# Package: MicroBioMeta (via r-universe)

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**Title** Analysis and Visualization of Microbiome Diversity, Composition, and Differential Abundance

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**Description** The goal of MicroBioMeta is to support beginners in microbiome data analysis, whether working with metagenomic or metabarcoding datasets. It enables users with limited experience in R to explore, visualize, and analyze results with minimal coding and customization. This helps save time on scripting, while still producing statistically robust results and publication-ready figures in an intuitive and accessible way.

**License** Artistic-2.0

**Depends** R (>= 4.5.0)

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abundance_bar_plot *Relative abundance barplot*

## Description

Generates a bar plot of relative abundance (%) for the most abundant taxa groups across samples or sample groups.

## Usage

```
abundance_bar_plot(
  table,
  metadata = NULL,
  taxonomy_db = "silva",
  level = "genus",
  x_col = NULL,
  facet_by = NULL,
  width_equal = FALSE,
  label = "taxonomy",
  top_n = 15,
  x_axis_title = "Samples",
  y_axis_title = "Relative abundance (%)",
  x_label_angle = 0,
  strip_text_bold = FALSE,
  strip_color = "grey",
  palette = "default",
  taxa_colors = NULL,
  taxa_order = "ascending",
  aspect_ratio = NULL,
  add_remained = FALSE,
  save_table = FALSE,
  table_filename = "relative_abundance.txt"
)
```

## Arguments

| | |
|---|---|
| `table` | A data frame with taxa in rows and samples in columns. The last column must be named `taxonomy`, containing full taxonomic strings. |
| `metadata` | A data frame containing sample metadata. Its first column must hold the sample IDs (the column names of `table`). Optional: if `NULL` (default), one bar is drawn per sample. |
| `taxonomy_db` | Character. Database the taxonomy strings come from: `"silva"` (default), `"gg2"` (Greengenes2, also `"gg"`), `"unite"` or `"Kraken2"` (also `"kraken"`). Case-insensitive. |
| `level` | Character. Taxonomic level: `"kingdom"`, `"phylum"`, `"class"`, `"order"`, `"family"`, `"genus"` (default) or `"species"`. Case-insensitive. |

| | |
|---|---|
| x_col | Character. Name of the column in metadata for the x-axis. If NULL (default), one bar per sample. |
| facet_by | Character. Name of the column in metadata to facet the plot by. Optional; NULL (default) for no facets. |
| width_equal | Logical. If TRUE, all bars have equal width regardless of sample count per group. Default FALSE. |
| label | Character. Title of the taxa legend. Default "taxonomy". |
| top_n | Integer. Number of most abundant taxa to show. Default 15. |
| x_axis_title | Character. Title of the x-axis. Default "Samples". |
| y_axis_title | Character. Title of the y-axis. Default "Relative abundance (%)". |
| x_label_angle | Numeric. Rotation (degrees) of the x-axis labels. Default 0 (horizontal); use 45 or 90 if they overlap. |
| strip_text_bold | Logical. If TRUE, the facet strip labels are bold. Default FALSE. |
| strip_color | Character. Background color of the facet strips (used when facet_by is set). Default "grey". |
| palette | Character. Palette for the taxa: "default" (bright colors, the original MicroBioMeta palette), "brewer" (the qualitative RColorBrewer palettes Accent, Dark2, Paired, Pastel1, Pastel2, Set1, Set2 and Set3 joined, without greys; not colorblind-friendly) or "safe" (colorblind-friendly "Safe" palette, 11 colors; with more taxa the colors are interpolated and are no longer fully colorblind-safe). The most abundant taxon gets the first color. Default "default". |
| taxa_colors | Optional named character vector of colors, one per taxon (names = taxa as shown in the legend). Taxa not named keep the palette colors. Default NULL. |
| taxa_order | Character. Order of the taxa in the bars and the legend: "ascending" (default; the most abundant taxon at the bottom of both) or "descending" (the most abundant taxon at the top of both). |
| aspect_ratio | Numeric. Aspect ratio (height/width) of each panel. Default NULL (automatic). |
| add_remained | Logical. If TRUE, adds an "Other" category with the sum of the remaining taxa. Default FALSE. |
| save_table | Logical. If TRUE, saves the relative-abundance table as a tab-delimited file. Default FALSE. |
| table_filename | Character. Name or path of the saved file (used when save_table = TRUE). Default "relative_abundance.txt". |

## Details

- Relative abundances are calculated per sample (%).
- Taxa are collapsed to the taxonomic level; taxa not resolved to that level are shown as "other ".
- Only the top top_n taxa are shown; others are filtered out.
- Samples are grouped and ordered according to x_col.
- Optional faceting by facet_by if provided.
- Features assigned only to a kingdom/domain (e.g. "d__Bacteria;__;__;__;__;__") or "Unassigned" are removed.

## Value

A ggplot object with the stacked bar plot of relative abundances.

## Examples

```
table_path <- system.file("extdata", "subset_table_bacteria.txt", package = "MicroBioMeta")
table <- read.delim(table_path, row.names = 1, check.names = FALSE)

metadata_path <- system.file("extdata", "subset_metadata_bacteria.txt", package = "MicroBioMeta")
metadata <- read.delim(metadata_path, check.names = FALSE)

abundance_bar_plot(
    table = table,
    metadata = metadata,
    taxonomy_db = "silva",
    level = "genus",
    x_col = "Location",
    label = "Genus",
    facet_by = "Treatment",
    top_n = 30,
    add_remained = TRUE
)
```

---

# abundance_heatmap_plot

*Heatmap of relative abundance*

---

## Description

Creates a heatmap (with ComplexHeatmap) of the relative abundance of the most abundant features (ASVs, OTUs or taxa).

## Usage

```
abundance_heatmap_plot(
  table,
  metadata = NULL,
  condition1 = NULL,
  condition2 = NULL,
  condition3 = NULL,
  colors_condition1 = NULL,
  colors_condition2 = NULL,
  colors_condition3 = NULL,
  name_legend_condition1 = NULL,
  name_legend_condition2 = NULL,
  name_legend_condition3 = NULL,
  top_n = 15,
  exclude_unclassified = TRUE,
```

```
  cluster = TRUE,
  show_column_names = TRUE,
  cell_size = NULL,
  annotation_height = NULL,
  save_table = FALSE,
  table_filename = "abundance_heatmap_table.txt",
  feature_prefix = "",
  max_label_length = 35,
  composite_names = TRUE,
  draw = TRUE
)
```

## Arguments

| | |
|---|---|
| table | A data frame with taxa in rows and samples in columns. The last column must be named taxonomy, containing full taxonomic strings. |
| metadata | A data frame containing sample metadata. Its first column must hold the sample IDs (the column names of table). Optional: if NULL (default), the heatmap has no sample annotations. |
| condition1 | Character. Name of the column in metadata for the first column annotation. Optional; NULL (default) for none. |
| condition2 | Character. Name of the column in metadata for the second column annotation. Optional; NULL (default) for none. |
| condition3 | Character. Name of the column in metadata for the third column annotation. Optional; NULL (default) for none. |
| colors_condition1 | Optional character vector of colors for condition1. If named, colors are matched to the values by name (e.g. c(Roots = "#009E73", Rhizosphere = "#56B4E9")); otherwise they are assigned in alphabetical order of the values. If NULL (default), the colorblind-friendly Okabe-Ito palette is used. |
| colors_condition2 | Optional character vector of colors for condition2 (same rules as colors_condition1). If NULL (default), colors of the colorblind-friendly "Safe" palette are used, which contrast with those of condition1. |
| colors_condition3 | Optional character vector of colors for condition3 (same rules as colors_condition1). If NULL (default), a third set of colorblind-friendly colors is used. |
| name_legend_condition1 | Character. Title of the legend of condition1. If NULL (default), the name of condition1 is used. |
| name_legend_condition2 | Character. Title of the legend of condition2. If NULL (default), the name of condition2 is used. |
| name_legend_condition3 | Character. Title of the legend of condition3. If NULL (default), the name of condition3 is used. |
| top_n | Integer. Number of most abundant features to show. Default 15. |

`exclude_unclassified`
: Logical. If `TRUE` (default), taxa with no classification at any level ("Unclassified") are dropped before selecting the `top_n` most abundant features, so all the rows are identified taxa. Use `FALSE` to keep them.

`cluster`
: Logical. If `TRUE` (default), the rows are clustered; if `FALSE`, they are ordered by abundance.

`show_column_names`
: Logical. If `TRUE` (default), the sample names are shown.

`cell_size`
: Numeric. Side, in millimeters, of each (square) cell. If `NULL` (default), it is set automatically.

`annotation_height`
: Numeric. Height, in millimeters, of each column annotation bar (`condition1`, `condition2`, `condition3`). If `NULL` (default), the cell height is used.

