Phyloseq

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#load required packages

```r
library(phyloseq)
library(dplyr)
```

```
##
## Attaching package: 'dplyr'

## The following objects are masked from 'package:stats':
##
##     filter, lag

## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
```

```r
library(BiMiCo)
library(ggplot2)
library(devtools)
```

```
## Loading required package: usethis
```

```r
library(MicEco)
library(vegan)
```

```
## Loading required package: permute

##
## Attaching package: 'permute'

## The following object is masked from 'package:devtools':
##
##     check

## Loading required package: lattice

## This is vegan 2.6-4
```

#load taxa and seqtab.nochim

```r
load("RData/taxa.RData")
load("RData/seqtab.nochim.RData")
```

#import metadata

```r
metadata <- read.csv("sample-metadata.csv", header=TRUE, row.names = 1)
```

#create phylseq object

```r
#make sure the seqtab.nochim and taxa objects are loaded
physeq <- phyloseq(otu_table(seqtab.nochim, taxa_are_rows=FALSE), sample_data(metadata),tax_table(taxa)
physeq
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 771 taxa and 34 samples ]
## sample_data() Sample Data:       [ 34 samples by 9 sample variables ]
## tax_table()   Taxonomy Table:    [ 771 taxa by 7 taxonomic ranks ]
```

#transform sample counts

```r
#convert from raw to abundance so its easier to comapre
physeq <- transform_sample_counts(physeq, function(abund) 1*(abund>0))

#visualize to data
physeq
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 771 taxa and 34 samples ]
## sample_data() Sample Data:       [ 34 samples by 9 sample variables ]
## tax_table()   Taxonomy Table:    [ 771 taxa by 7 taxonomic ranks ]
```

#remove the sequence itselt and replace with ASV

```r
##this allows it to be easier to read, replaces the raw data
dna <- Biostrings::DNAStringSet(taxa_names(physeq))
names(dna) <- taxa_names(physeq)
physeq <- merge_phyloseq(physeq, dna)
taxa_names(physeq) <- paste0("ASV", seq(ntaxa(physeq)))
physeq
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 771 taxa and 34 samples ]
## sample_data() Sample Data:       [ 34 samples by 9 sample variables ]
## tax_table()   Taxonomy Table:    [ 771 taxa by 7 taxonomic ranks ]
## refseq()      DNAStringSet:      [ 771 reference sequences ]
```

#remove mitochondria and phloroplast mathces, remove all non bacterial sequences

```r
#stictly use bacteria 16S rRNA,
physeq <- physeq %>% subset_taxa( Family!= "Mitochondria" | is.na(Family) & Order!="Chloroplast" | is.na
physeq
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 742 taxa and 34 samples ]
## sample_data() Sample Data:       [ 34 samples by 9 sample variables ]
## tax_table()   Taxonomy Table:    [ 742 taxa by 7 taxonomic ranks ]
## refseq()      DNAStringSet:      [ 742 reference sequences ]
```

#remove all non bacterial sequences

```r
physeq<-rm_nonbac(physeq)
physeq
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 737 taxa and 34 samples ]
## sample_data() Sample Data:       [ 34 samples by 9 sample variables ]
## tax_table()   Taxonomy Table:    [ 737 taxa by 7 taxonomic ranks ]
## refseq()      DNAStringSet:      [ 737 reference sequences ]
```

#save physeq objects to load later

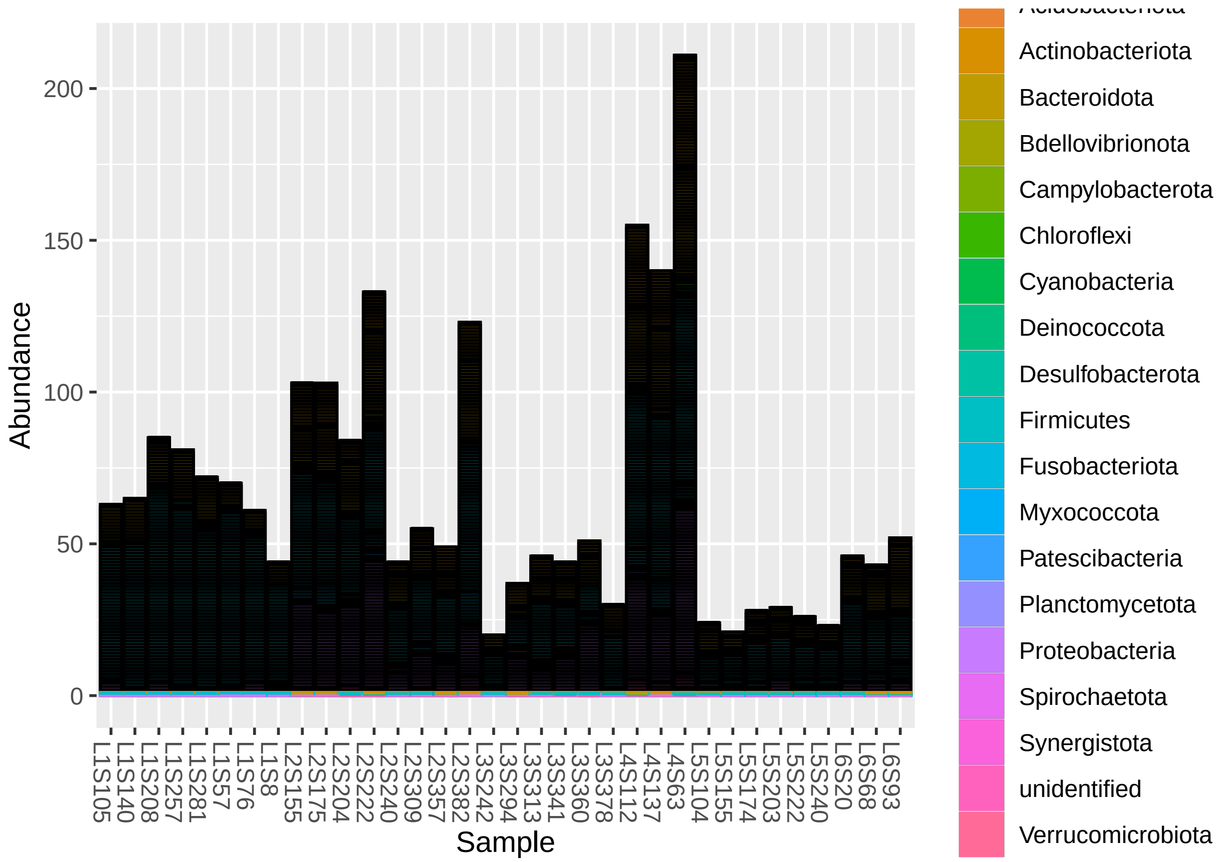
```r
save(physeq, file= "RData/physeq.RData")
```

