

HMM search output analyses

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

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2020-01-20

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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

But before starting to draw any figures, let's define some plot variables which will be used for all the plots.

```
plot_title_justification <- 0.5  
figure_width      <- 1000  
figure_height     <- 750  
plot_title_size   <- 22  
axis_title_size   <- 18  
axis_text_size    <- 16  
plot_resolution   <- 320  
legend_text_size  <- 16  
legend_title_size <- 18
```

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

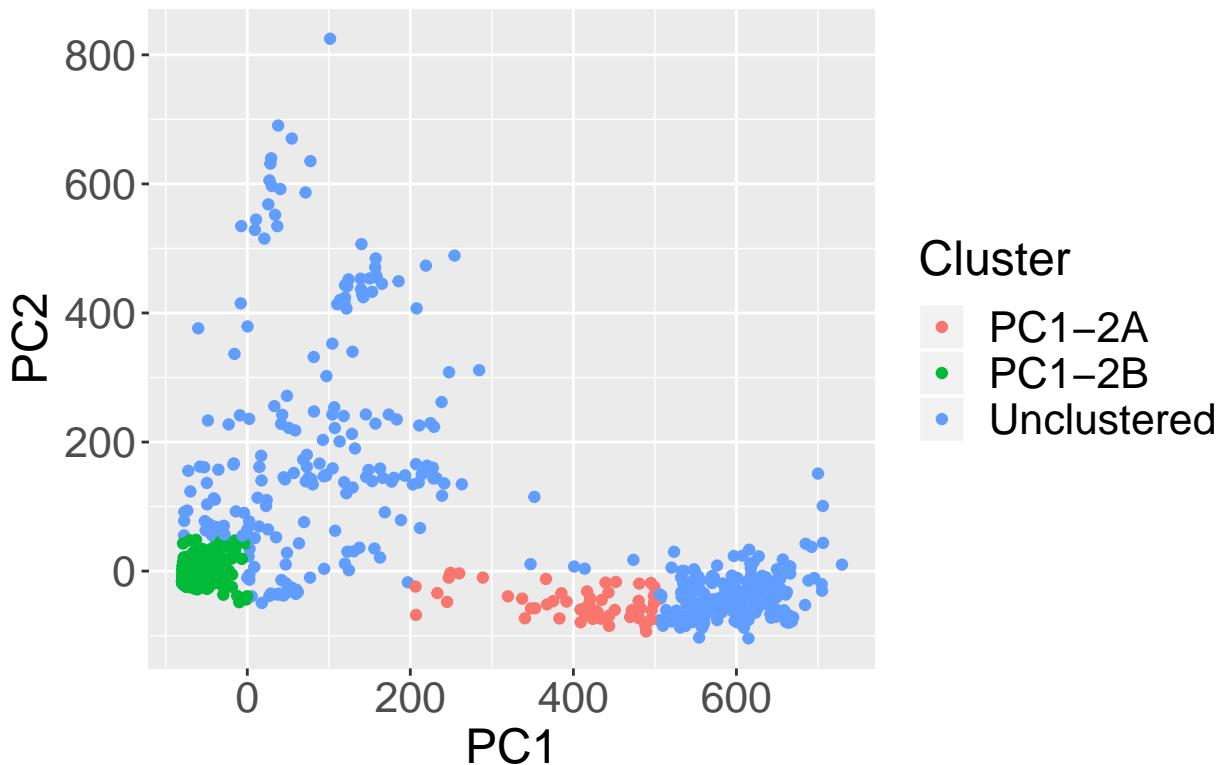
```
PC12s <- tibble(fOTUs = names(fOTUsHMM_pca$x[,1]),  
                 PC1 = unname(fOTUsHMM_pca$x[,1]),  
                 PC2 = unname(fOTUsHMM_pca$x[,2]),  
                 ) %>%  
  mutate(Cluster = case_when(PC1 >= -100 & PC1 <= 0 & PC2 >= -50 & PC2 <= 50 ~ 'PC1-2B',  
                             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) + ggtitle(title) +
theme(plot.title = element_text(hjust = plot_title_justification,
                                size = plot_title_size),
      axis.title=element_text( size = axis_title_size)) +
theme(legend.text=element_text( size = legend_text_size)) +
theme(legend.title=element_text(size = legend_title_size)) +
theme(axis.text=element_text( size = axis_text_size))