`save_table`
: Logical. If `TRUE`, saves the abundance table shown in the heatmap as a tab-delimited file. Default `FALSE`.

`table_filename`
: Character. Name or path of the saved file (used when `save_table = TRUE`). Default `"abundance_heatmap_table.txt"`.

`feature_prefix`
: Character. Prefix of the row labels, before the row number (e.g. `feature_prefix = "ASV"` labels the rows `"ASV1"`, `"ASV2"`...). Default `""` (rows labeled `"1"`, `"2"`...).

`max_label_length`
: Integer. Taxon names longer than this many characters are cut with an ellipsis in the row labels. `NULL` never cuts them. Default `35`.

`composite_names`
: Logical. If `TRUE` (default), SILVA composite names of three or more genera are labeled with the last genus plus "group" (e.g. "Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium" becomes "Rhizobium group"); two-genus names (e.g. "Escherichia-Shigella") are kept whole. The full names are kept in the saved table.

`draw`
: Logical. If `TRUE` (default), the heatmap is drawn on the current device. Use `FALSE` to only build the returned grob without drawing it, e.g. to combine it with other plots; then, unless `cell_size` is given, cells stretch to fill the panel they are placed in instead of having a fixed size.

## Value

Invisibly, a `gTree` (grid grob) with the heatmap of the `top_n` most abundant features. Printing it (e.g. typing its name) draws the heatmap; it can also be combined with other plots (e.g. `cowplot::plot_grid()`, `patchwork::wrap_elements()`).

## Examples

```
table_path <- system.file("extdata", "subset_table_bacteria.txt", package = "MicroBioMeta")
table <- read.delim(table_path, row.names = 1, check.names = FALSE)
metadata_path <- system.file("extdata", "subset_metadata_bacteria.txt", package = "MicroBioMeta")
metadata <- read.delim(metadata_path, check.names = FALSE)
```

```
abundance_heatmap_plot(
    table                 = table,
    metadata              = metadata,
    condition1            = "Location",
    condition2            = "Treatment",
    condition3            = "Plot",
    top_n                 = 50,
    cluster               = TRUE,
    show_column_names     = FALSE,
    name_legend_condition1 = "Location",
    name_legend_condition2 = "Treatment",
    name_legend_condition3 = "Plot"
)

heat <- abundance_heatmap_plot(
    table = table,
    metadata = metadata,
    condition1 = "Location",
    condition2 = "Treatment",
    top_n = 20,
    show_column_names = FALSE,
    colors_condition1 = c(Rhizosphere = "#56B4E9", Roots = "#009E73"),
    colors_condition2 = c(
        Control = "#CC6677",
        Moderate_drought = "#332288",
        Severe_drought = "#999933"
    ),
    name_legend_condition1 = "Compartment",
    feature_prefix = "ASV",
    max_label_length = 30,
    draw = FALSE
)
heat # printing the returned object draws the heatmap
```

---

abundance_sankey_plot *Sankey diagram of relative abundances*

---

## Description

Creates an interactive Sankey diagram (with networkD3) of how the relative abundance flows across taxonomic ranks.

## Usage

```
abundance_sankey_plot(
  table,
  output_file = NULL,
  maxn = 25,
  taxRanks = c("D", "K", "P", "C", "O", "F", "G", "S"),
```

```
  taxonomy_db = "silva",
  width = NULL,
  height = NULL,
  save_table = FALSE,
  table_filename = "sankey_nodes_links.txt"
)
```

## Arguments

| | |
|---|---|
| table | A data frame with taxa in rows and samples in columns. The last column must be named taxonomy, containing full taxonomic strings. |
| output_file | Character or NULL. Path of an HTML file to save the interactive diagram to. If NULL (default), nothing is written to disk; the diagram is only returned. |
| maxn | Integer. Maximum number of taxa per rank. Default 25. At each rank the most abundant taxa are chosen among the children of the taxa kept at the previous rank, so every flow follows the hierarchy without skipping columns. |
| taxRanks | Character vector of the ranks to show, as one-letter codes: "D" (domain), "K" (kingdom), "P", "C", "O", "F", "G" and "S". Default all of them. |
| taxonomy_db | Character. Database the taxonomy strings come from: "silva" (default), "gg2" (Greengenes2, also "gg"), "unite" or "Kraken2" (also "kraken"). Case-insensitive. |
| width, height | Numeric or NULL. Width and height of the diagram in pixels. If NULL (default), the widget's default size is used (it fills the available width). |
| save_table | Logical. If TRUE, saves the Sankey nodes and links (one table, with a table_type column: "node" or "link") as a tab-delimited file. Default FALSE. |
| table_filename | Character. Name or path of the saved file (used when save_table = TRUE). Default "sankey_nodes_links.txt". |

## Value

Invisibly returns the Sankey diagram object (an htmlwidget that is shown when printed). If output_file is given, it is also saved as an HTML file.

## Examples

```
table_path <- system.file("extdata", "subset_table_bacteria.txt", package = "MicroBioMeta")
table <- read.delim(table_path, row.names = 1, check.names = FALSE)

abundance_sankey_plot(
    table        = table,
    output_file  = file.path(tempdir(), "sankey_output.html"),
    maxn         = 10,
    taxRanks     = c("P", "C", "G", "S"),
    taxonomy_db  = "silva"
)
```

`aldex_heatmap_plot` *ALDEx2 differential abundance heatmap*

## Description

Runs ALDEx2 between two groups and shows the differentially abundant taxa as a heatmap (with ComplexHeatmap) of their CLR values, annotated with the effect size and the p-value.

## Usage

```
aldex_heatmap_plot(
  table,
  metadata,
  group_col,
  effect_threshold = 0,
  pval_threshold = 0.05,
  p_adjust_method = "BH",
  mc_samples = 128,
  cluster_rows = FALSE,
  cluster_columns = FALSE,
  heatmap_colors = NULL,
  effect_colors = c("#009E73", "white", "#CC79A7"),
 pvalue_colors = c(`<0.001` = "#000000", `<0.01` = "#D55E00", `<0.05` = "#F0E442",
    `>0.05` = "grey85"),
  group_colors = NULL,
  save_table = FALSE,
  table_filename = "aldex_pval_effect.txt",
  draw = TRUE
)
```

## Arguments

| | |
|---|---|
| `table` | A data frame with taxa in rows and samples in columns. The last column must be named `taxonomy`, containing full taxonomic strings. |
| `metadata` | A data frame containing sample metadata. Its first column must hold the sample IDs (the column names of `table`). |
| `group_col` | Character. Name of the column in `metadata` that defines the groups. It must have exactly two groups. |
| `effect_threshold` | Numeric. Minimum absolute effect size to retain. Default `0` (no effect-size filter), so by default the heatmap shows the same taxa that `aldex_volcano_plot` colors as significant. |
| `pval_threshold` | Numeric or `NULL`. P-value cutoff (after `p_adjust_method`) to keep only significant taxa. Default `0.05`. If `NULL`, only `effect_threshold` is applied. |

p_adjust_method
: Character. "BH" (default) or "none": use the Benjamini-Hochberg adjusted p-values of ALDEx2 (wi.eBH) or the raw ones (wi.ep) for pval_threshold and the p-value annotation. ALDEx2 only computes the BH correction.

mc_samples
: Integer. Number of ALDEx2 Monte Carlo instances. Default 128 (ALDEx2's default), recommended for final analyses. Fewer instances are faster but the p-values and effect sizes are less stable (e.g. 16 for a quick look); call set.seed() before the function to make the result reproducible.

cluster_rows
: Logical. Cluster heatmap rows (default FALSE).

cluster_columns
: Logical. Cluster heatmap columns (default FALSE).

heatmap_colors
: Controls the color scale of the main heatmap body (CLR values). Three options: NULL (default, same as "viridis") uses a sequential colorblind-friendly viridis scale (the same family used in abundance_heatmap_plot), with range computed automatically from the data; a preset name string: "viridis" or one of the diverging presets "BuOr" (blue-orange), "BuVm" (blue-vermillion), "BuPk" (blue-pink), "GnPk" (green-pink); or a circlize::colorRamp2 function for full manual control.

effect_colors
: Three colors for the effect size annotation strip: negative, zero and positive effect (mapped to -1.5, 0 and 1.5). Default c("#009E73", "white", "#CC79A7") (green-white-pink, colorblind-friendly, distinct from the heatmap body and p-value defaults). A circlize::colorRamp2() function is also accepted.

pvalue_colors
: Named vector of colors for the p-value annotation strip, with names "<0.001", "<0.01", "<0.05" and ">0.05". Default black/vermillion/yellow/grey (distinct from the heatmap body and effect size defaults). A list with one such vector named "p-value" is also accepted.

group_colors
: Optional character vector of colors for the group annotation, one per group, named after the groups or in their order. If NULL (default), the colorblind-friendly Okabe-Ito palette is used (orange/blue for two groups).

save_table
: Logical. If TRUE, saves the ALDEx2 results of the plotted taxa (effect size, diff.btw and p-value category) as a tab-delimited file. Default FALSE. effect and diff.btw are saved as ALDEx2 returns them (second group in alphabetical order minus the first); the higher_in column says in which group each taxon is higher.

table_filename
: Character. Name or path of the saved file (used when save_table = TRUE). Default "aldex_pval_effect.txt".

draw
: Logical. If TRUE (default), the heatmap is drawn on the current device. Use FALSE to only build the returned grob without drawing it.

