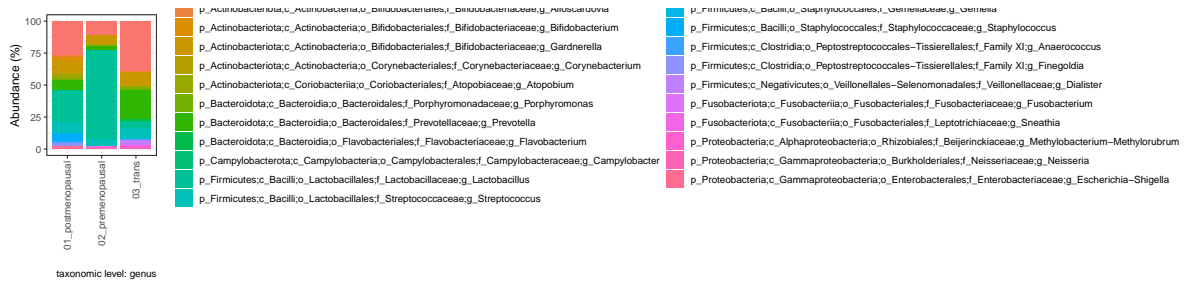
familyplot



```
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
	NugentScore	Testosterone	Estradiole	DurationMenopause			
22010-0001	7-10	NA	NA		11		
22010-0002	0-3	NA	NA		2		

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

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

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

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

```
2023-11-07 18:59:39.727922 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/asv_menopause-duration/maaslin2.1
2023-11-07 18:59:44.503551 INFO::Writing function arguments to log file
2023-11-07 18:59:44.508444 INFO::Verifying options selected are valid
2023-11-07 18:59:44.528465 INFO::Determining format of input files
2023-11-07 18:59:44.528856 INFO::Input format is data samples as rows and metadata samples as
2023-11-07 18:59:44.571932 INFO::Formula for fixed effects: expr ~ DurationMenopause
2023-11-07 18:59:44.572398 INFO::Filter data based on min abundance and min prevalence
2023-11-07 18:59:44.572631 INFO::Total samples in data: 25
2023-11-07 18:59:44.572851 INFO::Min samples required with min abundance for a feature not t
2023-11-07 18:59:44.607761 INFO::Total filtered features: 8831
2023-11-07 18:59:44.609479 INFO::Filtered feature names from abundance and prevalence filter
2023-11-07 18:59:44.611487 INFO::Total filtered features with variance filtering: 0
2023-11-07 18:59:44.611725 INFO::Filtered feature names from variance filtering:
2023-11-07 18:59:44.611939 INFO::Running selected normalization method: TSS
```

```

2023-11-07 18:59:44.612833 INFO::Applying z-score to standardize continuous metadata
2023-11-07 18:59:44.61573 INFO::Running selected transform method: LOG
2023-11-07 18:59:44.616143 INFO::Running selected analysis method: LM
2023-11-07 18:59:44.619776 INFO::Fitting model to feature number 1, ASV14
2023-11-07 18:59:44.621562 INFO::Fitting model to feature number 2, ASV16
2023-11-07 18:59:44.623968 INFO::Counting total values for each feature
2023-11-07 18:59:44.624522 INFO::Writing filtered data to file results/maaslin2/asv_menopause-
2023-11-07 18:59:44.625761 INFO::Writing filtered, normalized data to file results/maaslin2/
2023-11-07 18:59:44.626237 INFO::Writing filtered, normalized, transformed data to file resu
2023-11-07 18:59:44.626712 WARNING::Deleting existing residuals file: results/maaslin2/asv_m
2023-11-07 18:59:44.627114 INFO::Writing residuals to file results/maaslin2/asv_menopause-dur
2023-11-07 18:59:44.627703 WARNING::Deleting existing fitted file: results/maaslin2/asv_menop
2023-11-07 18:59:44.628037 INFO::Writing fitted values to file results/maaslin2/asv_menopause
2023-11-07 18:59:44.629236 INFO::Writing all results to file (ordered by increasing q-values)
2023-11-07 18:59:44.629764 INFO::Writing the significant results (those which are less than c
2023-11-07 18:59:44.630113 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2023-11-07 18:59:44.630929 INFO::Writing association plots (one for each significant associat
[1] "There are no associations to plot!"

```

```

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

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

```

```

2023-11-07 18:59:50.150783 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_menopause-duration/maaslin2
2023-11-07 18:59:50.319922 INFO::Writing function arguments to log file
2023-11-07 18:59:50.321962 INFO::Verifying options selected are valid
2023-11-07 18:59:50.322202 INFO::Determining format of input files
2023-11-07 18:59:50.322418 INFO::Input format is data samples as rows and metadata samples as
2023-11-07 18:59:50.324122 INFO::Formula for fixed effects: expr ~ DurationMenopause
2023-11-07 18:59:50.324379 INFO::Filter data based on min abundance and min prevalence
2023-11-07 18:59:50.324596 INFO::Total samples in data: 25
2023-11-07 18:59:50.324797 INFO::Min samples required with min abundance for a feature not t

```

```

2023-11-07 18:59:50.326213 INFO::Total filtered features: 294
2023-11-07 18:59:50.326509 INFO::Filtered feature names from abundance and prevalence filter:
2023-11-07 18:59:50.326935 INFO::Total filtered features with variance filtering: 0
2023-11-07 18:59:50.32717 INFO::Filtered feature names from variance filtering:
2023-11-07 18:59:50.327386 INFO::Running selected normalization method: TSS
2023-11-07 18:59:50.32774 INFO::Applying z-score to standardize continuous metadata
2023-11-07 18:59:50.330095 INFO::Running selected transform method: LOG
2023-11-07 18:59:50.330478 INFO::Running selected analysis method: LM
2023-11-07 18:59:50.330756 INFO::Fitting model to feature number 1, ASV16
2023-11-07 18:59:50.331768 INFO::Fitting model to feature number 2, ASV82
2023-11-07 18:59:50.332604 INFO::Fitting model to feature number 3, ASV271
2023-11-07 18:59:50.333442 INFO::Fitting model to feature number 4, ASV526
2023-11-07 18:59:50.336073 INFO::Counting total values for each feature
2023-11-07 18:59:50.336681 INFO::Writing filtered data to file results/maaslin2/genus_menopause-
2023-11-07 18:59:50.337682 INFO::Writing filtered, normalized data to file results/maaslin2/g
2023-11-07 18:59:50.33839 INFO::Writing filtered, normalized, transformed data to file resul
2023-11-07 18:59:50.338943 WARNING::Deleting existing residuals file: results/maaslin2/genus
2023-11-07 18:59:50.339366 INFO::Writing residuals to file results/maaslin2/genus_menopause-c
2023-11-07 18:59:50.339771 WARNING::Deleting existing fitted file: results/maaslin2/genus_mer
2023-11-07 18:59:50.340245 INFO::Writing fitted values to file results/maaslin2/genus_menopa
2023-11-07 18:59:50.340604 INFO::Writing all results to file (ordered by increasing q-values)
2023-11-07 18:59:50.34113 INFO::Writing the significant results (those which are less than o
2023-11-07 18:59:50.341464 INFO::Writing heatmap of significant results to file: results/maas
[1] "There are no associations to plot!"
2023-11-07 18:59:50.34191 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")

```

```

2023-11-07 18:59:53.818171 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/family_menopause-duration/maaslin
2023-11-07 18:59:53.879861 INFO::Writing function arguments to log file

```

```

2023-11-07 18:59:53.881921 INFO::Verifying options selected are valid
2023-11-07 18:59:53.882163 INFO::Determining format of input files
2023-11-07 18:59:53.882384 INFO::Input format is data samples as rows and metadata samples as columns
2023-11-07 18:59:53.883437 INFO::Formula for fixed effects: expr ~ DurationMenopause
2023-11-07 18:59:53.883695 INFO::Filter data based on min abundance and min prevalence
2023-11-07 18:59:53.883904 INFO::Total samples in data: 25
2023-11-07 18:59:53.884102 INFO::Min samples required with min abundance for a feature not met
2023-11-07 18:59:53.884807 INFO::Total filtered features: 111
2023-11-07 18:59:53.885039 INFO::Filtered feature names from abundance and prevalence filtering: 111
2023-11-07 18:59:53.885366 INFO::Total filtered features with variance filtering: 0
2023-11-07 18:59:53.885578 INFO::Filtered feature names from variance filtering: 0
2023-11-07 18:59:53.885774 INFO::Running selected normalization method: TSS
2023-11-07 18:59:53.886112 INFO::Applying z-score to standardize continuous metadata
2023-11-07 18:59:53.888342 INFO::Running selected transform method: LOG
2023-11-07 18:59:53.888684 INFO::Running selected analysis method: LM
2023-11-07 18:59:53.888893 INFO::Fitting model to feature number 1, ASV16
2023-11-07 18:59:53.889923 INFO::Fitting model to feature number 2, ASV82
2023-11-07 18:59:53.890774 INFO::Fitting model to feature number 3, ASV120
2023-11-07 18:59:53.891594 INFO::Fitting model to feature number 4, ASV271
2023-11-07 18:59:53.893944 INFO::Counting total values for each feature
2023-11-07 18:59:53.894513 INFO::Writing filtered data to file results/maaslin2/family_menopause/
2023-11-07 18:59:53.895107 INFO::Writing filtered, normalized data to file results/maaslin2/family_menopause/
2023-11-07 18:59:53.895615 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/family_menopause/
2023-11-07 18:59:53.896124 WARNING::Deleting existing residuals file: results/maaslin2/family_menopause/
2023-11-07 18:59:53.896547 INFO::Writing residuals to file results/maaslin2/family_menopause/
2023-11-07 18:59:53.896953 WARNING::Deleting existing fitted file: results/maaslin2/family_menopause/
2023-11-07 18:59:53.897434 INFO::Writing fitted values to file results/maaslin2/family_menopause/
2023-11-07 18:59:53.897784 INFO::Writing all results to file (ordered by increasing q-values)
2023-11-07 18:59:53.898325 INFO::Writing the significant results (those which are less than 0.05)
2023-11-07 18:59:53.898661 INFO::Writing heatmap of significant results to file: results/maaslin2/family_menopause/
[1] "There are no associations to plot!"
2023-11-07 18:59:53.899102 INFO::Writing association plots (one for each significant association)
[1] "There are no associations to plot!"

```