#load physeq objects to start here

```r
load("RData/physeq.RData")
```

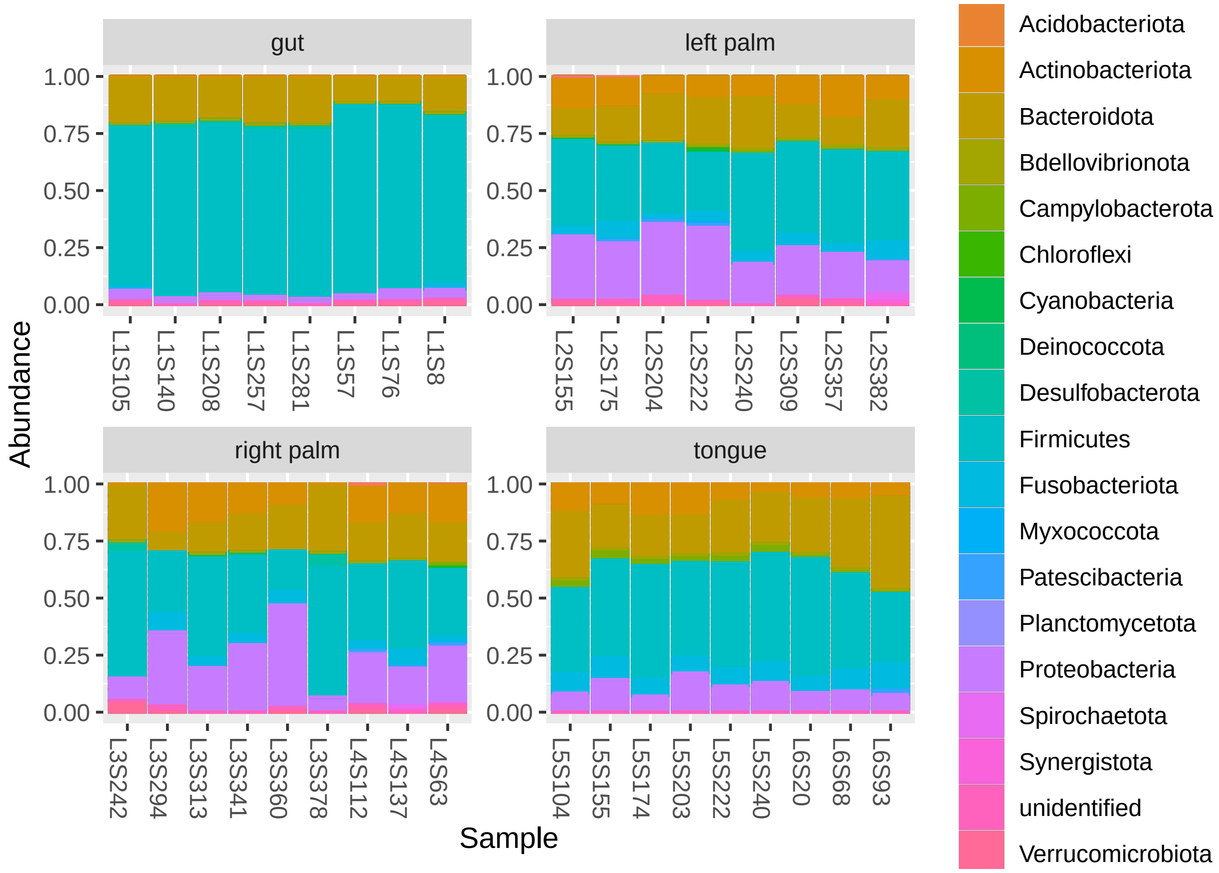
#plot bar grpah based on phylum

```r
plot_bar(physeq, fill = "Phylum") + geom_bar(aes(color=Phylum, fill=Phylum), stat="identity", position=
```



