# deseq2_analysis.R

*arisely*

*Fri Oct 06 14:11:23 2017*

```
## R script for publication: "Active migration associated with repeatable and specific changes to gut microbiota in migratory shorebirds"
# Alice Risely, David Waite, Beata Ujvari, Bethany Hoye, & Marcel Klaassen

##R version 3.4.1

##workflows:
#https://f1000research.com/articles/5-1492/v1
#http://deneflab.github.io/MicrobeMiseq/demos/mothur_2_phyloseq.html
#DESeq2
#https://www.bioconductor.org/packages/devel/bioc/vignettes/phyloseq/inst/doc/phyloseq-mixture-models.html


##good threads on data transformation
#https://github.com/joey711/phyloseq/issues/492
#https://github.com/joey711/phyloseq/issues/283

#To download phyloseq and DESeq2 from bioconductor run following two lines:

#source('http://bioconductor.org/biocLite.R')
#biocLite('phyloseq')
#biocLite("DESeq2")

#It might mess up some other packages, so packages may need reinstalling (either from CRAN or Bioconductor) as errors come up when loading phyloseq and DESeq2 into library
#I had to reinstall ~10 packages

##setwd() somewhere and download all github files to this location

library(phyloseq)
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, cbind, colMeans,
##     colnames, colSums, do.call, duplicated, eval, evalq, Filter,
##     Find, get, grep, grepl, intersect, is.unsorted, lapply,
##     lengths, Map, mapply, match, mget, order, paste, pmax,
##     pmax.int, pmin, pmin.int, Position, rank, rbind, Reduce,
##     rowMeans, rownames, rowSums, 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
```

```
## 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
```

```
## Warning: package 'matrixStats' was built under R version 3.4.2
```

```
##
## Attaching package: 'matrixStats'
```

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

```
##
## Attaching package: 'DelayedArray'
```

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

```
## The following object is masked from 'package:base':
##
##     apply
```

```
##other packages you may need, althoguh I think a few are included with the phyloseq package so you may not need to load them

library(ggplot2)
```

```
## Warning: package 'ggplot2' was built under R version 3.4.2
```

```
library(vegan)
```

```
## Warning: package 'vegan' was built under R version 3.4.2
```

```
## Loading required package: permute
```

```
## Warning: package 'permute' was built under R version 3.4.2
```

```
## Loading required package: lattice
```

```
## This is vegan 2.4-4
```

```
library(dplyr)
```

```
## Warning: package 'dplyr' was built under R version 3.4.2
```

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

```
## The following object is masked from 'package:matrixStats':
##
##     count
```

```
## The following object is masked from 'package:Biobase':
##
##     combine
```

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

```
## The following object is masked from 'package:GenomeInfoDb':
##
##     intersect
```

```
## The following objects are masked from 'package:IRanges':
##
##     collapse, desc, intersect, setdiff, slice, union
```

```
## The following objects are masked from 'package:S4Vectors':
##
##     first, intersect, rename, setdiff, setequal, union
## The following objects are masked from 'package:BiocGenerics':
##
##     combine, intersect, setdiff, union
```

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

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

```
library(scales)
```

```
## Warning: package 'scales' was built under R version 3.4.2
```

```
library(grid)
library(reshape2)
```

```
## Warning: package 'reshape2' was built under R version 3.4.2
```

```
library(ape)
library(gridExtra)
```

```
## Warning: package 'gridExtra' was built under R version 3.4.2
```

```
##
## Attaching package: 'gridExtra'
```

```
## The following object is masked from 'package:dplyr':
##
##     combine
```

```
## The following object is masked from 'package:Biobase':
##
##     combine
```

```
## The following object is masked from 'package:BiocGenerics':
##
##     combine
```

```
library(ade4)
```

```
## Warning: package 'ade4' was built under R version 3.4.2
```

```
##
## Attaching package: 'ade4'
```

```
## The following object is masked from 'package:GenomicRanges':
##
##     score
```

```
## The following object is masked from 'package:IRanges':
##
##     score
```

```
## The following object is masked from 'package:BiocGenerics':
##
##     score
```

```
library(plyr)
```

```
## Warning: package 'plyr' was built under R version 3.4.2
```

```
## ------------------------------------------------------------------------
```

```
## You have loaded plyr after dplyr - this is likely to cause problems.
## If you need functions from both plyr and dplyr, please load plyr first, then dplyr:
## library(plyr); library(dplyr)
```

```
## ------------------------------------------------------------------------
```

```
## 
## Attaching package: 'plyr'
```

```
## The following objects are masked from 'package:dplyr':
## 
##     arrange, count, desc, failwith, id, mutate, rename, summarise,
##     summarize
```

```
## The following object is masked from 'package:matrixStats':
## 
##     count
```

```
## The following object is masked from 'package:IRanges':
## 
##     desc
```

```
## The following object is masked from 'package:S4Vectors':
## 
##     rename
```

```
library(tidyr)
```

```
## Warning: package 'tidyr' was built under R version 3.4.2
```

```
## 
## Attaching package: 'tidyr'
```

```
## The following object is masked from 'package:reshape2':
## 
##     smiths
```

```
## The following object is masked from 'package:S4Vectors':
## 
##     expand
```

```
library(data.table)
```

```
## 
## Attaching package: 'data.table'
```

```
## The following objects are masked from 'package:reshape2':
## 
##     dcast, melt
```

```
## The following objects are masked from 'package:dplyr':
## 
##     between, first, last
```

```
## The following object is masked from 'package:SummarizedExperiment':
## 
##     shift
## The following object is masked from 'package:GenomicRanges':
## 
##     shift
```

```
## The following object is masked from 'package:IRanges':
## 
##     shift
```

```
## The following objects are masked from 'package:S4Vectors':
## 
##     first, second
```

```r
library(stringr)
```

```
## Warning: package 'stringr' was built under R version 3.4.2
```

```r
source("miseqR.R")

theme_set(theme_bw())


################# ONLY OTUS WITH OVER 10 SEQUENCES (AND WITH PHYLO TREE) ######################################
############################

setwd("C:\\Users\\arisely\\Dropbox\\PhD\\Microbiome\\CHAPTER 3 - PHYSIOLOGY\\ANALYSIS\\R\\Phyloseq analysis")

# Assign variables for imported data

sharedfile = "shorebird.microbiome.shared"
taxfile = "shorebird.microbiome.taxonomy"

##import mothur shared and tax files

mothur_data <- import_mothur(mothur_shared_file = sharedfile,
                             mothur_constaxonomy_file = taxfile)

##import metadata
map <- read.csv("shorebird.metadata.csv", header=T, row.names=1)

##make meta data into phyloseq format

map <- sample_data(map)
str(map)
```

```
## 'data.frame':    188 obs. of  21 variables:
## Formal class 'sample_data' [package "phyloseq"] with 4 slots
##   ..@ .Data    :List of 21
##   .. ..$ : Factor w/ 180 levels "8546","8548",..: 1 2 3 4 5 6 7 8 9 10 ...
##   .. ..$ : Factor w/ 6 levels "Curlew sandpiper",..: 4 4 4 4 4 4 4 4 4 4 ...
##   .. ..$ : Factor w/ 5 levels "","Broome","Flinders",..: 2 2 2 2 2 2 2 2 2 2 ...
##   .. ..$ : Factor w/ 7 levels "","August","December",..: 2 2 2 2 2 2 2 2 2 2 ...
##   .. ..$ : Factor w/ 6 levels "","Adult","Juvenile",..: 5 5 2 5 5 5 5 5 5 2 ...
##   .. ..$ : Factor w/ 4 levels "","one","three",..: 4 4 4 4 4 4 4 4 4 4 ...
##   .. ..$ : Factor w/ 161 levels "","3611876","3648481",..: 20 21 10 22 23 11 12 13 14 15 ...
##   .. ..$ : Factor w/ 3 levels "bird","env","neg": 1 1 1 1 1 1 1 1 1 1 ...
##   .. ..$ : Factor w/ 3 levels "na","No","Yes": 2 2 2 2 2 2 2 2 2 2 ...
##   .. ..$ : Factor w/ 2 levels "No","Yes": 1 1 1 1 1 1 1 1 1 1 ...
##   .. ..$ : int  NA NA NA NA NA NA NA NA NA NA ...
##   .. ..$ : Factor w/ 2 levels "No","Yes": 1 1 1 1 1 1 1 1 1 1 ...
##   .. ..$ : Factor w/ 2 levels "No","Yes": 1 1 1 1 1 1 1 1 1 1 ...
##   .. ..$ : int  NA NA NA NA NA NA NA NA NA NA ...
##   .. ..$ : num  NA NA NA NA NA NA NA NA NA NA ...
##   .. ..$ : num  NA NA NA NA NA NA NA NA NA NA ...
##   .. ..$ : Factor w/ 3 levels "","sink","source": 1 1 1 1 1 1 1 1 1 1 ...
##   .. ..$ : Factor w/ 4 levels "","env","gut",..: 1 1 1 1 1 1 1 1 1 1 ...
##   .. ..$ : Factor w/ 10 levels "1/04/2017","1/10/2016",..: 6 6 6 6 6 6 6 6 6 6 ...
##   .. ..$ : int  2 2 2 2 2 2 2 2 2 2 ...
##   .. ..$ : Factor w/ 9 levels "","Broome-curlewsand-adult",..: 5 5 4 5 5 5 5 5 5 4 ...
##   ..@ names    : chr  "Group" "species" "site" "month" ...
##   ..@ row.names: chr  "8546" "8548" "8549" "8551" ...
##   ..@ .S3Class : chr "data.frame"
```

```
head(map)
```

```
##      Group          species   site  month         age group bird_id type
## 8546  8546 Red-necked stint Broome August Second year   two 3674227 bird
## 8548  8548 Red-necked stint Broome August Second year   two 3674229 bird
## 8549  8549 Red-necked stint Broome August       Adult   two 3671906 bird
## 8551  8551 Red-necked stint Broome August Second year   two 3674231 bird
## 8552  8552 Red-necked stint Broome August Second year   two 3674232 bird
## 8556  8556 Red-necked stint Broome August Second year   two 3674214 bird
##      Recap recap_delete Recap_num replicate remove_replicate weight wing
## 8546    No           No        NA        No               No     NA   NA
## 8548    No           No        NA        No               No     NA   NA
## 8549    No           No        NA        No               No     NA   NA
## 8551    No           No        NA        No               No     NA   NA
## 8552    No           No        NA        No               No     NA   NA
## 8556    No           No        NA        No               No     NA   NA
##      hb SourceSink Env       Date Plate          migration
## 8546 NA                22/08/2015     2    Broome-stint-SY
## 8548 NA                22/08/2015     2    Broome-stint-SY
## 8549 NA                22/08/2015     2 Broome-stint-adult
## 8551 NA                22/08/2015     2    Broome-stint-SY
## 8552 NA                22/08/2015     2    Broome-stint-SY
## 8556 NA                22/08/2015     2    Broome-stint-SY
```

```
tail(map)
```

```
##          Group species     site    month age group bird_id type Recap
## F1   Flind_env     env Flinders    March  na             na  env    No
## F2   Flind_env     env Flinders    March  na             na  env    No
## Neg       Neg1     neg       na       na  na             na  neg    na
## Neg2      Neg2     neg                                      neg    No
## WTP1   WTP_env     env      WTP December  na             na  env    No
## WTP2   WTP_env     env      WTP December  na             na  env    No
##      recap_delete Recap_num replicate remove_replicate weight wing hb
## F1             No        NA       Yes               No     NA   NA NA
## F2             No        NA       Yes               No     NA   NA NA
## Neg            No        NA        No               No     NA   NA NA
## Neg2           No        NA        No               No     NA   NA NA
## WTP1           No        NA        No               No     NA   NA NA
## WTP2           No        NA        No               No     NA   NA NA
##      SourceSink Env       Date Plate migration
## F1         sink env 11/03/2016     1
## F2         sink env 11/03/2016     1
## Neg        sink neg  1/10/2016     1
## Neg2                 1/04/2017     2
## WTP1       sink env 29/12/2015     1
## WTP2       sink env 29/12/2015     1
```

```
##merge the metadata into the phyloseq object

moth_merge <- merge_phyloseq(mothur_data, map)

##import phylogenetic tree

tree<-read.tree("shorebird.microbiome.tree")

##make tree into phyloseq format

tree<-phy_tree(tree)

##merge tree into current phyloseq object so all object now contained in moth_merge

moth_merge <-merge_phyloseq(moth_merge, tree)

moth_merge
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 8791 taxa and 188 samples ]
## sample_data() Sample Data:       [ 188 samples by 21 sample variables ]
## tax_table()   Taxonomy Table:    [ 8791 taxa by 6 taxonomic ranks ]
## phy_tree()    Phylogenetic Tree: [ 8791 tips and 8789 internal nodes ]
```

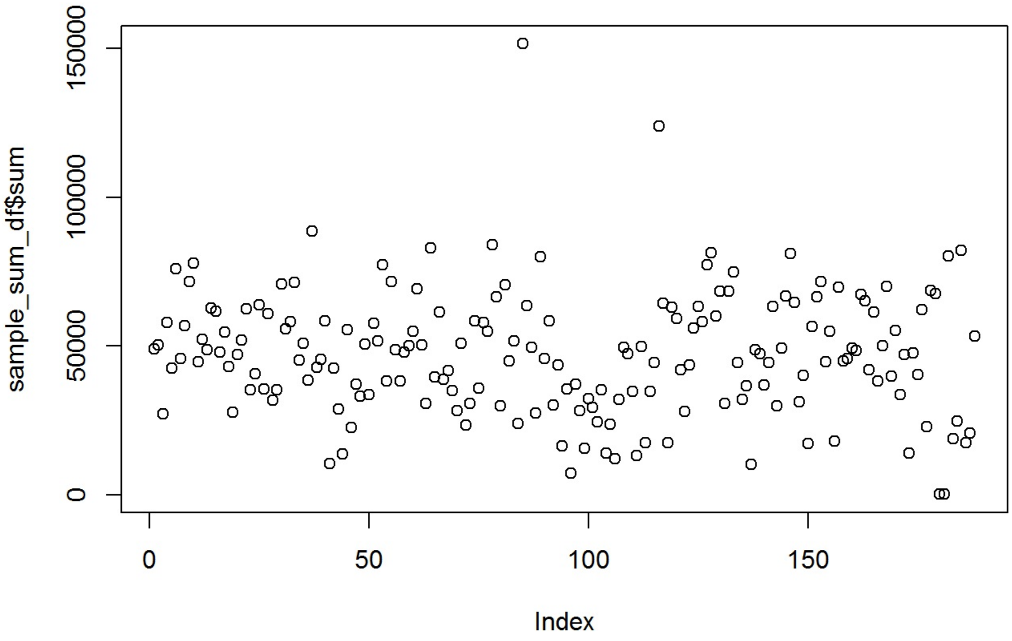
```
##everything looks fie and no errors!

##rename columns to relevant taxonomic group

colnames(tax_table(moth_merge))
```

```
## [1] "Rank1" "Rank2" "Rank3" "Rank4" "Rank5" "Rank6"
```

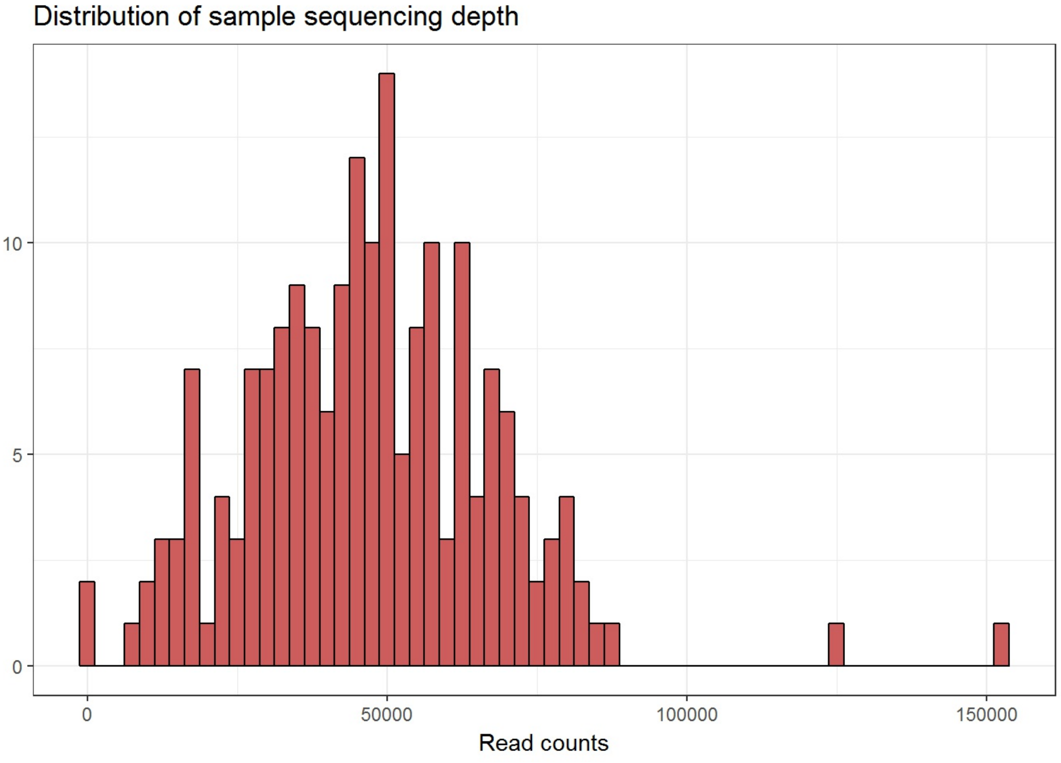
```
colnames(tax_table(moth_merge)) <- c("Kingdom", "Phylum", "Class",
                                     "Order", "Family", "Genus")

data_all <- moth_merge %>%
  subset_taxa(
    Kingdom == "Bacteria" &
      Family  != "mitochondria" &
      Class   != "Chloroplast"
  )

data_all
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 8791 taxa and 188 samples ]
## sample_data() Sample Data:       [ 188 samples by 21 sample variables ]
## tax_table()   Taxonomy Table:    [ 8791 taxa by 6 taxonomic ranks ]
## phy_tree()    Phylogenetic Tree: [ 8791 tips and 8789 internal nodes ]
```

```
##################################################################look at read library


sample_sum_df <- data.frame(sum = sample_sums(data_all))
plot(sample_sum_df$sum)
```



```
ggplot(sample_sum_df, aes(x = sum)) +
  geom_histogram(color = "black", fill = "indianred", binwidth = 2500) +
  ggtitle("Distribution of sample sequencing depth") +
  xlab("Read counts") +
  ylab("Frequency")+
  theme(axis.title.y = element_blank())
```



```
###############################################################################################################
###############

##subset samples to just birds (there are environmental samples in here too)

data_all<-data_all %>%
  subset_samples(Group != "Env_Broome_A")

data_all<-data_all %>%
  subset_samples(Group != "Env_Broome_E")


data_all<-prune_taxa(taxa_sums(data_all)>0, data_all)

## remove OTUs that are common in the neg controls (Neg = just lab control, Neg2 = field and lab control)
neg_control<-data_all %>% subset_samples(type == "neg")

neg_control <- prune_taxa(taxa_sums(neg_control) > 5, neg_control)
neg_control
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 97 taxa and 2 samples ]
## sample_data() Sample Data:       [ 2 samples by 21 sample variables ]
## tax_table()   Taxonomy Table:    [ 97 taxa by 6 taxonomic ranks ]
## phy_tree()    Phylogenetic Tree: [ 97 tips and 96 internal nodes ]
```

```
## 97 OTUs which have over 1 reads

## we should get rid of these

badtaxa<-taxa_names(neg_control)
alltaxa<-taxa_names(data_all)
alltaxa1 <- alltaxa[!(alltaxa %in% badtaxa)]

data_all = prune_taxa(alltaxa1, data_all)

##get rid of negative control altogether

data_all<-data_all%>%subset_samples(species!="Neg")

#delete replicates

data_all<-data_all%>%subset_samples(remove_replicate=="No")

## just birds

birds<-data_all%>%subset_samples(type=="bird")
birds <- prune_taxa(taxa_sums(birds) > 0, birds)


#####################################

## data has more samples than we need. We are going to prune the dataset to just the relevant samples for this
analysis

## for migration analysis (migrant/resident comparison)

migration<-birds%>%subset_samples(species=="Red-necked stint"|species=="Curlew sandpiper")
migration<-migration%>%subset_samples(month=="August"|month=="September")
migration <- prune_taxa(taxa_sums(migration) > 0, migration)
migration
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 5262 taxa and 77 samples ]
## sample_data() Sample Data:       [ 77 samples by 21 sample variables ]
## tax_table()   Taxonomy Table:    [ 5262 taxa by 6 taxonomic ranks ]
## phy_tree()    Phylogenetic Tree: [ 5262 tips and 5260 internal nodes ]
## 77 samples, 5262 OTUs

############ DESEq 2 ###############

## Will do this analysis first (for Fig2 and Table S2) before rarefying data

## Analyse differential abundances between migrants and residents for each group

## subset RNS (Red-necked stint) in Broome

rns_br<-migration%>%subset_samples(species=="Red-necked stint")
rns_br<-rns_br%>%subset_samples(site=="Broome")
rns_br <- prune_taxa(taxa_sums(rns_br) > 0, rns_br)

########################

##the following analysis is from the Phyloseq-DESeq2 vignette, linked at top of script.

diagdds = phyloseq_to_deseq2(rns_br, ~ age)
```