```

PCA of e-values of matches in fOTUs



```

ggsave(filename = "../visualisations/HMMsearch/pca_hmmsearch_max_PC1-2.png",
       device = png,
       width = figure_width,
       height = figure_height,
       units = "in",
       limitsize = FALSE,
       dpi = plot_resolution)

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')) %>%

```

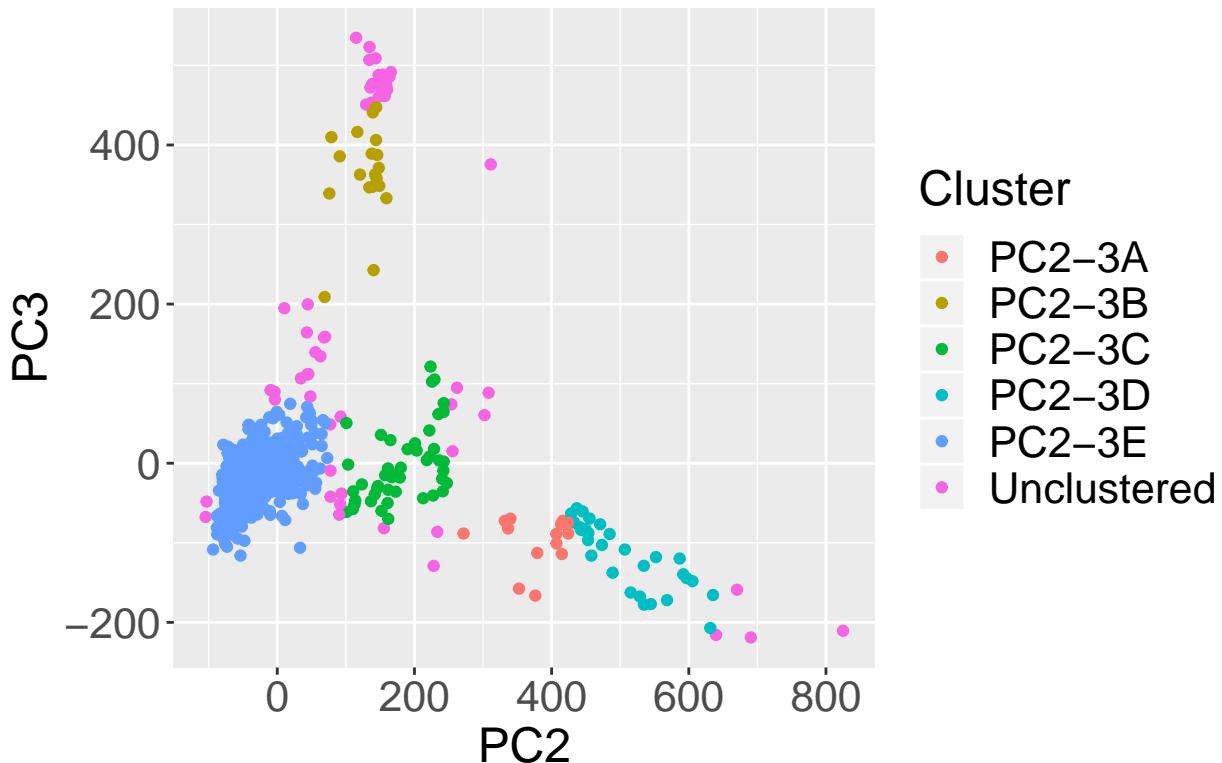
```

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) + ggttitle(title) +
theme(plot.title = element_text(hjust = plot_title_justification,
                                size = plot_title_size),
      axis.title=element_text( size = axis_title_size)) +
theme(legend.text=element_text( size = legend_text_size)) +
theme(legend.title=element_text(size = legend_title_size)) +
theme(axis.text=element_text( size = axis_text_size))

```

PCA of e–values of matches in fOTUs



```

ggsave(filename = "../visualisations/HMMsearch/pca_hmmsearch_max_PC2-3.png",
       device = png,
       width = figure_width,
       height = figure_height,
       units = "in",
       limitsize = FALSE,
       dpi = plot_resolution)

PC34s <- tibble(fOTUs = names(fOTUsHMM_pca$x[,1]),
                 PC3 = unname(fOTUsHMM_pca$x[,3]),

