

HMM search output analyses

Executing HMM search output analyses with max e-val:s

LJM

2020-01-09

Contents

1 Load libraries	1
2 Read intermediary csv file	2
3 Exchange NA, Inf and -Inf with 0	2
4 Move fOTU names to row names and execute PCA	2
5 Visualise the results	2
5.1 PCA plots	2
5.2 Heatmaps	6
6 NMDS	8
7 Save/Load R data	8
8 Remove outlier(s)	10
9 Rerun NMDS with outliers removed	10
10 Save/Load R data	10
11 Visualise the filtered data	12
12 Create lists of fOTUs of interest	13
12.1 PCA lists	13
12.2 NMDS lists	16
13 Session info	18
14 References	19

This document contains the final analyses and visualisations of HMMer search with eggNOG viral HMM profiles on fOTUs.

1 Load libraries

```
library(tidyverse)
#library(devtools)
#install_github("vqv/ggbiplot")
library(ggbiplot)
#install.packages("vegan")
library(vegan)
```

2 Read intermediary csv file

In the intermediary csv file the $-\log_{10}(\text{e-val})$:s are the maximum e-values among all the hits to the fOTUs instead (and in contrast to the previous run in file 2019-11-25_hmmsearch_output_analyses.Rmd) of the average of all the HMMsearch hits to the fOTUs.

```
columns <- paste("c", strrep("d", 8315), sep = "")  
fOTUsHMM <- read_csv(file = "../analyses/HMMsearch/max_fOTUsHMM.csv", col_types = columns)
```

3 Exchange NA, Inf and -Inf with 0

```
is.na(fOTUsHMM) <- sapply(fOTUsHMM, is.infinite)  
fOTUsHMM[is.na(fOTUsHMM)] <- 0
```

4 Move fOTU names to row names and execute PCA

```
fOTUsHMM_pca <- fOTUsHMM %>%  
  column_to_rownames(var = "fOTU_name") %>%  
  prcomp(.,  
         center = TRUE)
```

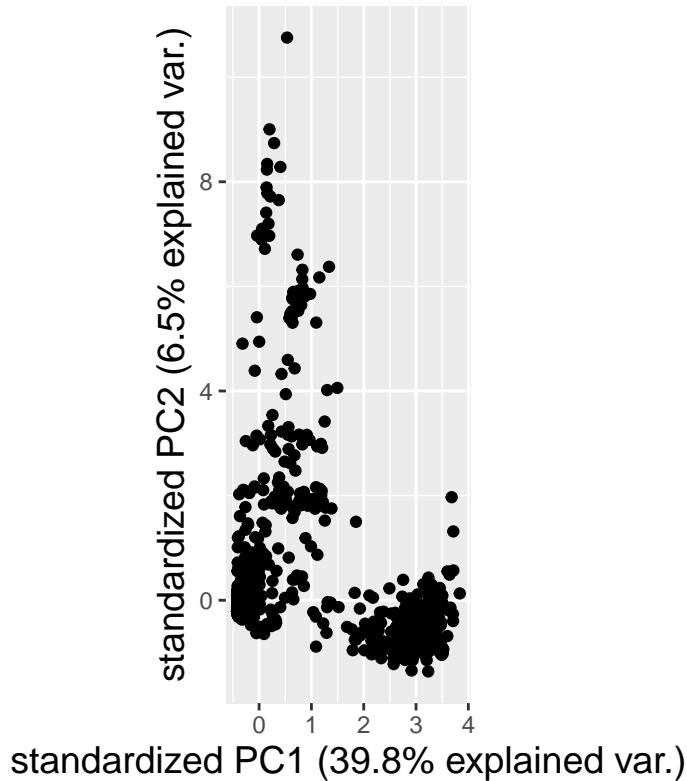
5 Visualise the results

5.1 PCA plots

These PCA plots can be used to visually determine potential groupings.

```
file_name <- "../visualisations/HMMsearch/ggbiplot_max_PC1-2.png"  
png(filename = file_name, width = 1000, height = 689)  
ggbiplot(fOTUsHMM_pca, var.axes = F, choices = 1:2) + ggtitle("PCA of fOTU wise HMM data") +  
  theme(plot.title = element_text(hjust = 0.5, size = 18), axis.title=element_text(size=14))  
dev.off()  
  
## pdf  
## 2  
ggbiplot(fOTUsHMM_pca, var.axes = F, choices = 1:2) + ggtitle("PCA of fOTU wise HMM data") +  
  theme(plot.title = element_text(hjust = 0.5, size = 18), axis.title=element_text(size=14))
```

