

Initial Analysis

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```
library(phyloseq)
library(qiime2R)
library(tidyverse)
```

```
## -- Attaching packages -----
## v ggplot2 3.3.2      v purrr  0.3.4
## v tibble  3.0.1      v dplyr  1.0.0
## v tidyr   1.1.0      v stringr 1.4.0
## v readr   1.3.1      v forcats 0.5.0

## -- Conflicts -----
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
```

```
library(magrittr)
```

```
##
## Attaching package: 'magrittr'

## The following object is masked from 'package:purrr':
##
##   set_names

## The following object is masked from 'package:tidyr':
##
##   extract
```

```
library(microbiome)
```

```
##
## microbiome R package (microbiome.github.com)
##
##
## Copyright (C) 2011-2020 Leo Lahti,
##   Sudarshan Shetty et al. <microbiome.github.io>
##
## Attaching package: 'microbiome'

## The following object is masked from 'package:ggplot2':
##
##   alpha

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

```

##      transform
library(vegan)

## Loading required package: permute
## Loading required package: lattice
## This is vegan 2.5-6
##
## Attaching package: 'vegan'
## The following object is masked from 'package:microbiome':
##
##      diversity
library(symbioterR)

hiveMB_1 <- qza_to_phyloseq(features = "QIIME_Processing_1/feat_tab.qza",
  taxonomy = "QIIME_Processing_1/taxonomy.qza")

tax_1 <- read_qza("QIIME_Processing_1/taxonomy.qza")
tax_table(hiveMB_1) <- as.character(tax_1$data$Taxon) %>%
