Short Tutorials for Metagenomic Analysis

This manual describes metagenomic analysis with the `matR` package (Metagenomic Analysis Tools for R). The sections form a progressive set, but can also be rearranged, and many can be treated as independent 10-15 minute tutorials. If this software helps your work, please cite us: *Daniel T. Braithwaite and Kevin P. Keegan (2013). matR: Metagenomics Analysis Tools for R. R package version 0.9.9.*

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Contents

1 Preliminaries

1.1 Obtaining and Installing R

R is free software, easily downloaded from the R Project Homepage: `http://www.r-project.org`. Binary versions are available for Mac and Windows systems, and source code for Linux. Download and install the version appropriate for your system.

Users who already have R should *update their version.* R and its extensions are frequently updated. Keeping current is important to avoid nuisance errors.

Add-on packages for many purposes, contributed by many people, are a great strength of R. For example, see this list of packages, organized by application area: `http://cran.r-project.org/web/views/`. For a repository dedicated entirely to biological functionality, see: `http://www.bioconductor.org`.

Now install `matR`, the MG-RAST interface add-on package. For this, use:

```
> install.packages("matR", repo="http://dunkirk.mcs.anl.gov/~braithwaite/R", type="source")
```

Open an R session. Use the following command to load the `matR` package (you would use a similar command to load any other package):

```
> library(matR)
```

`matR` relies on various other packages. To install these, follow the instructions provided by running this function:

```
> dependencies()
```

At the time of this writing, the packages relied on by `matR` are: `RJSONIO`, `ecodist`, `gplots`, `scatterplot3d`. If the `dependencies` function doesn't complete successfully, these need to be installed one at a time, as follows:

```
> install.packages("RJSONIO")
> install.packages("ecodist")
> install.packages("gplots")
> install.packages("scatterplot3d")
```

Now your R environment is ready to go!

1.2 Introduction to R

Here we review some basics of working with data in R, but the treatment is necessarily brief. For detailed R language tutorials, try: `http://www.ats.ucla.edu/stat/r`.

For us, two kinds of data objects are essential in R: `matrix` and `data.frame`. First, we create a `matrix`. The function `sample` just creates a random permutation, as shown.

```
> sample(1:200)

  [1] 101 147  42  41  81  21  99  25 169 117  44  26 110 185 189  98 163 197
 [19]  17  73 173 182  28  36 187 157 105 178 113  67 114 134  63  84 153 151
 [37] 188   6 122  57 196 152 132 143 140 124   5 135  90  61 184  89 190 200
 [55]  49 128 123 179  15 175  52 145  71   1  11 116  40  23 125 160  87   8
 [73] 115  48  27  18 159 139  30 142  10 121  83 127 161 168 199  66 193  64
 [91]  47 141 164  92 154  32 129  38 167  82  24  68 130 108  86 102  76 155
[109] 180 120 106 165  54   9 133  34  53  80   7  94 109  78 174 150  59   4
[127]  22  60  70 162  77 103  50 104  16  43 131 191  20 118  13 146 111 170
[145] 138 107  93  65 166 186 194 176   2  39 156 119  69  31  33 181 144  19
[163]  56  51  72 112  12  62 198 126 137  45 171  37  74 100  46 183  91  75
[181]  85  88 172  29  79  96 136 158 177  95  35  55 192  97   3 195  58 149
[199] 148  14

> m <- matrix(sample (1:200), nrow=20, ncol=10)
> m

      [,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8] [,9] [,10]
 [1,]   46  110  111   94  162  132   62   68   63    52
 [2,]  150   71  176  169   96  148    5   82  135   140
 [3,]  145   76  197   54   58   29   48  190    4   193
 [4,]  120   14   92  192  144   53   59   81    2   185
 [5,]  125  184   15   25  165   77  157  108  172     3
 [6,]  196  126   16   17  102   83   44  134  129    67
 [7,]   36    8   80   85   61   50  109   65   51   188
 [8,]  112  194  118  131   84  186  199  170  163   178
 [9,]  137   60  139  107  106  164   90   12   57    34
[10,]  149  146  161   55  173  200  101   11   91     6
[11,]   87   18   47  156  119   42  143   86   28   166
[12,]   32  177  133  116  187   40  103  105   38    43
[13,]  121   27  174  123   79  168   37  151   72    98
[14,]  104   74  127   24  158   89   22  124  142    21
[15,]   69   49  191   88  183  138    7   75  147   113
[16,]  154  189   93   56   30  114  117   19   66   175
[17,]  152  128  122  153  198   97  171   78   99    64
[18,]   26  155  160  181  141   41  167    1   31   179
[19,]   20  130   10   45  180   73  100  182   70    33
[20,]  115  136   13   23   95    9   35   39  195   159
```