```

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

adiv_menopause = plot_richness(ps_menopause_abs, measures = c("Observed", "Shannon", "InvSimpson"))

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

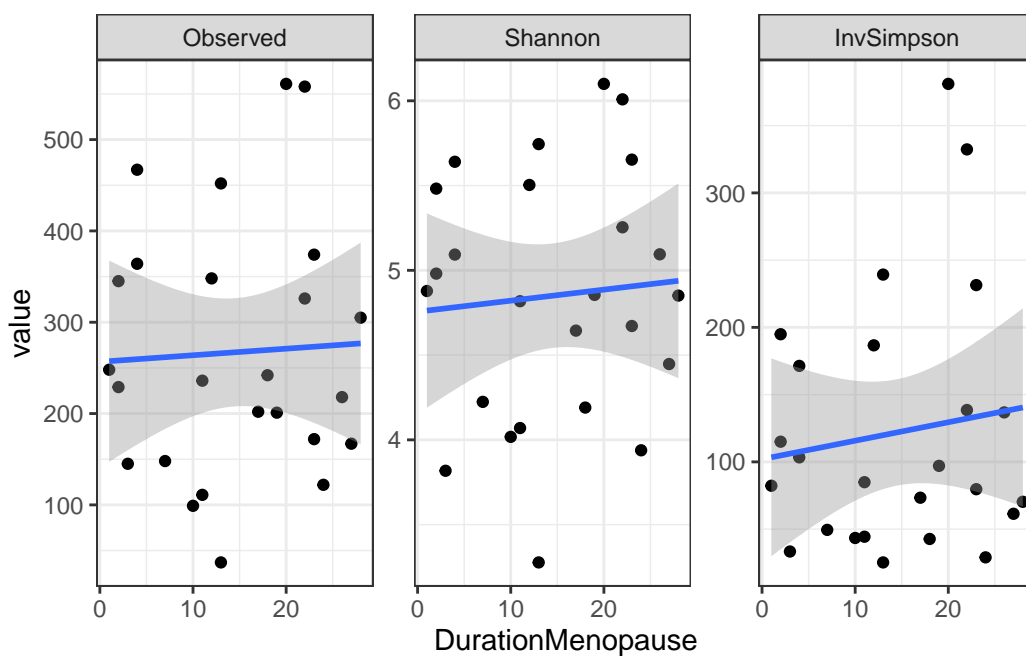
```



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

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

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



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

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

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

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

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

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

6.0.2 Duration of gender-affirming hormone therapy (GAHT)

This analysis is only performed in the TRANS group.

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

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

```
2023-11-07 18:59:54.221031 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/asv_GHAT_Length/maaslin2.log"
2023-11-07 18:59:58.875591 INFO::Writing function arguments to log file
2023-11-07 18:59:58.877706 INFO::Verifying options selected are valid
2023-11-07 18:59:58.877951 INFO::Determining format of input files
2023-11-07 18:59:58.878172 INFO::Input format is data samples as rows and metadata samples as columns
2023-11-07 18:59:58.909596 INFO::Formula for fixed effects: expr ~ GHAT_Length
2023-11-07 18:59:58.910029 INFO::Filter data based on min abundance and min prevalence
2023-11-07 18:59:58.910249 INFO::Total samples in data: 25
2023-11-07 18:59:58.910467 INFO::Min samples required with min abundance for a feature not to be filtered: 3
2023-11-07 18:59:58.943084 INFO::Total filtered features: 8824
```

```

2023-11-07 18:59:58.9445 INFO::Filtered feature names from abundance and prevalence filtering
2023-11-07 18:59:58.946471 INFO::Total filtered features with variance filtering: 0
2023-11-07 18:59:58.946711 INFO::Filtered feature names from variance filtering:
2023-11-07 18:59:58.946923 INFO::Running selected normalization method: TSS
2023-11-07 18:59:58.947309 INFO::Applying z-score to standardize continuous metadata
2023-11-07 18:59:58.949608 INFO::Running selected transform method: LOG
2023-11-07 18:59:58.949986 INFO::Running selected analysis method: LM
2023-11-07 18:59:58.950245 INFO::Fitting model to feature number 1, ASV54
2023-11-07 18:59:58.951257 INFO::Fitting model to feature number 2, ASV55
2023-11-07 18:59:58.952102 INFO::Fitting model to feature number 3, ASV57
2023-11-07 18:59:58.952946 INFO::Fitting model to feature number 4, ASV58
2023-11-07 18:59:58.953767 INFO::Fitting model to feature number 5, ASV59
2023-11-07 18:59:58.95458 INFO::Fitting model to feature number 6, ASV60
2023-11-07 18:59:58.955406 INFO::Fitting model to feature number 7, ASV61
2023-11-07 18:59:58.956221 INFO::Fitting model to feature number 8, ASV62
2023-11-07 18:59:58.957038 INFO::Fitting model to feature number 9, ASV64
2023-11-07 18:59:58.959601 INFO::Counting total values for each feature
2023-11-07 18:59:58.960246 INFO::Writing filtered data to file results/maaslin2/asv_GHAT_Length
2023-11-07 18:59:58.960908 INFO::Writing filtered, normalized data to file results/maaslin2/asv_GHAT_Length
2023-11-07 18:59:58.961478 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/asv_GHAT_Length
2023-11-07 18:59:58.962305 WARNING::Deleting existing residuals file: results/maaslin2/asv_GHAT_Length
2023-11-07 18:59:58.962706 INFO::Writing residuals to file results/maaslin2/asv_GHAT_Length
2023-11-07 18:59:58.963119 WARNING::Deleting existing fitted file: results/maaslin2/asv_GHAT_Length
2023-11-07 18:59:58.963472 INFO::Writing fitted values to file results/maaslin2/asv_GHAT_Length
2023-11-07 18:59:58.963846 INFO::Writing all results to file (ordered by increasing q-values)
2023-11-07 18:59:58.964402 INFO::Writing the significant results (those which are less than 0.05)
2023-11-07 18:59:58.964791 INFO::Writing heatmap of significant results to file: results/maaslin2/asv_GHAT_Length
[1] "There is not enough metadata in the associations to create a heatmap plot. Please review the metadata"
2023-11-07 18:59:58.965319 INFO::Writing association plots (one for each significant association)
2023-11-07 18:59:58.965859 INFO::Plotting associations from most to least significant, grouped by metadata number
2023-11-07 18:59:58.966133 INFO::Plotting data for metadata number 1, GHAT_Length
2023-11-07 18:59:58.96661 INFO::Creating scatter plot for continuous data, GHAT_Length vs ASV54

```

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

```

2023-11-07 18:59:59.046083 INFO::Creating scatter plot for continuous data, GHAT_Length vs ASV55

```

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

```

2023-11-07 18:59:59.110092 INFO::Creating scatter plot for continuous data, GHAT_Length vs ASV57

```

```

2023-11-07 18:59:59.169425 INFO::Creating scatter plot for continuous data, GHAT_Length vs ASV58

```

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

2023-11-07 18:59:59.2342 INFO::Creating scatter plot for continuous data, GHAT_Length vs ASV

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

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

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

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

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

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

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

	Genus	Species
1	Prevotella	<NA>
2	Prevotella	<NA>
3	Prevotella	<NA>
4	Prevotella	<NA>
5	Prevotella	<NA>
6	Prevotella	<NA>
7	Prevotella	<NA>
8	Prevotella	<NA>
9	Prevotella	<NA>

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