#create a barplot of relative abundance

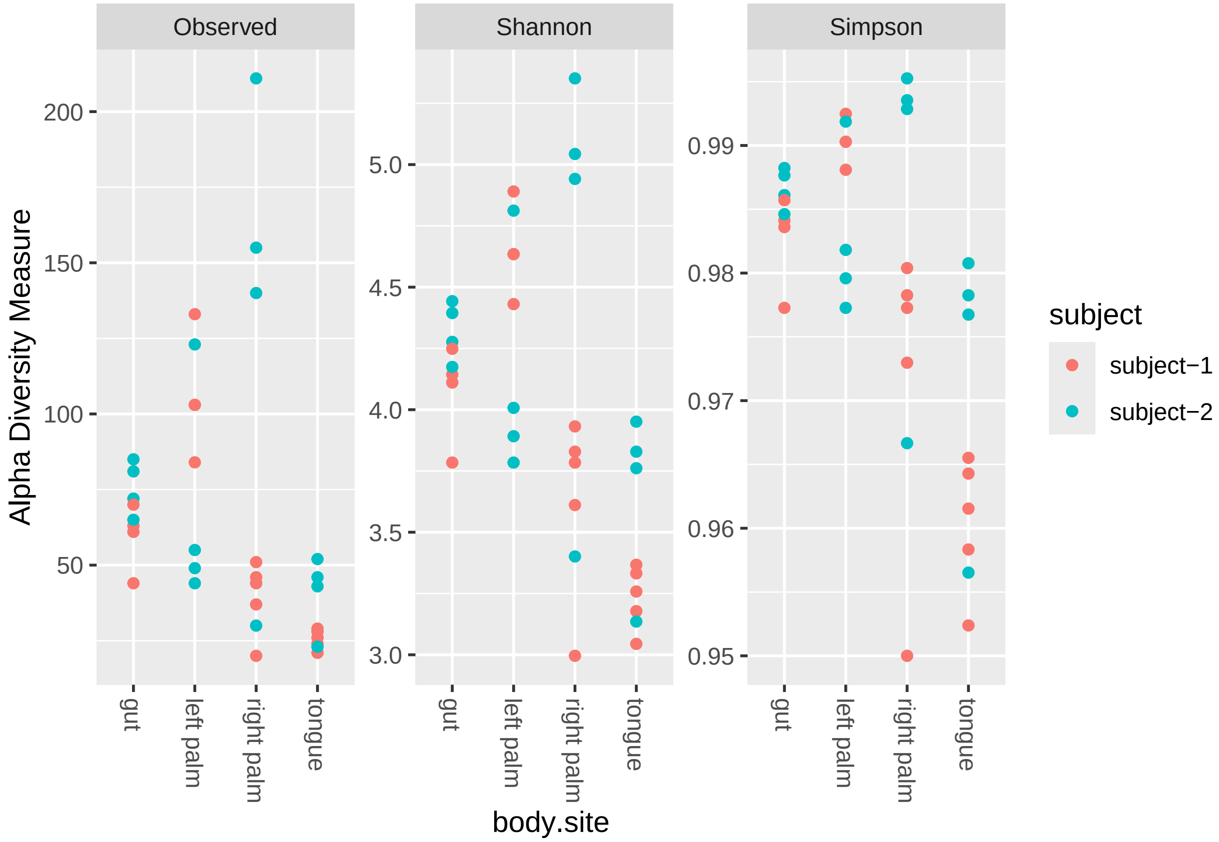
```r
#convert to relative abundance
physeq_relabund <- transform_sample_counts(physeq, function(x) x / sum(x))

#barplot
plot_bar(physeq_relabund, fill = "Phylum") + geom_bar(aes(color=Phylum, fill=Phylum), stat="identity", 
```



```
##can change based on the column name in metadata in facet_wrap(~columnName)
```

#plot alpha diversity based on body site

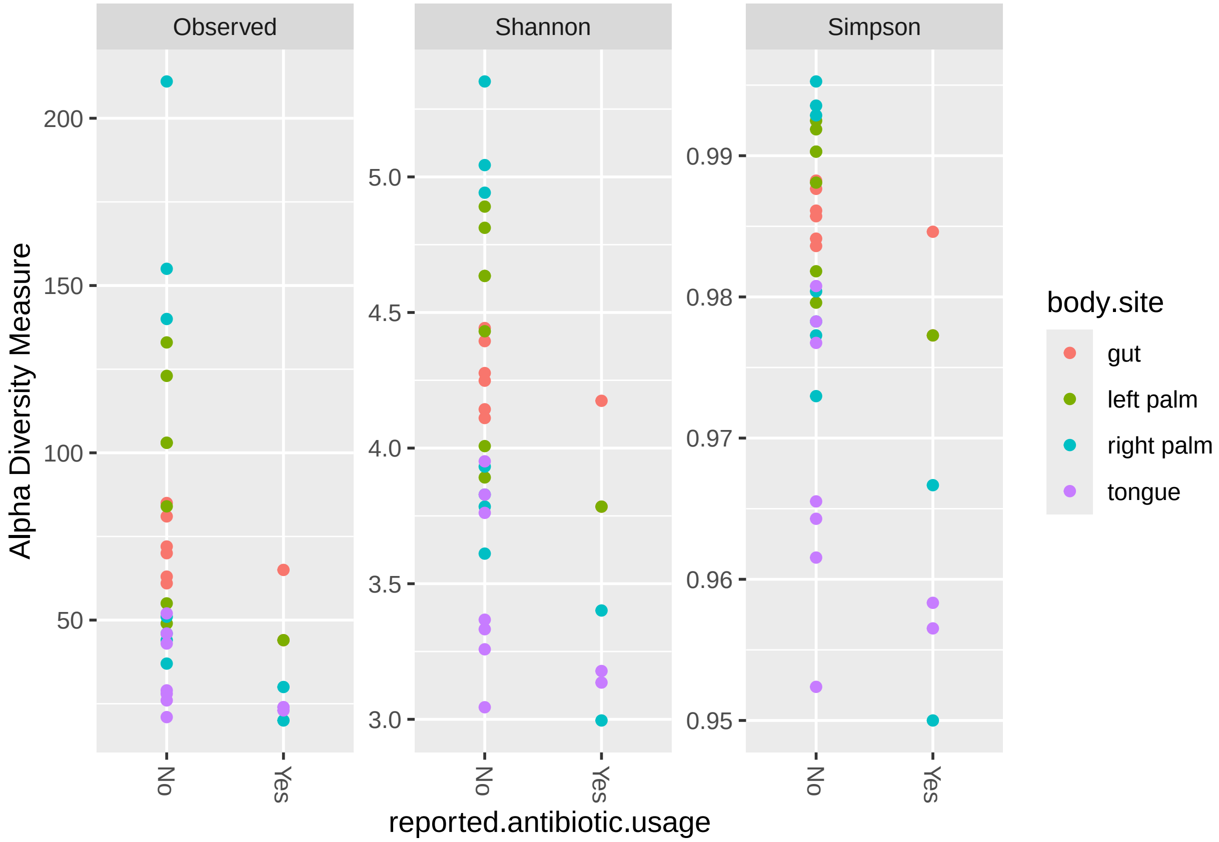
```
plot_richness(physeq, x="body.site", color= "subject", measures=c("Observed", "Simpson", "Shannon"))
```



```
##Simpson(less sensitive, will be more custered together) and Shannon(more sensitive to rare taxa) take
```