The `apply` function, below, applies the function specified by its last argument (in this case, `mean`) along the dimension of `m` specified by the second argument. So here we calculate the row means and then the column means of `m`.

```
> apply(m,1,mean)

 [1]  90 117  99  94 103  91  73 154  91 109  89  97 105  88 106 101 126 108  84
[20]  82
```

```
> apply(m,2,mean)

 [1] 105 104 109  95 126  97  89  89  88 105
```

Generally speaking, a `data.frame` is different from a `matrix` because it may contain non-numeric data. So, now we create a `data.frame` consisting of the *column means* and *column standard deviations* of `m`, but also containing a third, descriptive column.

```
> df <- data.frame(mu=apply(m,2,mean), sigma=apply(m,2,sd))
> df$sample <- paste("sample", LETTERS[1:10], sep = "-")
> df

    mu sigma   sample
1  105    51 sample-A
2  104    62 sample-B
3  109    62 sample-C
4   95    57 sample-D
5  126    50 sample-E
6   97    57 sample-F
7   89    58 sample-G
8   89    57 sample-H
9   88    57 sample-I
10 105    70 sample-J
```

Suppose we wanted to reorder the columns. Flexible indexing of objects is a great strength of R. Here we *replace* the first and third columns of `df` with (respectively) its own third and first columns — effectively, reordering them.

```
> df [c(1,3)] <- df [c(3,1)]
> df

         mu sigma sample
1  sample-A    51    105
2  sample-B    62    104
3  sample-C    62    109
4  sample-D    57     95
5  sample-E    50    126
6  sample-F    57     97
7  sample-G    58     89
8  sample-H    57     89
9  sample-I    57     88
10 sample-J    70    105
```

That almost worked, but notice that while the data moved, the column *labels* did not. It is possible to refer directly to the row and column labels of a `matrix` or `data.frame`, as follows.

```
> rownames(df)

 [1] "1"  "2"  "3"  "4"  "5"  "6"  "7"  "8"  "9"  "10"

> colnames(df)

[1] "mu"     "sigma"  "sample"
```

Now we finish by correcting the column labels.

```
> colnames(df) [c(1,3)] <- colnames(df) [c(3,1)]
> df
```

```
     sample sigma  mu
1  sample-A    51 105
2  sample-B    62 104
3  sample-C    62 109
4  sample-D    57  95
5  sample-E    50 126
6  sample-F    57  97
7  sample-G    58  89
8  sample-H    57  89
9  sample-I    57  88
10 sample-J    70 105
```

Here are some commands for viewing the first elements, last elements, and overall structure of large objects.

```
> head(m)
```

```
     [,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8] [,9] [,10]
[1,]   46  110  111   94  162  132   62   68   63    52
[2,]  150   71  176  169   96  148    5   82  135   140
[3,]  145   76  197   54   58   29   48  190    4   193
[4,]  120   14   92  192  144   53   59   81    2   185
[5,]  125  184   15   25  165   77  157  108  172     3
[6,]  196  126   16   17  102   83   44  134  129    67
```

```
> tail(m)
```

```
      [,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8] [,9] [,10]
[15,]   69   49  191   88  183  138    7   75  147   113
[16,]  154  189   93   56   30  114  117   19   66   175
[17,]  152  128  122  153  198   97  171   78   99    64
[18,]   26  155  160  181  141   41  167    1   31   179
[19,]   20  130   10   45  180   73  100  182   70    33
[20,]  115  136   13   23   95    9   35   39  195   159
```

```
> str(m)
```

