Microbial Biogeography of Restroom Surfaces

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Setup

Load packages.

```
library(plyr)
library(dplyr)
```

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

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

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

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

```
library(tidyr)
library(tibble)
library(ggplot2)
library(ggdendro)
library(RColorBrewer)
```

Set a general *ggplot2* theme to use throughout.

```
th <- theme_classic() +
    theme(panel.grid.major=element_line(size=0.5, linetype="solid", colour="#eeeeee"),
    panel.grid.minor=element_line(size=0.25, linetype="solid", colour="#eeeeee"))
theme_set(th)
```

Read data and prepare data

Read the OTU table and cast counts to numeric, since cor() and others require numeric data.

```
# read otu table and cast counts to numeric, cor() and others require numeric data
otu <- read.delim("1335.vdb.tab", header=TRUE) %>%
    mutate_if(is.integer, as.numeric)
str(otu)
```

```
## 'data.frame':    4105 obs. of  16 variables:
##  $ OTUNumber            : chr  "Otu00001" "Otu00002" "Otu00003" "Otu00004" ...
```

```
##  $ OTUConTaxonomy       : chr  "Bacteria(100);Actinobacteria(100);Actinobacteria(100);Actinomycetales
##  $ door_in_1            : num  1381 20 8 6 0 ...
##  $ door_in_2            : num  1032 17 47 27 29 ...
##  $ faucet_handle_1      : num  335 1686 6 5 0 ...
##  $ faucet_handle_2      : num  577 3 418 6 648 160 11 31 0 116 ...
##  $ sink_floor_1         : num  38 411 15 452 2 8 119 0 8 5 ...
##  $ sink_floor_2         : num  36 564 8 275 8 8 243 7 5 5 ...
##  $ soap_dispenser_1     : num  1115 128 84 63 3 ...
##  $ stall_in_1           : num  598 9 45 0 32 115 10 225 0 0 ...
##  $ toilet_floor_1       : num  45 430 6 236 1 24 112 4 14 11 ...
##  $ toilet_floor_2       : num  87 707 30 251 5 7 234 3 13 11 ...
##  $ toilet_flush_handle_1: num  13 0 3 9 0 3 4 14 0 2 ...
##  $ toilet_flush_handle_2: num  329 25 283 51 206 130 10 49 17 61 ...
##  $ toilet_seat_1        : num  76 258 41 5 7 32 29 56 32 54 ...
##  $ toilet_seat_2        : num  156 0 1059 3 95 ...
```

Preliminary analysis and data manipulation

First, get the names of phyla and taxa by splitting the *OTUConTaxonomy* column, containing the assigned taxonomy for each OTU.

```
# for each row of the otu table
tax <- apply(otu, 1, function(x)
    # select OTUConTaxonomy column
    x[2] %>%
        # split strings and get the result, returned inside a list
        strsplit(";") %>%
        # subset the list
        "[["(1) %>%
        # remove assignment score from the name
        sub(pattern="\\(.*", replacement="")
)
# transpose so OTUs are again the rows
tax <- t(tax)
str(tax)
```

```
##  chr [1:4105, 1:6] "Bacteria" "Bacteria" "Bacteria" "Bacteria" "Bacteria" ...
```

```
# bind the new phylum and taxon column to the original table
otu <- cbind(otu, data.frame(Genus=tax[,2], Taxon=tax[,6]))
str(otu)
```

```
## 'data.frame':    4105 obs. of  18 variables:
##  $ OTUNumber            : chr  "Otu00001" "Otu00002" "Otu00003" "Otu00004" ...
##  $ OTUConTaxonomy       : chr  "Bacteria(100);Actinobacteria(100);Actinobacteria(100);Actinomycetales
##  $ door_in_1            : num  1381 20 8 6 0 ...
##  $ door_in_2            : num  1032 17 47 27 29 ...
##  $ faucet_handle_1      : num  335 1686 6 5 0 ...
##  $ faucet_handle_2      : num  577 3 418 6 648 160 11 31 0 116 ...
##  $ sink_floor_1         : num  38 411 15 452 2 8 119 0 8 5 ...
##  $ sink_floor_2         : num  36 564 8 275 8 8 243 7 5 5 ...
##  $ soap_dispenser_1     : num  1115 128 84 63 3 ...
##  $ stall_in_1           : num  598 9 45 0 32 115 10 225 0 0 ...
##  $ toilet_floor_1       : num  45 430 6 236 1 24 112 4 14 11 ...
##  $ toilet_floor_2       : num  87 707 30 251 5 7 234 3 13 11 ...
##  $ toilet_flush_handle_1: num  13 0 3 9 0 3 4 14 0 2 ...
```

```
##  $ toilet_flush_handle_2: num  329 25 283 51 206 130 10 49 17 61 ...
##  $ toilet_seat_1        : num  76 258 41 5 7 32 29 56 32 54 ...
##  $ toilet_seat_2        : num  156 0 1059 3 95 ...
##  $ Genus                : chr  "Actinobacteria" "Proteobacteria" "Actinobacteria" "Actinobacteria" .
##  $ Taxon                : chr  "Propionibacterium" "Enhydrobacter" "Corynebacterium" "Arthrobacter"
```

For analysis further on, extract the only the counts from the table, and convert to a matrix: handy for some of the computations.

```
otumat <- otu %>%
    # the counts are the only numeric columns
    select_if(is.numeric) %>%
    as.matrix
str(otumat)
```

```
##  num [1:4105, 1:14] 1381 20 8 6 0 ...
##  - attr(*, "dimnames")=List of 2
##   ..$ : NULL
##   ..$ : chr [1:14] "door_in_1" "door_in_2" "faucet_handle_1" "faucet_handle_2" ...
```