## Value

Invisibly, a gTree (grid grob) with the heatmap. Printing it (e.g. typing its name) draws the heatmap; it can also be combined with other plots (e.g. cowplot::plot_grid(), patchwork::wrap_elements()).

## Examples

```
table_path <- system.file("extdata", "subset_table_bacteria.txt", package = "MicroBioMeta")
table <- read.delim(table_path, row.names = 1, check.names = FALSE)

metadata_path <- system.file("extdata", "subset_metadata_bacteria.txt", package = "MicroBioMeta")
metadata <- read.delim(metadata_path, check.names = FALSE)

# group_col must have exactly two groups; Location has two
# (Rhizosphere and Roots) in the bundled example data. Here taxa are
# filtered by effect size alone (pval_threshold = NULL), since this small
# (46-sample) dataset has few taxa with significant p-values.
aldex_heatmap_plot(
    table            = table,
    metadata         = metadata,
    group_col        = "Location",
    mc_samples       = 16,
    effect_threshold = 0.5,
    pval_threshold   = NULL
)

# All the colors have colorblind-friendly defaults, but each can be set by
# hand: group_colors (difference bars, named after the groups),
# effect_colors (negative, zero and positive effect size), pvalue_colors
# (one color per p-value class) and heatmap_colors (median clr values)

aldex_heatmap_plot(
    table = table,
    metadata = metadata,
    group_col = "Location",
    mc_samples = 16,
    effect_threshold = 0.5,
    pval_threshold = NULL,
    group_colors = c(Rhizosphere = "#56B4E9", Roots = "#009E73"),
    effect_colors = c("#0072B2", "white", "#E69F00"),
    pvalue_colors = c(
        "<0.001" = "black", "<0.01" = "grey30",
        "<0.05" = "grey60", ">0.05" = "grey90"
    ),
    heatmap_colors = "BuOr"
)
```

---

`aldex_volcano_plot` *ALDEx2 volcano or effect size plot*

---

## Description

Runs ALDEx2 between two groups and plots the results as a volcano plot or an effect size plot.

## Usage

```
aldex_volcano_plot(
  table,
  metadata,
  group_col,
  type = "volcano",
  mc_samples = 128,
  col_inf = "#56B4E9",
  col_sup = "#E69F00",
  threshold_lower = -1.5,
  threshold_upper = 1.5,
  cond = NULL,
  pval_threshold = 0.05,
  p_adjust_method = "BH",
  show_labels = TRUE,
  taxa = NULL,
  x_axis_title = NULL,
  y_axis_title = NULL,
  label_size = 3.5,
  filter_uncultured = FALSE,
  save_table = FALSE,
  table_filename = "aldex_pval_effect.txt"
)
```

## Arguments

| | |
|---|---|
| `table` | A data frame with taxa in rows and samples in columns. The last column must be named `taxonomy`, containing full taxonomic strings. |
| `metadata` | A data frame containing sample metadata. Its first column must hold the sample IDs (the column names of `table`). |
| `group_col` | Character. Name of the column in `metadata` that defines the groups. It must have exactly two groups. |
| `type` | Type of plot to generate: `"volcano"` (default, volcano plot) or `"effect"` (effect-size plot). Case-insensitive. |
| `mc_samples` | Integer. Number of ALDEx2 Monte Carlo instances. Default `128` (ALDEx2's default), recommended for final analyses. Fewer instances are faster but the p-values and effect sizes are less stable (e.g. `16` for a quick look); call `set.seed()` before the function to make the result reproducible. |
| `col_inf` | Character. Color of the taxa lower in `cond`. Default `"#56B4E9"` (Okabe-Ito blue). |
| `col_sup` | Character. Color of the taxa higher in `cond`. Default `"#E69F00"` (Okabe-Ito orange). |
| `threshold_lower` | Numeric. Lower cutoff on the x-axis (effect size or difference between groups), drawn as a dashed line. Default `-1.5`. |

threshold_upper
: Numeric. Upper cutoff on the x-axis (effect size or difference between groups), drawn as a dashed line. Default 1.5.

cond
: Name of the reference condition for the plot labels: positive values on the x-axis mean higher in cond. Default NULL, the condition of the first sample in table.

pval_threshold
: Numeric. P-value cutoff (after p_adjust_method), drawn as the dashed horizontal line. Default 0.05.

p_adjust_method
: Character. "BH" (default) or "none": use the Benjamini-Hochberg adjusted p-values of ALDEx2 (wi.eBH) or the raw ones (wi.ep) for the y-axis and pval_threshold. ALDEx2 only computes the BH correction.

show_labels
: Logical. If TRUE (default), shows the "Higher in"/"Lower in" cond labels (only for type = "effect").

taxa
: Optional data frame with columns Feature.ID and Taxon, used to label the taxa. If NULL (default), the taxonomy column of table is used.

x_axis_title, y_axis_title
: Titles for the x- and y-axis. Default NULL: "Effect size" ("effect") or log2 fold change ("volcano") on x, and the (adjusted) p-value on y.

label_size
: Numeric. Font size of the taxon labels drawn on significant points in the "volcano" plot. Default 3.5.

filter_uncultured
: Logical. If TRUE, taxa whose name contains "uncultured"/"unculture" are still plotted as points but not labelled, keeping the volcano plot's text annotations to named taxa. Default FALSE.

save_table
: Logical. If TRUE, saves the ALDEx2 results table as a tab-delimited file. Default FALSE. effect and diff.btw are saved as ALDEx2 returns them (second group in alphabetical order minus the first); in the plot, positive means higher in cond.

table_filename
: Character. Name or path of the saved file (used when save_table = TRUE). Default "aldex_pval_effect.txt".

## Value

A ggplot object with the selected plot.

## Examples

```
table_path <- system.file("extdata", "subset_table_bacteria.txt", package = "MicroBioMeta")
table <- read.delim(table_path, row.names = 1, check.names = FALSE)

metadata_path <- system.file("extdata", "subset_metadata_bacteria.txt", package = "MicroBioMeta")
metadata <- read.delim(metadata_path, check.names = FALSE)

# group_col must have exactly two groups; Location has two
# (Rhizosphere and Roots) in the bundled example data
aldex_volcano_plot(
    table = table,
    metadata = metadata,
    group_col = "Location",
```

```
    mc_samples = 16,
    type = "effect",
    col_inf = "#56B4E9",
    col_sup = "#E69F00",
    threshold_lower = -0.5,
    threshold_upper = 0.5,
    cond = "Rhizosphere",
    show_labels = TRUE
)
```

---

alpha_decay_plot *Alpha diversity along a continuous gradient*

---

## Description

Computes Hill numbers (q = 0, 1, 2) from table and plots them against a continuous metadata variable (e.g. distance to urban center, elevation, pH). It reports the Spearman rank correlation coefficient (rho, or Pearson r), its p-value, and optionally the linear regression $R^2$ and slope.