  strsplit(";") %>%
  do.call(what = qpcR::rbind.na) %>%
  set_colnames(c("Kingdom", "Phylum", "Class", "Order", "Family",
    "Genus", "Species")) %>%
  data.frame() %>%
  lapply(str_replace_all, "D_._", "") %>%
  as.data.frame() %>%
  set_rownames(tax_1$data$Feature.ID) %>%
  as.matrix()

hiveMB_2 <- qza_to_phyloseq(features = "QIIME_Processing_2/feat_tab.qza",
  taxonomy = "QIIME_Processing_2/taxonomy.qza")

tax_2 <- read_qza("QIIME_Processing_2/taxonomy.qza")
tax_table(hiveMB_2) <- as.character(tax_2$data$Taxon) %>%
  strsplit(";") %>%
  do.call(what = qpcR::rbind.na) %>%
  set_colnames(c("Kingdom", "Phylum", "Class", "Order", "Family",
    "Genus", "Species")) %>%
  data.frame() %>%
  lapply(str_replace_all, "D_._", "") %>%
  as.data.frame() %>%
  set_rownames(tax_2$data$Feature.ID) %>%
  as.matrix()

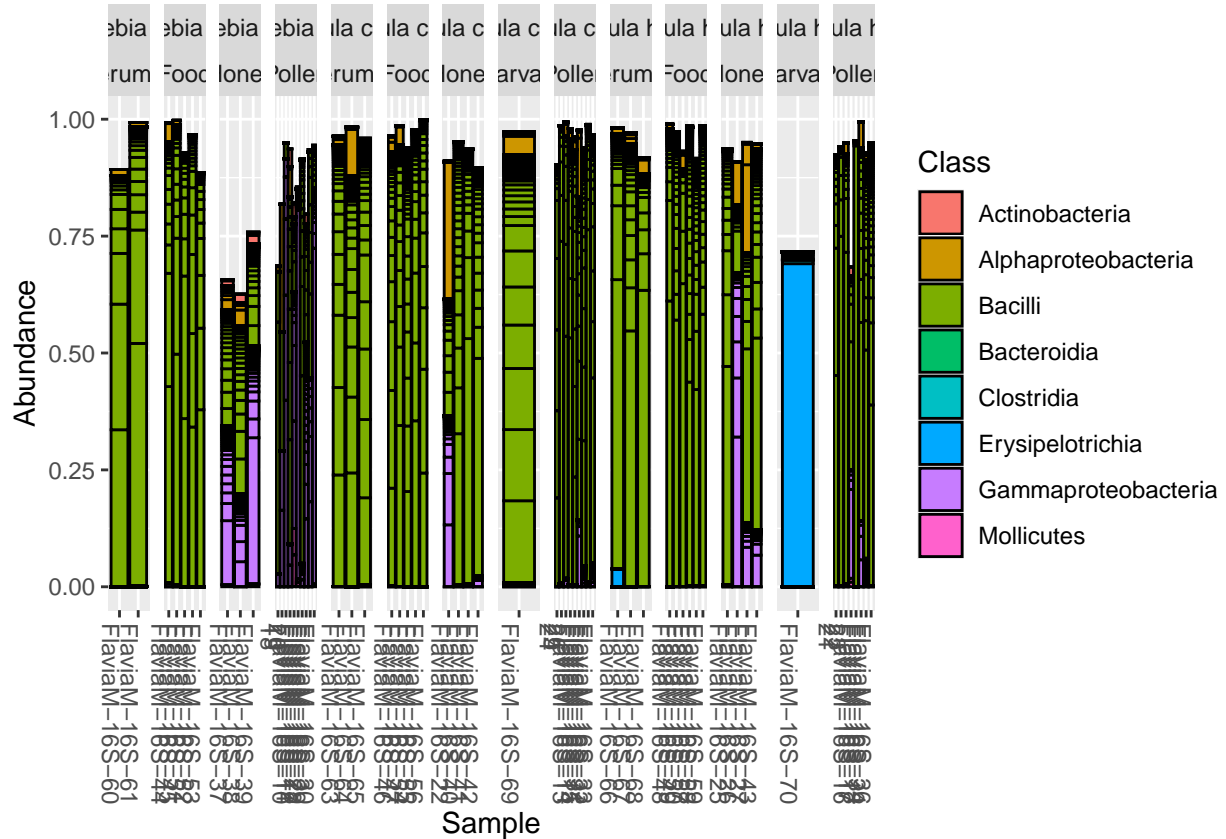
hiveMB <- merge_phyloseq(hiveMB_1, hiveMB_2)

sample_data(hiveMB) <- read.csv("Metadata_03082020.csv") %>%
  column_to_rownames("ID")

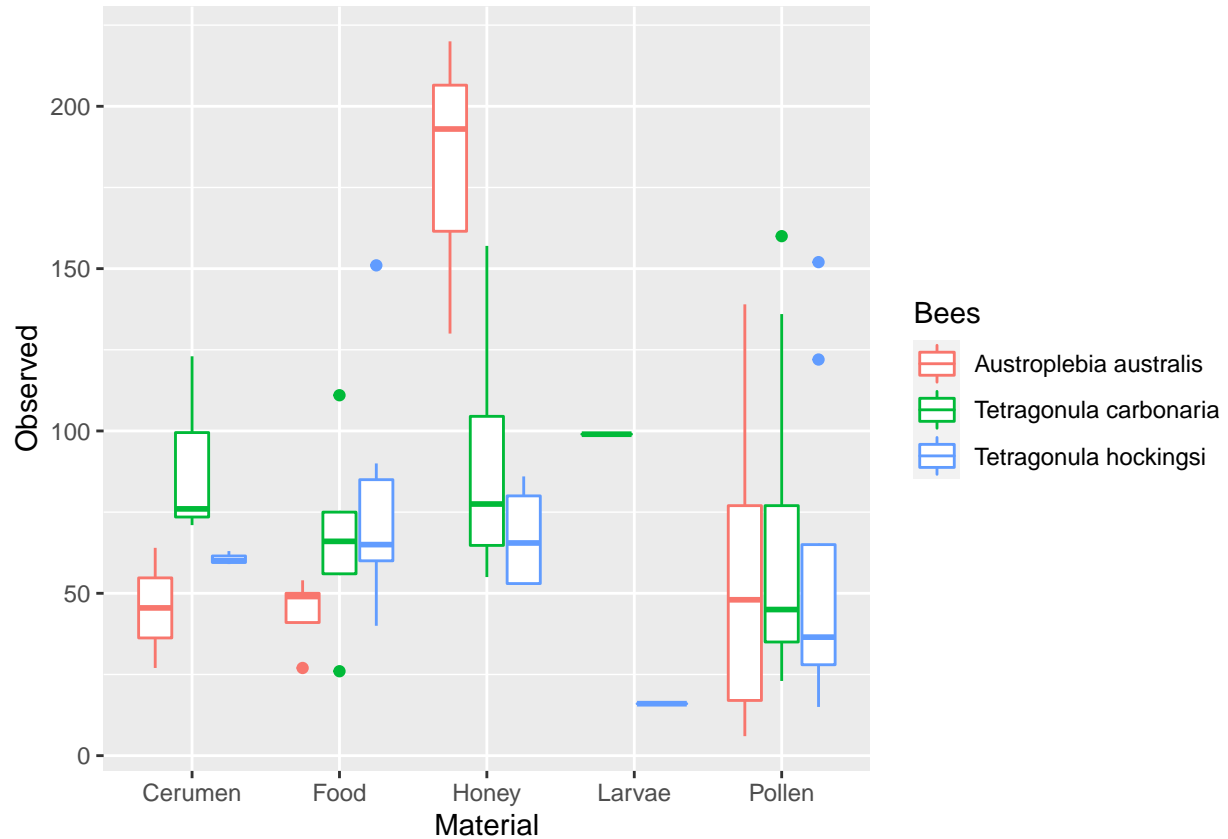
```

```
hiveMB <- removeNonbacterial(hiveMB)
```

```
makeObservationsRelative(hiveMB) %>%
  prevalenceFilter(threshold = 0.05) %>%
  plot_bar(fill = "Class") + facet_wrap(~ Bees + Material, scales = "free_x", nrow = 1)
```



```
alphaDiv <- estimate_richness(hiveMB, measures = c("Observed")) %>%
  cbind(data.frame(sample_data(hiveMB)))
ggplot(alphaDiv, aes(x = Material, y = Observed, color = Bees)) + geom_boxplot()
```