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

```
# calculate geometric means prior to estimate size factors
gm_mean = function(x, na.rm=TRUE){
  exp(sum(log(x[x > 0]), na.rm=na.rm) / length(x))
}
geoMeans = apply(counts(diagdds), 1, gm_mean)
diagdds = estimateSizeFactors(diagdds, geoMeans = geoMeans)
diagdds = DESeq(diagdds, fitType="local")
```

```
## using pre-existing size factors
```

```
## estimating dispersions
```

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

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

```
## final dispersion estimates
```

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

```
## -- replacing outliers and refitting for 985 genes
## -- DESeq argument 'minReplicatesForReplace' = 7
## -- original counts are preserved in counts(dds)
```

```
## estimating dispersions
```

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

```
#diagdds = DESeq(diagdds, fitType="parametric")

res = results(diagdds, cooksCutoff = FALSE)
alpha = 0.01
sigtab = res[which(res$padj < alpha), ]
sigtab_rns.br = cbind(as(sigtab, "data.frame"), as(tax_table(rns_br)[rownames(sigtab), ], "matrix"))
head(sigtab_rns.br)
```

```
##              baseMean log2FoldChange    lfcSE      stat       pvalue
## Otu000765    8.943807       6.451209 1.473355  4.378585 1.194523e-05
## Otu000279   12.561703       6.487397 1.733635  3.742078 1.825051e-04
## Otu000053  448.890074       4.714085 1.207552  3.903836 9.468006e-05
## Otu000013 1452.245588     -10.263594 1.174882 -8.735852 2.418274e-18
## Otu000117   43.826023      -8.519601 1.381634 -6.166322 6.989664e-10
## Otu000009  451.397495     -11.616548 1.627590 -7.137267 9.520456e-13
##                   padj  Kingdom         Phylum               Class
## Otu000765 5.492657e-04 Bacteria Proteobacteria Gammaproteobacteria
## Otu000279 4.994002e-03 Bacteria Proteobacteria Gammaproteobacteria
## Otu000053 3.562337e-03 Bacteria  Bacteroidetes    Sphingobacteriia
## Otu000013 3.639502e-16 Bacteria Actinobacteria      Actinobacteria
## Otu000117 5.259722e-08 Bacteria Actinobacteria      Actinobacteria
## Otu000009 9.552191e-11 Bacteria Actinobacteria      Actinobacteria
##                        Order                         Family
## Otu000765    Pseudomonadales                  Moraxellaceae
## Otu000279    Pseudomonadales                  Moraxellaceae
## Otu000053 Sphingobacteriales               Chitinophagaceae
## Otu000013  Corynebacteriales             Corynebacteriaceae
## Otu000117  Corynebacteriales             Corynebacteriaceae
## Otu000009  Corynebacteriales Corynebacteriales_unclassified
##                                      Genus
## Otu000765                    Acinetobacter
## Otu000279                    Acinetobacter
## Otu000053                    Vibrionimonas
## Otu000013 Corynebacteriaceae_unclassified
## Otu000117 Corynebacteriaceae_unclassified
## Otu000009  Corynebacteriales_unclassified
```

```
sigtab_rns.br<- sigtab_rns.br[order(sigtab_rns.br$log2FoldChange),]

#add variable to end
sigtab_rns.br$Group<-"Red-necked stint Broome"
#########


###############################################################################################

##RNS in Vic

rns_vic<-migration%>%subset_samples(species=="Red-necked stint")
rns_vic<-rns_vic%>%subset_samples(month=="September")
rns_vic <- prune_taxa(taxa_sums(rns_vic) > 0, rns_vic)

########################

diagdds = phyloseq_to_deseq2(rns_vic, ~ age)
```

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

```
# calculate geometric means prior to estimate size factors
gm_mean = function(x, na.rm=TRUE){
  exp(sum(log(x[x > 0]), na.rm=na.rm) / length(x))
}
geoMeans = apply(counts(diagdds), 1, gm_mean)
diagdds = estimateSizeFactors(diagdds, geoMeans = geoMeans)
diagdds = DESeq(diagdds, fitType="local")
```

```
## using pre-existing size factors
```

```
## estimating dispersions
```

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

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

```
## final dispersion estimates
## fitting model and testing
```

```
## -- replacing outliers and refitting for 1034 genes
## -- DESeq argument 'minReplicatesForReplace' = 7
## -- original counts are preserved in counts(dds)
```

```
## estimating dispersions
```

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

```
#diagdds = DESeq(diagdds, fitType="parametric")

res = results(diagdds, cooksCutoff = FALSE)
alpha = 0.01
sigtab = res[which(res$padj < alpha), ]
sigtab_rns.vic = cbind(as(sigtab, "data.frame"), as(tax_table(rns_vic)[rownames(sigtab), ], "matrix"))
head(sigtab_rns.vic)
```

```
##             baseMean log2FoldChange     lfcSE      stat       pvalue
## Otu000013  310.74839      -7.603356 0.9299704 -8.175912 2.936371e-16
## Otu000117   11.77106      -6.999681 1.2864956 -5.440890 5.301508e-08
## Otu000009 6020.41474      -9.169087 0.9653096 -9.498597 2.127372e-21
## Otu000575   17.49598      -7.299684 1.2980066 -5.623765 1.868398e-08
## Otu000265   12.17622      -7.131328 1.6612481 -4.292753 1.764711e-05
## Otu000757   13.11934      -6.617123 1.3422332 -4.929936 8.225670e-07
##                   padj  Kingdom         Phylum          Class
## Otu000013 1.642900e-13 Bacteria Actinobacteria Actinobacteria
## Otu000117 1.483097e-05 Bacteria Actinobacteria Actinobacteria
## Otu000009 2.380529e-18 Bacteria Actinobacteria Actinobacteria
## Otu000575 6.969125e-06 Bacteria Actinobacteria Actinobacteria
## Otu000265 2.194124e-03 Bacteria Actinobacteria Actinobacteria
## Otu000757 1.534087e-04 Bacteria Actinobacteria Actinobacteria
##                       Order                         Family
## Otu000013 Corynebacteriales             Corynebacteriaceae
## Otu000117 Corynebacteriales             Corynebacteriaceae
## Otu000009 Corynebacteriales Corynebacteriales_unclassified
## Otu000575 Corynebacteriales             Corynebacteriaceae
## Otu000265 Corynebacteriales             Corynebacteriaceae
## Otu000757 Corynebacteriales             Corynebacteriaceae
##                                     Genus
## Otu000013 Corynebacteriaceae_unclassified
## Otu000117 Corynebacteriaceae_unclassified
## Otu000009  Corynebacteriales_unclassified
## Otu000575 Corynebacteriaceae_unclassified
## Otu000265 Corynebacteriaceae_unclassified
## Otu000757                 Corynebacterium
```

```
sigtab_rns.vic<- sigtab_rns.vic[order(sigtab_rns.vic$log2FoldChange),]

sigtab_rns.vic$Group<-"Red-necked stint Flinders"

####################################################

#curlew sandpiper

cs<-migration%>%subset_samples(species=="Curlew sandpiper")
cs <- prune_taxa(taxa_sums(cs) > 0, cs)

#####################

diagdds = phyloseq_to_deseq2(cs, ~ age)
```

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

```
# calculate geometric means prior to estimate size factors
gm_mean = function(x, na.rm=TRUE){
  exp(sum(log(x[x > 0]), na.rm=na.rm) / length(x))
}
geoMeans = apply(counts(diagdds), 1, gm_mean)
diagdds = estimateSizeFactors(diagdds, geoMeans = geoMeans)
diagdds = DESeq(diagdds, fitType="local")
```

```
## using pre-existing size factors
```

```
## estimating dispersions
```

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

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

```
## final dispersion estimates
```

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

```
## -- replacing outliers and refitting for 509 genes
## -- DESeq argument 'minReplicatesForReplace' = 7
## -- original counts are preserved in counts(dds)
```

```
## estimating dispersions
```

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

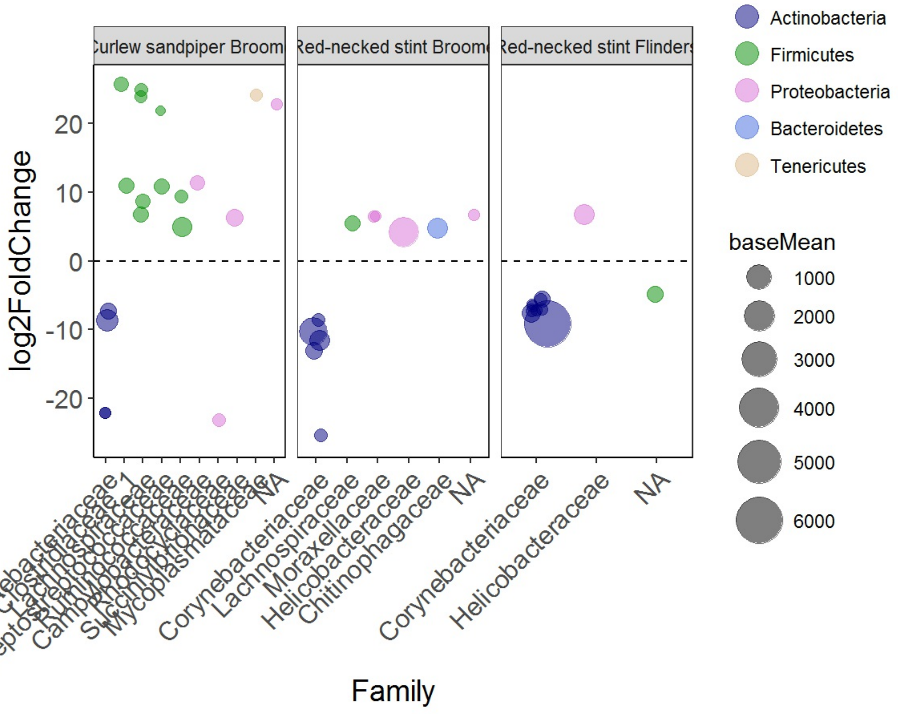
```
#diagdds = DESeq(diagdds, fitType="parametric")

res = results(diagdds, cooksCutoff = FALSE)
alpha = 0.01
sigtab = res[which(res$padj < alpha), ]
sigtab_cs = cbind(as(sigtab, "data.frame"), as(tax_table(cs)[rownames(sigtab), ], "matrix"))
head(sigtab_cs)
```

```
##            baseMean log2FoldChange    lfcSE      stat       pvalue
## Otu000319  36.83220     -23.122692 3.172221 -7.289117 3.119939e-13
## Otu000017 211.66346       6.298750 1.498019  4.204720 2.614058e-05
## Otu000023 648.40735      -8.741014 1.963501 -4.451749 8.517358e-06
## Otu000134  17.35786     -22.100447 3.161498 -6.990498 2.739122e-12
## Otu000157  17.97271     -22.155554 3.172759 -6.983056 2.888269e-12
## Otu000028 177.39850      -7.319617 2.008101 -3.645044 2.673464e-04
##                   padj  Kingdom         Phylum               Class
## Otu000319 1.164777e-11 Bacteria Proteobacteria  Betaproteobacteria
## Otu000017 4.182493e-04 Bacteria Proteobacteria Gammaproteobacteria
## Otu000023 1.467606e-04 Bacteria Actinobacteria      Actinobacteria
## Otu000134 7.188580e-11 Bacteria Actinobacteria      Actinobacteria
## Otu000157 7.188580e-11 Bacteria Actinobacteria      Actinobacteria
## Otu000028 3.522682e-03 Bacteria Actinobacteria      Actinobacteria
##                       Order              Family
## Otu000319     Rhodocyclales      Rhodocyclaceae
## Otu000017     Aeromonadales Succinivibrionaceae
## Otu000023 Corynebacteriales  Corynebacteriaceae
## Otu000134 Corynebacteriales  Corynebacteriaceae
## Otu000157 Corynebacteriales  Corynebacteriaceae
## Otu000028 Corynebacteriales  Corynebacteriaceae
##                                      Genus
## Otu000319                Methyloversatilis
## Otu000017 Succinivibrionaceae_unclassified
## Otu000023  Corynebacteriaceae_unclassified
## Otu000134  Corynebacteriaceae_unclassified
## Otu000157                  Corynebacterium
## Otu000028                             <NA>
```

```
sigtab_cs<- sigtab_cs[order(sigtab_cs$log2FoldChange),]

sigtab_cs$Group<-"Curlew sandpiper Broome"



##########################################

##plot all together
##bind objects

deseq2<-rbind(sigtab_rns.br, sigtab_rns.vic, sigtab_cs)

#edit small details for figure

deseq2[3,11]<-"Corynebacteriaceae"
deseq2[12,11]<-"Corynebacteriaceae"
revalue(deseq2$Family, c("Gammaproteobacteria_unclassified"="Gammaproteobacteria uncl."))
```

```
##                 Otu000118                 Otu000028
##        Corynebacteriaceae        Corynebacteriaceae
##                 Otu000009                 Otu000013
##        Corynebacteriaceae        Corynebacteriaceae
##                 Otu000117                 Otu000011
##        Corynebacteriaceae         Helicobacteraceae
##                 Otu000053                 Otu000106
##          Chitinophagaceae           Lachnospiraceae
##                 Otu000765                 Otu000279
##             Moraxellaceae             Moraxellaceae
##                 Otu000358                 Otu000009
## Gammaproteobacteria uncl.        Corynebacteriaceae
##                 Otu000013                 Otu000575
##        Corynebacteriaceae        Corynebacteriaceae
##                 Otu000265                 Otu000117
##        Corynebacteriaceae        Corynebacteriaceae
##                 Otu000757                 Otu000769
##        Corynebacteriaceae        Corynebacteriaceae
##                 Otu000136                 Otu000028
##        Corynebacteriaceae        Corynebacteriaceae
##                 Otu000020                 Otu000006
##           Enterococcaceae         Helicobacteraceae
##                 Otu000319                 Otu000157
##            Rhodocyclaceae        Corynebacteriaceae
##                 Otu000134                 Otu000023
##        Corynebacteriaceae        Corynebacteriaceae
##                 Otu000028                 Otu000044
##        Corynebacteriaceae           Ruminococcaceae
##                 Otu000017                 Otu000070
##       Succinivibrionaceae           Lachnospiraceae
##                 Otu000124                 Otu000126
##           Lachnospiraceae           Ruminococcaceae
##                 Otu000227                 Otu000208
##     Peptostreptococcaceae          Clostridiaceae_1
##                 Otu000078                 Otu000628
##        Campylobacteraceae     Peptostreptococcaceae
##                 Otu000358                 Otu000343
## Gammaproteobacteria uncl.           Lachnospiraceae
##                 Otu000094                 Otu000316
##          Mycoplasmataceae           Lachnospiraceae
##                 Otu000183
##          Clostridiaceae_1
## 15 Levels: Chitinophagaceae ... Succinivibrionaceae
```

```
deseq2$Family<-factor(deseq2$Family, levels = c("Corynebacteriaceae",
                                                 "Clostridiaceae_1","Lachnospiraceae" ,"Peptostreptococcaceae",
  "Ruminococcaceae",

                                                  "Moraxellaceae",
                                                  "Campylobacteraceae"  ,
                                                  "Helicobacteraceae" ,
                                                  "Rhodocyclaceae"   ,
                                                  "Gammaproteobacteria uncl.",
                                                  "Succinivibrionaceae",
                                                  "Chitinophagaceae" ,
                                                  "Mycoplasmataceae"))

deseq2$Phylum<-factor(deseq2$Phylum, levels = c("Actinobacteria","Firmicutes", "Proteobacteria","Bacteroidetes"
,"Tenericutes"))



#Figure 2

ggplot(deseq2, aes(x=Family, y=log2FoldChange, color=Phylum)) +
  geom_jitter(aes(size = baseMean), width = 0.2, alpha = 0.5)+ scale_size(range = c(2,10))+
  theme(axis.text.x = element_text(angle = 45, hjust = 1))+
  geom_hline(yintercept = 0, linetype = 2)+facet_grid(.~Group, scales = "free")+ylim(-26,26)+
  scale_color_manual(values = c("navyblue","green4","orchid","royalblue","burlywood"))+
  theme( axis.text = element_text(size=12), axis.title=element_text(size=14))+
  theme(plot.margin=unit(c(0.5,1,1,2),"cm"))+guides(color = guide_legend(override.aes = list(size=5)))+
  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(),
        panel.background = element_blank(), axis.line = element_line(colour = "black"))
```



```
################################################################################

##Now rest of analyses

migration1 <-migration

##rarefy
migration <-rarefy_even_depth(migration1, sample.size = min(sample_sums(migration1)),
                          rngseed = 3, replace = TRUE, trimOTUs = TRUE, verbose = TRUE)
```

```
## `set.seed(3)` was used to initialize repeatable random subsampling.
```

```
## Please record this for your records so others can reproduce.
## Try `set.seed(3); .Random.seed` for the full vector
```

```
## ...
```

```
## 856OTUs were removed because they are no longer 
## present in any sample after random subsampling
```

```
## ...
```

```
migration
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 4406 taxa and 77 samples ]
## sample_data() Sample Data:       [ 77 samples by 21 sample variables ]
## tax_table()   Taxonomy Table:    [ 4406 taxa by 6 taxonomic ranks ]
## phy_tree()    Phylogenetic Tree: [ 4406 tips and 4404 internal nodes ]
```

```
migration2 <- migration1 %>%
  scale_reads(n=5815) ##scale to smallest library size

#migration1 = unrarefied
#migration = rarefied
#migration2 = scaled

#using rarefied data, but can test with others - makes no difference

##########################################  Prevalence  ##############################################
##prevance whole group

prev0 = apply(X = otu_table(migration),
              MARGIN = ifelse(taxa_are_rows(migration), yes = 1, no = 2),
              FUN = function(x){sum(x > 0)})
prevdf = data.frame(Prevalence = prev0,
                    TotalAbundance = taxa_sums(migration),
                    tax_table(migration))
keepPhyla = table(prevdf$Phylum)[(table(prevdf$Phylum) > 5)]
prevdf1 = subset(prevdf, Phylum %in% names(keepPhyla))

prevdf$Prevalence<-(prevdf$Prevalence/77)*100


head(prevdf)
```

```
##           Prevalence TotalAbundance  Kingdom         Phylum
## Otu001177   1.298701              3 Bacteria Proteobacteria
## Otu000824   3.896104             15 Bacteria Proteobacteria
## Otu004711   2.597403              7 Bacteria Proteobacteria
## Otu005756   1.298701              4 Bacteria Proteobacteria
## Otu007032   1.298701              2 Bacteria Proteobacteria
## Otu001047   3.896104             17 Bacteria Proteobacteria
##                         Class           Order           Family
## Otu001177 Alphaproteobacteria Rhodobacterales Rhodobacteraceae
## Otu000824 Alphaproteobacteria Rhodobacterales Rhodobacteraceae
## Otu004711 Alphaproteobacteria Rhodobacterales Rhodobacteraceae
## Otu005756 Alphaproteobacteria Rhodobacterales Rhodobacteraceae
## Otu007032 Alphaproteobacteria Rhodobacterales Rhodobacteraceae
## Otu001047 Alphaproteobacteria Rhodobacterales Rhodobacteraceae
##                                   Genus
## Otu001177              Rubellimicrobium
## Otu000824                      Wenxinia
## Otu004711 Rhodobacteraceae_unclassified
## Otu005756                          <NA>
## Otu007032                          <NA>
## Otu001047                Pseudoruegeria
```

```
str(prevdf)
```

```
## 'data.frame':    4406 obs. of  8 variables:
##  $ Prevalence    : num  1.3 3.9 2.6 1.3 1.3 ...
##  $ TotalAbundance: num  3 15 7 4 2 17 1 1 3 3 ...
##  $ Kingdom       : Factor w/ 1 level "Bacteria": 1 1 1 1 1 1 1 1 1 1 ...
##  $ Phylum        : Factor w/ 27 levels "Acidobacteria",..: 21 21 21 21 21 21 21 21 21 21 ...
##  $ Class         : Factor w/ 75 levels "Acidimicrobiia",..: 4 4 4 4 4 4 4 4 4 4 ...
##  $ Order         : Factor w/ 150 levels "Acidimicrobiales",..: 110 110 110 110 110 110 110 110 110 110 ...
##  $ Family        : Factor w/ 270 levels "0319-6G20","Acetobacteraceae",..: 203 203 203 203 203 203 203 203 2
03 203 ...
##  $ Genus         : Factor w/ 554 levels "0319-6G20_ge",..: 443 548 422 NA NA 407 407 422 422 NA ...
```

```
summary(prevdf$Prevalence)
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
##   1.299   1.299   2.597   4.638   3.896  93.506
```

