

Package ‘microeco’

November 15, 2022

Type Package

Title Microbial Community Ecology Data Analysis

Version 0.13.0

Author Chi Liu [aut, cre],

Felipe R. P. Mansoldo [ctb],
Umer Zeeshan Ijaz [ctb],
Chenhao Li [ctb],
Yang Cao [ctb],
Minjie Yao [ctb],
Xiangzhen Li [ctb]

Maintainer Chi Liu <liuchi0426@126.com>

Description A series of statistical and plotting approaches in microbial community ecology based on the R6 class. The classes are designed for data preprocessing, taxa abundance plotting, alpha diversity analysis, beta diversity analysis, differential abundance test, null model analysis, network analysis, machine learning, environmental data analysis and functional analysis.

URL <https://github.com/ChiLiubio/microeco>

Depends R (>= 3.5.0)

Imports R6, stats, ape, vegan, rlang, data.table, magrittr, dplyr,
tibble, scales, grid, ggplot2, RColorBrewer, reshape2

Suggests GUniFrac, MASS, ggpibr, randomForest, ggdendro, ggrepel,
agridolae, gridExtra, picante, pheatmap, igraph, rgeff, mice,
GGally

License GPL-3

LazyData true

Encoding UTF-8

NeedsCompilation no

Repository CRAN

Date/Publication 2022-11-15 19:30:13 UTC

RoxygenNote 7.2.1

R topics documented:

clone	2
color_palette_20	3
dataset	3
dropallfactors	4
env_data_16S	5
fungi_func_FungalTraits	5
fungi_func_FUNGuild	5
microtable	6
otu_table_16S	15
otu_table_ITS	15
phylo_tree_16S	16
prok_func_FAPROTAX	16
prok_func_NJC19_list	16
sample_info_16S	17
sample_info_ITS	17
Tax4Fun2_KEGG	17
taxonomy_table_16S	17
taxonomy_table_ITS	18
tidy_taxonomy	18
trans_abund	19
trans_alpha	27
trans_beta	31
trans_classifier	37
trans_diff	44
trans_env	52
trans_func	66
trans_network	72
trans_nullmodel	86
trans_venn	96

Index

101

clone

Copy an R6 class object completely

Description

Copy an R6 class object completely

Usage

```
clone(x, deep = TRUE)
```

Arguments

x	R6 class object
deep	default TRUE; deep copy

Value

identical but unrelated R6 object.

Examples

```
data("dataset")
clone(dataset)
```

color_palette_20 *A color palette for 20 elements.*

Description

This is one palette option for users who have ≤ 20 elements to plot. The palettes of RColorBrewer package provide at most 12 discrete colors, such as "Set3" and "Paired". This palette is adapted from D3.js library and can be used as a supplement.

Usage

```
color_palette_20
```

Format

An object of class character of length 20.

dataset *The dataset in the microeco package*

Description

The dataset is structured with microtable class for the demonstration of examples and tutorials.

Usage

```
data(dataset)
```

Format

An R6 class object

Details

- sample_table: sample information table
- otu_table: species-community abundance table
- tax_table: taxonomic table
- phylo_tree: phylogenetic tree
- taxa_abund: taxa abundance list with several tables for Phylum...Genus
- alpha_diversity: alpha diversity table
- beta_diversity: list with several beta diversity distance matrix

dropallfactors

Remove all factors in a data frame

Description

Remove all factors in a data frame

Usage

```
dropallfactors(x, unfac2num = FALSE, char2num = FALSE)
```

Arguments

x	data frame
unfac2num	default FALSE; whether try to convert all character to numeric; if FALSE, only try to convert column with factor attribute. Note that this can only transform the columns that may be transformed to numeric without using factor.
char2num	default FALSE; whether force all the character to be numeric class by using factor as an intermediate.

Value

data frame without factor

Examples

```
data("taxonomy_table_16S")
taxonomy_table_16S[, 1] <- as.factor(taxonomy_table_16S[, 1])
str(dropallfactors(taxonomy_table_16S))
```

env_data_16S

The environmental factors for the 16S dataset in the microeco package

Description

The environmental factors for the 16S dataset in the microeco package

Usage

```
data(env_data_16S)
```

fungi_func_FungalTraits

The FungalTraits database for fungi trait identification in the microeco package

Description

The FungalTraits database for fungi trait identification in the microeco package

Usage

```
data(fungi_func_FungalTraits)
```

fungi_func_FUNGuild

The FUNGuild database for fungi trait identification in the microeco package

Description

The FUNGuild database for fungi trait identification in the microeco package

Usage

```
data(fungi_func_FUNGuild)
```

microtable*Create microtable object to store and manage all the basic files.*

Description

This class is a wrapper for a series of operations on the original files and the basic manipulations, including the microtable object creation, data reduction, data rarefaction based on Paul et al. (2013) <doi:10.1371/journal.pone.0061217>, taxa abundance calculation, alpha and beta diversity calculation based on the An et al. (2019) <doi:10.1016/j.geoderma.2018.09.035> and Lozupone et al. (2005) <doi:10.1128/AEM.71.12.8228–8235.2005> and other basic operations.

Online tutorial website: https://chiliubio.github.io/microeco_tutorial/

Download tutorial: https://github.com/ChiLiubio/microeco_tutorial/releases

Format

`microtable`.

Methods

Public methods:

- `microtable$new()`
- `microtable$filter_pollution()`
- `microtable$rarefy_samples()`
- `microtable$tidy_dataset()`
- `microtable$add_rownames2taxonomy()`
- `microtable$cal_abund()`
- `microtable$save_abund()`
- `microtable$sample_sums()`
- `microtable$taxa_sums()`
- `microtable$sample_names()`
- `microtable$taxa_names()`
- `microtable$rename_taxa()`
- `microtable$merge_samples()`
- `microtable$merge_taxa()`
- `microtable$cal_alphaDiv()`
- `microtable$save_alphaDiv()`
- `microtable$cal_betaDiv()`
- `microtable$save_betaDiv()`
- `microtable$print()`
- `microtable$clone()`

Method `new()`:

Usage:

```

microtable$new(
  otu_table,
  sample_table = NULL,
  tax_table = NULL,
  phylo_tree = NULL,
  rep_fasta = NULL,
  auto_tidy = FALSE
)

```

Arguments:

- otu_table data.frame; necessary; The feature abundance table; rownames are features (e.g. OTUs/ASVs/species/genes); column names are samples.
- sample_table data.frame; default NULL; The sample information table; rownames are samples; columns are sample metadata; If not provided, the function can generate a table automatically according to the sample names in otu_table.
- tax_table data.frame; default NULL; The taxonomic information table; rownames are features; column names are taxonomic classes.
- phylo_tree phylo; default NULL; The phylogenetic tree; use read.tree function in ape package for input.
- rep_fasta list or DNAStringSet; default NULL; The representative sequences; use read.fasta function in seqinr package or readDNAStringSet function in Biostrings package for input.
- auto_tidy default FALSE; Whether trim the files in the microtable object automatically; If TRUE, running the functions in microtable class can invoke the tidy_dataset function automatically.

Returns: an object of class microtable with the following components:

- sample_table The sample information table.
- otu_table The feature table.
- tax_table The taxonomic table.
- phylo_tree The phylogenetic tree.
- rep_fasta The representative sequence.
- taxa_abund default NULL; use cal_abund function to calculate.
- alpha_diversity default NULL; use cal_alpha function to calculate.
- beta_diversity default NULL; use cal_beta function to calculate.

Examples:

```

data(otu_table_16S)
data(taxonomy_table_16S)
data(sample_info_16S)
data(phylo_tree_16S)
dataset <- microtable$new(otu_table = otu_table_16S)
dataset <- microtable$new(sample_table = sample_info_16S, otu_table = otu_table_16S,
  tax_table = taxonomy_table_16S, phylo_tree = phylo_tree_16S)
# trim the files in the dataset
dataset$tidy_dataset()

```

Method filter_pollution(): Filter the taxa considered as pollution from microtable\$tax_table. This operation will remove any line of the microtable\$tax_table containing any the word in taxa parameter regardless of word case.

Usage:

```
microtable$filter_pollution(taxa = c("mitochondria", "chloroplast"))
```

Arguments:

`taxa` default: `c("mitochondria", "chloroplast")`; filter mitochondria and chloroplast, or others as needed.

Returns: None

Examples:

```
dataset$filter_pollution(taxa = c("mitochondria", "chloroplast"))
```

Method `rarefy_samples()`: Rarefy communities to make all samples have same species number. See also `rrarefy` of vegan package for the alternative method.

Usage:

```
microtable$rarefy_samples(sample.size = NULL, rngseed = 123, replace = TRUE)
```

Arguments:

`sample.size` default: `NULL`; species number, If not provided, use minimum number of all samples.

`rngseed` random seed; default: 123.

`replace` default: `TRUE`; See `sample` for the random sampling.

Returns: None; rarefied dataset.

Examples:

```
\donttest{
dataset$rarefy_samples(sample.size = min(dataset$sample_sums()), replace = TRUE)
}
```

Method `tidy_dataset()`: Trim all the data in the `microtable` object to make taxa and samples consistent. So the results are intersections.

Usage:

```
microtable$tidy_dataset(main_data = FALSE)
```

Arguments:

`main_data` default `FALSE`; if `TRUE`, only basic data in `microtable` object is trimmed. Otherwise, all data, including `taxa_abund`, `alpha_diversity` and `beta_diversity`, are all trimmed.

Returns: None, object of `microtable` itself cleaned up.

Examples:

```
dataset$tidy_dataset(main_data = TRUE)
```

Method `add_rownames2taxonomy()`: Add the rownames of `microtable$tax_table` as its last column. This is especially useful when the rownames of `microtable$tax_table` are required as a taxonomic level for the taxonomic abundance calculation and biomarker identification.

Usage:

```
microtable$add_rownames2taxonomy(use_name = "OTU")
```

Arguments:

`use_name` default "OTU"; The column name used in the `tax_table`.

Returns: NULL, a new tax_table stored in the object.

Examples:

```
\donttest{
dataset$add_rownames2taxonomy()
}
```

Method `cal_abund()`: Calculate the taxonomic abundance at each taxonomic rank.

Usage:

```
microtable$cal_abund(
  select_cols = NULL,
  rel = TRUE,
  split_group = FALSE,
  split_by = "&&",
  split_column = NULL
)
```

Arguments:

`select_cols` default NULL; numeric vector or character vector of colnames of `microtable$tax_table`; used to select columns to merge and calculate abundances. This is very useful if there are commented columns or some columns with multiple structure that cannot be used directly.

`rel` default TRUE; if TRUE, relative abundance is used; if FALSE, absolute abundance will be summed.

`split_group` default FALSE; if TRUE, split the rows to multiple rows according to one or more columns in `tax_table`. Very useful when multiple mapping info exist.

`split_by` default "&&"; Separator delimiting collapsed values; only useful when `split_group` = TRUE; see `sep` in `separate_rows` function.

`split_column` default NULL; character vector or list; only useful when `split_group` = TRUE; character vector: fixed column or columns used for the splitting in `tax_table` in each abundance calculation; list: containing more character vectors to assign the column names to each calculation, such as `list(c("Phylum"), c("Phylum", "Class"))`.

Returns: taxa_abund in object.

Examples:

```
\donttest{
dataset$cal_abund()
}
```

Method `save_abund()`: Save taxonomic abundance to the computer local place.

Usage:

```
microtable$save_abund(dirpath = "taxa_abund")
```

Arguments:

`dirpath` default "taxa_abund"; directory name to save the taxonomic abundance files.

Examples:

```
\dontrun{
dataset$save_abund(dirpath = "taxa_abund")
}
```

Method `sample_sums()`: Sum the species number for each sample.

Usage:

```
microtable$sample_sums()
```

Returns: species number of samples.

Examples:

```
\donttest{
dataset$sample_sums()
}
```

Method `taxa_sums()`: Sum the species number for each taxa.

Usage:

```
microtable$taxa_sums()
```

Returns: species number of taxa.

Examples:

```
\donttest{
dataset$taxa_sums()
}
```

Method `sample_names()`: Show sample names.

Usage:

```
microtable$sample_names()
```

Returns: sample names.

Examples:

```
\donttest{
dataset$sample_names()
}
```

Method `taxa_names()`: Show taxa names of tax_table.

Usage:

```
microtable$taxa_names()
```

Returns: taxa names.

Examples:

```
\donttest{
dataset$taxa_names()
}
```

Method `rename_taxa()`: Rename the taxa, including the rownames of otu_table, rownames of tax_table, tip labels of phylo_tree and rep_fasta.

Usage:

```
microtable$rename_taxa(newname_prefix = "ASV_")
```

Arguments:

`newname_prefix` default "ASV_"; the prefix of new names; new names will be `newname_prefix` + numbers according to the rowname order of otu_table.

Returns: renamed dataset.

Examples:

```
\donttest{
dataset$rename_taxa()
}
```

Method `merge_samples()`: Merge samples according to specific group to generate a new microtable.

Usage:

```
microtable$merge_samples(use_group)
```

Arguments:

`use_group` the group column in `sample_table`.

Returns: a new merged microtable object.

Examples:

```
\donttest{
dataset$merge_samples(use_group = "Group")
}
```

Method `merge_taxa()`: Merge taxa according to specific taxonomic rank to generate a new microtable.

Usage:

```
microtable$merge_taxa(taxa = "Genus")
```

Arguments:

`taxa` the specific rank in `tax_table`.

Returns: a new merged microtable object.

Examples:

```
\donttest{
dataset$merge_taxa(taxa = "Genus")
}
```

Method `cal_alphaDiv()`: Calculate alpha diversity in microtable object.

Usage:

```
microtable$cal_alphaDiv(measures = NULL, PD = FALSE)
```

Arguments:

`measures` default `NULL`; one or more indexes from "Observed", "Coverage", "Chao1", "ACE", "Shannon", "Simpson", "InvSimpson", "Fisher", "PD"; If `null`, use all those measures. 'Shannon', 'Simpson' and 'InvSimpson' are calculated based on `vegan::diversity` function; 'Chao1' and 'ACE' depend on the function `vegan::estimateR`; 'PD' depends on the function `picante::pd`.

`PD` `TRUE` or `FALSE`, whether phylogenetic tree should be calculated, default `FALSE`.

Returns: alpha_diversity stored in object.

Examples:

```
\donttest{
dataset$cal_alphaalphadiv(measures = NULL, PD = FALSE)
class(dataset$alpha_diversity)
}
```

Method `save_alphaalphadiv()`: Save alpha diversity table to the computer.

Usage:

```
microtable$save_alphaalphadiv(dirpath = "alpha_diversity")
```

Arguments:

`dirpath` default "alpha_diversity"; directory name to save the `alpha_diversity.csv` file.

Method `cal_betaalphadiv()`: Calculate beta diversity in microtable object, including Bray-Curtis, Jaccard, and UniFrac. See An et al. (2019) <doi:10.1016/j.geoderma.2018.09.035> and Lozupone et al. (2005) <doi:10.1128/AEM.71.12.8228–8235.2005>.

Usage:

```
microtable$cal_betaalphadiv(method = NULL, unifrac = FALSE, binary = FALSE, ...)
```

Arguments:

`method` default NULL; a character vector with one or more elements; If default, "bray" and "jaccard" will be used; see `vegdist` function and `method` parameter in `vegan` package.

`unifrac` default FALSE; TRUE or FALSE, whether unifrac index should be calculated.

`binary` default FALSE; TRUE is used for jaccard and unweighted unifrac; optional for other indexes.

... parameters passed to `vegdist` function.

Returns: beta_diversity stored in object.

Examples:

```
\donttest{
dataset$cal_betaalphadiv(unifrac = FALSE)
class(dataset$beta_diversity)
}
```

Method `save_betaalphadiv()`: Save beta diversity matrix to the computer.

Usage:

```
microtable$save_betaalphadiv(dirpath = "beta_diversity")
```

Arguments:

`dirpath` default "beta_diversity"; directory name to save the beta diversity matrix files.

Method `print()`: Print the microtable object.

Usage:

```
microtable$print()
```

Method `clone()`: The objects of this class are cloneable with this method.

Usage:

```
microtable$clone(deep = FALSE)
```

Arguments:

`deep` Whether to make a deep clone.

Examples

```
## -----
## Method `microtable$new`
## -----  
  
data(otu_table_16S)
data(taxonomy_table_16S)
data(sample_info_16S)
data(phylo_tree_16S)
dataset <- microtable$new(otu_table = otu_table_16S)
dataset <- microtable$new(sample_table = sample_info_16S, otu_table = otu_table_16S,
  tax_table = taxonomy_table_16S, phylo_tree = phylo_tree_16S)
# trim the files in the dataset
dataset$tidy_dataset()  
  
## -----
## Method `microtable$filter_pollution`
## -----  
  
dataset$filter_pollution(taxa = c("mitochondria", "chloroplast"))  
  
## -----
## Method `microtable$rarefy_samples`
## -----  
  
dataset$rarefy_samples(sample.size = min(dataset$sample_sums()), replace = TRUE)  
  
## -----
## Method `microtable$tidy_dataset`
## -----  
  
dataset$tidy_dataset(main_data = TRUE)  
  
## -----
## Method `microtable$add_rownames2taxonomy`
## -----  
  
dataset$add_rownames2taxonomy()  
  
## -----
## Method `microtable$cal_abund`
## -----  
  
dataset$cal_abund()
```

```
## -----
## Method `microtable$save_abund`
## -----  
  
## Not run:  
dataset$save_abund(dirpath = "taxa_abund")  
  
## End(Not run)  
  
## -----
## Method `microtable$sample_sums`
## -----  
  
dataset$sample_sums()  
  
## -----
## Method `microtable$taxa_sums`
## -----  
  
dataset$taxa_sums()  
  
## -----
## Method `microtable$sample_names`
## -----  
  
dataset$sample_names()  
  
## -----
## Method `microtable$taxa_names`
## -----  
  
dataset$taxa_names()  
  
## -----
## Method `microtable$rename_taxa`
## -----  
  
dataset$rename_taxa()  
  
## -----
## Method `microtable$merge_samples`
## -----
```

```
dataset$merge_samples(use_group = "Group")

## -----
## Method `microtable$merge_taxa`
## -----


dataset$merge_taxa(taxa = "Genus")

## -----
## Method `microtable$cal_alphaalphadiv`
## -----


dataset$cal_alphaalphadiv(measures = NULL, PD = FALSE)
class(dataset$alpha_diversity)

## -----
## Method `microtable$cal_betaalphadiv`
## -----


dataset$cal_betaalphadiv(unifrac = FALSE)
class(dataset$beta_diversity)
```

otu_table_16S

The OTU table of the 16S dataset in the microeco package

Description

The OTU table of the 16S dataset in the microeco package

Usage

```
data(otu_table_16S)
```

otu_table_ITS

The OTU table of the ITS dataset in the microeco package

Description

The OTU table of the ITS dataset in the microeco package

Usage

```
data(otu_table_ITS)
```

phylo_tree_16S *The phylogenetic tree of 16S dataset in the microeco package*

Description

The phylogenetic tree of 16S dataset in the microeco package

Usage

```
data(phylo_tree_16S)
```

prok_func_FAPROTAX *The modified FAPROTAX trait database in the microeco package*

Description

The modified FAPROTAX trait database in the microeco package

Usage

```
data(prok_func_FAPROTAX)
```

prok_func_NJC19_list *The modified NJC19 database in the microeco package*

Description

The modified NJC19 database in the microeco package

Usage

```
data(prok_func_NJC19_list)
```

sample_info_16S *The sample information of 16S dataset in the microeco package*

Description

The sample information of 16S dataset in the microeco package

Usage

```
data(sample_info_16S)
```

sample_info_ITS *The sample information of ITS dataset in the microeco package*

Description

The sample information of ITS dataset in the microeco package

Usage

```
data(sample_info_ITS)
```

Tax4Fun2_KEGG *The KEGG data files used in the cal_tax4fun2 function of trans_func class.*

Description

The KEGG data files used in the cal_tax4fun2 function of trans_func class.

Usage

```
data(Tax4Fun2_KEGG)
```

taxonomy_table_16S *The taxonomic information of 16S dataset in the microeco package*

Description

The taxonomic information of 16S dataset in the microeco package

Usage

```
data(taxonomy_table_16S)
```

<code>taxonomy_table_ITS</code>	<i>The taxonomic information of ITS dataset in the microeco package</i>
---------------------------------	---

Description

The taxonomic information of ITS dataset in the microeco package

Usage

```
data(taxonomy_table_ITS)
```

<code>tidy_taxonomy</code>	<i>Clean up the taxonomic table to make taxonomic assignments consistent.</i>
----------------------------	---

Description

Clean up the taxonomic table to make taxonomic assignments consistent.