PCA of fOTU wise HMM data

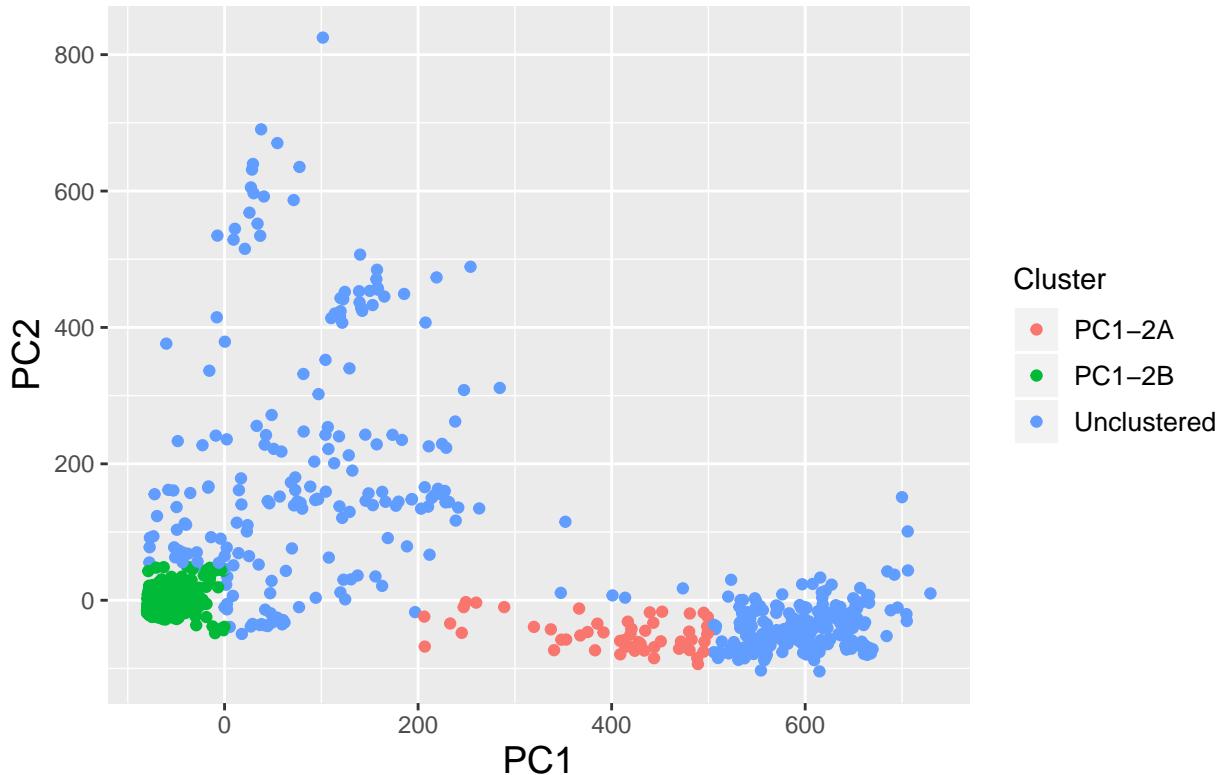


Here above the plot is slightly squashed but the following aren't:

```
PC12s <- tibble(fOTUs = names(fOTUsHMM_pca$x[,1]),
                 PC1 = unname(fOTUsHMM_pca$x[,1]),
                 PC2 = unname(fOTUsHMM_pca$x[,2]),
                 ) %>%
  mutate(Cluster = case_when(PC1 >= -100 & PC1 <= 0 & PC2 >= -50 & PC2 <= 50 ~ 'PC1-2B',
                             PC1 >= 200 & PC1 <= 500 & PC2 >= -100 & PC2 <= 0 ~ 'PC1-2A',
                             TRUE ~ 'Unclustered')) %>%
  mutate_at(4, as.factor)

title <- "PCA of e-values of matches in fOTUs"
qplot(PC1,
       PC2,
       data = PC12s,
       geom = c("point"),
       group = Cluster,
       color = Cluster,
       fill = Cluster) + ggtile(title) +
theme(plot.title = element_text(hjust = 0.5, size = 18),
      axis.title=element_text(size=14)) +
theme(legend.text=element_text(size=10))
```

PCA of e-values of matches in fOTUs



```

ggsave(filename = "../visualisations/HMMsearch/pca_hmmsearch_max_PC1-2.png",
       device = png,
       width = 1000,
       height = 750,
       units = "in",
       limitsize = FALSE,
       dpi = 320)

PC23s <- tibble(fOTUs = names(fOTUsHMM_pca$x[,1]),
                 PC2 = unname(fOTUsHMM_pca$x[,2]),
                 PC3 = unname(fOTUsHMM_pca$x[,3])) %>%
  mutate(Cluster = case_when(PC2 >= -100 & PC2 <= 75 & PC3 >= -150 & PC3 <= 80 ~ 'PC2-3E',
                             PC2 >= 100 & PC2 <= 250 & PC3 >= -80 & PC3 <= 125 ~ 'PC2-3C',
                             PC2 >= 250 & PC2 <= 425 & PC3 >= -200 & PC3 <= 0 ~ 'PC2-3A',
                             PC2 >= 400 & PC2 <= 650 & PC3 >= -210 & PC3 <= 80 ~ 'PC2-3D',
                             PC2 >= 0 & PC2 <= 200 & PC3 >= 200 & PC3 <= 450 ~ 'PC2-3B',
                             TRUE ~ 'Unclustered')) %>%
  mutate_at(4, as.factor)

title <- "PCA of e-values of matches in fOTUs"
qplot(PC2,
      PC3,
      data = PC23s,
      geom = c("point"),
      group = Cluster,
      color = Cluster,
      fill = Cluster) + ggtitle(title) +

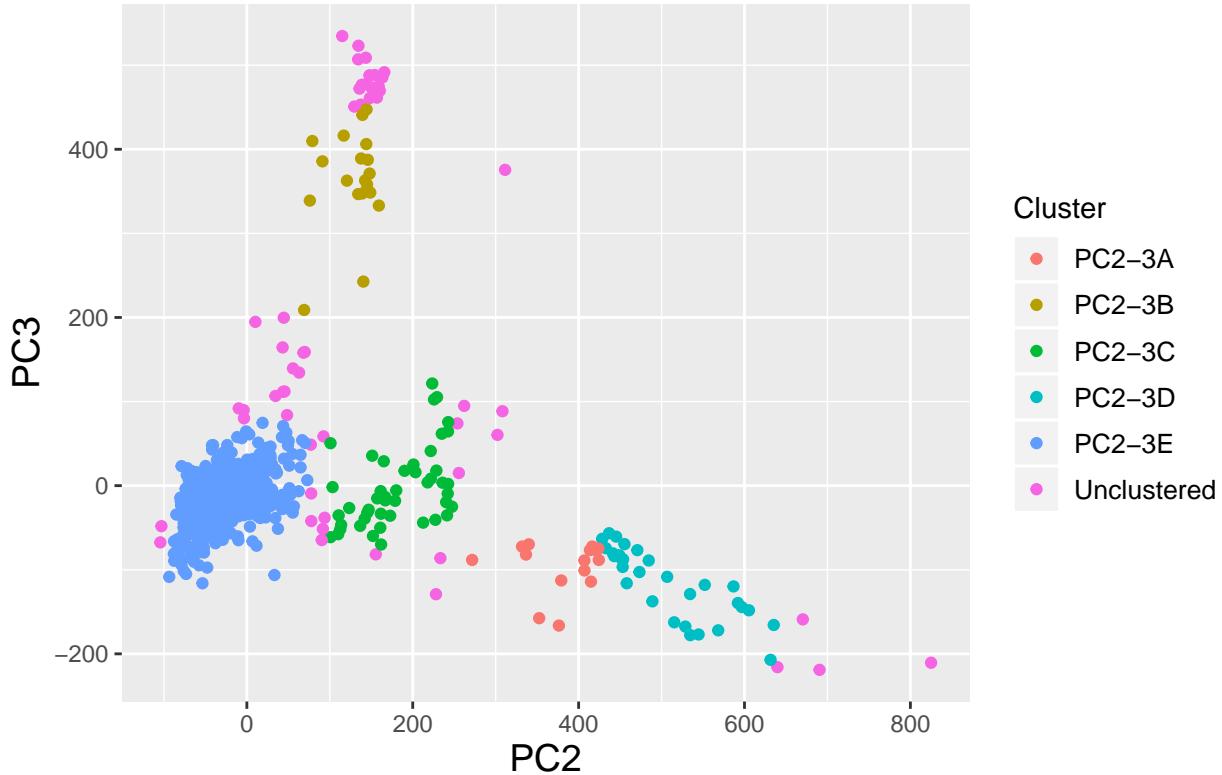
```

```

theme(plot.title = element_text(hjust = 0.5, size = 18),
      axis.title=element_text(size=14)) +
theme(legend.text=element_text(size=10))