```

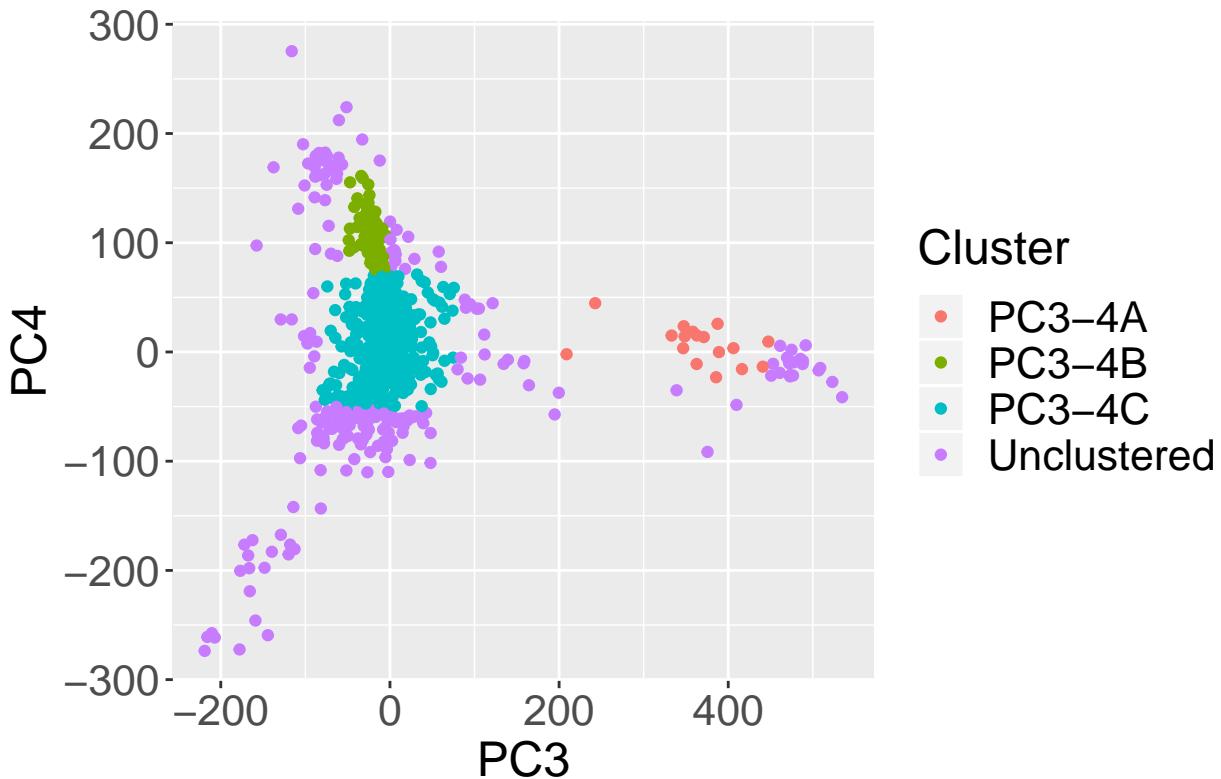
```

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 = plot_title_justification,
                                  size = plot_title_size),
        axis.title=element_text( size = axis_title_size)) +
  theme(legend.text=element_text( size = legend_text_size)) +
  theme(legend.title=element_text(size = legend_title_size)) +
  theme(axis.text=element_text( size = axis_text_size))

```

PCA of e-values of matches in fOTUs



```

ggsave(filename = "../visualisations/HMMsearch/pca_hmmsearch_max_PC3-4.png",
       device = png,
       width = figure_width,
       height = figure_height,
       units = "in",

```

```

    limitsize = FALSE,
    dpi = plot_resolution)

