

# Analyses\_and\_Plots

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```
library("ggplot2") library("dplyr") library("vegan") library("DESeq2") library("knitr")
```

```
## While the p-value for ANOVA of alphadiv~Treatment isn't extremely low ( $Pr(>F) \sim 0.13$ ), Shannon~Treatment  
## Should maybe look at mean #ofgenera observed as a function of radiation level. Considering the low c
```

```
library("phyloseq")
```

```
sample_data(physeq1)$Alphadiv <- estimate_richness(physeq1, split=TRUE, measures=c("Shannon"))
```

```
## Warning in estimate_richness(physeq1, split = TRUE, measures = c("Shannon")): The data you have provided
```

```
## any singletons. This is highly suspicious. Results of richness
```

```
## estimates (for example) are probably unreliable, or wrong, if you have already
```

```
## trimmed low-abundance taxa from the data.
```

```
##
```

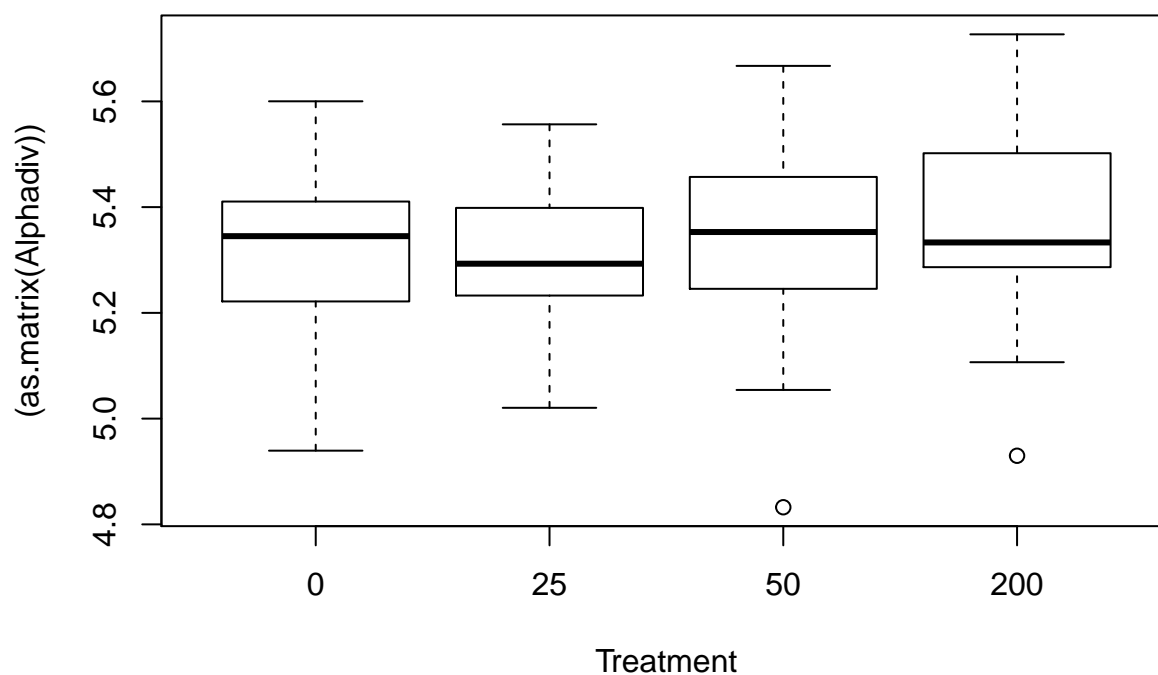
```
## We recommended that you find the un-trimmed data and retry.
```

```
anova_df <- data.frame(sample_data(physeq1))
```

```
aov_results <- aov(as.matrix(Alphadiv) ~ Treatment*Sex, data=anova_df)
```

```
anova_results <- anova(aov_results)
```

```
some_plot <- boxplot((as.matrix(Alphadiv))~Treatment, data=anova_df)
```



```
print(anova_results)
```

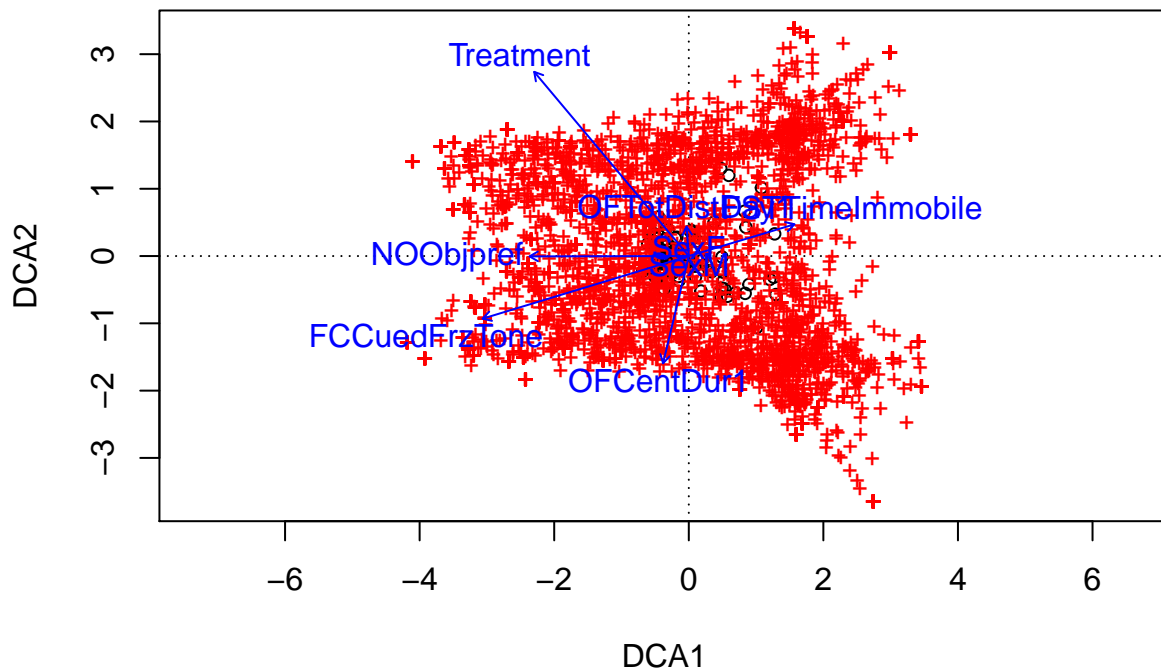
```
## Analysis of Variance Table
##
## Response: as.matrix(Alphadiv)
##           Df Sum Sq Mean Sq F value Pr(>F)
## Treatment    1 0.06374 0.063736   2.3496 0.12871
## Sex           1 0.00272 0.002719   0.1002 0.75227
## Treatment:Sex 1 0.09758 0.097576   3.5971 0.06099 .
## Residuals    93 2.52278 0.027127
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

## Loading required package: permute

## Loading required package: lattice

## This is vegan 2.5-5

## Warning in decorana(data.frame(otu_table(physeq1))): some species were
## removed because they were missing in the data
```

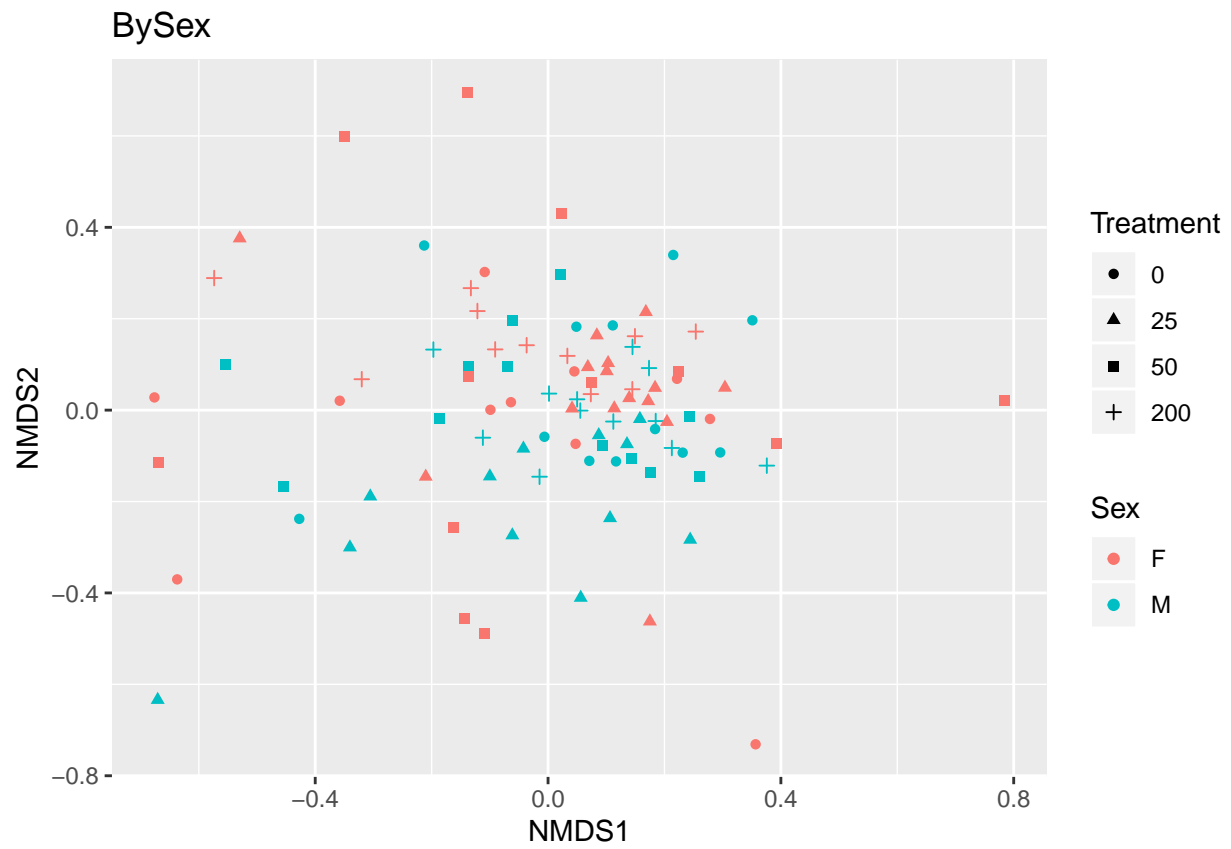


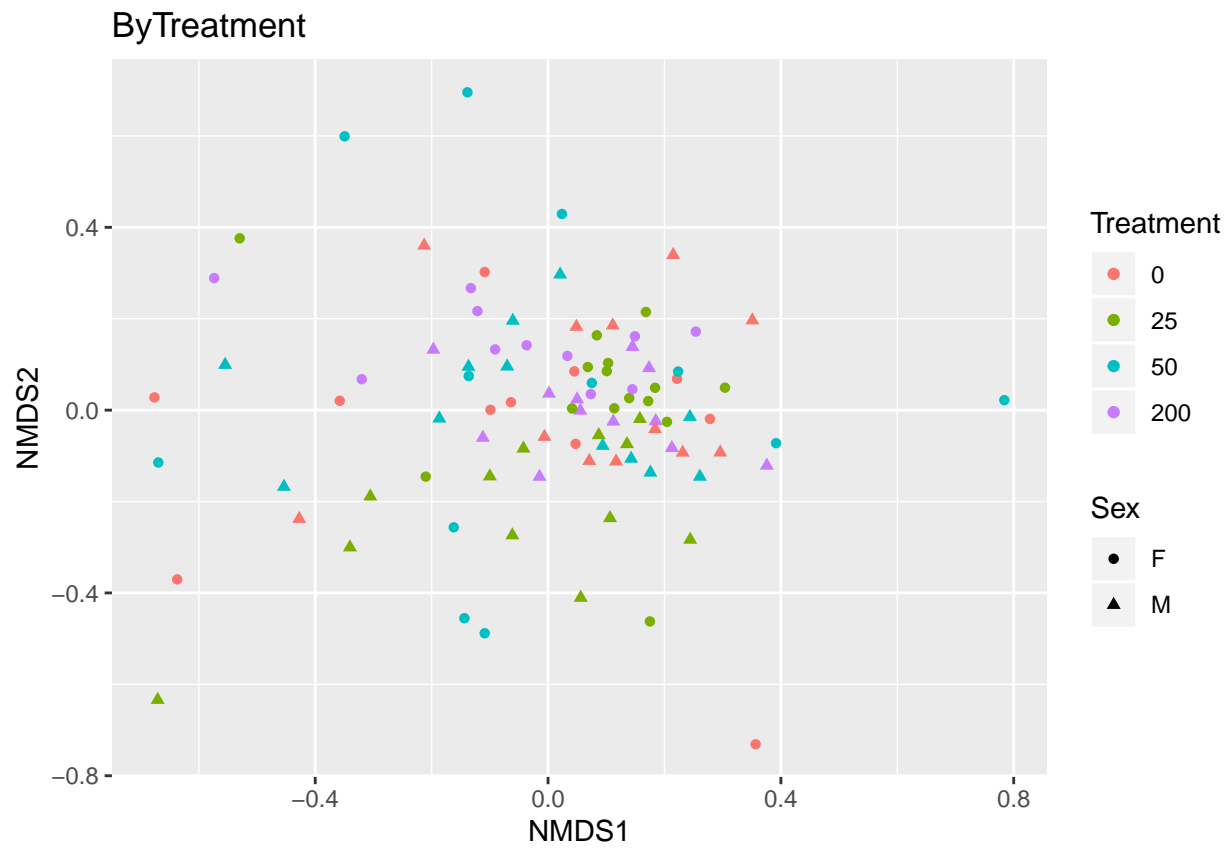
```
## Square root transformation
```

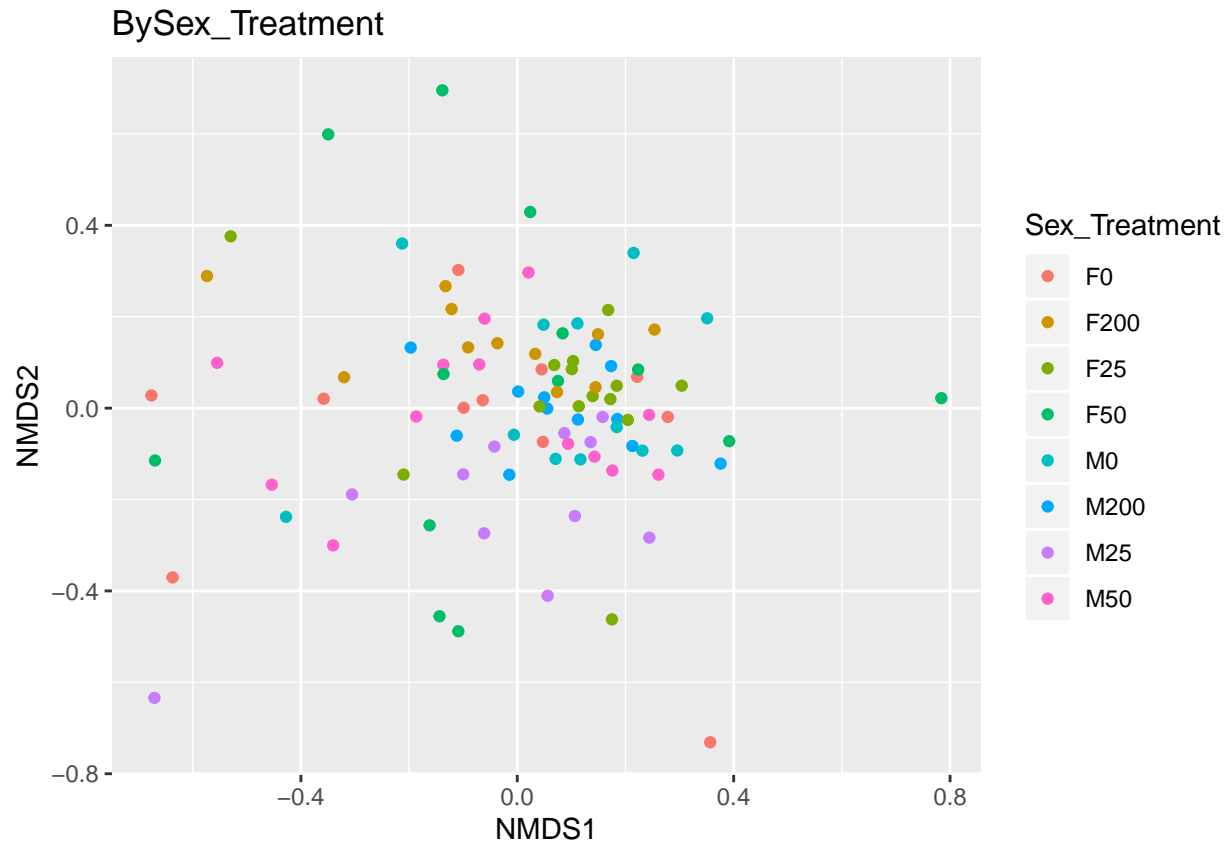
```