```

PCA of e-values of matches in fOTUs



```

ggsave(filename = ".../visualisations/HMMsearch/pca_hmmsearch_max_PC2-3.png",
       device = png,
       width = 1000,
       height = 750,
       units = "in",
       limitsize = FALSE,
       dpi = 320)

PC34s <- tibble(fOTUs = names(fOTUsHMM_pca$x[,1]),
                 PC3 = unname(fOTUsHMM_pca$x[,3]),
                 PC4 = unname(fOTUsHMM_pca$x[,4])) %>%
  mutate(Cluster = case_when(PC3 >= -80 & PC3 <= 80 & PC4 >= -50 & PC4 <= 75 ~ 'PC3-4C',
                             PC3 >= -50 & PC3 <= 0 & PC4 >= 75 & PC4 <= 175 ~ 'PC3-4B',
                             PC3 >= 200 & PC3 <= 450 & PC4 >= -25 & PC4 <= 50 ~ 'PC3-4A',
                             TRUE ~ 'Unclustered')) %>%
  mutate_at(4, as.factor)

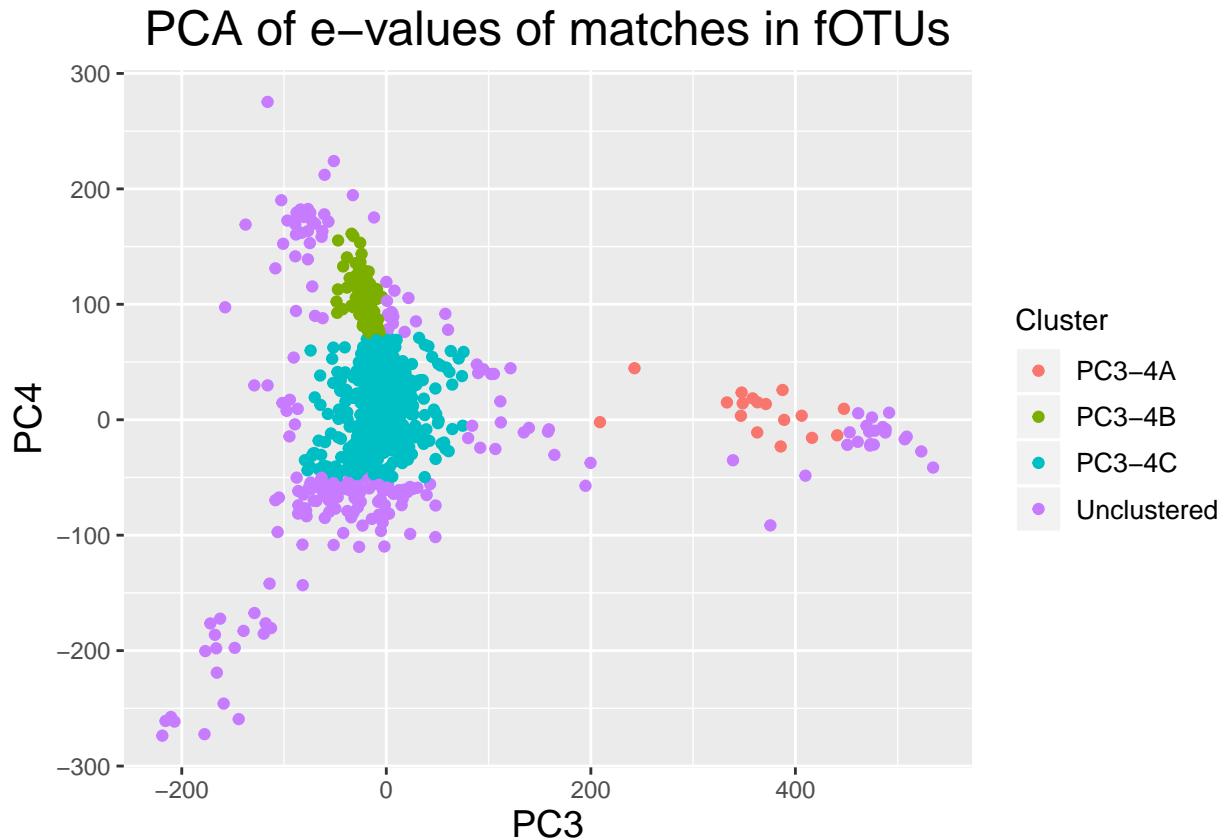
title <- "PCA of e-values of matches in fOTUs"
qplot(PC3,
      PC4,
      data = PC34s,
      geom = c("point"),
      group = Cluster,

```

```

    color = Cluster,
    fill = Cluster) + ggtitle(title) +
  theme(plot.title = element_text(hjust = 0.5, size = 18),
        axis.title=element_text(size=14)) +
  theme(legend.text=element_text(size=10))

```



```

ggsave(filename = "../visualisations/HMMsearch/pca_hmmsearch_max_PC3-4.png",
       device = png,
       width = 1000,
       height = 750,
       units = "in",
       limitsize = FALSE,
       dpi = 320)

