

MBVagTrans Results

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1 Introduction

Include here metadata summaries and summary of phyloseq object

2 PBS empty control

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

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

Taxonomy	Abundance
p_Firmicutes;f_Lactobacillaceae;g_Lactobacillus	30.57
p_Actinobacteriota;f_Bifidobacteriaceae;g_Bifidobacterium	16.69
p_Actinobacteriota;f_Bifidobacteriaceae;g_Gardnerella	12.40
p_Proteobacteria;f_Moraxellaceae;g_Enhydrobacter	5.80
p_Firmicutes;f_Family XI;g_Anaerococcus	4.72
p_Actinobacteriota;f_Bifidobacteriaceae;g_Alloscardovia	3.61
p_Firmicutes;f_Streptococcaceae;g_Lactococcus	3.41
p_Bacteroidota;f_Prevotellaceae;g_Prevotella	3.37
p_Firmicutes;f_Streptococcaceae;g_Streptococcus	3.05
p_Proteobacteria;f_Moraxellaceae;g_Acinetobacter	2.45
p_Actinobacteriota;f_Atopobiaceae;g_Atopobium	2.16
p_Firmicutes;f_Family XI;g_Finegoldia	1.55
p_Firmicutes;f_Staphylococcaceae;g_Staphylococcus	1.31
p_Actinobacteriota;f_Propionibacteriaceae;g_Cutibacterium	1.17
p_Firmicutes;f_Lachnospiraceae;g_Anaerostipes	1.17
p_Firmicutes;f_Ruminococcaceae;g_Faecalibacterium	1.08
p_Actinobacteriota;f_Corynebacteriaceae;g_Corynebacterium	0.92
p_Actinobacteriota;f_Actinomycetaceae;g_NA	0.69
p_Proteobacteria;f_Xanthobacteraceae;g_Bradyrhizobium	0.66
p_Actinobacteriota;f_Actinomycetaceae;g_Varibaculum	0.59
p_Firmicutes;f_Family XI;g_Ezakiella	0.59
p_Firmicutes;f_Enterococcaceae;g_Enterococcus	0.58
p_Actinobacteriota;f_Corynebacteriaceae;g_NA	0.54
p_Actinobacteriota;f_Pseudonocardiaceae;g_Prauserella	0.54
p_Firmicutes;f_Marinococcaceae;g_Alteribacillus	0.39

```
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

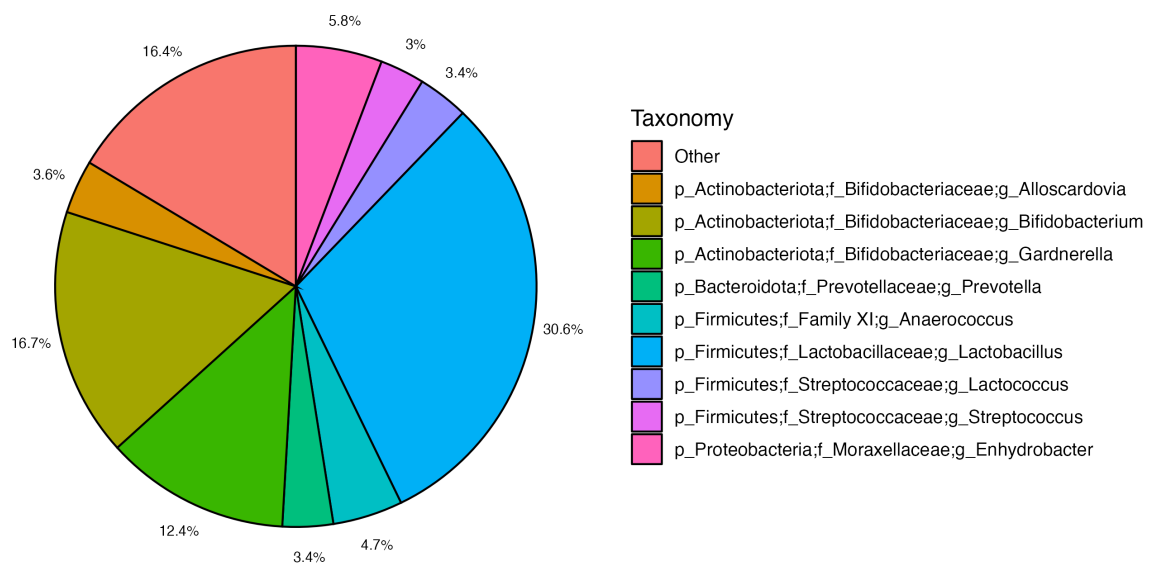


Figure 1: Taxonomic composition of PBS sample

measure.

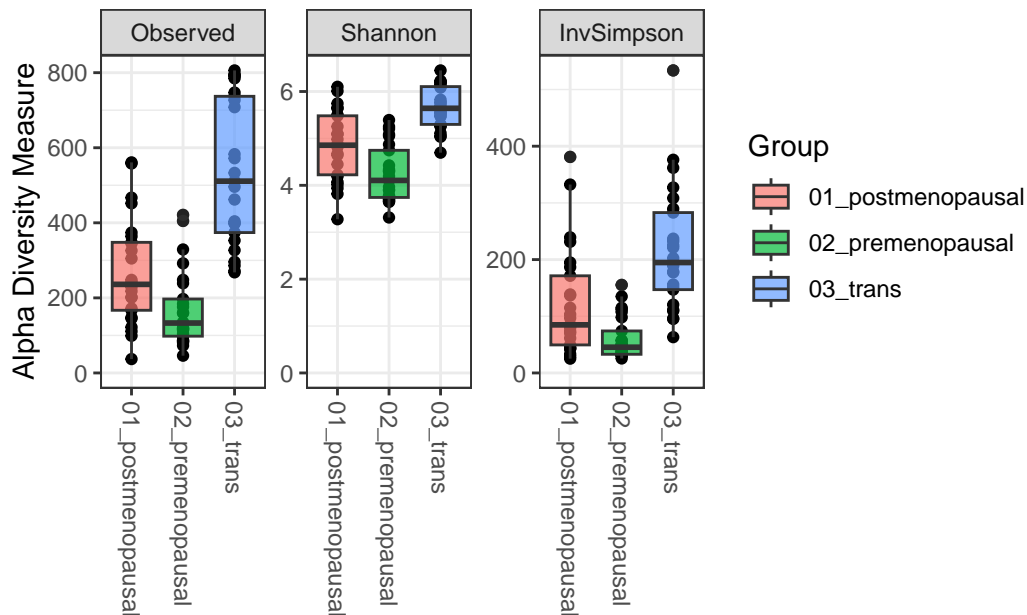
In this dataset, alpha diversity is markedly different between the group of interest (i.e. *03_trans*) and the two control groups. However, the intervention group is more similar to the first control group (i.e. *postmenopausal women*).

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

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 GHAT within patient group

```

adiv$data %>%
  filter(grp == "03_trans") %>%
  group_by(variable, grp) %>%
  cor_test(value, GHAT_Length) %>%
  select(grp, variable, cor, statistic, p, conf.low, conf.high) %>%
  kable()

```

grp	variable	cor	statistic	p	conf.low	conf.high
03_trans	Observed	-0.29	-1.450119	0.1610	-0.6143064	0.1193512
03_trans	Shannon	-0.50	-2.760257	0.0111	-0.7467711	-0.1291582
03_trans	InvSimpson	-0.35	-1.816990	0.0823	-0.6573940	0.0474853

In this analysis, a significant effect on Shannon index with length of GHAT 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()