```

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

```

```

2023-11-07 19:00:05.722891 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_GHAT_Length/maaslin2.log"
2023-11-07 19:00:05.886705 INFO::Writing function arguments to log file
2023-11-07 19:00:05.88872 INFO::Verifying options selected are valid
2023-11-07 19:00:05.888965 INFO::Determining format of input files
2023-11-07 19:00:05.889175 INFO::Input format is data samples as rows and metadata samples as columns
2023-11-07 19:00:05.890874 INFO::Formula for fixed effects: expr ~ GHAT_Length
2023-11-07 19:00:05.891128 INFO::Filter data based on min abundance and min prevalence
2023-11-07 19:00:05.891333 INFO::Total samples in data: 25
2023-11-07 19:00:05.891543 INFO::Min samples required with min abundance for a feature not to be filtered: 5
2023-11-07 19:00:05.892936 INFO::Total filtered features: 288
2023-11-07 19:00:05.893217 INFO::Filtered feature names from abundance and prevalence filtering:
2023-11-07 19:00:05.893681 INFO::Total filtered features with variance filtering: 0
2023-11-07 19:00:05.893902 INFO::Filtered feature names from variance filtering:
2023-11-07 19:00:05.894103 INFO::Running selected normalization method: TSS
2023-11-07 19:00:05.894488 INFO::Applying z-score to standardize continuous metadata
2023-11-07 19:00:05.897284 INFO::Running selected transform method: LOG
2023-11-07 19:00:05.89771 INFO::Running selected analysis method: LM
2023-11-07 19:00:05.897979 INFO::Fitting model to feature number 1, ASV14
2023-11-07 19:00:05.898973 INFO::Fitting model to feature number 2, ASV55
2023-11-07 19:00:05.899811 INFO::Fitting model to feature number 3, ASV66
2023-11-07 19:00:05.900622 INFO::Fitting model to feature number 4, ASV87
2023-11-07 19:00:05.901421 INFO::Fitting model to feature number 5, ASV120
2023-11-07 19:00:05.902225 INFO::Fitting model to feature number 6, ASV205
2023-11-07 19:00:05.90303 INFO::Fitting model to feature number 7, ASV241
2023-11-07 19:00:05.90383 INFO::Fitting model to feature number 8, ASV443
2023-11-07 19:00:05.904625 INFO::Fitting model to feature number 9, ASV568
2023-11-07 19:00:05.905404 INFO::Fitting model to feature number 10, ASV940
2023-11-07 19:00:05.907997 INFO::Counting total values for each feature
2023-11-07 19:00:05.908675 INFO::Writing filtered data to file results/maaslin2/genus_GHAT_Length/maaslin2_filtered_data.csv
2023-11-07 19:00:05.909417 INFO::Writing filtered, normalized data to file results/maaslin2/genus_GHAT_Length/maaslin2_normalized_data.csv
2023-11-07 19:00:05.910056 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/genus_GHAT_Length/maaslin2_transformed_data.csv
2023-11-07 19:00:05.91068 WARNING::Deleting existing residuals file: results/maaslin2/genus_GHAT_Length/maaslin2_residuals.csv
2023-11-07 19:00:05.911102 INFO::Writing residuals to file results/maaslin2/genus_GHAT_Length/maaslin2_residuals.csv
2023-11-07 19:00:05.911557 WARNING::Deleting existing fitted file: results/maaslin2/genus_GHAT_Length/maaslin2_fitted_values.csv
2023-11-07 19:00:05.911889 INFO::Writing fitted values to file results/maaslin2/genus_GHAT_Length/maaslin2_fitted_values.csv

```

```

2023-11-07 19:00:05.912278 INFO::Writing all results to file (ordered by increasing q-values)
2023-11-07 19:00:05.912813 INFO::Writing the significant results (those which are less than 0.2)
2023-11-07 19:00:05.91317 INFO::Writing heatmap of significant results to file: results/maaslin_res_genus_ghat
[1] "There is not enough metadata in the associations to create a heatmap plot. Please review the metadata"
2023-11-07 19:00:05.913673 INFO::Writing association plots (one for each significant association)
2023-11-07 19:00:05.914197 INFO::Plotting associations from most to least significant, grouped by metadata number
2023-11-07 19:00:05.914464 INFO::Plotting data for metadata number 1, GHAT_Length
2023-11-07 19:00:05.914925 INFO::Creating scatter plot for continuous data, GHAT_Length vs Abundance

```

```

2023-11-07 19:00:05.972192 INFO::Creating scatter plot for continuous data, GHAT_Length vs Abundance

```

```

2023-11-07 19:00:06.031102 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>

```

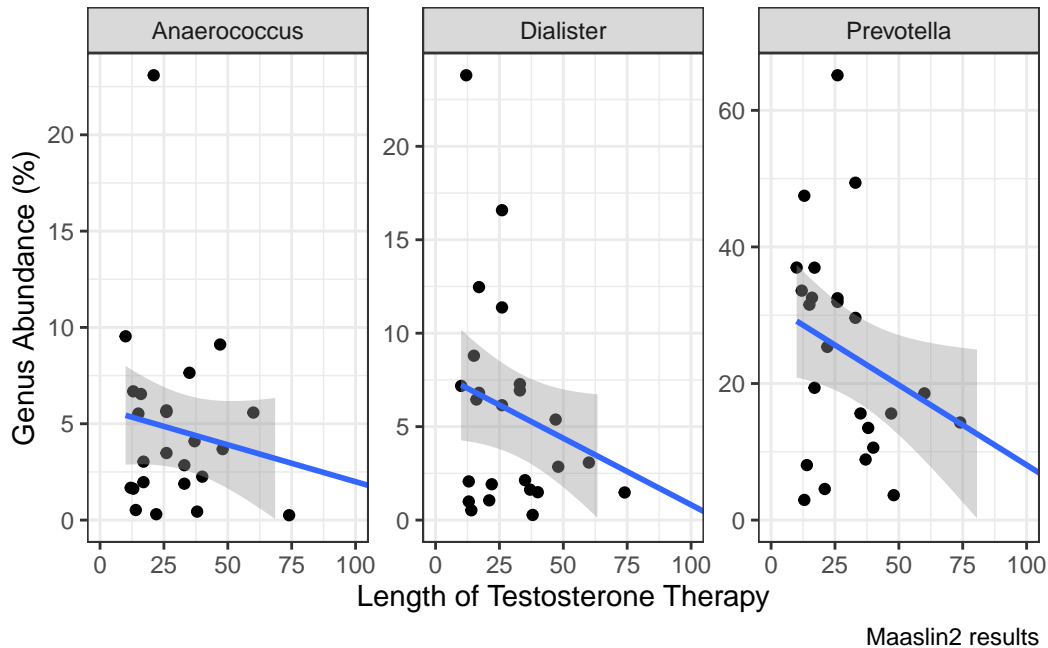
# plot correlation for these 3 ASVs
psmelt_trans_genus = psmelt(ps_trans_genus)
maaslin_res_genus_ghat = ggplot(filter(psmelt_trans_genus, OTU %in% filter(genus_GHAT$results, qval < 0.2)$feature)) +
  aes(x = GHAT_Length, y = Abundance)) +
  geom_point() +
  geom_smooth(method = "lm") +
  facet_wrap(~Genus,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

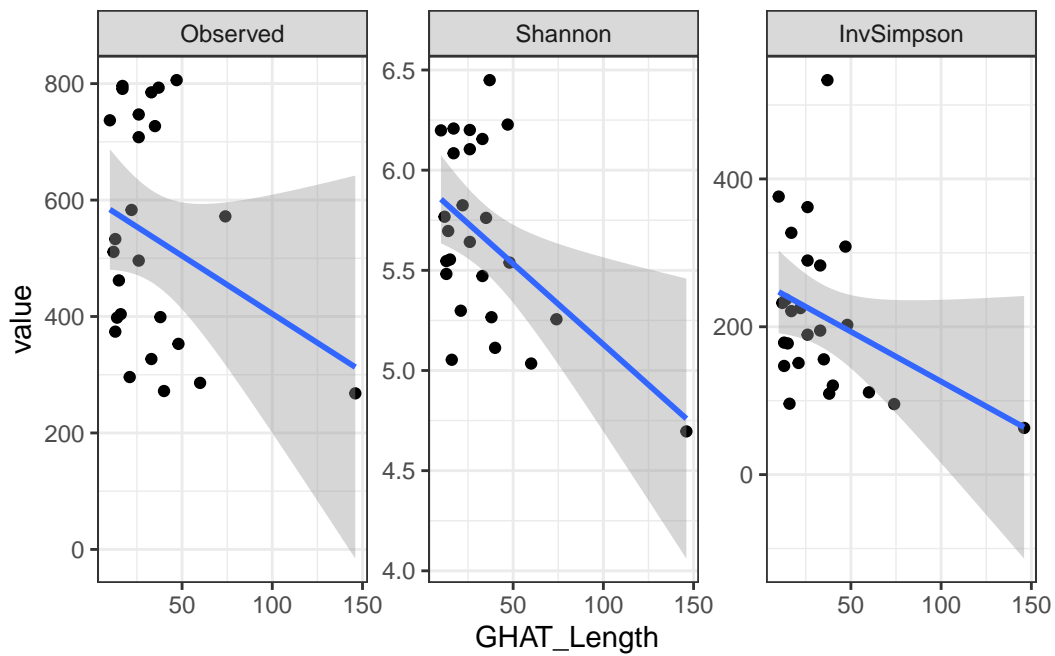
```