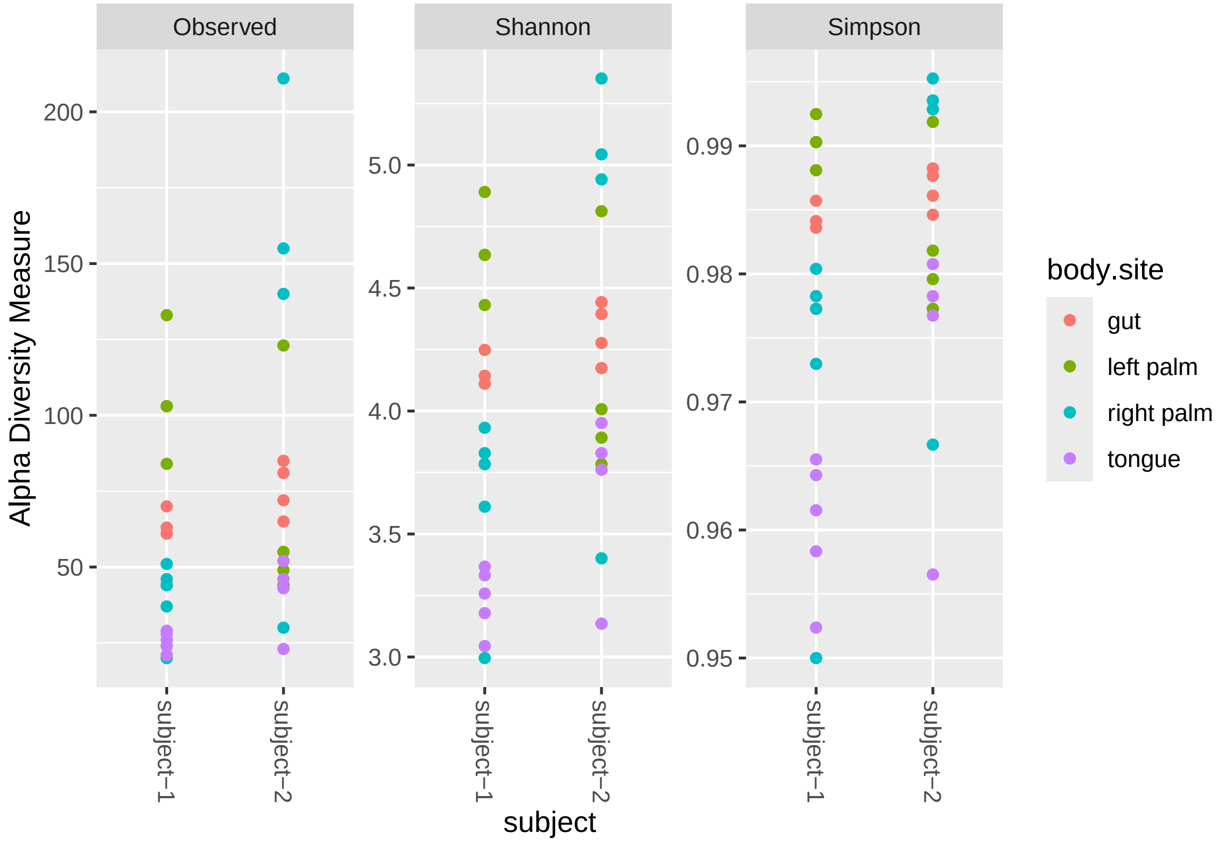
#pplot alpha diversity based on reported.antibiotic.usage

```
plot_richness(physeq, x="reported.antibiotic.usage", color= "body.site", measures=c("Observed", "Simpso
```



#plot alpha diversity based on subject

```
plot_richness(physeq, x="subject", color= "body.site", measures=c("Observed", "Simpson", "Shannon"))
```



#test for normailoty

```
alpha <- estimate_richness(physeq, measures=c("Observed", "Simpson", "Shannon"))

#alternative = the data is normal distributed
#null = the data is not normal distributed

#Shapiro-wilk - used when it has fewer than 50 samples
observed <- shapiro.test(alpha$Observed)
shannon <- shapiro.test(alpha$Shannon)
simpson <- shapiro.test(alpha$Simpson)

#print
print(observed)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  alpha$Observed
## W = 0.85439, p-value = 0.0003513
```

```
print(shannon)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  alpha$Shannon
## W = 0.97517, p-value = 0.617
```

```
print(simpson)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  alpha$Simpson
## W = 0.91749, p-value = 0.01373
```

#create data frames for statistical analyses

```
#extract sample information from the physeq object
samples <- sample_data(physeq)

#if sample is a phyloseq sample_Data, convert it to a data frame
if (class(samples) == "sample_data") {
  samples <- data.frame(sample_data(samples))
  }

#add a column to alpha with sample names
alpha$sample <- rownames(alpha)

#merge alpha diversity data and sample(meta) data
alpha <- merge(alpha, samples, by = "sample")
```

#perform statistics based on subject

```
#perform t/wilcox tests for each biodiversity index
test_observed <- wilcox.test(Observed ~ subject, data = alpha)
```

```
## Warning in wilcox.test.default(x = DATA[[1L]], y = DATA[[2L]], ...): cannot
## compute exact p-value with ties
```

```
test_simpson <- wilcox.test(Simpson ~ subject, data = alpha)
```

```
## Warning in wilcox.test.default(x = DATA[[1L]], y = DATA[[2L]], ...): cannot
## compute exact p-value with ties
```

```
test_shannon <- t.test(Shannon ~ subject, data = alpha)

#print results
print(test_observed)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  Observed by subject
## W = 96.5, p-value = 0.1047
## alternative hypothesis: true location shift is not equal to 0
```

```
print(test_simpson)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  Simpson by subject
## W = 96.5, p-value = 0.1047
## alternative hypothesis: true location shift is not equal to 0
```

```r
print(test_shannon)
```

```
##
##  Welch Two Sample t-test
##
## data:  Shannon by subject
## t = -1.7373, df = 31.125, p-value = 0.09223
## alternative hypothesis: true difference in means between group subject-1 and group subject-2 is not
## 95 percent confidence interval:
##  -0.77161834  0.06168674
## sample estimates:
## mean in group subject-1 mean in group subject-2
##                3.844933                4.199899
```

###to change text, select chunk > edit > find >click in selection > replace these tabs with what you ant to change; find - replace > all