## Wisconsin double standardization
## Run 0 stress 0.2327549
## Run 1 stress 0.2489618
## Run 2 stress 0.2373731
## Run 3 stress 0.2366082
## Run 4 stress 0.2407472
## Run 5 stress 0.2407104
## Run 6 stress 0.2561073
## Run 7 stress 0.2431509
## Run 8 stress 0.2473315
## Run 9 stress 0.231759
## ... New best solution
## ... Procrustes: rmse 0.03149534  max resid 0.2455192
## Run 10 stress 0.2402663
## Run 11 stress 0.2427163
## Run 12 stress 0.23963
## Run 13 stress 0.2468628
## Run 14 stress 0.2441619
## Run 15 stress 0.2436797
## Run 16 stress 0.2365216
## Run 17 stress 0.2344287
## Run 18 stress 0.2324491
## Run 19 stress 0.2405164
## Run 20 stress 0.2342434
## *** No convergence -- monoMDS stopping criteria:
##      1: no. of iterations >= maxit
##     19: stress ratio > sratmax

```







```
## Using adonis to evaluate the importance of covariates in explaining variation in the ordination prod
library("phyloseq")
library("vegan")
set.seed(1)
physeq1 <- rarefy_even_depth(physeq1)
```

```
## You set `rngseed` to FALSE. Make sure you've set & recorded
## the random seed of your session for reproducibility.
## See `?set.seed`
```

```
## ...
```

```
## 620TUs were removed because they are no longer
## present in any sample after random subsampling
```

```
## ...
```

```
physeq1.dist <- vegdist(otu_table(physeq1), method="bray")
sampledf <- data.frame(sample_data(physeq1))
set.seed(1)
perm.results.treatment <- adonis(physeq1.dist ~ Treatment*Sex, data=sampledf, permutations=5000)
print(perm.results.treatment)
```

```
##
```

```
## Call:
## adonis(formula = physeq1.dist ~ Treatment * Sex, data = sampledf,      permutations = 5000)
##
## Permutation: free
## Number of permutations: 5000
##
## Terms added sequentially (first to last)
##
##              Df SumsOfSqs MeanSqs F.Model    R2    Pr(>F)
## Treatment      3    0.9693 0.32312  1.5485 0.04629 0.0002000 ***
## Sex            1    0.5456 0.54556  2.6145 0.02605 0.0002000 ***
## Treatment:Sex  3    0.8565 0.28551  1.3682 0.04090 0.0005999 ***
## Residuals     89   18.5712 0.20867          0.88677
## Total         96   20.9426          1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

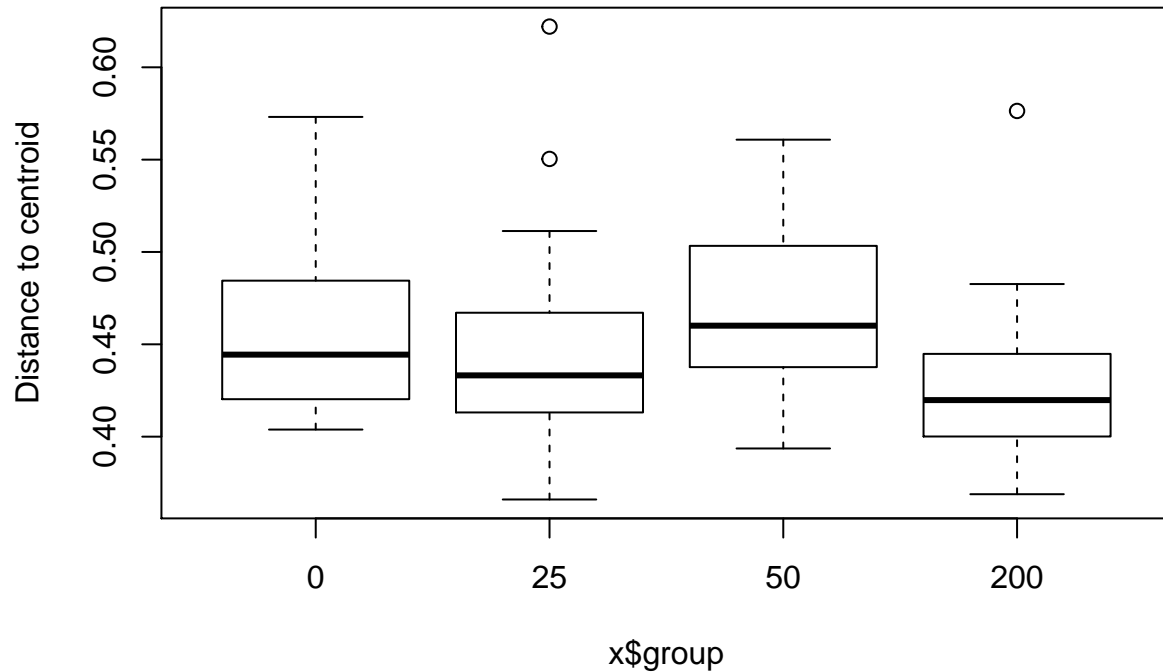
```
## We ran through possible permutations and options for model selection using AIC in ordistep after bui
sampledf <- data.frame(sample_data(physeq1))
physeq1.capsca <- capscale(physeq1.dist ~ Treatment*Sex, sampledf, dist="bray")
set.seed(1)
mod <- ordistep(physeq1.capsca, perm.max = 5000, trace = T, direction = "both")
```

```
##
## Start: physeq1.dist ~ Treatment * Sex
##
##              Df    AIC      F Pr(>F)
## - Treatment:Sex 3 296.76 1.3683 0.005 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
print(mod)
```

```
## Call: capscale(formula = physeq1.dist ~ Treatment * Sex, data =
## sampledf, distance = "bray")
##
##              Inertia Proportion Rank
## Total         20.9426      1.0000
## Constrained    2.3714      0.1132    7
## Unconstrained 18.5712      0.8868   89
## Inertia is squared Bray distance
##
## Eigenvalues for constrained axes:
##   CAP1  CAP2  CAP3  CAP4  CAP5  CAP6  CAP7
## 0.6070 0.4339 0.3655 0.2915 0.2559 0.2182 0.1994
##
## Eigenvalues for unconstrained axes:
##   MDS1  MDS2  MDS3  MDS4  MDS5  MDS6  MDS7  MDS8
## 1.4281 0.7499 0.6545 0.5785 0.5111 0.4858 0.4447 0.4277
## (Showing 8 of 89 unconstrained eigenvalues)
```

```
## The graphical results of this suggest that there might be a difference in mean betadispersion, with
## Do I need to correct for multiple pairwise comparisons?
treatments <- factor(sample_data(physeq1)$Treatment)
betadispersion <- betadisper(physeq1.dist, treatments, type=c("centroid"))
boxplot(betadispersion)
```



```
set.seed(1)
testbetad <- permutest(betadispersion, pairwise=TRUE, permutations=5000)
print(testbetad)
```

```
##
## Permutation test for homogeneity of multivariate dispersions
## Permutation: free
## Number of permutations: 5000
##
## Response: Distances
##      Df  Sum Sq  Mean Sq    F N.Perm Pr(>F)
## Groups   3 0.024786 0.0082620 3.3132  5000 0.0218 *
## Residuals 93 0.231907 0.0024936
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Pairwise comparisons:
## (Observed p-value below diagonal, permuted p-value above diagonal)
##      0      25      50      200
```



```
## 0          0.3157369 0.5686863 0.0214
## 25 0.3181555          0.1003799 0.1886
## 50 0.5693164 0.0996424          0.0016
## 200 0.0230888 0.1872353 0.0024643
```

```
beta_disp_tukey_result <- TukeyHSD(betadisposition, ordered=FALSE, conf.level=0.95)
```

```
library("phyloseq")
taxa_with_deseq <- function(treatdds.deseq, physeq1){
  sigtab <- NULL
  ##treatdds = phyloseq_to_deseq2(phyloseq_object, ~"some_covariate")
  treatdds.deseq = DESeq(treatdds)
  res = results(treatdds.deseq, cooksCutoff=FALSE)
  res = data.frame(res)
  alpha = 0.1
  remove_nas <- is.na(res$adj)
  sigtab <- subset(res, remove_nas)
  res <- subset(res, !is.na(res$padj))
  sigtab <- subset(res, res$padj < alpha)
  if (nrow(sigtab)>0){
    sigtab = cbind(as(sigtab, "data.frame"), as(tax_table(physeq1)[rownames(sigtab), ], "matrix"))
    return(sigtab)
  } else {
    return("No significant taxa were identified using the specified formula")
  }
}
```

```
## This first step agglomerates to a chosen level, then you can use the workthrough below to continue t
## Covariates = Sex+Treatment+FSTTimeImmobile+NOObjpref+OFCentDur1+FCCuedFrzTone+OFTotDistDay1
library(DESeq2)
```

```
## Loading required package: S4Vectors
```

```
## Loading required package: stats4
```

```
## Loading required package: BiocGenerics
```

```
## Loading required package: parallel
```

```
##
```

```
## Attaching package: 'BiocGenerics'
```

```
## The following objects are masked from 'package:parallel':
```

```
##
```

```
## clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
## clusterExport, clusterMap, parApply, parCapply, parLapply,
## parLapplyLB, parRapply, parSapply, parSapplyLB
```

```
## The following objects are masked from 'package:stats':
```

```
##
```

```
## IQR, mad, sd, var, xtabs
```

```

## The following objects are masked from 'package:base':
##
##   anyDuplicated, append, as.data.frame, basename, cbind,
##   colnames, dirname, do.call, duplicated, eval, evalq, Filter,
##   Find, get, grep, grepl, intersect, is.unsorted, lapply, Map,
##   mapply, match, mget, order, paste, pmax, pmax.int, pmin,
##   pmin.int, Position, rank, rbind, Reduce, rownames, sapply,
##   setdiff, sort, table, tapply, union, unique, unsplit, which,
##   which.max, which.min
##
## Attaching package: 'S4Vectors'
##
## The following object is masked from 'package:base':
##
##   expand.grid
##
## Loading required package: IRanges
##
## Attaching package: 'IRanges'
##
## The following object is masked from 'package:phyloseq':
##
##   distance
##
## The following object is masked from 'package:grDevices':
##
##   windows
##
## Loading required package: GenomicRanges
##
## Loading required package: GenomeInfoDb
##
## Loading required package: SummarizedExperiment
##
## Loading required package: Biobase
##
## Welcome to Bioconductor
##
##   Vignettes contain introductory material; view with
##   'browseVignettes()'. To cite Bioconductor, see
##   'citation("Biobase)"', and for packages 'citation("pkgname)"'.
##
## Attaching package: 'Biobase'
##
## The following object is masked from 'package:phyloseq':
##
##   sampleNames