Usage

```
tidy_taxonomy(
  taxonomy_table,
  column = "all",
  pattern = c(".*uncultur.*", ".*unknown.*", ".*unidentif.*", ".*unclassified.*",
             ".*No blast hit.*", ".*sp\\.$", ".*metagenome.*", ".*cultivar.*", ".*archaeon$",
             "__synthetic.*", ".*\\sbacterium$", ".*bacterium\\s.*", ".*Incertae.sedis.*"),
  replacement = "",
  ignore.case = TRUE,
  na_fill = ""
)
```

Arguments

<code>taxonomy_table</code>	a data.frame with taxonomic information.
<code>column</code>	default "all"; "all" or a number; 'all' represents cleaning up all the columns; a number represents cleaning up this column.
<code>pattern</code>	default see the function parameter; the characters (regular expression) to be cleaned up or replaced; cleaned up when parameter replacement = "", replaced when parameter replacement has something; Note that the capital and small letters are not distinguished.
<code>replacement</code>	default ""; the characters used to replace the character in pattern parameter.
<code>ignore.case</code>	default TRUE; if FALSE, the pattern matching is case sensitive and if TRUE, case is ignored during matching.
<code>na_fill</code>	default ""; used to replace the NA.

Format

`data.frame` object.

Value

taxonomic table.

Examples

```
data("taxonomy_table_16S")
tidy_taxonomy(taxonomy_table_16S)
```

trans_abund

Create trans_abund object for plotting taxonomic abundance.

Description

This class is a wrapper for the taxonomic abundance transformations and plotting. The transformed data style is the long-format for ggplot2 plotting. The plotting methods include bar plot, boxplot, heatmap and pie chart.

Methods**Public methods:**

- `trans_abund$new()`
- `trans_abund$plot_bar()`
- `trans_abund$plot_heatmap()`
- `trans_abund$plot_box()`
- `trans_abund$plot_line()`
- `trans_abund$plot_pie()`
- `trans_abund$print()`
- `trans_abund$clone()`

Method new():

Usage:

```
trans_abund$new(
  dataset = NULL,
  taxrank = "Phylum",
  show = 0,
  ntaxa = 10,
  groupmean = NULL,
  delete_full_prefix = TRUE,
  delete_part_prefix = FALSE,
  prefix = NULL,
  use_percentage = TRUE,
  input_taxaname = NULL
)
```

Arguments:

`dataset` default NULL; the object of `microtable` class.
`taxrank` default "Phylum"; taxonomic rank.
`show` default 0; the relative abundance threshold used for filtering.
`ntaxa` default 10; how many taxa will be used, ordered by abundance from high to low; this parameter does not conflict with the parameter `show`; both can be used.
`groupmean` default NULL; calculating mean abundance for each group; select a group column name in `microtable$sample_table`.
`delete_full_prefix` default TRUE; whether delete both the prefix of taxonomy and the character in front of them.
`delete_part_prefix` default FALSE; whether only delete the prefix of taxonomy.
`prefix` default NULL; character string; can be used when `delete_full_prefix = T` or `delete_part_prefix = T`; default NULL represents using the "letter+__", e.g. "k__" for Phylum level; Please alter this parameter when the prefix is not standard.
`use_percentage` default TRUE; show the abundance percentage.
`input_taxaname` default NULL; character vector; input taxa names for selecting some taxa.

Returns: `data_abund` stored in the object.

Examples:

```
\donttest{
data(dataset)
t1 <- trans_abund$new(dataset = dataset, taxrank = "Phylum", ntaxa = 10)
}
```

Method `plot_bar()`: Bar plot.

Usage:

```
trans_abund$plot_bar(
  color_values = RColorBrewer::brewer.pal(12, "Paired"),
  bar_type = "full",
  others_color = "grey90",
  facet = NULL,
  facet2 = NULL,
  facet3 = NULL,
  facet4 = NULL,
  order_facet = NULL,
  order_x = NULL,
  x_axis_name = NULL,
  barwidth = NULL,
  use_alluvium = FALSE,
  clustering = FALSE,
  facet_color = "grey95",
  strip_text = 11,
  legend_text_italic = FALSE,
  xtext_type_hor = TRUE,
  xtext_size = 10,
  xtext_keep = TRUE,
  xtitle_keep = TRUE,
```

```

    ytitle_size = 17,
    ylab_title = NULL
)
Arguments:
color_values default RColorBrewer::brewer.pal(12, "Paired"); colors palette for the plotting.
bar_type default "full"; "full" or "notfull"; if full, the total abundance sum to 1 or 100 percentage.
others_color default "grey90"; the color for "others" taxa.
facet default NULL; if using facet, providing a group column name of sample_table, such as, "Group".
facet2 default NULL; the second facet, used with facet parameter together; facet2 should have a finer scale; use this parameter, please first install package ggh4x using install.packages("ggh4x").
facet3 default NULL; the third facet, used with facet and facet2 parameter together.
facet4 default NULL; the fourth facet, used with facet, facet2 and facet3 parameter together.
order_facet NULL; vector; used to order the facet, such as, c("Group1", "Group3", "Group2");
If multiple facets are used, please manually assign factors in sample_table of microtable object.
order_x default NULL; vector; used to order the sample names in x axis; must be the samples vector, such as, c("S1", "S3", "S2").
x_axis_name NULL; a character string; a column name of sample_table in dataset; used to show the sample names in x axis.
barwidth default NULL; bar width, see width in geom\_bar.
use_alluvium default FALSE; whether add alluvium plot
clustering default FALSE; whether order samples by the clustering
facet_color default "grey95"; facet background color.
strip_text default 11; facet text size.
legend_text_italic default FALSE; whether use italic in legend.
xtext_type_hor default TRUE; x axis text horizontal, if FALSE; text slant.
xtext_size default 10; x axis text size.
xtext_keep default TRUE; whether retain x text.
xtitle_keep default TRUE; whether retain x title.
ytitle_size default 17; y axis title size.
ylab_title default NULL; y axis title.
```

Returns: ggplot2 plot.

Examples:

```
\donttest{
t1$plot_bar(facet = "Group", xtext_keep = FALSE)
}
```

Method `plot_heatmap()`: Plot the heatmap.

Usage:

```

trans_abund$plot_heatmap(
  color_values = rev(RColorBrewer::brewer.pal(n = 11, name = "RdYlBu")),
  facet = NULL,
  facet2 = NULL,
  facet3 = NULL,
  facet4 = NULL,
  order_facet = NULL,
  x_axis_name = NULL,
  order_x = NULL,
  withmargin = TRUE,
  plot_numbers = FALSE,
  plot_text_size = 4,
  plot_breaks = NULL,
  margincolor = "white",
  plot_colorscale = "log10",
  min_abundance = 0.01,
  max_abundance = NULL,
  strip_text = 11,
  xtext_size = 10,
  ytext_size = 11,
  xtext_keep = TRUE,
  xtitle_keep = TRUE,
  grid_clean = TRUE,
  xtext_type_hor = TRUE,
  legend_title = "% Relative\nAbundance",
  pheatmap = FALSE,
  ...
)}

```

Arguments:

color_values default rev(RColorBrewer::brewer.pal(n = 11, name = "RdYlBu")); colors palette for the plotting.

facet default NULL; a character string; if using facet, provide a column name in *sample_table*, such as "Group".

facet2 default NULL; the second facet, used with facet parameter together; *facet2* should have a finer scale; use this parameter, please first install package ggh4x.

facet3 default NULL; the third facet, used with facet and *facet2* parameter together.

facet4 default NULL; the fourth facet, used with facet, *facet2* and *facet3* parameter together.

order_facet NULL; vector; used to order the facet, such as, c("Group1", "Group3", "Group2").

x_axis_name NULL; a character string; a column name of *sample_table* used to show the sample names in x axis.

order_x default NULL; vector; used to order the sample names in x axis; must be the samples vector, such as, c("S1", "S3", "S2").

withmargin default TRUE; whether retain the tile margin.

plot_numbers default FALSE; whether plot the number in heatmap.

plot_text_size default 4; If *plot_numbers* TRUE, text size in plot.

plot_breaks default NULL; The legend breaks.

margincolor default "white"; If withmargin TRUE, use this as the margin color.
 plot_colorscale default "log10"; color scale.
 min_abundance default .01; the minimum abundance percentage in plot.
 max_abundance default NULL; the maximum abundance percentage in plot, NULL represent
 the max percentage.
 strip_text default 11; facet text size.
 xtext_size default 10; x axis text size.
 ytext_size default 11; y axis text size.
 xtext_keep default TRUE; whether retain x text.
 xtitle_keep default TRUE; whether retain x title.
 grid_clean default TRUE; whether remove grid lines.
 xtext_type_hor default TRUE; x axis text horizontal, if FALSE; text slant.
 legend_title default "% Relative\nAbundance"; legend title text.
 pheatmap default FALSE; whether use pheatmap package to plot the heatmap.
 ... paremeters pass to pheatmap when pheatmap = TRUE.

Returns: ggplot2 plot or grid plot based on pheatmap.

Examples:

```
\donttest{
t1 <- trans_abund$new(dataset = dataset, taxrank = "Genus", ntaxa = 40)
t1$plot_heatmap(facet = "Group", xtext_keep = FALSE, withmargin = FALSE)
}
```

Method plot_box(): Box plot.

Usage:

```
trans_abund$plot_box(
  color_values = RColorBrewer::brewer.pal(8, "Dark2"),
  group = NULL,
  show_point = FALSE,
  point_color = "black",
  point_size = 3,
  point_alpha = 0.3,
  plot_flip = FALSE,
  boxfill = TRUE,
  middlecolor = "grey95",
  middlesize = 1,
  xtext_type_hor = FALSE,
  xtext_size = 10,
  xtext_keep = TRUE,
  xtitle_keep = TRUE,
  ytitle_size = 17,
  ...
)
```

Arguments:

color_values default RColorBrewer::brewer.pal(8, "Dark2"); colors palette for the plotting.

```

group default NULL; a column name of sample table to show abundance across groups.
show_point default FALSE; whether show points in plot.
point_color default "black"; If show_point TRUE; use the color
point_size default 3; If show_point TRUE; use the size
point_alpha default .3; If show_point TRUE; use the transparency.
plot_flip default FALSE; Whether rotate plot.
boxfill default TRUE; Whether fill the box with colors.
middlecolor default "grey95"; The middle line color.
middlesize default 1; The middle line size.
xtext_type_hor default TRUE; x axis text horizontal, if FALSE; text slant.
xtext_size default 10; x axis text size.
xtext_keep default TRUE; whether retain x text.
xtitle_keep default TRUE; whether retain x title.
ytitle_size default 17; y axis title size.
... parameters pass to geom\_boxplot.

```

Returns: ggplot2 plot.

Examples:

```
\donttest{
t1$plot_box(group = "Group")
}
```

Method `plot_line()`: Plot the line chart.

Usage:

```

trans_abund$plot_line(
  color_values = RColorBrewer::brewer.pal(8, "Dark2"),
  plot_SE = TRUE,
  position = position_dodge(0.1),
  errorbar_size = 1,
  errorbar_width = 0.1,
  point_size = 3,
  point_alpha = 0.8,
  line_size = 0.8,
  line_alpha = 0.8,
  line_type = 1,
  xtext_type_hor = FALSE,
  xtext_size = 10,
  ytitle_size = 17
)

```

Arguments:

```

color_values default RColorBrewer::brewer.pal(8, "Dark2"); colors palette for the plotting.
plot_SE default TRUE; TRUE: plot the errorbar with mean±se; FALSE: plot the errorbar with mean±sd.
position default position_dodge(0.1); Position adjustment, either as a string (such as "identity"), or the result of a call to a position adjustment function.

```

```

errorbar_size default 1; errorbar size.
errorbar_width default 0.1; errorbar width.
point_size default 3; point size for taxa.
point_alpha default 0.8; point transparency.
line_size default 0.8; line size.
line_alpha default 0.8; line transparency.
line_type default 1; an integer; line type.
xtext_type_hor default TRUE; x axis text horizontal, if FALSE; text slant.
xtext_size default 10; x axis text size.
ytitle_size default 17; y axis title size.

```

Returns: ggplot2 plot.

Examples:

```
\donttest{
t1 <- trans_abund$new(dataset = dataset, taxrank = "Genus", ntaxa = 5)
t1$plot_line(point_size = 3)
t1 <- trans_abund$new(dataset = dataset, taxrank = "Genus", ntaxa = 5, groupmean = "Group")
t1$plot_line(point_size = 5, errorbar_size = 1, xtext_type_hor = TRUE)
}
```

Method `plot_pie()`: Plot pie chart.

Usage:

```
trans_abund$plot_pie(
  color_values = RColorBrewer::brewer.pal(8, "Dark2"),
  facet_nrow = 1,
  strip_text = 11,
  legend_text_italic = FALSE
)
```

Arguments:

```

color_values default RColorBrewer::brewer.pal(8, "Dark2"); colors palette for the plotting.
facet_nrow default 1; how many rows in the plot.
strip_text default 11; sample title size.
legend_text_italic default FALSE; whether use italic in legend.

```

Returns: ggplot2 plot.

Examples:

```
\donttest{
t1 <- trans_abund$new(dataset = dataset, taxrank = "Phylum", ntaxa = 6, groupmean = "Group")
t1$plot_pie(facet_nrow = 1)
}
```

Method `print()`: Print the trans_abund object.

Usage:

```
trans_abund$print()
```

Method clone(): The objects of this class are cloneable with this method.

Usage:

```
trans_abund$clone(deep = FALSE)
```

Arguments:

deep Whether to make a deep clone.

Examples

```
## -----
## Method `trans_abund$new`
## -----


data(dataset)
t1 <- trans_abund$new(dataset = dataset, taxrank = "Phylum", ntaxa = 10)

## -----
## Method `trans_abund$plot_bar`
## -----


t1$plot_bar(facet = "Group", xtext_keep = FALSE)

## -----
## Method `trans_abund$plot_heatmap`
## -----


t1 <- trans_abund$new(dataset = dataset, taxrank = "Genus", ntaxa = 40)
t1$plot_heatmap(facet = "Group", xtext_keep = FALSE, withmargin = FALSE)

## -----
## Method `trans_abund$plot_box`
## -----


t1$plot_box(group = "Group")

## -----
## Method `trans_abund$plot_line`
## -----


t1 <- trans_abund$new(dataset = dataset, taxrank = "Genus", ntaxa = 5)
t1$plot_line(point_size = 3)
t1 <- trans_abund$new(dataset = dataset, taxrank = "Genus", ntaxa = 5, groupmean = "Group")
```

```
t1$plot_line(point_size = 5, errorbar_size = 1, xtext_type_hor = TRUE)

## -----
## Method `trans_abund$plot_pie`
## -----
```

t1 <- trans_abund\$new(dataset = dataset, taxrank = "Phylum", ntaxa = 6, groupmean = "Group")
t1\$plot_pie(facet_nrow = 1)

trans_alpha*Create trans_alpha object for alpha diversity statistics and plotting.***Description**

This class is a wrapper for a series of alpha diversity related analysis, including the statistics and plotting based on An et al. (2019) <doi:10.1016/j.geoderma.2018.09.035> and Paul et al. (2013) <doi:10.1371/journal.pone.0061217>.

Methods**Public methods:**

- `trans_alpha$new()`
- `trans_alpha$cal_diff()`
- `trans_alpha$plot_alpha()`
- `trans_alpha$print()`
- `trans_alpha$clone()`

Method new():*Usage:*`trans_alpha$new(dataset = NULL, group = NULL, by_group = NULL, order_x = NULL)`*Arguments:*`dataset` the object of `microtable` Class.`group` default NULL; the sample column used for the statistics; If provided, can return `data_stat`.`by_group` default NULL; perform the differential test among groups (`group` parameter) within each group (`by_group` parameter).`order_x` default NULL; a `sample_table` column name or a vector containing sample names; if provided, order samples by using factor.*Returns:* `data_alpha` and `data_stat` stored in the object.*Examples:*

```
\donttest{
data(dataset)
t1 <- trans_alpha$new(dataset = dataset, group = "Group")
}
```

Method cal_diff(): Test the difference of alpha diversity.

Usage:

```
trans_alpha$cal_diff(
  method = c("KW", "KW_dunn", "wilcox", "t.test", "anova", "scheirerRayHare")[1],
  measure = NULL,
  p_adjust_method = "fdr",
  formula = NULL,
  ...
)
```

Arguments:

method default "KW"; see the following available options:

'**KW**' Kruskal-Wallis Rank Sum Test for all groups (>= 2)

'**KW_dunn**' Dunn's Kruskal-Wallis Multiple Comparisons, see dunnTest function in FSA package

'**wilcox**' Wilcoxon Rank Sum and Signed Rank Tests for all paired groups

'**t.test**' Student's t-Test for all paired groups

'**anova**' Duncan's new multiple range test for one-way anova; see duncan.test function of agricolae package. For multi-factor anova, see **aov**

'**scheirerRayHare**' Scheirer Ray Hare test for nonparametric test used for a two-way factorial experiment; see scheirerRayHare function of rcompanion package

measure default NULL; a vector; if null, all indexes will be calculated; see names of microtable\$alpha_diversity, e.g. Observed, Chao1, ACE, Shannon, Simpson, InvSimpson, Fisher, Coverage, PD.

p_adjust_method default "fdr"; p.adjust method; see method parameter of p.adjust function for available options; NULL can disuse the p value adjustment.

formula default NULL; applied to two-way or multi-factor anova analysis when method = "anova" or "scheirerRayHare"; specified set for independent variables, i.e. the latter part of the formula in **aov**, such as 'block + N*P*K'.

... parameters passed to kruskal.test or wilcox.test function (method = "KW") or dunnTest function of FSA package (method = "KW_dunn") or agricolae::duncan.test (method = "anova").

Returns: res_diff in object with the format data.frame. In the data frame, 'Group' column means that the group has the maximum median or mean value across the test groups; For non-parametric methods, maximum median value; For t.test, maximum mean value.

Examples:

```
\donttest{
t1$cal_diff(method = "KW")
t1$cal_diff(method = "anova")
t1 <- trans_alpha$new(dataset = dataset, group = "Type", by_group = "Group")
t1$cal_diff(method = "anova")
}
```

Method plot_alpha(): Plotting the alpha diversity.

Usage:

```

trans_alpha$plot_alpha(
  color_values = RColorBrewer::brewer.pal(8, "Dark2"),
  measure = "Shannon",
  group = NULL,
  add_sig = TRUE,
  add_sig_label = "Significance",
  add_sig_text_size = 3.88,
  use_boxplot = TRUE,
  boxplot_add = "jitter",
  order_x_mean = FALSE,
  y_start = 1.01,
  y_increase = 0.05,
  xtext_angle = NULL,
  xtext_size = 15,
  ytitle_size = 17,
  barwidth = 0.9,
  ...
)

```

Arguments:

color_values default RColorBrewer::brewer.pal(8, "Dark2"); color palette for groups.
 measure default Shannon; alpha diversity measurement; see names of alpha_diversity of dataset,
 e.g., Observed, Chao1, ACE, Shannon, Simpson, InvSimpson, Fisher, Coverage, PD.
 group default NULL; group name used for the plot.
 add_sig default TRUE; whether add significance label using the result of cal_diff function, i.e.
 object\$res_diff; This is mainly designed to add post hoc test of anova or Dunn's Kruskal-
 Wallis Multiple Comparisons to make the label adding easy.
 add_sig_label default "Significance"; select a colname of object\$res_diff for the label
 text, such as 'P.adj' or 'Significance'.
 add_sig_text_size default 3.88; the size of text in added label.
 use_boxplot default TRUE; TRUE: boxplot; FALSE: mean-se plot.
 boxplot_add default "jitter"; points type, see the add parameter in ggpibr::ggboxplot.
 order_x_mean default FALSE; whether order x axis by the means of groups from large to
 small.
 y_start default 1.01; the y axis value from which to add the label; the default 1.01 means 1.01
 * max(values).
 y_increase default 0.05; the increasing y axis space to add label; the default 0.05 means 0.05
 * y_start; this parameter is also used to label the letters of anova result with the fixed (1 +
 y_increase) * y_start space.
 xtext_angle default NULL; number (e.g. 30) used to make x axis text generate angle.
 xtext_size default 15; x axis text size.
 ytitle_size default 17; y axis title size.
 barwidth default 0.9; the bar width in plot; applied when by_group is not NULL.
 ... parameters pass to ggpibr::ggboxplot function.

Returns: ggplot.

Examples:

```
\donttest{
t1 <- trans_alpha$new(dataset = dataset, group = "Group")
t1$cal_diff(method = "wilcox")
t1$plot_alpha(measure = "Shannon", add_sig = TRUE)
t1 <- trans_alpha$new(dataset = dataset, group = "Type", by_group = "Group")
t1$cal_diff(method = "wilcox")
t1$plot_alpha(measure = "Shannon", add_sig = TRUE)
}
```

Method `print()`: Print the `trans_alpha` object.

Usage:

```
trans_alpha$print()
```

Method `clone()`: The objects of this class are cloneable with this method.

Usage:

```
trans_alpha$clone(deep = FALSE)
```

Arguments:

`deep` Whether to make a deep clone.

Examples

```
## -----
## Method `trans_alpha$new`
## -----


data(dataset)
t1 <- trans_alpha$new(dataset = dataset, group = "Group")

## -----
## Method `trans_alpha$cal_diff`
## -----


t1$cal_diff(method = "KW")
t1$cal_diff(method = "anova")
t1 <- trans_alpha$new(dataset = dataset, group = "Type", by_group = "Group")
t1$cal_diff(method = "anova")

## -----
## Method `trans_alpha$plot_alpha`
## -----


t1 <- trans_alpha$new(dataset = dataset, group = "Group")
t1$cal_diff(method = "wilcox")
t1$plot_alpha(measure = "Shannon", add_sig = TRUE)
```

```
t1 <- trans_alpha$new(dataset = dataset, group = "Type", by_group = "Group")
t1$cal_diff(method = "wilcox")
t1$plot_alpha(measure = "Shannon", add_sig = TRUE)
```

trans_beta

Create trans_beta object for beta-diversity analysis based on the distance matrix

Description

This class is a wrapper for a series of beta-diversity related analysis, including several ordination calculations and plotting based on An et al. (2019) <doi:10.1016/j.geoderma.2018.09.035>, group distance comparision, clustering, perMANOVA based on Anderson al. (2008) <doi:10.1111/j.1442-9993.2001.01070.pp.x> and PERMDISP.