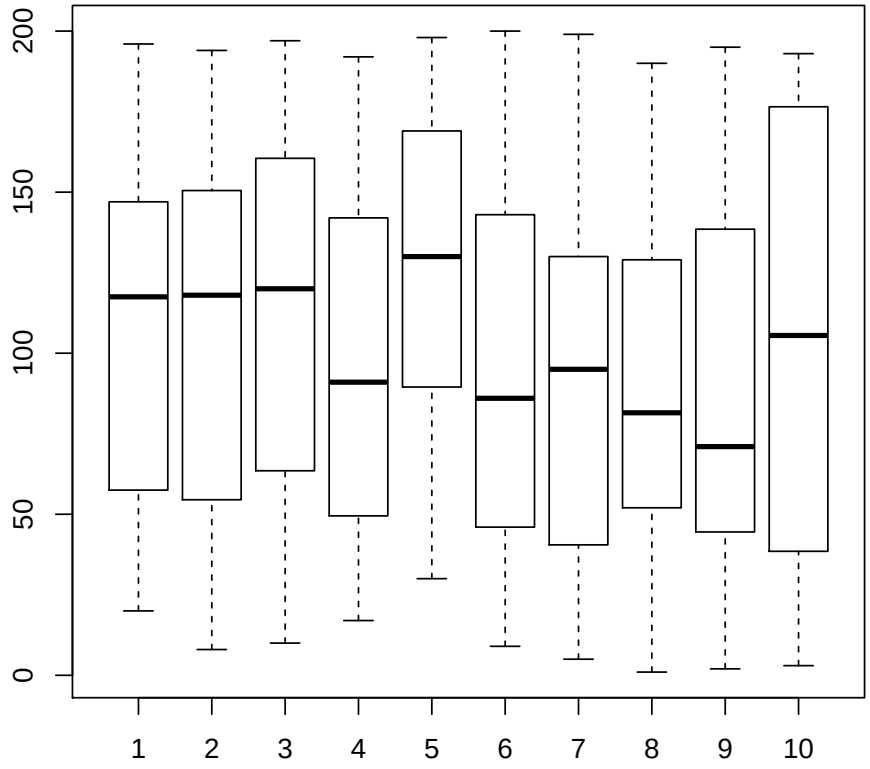
```
 int [1:20, 1:10] 46 150 145 120 125 196 36 112 137 149 ...
```

```
> str(df)
```

```
'data.frame':        10 obs. of  3 variables:
 $ sample: chr  "sample-A" "sample-B" "sample-C" "sample-D" ...
 $ sigma : num  50.9 61.8 61.7 56.5 49.5 ...
 $ mu    : num  104.8 103.6 108.8 94.7 126 ...
```

Finally, any introduction to R should show how it easily renders statistical graphics, as with this boxplot of the columns of `m`.

```
> boxplot(m)
```



There is a lot more to R, but the subset of commands shown here, together with the help tutorial (which is next), already enable many things!

1.3 Using R Help

In R, as with any system, it's important to know how to use the help.

First, locate the one-page quick reference for all `matR` commands:

```
> vignette("matR-quick-reference")
```

If that doesn't work, the quick reference is also available at: `http://dunkirk.mcs.anl.gov/~braithwaite/library/matR/doc/matR-quick-reference.pdf`. It may be handy to print a copy.

Help on any R command is available with:

```
> ?command
```

For example, try:

```
> ?mean
> ?sample
> ?apply
```

For keyword-based help, use the double question mark, as in these examples:

```
> ??random
> ??plot
```

Finally, to retrieve an index of all help topics *for a specific package*, use this command, replacing `matR` with the name of the relevant package:

```
> library(help="matR")
```

`matR` is updated regularly. For a summary of the latest changes, see:

```
> vignette("matR-change-log")
```

The same document is also available at: `http://dunkirk.mcs.anl.gov/~braithwaite/library/matR/doc/matR-change-log.pdf`.