```

```
## Loading required package: DelayedArray

## Loading required package: matrixStats

##
## Attaching package: 'matrixStats'

## The following objects are masked from 'package:Biobase':
##
##     anyMissing, rowMedians

## Loading required package: BiocParallel

##
## Attaching package: 'DelayedArray'

## The following objects are masked from 'package:matrixStats':
##
##     colMaxs, colMins, colRanges, rowMaxs, rowMins, rowRanges

## The following objects are masked from 'package:base':
##
##     aperm, apply, rowsum
```

```
set.seed(1)
phyloseq_object <- physeq1
physeq_genus <- tax_glom(physeq, taxrank="Genus")
##This is a basic workthrough that runs DESeq2 for analysis of taxa analysis. One thing that that I sho
## A couple limitations
sigtab <- NULL
treatdds = phyloseq_to_deseq2(physeq_genus, ~OFTotDistDay1)
```

```
## converting counts to integer mode
```

```
treatdds.deseq = DESeq(treatdds)
```

```
## estimating size factors
```

```
## estimating dispersions
```

```
## gene-wise dispersion estimates
```

```
## mean-dispersion relationship
```

```
## final dispersion estimates
```

```
## fitting model and testing
```

```

res = results(treatdds.deseq, cooksCutoff=FALSE)
res = data.frame(res)
alpha = 0.1
remove_nas <- is.na(res$adj)
sigtab <- subset(res, remove_nas)
res <- subset(res, !is.na(res$padj))
sigtab <- subset(res, res$padj < alpha)
if (nrow(sigtab)>0){
  sigtab = cbind(as(sigtab, "data.frame"), as(tax_table(physeq1)[rownames(sigtab), ], "matrix"))
  print(sigtab)
} else {
  print("No significant taxa were identified using the specified formula")
}

```

```
## [1] "No significant taxa were identified using the specified formula"
```

```

## Generate a couple of sigtab tables that reflect significant taxa according to behavioral covariates
## After generating the taxa tables, see which taxa might be common between sets
## Get a feel for the taxa present by looking at microbes observed in Keaton's work, and what might have

```

```
phyloseq_object1 = rarefy_even_depth(phyloseq_object)
```

```

## You set `rngseed` to FALSE. Make sure you've set & recorded
## the random seed of your session for reproducibility.
## See `?set.seed`

```

```
## ...
```

```

## 350TUs were removed because they are no longer
## present in any sample after random subsampling

```

```
## ...
```

```

asv_table=(otu_table(phyloseq_object1))
sample_data_to_smash=sample_data(phyloseq_object1)
taxa_table_plus_metadata <- data.frame(cbind(data.frame(asv_table), data.frame(sample_data_to_smash)))
##plotting_taxa <- ggplot(taxa_table_plus_metadata, aes(x=Treatment, y=ASV92)) + geom_smooth()##+ geom_
##plotting_taxa

```

```

## DESeq2 analysis using the specified covariate (ASV level)
treatdds = phyloseq_to_deseq2(phyloseq_object, ~OFTotDistDay1)

```

```
## converting counts to integer mode
```

```

## Generated table containing ASVs
OFTotDistDay1_ASVs <- taxa_with_deseq(treatdds, physeq1)

```

```
## estimating size factors
```

```
## estimating dispersions
```

```

## gene-wise dispersion estimates

## mean-dispersion relationship

## final dispersion estimates

## fitting model and testing

print(OFTotDistDay1_ASVs)

## [1] "No significant taxa were identified using the specified formula"

## DESeq2 genus level analysis using OFTotDistDay1
treatdds = phyloseq_to_deseq2(phyloseq_genus, ~OFTotDistDay1)

## converting counts to integer mode

## Genus level table
OFTotDistDay1_genus <- taxa_with_deseq(treatdds, phyloseq_genus)

## estimating size factors

## estimating dispersions

## gene-wise dispersion estimates

## mean-dispersion relationship

## final dispersion estimates

## fitting model and testing

print(OFTotDistDay1_genus)

## [1] "No significant taxa were identified using the specified formula"

## DESeq2 analysis using the specified covariate (ASV level)
treatdds = phyloseq_to_deseq2(phyloseq_object, ~FSTTimeImmobile)

## converting counts to integer mode

## Generated table containing ASVs
FSTTimeImmobile_ASVs <- taxa_with_deseq(treatdds, physeq1)

## estimating size factors

## estimating dispersions

```

```

## gene-wise dispersion estimates

## mean-dispersion relationship

## final dispersion estimates

## fitting model and testing

## 1 rows did not converge in beta, labelled in mcols(object)$betaConv. Use larger maxit argument with

## -- replacing outliers and refitting for 2746 genes
## -- DESeq argument 'minReplicatesForReplace' = 7
## -- original counts are preserved in counts(dds)

## estimating dispersions

## fitting model and testing

## 1 rows did not converge in beta, labelled in mcols(object)$betaConv. Use larger maxit argument with

print(FSTTimeImmobile_ASVs)

```

	baseMean	log2FoldChange	lfcSE	stat	pvalue
## ASV1765	0.2952101	0.3943297	0.07997683	4.930549	8.199882e-07
## ASV1790	0.6332132	-1.8219435	0.07989469	-22.804312	4.154993e-115
## ASV1813	0.6727890	-1.8338704	0.07989464	-22.953610	1.356088e-116
## ASV1815	0.7129159	-0.2884507	0.07955010	-3.626025	2.878171e-04
## ASV1911	0.6351867	-7.2342363	0.07994067	-90.495069	0.000000e+00
## ASV1932	0.6568408	-7.2859773	0.07994066	-91.142324	0.000000e+00
## ASV1941	0.6035313	-1.8124316	0.07989474	-22.685244	6.265873e-114
## ASV2054	0.5045918	-1.7771233	0.07989493	-22.243255	1.310930e-109
## ASV2122	0.2767594	0.3933039	0.07997717	4.917702	8.756587e-07
## ASV2231	0.2829096	0.3936553	0.07997705	4.922103	8.561918e-07
## ASV2327	0.3753376	-7.0656075	0.07994089	-88.385404	0.000000e+00
## ASV2394	0.4650160	-1.7610072	0.07989503	-22.041512	1.152256e-107
## ASV2424	0.3363945	-1.6973024	0.07989552	-21.244025	3.743926e-100
## ASV2705	0.2077731	-1.6030537	0.07989661	-20.064102	1.520126e-89
## ASV2731	0.2275610	-1.6207956	0.07989636	-20.286225	1.701718e-91
## ASV2772	0.3561824	-1.7085344	0.07989542	-21.384635	1.857351e-101
## ASV2797	0.3392474	-7.0118955	0.07994094	-87.713445	0.000000e+00
## ASV2836	0.1082705	-6.5613023	0.07994220	-82.075581	0.000000e+00
## ASV2941	0.2176670	-1.6121223	0.07989648	-20.177639	1.539260e-90
## ASV3021	0.2598491	-6.9152715	0.07994112	-86.504559	0.000000e+00
## ASV3067	0.1978791	-1.5934871	0.07989676	-19.944328	1.678912e-88
## ASV3172	0.2077731	-1.6030537	0.07989661	-20.064102	1.520126e-89
## ASV3304	0.1154885	-6.5785984	0.07994208	-82.292058	0.000000e+00
## ASV3330	0.1187275	-1.4944099	0.07989877	-18.703791	4.610671e-78
## ASV3479	0.1154885	-6.5785984	0.07994208	-82.292058	0.000000e+00
## ASV3480	0.1154885	-6.5785984	0.07994208	-82.292058	0.000000e+00
## ASV3530	0.1187275	-1.4944099	0.07989877	-18.703791	4.610671e-78
## ASV3688	0.0721803	-6.3967893	0.07994312	-80.016759	0.000000e+00

```

## ASV3788 0.0721803      -6.3967893 0.07994312 -80.016759 0.000000e+00
##          padj  Kingdom          Phylum          Class
## ASV1765 1.223420e-04 Bacteria Bacteroidetes Bacteroidia
## ASV1790 1.250333e-112 Bacteria Firmicutes Clostridia
## ASV1813 4.420848e-114 Bacteria Bacteroidetes Bacteroidia
## ASV1815 3.882554e-02 Bacteria Bacteroidetes Bacteroidia
## ASV1911 0.000000e+00 Bacteria Bacteroidetes Bacteroidia
## ASV1932 0.000000e+00 Bacteria Firmicutes Clostridia
## ASV1941 1.750864e-111 Bacteria Firmicutes Clostridia
## ASV2054 3.418906e-107 Bacteria Firmicutes Clostridia
## ASV2122 1.223420e-04 Bacteria Firmicutes Clostridia
## ASV2231 1.223420e-04 Bacteria Bacteroidetes Bacteroidia
## ASV2327 0.000000e+00 Bacteria Firmicutes Clostridia
## ASV2394 2.817265e-105 Bacteria Firmicutes Clostridia
## ASV2424 8.136800e-98 Bacteria Firmicutes Clostridia
## ASV2705 2.703061e-87 Bacteria Firmicutes Clostridia
## ASV2731 3.503748e-89 Bacteria Firmicutes Clostridia
## ASV2772 4.274093e-99 Bacteria Firmicutes Clostridia
## ASV2797 0.000000e+00 Bacteria Firmicutes Erysipelotrichia
## ASV2836 0.000000e+00 Bacteria Tenericutes Mollicutes
## ASV2941 3.010792e-88 Bacteria Firmicutes Clostridia
## ASV3021 0.000000e+00 Bacteria Bacteroidetes Bacteroidia
## ASV3067 2.855610e-86 Bacteria Firmicutes Clostridia
## ASV3172 2.703061e-87 Bacteria Firmicutes Clostridia
## ASV3304 0.000000e+00 Bacteria Firmicutes Clostridia
## ASV3330 7.214777e-76 Bacteria Bacteroidetes Bacteroidia
## ASV3479 0.000000e+00 Bacteria Tenericutes Mollicutes
## ASV3480 0.000000e+00 Bacteria Bacteroidetes Bacteroidia
## ASV3530 7.214777e-76 Bacteria Firmicutes Clostridia
## ASV3688 0.000000e+00 Bacteria Firmicutes Clostridia
## ASV3788 0.000000e+00 Bacteria Bacteroidetes Bacteroidia
##          Order          Family
## ASV1765 Bacteroidales Muribaculaceae
## ASV1790 Clostridiales Lachnospiraceae
## ASV1813 Bacteroidales Muribaculaceae
## ASV1815 Bacteroidales Muribaculaceae
## ASV1911 Bacteroidales Muribaculaceae
## ASV1932 Clostridiales Lachnospiraceae
## ASV1941 Clostridiales Lachnospiraceae
## ASV2054 Clostridiales Lachnospiraceae
## ASV2122 Clostridiales Peptostreptococcaceae
## ASV2231 Bacteroidales Muribaculaceae
## ASV2327 Clostridiales Lachnospiraceae
## ASV2394 Clostridiales Ruminococcaceae
## ASV2424 Clostridiales Lachnospiraceae
## ASV2705 Clostridiales Ruminococcaceae
## ASV2731 Clostridiales Lachnospiraceae
## ASV2772 Clostridiales Lachnospiraceae
## ASV2797 Erysipelotrichales Erysipelotrichaceae
## ASV2836 Mollicutes_RF39 <NA>
## ASV2941 Clostridiales Lachnospiraceae
## ASV3021 Bacteroidales Muribaculaceae
## ASV3067 Clostridiales Ruminococcaceae
## ASV3172 Clostridiales Lachnospiraceae