```

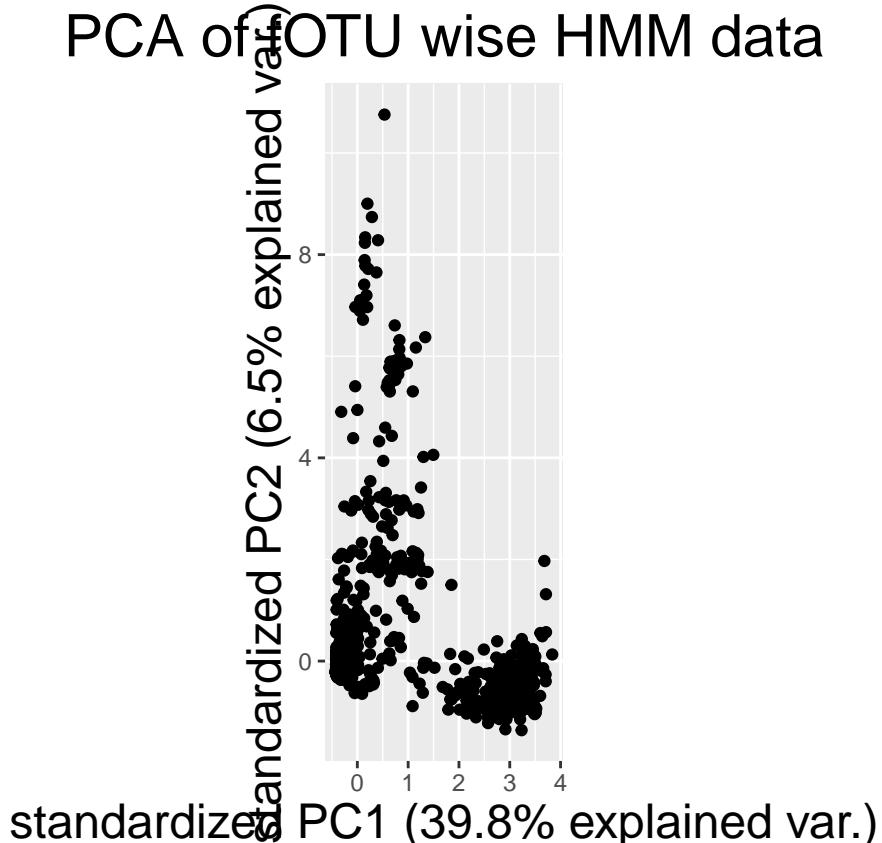
These PCA plots can be used to visually determine potential groupings.

```

file_name <- "../visualisations/HMMsearch/ggbiplot_max_PC1-2.png"
png(filename = file_name,
    width = figure_width,
    height = figure_height)
ggbiplot(fOTUsHMM_pca,
    var.axes = F,
    choices = 1:2) +
  ggtitle("PCA of fOTU wise HMM data") +
  theme(plot.title = element_text(hjust = plot_title_justification,
                                  size = plot_title_size),
        axis.title=element_text(size = axis_title_size))
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 = plot_title_justification,
                                  size = plot_title_size),
        axis.title=element_text(size = axis_title_size))