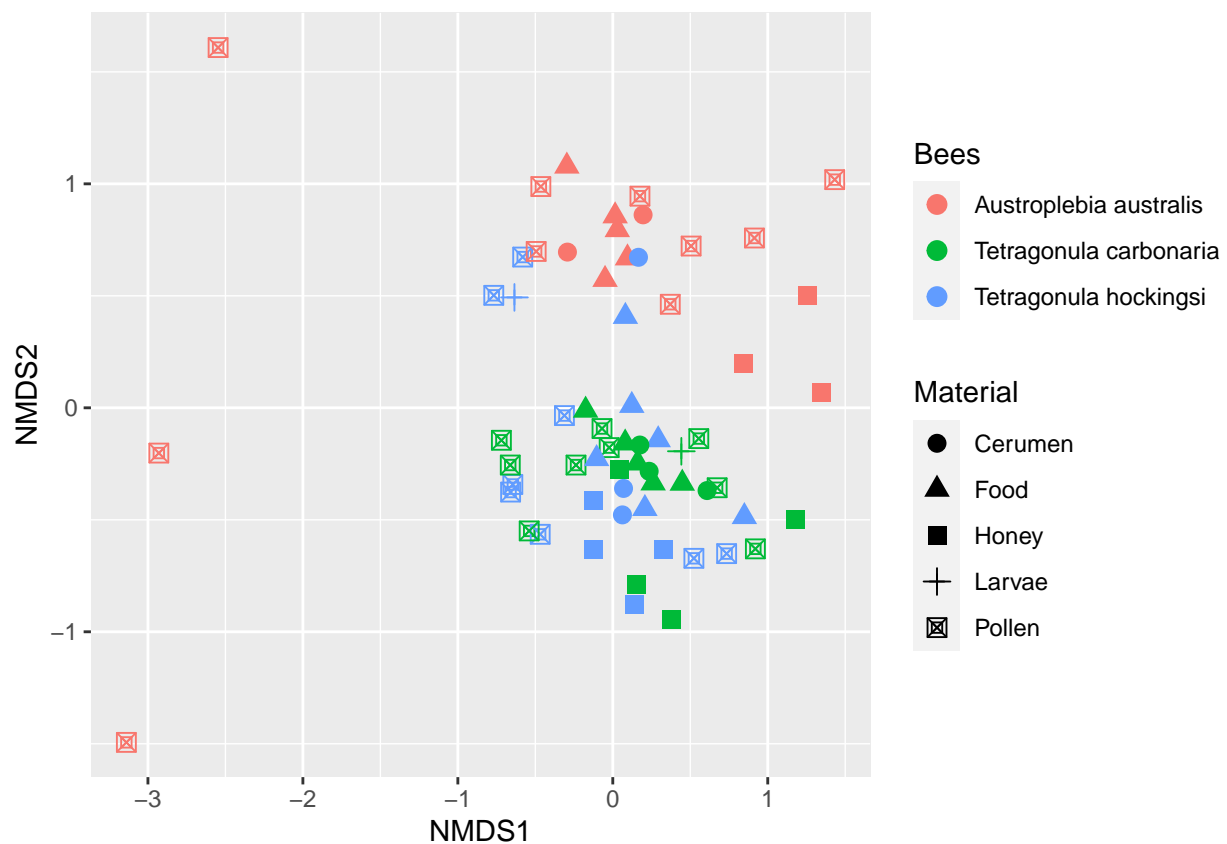
```
hiveMB <- transform(hiveMB, "clr")
otu_table(hiveMB)[otu_table(hiveMB) <= 0] <- 0

ord <- ordinate(hiveMB, distance = "bray", method = "NMDS")

## Wisconsin double standardization
## Run 0 stress 0.1975073
## Run 1 stress 0.1792445
## ... New best solution
## ... Procrustes: rmse 0.08878056 max resid 0.3129829
## Run 2 stress 0.1984643
## Run 3 stress 0.1767967
## ... New best solution
## ... Procrustes: rmse 0.02579431 max resid 0.08901046
## Run 4 stress 0.1768018
## ... Procrustes: rmse 0.000583197 max resid 0.002737982
## ... Similar to previous best
## Run 5 stress 0.1824837
## Run 6 stress 0.1763814
## ... New best solution
## ... Procrustes: rmse 0.01552371 max resid 0.05670576
## Run 7 stress 0.1988439
## Run 8 stress 0.1763114
## ... New best solution
## ... Procrustes: rmse 0.008162403 max resid 0.03649033
## Run 9 stress 0.1965415
## Run 10 stress 0.176388
```

```
## ... Procrustes: rmse 0.005753602  max resid 0.03233328
## Run 11 stress 0.1798948
## Run 12 stress 0.179392
## Run 13 stress 0.1791805
## Run 14 stress 0.1768018
## ... Procrustes: rmse 0.01053242  max resid 0.05014851
## Run 15 stress 0.1988445
## Run 