1.4 Exporting and Importing Data; Saving Images

This tutorial explains how to get images out of R for publications, how to bring data into R from formats such as csv, tsv, or biom; and how to save data for use in future R sessions, in Excel, or with other programs.

matR provides a function, `asFile()`, that conveniently exports several kinds of object in a default format. It's not flexible but may be adequate for many purposes. Try it on any vector or matrix object:

```
> asFile(cc$raw, file="saved_matrix.txt")
```

`write.table()` and `read.table()` are the workhorse commands for exporting and importing any kind of tabular data. They have many options, as well as variants such as `read.csv()`. The following examples show the most common options. These functions are very flexible, though, so consult the help system to learn more.

```
> cc <- collection("4441679.3 4441680.3 4441682.3")
> write.table(cc$raw, file="data.txt", sep="\t")
> x <- read.table(file="data.txt")
> x
```

The functions `save()` and `load()` store R objects in a binary format for use in later R sessions. (By convention, these files end with `.Rda`.) This is helpful, for example, to store a metagenome collection or the result of an analysis that is computation-intensive. Here are some examples:

```
> cc <- collection("4441679.3 4441680.3 4441682.3")
> p <- pco(cc)
> ls()
> save(cc, p, file="saved_data.Rda")
> rm(cc, p)
> ls()
> load(file="saved_data.Rda")
> ls()
```

There is an easy method to export images from an R session. First develop the exact commands to produce the desired image interactively. For instance, suppose we want to export the following PCoA.

```
> pco(Waters, main="functional level 3", col=c(rep("red",12),rep("blue",12)))
```

To produce a pdf file, simply amend the code in this way.

```
> pdf(filename="my_pco.pdf", width=5, height=5)
> pco(Waters, main="functional level 3", col=c(rep("red",12),rep("blue",12)))
> dev.off()
```

The function `pdf()` can be replaced with others, such as `png()`. For more detail, consult the help system.

1.5 Data Type Conversions (including BIOM)

In most programming languages, it is important to know the kind (or type or class) of data objects. This can be a vexed subject in R. Our purposes require: `vector`, `matrix`, `data.frame`, `list`, `collection`, and `BIOM`.

2 Examples

2.1 Functional Comparison of Lean and Obese Mouse

2.2 HMP Samples with External Metadata

2.3 Variability of Clustering by Annotation Source

2.4 Parallel Coordinates of Brazilian Coastal Samples

2.5 Where to Find More

A gallery of additional simple examples is maintained at: `http://dunkirk.mcs.anl.gov/~braithwaite`.

3 Basics

3.1 Data in an Annotation Matrix

The columns of a `matR` matrix are labeled by sample, and rows are labeled by annotation. The annotations may be taxonomic or functional, at various hierarchy levels. Often, the matrix entries are raw counts of annotations per sample. So an "OTU table" is just one kind of `matR` matrix.

The matrix may also contain other quantities such as (for instance) normalized abundance counts, or average read length of annotated sequences, per annotation and per sample. Matrix entries may also be qualified or limited. For example, counts may be requested only from a particular annotation database.

Suppose you have selected a particular set of metagenomes. Next, in order to retrieve related data, you have to specify exactly what data you want. Such a description is called a `view` of the data, and it is spelled out with predefined options. Here are some examples of `views`:

```
> c(level="level1")
> c(annot="organism",level="phylum")
> c(entry="normed.counts",source="NOG")
```

The first line indicates counts per functional annotation at level 1 of the Subsystems hierarchy. The second indicates counts of *taxonomic* annotations at phylum level from the M5RNA database. The third indicates *normalized* counts of functional annotations from only the NOG database.

The options for data `views` are listed and fully described in the `matR` package itself. Examine these objects at the R prompt just by typing their names:

```
> view.descriptions
> view.parameters
> view.defaults
```

The last one, `view.defaults`, shows what data is retrieved if you don't choose explicitly.