```

5.2 Heatmaps

```

dim(fOTUsHMM_pca)

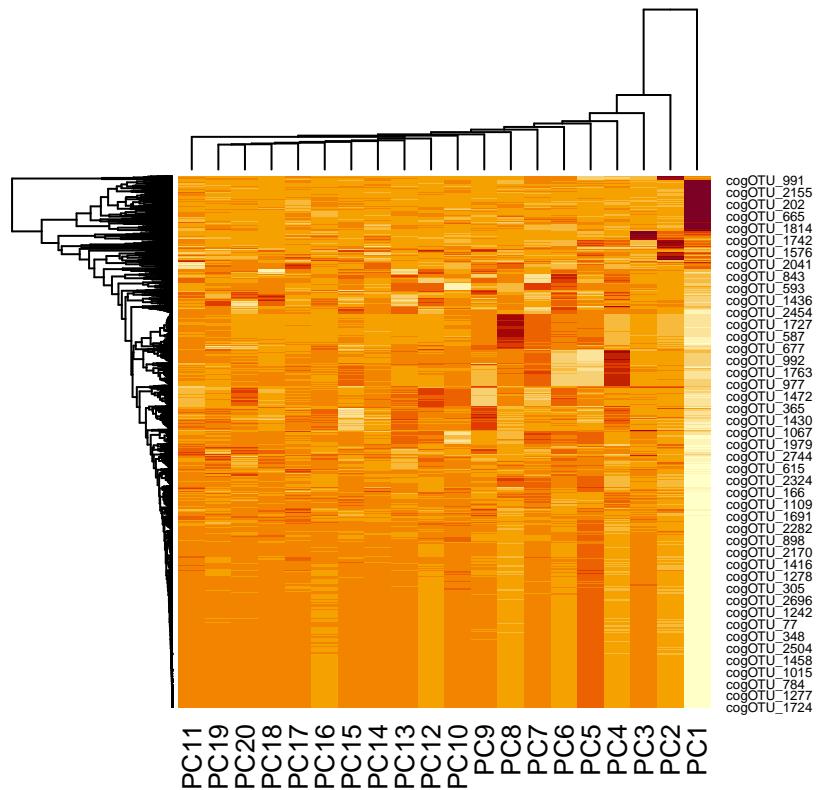
## NULL

prediction <- predict(fOTUsHMM_pca) [,1:20]
file_name <- "../visualisations/HMMsearch/heatmap_max_20_first_PCs.png"
png(filename = file_name)
heatmap(as.matrix(prediction))
dev.off()

## pdf
## 2

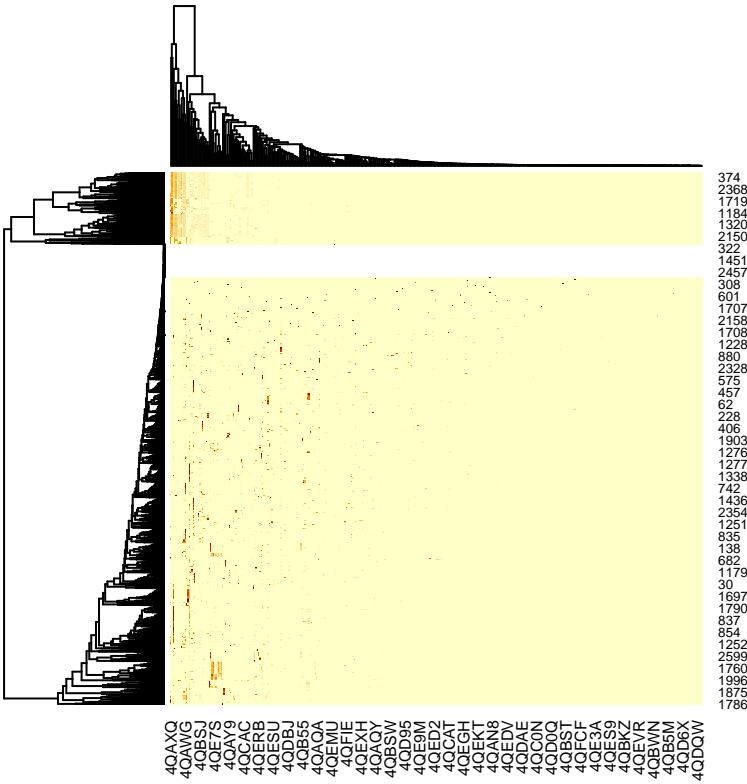
```

```
heatmap(as.matrix(prediction))
```



```
file_name <- ".../visualisations/HMMsearch/heatmap_max_400_first_fOTUs.png"  
png(filename = file_name)  
heatmap(as.matrix(fOTUsHMM[,-1])[,1:400])  
dev.off()
```

```
## pdf  
## 2  
heatmap(as.matrix(fOTUsHMM[,-1])[,1:400])
```



6 NMDS

Let's perform non-metric multidimensional scaling:

```
width <- dim(fOTUsHMM)[2]
ord <- metaMDS(fOTUsHMM[, 2:width])
```

7 Save/Load R data

Save the data once again to speed-up things.

```
#saveRDS(ord, file = ".../analyses/HMMsearch/R_data/max_ord.rds")
ord <- readRDS(file = ".../analyses/HMMsearch/R_data/max_ord.rds")
```