```

```

## ASV3304      Clostridiales      Ruminococcaceae
## ASV3330      Bacteroidales      <NA>
## ASV3479      Mollicutes_RF39    <NA>
## ASV3480      Bacteroidales      Rikenellaceae
## ASV3530      Clostridiales      Lachnospiraceae
## ASV3688      Clostridiales      Lachnospiraceae
## ASV3788      Bacteroidales      Muribaculaceae
##
##                                     Genus Species
## ASV1765                                     <NA> <NA>
## ASV1790 Lachnospiraceae_NK4A136_group <NA>
## ASV1813                                     <NA> <NA>
## ASV1815                                     <NA> <NA>
## ASV1911      Muribaculum          <NA>
## ASV1932                                     <NA> <NA>
## ASV1941                                     <NA> <NA>
## ASV2054      Lachnospiraceae_UCG-001 <NA>
## ASV2122      Romboutsia          <NA>
## ASV2231                                     <NA> <NA>
## ASV2327                                     <NA> <NA>
## ASV2394      Pseudoflavonifractor <NA>
## ASV2424      UC5-1-2E3           <NA>
## ASV2705      Ruminococcaceae_UCG-014 <NA>
## ASV2731      Acetatifactor       <NA>
## ASV2772      <NA>                <NA>
## ASV2797      Faecalibaculum      <NA>
## ASV2836      <NA>                <NA>
## ASV2941      <NA>                <NA>
## ASV3021      <NA>                <NA>
## ASV3067      Butyricicoccus       <NA>
## ASV3172      <NA>                <NA>
## ASV3304      Ruminiclostridium    <NA>
## ASV3330      <NA>                <NA>
## ASV3479      <NA>                <NA>
## ASV3480      Alistipes            <NA>
## ASV3530      <NA>                <NA>
## ASV3688      ASF356              <NA>
## ASV3788      <NA>                <NA>

```

```

## DESeq2 genus level analysis using FSTTimeImmobile
treatdds = phyloseq_to_deseq2(physeq_genus, ~FSTTimeImmobile)

```

```

## converting counts to integer mode

```

```

## Genus level table
FSTTimeImmobile_genus <- taxa_with_deseq(treatdds, physeq_genus)

```

```

## estimating size factors

```

```

## estimating dispersions

```

```

## gene-wise dispersion estimates

```

```

## mean-dispersion relationship

```



```

## final dispersion estimates

## fitting model and testing

## -- replacing outliers and refitting for 21 genes
## -- DESeq argument 'minReplicatesForReplace' = 7
## -- original counts are preserved in counts(dds)

## estimating dispersions

## fitting model and testing

print(FSTTimeImmobile_genus)

## [1] "No significant taxa were identified using the specified formula"

## Convert to deseq2 object using the specified covariate
treatdds = phyloseq_to_deseq2(phyloseq_object, ~NOObjpref)

## converting counts to integer mode

## Generated table containing ASVs
NOObjpref_ASVs <- taxa_with_deseq(treatdds, physeq1)

## estimating size factors

## estimating dispersions

## gene-wise dispersion estimates

## mean-dispersion relationship

## final dispersion estimates

## fitting model and testing

print(NOObjpref_ASVs)

##           baseMean log2FoldChange      lfcSE      stat      pvalue
## ASV500  6.20788724      0.4592145 0.09288720   4.943787 7.661945e-07
## ASV1243 1.50968017     -1.1585948 0.09338983  -12.406006 2.424473e-35
## ASV1557 0.60350292     -1.3496235 0.09345427  -14.441539 2.834433e-47
## ASV1636 0.57481768      0.3479243 0.09346714   3.722424 1.973193e-04
## ASV1751 0.43586322     -1.3173552 0.09345456  -14.096211 4.007170e-45
## ASV1800 0.81250650      0.3568153 0.09342789   3.819152 1.339113e-04
## ASV1894 0.60865496      0.3501445 0.09345804   3.746543 1.792886e-04
## ASV1921 0.64554313      0.3509319 0.09345508   3.755087 1.732815e-04
## ASV2232 0.41574645     -1.3118891 0.09345461  -14.037714 9.162091e-45
## ASV2255 0.32186822     -1.2937254 0.09345493  -13.843309 1.396284e-43

```

```

## ASV2603 0.26151793 -1.2804361 0.09345524 -13.701062 1.000504e-42
## ASV2775 0.19446205 -1.2576574 0.09345583 -13.457238 2.791573e-41
## ASV2813 0.20116764 -1.2616930 0.09345576 -13.500431 1.554639e-41
## ASV2853 0.17434529 -1.2577397 0.09345610 -13.458080 2.759939e-41
## ASV2888 0.14081735 -1.2567050 0.09345671 -13.446922 3.209578e-41
## ASV2916 0.17434529 -1.2577397 0.09345610 -13.458080 2.759939e-41
## ASV2917 0.13411176 -1.2433619 0.09345687 -13.304126 2.190409e-40
## ASV2946 0.16763970 -1.2316491 0.09345620 -13.178891 1.160840e-39
## ASV3043 0.12740617 -1.2333811 0.09345705 -13.197305 9.093039e-40
## ASV3044 0.14752294 -1.2570388 0.09345656 -13.450514 3.057412e-41
## ASV3088 0.15422852 -1.2521735 0.09345643 -13.398473 6.171977e-41
## ASV3189 0.12070058 -1.2375082 0.09345724 -13.241438 5.057147e-40
## ASV3248 0.15422852 -1.2521735 0.09345643 -13.398473 6.171977e-41
## ASV3249 0.10058382 -1.2405694 0.09345798 -13.274088 3.272295e-40
## ASV3440 0.08046706 -1.2109532 0.09345909 -12.957041 2.143574e-38
##
##          padj Kingdom      Phylum      Class
## ASV500  1.469634e-04 Bacteria Bacteroidetes Bacteroidia
## ASV1243 4.882889e-33 Bacteria Firmicutes Clostridia
## ASV1557 1.141709e-43 Bacteria Bacteroidetes Bacteroidia
## ASV1636 3.179208e-02 Bacteria Bacteroidetes Bacteroidia
## ASV1751 8.070440e-42 Bacteria Firmicutes Clostridia
## ASV1800 2.451794e-02 Bacteria Bacteroidetes Bacteroidia
## ASV1894 3.009060e-02 Bacteria Bacteroidetes Bacteroidia
## ASV1921 3.009060e-02 Bacteria Firmicutes Clostridia
## ASV2232 1.230163e-41 Bacteria Firmicutes Clostridia
## ASV2255 1.406058e-40 Bacteria Firmicutes Clostridia
## ASV2603 8.060061e-40 Bacteria Firmicutes Clostridia
## ASV2775 1.175289e-38 Bacteria Firmicutes Clostridia
## ASV2813 1.043681e-38 Bacteria Firmicutes Clostridia
## ASV2853 1.175289e-38 Bacteria Firmicutes Clostridia
## ASV2888 1.175289e-38 Bacteria Firmicutes Clostridia
## ASV2916 1.175289e-38 Bacteria Firmicutes Clostridia
## ASV2917 6.302119e-38 Bacteria Firmicutes Clostridia
## ASV2946 2.597702e-37 Bacteria Firmicutes Clostridia
## ASV3043 2.154515e-37 Bacteria Firmicutes Clostridia
## ASV3044 1.175289e-38 Bacteria Firmicutes Erysipelotrichia
## ASV3088 1.912363e-38 Bacteria Firmicutes Clostridia
## ASV3189 1.273137e-37 Bacteria Firmicutes Erysipelotrichia
## ASV3248 1.912363e-38 Bacteria Tenericutes Mollicutes
## ASV3249 8.787204e-38 Bacteria Firmicutes Clostridia
## ASV3440 4.544377e-36 Bacteria Firmicutes Clostridia
##
##          Order      Family
## ASV500      Bacteroidales Muribaculaceae
## ASV1243      Clostridiales Lachnospiraceae
## ASV1557      Bacteroidales Muribaculaceae
## ASV1636      Bacteroidales Muribaculaceae
## ASV1751      Clostridiales Lachnospiraceae
## ASV1800      Bacteroidales Muribaculaceae
## ASV1894      Bacteroidales Bacteroidaceae
## ASV1921      Clostridiales Lachnospiraceae
## ASV2232      Clostridiales Lachnospiraceae
## ASV2255      Clostridiales Lachnospiraceae
## ASV2603      Clostridiales Lachnospiraceae
## ASV2775      Clostridiales Ruminococcaceae