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()

```

```

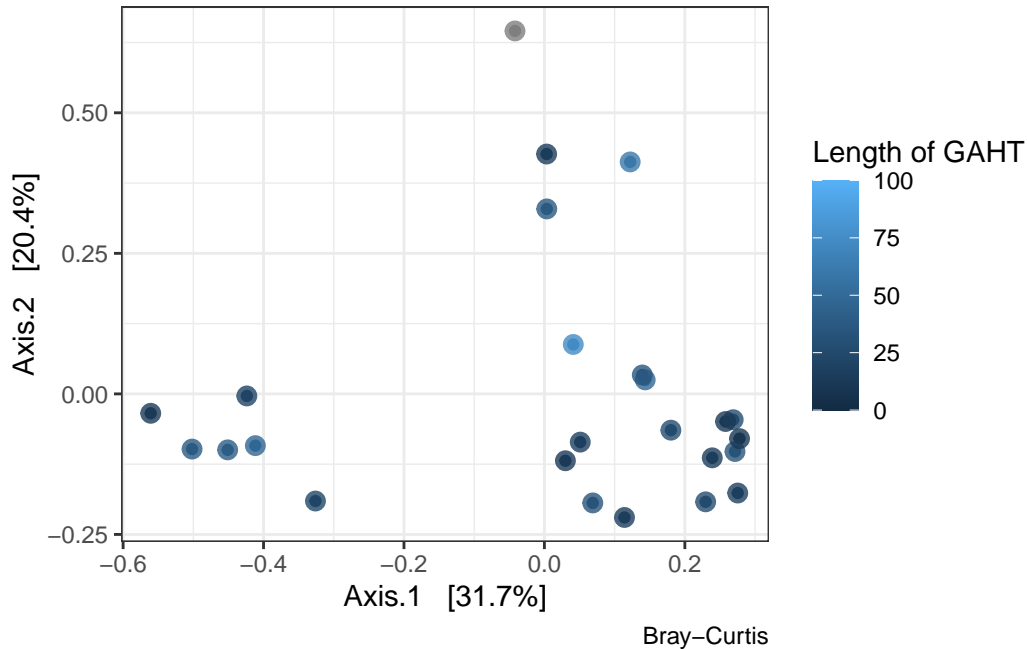
Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
10.00  16.00   26.00   33.56  38.00  146.00

```

```

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
```

```
2023-11-07 19:00:12.510541 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_Testosterone/maaslin2.log"
2023-11-07 19:00:12.668838 INFO::Writing function arguments to log file
2023-11-07 19:00:12.671066 INFO::Verifying options selected are valid
```

```

2023-11-07 19:00:12.671312 INFO::Determining format of input files
2023-11-07 19:00:12.671541 INFO::Input format is data samples as rows and metadata samples as
2023-11-07 19:00:12.673349 INFO::Formula for fixed effects: expr ~ Testosterone
2023-11-07 19:00:12.673617 INFO::Filter data based on min abundance and min prevalence
2023-11-07 19:00:12.673845 INFO::Total samples in data: 25
2023-11-07 19:00:12.674057 INFO::Min samples required with min abundance for a feature not to
2023-11-07 19:00:12.675472 INFO::Total filtered features: 292
2023-11-07 19:00:12.675759 INFO::Filtered feature names from abundance and prevalence filter:
2023-11-07 19:00:12.676205 INFO::Total filtered features with variance filtering: 0
2023-11-07 19:00:12.676433 INFO::Filtered feature names from variance filtering:
2023-11-07 19:00:12.676639 INFO::Running selected normalization method: TSS
2023-11-07 19:00:12.677015 INFO::Applying z-score to standardize continuous metadata
2023-11-07 19:00:12.67939 INFO::Running selected transform method: LOG
2023-11-07 19:00:12.679779 INFO::Running selected analysis method: LM
2023-11-07 19:00:12.680039 INFO::Fitting model to feature number 1, ASV14
2023-11-07 19:00:12.68123 INFO::Fitting model to feature number 2, ASV54
2023-11-07 19:00:12.682158 INFO::Fitting model to feature number 3, ASV66
2023-11-07 19:00:12.683041 INFO::Fitting model to feature number 4, ASV87
2023-11-07 19:00:12.683918 INFO::Fitting model to feature number 5, ASV205
2023-11-07 19:00:12.684827 INFO::Fitting model to feature number 6, ASV568
2023-11-07 19:00:12.687528 INFO::Counting total values for each feature
2023-11-07 19:00:12.688188 INFO::Writing filtered data to file results/maaslin2/genus_Testosterone
2023-11-07 19:00:12.688842 INFO::Writing filtered, normalized data to file results/maaslin2/genus_Testosterone
2023-11-07 19:00:12.689395 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/genus_Testosterone
2023-11-07 19:00:12.689978 WARNING::Deleting existing residuals file: results/maaslin2/genus_Testosterone
2023-11-07 19:00:12.690394 INFO::Writing residuals to file results/maaslin2/genus_Testosterone
2023-11-07 19:00:12.690814 WARNING::Deleting existing fitted file: results/maaslin2/genus_Testosterone
2023-11-07 19:00:12.69119 INFO::Writing fitted values to file results/maaslin2/genus_Testosterone
2023-11-07 19:00:12.691604 INFO::Writing all results to file (ordered by increasing q-values)
2023-11-07 19:00:12.692167 INFO::Writing the significant results (those which are less than 0.05)
2023-11-07 19:00:12.692519 INFO::Writing heatmap of significant results to file: results/maaslin2/genus_Testosterone
[1] "There are no associations to plot!"
2023-11-07 19:00:12.692974 INFO::Writing association plots (one for each significant association)
[1] "There are no associations to plot!"

```

No significant associations.

6.0.4 Duration of GnRH therapy in TRANS group

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

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

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

```
2023-11-07 19:00:12.699004 WARNING::Input is a matrix, passing through as.data.frame() .  
[1] "Warning: Deleting existing log file: results/maaslin2/genus_DurationGNRH/maaslin2.log"  
2023-11-07 19:00:12.863468 INFO::Writing function arguments to log file  
2023-11-07 19:00:12.866194 INFO::Verifying options selected are valid  
2023-11-07 19:00:12.866524 INFO::Determining format of input files  
2023-11-07 19:00:12.866808 INFO::Input format is data samples as rows and metadata samples as columns  
2023-11-07 19:00:12.868821 INFO::Formula for fixed effects: expr ~ Duration_GNRH  
2023-11-07 19:00:12.869234 INFO::Filter data based on min abundance and min prevalence  
2023-11-07 19:00:12.869491 INFO::Total samples in data: 25  
2023-11-07 19:00:12.86972 INFO::Min samples required with min abundance for a feature not to be filtered: 5  
2023-11-07 19:00:12.871562 INFO::Total filtered features: 292  
2023-11-07 19:00:12.872112 INFO::Filtered feature names from abundance and prevalence filtering: 292  
2023-11-07 19:00:12.872677 INFO::Total filtered features with variance filtering: 0  
2023-11-07 19:00:12.872943 INFO::Filtered feature names from variance filtering:  
2023-11-07 19:00:12.873169 INFO::Running selected normalization method: TSS  
2023-11-07 19:00:12.873581 INFO::Applying z-score to standardize continuous metadata  
2023-11-07 19:00:12.876244 INFO::Running selected transform method: LOG  
2023-11-07 19:00:12.876713 INFO::Running selected analysis method: LM  
2023-11-07 19:00:12.876991 INFO::Fitting model to feature number 1, ASV14  
2023-11-07 19:00:12.878138 INFO::Fitting model to feature number 2, ASV54  
2023-11-07 19:00:12.879174 INFO::Fitting model to feature number 3, ASV66  
2023-11-07 19:00:12.880154 INFO::Fitting model to feature number 4, ASV87  
2023-11-07 19:00:12.881037 INFO::Fitting model to feature number 5, ASV205  
2023-11-07 19:00:12.881906 INFO::Fitting model to feature number 6, ASV568  
2023-11-07 19:00:12.884879 INFO::Counting total values for each feature
```

```

2023-11-07 19:00:12.885568 INFO::Writing filtered data to file results/maaslin2/genus_DurationGNI
2023-11-07 19:00:12.886601 INFO::Writing filtered, normalized data to file results/maaslin2/genus_DurationGNI
2023-11-07 19:00:12.887461 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/genus_DurationGNI
2023-11-07 19:00:12.888367 WARNING::Deleting existing residuals file: results/maaslin2/genus_DurationGNI
2023-11-07 19:00:12.888966 INFO::Writing residuals to file results/maaslin2/genus_DurationGNI
2023-11-07 19:00:12.889615 WARNING::Deleting existing fitted file: results/maaslin2/genus_DurationGNI
2023-11-07 19:00:12.890123 INFO::Writing fitted values to file results/maaslin2/genus_DurationGNI
2023-11-07 19:00:12.890559 INFO::Writing all results to file (ordered by increasing q-values)
2023-11-07 19:00:12.891217 INFO::Writing the significant results (those which are less than 0.05)
2023-11-07 19:00:12.891564 INFO::Writing heatmap of significant results to file: results/maaslin2/genus_DurationGNI
[1] "There are no associations to plot!"
2023-11-07 19:00:12.892025 INFO::Writing association plots (one for each significant association)
[1] "There are no associations to plot!"

```