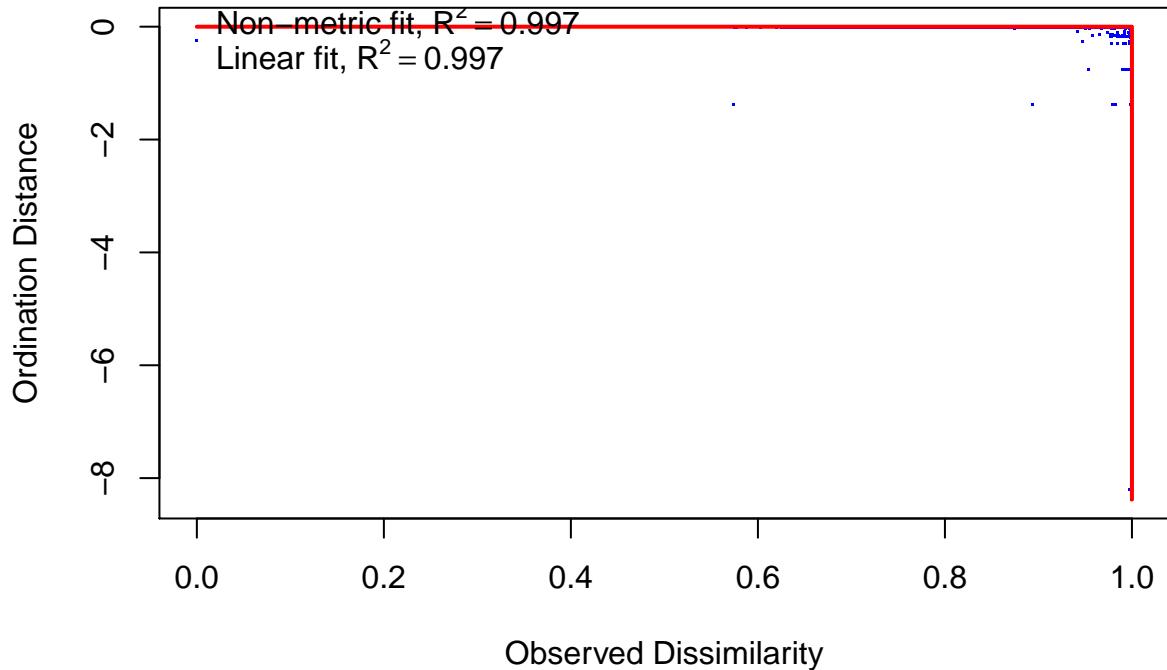
How well does the 2-D representation of multidimensional space represent the actual multidimensional space is done by using statistic called stress. Stress < 0.1 indicates that the 2-D representation is a good representation of the multi-D space. Let's check that out now:

```
ord$stress
```

```
## [1] 0.05091335
```

This is quite low which is great! Let's now plot out the relationship between the ordination dissimilarities and the distances in the ordination space:

```
stressplot(ord)
```

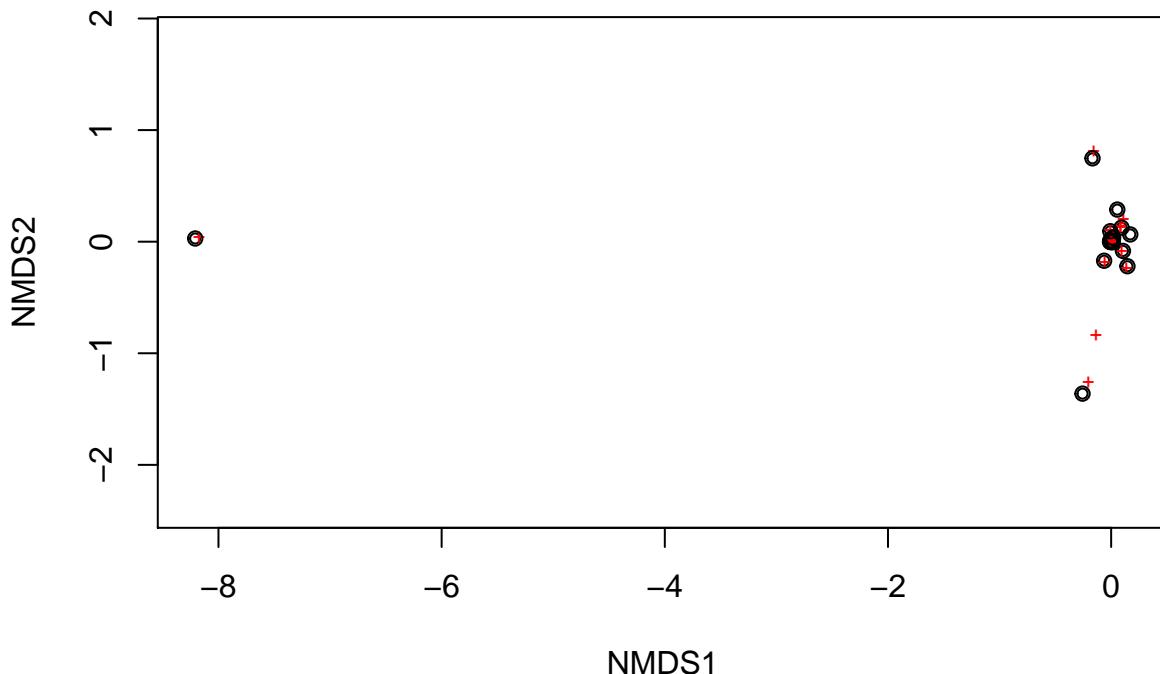


Observed dissimilarity of 1 means the fOTU doesn't have any HMMs incommon with the others.

Let's plot out the ordination space in 2-d:

```
file_name <- ".../visualisations/HMMsearch/nmds_ordiplot_all_data.png"
png(filename = file_name)
ordiplot(ord)
points(ord)
dev.off()

## pdf
## 2
ordiplot(ord)
points(ord)
```



8 Remove outlier(s)

These are just removed so that it's easier to see the better the whole data.

```
NMDS_points <- as_tibble(ord$points) %>%
  filter(MDS1 > -2)
```

9 Rerun NMDS with outliers removed

Let's perform non-metric multidimensional scaling again:

```
width <- dim(NMDS_points)[2]
ord <- metaMDS(NMDS_points[,2:width],)
```