16 stress 0.1794633
## Run 17 stress 0.1976074
## Run 18 stress 0.1767494
## ... Procrustes: rmse 0.007332822  max resid 0.04213818
## Run 19 stress 0.2040346
## Run 20 stress 0.1763949
## ... Procrustes: rmse 0.00599557  max resid 0.03361826
## *** No convergence -- monoMDS stopping criteria:
##      1: no. of iterations >= maxit
##     19: stress ratio > sratmax
```

```
plot_ordination(hiveMB, ord, color = "Bees", shape = "Material") +
  geom_point(size = 3)
```



```
dist <- phyloseq::distance(hiveMB, method = "bray")
adonis(dist ~ Bees + Material + Hive_ID, as(sample_data(hiveMB), "data.frame"))
```

```
##
## Call:
## adonis(formula = dist ~ Bees + Material + Hive_ID, data = as(sample_data(hiveMB), "data.frame"))
```

```
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##           Df SumsOfSqs MeanSqs F.Model    R2 Pr(>F)
## Bees       2     3.4246 1.71230  6.7357 0.15940 0.001 ***
## Material   4     2.7422 0.68555  2.6967 0.12763 0.001 ***
## Hive_ID   10     3.3700 0.33700  1.3257 0.15686 0.002 **
## Residuals 47    11.9480 0.25421          0.55611
## Total     63    21.4848          1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Session Info