#perform statistics based on reported.anitbiotic.usage

```r
#perform t/wilcox tests for each biodiversity index
test_observed <- wilcox.test(Observed ~ reported.antibiotic.usage, data = alpha)
```

```
## Warning in wilcox.test.default(x = DATA[[1L]], y = DATA[[2L]], ...): cannot
## compute exact p-value with ties
```

```r
test_simpson <- wilcox.test(Simpson ~ reported.antibiotic.usage, data = alpha)
```

```
## Warning in wilcox.test.default(x = DATA[[1L]], y = DATA[[2L]], ...): cannot
## compute exact p-value with ties
```

```r
test_shannon <- t.test(Shannon ~ reported.antibiotic.usage, data = alpha)

#print results
print(test_observed)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  Observed by reported.antibiotic.usage
## W = 155, p-value = 0.01057
## alternative hypothesis: true location shift is not equal to 0
```

```r
print(test_simpson)
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  Simpson by reported.antibiotic.usage
## W = 155, p-value = 0.01057
## alternative hypothesis: true location shift is not equal to 0
```

```r
print(test_shannon)
```

```
##
##  Welch Two Sample t-test
##
## data:  Shannon by reported.antibiotic.usage
## t = 3.3002, df = 12.383, p-value = 0.006097
## alternative hypothesis: true difference in means between group No and group Yes is not equal to 0
## 95 percent confidence interval:
##  0.2233954 1.0828476
## sample estimates:
##  mean in group No mean in group Yes
##          4.146442          3.493321
```

#test for body site

```r
kruskal.test(Simpson ~ body.site, data=alpha)
```

```
## 
##  Kruskal-Wallis rank sum test
## 
## data:  Simpson by body.site
## Kruskal-Wallis chi-squared = 13.435, df = 3, p-value = 0.003785
```

```r
pairwise.wilcox.test(alpha$Simpson, alpha$body.site, p.adjust.method = "holm")
```

```
## Warning in wilcox.test.default(xi, xj, paired = paired, ...): cannot compute
## exact p-value with ties

## Warning in wilcox.test.default(xi, xj, paired = paired, ...): cannot compute
## exact p-value with ties

## Warning in wilcox.test.default(xi, xj, paired = paired, ...): cannot compute
## exact p-value with ties

## Warning in wilcox.test.default(xi, xj, paired = paired, ...): cannot compute
## exact p-value with ties

## Warning in wilcox.test.default(xi, xj, paired = paired, ...): cannot compute
## exact p-value with ties

## 
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction 
## 
## data:  alpha$Simpson and alpha$body.site 
## 
##            gut    left palm right palm
## left palm  1.0000 -         -         
## right palm 1.0000 1.0000    -         
## tongue     0.0020 0.0088    0.2805    
## 
## P value adjustment method: holm
```

```r
kruskal.test(Observed ~ body.site, data=alpha)
```

```
## 
##  Kruskal-Wallis rank sum test
## 
## data:  Observed by body.site
## Kruskal-Wallis chi-squared = 13.435, df = 3, p-value = 0.003785
```

```r
pairwise.wilcox.test(alpha$Observed, alpha$body.site, p.adjust.method = "holm")
```

```
## Warning in wilcox.test.default(xi, xj, paired = paired, ...): cannot compute
## exact p-value with ties
## Warning in wilcox.test.default(xi, xj, paired = paired, ...): cannot compute
## exact p-value with ties

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## exact p-value with ties

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## exact p-value with ties

## Warning in wilcox.test.default(xi, xj, paired = paired, ...): cannot compute
## exact p-value with ties

## 
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction 
## 
## data:  alpha$Observed and alpha$body.site 
## 
##            gut    left palm right palm
## left palm  1.0000 -         -         
## right palm 1.0000 1.0000    -         
## tongue     0.0020 0.0088    0.2805    
## 
## P value adjustment method: holm
```

```
shannonanova <- aov(Shannon ~ body.site, data=alpha)
summary(shannonanova)
```

```
##             Df Sum Sq Mean Sq F value  Pr(>F)   
## body.site    3  4.523  1.5076     5.8 0.00299 **
## Residuals   30  7.797  0.2599                   
## ---
## Signif. codes:  0 ’***’ 0.001 ’**’ 0.01 ’*’ 0.05 ’.’ 0.1 ’ ’ 1
```

```
TukeyHSD(shannonanova)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
## 
## Fit: aov(formula = Shannon ~ body.site, data = alpha)
## 
## $body.site
##                             diff        lwr         upr     p adj
## left palm-gut         0.18891301 -0.5042094  0.88203538 0.8796872
## right palm-gut       -0.09803036 -0.7716242  0.57556350 0.9786056
## tongue-gut           -0.76832784 -1.4419217 -0.09473398 0.0205658
## right palm-left palm -0.28694338 -0.9605372  0.38665049 0.6570793
## tongue-left palm     -0.95724086 -1.6308347 -0.28364699 0.0029520
## tongue-right palm    -0.67029748 -1.3237795 -0.01681544 0.0427299
```