Next, we'll need a the summed counts by taxon across OTUs, and get the top 19 taxons in terms of abundance. The very first element is actually *unclassified*, the unassigned OTUs, and we'll discard it.

```
# this same construct will be used later:
# take the numeric count matrix
taxtotals <- otumat %>%
    # for each of its columns (sites)
    apply(2, function(x)
        # for each unique taxon (rows)
        sapply(unique(otu$Taxon), function(t)
            # sum its abundance
            sum(x[otu$Taxon == t])
        )
    # this returns total abundance per taxon per site
    ) %>%
    # sum abundance across sites => table with total abundance per taxon
    apply(1, sum)
str(taxtotals)
```

```
##  Named num [1:357] 6003 4315 4941 1458 1589 ...
##  - attr(*, "names")= chr [1:357] "Propionibacterium" "Enhydrobacter" "Corynebacterium" "Arthrobacter
```

```
# order taxons by descending abundance
taxtotals <- taxtotals[order(-taxtotals)]
# then extract the top 19 taxons (removing unclassified)
toptax <- taxtotals[2:20]
toptax
```

```
## Propionibacterium   Corynebacterium     Enhydrobacter    Staphylococcus
##              6003              4941              4315              1589
##      Arthrobacter      Sphingomonas     Acinetobacter       Pseudomonas
##              1458              1242              1140               805
##     Aquabacterium      Anaerococcus       Roseomonas     Streptococcus
##               787               674               536               535
##        Finegoldia  Chryseobacterium          Kocuria      Hymenobacter
##               434               382               374               345
##    Friedmanniella  Methylobacterium       Sphingobium
```

```
##                340                305                300
```

Phylum composition by site

We'll first expore phylum composition by site. Using the previous manipulations, we'll first compute a slightly prettier color palette for each phylum, then output a stacked percent barchart, showing relative proportion of each phylum in each site.

Color palette

Here, we use ColorBrewer palettes for good constracts and for categorical data, since those sets have the most colors. We have more phylum (18) than colors in the set (12), so we'll interpolate some additional colors.

```
# number of unique phylum, the number of colors needed
ngenus <- length(unique(otu$Genus))
# extract the colors of the Set1 brewer color palette
# this gives a harmless warning since we request too many colors
pal.g <- brewer.pal(ngenus, "Set1")
```

```
## Warning in brewer.pal(ngenus, "Set1"): n too large, allowed maximum for palette Set1 is 9
## Returning the palette you asked for with that many colors
```

```
# interpolate for the remaining elements
# colorRampPalette returns a function which we use directly to extract our colors
cmap.g <- colorRampPalette(pal.g, bias=1, interpolate="spline")(ngenus)
cmap.g
```

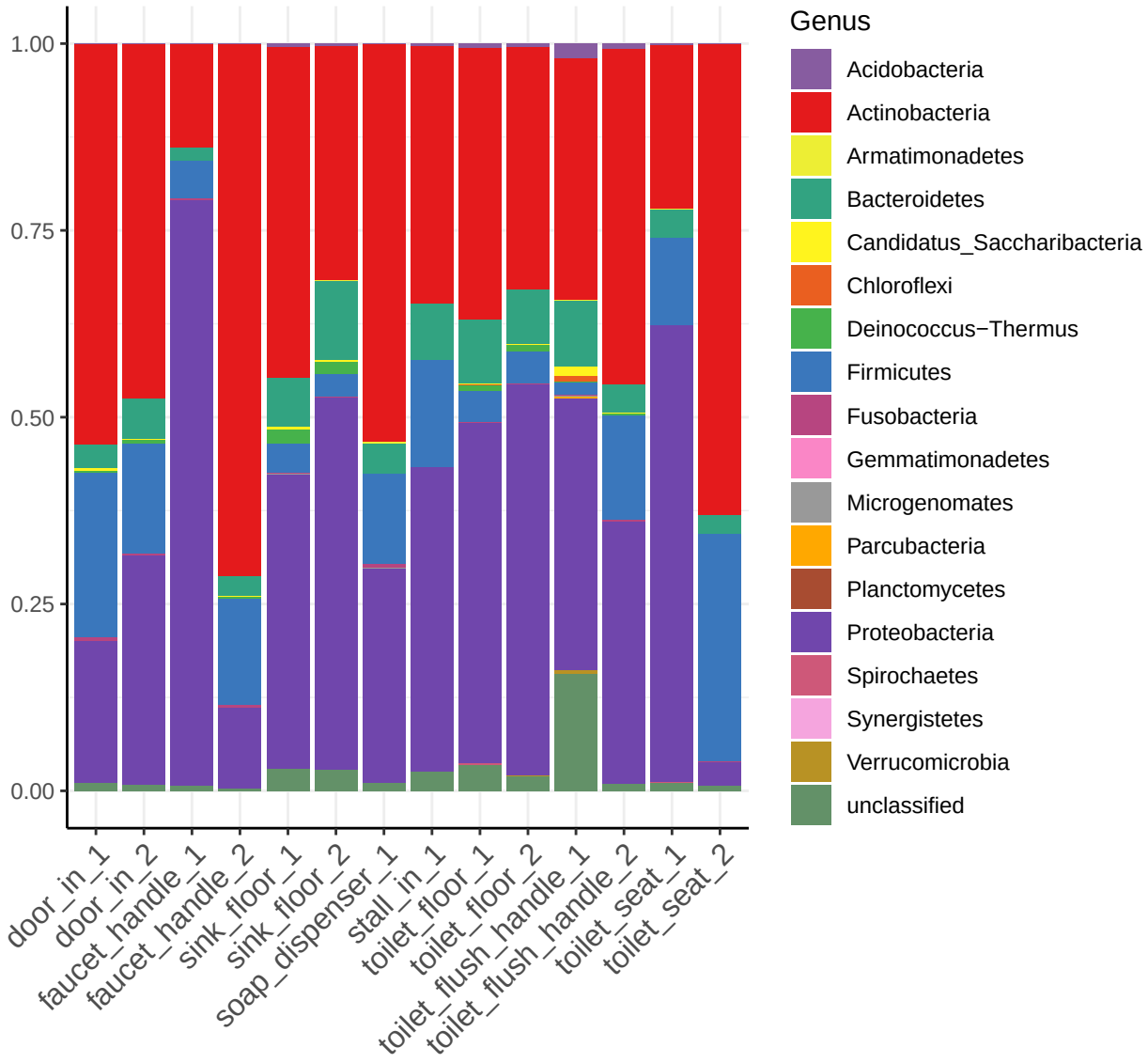
```
##  [1] "#E41A1C" "#7046AC" "#3A77BC" "#32A381" "#46B24B" "#639168" "#875CA0"
##  [8] "#B74580" "#EA5F20" "#FFA800" "#FFF41E" "#EDEE34" "#B89224" "#A84C31"
## [15] "#CE5879" "#FA86C6" "#F5A5DE" "#999999"
```

Plot

We have to transform our OTU table into long format for use with *ggplot2*. Since we don't reuse this result, we'll just pipe it directly to *ggplot()*.