```

variable	group1	group2	estimate	p.adj	p.adj.signif	conf.low	conf.high
Observed	0-3	4-6	212.0285714	0.006260	**	47.2238381	376.8333047
Observed	0-3	7-10	-	0.993000	ns	-	277.0880905
			31.2333333			339.5547572	
Observed	0-3	no bacteria	200.6000000	0.001040	**	66.8042429	334.3957571
Observed	4-6	7-10	-	0.207000	ns	-	80.6805011
			243.2619048			567.2043106	
Observed	4-6	no bacteria	-	0.998000	ns	-	155.2384214
			11.4285714			178.0955642	
Observed	7-10	no bacteria	231.8333333	0.208000	ns	-	541.1541810
						77.4875143	
Shannon	0-3	4-6	0.9730116	0.000542	***	0.3542272	1.5917961
Shannon	0-3	7-10	0.0280937	1.000000	ns	-1.1295460	1.1857334
Shannon	0-3	no bacteria	0.8511314	0.000175	***	0.3487749	1.3534879

variable	group1	group2	estimate	p.adj	p.adj.signif	conf.low	conf.high
Shannon	4-6	7-10	-0.9449179	0.182000	ns	-2.1612089	0.2713731
Shannon	4-6	no bacteria	-0.1218802	0.956000	ns	-0.7476568	0.5038964
Shannon	7-10	no bacteria	0.8230377	0.253000	ns	-0.3383545	1.9844299
InvSimpson0-3		4-6	93.8017175	0.022000	*	10.0658912	177.5375438
InvSimpson0-3		7-10	-9.9424700	0.998000	ns	-	146.7129165
						166.5978564	
InvSimpson0-3		no bacteria	84.0085749	0.009320	**	16.0281350	151.9890149
InvSimpson4-6		7-10	-	0.353000	ns	-	60.8480816
			103.7441875			268.3364566	
InvSimpson4-6		no bacteria	-9.7931426	0.990000	ns	-	74.8888813
						94.4751664	
InvSimpson7-10		no bacteria	93.9510449	0.401000	ns	-	251.1142297
						63.2121399	

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 10 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)

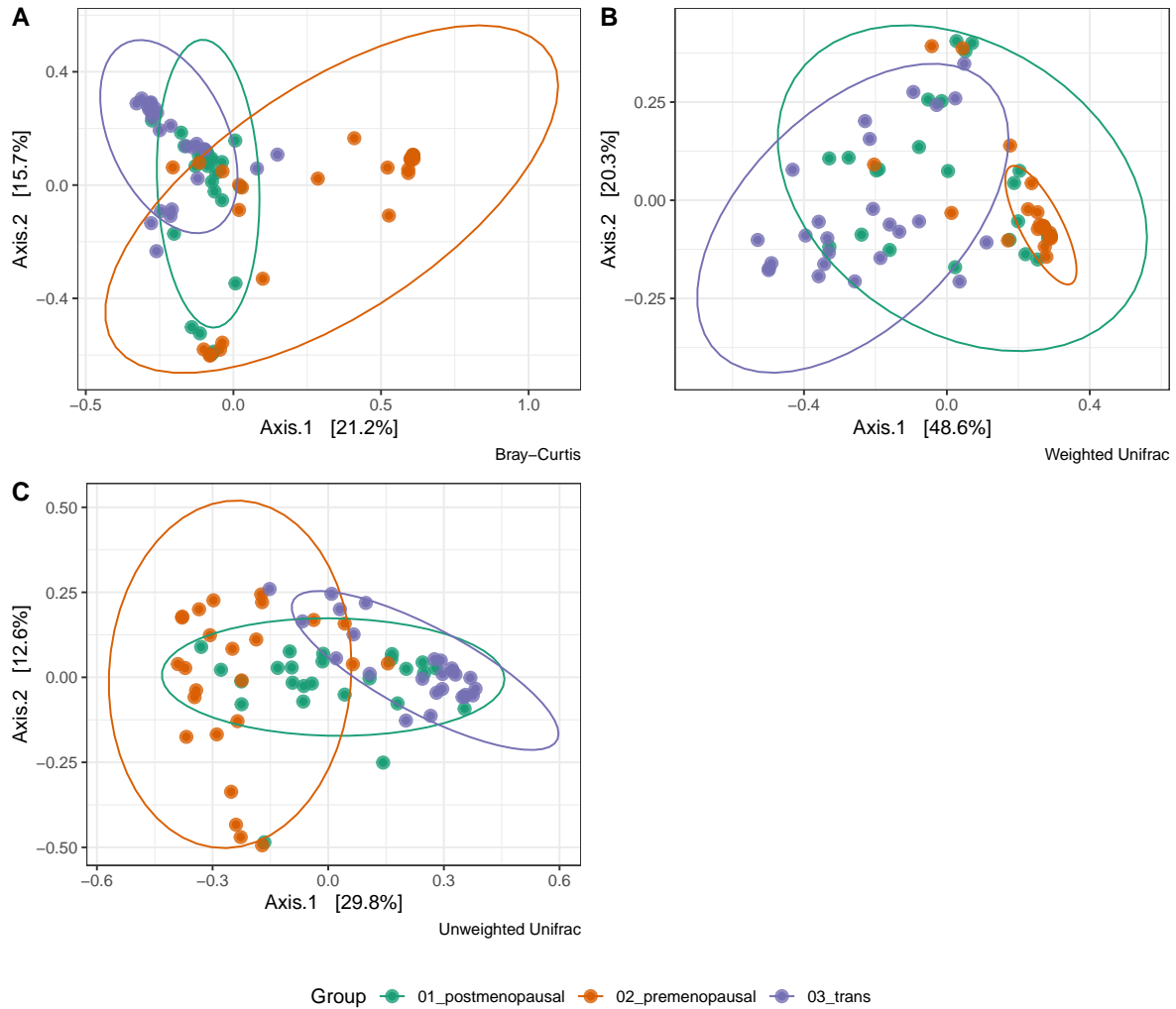
```

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, caption = "Bray-Curtis")
```

Table 6: Bray-Curtis

pairs	Df	SumsOfSqF.Model		R2	p.value	p.adjusted	sig
01_postmenopausal vs 02_premenopausal	1	1.803355	4.965357	0.0937473	0.001	0.003	*
01_postmenopausal vs 03_trans	1	1.411296	3.997345	0.0768759	0.001	0.003	*
02_premenopausal vs 03_trans	1	3.513273	11.173325	0.1888237	0.001	0.003	*

```
kable(adonis_pw_uf, caption = "Unweigthed Unifrac")
```

Table 7: Unweigthed Unifrac

pairs	Df	SumsOfSqF.Model		R2	p.value	p.adjusted	sig
01_postmenopausal vs 02_premenopausal	1	1.0955045	5.754483	0.1070512	0.001	0.003	*
01_postmenopausal vs 03_trans	1	0.8878037	5.900930	0.1094773	0.001	0.003	*
02_premenopausal vs 03_trans	1	2.5588959	16.713153	0.2582652	0.001	0.003	*

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

Table 8: Weighted Unifrac

pairs	Df	SumsOfSqF.Model		R2	p.value	p.adjusted	sig
01_postmenopausal vs 02_premenopausal	1	0.5833471	6.608171	0.1210107	0.001	0.003	*
01_postmenopausal vs 03_trans	1	0.9949649	8.073006	0.1439731	0.001	0.003	*
02_premenopausal vs 03_trans	1	2.5629809	30.132808	0.3856614	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	negLog10Pvalue	negLog10Padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV174	3574.694	1.523	0.0720	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV267	7164.210	1.452	0.8950	0.0040	0.013	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV367	1723.754	1.422	2.6390	0.0080	0.023	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV464	6094.388	1.475	2.9750	0.0030	0.010	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV558	9674.588	1.502	3.0550	0.0020	0.009	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV662	1674.442	1.443	3.0690	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV758	4584.132	1.432	2.8830	0.0040	0.013	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV858	5634.669	1.500	3.1120	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV956	4434.629	1.483	3.1160	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV106	4324.277	1.501	2.8500	0.0040	0.014	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV136	1334.037	1.430	2.8100	0.0050	0.016	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV136	2984.465	1.510	2.9580	0.0030	0.011	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV153	1694.740	1.441	3.2890	0.0010	0.005	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV151	2413.860	1.451	2.6600	0.0080	0.022	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus