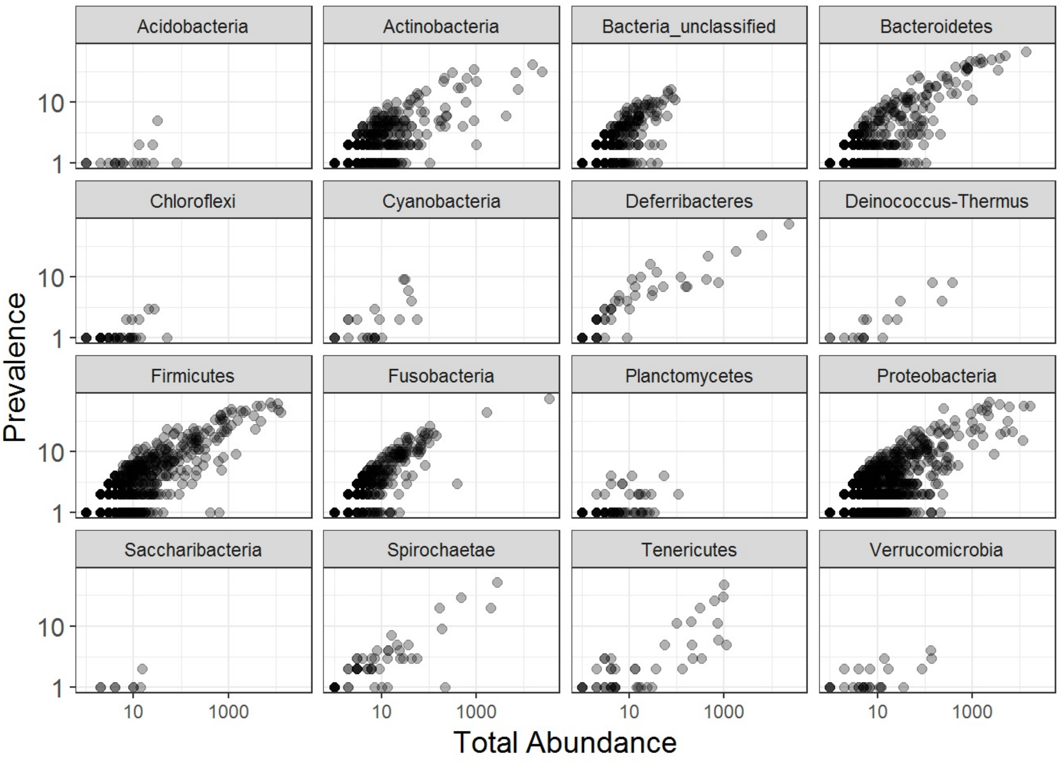
```
###only keep those phyla which occur in at least 5 samples



# Define prevalence threshold as 5% of total samples
prevalenceThreshold = 0.05 * nsamples(migration)
prevalenceThreshold
```

```
## [1] 3.85
```

```
migration1 = prune_taxa((prev0 > prevalenceThreshold), migration)
migration1
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 1016 taxa and 77 samples ]
## sample_data() Sample Data:       [ 77 samples by 21 sample variables ]
## tax_table()   Taxonomy Table:    [ 1016 taxa by 6 taxonomic ranks ]
## phy_tree()    Phylogenetic Tree: [ 1016 tips and 1015 internal nodes ]
```

```
ggplot(prevdf1, aes(TotalAbundance, Prevalence)) +
  geom_point(size = 2, alpha = 0.3) +
  scale_y_log10() + scale_x_log10() +
  xlab("Total Abundance") +
  facet_wrap(~Phylum)+theme_bw() +
  theme(  axis.text.y = element_text(size=12), axis.title=element_text(size=14))
```



```
#write.csv(prevdf, "prevalence_all_samples.csv")

####################################################

##table S1

#Subset each group seperately to get top 10 OTUs
# b = broome, f = flinders, a = adult/migrants, sy = second year/resident

curlew.b<-migration%>%subset_samples(species=="Curlew sandpiper"& site=="Broome")
curlew.b<- prune_taxa(taxa_sums(curlew.b) > 0, curlew.b)
curlew.b.a<-curlew.b%>%subset_samples(age=="Adult")
curlew.b.a<- prune_taxa(taxa_sums(curlew.b.a) > 0, curlew.b.a)
curlew.b.sy<-curlew.b%>%subset_samples(age=="Second year")
curlew.b.sy<- prune_taxa(taxa_sums(curlew.b.sy) > 0, curlew.b.sy)

stint.b<-migration%>%subset_samples(site=="Broome" & species=="Red-necked stint")
stint.b<- prune_taxa(taxa_sums(stint.b) > 0, stint.b)
stint.b.a<-stint.b%>%subset_samples(age=="Adult")
stint.b.a<- prune_taxa(taxa_sums(stint.b.a) > 0, stint.b.a)
stint.b.sy<-stint.b%>%subset_samples(age=="Second year")
stint.b.sy<- prune_taxa(taxa_sums(stint.b.sy) > 0, stint.b.sy)

stint.f<-migration%>%subset_samples(month=="September")
stint.f<-stint.f%>%subset_samples(species=="Red-necked stint")
stint.f<- prune_taxa(taxa_sums(stint.f) > 0, stint.f)
stint.f.sy<-stint.f%>%subset_samples(age=="Second year")

stint.f.sy<- prune_taxa(taxa_sums(stint.f.sy) > 0, stint.f.sy)
stint.f.a<-stint.f%>%subset_samples(age=="Adult")
stint.f.a<- prune_taxa(taxa_sums(stint.f.a) > 0, stint.f.a)

######## TABLE S1A)

# broome curlew sand migrants

prev0 = apply(X = otu_table(curlew.b.a),
              MARGIN = ifelse(taxa_are_rows(curlew.b.a), yes = 1, no = 2),
              FUN = function(x){sum(x > 0)})
prevdf = data.frame(Prevalence = prev0,
                    TotalAbundance = taxa_sums(curlew.b.a),
                    tax_table(curlew.b.a))


prevdf$RelAbund<-prevdf$TotalAbundance/sum(prevdf$TotalAbundance)
prevdf$RelPrev<-prevdf$Prevalence/12
prevdf<-prevdf[order(-prevdf$RelAbund),]
head(prevdf)
```

```
##           Prevalence TotalAbundance  Kingdom          Phylum
## Otu000023          9           7688 Bacteria  Actinobacteria
## Otu000027          8           5928 Bacteria  Proteobacteria
## Otu000002         11           4683 Bacteria    Fusobacteria
## Otu000058          4           4295 Bacteria  Actinobacteria
## Otu000010         10           3326 Bacteria Deferribacteres
## Otu000028          7           2829 Bacteria  Actinobacteria
##                         Class             Order              Family
## Otu000023      Actinobacteria Corynebacteriales  Corynebacteriaceae
## Otu000027 Gammaproteobacteria     Aeromonadales Succinivibrionaceae
## Otu000002       Fusobacteriia   Fusobacteriales    Fusobacteriaceae
## Otu000058      Actinobacteria Corynebacteriales  Corynebacteriaceae
## Otu000010     Deferribacteres Deferribacterales  Deferribacteraceae
## Otu000028      Actinobacteria Corynebacteriales  Corynebacteriaceae
##                                     Genus   RelAbund   RelPrev
## Otu000023 Corynebacteriaceae_unclassified 0.11017484 0.7500000
## Otu000027              Anaerobiospirillum 0.08495271 0.6666667
## Otu000002                   Cetobacterium 0.06711092 0.9166667
## Otu000058 Corynebacteriaceae_unclassified 0.06155059 0.3333333
## Otu000010                   Mucispirillum 0.04766409 0.8333333
## Otu000028                            <NA> 0.04054170 0.5833333
```

```
#write.csv(prevdf, "curlew.b.a.csv")

######################

# broome curlew sand residents

prev0 = apply(X = otu_table(curlew.b.sy),
              MARGIN = ifelse(taxa_are_rows(curlew.b.sy), yes = 1, no = 2),
              FUN = function(x){sum(x > 0)})
prevdf = data.frame(Prevalence = prev0,
                    TotalAbundance = taxa_sums(curlew.b.sy),
                    tax_table(curlew.b.sy))


prevdf$RelAbund<-prevdf$TotalAbundance/sum(prevdf$TotalAbundance)
prevdf$RelPrev<-prevdf$Prevalence/6
prevdf<-prevdf[order(-prevdf$RelAbund),]
head(prevdf)
```

```
##           Prevalence TotalAbundance  Kingdom          Phylum
## Otu000002          5           4223 Bacteria    Fusobacteria
## Otu000010          5           3457 Bacteria Deferribacteres
## Otu000018          6           2711 Bacteria      Firmicutes
## Otu000016          6           2427 Bacteria   Bacteroidetes
## Otu000034          6           2238 Bacteria      Firmicutes
## Otu000008          5           1973 Bacteria      Firmicutes
##                      Class              Order              Family
## Otu000002    Fusobacteriia    Fusobacteriales    Fusobacteriaceae
## Otu000010  Deferribacteres  Deferribacterales  Deferribacteraceae
## Otu000018       Clostridia      Clostridiales     Lachnospiraceae
## Otu000016      Bacteroidia      Bacteroidales      Bacteroidaceae
## Otu000034 Erysipelotrichia Erysipelotrichales Erysipelotrichaceae
## Otu000008          Bacilli    Lactobacillales     Enterococcaceae
##                                      Genus   RelAbund   RelPrev
## Otu000002                    Cetobacterium 0.12103755 0.8333333
## Otu000010                    Mucispirillum 0.09908283 0.8333333
## Otu000018                     Tyzzerella_3 0.07770135 1.0000000
## Otu000016                      Bacteroides 0.06956148 1.0000000
## Otu000034 Erysipelotrichaceae_unclassified 0.06414445 1.0000000
## Otu000008                    Catellicoccus 0.05654915 0.8333333
```

```
#write.csv(prevdf, "curlew.b.sy.csv")

########################################################

##table S1b)

# broome stint migrants

prev0 = apply(X = otu_table(stint.b.a),
              MARGIN = ifelse(taxa_are_rows(stint.b.a), yes = 1, no = 2),
              FUN = function(x){sum(x > 0)})
prevdf = data.frame(Prevalence = prev0,
                    TotalAbundance = taxa_sums(stint.b.a),
                    tax_table(stint.b.a))


prevdf$RelAbund<-prevdf$TotalAbundance/sum(prevdf$TotalAbundance)
prevdf$RelPrev<-prevdf$Prevalence/13
prevdf<-prevdf[order(-prevdf$RelAbund),]
head(prevdf)
```

```
##           Prevalence TotalAbundance  Kingdom          Phylum
## Otu000013         11           8616 Bacteria  Actinobacteria
## Otu000002         13           7614 Bacteria    Fusobacteria
## Otu000017         12           5778 Bacteria  Proteobacteria
## Otu000009          6           5195 Bacteria  Actinobacteria
## Otu000010         13           3510 Bacteria Deferribacteres
## Otu000016         12           3106 Bacteria   Bacteroidetes
##                         Class             Order
## Otu000013      Actinobacteria Corynebacteriales
## Otu000002       Fusobacteriia   Fusobacteriales
## Otu000017 Gammaproteobacteria     Aeromonadales
## Otu000009      Actinobacteria Corynebacteriales
## Otu000010     Deferribacteres Deferribacterales
## Otu000016         Bacteroidia     Bacteroidales
##                                   Family                            Genus
## Otu000013             Corynebacteriaceae  Corynebacteriaceae_unclassified
## Otu000002               Fusobacteriaceae                    Cetobacterium
## Otu000017            Succinivibrionaceae Succinivibrionaceae_unclassified
## Otu000009 Corynebacteriales_unclassified   Corynebacteriales_unclassified
## Otu000010             Deferribacteraceae                    Mucispirillum
## Otu000016                 Bacteroidaceae                      Bacteroides
##             RelAbund   RelPrev
## Otu000013 0.11397579 0.8461538
## Otu000002 0.10072095 1.0000000
## Otu000017 0.07643363 0.9230769
## Otu000009 0.06872148 0.4615385
## Otu000010 0.04643164 1.0000000
## Otu000016 0.04108737 0.9230769
```

```
#write.csv(prevdf, "stint.b.a.csv")
########################

## broome stint resident

prev0 = apply(X = otu_table(stint.b.sy),
              MARGIN = ifelse(taxa_are_rows(stint.b.sy), yes = 1, no = 2),
              FUN = function(x){sum(x > 0)})
prevdf = data.frame(Prevalence = prev0,
                    TotalAbundance = taxa_sums(stint.b.sy),
                    tax_table(stint.b.sy))


prevdf$RelAbund<-prevdf$TotalAbundance/sum(prevdf$TotalAbundance)
prevdf$RelPrev<-prevdf$Prevalence/16
prevdf<-prevdf[order(-prevdf$RelAbund),]
head(prevdf)
```

```
##           Prevalence TotalAbundance  Kingdom          Phylum
## Otu000010         16           9725 Bacteria Deferribacteres
## Otu000011         15           7912 Bacteria  Proteobacteria
## Otu000020         10           5754 Bacteria      Firmicutes
## Otu000002         15           4614 Bacteria    Fusobacteria
## Otu000018          9           4421 Bacteria      Firmicutes
## Otu000036         15           4284 Bacteria  Proteobacteria
##                           Class             Order              Family
## Otu000010       Deferribacteres Deferribacterales  Deferribacteraceae
## Otu000011 Epsilonproteobacteria Campylobacterales   Helicobacteraceae
## Otu000020               Bacilli   Lactobacillales     Enterococcaceae
## Otu000002         Fusobacteriia   Fusobacteriales    Fusobacteriaceae
## Otu000018            Clostridia     Clostridiales     Lachnospiraceae
## Otu000036   Alphaproteobacteria       Rhizobiales Methylobacteriaceae
##                      Genus   RelAbund RelPrev
## Otu000010    Mucispirillum 0.10452494  1.0000
## Otu000011     Helicobacter 0.08503869  0.9375
## Otu000020    Catellicoccus 0.06184437  0.6250
## Otu000002    Cetobacterium 0.04959157  0.9375
## Otu000018     Tyzzerella_3 0.04751720  0.5625
## Otu000036 Methylobacterium 0.04604471  0.9375
```

```
#write.csv(prevdf, "stint.b.sy.csv")

#######################

#Table S1c)

## vic stint migrants

prev0 = apply(X = otu_table(stint.f.a),
              MARGIN = ifelse(taxa_are_rows(stint.f.a), yes = 1, no = 2),
              FUN = function(x){sum(x > 0)})
prevdf = data.frame(Prevalence = prev0,
                    TotalAbundance = taxa_sums(stint.f.a),
                    tax_table(stint.f.a))

prevdf$RelAbund<-prevdf$TotalAbundance/sum(prevdf$TotalAbundance)
prevdf$RelPrev<-prevdf$Prevalence/15
prevdf<-prevdf[order(-prevdf$RelAbund),]

head(prevdf)
```

```
##           Prevalence TotalAbundance  Kingdom         Phylum
## Otu000009         14          19124 Bacteria Actinobacteria
## Otu000002         14           6743 Bacteria   Fusobacteria
## Otu000011         13           5365 Bacteria Proteobacteria
## Otu000020         10           4261 Bacteria     Firmicutes
## Otu000013         14           3865 Bacteria Actinobacteria
## Otu000014         15           2560 Bacteria     Firmicutes
##                           Class             Order
## Otu000009        Actinobacteria Corynebacteriales
## Otu000002         Fusobacteriia   Fusobacteriales
## Otu000011 Epsilonproteobacteria Campylobacterales
## Otu000020               Bacilli   Lactobacillales
## Otu000013        Actinobacteria Corynebacteriales
## Otu000014               Bacilli   Lactobacillales
##                                   Family                           Genus
## Otu000009 Corynebacteriales_unclassified  Corynebacteriales_unclassified
## Otu000002               Fusobacteriaceae                   Cetobacterium
## Otu000011              Helicobacteraceae                    Helicobacter
## Otu000020                Enterococcaceae                   Catellicoccus
## Otu000013             Corynebacteriaceae Corynebacteriaceae_unclassified
## Otu000014                Enterococcaceae                   Catellicoccus
##             RelAbund   RelPrev
## Otu000009 0.21924907 0.9333333
## Otu000002 0.07730582 0.9333333
## Otu000011 0.06150760 0.8666667
## Otu000020 0.04885067 0.6666667
## Otu000013 0.04431069 0.9333333
## Otu000014 0.02934938 1.0000000
```

```
#write.csv(prevdf, "stint.f.a.csv")

#############################

##vic stint resident

prev0 = apply(X = otu_table(stint.f.sy),
              MARGIN = ifelse(taxa_are_rows(stint.f.sy), yes = 1, no = 2),
              FUN = function(x){sum(x > 0)})
prevdf = data.frame(Prevalence = prev0,
                    TotalAbundance = taxa_sums(stint.f.sy),
                    tax_table(stint.f.sy))

prevdf$RelAbund<-prevdf$TotalAbundance/sum(prevdf$TotalAbundance)
prevdf$RelPrev<-prevdf$Prevalence/15
prevdf<-prevdf[order(-prevdf$RelAbund),]
head(prevdf)
```

```
##           Prevalence TotalAbundance  Kingdom         Phylum
## Otu000006          7          11596 Bacteria Proteobacteria
## Otu000002         14           6271 Bacteria   Fusobacteria
## Otu000008         15           4866 Bacteria     Firmicutes
## Otu000031          8           4102 Bacteria     Firmicutes
## Otu000039          3           3774 Bacteria Proteobacteria
## Otu000014         13           2963 Bacteria     Firmicutes
##                           Class              Order              Family
## Otu000006 Epsilonproteobacteria  Campylobacterales   Helicobacteraceae
## Otu000002         Fusobacteriia    Fusobacteriales    Fusobacteriaceae
## Otu000008               Bacilli    Lactobacillales     Enterococcaceae
## Otu000031      Erysipelotrichia Erysipelotrichales Erysipelotrichaceae
## Otu000039   Gammaproteobacteria  Enterobacteriales  Enterobacteriaceae
## Otu000014               Bacilli    Lactobacillales     Enterococcaceae
##                                      Genus   RelAbund   RelPrev
## Otu000006   Helicobacteraceae_unclassified 0.13294354 0.4666667
## Otu000002                    Cetobacterium 0.07189453 0.9333333
## Otu000008                    Catellicoccus 0.05578676 1.0000000
## Otu000031 Erysipelotrichaceae_unclassified 0.04702780 0.5333333
## Otu000039             Escherichia-Shigella 0.04326741 0.2000000
## Otu000014                    Catellicoccus 0.03396962 0.8666667
```

```
#write.csv(prevdf, "stint.f.sy.csv")



#############################################stacked barplots #################################################
################

#Figure 1)

#################### migration

migration_phylum <- migration %>%
  tax_glom(taxrank = "Phylum") %>%                     # agglomerate at phylum level
  transform_sample_counts(function(x) {x/sum(x)} ) %>% # Transform to rel. abundance
  psmelt() %>%                                         # Melt to long format
  arrange(Phylum)                                      # Sort data frame alphabetically by phylum

#filter(Abundance > 0.05) %>%                         # Filter out low abundance taxa
head(migration_phylum)
```

```
##         OTU Sample   Abundance Group          species     site     month
## 1 Otu000944   8680 0.016852966  8680 Red-necked stint Flinders September
## 2 Otu000944   8607 0.004815133  8607 Red-necked stint   Broome    August
## 3 Otu000944   8667 0.004815133  8667 Red-necked stint Flinders September
## 4 Otu000944   8639 0.003955288  8639 Red-necked stint Flinders September
## 5 Otu000944   8560 0.003439381  8560 Red-necked stint   Broome    August
## 6 Otu000944   8566 0.002063629  8566 Red-necked stint   Broome    August
##           age group bird_id type Recap recap_delete Recap_num replicate
## 1       Adult three 3685834 bird    No           No        NA        No
## 2       Adult   two 3678451 bird    No           No        NA        No
## 3 Second year three 3686272 bird    No           No        NA        No
## 4       Adult three 3686361 bird   Yes          Yes         1       Yes
## 5       Adult   two 3674218 bird    No           No        NA        No
## 6 Second year   two 3674234 bird    No           No        NA        No
##   remove_replicate weight wing hb SourceSink Env       Date Plate
## 1               No     29   NA NA     source gut 20/09/2015     1
## 2               No     NA   NA NA                29/08/2015     2
## 3               No     28   NA NA     source gut 20/09/2015     1
## 4               No     31   NA NA     source gut 20/09/2015     1
## 5               No     NA   NA NA                22/08/2015     2
## 6               No     NA   NA NA                22/08/2015     2
##              migration  Kingdom        Phylum
## 1 Flinders-stint-adult Bacteria Acidobacteria
## 2   Broome-stint-adult Bacteria Acidobacteria
## 3    Flinders-stint-SY Bacteria Acidobacteria
## 4 Flinders-stint-adult Bacteria Acidobacteria
## 5   Broome-stint-adult Bacteria Acidobacteria
## 6      Broome-stint-SY Bacteria Acidobacteria
```

```
######################

migration_phylum$Phylum<-factor(migration_phylum$Phylum)
#broome_phylum$Order<-factor(broome_phylum$Order)
unique(migration_phylum$Phylum )
```

```
##  [1] Acidobacteria            Actinobacteria
##  [3] Armatimonadetes          Bacteria_unclassified
##  [5] Bacteroidetes            Chloroflexi
##  [7] Cyanobacteria            Deferribacteres
##  [9] Deinococcus-Thermus      FBP
## [11] Fibrobacteres            Firmicutes
## [13] Fusobacteria             Gemmatimonadetes
## [15] Gracilibacteria          Lentisphaerae
## [17] Microgenomates           Parcubacteria
## [19] Peregrinibacteria        Planctomycetes
## [21] Proteobacteria           Saccharibacteria
## [23] Spirochaetae             SR1_(Absconditabacteria)
## [25] Tenericutes              TM6_(Dependentiae)
## [27] Verrucomicrobia
## 27 Levels: Acidobacteria Actinobacteria ... Verrucomicrobia
```