Methods

Public methods:

- `trans_beta$new()`
- `trans_beta$cal_ordination()`
- `trans_beta$plot_ordination()`
- `trans_beta$cal_manova()`
- `trans_beta$cal_betadisper()`
- `trans_beta$cal_group_distance()`
- `trans_beta$cal_group_distance_diff()`
- `trans_beta$plot_group_distance()`
- `trans_beta$plot_clustering()`
- `trans_beta$clone()`

Method new():

Usage:

```
trans_beta$new(dataset = NULL, measure = NULL, group = NULL)
```

Arguments:

`dataset` the object of `microtable` class.

`measure` default NULL; bray, jaccard, wei_unifrac or unwei_unifrac, or other name of matrix you add in `microtable$beta_diversity`; used for ordination, manova or group distance. The measure must be one of names of `microtable$beta_diversity` list. Please see `microtable$cal_betadiv` function for more details.

`group` default NULL; sample group used for manova, betadisper or group distance.

`Returns:` parameters stored in the object.

Examples:

```
data(dataset)
t1 <- trans_beta$new(dataset = dataset, measure = "bray", group = "Group")
```

Method `cal_ordination()`: Ordination based on An et al. (2019) <doi:10.1016/j.geoderma.2018.09.035>.

Usage:

```
trans_beta$cal_ordination(
  ordination = "PCoA",
  ncomp = 3,
  trans_otu = FALSE,
  scale_species = FALSE
)
```

Arguments:

`ordination` default "PCoA"; "PCA", "PCoA" or "NMDS". PCA: principal component analysis; PCoA: principal coordinates analysis; NMDS: non-metric multidimensional scaling.
`ncomp` default 3; the returned dimensions.

`trans_otu` default FALSE; whether species abundance will be square transformed, used for PCA.

`scale_species` default FALSE; whether species loading in PCA will be scaled.

Returns: `res_ordination` stored in the object.

Examples:

```
t1$cal_ordination(ordination = "PCoA")
```

Method `plot_ordination()`: Plotting the ordination result based on An et al. (2019) <doi:10.1016/j.geoderma.2018.09.0.

Usage:

```
trans_beta$plot_ordination(
  plot_type = "point",
  color_values = RColorBrewer::brewer.pal(8, "Dark2"),
  shape_values = c(16, 17, 7, 8, 15, 18, 11, 10, 12, 13, 9, 3, 4, 0, 1, 2, 14),
  plot_color = NULL,
  plot_shape = NULL,
  plot_group_order = NULL,
  add_sample_label = NULL,
  point_size = 3,
  point_alpha = 0.8,
  centroid_segment_alpha = 0.6,
  centroid_segment_size = 1,
  centroid_segment_linetype = 3,
  ellipse_chull_fill = TRUE,
  ellipse_chull_alpha = 0.1,
  ellipse_level = 0.9,
  ellipse_type = "t"
)
```

Arguments:

`plot_type` default "point"; one or more elements of "point", "ellipse", "chull" and "centroid".

'point' add point

'ellipse' add confidence ellipse for points of each group

'chull' add convex hull for points of each group

'centroid' add centroid line of each group

```

color_values default RColorBrewer::brewer.pal(8, "Dark2"); colors palette for different
groups.
shape_values default c(16, 17, 7, 8, 15, 18, 11, 10, 12, 13, 9, 3, 4, 0, 1, 2, 14); a vector for
point shape types of groups, see ggplot2 tutorial.
plot_color default NULL; a colname of sample_table to assign colors to different groups in
plot.
plot_shape default NULL; a colname of sample_table to assign shapes to different groups in
plot.
plot_group_order default NULL; a vector used to order the groups in the legend of plot.
add_sample_label default NULL; the column name in sample table, if provided, show the
point name in plot.
point_size default 3; point size in plot when "point" is in plot_type.
point_alpha default .8; point transparency in plot when "point" is in plot_type.
centroid_segment_alpha default 0.6; segment transparency in plot when "centroid" is in
plot_type.
centroid_segment_size default 1; segment size in plot when "centroid" is in plot_type.
centroid_segment_linetype default 3; the line type related with centroid in plot when "cen-
troid" is in plot_type.
ellipse_chull_fill default TRUE; whether fill colors to the area of ellipse or chull.
ellipse_chull_alpha default 0.1; color transparency in the ellipse or convex hull depending
on whether "ellipse" or "centroid" is in plot_type.
ellipse_level default .9; confidence level of ellipse when "ellipse" is in plot_type.
ellipse_type default "t"; ellipse type when "ellipse" is in plot_type; see type in stat\_ellipse.

```

Returns: ggplot.

Examples:

```

t1$plot_ordination(plot_type = "point")
t1$plot_ordination(plot_color = "Group", plot_shape = "Group", plot_type = "point")
t1$plot_ordination(plot_color = "Group", plot_type = c("point", "ellipse"))
t1$plot_ordination(plot_color = "Group", plot_type = c("point", "chull"))
t1$plot_ordination(plot_color = "Group", plot_type = c("point", "centroid"),
  centroid_segment_linetype = 1)

```

Method cal_manova(): Calculate perMANOVA based on <doi:10.1111/j.1442-9993.2001.01070.pp.x> and R vegan adonis2 function.

Usage:

```

trans_beta$cal_manova(
  manova_all = TRUE,
  manova_set = NULL,
  group = NULL,
  p_adjust_method = "fdr",
  ...
)

```

Arguments:

manova_all default TRUE; TRUE represents test for all the groups, i.e. the overall test; FALSE represents test for all the paired groups.

`manova_set` default NULL; other specified group set for manova, such as "Group + Type" and "Group*Type"; see also [adonis2](#).
`group` default NULL; a column name of sample_table used for manova. If NULL, search group stored in the object.
`p_adjust_method` default "fdr"; p.adjust method when `manova_all = FALSE`; see method parameter of `p.adjust` function for available options.
... parameters passed to [adonis2](#) function of vegan package.

Returns: `res_manova` stored in object.

Examples:

```
t1$cal_manova(manova_all = TRUE)
```

Method `cal_betadisper()`: A wrapper for `betadisper` function in vegan package for multivariate homogeneity test of groups dispersions.

Usage:

```
trans_beta$cal_betadisper(...)
```

Arguments:

... parameters passed to [betadisper](#) function.

Returns: `res_betadisper` stored in object.

Examples:

```
t1$cal_betadisper()
```

Method `cal_group_distance()`: Transform sample distances within groups or between groups.

Usage:

```
trans_beta$cal_group_distance(within_group = TRUE, by_group = NULL)
```

Arguments:

`within_group` default TRUE; whether transform sample distance within groups, if FALSE, transform sample distance between any two groups.

`by_group` default NULL; one colname name of sample_table in microtable object. If provided, transform distances by the provided `by_group` parameter. This is especially useful for ordering and filtering values further. When `within_group = TRUE`, `by_group` parameter is the format of paired groups. When `within_group = FALSE`, `by_group` parameter is the format same with the group information in sample_table.

Returns: `res_group_distance` stored in object.

Examples:

```
\donttest{
t1$cal_group_distance(within_group = TRUE)
}
```

Method `cal_group_distance_diff()`: Differential test of distances among groups.

Usage:

```
trans_beta$cal_group_distance_diff(comp_bygroup = FALSE, ...)
```

Arguments:

`comp_bygroup` default FALSE; whether perform the differential test using `by_group` column coming from the return of function `cal_group_distance`.

... parameters passed to `cal_diff` function of `trans_alpha` class.

Returns: `res_group_distance_diff` stored in object.

Examples:

```
\donttest{
t1$cal_group_distance_diff()
}
```

Method `plot_group_distance()`: Plotting the distance between samples within or between groups.

Usage:

```
trans_beta$plot_group_distance(plot_group_order = NULL, ...)
```

Arguments:

`plot_group_order` default NULL; a vector used to order the groups in the plot.

... parameters (except measure) passed to `plot_alpha` function of `trans_alpha` class.

Returns: ggplot.

Examples:

```
\donttest{
t1$plot_group_distance()
}
```

Method `plot_clustering()`: Plotting clustering result based on the `ggdendro` package.

Usage:

```
trans_beta$plot_clustering(
  use_colors = RColorBrewer::brewer.pal(8, "Dark2"),
  measure = NULL,
  group = NULL,
  replace_name = NULL
)
```

Arguments:

`use_colors` colors for presentation.

`measure` default NULL; beta diversity index; If NULL, using the measure when creating object
`group` default NULL; if provided, use this group to assign color.

`replace_name` default NULL; if provided, use this as label.

Returns: ggplot.

Examples:

```
t1$plot_clustering(group = "Group", replace_name = c("Saline", "Type"))
```

Method `clone()`: The objects of this class are cloneable with this method.

Usage:

```
trans_beta$clone(deep = FALSE)
```

Arguments:

`deep` Whether to make a deep clone.

Examples

```

## -----
## Method `trans_beta$new`
## -----


data(dataset)
t1 <- trans_beta$new(dataset = dataset, measure = "bray", group = "Group")

## -----
## Method `trans_beta$cal_ordination`
## -----


t1$cal_ordination(ordination = "PCoA")

## -----
## Method `trans_beta$plot_ordination`
## -----


t1$plot_ordination(plot_type = "point")
t1$plot_ordination(plot_color = "Group", plot_shape = "Group", plot_type = "point")
t1$plot_ordination(plot_color = "Group", plot_type = c("point", "ellipse"))
t1$plot_ordination(plot_color = "Group", plot_type = c("point", "chull"))
t1$plot_ordination(plot_color = "Group", plot_type = c("point", "centroid"),
                    centroid_segment_linetype = 1)

## -----
## Method `trans_beta$cal_manova`
## -----


t1$cal_manova(manova_all = TRUE)

## -----
## Method `trans_beta$cal_betadisper`
## -----


t1$cal_betadisper()

## -----
## Method `trans_beta$cal_group_distance`
## -----


t1$cal_group_distance(within_group = TRUE)

## -----
## Method `trans_beta$cal_group_distance_diff`
## -----


t1$cal_group_distance_diff()

```

```
## -----
## Method `trans_beta$plot_group_distance`
## -----  
  
t1$plot_group_distance()  
  
## -----
## Method `trans_beta$plot_clustering`
## -----  
  
t1$plot_clustering(group = "Group", replace_name = c("Saline", "Type"))
```

trans_classifier *Create trans_classifier object for machine-learning-based model prediction.*

Description

This class is a wrapper for methods of machine-learning-based classification models, including data pre-processing, feature selection, data split, model training, prediction, confusionMatrix and ROC (Receiver Operator Characteristic) or PR (Precision-Recall) curve.

Author(s): Felipe Mansoldo and Chi Liu

Methods

Public methods:

- `trans_classifier$new()`
- `trans_classifier$cal_preProcess()`
- `trans_classifier$cal_feature_sel()`
- `trans_classifier$cal_split()`
- `trans_classifier$set_trainControl()`
- `trans_classifier$cal_train()`
- `trans_classifier$cal_feature_imp()`
- `trans_classifier$cal_predict()`
- `trans_classifier$plot_confusionMatrix()`
- `trans_classifier$cal_ROC()`
- `trans_classifier$plot_ROC()`
- `trans_classifier$clone()`

Method new(): Create the trans_classifier object.

Usage:

```
trans_classifier$new(
  dataset = NULL,
  x.predictors = "all",
  y.response = NULL,
  n.cores = 1
)
```

Arguments:

dataset the object of [microtable](#) Class.

x.predictors default "all"; character string or data.frame; a character string represents selecting the corresponding data from microtable\$taxa_abund; data.frame represents other customized data. See the following available options:

- '**all**' use all the taxa stored in microtable\$taxa_abund
- '**Genus**' use Genus level table in microtable\$taxa_abund, or other specific taxonomic rank, e.g. 'Phylum'

other input must be a data.frame; It should have the same format with the data.frame in microtable\$taxa_abund, i.e. rows are features; cols are samples with same names in sample_table

y.response default NULL; the response variable in sample_table.

n.cores default 1; the CPU thread used.

Returns: data_feature and data_response in the object.

Examples:

```
\donttest{
data(dataset)
t1 <- trans_classifier$new(
dataset = dataset,
x.predictors = "Genus",
y.response = "Group")
}
```

Method cal_preProcess(): Pre-process (centering, scaling etc.) of the feature data based on the caret::preProcess function. See <https://topepo.github.io/caret/pre-processing.html> for more details.

Usage:

```
trans_classifier$cal_preProcess(...)
```

Arguments:

... parameters pass to preProcess function of caret package.

Returns: converted data_feature in the object.

Examples:

```
\dontrun{
t1$cal_preProcess(method = c("center", "scale", "nzv"))
}
```

Method cal_feature_sel(): Perform feature selection. See <https://topepo.github.io/caret/feature-selection-overview.html> for more details.

Usage:

```
trans_classifier$cal_feature_sel(  
  boruta.maxRuns = 300,  
  boruta.pValue = 0.01,  
  boruta.repetitions = 4,  
  ...  
)
```

Arguments:

boruta.maxRuns default 300; maximal number of importance source runs; passed to the maxRuns parameter in Boruta function of Boruta package.

boruta.pValue default 0.01; p value passed to the pValue parameter in Boruta function of Boruta package.

boruta.repetitions default 4; repetition runs for the feature selection.

... parameters pass to Boruta function of Boruta package.

Returns: optimized data_feature in the object.

Examples:

```
\dontrun{  
t1$cal_feature_sel(boruta.maxRuns = 300, boruta.pValue = 0.01)  
}
```

Method cal_split(): Split data for training and testing.

Usage:

```
trans_classifier$cal_split(prop.train = 3/4)
```

Arguments:

prop.train default 3/4; the ratio of the dataset used for the training.

Returns: data_train and data_test in the object.

Examples:

```
\dontrun{  
t1$cal_split(prop.train = 3/4)  
}
```

Method set_trainControl(): Control parameters for the following training. See trainControl function of caret package for details.

Usage:

```
trans_classifier$set_trainControl(  
  method = "repeatedcv",  
  classProbs = TRUE,  
  savePredictions = TRUE,  
  ...  
)
```

Arguments:

method default 'repeatedcv'; 'repeatedcv': Repeated k-Fold cross validation; see method parameter in trainControl function of caret package for available options.

```
classProbs default TRUE; should class probabilities be computed for classification models?;
see classProbs parameter in caret::trainControl function.
savePredictions default TRUE; see savePredictions parameter in caret::trainControl function
... parameters pass to trainControl function of caret package.
```

Returns: trainControl in the object.

Examples:

```
\dontrun{
t1$set_trainControl(method = 'repeatedcv')
}
```

Method cal_train(): Run the model training.

Usage:

```
trans_classifier$cal_train(
  method = "rf",
  metric = "Accuracy",
  max.mtry = 2,
  max.ntree = 200,
  ...
)
```

Arguments:

```
method default "rf"; "rf": random forest; see method in caret::train function for other options.
metric default "Accuracy"; see metric in caret::train function for other options.
max.mtry default 2; for method = "rf"; maximum mtry used for the tunegrid to do hyperparameter tuning to optimize the model.
max.ntree default 200; for method = "rf"; maximum number of trees used to optimize the model.
... parameters pass to train function of caret package.
```

Returns: res_train in the object.

Examples:

```
\dontrun{
# random forest
t1$cal_train(method = "rf")
# Support Vector Machines with Radial Basis Function Kernel
t1$cal_train(method = "svmRadial", tuneLength = 15)
}
```

Method cal_feature_imp(): Get feature importance from the training model.

Usage:

```
trans_classifier$cal_feature_imp(...)
```

Arguments:

... parameters pass to varImp function of caret package.

Returns: res_feature_imp in the object. One row for each predictor variable. The column(s) are different importance measures.

Examples:

```
\dontrun{
t1$cal_feature_imp()
}
```

Method cal_predict(): Run the prediction.

Usage:

```
trans_classifier$cal_predict(positive_class = NULL)
```

Arguments:

positive_class default NULL; see positive parameter in confusionMatrix function of caret package; If positive_class is NULL, use the first group in data as the positive class automatically.

Returns: res_predict, res_confusion_fit and res_confusion_stats stored in the object.

Examples:

```
\dontrun{
t1$cal_predict()
}
```

Method plot_confusionMatrix(): Plot the cross-tabulation of observed and predicted classes with associated statistics.

Usage:

```
trans_classifier$plot_confusionMatrix(
  plot_confusion = TRUE,
  plot_statistics = TRUE
)
```

Arguments:

plot_confusion default TRUE; whether plot the confusion matrix.
plot_statistics default TRUE; whether plot the statistics.

Returns: ggplot object.

Examples:

```
\dontrun{
t1$plot_confusionMatrix()
}
```

Method cal_ROC(): Get ROC (Receiver Operator Characteristic) curve data and the performance data.

Usage:

```
trans_classifier$cal_ROC(input = "pred")
```

Arguments:

input default "pred"; 'pred' or 'train'; 'pred' represents using prediction results; 'train' represents using training results.

Returns: a list res_ROC stored in the object.

Examples:

```
\dontrun{
t1$cal_ROC()
}
```

Method `plot_ROC()`: Plot ROC curve.

Usage:

```
trans_classifier$plot_ROC(
  plot_type = c("ROC", "PR")[1],
  plot_group = "all",
  color_values = RColorBrewer::brewer.pal(8, "Dark2"),
  add_AUC = TRUE,
  plot_method = FALSE,
  ...
)
```

Arguments:

`plot_type` default `c("ROC", "PR")[1]`; 'ROC' represents ROC (Receiver Operator Characteristic) curve; 'PR' represents PR (Precision-Recall) curve.

`plot_group` default "all"; 'all' represents all the classes in the model; 'add' represents all adding micro-average and macro-average results, see https://scikit-learn.org/stable/auto_examples/model_selection/p other options should be one or more class names, same with the names in Group column of `res_ROC$res_roc` from `cal_ROC` function.

`color_values` default `RColorBrewer::brewer.pal(8, "Dark2")`; colors used in the plot.

`add_AUC` default `TRUE`; whether add AUC in the legend.

`plot_method` default `FALSE`; If `TRUE`, show the method in the legend though only one method is found.

... parameters pass to `geom_path` function of `ggplot2` package.

Returns: `ggplot2` object.

Examples:

```
\dontrun{
t1$plot_ROC(size = 1, alpha = 0.7)
}
```

Method `clone()`: The objects of this class are cloneable with this method.

Usage:

```
trans_classifier$clone(deep = FALSE)
```

Arguments:

`deep` Whether to make a deep clone.

Examples

```
## -----
## Method `trans_classifier$new`
## -----
```

```
data(dataset)
t1 <- trans_classifier$new(
  dataset = dataset,
  x.predictors = "Genus",
  y.response = "Group")

## -----
## Method `trans_classifier$cal_preProcess`
## -----

## Not run:
t1$cal_preProcess(method = c("center", "scale", "nzv"))

## End(Not run)

## -----
## Method `trans_classifier$cal_feature_sel`
## -----

## Not run:
t1$cal_feature_sel(boruta.maxRuns = 300, boruta.pValue = 0.01)

## End(Not run)

## -----
## Method `trans_classifier$cal_split`
## -----

## Not run:
t1$cal_split(prop.train = 3/4)

## End(Not run)

## -----
## Method `trans_classifier$set_trainControl`
## -----

## Not run:
t1$set_trainControl(method = 'repeatedcv')

## End(Not run)

## -----
## Method `trans_classifier$cal_train`
## -----

## Not run:
# random forest
t1$cal_train(method = "rf")
# Support Vector Machines with Radial Basis Function Kernel
t1$cal_train(method = "svmRadial", tuneLength = 15)
```

```

## End(Not run)

## -----
## Method `trans_classifier$cal_feature_imp`
## -----


## Not run:
t1$cal_feature_imp()

## End(Not run)

## -----
## Method `trans_classifier$cal_predict`
## -----


## Not run:
t1$cal_predict()

## End(Not run)

## -----
## Method `trans_classifier$plot_confusionMatrix`
## -----


## Not run:
t1$plot_confusionMatrix()

## End(Not run)

## -----
## Method `trans_classifier$cal_ROC`
## -----


## Not run:
t1$cal_ROC()

## End(Not run)

## -----
## Method `trans_classifier$plot_ROC`
## -----


## Not run:
t1$plot_ROC(size = 1, alpha = 0.7)

## End(Not run)

```

trans_diff

Create trans_diff object for the differential analysis on the taxonomic abundance

Description

This class is a wrapper for a series of differential abundance test and indicator analysis methods, including LEfSe based on the Segata et al. (2011) <doi:10.1186/gb-2011-12-6-r60>, random forest <doi:10.1016/j.geoderma.2018.09.035>, metastat based on White et al. (2009) <doi:10.1371/journal.pcbi.1000352>, non-parametric Kruskal-Wallis Rank Sum Test, Dunn's Kruskal-Wallis Multiple Comparisons based on the FSA package, Wilcoxon Rank Sum and Signed Rank Tests, t-test, anova, Scheirer Ray Hare test, R package metagenomeSeq Paulson et al. (2013) <doi:10.1038/nmeth.2658>, R package ANCOMBC <doi:10.1038/s41467-020-17041-7> and R package ALDEx2 <doi:10.1371/journal.pone.0067019; 10.1186/2049-2618-2-15>.