10 Save/Load R data

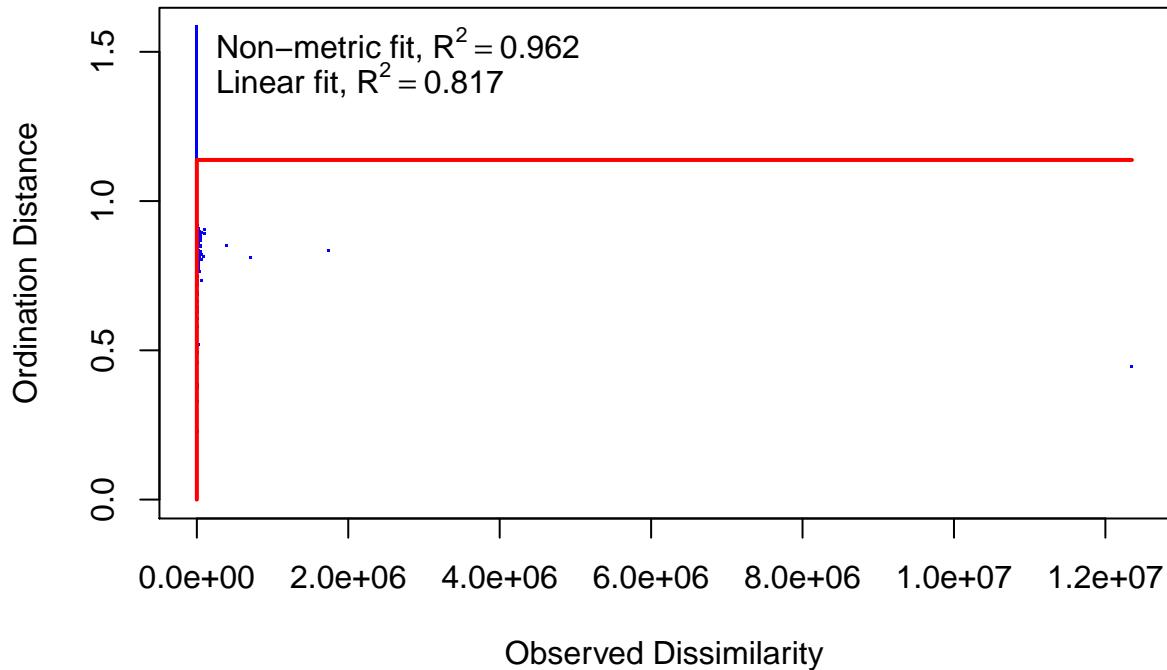
Save the data once again to speed-up things.

```
#saveRDS(ord, file = "../analyses/HMMsearch/R_data/max_ord_outlier_filtered.rds")
ord <- readRDS(file = "../analyses/HMMsearch/R_data/max_ord_outlier_filtered.rds")
```

How well does the 2-D representation of multidimensional space represent the actual multidimensional space is done by using statistic called stress. Stress < 0.1 indicates that the 2-D representation is a good representation of the multi-D space. This time the stress value was: 0.1949408 indicating that it wasn't super good.

Here is the stress plot for this new NMDS:

```
stressplot(ord)
```



```

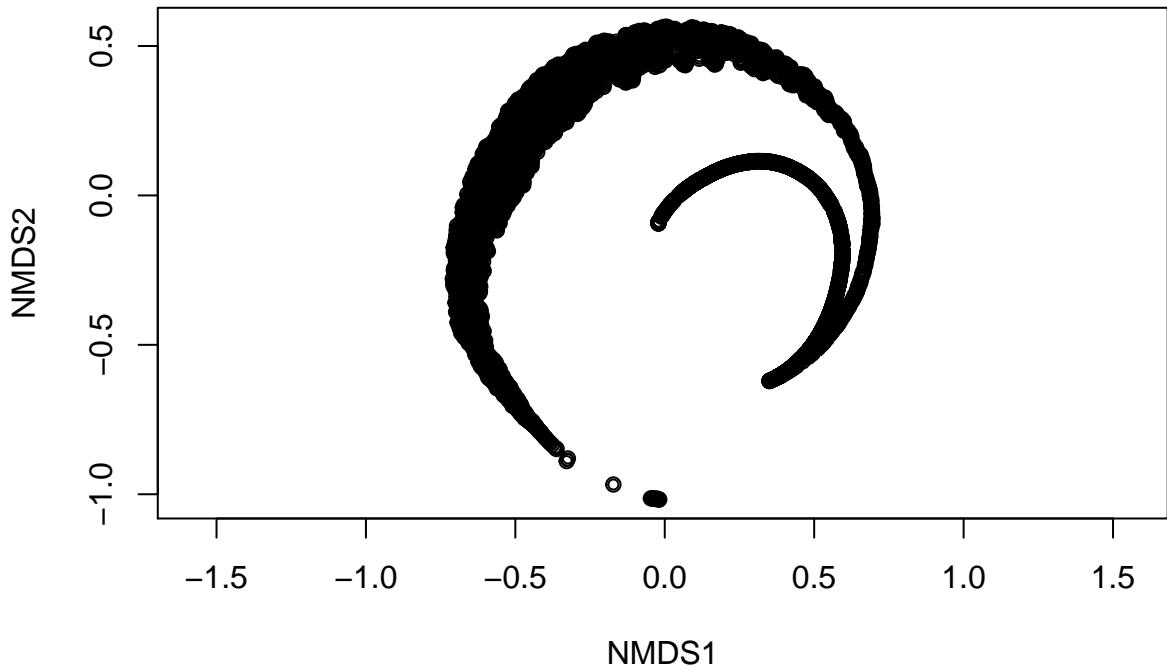
file_name <- ".../visualisations/HMMsearch/nmds_ordiplot_all_data_outlier_filtered.png"
png(filename = file_name)
ordiplot(ord)

## species scores not available
points(ord)
dev.off()

## pdf
## 2
ordiplot(ord)

## species scores not available
points(ord)

```



11 Visualise the filtered data

```

title <- "NMDS, all from MDS1 > -2"
qplot(MDS1,
      MDS2,
      data = NMDS_points,
      geom = c("point", "density2d")) + ggtitle(title) +
      theme(plot.title = element_text(hjust = 0.5, size = 18),
            axis.title=element_text(size=14))

path <- "../visualisations/HMMsearch/nmds_hmmsearch_max_MDS1_greater_than_-2.png"

ggsave(filename = path,
       device = png,
       width = 1000,
       height = 750,
       units = "in",
       limitsize = FALSE,
       dpi = 320)

# Let's zoom in to the main cluster
zoom <- coord_cartesian(xlim = c(-0.02,0.02))

title <- "NMDS, -0.02 < MDS1 < 0.02"
qplot(MDS1,
      MDS2,
      data = NMDS_points,
      geom = c("point", "density2d")) + ggtitle(title) + zoom +
      theme(plot.title = element_text(hjust = 0.5, size = 18),
            axis.title=element_text(size=14))

```

```

path <- "../visualisations/HMMsearch/nmds_hmmsearch_max_MDS1_greater_than_-0_02_and_less_than_0_02.png"

ggsave(filename = path,
       device = png,
       width = 1000,
       height = 750,
       units = "in",
       limitsize = FALSE,
       dpi = 320)

```

12 Create lists of fOTUs of interest

12.1 PCA lists

Based on the previewing of PCA plots in 5.1 we can now manually curate a lists of fOTUs approximately based on clustering in them.