## Usage

```
alpha_decay_plot(
  table,
  metadata,
  cont_var,
  group_col = NULL,
  method = "spearman",
  show_lm_stats = TRUE,
  facet_orientation = "horizontal",
  palette = "colorb",
  group_colors = NULL,
  x_axis_title = NULL,
  y_axis_title = "Effective number of features",
  title = NULL,
  show_legend = TRUE,
  legend_position = "bottom",
  free_y = TRUE,
  point_size = 2,
  line_width = 0.9,
  point_alpha = 0.8,
  annotation_size = 3.5,
  panel_label_case = "upper",
  panel_labels = NULL,
  panel_label_bold = TRUE,
  strip_text_bold = FALSE
)
```

## Arguments

| | |
|---|---|
| table | A data frame with taxa in rows and samples in columns. The last column must be named taxonomy, containing full taxonomic strings. |
| metadata | A data frame containing sample metadata. Its first column must hold the sample IDs (the column names of table). |
| cont_var | Character. Name of the continuous variable column in metadata to place on the x-axis (e.g. "dist_km"). |
| group_col | Character. Name of the column in metadata that defines the groups, used to color the points and fit one regression line per group. Optional; NULL (default) for no groups. |
| method | Character. Correlation method for the statistic annotation. One of "spearman" (default) or "pearson". |
| show_lm_stats | Logical. If TRUE (default), adds $R^2$ and slope from the linear model to the annotation label. |
| facet_orientation | Character. "horizontal" (default) puts the three q0/q1/q2 panels in a row; "vertical" in a column. |
| palette | Character. Palette name: "colorb" (default, colorblind-friendly Okabe-Ito), "safe" (colorblind-friendly CARTO "Safe"), "earth" (CARTO "Earth", brown to teal; best for two or ordered groups), "grey", "viridis" or "brewer". |
| group_colors | Optional character vector of colors, one per group, named after the groups or in their order. Overrides palette. |
| x_axis_title | Character. Title of the x-axis. If NULL (default), the name of cont_var is used. |
| y_axis_title | Character. Title of the y-axis. Default "Effective number of features". |
| title | Character. Plot title. NULL (default) shows no title. |
| show_legend | Logical. If TRUE (default), the legend is shown. |
| legend_position | Character. Position of the legend: "bottom" (default), "top", "right" or "left". |
| free_y | Logical. If TRUE, the y-axis scale is free across facets. Default TRUE. |
| point_size | Numeric. Size of the points. Default 2. |
| line_width | Numeric. Width of regression lines. Default 0.9. |
| point_alpha | Numeric (0-1). Transparency of the points. Default 0.8. |
| annotation_size | Numeric. Font size for the stats annotation. Default 3.5. |
| panel_label_case | Character. Case of the panel tags: "upper" (default; A, B, C) or "lower" (a, b, c). Ignored if panel_labels is given. |
| panel_labels | Optional character vector of custom panel tags, one per panel (q0, q1, q2), used as-is (e.g. c("(a)", "(b)", "(c)")). Overrides panel_label_case. |
| panel_label_bold | Logical. If TRUE (default), panel tags are bold. Set to FALSE for journals that require plain (non-bold) panel tags. |
| strip_text_bold | Logical. If TRUE, the facet strip labels are bold. Default FALSE. |

## Value

A patchwork object with the three q0/q1/q2 panels, tagged as set by panel_label_case or panel_labels. It can still be modified: p & theme(...) changes every panel, p[[2]] + labs(...) a single one.

## Examples

```
table_path <- system.file("extdata", "subset_table_bacteria.txt", package = "MicroBioMeta")
table <- read.delim(table_path, row.names = 1, check.names = FALSE)

metadata_path <- system.file("extdata", "subset_metadata_bacteria.txt", package = "MicroBioMeta")
metadata <- read.delim(metadata_path, check.names = FALSE)

loc_dist <- data.frame(Loc = 1:7, dist_km = seq(0, 12, length.out = 7))
metadata$dist_km <- loc_dist$dist_km[match(metadata$Loc, loc_dist$Loc)] +
    stats::rnorm(nrow(metadata), sd = 0.3)

# All samples, no grouping. Always tagged A/B/C (see panel_label_case and
# panel_labels to customize)
alpha_decay_plot(
    table        = table,
    metadata     = metadata,
    cont_var     = "dist_km",
    x_axis_title = "Distance (km)"
)

# Separate regression lines by location
alpha_decay_plot(
    table        = table,
    metadata     = metadata,
    cont_var     = "dist_km",
    x_axis_title = "Distance (km)",
    group_col    = "Location"
)
```

---

# alpha_diversity_plot *Alpha diversity indices plot*

---

## Description

Computes the Chao1, Shannon and Simpson indices of each sample (or Faith's phylogenetic diversity or Rao's quadratic entropy, see diversity) and plots them as box plots or bar plots, one panel per index, optionally faceted by one or two metadata variables and with statistical comparisons between groups.

## Usage

```
alpha_diversity_plot(
  table,
  metadata,
```

```
  type = "boxplot",
  stat = NULL,
  p_adjust_method = "holm",
  x_col,
  fill_col,
  facet_by = NULL,
  facet_by2 = NULL,
  facet_orientation = "horizontal",
  palette = "colorb",
  group_colors = NULL,
  n_cols = NULL,
  n_rows = NULL,
  strip_color = "grey",
  show_legend = TRUE,
  title = NULL,
  legend_title = NULL,
  legend_position = "bottom",
  x_axis_title = NULL,
  y_axis_title = NULL,
  free_y = FALSE,
  rarefy_depth = NULL,
  panel_label_case = "upper",
  panel_labels = NULL,
  panel_label_bold = TRUE,
  x_label_angle = 0,
  strip_text_bold = FALSE,
  aspect_ratio = NULL,
  save_table = FALSE,
  table_filename = "diversity.txt",
  diversity = "taxonomic",
  tree = NULL,
  traits = NULL
)
```

## Arguments

| | |
|---|---|
| table | A data frame with taxa in rows and samples in columns. The last column must be named taxonomy, containing full taxonomic strings. |
| metadata | A data frame containing sample metadata. Its first column must hold the sample IDs (the column names of table). |
| type | Type of plot: "boxplot" (default) or "barplot". Case-insensitive. |
| stat | Character or NULL. Statistical test to compare the groups within each panel. "wilcox.test" or "t.test" compare every pair of groups, each with its own bracket and p-value (ggpubr::stat_pwc()); "kruskal.test" or "anova" give one global p-value per panel. Default NULL (no test shown). |
| p_adjust_method | Character. Multiple-testing correction for the pairwise tests (stat = "wilcox.test" or "t.test"), applied within each panel, any method of stats::p.adjust(). |

Default "holm"; "none" uses the raw p-values.

x_col Character. Name of the column in metadata for the x-axis.

fill_col Character. Name of the column in metadata for the fill color.

facet_by Character. Name of the column in metadata to facet the plot by. Optional; NULL (default) for no facets.

facet_by2 Character. Name of a second column in metadata to facet by. Optional; NULL (default) for none.

facet_orientation
Character. "horizontal" (default) puts the facet_by facets in columns; "vertical" in rows. Case-insensitive.

palette Character. Palette name: "colorb" (default, colorblind-friendly Okabe-Ito), "safe" (colorblind-friendly CARTO "Safe"), "earth" (CARTO "Earth", brown to teal; best for two or ordered groups), "grey", "viridis" or "brewer". Case-insensitive.

group_colors Optional character vector of colors, one per group, named after the groups or in their order. Overrides palette.

n_cols Integer. Number of columns of the facet grid. If NULL (default), set automatically.

n_rows Integer. Number of rows of the facet grid. If NULL (default), set automatically.

strip_color Character. Background color of the facet strips (used when facet_by is set). Default "grey".

show_legend Logical. If TRUE (default), the legend is shown.

title Character. Plot title. NULL (default) shows no title.

legend_title Character. Title of the legend. If NULL (default), the legend has no title.

legend_position
Character. Position of the legend: "bottom" (default), "top", "right" or "left".

x_axis_title Character. Title of the x-axis. Default NULL (no title).

y_axis_title Character. Title of the y-axis. Default NULL: "Diversity measure", "Branch length" (Faith PD) or "Mean functional distance" (Rao Q).

free_y Logical. Each index always has its own y-axis scale (Chao1, Shannon and Simpson have very different ranges). If FALSE (default), that scale is shared by all the panels of the same index, so the groups can be compared across facets. If TRUE, every panel gets its own scale.

rarefy_depth Integer. If given, the samples are rarefied to this depth (vegan::rrarefy()) before computing the indices. Default NULL (no rarefaction).

panel_label_case
Character. Case of the panel tags (added to every panel): "upper" (default; A, B, C) or "lower" (a, b, c). Ignored if panel_labels is given.

panel_labels Optional character vector of custom panel tags, one per panel, used as-is (e.g. c("(a)", "(b)", "(c)")). Overrides panel_label_case.

panel_label_bold
: Logical. If TRUE (default), panel tags are bold. Set to FALSE for journals that require plain (non-bold) panel tags.

x_label_angle
: Numeric. Rotation (degrees) of the x-axis labels. Default 0 (horizontal); use 45 or 90 if they overlap.

strip_text_bold
: Logical. If TRUE, the facet strip labels are bold. Default FALSE.

aspect_ratio
: Numeric. Aspect ratio (height/width) of each panel. Default NULL (automatic).

save_table
: Logical. If TRUE, saves the diversity table as a tab-delimited file. Default FALSE.

table_filename
: Character. Name or path of the saved file (used when save_table = TRUE). Default "diversity.txt".

diversity
: Character. "taxonomic" (default; Chao1, Shannon and Simpson), "phylogenetic" (Faith's PD, the summed branch length up to the root; needs tree) or "functional" (Rao's Q, the mean Gower distance between two random reads; needs traits). Case-insensitive.

tree
: A rooted phylo tree whose tip labels are the taxa of table. Used when diversity = "phylogenetic".

traits
: A data frame with taxa in rows and traits in columns. Used when diversity = "functional".