Finally, we save the *ggplot* object to a png file. Rendering to pdf had different results on various machines, as could possibly be seen in this document.

```
# stacked percent barchart
bar.g <- otu %>%
    # get count columns and pivot to long format (similar to melt in reshape2)
    # tidyverse matches() is used to select the columns via a regex
    # site column will contains the sample site, and abundance the abundance values
    pivot_longer(cols=matches("^[a-z]", ignore.case=FALSE), names_to="site", values_to="abundance") %>%
    # x=site, y=abundance and use phylum as group colors for the bar's fill
    ggplot(aes(site, abundance, fill=Genus)) +
        # output percent stacked barchart
        geom_bar(position="fill", stat="identity") +
        # rotate xlab and remove x title
        theme(axis.text.x=element_text(angle=45, vjust=1, size=12, hjust=1),
            axis.title=element_blank()) +
        # manually set the colors for each phylum from the palette
        scale_fill_manual(values=cmap.g[order(unique(otu$Genus))])
bar.g
```




```
# save it
ggsave("plots/stacked.genus.by.site.png", bar.g, width=12, height=10)
```

Taxonomic composition by site

Next, we'll do the same, but this time for taxa. These are more precise and familiar and can provide interesting results.

To make a prettier and more informative plot, there are several complicated steps involved.

To easily view which phylum the taxa belong to, we'd like to group them visually. An intuitive way to do this is to make gradients of one color for each phylum, since there are no more than a few of them, and the taxa are few enough that there still will be enough contrast to distinguish each of them.

To this end, we need to create the color palette manually for each, but in a scriptable and extensible way.

Creating the color palettes

We'll have to build a correspondence table with the top 19 taxa found earlier.

First, we extract the phylum and taxon columns from the taxonomy table computed earlier.

```
# filter the taxonomy table by the names of the top taxa, get phylum (2) and taxon (6) columns
corr <- tax[tax[,6] %in% names(toptax),][,c(2,6)]
# only take unique rows, then convert to data frame and set appropriate column names
corr <- unique(corr) %>%
    as.data.frame %>%
    # "family" seems more appropriate than "genus" here
    rename(Family=V1, Taxon=V2)
corr
```

```
##            Family             Taxon
## 1  Actinobacteria Propionibacterium
## 2  Proteobacteria     Enhydrobacter
## 3  Actinobacteria   Corynebacterium
## 4  Actinobacteria      Arthrobacter
## 5      Firmicutes    Staphylococcus
## 6  Proteobacteria     Acinetobacter
## 7  Proteobacteria     Aquabacterium
## 8  Proteobacteria      Sphingomonas
## 9      Firmicutes        Finegoldia
## 10 Proteobacteria       Pseudomonas
## 11  Bacteroidetes  Chryseobacterium
## 12 Proteobacteria        Roseomonas
## 13 Actinobacteria           Kocuria
## 14     Firmicutes     Streptococcus
## 15 Actinobacteria    Friedmanniella
## 16     Firmicutes      Anaerococcus
## 17 Proteobacteria       Sphingobium
## 18 Proteobacteria  Methylobacterium
## 19  Bacteroidetes      Hymenobacter
```

Next, we use ordered factors to force ordering by phylum, then taxon. This is necessary to preserve this order later since we further manipulate the tables, and have to make sure colors are correctly attributed.

```
# take our correspondence table
corr <- corr %>%
    # convert the family column to an ordered factor according to abundance and importance
    # the levels are from what was obtained above
    mutate(Family=factor(Family,
        levels=c("Proteobacteria", "Actinobacteria", "Firmicutes", "Bacteroidetes"),
        ordered=TRUE)) %>%
    # sort by family then taxon
    arrange(Family, Taxon) %>%
    # now freeze the new taxon order
    mutate(Taxon=factor(Taxon, levels=unique(Taxon), ordered=TRUE))
corr
```

```
##            Family             Taxon
## 1  Proteobacteria     Acinetobacter
## 2  Proteobacteria     Aquabacterium
## 3  Proteobacteria     Enhydrobacter
## 4  Proteobacteria  Methylobacterium
## 5  Proteobacteria       Pseudomonas
## 6  Proteobacteria        Roseomonas
## 7  Proteobacteria       Sphingobium
```

```
## 8  Proteobacteria      Sphingomonas
## 9  Actinobacteria      Arthrobacter
## 10 Actinobacteria   Corynebacterium
## 11 Actinobacteria    Friedmanniella
## 12 Actinobacteria           Kocuria
## 13 Actinobacteria Propionibacterium
## 14     Firmicutes      Anaerococcus
## 15     Firmicutes        Finegoldia
## 16     Firmicutes    Staphylococcus
## 17     Firmicutes     Streptococcus
## 18  Bacteroidetes  Chryseobacterium
## 19  Bacteroidetes      Hymenobacter
```

Finally, we can attribute color gradients for each phylum, making sure each gets a different base color.

```
# add a new column with the colors for the ordering
corr$col <- c(
    # blue, red, green and violet/pink gradients
    # like before, we directly call the function returned with the appropriate number of colors we need
    colorRampPalette(c("lightblue", "darkblue"))(8),
    colorRampPalette(c("#ffff66", "red", "darkred"))(5),
    colorRampPalette(c("lightgreen", "darkgreen"))(4),
    colorRampPalette(c("violet", "blue"))(8)[1:2]
)
corr
```