Let's first create file names:

```

# Path to files
path <- "../analyses/HMMsearch/lists_of_PCA_clusters/"

# Names of files
PC12a_filename <- paste(path,
                         "fOTU_max_PC1-and-2_x--100-to-0-and-y--50-to-+50.txt",
                         sep = "",
                         collapse = NULL)
PC12b_filename <- paste(path,
                         "fOTU_max_PC1-and-2_x-+200-to-+500-and-y--100-to-0.txt",
                         sep = "",
                         collapse = NULL)

PC23a_filename <- paste(path,
                         "fOTU_max_PC2-and-3_x--100-to-+75-and-y--150-to-+80.txt",
                         sep = "",
                         collapse = NULL)
PC23b_filename <- paste(path,
                         "fOTU_max_PC2-and-3_x-+100-to-+250-and-y--80-to-+125.txt",
                         sep = "",
                         collapse = NULL)
PC23c_filename <- paste(path,
                         "fOTU_max_PC2-and-3_x-+250-to-+425-and-y--200-to-0.txt",
                         sep = "",
                         collapse = NULL)
PC23d_filename <- paste(path,
                         "fOTU_max_PC2-and-3_x-+400-to-+650-and-y--210-to--80.txt",
                         sep = "",
                         collapse = NULL)
PC23e_filename <- paste(path,
                         "fOTU_max_PC2-and-3_x-0-to-+200-and-y-+200-to-+450.txt",
                         sep = "",
                         collapse = NULL)

```

```

PC34a_filename <- paste(path,
                        "fOTU_max_PC3-and-4_x--80-to-+80-and-y--50-to-+75.txt",
                        sep = "",
                        collapse = NULL)
PC34b_filename <- paste(path,
                        "fOTU_max_PC3-and-4_x--50-to-0-and-y-+75-to-+175.txt",
                        sep = "",
                        collapse = NULL)
PC34c_filename <- paste(path,
                        "fOTU_max_PC3-and-4_x-+200-to-+450-and-y--25-to-+50.txt",
                        sep = "",
                        collapse = NULL)

```

and then we'll filter tibbles based on their coordinates with a simple function:

```

# This function filters a tibble based on given limit values
filterator <- function(data,
                        x_low = -9999,
                        x_high = 9999,
                        y_low = -9999,
                        y_high = 9999){

  data %>%
    filter(data[,2] > x_low & data[,2] < x_high & data[,3] > y_low & data[,3] < y_high) #%>%
    #select(1)
}

```

and the actual filtering:

```

PC12_cluster1 <- filterator(PC12s,-100,0,-50,50)
PC12_cluster2 <- filterator(PC12s,200,500,-100,0)

PC23_cluster1 <- filterator(PC23s,-100,75,-150,80)
PC23_cluster2 <- filterator(PC23s,100,250,-80,125)
PC23_cluster3 <- filterator(PC23s,250,425,-200,0)
PC23_cluster4 <- filterator(PC23s,400,650,-210,80)
PC23_cluster5 <- filterator(PC23s,0,200,200,450)

PC34_cluster1 <- filterator(PC34s,-80,80,-50,75)
PC34_cluster2 <- filterator(PC34s,-50,0,75,175)
PC34_cluster3 <- filterator(PC34s,200,450,-25,50)

```

Lastly, we'll just write the files:

```

# PC1-PC2 manually curated clusters
write_delim(PC12_cluster1,
            PC12a_filename,
            delim = "",
            na = "NA",
            append = FALSE,
            col_names = FALSE,
            quote_escape = "double")
write_delim(PC12_cluster2,
            PC12b_filename,
            delim = "",
            na = "NA",

```

```

append = FALSE,
col_names = FALSE,
quote_escape = "double")

# PC2-PC3 manually curated clusters
write_delim(PC23_cluster1,
PC23a_filename,
delim = "",
na = "NA",
append = FALSE,
col_names = FALSE,
quote_escape = "double")
write_delim(PC23_cluster2,
PC23b_filename,
delim = "",
na = "NA",
append = FALSE,
col_names = FALSE,
quote_escape = "double")
write_delim(PC23_cluster3,
PC23c_filename,
delim = "",
na = "NA",
append = FALSE,
col_names = FALSE,
quote_escape = "double")
write_delim(PC23_cluster4,
PC23d_filename,
delim = "",
na = "NA",
append = FALSE,
col_names = FALSE,
quote_escape = "double")
write_delim(PC23_cluster5,
PC23e_filename,
delim = "",
na = "NA",
append = FALSE,
col_names = FALSE,
quote_escape = "double")

# PC3-PC4 manually curated clusters
write_delim(PC34_cluster1,
PC34a_filename,
delim = "",
na = "NA",
append = FALSE,
col_names = FALSE,
quote_escape = "double")
write_delim(PC34_cluster2,
PC34b_filename,
delim = "",
na = "NA",

```

```

    append = FALSE,
    col_names = FALSE,
    quote_escape = "double")
write_delim(PC34_cluster3,
            PC34c_filename,
            delim = "",
            na = "NA",
            append = FALSE,
            col_names = FALSE,
            quote_escape = "double")

```

12.2 NMDS lists

Based on the previewing of NMDS plots in 11 we can now manually curate a lists of fOTUs approximately based on various putative clusters in them.

