

# Initial Analysis

Boyd Tarlinton

18/06/2020

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

```
## -- Attaching packages -----
## v ggplot2 3.3.1      v purrr  0.3.4
## v tibble  3.0.1      v dplyr  0.8.5
## 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)
```

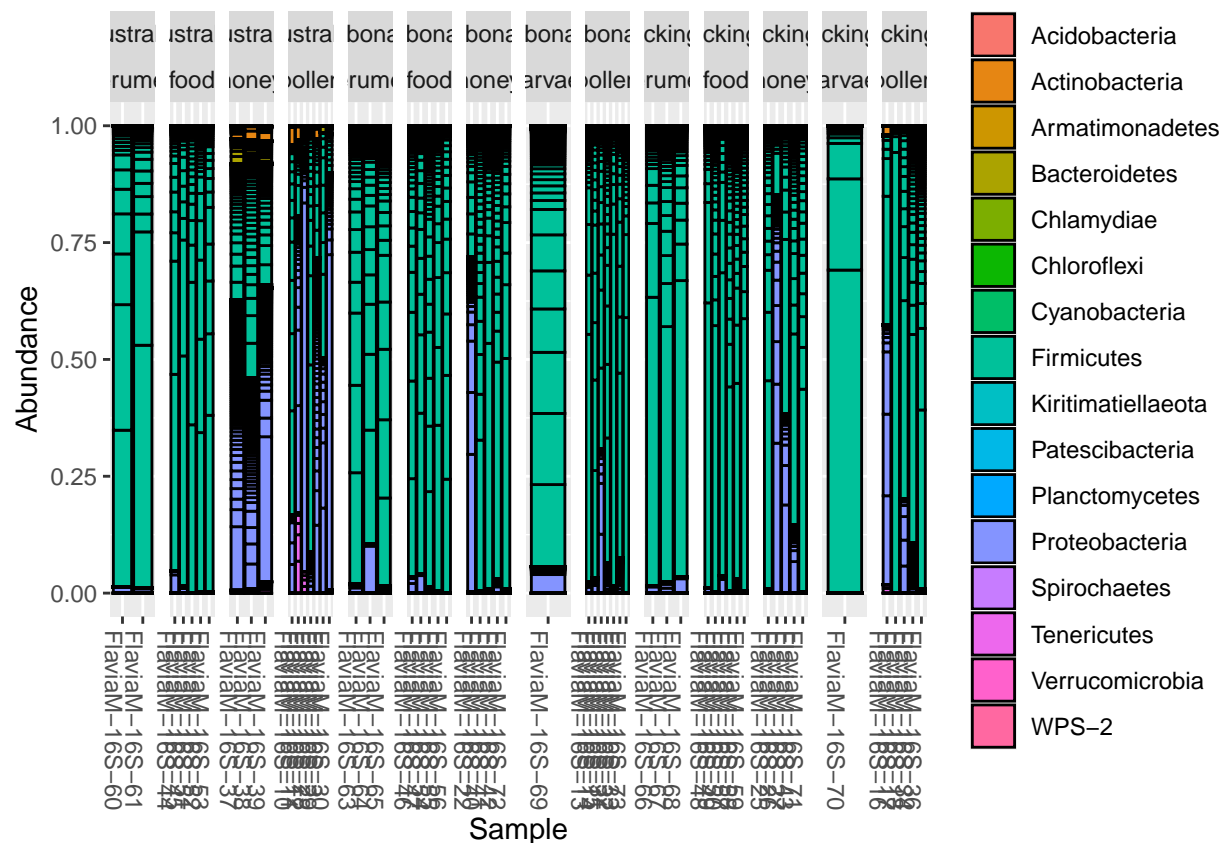
```
hiveMB <- qza_to_phyloseq(
  "QIIME_Processing/feat_tab.qza",
  "QIIME_Processing/phylogeny.qza",
  "QIIME_Processing/taxonomy.qza")

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