## Value

A ggplot object.

## Examples

```
table_path <- system.file("extdata", "subset_table_bacteria.txt", package = "MicroBioMeta")
table <- read.delim(table_path, row.names = 1, check.names = FALSE)

metadata_path <- system.file("extdata", "subset_metadata_bacteria.txt", package = "MicroBioMeta")
metadata <- read.delim(metadata_path, check.names = FALSE)

# Panel tags (A/B/C) are always added (see panel_label_case and
# panel_labels to customize their case/format); stat additionally
# overlays p-value annotations on each panel
alpha_diversity_plot(
  table            = table,
  metadata         = metadata,
  type             = "barplot",
  x_col            = "Location",
  fill_col         = "Location",
  facet_by         = "Treatment",
  free_y           = TRUE,
  legend_position  = "top",
  stat             = "t.test",
  panel_label_case = "upper"
)
```

`alpha_hill_corr_plot` *Alpha diversity vs sequencing depth*

## Description

Computes the Hill numbers (q = 0, 1, 2) of each sample and plots each against the sequencing depth (total reads), with a regression line and the correlation coefficient, combining the three panels in one figure.

## Usage

```
alpha_hill_corr_plot(
  table,
  method = "spearman",
  facet_orientation = "horizontal",
  title = "auto",
  panel_label_case = "upper",
  panel_labels = NULL,
  panel_label_bold = TRUE,
  save_table = FALSE,
  table_filename = "hill.txt",
  x_axis_title = "Sequencing depth (number of reads)"
)
```

## Arguments

| | |
|---|---|
| `table` | A data frame with taxa in rows and samples in columns. The last column must be named `taxonomy`, containing full taxonomic strings. |
| `method` | Character. Correlation method passed to `ggpubr::stat_cor`. One of `"spearman"` (default, rank-based and robust to non-linear/non-normal relationships), `"pearson"`, or `"kendall"`. |
| `facet_orientation` | Character. `"horizontal"` (default) puts the three q0/q1/q2 panels in a row; `"vertical"` in a column. |
| `title` | Character. Plot title. `"auto"` (default) shows `"Alpha diversity vs sequencing depth"`; `NULL` shows no title; any other text is used as the title. |
| `panel_label_case` | Character. Case of the panel tags: `"upper"` (default; A, B, C) or `"lower"` (a, b, c). Ignored if `panel_labels` is given. |
| `panel_labels` | Optional character vector of custom panel tags, one per panel (q0, q1, q2), used as-is (e.g. `c("(a)", "(b)", "(c)")`). Overrides `panel_label_case`. |
| `panel_label_bold` | Logical. If `TRUE` (default), panel tags are bold. Set to `FALSE` for journals that require plain (non-bold) panel tags. |
| `save_table` | Logical. If `TRUE`, saves the Hill numbers table as a tab-delimited file. Default `FALSE`. |

table_filename Character. Name or path of the saved file (used when save_table = TRUE). Default "hill.txt".

x_axis_title Character. Title of the x-axis of the three panels. Default "Sequencing depth (number of reads)".

## Value

A patchwork object with the three q0/q1/q2 panels.

## Examples

```
table_path <- system.file("extdata", "subset_table_bacteria.txt", package = "MicroBioMeta")
table <- read.delim(table_path, row.names = 1, check.names = FALSE)

# Always tagged A/B/C (see panel_label_case and panel_labels to customize)
alpha_hill_corr_plot(
    table             = table,
    facet_orientation = "horizontal"
)

## Using Pearson correlation instead of the default Spearman
alpha_hill_corr_plot(
    table  = table,
    method = "pearson"
)
```

---

# alpha_hill_plot *Hill numbers alpha diversity plot*

---

## Description

Computes the Hill numbers (q = 0, 1, 2) of each sample and plots them as box plots or bar plots, one panel per order q, optionally faceted by one or two metadata variables and with statistical comparisons between groups.

## Usage

```
alpha_hill_plot(
  table,
  metadata,
  type = "boxplot",
  stat = NULL,
  p_adjust_method = "holm",
  x_col,
  fill_col,
  facet_by = NULL,
  facet_by2 = NULL,
  facet_orientation = "horizontal",
```

```
  palette = "colorb",
  group_colors = NULL,
  n_cols = NULL,
  n_rows = NULL,
  strip_color = "grey",
  show_legend = TRUE,
  title = NULL,
  legend_title = NULL,
  legend_position = "bottom",
  x_axis_title = NULL,
  y_axis_title = NULL,
  free_y = FALSE,
  panel_label_case = "upper",
  panel_labels = NULL,
  panel_label_bold = TRUE,
  x_label_angle = 0,
  strip_text_bold = FALSE,
  aspect_ratio = NULL,
  save_table = FALSE,
  table_filename = "hill.txt",
  diversity = "taxonomic",
  tree = NULL,
  traits = NULL,
  tau = NULL
)
```

## Arguments

| | |
|---|---|
| table | A data frame with taxa in rows and samples in columns. The last column must be named taxonomy, containing full taxonomic strings. |
| metadata | A data frame containing sample metadata. Its first column must hold the sample IDs (the column names of table). |
| type | Type of plot: "boxplot" (default) or "barplot". Case-insensitive. |
| stat | Character or NULL. Statistical test to compare the groups within each panel. "wilcox.test" or "t.test" compare every pair of groups, each with its own bracket and p-value (ggpubr::stat_pwc()); "kruskal.test" or "anova" give one global p-value per panel. Default NULL (no test shown). |
| p_adjust_method | Character. Multiple-testing correction for the pairwise tests (stat = "wilcox.test" or "t.test"), applied within each panel, any method of stats::p.adjust(). Default "holm"; "none" uses the raw p-values. |
| x_col | Character. Name of the column in metadata for the x-axis. |
| fill_col | Character. Name of the column in metadata for the fill color. |
| facet_by | Character. Name of the column in metadata to facet the plot by. Optional; NULL (default) for no facets. |
| facet_by2 | Character. Name of a second column in metadata to facet by. Optional; NULL (default) for none. |

facet_orientation
: Character. "horizontal" (default) puts the facet_by facets in columns; "vertical" in rows. Case-insensitive.

palette
: Character. Palette name: "colorb" (default, colorblind-friendly Okabe-Ito), "safe" (colorblind-friendly CARTO "Safe"), "earth" (CARTO "Earth", brown to teal; best for two or ordered groups), "grey", "viridis" or "brewer". Case-insensitive.

group_colors
: Optional character vector of colors, one per group, named after the groups or in their order. Overrides palette.

n_cols
: Integer. Number of columns of the facet grid. If NULL (default), set automatically.

n_rows
: Integer. Number of rows of the facet grid. If NULL (default), set automatically.

strip_color
: Character. Background color of the facet strips (used when facet_by is set). Default "grey".

show_legend
: Logical. If TRUE (default), the legend is shown.

title
: Character. Plot title. NULL (default) shows no title.

legend_title
: Character. Title of the legend. If NULL (default), the legend has no title.

legend_position
: Character. Position of the legend: "bottom" (default), "top", "right" or "left".

x_axis_title
: Character. Title of the x-axis. Default NULL (no title).

y_axis_title
: Character. Title of the y-axis. Default NULL: the unit of diversity: "Effective number of features", "Effective number of lineages" or "Effective number of functional groups".

free_y
: Logical. Each order q always has its own y-axis scale (q0 is usually much larger than q2). If FALSE (default), that scale is shared by all the panels of the same order, so the groups can be compared across facets. If TRUE, every panel gets its own scale.

panel_label_case
: Character. Case of the panel tags (added to every panel): "upper" (default; A, B, C) or "lower" (a, b, c). Ignored if panel_labels is given.

panel_labels
: Optional character vector of custom panel tags, one per panel, used as-is (e.g. c("(a)", "(b)", "(c)")). Overrides panel_label_case.

panel_label_bold
: Logical. If TRUE (default), panel tags are bold. Set to FALSE for journals that require plain (non-bold) panel tags.

x_label_angle
: Numeric. Rotation (degrees) of the x-axis labels. Default 0 (horizontal); use 45 or 90 if they overlap.

strip_text_bold
: Logical. If TRUE, the facet strip labels are bold. Default FALSE.

aspect_ratio
: Numeric. Aspect ratio (height/width) of each panel. Default NULL (automatic).

save_table
: Logical. If TRUE, saves the Hill numbers table as a tab-delimited file. Default FALSE.

table_filename Character. Name or path of the saved file (used when save_table = TRUE). Default "hill.txt".

diversity Character. "taxonomic" (default, Hill numbers of the abundances), "phylogenetic" (needs tree) or "functional" (needs traits), computed with hilldiv3::hilldiv(). Phylogenetic values are effective numbers of lineages read at one common tree depth (reference = "pool"), so a sample can fall slightly below

1. Functional values are effective numbers of functional groups at the threshold tau. Case-insensitive.

tree A rooted phylo tree (e.g. from ape::read.tree()) whose tip labels are the taxa of table. Used when diversity = "phylogenetic".

traits A data frame with taxa in rows and traits in columns (e.g. the EC or KO copy numbers predicted per ASV by PICRUSt2), converted to Gower distances with hilldiv3::traits2dist(). Used when diversity = "functional".

tau Numeric. Threshold of functional distinctiveness: taxa at this distance or more count as different functional groups. NULL (default) uses the mean distance between two reads of the pooled samples (Chao et al. 2019). Used when diversity = "functional".