ASV	baseMean	log2FoldChange	negLog10Pvalue	negLog10FDR	Kingdom	Phylum	Class	Order	Family	Genus		
ASV17	7.3954	2.291	1.482	2.8950	0.0040	0.013	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV18	8.6634	2.439	1.440	3.0830	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV19	7.2534	2.649	1.477	3.1480	0.0020	0.007	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV20	6.1774	2.292	1.395	3.0770	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV21	3.1014	2.616	1.455	3.1720	0.0020	0.007	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV22	1.9824	2.235	1.520	2.7860	0.0050	0.017	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV23	2.9054	2.446	1.382	3.2170	0.0010	0.006	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV30	0.8994	2.442	1.414	3.1410	0.0020	0.007	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV31	9.2904	2.235	1.492	2.8320	0.0050	0.015	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV33	9.1614	2.514	1.461	3.0890	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV37	7.8594	2.511	1.497	3.0120	0.0030	0.009	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV38	8.1474	2.606	1.479	3.1150	0.0020	0.008	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV40	3.3044	2.236	1.472	2.8720	0.0040	0.014	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV53	8.312	-	1.354	-	0.0140	0.034	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.329		2.459									
ASV53	7.625	-	1.333	-	0.0160	0.039	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.196		2.398									
ASV53	3.608	-	1.336	-	0.0130	0.033	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.317		2.483									
ASV53	4.361	-	1.466	-	0.0230	0.050	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.341		2.279									
ASV53	2.825	-	1.483	-	0.0060	0.018	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	4.053		2.733									
ASV61	2.056	-	1.415	-	0.0050	0.016	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.957		2.796									
ASV62	1.648	-	1.379	-	0.0050	0.017	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.842		2.787									
ASV63	1.023	-	1.384	-	0.0120	0.031	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.482		2.517									
ASV67	6.851	-	1.404	-	0.0030	0.012	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	4.106		2.924									
ASV68	7.069	-	1.465	-	0.0090	0.026	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.805		2.597									
ASV68	6.421	-	1.399	-	0.0090	0.024	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.679		2.629									
ASV72	6.234	-	1.342	-	0.0150	0.036	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.269		2.437									
ASV72	5.051	-	1.505	-	0.0200	0.045	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.510		2.333									
ASV72	2.871	-	1.379	-	0.0150	0.037	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.350		2.429									

ASV	baseMean	log2FoldChange	negLog10Padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV72	22.816	-	1.468	-	0.0100.028	Bacteria	Bacteroidia	Bacteroidales	Prevotella
	3.757		2.560					Prevotella	
ASV82	22.716	-	1.432	-	0.0220.049	Bacteria	Bacteroidia	Bacteroidales	Prevotella
	3.270		2.283					Prevotella	
ASV82	22.808	-	1.500	-	0.0050.017	Bacteria	Bacteroidia	Bacteroidales	Prevotella
	4.167		2.778					Prevotella	
ASV82	22.674	-	1.453	-	0.0140.034	Bacteria	Bacteroidia	Bacteroidales	Prevotella
	3.577		2.462					Prevotella	
ASV92	20.463	-	1.440	-	0.0180.043	Bacteria	Bacteroidia	Bacteroidales	Prevotella
	3.392		2.356					Prevotella	
ASV102	20.793	-	1.414	-	0.0030.009	Bacteria	Bacteroidia	Bacteroidales	Prevotella
	4.268		3.018					Prevotella	
ASV122	22.144	-	1.444	-	0.0120.032	Bacteria	Bacteroidia	Bacteroidales	Prevotella
	3.610		2.500					Prevotella	
ASV122	20.759	-	1.455	-	0.0220.049	Bacteria	Firmicutes	Clostridia	Reptostreptococcaceae
	3.325		2.285				Tissierellales	XI	Finegoldia
ASV122	20.523	-	1.743	-	0.0020.008	Bacteria	Firmicutes	Bacilli	Lactobacillales
	5.367		3.079					Streptococcaceae	Streptococcus
ASV132	20.781	-	1.443	-	0.0190.043	Bacteria	Bacteroidia	Bacteroidales	Prevotella
	3.399		2.355					Prevotella	
ASV132	20.041	-	1.606	-	0.0040.014	Bacteria	Firmicutes	Bacilli	Lactobacillales
	4.593		2.860					Streptococcaceae	Streptococcus
ASV142	20.964	-	1.656	-	0.0000.000	Bacteria	Firmicutes	Bacilli	Lactobacillales
	7.262		4.385					Streptococcaceae	Streptococcus
ASV152	20.289	-	1.779	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales
	7.158		4.024					Streptococcaceae	Streptococcus
ASV152	20.947	-	1.469	-	0.0010.004	Bacteria	Bacteroidia	Bacteroidales	Prevotella
	4.982		3.391					Prevotella	
ASV162	20.189	-	1.455	-	0.0000.002	Bacteria	Bacteroidia	Bacteroidales	Prevotella
	5.168		3.551					Prevotella	
ASV162	20.436	-	1.351	-	0.0000.000	Bacteria	Bacteroidia	Bacteroidales	Prevotella
	7.124		5.272					Prevotella	
ASV162	20.300	-	1.721	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales
	6.944		4.035					Streptococcaceae	Streptococcus
ASV172	20.624	-	1.457	-	0.0010.004	Bacteria	Bacteroidia	Bacteroidales	Prevotella
	4.849		3.329					Prevotella	
ASV172	20.120	-	1.770	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales
	6.800		3.842					Streptococcaceae	Streptococcus
ASV182	20.069	-	1.452	-	0.0000.002	Bacteria	Bacteroidia	Bacteroidales	Prevotella
	5.346		3.683					Prevotella	

ASV	baseMean	log2FoldChange	negLog10Pvalue	negLog10Pvalueadj	Kingdom	Phylum	Class	Order	Family	Genus
ASV192	1.983	-	1.651	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcus
	6.993		4.237							
ASV196	1.878	-	1.413	-	0.0000.001	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae
	5.670		4.011							
ASV197	1.760	4.556	1.883	2.420	0.0160.037	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae
ASV199	1.687	-	1.711	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcus
	6.759		3.949							
ASV203	1.564	-	1.871	-	0.0140.034	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae
	4.616		2.467							
ASV209	1.992	-	1.838	-	0.0000.002	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcus
	6.638		3.612							
ASV210	1.669	-	2.409	-	0.0140.034	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcus
	5.944		2.467							
ASV214	1.706	-	1.850	-	0.0000.002	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcus
	6.698		3.620							
ASV220	1.907	-	1.648	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcus
	6.788		4.120							
ASV222	1.862	-	1.518	-	0.0000.001	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae
	5.804		3.824							
ASV223	1.076	-	1.431	-	0.0000.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae
	6.698		4.679							
ASV223	1.003	-	1.422	-	0.0000.001	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae
	5.895		4.146							
ASV230	1.649	-	1.644	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcus
	6.779		4.124							
ASV232	1.725	-	1.440	-	0.0000.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae
	6.607		4.588							
ASV232	1.549	-	1.699	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcus
	6.767		3.983							
ASV242	1.202	-	1.437	-	0.0000.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae
	6.970		4.851							
ASV247	1.258	-	1.441	-	0.0220.049	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Finegoldia
	3.295		2.286							
ASV250	1.985	-	1.770	-	0.0110.029	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae
	4.498		2.542							
ASV257	1.602	6.402	2.096	3.055	0.0020.009	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae
ASV258	1.211	-	1.749	-	0.0130.034	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae
	4.327		2.474							
ASV262	1.064	-	2.078	-	0.0090.025	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcus
	5.407		2.602							