3.2 Metagenome Collections

Metagenome data is always retrieved by constructing a `collection`. The samples of interest must be identified by ID. Here are some examples.

```
> IDs <- c(gut1="4441695.3", gut2="4441696.3")
> cc <- collection(IDs)
> dd <- collection("4441679.3 4441680.3 4441682.3 4441695.3 4441696.3 4440463.3 4440464.3")
> ee <- collection(file="test-IDs.txt")
```

In the first example, the samples are given names. The last example reads a list of IDs from a text file. IDs in files should be whitespace-separated. The file may also contain names in a first column and IDs in a second column. In addition to metagenome IDs, project IDs may be used. The effect is to request all metagenomes from that project. Project IDs should begin with `"mgp"`.

Choosing samples is only half the story: various data pertaining to those samples can be requested. In each of the following examples, each part of the `collection` function names and describes a distinct `view` of the data, as discussed above.

```
> collection(IDs,
+   raw=c(entry="count"),
+   nrm=c(entry="normed.counts"))
> collection(IDs,
+   L1=c(level="level1"), L2=c(level="level2"),
+   L3=c(level="level3"), L4=c(level="function"))
> collection(IDs,
+   nog=c(source="NOG"),
+   cog=c(source="COG"),
+   ko=c(source="KO"))
> collection(IDs,
+   lca=c(annot="organism", hit="lca"),
+   repr=c(annot="organism", hit="single"),
+   all=c(annot="organism", hit="all"))
```

A handy techniques is to make lists of `views`:

```
> top.levels <- list(
+   L1=c(level="level1"),
+   L2=c(level="level2"))
> all.ontologies <- list(
+   nog=c(source="NOG"),
+   cog=c(source="COG"),
+   ko=c(source="KO"),
+   sub=c(source="Subsystems"))
> all.count.methods <- list(
+   lca=c(annot="organism", hit="lca"),
+   repr=c(annot="organism", hit="single"),
+   all=c(annot="organism", hit="all"))
```

Such lists can then be used (and reused) as follows:

```
> cc <- collection (guts, top.levels)
> dd <- collection (guts, all.ontologies)
> ee <- collection (guts, all.count.methods)
```

The matrix of data corresponding to a `view` is accessed with `$` plus the appropriate name:

```
> cc$L1
> dd$nog
> ee$all
```

views can be specified when a collection is constructed, as shown above, and can also be added to an existing collection in this way:

```
> dd$cog <- c(source="COG")
```

Various common sense functions apply to collections:

```
> samples(cc)      # show metagenomes in the collection
> projects(cc)     # show projects in the collection
> names(cc)        # show names of metagenomes
> views(cc)        # show the data views in the collection
> viewnames(cc)    # show just the names of the views
> groups(cc)       # show grouping of metagenomes (if assigned)
> metadata(cc)     # access metadata
```

(For more about metadata, see below.) Values may be assigned to names, viewnames, and groups, as with:

```
> names(cc) <- c("new.name.1", "new.name.2")
```

Within each view, the names of annotations are accessed with rownames. Annotation names are hierarchical, and the sep parameter affects how the hierarchy is presented. There are four alternatives:

```
> rownames(Guts, view="raw", sep=NULL)
> rownames(Guts, view="raw", sep=FALSE)
> rownames(Guts, view="raw", sep=TRUE)
> rownames(Guts, view="raw", sep="\t")
```

The corresponding results are: annotations named by terminal hierarchy level only; a matrix of annotation names with one column per hierarchy level; annotations named by semicolon-separated concatenation of all hierarchy level names; same as previous, but with specified separator character.

Subsets may be taken of collections, as of other objects in R. Here we extract the first three samples of dd into a new collection.

```
> ff <- dd[1:3]
```

3.3 Using Metadata

Collections have metadata elements, which are named. The names of elements reflect the hierarchical nature of metadata. To see all metadata of the collection `Guts`, which is prepackaged with `matR`, simply enter:

```
> metadata(Guts)
```

Analyses usually require picking out specific metadata elements, and metadata can be indexed for that purpose. Metadata indexing is by element name(s), and an arbitrary number of indices may be specified. This is best understood by example. First, we use *one index* of *length one* to get all metadata from one sample of the collection:

```
> metadata(Guts)["4440464.3"]
```

Here is an example of metadata indexing using *two indices*, each of *length one*, to get sampling location information for all samples.

```
> metadata(Guts)["latitude", "longitude"]
```