## Value

A ggplot object.

## Examples

```
table_path <- system.file("extdata", "subset_table_bacteria.txt", package = "MicroBioMeta")
table <- read.delim(table_path, row.names = 1, check.names = FALSE)

metadata_path <- system.file("extdata", "subset_metadata_bacteria.txt", package = "MicroBioMeta")
metadata <- read.delim(metadata_path, check.names = FALSE)

# Panel tags (A/B/C) are always added (see panel_label_case and
# panel_labels to customize their case/format); stat additionally
# overlays p-value annotations on each panel
alpha_hill_plot(
  table            = table,
  metadata         = metadata,
  type             = "boxplot",
  x_col            = "Location",
  fill_col         = "Location",
  facet_by         = "Treatment",
  legend_position  = "top",
  stat             = "kruskal.test",
  panel_label_case = "upper"
)
```

ancombc_plot *ANCOMBC2 differential abundance bar/heatmap plot*

## Description

Runs ANCOMBC2 on a table and metadata, then visualizes differentially abundant taxa.

## Usage

```
ancombc_plot(
  table,
  metadata,
  group_col,
  level = "genus",
  min_prevalence = 0.1,
  p_adjust_method = "holm",
  formula = NULL,
  rand_formula = NULL,
  ref_level = NULL,
  diverging_palette = "BuOr",
  x_axis_title = NULL,
  y_axis_title = NULL,
  bar_colors = c("#56B4E9", "#E69F00"),
  save_table = FALSE,
  table_filename = "ancombc_results.txt"
)
```

## Arguments

| | |
|---|---|
| table | A data frame with taxa in rows and samples in columns. The last column must be named taxonomy, containing full taxonomic strings. |
| metadata | A data frame containing sample metadata. Its first column must hold the sample IDs (the column names of table). |
| group_col | Character. Name of the column in metadata that defines the groups. If it is numeric, it is treated as a continuous covariate. |
| level | Character or NULL. Taxonomic level to agglomerate to before running ancombc2 (e.g. "genus", "family"; case-insensitive). Default "genus". Pass NULL to skip agglomeration and run ANCOMBC2 directly on the ASV/OTU/species-level table (rows of table, as-is). |
| min_prevalence | Numeric. Prevalence cut-off passed to ancombc2 (default 0.1). Lower values retain more taxa. |
| p_adjust_method | Character. Multiple-testing correction for the ANCOMBC2 p-values of the taxa (passed to ancombc2()), any method of stats::p.adjust(). Default "holm"; "none" uses the raw p-values. Use "BH" for a less strict correction with few samples. |

formula — Character. Right-hand side of the fixed-effects formula passed to ancombc2 (e.g. "group + age"). If NULL (default) group_col is used alone.

rand_formula — Character. Random-effects formula passed to ancombc2 for mixed models (e.g. "~ 1 | subject_id"). Default NULL.

ref_level — Character. Reference level of group_col, the baseline of the comparisons. If NULL (default), the first factor level is used (e.g. ref_level = "P2" compares every other group against P2).

diverging_palette — Character. Colorblind-friendly diverging palette of the heatmap (3 or more groups): "BuOr" (blue-orange, default), "BuVm" (blue-vermillion), "BuPk" (blue-pink), "GnPk" (green-pink) or "PuYl" (purple-yellow). Not used by the bar plot, which uses bar_colors.

x_axis_title, y_axis_title — Titles for the x- and y-axis. Default NULL: the log fold change of the comparison on x of the bar plots, and no title otherwise.

bar_colors — Character vector of two colors for the bar plot: the first for the taxa higher in the non-reference group, the second for those higher in the reference group. Default c("#56B4E9", "#E69F00").

save_table — Logical. If TRUE, saves the ANCOMBC2 results table (log fold change, standard error, p- and q-values and diff_ of each term) as a tab-delimited file. Default FALSE.

table_filename — Character. Name or path of the saved file (used when save_table = TRUE). Default "ancombc_results.txt".

## Value

A ggplot object: a bar plot (2 groups) or a heatmap (3 or more groups).

## Examples

```
table_path <- system.file("extdata", "subset_table_bacteria.txt", package = "MicroBioMeta")
table <- read.delim(table_path, row.names = 1, check.names = FALSE)

metadata_path <- system.file("extdata", "subset_metadata_bacteria.txt", package = "MicroBioMeta")
metadata <- read.delim(metadata_path, check.names = FALSE)


ancombc_plot(
    table = table,
    metadata = metadata,
    group_col = "Location",
    min_prevalence = 0.3,
    p_adjust_method = "BH"
)
```

`beta_decay_plot` *Distance-decay of community similarity plot*

## Description

Computes the pairwise community dissimilarity (Jaccard, Morisita-Horn, Bray-Curtis or another vegdist method) and the pairwise geographic distance from the sample coordinates in metadata, and tests their relationship with a Mantel test.

## Usage

```
beta_decay_plot(
  table,
  metadata,
  lat_col,
  lon_col,
  group_col = NULL,
  palette = "colorb",
  distance = "jaccard",
  method = "spearman",
  permutations = 999,
  show_lm_stats = TRUE,
  point_color = "black",
  line_color = "#D55E00",
  point_size = 1,
  point_alpha = 0.5,
  annotation_size = 3.5,
  x_axis_title = "Spatial distance (km)",
  y_axis_title = NULL,
  title = NULL
)
```

## Arguments

| | |
|---|---|
| `table` | A data frame with taxa in rows and samples in columns. The last column must be named `taxonomy`, containing full taxonomic strings. |
| `metadata` | A data frame containing sample metadata. Its first column must hold the sample IDs (the column names of `table`). It must also have the latitude and longitude columns. |
| `lat_col` | Character. Name of the latitude column in `metadata` (decimal degrees). |
| `lon_col` | Character. Name of the longitude column in `metadata` (decimal degrees). |
| `group_col` | Character. Name of the column in `metadata` that defines the groups. If given, only pairs of samples from the same group are kept, with one color per group. Optional; `NULL` (default) for no groups. |

| | |
|---|---|
| palette | Palette name ("colorb" (default), "safe", "earth", "grey", "viridis" or "brewer") or a vector of colors, one per group. Only used when group_col is given. |
| distance | Character. Dissimilarity metric passed to vegan::vegdist. Common options: "jaccard" (default), "horn" (Morisita-Horn / Hill q = 1 analogue), "bray" (Bray-Curtis). Any method accepted by vegdist is valid. Case-insensitive. |
| method | Character. Correlation method for the Mantel test: "spearman" (default) or "pearson". Case-insensitive. |
| permutations | Integer. Number of permutations for the Mantel test. Default 999. |
| show_lm_stats | Logical. If TRUE (default), adds $R^2$ to the annotation label in addition to the Mantel r, p-value, and slope. |
| point_color | Character. Color of scatter points. Default "black". |
| line_color | Character. Color of the regression line. Default "#D55E00". |
| point_size | Numeric. Size of the points. Default 1. |
| point_alpha | Numeric (0-1). Transparency of the points. Default 0.5. |
| annotation_size | Numeric. Font size for the stats annotation. Default 3.5. |
| x_axis_title | Character. Title of the x-axis. Default "Spatial distance (km)". |
| y_axis_title | Character. Title of the y-axis. If NULL (default), it is built automatically. |
| title | Character. Plot title. NULL (default) shows no title. |

## Value

A ggplot object.