ASV	baseMean	log2FoldChange	negLog10Pval	adjPval	Kingdom	Phylum	Class	Order	Family	Genus
ASV262753	-	1.410	-	0.0000.002	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	5.113		3.626							
ASV262502	-	1.836	-	0.0000.002	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.663		3.629							
ASV266957	-	1.387	-	0.0000.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	7.244		5.222							
ASV270388	-	1.495	-	0.0000.001	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	5.665		3.789							
ASV272277	-	1.705	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.448		3.781							
ASV274888	-	2.078	-	0.0110.029	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	5.302		2.551							
ASV276402	-	1.761	-	0.0000.002	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.603		3.749							
ASV277702	-	1.666	-	0.0060.017	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
	4.607		2.765							
ASV301231	-	1.454	-	0.0000.000	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.591		4.532							
ASV302878	4.772	1.962	2.433	0.0150.036	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV308355	-	2.077	-	0.0170.040	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	4.951		2.384							
ASV309450	-	1.770	-	0.0020.007	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
	5.598		3.162							
ASV309581	-	1.646	-	0.0000.001	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.538		3.973							
ASV309649	-	1.439	-	0.0130.033	Bacteria	Firmicutes	Clostridia	Reptostreptococcales-Tissierellales	Familia XI	Finegoldia
	3.570		2.481							
ASV3098117	-	1.502	-	0.0110.029	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	3.819		2.543							
ASV3099167	-	1.821	-	0.0000.003	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.434		3.534							
ASV310472	-	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							
ASV320222	-	1.732	-	0.0150.037	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister
	4.198		2.424							
ASV322399	-	2.756	-	0.0000.000	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	24.683		8.956							
ASV326260	-	1.709	-	0.0000.000	Bacteria	Firmicutes	Clostridia	Reptostreptococcales-Tissierellales	Familia XI	Fenollaria
	7.621		4.459							

ASV	baseMean	log2FoldChange	negLog10Pvalue	negLog10FDR	padj	Kingdom	Phylum	Class	Order	Family	Genus
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								
ASV336987	4.962	1.951	2.544	0.0110.029		Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV337530	-	1.518	-	0.0000.000		Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.688		4.405								
ASV33892	-	1.426	-	0.0000.000		Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.879		4.824								
ASV339595	-	1.863	-	0.0180.042		Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	4.410		2.368								
ASV339607	-	1.826	-	0.0000.002		Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.464		3.540								
ASV339713	-	1.399	-	0.0000.000		Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.310		4.512								
ASV339705	-	1.652	-	0.0000.001		Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Anaerococcus
	6.673		4.038								
ASV371138	-	1.757	-	0.0020.009		Bacteria	Firmicutes	Negativibacteria	Veillonellales	Veillonellaceae	Dialister
	5.341		3.040								
ASV37526	6.086	2.014	3.022	0.0030.009		Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV37772	-	1.865	-	0.0070.019		Bacteria	Firmicutes	Negativibacteria	Veillonellales	Veillonellaceae	Dialister
	5.068		2.717								
ASV378028	-	1.439	-	0.0000.001		Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	5.967		4.148								
ASV381733	-	1.749	-	0.0000.001		Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Fenollaria
	7.241		4.141								
ASV38926	-	2.147	-	0.0090.026		Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	5.573		2.596								
ASV40266	-	2.067	-	0.0160.038		Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	4.984		2.412								
ASV40730	-	2.259	-	0.0220.049		Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	5.167		2.288								
ASV40886	-	1.462	-	0.0000.000		Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	7.156		4.896								
ASV41246	-	1.879	-	0.0010.006		Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.049		3.220								

ASV	baseMean	log2FoldChange	negLog10Pval	adjPval	Kingdom	Phylum	Class	Order	Family	Genus
ASV41926	-	2.168	-	0.0070.019	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Alioscandia
	5.876		2.710							
ASV42365	-	1.467	-	0.0000.001	Bacteria	Bacteroidetes	Bacteroidetes	Bacteroidales	Prevotellaceae	Prevotella
	6.250		4.261							
ASV42561	-	2.022	-	0.0060.017	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Alioscandia
	5.611		2.775							
ASV43018	-	1.845	-	0.0000.001	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Fenollaria
	7.522		4.078					Tissierellales	XI	
ASV43228	-	1.778	-	0.0000.001	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Fenollaria
	7.325		4.119					Tissierellales	XI	
ASV43438	-	1.714	-	0.0170.039	Bacteria	Firmicutes	Negativitellae	Veillonellales	Veillonellaceae	Dialister
	4.107		2.395					Selenomonadales		
ASV43744	-	1.932	-	0.0060.017	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Alioscandia
	5.359		2.774							
ASV43770	-	1.869	-	0.0000.001	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Fenollaria
	7.384		3.951					Tissierellales	XI	
ASV43997	-	1.545	-	0.0000.000	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Peptoniphilus
	6.882		4.456					Tissierellales	XI	
ASV43995	-	1.735	-	0.0000.003	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	6.066		3.496							
ASV45072	-	1.957	-	0.0000.002	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Fenollaria
	7.278		3.719					Tissierellales	XI	
ASV45130	-	2.046	-	0.0120.031	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	5.133		2.508							
ASV45956	-	1.965	-	0.0000.002	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Fenollaria
	7.223		3.676					Tissierellales	XI	
ASV46787	-	2.131	-	0.0190.043	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	5.002		2.347							
ASV46952	-	2.223	-	0.0200.045	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
	5.177		2.329							
ASV46141	-	1.427	-	0.0000.000	Bacteria	Bacteroidetes	Bacteroidetes	Bacteroidales	Prevotellaceae	Prevotella
	7.008		4.910							
ASV46741	4.968	1.998	2.486	0.0130.033	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV46544	-	1.918	-	0.0050.015	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Alioscandia
	5.413		2.823							
ASV46254	-	1.821	-	0.0020.009	Bacteria	Firmicutes	Negativitellae	Veillonellales	Veillonellaceae	Dialister
	5.572		3.060					Selenomonadales		
ASV46615	-	1.743	-	0.0020.007	Bacteria	Firmicutes	Negativitellae	Veillonellales	Veillonellaceae	Dialister
	5.458		3.131					Selenomonadales		
ASV47264	-	1.947	-	0.0000.002	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Fenollaria
	7.143		3.668					Tissierellales	XI	