```

```

## ASV2813      Clostridiales      Ruminococcaceae
## ASV2853      Clostridiales      Lachnospiraceae
## ASV2888      Clostridiales      Lachnospiraceae
## ASV2916      Clostridiales      Lachnospiraceae
## ASV2917      Clostridiales      Ruminococcaceae
## ASV2946      Clostridiales      Lachnospiraceae
## ASV3043      Clostridiales Clostridiales_vadinBB60_group
## ASV3044 Erysipelotrichales      Erysipelotrichaceae
## ASV3088      Clostridiales      <NA>
## ASV3189 Erysipelotrichales      Erysipelotrichaceae
## ASV3248      Mollicutes_RF39      <NA>
## ASV3249      Clostridiales      Ruminococcaceae
## ASV3440      Clostridiales      Ruminococcaceae
##
##                               Genus Species
## ASV500              <NA> <NA>
## ASV1243              <NA> <NA>
## ASV1557              <NA> <NA>
## ASV1636              <NA> <NA>
## ASV1751              <NA> <NA>
## ASV1800              <NA> <NA>
## ASV1894      Bacteroides <NA>
## ASV1921              <NA> <NA>
## ASV2232              <NA> <NA>
## ASV2255              <NA> <NA>
## ASV2603              <NA> <NA>
## ASV2775      Pseudoflavonifractor <NA>
## ASV2813      Ruminiclostridium <NA>
## ASV2853              <NA> <NA>
## ASV2888              <NA> <NA>
## ASV2916      Acetatifactor <NA>
## ASV2917      Ruminiclostridium <NA>
## ASV2946      UC5-1-2E3 <NA>
## ASV3043              <NA> <NA>
## ASV3044      Erysipelatoclostridium <NA>
## ASV3088              <NA> <NA>
## ASV3189      Candidatus_Stoquefichus <NA>
## ASV3248              <NA> <NA>
## ASV3249      Ruminococcaceae_UCG-014 <NA>
## ASV3440      Hydrogenoanaerobacterium <NA>

```

```

## DESeq2 analysis using NOObjpref
treatdds = phyloseq_to_deseq2(physeq_genus, ~NOObjpref)

```

```
## converting counts to integer mode
```

```

## Genus level table
NOObjpref_genus <- taxa_with_deseq(treatdds, physeq_genus)

```

```
## estimating size factors
```

```
## estimating dispersions
```

```
## gene-wise dispersion estimates
```

```

## mean-dispersion relationship

## final dispersion estimates

## fitting model and testing

print(NOObjpref_genus)

## [1] "No significant taxa were identified using the specified formula"

## Convert to deseq2 object using the specified covariate
treatdds = phyloseq_to_deseq2(phyloseq_object, ~OFCentDur1)

## converting counts to integer mode

## Generated table containing ASVs
OFCentDur1_ASVs <- taxa_with_deseq(treatdds, physeq1)

## estimating size factors

## estimating dispersions

## gene-wise dispersion estimates

## mean-dispersion relationship

## final dispersion estimates

## fitting model and testing

print(OFCentDur1_ASVs)

##           baseMean log2FoldChange      lfcSE      stat      pvalue
## ASV1721 0.53495319      1.2165659 0.2138573 5.688681 1.280245e-08
## ASV1862 0.63371378      1.2246116 0.2138503 5.726490 1.025299e-08
## ASV1903 0.36930268      0.8755349 0.2141130 4.089126 4.330011e-05
## ASV2009 0.39102637      0.8773798 0.2141126 4.097750 4.171860e-05
## ASV2029 0.31137285      0.8700311 0.2141142 4.063397 4.836368e-05
## ASV2273 0.28964916      0.8676969 0.2141148 4.052484 5.067669e-05
## ASV2305 0.33309653      0.8722056 0.2141137 4.073563 4.629937e-05
## ASV2306 0.24620179      0.8624605 0.2141163 4.028000 5.625324e-05
## ASV2371 0.19551318      0.8550288 0.2141188 3.993245 6.517515e-05
## ASV2414 0.28240793      0.8668832 0.2141150 4.048680 5.150739e-05
## ASV2523 0.28240793      0.8668832 0.2141150 4.048680 5.150739e-05
## ASV2803 0.14482458      0.8453467 0.2141229 3.947951 7.882309e-05
## ASV3075 0.10861844      0.8360500 0.2141280 3.904440 9.444387e-05
## ASV3104 0.13758335      0.8436916 0.2141237 3.940206 8.141176e-05
## ASV3261 0.13758335      0.8436916 0.2141237 3.940206 8.141176e-05
## ASV3385 0.11585966      0.8381377 0.2141268 3.914213 9.069964e-05

```

##	ASV3451	0.07965352	0.8259917	0.2141352	3.857337	1.146292e-04
##	ASV3489	0.06517106	0.8194667	0.2141411	3.826761	1.298404e-04
##	ASV3490	0.04344737	0.8062047	0.2141567	3.764555	1.668457e-04
##	ASV3491	0.07965352	0.8259917	0.2141352	3.857337	1.146292e-04
##	ASV3595	0.07965352	0.8259917	0.2141352	3.857337	1.146292e-04
##	ASV3663	0.05792983	0.8156218	0.2141450	3.808736	1.396792e-04
##	ASV3739	0.03620615	0.8002043	0.2141658	3.736377	1.866905e-04
##		padj	Kingdom	Phylum		Class
##	ASV1721	2.578414e-05	Bacteria	Bacteroidetes		Bacteroidia
##	ASV1862	2.578414e-05	Bacteria	Bacteroidetes		Bacteroidia
##	ASV1903	2.265880e-02	Bacteria	Firmicutes		Clostridia
##	ASV2009	2.265880e-02	Bacteria	Firmicutes		Clostridia
##	ASV2029	2.265880e-02	Bacteria	Firmicutes		Clostridia
##	ASV2273	2.265880e-02	Bacteria	Proteobacteria	Deltaproteobacteria	
##	ASV2305	2.265880e-02	Bacteria	Firmicutes		Clostridia
##	ASV2306	2.265880e-02	Bacteria	Firmicutes		Clostridia
##	ASV2371	2.342333e-02	Bacteria	Firmicutes		Clostridia
##	ASV2414	2.265880e-02	Bacteria	Firmicutes		Clostridia
##	ASV2523	2.265880e-02	Bacteria	Firmicutes		Clostridia
##	ASV2803	2.342333e-02	Bacteria	Firmicutes		Clostridia
##	ASV3075	2.377625e-02	Bacteria	Firmicutes		Clostridia
##	ASV3104	2.342333e-02	Bacteria	<NA>		<NA>
##	ASV3261	2.342333e-02	Bacteria	Firmicutes		Clostridia
##	ASV3385	2.377625e-02	Bacteria	Firmicutes		Clostridia
##	ASV3451	2.430140e-02	Bacteria	Firmicutes		Clostridia
##	ASV3489	2.614986e-02	Bacteria	Tenericutes		Mollicutes
##	ASV3490	3.054792e-02	Bacteria	Firmicutes		Clostridia
##	ASV3491	2.430140e-02	Bacteria	Bacteroidetes		Bacteroidia
##	ASV3595	2.430140e-02	Bacteria	Firmicutes		Clostridia
##	ASV3663	2.679179e-02	Bacteria	Firmicutes		Clostridia
##	ASV3739	3.269518e-02	Bacteria	Firmicutes	Erysipelotrichia	
##			Order	Family		
##	ASV1721		Bacteroidales	Muribaculaceae		
##	ASV1862		Bacteroidales	Rikenellaceae		
##	ASV1903		Clostridiales	Lachnospiraceae		
##	ASV2009		Clostridiales	Lachnospiraceae		
##	ASV2029		Clostridiales	Lachnospiraceae		
##	ASV2273	Desulfovibrionales		Desulfovibrionaceae		
##	ASV2305		Clostridiales	Lachnospiraceae		
##	ASV2306		Clostridiales	Ruminococcaceae		
##	ASV2371		Clostridiales	Lachnospiraceae		
##	ASV2414		Clostridiales	Lachnospiraceae		
##	ASV2523		Clostridiales	Ruminococcaceae		
##	ASV2803		Clostridiales	Lachnospiraceae		
##	ASV3075		Clostridiales	Lachnospiraceae		
##	ASV3104		<NA>	<NA>		
##	ASV3261		Clostridiales	Ruminococcaceae		
##	ASV3385		Clostridiales	Ruminococcaceae		
##	ASV3451		Clostridiales	Lachnospiraceae		
##	ASV3489	Mollicutes_RF39		<NA>		
##	ASV3490		Clostridiales	Lachnospiraceae		
##	ASV3491		Bacteroidales	Muribaculaceae		
##	ASV3595		Clostridiales	Lachnospiraceae		
##	ASV3663		Clostridiales	Ruminococcaceae		

```
## ASV3739 Erysipelotrichales Erysipelotrichaceae
##                               Genus Species
## ASV1721                       <NA>  <NA>
## ASV1862  Rikenellaceae_RC9_gut_group  <NA>
## ASV1903                       <NA>  <NA>
## ASV2009          Lachnoclostridium  <NA>
## ASV2029                       <NA>  <NA>
## ASV2273                       <NA>  <NA>
## ASV2305                       UC5-1-2E3  <NA>
## ASV2306  Ruminococcaceae_UCG-014  <NA>
## ASV2371                       <NA>  <NA>
## ASV2414                       <NA>  <NA>
## ASV2523          Pseudoflavonifractor  <NA>
## ASV2803                       <NA>  <NA>
## ASV3075                       <NA>  <NA>
## ASV3104                       <NA>  <NA>
## ASV3261          Ruminiclostridium  <NA>
## ASV3385  Ruminococcaceae_NK4A214_group  <NA>
## ASV3451                       <NA>  <NA>
## ASV3489                       <NA>  <NA>
## ASV3490          Tyzzerella_3  <NA>
## ASV3491          CAG-873  <NA>
## ASV3595                       <NA>  <NA>
## ASV3663  Ruminococcaceae_UCG-014  <NA>
## ASV3739          Dubosiella  <NA>
```

```
## DESeq2 analysis using OFCentDur1
```

```
treatdds = phyloseq_to_deseq2(physeq_genus, ~OFCentDur1)
```

```
## converting counts to integer mode
```

```
## Genus level table
```

```
OFCentDur1_genus <- taxa_with_deseq(treatdds, physeq_genus)
```

```
## estimating size factors
```

```
## estimating dispersions
```

```
## gene-wise dispersion estimates
```

```
## mean-dispersion relationship
```

```
## final dispersion estimates
```

```
## fitting model and testing
```

```
print(OFCentDur1_genus)
```

```
## [1] "No significant taxa were identified using the specified formula"
```

```
## Convert to deseq2 object using the specified covariate
treatdds = phyloseq_to_deseq2(phyloseq_object, ~Treatment)
```

```
## converting counts to integer mode
```

```
## Generated table containing ASVs
Treatment_ASVs <- taxa_with_deseq(treatdds, physeq1)
```

```
## estimating size factors
```

```
## estimating dispersions
```

```
## gene-wise dispersion estimates
```

```
## mean-dispersion relationship
```

```
## final dispersion estimates
```

```
## fitting model and testing
```

```
## -- replacing outliers and refitting for 2661 genes
## -- DESeq argument 'minReplicatesForReplace' = 7
## -- original counts are preserved in counts(dds)
```

```
## estimating dispersions
```

```
## fitting model and testing
```

```
print(Treatment_ASVs)
```