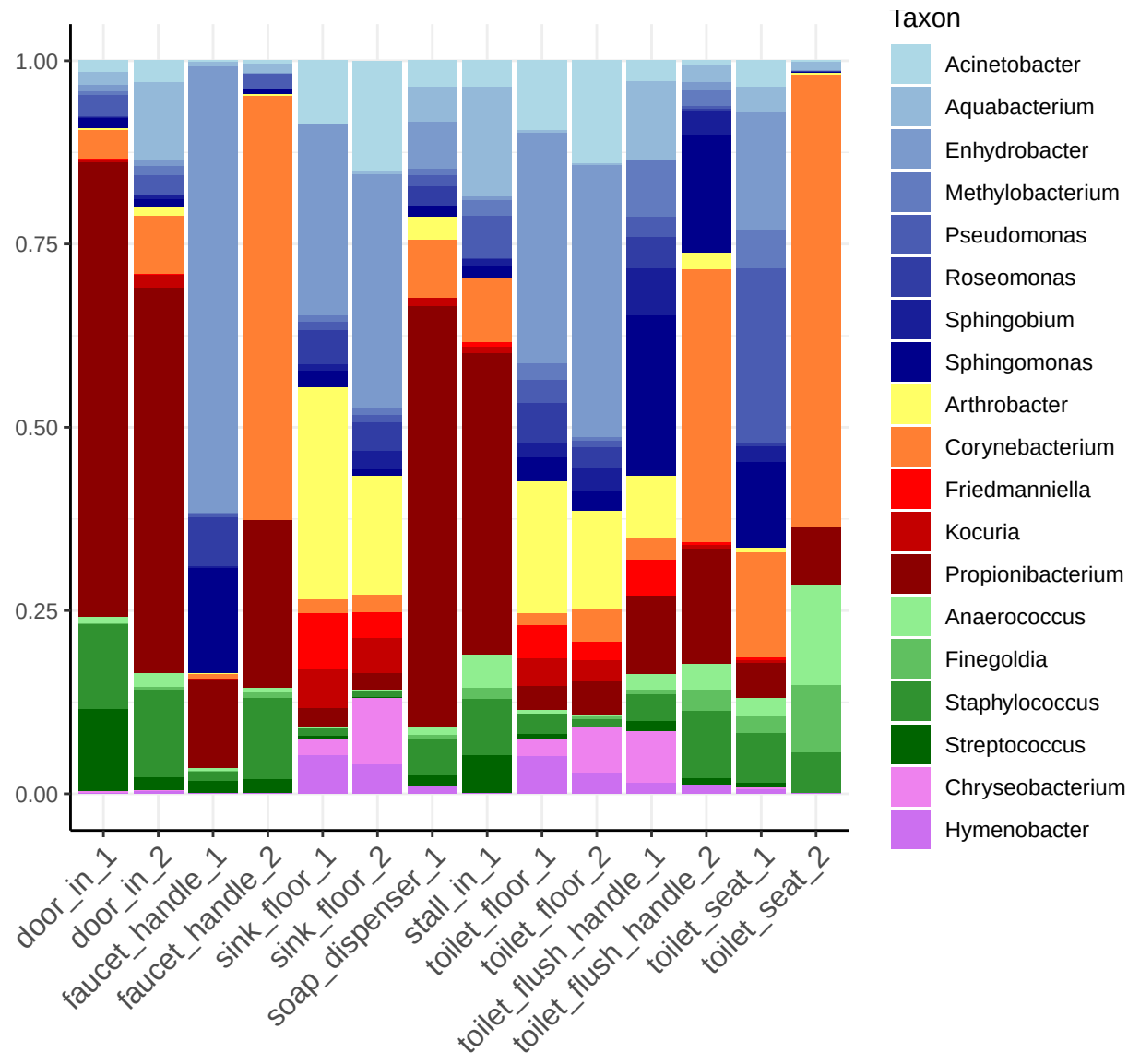
```
##            Family             Taxon     col
## 1  Proteobacteria     Acinetobacter #ADD8E6
## 2  Proteobacteria     Aquabacterium #94B9D9
## 3  Proteobacteria     Enhydrobacter #7B9ACC
## 4  Proteobacteria  Methylobacterium #627BBF
## 5  Proteobacteria       Pseudomonas #4A5CB2
## 6  Proteobacteria        Roseomonas #313DA4
## 7  Proteobacteria       Sphingobium #181E98
## 8  Proteobacteria      Sphingomonas #00008B
## 9  Actinobacteria      Arthrobacter #FFFF66
## 10 Actinobacteria   Corynebacterium #FF7F33
## 11 Actinobacteria    Friedmanniella #FF0000
## 12 Actinobacteria           Kocuria #C40000
## 13 Actinobacteria Propionibacterium #8B0000
## 14     Firmicutes      Anaerococcus #90EE90
## 15     Firmicutes        Finegoldia #60C060
## 16     Firmicutes    Staphylococcus #309230
## 17     Firmicutes     Streptococcus #006400
## 18  Bacteroidetes  Chryseobacterium #EE82EE
## 19  Bacteroidetes      Hymenobacter #CC6FF0
```

Stacked percent barchart

We can now output the final plot using the colors we computed, the same way as before.

```
bar.t <- otu %>%
    # remove columns we don't care about
    select(-Genus, -starts_with("OTU")) %>%
    # extract rows for only top taxa
    filter(Taxon %in% names(toptax)) %>%
```

```
    # convert to long format for ggplot as before
    pivot_longer(cols=matches("^[a-t]", ignore.case=FALSE), names_to="site", values_to="abundance") %>%
    # convert to ordered factor as with the correspondence table, using its order
    # counterintuitive and timeconsuming to figure out, but very important to avoid any discrepancies..
    mutate(Taxon=factor(Taxon, levels=levels(corr$Taxon), ordered=TRUE)) %>%
    ggplot(aes(site, abundance, fill=Taxon)) +
        # stacked percent barchart
        geom_bar(position="fill", stat="identity") +
        # set our colors
        scale_fill_manual(values=corr$col) +
        # rotate x legend, remove x axis label
        theme(axis.text.x=element_text(angle=45, vjust=1, size=12, hjust=1),
            axis.title=element_blank())
bar.t
```




```
# save the plot
ggsave("plots/stacked.taxon.by.site.png", bar.t, width=12, height=10)
```

Phylum correlation map

It is of interest to evaluate links between phylum in our data. This can be done by computing the correlation between phylum, then visualizing it as a sort of heatmap.