```
# no significant associations
```

No significant associations.

6.0.5 Cycle dependency in premenopausal group

```

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

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

```

```

2023-11-07 19:00:12.945943 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/asv_cycle_premeno/maaslin2.log"
2023-11-07 19:00:17.618987 INFO::Writing function arguments to log file
2023-11-07 19:00:17.621825 INFO::Verifying options selected are valid
2023-11-07 19:00:17.622107 INFO::Determining format of input files
2023-11-07 19:00:17.622357 INFO::Input format is data samples as rows and metadata samples as columns
2023-11-07 19:00:17.654221 INFO::Formula for fixed effects: expr ~ CycleDaySampling
2023-11-07 19:00:17.65469 INFO::Filter data based on min abundance and min prevalence
2023-11-07 19:00:17.654916 INFO::Total samples in data: 25

```

```

2023-11-07 19:00:17.655124 INFO::Min samples required with min abundance for a feature not t
2023-11-07 19:00:17.693918 INFO::Total filtered features: 8808
2023-11-07 19:00:17.695431 INFO::Filtered feature names from abundance and prevalence filter
2023-11-07 19:00:17.697509 INFO::Total filtered features with variance filtering: 0
2023-11-07 19:00:17.697759 INFO::Filtered feature names from variance filtering:
2023-11-07 19:00:17.697976 INFO::Running selected normalization method: TSS
2023-11-07 19:00:17.698499 INFO::Applying z-score to standardize continuous metadata
2023-11-07 19:00:17.700903 INFO::Running selected transform method: LOG
2023-11-07 19:00:17.701371 INFO::Running selected analysis method: LM
2023-11-07 19:00:17.701638 INFO::Fitting model to feature number 1, ASV1
2023-11-07 19:00:17.702686 INFO::Fitting model to feature number 2, ASV2
2023-11-07 19:00:17.703536 INFO::Fitting model to feature number 3, ASV3
2023-11-07 19:00:17.70436 INFO::Fitting model to feature number 4, ASV4
2023-11-07 19:00:17.705173 INFO::Fitting model to feature number 5, ASV5
2023-11-07 19:00:17.70613 INFO::Fitting model to feature number 6, ASV6
2023-11-07 19:00:17.707045 INFO::Fitting model to feature number 7, ASV7
2023-11-07 19:00:17.707895 INFO::Fitting model to feature number 8, ASV8
2023-11-07 19:00:17.708729 INFO::Fitting model to feature number 9, ASV9
2023-11-07 19:00:17.709576 INFO::Fitting model to feature number 10, ASV10
2023-11-07 19:00:17.710459 INFO::Fitting model to feature number 11, ASV11
2023-11-07 19:00:17.711323 INFO::Fitting model to feature number 12, ASV12
2023-11-07 19:00:17.712134 INFO::Fitting model to feature number 13, ASV13
2023-11-07 19:00:17.712934 INFO::Fitting model to feature number 14, ASV14
2023-11-07 19:00:17.713754 INFO::Fitting model to feature number 15, ASV15
2023-11-07 19:00:17.714555 INFO::Fitting model to feature number 16, ASV16
2023-11-07 19:00:17.715374 INFO::Fitting model to feature number 17, ASV17
2023-11-07 19:00:17.716336 INFO::Fitting model to feature number 18, ASV18
2023-11-07 19:00:17.717307 INFO::Fitting model to feature number 19, ASV19
2023-11-07 19:00:17.718181 INFO::Fitting model to feature number 20, ASV20
2023-11-07 19:00:17.719071 INFO::Fitting model to feature number 21, ASV21
2023-11-07 19:00:17.71996 INFO::Fitting model to feature number 22, ASV22
2023-11-07 19:00:17.720771 INFO::Fitting model to feature number 23, ASV23
2023-11-07 19:00:17.721725 INFO::Fitting model to feature number 24, ASV27
2023-11-07 19:00:17.722615 INFO::Fitting model to feature number 25, ASV32
2023-11-07 19:00:17.72586 INFO::Counting total values for each feature
2023-11-07 19:00:17.726742 INFO::Writing filtered data to file results/maaslin2/asv_cycle_pr
2023-11-07 19:00:17.727669 INFO::Writing filtered, normalized data to file results/maaslin2/a
2023-11-07 19:00:17.728476 INFO::Writing filtered, normalized, transformed data to file resu
2023-11-07 19:00:17.729376 WARNING::Deleting existing residuals file: results/maaslin2/asv_cy
2023-11-07 19:00:17.729817 INFO::Writing residuals to file results/maaslin2/asv_cycle_premen
2023-11-07 19:00:17.730352 WARNING::Deleting existing fitted file: results/maaslin2/asv_cycl
2023-11-07 19:00:17.7307 INFO::Writing fitted values to file results/maaslin2/asv_cycle_prem
2023-11-07 19:00:17.731105 INFO::Writing all results to file (ordered by increasing q-values)

```

```

2023-11-07 19:00:17.731742 INFO::Writing the significant results (those which are less than 0.05) to file: results/maaslin2/genus_cycle_premeno/maaslin2.log
2023-11-07 19:00:17.732106 INFO::Writing heatmap of significant results to file: results/maaslin2/genus_cycle_premeno/maaslin2_heatmap.png
[1] "There are no associations to plot!"
2023-11-07 19:00:17.732554 INFO::Writing association plots (one for each significant association) to file: results/maaslin2/genus_cycle_premeno/maaslin2_association_plots/
[1] "There are no associations to plot!"

```

```

ps_pre_genus = tax_glom(ps_pre, "Genus")

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

```

```

2023-11-07 19:00:23.725377 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_cycle_premeno/maaslin2.log"
2023-11-07 19:00:23.89215 INFO::Writing function arguments to log file
2023-11-07 19:00:23.894571 INFO::Verifying options selected are valid
2023-11-07 19:00:23.894818 INFO::Determining format of input files
2023-11-07 19:00:23.895033 INFO::Input format is data samples as rows and metadata samples as columns
2023-11-07 19:00:23.896742 INFO::Formula for fixed effects: expr ~ CycleDaySampling
2023-11-07 19:00:23.897006 INFO::Filter data based on min abundance and min prevalence
2023-11-07 19:00:23.897212 INFO::Total samples in data: 25
2023-11-07 19:00:23.897415 INFO::Min samples required with min abundance for a feature not to be filtered: 3
2023-11-07 19:00:23.898803 INFO::Total filtered features: 296
2023-11-07 19:00:23.899094 INFO::Filtered feature names from abundance and prevalence filtering: 296
2023-11-07 19:00:23.899502 INFO::Total filtered features with variance filtering: 0
2023-11-07 19:00:23.899719 INFO::Filtered feature names from variance filtering: 0
2023-11-07 19:00:23.899926 INFO::Running selected normalization method: TSS
2023-11-07 19:00:23.900281 INFO::Applying z-score to standardize continuous metadata
2023-11-07 19:00:23.902566 INFO::Running selected transform method: LOG
2023-11-07 19:00:23.902911 INFO::Running selected analysis method: LM
2023-11-07 19:00:23.903175 INFO::Fitting model to feature number 1, ASV1
2023-11-07 19:00:23.904166 INFO::Fitting model to feature number 2, ASV66
2023-11-07 19:00:23.906457 INFO::Counting total values for each feature
2023-11-07 19:00:23.907009 INFO::Writing filtered data to file results/maaslin2/genus_cycle_premeno/maaslin2_filtered_data.csv
2023-11-07 19:00:23.908011 INFO::Writing filtered, normalized data to file results/maaslin2/genus_cycle_premeno/maaslin2_normalized_data.csv
2023-11-07 19:00:23.908482 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/genus_cycle_premeno/maaslin2_transformed_data.csv
2023-11-07 19:00:23.90895 WARNING::Deleting existing residuals file: results/maaslin2/genus_cycle_premeno/maaslin2_residuals.csv

```

```

2023-11-07 19:00:23.909601 INFO::Writing residuals to file results/maaslin2/genus_cycle_prema
2023-11-07 19:00:23.911167 WARNING::Deleting existing fitted file: results/maaslin2/genus_cy
2023-11-07 19:00:23.911752 INFO::Writing fitted values to file results/maaslin2/genus_cycle_
2023-11-07 19:00:23.912157 INFO::Writing all results to file (ordered by increasing q-values)
2023-11-07 19:00:23.912852 INFO::Writing the significant results (those which are less than 0
2023-11-07 19:00:23.913315 INFO::Writing heatmap of significant results to file: results/maas
[1] "There is not enough metadata in the associations to create a heatmap plot. Please review
2023-11-07 19:00:23.913872 INFO::Writing association plots (one for each significant associat
2023-11-07 19:00:23.914407 INFO::Plotting associations from most to least significant, group
2023-11-07 19:00:23.914693 INFO::Plotting data for metadata number 1, CycleDaySampling
2023-11-07 19:00:23.915193 INFO::Creating scatter plot for continuous data, CycleDaySampling