##	baseMean	log2FoldChange	lfcSE	stat	pvalue
## ASV231	19.452021	22.60109	2.266601	9.971357	2.034310e-23
## ASV232	4.282183	-23.30934	4.212114	-5.533881	3.132209e-08
## ASV299	8.217898	21.82570	3.040557	7.178193	7.063897e-13
## ASV313	7.172216	-23.53595	4.211867	-5.588009	2.296874e-08
## ASV324	12.038005	-23.80201	2.593344	-9.178114	4.387066e-20
## ASV331	5.428083	-23.59893	4.212008	-5.602776	2.109461e-08
## ASV337	12.472891	-24.23369	2.852470	-8.495684	1.967711e-17
## ASV353	11.669592	22.05754	4.125537	5.346586	8.962857e-08
## ASV377	10.208945	22.17057	2.980220	7.439238	1.012680e-13
## ASV384	9.082391	21.31724	3.240717	6.577939	4.770137e-11
## ASV387	7.633003	21.61720	3.541228	6.104436	1.031643e-09
## ASV397	10.900271	21.74084	3.338417	6.512321	7.399828e-11
## ASV411	9.162846	-23.94111	3.031450	-7.897579	2.843736e-15
## ASV429	12.046382	-24.83252	3.666838	-6.772191	1.268464e-11
## ASV434	7.680128	21.22428	3.765960	5.635822	1.742250e-08
## ASV436	9.773793	19.42119	3.529868	5.501960	3.755913e-08
## ASV520	6.671459	19.87999	3.449390	5.763334	8.246853e-09
## ASV552	6.355310	20.13960	3.918113	5.140126	2.745538e-07

```

## ASV577 5.189219 -23.36503 4.212030 -5.547213 2.902592e-08
## ASV589 6.905267 17.75115 3.596291 4.935960 7.975760e-07
## padj Kingdom Phylum Class
## ASV231 4.780628e-20 Bacteria Bacteroidetes Bacteroidia
## ASV232 4.600432e-06 Bacteria Bacteroidetes Bacteroidia
## ASV299 2.766693e-10 Bacteria Firmicutes Clostridia
## ASV313 3.855467e-06 Bacteria Firmicutes Clostridia
## ASV324 5.154803e-17 Bacteria Bacteroidetes Bacteroidia
## ASV331 3.813256e-06 Bacteria Firmicutes Clostridia
## ASV337 1.541374e-14 Bacteria Firmicutes Clostridia
## ASV353 1.170151e-05 Bacteria Firmicutes Erysipelotrichia
## ASV377 4.759596e-11 Bacteria Bacteroidetes Bacteroidia
## ASV384 1.401228e-08 Bacteria Bacteroidetes Bacteroidia
## ASV387 2.424360e-07 Bacteria Firmicutes Bacilli
## ASV397 1.932177e-08 Bacteria Firmicutes Clostridia
## ASV411 1.670695e-12 Bacteria Bacteroidetes Bacteroidia
## ASV429 4.258416e-09 Bacteria Firmicutes Clostridia
## ASV434 3.411907e-06 Bacteria Firmicutes Clostridia
## ASV436 5.191997e-06 Bacteria Firmicutes Clostridia
## ASV520 1.761828e-06 Bacteria Firmicutes Clostridia
## ASV552 3.395797e-05 Bacteria Firmicutes Erysipelotrichia
## ASV577 4.547394e-06 Bacteria Bacteroidetes Bacteroidia
## ASV589 9.371518e-05 Bacteria Firmicutes Clostridia
## Order Family
## ASV231 Bacteroidales Rikenellaceae
## ASV232 Bacteroidales Muribaculaceae
## ASV299 Clostridiales Lachnospiraceae
## ASV313 Clostridiales Lachnospiraceae
## ASV324 Bacteroidales Rikenellaceae
## ASV331 Clostridiales Lachnospiraceae
## ASV337 Clostridiales Lachnospiraceae
## ASV353 Erysipelotrichales Erysipelotrichaceae
## ASV377 Bacteroidales Rikenellaceae
## ASV384 Bacteroidales Muribaculaceae
## ASV387 Lactobacillales Lactobacillaceae
## ASV397 Clostridiales Lachnospiraceae
## ASV411 Bacteroidales Prevotellaceae
## ASV429 Clostridiales Ruminococcaceae
## ASV434 Clostridiales Lachnospiraceae
## ASV436 Clostridiales Lachnospiraceae
## ASV520 Clostridiales Lachnospiraceae
## ASV552 Erysipelotrichales Erysipelotrichaceae
## ASV577 Bacteroidales Muribaculaceae
## ASV589 Clostridiales Lachnospiraceae
## Genus Species
## ASV231 Alistipes <NA>
## ASV232 <NA> <NA>
## ASV299 <NA> <NA>
## ASV313 <NA> <NA>
## ASV324 Alistipes <NA>
## ASV331 <NA> <NA>
## ASV337 <NA> <NA>
## ASV353 Dubosiella <NA>
## ASV377 Alistipes <NA>

```



```
## ASV384                <NA>    <NA>
## ASV387                Lactobacillus <NA>
## ASV397                <NA>    <NA>
## ASV411 Prevotellaceae_NK3B31_group <NA>
## ASV429                <NA>    <NA>
## ASV434                <NA>    <NA>
## ASV436 Lachnospiraceae_NK4A136_group <NA>
## ASV520                <NA>    <NA>
## ASV552                Dubosiella <NA>
## ASV577                <NA>    <NA>
## ASV589                <NA>    <NA>
```

```
## DESeq2 analysis using Treatment
```

```
treatdds = phyloseq_to_deseq2(physeq_genus, ~Treatment)
```

```
## converting counts to integer mode
```

```
## the design formula contains a numeric variable with integer values,
## specifying a model with increasing fold change for higher values.
## did you mean for this to be a factor? if so, first convert
## this variable to a factor using the factor() function
```

```
## Genus level table
```

```
Treatment_genus <- taxa_with_deseq(treatdds, physeq_genus)
```

```
## estimating size factors
```

```
## estimating dispersions
```

```
## gene-wise dispersion estimates
```

```
## mean-dispersion relationship
```

```
## final dispersion estimates
```

```
## fitting model and testing
```

```
## -- replacing outliers and refitting for 18 genes
## -- DESeq argument 'minReplicatesForReplace' = 7
## -- original counts are preserved in counts(dds)
```

```
## estimating dispersions
```

```
## fitting model and testing
```

```
print(Treatment_genus)
```

```
##          baseMean log2FoldChange      lfcSE      stat      pvalue
## ASV656 31.87192      -0.01927555 0.005205118 -3.703192 0.0002129035
##          padj  Kingdom      Phylum      Class      Order
## ASV656 0.01809679 Bacteria Tenericutes Mollicutes Anaeroplasmatales
##          Family      Genus Species
## ASV656 Anaeroplasmataceae Anaeroplasma      <NA>
```

```
## Convert to deseq2 object using the specified covariate
treatdds = phyloseq_to_deseq2(phyloseq_object, ~Sex)
```

```
## converting counts to integer mode
```

```
## Warning in DESeqDataSet(se, design = design, ignoreRank): some variables in
## design formula are characters, converting to factors
```

```
## Generated table containing ASVs
Sex_ASVs <- taxa_with_deseq(treatdds, physeq1)
```

```
## estimating size factors
```

```
## estimating dispersions
```

```
## gene-wise dispersion estimates
```

```
## mean-dispersion relationship
```

```
## final dispersion estimates
```

```
## fitting model and testing
```

```
## -- replacing outliers and refitting for 2888 genes
## -- DESeq argument 'minReplicatesForReplace' = 7
## -- original counts are preserved in counts(dds)
```

```
## estimating dispersions
```

```
## fitting model and testing
```

```
print(Sex_ASVs)
```

```
##          baseMean log2FoldChange      lfcSE      stat      pvalue
## ASV260 16.70038      -7.417883 1.532407 -4.840675 1.293991e-06
## ASV265 15.31345      -7.292913 1.460644 -4.992943 5.946624e-07
## ASV269 13.87368      -7.150633 1.455337 -4.913387 8.951612e-07
## ASV298 12.40779      23.055573 1.885030 12.230878 2.126269e-34
##          padj  Kingdom      Phylum      Class
## ASV260 7.388690e-04 Bacteria Firmicutes Erysipelotrichia
## ASV265 6.791045e-04 Bacteria Firmicutes Erysipelotrichia
## ASV269 6.815160e-04 Bacteria Firmicutes Erysipelotrichia
```

```
## ASV298 4.856399e-31 Bacteria Bacteroidetes Bacteroidia
## Order Family Genus
## ASV260 Erysipelotrichales Erysipelotrichaceae Turicibacter
## ASV265 Erysipelotrichales Erysipelotrichaceae Turicibacter
## ASV269 Erysipelotrichales Erysipelotrichaceae Turicibacter
## ASV298 Bacteroidales Prevotellaceae Prevotellaceae_NK3B31_group
## Species
## ASV260 <NA>
## ASV265 <NA>
## ASV269 <NA>
## ASV298 <NA>
```

```
## DESeq2 analysis using Sex
```

```
treatdds = phyloseq_to_deseq2(physeq_genus, ~Sex)
```

```
## converting counts to integer mode
```

```
## Warning in DESeqDataSet(se, design = design, ignoreRank): some variables in
## design formula are characters, converting to factors
```

```
## Genus level table
```

```
Sex_genus <- taxa_with_deseq(treatdds, physeq_genus)
```

```
## estimating size factors
```

```
## estimating dispersions
```

```
## gene-wise dispersion estimates
```

```
## mean-dispersion relationship
```

```
## final dispersion estimates
```

```
## fitting model and testing
```

```
## -- replacing outliers and refitting for 20 genes
```

```
## -- DESeq argument 'minReplicatesForReplace' = 7
```

```
## -- original counts are preserved in counts(dds)
```

```
## estimating dispersions
```

```
## fitting model and testing
```

```
print(Sex_genus)
```

```
## baseMean log2FoldChange lfcSE stat pvalue
## ASV7 2508.592285 0.4027100 0.1528421 2.634810 8.418438e-03
## ASV44 2120.396628 -0.4422172 0.1071861 -4.125696 3.696156e-05
## ASV87 739.792834 -0.5299846 0.1878351 -2.821543 4.779329e-03
```

```

## ASV128 316.072736 -0.9304670 0.2132914 -4.362422 1.286303e-05
## ASV192 200.804720 -2.5341245 0.6353693 -3.988427 6.651277e-05
## ASV260 146.200684 -8.4655991 0.8014124 -10.563349 4.406532e-26
## ASV288 162.723032 2.4488858 0.7522005 3.255629 1.131414e-03
## ASV319 103.343562 -0.7827188 0.3133416 -2.497973 1.249057e-02
## ASV867 10.634969 23.4918836 1.8294884 12.840685 9.701288e-38
## ASV987 9.299354 -24.7213461 2.1052376 -11.742782 7.691428e-32
## ASV1574 18.113569 -1.9675515 0.8004211 -2.458145 1.396566e-02
##      padj Kingdom      Phylum      Class
## ASV7 7.295979e-02 Bacteria Bacteroidetes Bacteroidia
## ASV44 5.766004e-04 Bacteria Bacteroidetes Bacteroidia
## ASV87 4.659845e-02 Bacteria Proteobacteria Gammaproteobacteria
## ASV128 2.508292e-04 Bacteria Bacteroidetes Bacteroidia
## ASV192 8.646660e-04 Bacteria Firmicutes Erysipelotrichia
## ASV260 1.145698e-24 Bacteria Firmicutes Erysipelotrichia
## ASV288 1.260719e-02 Bacteria Bacteroidetes Bacteroidia
## ASV319 9.742641e-02 Bacteria Bacteroidetes Bacteroidia
## ASV867 7.567004e-36 Bacteria Tenericutes Mollicutes
## ASV987 2.999657e-30 Bacteria Firmicutes Clostridia
## ASV1574 9.902924e-02 Bacteria Firmicutes Clostridia
##      Order      Family
## ASV7 Bacteroidales Prevotellaceae
## ASV44 Bacteroidales Bacteroidaceae
## ASV87 Betaproteobacteriales Burkholderiaceae
## ASV128 Bacteroidales Muribaculaceae
## ASV192 Erysipelotrichales Erysipelotrichaceae
## ASV260 Erysipelotrichales Erysipelotrichaceae
## ASV288 Bacteroidales Prevotellaceae
## ASV319 Bacteroidales Tannerellaceae
## ASV867 Mycoplasmatales Mycoplasmataceae
## ASV987 Clostridiales Peptostreptococcaceae
## ASV1574 Clostridiales Lachnospiraceae
##      Genus Species
## ASV7 Prevotellaceae_UCG-001 <NA>
## ASV44 Bacteroides <NA>
## ASV87 Parasutterella <NA>
## ASV128 Muribaculum <NA>
## ASV192 Faecalibaculum <NA>
## ASV260 Turicibacter <NA>
## ASV288 Prevotellaceae_NK3B31_group <NA>
## ASV319 Parabacteroides <NA>
## ASV867 Mycoplasma <NA>
## ASV987 Romboutsia <NA>
## ASV1574 ASF356 <NA>