ASV	baseMean	log2FoldChange	negLog10PValue	negLog10AdjPValue	Kingdom	Phylum	Class	Order	Family	Genus
ASV49702	-	1.548	-	0.0000.000	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecalibacterium	Peptoniphilus
	6.716		4.339					Tissierellales	XI	
ASV50856	-	1.418	-	0.0000.001	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecalibacterium	Peptoniphilus
	5.438		3.835					Tissierellales	XI	
ASV52334	-	2.397	-	0.0170.041	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacterium	Alioscandia
	5.696		2.376							
ASV53165	-	2.186	-	0.0130.032	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacterium	Alioscandia
	5.449		2.493							
ASV51299	-	1.815	-	0.0000.001	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecalibacterium	Fenollaria
	6.993		3.852					Tissierellales	XI	
ASV51306	-	1.939	-	0.0000.002	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecalibacterium	Fenollaria
	7.154		3.690					Tissierellales	XI	
ASV51261	-	1.881	-	0.0000.002	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecalibacterium	Fenollaria
	7.004		3.724					Tissierellales	XI	
ASV52795	-	1.630	-	0.0030.010	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecalibacterium	Peptoniphilus
	4.893		3.002					Tissierellales	XI	
ASV53280	-	1.699	-	0.0000.002	Bacteria	Firmicutes	Negativibacteriales	Veillonellales	Veillonella	Dialister
	6.016		3.542					Selenomonadales		
ASV51311	-	1.511	-	0.0000.001	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecalibacterium	Peptoniphilus
	6.394		4.231					Tissierellales	XI	
ASV51609	-	1.770	-	0.0000.002	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	6.358		3.592							
ASV51891	-	1.447	-	0.0000.001	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecalibacterium	Peptoniphilus
	6.114		4.225					Tissierellales	XI	
ASV51332	-	1.345	-	0.0000.000	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecalibacterium	Peptoniphilus
	6.632		4.929					Tissierellales	XI	
ASV51997	-	1.708	-	0.0000.002	Bacteria	Firmicutes	Negativibacteriales	Veillonellales	Veillonella	Dialister
	6.177		3.617					Selenomonadales		
ASV51039	-	1.779	-	0.0010.003	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecalibacterium	Peptoniphilus
	6.099		3.428					Tissierellales	XI	
ASV51422	-	1.739	-	0.0000.002	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	6.426		3.695							
ASV51559	-	1.602	-	0.0000.001	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	6.548		4.088							
ASV51796	-	1.518	-	0.0000.000	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecalibacterium	Peptoniphilus
	6.705		4.416					Tissierellales	XI	
ASV51753	-	1.792	-	0.0030.010	Bacteria	Bacteroidetes	Bacteroidetes	Bacteroidales	Prevotellaceae	Prevotella
	5.335		2.977							
ASV51531	-	1.795	-	0.0000.001	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Faecalibacterium	Anaerococcus
	6.801		3.790					Tissierellales	XI	

ASV	baseMean	log2FoldChange	negLog10Padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV57303	-	1.929	- 0.0010.006	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	6.260	3.245							
ASV58659	-	1.831	- 0.0000.002	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Fenollaria
	6.749	3.687					Tissierellales	XI	
ASV58174	-	2.082	- 0.0100.027	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacter	Alioscandia
	5.372	2.581							
ASV58581	-	2.021	- 0.0020.007	Bacteria	Bacteroidetes	Bacteroidetes	Bacteroidales	Prevotellaceae	Prevotella
	6.373	3.153							
ASV58837	-	1.817	- 0.0000.002	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Fenollaria
	6.718	3.698					Tissierellales	XI	
ASV59713	-	2.022	- 0.0010.004	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Fenollaria
	6.738	3.332					Tissierellales	XI	
ASV59754	-	1.984	- 0.0020.009	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Anaerococcus
	6.003	3.026					Tissierellales	XI	
ASV60773	-	1.497	- 0.0000.001	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Peptoniphilus
	6.334	4.231					Tissierellales	XI	
ASV60965	-	1.833	- 0.0010.004	Bacteria	Bacteroidetes	Bacteroidetes	Bacteroidales	Prevotellaceae	Prevotella
	6.142	3.350							
ASV61423	-	2.005	- 0.0020.007	Bacteria	Bacteroidetes	Bacteroidetes	Bacteroidales	Prevotellaceae	Prevotella
	6.314	3.150							
ASV61674	-	1.962	- 0.0010.004	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Anaerococcus
	6.636	3.382					Tissierellales	XI	
ASV61806	-	1.792	- 0.0000.001	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Fenollaria
	6.887	3.843					Tissierellales	XI	
ASV62350	-	1.499	- 0.0000.000	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Peptoniphilus
	6.543	4.365					Tissierellales	XI	
ASV62583	-	2.027	- 0.0020.008	Bacteria	Bacteroidetes	Bacteroidetes	Bacteroidales	Prevotellaceae	Prevotella
	6.315	3.116							
ASV63387	-	2.019	- 0.0020.007	Bacteria	Bacteroidetes	Bacteroidetes	Bacteroidales	Prevotellaceae	Prevotella
	6.332	3.136							
ASV63766	-	2.645	- 0.0170.040	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces
	6.323	2.391							
ASV63794	-	1.947	- 0.0010.003	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Fenollaria
	6.717	3.450					Tissierellales	XI	
ASV64166	-	1.646	- 0.0000.002	Bacteria	Firmicutes	Negativibacteria	Veillonellales	Veillonellaceae	Dialister
	6.007	3.649					Selenomonadales		
ASV65184	-	1.900	- 0.0010.003	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Anaerococcus
	6.585	3.466					Tissierellales	XI	
ASV65730	-	1.880	- 0.0000.002	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Fenollaria
	6.689	3.558					Tissierellales	XI	

ASV	baseMean	log2FoldChange	negLog10Padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV65746	-	1.530	- 0.0000.002	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Peptoniphilus
	5.438	3.554					Tissierellales	XI	
ASV65900	-	1.948	- 0.0010.004	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Anaerococcus
	6.577	3.376					Tissierellales	XI	
ASV67224	-	1.908	- 0.0000.003	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Fenollaria
	6.721	3.523					Tissierellales	XI	
ASV67364	-	2.197	- 0.0070.020	Bacteria	Actinobacteria	Actinomycetales	Actinomycetales	Actinomycetales	Actinomycetales
	5.939	2.702							
ASV67709	-	1.601	- 0.0010.003	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Peptoniphilus
	5.480	3.422					Tissierellales	XI	
ASV68746	-	1.937	- 0.0010.005	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Anaerococcus
	6.415	3.312					Tissierellales	XI	
ASV68334	-	1.494	- 0.0000.001	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Peptoniphilus
	6.197	4.147					Tissierellales	XI	
ASV68347	-	2.281	- 0.0060.017	Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospirales	Lachnospirales
	6.315	2.768							
ASV68792	-	2.283	- 0.0140.034	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Anaerococcus
	5.607	2.457					Tissierellales	XI	
ASV69841	-	1.929	- 0.0010.005	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellales	Prevotellales
	6.274	3.253							
ASV69959	-	1.873	- 0.0000.002	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Fenollaria
	6.653	3.552					Tissierellales	XI	
ASV69363	-	1.513	- 0.0000.001	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Peptoniphilus
	5.828	3.852					Tissierellales	XI	
ASV70250	-	1.741	- 0.0010.006	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellales	Veillonellales
	5.609	3.221					Selenomonadales		
ASV70736	-	1.976	- 0.0020.008	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	6.176	3.125							
ASV70884	-	1.766	- 0.0000.001	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Fenollaria
	6.665	3.774					Tissierellales	XI	
ASV70937	-	1.848	- 0.0000.002	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Fenollaria
	6.680	3.615					Tissierellales	XI	
ASV71362	-	2.162	- 0.0070.020	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Anaerococcus
	5.838	2.700					Tissierellales	XI	
ASV71748	-	1.705	- 0.0000.001	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellales	Prevotellales
	7.086	4.156							
ASV71620	-	1.917	- 0.0010.006	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellales	Prevotellales
	6.228	3.249							
ASV72423	-	2.056	- 0.0020.007	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Anaerococcus
	6.512	3.168					Tissierellales	XI	