Methods

Public methods:

- `trans_diff$new()`
- `trans_diff$plot_diff_abund()`
- `trans_diff$plot_diff_bar()`
- `trans_diff$plot_diff_cladogram()`
- `trans_diff$print()`
- `trans_diff$clone()`

Method new():

Usage:

```
trans_diff$new(
  dataset = NULL,
  method = c("lefse", "rf", "metastat", "metagenomeSeq", "KW", "KW_dunn", "wilcox",
            "t.test", "anova", "scheirerRayHare", "ANCOMBC", "ALDEx2_t", "ALDEx2_kw")[1],
  group = NULL,
  taxa_level = "all",
  filter_thres = 0,
  alpha = 0.05,
  p_adjust_method = "fdr",
  lefse_subgroup = NULL,
  lefse_min_subsam = 10,
  lefse_norm = 1e+06,
  nresam = 0.6667,
  boots = 30,
  rf_ntree = 1000,
  group_choose_paired = NULL,
  metagenomeSeq_count = 1,
  ALDEx2_sig = c("wi.eBH", "kw.eBH"),
  ...
)
```

Arguments:

`dataset` the object of `microtable` Class.

`method` default "lefse"; see the following available options:

'`lefse`' LEfSe method based on Segata et al. (2011) <doi:10.1186/gb-2011-12-6-r60>

'rf' random forest and non-parametric test method based on An et al. (2019) <doi:10.1016/j.geoderma.2018.09.035>
'metastat' Metastat method for all paired groups based on White et al. (2009) <doi:10.1371/journal.pcbi.1000352>
'metagenomeSeq' zero-inflated log-normal model-based differential test method from metagenomeSeq package.
'KW' KW: Kruskal-Wallis Rank Sum Test for all groups (>= 2)
'KW_dunn' Dunn's Kruskal-Wallis Multiple Comparisons when group number > 2; see dunnTest function in FSA package
'wilcox' Wilcoxon Rank Sum and Signed Rank Tests for all paired groups
't.test' Student's t-Test for all paired groups
'anova' ANOVA for one-way or multi-factor analysis; see cal_diff function of trans_alpha class
'scheirerRayHare' Scheirer Ray Hare test for nonparametric test used for a two-way factorial experiment; see scheirerRayHare function of rcompanion package
'ANCOMBC' Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC); call ancombc2 function from ANCOMBC package; only support the case that group is same with fix_formula parameter in order to get well-organized output table; For more flexible usages, please see and use ancombc2 function directly; Reference: <doi:10.1038/s41467-020-17041-7>; Require ANCOMBC package to be installed (<https://bioconductor.org/packages/release/bioc/html/ANCOMBC.html>)
'ALDEx2_t' runs Welch's t and Wilcoxon tests with ALDEx2 package; see also the test parameter in ALDEx2::aldex function; ALDEx2 uses the centred log-ratio (clr) transformation and estimates per-feature technical variation within each sample using Monte-Carlo instances drawn from the Dirichlet distribution; Reference: <doi:10.1371/journal.pone.0067019> and <doi:10.1186/2049-2618-2-15>; require ALDEx2 package to be installed (<https://bioconductor.org/packages/release/bioc/html/ALDEx2.html>)
'ALDEx2_kw' runs Kruskal-Wallace and generalized linear model (glm) test with ALDEx2 package; see also the test parameter in ALDEx2::aldex function.
group default NULL; sample group used for the comparision; a colname of input microtable\$sample_table; It is necessary when method is not "anova" or method is "anova" but formula is not provided.
Once group is provided, the return res_abund will have mean and sd values for group.
taxa_level default "all"; 'all' represents using abundance data at all taxonomic ranks; For testing at a specific rank, provide taxonomic rank name, such as "Genus". If the provided taxonomic name is neither 'all' nor a colname in tax_table of input dataset, the function will use the features in input microtable\$otu_table automatically.
filter_thres default 0; the relative abundance threshold, such as 0.0005; only useful when method != "metastat".
alpha default 0.05; differential significance threshold for method = "lefs" or "rf"; used to select taxa with significance across groups.
p_adjust_method default "fdr"; p.adjust method; see method parameter of p.adjust function for other available options; NULL mean disuse the p value adjustment; So when p_adjust_method = NULL, P.adj is same with P.unadj.
lefs_subgroup default NULL; sample sub group used for sub-comparision in lefs; Segata et al. (2011) <doi:10.1186/gb-2011-12-6-r60>.
lefs_min_subsam default 10; sample numbers required in the subgroup test.
lefs_norm default 1000000; scale value in lefs.
nresam default 0.6667; sample number ratio used in each bootstrap for method = "lefs" or "rf".

boots default 30; bootstrap test number for method = "lefse" or "rf".
 rf_ntree default 1000; see ntree in randomForest function of randomForest package when method = "rf".
 group_choose_paired default NULL; a vector used for selecting the required groups for paired testing, only used for method = "metastat" or "metagenomeSeq".
 metagenomeSeq_count default 1; Filter features to have at least 'counts' counts.; see the count parameter in MRcoefs function of metagenomeSeq package.
 ALDEx2_sig default c("wi.eBH", "kw.eBH"); which column of the final result is used as the significance asterisk assignment; applied to method = "ALDEx2_t" or "ALDEx2_kw"; the first element is provided to "ALDEx2_t"; the second is provided to "ALDEx2_kw"; for "ALDEx2_t", the available choice is "wi.eBH" (Expected Benjamini-Hochberg corrected P value of Wilcoxon test) and "we.eBH" (Expected BH corrected P value of Welch's t test); for "ALDEx2_kw"; for "ALDEx2_t", the available choice is "kw.eBH" (Expected BH corrected P value of Kruskal-Wallace test) and "glm.eBH" (Expected BH corrected P value of glm test).
 ... parameters passed to cal_diff function of trans_alpha class when method is one of "KW", "KW_dunn", "wilcox", "t.test" and "anova"; passed to ANCOMBC::ancombc function when method is "ANCOMBC" (except tax_level, global and fix_formula parameters); passed to ALDEx2::aldex function when method = "ALDEx2_t" or "ALDEx2_kw".

Returns: res_diff and res_abund.

res_abund includes mean abundance of each taxa (Mean), standard deviation (SD), standard error (SE) and sample number (N) in the group (Group).

res_diff is the detailed differential test result, containing:

"Comparison": The groups for the comparison, maybe all groups or paired groups. If this column is not found, all groups used;

"Group": Which group has the maximum median or mean value across the test groups; For non-parametric methods, median value; For t.test, mean value;

"Taxa": which taxa is used in this comparison;

"Method": Test method used in the analysis depending on the method input;

"LDA" or "MeanDecreaseGini": LDA: linear discriminant score in LEfSe; MeanDecreaseGini: mean decreasing gini index in random forest;

"P.unadj" and "P.adj": raw p value; P.adj: adjusted p value;

"qvalue": qvalue for metastat analysis.

Examples:

```
\donttest{
data(dataset)
t1 <- trans_diff$new(dataset = dataset, method = "lefse", group = "Group")
t1 <- trans_diff$new(dataset = dataset, method = "rf", group = "Group")
t1 <- trans_diff$new(dataset = dataset, method = "metastat", group = "Group", tax_level = "Genus")
t1 <- trans_diff$new(dataset = dataset, method = "wilcox", group = "Group")
}
```

Method plot_diff_abund(): Plot the abundance of differential taxa

Usage:

```
trans_diff$plot_diff_abund(
  use_number = 1:20,
  color_values = RColorBrewer::brewer.pal(8, "Dark2"),
```

```

    select_group = NULL,
    select_taxa = NULL,
    simplify_names = TRUE,
    keep_prefix = TRUE,
    group_order = NULL,
    barwidth = 0.9,
    use_se = TRUE,
    add_sig = FALSE,
    add_sig_label = "Significance",
    add_sig_label_color = "black",
    add_sig_tip_length = 0.01,
    y_start = 1.01,
    y_increase = 0.05,
    text_y_size = 10,
    coord_flip = TRUE,
    ...
)

```

Arguments:

`use_number` default 1:20; numeric vector; the taxa numbers (1:n) selected in the plot; If the n is larger than the number of total significant taxa, automatically use all the taxa.
`color_values` default RColorBrewer::brewer.pal(8, "Dark2"); colors palette.
`select_group` default NULL; this is used to select the paired groups. This parameter is especially useful when the comparison methods is applied to paired groups; The input select_group must be one of `objectres_diffComparison`.
`select_taxa` default NULL; character vector to provide taxa names. The taxa names should be same with the names shown in the plot, not the 'Taxa' column names in `objectres_diffTaxa`.
`simplify_names` default TRUE; whether use the simplified taxonomic name.
`keep_prefix` default TRUE; whether retain the taxonomic prefix.
`group_order` default NULL; a vector to order groups, i.e. reorder the legend and colors in plot; If NULL, the function can first check whether the group column of `sample_table` is factor. If yes, use the levels in it. If provided, overlook the levels in the group of `sample_table`.
`barwidth` default 0.9; the bar width in plot.
`use_se` default TRUE; whether use SE in plot, if FALSE, use SD.
`add_sig` default FALSE; whether add the significance label to the plot.
`add_sig_label` default "Significance"; select a colname of `object$res_diff` for the label text, such as 'P.adj' or 'Significance'.
`add_sig_label_color` default "black"; the color for the label text when `add_sig` = TRUE.
`add_sig_tip_length` default 0.01; the tip length for the added line when `add_sig` = TRUE.
`y_start` default 1.01; the y axis position from which to add the label; the default 1.01 means 1.01 * Value; For method != "anova", all the start positions are same, i.e. Value = max(Mean+SD or Mean+SE); For method = "anova"; the stat position is calculated for each point, i.e. Value = Mean+SD or Mean+SE.
`y_increase` default 0.05; the increasing y axis space to add label for paired groups; the default 0.05 means 0.05 * `y_start` * Value; In addition, this parameter is also used to label the letters of anova result with the fixed (1 + `y_increase`) * `y_start` * Value.
`text_y_size` default 10; the size for the y axis text.

`coord_flip` default TRUE; whether flip cartesian coordinates so that horizontal becomes vertical, and vertical, horizontal.

... parameters passed to `ggsignif::stat_signif` when `add_sig = TRUE`.

Returns: ggplot.

Examples:

```
\donttest{
t1 <- trans_diff$new(dataset = dataset, method = "anova", group = "Group", taxa_level = "Genus")
t1$plot_diff_abund(use_number = 1:10)
t1$plot_diff_abund(use_number = 1:10, add_sig = TRUE)
t1 <- trans_diff$new(dataset = dataset, method = "wilcox", group = "Group")
t1$plot_diff_abund(use_number = 1:20)
t1$plot_diff_abund(use_number = 1:20, add_sig = TRUE)
t1 <- trans_diff$new(dataset = dataset, method = "lefse", group = "Group")
t1$plot_diff_abund(use_number = 1:20)
t1$plot_diff_abund(use_number = 1:20, add_sig = TRUE)
}
```

Method `plot_diff_bar()`: Bar plot for indicator index, such as LDA score and P value.

Usage:

```
trans_diff$plot_diff_bar(
  color_values = RColorBrewer::brewer.pal(8, "Dark2"),
  use_number = 1:10,
  threshold = NULL,
  select_group = NULL,
  simplify_names = TRUE,
  keep_prefix = TRUE,
  group_order = NULL,
  axis_text_y = 12,
  plot_vertical = TRUE,
  ...
)
```

Arguments:

`color_values` default `RColorBrewer::brewer.pal(8, "Dark2")`; colors palette for different groups.

`use_number` default 1:10; numeric vector; the taxa numbers used in the plot, i.e. 1:n.

`threshold` default NULL; threshold value of indicators for selecting taxa, such as 3 for LDA score of LEfSe.

`select_group` default NULL; this is used to select the paired group when multiple comparisons are generated; The input `select_group` must be one of `objectres_diffComparison`.

`simplify_names` default TRUE; whether use the simplified taxonomic name.

`keep_prefix` default TRUE; whether retain the taxonomic prefix.

`group_order` default NULL; a vector to order the legend and colors in plot; If NULL, the function can first check whether the group column of `microtable$sample_table` is factor. If yes, use the levels in it. If provided, this parameter can overwrite the levels in the group of `microtable$sample_table`.

`axis_text_y` default 12; the size for the y axis text.

plot_vertical default TRUE; whether use vertical bar plot or horizontal.

... parameters pass to `geom_bar`

Returns: ggplot.

Examples:

```
\donttest{
t1$plot_diff_bar(use_number = 1:20)
}
```

Method `plot_diff_cladogram()`: Plot the cladogram using taxa with significant difference.

Usage:

```
trans_diff$plot_diff_cladogram(
  color = RColorBrewer::brewer.pal(8, "Dark2"),
  group_order = NULL,
  use_taxa_num = 200,
  filter_taxa = NULL,
  use_feature_num = NULL,
  clade_label_level = 4,
  select_show_labels = NULL,
  only_select_show = FALSE,
  sep = "|",
  branch_size = 0.2,
  alpha = 0.2,
  clade_label_size = 2,
  clade_label_size_add = 5,
  clade_label_size_log = exp(1),
  node_size_scale = 1,
  node_size_offset = 1,
  annotation_shape = 22,
  annotation_shape_size = 5
)
```

Arguments:

`color` default `RColorBrewer::brewer.pal(8, "Dark2")`; color palette used in the plot.

`group_order` default `NULL`; a vector to order the legend in plot; If `NULL`, the function can first check whether the group column of `sample_table` is factor. If yes, use the levels in it. If provided, this parameter can overwrite the levels in the group of `sample_table`. If the number of provided `group_order` is less than the number of groups in `res_diff$Group`, the function will select the groups of `group_order` automatically.

`use_taxa_num` default 200; integer; The taxa number used in the background tree plot; select the taxa according to the mean abundance .

`filter_taxa` default `NULL`; The mean relative abundance used to filter the taxa with low abundance.

`use_feature_num` default `NULL`; integer; The feature number used in the plot; select the features according to the LDA score (`method = "lefsse"`) or `MeanDecreaseGini` (`method = "rf"`) from high to low.

`clade_label_level` default 4; the taxonomic level for marking the label with letters, root is the largest.

select_show_labels default NULL; character vector; The features to show in the plot with full label names, not the letters.
 only_select_show default FALSE; whether only use the the select features in the parameter select_show_labels.
 sep default "|"; the seperate character in the taxonomic information.
 branch_size default 0.2; numeric, size of branch.
 alpha default 0.2; shading of the color.
 clade_label_size default 2; basic size for the clade label; please also see clade_label_size_add and clade_label_size_log.
 clade_label_size_add default 5; added basic size for the clade label; see the formula in clade_label_size_log parameter.
 clade_label_size_log default exp(1); the base of log function for added size of the clade label; the size formula: clade_label_size + log(clade_label_level + clade_label_size_add, base = clade_label_size_log); so use clade_label_size_log, clade_label_size_add and clade_label_size can totally control the label size for different taxonomic levels.
 node_size_scale default 1; scale for the node size.
 node_size_offset default 1; offset for the node size.
 annotation_shape default 22; shape used in the annotation legend.
 annotation_shape_size default 5; size used in the annotation legend.

Returns: ggplot.

Examples:

```
\donttest{
t1$plot_diff_cladogram(use_taxa_num = 100, use_feature_num = 30, select_show_labels = NULL)
}
```

Method print(): Print the trans_alpha object.

Usage:

```
trans_diff$print()
```

Method clone(): The objects of this class are cloneable with this method.

Usage:

```
trans_diff$clone(deep = FALSE)
```

Arguments:

deep Whether to make a deep clone.

Examples

```
## -----
## Method `trans_diff$new`
## -----  
  

data(dataset)
t1 <- trans_diff$new(dataset = dataset, method = "lefse", group = "Group")
t1 <- trans_diff$new(dataset = dataset, method = "rf", group = "Group")
```

```

t1 <- trans_diff$new(dataset = dataset, method = "metastat", group = "Group", taxa_level = "Genus")
t1 <- trans_diff$new(dataset = dataset, method = "wilcox", group = "Group")

## -----
## Method `trans_diff$plot_diff_abund`
## -----


t1 <- trans_diff$new(dataset = dataset, method = "anova", group = "Group", taxa_level = "Genus")
t1$plot_diff_abund(use_number = 1:10)
t1$plot_diff_abund(use_number = 1:10, add_sig = TRUE)
t1 <- trans_diff$new(dataset = dataset, method = "wilcox", group = "Group")
t1$plot_diff_abund(use_number = 1:20)
t1$plot_diff_abund(use_number = 1:20, add_sig = TRUE)
t1 <- trans_diff$new(dataset = dataset, method = "lefsse", group = "Group")
t1$plot_diff_abund(use_number = 1:20)
t1$plot_diff_abund(use_number = 1:20, add_sig = TRUE)

## -----
## Method `trans_diff$plot_diff_bar`
## -----


t1$plot_diff_bar(use_number = 1:20)

## -----
## Method `trans_diff$plot_diff_cladogram`
## -----


t1$plot_diff_cladogram(use_taxa_num = 100, use_feature_num = 30, select_show_labels = NULL)

```

trans_env

Create trans_env object for the analysis of the effects of environmental factors on communities.

Description

This class is a wrapper for a series of operations associated with environmental measurements, including redundancy analysis, mantel test, correlation analysis and linear fitting based on An et al. (2019) <doi:10.1016/j.geoderma.2018.09.035>.

Methods

Public methods:

- [trans_env\\$new\(\)](#)

- `trans_env$cal_diff()`
- `trans_env$plot_diff()`
- `trans_env$cal_autocor()`
- `trans_env$cal_ordination()`
- `trans_env$cal_ordination_envsquare()`
- `trans_env$trans_ordination()`
- `trans_env$plot_ordination()`
- `trans_env$cal_mantel()`
- `trans_env$cal_cor()`
- `trans_env$plot_cor()`
- `trans_env$plot_scatterfit()`
- `trans_env$print()`
- `trans_env$clone()`

Method `new()`:

Usage:

```
trans_env$new(
  dataset = NULL,
  env_cols = NULL,
  add_data = NULL,
  character2numeric = TRUE,
  complete_na = FALSE
)
```

Arguments:

`dataset` the object of `microtable` Class.

`env_cols` default `NULL`; either numeric vector or character vector to select columns in `microtable$sample_table`, i.e. `dataset$sample_table`. This parameter should be used in the case that all the required environmental data is in `sample_table` of your `microtable` object. Otherwise, please use `add_data` parameter.

`add_data` default `NULL`; `data.frame` format; provide the environmental data in the format `data.frame`; rownames should be sample names. This parameter should be used when the your `microtable$sample_table` object has no environmental data. Under this circumstance, the `env_cols` parameter can not be used because no data can be selected.

`character2numeric` default `TRUE`; whether transform the characters or factors to numeric attributes.

`complete_na` default `FALSE`; Whether fill the NA (missing value) in the environmental data; If `TRUE`, the function can run the interpolation with the `mice` package; to use this parameter, please first install `mice` package.

Returns: `data_env` stored in the `trans_env` object.

Examples:

```
data(dataset)
data(env_data_16S)
t1 <- trans_env$new(dataset = dataset, add_data = env_data_16S[, 4:11])
```

Method `cal_diff()`: Test the difference of environmental variable across groups.

Usage:

```
trans_env$cal_diff(
  group = NULL,
  by_group = NULL,
  method = c("KW", "KW_dunn", "wilcox", "t.test", "anova", "scheirerRayHare")[1],
  ...
)
```

Arguments:

group default NULL; a colname of sample_table used to compare values across groups.
 by_group default NULL; perform the differential test among groups (group parameter) within each group (by_group parameter).
 method default "KW"; see the following available options:

- '**KW**' KW: Kruskal-Wallis Rank Sum Test for all groups (≥ 2)
- '**KW_dunn**' Dunn's Kruskal-Wallis Multiple Comparisons, see dunnTest function in FSA package
- '**wilcox**' Wilcoxon Rank Sum and Signed Rank Tests for all paired groups
- '**t.test**' Student's t-Test for all paired groups
- '**anova**' Duncan's new multiple range test for one-way anova; see duncan.test function of agricolae package. For multi-factor anova, see aov
- '**scheirerRayHare**' Scheirer Ray Hare test for nonparametric test used for a two-way factorial experiment; see scheirerRayHare function of rcompanion package
- ... parameters passed to cal_diff function of [trans_alpha](#) class.

Returns: res_diff in object. In the data frame, 'Group' column means that the group has the maximum median or mean value across the test groups; For non-parametric methods, maximum median value; For t.test, maximum mean value.

Examples:

```
\donttest{
t1$cal_diff(group = "Group", method = "KW")
t1$cal_diff(group = "Group", method = "KW_dunn")
t1$cal_diff(group = "Group", method = "anova")
}
```

Method `plot_diff()`: Plotting values of environmental variables across groups and add the significance label.