Preparing the data

We first need to extract the totals for each phylum by site, since that's what we'll be calculating the correlation matrix on.

```
# use the abundance matrix computed at the beginning, cor() needs numerics
gentotals <- otumat %>%
    # for each site (column)
        apply(2, function(x)
        # for each OTU (row),
        # sum abundances of OTUs assigned to each individual phylum
                sapply(unique(otu$Genus), function(g)
                        sum(x[otu$Genus == g])
                )
        )
head(gentotals)
```

```
##                     door_in_1 door_in_2 faucet_handle_1 faucet_handle_2
## Actinobacteria           1669      1483             434            2224
## Proteobacteria            593       960            2445             340
## Firmicutes                686       457             155             440
## Bacteroidetes             100       170              54              84
## Deinococcus-Thermus        10        20               0              12
## unclassified               30        22              19               6
##                     sink_floor_1 sink_floor_2 soap_dispenser_1 stall_in_1
## Actinobacteria              1382          978             1665       1077
## Proteobacteria              1229         1555              897       1272
## Firmicutes                   123           94              378        446
## Bacteroidetes                201          333              127        235
## Deinococcus-Thermus           59           50                0          0
## unclassified                  89           85               30         78
##                     toilet_floor_1 toilet_floor_2 toilet_flush_handle_1
## Actinobacteria                1133           1013                  1006
## Proteobacteria                1419           1634                  1133
## Firmicutes                     130            131                    51
## Bacteroidetes                  267            228                   276
## Deinococcus-Thermus             23             28                     5
## unclassified                   106             60                   486
##                     toilet_flush_handle_2 toilet_seat_1 toilet_seat_2
## Actinobacteria                       1400           685          1968
## Proteobacteria                       1096          1906           100
## Firmicutes                            433           363           946
## Bacteroidetes                         120           117            80
## Deinococcus-Thermus                    10             2             1
## unclassified                           26            32            19
```

There is a high number of OTUs with single counts across sites, and many unassigned ones. Similarly, there are phylums with only a few corresponding rows, and very low counts. We could still compute a correlation with the rest, but it would be difficult to interpret. We set the threshold as low as possible. Two phylum are removed.

```
# calculate a frequency table
genfreq <- table(otu$Genus)
genfreq
```

```
##
##               Acidobacteria             Actinobacteria
##                          79                       1337
##            Armatimonadetes              Bacteroidetes
##                           6                        509
## Candidatus_Saccharibacteria                Chloroflexi
##                          44                         14
##         Deinococcus-Thermus                 Firmicutes
##                          35                        381
##                Fusobacteria            Gemmatimonadetes
##                          13                          7
##             Microgenomates               Parcubacteria
##                           2                          6
##             Planctomycetes              Proteobacteria
##                           5                       1113
##                Spirochaetes               Synergistetes
##                           3                          1
##            Verrucomicrobia                unclassified
##                          14                        536
```

```
# filter the most infrequent phylum
gentotals <- gentotals[!rownames(gentotals) %in% names(genfreq)[genfreq < 5],]
head(gentotals)
```

```
##                     door_in_1 door_in_2 faucet_handle_1 faucet_handle_2
## Actinobacteria           1669      1483             434            2224
## Proteobacteria            593       960            2445             340
## Firmicutes                686       457             155             440
## Bacteroidetes             100       170              54              84
## Deinococcus-Thermus        10        20               0              12
## unclassified               30        22              19               6
##                     sink_floor_1 sink_floor_2 soap_dispenser_1 stall_in_1
## Actinobacteria              1382          978             1665       1077
## Proteobacteria              1229         1555              897       1272
## Firmicutes                   123           94              378        446
## Bacteroidetes                201          333              127        235
## Deinococcus-Thermus           59           50                0          0
## unclassified                  89           85               30         78
##                     toilet_floor_1 toilet_floor_2 toilet_flush_handle_1
## Actinobacteria                1133           1013                  1006
## Proteobacteria                1419           1634                  1133
## Firmicutes                     130            131                    51
## Bacteroidetes                  267            228                   276
## Deinococcus-Thermus             23             28                     5
## unclassified                   106             60                   486
##                     toilet_flush_handle_2 toilet_seat_1 toilet_seat_2
## Actinobacteria                       1400           685          1968
## Proteobacteria                       1096          1906           100
## Firmicutes                            433           363           946
## Bacteroidetes                         120           117            80
## Deinococcus-Thermus                    10             2             1
```

```
## unclassified                          26            32            19
```

Computing correlation and ordering

We'll first compute an ordinary correlation matrix between phylum, which are the rows of the total abundance matrix.

```
gencor <- cor(t(gentotals))
```

Next, we'd like to group highly correlated phylum together. An easy way is to perform hierarchical clustering and use the clustering's order. We even get a dendrogram for easier visualization that way.