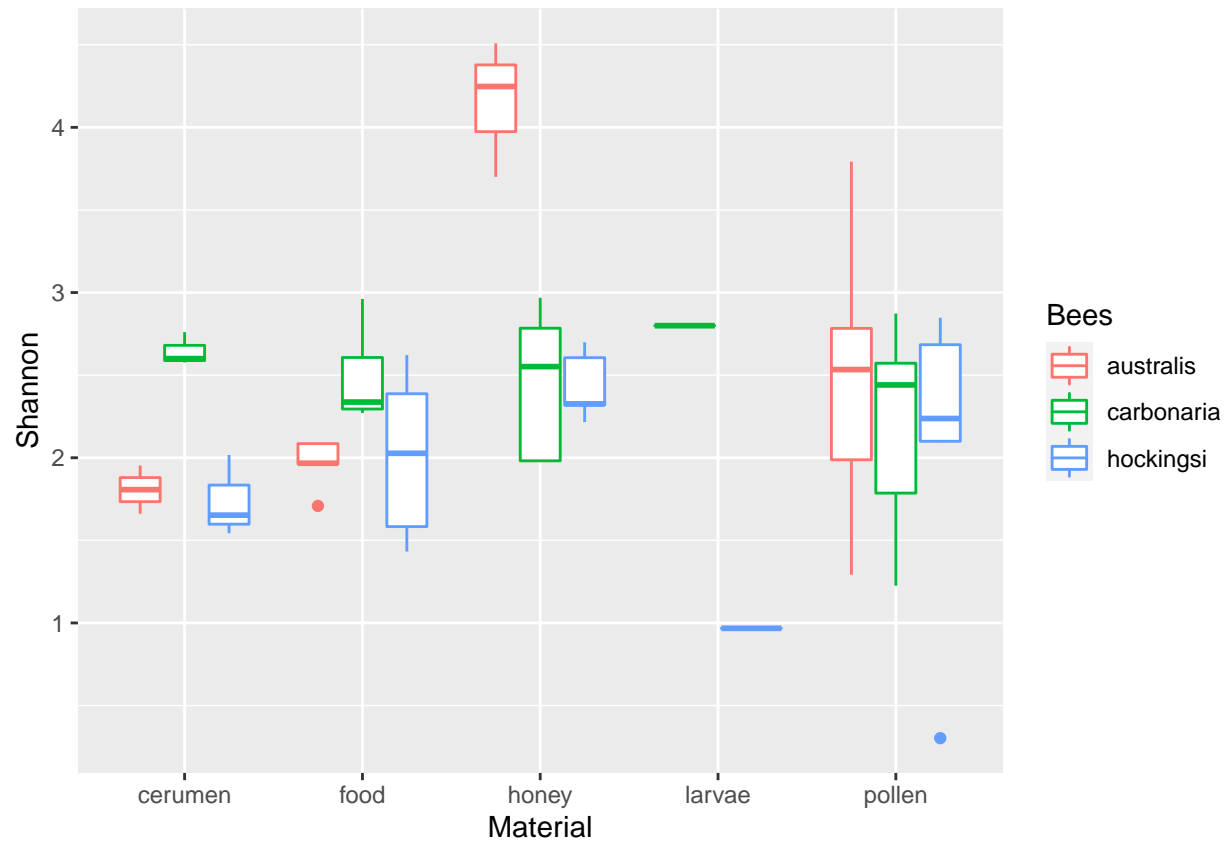
tax <- read_qza("QIIME_Processing/taxonomy.qza")
tax_table(hiveMB) <- as.character(tax$data$Taxon) %>%
  strsplit(";") %>%
  do.call(what = qpcR::rbind.na) %>%
  set_colnames(c("Kingdom", "Phylum", "Class", "Order", "Family",
                 "Genus", "Species")) %>%
  data.frame() %>%
  lapply(str_replace_all,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

hiveMB <- removeNonbacterial(hiveMB)

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

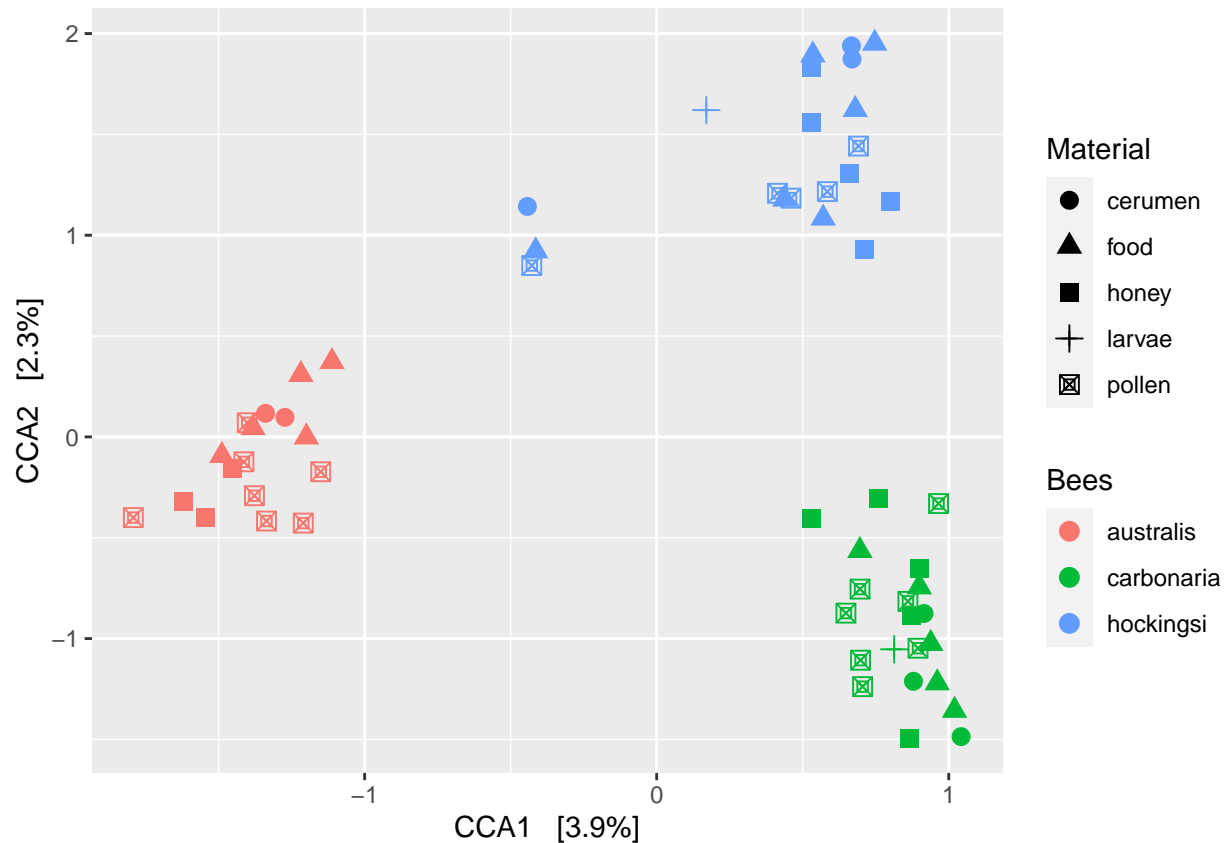