Usage:

```
trans_env$plot_diff(...)
```

Arguments:

... parameters passed to plot_alpha of [trans_alpha](#) class. Please see `trans_alpha$plot_alpha` function for all the available parameters.

Method `cal_autocor()`: Calculate the autocorrelations among environmental variables and plot the result.

Usage:

```
trans_env$cal_autocor(
  group = NULL,
  color_values = RColorBrewer::brewer.pal(8, "Dark2"),
  alpha = 0.8,
  ...
)
```

Arguments:

group default NULL; a colname of sample_table; used to perform calculations for different groups.

color_values default RColorBrewer::brewer.pal(8, "Dark2"); colors palette.

alpha default 0.8; the alpha value to add transparency in colors; useful when group is not NULL.

... default parameters passed to GGally::ggpairs.

Returns: ggmatrix.

Examples:

```
\dontrun{
# Spearman correlation
t1$cal_autocor(upper = list(continuous = GGally::wrap("cor", method= "spearman")))
}
```

Method cal_ordination(): Redundancy analysis (RDA) and Correspondence Analysis (CCA) based on the vegan package.

Usage:

```
trans_env$cal_ordination(
  method = c("RDA", "dbRDA", "CCA")[1],
  feature_sel = FALSE,
  taxa_level = NULL,
  taxa_filter_thres = NULL,
  use_measure = NULL,
  add_matrix = NULL,
  ...
)
```

Arguments:

method default c("RDA", "dbRDA", "CCA")[1]; the ordination method.

feature_sel default FALSE; whether perform the feature selection based on forward selection method.

taxa_level default NULL; If use RDA or CCA, provide the taxonomic rank, such as "Phylum" or "Genus"; If use otu_table; please input "OTU".

taxa_filter_thres default NULL; If want to filter taxa, provide the relative abundance threshold.

use_measure default NULL; a name of beta diversity matrix; only useful when parameter method = "dbRDA"; If not provided, use the first beta diversity matrix automatically.

add_matrix default NULL; additional distance matrix provided, when the user does not want to use the beta diversity matrix within the dataset; only available when method = "dbRDA".

... paremeters pass to dbrda or rda or cca function according to the input of method.

Returns: res_ordination, res_ordination_R2, res_ordination_terms and res_ordination_axis in object.

Examples:

```
\donttest{
t1$cal_ordination(method = "dbRDA", use_measure = "bray")
t1$cal_ordination(method = "RDA", taxa_level = "Genus")
t1$cal_ordination(method = "CCA", taxa_level = "Genus")
}
```

Method cal_ordination_envsquare(): Fits each environmental vector onto the ordination to obtain the contribution of each variable.

Usage:

```
trans_env$cal_ordination_envsquare(...)
```

Arguments:

... the parameters passing to vegan::envfit function.

Returns: res_ordination_envsquare in object.

Examples:

```
\donttest{
t1$cal_ordination_envsquare()
}
```

Method trans_ordination(): transform ordination result for the following plotting.

Usage:

```
trans_env$trans_ordination(
  show_taxa = 10,
  adjust_arrow_length = FALSE,
  min_perc_env = 0.1,
  max_perc_env = 0.8,
  min_perc_tax = 0.1,
  max_perc_tax = 0.8
)
```

Arguments:

show_taxa default 10; taxa number shown in the plot.

adjust_arrow_length default FALSE; whether adjust the arrow length to be clearer.

min_perc_env default 0.1; used for scaling up the minimum of env arrow; multiply by the maximum distance between samples and origin.

max_perc_env default 0.8; used for scaling up the maximum of env arrow; multiply by the maximum distance between samples and origin.

min_perc_tax default 0.1; used for scaling up the minimum of tax arrow; multiply by the maximum distance between samples and origin.

max_perc_tax default 0.8; used for scaling up the maximum of tax arrow; multiply by the maximum distance between samples and origin.

Returns: res_ordination_trans in object.

Examples:

```
\donttest{
t1$trans_ordination(adjust_arrow_length = TRUE, min_perc_env = 0.1, max_perc_env = 1)
}
```

Method `plot_ordination()`: plot ordination result.

Usage:

```
trans_env$plot_ordination(
  plot_color = NULL,
  plot_shape = NULL,
  color_values = RColorBrewer::brewer.pal(8, "Dark2"),
  shape_values = c(16, 17, 7, 8, 15, 18, 11, 10, 12, 13, 9, 3, 4, 0, 1, 2, 14),
  env_text_color = "black",
  env_arrow_color = "grey30",
  taxa_text_color = "firebrick1",
  taxa_arrow_color = "firebrick1",
  env_text_size = 3.7,
  taxa_text_size = 3,
  taxa_text_italic = TRUE,
  plot_type = "point",
  point_size = 3,
  point_alpha = 0.8,
  centroid_segment_alpha = 0.6,
  centroid_segment_size = 1,
  centroid_segment_linetype = 3,
  ellipse_chull_fill = TRUE,
  ellipse_chull_alpha = 0.1,
  ellipse_level = 0.9,
  ellipse_type = "t",
  add_sample_label = NULL,
  env_nudge_x = NULL,
  env_nudge_y = NULL,
  taxa_nudge_x = NULL,
  taxa_nudge_y = NULL,
  ...
)
```

Arguments:

`plot_color` default `NULL`; a colname of `sample_table` to assign colors to different groups in plot.

`plot_shape` default `NULL`; a colname of `sample_table` to assign shapes to different groups in plot.

`color_values` default `RColorBrewer::brewer.pal(8, "Dark2")`; color pallete for different groups.

`shape_values` default `c(16, 17, 7, 8, 15, 18, 11, 10, 12, 13, 9, 3, 4, 0, 1, 2, 14)`; a vector for point shape types of groups, see `ggplot2` tutorial.

`env_text_color` default `"black"`; environmental variable text color.

`env_arrow_color` default `"grey30"`; environmental variable arrow color.

`taxa_text_color` default `"firebrick1"`; taxa text color.

```

taxa_arrow_color default "firebrick1"; taxa arrow color.
env_text_size default 3.7; environmental variable text size.
taxa_text_size default 3; taxa text size.
taxa_text_italic default TRUE; "italic"; whether use "italic" style for the taxa text in the
plot.
plot_type default "point"; one or more elements of "point", "ellipse", "chull" and "centroid".
  'point' add point
  'ellipse' add confidence ellipse for points of each group
  'chull' add convex hull for points of each group
  'centroid' add centroid line of each group
point_size default 3; point size in plot when "point" is in plot_type.
point_alpha default .8; point transparency in plot when "point" is in plot_type.
centroid_segment_alpha default 0.6; segment transparency in plot when "centroid" is in
plot_type.
centroid_segment_size default 1; segment size in plot when "centroid" is in plot_type.
centroid_segment_linetype default 3; an integer; the line type related with centroid in plot
when "centroid" is in plot_type.
ellipse_chull_fill default TRUE; whether fill colors to the area of ellipse or chull.
ellipse_chull_alpha default 0.1; color transparency in the ellipse or convex hull depending
on whether "ellipse" or "centroid" is in plot_type.
ellipse_level default .9; confidence level of ellipse when "ellipse" is in plot_type.
ellipse_type default "t"; ellipse type when "ellipse" is in plot_type; see type in stat\_ellipse.
add_sample_label default NULL; the column name in sample table, if provided, show the
point name in plot.
env_nudge_x default NULL; numeric vector to adjust the env text x axis position; passed to
nudge_x parameter of ggrepel::geom_text_repel function; default NULL represents au-
tomatic adjustment; the length must be same with the row number of object$res_ordination_trans$df_arrows.
For example, if there are 5 env variables, env_nudge_x should be something like c(0.1,
0, -0.2, 0, 0). Note that this parameter and env_nudge_y is generally used when the
automatic text adjustment is not very well.
env_nudge_y default NULL; numeric vector to adjust the env text y axis position; passed to
nudge_y parameter of ggrepel::geom_text_repel function; default NULL represents auto-
matic adjustment; the length must be same with the row number of object$res_ordination_trans$df_arrows.
For example, if there are 5 env variables, env_nudge_y should be something like c(0.1, 0,
-0.2, 0, 0).
taxa_nudge_x default NULL; numeric vector to adjust the taxa text x axis position; passed to
nudge_x parameter of ggrepel::geom_text_repel function; default NULL represents auto-
matic adjustment; the length must be same with the row number of object$res_ordination_trans$df_arrows_sp.
For example, if 3 taxa are shown, taxa_nudge_x should be something like c(0.3, -0.2,
0).
taxa_nudge_y default NULL; numeric vector to adjust the taxa text y axis position; passed to
nudge_y parameter of ggrepel::geom_text_repel function; default NULL represents auto-
matic adjustment; the length must be same with the row number of object$res_ordination_trans$df_arrows_sp.
For example, if 3 taxa are shown, taxa_nudge_y should be something like c(-0.2, 0, 0.4).
... parameters pass to geom_point for controlling sample points.

```

Returns: ggplot object.

Examples:

```
\donttest{
t1$cal_ordination(method = "RDA")
t1$trans_ordination(adjust_arrow_length = TRUE, max_perc_env = 1.5)
t1$plot_ordination(plot_color = "Group")
t1$plot_ordination(plot_color = "Group", plot_shape = "Group", plot_type = c("point", "ellipse"))
t1$plot_ordination(plot_color = "Group", plot_type = c("point", "chull"))
t1$plot_ordination(plot_color = "Group", plot_type = c("point", "centroid"),
  centroid_segment_linetype = 1)
t1$plot_ordination(plot_color = "Group", env_nudge_x = c(0.4, 0, 0, 0, 0, -0.2, 0, 0),
  env_nudge_y = c(0.6, 0, 0.2, 0.5, 0, 0.1, 0, 0.2))
}
```

Method cal_mantel(): Mantel test between beta diversity matrix and environmental data.

Usage:

```
trans_env$cal_mantel(
  select_env_data = NULL,
  partial_mantel = FALSE,
  add_matrix = NULL,
  use_measure = NULL,
  method = "pearson",
  p_adjust_method = "fdr",
  by_group = NULL,
  ...
)
```

Arguments:

`select_env_data` default NULL; numeric or character vector to select columns in `data_env`; if not provided, automatically select the columns with numeric attributes.
`partial_mantel` default FALSE; whether use partial mantel test; If TRUE, use other measurements as the zdis.
`add_matrix` default NULL; additional distance matrix provided, if you donot want to use the beta diversity matrix in the dataset.
`use_measure` default NULL; name of beta diversity matrix. If necessary and not provided, use the first beta diversity matrix.
`method` default "pearson"; one of "pearson", "spearman" and "kendall"; correlation method; see method parameter in `vegan::mantel` function.
`p_adjust_method` default "fdr"; `p.adjust` method; see method parameter of `p.adjust` function for available options.
`by_group` default NULL; one column name or number in `sample_table`; used to perform mantel test for different groups separately.
... paremeters pass to `mantel` of `vegan` package.

Returns: `res_mantel` in object.

Examples:

```
\donttest{
t1$cal_mantel(use_measure = "bray")
t1$cal_mantel(partial_mantel = TRUE, use_measure = "bray")
}
```

Method cal_cor(): Calculating the correlations between taxa abundance and environmental variables. Actually, it can also be used for calculating other correlation between any two variables from two tables.

Usage:

```
trans_env$cal_cor(
  use_data = c("Genus", "all", "other")[1],
  select_env_data = NULL,
  cor_method = c("pearson", "spearman", "kendall")[1],
  p_adjust_method = "fdr",
  p_adjust_type = c("Type", "Taxa", "Env")[3],
  add_abund_table = NULL,
  by_group = NULL,
  use_taxa_num = NULL,
  other_taxa = NULL,
  group_use = NULL,
  group_select = NULL,
  taxa_name_full = TRUE
)
```

Arguments:

`use_data` default "Genus"; "Genus", "all" or "other"; "Genus" or other taxonomic name: use genus or other taxonomic abundance table in `taxa_abund`; "all": use all merged taxa abundance table; "other": provide additional taxa name with `other_taxa` parameter which is necessary.

`select_env_data` default NULL; numeric or character vector to select columns in `data_env`; if not provided, automatically select the columns with numeric attributes.

`cor_method` default "pearson"; "pearson", "spearman" or "kendall"; correlation method.

`p_adjust_method` default "fdr"; `p.adjust` method; see method parameter of `p.adjust` function for available options.

`p_adjust_type` default "Env"; "Type", "Taxa" or "Env"; `p.adjust` type; Env: environmental data; Taxa: taxa data; Type: group used.

`add_abund_table` default NULL; additional data table to be used. Samples must be rows.

`by_group` default NULL; one column name or number in `sample_table`; calculate correlations for different groups separately.

`use_taxa_num` default NULL; integer; a number used to select high abundant taxa; only useful when `use_data` parameter is a taxonomic level, e.g., "Genus".

`other_taxa` default NULL; character vector containing a series of taxa names; used when `use_data = "other"`; the provided names should be standard full names used to select taxa from all the tables in `taxa_abund` list of the `microtable` object; please see the example.

`group_use` default NULL; numeric or character vector to select one column in `sample_table` for selecting samples; together with `group_select`.

`group_select` default NULL; the group name used; remain samples within the group.

`taxa_name_full` default TRUE; Whether use the complete taxonomic name of taxa.

Returns: `res_cor` in object.

Examples:

```
\donttest{
t2 <- trans_diff$new(dataset = dataset, method = "rf", group = "Group", rf_taxa_level = "Genus")
t1 <- trans_env$new(dataset = dataset, add_data = env_data_16S[, 4:11])
t1$cal_cor(use_data = "other", p_adjust_method = "fdr", other_taxa = t2$res_diff$Taxa[1:40])
}
```

Method `plot_cor()`: Plot correlation heatmap.

Usage:

```
trans_env$plot_cor(
  color_vector = c("#053061", "white", "#A50026"),
  color_palette = NULL,
  pheatmap = FALSE,
  filter_feature = NULL,
  ylab_type_italic = FALSE,
  keep_full_name = FALSE,
  keep_prefix = TRUE,
  text_y_order = NULL,
  text_x_order = NULL,
  font_family = NULL,
  cluster_ggplot = "none",
  cluster_height_rows = 0.2,
  cluster_height_cols = 0.2,
  text_y_position = "right",
  mylabels_x = NULL,
  ...
)
```

Arguments:

`color_vector` default `c("#053061", "white", "#A50026")`; colors with only three values representing low, middle and high value.

`color_palette` default `NULL`; a customized palette with more color values; if provided, use it instead of `color_vector`.

`pheatmap` default `FALSE`; whether use `pheatmap` package to plot the heatmap.

`filter_feature` default `NULL`; character vector; used to filter features that only have significance labels in the `filter_feature` vector. For example, `filter_feature = ""` can be used to filter features that only have "", no any "*".

`ylab_type_italic` default `FALSE`; whether use italic type for y lab text.

`keep_full_name` default `FALSE`; whether use the complete taxonomic name.

`keep_prefix` default `TRUE`; whether retain the taxonomic prefix.

`text_y_order` default `NULL`; character vector; provide customized text order for y axis; shown in the plot from the top down.

`text_x_order` default `NULL`; character vector; provide customized text order for x axis.

`font_family` default `NULL`; font family used in `ggplot2`; only available when `pheatmap = FALSE`.

```

cluster_ggplot default "none"; add clustering dendrogram for ggplot2 based heatmap; available options: "none", "row", "col" or "both". "none": no any clustering used; "row": add clustering for rows; "col": add clustering for columns; "both": add clustering for both rows and columns. Only available when pheatmap = FALSE.
cluster_height_rows default 0.2, the dendrogram plot height for rows; available when cluster_ggplot != "none".
cluster_height_cols default 0.2, the dendrogram plot height for columns; available cluster_ggplot != "none".
text_y_position default "right"; "left" or "right"; the y axis text position; ggplot2 based heatmap.
mylabels_x default NULL; provide x axis text labels additionally; only available when pheatmap = TRUE.
... parameters pass to ggplot2::geom_tile or pheatmap, depending on the pheatmap parameter is FALSE or TRUE.

```

Returns: plot.

Examples:

```
\donttest{
t1$plot_cor(pheatmap = FALSE)
}
```

Method `plot_scatterfit()`: Scatter plot and add fitted line. The most important thing is to make sure that the input x and y have correponding sample orders. If one of x and y is a matrix, the other will be also transformed to matrix with Euclidean distance. Then, both of them are transformed to be vectors. If x or y is a vector with a single value, x or y will be assigned according to the column selection of the `data_env` inside.

Usage:

```
trans_env$plot_scatterfit(
  x = NULL,
  y = NULL,
  group = NULL,
  group_order = NULL,
  color_values = RColorBrewer::brewer.pal(8, "Dark2"),
  shape_values = NULL,
  type = c("cor", "lm")[1],
  cor_method = "pearson",
  label_sep = ";",
  label.x.npc = "left",
  label.y.npc = "top",
  label.x = NULL,
  label.y = NULL,
  x_axis_title = "",
  y_axis_title = "",
  point_size = 5,
  point_alpha = 0.6,
  line_size = 0.8,
  line_alpha = 1,
  line_color = "black",
```

```

line_se = TRUE,
line_se_color = "grey70",
pvalue_trim = 4,
cor_coef_trim = 3,
lm_fir_trim = 2,
lm_sec_trim = 2,
lm_squ_trim = 2,
...
)

```

Arguments:

- x default NULL; a single numeric or character value or a vector or a distance matrix used for the x axis. If x is a single value, it will be used to select the column of data_env inside. If x is a distance matrix, it will be transformed to be a vector.
- y default NULL; a single numeric or character value or a vector or a distance matrix used for the y axis. If y is a single value, it will be used to select the column of data_env inside. If y is a distance matrix, it will be transformed to be a vector.
- group default NULL; a character vector; if length is 1, must be a colname of dataset\$sample_table; Otherwise, group should be a vector with same length of x/y (for vector) or ncol of x/y (for matrix).
- group_order default NULL; a vector to order groups, i.e. reorder the legend and colors in plot when group is not NULL; If group_order is NULL and group is provided, the function can first check whether the group column of dataset\$sample_table is factor. If provided, overlook the levels in the group of dataset\$sample_table.
- color_values default RColorBrewer::brewer.pal(8, "Dark2"); color pallete for different groups.
- shape_values default NULL; a numeric vector for point shape types of groups when group is not NULL, see ggplot2 tutorial.
- type default c("cor", "lm")[1]; "cor": correlation; "lm" for regression.
- cor_method default "pearson"; one of "pearson", "kendall" and "spearman"; correlation method.
- label_sep default ":"; the separator string between different label parts.
- label.x.npc default "left"; can be numeric or character vector of the same length as the number of groups and/or panels. If too short they will be recycled.
- numeric** value should be between 0 and 1. Coordinates to be used for positioning the label, expressed in "normalized parent coordinates"
- character** allowed values include: i) one of c('right', 'left', 'center', 'centre', 'middle') for x-axis; ii) and one of c('bottom', 'top', 'center', 'centre', 'middle') for y-axis.
- label.y.npc default "top"; same usage with label.x.npc; see also label.y.npc parameter of ggpubr::stat_cor package.
- label.x default NULL; x axis absolute position for adding the statictic label.
- label.y default NULL; x axis absolute position for adding the statictic label.
- x_axis_title default ""; the title of x axis.
- y_axis_title default ""; the title of y axis.
- point_size default 5; point size value.
- point_alpha default 0.6; alpha value for the point color transparency.
- line_size default 0.8; line size value.

```

line_alpha default 1; alpha value for the line color transparency.
line_color default "black"; fitted line color only useful when group = NULL.
line_se default TRUE; Whether show the confidence interval for the fitting.
line_se_color default "grey70"; the color to fill the confidence interval when line_se =
    TRUE.
pvalue_trim default 4; trim the decimal places of p value.
cor_coef_trim default 3; trim the decimal places of correlation coefficient.
lm_fir_trim default 2; trim the decimal places of regression first coefficient.
lm_sec_trim default 2; trim the decimal places of regression second coefficient.
lm_squ_trim default 2; trim the decimal places of regression R square.
... other arguments to pass to geom_text or geom_label.

```

Returns: ggplot.

Examples:

```
\donttest{
t1$plot_scatterfit(x = 1, y = 2, type = "cor")
t1$plot_scatterfit(x = 1, y = 2, type = "lm", point_alpha = .3)
t1$plot_scatterfit(x = "pH", y = "TOC", type = "lm", group = "Group", line_se = FALSE)
t1$plot_scatterfit(x =
  dataset$beta_diversity$bray[rownames(t1$data_env), rownames(t1$data_env)], y = "pH")
}
```

Method print(): Print the trans_env object.

Usage:

```
trans_env$print()
```

Method clone(): The objects of this class are cloneable with this method.

Usage:

```
trans_env$clone(deep = FALSE)
```

Arguments:

deep Whether to make a deep clone.