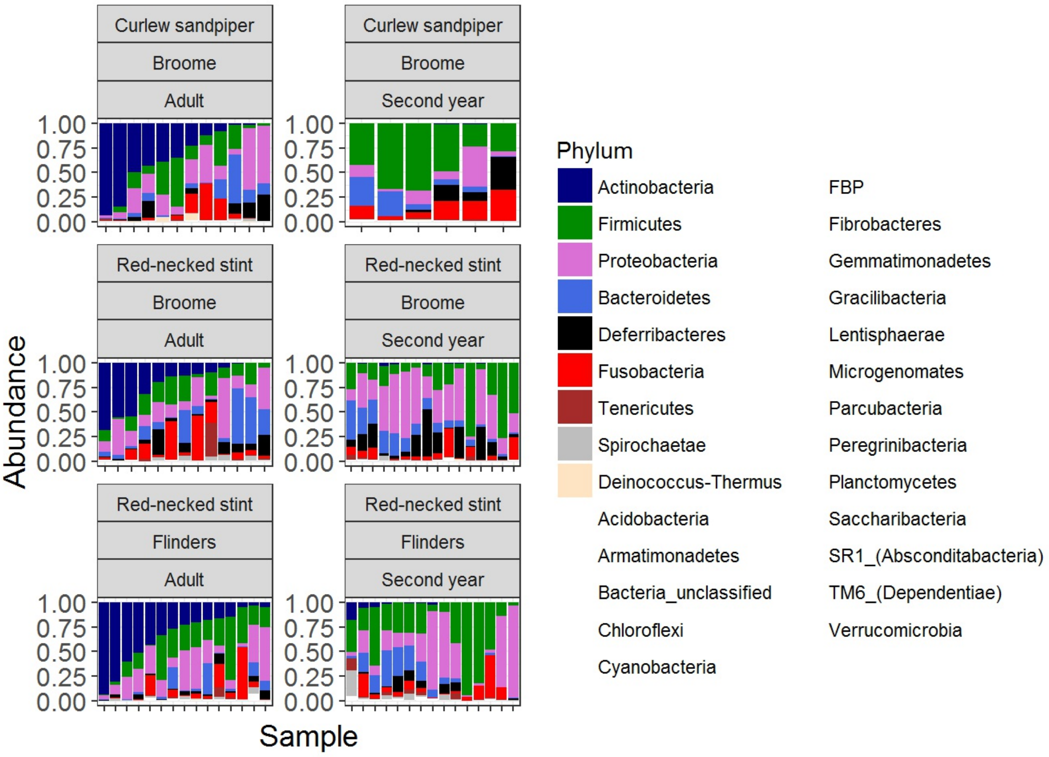
```
migration_phylum$Phylum<-factor(migration_phylum$Phylum)


unique(migration_phylum$Phylum)
```

```
##  [1] Acidobacteria            Actinobacteria
##  [3] Armatimonadetes          Bacteria_unclassified
##  [5] Bacteroidetes            Chloroflexi
##  [7] Cyanobacteria            Deferribacteres
##  [9] Deinococcus-Thermus      FBP
## [11] Fibrobacteres            Firmicutes
## [13] Fusobacteria             Gemmatimonadetes
## [15] Gracilibacteria          Lentisphaerae
## [17] Microgenomates           Parcubacteria
## [19] Peregrinibacteria        Planctomycetes
## [21] Proteobacteria           Saccharibacteria
## [23] Spirochaetae             SR1_(Absconditabacteria)
## [25] Tenericutes              TM6_(Dependentiae)
## [27] Verrucomicrobia
## 27 Levels: Acidobacteria Actinobacteria ... Verrucomicrobia
```

```
migration_phylum$Phylum<-factor(migration_phylum$Phylum, level = c("Actinobacteria",
                                                                   "Firmicutes",
                                                                   "Proteobacteria",
                                                                   "Bacteroidetes",
                                                                   "Deferribacteres",
                                                                   "Fusobacteria",
                                                                   "Tenericutes",
                                                                   "Spirochaetae" ,
                                                                   "Deinococcus-Thermus",
                                                                   "Acidobacteria",
                                                                   "Armatimonadetes",
                                                                   "Bacteria_unclassified",
                                                                   "Chloroflexi",
                                                                   "Cyanobacteria",
                                                                   "FBP",
                                                                   "Fibrobacteres",
                                                                   "Gemmatimonadetes",
                                                                   "Gracilibacteria",
                                                                   "Ignavibacteriae",
                                                                   "Lentisphaerae",
                                                                   "Microgenomates",
                                                                   "Parcubacteria",
                                                                   "Peregrinibacteria",
                                                                   "Planctomycetes",
                                                                   "Saccharibacteria",
                                                                   "SR1_(Absconditabacteria)",
                                                                   "TM6_(Dependentiae)",
                                                                   "Verrucomicrobia"))




unique(migration_phylum$Sample)
```

```
##  [1] "8680" "8607" "8667" "8639" "8560" "8566" "8645" "8662" "8558" "8559"
## [11] "8610" "8632" "8628" "8563" "8581" "8671" "8549" "8552" "8548" "8660"
## [21] "8601" "8604" "8546" "8605" "8612" "8658" "8556" "8570" "8551" "8659"
## [31] "8657" "8646" "8665" "8629" "8655" "8627" "8633" "8609" "8616" "8649"
## [41] "8589" "8606" "8586" "8575" "8644" "8557" "8571" "8677" "8672" "8663"
## [51] "8630" "8689" "8565" "8569" "8615" "8564" "8590" "8561" "8682" "8583"
## [61] "8634" "8683" "8685" "8588" "8675" "8674" "8613" "8626" "8688" "8686"
## [71] "8578" "8625" "8573" "8618" "8684" "8679" "8638"
```

```
colors <- c("navyblue","green4","orchid","royalblue","black","red","brown","grey","bisque",
            "white","white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white")


names(colors) <- levels(migration_phylum$Phylum)
colScale <- scale_fill_manual(name = "Phylum",values = colors)

migration_phylum$Sample<-factor(migration_phylum$Sample, level = c("8682", "8629", "8615", "8685", "8610", "866
2", "8560", "8607", "8671", "8589", "8657", "8573", "8625", "8627", "8639", "8549", "8645", "8632", "8660", "86
49", "8601",
                                                                   "8658", "8638", "8646", "8634", "8606", "861
2", "8581", "8618", "8609", "8628", "8672", "8605", "8689", "8659", "8616", "8626", "8604", "8569", "8677", "85
88", "8675",
                                                                   "8551", "8613", "8546", "8683", "8590", "855
7", "8558", "8674", "8559", "8680", "8630", "8563", "8586", "8556", "8561", "8644", "8667", "8566", "8665", "86
33", "8564",
                                                                   "8583", "8575", "8571", "8565", "8578", "857
0", "8684", "8688", "8655", "8552", "8548", "8679", "8663", "8686"))

##Figure 1a

ggplot(migration_phylum, aes(x = Sample, y = Abundance, fill = Phylum)) +

  geom_bar(stat = "identity") +
  theme(axis.text.x = element_blank(), axis.text.y = element_text(size=12), axis.title=element_text(size=14))+
  facet_wrap(species~site~age, scales="free", nrow=3, ncol=2)+colScale
```



```
###########repeat but for Family Fig 1b ##############################

migration_family <- migration %>%
  tax_glom(taxrank = "Family") %>%                     # agglomerate at phylum level
  transform_sample_counts(function(x) {x/sum(x)} ) %>% # Transform to rel. abundance
  psmelt() %>%                                         # Melt to long format                      # Filter out
 low abundance taxa
  arrange(Family)                                      # Sort data frame alphabetically by family
#
head(migration_family)
```