For this kind of data, Pearson distance, defined as 1 - Pearson correlation is appropriate, and Ward linkage method works very well. As indicated in the manual, *ward.D2* must be used, since our distances are not squared (or positive integers).

```
# perform the clustering
hcl <- hclust(as.dist(1 - gencor), method="ward.D2")
# extract order
horder <- rownames(gentotals)[hcl$order]
horder
```

```
##  [1] "Fusobacteria"                "Actinobacteria"
##  [3] "Firmicutes"                  "Acidobacteria"
##  [5] "Candidatus_Saccharibacteria" "unclassified"
##  [7] "Chloroflexi"                 "Parcubacteria"
##  [9] "Verrucomicrobia"             "Armatimonadetes"
## [11] "Planctomycetes"              "Proteobacteria"
## [13] "Bacteroidetes"               "Deinococcus-Thermus"
## [15] "Gemmatimonadetes"
```

```
# convert to data appropriate for ggplot2, which gives us segment coordinates for drawing it directly o
dhcl <- dendro_data(hcl)
# reorder the matrix
gencor <- gencor[horder,horder]
gencor
```

```
##                             Fusobacteria Actinobacteria Firmicutes
## Fusobacteria                   1.0000000     0.52231230  0.4185781
## Actinobacteria                 0.5223123     1.00000000  0.6388938
## Firmicutes                     0.4185781     0.63889378  1.0000000
## Acidobacteria                 -0.4434068    -0.30588683 -0.5067274
## Candidatus_Saccharibacteria   -0.2770136    -0.14812119 -0.4046593
## unclassified                  -0.4212899    -0.25654800 -0.4637358
## Chloroflexi                   -0.2780714    -0.18032203 -0.3610519
## Parcubacteria                 -0.2832168    -0.18421438 -0.3532849
## Verrucomicrobia               -0.3035044    -0.19970302 -0.3837630
## Armatimonadetes                0.1685259    -0.04009420 -0.1773478
## Planctomycetes                 0.1167320     0.04730139 -0.3280876
## Proteobacteria                -0.3880156    -0.95048641 -0.7020287
## Bacteroidetes                 -0.6488839    -0.33141043 -0.6172501
## Deinococcus-Thermus           -0.3478978    -0.03705856 -0.4772676
## Gemmatimonadetes              -0.4600615    -0.29742251 -0.6147621
##                             Acidobacteria Candidatus_Saccharibacteria
## Fusobacteria                  -0.44340677                 -0.27701357
## Actinobacteria                -0.30588683                 -0.14812119
## Firmicutes                    -0.50672741                 -0.40465930
```

```
## Acidobacteria                  1.00000000                  0.89946659
## Candidatus_Saccharibacteria    0.89946659                  1.00000000
## unclassified                   0.93818310                  0.94984972
## Chloroflexi                    0.91273061                  0.92724326
## Parcubacteria                  0.89476947                  0.92432650
## Verrucomicrobia                0.90620983                  0.93297623
## Armatimonadetes                0.25579650                  0.30828409
## Planctomycetes                -0.09441804                 -0.09806426
## Proteobacteria                 0.12659445                 -0.03124084
## Bacteroidetes                  0.55391175                  0.45166935
## Deinococcus-Thermus            0.07832152                  0.11399123
## Gemmatimonadetes               0.53248997                  0.61152430
##                           unclassified Chloroflexi Parcubacteria
## Fusobacteria              -0.421289933 -0.27807140  -0.283216820
## Actinobacteria            -0.256547998 -0.18032203  -0.184214384
## Firmicutes                -0.463735784 -0.36105188  -0.353284937
## Acidobacteria              0.938183100  0.91273061   0.894769467
## Candidatus_Saccharibacteria  0.949849717  0.92724326   0.924326496
## unclassified               1.000000000  0.97348566   0.965168777
## Chloroflexi                0.973485655  1.00000000   0.986298372
## Parcubacteria              0.965168777  0.98629837   1.000000000
## Verrucomicrobia            0.977759885  0.99220269   0.995948122
## Armatimonadetes            0.381925400  0.41771348   0.405919159
## Planctomycetes            -0.057795937 -0.12639133  -0.164933673
## Proteobacteria             0.058866631 -0.01150569  -0.006459295
## Bacteroidetes              0.551161910  0.38691522   0.367732760
## Deinococcus-Thermus        0.003587177 -0.15368927  -0.148534166
## Gemmatimonadetes           0.526809969  0.40981432   0.450818707
##                           Verrucomicrobia Armatimonadetes Planctomycetes
## Fusobacteria                -0.303504411     0.168525863     0.11673201
## Actinobacteria              -0.199703018    -0.040094201     0.04730139
## Firmicutes                  -0.383762989    -0.177347837    -0.32808762
## Acidobacteria                0.906209829     0.255796504    -0.09441804
## Candidatus_Saccharibacteria  0.932976227     0.308284087    -0.09806426
## unclassified                 0.977759885     0.381925400    -0.05779594
## Chloroflexi                  0.992202693     0.417713478    -0.12639133
## Parcubacteria                0.995948122     0.405919159    -0.16493367
## Verrucomicrobia              1.000000000     0.404932208    -0.13282189
## Armatimonadetes              0.404932208     1.000000000     0.57522374
## Planctomycetes              -0.132821888     0.575223742     1.00000000
## Proteobacteria               0.006981966     0.007270898     0.06327063
## Bacteroidetes                0.420532471     0.049344296     0.29492361
## Deinococcus-Thermus         -0.113379200    -0.349764842     0.24955112
## Gemmatimonadetes             0.448273872    -0.015339300     0.03750000
##                           Proteobacteria Bacteroidetes Deinococcus-Thermus
## Fusobacteria               -0.388015555    -0.6488839       -0.347897839
## Actinobacteria             -0.950486412    -0.3314104       -0.037058560
## Firmicutes                 -0.702028717    -0.6172501       -0.477267605
## Acidobacteria               0.126594453     0.5539118        0.078321521
## Candidatus_Saccharibacteria -0.031240835     0.4516693        0.113991226
## unclassified                0.058866631     0.5511619        0.003587177
## Chloroflexi                -0.011505694     0.3869152       -0.153689265
## Parcubacteria              -0.006459295     0.3677328       -0.148534166
## Verrucomicrobia             0.006981966     0.4205325       -0.113379200
```

```
## Armatimonadetes                0.007270898     0.0493443        -0.349764842
## Planctomycetes                 0.063270633     0.2949236         0.249551121
## Proteobacteria                 1.000000000     0.2248817         0.118933720
## Bacteroidetes                  0.224881731     1.0000000         0.556154831
## Deinococcus-Thermus            0.118933720     0.5561548         1.000000000
## Gemmatimonadetes               0.282512065     0.3833599         0.606763948
##                              Gemmatimonadetes
## Fusobacteria                      -0.4600615
## Actinobacteria                    -0.2974225
## Firmicutes                        -0.6147621
## Acidobacteria                      0.5324900
## Candidatus_Saccharibacteria        0.6115243
## unclassified                       0.5268100
## Chloroflexi                        0.4098143
## Parcubacteria                      0.4508187
## Verrucomicrobia                    0.4482739
## Armatimonadetes                   -0.0153393
## Planctomycetes                     0.0375000
## Proteobacteria                     0.2825121
## Bacteroidetes                      0.3833599
## Deinococcus-Thermus                0.6067639
## Gemmatimonadetes                   1.0000000
```