An alternative form returns the same output in a more convenient form.

```
> metadata(Guts)["latitude", "longitude", bygroup=TRUE]
```

In this variant NA is placed when a field is missing, as in the next example.

```
> metadata(Guts)["host_common_name", "disease", ".age", bygroup=TRUE]
```

The next example obtains the entire environmental package from one metagenome using *one index* of *length two*. Only metadata fields matching *both* strings are selected:

```
> metadata(Guts)[c("4440464.3","env_package.data")]
```

Finally, this example uses *three indices* all of *length two* to select miscellaneous elements:

```
> metadata(Guts)[c("env","temp"), c("4440464.3","PI_organization"), c("0464","biome")]
```

Actually, metadata can be handled independently of annotation data. This saves time when annotation data is not needed. Metadata can be retrieved by sample, just as with the `collection` function:

```
> mm <- metadata("4441679.3 4441680.3 4441682.3 4441695.3 4441696.3")
```

Now `mm` can be used just as `metadata(Guts)` was used above.

4 Analysis

- matR provides new analysis methods as well as customized versions of functions included in base R and contributed packages. The latter are gratefully acknowledged: qvalue, ecodist, gplots2.
- matR functions build on existing functions by adding features and helpful defaults. Options to existing functions usually also apply to matR versions. The former are directly available to users who want more control, of course.
- (Some analyses have graphical representations, and others do not. A universal function, render(), visualizes the results of analysis computations. This functionality enables fast re-visualization (with modified parameters) of costly computations. However, the implementation is not yet complete.)
- As discussed earlier, a matrix within a collection is called a view and can be extracted with $. Conversely, a standalone matrix can be converted into a collection with class coercion via as(my_matrix, "collection"). Since some functions below apply to a matrix, and others to a collection, these conversions are important to understand.
- Some functions accept a grouping, which can be specified by any vector equal in length to the number of samples (columns). collection functions usually accept the parameters view and rows, which determine what part of the collection is analyzed.
- More detail on inputs, options, and outputs is given below. matrix functions are discussed first, then collection functions.

4.1 Singleton Removal and Normalization

It's a good idea to ignore abundance counts of one (singletons). The remove.singletons() function accomplishes that. Also, abundance values that have been normalized can be more meaningful than raw counts. For that matR includes the function normalize().

```
> cc <- collection(...)
> ns <- remove.singletons(cc$raw)
> nrm <- normalize(r)
```

Options to both functions are detailed in the help system.

4.2 Distance between Samples and Groups

matR extends the base R function dist in several ways. Additional metrics / dissimilarities can be selected with the method parameter. For metagenomic analysis, the parameter bycol is usually appropriate, to compute distance between columns rather than rows. With groups specified, a square matrix of intra- and inter-group mean pairwise distances is returned.

```
> dist(m, method="bray-curtis", bycol=TRUE)
> dist(m, groups=c(1,1,1,2,2,2,3,3,4,4,4,4), bycol=TRUE)
```

With an additional vector specified, its distance to each row or column is computed. When groups are also specified, mean pairwise distances from the vector to each group are computed.

```
> dist(m, y, bycol=TRUE)
> dist(m, y, groups=c(1,1,1,2,2,2,3,3,4,4,4,4), bycol=TRUE)
```

See the help system for more detail.

4.3 Statistical Significance Tests

The function `sigtest` is a convenient interface to apply any of several statistical signifance tests to annotations (rows) of a matrix. The specified test is applied, given a grouping of samples (columns), to each annotation (row). The tests typically test the null hypothesis that the group means of annotation abundances (whether raw or normalized) are the same. Qvalue testing can be applied to the multiple tests, but must be explicitly requested. As with all other function below, the components of the analysis results are returned in a list.

```
> sigtest (m, groups=c(1,1,1,2,2,2,3,3,4,4,4,4), test="Kruskal-Wallis")
> sigtest (m, groups=c(1,1,1,2,2,2,3,3,4,4,4,4), test="Kruskal-Wallis", qvalue=TRUE)
> sigtest (m, groups=c(1,1,1,2,2,2,3,3,4,4,4,4), test="Kruskal-Wallis", qvalue=TRUE, fdr.level=0.01)
```