#load physeq objects to start here

```
load("RData/physeq.RData")
```

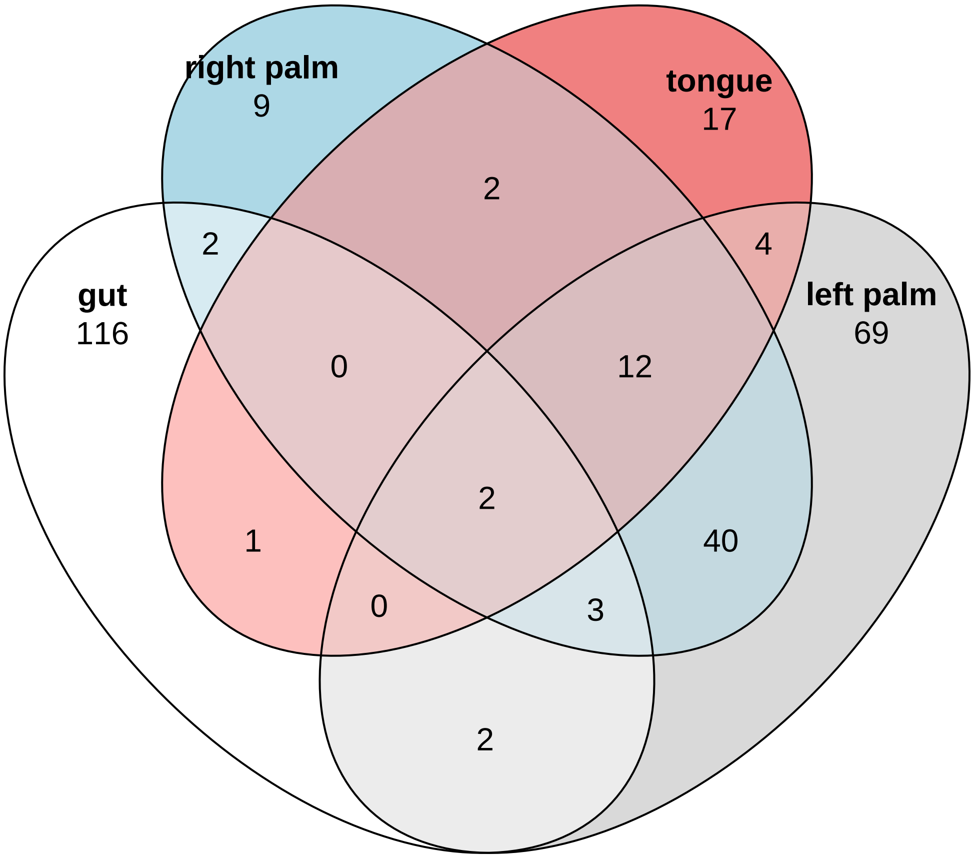
#remove taxa with relative abundance <0.05%

```r
minTotRelAbun = .00005
x = taxa_sums(physeq)
keepTaxa = (x / sum(x)) > minTotRelAbun
physeqprune = prune_taxa(keepTaxa, physeq)
physeqprune
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 737 taxa and 34 samples ]
## sample_data() Sample Data:       [ 34 samples by 9 sample variables ]
## tax_table()   Taxonomy Table:    [ 737 taxa by 7 taxonomic ranks ]
## refseq()      DNAStringSet:      [ 737 reference sequences ]
```

#nuber of shared ASVs body.site (found in 25% or more)

```r
#create a ven diagram showing the different categories and what they share
bodysite=ps_venn(
physeqprune,
"body.site",
fraction = .25,
weight = FALSE,
relative = TRUE,
plot = TRUE)
bodysite
```



#bray curtis caculation, 0; exactly the same, 1; very diverse

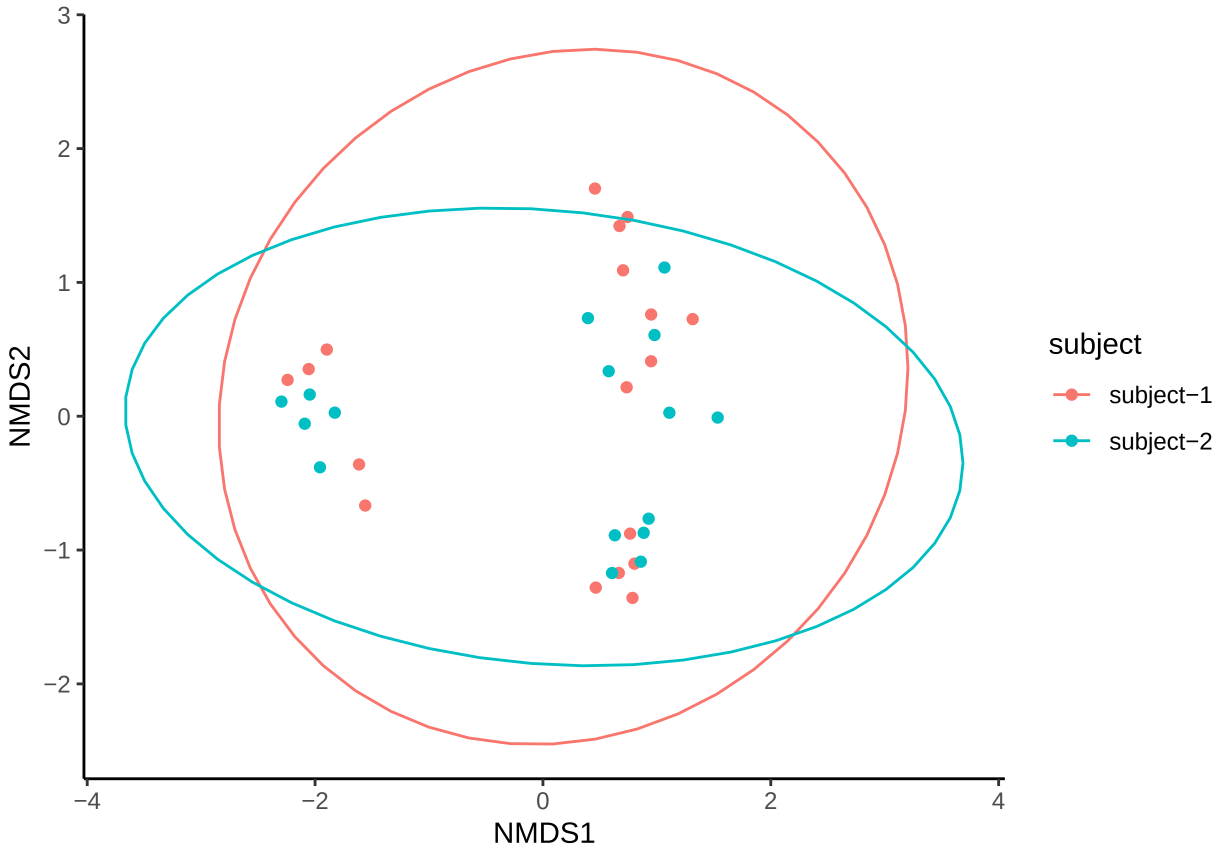
```r
set.seed(666)
dist = phyloseq::distance(physeqprune, method="bray", weighted=TRUE)
```

```
ordination = ordinate(physeqprune, method="NMDS", distance=dist)
```

```
## Run 0 stress 0.08766934
## Run 1 stress 0.08742315
## ... New best solution
## ... Procrustes: rmse 0.007883637  max resid 0.02695926
## Run 2 stress 0.08766934
## ... Procrustes: rmse 0.007888623  max resid 0.0269594
## Run 3 stress 0.08766933
## ... Procrustes: rmse 0.007888525  max resid 0.02696067
## Run 4 stress 0.08682979
## ... New best solution
## ... Procrustes: rmse 0.009820118  max resid 0.03764741
## Run 5 stress 0.08766933
## Run 6 stress 0.08682979
## ... New best solution
## ... Procrustes: rmse 1.682291e-06  max resid 5.964775e-06
## ... Similar to previous best
## Run 7 stress 0.08682979
## ... New best solution
## ... Procrustes: rmse 1.239842e-06  max resid 3.94404e-06
## ... Similar to previous best
## Run 8 stress 0.08682979
## ... New best solution
## ... Procrustes: rmse 1.562855e-06  max resid 4.551113e-06
## ... Similar to previous best
## Run 9 stress 0.08682979
## ... Procrustes: rmse 2.392283e-06  max resid 9.91919e-06
## ... Similar to previous best
## Run 10 stress 0.08742315
## Run 11 stress 0.08682979
## ... Procrustes: rmse 2.267872e-06  max resid 5.216871e-06
## ... Similar to previous best
## Run 12 stress 0.08682979
## ... New best solution
## ... Procrustes: rmse 1.285443e-06  max resid 3.260187e-06
## ... Similar to previous best
## Run 13 stress 0.08942864
## Run 14 stress 0.08682979
## ... Procrustes: rmse 3.566817e-06  max resid 1.206664e-05
## ... Similar to previous best
## Run 15 stress 0.08682979
## ... Procrustes: rmse 3.008016e-06  max resid 1.162141e-05
## ... Similar to previous best
## Run 16 stress 0.08747913
## Run 17 stress 0.08682979
## ... New best solution
## ... Procrustes: rmse 1.848555e-06  max resid 7.543773e-06
## ... Similar to previous best
## Run 18 stress 0.08742337
## Run 19 stress 0.08747857
## Run 20 stress 0.08682979
## ... Procrustes: rmse 1.267083e-06  max resid 4.162071e-06
## ... Similar to previous best
## *** Best solution repeated 2 times
```