We then remove redundant data by removing the top triangle of the matrix.

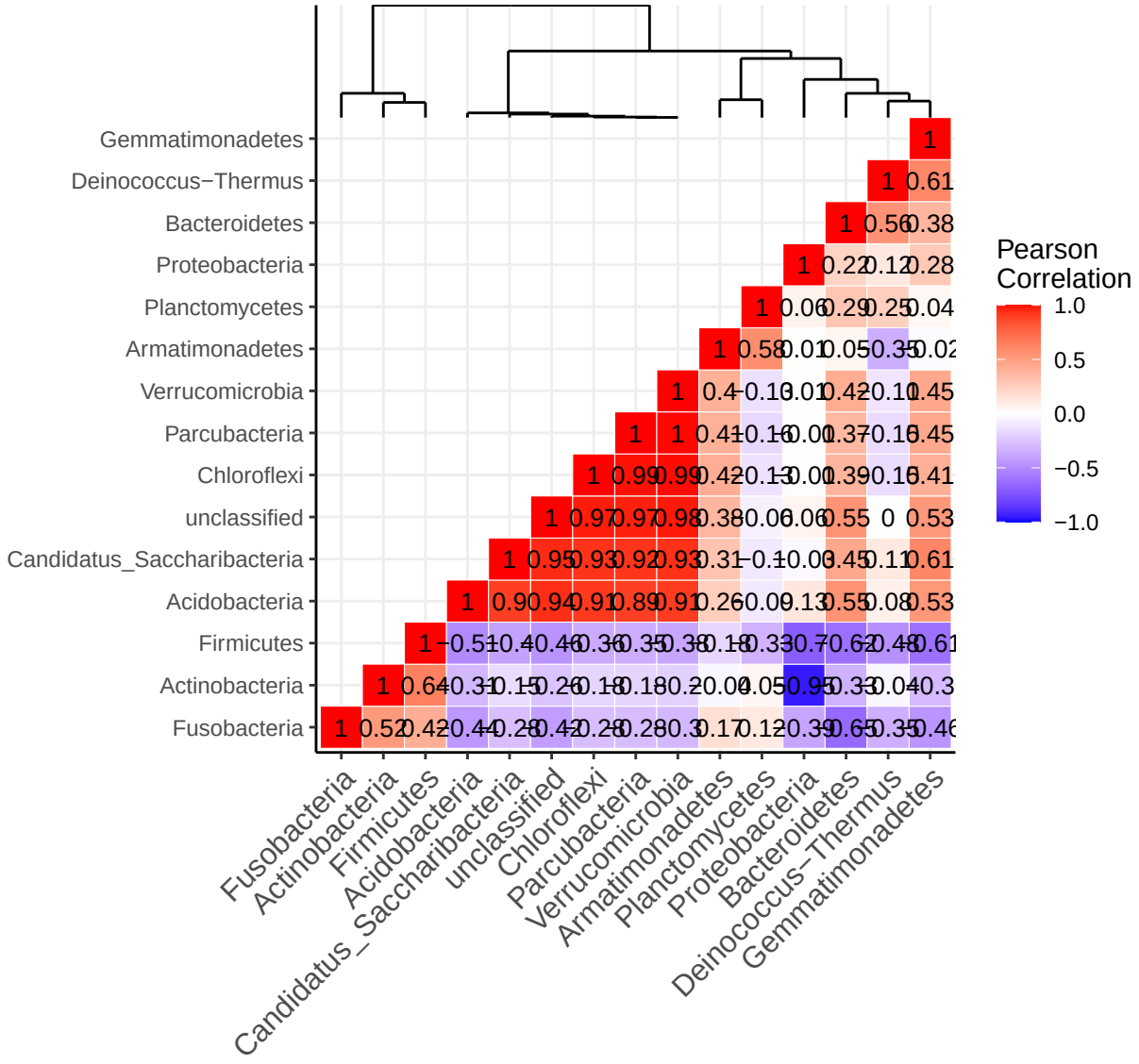
```
# lower.tri selects the matrix' lower triangle, and we just remove all the data
gencor[lower.tri(gencor)] <- NA
```