Examples

```
## -----
## Method `trans_env$new`
## -----
data(dataset)
data(env_data_16S)
t1 <- trans_env$new(dataset = dataset, add_data = env_data_16S[, 4:11])

## -----
## Method `trans_env$cal_diff`
## -----
```

```
t1$cal_diff(group = "Group", method = "KW")
t1$cal_diff(group = "Group", method = "KW_dunn")
t1$cal_diff(group = "Group", method = "anova")

## -----
## Method `trans_env$cal_autocor`
## -----


## Not run:
# Spearman correlation
t1$cal_autocor(upper = list(continuous = GGally::wrap("cor", method= "spearman")))

## End(Not run)

## -----
## Method `trans_env$cal_ordination`
## -----


t1$cal_ordination(method = "dbRDA", use_measure = "bray")
t1$cal_ordination(method = "RDA", taxa_level = "Genus")
t1$cal_ordination(method = "CCA", taxa_level = "Genus")

## -----
## Method `trans_env$cal_ordination_envsquare`
## -----


t1$cal_ordination_envsquare()

## -----
## Method `trans_env$trans_ordination`
## -----


t1$trans_ordination(adjust_arrow_length = TRUE, min_perc_env = 0.1, max_perc_env = 1)

## -----
## Method `trans_env$plot_ordination`
## -----


t1$cal_ordination(method = "RDA")
t1$trans_ordination(adjust_arrow_length = TRUE, max_perc_env = 1.5)
t1$plot_ordination(plot_color = "Group")
t1$plot_ordination(plot_color = "Group", plot_shape = "Group", plot_type = c("point", "ellipse"))
t1$plot_ordination(plot_color = "Group", plot_type = c("point", "chull"))
t1$plot_ordination(plot_color = "Group", plot_type = c("point", "centroid"),
```

```

centroid_segment_linetype = 1)
t1$plot_ordination(plot_color = "Group", env_nudge_x = c(0.4, 0, 0, 0, 0, -0.2, 0, 0),
env_nudge_y = c(0.6, 0, 0.2, 0.5, 0, 0.1, 0, 0.2))

## -----
## Method `trans_env$cal_mantel`
## -----


t1$cal_mantel(use_measure = "bray")
t1$cal_mantel(partial_mantel = TRUE, use_measure = "bray")

## -----
## Method `trans_env$cal_cor`
## -----


t2 <- trans_diff$new(dataset = dataset, method = "rf", group = "Group", rf_taxa_level = "Genus")
t1 <- trans_env$new(dataset = dataset, add_data = env_data_16S[, 4:11])
t1$cal_cor(use_data = "other", p_adjust_method = "fdr", other_taxa = t2$res_diff$Taxa[1:40])

## -----
## Method `trans_env$plot_cor`
## -----


t1$plot_cor(pheatmap = FALSE)

## -----
## Method `trans_env$plot_scatterfit`
## -----


t1$plot_scatterfit(x = 1, y = 2, type = "cor")
t1$plot_scatterfit(x = 1, y = 2, type = "lm", point_alpha = .3)
t1$plot_scatterfit(x = "pH", y = "TOC", type = "lm", group = "Group", line_se = FALSE)
t1$plot_scatterfit(x =
dataset$beta_diversity$bray[rownames(t1$data_env), rownames(t1$data_env)], y = "pH")

```

trans_func*Create trans_func object for functional analysis.***Description**

This class is a wrapper for a series of functional analysis on species and communities, including the prokaryotes function identification based on Louca et al. (2016) <doi:10.1126/science.aaf4507>

and Lim et al. (2020) <10.1038/s41597-020-0516-5>, or fungi function identification based on Nguyen et al. (2016) <10.1016/j.funeco.2015.06.006> and Polme et al. (2020) <doi:10.1007/s13225-020-00466-2>; functional redundancy calculation and metabolic pathway abundance prediction Abhauer et al. (2015) <10.1093/bioinformatics/btv287>.

Active bindings

`func_group_list` store and show the function group list

Methods

Public methods:

- `trans_func$new()`
- `trans_func$cal_spe_func()`
- `trans_func$cal_spe_func_perc()`
- `trans_func$show_prok_func()`
- `trans_func$plot_spe_func_perc()`
- `trans_func$cal_tax4fun()`
- `trans_func$cal_tax4fun2()`
- `trans_func$cal_tax4fun2_FRI()`
- `trans_func$print()`
- `trans_func$clone()`

Method new(): Create the `trans_func` object. This function can identify the data type for Prokaryotes or Fungi automatically.

Usage:

```
trans_func$new(dataset = NULL)
```

Arguments:

`dataset` the object of `microtable` Class.

Returns: `for_what`: "prok" or "fungi" or NA, "prok" represent prokaryotes. "fungi" represent fungi. NA stand for not identified according to the Kingdom information, at this time, if you want to use the functions to identify species traits, you need provide "prok" or "fungi" manually, e.g. `dataset$for_what <- "prok"`.

Examples:

```
data(dataset)
t1 <- trans_func$new(dataset = dataset)
```

Method cal_spe_func(): Confirm traits of each feature by matching the taxonomic assignments to the functional database.

Usage:

```
trans_func$cal_spe_func(
  prok_database = c("FAPROTAX", "NJC19")[1],
  fungi_database = c("FUNGuild", "FungalTraits")[1]
)
```

Arguments:

`prok_database` default "FAPROTAX"; "FAPROTAX" or "NJC19"; select a prokaryotic trait database; see the details:
'FAPROTAX' FAPROTAX v1.2.4; Reference: Louca et al. (2016). Decoupling function and taxonomy in the global ocean microbiome. *Science*, 353(6305), 1272. <[doi:10.1126/science.aaf4507](https://doi.org/10.1126/science.aaf4507)>
'NJC19' NJC19: Lim et al. (2020). Large-scale metabolic interaction network of the mouse and human gut microbiota. *Scientific Data*, 7(1). <[10.1038/s41597-020-0516-5](https://doi.org/10.1038/s41597-020-0516-5)>
`fungi_database` default "FUNGuild"; "FUNGuild" or "FungalTraits"; select a fungal trait database; see the details:
'FUNGuild' Nguyen et al. (2016) FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*, 20(1), 241-248, <[doi:10.1016/j.funeco.2015.06.006](https://doi.org/10.1016/j.funeco.2015.06.006)>
'FungalTraits' version: FungalTraits_1.2_ver_16Dec_2020V.1.2; Polme et al. Fungal-Traits: a user-friendly traits database of fungi and fungus-like stramenopiles. *Fungal Diversity* 105, 1-16 (2020). <[doi:10.1007/s13225-020-00466-2](https://doi.org/10.1007/s13225-020-00466-2)>

Returns: `res_spe_func` stored in object.

Examples:

```
\donttest{
t1$cal_spe_func(prok_database = "FAPROTAX")
t1$cal_spe_func(fungi_database = "FungalTraits")
}
```

Method `cal_spe_func_perc()`: Calculating the percentages of species with specific trait in communities. The percentages of the taxa with specific trait can reflect the potential of the corresponding function in the community. So this method is a simple calculation of functional redundancy without the consideration of phylogenetic distance among taxa.

Usage:

```
trans_func$cal_spe_func_perc(abundance_weighted = FALSE)
```

Arguments:

`abundance_weighted` default FALSE; whether use abundance of taxa. If FALSE, calculate the functional population percentage. If TRUE, calculate the functional individual percentage.

Returns: `res_spe_func_perc` stored in the object.

Examples:

```
\donttest{
t1$cal_spe_func_perc(abundance_weighted = TRUE)
}
```

Method `show_prok_func()`: Show the annotation information for a function of prokaryotes from FAPROTAX database.

Usage:

```
trans_func$show_prok_func(use_func = NULL)
```

Arguments:

`use_func` default NULL; the function name.

Returns: None.

Examples:

```
\donttest{
t1$show_prok_func(use_func = "methanotrophy")
}
```

Method plot_spe_func_perc(): Plot the percentages of species with specific trait in communities or network modules.

Usage:

```
trans_func$plot_spe_func_perc(
  filter_func = NULL,
  use_group_list = TRUE,
  add_facet = TRUE,
  order_x = NULL,
  color_gradient_low = "#00008B",
  color_gradient_high = "#9E0142"
)
```

Arguments:

filter_func default NULL; a vector of function names used to show in the plot.

use_group_list default TRUE; If TRUE, use default group list; If user want to use personalized group list, please first set trans_func\$func_group_list object with a list of group names and functions.

add_facet default TRUE; whether use group names as the facets in the plot, see trans_func\$func_group_list object.

order_x default NULL; character vector; to sort the x axis text; can be also used to select partial samples to show.

color_gradient_low default "#00008B"; the color used as the low end in the color gradient.

color_gradient_high default "#9E0142"; the color used as the high end in the color gradient.

Returns: ggplot2.

Examples:

```
\donttest{
t1$plot_spe_func_perc(use_group_list = TRUE)
}
```

Method cal_tax4fun(): Predict functional potential of communities using tax4fun. please cite: Tax4Fun: Predicting functional profiles from metagenomic 16S rRNA data. Bioinformatics, 31(17), 2882-2884, <doi:10.1093/bioinformatics/btv287>. Note that this function requires a standard prefix in taxonomic table with double underscores (e.g. g__).

Usage:

```
trans_func$cal_tax4fun(keep_tem = FALSE, folderReferenceData = NULL)
```

Arguments:

keep_tem default FALSE; whether keep the intermediate file, that is, the feature table in local place.

folderReferenceData default NULL; the folder, see <http://tax4fun.gobics.de/> and Tax4Fun function in Tax4Fun package.

Returns: tax4fun_KO and tax4fun_path in object.

Method cal_tax4fun2(): Predict functional potential of communities with Tax4Fun2 method. The function was adapted from the raw Tax4Fun2 package to make it compatible with the mi-crotble object. Pleas cite: Tax4Fun2: prediction of habitat-specific functional profiles and functional redundancy based on 16S rRNA gene sequences. Environmental Microbiome 15, 11 (2020). <doi:10.1186/s40793-020-00358-7>

Usage:

```
trans_func$cal_tax4fun2(
  blast_tool_path = NULL,
  path_to_reference_data = "Tax4Fun2_ReferenceData_v2",
  path_to_temp_folder = NULL,
  database_mode = "Ref99NR",
  normalize_by_copy_number = T,
  min_identity_to_reference = 97,
  use_uproc = T,
  num_threads = 1,
  normalize_pathways = F
)
```

Arguments:

blast_tool_path default NULL; the folder path, e.g., ncbi-blast-2.5.0+/bin ; blast tools folder downloaded from "ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+" ; e.g., ncbi-blast-2.5.0+-x64-win64.tar.gz for windows system; if blast_tool_path is NULL, search the tools in the environmental path variable.

path_to_reference_data default "Tax4Fun2_ReferenceData_v2"; the path that points to files used in the prediction; The directory must contain the Ref99NR or Ref100NR folder; download Ref99NR.zip from <https://cloudstor.aarnet.edu.au/plus/s/DkoZIyZpMNbrzSw/download> or Ref100NR.zip from <https://cloudstor.aarnet.edu.au/plus/s/jIByczak9ZAFUB4/download>.

path_to_temp_folder default NULL; The temporary folder to store the logfile, intermediate file and result files; if NULL, use the default temporary in the computer system.

database_mode default 'Ref99NR'; "Ref99NR" or "Ref100NR"; Ref99NR: 99% clustering reference database; Ref100NR: no clustering.

normalize_by_copy_number default TRUE; whether normalize the result by the 16S rRNA copy number in the genomes.

min_identity_to_reference default 97; the sequences identity threshold used for finding the nearest species.

use_uproc default TRUE; whether use UProC to functionally annotate the genomes in the reference data.

num_threads default 1; the threads used in the blastn.

normalize_pathways default FALSE; Different to Tax4Fun, when converting from KEGG functions to KEGG pathways, Tax4Fun2 does not equally split KO gene abundances between pathways a functions is affiliated to. The full predicted abundance is affiliated to each pathway. Use TRUE to split the abundances (default is FALSE).

Returns: res_tax4fun2_KO and res_tax4fun2_pathway in object.

Examples:

```
\dontrun{
t1$cal_tax4fun2(blast_tool_path = "ncbi-blast-2.5.0+/bin",
```

```
    path_to_reference_data = "Tax4Fun2_ReferenceData_v2")
}
```

Method cal_tax4fun2_FRI(): Calculate (multi-) functional redundancy index (FRI) of prokaryotic community with Tax4Fun2 method. This function is used to calculating aFRI and rFRI use the intermediate files generated by the function cal_tax4fun2(). please also cite: Tax4Fun2: prediction of habitat-specific functional profiles and functional redundancy based on 16S rRNA gene sequences. Environmental Microbiome 15, 11 (2020). <doi:10.1186/s40793-020-00358-7>

Usage:

```
trans_func$cal_tax4fun2_FRI()
```

Returns: res_tax4fun2_aFRI and res_tax4fun2_rFRI in object.

Examples:

```
\dontrun{
t1$cal_tax4fun2_FRI()
}
```

Method print(): Print the trans_func object.

Usage:

```
trans_func$print()
```

Method clone(): The objects of this class are cloneable with this method.

Usage:

```
trans_func$clone(deep = FALSE)
```

Arguments:

deep Whether to make a deep clone.

Examples

```
## -----
## Method `trans_func$new`
## -----


data(dataset)
t1 <- trans_func$new(dataset = dataset)

## -----
## Method `trans_func$cal_spe_func`
## -----


t1$cal_spe_func(prok_database = "FAPROTAX")
t1$cal_spe_func(fungi_database = "FungalTraits")

## -----
## Method `trans_func$cal_spe_func_perc`
## -----
```

```

t1$cal_spe_func_perc(abundance_weighted = TRUE)

## -----
## Method `trans_func$show_prok_func`
## -----


t1$show_prok_func(use_func = "methanotrophy")

## -----
## Method `trans_func$plot_spe_func_perc`
## -----


t1$plot_spe_func_perc(use_group_list = TRUE)

## -----
## Method `trans_func$cal_tax4fun2`
## -----


## Not run:
t1$cal_tax4fun2(blast_tool_path = "ncbi-blast-2.5.0+/bin",
                 path_to_reference_data = "Tax4Fun2_ReferenceData_v2")

## End(Not run)

## -----
## Method `trans_func$cal_tax4fun2_FRI`
## -----


## Not run:
t1$cal_tax4fun2_FRI()

## End(Not run)

```

trans_network*Create trans_network object for co-occurrence network analysis.***Description**

This class is a wrapper for a series of network analysis methods, including the network construction, network attributes analysis, eigengene analysis, network subsetting, node and edge properties, network visualization and other network operations.

Methods

Public methods:

- `trans_network$new()`
- `trans_network$cal_network()`
- `trans_network$cal_module()`
- `trans_network$save_network()`
- `trans_network$cal_network_attr()`
- `trans_network$get_node_table()`
- `trans_network$get_edge_table()`
- `trans_network$get_adjacency_matrix()`
- `trans_network$plot_network()`
- `trans_network$cal_eigen()`
- `trans_network$plot_taxa_roles()`
- `trans_network$subset_network()`
- `trans_network$cal_powerlaw()`
- `trans_network$cal_sum_links()`
- `trans_network$plot_sum_links()`
- `trans_network$trans_comm()`
- `trans_network$print()`
- `trans_network$clone()`

Method new(): Create the `trans_network` object, store the important intermediate data and calculate correlations if `cor_method` parameter is not NULL.

Usage:

```
trans_network$new(
  dataset = NULL,
  cor_method = NULL,
  use_WGCNA_pearson_spearman = FALSE,
  use_NetCoMi_pearson_spearman = FALSE,
  use_sparcc_method = c("NetCoMi", "SpiecEasi")[1],
  taxa_level = "OTU",
  filter_thres = 0,
  nThreads = 1,
  SparCC_simu_num = 100,
  env_cols = NULL,
  add_data = NULL,
  ...
)
```

Arguments:

`dataset` the object of `microtable` Class.
`cor_method` default NULL; NULL or one of "bray", "pearson", "spearman", "bicor", "sparcc", "cclasso" and "ccrepe"; All the methods refered to NetCoMi package are performed based on `netConstruct` function of NetCoMi package and require NetCoMi to be installed from Github (<https://github.com/stefpeschel/NetCoMi>); For the algorithm details, please see Peschel et al. 2020 Brief. Bioinform <doi: 10.1093/bib/bbaa290>;

NULL NULL denotes non-correlation network, i.e. do not use correlation-based network.
 If so, the return `res_cor_p` list will be NULL, and COR option in `network_method` parameter of `cal_network` function can not be used.

'bray' 1-B, where B is Bray–Curtis dissimilarity; based on `vegdist` function

'pearson' Pearson correlation; If `use_WGCNA_pearson_spearman` and `use_NetCoMi_pearson_spearman` are both FALSE, use the function `cor.test` in R; `use_WGCNA_pearson_spearman = TRUE` invoke `corAndPvalue` function of WGCNA package; `use_NetCoMi_pearson_spearman = TRUE` invoke `netConstruct` function of NetCoMi package

'spearman' Spearman correlation; other details are same with the 'pearson' option

'bicor' Calculate biweight midcorrelation efficiently for matrices based on `WGCNA::bicor` function; require WGCNA and NetCoMi packages to be installed

'sparcc' SparCC algorithm (Friedman & Alm, PLoS Comp Biol, 2012, <doi:10.1371/journal.pcbi.1002687>);
 use NetCoMi package when `use_sparcc_method = "NetCoMi"`; use SpiecEasi package when `use_sparcc_method = "SpiecEasi"` and require SpiecEasi to be installed from Github (<https://github.com/zdk123/SpiecEasi>)

'cclasso' Correlation inference of Composition data through Lasso method based on `netConstruct` function of NetCoMi package; for details, see `NetCoMi::cclasso` function

'ccrepe' Calculates compositionality-corrected p-values and q-values for compositional data using an arbitrary distance metric based on `NetCoMi::netConstruct` function; also see `NetCoMi::ccrepe` function

`use_WGCNA_pearson_spearman` default FALSE; whether use WGCNA package to calculate correlation when `cor_method = "pearson"` or `"spearman"`.

`use_NetCoMi_pearson_spearman` default FALSE; whether use NetCoMi package to calculate correlation when `cor_method = "pearson"` or `"spearman"`. The important difference between NetCoMi and others is the features of zero handling and data normalization; See <doi: 10.1093/bib/bbaa290>.

`use_sparcc_method` default c("NetCoMi", "SpiecEasi")[1]; use NetCoMi package or SpiecEasi package to perform SparCC when `cor_method = "sparcc"`.

`taxa_level` default "OTU"; taxonomic rank; 'OTU' denotes using feature table directly; other available options should be one of the colnames of `tax_table` of input dataset.

`filter_thres` default 0; the relative abundance threshold.

`nThreads` default 1; the CPU thread number; available when `use_WGCNA_pearson_spearman = TRUE` or `use_sparcc_method = "SpiecEasi"`.

`SparCC_simu_num` default 100; SparCC simulation number for bootstrap when `use_sparcc_method = "SpiecEasi"`.

`env_cols` default NULL; numeric or character vector to select the column names of environmental data in `dataset$sample_table`; the environmental data can be used in the correlation network (as the nodes) or FlashWeave network.

`add_data` default NULL; provide environmental table additionally instead of `env_cols` parameter; rownames must be sample names.

... parameters pass to `NetCoMi::netConstruct` for other operations, such as zero handling and/or data normalization when `cor_method` and other parameters refer to NetCoMi package.

Returns: `res_cor_p` list with the correlation (association) matrix and p value matrix. Note that when `cor_method` and other parameters refer to NetCoMi package, the p value table are all zero as the significant associations have been selected.

Examples:

```
\donttest{
data(dataset)
# for correlation network
t1 <- trans_network$new(dataset = dataset, cor_method = "pearson",
taxa_level = "OTU", filter_thres = 0.0002)
# for non-correlation network
t1 <- trans_network$new(dataset = dataset, cor_method = NULL)
}
```

Method cal_network(): Construct network based on the correlation method or SpiecEasi package or julia FlashWeave package or beemStatic package.

Usage:

```
trans_network$cal_network(
network_method = c("COR", "SpiecEasi", "gcoda", "FlashWeave", "beemStatic")[1],
COR_p_thres = 0.01,
COR_p_adjust = "fdr",
COR_weight = TRUE,
COR_cut = 0.6,
COR_optimization = FALSE,
COR_optimization_low_high = c(0.01, 0.8),
COR_optimization_seq = 0.01,
SpiecEasi_method = "mb",
FlashWeave_tempdir = NULL,
FlashWeave_meta_data = FALSE,
FlashWeave_other_para = "alpha=0.01,sensitive=true,heterogeneous=true",
beemStatic_t_strength = 0.001,
beemStatic_t_stab = 0.8,
add_taxa_name = "Phylum",
delete_unlinked_nodes = TRUE,
username_rawtaxa_when_taxalevel_notOTU = FALSE,
...
)
```

Arguments:

network_method default "COR"; "COR", "SpiecEasi", "gcoda", "FlashWeave" or "beemStatic";

The option details:

'COR' correlation-based network; use the correlation and p value matrixes in object\$res_cor_p returned from trans_network\$new; See Deng et al. (2012) <doi:10.1186/1471-2105-13-113> for other details

'SpiecEasi' SpiecEasi network; relies on algorithms for sparse neighborhood and inverse covariance selection; belong to the category of conditional dependence and graphical models; see <https://github.com/zdk123/SpiecEasi> for installing the R package; see Kurtz et al. (2015) <doi:10.1371/journal.pcbi.1004226> for the algorithm details

'gcoda' hypothesize the logistic normal distribution of microbiome data; use penalized maximum likelihood method to estimate the sparse structure of inverse covariance for latent normal variables to address the high dimensionality of the microbiome data; belong to the category of conditional dependence and graphical models; depend on the

R NetCoMi package <https://github.com/stefpeschel/NetCoMi>; see FANG et al. (2017) <doi:10.1089/cmb.2017.0054> for the algorithm details

'FlashWeave' FlashWeave network; Local-to-global learning framework; belong to the category of conditional dependence and graphical models; good performance on heterogeneous datasets to find direct associations among taxa; see <https://github.com/meringlab/FlashWeave.jl> for installing julia language and FlashWeave package; julia must be in the computer system env path, otherwise the program can not find julia; see Tackmann et al. (2019) <doi:10.1016/j.cels.2019.08.002> for the algorithm details

'beemStatic' beemStatic network; extend generalized Lotka-Volterra model to cases of cross-sectional datasets to infer interaction among taxa based on expectation-maximization algorithm; see <https://github.com/CSB5/BEEM-static> for installing the R package; see Li et al. (2021) <doi:10.1371/journal.pcbi.1009343> for algorithm details

COR_p_thres default 0.01; the p value threshold for the correlation-based network.