Let's first create file names for the putative clusters:

```

# Path to files
path <- "../analyses/HMMsearch/lists_of_clusters/"

# Names of files
NMDSa_filename <- paste(path,
                         "fOTU_max_NMDS_x--10-to--7.txt",
                         sep = "",
                         collapse = NULL)
NMDSb_filename <- paste(path,
                         "fOTU_max_NMDS_x--1-to--0_2.txt",
                         sep = "",
                         collapse = NULL)
NMDSc_filename <- paste(path,
                         "fOTU_max_NMDS_x--0_2-to--0.05txt",
                         sep = "",
                         collapse = NULL)
NMDSd_filename <- paste(path,
                         "fOTU_max_NMDS_x--0_02-to-0.txt",
                         sep = "",
                         collapse = NULL)
NMDSe_filename <- paste(path,
                         "fOTU_max_NMDS_x-0-to-+0_01.txt",
                         sep = "",
                         collapse = NULL)
NMDSf_filename <- paste(path,
                         "fOTU_max_NMDS_x-+0_01-to-+0_02.txt",
                         sep = "",
                         collapse = NULL)
NMDSg_filename <- paste(path,
                         "fOTU_max_NMDS_x-+0_05-to-+9999.txt",
                         sep = "",
                         collapse = NULL)

```

Now we need to have a data frame with 3 columns, first of which contains the fOTU names. This so because then the already made function `filterator`'s indexing is correct.

```

unfiltered <- as_tibble(ord$points)
# Prepend the fOTU_names before first column
unfiltered <- cbind(select(fOTUsHMM, 1),
                     unfiltered)

```

and execute the actual filtering:

```

# The outlier
NMDS_cluster1 <- filterator(unfiltered,
                             x_low = -10,
                             x_high = -7)

# And the rest
NMDS_cluster2 <- filterator(unfiltered,
                             x_low = -1,
                             x_high = -0.2)
NMDS_cluster3 <- filterator(unfiltered,
                             x_low = -0.2,
                             x_high = -0.05)
NMDS_cluster4 <- filterator(unfiltered,
                             x_low = -0.02,
                             x_high = 0)
NMDS_cluster5 <- filterator(unfiltered,
                             x_low = 0,
                             x_high = 0.01)
NMDS_cluster6 <- filterator(unfiltered,
                             x_low = 0.01,
                             x_high = 0.02)
NMDS_cluster7 <- filterator(unfiltered,
                             x_low = 0.05)

```

Lastly, we'll just write the cluster list files:

```

write_delim(NMDS_cluster1,
            NMDSa_filename,
            delim = "",
            na = "NA",
            append = FALSE,
            col_names = FALSE,
            quote_escape = "double")
write_delim(NMDS_cluster2,
            NMDSb_filename,
            delim = "",
            na = "NA",
            append = FALSE,
            col_names = FALSE,
            quote_escape = "double")
write_delim(NMDS_cluster3,
            NMDSc_filename,
            delim = "",
            na = "NA",
            append = FALSE,
            col_names = FALSE,
            quote_escape = "double")
write_delim(NMDS_cluster4,
            NMDSd_filename,
            
```

```

    delim = "",
    na = "NA",
    append = FALSE,
    col_names = FALSE,
    quote_escape = "double")
write_delim(NMDS_cluster5,
            NMDSe_filename,
            delim = "",
            na = "NA",
            append = FALSE,
            col_names = FALSE,
            quote_escape = "double")
write_delim(NMDS_cluster6,
            NMDSf_filename,
            delim = "",
            na = "NA",
            append = FALSE,
            col_names = FALSE,
            quote_escape = "double")
write_delim(NMDS_cluster7,
            NMDSg_filename,
            delim = "",
            na = "NA",
            append = FALSE,
            col_names = FALSE,
            quote_escape = "double")

```

13 Session info

```

sessionInfo()

## R version 3.6.1 (2019-07-05)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Debian GNU/Linux 9 (stretch)
##
## Matrix products: default
## BLAS/LAPACK: /usr/lib/libopenblas-r0.2.19.so
##
## locale:
## [1] LC_CTYPE=C.UTF-8          LC_NUMERIC=C           LC_TIME=C.UTF-8
## [4] LC_COLLATE=C.UTF-8        LC_MONETARY=C.UTF-8   LC_MESSAGES=C
## [7] LC_PAPER=C.UTF-8         LC_NAME=C             LC_ADDRESS=C
## [10] LC_TELEPHONE=C          LC_MEASUREMENT=C.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] grid      stats     graphics grDevices utils     datasets  methods
## [8] base
##
## other attached packages:
## [1] vegan_2.5-6    lattice_0.20-38  permute_0.9-5   ggbiplot_0.55
## [5] scales_1.0.0   plyr_1.8.4    forcats_0.4.0   stringr_1.4.0
## [9] dplyr_0.8.3    purrr_0.3.2    readr_1.3.1    tidyverse_1.2.1
## [13] tibble_2.1.3   ggplot2_3.2.1   tidyverse_1.2.1

```

```

##
## loaded via a namespace (and not attached):
## [1] tidyselect_0.2.5 xfun_0.9      splines_3.6.1   haven_2.1.1
## [5] colorspace_1.4-1 generics_0.0.2 vctrs_0.2.0     htmltools_0.3.6
## [9] mgcv_1.8-28    yaml_2.2.0     rlang_0.4.0     pillar_1.4.2
## [13] glue_1.3.1     withr_2.1.2     modelr_0.1.5   readxl_1.3.1
## [17] munsell_0.5.0  gtable_0.3.0   cellranger_1.1.0 rvest_0.3.4
## [21] evaluate_0.14 labeling_0.3   knitr_1.24     parallel_3.6.1
## [25] broom_0.5.2    Rcpp_1.0.2     backports_1.1.4 jsonlite_1.6
## [29] hms_0.5.1      digest_0.6.20  stringi_1.4.3   bookdown_0.13
## [33] cli_1.1.0      tools_3.6.1   magrittr_1.5   lazyeval_0.2.2
## [37] cluster_2.1.0  crayon_1.3.4  pkgconfig_2.0.2 zeallot_0.1.0
## [41] Matrix_1.2-17  MASS_7.3-51.4 xml2_1.2.2     lubridate_1.7.4
## [45] assertthat_0.2.1 rmarkdown_1.15 httr_1.4.1     rstudioapi_0.10
## [49] R6_2.4.0       nlme_3.1-140  compiler_3.6.1

```

14 References