```
alphaDiv <- estimate_richness(hiveMB, measures = c("Shannon")) %>%
  cbind(data.frame(sample_data(hiveMB)))
ggplot(alphaDiv, aes(x = Material, y = Shannon, color = Bees)) + geom_boxplot()
```



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

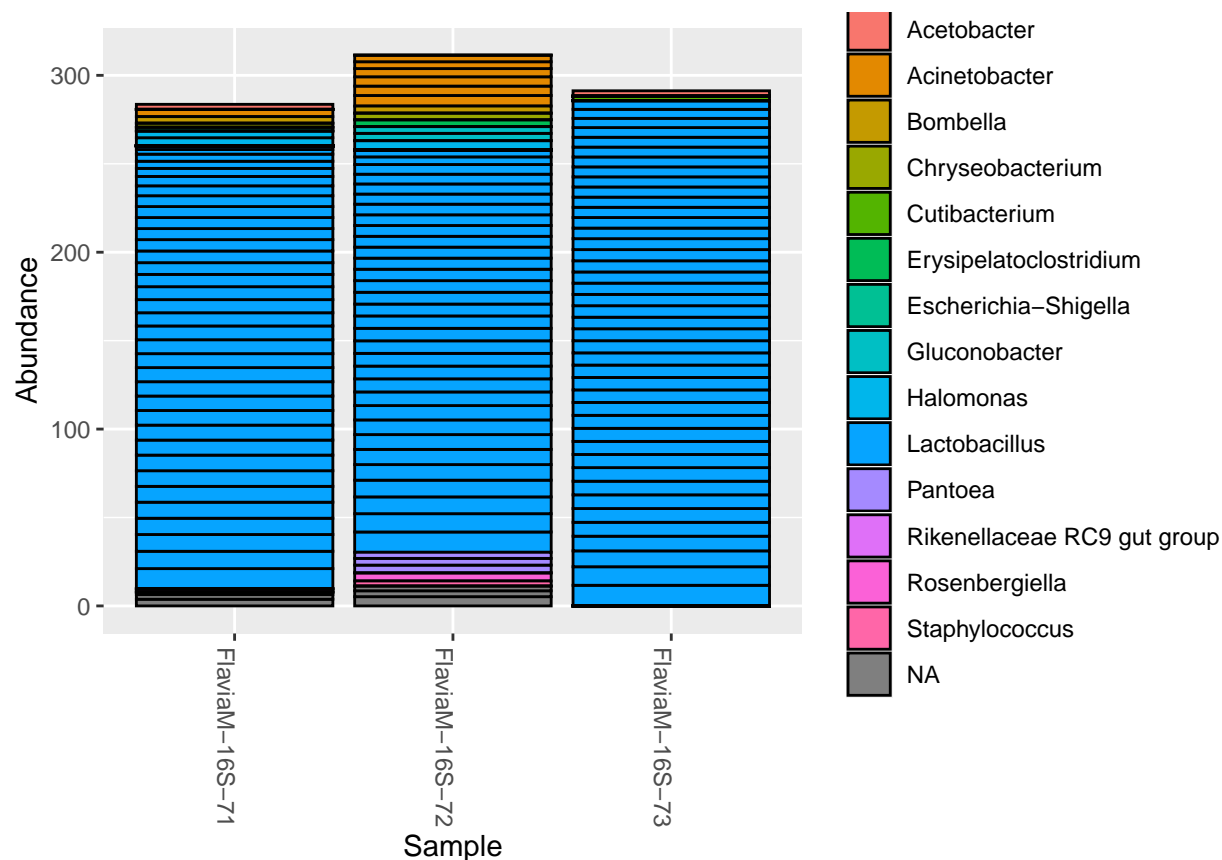
ord <- ordinate(hiveMB, distance = "wunifrac", method = "CCA", ~ Bees)
plot_ordination(hiveMB, ord, color = "Bees", shape = "Material") +
  geom_point(size = 3)
```