```
sessionInfo()
```

```
## R version 4.0.2 (2020-06-22)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 18363)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=English_Australia.1252 LC_CTYPE=English_Australia.1252
## [3] LC_MONETARY=English_Australia.1252 LC_NUMERIC=C
## [5] LC_TIME=English_Australia.1252
##
## attached base packages:
## [1] stats      graphics  grDevices  utils      datasets  methods    base
##
## other attached packages:
## [1] symbioteR_0.0.0.9000 vegan_2.5-6          lattice_0.20-41
## [4] permute_0.9-5          microbiome_1.11.0    magrittr_1.5
## [7] forcats_0.5.0          stringr_1.4.0        dplyr_1.0.0
## [10] purrr_0.3.4            readr_1.3.1          tidyr_1.1.0
## [13] tibble_3.0.1           ggplot2_3.3.2        tidyverse_1.3.0
## [16] qiime2R_0.99.13        phyloseq_1.33.0
##
## loaded via a namespace (and not attached):
## [1] Rtsne_0.15              colorspace_1.4-1      ellipsis_0.3.1
## [4] htmlTable_2.0.0         XVector_0.29.3        base64enc_0.1-3
## [7] fs_1.4.1                rstudioapi_0.11       farver_2.0.3
## [10] fansi_0.4.1            lubridate_1.7.9       xml2_1.3.2
## [13] codetools_0.2-16       splines_4.0.2         robustbase_0.93-6
## [16] knitr_1.29             ade4_1.7-15           Formula_1.2-3
## [19] jsonlite_1.6.1         broom_0.5.6           cluster_2.1.0
## [22] dbplyr_1.4.4           png_0.1-7             shiny_1.5.0
## [25] compiler_4.0.2         http_1.4.1            backports_1.1.6
## [28] fastmap_1.0.1          assertthat_0.2.1      Matrix_1.2-18
## [31] cli_2.0.2              later_1.1.0.1         acepack_1.4.1
## [34] htmltools_0.5.0        tools_4.0.2           igraph_1.2.5
```

## [37] gtable_0.3.0	glue_1.4.1	reshape2_1.4.4
## [40] Rcpp_1.0.4.6	Biobase_2.49.0	cellranger_1.1.0
## [43] vctrs_0.3.1	Biostrings_2.57.2	rhdf5filters_1.1.0
## [46] multtest_2.45.0	ape_5.4	nlme_3.1-148
## [49] iterators_1.0.12	crosstalk_1.1.0.1	xfun_0.15
## [52] rvest_0.3.5	miniUI_0.1.1.1	mime_0.9
## [55] lifecycle_0.2.0	DEoptimR_1.0-8	zlibbioc_1.35.0
## [58] MASS_7.3-51.6	scales_1.1.1	hms_0.5.3
## [61] promises_1.1.1	parallel_4.0.2	biomformat_1.17.0
## [64] rhdf5_2.33.3	RColorBrewer_1.1-2	yaml_2.2.1
## [67] gridExtra_2.3	qpcR_1.4-1	rpart_4.1-15
## [70] latticeExtra_0.6-29	stringi_1.4.6	S4Vectors_0.27.12
## [73] foreach_1.5.0	checkmate_2.0.0	BiocGenerics_0.35.4
## [76] manipulateWidget_0.10.1	rlang_0.4.6	pkgconfig_2.0.3
## [79] rgl_0.100.54	evaluate_0.14	Rhdf5lib_1.11.2
## [82] labeling_0.3	htmlwidgets_1.5.1	tidyselect_1.1.0
## [85] plyr_1.8.6	R6_2.4.1	IRanges_2.23.10
## [88] generics_0.0.2	Hmisc_4.4-0	DBI_1.1.0
## [91] pillar_1.4.4	haven_2.3.1	foreign_0.8-80
## [94] withr_2.2.0	mgcv_1.8-31	survival_3.1-12
## [97] nnet_7.3-14	modelr_0.1.8	crayon_1.3.4
## [100] rmarkdown_2.3	jpeg_0.1-8.1	grid_4.0.2
## [103] readxl_1.3.1	minpack.lm_1.2-1	data.table_1.12.8
## [106] blob_1.2.1	webshot_0.5.2	reprex_0.3.0
## [109] digest_0.6.25	xtable_1.8-4	httpuv_1.5.4
## [112] stats4_4.0.2	munsell_0.5.0	