## Examples

```
table_path <- system.file("extdata", "subset_table_bacteria.txt", package = "MicroBioMeta")
table <- read.delim(table_path, row.names = 1, check.names = FALSE)

metadata_path <- system.file("extdata", "subset_metadata_bacteria.txt", package = "MicroBioMeta")
metadata <- read.delim(metadata_path, check.names = FALSE)

loc_coords <- data.frame(
    Loc = 1:7,
    lat = 19.0 + seq(0, 0.6, length.out = 7),
    lon = -99.0 + seq(0, 0.6, length.out = 7)
)
metadata$lat <- loc_coords$lat[match(metadata$Loc, loc_coords$Loc)]
metadata$lon <- loc_coords$lon[match(metadata$Loc, loc_coords$Loc)]

# Jaccard + Spearman Mantel (default)
beta_decay_plot(
    table    = table,
    metadata = metadata,
    lat_col  = "lat",
    lon_col  = "lon"
```

```
)

# Horn dissimilarity + Spearman Mantel
beta_decay_plot(
    table    = table,
    metadata = metadata,
    lat_col  = "lat",
    lon_col  = "lon",
    distance = "horn",
    method   = "spearman"
)

# Separate Mantel test per location
beta_decay_plot(
    table     = table,
    metadata  = metadata,
    lat_col   = "lat",
    lon_col   = "lon",
    group_col = "Location"
)
```

---

`beta_dissimilarity_plot` *Beta diversity boxplot*

---

## Description

Computes the pairwise beta diversity between samples (shared features, turnover or nestedness) and plots it as box plots, one box per pair of groups.

## Usage

```
beta_dissimilarity_plot(
  table,
  metadata,
  comparison_condition1 = NULL,
  condition1_col,
  condition2_col = NULL,
  facet_colors = NULL,
  group_colors = NULL,
  x_axis_title = "Condition",
  y_axis_title = NULL,
  partition = c("shared", "turnover", "nestedness"),
  family = c("sorensen", "jaccard"),
  stat = NULL,
  p_adjust_method = "holm",
  show_x_labels = TRUE,
  x_label_angle = 0,
```

```
  strip_text_bold = FALSE,
  strip_text_color = "black",
  aspect_ratio = NULL,
  save_table = FALSE,
  table_filename = "betadiv_table.txt"
)
```

## Arguments

| | |
|---|---|
| table | A data frame with taxa in rows and samples in columns. The last column must be named taxonomy, containing full taxonomic strings. |
| metadata | A data frame containing sample metadata. Its first column must hold the sample IDs (the column names of table). |
| comparison_condition1 | Optional character vector with the pairs of groups of condition1_col to show (e.g. "Rhizosphere_vs_Roots"; the order of the two groups does not matter). If NULL (default), all pairs are shown. |
| condition1_col | Character. Name of the column in metadata whose groups are compared: each box is one pair of groups. |
| condition2_col | Character. Name of a second column in metadata. If given, only pairs of samples with the same value are kept, and the plot is faceted by it. Optional; NULL (default) for none. |
| facet_colors | Optional character vector of background colors for the facet strips. If NULL (default), a neutral "grey85" is used. |
| group_colors | Optional character vector of colors, one per comparison on the x-axis, named after them or in their order. If NULL (default), the colorblind-friendly Okabe-Ito palette is used. |
| x_axis_title | Character. Title of the x-axis. Default "Condition". |
| y_axis_title | Character. Title of the y-axis. If NULL (default), it is built automatically. |
| partition | Type of beta diversity to compute: "shared" (default), "turnover", or "nestedness". Case-insensitive. |
| family | Dissimilarity family for the turnover/nestedness partition: "sorensen" (default) or "jaccard". Case-insensitive. |
| stat | Character or NULL. Statistical test to compare the boxes within each panel. "wilcox.test" or "t.test" compare every pair of boxes, each with its own bracket and p-value (ggpubr::stat_pwc()); "kruskal.test" or "anova" give one global p-value per panel (ggpubr::stat_compare_means()). Default NULL (no test shown). |
| p_adjust_method | Character. Multiple-testing correction for the pairwise tests (stat = "wilcox.test" or "t.test"), applied within each panel, any method of stats::p.adjust(). Default "holm"; "none" uses the raw p-values. |
| show_x_labels | Logical. If TRUE (default), the x-axis labels are shown. |
| x_label_angle | Numeric. Rotation (degrees) of the x-axis labels (when show_x_labels = TRUE). Default 0 (horizontal); use 45 or 90 if they overlap. |

strip_text_bold
: Logical. If TRUE, the facet strip labels are bold. Default FALSE.

strip_text_color
: Color of the facet strip labels, which sit on the facet_colors backgrounds. Default "black" (the default facet_colors is a light "grey85"; pass "white" if you supply darker facet_colors).

aspect_ratio
: Numeric. Aspect ratio (height/width) of each panel. Default NULL (automatic).

save_table
: Logical. If TRUE, saves the beta diversity table as a tab-delimited file. Default FALSE.

table_filename
: Character. Name or path of the saved file (used when save_table = TRUE). Default "betadiv_table.txt".

## Value

A ggplot object.

## Examples

```
table_path <- system.file("extdata", "subset_table_bacteria.txt", package = "MicroBioMeta")
table <- read.delim(table_path, row.names = 1, check.names = FALSE)

metadata_path <- system.file("extdata", "subset_metadata_bacteria.txt", package = "MicroBioMeta")
metadata <- read.delim(metadata_path, check.names = FALSE)

beta_dissimilarity_plot(
    table = table,
    metadata = metadata,
    comparison_condition1 = c("Rhizosphere_vs_Roots"),
    condition1_col = "Location",
    condition2_col = "Treatment",
    x_axis_title = "Samples",
    partition = "shared",
    family = "sorensen"
)
```

---

# beta_ord_plot *Beta diversity ordination plot*

---

## Description

Computes the beta diversity between samples with several distance metrics (including the compositional CLR/Aitchison distance via ALDEx2) and plots a PCA, PCoA or NMDS ordination.

## Usage

```
beta_ord_plot(
  table,
  metadata,
  distance = "compositional",
  mc_samples = 1,
  ordination = "PCA",
  group_col = NULL,
  palette = "colorb",
  shape_col = NULL,
  legend_title = NULL,
  taxonomy_db = "silva",
  arrows_size = 10,
  top_n = 5,
  title = "auto",
  save_table = FALSE,
  table_filename = "ordination_scores.txt",
  q = 1,
  diversity = "taxonomic",
  tree = NULL,
  traits = NULL,
  tau = NULL
)
```

## Arguments

| | |
|---|---|
| table | A data frame with taxa in rows and samples in columns. The last column must be named taxonomy, containing full taxonomic strings. |
| metadata | A data frame containing sample metadata. Its first column must hold the sample IDs (the column names of table). |
| distance | Distance method: one of "euclidean", "bray", "jaccard", "sorensen", "compositional" (default; CLR/Aitchison via ALDEx2), "aitchison", "robust.aitchison", or the Hill-number dissimilarities "U", "C", "V" or "S" of hilldiv3::hillpair() (with PCoA or NMDS; see q). Case-insensitive. Note: ordination = "PCA" requires distance = "compositional". |
| mc_samples | Number of ALDEx2 Monte Carlo instances used when distance = "compositional". With 1 (default) the clr values of one random instance are used: fast, but the result changes between runs (use set.seed()). With more, the clr values are averaged across instances, which gives an almost identical result in every run; 128 (ALDEx2's default) is suggested for final analyses, and takes longer. Ignored for other distances. |
| ordination | Ordination method: one of "PCA" (default), "PCoA", or "NMDS". Case-insensitive. |
| group_col | Character. Name of the column in metadata used to color the points; if it is numeric, a continuous color scale is used. Optional; NULL (default) for no groups. |
| palette | Either a palette **name** or a **vector of fixed colors**; which scale it produces depends on whether group_col is discrete or continuous. |

- Named, discrete group_col: one of "colorb" (default; qualitative colorblind-friendly palette), "safe" (colorblind-friendly CARTO "Safe"), "earth" (CARTO "Earth", brown to teal; best for two or ordered groups), "grey", "viridis", or "brewer" ("Set2").
- Named, continuous group_col: "viridis" (default; option = "cividis") or "gradient"
- Vector of colors, discrete group_col: used as-is, one color per level (scale_*_manual).
- Vector of colors, continuous group_col: used as gradient stops (scale_*_gradientn).

shape_col — Character. Name of the column in metadata used for the point shapes. Optional; NULL (default) for one shape.

legend_title — Character. Title of the legend. If NULL (default), the name of group_col is used.

taxonomy_db — Character. Database the taxonomy strings come from: "silva" (default), "gg2" (Greengenes2, also "gg"), "unite" or "Kraken2" (also "kraken"). Case-insensitive. Only used when ordination = "PCA".

arrows_size — Numeric. Size/length scaling factor for biplot arrows. Default 10.

top_n — Integer. Number of taxa that contribute most to the PCA, drawn as arrows. Default 5.

title — Character. Plot title. "auto" (default) shows "Ordination – <distance>"; NULL shows no title; any other text is used as the title.

save_table — Logical. If TRUE, saves the ordination scores and loadings (one table) as a tab-delimited file. Default FALSE.

table_filename — Character. Name or path of the saved file (used when save_table = TRUE). Default "ordination_scores.txt".

q — Numeric. Order of the Hill-number dissimilarities "U", "C", "V" and "S": 0 (presence/absence), 1 (default) or 2 (dominant taxa). Ignored by the other distances.

diversity — Character. What the Hill-number dissimilarities use: "taxonomic" (default), "phylogenetic" (needs tree) or "functional" (needs traits). Case-insensitive.

tree — A rooted phylo tree whose tip labels are the taxa of table. Used when diversity = "phylogenetic".

traits — A data frame with taxa in rows and traits in columns. Used when diversity = "functional".

tau — Numeric. Threshold of functional distinctiveness: taxa at this distance or more count as different functional groups. NULL (default) uses the mean distance between two reads of the pooled samples (Chao et al. 2019). Used when diversity = "functional".