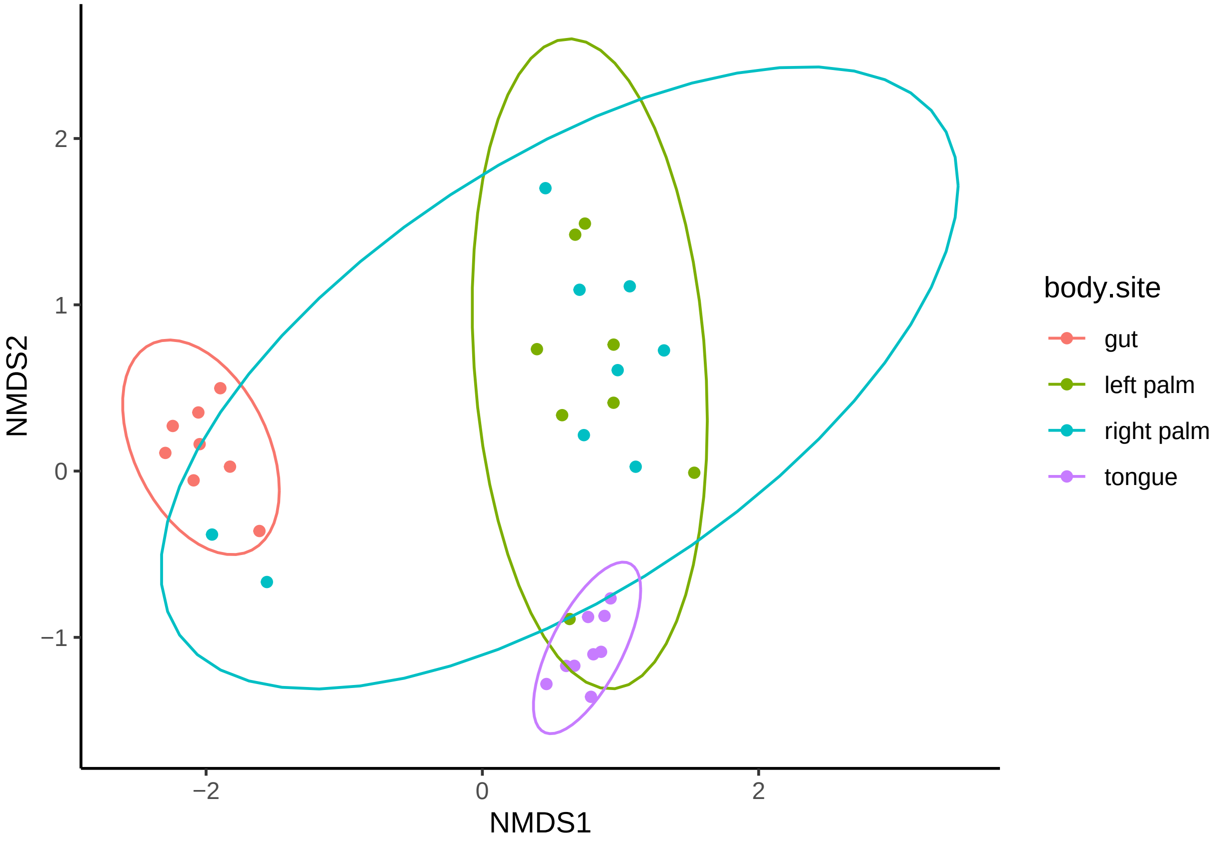
#bray curtis subject plot

```
braysubject=plot_ordination(physeqprune, ordination, color="subject") +
  theme_classic() +
  theme(strip.background = element_blank()) + stat_ellipse(aes(group=subject))
braysubject
```