```



5.2 Heatmaps

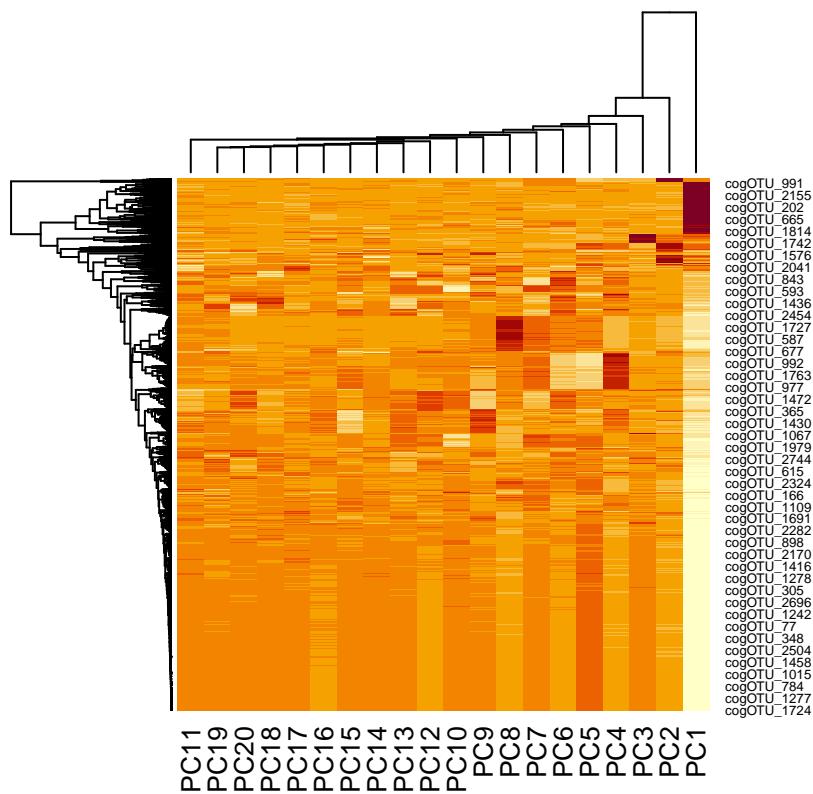
```
dim(fOTUsHMM_pca)

## 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

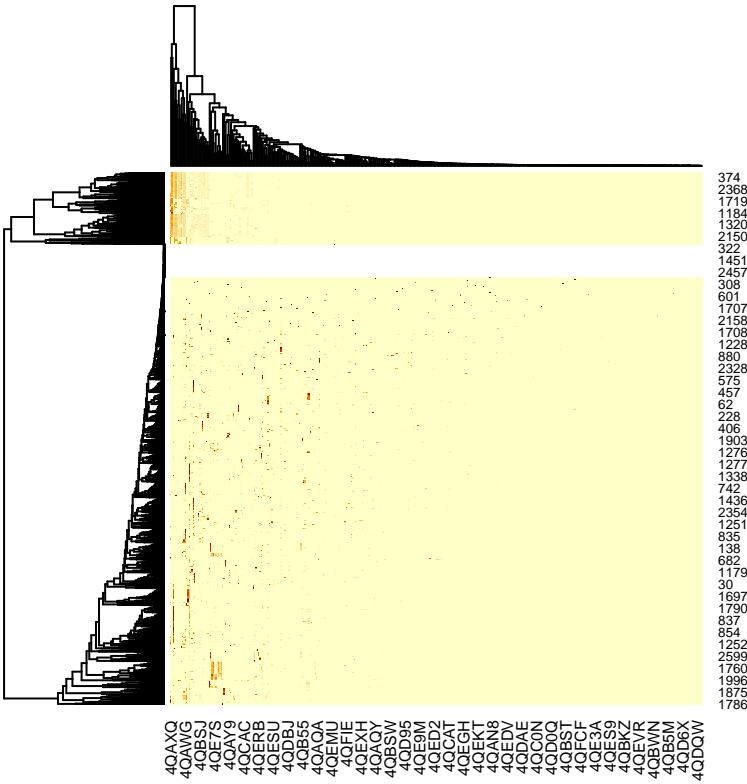
heatmap(as.matrix(prediction))
```



```
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")
```

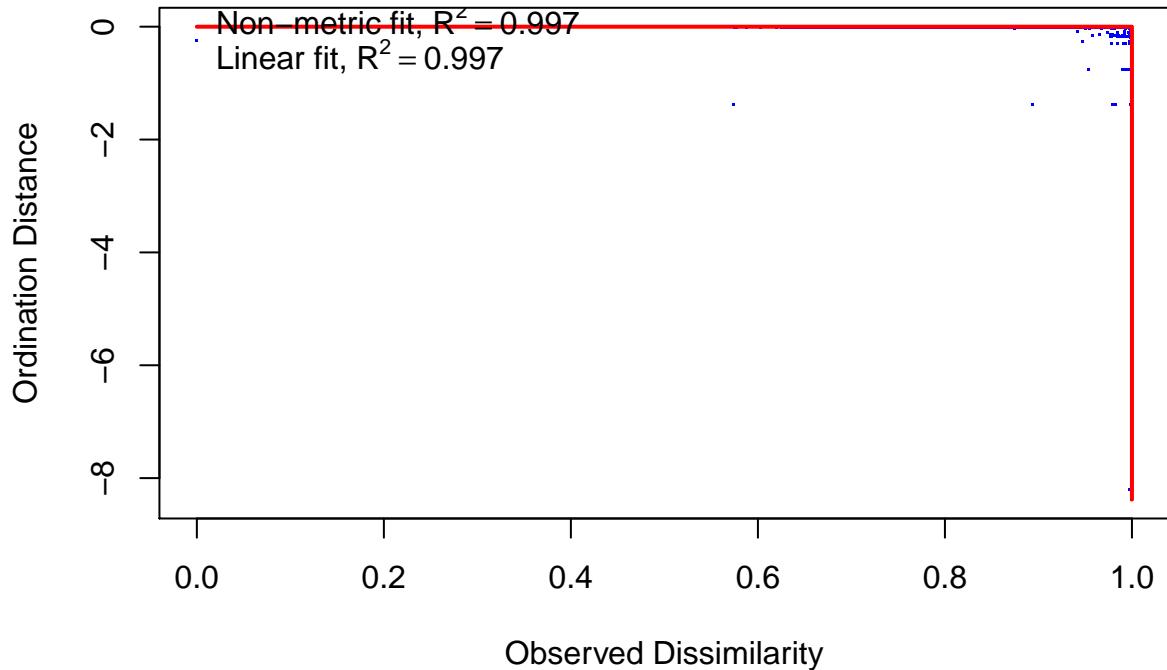
How well does the 2-D representation of multidimensional space represent the actual multidimensional space is done by using statistic called stress. Stress < 0.1 indicates that the 2-D representation is a good representation of the multi-D space. Let's check that out now:

```
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)
```