ASV	baseMean	log2FoldChange	negLog10Padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV72854	-	1.773	- 0.0000.001	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	6.890	3.885							
ASV741591	-	1.882	- 0.0000.003	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Fenollaria
	6.612	3.513					Tissierellales	XI	
ASV74115	-	1.880	- 0.0000.003	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Fenollaria
	6.597	3.509					Tissierellales	XI	
ASV73510	-	1.816	- 0.0020.009	Bacteria	Firmicutes	Negativibacter	Veillonellales	Veillonellaceae	Dialister
	5.506	3.032					Selenomonadales		
ASV74657	-	1.893	- 0.0080.022	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	5.022	2.653							
ASV73256	-	2.189	- 0.0210.047	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Anaerococcus
	5.046	2.305					Tissierellales	XI	
ASV75390	-	1.613	- 0.0000.002	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	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							
ASV73894	-	2.160	- 0.0050.017	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Anaerococcus
	6.007	2.781					Tissierellales	XI	
ASV74659	-	1.601	- 0.0000.001	Bacteria	Firmicutes	Negativibacter	Veillonellales	Veillonellaceae	Dialister
	6.144	3.837					Selenomonadales		
ASV73485	-	2.223	- 0.0060.018	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Anaerococcus
	6.092	2.740					Tissierellales	XI	
ASV74951	-	1.502	- 0.0000.001	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Peptoniphilus
	5.833	3.884					Tissierellales	XI	
ASV73329	-	2.228	- 0.0070.019	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Anaerococcus
	6.056	2.718					Tissierellales	XI	
ASV73409	-	2.093	- 0.0050.016	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	5.862	2.800							
ASV74937	-	2.053	- 0.0030.010	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Anaerococcus
	6.118	2.980					Tissierellales	XI	
ASV74980	-	2.066	- 0.0020.009	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	6.300	3.049							
ASV74676	-	1.662	- 0.0000.002	Bacteria	Firmicutes	Negativibacter	Veillonellales	Veillonellaceae	Dialister
	6.110	3.675					Selenomonadales		
ASV80402	-	2.285	- 0.0080.022	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Ezakiella
	6.101	2.670					Tissierellales	XI	
ASV80365	-	1.794	- 0.0010.003	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	6.153	3.430							

ASV	baseMean	log2FoldChange	negLog10Padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV80564	-	2.401	- 0.0160.039	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Anaerococcus
	5.775	2.405					Tissierellales	XI	
ASV80930	-	2.044	- 0.0030.010	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Anaerococcus
	6.075	2.973					Tissierellales	XI	
ASV81568	-	1.684	- 0.0010.004	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Peptoniphilus
	5.638	3.348					Tissierellales	XI	
ASV82017	-	1.919	- 0.0010.005	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Fenollaria
	6.309	3.288					Tissierellales	XI	
ASV82351	-	2.276	- 0.0090.025	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Parvimonas
	5.944	2.612					Tissierellales	XI	
ASV82620	-	1.975	- 0.0020.008	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.175	3.127							
ASV82773	-	1.662	- 0.0000.002	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
	6.128	3.686					Selenomonadales		
ASV83485	-	1.742	- 0.0010.005	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Peptoniphilus
	5.719	3.283					Tissierellales	XI	
ASV83900	-	2.198	- 0.0060.017	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Anaerococcus
	6.097	2.775					Tissierellales	XI	
ASV84592	-	1.689	- 0.0010.003	Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister
	5.777	3.420					Selenomonadales		
ASV84710	-	1.589	- 0.0010.004	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Peptoniphilus
	5.344	3.363					Tissierellales	XI	
ASV84906	-	2.265	- 0.0100.020	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Anaerococcus
	5.865	2.589					Tissierellales	XI	
ASV85088	-	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							
ASV84710	-	2.056	- 0.0040.013	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Anaerococcus
	5.918	2.879					Tissierellales	XI	
ASV83970	-	2.171	- 0.0080.022	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Anaerococcus
	5.789	2.667					Tissierellales	XI	
ASV84604	-	2.177	- 0.0060.018	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Ezakiella
	5.980	2.747					Tissierellales	XI	
ASV90242	-	2.173	- 0.0060.018	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	5.982	2.753							
ASV90553	-	1.739	- 0.0000.001	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.616	3.804							
ASV91221	-	1.735	- 0.0020.009	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Peptoniphilus
	5.250	3.026					Tissierellales	XI	

ASV	baseMean	log2FoldChange	negLog10Padj	Kingdom	Phylum	Class	Order	Family	Genus
ASV92347	-	1.746	- 0.0010.003	Bacteria	Firmicutes	Negativicoccus	Veillonellales	Veillonellaceae	Dialister
	6.074	3.478					Selenomonadales		
ASV92492	-	1.759	- 0.0000.002	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
	6.428	3.655							
ASV93552	-	2.030	- 0.0030.011	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Ezakiella
	6.020	2.966					Tissierellales	XI	
ASV94892	4.227	1.821	2.3220.0200.046	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
ASV95877	-	2.069	- 0.0050.016	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Anaerococcus
	5.817	2.811					Tissierellales	XI	
ASV97514	-	2.298	- 0.0200.045	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Parvimonas
	5.353	2.329					Tissierellales	XI	
ASV98347	-	2.364	- 0.0140.034	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Anaerococcus
	5.816	2.461					Tissierellales	XI	
ASV13239	-	2.287	- 0.0120.031	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Ezakiella
	5.743	2.511					Tissierellales	XI	
ASV13364	-	2.057	- 0.0040.015	Bacteria	Campylobacter	Campylobacter	Campylobacter	Campylobacter	Campylobacter
	5.855	2.846							
ASV13458	-	2.368	- 0.0120.031	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Parvimonas
	5.942	2.509					Tissierellales	XI	
ASV13861	-	2.037	- 0.0030.012	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Ezakiella
	5.963	2.927					Tissierellales	XI	
ASV14280	-	2.097	- 0.0050.016	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Anaerococcus
	5.882	2.805					Tissierellales	XI	
ASV14324	-	2.306	- 0.0190.044	Bacteria	Firmicutes	Clostridia	Reptostreptococcales	Familia	Anaerococcus
	5.388	2.337					Tissierellales	XI	

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

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

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

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

ASV base	Log2	Fold	KS	Signif	p	val	adj	Kingd	Phylum	Class	Order	Family	Genus	Species
ASV26	2.234	-	1.350	-	0.0010	0.035		Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	NA
	4.408		3.264											
ASV22	2.871	-	1.395	-	0.0000	0.026		Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	NA
	5.000		3.584											
ASV22	2.716	-	1.446	-	0.0010	0.034		Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	NA
	4.845		3.350											
ASV100	793	-	1.432	-	0.0000	0.026		Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	NA
	5.282		3.689											
ASV25	811	-	1.776	-	0.0000	0.026		Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister	NA
	6.944		3.910											
ASV30	450	-	1.785	-	0.0000	0.026		Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister	NA
	6.873		3.851											
ASV32	022	-	1.754	-	0.0010	0.035		Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister	NA
	5.740		3.272											
ASV37	138	-	1.771	-	0.0000	0.026		Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister	NA
	6.614		3.734											
ASV39	045	-	1.858	-	0.0000	0.027		Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister	NA
	6.536		3.517											
ASV43	012	-	1.856	-	0.0010	0.034		Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister	NA
	6.279		3.383											
ASV43	838	-	1.739	-	0.0000	0.026		Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister	NA
	6.417		3.691											
ASV45	659	-	1.784	-	0.0000	0.026		Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister	NA
	6.421		3.599											
ASV45	241	-	1.843	-	0.0010	0.034		Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister	NA
	6.084		3.301											
ASV45	854	-	1.831	-	0.0010	0.034		Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister	NA
	6.153		3.361											
ASV45	015	-	1.754	-	0.0000	0.026		Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister	NA
	6.246		3.560											
ASV50	091	-	1.896	-	0.0010	0.037		Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister	NA
	6.125		3.231											
ASV51	038	-	1.828	-	0.0010	0.034		Bacteria	Firmicutes	Negativicutes	Veillonellales	Veillonellaceae	Dialister	NA
	6.040		3.304											
ASV56	753	-	1.804	-	0.0010	0.034		Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	NA
	6.083		3.373											
ASV75	639	-	1.562	-	0.0010	0.034		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
Finegoldia	03_trans	0.6289195	0.2773376	1.3062089	0.000000	6.1532654
Howardella	01_postmenopausal	D.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	f.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	D.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	l.8298909	0.3984946	2.8734076	0.000000	10.4592997