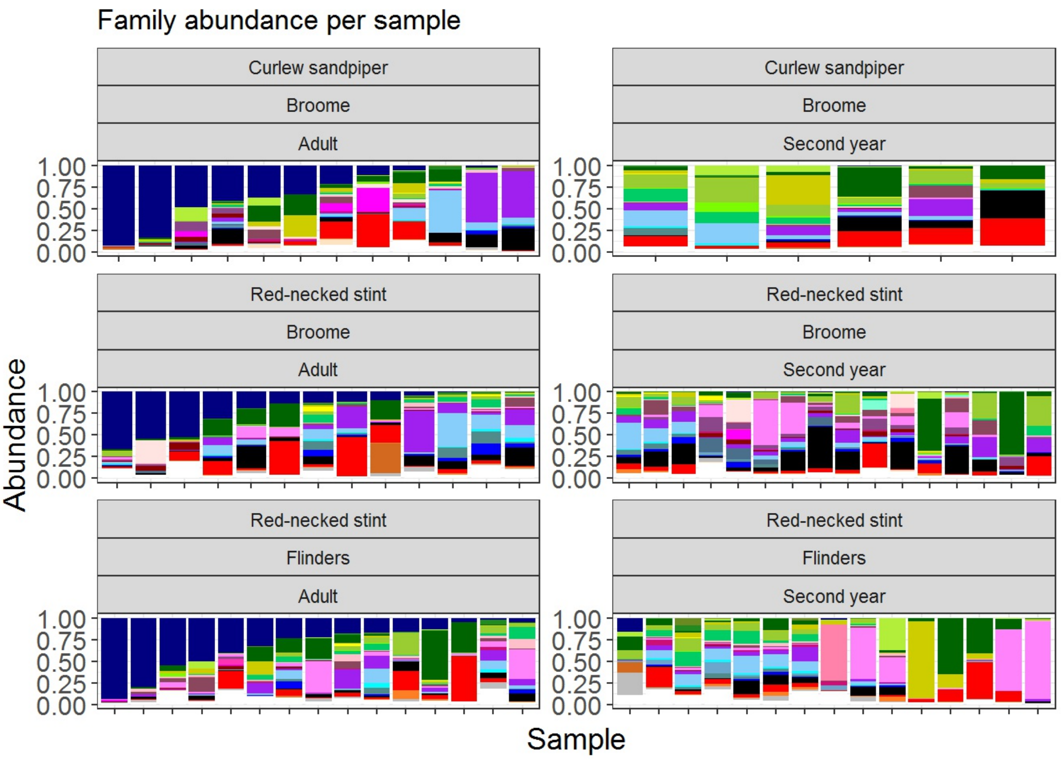
```
##         OTU Sample   Abundance Group          species   site  month
## 1 Otu000437   8558 0.003439381  8558 Red-necked stint Broome August
## 2 Otu000437   8563 0.002407567  8563 Red-necked stint Broome August
## 3 Otu000437   8552 0.001031814  8552 Red-necked stint Broome August
## 4 Otu000437   8564 0.001031814  8564 Red-necked stint Broome August
## 5 Otu000437   8556 0.000000000  8556 Red-necked stint Broome August
## 6 Otu000437   8583 0.000000000  8583 Curlew sandpiper Broome August
##           age group bird_id type Recap recap_delete Recap_num replicate
## 1 Second year   two 3674216 bird    No           No        NA        No
## 2 Second year   two 3674221 bird    No           No        NA        No
## 3 Second year   two 3674232 bird    No           No        NA        No
## 4 Second year   two 3674222 bird    No           No        NA        No
## 5 Second year   two 3674214 bird    No           No        NA        No
## 6 Second year   one 4267072 bird    No           No        NA        No
##   remove_replicate weight wing hb SourceSink Env       Date Plate
## 1               No     NA   NA NA                22/08/2015     2
## 2               No     NA   NA NA                22/08/2015     2
## 3               No     NA   NA NA                22/08/2015     2
## 4               No     NA   NA NA                22/08/2015     2
## 5               No     NA   NA NA                22/08/2015     2
## 6               No     NA   NA NA                22/08/2015     2
##              migration  Kingdom         Phylum               Class
## 1      Broome-stint-SY Bacteria Proteobacteria Deltaproteobacteria
## 2      Broome-stint-SY Bacteria Proteobacteria Deltaproteobacteria
## 3      Broome-stint-SY Bacteria Proteobacteria Deltaproteobacteria
## 4      Broome-stint-SY Bacteria Proteobacteria Deltaproteobacteria
## 5      Broome-stint-SY Bacteria Proteobacteria Deltaproteobacteria
## 6 Broome-curlewsand-SY Bacteria Proteobacteria Deltaproteobacteria
##           Order    Family
## 1 Oligoflexales 0319-6G20
## 2 Oligoflexales 0319-6G20
## 3 Oligoflexales 0319-6G20
## 4 Oligoflexales 0319-6G20
## 5 Oligoflexales 0319-6G20
## 6 Oligoflexales 0319-6G20
```

```
migration_family$Family<-factor(migration_family$Family)
#broome_phylum$Order<-factor(broome_phylum$Order)
unique(migration_family$Family)
```

```
##   [1] 0319-6G20                          Acetobacteraceae
##   [3] Acidaminococcaceae                 Acidimicrobiaceae
##   [5] Acidimicrobiales_unclassified      Acidobacteriaceae_(Subgroup_1)
##   [7] Actinobacteria_unclassified        Actinomycetaceae
##   [9] Aeromonadaceae                     Aeromonadales_unclassified
##  [11] Alcaligenaceae                     Alphaproteobacteria_unclassified
##  [13] Alteromonadaceae                   Anaerolineaceae
##  [15] Anaeroplasmataceae                 Anaplasmataceae
##  [17] Archangiaceae                      Ardenticatenales_fa
##  [19] Arenicellaceae                     Armatimonadales_unclassified
##  [21] Aurantimonadaceae                  Bacillaceae
##  [23] Bacilli_unclassified               Bacteria_unclassified
##  [25] Bacteriovoracaceae                 Bacteroidaceae
##  [27] Bacteroidales_S24-7_group          Bacteroidales_unclassified
##  [29] Bacteroidetes_unclassified         BD2-11_terrestrial_group_fa
##  [31] BD7-8_marine_group_fa              Bdellovibrionaceae
##  [33] Beijerinckiaceae                   Bifidobacteriaceae
##  [35] Blastocatellaceae_(Subgroup_4)     Brachyspiraceae
##  [37] Bradymonadaceae                    Bradyrhizobiaceae
##  [39] Brevibacteriaceae                  Brucellaceae
##  [41] Burkholderiaceae                   Burkholderiales_unclassified
##  [43] Caldilineaceae                     Campylobacteraceae
##  [45] Campylobacterales_unclassified     Cardiobacteriaceae
##  [47] Carnobacteriaceae                  Caulobacteraceae
##  [49] Cellulomonadaceae                  Cellvibrionaceae
##  [51] Cellvibrionales_unclassified       Chitinophagaceae
##  [53] Chloroflexaceae                    Chloroflexi_unclassified
##  [55] Chromatiaceae                      Clostridia_unclassified
##  [57] Clostridiaceae_1                   Clostridiaceae_4
##  [59] Clostridiales_unclassified         Clostridiales_vadinBB60_group
##  [61] Comamonadaceae                     Coriobacteriaceae
##  [63] Corynebacteriaceae                 Corynebacteriales_unclassified
##  [65] Coxiellaceae                       Cryomorphaceae
##  [67] CS-B046_fa                         Cyanobacteria_unclassified
##  [69] Cyclobacteriaceae                  Cytophagaceae
##  [71] Cytophagales_unclassified          d142_fa
##  [73] DA111                              DBS1
##  [75] Deferribacteraceae                 Deinococcaceae
##  [77] Deltaproteobacteria_unclassified   Dermabacteraceae
##  [79] Dermacoccaceae                     Dermatophilaceae
##  [81] Desulfobacteraceae                 Desulfobulbaceae
##  [83] Desulfovibrionaceae                Desulfovibrionales_unclassified
##  [85] Desulfuromonadaceae                DEV007
##  [87] Dietziaceae                        Ectothiorhodospiraceae
##  [89] Eel-36e1D6                         Elev-16S-1332
##  [91] Enterobacteriaceae                 Enterococcaceae
##  [93] Entomoplasmatales_Incertae_Sedis   Epsilonproteobacteria_unclassified
##  [95] Erysipelotrichaceae                Erythrobacteraceae
##  [97] Eubacteriaceae                     Euzebyaceae
##  [99] EV818SWSAP88                       Family_XI
## [101] Family_XII                         Family_XIII
## [103] FamilyI                            FamilyII
## [105] FBP_fa                             FD035
## [107] Firmicutes_unclassified            Flammeovirgaceae
## [109] Flavobacteriaceae                  Flavobacteriales_unclassified
## [111] Fusobacteriaceae                   Fusobacteriales_unclassified
## [113] Gammaproteobacteria_unclassified   Gastranaerophilales_fa
## [115] Gemmatimonadaceae                  Geodermatophilaceae
## [117] Gitt-GS-136_fa                     Gracilibacteria_fa
## [119] Granulosicoccaceae                 Hahellaceae
## [121] Halanaerobiales_unclassified       Haliangiaceae
## [123] Halieaceae                         Halomonadaceae
## [125] Helicobacteraceae                  Herpetosiphonaceae
## [127] HOC36_fa                           Holosporaceae
## [129] Hydrogenophilaceae                 Hyphomicrobiaceae
## [131] Hyphomonadaceae                    Intrasporangiaceae
## [133] JG30-KF-CM45_fa                    JG34-KF-361
## [135] JTB255_marine_benthic_group        KD4-96_fa
## [137] KF-JG30-B3                         Kineosporiaceae
## [139] Lachnospiraceae                    Lactobacillaceae
## [141] Lactobacillales_unclassified       LD29
## [143] Legionellaceae                     Lentisphaeraceae
## [145] Leptospiraceae                     Leptotrichiaceae
## [147] Leuconostocaceae                   Listeriaceae
## [149] Longimicrobiaceae                  Methylobacteriaceae
## [151] Methylophilaceae                   MgMjR-022
## [153] Microbacteriaceae                  Micrococcaceae
## [155] Micrococcales_unclassified         Microgenomates_unclassified
## [157] Milano-WF1B-44_fa                  Mollicutes_unclassified
## [159] Moraxellaceae                      MSB-1E8
## [161] Mycobacteriaceae                   Mycoplasmataceae
## [163] NB1-n_fa                           Neisseriaceae
## [165] Nitriliruptoraceae                 Nitrosomonadaceae
## [167] Nocardiaceae                       Nocardioidaceae
## [169] NS9_marine_group                   Obscuribacterales_fa
## [171] Oceanospirillaceae                 Oceanospirillales_unclassified
## [173] Oligoflexaceae                     OM1_clade
## [175] OM182_clade                        OM190_fa
## [177] Oxalobacteraceae                   Paenibacillaceae
## [179] Parcubacteria_fa                   Parvularculaceae
## [181] Pasteurellaceae                    PeM15_fa
## [183] Peptostreptococcaceae              Peregrinibacteria_fa
## [185] Phycisphaeraceae                   Phyllobacteriaceae
## [187] Pla3_lineage_fa                    Planctomycetaceae
## [189] Planococcaceae                     Porphyromonadaceae
## [191] Prevotellaceae                     Propionibacteriaceae
## [193] Proteobacteria_unclassified        Pseudoalteromonadaceae
## [195] Pseudomonadaceae                   Pseudonocardiaceae
## [197] Psychromonadaceae                  Puniceicoccaceae
## [199] PYR10d3_fa                         Rhizobiaceae
## [201] Rhizobiales_Incertae_Sedis         Rhizobiales_unclassified
## [203] Rhodobacteraceae                   Rhodobiaceae
## [205] Rhodocyclaceae                     Rhodospirillaceae
## [207] Rhodospirillales_Incertae_Sedis    Rhodospirillales_unclassified
## [209] Rhodothermaceae                    Rickettsiaceae
## [211] Rickettsiales_Incertae_Sedis       Rickettsiales_unclassified
## [213] Rikenellaceae                      Rs-D42
## [215] Rubritaleaceae                     Ruminococcaceae
## [217] S0134_terrestrial_group_fa         S085_fa
## [219] Saccharibacteria_fa                Sandaracinaceae
## [221] Saprospiraceae                     SAR116_clade
## [223] SAR324_clade(Marine_group_B)_fa    Shewanellaceae
## [225] Solibacteraceae_(Subgroup_3)       Solirubrobacteraceae
## [227] Sphingobacteriaceae                Sphingobacteriales_unclassified
## [229] Sphingomonadaceae                  Sphingomonadales_unclassified
## [231] Spirochaetaceae                    Spirochaetales_unclassified
## [233] Spongiibacteraceae                 SR1_(Absconditabacteria)_fa
## [235] Staphylococcaceae                  Streptococcaceae
## [237] Streptomycetaceae                  Subgroup_21_fa
## [239] Subgroup_22_fa                     Subgroup_23_fa
## [241] Subgroup_6_fa                      Subgroup_7_fa
## [243] Succinivibrionaceae                Surface_1
## [245] Sva0071_fa                         Sva0725
## [247] Sva0996_marine_group               Sva1033
## [249] Syntrophaceae                      T9d
## [251] Tepidisphaeraceae                  Thermoactinomycetaceae
## [253] Thiotrichaceae                     TM6_(Dependentiae)_fa
## [255] TRA3-20_fa                         Trueperaceae
## [257] uncultured                         uncultured_fa
## [259] Unknown_Family                     Veillonellaceae
## [261] Verrucomicrobia_fa                 Verrucomicrobia_unclassified
## [263] Verrucomicrobiaceae                Verrucomicrobiales_unclassified
## [265] Vibrionaceae                       X35_fa
## [267] Xanthobacteraceae                  Xanthomonadaceae
## [269] Xanthomonadales_Incertae_Sedis     Xanthomonadales_unclassified
## 270 Levels: 0319-6G20 Acetobacteraceae ... Xanthomonadales_unclassified
```

```
#write.csv(migration_family,"family.csv")

##all common Corynebacteriales_unclassified are genus Corynebacterium (we ran sequences through ARB):

migration_family$Family[migration_family$Family=="Corynebacteriales_unclassified"]<-"Corynebacteriaceae"


##########plot
```

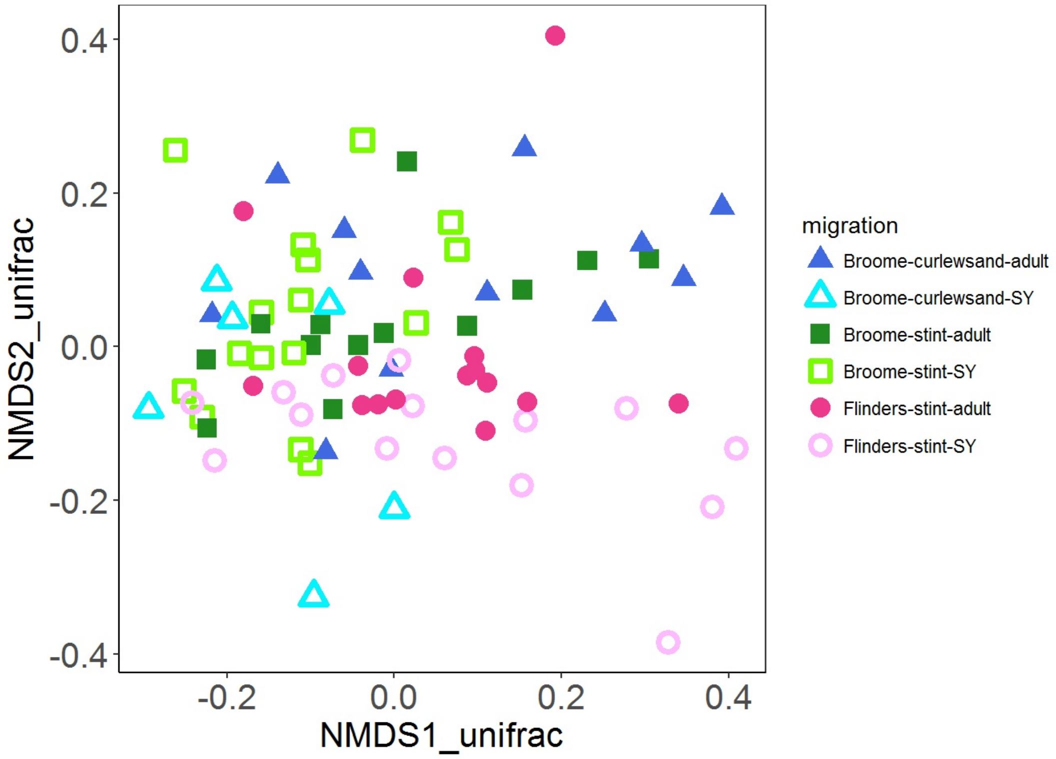
```
migration_family$Family<-factor(migration_family$Family, level = c("Corynebacteriaceae",
                                                                   "Acidaminococcaceae","Clostridiaceae_1","Clo
stridiales_unclassified" , "Enterococcaceae" ,"Erysipelotrichaceae"  ,"Firmicutes_unclassified" , "Lachnospirac
eae"  ,"Peptostreptococcaceae" , "Ruminococcaceae" , "Staphylococcaceae" ,
                                                                   "Comamonadaceae", "Desulfovibrionaceae","Ent
erobacteriaceae","Gammaproteobacteria_unclassified" ,"Helicobacteraceae"  ,  "Methylobacteriaceae" ,"Oxalobacte
raceae" ,"Rhizobiales_unclassified" , "Rhodobacteraceae",   "Sphingomonadaceae" , "Succinivibrionaceae",
                                                                   "Bacteroidaceae" , "Bacteroidales_unclassifi
ed" ,"Chitinophagaceae" ,"Flavobacteriaceae","Porphyromonadaceae","Rikenellaceae" ,
                                                                   "Deferribacteraceae" ,
                                                                   "Fusobacteriaceae",
                                                                   "Anaeroplasmataceae", "Mycoplasmataceae",
                                                                   "Brachyspiraceae" ,
                                                                   "Deinococcaceae",

                                                                   "0319-6G20",
                                                                   "Acetobacteraceae",

                                                                   "Acidimicrobiaceae",
                                                                   "Acidimicrobiales_unclassified",
                                                                   "Acidobacteriaceae_(Subgroup_1)",
                                                                   "Actinobacteria_unclassified",
                                                                   "Actinomycetaceae",
                                                                   "Aeromonadaceae",
                                                                   "Aeromonadales_unclassified",
                                                                   "Alcaligenaceae",
                                                                   "Alphaproteobacteria_unclassified",
                                                                   "Alteromonadaceae",
                                                                   "Anaerolineaceae",

                                                                   "Anaplasmataceae",
                                                                   "Ardenticatenales_fa",
                                                                   "Arenicellaceae",
                                                                   "Armatimonadales_unclassified",
                                                                   "Aurantimonadaceae",
                                                                   "Bacillaceae",
                                                                   "Bacilli_unclassified",
                                                                   "Bacteria_unclassified",
                                                                   "Bacteriovoracaceae",

                                                                   "Bacteroidales_S24-7_group",

                                                                   "Bacteroidetes_unclassified",
                                                                   "Bartonellaceae",
                                                                   "BD2-11_terrestrial_group_fa",
                                                                   "BD7-8_marine_group_fa",
                                                                   "Bdellovibrionaceae",
                                                                   "Beijerinckiaceae",
                                                                   "Bifidobacteriaceae",
                                                                   "Blastocatellaceae_(Subgroup_4)",

                                                                   "Bradymonadaceae",
                                                                   "Bradymonadales_fa",
                                                                   "Bradyrhizobiaceae",
                                                                   "Brevibacteriaceae",
                                                                   "Brucellaceae",
                                                                   "Burkholderiaceae",
                                                                   "Burkholderiales_unclassified",
                                                                   "Caldilineaceae",
                                                                   "Campylobacteraceae",
                                                                   "Campylobacterales_unclassified",
                                                                   "Cardiobacteriaceae",
                                                                   "Carnobacteriaceae",
                                                                   "Caulobacteraceae",
                                                                   "Cellulomonadaceae",
                                                                   "Cellvibrionaceae",
                                                                   "Cellvibrionales_unclassified",

                                                                   "Chloroflexaceae",
                                                                   "Chloroflexi_unclassified",
                                                                   "Chromatiaceae",
                                                                   "Clostridia_unclassified",

                                                                   "Clostridiaceae_4",

                                                                   "Clostridiales_vadinBB60_group",
"Coriobacteriaceae",

"Coxiellaceae",
"Cryomorphaceae",
"CS-B046_fa",
"Cyanobacteria_unclassified",
"Cyclobacteriaceae",
"Cytophagaceae",
"Cytophagales_unclassified",
"d142_fa",
"DA111",
"DBS1",


"Deltaproteobacteria_unclassified",
"Dermabacteraceae",
"Dermacoccaceae",
"Dermatophilaceae",
"Desulfobacteraceae",
"Desulfobulbaceae",

"Desulfovibrionales_unclassified",
"Desulfuromonadaceae",
"Desulfuromonadales_unclassified",
"DEV007",
"Dietziaceae",
"Eel-36e1D6",
"Elev-16S-1332",


"Entomoplasmatales_Incertae_Sedis",
"Epsilonproteobacteria_unclassified",

"Erythrobacteraceae",
"Eubacteriaceae",
"Euzebyaceae",
"Family_XI",
"Family_XII",
"Family_XIII",
"FamilyI",
"FamilyII",
"FBP_fa",
"FD035",

"Flammeovirgaceae",

"Flavobacteriales_unclassified",

"Fusobacteriales_unclassified",

"Gastranaerophilales_fa",
"Gemmatimonadaceae",
"Geodermatophilaceae",
"Gitt-GS-136_fa",
"Gracilibacteria_fa",
"Granulosicoccaceae",
"Hahellaceae",
"Halanaerobiales_unclassified",
"Haliangiaceae",
"Halieaceae",
"Halomonadaceae",

"Herpetosiphonaceae",
"HOC36_fa",
"Holosporaceae",
"Hydrogenophilaceae",
"Hyphomicrobiaceae",
"Hyphomonadaceae",
"Iamiaceae",
"Intrasporangiaceae",
"JG30-KF-CM45_fa",
"JG34-KF-361",
"JTB255_marine_benthic_group",
"KD4-96_fa",
"KF-JG30-B3",
"Kineosporiaceae",

"Lactobacillaceae",
"Lactobacillales_unclassified",
"LD29",
"Legionellaceae",
"Lentisphaeraceae",
"Leptospiraceae",
"Leptotrichiaceae",
"Leuconostocaceae",
"Listeriaceae",
"Longimicrobiaceae",

"Methylophilaceae",
"MgMjR-022",
"Microbacteriaceae",
"Micrococcaceae",
"Micrococcales_unclassified",
"Microgenomates_unclassified",
"Milano-WF1B-44_fa",
"Mollicutes_unclassified",
"Moraxellaceae",
"MSB-1E8",
"Mycobacteriaceae",

"NB1-n_fa",
"Neisseriaceae",
"Nitriliruptoraceae",
"Nitrosomonadaceae",
"Nocardiaceae",
"Nocardioidaceae",
"NS9_marine_group",
"Obscuribacterales_fa",
"Oceanospirillaceae",
"Oceanospirillales_unclassified",
"Oligoflexaceae",
"OM1_clade",
"OM182_clade",
"OM190_fa",

"Paenibacillaceae",
"Parcubacteria_fa",
"Parvularculaceae",
"Pasteurellaceae",
"PeM15_fa",

"Peregrinibacteria_fa",
"Phycisphaeraceae",
"Phyllobacteriaceae",
"Pla3_lineage_fa",
"Planctomycetaceae",
"Planococcaceae",

"Prevotellaceae",
"Propionibacteriaceae",
"Proteobacteria_unclassified",
"Pseudoalteromonadaceae",
"Pseudomonadaceae",
"Pseudonocardiaceae",
"Psychromonadaceae",
"Puniceicoccaceae",
"PYR10d3_fa",
"Rhizobiaceae",
"Rhizobiales_Incertae_Sedis",


"Rhodobiaceae",
"Rhodocyclaceae",
"Rhodospirillaceae",
"Rhodospirillales_Incertae_Sedis",
"Rhodospirillales_unclassified",
"Rhodothermaceae",
"Rickettsiaceae",
"Rickettsiales_Incertae_Sedis",
"Rickettsiales_unclassified",
                                                                   "Rs-D42",
                                                                   "Rubritaleaceae",

                                                                   "S0134_terrestrial_group_fa",
                                                                   "S085_fa",
                                                                   "Saccharibacteria_fa",
                                                                   "Sandaracinaceae",
                                                                   "Saprospiraceae",
                                                                   "SAR116_clade",
                                                                   "SAR324_clade(Marine_group_B)_fa",
                                                                   "Shewanellaceae",
                                                                   "Solibacteraceae_(Subgroup_3)",
                                                                   "Solirubrobacteraceae",
                                                                   "Sphingobacteriaceae",
                                                                   "Sphingobacteriales_unclassified",

                                                                   "Sphingomonadales_unclassified",
                                                                   "Spirochaetaceae",
                                                                   "Spirochaetales_unclassified",
                                                                   "Spongiibacteraceae",
                                                                   "SR-FBR-L83",
                                                                   "SR1_(Absconditabacteria)_fa",

                                                                   "Streptococcaceae",
                                                                   "Streptomycetaceae",
                                                                   "Subgroup_21_fa",
                                                                   "Subgroup_22_fa",
                                                                   "Subgroup_23_fa",
                                                                   "Subgroup_6_fa",
                                                                   "Subgroup_7_fa",

                                                                   "Surface_1",
                                                                   "Sva0071_fa",
                                                                   "Sva0725",
                                                                   "Sva0996_marine_group",
                                                                   "Sva1033",
                                                                   "Syntrophaceae",
                                                                   "T9d",
                                                                   "Tepidisphaeraceae",
                                                                   "Thermoactinomycetaceae",
                                                                   "Thiotrichaceae",
                                                                   "TM146",
                                                                   "TM6_(Dependentiae)_fa",
                                                                   "TRA3-20_fa",
                                                                   "Trueperaceae",
                                                                   "uncultured",
                                                                   "uncultured_fa",
                                                                   "Unknown_Family",
                                                                   "Veillonellaceae",
                                                                   "Verrucomicrobia_fa",
                                                                   "Verrucomicrobia_unclassified",
                                                                   "Verrucomicrobiaceae",
                                                                   "Verrucomicrobiales_unclassified",
                                                                   "Vibrionaceae",
                                                                   "X35_fa",
                                                                   "Xanthobacteraceae",
                                                                   "Xanthomonadaceae",
                                                                   "Xanthomonadales_Incertae_Sedis",
                                                                   "Xanthomonadales_unclassified"))





##only families that make up more than 5% total abundance are coloured. The rest are coloured white

colors <- c("navy",
            "olivedrab4","olivedrab2","forestgreen","darkgreen","yellow3","yellow1","yellowgreen","lawngreen","
springgreen3","aquamarine",
            "mistyrose","pink","palevioletred1","palevioletred4", "orchid1","orchid4", "maroon1","magenta","dee
ppink3","red4","purple",

            "lightskyblue", "cyan","skyblue4", "skyblue3","darkslategray4","blue",
            "black",
            "red",
            "chocolate1","chocolate",
            "grey",
            "bisque",
            "white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white","white","white",
            "white","white","white","white","white","white","white","white","white","white")


names(colors) <- levels(migration_family$Family)
colScale <- scale_fill_manual(name = "Family",values = colors)


migration_family$Sample<-factor(migration_family$Sample, level = c("8682", "8629", "8615", "8685", "8610", "866
2", "8560", "8607", "8671", "8589", "8657", "8573", "8625", "8627", "8639", "8549", "8645", "8632", "8660", "86
49", "8601",
                                                                   "8658", "8638", "8646", "8634", "8606", "861
2", "8581", "8618", "8609", "8628", "8672", "8605", "8689", "8659", "8616", "8626", "8604", "8569", "8677", "85
88", "8675",
                                                                   "8551", "8613", "8546", "8683", "8590", "855
7", "8558", "8674", "8559", "8680", "8630", "8563", "8586", "8556", "8561", "8644", "8667", "8566", "8665", "86
33", "8564",
                                                                   "8583", "8575", "8571", "8565", "8578", "857
0", "8684", "8688", "8655", "8552", "8548", "8679", "8663", "8686"))





ggplot(migration_family, aes(x = Sample, y = Abundance, fill = Family)) +

  #Fig 1b)
  geom_bar(stat = "identity", position="fill") +
  theme(axis.text.x = element_blank(), axis.text.y = element_text(size=12), axis.title=element_text(size=14))+
  ggtitle("Family abundance per sample")+facet_wrap(species~site~age, scales="free", nrow=3, ncol=2)+colScale +
 theme(legend.position="none")
```