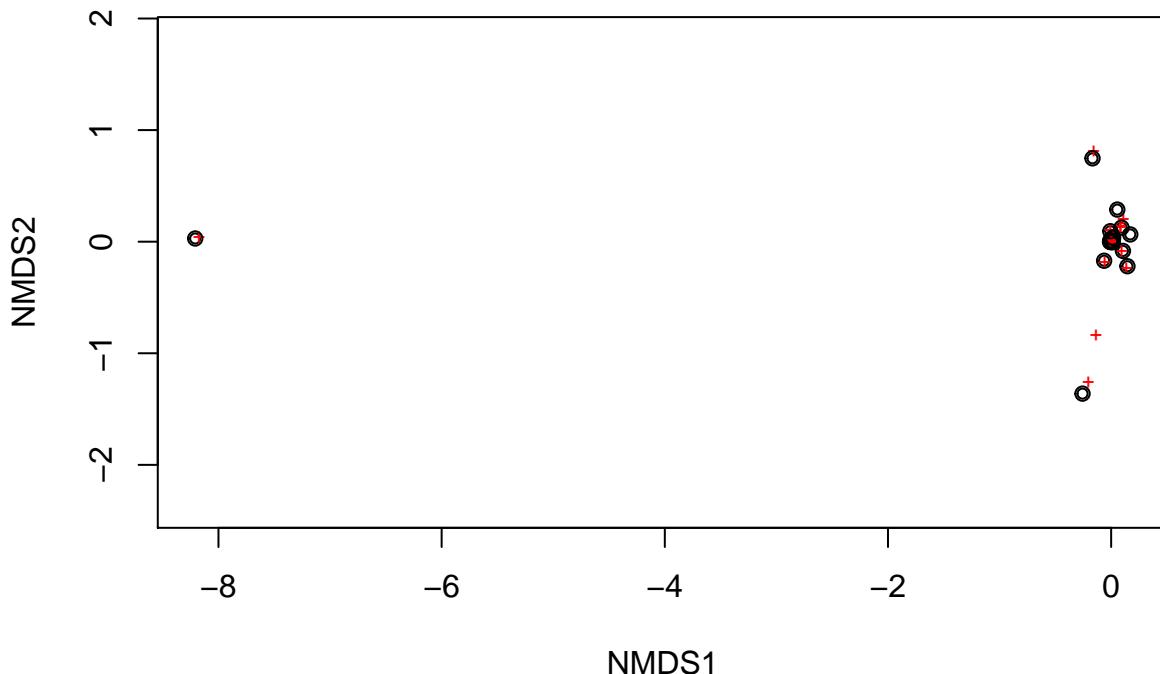


Observed dissimilarity of 1 means the fOTU doesn't have any HMMs incommon with the others.

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 Find and filter out the outlier(s)