2023-11-07 19:00:23.973035 INFO::Creating scatter plot for continuous data, CycleDaySampling

```

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

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

```

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

```

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

```

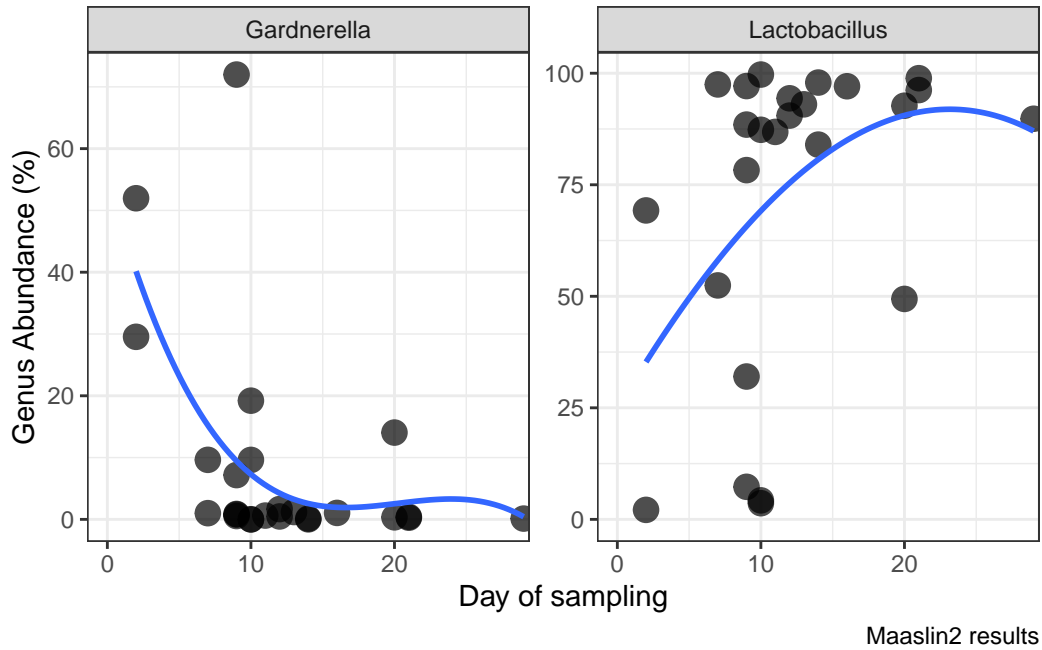
psmelt_pre_genus = psmelt(ps_pre_genus)

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

```



```
maaslin_res_genus_cycleday
```



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

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

6.0.6 Influence of sexual activity on microbiome composition

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

phyloseq-class experiment-level object

```

otu_table()   OTU Table:           [ 298 taxa and 72 samples ]
sample_data() Sample Data:         [ 72 samples by 14 sample variables ]
tax_table()   Taxonomy Table:      [ 298 taxa by 7 taxonomic ranks ]
phy_tree()    Phylogenetic Tree:   [ 298 tips and 297 internal nodes ]
refseq()      DNASTringSet:        [ 298 reference sequences ]

```

```

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

```

```

2023-11-07 19:00:32.490987 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_SexuallyActive/maaslin2.log"
2023-11-07 19:00:32.655869 INFO::Writing function arguments to log file
2023-11-07 19:00:32.65827 INFO::Verifying options selected are valid
2023-11-07 19:00:32.658555 INFO::Determining format of input files
2023-11-07 19:00:32.658791 INFO::Input format is data samples as rows and metadata samples as columns
2023-11-07 19:00:32.660649 INFO::Formula for fixed effects: expr ~ SexuallyActive
2023-11-07 19:00:32.660943 INFO::Factor detected for categorical metadata 'SexuallyActive'. Processing as factor
2023-11-07 19:00:32.661148 INFO::Filter data based on min abundance and min prevalence
2023-11-07 19:00:32.661353 INFO::Total samples in data: 72
2023-11-07 19:00:32.661548 INFO::Min samples required with min abundance for a feature not to be filtered: 5
2023-11-07 19:00:32.66307 INFO::Total filtered features: 294
2023-11-07 19:00:32.663366 INFO::Filtered feature names from abundance and prevalence filtering:
2023-11-07 19:00:32.663762 INFO::Total filtered features with variance filtering: 0
2023-11-07 19:00:32.663989 INFO::Filtered feature names from variance filtering:
2023-11-07 19:00:32.664199 INFO::Running selected normalization method: TSS
2023-11-07 19:00:32.664617 INFO::Applying z-score to standardize continuous metadata
2023-11-07 19:00:32.666995 INFO::Running selected transform method: LOG
2023-11-07 19:00:32.667398 INFO::Running selected analysis method: LM
2023-11-07 19:00:32.667664 INFO::Fitting model to feature number 1, ASV1
2023-11-07 19:00:32.668819 INFO::Fitting model to feature number 2, ASV54
2023-11-07 19:00:32.669806 INFO::Fitting model to feature number 3, ASV66
2023-11-07 19:00:32.67073 INFO::Fitting model to feature number 4, ASV87
2023-11-07 19:00:32.673231 INFO::Counting total values for each feature
2023-11-07 19:00:32.673818 INFO::Writing filtered data to file results/maaslin2/genus_SexuallyActive/maaslin2_filtered_data.csv
2023-11-07 19:00:32.674701 INFO::Writing filtered, normalized data to file results/maaslin2/genus_SexuallyActive/maaslin2_normalized_data.csv
2023-11-07 19:00:32.675359 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/genus_SexuallyActive/maaslin2_transformed_data.csv

```

```

2023-11-07 19:00:32.676029 WARNING::Deleting existing residuals file: results/maaslin2/genus_SexuallyActive
2023-11-07 19:00:32.676428 INFO::Writing residuals to file results/maaslin2/genus_SexuallyActive
2023-11-07 19:00:32.676861 WARNING::Deleting existing fitted file: results/maaslin2/genus_SexuallyActive
2023-11-07 19:00:32.677187 INFO::Writing fitted values to file results/maaslin2/genus_SexuallyActive
2023-11-07 19:00:32.677545 INFO::Writing all results to file (ordered by increasing q-values)
2023-11-07 19:00:32.678075 INFO::Writing the significant results (those which are less than 0.05)
2023-11-07 19:00:32.678452 INFO::Writing heatmap of significant results to file: results/maaslin2/genus_SexuallyActive
[1] "There is not enough metadata in the associations to create a heatmap plot. Please review the metadata"
2023-11-07 19:00:32.678966 INFO::Writing association plots (one for each significant association)
2023-11-07 19:00:32.679511 INFO::Plotting associations from most to least significant, grouped by metadata number
2023-11-07 19:00:32.679777 INFO::Plotting data for metadata number 1, SexuallyActive
2023-11-07 19:00:32.68029 INFO::Creating boxplot for categorical data, SexuallyActive vs ASV54

2023-11-07 19:00:32.733361 INFO::Creating boxplot for categorical data, SexuallyActive vs ASV87

```

```

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

```

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

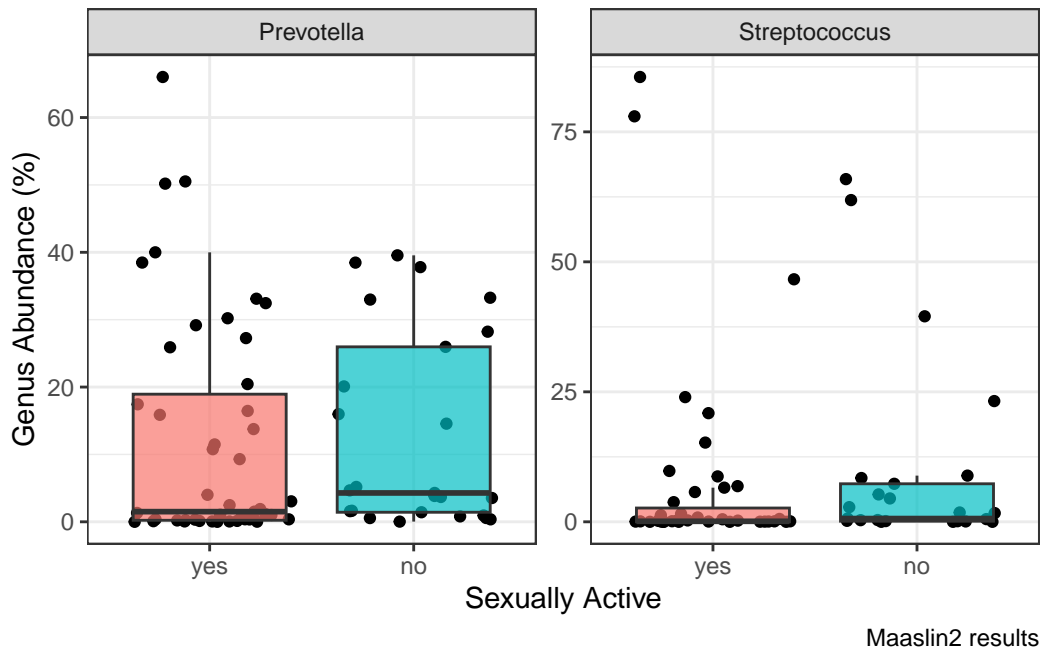
```

# plot sexuallyActive vs these 2 genera
psmelt_rel_genus_sexuallyactive = psmelt(ps_rel_genus_sexuallyactive)
maaslin_res_genus_sexactive = ggplot(filter(psmelt_rel_genus_sexuallyactive, OTU %in% filter(tax_table(ps_rel_genus_sexuallyactive), ASV %in% ASV54 | ASV87))) +
  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 13 rows containing missing values (``geom_point()``).