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

```
##
## Call:
## adonis(formula = dist ~ Bees + Material, 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     3.1383  1.56914  6.2366 0.16910  0.001 ***
## Material      4     2.5886  0.64714  2.5721 0.13948  0.001 ***
## Residuals    51    12.8317  0.25160          0.69142
## Total        57    18.5585          1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
fermS <- subset_samples(hiveMB, Fermentation == "S")
fermS <- prune_taxa(x = fermS, taxa_sums(fermS) > 0)
plot_bar(fermS, fill = "Genus")
```



## Session Info

```
sessionInfo()
```

```
## R version 4.0.0 (2020-04-24)
## 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_0.8.5
## [10] purrr_0.3.4           readr_1.3.1          tidyr_1.1.0
## [13] tibble_3.0.1          ggplot2_3.3.1        tidyverse_1.3.0
## [16] qiime2R_0.99.13       phyloseq_1.32.0
##
```

```

## loaded via a namespace (and not attached):
## [1] Rtsne_0.15 colorspace_1.4-1 ellipsis_0.3.1
## [4] htmlTable_1.13.3 XVector_0.28.0 base64enc_0.1-3
## [7] fs_1.4.1 rstudioapi_0.11 farver_2.0.3
## [10] fansi_0.4.1 lubridate_1.7.8 xml2_1.3.2
## [13] codetools_0.2-16 splines_4.0.0 robustbase_0.93-6
## [16] knitr_1.28 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_1.4.0.2
## [25] compiler_4.0.0 httr_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_1.0.0 acepack_1.4.1
## [34] htmltools_0.4.0 tools_4.0.0 igraph_1.2.5
## [37] gtable_0.3.0 glue_1.4.1 reshape2_1.4.4
## [40] Rcpp_1.0.4.6 Biobase_2.48.0 cellranger_1.1.0
## [43] vctrs_0.3.0 Biostrings_2.56.0 multtest_2.44.0
## [46] ape_5.3 nlme_3.1-147 iterators_1.0.12
## [49] crosstalk_1.1.0.1 xfun_0.14 rvest_0.3.5
## [52] miniUI_0.1.1.1 mime_0.9 lifecycle_0.2.0
## [55] DEoptimR_1.0-8 zlibbioc_1.34.0 MASS_7.3-51.5
## [58] scales_1.1.1 hms_0.5.3 promises_1.1.0
## [61] parallel_4.0.0 biomformat_1.17.0 rhdf5_2.32.0
## [64] RColorBrewer_1.1-2 yaml_2.2.1 gridExtra_2.3
## [67] qpcR_1.4-1 rpart_4.1-15 latticeExtra_0.6-29
## [70] stringi_1.4.6 S4Vectors_0.26.0 foreach_1.5.0
## [73] checkmate_2.0.0 BiocGenerics_0.34.0 manipulateWidget_0.10.1
## [76] rlang_0.4.6 pkgconfig_2.0.3 rgl_0.100.54
## [79] evaluate_0.14 Rhdf5lib_1.10.0 labeling_0.3
## [82] htmlwidgets_1.5.1 tidyselect_1.1.0 plyr_1.8.6
## [85] R6_2.4.1 IRanges_2.22.1 generics_0.0.2
## [88] Hmisc_4.4-0 DBI_1.1.0 pillar_1.4.4
## [91] haven_2.3.0 foreign_0.8-78 withr_2.2.0
## [94] mgcv_1.8-31 survival_3.1-12 nnet_7.3-13
## [97] modelr_0.1.8 crayon_1.3.4 rmarkdown_2.1
## [100] jpeg_0.1-8.1 grid_4.0.0 readxl_1.3.1
## [103] minpack.lm_1.2-1 data.table_1.12.8 blob_1.2.1
## [106] webshot_0.5.2 reprex_0.3.0 digest_0.6.25
## [109] xtable_1.8-4 httpuv_1.5.2 stats4_4.0.0
## [112] munsell_0.5.0

```