4.4 Randomization Tests

The function `randomize` facilitates randomization (or permutation) analyses. It returns the result of applying any given summary function to each of a specified number of random permutations of a matrix. Several different randomization methods are implemented.

```
> randomize (m)
> randomize (m, n=10, method="sample")
> randomize (m, n=10, method="rowwise", FUN=mean)
> randomize (m, n=10, method="dataset", FUN=colSums, na.rm=TRUE)
> randomize (m, n=10, method="complete", FUN=function (m) apply (m, MARGIN=2, hist, plot=FALSE))
```

`sample` randomization randomly permutes the entries of each column. `rowwise` randomization randomly permutes the entries of each row. `dataset` randomization randomly permutes entries across the entire matrix. `complete` randomization randomly reassigns each (unit) annotation count.

4.5 Boxplots of Diversity

Boxplots are useful to summarize the distribution of annotation counts in samples of a collection. Boxplots are produced by the `render` function applied to a collection, since they illustrate data so directly. As with other functions below that apply to collections, a `view` may be specified or omitted.

```
> render(Waters)
> render (Waters, notch = TRUE, pch = 19, cex = 0.5, names = names (waters),
+ main = "Annotation Diversity at Function Level 3", cex.axis = 1.1)
```

For applicable graphical parameters, see `?base::boxplot`. The most useful are `main`, `names`, `notch`, and `outline`.

4.6 Principal Coordinates

The `pco` function also operates on a collection object. `rows` can be used to limit the analysis to specified annotations. `comp` specifies which principal components (1, 2, or 3 may be selectd) to plot, and `method` specifies the metric / dissimilarity used (as in `dist`).

```
> pco(cc)
> col <- factor (metadata (cc) ["biome"])
> levels (col) <- c ("#1F78B4", "#E31A1C", "#B15928")
> col.vec <- as.character (col)
> pco (cc, view="norm", comp = c (2,3,4), sub = "Principal Coordinates 2 to 4", cex.sub = 1.5,
+ main = "", color = col.vec, labels = "", cex = 1.5, lty.hplot="dashed",
+ mar = c (5,5,0,3))
```

The most important graphical parameters are `col` (for 2-d plots), `color` (for 3-d plots), `labels`, and `main`.
For others, see `?graphics::points`, `?graphics::text`, and `?scatterplot3d::scatterplot3d`.

4.7 Heatmap-Dendrograms

`heatmap` applies to collections and accepts optional parameters `view` and `rows`, as well.

```
> cc <- collection("....", n1 = c(entry="ns.normed.counts", level="level1"), raw=default.views$raw)
> test.result <- sigtest(cc$n1, "Kruskal")
> red.yellow <- rgb (colorRamp(c ("#FFFFCC", "#800026")) (seq(0, 1, length = 20)), max = 255)
> heatmap(cc)
> heatmap(cc, view="n1", rows=test.result$significant, main="significant annotations only", labRow=NA, l
```

Some common graphical parameters are illustrated above. See `?gplots::heatmap.2` for more possibilities.

4.8 Parallel Coordinates

5 Miscellaneous

5.1 API Calls for Extended Functionality

The full functionality of the MG-RAST API is available through `matR`. For API details, see `http://api.metagenomics.anl.gov`.

Many API resources are available with a convenient syntax using the mid-level interface function, `mGet`.

```
> mGet("metagenome_statistics", "mgm4472882.3")
```

For more control, use the low-level function `callRaw`. This function simply prepends the API server name and appends the session authorization key (if set) to its argument.

```
> callRaw("metagenome_statistics/mgm4472882.3")
```

Most API resources are returned as JSON objects and automatically parsed by `mGet` (or `callRaw`) into a list structure. JSON text can be retained with `parse=FALSE`.

5.2 Using `matR` within an iPython Notebook

`matR` is easily invoked from iPython Notebook to leverage the many advantages of that scripting environment.

5.3 Other Packages: `ggplot2`, `vegan`, `picante`

`matR` interacts easily with other R software for graphics and analysis.