COR_p_adjust default "fdr"; p value adjustment method, see method of p.adjust function for available options.

COR_weight default TRUE; whether use correlation coefficient as the weight of edges; FALSE represents weight = 1 for all edges.

COR_cut default 0.6; correlation coefficient threshold for the correlation network.

COR_optimization default FALSE; whether use random matrix theory (RMT) based method to determine the correlation coefficient; see <https://doi.org/10.1186/1471-2105-13-113>

COR_optimization_low_high default c(0.01, 0.8); the low and high value threshold used for the RMT optimization; only useful when COR_optimization = TRUE.

COR_optimization_seq default 0.01; the interval of correlation coefficient used for RMT optimization; only useful when COR_optimization = TRUE.

SpiecEasi_method default "mb"; either 'glasso' or 'mb'; see spiec.easi function in package SpiecEasi and <https://github.com/zdk123/SpiecEasi>.

FlashWeave_tempdir default NULL; The temporary directory used to save the temporary files for running FlashWeave; If not assigned, use the system user temp.

FlashWeave_meta_data default FALSE; whether use env data for the optimization, If TRUE, the function automatically find the object\$env_data in the object and generate a file for meta_data_path parameter of FlashWeave.

FlashWeave_other_para default "alpha=0.01, sensitive=true, heterogeneous=true"; the parameters used for FlashWeave; user can change the parameters or add more according to FlashWeave help document; An exception is meta_data_path parameter as it is generated based on the data inside the object, see FlashWeave_meta_data parameter for the description.

beemStatic_t_strength default 0.001; for network_method = "beemStatic"; the threshold used to limit the number of interactions (strength); same with the t.strength parameter in showInteraction function of beemStatic package.

beemStatic_t_stab default 0.8; for network_method = "beemStatic"; the threshold used to limit the number of interactions (stability); same with the t.stab parameter in showInteraction function of beemStatic package.

add_taxa_name default "Phylum"; one or more taxonomic rank name; used to add taxonomic rank name to network node properties.

delete_unlinked_nodes default TRUE; whether delete the nodes without any link.

```

username_rawtaxa_when_taxalevel_notOTU default FALSE; whether replace the name of nodes
using the taxonomic information.
... parameters pass to SpiecEasi::spiec.easi when network_method = "SpiecEasi"; pass
to NetCoMi::netConstruct when network_method = "gcoda"; pass to beemStatic::func.EM
when network_method = "beemStatic".

```

Returns: res_network stored in object.

Examples:

```
\dontrun{
# for correlation network
t1 <- trans_network$new(dataset = dataset, cor_method = "pearson",
taxa_level = "OTU", filter_thres = 0.001)
t1$cal_network(COR_p_thres = 0.05, COR_cut = 0.6)
t1 <- trans_network$new(dataset = dataset, cor_method = NULL, filter_thres = 0.003)
t1$cal_network(network_method = "SpiecEasi", SpiecEasi_method = "mb")
t1 <- trans_network$new(dataset = dataset, cor_method = NULL, filter_thres = 0.005)
t1$cal_network(network_method = "beemStatic")
t1 <- trans_network$new(dataset = dataset, cor_method = NULL, filter_thres = 0.001)
t1$cal_network(network_method = "FlashWeave")
}
```

Method cal_module(): Calculate network modules and add module names to the network node properties.

Usage:

```
trans_network$cal_module(
  method = "cluster_fast_greedy",
  module_name_prefix = "M"
)
```

Arguments:

method default "cluster_fast_greedy"; the method used to find the optimal community structure of a graph; the following are available functions (options) from igraph package: "cluster_fast_greedy", "cluster_optimal", "cluster_edge_betweenness", "cluster_infomap", "cluster_label_prop", "cluster_leading_eigen", "cluster_louvain", "cluster_springlass", "cluster_walktrap". For the details of these functions, see the help document, such as `help(cluster_fast_greedy)`. Note that the default "cluster_fast_greedy" method can only be used for undirected network. If the user selects network_method = "beemStatic" in cal_network function or provides other directed network, please use cluster_optimal or others for the modules identification. module_name_prefix default "M"; the prefix of module names; module names are made of the module_name_prefix and numbers; numbers are assigned according to the sorting result of node numbers in modules with decreasing trend.

Returns: res_network with modules, stored in object.

Examples:

```
\donttest{
t1 <- trans_network$new(dataset = dataset, cor_method = "pearson",
taxa_level = "OTU", filter_thres = 0.0002)
t1$cal_network(COR_p_thres = 0.01, COR_cut = 0.6)
t1$cal_module(method = "cluster_fast_greedy")
}
```

Method `save_network()`: Save network as gexf style, which can be opened by Gephi (<https://gephi.org/>).

Usage:

```
trans_network$save_network(filepath = "network.gexf")
```

Arguments:

`filepath` default "network.gexf"; file path to save the network.

Returns: None

Examples:

```
\dontrun{
t1$save_network(filepath = "network.gexf")
}
```

Method `cal_network_attr()`: Calculate network properties.

Usage:

```
trans_network$cal_network_attr()
```

Returns: `res_network_attr` stored in object.

Examples:

```
\donttest{
t1$cal_network_attr()
}
```

Method `get_node_table()`: Get the node property table. The properties may include the node names, modules allocation, degree, betweenness, abundance, taxonomy, within-module connectivity and among-module connectivity <doi:10.1016/j.geoderma.2022.115866>.

Authors: Chi Liu, Umer Zeeshan Ijaz

Usage:

```
trans_network$get_node_table(node_roles = TRUE)
```

Arguments:

`node_roles` default TRUE; whether calculate node roles, i.e. Module hubs, Network hubs, Connectors and Peripherals <doi:10.1016/j.geoderma.2022.115866>.

Returns: `res_node_table` in object; Abundance expressed as a percentage; z denotes within-module connectivity; p denotes among-module connectivity.

Examples:

```
\donttest{
t1$get_node_table(node_roles = TRUE)
}
```

Method `get_edge_table()`: Get the edge property table, including connected nodes, label and weight.

Usage:

```
trans_network$get_edge_table()
```

Returns: `res_edge_table` in object.

Examples:

```
\donttest{
t1$get_edge_table()
}
```

Method `get_adjacency_matrix()`: Get the adjacency matrix from the network graph.

Usage:

```
trans_network$get_adjacency_matrix(...)
```

Arguments:

... parameters passed to `as_adjacency_matrix` function of `igraph` package.

Returns: `res_adjacency_matrix` in object.

Examples:

```
\donttest{
t1$get_adjacency_matrix(attr = "weight")
}
```

Method `plot_network()`: Plot the network based on a series of methods from other packages, such as `igraph`, `ggraph` and `networkD3`. The `networkD3` package provides dynamic network. It is especially useful for a glimpse of the whole network structure and finding the interested nodes and edges in a large network. In contrast, the `igraph` and `ggraph` methods are suitable for relatively small network.

Usage:

```
trans_network$plot_network(
  method = c("igraph", "ggraph", "networkD3")[1],
  node_label = "name",
  node_color = NULL,
  ggraph_layout = "fr",
  ggraph_node_size = 2,
  ggraph_text_color = NULL,
  ggraph_text_size = 3,
  networkD3_node_legend = TRUE,
  networkD3_zoom = TRUE,
  ...
)
```

Arguments:

`method` default "igraph"; The available options:

'igraph' call `plot.igraph` function in `igraph` package for a static network; see `plot.igraph` for the parameters

'ggraph' call `ggraph` function in `ggraph` package for a static network

'networkD3' use `forceNetwork` function in `networkD3` package for a dynamic network; see `forceNetwork` function for the parameters

`node_label` default "name"; node label shown in the plot for `method = "ggraph"` or `method = "networkD3"`; Please see the column names of `object$res_node_table`, which is the returned table of function `object$get_node_table`; User can select other column names in `res_node_table`.

`node_color` default NULL; node color assignment for `method = "ggraph"` or `method = "networkD3"`; Select a column name of `object$res_node_table`, such as "module".

```

ggraph_layout default "fr"; for method = "ggraph"; see layout parameter of create_layout
    function in ggraph package.
ggraph_node_size default 2; for method = "ggraph"; the node size.
ggraph_text_color default NULL; for method = "ggraph"; a column name of object$res_node_table;
    User can select other column names or change the content of object$res_node_table.
ggraph_text_size default 3; for method = "ggraph"; the node label text size.
networkD3_node_legend default TRUE; used for method = "networkD3"; logical value to enable
    node colour legends; Please see the legend parameter in networkD3::forceNetwork
    function.
networkD3_zoom default TRUE; used for method = "networkD3"; logical value to enable (TRUE)
    or disable (FALSE) zooming; Please see the zoom parameter in networkD3::forceNetwork
    function.
... parameters passed to plot.igraph function when method = "igraph" or forceNetwork
    function when method = "networkD3".

```

Returns: network plot.

Examples:

```
\donttest{
t1$plot_network(method = "igraph", layout = layout_with_kk)
t1$plot_network(method = "ggraph", node_color = "module")
t1$plot_network(method = "networkD3", node_color = "module")
}
```

Method cal_eigen(): Calculate eigengenes of modules, i.e. the first principal component based on PCA analysis, and the percentage of variance <doi:10.1186/1471-2105-13-113>.

Usage:

```
trans_network$cal_eigen()
```

Returns: res_eigen and res_eigen_expla in object.

Examples:

```
\donttest{
t1$cal_eigen()
}
```

Method plot_taxa_roles(): Plot the classification and importance of nodes, see object\$res_node_table for the variable names used in the parameters.

Usage:

```
trans_network$plot_taxa_roles(
  use_type = c(1, 2)[1],
  roles_color_background = FALSE,
  roles_color_values = NULL,
  add_label = FALSE,
  add_label_group = "Network hubs",
  add_label_text = "name",
  label_text_size = 4,
  label_text_color = "grey50",
  label_text_italic = FALSE,
```

```

plot_module = FALSE,
x_lim = c(0, 1),
use_level = "Phylum",
show_value = c("z", "p"),
show_number = 1:10,
plot_color = "Phylum",
plot_shape = "taxa_roles",
plot_size = "Abundance",
color_values = RColorBrewer::brewer.pal(12, "Paired"),
shape_values = c(16, 17, 7, 8, 15, 18, 11, 10, 12, 13, 9, 3, 4, 0, 1, 2, 14),
...
)

```

Arguments:

use_type default 1; 1 or 2; 1 represents taxa roles area plot; 2 represents the layered plot with taxa as x axis.

roles_color_background default FALSE; for use_type=1; TRUE: use background colors for each area; FALSE: use classic point colors.

roles_color_values default NULL; for use_type=1; color palette for background or points.

add_label default FALSE; for use_type = 1; whether add labels for the points.

add_label_group default "Network hubs"; If add_label = TRUE; which part of tax_roles is used to show labels; character vectors.

add_label_text default "name"; If add_label = TRUE; which column of object\$res_node_table is used to label the text.

label_text_size default 4; The text size of the label.

label_text_color default "grey50"; The text color of the label.

label_text_italic default FALSE; whether use italic style for the label text.

plot_module default FALSE; for use_type=1; whether plot the modules information.

x_lim default c(0, 1); for use_type=1; x axis range when roles_color_background = FALSE.

use_level default "Phylum"; for use_type=2; used taxonomic level in x axis.

show_value default c("z", "p"); for use_type=2; used variable in y axis.

show_number default 1:10; for use_type=2; showed number in x axis, sorting according to the nodes number.

plot_color default "Phylum"; for use_type=2; used variable for color.

plot_shape default "taxa_roles"; for use_type=2; used variable for shape.

plot_size default "Abundance"; for use_type=2; used for point size; a fixed number (e.g. 5) is also available.

color_values default RColorBrewer::brewer.pal(12, "Paired"); for use_type=2; color vector

shape_values default c(16, 17, 7, 8, 15, 18, 11, 10, 12, 13, 9, 3, 4, 0, 1, 2, 14); for use_type=2; shape vector, see ggplot2 tutorial for the shape meaning.

... parameters pass to geom_point.

Returns: ggplot.

Examples:

```
\donttest{
t1$plot_taxa_roles(roles_color_background = FALSE)
}
```

Method `subset_network()`: Subset of the network.

Usage:

```
trans_network$subset_network(node = NULL, edge = NULL, rm_single = TRUE)
```

Arguments:

`node` default `NULL`; provide the node names that you want to use in the sub-network.

`edge` default `NULL`; provide the edge name needed; must be one of `"+"` or `"-"` .

`rm_single` default `TRUE`; whether remove the nodes without any edge in the sub-network.

Returns: a new network

Examples:

```
\donttest{
t1$subset_network(node = t1$res_node_table %>% base::subset(module == "M1") %>%
  rownames, rm_single = TRUE)
# return a sub network that contains all nodes of module M1
}
```

Method `cal_powerlaw()`: Fit degrees to a power law distribution. First, perform a bootstrapping hypothesis test to determine whether degrees follow a power law distribution. If the distribution follows power law, then fit degrees to power law distribution and return the parameters.

Usage:

```
trans_network$cal_powerlaw(...)
```

Arguments:

... parameters pass to `fit_power_law` function in `igraph` package.

Returns: `res_powerlaw_p` and `res_powerlaw_fit`; see `powerLaw::bootstrap_p` function for the bootstrapping p value details; see `igraph::fit_power_law` function for the power law fit return details.

Examples:

```
\donttest{
t1$cal_powerlaw()
}
```

Method `cal_sum_links()`: This function is used to sum the links number from one taxa to another or in the same taxa, for example, at Phylum level. This is very useful to fast see how many nodes are connected between different taxa or within the taxa.

Usage:

```
trans_network$cal_sum_links(taxa_level = "Phylum")
```

Arguments:

`taxa_level` default `"Phylum"`; taxonomic rank.

Returns: `res_sum_links_pos` and `res_sum_links_neg` in object.

Examples:

```
\donttest{
t1$cal_sum_links(taxa_level = "Phylum")
}
```

Method `plot_sum_links()`: Plot the summed linkages among taxa using chorddiag package <<https://github.com/mattflor/chorddiag>>.

Usage:

```
trans_network$plot_sum_links(
  plot_pos = TRUE,
  plot_num = NULL,
  color_values = NULL,
  ...
)
```

Arguments:

`plot_pos` default TRUE; If TRUE, plot the summed positive linkages; If FALSE, plot the summed negative linkages.

`plot_num` default NULL; number of taxa presented in the plot.

`color_values` default NULL; If not provided, use `microeco::color_palette_20` or `randomcoloR` package to generate random colors (for taxa > 20).

... parameters pass to `chorddiag::chorddiag` function.

Returns: chorddiag plot

Examples:

```
\dontrun{
test1$plot_sum_links(plot_pos = TRUE, plot_num = 10)
}
```

Method `trans_comm()`: Transform classified features to community-like microtable object for further analysis, such as module-taxa table.

Usage:

```
trans_network$trans_comm(use_col = "module", abundance = TRUE)
```

Arguments:

`use_col` default "module"; which column to use as the 'community'; must be one of the name of `res_node_table` from function `get_node_table`.

`abundance` default TRUE; whether sum abundance of taxa. TRUE: sum the abundance for a taxon across all samples; FALSE: sum the frequency for a taxon across all samples.

Returns: a new `microtable` class.

Examples:

```
\donttest{
t2 <- t1$trans_comm(use_col = "module")
}
```

Method `print()`: Print the trans_network object.

Usage:

```
trans_network$print()
```

Method `clone()`: The objects of this class are cloneable with this method.

Usage:

```
trans_network$clone(deep = FALSE)
```

Arguments:

`deep` Whether to make a deep clone.

Examples

```

## -----
## Method `trans_network$new`
## -----


data(dataset)
# for correlation network
t1 <- trans_network$new(dataset = dataset, cor_method = "pearson",
taxa_level = "OTU", filter_thres = 0.0002)
# for non-correlation network
t1 <- trans_network$new(dataset = dataset, cor_method = NULL)

## -----
## Method `trans_network$cal_network`
## -----


## Not run:
# for correlation network
t1 <- trans_network$new(dataset = dataset, cor_method = "pearson",
taxa_level = "OTU", filter_thres = 0.001)
t1$cal_network(COR_p_thres = 0.05, COR_cut = 0.6)
t1 <- trans_network$new(dataset = dataset, cor_method = NULL, filter_thres = 0.003)
t1$cal_network(network_method = "SpiecEasi", SpiecEasi_method = "mb")
t1 <- trans_network$new(dataset = dataset, cor_method = NULL, filter_thres = 0.005)
t1$cal_network(network_method = "beemStatic")
t1 <- trans_network$new(dataset = dataset, cor_method = NULL, filter_thres = 0.001)
t1$cal_network(network_method = "FlashWeave")

## End(Not run)

## -----
## Method `trans_network$cal_module`
## -----


t1 <- trans_network$new(dataset = dataset, cor_method = "pearson",
taxa_level = "OTU", filter_thres = 0.0002)
t1$cal_network(COR_p_thres = 0.01, COR_cut = 0.6)
t1$cal_module(method = "cluster_fast_greedy")

## -----
## Method `trans_network$save_network`
## -----


## Not run:
t1$save_network(filepath = "network.gexf")

## End(Not run)

```

```
## -----
## Method `trans_network$cal_network_attr`
## -----



t1$cal_network_attr()



## -----
## Method `trans_network$get_node_table`
## -----



t1$get_node_table(node_roles = TRUE)



## -----
## Method `trans_network$get_edge_table`
## -----



t1$get_edge_table()



## -----
## Method `trans_network$get_adjacency_matrix`
## -----



t1$get_adjacency_matrix(attr = "weight"



## -----
## Method `trans_network$plot_network`
## -----



t1$plot_network(method = "igraph", layout = layout_with_kk)
t1$plot_network(method = "ggraph", node_color = "module")
t1$plot_network(method = "networkD3", node_color = "module")



## -----
## Method `trans_network$cal_eigen`
## -----



t1$cal_eigen()



## -----
## Method `trans_network$plot_taxa_roles`
```

```
## -----  
  
t1$plot_taxa_roles(roles_color_background = FALSE)  
  
## -----  
## Method `trans_network$subset_network`  
## -----  
  
t1$subset_network(node = t1$res_node_table %>% base::subset(module == "M1") %>%  
  rownames, rm_single = TRUE)  
# return a sub network that contains all nodes of module M1  
  
## -----  
## Method `trans_network$cal_powerlaw`  
## -----  
  
t1$cal_powerlaw()  
  
## -----  
## Method `trans_network$cal_sum_links`  
## -----  
  
t1$cal_sum_links(taxa_level = "Phylum")  
  
## -----  
## Method `trans_network$plot_sum_links`  
## -----  
  
## Not run:  
test1$plot_sum_links(plot_pos = TRUE, plot_num = 10)  
  
## End(Not run)  
  
## -----  
## Method `trans_network$trans_comm`  
## -----  
  
t2 <- t1$trans_comm(use_col = "module")
```

`trans_nullmodel` *Create trans_nullmodel object for phylogeny- and taxonomy-based null model analysis.*

Description

This class is a wrapper for a series of null model related approaches, including the mantel correlogram analysis of phylogenetic signal, beta nearest taxon index (betaNTI), beta net relatedness index (betaNRI), NTI, NRI and RCbray calculations; See Stegen et al. (2013) <10.1038/ismej.2013.93> and Liu et al. (2017) <doi:10.1038/s41598-017-17736-w> for the algorithms and applications.

Methods

Public methods:

- `trans_nullmodel$new()`
- `trans_nullmodel$cal_mantel_corr()`
- `trans_nullmodel$plot_mantel_corr()`
- `trans_nullmodel$cal_betampd()`
- `trans_nullmodel$cal_betamtd()`
- `trans_nullmodel$cal_ses_betampd()`
- `trans_nullmodel$cal_ses_betamtd()`
- `trans_nullmodel$cal_rcbray()`
- `trans_nullmodel$cal_process()`
- `trans_nullmodel$cal_NRI()`
- `trans_nullmodel$cal_NTI()`
- `trans_nullmodel$cal_Cscore()`
- `trans_nullmodel$cal_tNST()`
- `trans_nullmodel$cal_tNST_test()`
- `trans_nullmodel$clone()`

Method new():

Usage:

```
trans_nullmodel$new(  
  dataset = NULL,  
  filter_thres = 0,  
  taxa_number = NULL,  
  group = NULL,  
  select_group = NULL,  
  env_cols = NULL,  
  add_data = NULL,  
  complete_na = FALSE  
)
```

Arguments:

`dataset` the object of `microtable` Class.