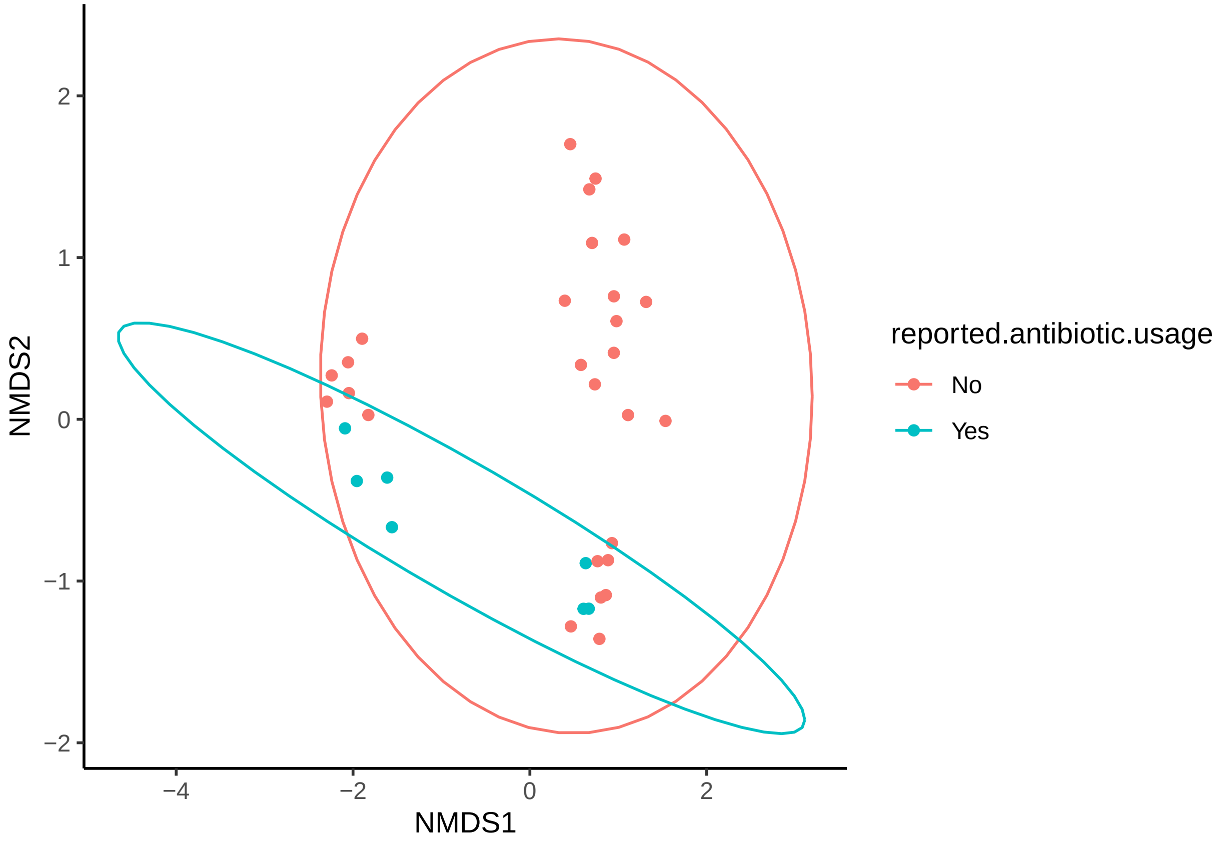
bray curtis body site plot

```
braybodysite=plot_ordination(physeqprune, ordination, color="body.site") +
  theme_classic() +
  theme(strip.background = element_blank()) + stat_ellipse(aes(group=body.site))
braybodysite
```



bray curtis antibiotic usage plot bray curtis body site plot

```
brayabusage=plot_ordination(physeqprune, ordination, color="reported.antibiotic.usage") +
  theme_classic() +
  theme(strip.background = element_blank()) + stat_ellipse(aes(group=reported.antibiotic.usage))
brayabusage
```



#bray curits subject statistics

```
adonis2(dist ~ sample_data(physeqprune)$subject)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = dist ~ sample_data(physeqprune)$subject)
##                                  Df SumOfSqs      R2      F Pr(>F)
## sample_data(physeqprune)$subject  1   0.5144 0.04432 1.4841  0.155
## Residual                         32  11.0912 0.95568
## Total                            33  11.6056 1.00000
```

#bray curtis body site statistics

```
adonis2(dist ~ sample_data(physeqprune)$body.site)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = dist ~ sample_data(physeqprune)$body.site)
##                                    Df SumOfSqs      R2      F Pr(>F)
## sample_data(physeqprune)$body.site  3   5.2363 0.45119 8.2212  0.001 ***
## Residual                           30   6.3693 0.54881
## Total                              33  11.6056 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
ps.disper<-betadisper(dist, sample_data(physeqprune)$body.site)
permutest(ps.disper, pair=TRUE)
```

```
##
## Permutation test for homogeneity of multivariate dispersions
## Permutation: free
## Number of permutations: 999
##
## Response: Distances
##           Df  Sum Sq  Mean Sq      F N.Perm Pr(>F)
## Groups     3 0.44201 0.147337 19.584    999  0.001 ***
## Residuals 30 0.22570 0.007523
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Pairwise comparisons:
## (Observed p-value below diagonal, permuted p-value above diagonal)
##                   gut  left palm right palm tongue
## gut                   1.6000e-02 1.0000e-03  0.006
## left palm  1.9713e-02            1.6500e-01  0.001
## right palm 1.1397e-03 1.7776e-01              0.001
## tongue     4.4439e-03 3.5078e-05 2.6079e-06
```

#bray curtis antibiotic usage statistics

```
adonis2(dist ~ sample_data(physeqprune)$reported.antibiotic.usage)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = dist ~ sample_data(physeqprune)$reported.antibiotic.usage)
##                                                    Df SumOfSqs     R2      F
## sample_data(physeqprune)$reported.antibiotic.usage  1   0.7555 0.0651 2.2283
## Residual                                           32  10.8501 0.9349
## Total                                              33  11.6056 1.0000
##                                                    Pr(>F)
## sample_data(physeqprune)$reported.antibiotic.usage  0.029 *
## Residual
## Total
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```