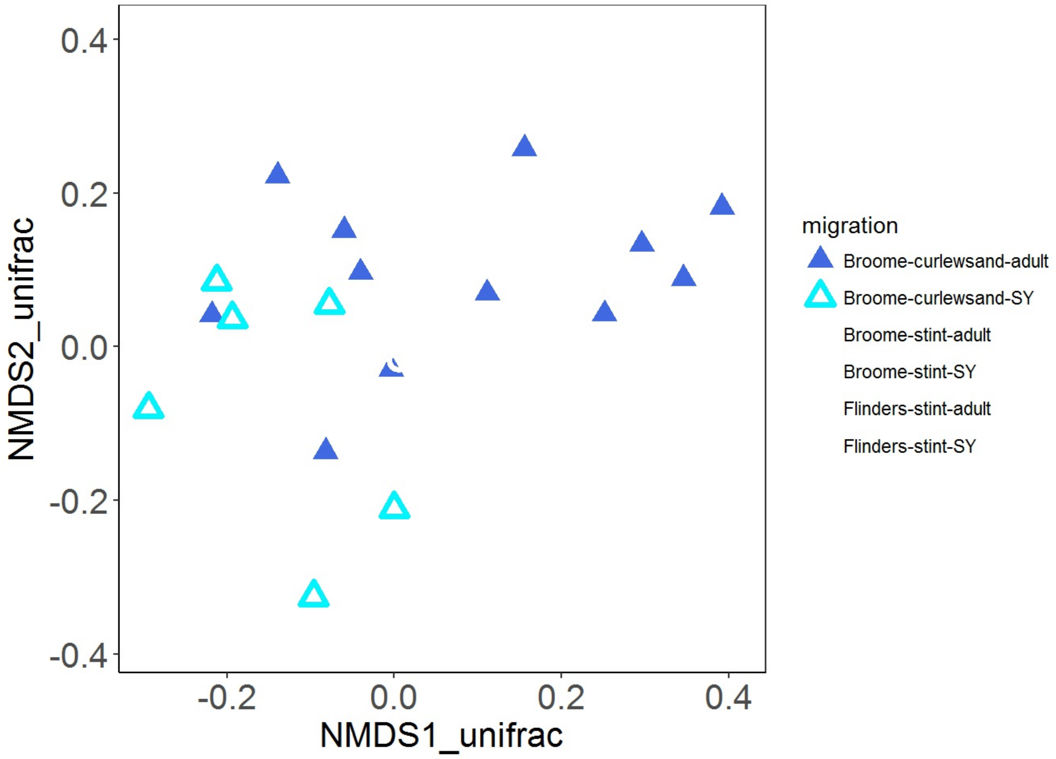
```
############################################### ORDINATION ANLYSIS############################################
#

##Figure S3)

set.seed(1)

migration_unifrac <- ordinate(
  physeq = migration,
  method = "NMDS",
  distance = "unifrac"
)
```

```
## Warning in UniFrac(physeq, ...): Randomly assigning root as -- Otu000566 --
## in the phylogenetic tree in the data you provided.
## Run 0 stress 0.2178487 
## Run 1 stress 0.2322504 
## Run 2 stress 0.2155708 
## ... New best solution
## ... Procrustes: rmse 0.06628759  max resid 0.2890222 
## Run 3 stress 0.2271085 
## Run 4 stress 0.2286939 
## Run 5 stress 0.2196326 
## Run 6 stress 0.2349434 
## Run 7 stress 0.2226841 
## Run 8 stress 0.2133707 
## ... New best solution
## ... Procrustes: rmse 0.02225117  max resid 0.1186602 
## Run 9 stress 0.2190638 
## Run 10 stress 0.2108544 
## ... New best solution
## ... Procrustes: rmse 0.06605822  max resid 0.2885335 
## Run 11 stress 0.2209799 
## Run 12 stress 0.2172837 
## Run 13 stress 0.22129 
## Run 14 stress 0.2105399 
## ... New best solution
## ... Procrustes: rmse 0.02824563  max resid 0.1835601 
## Run 15 stress 0.2231375 
## Run 16 stress 0.2185725 
## Run 17 stress 0.2123678 
## Run 18 stress 0.2131853 
## Run 19 stress 0.2172729 
## Run 20 stress 0.2181311 
## *** No convergence -- monoMDS stopping criteria:
##     20: stress ratio > sratmax
```

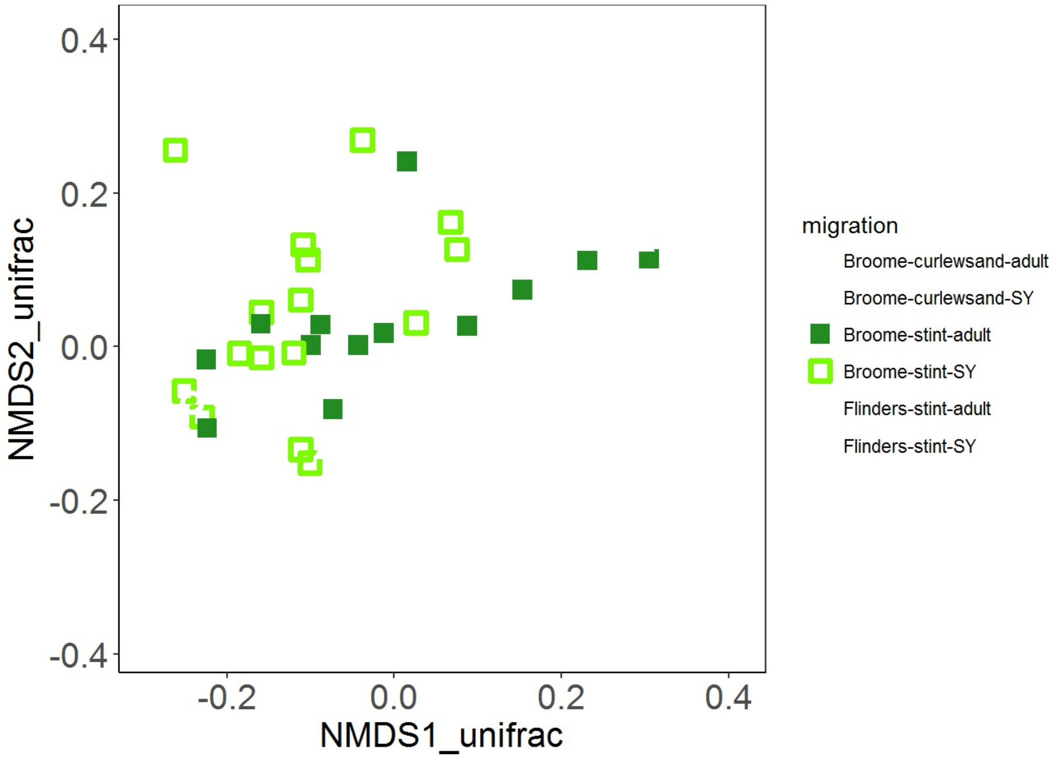
```
## add unifrac ordination axes to dataframe and plot

unifrac<-data.frame(migration_unifrac$points)
sample_data(migration)$NMDS1_unifrac<-unifrac$MDS1
sample_data(migration)$NMDS2_unifrac<-unifrac$MDS2

migration.df<-data.frame(sample_data(migration))

ggplot(data=migration.df, aes(x = NMDS1_unifrac, y = NMDS2_unifrac))+ geom_point(aes(colour = migration, shape
= migration), alpha = 1, size = 3, stroke = 2) +
  theme_bw()+scale_color_manual(values=c("royalblue","turquoise1",
                                         "forestgreen","lawngreen","violetred2","plum1"))+
  scale_shape_manual(values=c(17,2,15,0,16,1))+theme(panel.grid.major = element_blank(), panel.grid.minor = ele
ment_blank(),
                                                     panel.background = element_blank(), axis.line = element_li
ne(colour = "black"))+
  theme(axis.text=element_text(size=16), axis.title=element_text(size=16))
```



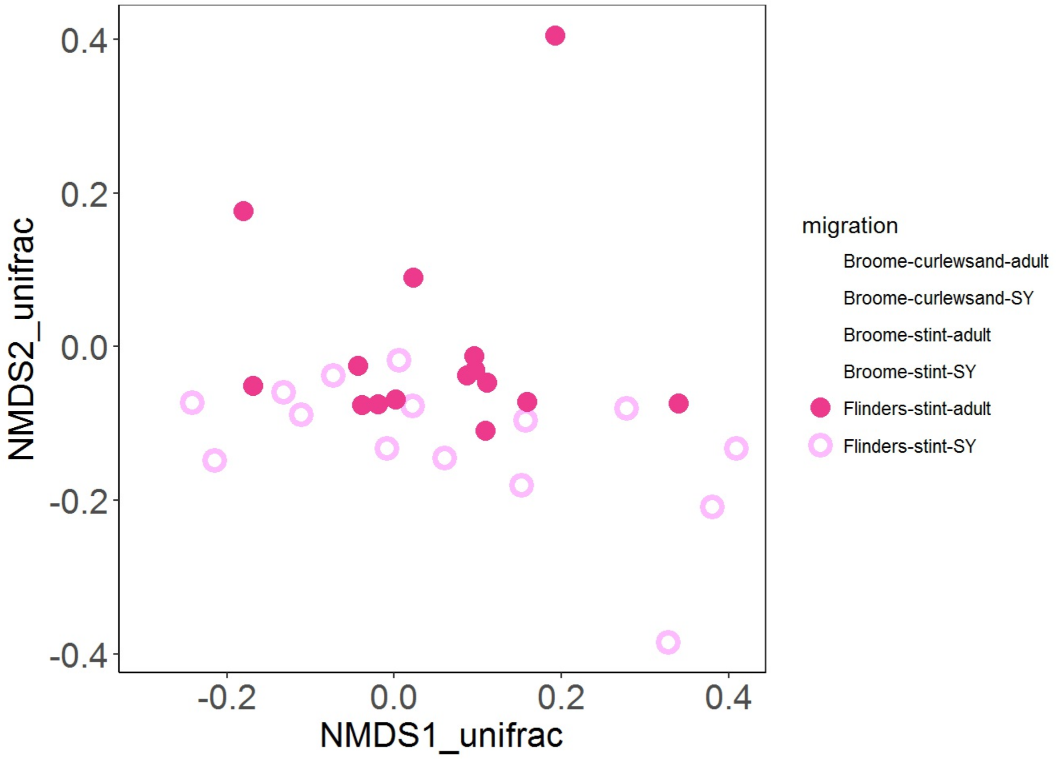
```
##################################

ggplot(data=migration.df, aes(x = NMDS1_unifrac, y = NMDS2_unifrac))+ geom_point(aes(colour = migration, shape
= migration), alpha = 1, size = 3, stroke = 2) +
  theme_bw()+scale_color_manual(values=c("royalblue","turquoise1",
                                         "white","white","white","white"))+
  scale_shape_manual(values=c(17,2,15,0,16,1))+theme(panel.grid.major = element_blank(), panel.grid.minor = ele
ment_blank(),
                                                     panel.background = element_blank(), axis.line = element_li
ne(colour = "black"))+
  theme(axis.text=element_text(size=16), axis.title=element_text(size=16))
```



```
##################################


ggplot(data=migration.df, aes(x = NMDS1_unifrac, y = NMDS2_unifrac))+ geom_point(aes(colour = migration, shape
= migration), alpha = 1, size = 3, stroke = 2) +
  theme_bw()+scale_color_manual(values=c("white","white",
                                         "forestgreen","lawngreen","white","white"))+
  scale_shape_manual(values=c(17,2,15,0,16,1))+theme(panel.grid.major = element_blank(), panel.grid.minor = ele
ment_blank(),
                                                     panel.background = element_blank(), axis.line = element_li
ne(colour = "black"))+
  theme(axis.text=element_text(size=16), axis.title=element_text(size=16))
```



```
##################################


ggplot(data=migration.df, aes(x = NMDS1_unifrac, y = NMDS2_unifrac))+ geom_point(aes(colour = migration, shape
= migration), alpha = 1, size = 3, stroke = 2) +
  theme_bw()+scale_color_manual(values=c("white","white",
                                         "white","white","violetred2","plum1"))+
  scale_shape_manual(values=c(17,2,15,0,16,1))+theme(panel.grid.major = element_blank(), panel.grid.minor = ele
ment_blank(),
                                                     panel.background = element_blank(), axis.line = element_li
ne(colour = "black"))+
  theme(axis.text=element_text(size=16), axis.title=element_text(size=16))
```



```
###################

##stats
migration_unifrac <- phyloseq::distance(migration, method = "unifrac")
```

```
## Warning in UniFrac(physeq, ...): Randomly assigning root as -- Otu004229 --
## in the phylogenetic tree in the data you provided.
```