```

```

## Convert to deseq2 object using the specified covariate
treatdds = phyloseq_to_deseq2(phyloseq_object, ~Treatment*Sex)

```

```
## converting counts to integer mode
```

```

## Warning in DESeqDataSet(se, design = design, ignoreRank): some variables in
## design formula are characters, converting to factors

```

```
## Generated table containing ASVs
Treatment_Sex_ASVs <- taxa_with_deseq(treatdds, physeq1)
```

```
## estimating size factors
```

```
## estimating dispersions
```

```
## gene-wise dispersion estimates
```

```
## mean-dispersion relationship
```

```
## final dispersion estimates
```

```
## fitting model and testing
```

```
## -- replacing outliers and refitting for 2212 genes
```

```
## -- DESeq argument 'minReplicatesForReplace' = 7
```

```
## -- original counts are preserved in counts(dds)
```

```
## estimating dispersions
```

```
## fitting model and testing
```

```
print(Treatment_Sex_ASVs)
```

##	baseMean	log2FoldChange	lfcSE	stat	pvalue
## ASV92	40.788278	-21.88526	4.256127	-5.142059	2.717444e-07
## ASV127	30.005054	-23.75793	3.899252	-6.092947	1.108505e-09
## ASV128	33.501420	-24.45868	4.747370	-5.152048	2.576571e-07
## ASV140	27.850127	21.57201	5.806267	3.715298	2.029644e-04
## ASV142	30.073764	-22.81454	4.258801	-5.357035	8.459888e-08
## ASV157	22.939508	19.54152	5.756690	3.394576	6.873507e-04
## ASV160	27.674229	-21.19735	5.374278	-3.944222	8.005960e-05
## ASV161	27.366101	-22.05439	5.009274	-4.402712	1.069062e-05
## ASV172	27.852568	23.82893	6.269147	3.800985	1.441220e-04
## ASV183	26.133628	22.14207	5.797501	3.819243	1.338617e-04
## ASV191	21.401364	-23.39099	4.010532	-5.832391	5.463882e-09
## ASV192	19.946870	18.63528	4.492636	4.147962	3.354483e-05
## ASV195	23.288142	20.96365	5.323544	3.937913	8.219355e-05
## ASV199	20.467950	18.93073	5.694065	3.324643	8.853188e-04
## ASV203	24.940862	-17.78342	5.754500	-3.090350	1.999210e-03
## ASV204	20.778784	-17.73669	4.810729	-3.686904	2.269990e-04
## ASV211	20.771199	21.06672	5.433026	3.877529	1.055226e-04
## ASV212	21.326545	-20.65006	5.334932	-3.870727	1.085113e-04
## ASV219	20.473814	-16.33240	4.640061	-3.519867	4.317628e-04
## ASV220	17.184595	-19.56264	3.757744	-5.205954	1.930026e-07
## ASV222	20.169315	-18.44906	4.008015	-4.603041	4.163658e-06
## ASV231	19.452021	16.60726	4.660717	3.563241	3.663048e-04
## ASV233	16.725268	-18.14924	6.191627	-2.931256	3.375950e-03
## ASV247	17.467786	23.71060	6.117667	3.875758	1.062931e-04

## ASV264	14.947258	-23.44920	6.661774	-3.519964	4.316059e-04
## ASV271	18.570251	23.32346	6.339944	3.678811	2.343240e-04
## ASV274	14.127895	-18.16153	6.837128	-2.656309	7.900107e-03
## ASV275	7.116703	-30.00000	8.433682	-3.557165	3.748785e-04
## ASV290	16.672297	23.08801	6.706274	3.442747	5.758368e-04
## ASV294	17.767055	-25.42870	7.011446	-3.626741	2.870210e-04
## ASV296	12.069258	20.65236	6.818787	3.028744	2.455725e-03
## ASV304	13.912721	-16.10510	5.443420	-2.958636	3.090043e-03
## ASV306	11.535194	30.00000	6.513100	4.606101	4.102885e-06
## ASV310	13.291837	22.26429	7.293079	3.052797	2.267190e-03
## ASV312	12.641837	-15.61060	5.892949	-2.649030	8.072310e-03
## ASV317	12.789149	21.40451	5.986788	3.575291	3.498383e-04
## ASV320	11.923869	-19.97904	6.975419	-2.864206	4.180560e-03
## ASV322	12.152548	-18.19519	6.051011	-3.006967	2.638688e-03
## ASV331	14.192278	21.87476	7.632462	2.866016	4.156734e-03
## ASV334	11.550795	20.48407	6.344076	3.228850	1.242889e-03
## ASV338	11.479858	-20.63103	6.914226	-2.983853	2.846437e-03
## ASV341	11.170571	18.30776	5.342702	3.426686	6.109953e-04
## ASV362	9.261070	-16.91949	6.364295	-2.658502	7.848878e-03
## ASV384	10.432443	-19.51685	6.438741	-3.031160	2.436165e-03
## ASV385	8.757346	30.00000	7.050990	4.254722	2.093090e-05
## ASV387	9.408915	-22.82341	7.689385	-2.968171	2.995777e-03
## ASV409	9.152697	18.83184	6.689765	2.815022	4.877388e-03
## ASV412	9.815802	-21.67432	5.442199	-3.982641	6.815366e-05
## ASV417	9.411239	22.96084	6.888078	3.333417	8.578620e-04
## ASV425	9.453970	-24.98572	7.879725	-3.170887	1.519741e-03
## ASV428	9.985177	-23.74258	7.556819	-3.141875	1.678697e-03
## ASV433	7.321738	30.00000	5.850934	5.127387	2.937914e-07
## ASV437	7.075914	-19.99844	5.860563	-3.412375	6.439950e-04
## ASV439	7.078955	-20.80996	5.318522	-3.912734	9.125701e-05
## ASV441	8.972203	19.36385	5.482299	3.532067	4.123244e-04
## ASV442	9.144735	-18.99541	6.667909	-2.848781	4.388709e-03
## ASV444	9.422055	-23.39056	5.638958	-4.148029	3.353496e-05
## ASV453	4.704482	-17.98533	6.258443	-2.873771	4.056028e-03
## ASV458	9.176012	-23.56381	8.334394	-2.827297	4.694277e-03
## ASV462	7.718984	-21.95486	6.259517	-3.507436	4.524469e-04
## ASV464	9.791481	-30.00000	6.438987	-4.659118	3.175677e-06
## ASV468	7.483057	-23.69893	6.976428	-3.397000	6.812904e-04
## ASV472	7.232254	-26.41714	7.677115	-3.441024	5.795162e-04
## ASV480	7.854096	-30.00000	6.701459	-4.476637	7.582796e-06
## ASV496	5.691803	30.00000	7.146855	4.197651	2.696980e-05
## ASV501	6.771012	19.77538	6.472832	3.055135	2.249593e-03
## ASV511	8.813910	-24.08101	7.455666	-3.229894	1.238360e-03
## ASV517	6.744208	16.70485	5.972067	2.797164	5.155343e-03
## ASV522	1.773877	30.00000	8.432665	3.557594	3.742668e-04
## ASV540	6.124648	-23.93052	6.889485	-3.473485	5.137457e-04
## ASV549	3.734182	30.00000	8.433113	3.557405	3.745363e-04
## ASV556	5.770589	-30.00000	8.432252	-3.557768	3.740189e-04
## ASV563	6.759062	30.00000	7.461455	4.020663	5.803446e-05
## ASV577	6.252781	-23.48907	8.412731	-2.792087	5.236934e-03
## ASV578	8.077157	30.00000	8.010084	3.745279	1.801933e-04
## ASV594	5.900691	-30.00000	8.431446	-3.558108	3.735350e-04
## ASV601	6.354541	-30.00000	7.008610	-4.280449	1.865165e-05
## ASV609	6.840244	-22.55258	7.061420	-3.193775	1.404256e-03

##	ASV759	4.248739	-30.00000	8.431934	-3.557903	3.738276e-04
##	ASV773	3.350300	-30.00000	8.431781	-3.557967	3.737358e-04
##	ASV860	3.416710	-30.00000	8.432009	-3.557871	3.738732e-04
##	ASV981	3.103861	-30.00000	8.432592	-3.557625	3.742229e-04
##		padj	Kingdom	Phylum	Class	
##	ASV92	4.125670e-05	Bacteria	Firmicutes	Clostridia	
##	ASV127	1.089660e-06	Bacteria	Firmicutes	Clostridia	
##	ASV128	4.125670e-05	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV140	6.879794e-03	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV142	2.772023e-05	Bacteria	Firmicutes	Clostridia	
##	ASV157	1.251233e-02	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV160	3.847441e-03	Bacteria	Firmicutes	Clostridia	
##	ASV161	8.757400e-04	Bacteria	Firmicutes	Clostridia	
##	ASV172	5.247110e-03	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV183	5.061001e-03	Bacteria	Firmicutes	Clostridia	
##	ASV191	2.685498e-06	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV192	1.939681e-03	Bacteria	Firmicutes	Erysipelotrichia	
##	ASV195	3.847441e-03	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV199	1.554051e-02	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV203	3.169714e-02	Bacteria	Firmicutes	Clostridia	
##	ASV204	7.430338e-03	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV211	4.266663e-03	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV212	4.266663e-03	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV219	9.226583e-03	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV220	4.125670e-05	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV222	4.092876e-04	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV231	8.569898e-03	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV233	4.674026e-02	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV247	4.266663e-03	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV264	9.226583e-03	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV271	7.430338e-03	Bacteria	Firmicutes	Clostridia	
##	ASV274	9.587414e-02	Bacteria	Actinobacteria	Actinobacteria	
##	ASV275	8.569898e-03	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV290	1.139329e-02	Bacteria	Proteobacteria	Deltaproteobacteria	
##	ASV294	8.569898e-03	Bacteria	Firmicutes	Clostridia	
##	ASV296	3.657543e-02	Bacteria	Firmicutes	Erysipelotrichia	
##	ASV304	4.339303e-02	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV306	4.092876e-04	Bacteria	Firmicutes	Clostridia	
##	ASV310	3.482261e-02	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV312	9.676927e-02	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV317	8.569898e-03	Bacteria	Firmicutes	Clostridia	
##	ASV320	5.553365e-02	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV322	3.871389e-02	Bacteria	Firmicutes	Clostridia	
##	ASV331	5.553365e-02	Bacteria	Firmicutes	Clostridia	
##	ASV334	2.106483e-02	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV338	4.114775e-02	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV341	1.177664e-02	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV362	9.587414e-02	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV384	3.657543e-02	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV385	1.469648e-03	Bacteria	Firmicutes	Clostridia	
##	ASV387	4.267897e-02	Bacteria	Firmicutes	Bacilli	
##	ASV409	6.226587e-02	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV412	3.526055e-03	Bacteria	Bacteroidetes	Bacteroidia	
##	ASV417	1.533233e-02	Bacteria	Proteobacteria	Deltaproteobacteria	