Correlation map

We can now output a correlation map similar similar to a heatmap. Colors are set as white for no correlation and red and blue for positive and negative correlation respectively. We add the dendrogram obtained before above it.

```
# use the ordered correlation matrix
cormap <- gencor %>%
    # convert to dataframe for ggplot
    as.data.frame %>%
    # get the rownames to use for grouping
    rownames_to_column("grp") %>%
    # convert to long format, remove missing values (upper triangle)
    pivot_longer(-grp, names_to="key", values_to="v", values_drop_na=TRUE) %>%
    # make sure the order is preserved like before, otherwise elements may be shifted seemingly at rand
    mutate(grp=factor(grp, levels=horder, ordered=TRUE),
        key=factor(key, levels=horder, ordered=TRUE)) %>%
    # pass it directly to ggplot; label will be the rounded correlation value within each cell
    ggplot(aes(key, grp, fill=v, label=round(v,2))) +
            # tilemap background
            geom_tile(color="white") +
            # set a blue to red gradient for [-1;1] correlation values
            scale_fill_gradient2(
                low="blue", high="red", mid="white",
                midpoint=0,
                limit=c(-1,1), space="Lab",
                name="Pearson\nCorrelation") +
```

```
            # rotate x axis for easier viewing, remove axis title
            theme(axis.text.x=element_text(angle=45, vjust=1, size=12, hjust=1),
                axis.title=element_blank()) +
            # make sure we get a square matrix
            coord_fixed() +
            # add correlation values
            geom_text(color="black", size=3.5) +
            # draw the dendrogram; we offset y coordinates to place it above the tilemap
            geom_segment(data=segment(dhcl), aes(x, y+nrow(gencor)+0.5, xend=xend, yend=yend+nrow(genco
cormap
```




```
# save it
ggsave("plots/cormap.pdf", cormap, width=12, height=10)
```

Principal component analysis

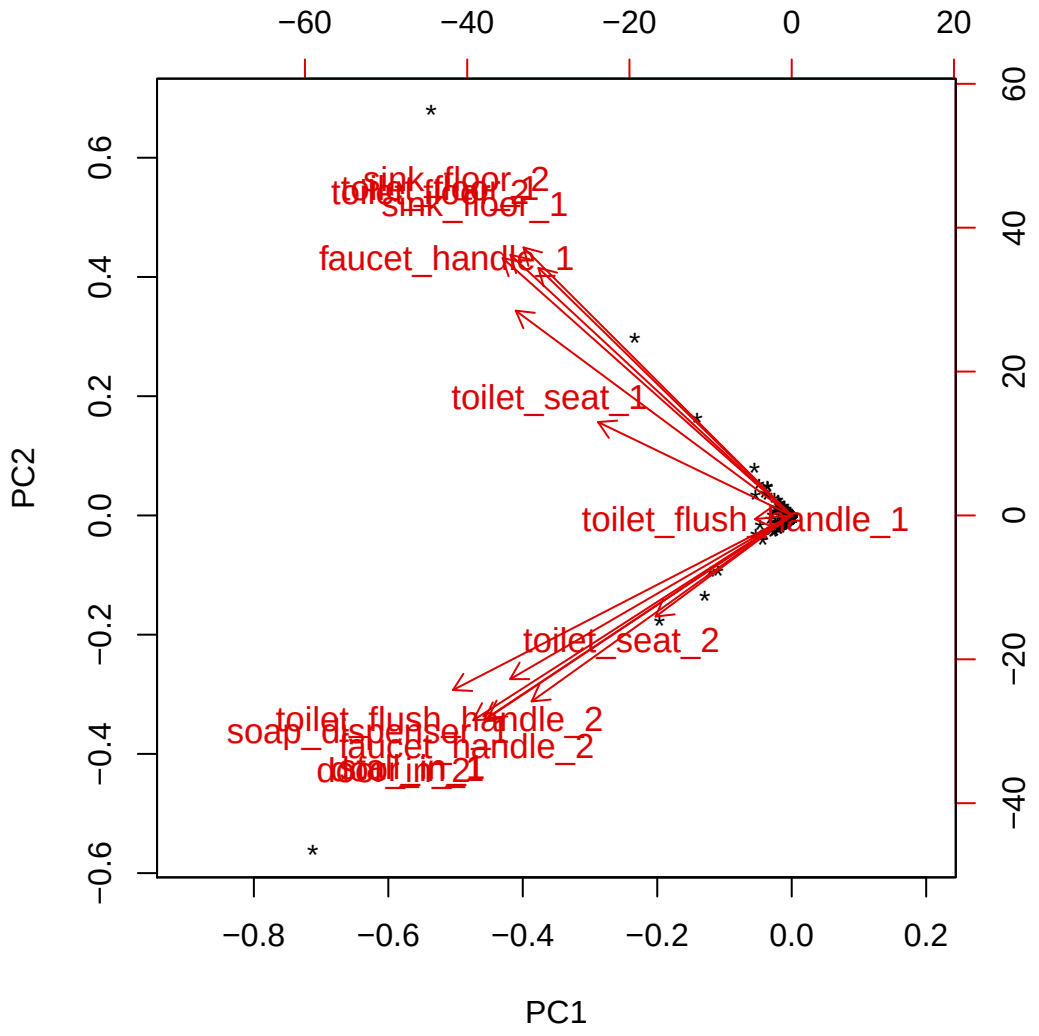
Finally, we can perform a simple PCA to evaluate if there is any grouping structure in the data.

```r
# compute pca on numeric matrix, scale the variables to unit variance as recommended
pca <- prcomp(otumat, scale=TRUE)
# percent of variance explained by each axis
pca$sdev ^ 2 / sum(pca$sdev ^ 2)
```

```
##  [1] 0.4057426457 0.2861426256 0.0988359817 0.0715174067 0.0565024752
##  [6] 0.0254300512 0.0230228818 0.0113783480 0.0093346932 0.0063051507
## [11] 0.0021608007 0.0018565269 0.0011851263 0.0005852864
```

```r
# 40.6% and 28.6% for PC1 and PC2
```

```r
# render base graphics plot
biplot(pca,
    # draw arrows for each eigenvector
    var.axes=TRUE,
    xlabs=rep("*", nrow(otumat)), cex=c(0.9,1.1),
    xlim=c(-0.9,0.2), expand=50,
    col=c("black", "#dd0000"))
```



```r
pdf("plots/pca.pdf", 12, 10)
# plot the pca
biplot(pca,
    # draw arrows for each eigenvector
    var.axes=TRUE,
    xlabs=rep("*", nrow(otumat)), cex=c(0.9,1.1),
    xlim=c(-0.9,0.2), expand=50,
    col=c("black", "#dd0000"))
dev.off()
```