`filter_thres` default 0; the relative abundance threshold.

`taxa_number` default NULL; how many taxa the user want to keep, if provided, `filter_thres` parameter will be forcible invalid.
`group` default NULL; which group column name in `sample_table` is selected.
`select_group` default NULL; the group name, used following the group to filter samples.
`env_cols` default NULL; number or name vector to select the environmental data in `dataset$sample_table`.
`add_data` default NULL; provide environmental data table additionally.
`complete_na` default FALSE; whether fill the NA in environmental data based on the method in `mice` package.

Returns: `data_comm` and `data_tree` in object.

Examples:

```
data(dataset)
data(env_data_16S)
t1 <- trans_nullmodel$new(dataset, filter_thres = 0.0005, add_data = env_data_16S)
```

Method `cal_mantel_corr()`: Calculate mantel correlogram.

Usage:

```
trans_nullmodel$cal_mantel_corr(
  use_env = NULL,
  break pts = seq(0, 1, 0.02),
  cutoff = FALSE,
  ...
)
```

Arguments:

`use_env` default NULL; numeric or character vector to select `env_data`; if provide multiple variables or NULL, use PCA (principal component analysis) to reduce dimensionality.

`break pts` default `seq(0, 1, 0.02)`; see `break pts` parameter in `mantel.correlog` of `vegan` package.

`cutoff` default FALSE; see `cutoff` parameter in `mantel.correlog`.

... parameters pass to `mantel.correlog`

Returns: `res_mantel_corr` in object.

Examples:

```
\donttest{
t1$cal_mantel_corr(use_env = "pH")
}
```

Method `plot_mantel_corr()`: Plot mantel correlogram.

Usage:

```
trans_nullmodel$plot_mantel_corr(point_shape = 22, point_size = 3)
```

Arguments:

`point_shape` default 22; the number for selecting point shape type; see `ggplot2` manual for the number meaning.

`point_size` default 3; the point size.

Returns: `ggplot`.

Examples:

```
\donttest{
t1$plot_mantel_corr()
}
```

Method `cal_betampd()`: Calculate betaMPD (mean pairwise distance). Same with `picante::comdist` function, but faster.

Usage:

```
trans_nullmodel$cal_betampd(abundance.weighted = TRUE)
```

Arguments:

`abundance.weighted` default TRUE; whether use abundance-weighted method.

Returns: `res_betampd` in object.

Examples:

```
\donttest{
t1$cal_betampd(abundance.weighted = TRUE)
}
```

Method `cal_betamntd()`: Calculate betaMNTD (mean nearest taxon distance). Same with `picante::comdistnt` package, but faster.

Usage:

```
trans_nullmodel$cal_betamntd(
  abundance.weighted = TRUE,
  exclude.conspecifics = FALSE,
  use_iCAMP = FALSE,
  use_iCAMP_force = TRUE,
  iCAMP_tempdir = NULL,
  ...
)
```

Arguments:

`abundance.weighted` default TRUE; whether use abundance-weighted method.

`exclude.conspecifics` default FALSE; see `exclude.conspecifics` parameter in `comdistnt` function of `picante` package.

`use_iCAMP` default FALSE; whether use `bmntd.big` function of `iCAMP` package to calculate betaMNTD. This method can store the phylogenetic distance matrix on the disk to lower the memory spending and perform the calculation parallelly.

`use_iCAMP_force` default FALSE; whether use `bmntd.big` function of `iCAMP` package automatically when the feature number is large.

`iCAMP_tempdir` default NULL; the temporary directory used to place the large tree file; If NULL; use the system user tempdir.

... parameters pass to `iCAMP::pdist.big` function.

Returns: `res_betamntd` in object.

Examples:

```
\donttest{
t1$cal_betamntd(abundance.weighted = TRUE)
}
```

Method `cal_ses_betampd()`: Calculate standardized effect size of betaMPD, i.e. beta net relatedness index (betaNRI).

Usage:

```
trans_nullmodel$cal_ses_betampd(
  runs = 1000,
  null.model = c("taxa.labels", "richness", "frequency", "sample.pool", "phylogeny.pool",
    "independentswap", "trialswap")[1],
  abundance.weighted = TRUE,
  iterations = 1000
)
```

Arguments:

`runs` default 1000; simulation runs.

`null.model` default "taxa.labels"; The available options include "taxa.labels", "richness", "frequency", "sample.pool", "phylogeny.pool", "independentswap" and "trialswap"; see `null.model` parameter of `ses.mntd` function in `picante` package for the algorithm details.

`abundance.weighted` default TRUE; whether use weighted abundance.

`iterations` default 1000; iteration number for part null models to perform; see `iterations` parameter of `picante::randomizeMatrix` function.

Returns: `res_ses_betampd` in object.

Examples:

```
\donttest{
# only run 50 times for the example; default 1000
t1$cal_ses_betampd(runs = 50, abundance.weighted = TRUE)
}
```

Method `cal_ses_betamntd()`: Calculate standardized effect size of betaMNTD, i.e. beta nearest taxon index (betaNTI).

Usage:

```
trans_nullmodel$cal_ses_betamntd(
  runs = 1000,
  null.model = c("taxa.labels", "richness", "frequency", "sample.pool", "phylogeny.pool",
    "independentswap", "trialswap")[1],
  abundance.weighted = TRUE,
  exclude.conspecifics = FALSE,
  use_iCAMP = FALSE,
  use_iCAMP_force = TRUE,
  iCAMP_tempdir = NULL,
  nworker = 2,
  iterations = 1000
)
```

Arguments:

`runs` default 1000; simulation number of null model.

`null.model` default "taxa.labels"; The available options include "taxa.labels", "richness", "frequency", "sample.pool", "phylogeny.pool", "independentswap" and "trialswap"; see `null.model` parameter of `ses.mntd` function in `picante` package for the algorithm details.

```

abundance.weighted default TRUE; whether use abundance-weighted method.
exclude.conspecifics default FALSE; see comdistnt in picante package.
use_iCAMP default FALSE; whether use bmntd.big function of iCAMP package to calculate
betaMNTD. This method can store the phylogenetic distance matrix on the disk to lower
the memory spending and perform the calculation parallelly.
use_iCAMP_force default FALSE; whether to make use_iCAMP to be TRUE when the feature
number is large.
iCAMP_tempdir default NULL; the temporary directory used to place the large tree file; If
NULL; use the system user tempdir.
nworker default 2; the CPU thread number.
iterations default 1000; iteration number for part null models to perform; see iterations pa-
rameter of picante::randomizeMatrix function.

```

Returns: res_ses_betamntd in object.

Examples:

```
\donttest{
# only run 50 times for the example; default 1000
t1$cal_ses_betamntd(runs = 50, abundance.weighted = TRUE, exclude.conspecifics = FALSE)
}
```

Method cal_rcbray(): Calculate Bray–Curtis-based Raup–Crick (RCbray).

Usage:

```
trans_nullmodel$cal_rcbray(
  runs = 1000,
  verbose = TRUE,
  null.model = "independentswap"
)
```

Arguments:

runs default 1000; simulation runs.
 verbose default TRUE; whether show the calculation process message.
 null.model default "independentswap"; see more available options in randomizeMatrix func-
 tion of picante package.

Returns: res_rcbray in object.

Examples:

```
\donttest{
# only run 50 times for the example; default 1000
t1$cal_rcbray(runs = 50)
}
```

Method cal_process(): Infer the ecological processes according to ses.betaMNTD ses.betaMPD
and rcbray.

Usage:

```
trans_nullmodel$cal_process(use_betamntd = TRUE)
```

Arguments:

use_betamntd default TRUE; whether use ses.betaMNTD; if false, use ses.betaMPD.

Returns: res_rcbray in object.

Examples:

```
\donttest{
t1$cal_process(use_betamntd = TRUE)
}
```

Method cal_NRI(): Calculates Nearest Relative Index (NRI), equivalent to -1 times the standardized effect size of MPD.

Usage:

```
trans_nullmodel$cal_NRI(
  null.model = "taxa.labels",
  abundance.weighted = FALSE,
  runs = 999,
  ...
)
```

Arguments:

null.model default "taxa.labels"; Null model to use; see null.model parameter in ses.mpd function of picante package for available options.

abundance.weighted default FALSE; Should mean nearest relative distances for each species be weighted by species abundance?

runs default 999; Number of randomizations.

... parameters pass to ses.mpd function in picante package.

Returns: res_NRI in object, equivalent to -1 times ses.mpd.

Examples:

```
\donttest{
# only run 50 times for the example; default 999
t1$cal_NRI(null.model = "taxa.labels", abundance.weighted = FALSE, runs = 50)
}
```

Method cal_NTI(): Calculates Nearest Taxon Index (NTI), equivalent to -1 times the standardized effect size of MNTD.

Usage:

```
trans_nullmodel$cal_NTI(
  null.model = "taxa.labels",
  abundance.weighted = FALSE,
  runs = 999,
  ...
)
```

Arguments:

null.model default "taxa.labels"; Null model to use; see null.model parameter in ses.mntd function of picante package for available options.

abundance.weighted default FALSE; Should mean nearest taxon distances for each species be weighted by species abundance?

runs default 999; Number of randomizations.

... parameters pass to ses.mntd function in picante package.

Returns: res_NTI in object, equivalent to -1 times ses.mntd.

Examples:

```
\donttest{
# only run 50 times for the example; default 999
t1$cal_NTI(null.model = "taxa.labels", abundance.weighted = TRUE, runs = 50)
}
```

Method cal_Cscore(): Calculates the (normalised) mean number of checkerboard combinations (C-score) using C.score function in bipartite package.

Usage:

```
trans_nullmodel$cal_Cscore(by_group = NULL, ...)
```

Arguments:

by_group default NULL; one column name or number in sample_table; calculate C-score for different groups separately.

... parameters pass to bipartite::C.score function.

Returns: vector.

Examples:

```
\dontrun{
t1$cal_Cscore(normalise = FALSE)
t1$cal_Cscore(by_group = "Group", normalise = FALSE)
}
```

Method cal_tNST(): Calculate normalized stochasticity ratio (NST) based on the tNST function of NST package.

Usage:

```
trans_nullmodel$cal_tNST(group, ...)
```

Arguments:

group a colname of sample_table; the function can select the data from sample_table to generate a one-column (n x 1) matrix and provide it to the group parameter of tNST function.

... parameters pass to NST::tNST function; see the documents of tNST function for more details.

Returns: .

Examples:

```
\dontrun{
t1$cal_tNST(group = "Group", dist.method = "bray", output.rand = TRUE, SES = TRUE)
}
```

Method cal_tNST_test(): Test the significance of NST difference between each pair of groups.

Usage:

```
trans_nullmodel$cal_tNST_test(method = "nst.boot", ...)
```

Arguments:

```
method default "nst.boot"; "nst.boot" or "nst.panova"; see NST::nst.boot function or NST::nst.panova function for the details.
... paremeters pass to NST::nst.boot when method = "nst.boot" or NST::nst.panova when method = "nst.panova"
```

Returns: .

Examples:

```
\dontrun{
t1$cal_tNST_test()
}
```

Method `clone()`: The objects of this class are cloneable with this method.

Usage:

```
trans_nullmodel$clone(deep = FALSE)
```

Arguments:

`deep` Whether to make a deep clone.

Examples

```
## -----
## Method `trans_nullmodel$new`
## -----


data(dataset)
data(env_data_16S)
t1 <- trans_nullmodel$new(dataset, filter_thres = 0.0005, add_data = env_data_16S)

## -----
## Method `trans_nullmodel$cal_mantel_corr`
## -----


t1$cal_mantel_corr(use_env = "pH")

## -----
## Method `trans_nullmodel$plot_mantel_corr`
## -----


t1$plot_mantel_corr()

## -----
## Method `trans_nullmodel$cal_betampd`
## -----


t1$cal_betampd(abundance.weighted = TRUE)
```

```
## -----
## Method `trans_nullmodel$cal_betamntd`
## -----



t1$cal_betamntd(abundance.weighted = TRUE)

## -----
## Method `trans_nullmodel$cal_ses_betampd`
## -----



# only run 50 times for the example; default 1000
t1$cal_ses_betampd(runs = 50, abundance.weighted = TRUE)

## -----
## Method `trans_nullmodel$cal_ses_betamntd`
## -----



# only run 50 times for the example; default 1000
t1$cal_ses_betamntd(runs = 50, abundance.weighted = TRUE, exclude.conspecifics = FALSE)

## -----
## Method `trans_nullmodel$cal_rcbray`
## -----



# only run 50 times for the example; default 1000
t1$cal_rcbray(runs = 50)

## -----
## Method `trans_nullmodel$cal_process`
## -----



t1$cal_process(use_betamntd = TRUE)

## -----
## Method `trans_nullmodel$cal_NRI`
## -----



# only run 50 times for the example; default 999
t1$cal_NRI(null.model = "taxa.labels", abundance.weighted = FALSE, runs = 50)
```

```

## -----
## Method `trans_nullmodel$cal_NTI`
## -----


# only run 50 times for the example; default 999
t1$cal_NTI(null.model = "taxa.labels", abundance.weighted = TRUE, runs = 50)

## -----
## Method `trans_nullmodel$cal_Cscore`
## -----


## Not run:
t1$cal_Cscore(normalise = FALSE)
t1$cal_Cscore(by_group = "Group", normalise = FALSE)

## End(Not run)

## -----
## Method `trans_nullmodel$cal_tNST`
## -----


## Not run:
t1$cal_tNST(group = "Group", dist.method = "bray", output.rand = TRUE, SES = TRUE)

## End(Not run)

## -----
## Method `trans_nullmodel$cal_tNST_test`
## -----


## Not run:
t1$cal_tNST_test()

## End(Not run)

```

trans_venn*Create trans_venn object.***Description**

This class is a wrapper for a series of venn analysis related methods, including venn result, 2- to 5-way venn diagram, more than 5-way petal plot and venn result transformations based on David et al. (2012) <doi:10.1128/AEM.01459-12>.

Methods**Public methods:**

- `trans_venn$new()`
- `trans_venn$plot_venn()`
- `trans_venn$plot_bar()`
- `trans_venn$trans_comm()`
- `trans_venn$print()`
- `trans_venn$clone()`

Method new():*Usage:*

```
trans_venn$new(
  dataset = NULL,
  sample_names = NULL,
  ratio = NULL,
  add_abund_table = NULL,
  name_joint = "&"
)
```

Arguments:

`dataset` the object of `microtable` Class.
`sample_names` default NULL; if provided, filter the samples.
`ratio` default NULL; NULL, "numratio" or "seqratio"; numratio: calculate number percentage;
 seqratio: calculate sequence percentage; NULL: no additional percentage.
`add_abund_table` default NULL; data.frame or matrix format; additional data provided instead of `dataset$otu_table`; Features must be rows.
`name_joint` default "&"; the joint mark for generating multi-sample names.

Returns: `data_details` and `data_summary` stored in `trans_venn` object.*Examples:*

```
\donttest{
data(dataset)
t1 <- dataset$merge_samples(use_group = "Group")
t1 <- trans_venn$new(dataset = t1, ratio = "numratio")
}
```

Method plot_venn(): Plot venn diagram.*Usage:*

```
trans_venn$plot_venn(
  color_circle = RColorBrewer::brewer.pal(8, "Dark2"),
  fill_color = TRUE,
  text_size = 4.5,
  text_name_size = 6,
  text_name_position = NULL,
  alpha = 0.3,
  linesize = 1.1,
  petal_plot = FALSE,
  petal_color = "#BEAED4",
  petal_color_center = "#BEBADA",
```

```

petal_a = 4,
petal_r = 1,
petal_use_lim = c(-12, 12),
petal_center_size = 40,
petal_move_xy = 4,
petal_move_k = 2.3,
petal_move_k_count = 1.3,
petal_text_move = 40,
other_text_show = NULL,
other_text_position = c(2, 2),
other_text_size = 5
)

```

Arguments:

```

color_circle default RColorBrewer::brewer.pal(8, "Dark2"); color pallete
fill_color default TRUE; whether fill the area color
text_size default 4.5; text size in plot
text_name_size default 6; name size in plot
text_name_position default NULL; name position in plot
alpha default .3; alpha for transparency
linesize default 1.1; cycle line size
petal_plot default FALSE; whether use petal plot.
petal_color default "#BEAED4"; color of the petals.
petal_color_center default "#BEBADA"; color of the center in the petal plot.
petal_a default 4; the length of the ellipse
petal_r default 1; scaling up the size of the ellipse
petal_use_lim default c(-12, 12); the width of the plot
petal_center_size default 40; petal center circle size
petal_move_xy default 4; the distance of text to circle
petal_move_k default 2.3; the distance of title to circle
petal_move_k_count default 1.3; the distance of data text to circle
petal_text_move default 40; the distance between two data text
other_text_show default NULL; other characters used to show in the plot
other_text_position default c(1, 1); the text position for text in other_text_show
other_text_size default 5; the text size for text in other_text_show

```

Returns: ggplot.

Examples:

```
\donttest{
t1$plot_venn()
}
```

Method `plot_bar()`: Plot the intersections using histogram. Especially useful when samples > 5.

Usage:

```
trans_venn$plot_bar(
  bottom_y_text_size = 12,
  up_y_title = "Intersection set",
  up_y_title_size = 15,
  up_y_text_size = 8,
  bar_color = "grey70",
  bar_fill = "grey70",
  point_size = 3,
  point_color = "black",
  bottom_height = 0.5
)
```

Arguments:

bottom_y_text_size default 12; y axis text size, i.e. sample name size, of bottom plot.
 up_y_title default "Intersection set"; y axis title of upper plot.
 up_y_title_size default 15; y axis title size of upper plot.
 up_y_text_size default 4; y axis text size of upper plot.
 bar_color default "grey70"; bar border color of upper plot.
 bar_fill default "grey70"; bar fill color of upper plot.
 point_size default 3; point size of bottom plot.
 point_color default "black"; point color of bottom plot.
 bottom_height default 0.5; bottom plot height.

Returns: a ggplot2 object.

Examples:

```
\donttest{
t2 <- t1$plot_bar()
}
```

Method trans_comm(): Transform venn result to community-like microtable object for further composition analysis.

Usage:

```
trans_venn$trans_comm(use_frequency = TRUE)
```

Arguments:

use_frequency default TRUE; whether only use OTUs occurrence frequency, i.e. presence/absence data; if FALSE, use abundance data.

Returns: a new [microtable](#) class.

Examples:

```
\donttest{
t2 <- t1$trans_comm(use_frequency = TRUE)
}
```

Method print(): Print the trans_venn object.

Usage:

```
trans_venn/print()
```

Method clone(): The objects of this class are cloneable with this method.

Usage:

```
trans_venn$clone(deep = FALSE)
```

Arguments:

deep Whether to make a deep clone.

Examples

```
## -----
## Method `trans_venn$new`
## -----



data(dataset)
t1 <- dataset$merge_samples(use_group = "Group")
t1 <- trans_venn$new(dataset = t1, ratio = "numratio")

## -----
## Method `trans_venn$plot_venn`
## -----


t1$plot_venn()

## -----
## Method `trans_venn$plot_bar`
## -----


t2 <- t1$plot_bar()

## -----
## Method `trans_venn$trans_comm`
## -----


t2 <- t1$trans_comm(use_frequency = TRUE)
```

Index

* **R6**
 dataset, 3
* **data.frame**
 env_data_16S, 5
 fungi_func_FungalTraits, 5
 fungi_func_FUNGuild, 5
 otu_table_16S, 15
 otu_table_ITS, 15
 phylo_tree_16S, 16
 prok_func_FAPROTAX, 16
 prok_func_NJC19_list, 16
 sample_info_16S, 17
 sample_info_ITS, 17
 taxonomy_table_16S, 17
 taxonomy_table_ITS, 18
* **datasets**
 color_palette_20, 3
* **list**
 Tax4Fun2_KEGG, 17
* **object**
 dataset, 3

adonis2, 34
aov, 28

betadisper, 34

clone, 2
color_palette_20, 3

data.frame, 19
dataset, 3
dropallfactors, 4

env_data_16S, 5

fungi_func_FungalTraits, 5
fungi_func_FUNGuild, 5

geom_bar, 21, 50
geom_boxplot, 24

mantel, 59
mantel.correlog, 88
micratable, 6, 20, 27, 31, 38, 45, 53, 67, 73, 83, 87, 97, 99

otu_table_16S, 15
otu_table_ITS, 15

phylo_tree_16S, 16
prok_func_FAPROTAX, 16
prok_func_NJC19_list, 16

rrarefy, 8

sample, 8
sample_info_16S, 17
sample_info_ITS, 17
stat_ellipse, 33, 58

Tax4Fun2_KEGG, 17
taxonomy_table_16S, 17
taxonomy_table_ITS, 18
tidy_taxonomy, 18
trans_abund, 19
trans_alpha, 27, 35, 54
trans_beta, 31
trans_classifier, 37
trans_diff, 44
trans_env, 52
trans_func, 66
trans_network, 72
trans_nullmodel, 86
trans_venn, 96

vegdist, 12