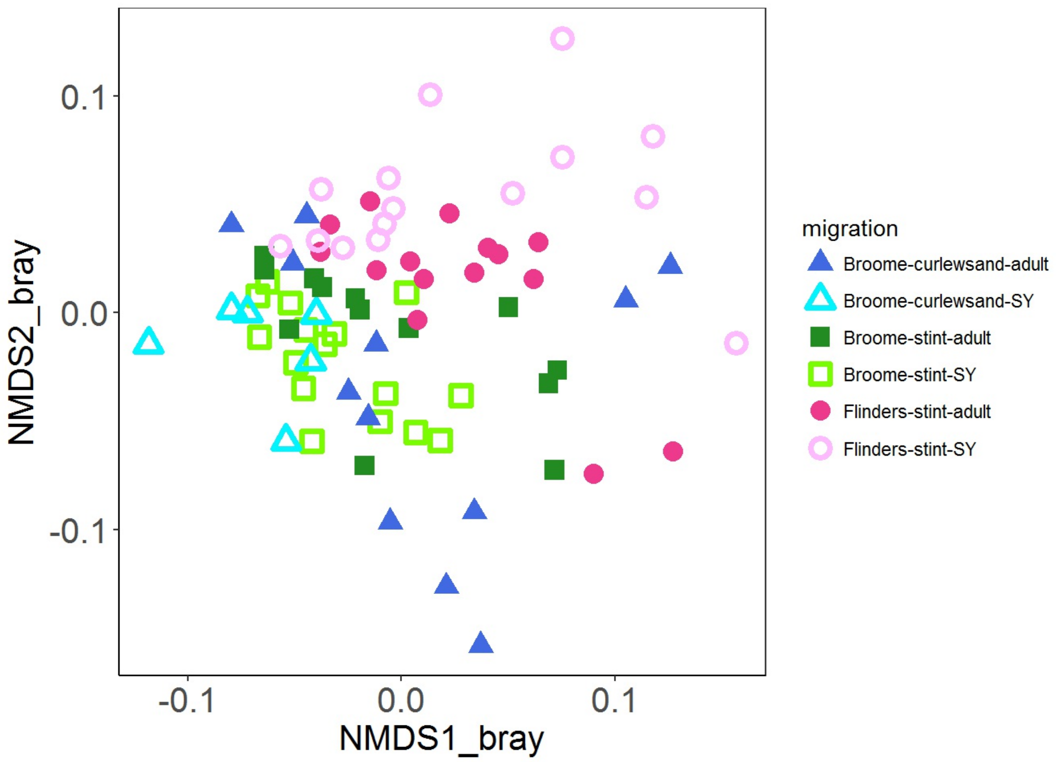
```
# make a data frame from the sample_data
sampledf <- data.frame(sample_data(migration))

# Adonis test
adonis(migration_unifrac ~ site+species+age, data = sampledf)
```

```
##
## Call:
## adonis(formula = migration_unifrac ~ site + species + age, data = sampledf)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##            Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## site        1    0.8129 0.81295  3.3509 0.04143  0.001 ***
## species     1    0.5368 0.53678  2.2126 0.02736  0.001 ***
## age         1    0.5606 0.56056  2.3106 0.02857  0.001 ***
## Residuals 73   17.7102 0.24261         0.90264
## Total     76   19.6205                 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

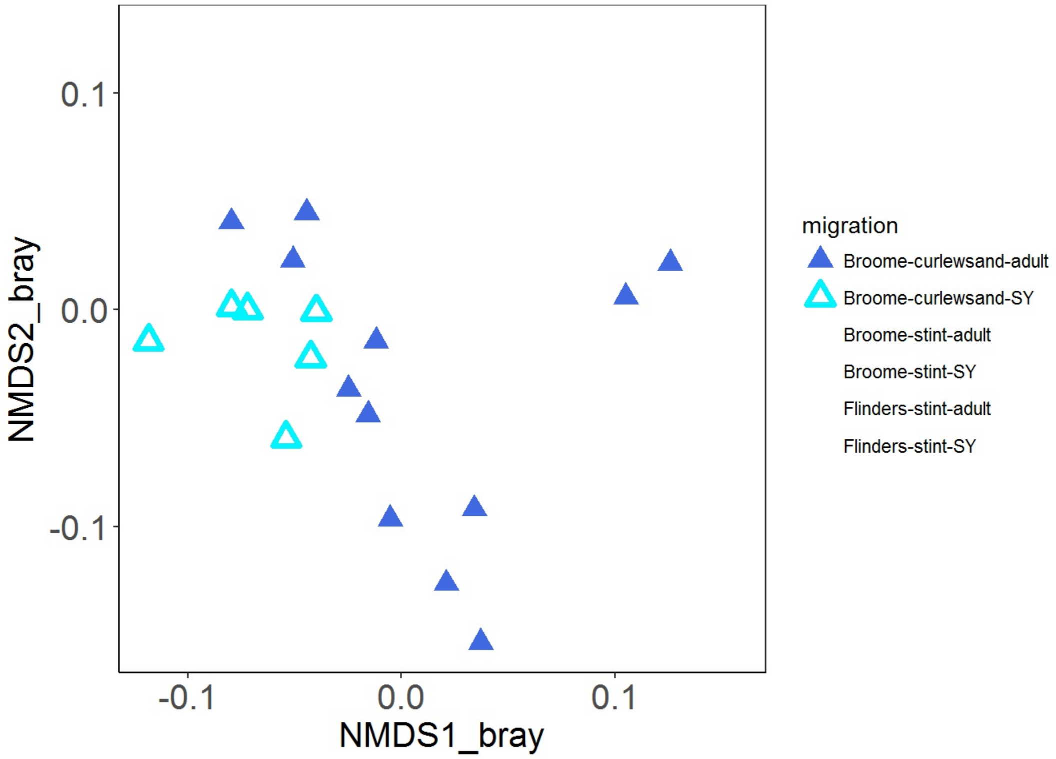
```
############

################################bray curtis

##Figure 3)

set.seed(1)

migration_bray <- ordinate(
  physeq = migration,
  method = "NMDS",
  distance = "bray"
)
```

```
## Square root transformation
## Wisconsin double standardization
## Run 0 stress 0.2453602
## Run 1 stress 0.2607537
## Run 2 stress 0.255154
## Run 3 stress 0.2561769
## Run 4 stress 0.2518526
## Run 5 stress 0.2718302
## Run 6 stress 0.2533695
## Run 7 stress 0.2584876
## Run 8 stress 0.2453605
## ... Procrustes: rmse 0.0003253219  max resid 0.002527825
## ... Similar to previous best
## Run 9 stress 0.261244
## Run 10 stress 0.2517643
## Run 11 stress 0.2439716
## ... New best solution
## ... Procrustes: rmse 0.04242624  max resid 0.3241008
## Run 12 stress 0.2649426
## Run 13 stress 0.2657925
## Run 14 stress 0.2448462
## Run 15 stress 0.243968
## ... New best solution
## ... Procrustes: rmse 0.0320396  max resid 0.2619141
## Run 16 stress 0.26698
## Run 17 stress 0.2443094
## ... Procrustes: rmse 0.04255965  max resid 0.2075804
## Run 18 stress 0.2705507
## Run 19 stress 0.2493743
## Run 20 stress 0.2500642
## *** No convergence -- monoMDS stopping criteria:
##     20: stress ratio > sratmax
```

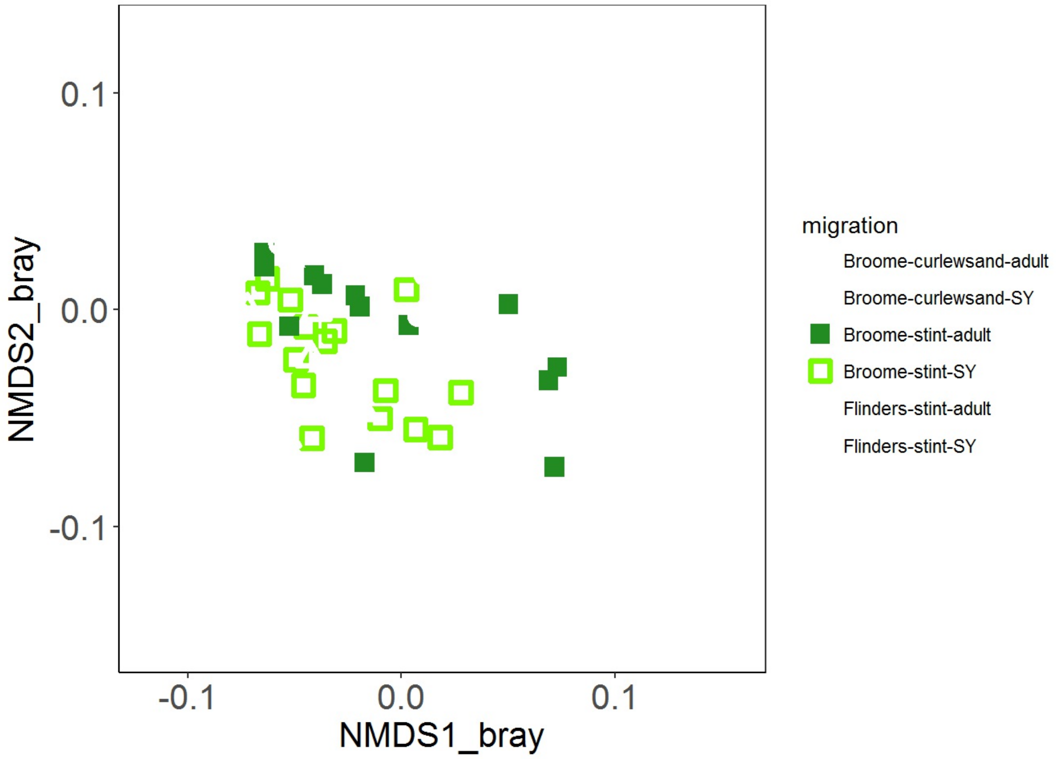
```
bray<-data.frame(migration_bray$points)
sample_data(migration)$NMDS1_bray<-bray$MDS1
sample_data(migration)$NMDS2_bray<-bray$MDS2

migration.df<-data.frame(sample_data(migration))

##plot

ggplot(data=migration.df, aes(x = NMDS1_bray, y = NMDS2_bray))+
  geom_point(aes(colour = migration, shape = migration), alpha = 1, size = 3, stroke = 2) +
  theme_bw()+scale_color_manual(values=c("royalblue","turquoise1",
                                         "forestgreen","lawngreen","violetred2","plum1"))+
  scale_shape_manual(values=c(17,2,15,0,16,1))+theme(panel.grid.major = element_blank(),
                                                     panel.grid.minor = element_blank(),
                                                     panel.background = element_blank(),
                                                     axis.line = element_line(colour = "black"))+
  theme(axis.text=element_text(size=16), axis.title=element_text(size=16))
```



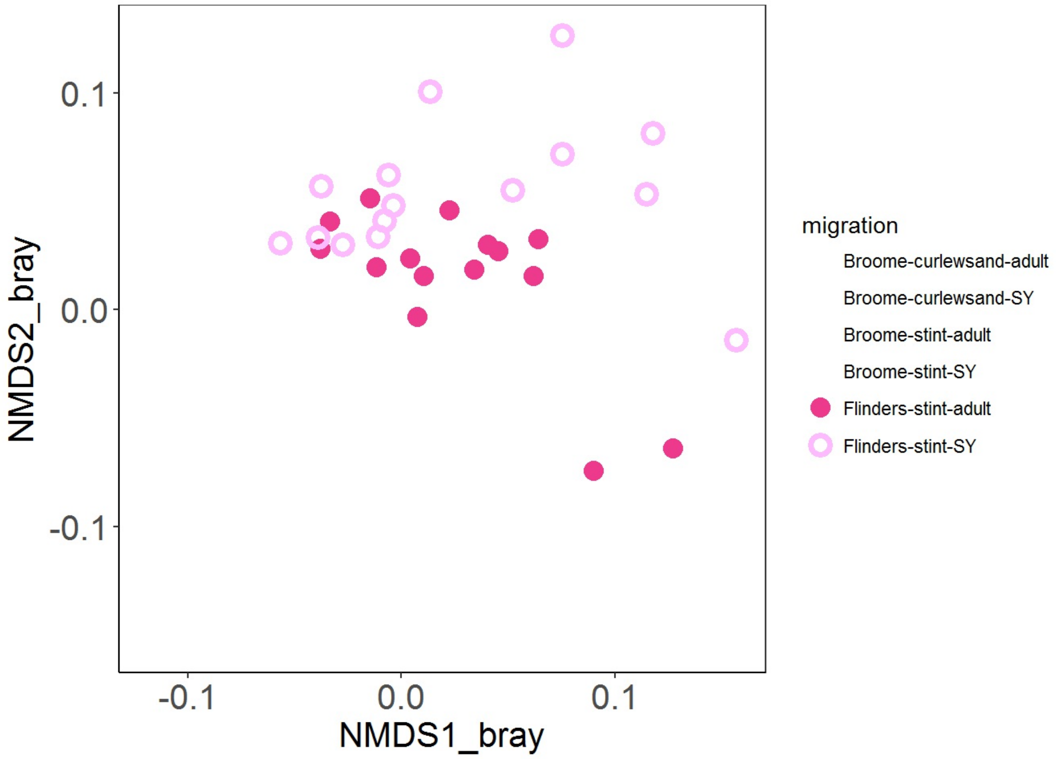
```
#########################################
##################################

ggplot(data=migration.df, aes(x = NMDS1_bray, y = NMDS2_bray))+
  geom_point(aes(colour = migration, shape = migration), alpha = 1, size = 3, stroke = 2) +
  theme_bw()+scale_color_manual(values=c("royalblue","turquoise1",
                                         "white","white","white","white"))+
  scale_shape_manual(values=c(17,2,15,0,16,1))+theme(panel.grid.major = element_blank(),
                                                     panel.grid.minor = element_blank(),
                                                     panel.background = element_blank(),
                                                     axis.line = element_line(colour = "black"))+
  theme(axis.text=element_text(size=16), axis.title=element_text(size=16))
```



```
##################################


ggplot(data=migration.df, aes(x = NMDS1_bray, y = NMDS2_bray))+
  geom_point(aes(colour = migration, shape = migration), alpha = 1, size = 3, stroke = 2) +
  theme_bw()+scale_color_manual(values=c("white","white",
                                       "forestgreen","lawngreen","white","white"))+
  scale_shape_manual(values=c(17,2,15,0,16,1))+theme(panel.grid.major = element_blank(),
                                                   panel.grid.minor = element_blank(),
                                                   panel.background = element_blank(),
                                                   axis.line = element_line(colour = "black"))+
  theme(axis.text=element_text(size=16), axis.title=element_text(size=16))
```



```
##################################


ggplot(data=migration.df, aes(x = NMDS1_bray, y = NMDS2_bray))+
  geom_point(aes(colour = migration, shape = migration), alpha = 1, size = 3, stroke = 2) +
  theme_bw()+scale_color_manual(values=c("white","white",
                                       "white","white","violetred2","plum1"))+
  scale_shape_manual(values=c(17,2,15,0,16,1))+theme(panel.grid.major = element_blank(),
                                                   panel.grid.minor = element_blank(),
                                                   panel.background = element_blank(),
                                                   axis.line = element_line(colour = "black"))+
  theme(axis.text=element_text(size=16), axis.title=element_text(size=16))
```



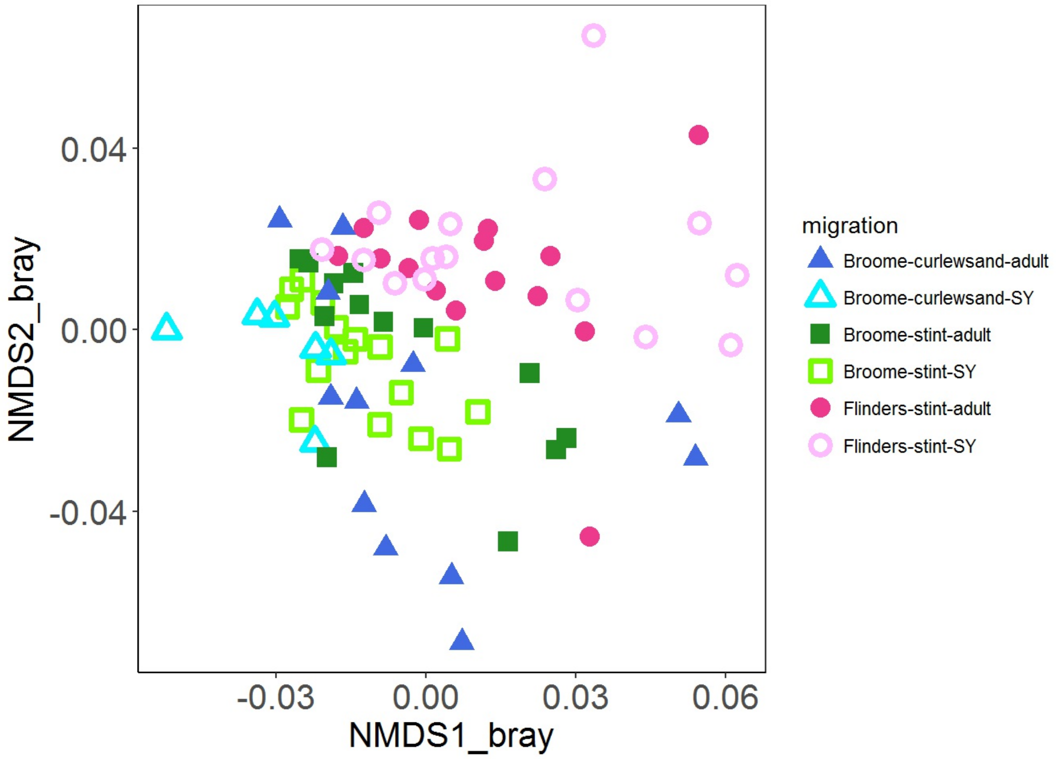
```
################################################

##stats

migration_bray <- phyloseq::distance(migration, method = "bray")


# make a data frame from the sample_data
sampledf <- data.frame(sample_data(migration))

# Adonis test
adonis(migration_bray ~ site+species+age, data = sampledf)
```

```
##
## Call:
## adonis(formula = migration_bray ~ site + species + age, data = sampledf)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##           Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## site       1    1.1939 1.19392  3.4024 0.04114  0.001 ***
## species    1    0.9877 0.98765  2.8146 0.03403  0.001 ***
## age        1    1.2225 1.22247  3.4837 0.04212  0.001 ***
## Residuals 73   25.6163 0.35091         0.88270
## Total     76   29.0203                 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
#############################################################



##remove corynebacteria to see if any other differences

migration1<-subset_taxa(migration, Order != "Corynebacteriales")
migration1<- prune_taxa(taxa_sums(migration1)>0, migration1)

set.seed(1)

migration_bray <- ordinate(
  physeq = migration1,
  method = "NMDS",
  distance = "bray"
)
```