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

```
NMDS_points <- fOTUsHMM[which(as_tibble(ord$points)$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

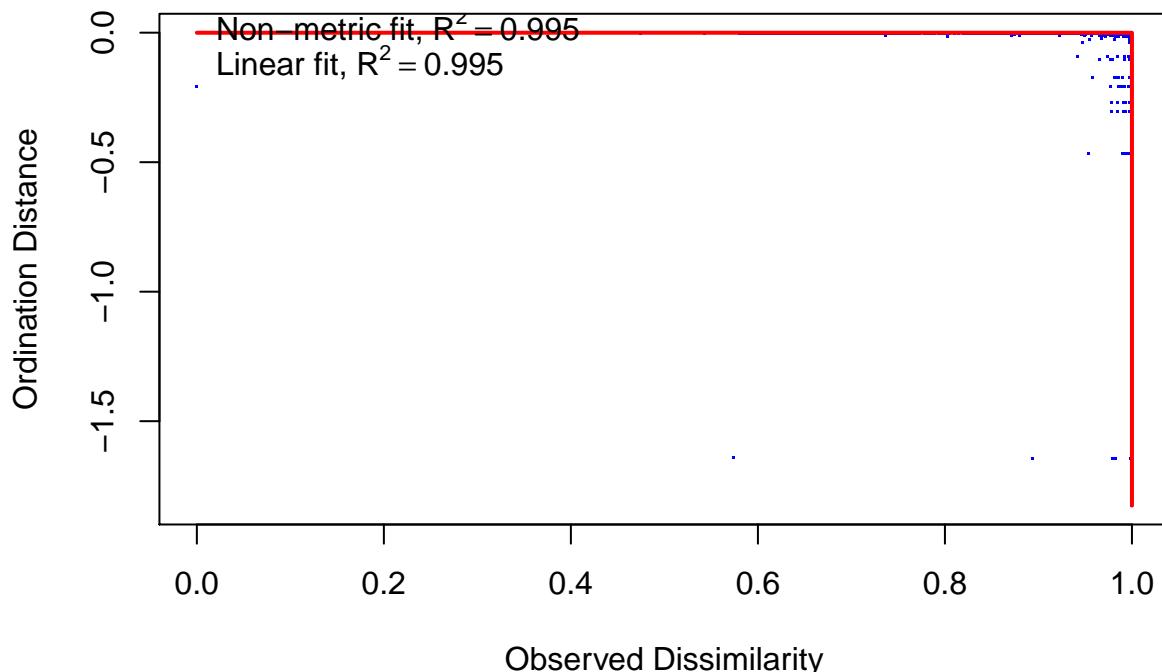
Save the data once again to speed-up things.

```
#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: 0.0674991 indicating that it wasn't super good.

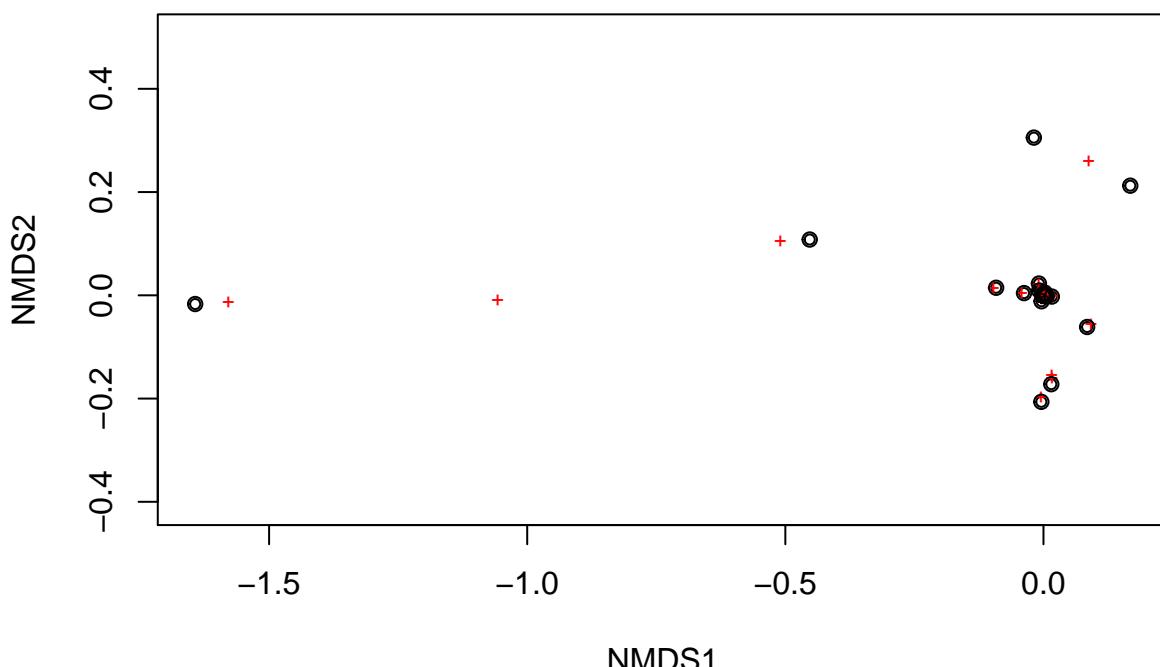
Here is the stress plot for this new NMDS:

```
stressplot(ord)
```