##	ASV425	2.489843e-02	Bacteria	Bacteroidetes	Bacteroidia
##	ASV428	2.705178e-02	Bacteria	Firmicutes	Clostridia
##	ASV433	4.125670e-05	Bacteria	Bacteroidetes	Bacteroidia
##	ASV437	1.217398e-02	Bacteria	Bacteroidetes	Bacteroidia
##	ASV439	4.077529e-03	Bacteria	Bacteroidetes	Bacteroidia
##	ASV441	9.211703e-03	Bacteria	Proteobacteria	Deltaproteobacteria
##	ASV442	5.752134e-02	Bacteria	Bacteroidetes	Bacteroidia
##	ASV444	1.939681e-03	Bacteria	Bacteroidetes	Bacteroidia
##	ASV453	5.537604e-02	Bacteria	Proteobacteria	<NA>
##	ASV458	6.071677e-02	Bacteria	Firmicutes	Bacilli
##	ASV462	9.462878e-03	Bacteria	Firmicutes	Bacilli
##	ASV464	3.902113e-04	Bacteria	Epsilonbacteraeota	Campylobacteri
##	ASV468	1.251233e-02	Bacteria	Bacteroidetes	Bacteroidia
##	ASV472	1.139329e-02	Bacteria	Bacteroidetes	Bacteroidia
##	ASV480	6.776262e-04	Bacteria	Bacteroidetes	Bacteroidia
##	ASV496	1.767421e-03	Bacteria	Bacteroidetes	Bacteroidia
##	ASV501	3.482261e-02	Bacteria	Bacteroidetes	Bacteroidia
##	ASV511	2.106483e-02	Bacteria	Bacteroidetes	Bacteroidia
##	ASV517	6.497055e-02	Bacteria	Proteobacteria	Deltaproteobacteria
##	ASV522	8.569898e-03	Bacteria	Bacteroidetes	Bacteroidia
##	ASV540	1.052108e-02	Bacteria	Firmicutes	Erysipelotrichi
##	ASV549	8.569898e-03	Bacteria	Firmicutes	Clostridia
##	ASV556	8.569898e-03	Bacteria	Firmicutes	Clostridia
##	ASV563	3.169326e-03	Bacteria	Bacteroidetes	Bacteroidia
##	ASV577	6.516336e-02	Bacteria	Bacteroidetes	Bacteroidia
##	ASV578	6.326071e-03	Bacteria	Firmicutes	Clostridia
##	ASV594	8.569898e-03	Bacteria	Firmicutes	Clostridia
##	ASV601	1.410352e-03	Bacteria	Bacteroidetes	Bacteroidia
##	ASV609	2.339634e-02	Bacteria	Actinobacteria	Actinobacteria
##	ASV759	8.569898e-03	Bacteria	Firmicutes	Clostridia
##	ASV773	8.569898e-03	Bacteria	Firmicutes	Clostridia
##	ASV860	8.569898e-03	Bacteria	Firmicutes	Clostridia
##	ASV981	8.569898e-03	Bacteria	Bacteroidetes	Bacteroidia
##			Order	Family	
##	ASV92		Clostridiales	Lachnospiraceae	
##	ASV127		Clostridiales	Lachnospiraceae	
##	ASV128		Bacteroidales	Muribaculaceae	
##	ASV140		Bacteroidales	Muribaculaceae	
##	ASV142		Clostridiales	Lachnospiraceae	
##	ASV157		Bacteroidales	Muribaculaceae	
##	ASV160		Clostridiales	Lachnospiraceae	
##	ASV161		Clostridiales	Lachnospiraceae	
##	ASV172		Bacteroidales	Muribaculaceae	
##	ASV183		Clostridiales	Lachnospiraceae	
##	ASV191		Bacteroidales	Rikenellaceae	
##	ASV192	Erysipelotrichales	Erysipelotrichaceae		
##	ASV195		Bacteroidales	Muribaculaceae	
##	ASV199		Bacteroidales	Muribaculaceae	
##	ASV203		Clostridiales	Lachnospiraceae	
##	ASV204		Bacteroidales	Muribaculaceae	
##	ASV211		Bacteroidales	Prevotellaceae	
##	ASV212		Bacteroidales	Muribaculaceae	
##	ASV219		Bacteroidales	Rikenellaceae	
##	ASV220		Bacteroidales	Prevotellaceae	



##	ASV222	Bacteroidales	Prevotellaceae
##	ASV231	Bacteroidales	Rikenellaceae
##	ASV233	Bacteroidales	Muribaculaceae
##	ASV247	Bacteroidales	Prevotellaceae
##	ASV264	Bacteroidales	Muribaculaceae
##	ASV271	Clostridiales	Lachnospiraceae
##	ASV274	Bifidobacteriales	Bifidobacteriaceae
##	ASV275	Bacteroidales	Muribaculaceae
##	ASV290	Desulfovibrionales	Desulfovibrionaceae
##	ASV294	Clostridiales	Lachnospiraceae
##	ASV296	Erysipelotrichales	Erysipelotrichaceae
##	ASV304	Bacteroidales	Rikenellaceae
##	ASV306	Clostridiales	Lachnospiraceae
##	ASV310	Bacteroidales	Muribaculaceae
##	ASV312	Bacteroidales	Rikenellaceae
##	ASV317	Clostridiales	Lachnospiraceae
##	ASV320	Bacteroidales	Muribaculaceae
##	ASV322	Clostridiales	Ruminococcaceae
##	ASV331	Clostridiales	Lachnospiraceae
##	ASV334	Bacteroidales	Muribaculaceae
##	ASV338	Bacteroidales	Bacteroidaceae
##	ASV341	Bacteroidales	Muribaculaceae
##	ASV362	Bacteroidales	Muribaculaceae
##	ASV384	Bacteroidales	Muribaculaceae
##	ASV385	Clostridiales	Lachnospiraceae
##	ASV387	Lactobacillales	Lactobacillaceae
##	ASV409	Bacteroidales	Muribaculaceae
##	ASV412	Bacteroidales	Prevotellaceae
##	ASV417	Desulfovibrionales	Desulfovibrionaceae
##	ASV425	Bacteroidales	Muribaculaceae
##	ASV428	Clostridiales	Lachnospiraceae
##	ASV433	Bacteroidales	Muribaculaceae
##	ASV437	Bacteroidales	Tannerellaceae
##	ASV439	Bacteroidales	Prevotellaceae
##	ASV441	Desulfovibrionales	Desulfovibrionaceae
##	ASV442	Bacteroidales	Muribaculaceae
##	ASV444	Bacteroidales	Bacteroidaceae
##	ASV453	<NA>	<NA>
##	ASV458	Lactobacillales	Lactobacillaceae
##	ASV462	Lactobacillales	Lactobacillaceae
##	ASV464	Campylobacterales	Helicobacteraceae
##	ASV468	Bacteroidales	Bacteroidaceae
##	ASV472	Bacteroidales	Prevotellaceae
##	ASV480	Bacteroidales	Muribaculaceae
##	ASV496	Bacteroidales	Rikenellaceae
##	ASV501	Bacteroidales	Rikenellaceae
##	ASV511	Bacteroidales	Muribaculaceae
##	ASV517	Desulfovibrionales	Desulfovibrionaceae
##	ASV522	Bacteroidales	Muribaculaceae
##	ASV540	Erysipelotrichales	Erysipelotrichaceae
##	ASV549	Clostridiales	Lachnospiraceae
##	ASV556	Clostridiales	Lachnospiraceae
##	ASV563	Bacteroidales	Muribaculaceae
##	ASV577	Bacteroidales	Muribaculaceae

##	ASV578	Clostridiales	Lachnospiraceae
##	ASV594	Clostridiales	Lachnospiraceae
##	ASV601	Bacteroidales	Muribaculaceae
##	ASV609	Bifidobacteriales	Bifidobacteriaceae
##	ASV759	Clostridiales	Ruminococcaceae
##	ASV773	Clostridiales	Lachnospiraceae
##	ASV860	Clostridiales	Lachnospiraceae
##	ASV981	Bacteroidales	Bacteroidaceae
##		Genus	Species
##	ASV92	Roseburia	<NA>
##	ASV127	<NA>	<NA>
##	ASV128	Muribaculum	<NA>
##	ASV140	Muribaculum	<NA>
##	ASV142	<NA>	<NA>
##	ASV157	Muribaculum	<NA>
##	ASV160	<NA>	<NA>
##	ASV161	Roseburia	<NA>
##	ASV172	Muribaculum	<NA>
##	ASV183	Lachnospiraceae_NK4A136_group	<NA>
##	ASV191	Alistipes	<NA>
##	ASV192	Faecalibaculum	<NA>
##	ASV195	<NA>	<NA>
##	ASV199	<NA>	<NA>
##	ASV203	Roseburia	<NA>
##	ASV204	<NA>	<NA>
##	ASV211	Prevotellaceae_UCG-001	<NA>
##	ASV212	<NA>	<NA>
##	ASV219	Alistipes	<NA>
##	ASV220	Alloprevotella	<NA>
##	ASV222	Alloprevotella	<NA>
##	ASV231	Alistipes	<NA>
##	ASV233	<NA>	<NA>
##	ASV247	Prevotellaceae_UCG-001	<NA>
##	ASV264	<NA>	<NA>
##	ASV271	<NA>	<NA>
##	ASV274	Bifidobacterium	<NA>
##	ASV275	Muribaculum	<NA>
##	ASV290	Desulfovibrio	<NA>
##	ASV294	<NA>	<NA>
##	ASV296	Dubosiella	<NA>
##	ASV304	Alistipes	<NA>
##	ASV306	Lachnospiraceae_NK4A136_group	<NA>
##	ASV310	<NA>	<NA>
##	ASV312	Alistipes	<NA>
##	ASV317	Roseburia	<NA>
##	ASV320	<NA>	<NA>
##	ASV322	Intestinimonas	<NA>
##	ASV331	<NA>	<NA>
##	ASV334	<NA>	<NA>
##	ASV338	Bacteroides	<NA>
##	ASV341	<NA>	<NA>
##	ASV362	<NA>	<NA>
##	ASV384	<NA>	<NA>
##	ASV385	Roseburia	<NA>

```

## ASV387          Lactobacillus  <NA>
## ASV409          <NA>          <NA>
## ASV412          Alloprevotella <NA>
## ASV417          Desulfovibrio  <NA>
## ASV425          <NA>          <NA>
## ASV428          <NA>          <NA>
## ASV433          <NA>          <NA>
## ASV437          Parabacteroides <NA>
## ASV439          Alloprevotella <NA>
## ASV441          <NA>          <NA>
## ASV442          <NA>          <NA>
## ASV444          Bacteroides    <NA>
## ASV453          <NA>          <NA>
## ASV458          Lactobacillus  <NA>
## ASV462          Lactobacillus  <NA>
## ASV464          Helicobacter   <NA>
## ASV468          Bacteroides    <NA>
## ASV472          Prevotellaceae_UCG-001 <NA>
## ASV480          <NA>          <NA>
## ASV496          Rikenellaceae_RC9_gut_group <NA>
## ASV501          Alistipes      <NA>
## ASV511          <NA>          <NA>
## ASV517          <NA>          <NA>
## ASV522          <NA>          <NA>
## ASV540          Faecalibaculum <NA>
## ASV549          28-4          <NA>
## ASV556          <NA>          <NA>
## ASV563          <NA>          <NA>
## ASV577          <NA>          <NA>
## ASV578          Lachnospiraceae_NK4A136_group <NA>
## ASV594          <NA>          <NA>
## ASV601          <NA>          <NA>
## ASV609          Bifidobacterium <NA>
## ASV759          Oscillibacter  <NA>
## ASV773          Lachnospiraceae_NK4A136_group <NA>
## ASV860          <NA>          <NA>
## ASV981          Bacteroides    <NA>

```

```

## DESeq2 analysis using Treatment*Sex
treatdds = phyloseq_to_deseq2(physeq_genus, ~Treatment*Sex)

```

```

## converting counts to integer mode

```

```

## Warning in DESeqDataSet(se, design = design, ignoreRank): some variables in
## design formula are characters, converting to factors

```

```

## the design formula contains a numeric variable with integer values,
## specifying a model with increasing fold change for higher values.
## did you mean for this to be a factor? if so, first convert
## this variable to a factor using the factor() function

```

```

## Genus level table
Treatment_Sex_genus <- taxa_with_deseq(treatdds, physeq_genus)

## estimating size factors

## estimating dispersions

## gene-wise dispersion estimates

## mean-dispersion relationship

## final dispersion estimates

## fitting model and testing

## -- replacing outliers and refitting for 25 genes
## -- DESeq argument 'minReplicatesForReplace' = 7
## -- original counts are preserved in counts(dds)

## estimating dispersions

## fitting model and testing

print(Treatment_Sex_genus)

## [1] "No significant taxa were identified using the specified formula"

```