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

Warning: Removed 14 rows containing missing values (``geom_point()``).

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

6.0.7 Influence of the duration of amenorrhea on microbiome composition

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

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

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

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

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

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

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

```
sample_data(ps_menopause)$DurationMenopauseMonths = sample_data(ps_menopause)$DurationMenopauseMonths
sample_data(ps_menopause)$DurationCombined = ifelse(is.na(sample_data(ps_menopause)$DurationMenopauseMonths),
  sample_data(ps_menopause)$DurationAmenorrhea, sample_data(ps_menopause)$DurationMenopauseMonths)

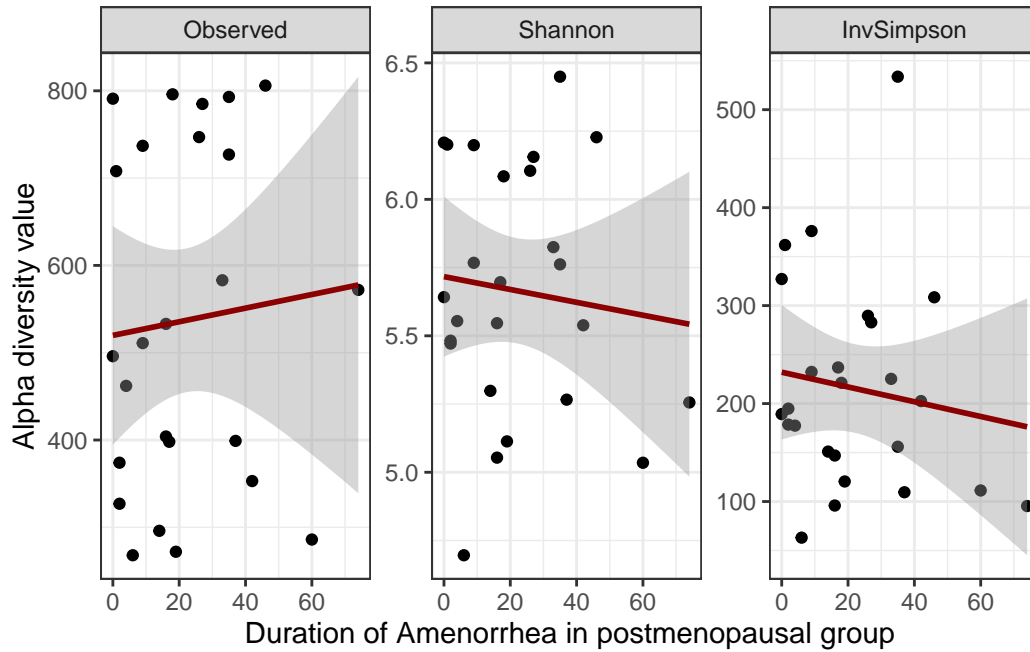
ps_menopause_rel = transform_sample_counts(ps_menopause, function(x) x/sum(x) * 100)
# Diversity analysis
adiv_menopause = plot_richness(ps_menopause, measures = c("Observed", "Shannon", "InvSimpson"))

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

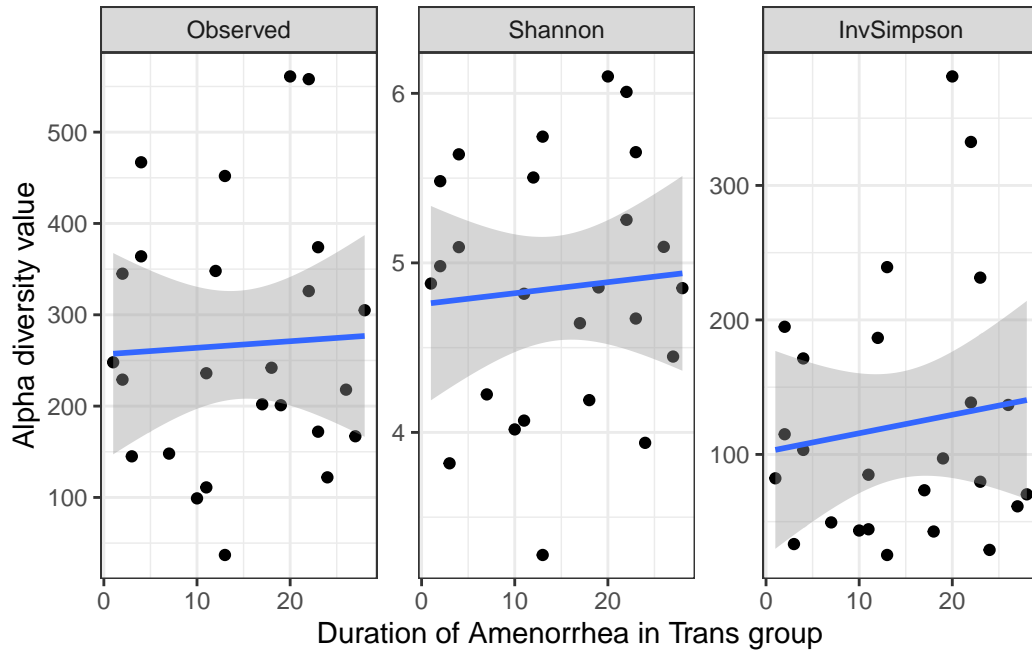
corr_trans = filter(adiv_menopause, grp == "03_trans") %>%
  group_by(variable) %>%
  rstatix::cor_test(value, DurationAmenorrhea)
kable(corr_trans)
```

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

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



```
ggplot(filter(adiv_menopause, grp == "01_postmenopausal"), aes(x = DurationMenopause, y = value)) +
  geom_point() +
  geom_smooth(method = "lm") +
  facet_wrap(~variable, scales = "free") +
  labs(x = "Duration of Amenorrhea in Trans group", y = "Alpha diversity value")
```



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

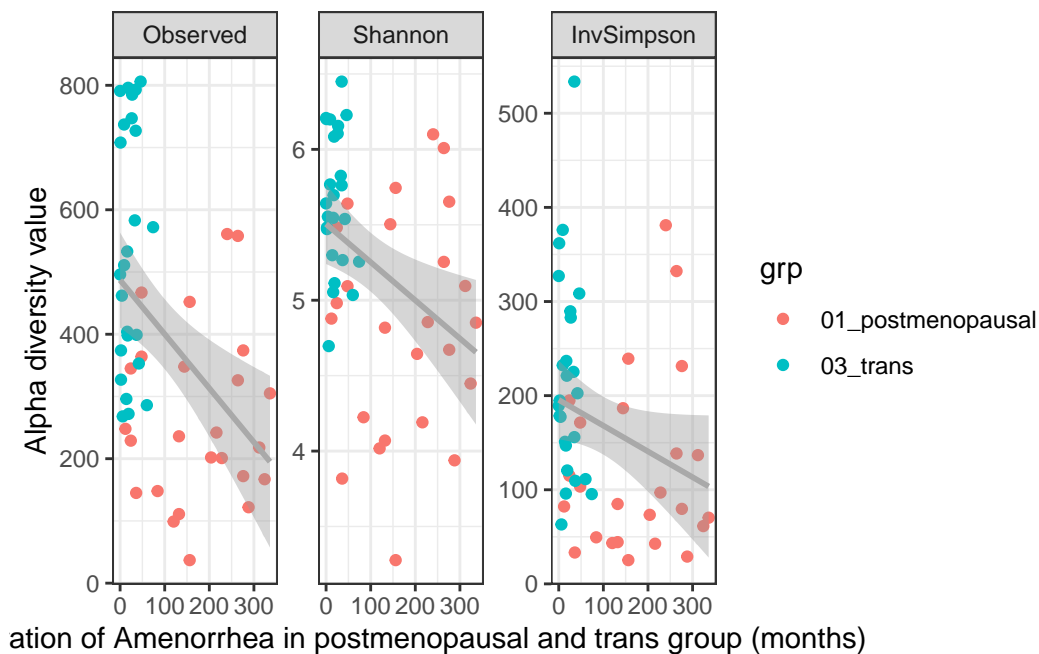
variable	var1	var2	cor	statistic	p	conf.low	conf.high	method
Observed	value	DurationMenopause	0.045	0.2158134	0.831	-0.3565088	0.4324048	Pearson
Shannon	value	DurationMenopause	0.078	0.3754553	0.711	-0.3271717	0.4590238	Pearson
InvSimpson	value	DurationMenopause	0.130	0.6169177	0.543	-0.2817497	0.4976290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