```
## Square root transformation
## Wisconsin double standardization
## Run 0 stress 0.2434372
## Run 1 stress 0.2520435
## Run 2 stress 0.2505532
## Run 3 stress 0.2414003
## ... New best solution
## ... Procrustes: rmse 0.05723422  max resid 0.3325888
## Run 4 stress 0.2407164
## ... New best solution
## ... Procrustes: rmse 0.04558418  max resid 0.3847929
## Run 5 stress 0.2636338
## Run 6 stress 0.2525901
## Run 7 stress 0.2554168
## Run 8 stress 0.2445606
## Run 9 stress 0.2592545
## Run 10 stress 0.2590405
## Run 11 stress 0.2401147
## ... New best solution
## ... Procrustes: rmse 0.04837525  max resid 0.3577633
## Run 12 stress 0.2539493
## Run 13 stress 0.248226
## Run 14 stress 0.2423354
## Run 15 stress 0.2504476
## Run 16 stress 0.2600155
## Run 17 stress 0.2488418
## Run 18 stress 0.2526451
## Run 19 stress 0.240373
## ... Procrustes: rmse 0.03543252  max resid 0.2868491
## Run 20 stress 0.2567804
## *** No convergence -- monoMDS stopping criteria:
##     20: stress ratio > sratmax
```

```
bray<-data.frame(migration_bray$points)
sample_data(migration1)$NMDS1_bray<-bray$MDS1
sample_data(migration1)$NMDS2_bray<-bray$MDS2

migration1.df<-data.frame(sample_data(migration1))

##plot

ggplot(data=migration1.df, aes(x = NMDS1_bray, y = NMDS2_bray))+
  geom_point(aes(colour = migration, shape = migration), alpha = 1, size = 3, stroke = 2) +
  theme_bw()+scale_color_manual(values=c("royalblue","turquoise1",
                                         "forestgreen","lawngreen","violetred2","plum1"))+
  scale_shape_manual(values=c(17,2,15,0,16,1))+theme(panel.grid.major = element_blank(),
                                                     panel.grid.minor = element_blank(),
                                                     panel.background = element_blank(),
                                                     axis.line = element_line(colour = "black"))+
  theme(axis.text=element_text(size=16), axis.title=element_text(size=16))
```

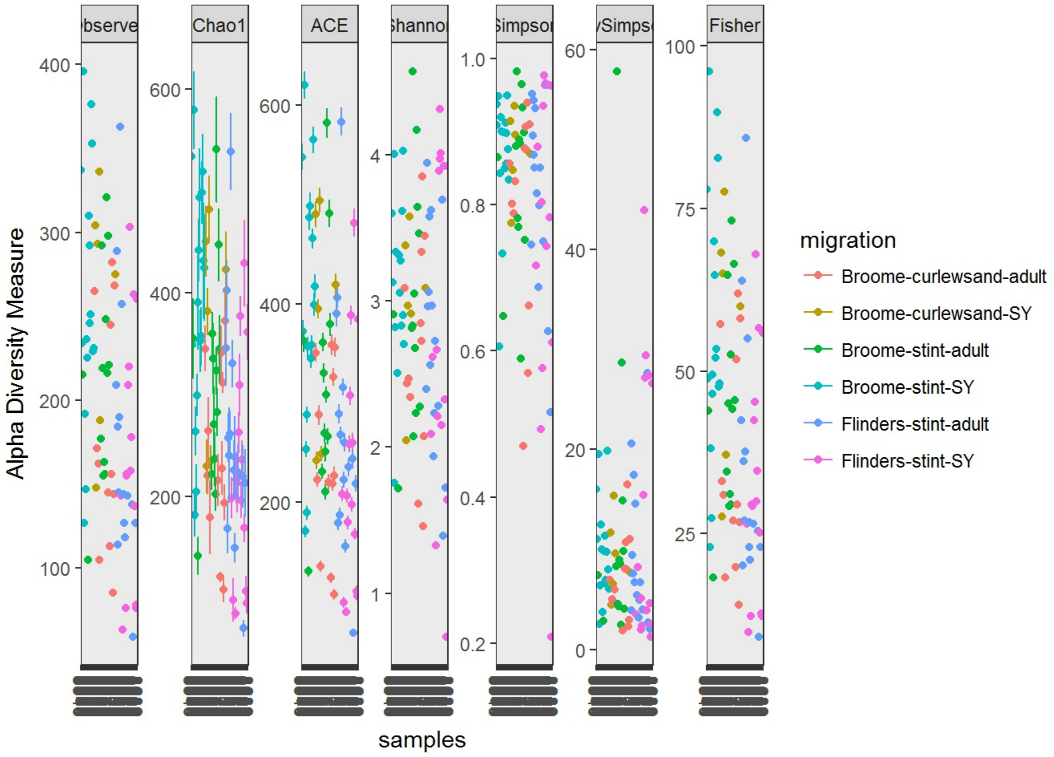


```
##stats

migration_bray <- phyloseq::distance(migration1, method = "bray")
#migration_bray <- phyloseq::distance(migration1, method = "unifrac")


# make a data frame from the sample_data
sampledf <- data.frame(sample_data(migration1))

# Adonis test
adonis(migration_bray ~ site+species+age, data = sampledf)
```

```
##
## Call:
## adonis(formula = migration_bray ~ site + species + age, data = sampledf)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##           Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## site       1    1.0202 1.02019  2.8284 0.03539  0.001 ***
## species    1    0.9369 0.93687  2.5974 0.03250  0.001 ***
## age        1    0.5401 0.54012  1.4974 0.01874  0.042 *
## Residuals 73   26.3305 0.36069         0.91338
## Total     76   28.8277                 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
##########################################################

############################################################################################

########################################################################################################

####alpha diversity
### Figure 3c)


plot_richness(migration, color = "migration")
```

```
## Warning: Removed 385 rows containing missing values (geom_errorbar).
```



bserve
Chao1
ACE
Shanno
Simpso
vSimps
Fisher
Alpha Diversity Measure
400
300
200
100
600
400
200
600
400
200
4
3
2
1
1.0
0.8
0.6
0.4
0.2
60
40
20
0
100
75
50
25
samples
migration
Broome-curlewsand-adult
Broome-curlewsand-SY
Broome-stint-adult
Broome-stint-SY
Flinders-stint-adult
Flinders-stint-SY

```
min_lib <- min(sample_sums(migration))

# Initialize matrices to store richness and evenness estimates
nsamp = nsamples(migration)
trials = 100

richness <- matrix(nrow = nsamp, ncol = trials)
row.names(richness) <- sample_names(migration)

evenness <- matrix(nrow = nsamp, ncol = trials)
row.names(evenness) <- sample_names(migration)


###

set.seed(3)

for (i in 1:100) {
  # Subsample
  r <-  rarefy_even_depth(migration, sample.size = min_lib, verbose = FALSE, replace = TRUE)

  # Calculate richness
  rich <- as.numeric(as.matrix(estimate_richness(r, measures = "Observed")))
  richness[ ,i] <- rich

  # Calculate evenness
  even <- as.numeric(as.matrix(estimate_richness(r, measures = "InvSimpson")))
  evenness[ ,i] <- even
}

SampleID <- row.names(richness)
mean <- apply(richness, 1, mean)
sd <- apply(richness, 1, sd)
measure <- rep("Richness", nsamp)
rich_stats <- data.frame(SampleID, mean, sd, measure)

SampleID <- row.names(evenness)
mean <- apply(evenness, 1, mean)
sd <- apply(evenness, 1, sd)
measure <- rep("Inverse Simpson", nsamp)
even_stats <- data.frame(SampleID, mean, sd, measure)

s <- data.frame(sample_data(migration))

alphadiv_rich_observed <- merge(rich_stats, s, by = "row.names")
alphadiv_even_inverse <- merge(even_stats, s, by = "row.names")

#summary stats

tapply(alphadiv_rich_observed$mean, alphadiv_rich_observed$migration, summary)
```

```
## $`Broome-curlewsand-adult`
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
##   74.05  116.02  132.22  152.53  210.12  251.35 
## 
## $`Broome-curlewsand-SY`
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
##   118.6   176.5   236.0   212.4   248.5   274.6 
## 
## $`Broome-stint-adult`
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
##   91.52  139.22  176.77  179.97  215.02  262.04 
## 
## $`Broome-stint-SY`
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
##   107.2   185.0   206.9   216.7   257.8   324.4 
## 
## $`Flinders-stint-adult`
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
##   52.94  103.52  118.65  145.55  162.16  294.01 
## 
## $`Flinders-stint-SY`
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
##   54.81   92.65  141.46  141.66  189.76  256.55
```

```
unique(alphadiv_rich_observed$migration)
```

```
## [1] Broome-stint-SY         Broome-stint-adult      Broome-curlewsand-adult
## [4] Broome-curlewsand-SY    Flinders-stint-adult    Flinders-stint-SY
## 6 Levels: Broome-curlewsand-adult ... Flinders-stint-SY
```

```
group1a<-subset(alphadiv_rich_observed, migration=="Broome-curlewsand-adult")
sd(group1a$mean)
```

```
## [1] 59.45963
```

```
group1b<-subset(alphadiv_rich_observed, migration=="Broome-curlewsand-SY")
sd(group1b$mean)
```

```
## [1] 60.1231
```

```
group2a<-subset(alphadiv_rich_observed, migration=="Broome-stint-adult")
sd(group2a$mean)
```

```
## [1] 53.34191
```

```
group2b<-subset(alphadiv_rich_observed, migration=="Broome-stint-SY")
sd(group2b$mean)
```

```
## [1] 62.84926
```

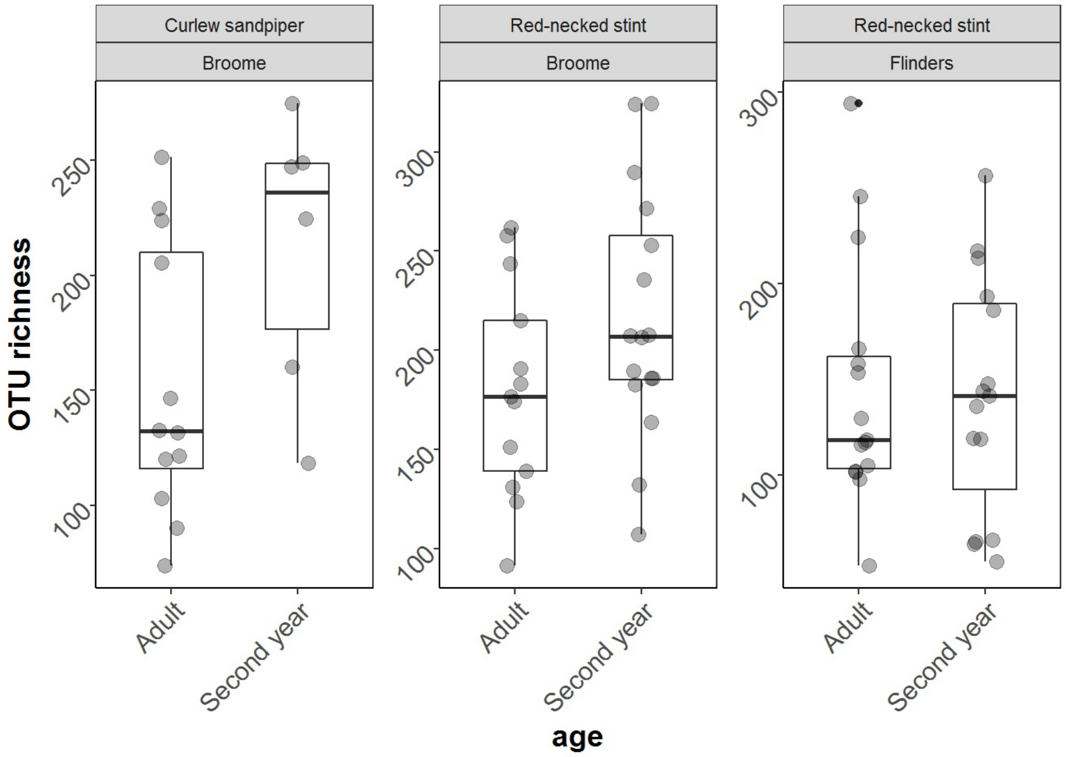
```
group3a<-subset(alphadiv_rich_observed, migration=="Flinders-stint-adult")
sd(group3a$mean)
```

```
## [1] 64.27512
```

```
group3b<-subset(alphadiv_rich_observed, migration=="Flinders-stint-SY")
sd(group3b$mean)
```

```
## [1] 62.57409
```

```
##observed richness

ggplot(alphadiv_rich_observed, aes(x = age, y = mean))+geom_boxplot(width=0.5)+
  geom_jitter(width=0.1, alpha = 0.3, size=3)+ylab("OTU richness")+
  theme(axis.text=element_text(size=12, angle=45, hjust=1), axis.title=element_text(size=14,face="bold"))+
  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(),
        panel.background = element_blank(), axis.line = element_line(colour = "black"))+
  facet_wrap(species~site, scales="free", nrow=1, ncol=3)
```



```
###################################################SHANNON

set.seed(3)

for (i in 1:100) {
  # Subsample
  r <-  rarefy_even_depth(migration, sample.size = min_lib, verbose = FALSE, replace = TRUE)

  # Calculate richness
  rich <- as.numeric(as.matrix(estimate_richness(r, measures = "Shannon")))
  richness[ ,i] <- rich

  # Calculate evenness
  even <- as.numeric(as.matrix(estimate_richness(r, measures = "InvSimpson")))
  evenness[ ,i] <- even
}

SampleID <- row.names(richness)
mean <- apply(richness, 1, mean)
sd <- apply(richness, 1, sd)
measure <- rep("Richness", nsamp)
rich_stats <- data.frame(SampleID, mean, sd, measure)

SampleID <- row.names(evenness)
mean <- apply(evenness, 1, mean)
sd <- apply(evenness, 1, sd)
measure <- rep("Inverse Simpson", nsamp)
even_stats <- data.frame(SampleID, mean, sd, measure)

s <- data.frame(sample_data(migration))

alphadiv_rich_observed <- merge(rich_stats, s, by = "row.names")
alphadiv_even_inverse <- merge(even_stats, s, by = "row.names")

#summary stats

tapply(alphadiv_rich_observed$mean, alphadiv_rich_observed$migration, summary)
```

```
## $`Broome-curlewsand-adult`
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
##   1.449   2.263   2.585   2.624   3.128   3.824 
## 
## $`Broome-curlewsand-SY`
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
##   2.032   2.908   3.000   2.971   3.279   3.545 
## 
## $`Broome-stint-adult`
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
##   1.708   2.257   2.814   2.936   3.433   4.546 
## 
## $`Broome-stint-SY`
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
##   1.744   2.803   3.176   3.112   3.381   3.990 
## 
## $`Flinders-stint-adult`
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
##   1.392   2.244   2.717   2.723   3.307   3.918 
## 
## $`Flinders-stint-SY`
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
##  0.6943  2.1060  2.6075  2.7683  3.8974  4.2952
```

```
unique(alphadiv_rich_observed$migration)
```

```
## [1] Broome-stint-SY         Broome-stint-adult      Broome-curlewsand-adult
## [4] Broome-curlewsand-SY    Flinders-stint-adult    Flinders-stint-SY      
## 6 Levels: Broome-curlewsand-adult ... Flinders-stint-SY
```

```
group1a<-subset(alphadiv_rich_observed, migration=="Broome-curlewsand-adult")
sd(group1a$mean)
```

```
## [1] 0.720232
```

```
group1b<-subset(alphadiv_rich_observed, migration=="Broome-curlewsand-SY")
sd(group1b$mean)
```

```
## [1] 0.5237586
```

```
group2a<-subset(alphadiv_rich_observed, migration=="Broome-stint-adult")
sd(group2a$mean)
```

```
## [1] 0.8243023
```

```
group2b<-subset(alphadiv_rich_observed, migration=="Broome-stint-SY")
sd(group2b$mean)
```

```
## [1] 0.5628283
```

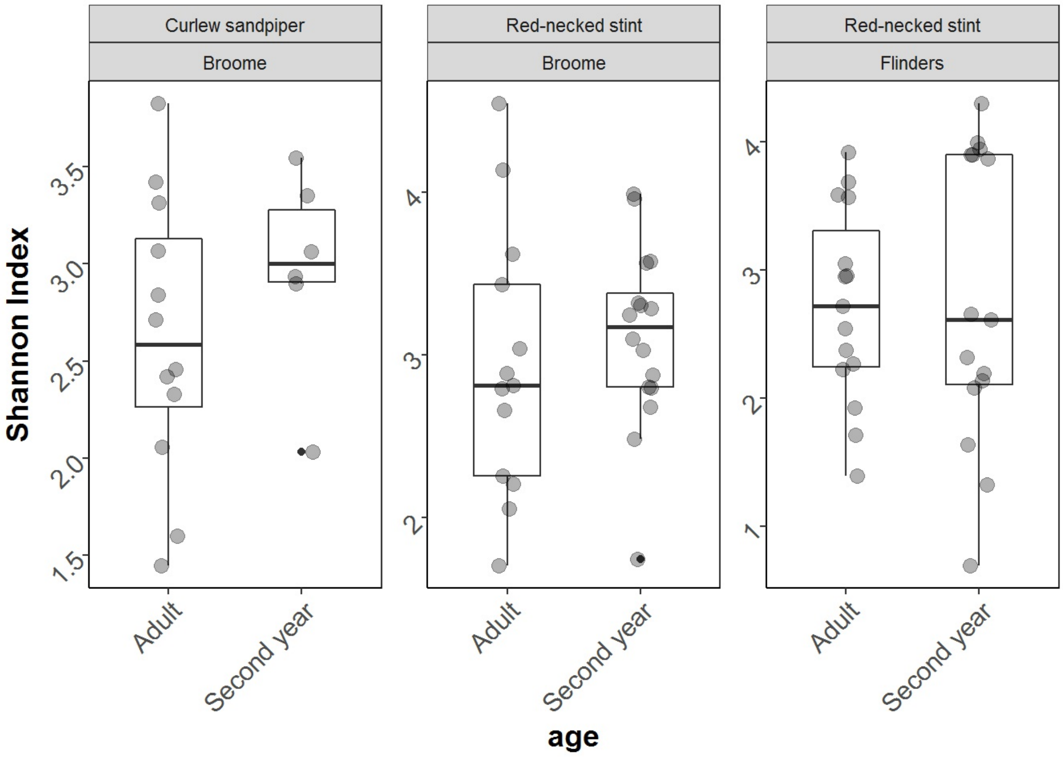
```
group3a<-subset(alphadiv_rich_observed, migration=="Flinders-stint-adult")
sd(group3a$mean)
```

```
## [1] 0.7589452
```

```
group3b<-subset(alphadiv_rich_observed, migration=="Flinders-stint-SY")
sd(group3b$mean)
```

```
## [1] 1.137082
```

```
##observed richness

ggplot(alphadiv_rich_observed, aes(x = age, y = mean))+geom_boxplot(width=0.5)+
  geom_jitter(width=0.1, alpha = 0.3, size=3)+ylab("Shannon Index")+
  theme(axis.text=element_text(size=12, angle=45, hjust=1), axis.title=element_text(size=14,face="bold"))+
  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(),
        panel.background = element_blank(), axis.line = element_line(colour = "black"))+
  facet_wrap(species~site, scales="free", nrow=1, ncol=3)
```



```
####################### END ###########################
```