Genus	grp	mean	median	sd	min	max
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	5.7637229	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)
```

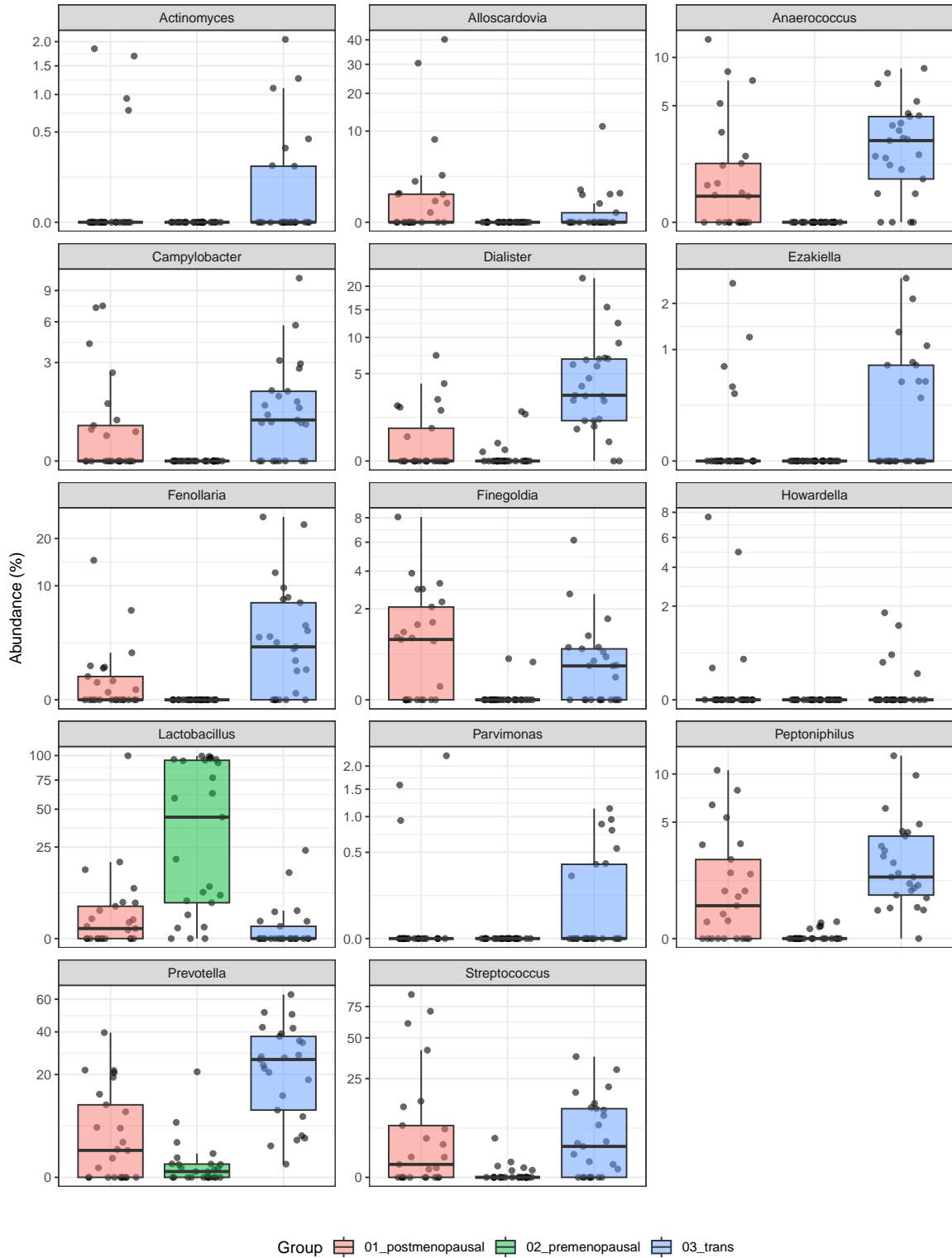
Genus	01_postmenopausal	02_premenopausal	03_trans
Actinomyces	0.21 ± 0.53	0 ± 0	0.22 ± 0.51
Alloscardovia	3.5 ± 9.83	0 ± 0	0.63 ± 2.21
Anaerococcus	1.69 ± 3.21	0 ± 0	2.72 ± 2.53
Campylobacter	0.96 ± 2.15	0 ± 0	1.4 ± 2.3
Dialister	0.82 ± 1.7	0.14 ± 0.42	4.98 ± 5.28
Ezakiella	0.21 ± 0.57	0 ± 0	0.45 ± 0.71
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 10 sample variables ]
tax_table() Taxonomy Table: [ 383 taxa by 7 taxonomic ranks ]
phy_tree() Phylogenetic Tree: [ 383 tips and 382 internal nodes ]
refseq() DNASTringSet: [ 383 reference sequences ]
```