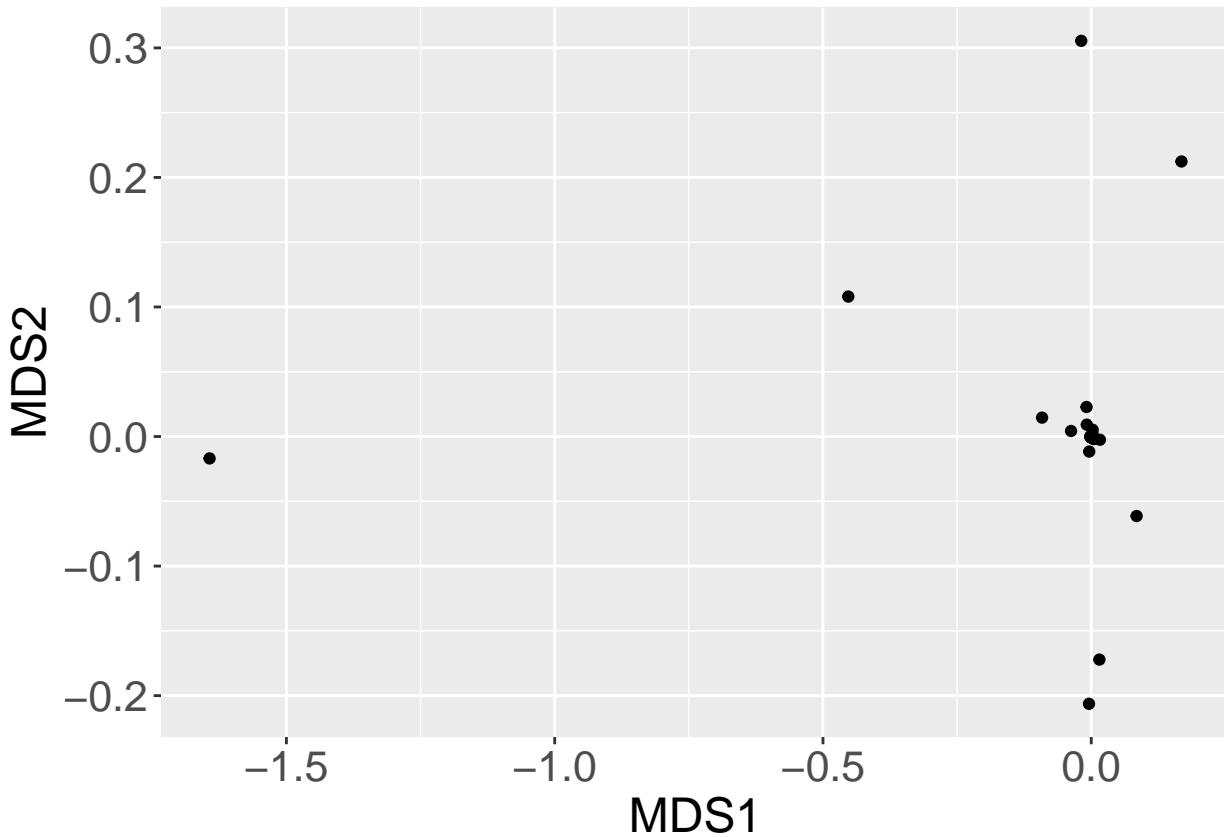
```
file_name <- ".../visualisations/HMMsearch/nmds_ordiplot_all_data_outlier_filtered.png"
png(filename = file_name)
ordiplot(ord)
points(ord)
dev.off()

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



11 Make a nicer figure of NMDS plot

```
NMDS_points <- as_tibble(ord[["points"]])  
  
qplot(MDS1,  
      MDS2,  
      data = NMDS_points,  
      geom = c("point")) +  
  theme(plot.title = element_text(hjust = plot_title_justification,  
                                  size   = plot_title_size),  
        axis.title=element_text( size   = axis_title_size)) +  
  theme(axis.text=element_text( size   = axis_text_size))
```



```
path <- "../visualisations/HMMsearch/nmds_hmmsearch_max_all_data.png"  
ggsave(filename = path,  
       device = png,  
       width = figure_width,  
       height = figure_height,  
       units = "in",  
       limitsize = FALSE,  
       dpi = plot_resolution)
```

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 ?? 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--0.05.txt",
                         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")

```

```

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    purrrr_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