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

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

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

```
ggplot(activ_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")

```

```

2023-11-07 19:00:39.992263 WARNING::Input is a matrix, passing through as.data.frame() .
[1] "Warning: Deleting existing log file: results/maaslin2/genus_MenopausedurationMonths/maaslin2.log"
2023-11-07 19:00:40.146002 INFO::Writing function arguments to log file
2023-11-07 19:00:40.148153 INFO::Verifying options selected are valid
2023-11-07 19:00:40.148411 INFO::Determining format of input files
2023-11-07 19:00:40.148641 INFO::Input format is data samples as rows and metadata samples as columns
2023-11-07 19:00:40.150463 INFO::Formula for fixed effects: expr ~ DurationCombined
2023-11-07 19:00:40.15074 INFO::Filter data based on min abundance and min prevalence
2023-11-07 19:00:40.150954 INFO::Total samples in data: 50
2023-11-07 19:00:40.151161 INFO::Min samples required with min abundance for a feature not to be filtered: 5
2023-11-07 19:00:40.152723 INFO::Total filtered features: 292
2023-11-07 19:00:40.153022 INFO::Filtered feature names from abundance and prevalence filtering: ASV14, ASV54, ASV82, ASV205, ASV210, ASV369
2023-11-07 19:00:40.153463 INFO::Total filtered features with variance filtering: 0
2023-11-07 19:00:40.153703 INFO::Filtered feature names from variance filtering:
2023-11-07 19:00:40.15392 INFO::Running selected normalization method: TSS
2023-11-07 19:00:40.154315 INFO::Applying z-score to standardize continuous metadata
2023-11-07 19:00:40.156616 INFO::Running selected transform method: LOG
2023-11-07 19:00:40.157014 INFO::Running selected analysis method: LM
2023-11-07 19:00:40.157281 INFO::Fitting model to feature number 1, ASV14
2023-11-07 19:00:40.158343 INFO::Fitting model to feature number 2, ASV54
2023-11-07 19:00:40.159301 INFO::Fitting model to feature number 3, ASV82
2023-11-07 19:00:40.16023 INFO::Fitting model to feature number 4, ASV205
2023-11-07 19:00:40.161081 INFO::Fitting model to feature number 5, ASV210
2023-11-07 19:00:40.161925 INFO::Fitting model to feature number 6, ASV369
2023-11-07 19:00:40.164804 INFO::Counting total values for each feature
2023-11-07 19:00:40.165653 INFO::Writing filtered data to file results/maaslin2/genus_MenopausedurationMonths/maaslin2_filtered_data.csv
2023-11-07 19:00:40.166747 INFO::Writing filtered, normalized data to file results/maaslin2/genus_MenopausedurationMonths/maaslin2_normalized_data.csv
2023-11-07 19:00:40.167442 INFO::Writing filtered, normalized, transformed data to file results/maaslin2/genus_MenopausedurationMonths/maaslin2_transformed_data.csv
2023-11-07 19:00:40.168168 WARNING::Deleting existing residuals file: results/maaslin2/genus_MenopausedurationMonths/maaslin2_residuals.csv
2023-11-07 19:00:40.168602 INFO::Writing residuals to file results/maaslin2/genus_MenopausedurationMonths/maaslin2_residuals.csv
2023-11-07 19:00:40.169071 WARNING::Deleting existing fitted file: results/maaslin2/genus_MenopausedurationMonths/maaslin2_fitted_values.csv
2023-11-07 19:00:40.169421 INFO::Writing fitted values to file results/maaslin2/genus_MenopausedurationMonths/maaslin2_fitted_values.csv
2023-11-07 19:00:40.169816 INFO::Writing all results to file (ordered by increasing q-values): results/maaslin2/genus_MenopausedurationMonths/maaslin2_results.csv
2023-11-07 19:00:40.170429 INFO::Writing the significant results (those which are less than 0.05) to file: results/maaslin2/genus_MenopausedurationMonths/maaslin2_significant_results.csv
2023-11-07 19:00:40.170835 INFO::Writing heatmap of significant results to file: results/maaslin2/genus_MenopausedurationMonths/maaslin2_heatmap.png
[1] "There is not enough metadata in the associations to create a heatmap plot. Please review the metadata."
2023-11-07 19:00:40.1714 INFO::Writing association plots (one for each significant association) to file: results/maaslin2/genus_MenopausedurationMonths/maaslin2_association_plots

```

```
2023-11-07 19:00:40.171994 INFO::Plotting associations from most to least significant, group
2023-11-07 19:00:40.172282 INFO::Plotting data for metadata number 1, DurationCombined
2023-11-07 19:00:40.172801 INFO::Creating scatter plot for continuous data, DurationCombined
```

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

```
2023-11-07 19:00:40.237519 INFO::Creating scatter plot for continuous data, DurationCombined
```

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

```
2023-11-07 19:00:40.303043 INFO::Creating scatter plot for continuous data, DurationCombined
```

```
2023-11-07 19:00:40.370367 INFO::Creating scatter plot for continuous data, DurationCombined
```

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

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

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

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

```
# 4 ASVs
```

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

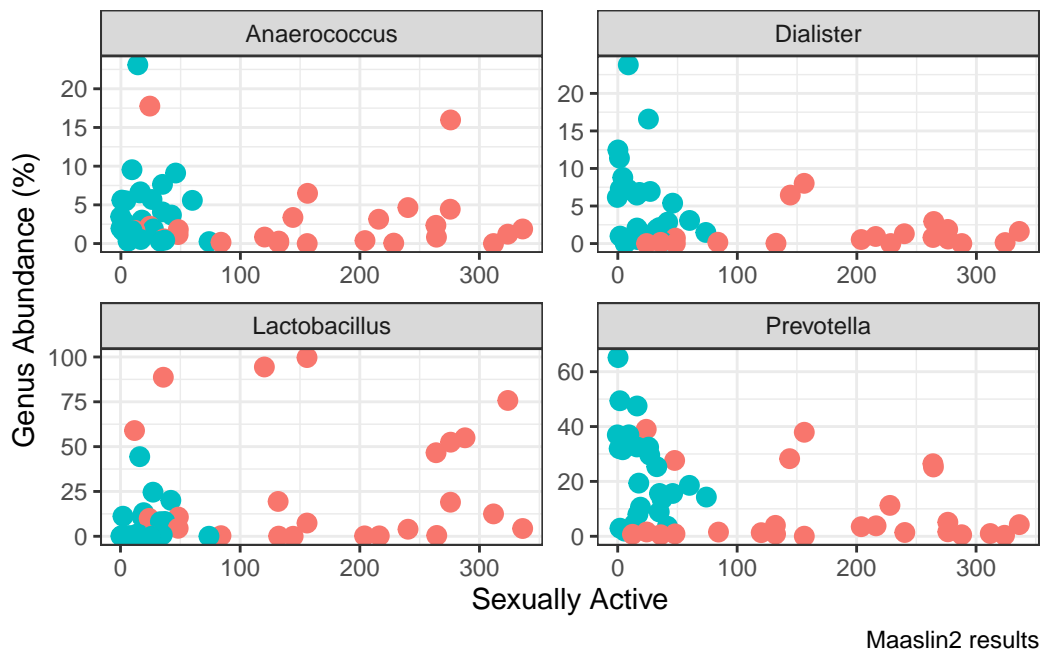
	ASV	Kingdom	Phylum	Class					
1	ASV14	Bacteria	Firmicutes	Bacilli					
2	ASV54	Bacteria	Bacteroidota	Bacteroidia					
3	ASV205	Bacteria	Firmicutes	Negativicutes					
4	ASV369	Bacteria	Firmicutes	Clostridia					
			Order	Family	Genus	Species			
1			Lactobacillales	Lactobacillaceae	Lactobacillus	<NA>	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

```
# Lactobacillus, dialister, Anaerococcus, Prevotella

# plot DurationCombined vs these 4 genera
psmelt_rel_genus_durationComb = psmelt(ps_menopause_rel_genus)
maaslin_res_genus_durationComb = ggplot(filter(psmelt_rel_genus_durationComb, OTU %in% fil
      aes(x = DurationCombined, y = Abundance,color = grp)) +
  geom_jitter(size = 3) +
  facet_wrap(~Genus, scales = "free") +
  scale_y_continuous(limits = function(x){c(0, max(0.1, x))}) +
  theme(legend.position = "none")+
  labs(x = "Sexually Active",
       y = "Genus Abundance (%)",
       caption = "Maaslin2 results")

maaslin_res_genus_durationComb
```

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



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

Warning: Removed 9 rows containing missing values (`geom_point()`).