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

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

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

phyloseq-class experiment-level object

```
otu_table() OTU Table: [ 151 taxa and 75 samples ]
sample_data() Sample Data: [ 75 samples by 10 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 10 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 10 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 10 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."

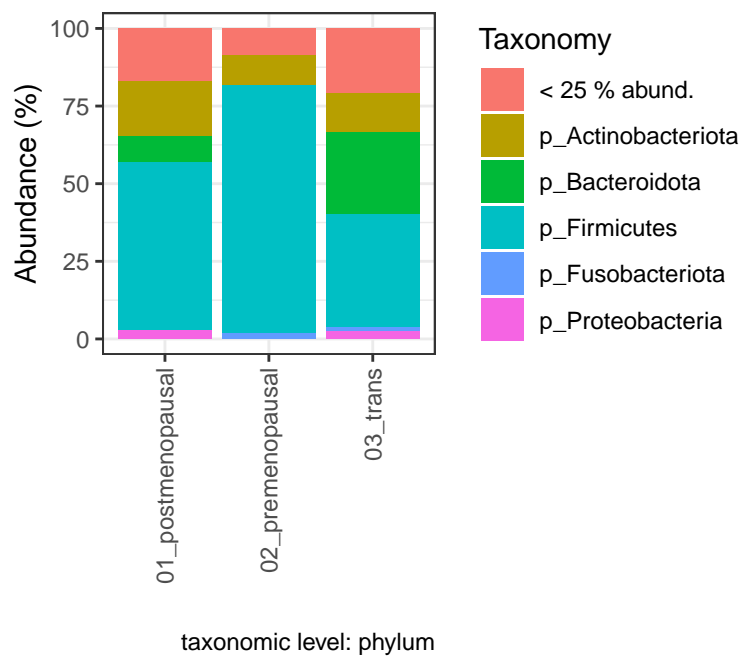
phylumplot = ggplot(phylum, aes(x = grp, y = Abundance/25, fill = Taxonomy)) +
  geom_col(position = "stack") +
```

```
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.

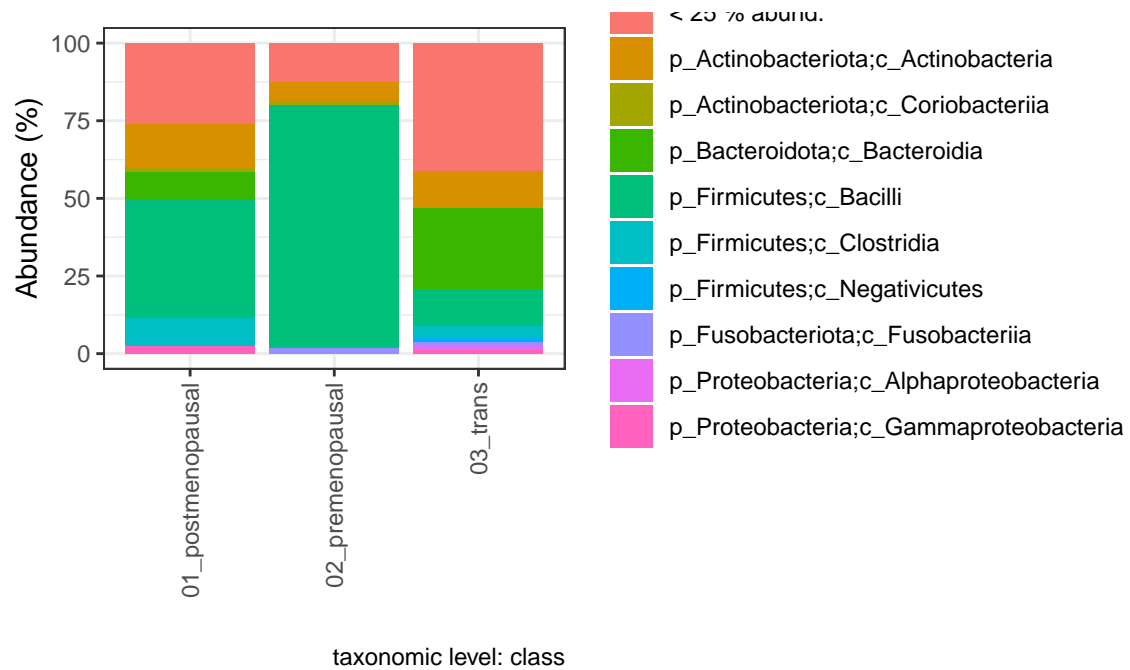
```
phylumplot
```



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

5.2 Class level

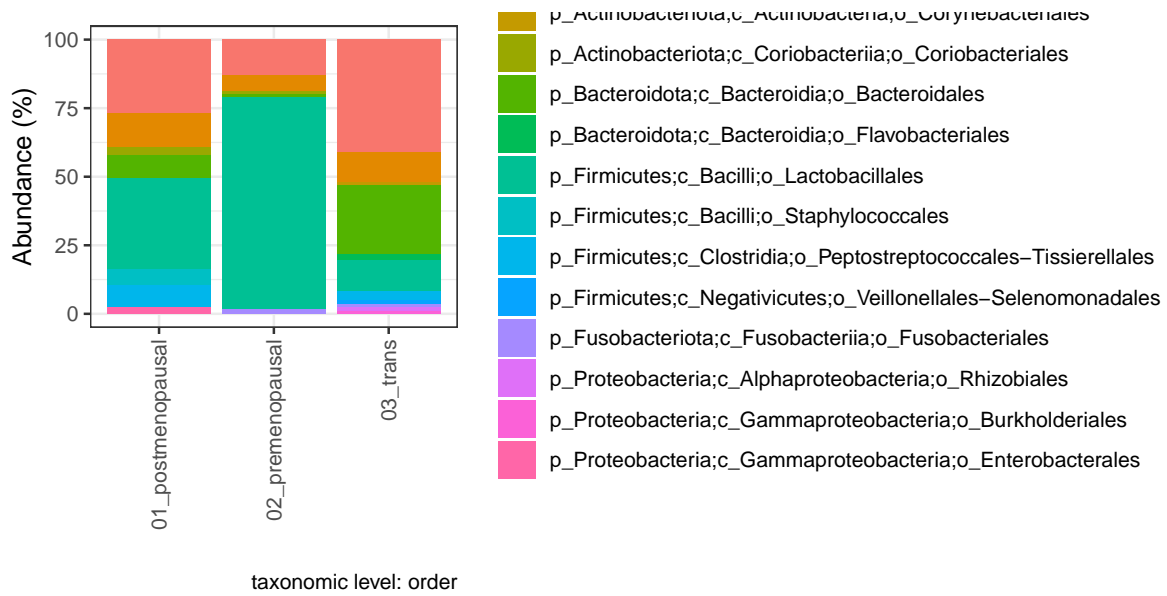
```
classplot
```

```
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

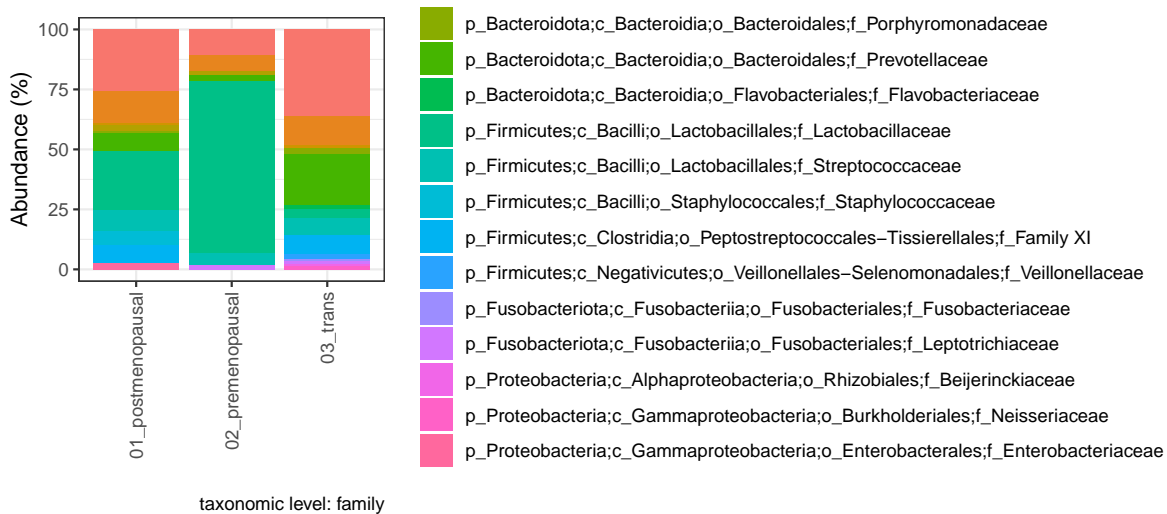
```
orderplot
```



```
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

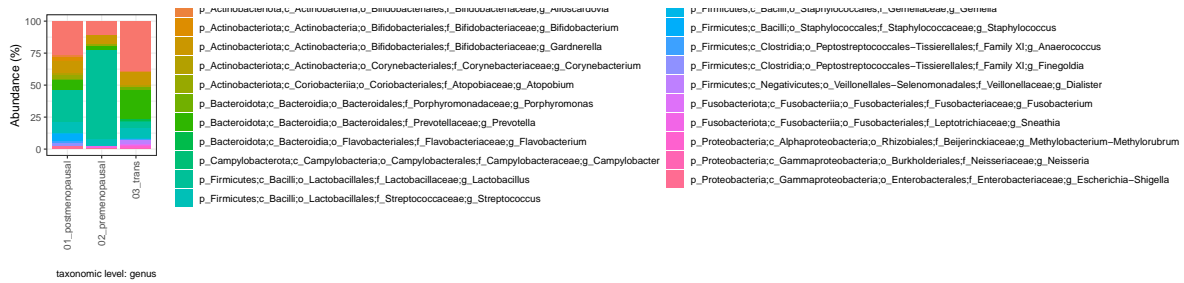
familyplot



```
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,
```