## Details

With distance = "compositional" and mc_samples = 1, the clr values come from one random Monte Carlo instance of ALDEx2::aldex.clr(); call set.seed() before the function to make the result reproducible, or use mc_samples = 128.

## Value

A ggplot object.

## Examples

```
table_path <- system.file("extdata", "subset_table_bacteria.txt", package = "MicroBioMeta")
table <- read.delim(table_path, row.names = 1, check.names = FALSE)

metadata_path <- system.file("extdata", "subset_metadata_bacteria.txt", package = "MicroBioMeta")
metadata <- read.delim(metadata_path, check.names = FALSE)

beta_ord_plot(
    table      = table,
    metadata   = metadata,
    distance   = "aitchison",
    ordination = "NMDS",
    group_col  = "Location",
    top_n      = 5
)
```

---

# beta_partition_ord_plot

*Beta diversity partition ordination*

---

## Description

Partitions the beta diversity (Jaccard or Sorensen) into its turnover and nestedness components with betapart, and plots a PCoA of each component (vegan::betadisper()) with the samples linked to their group centroid.

## Usage

```
beta_partition_ord_plot(
  table,
  metadata,
  family = "jaccard",
  group_col,
  shape_col = NULL,
  legend_title = NULL,
  point_size = 3,
  group_colors = NULL,
  panel_label_case = "upper",
  panel_labels = NULL,
  panel_label_bold = TRUE,
  save_table = FALSE,
  table_filename = "beta_partition"
)
```

## Arguments

| | |
|---|---|
| table | A data frame with taxa in rows and samples in columns. The last column must be named taxonomy, containing full taxonomic strings. |

| | |
|---|---|
| metadata | A data frame containing sample metadata. Its first column must hold the sample IDs (the column names of table). |
| family | Dissimilarity family for the partition: "jaccard" (default) or "sorensen". Case-insensitive. |
| group_col | Character. Name of the column in metadata that defines the groups. Required: the dispersion and the lines to the centroid are computed per group. |
| shape_col | Character. Name of the column in metadata used for the point shapes. Optional; NULL (default) for one shape. |
| legend_title | Character. Title of the legend. If NULL (default), the name of group_col is used. |
| point_size | Numeric. Size of the points. Default 3. |
| group_colors | Optional character vector of colors, one per group, named after the groups or in their order. If NULL (default), the colorblind-friendly Okabe-Ito palette is used. |
| panel_label_case | Character. Case of the panel tags: "upper" (default; A, B, C) or "lower" (a, b, c). Ignored if panel_labels is given. |
| panel_labels | Optional character vector of custom panel tags, one per panel (Jaccard/Sorensen, turnover, nestedness), used as-is (e.g. c("(a)", "(b)", "(c)")). Overrides panel_label_case. |
| panel_label_bold | Logical. If TRUE (default), panel tags are bold. Set to FALSE for journals that require plain (non-bold) panel tags. |
| save_table | Logical. If TRUE, saves the PCoA coordinates of the three ordinations as tab-delimited files. Default FALSE. |
| table_filename | Character. Base name or path of the saved files (used when save_table = TRUE): three files, <name>_jacs.txt, <name>_jtus.txt and <name>_jnes.txt. Default "beta_partition". |

## Value

A patchwork object with the three ordinations (Jaccard/Sorensen, turnover and nestedness).

## Examples

```
table_path <- system.file("extdata", "subset_table_bacteria.txt", package = "MicroBioMeta")
table <- read.delim(table_path, row.names = 1, check.names = FALSE)

metadata_path <- system.file("extdata", "subset_metadata_bacteria.txt", package = "MicroBioMeta")
metadata <- read.delim(metadata_path, check.names = FALSE)

beta_partition_ord_plot(
    table      = table,
    metadata   = metadata,
    group_col  = "Location",
    point_size = 4
)
```

beta_test_table *PERMANOVA or betadisper results table*

## Description

Runs a PERMANOVA (vegan::adonis2()) or a test of homogeneity of dispersions (vegan::betadisper()) on the distances between samples and returns the results as a table figure.

## Usage

```
beta_test_table(
  table,
  metadata,
  formula_str,
  distance = "euclidean",
  test = c("permanova", "betadisper"),
  permutations = 999,
  mc_samples = 1,
  strata_var = NULL,
  digits = 3,
  save_table = FALSE,
  table_filename = "beta_test_results.txt",
  q = 1,
  diversity = "taxonomic",
  tree = NULL,
  traits = NULL,
  tau = NULL
)
```

## Arguments

| | |
|---|---|
| table | A data frame with taxa in rows and samples in columns. The last column must be named taxonomy, containing full taxonomic strings. |
| metadata | A data frame containing sample metadata. Its first column must hold the sample IDs (the column names of table). |
| formula_str | Character. Right-hand side of the model formula, with columns of metadata (e.g. "Type_of_soil*Treatment"). |
| distance | Character. Distance metric: "compositional" (CLR with ALDEx2), "aitchison", "robust.aitchison" or any other method of vegan::vegdist() (e.g. "bray", "jaccard"), or the Hill-number dissimilarities "U", "C", "V" or "S" of hilldiv3::hillpair() (see q). Default "euclidean". Case-insensitive. Ignored when table is already a distance. |
| test | Statistical test to run: one of "permanova" (default) or "betadisper". Case-insensitive. |
| permutations | Integer. Number of permutations for the test. Default 999. |

| | |
|---|---|
| mc_samples | Number of ALDEx2 Monte Carlo instances used when distance = "compositional". With 1 (default) the clr values of one random instance are used: fast, but the result changes between runs (use set.seed()). With more, the clr values are averaged across instances, which gives an almost identical result in every run; 128 (ALDEx2's default) is suggested for final analyses, and takes longer. Ignored for other distances. |
| strata_var | Character. Name of a column in metadata within which the permutations are restricted (strata). Optional; NULL (default) for none. |
| digits | Integer. Decimal places of the numeric columns (except the p-value). Default 3. |
| save_table | Logical. If TRUE, saves the results table as a tab-delimited file. Default FALSE. |
| table_filename | Character. Name or path of the saved file (used when save_table = TRUE). Default "beta_test_results.txt". |
| q | Numeric. Order of the Hill-number dissimilarities "U", "C", "V" and "S": 0 (presence/absence), 1 (default) or 2 (dominant taxa). Ignored by the other distances. |
| diversity | Character. What the Hill-number dissimilarities use: "taxonomic" (default), "phylogenetic" (needs tree) or "functional" (needs traits). Case-insensitive. |
| tree | A rooted phylo tree whose tip labels are the taxa of table. Used when diversity = "phylogenetic". |
| traits | A data frame with taxa in rows and traits in columns. Used when diversity = "functional". |
| tau | Numeric. Threshold of functional distinctiveness: taxa at this distance or more count as different functional groups. NULL (default) uses the mean distance between two reads of the pooled samples (Chao et al. 2019). Used when diversity = "functional". |

## Details

The first column of metadata must hold the sample IDs; metadata rows are matched to the samples by ID, so their order doesn't matter. A precomputed distance is used as-is (distance is ignored). The p-values come from permutations; call set.seed() before the function to make the result reproducible.

## Value

A ggplot object (ggpubr::ggtexttable()) with the results table; significant p-values are in bold. Use save_table = TRUE to get the results as a tab-delimited file.

## Examples

```
table_path <- system.file("extdata", "subset_table_bacteria.txt", package = "MicroBioMeta")
table <- read.delim(table_path, row.names = 1, check.names = FALSE)

metadata_path <- system.file("extdata", "subset_metadata_bacteria.txt", package = "MicroBioMeta")
metadata <- read.delim(metadata_path, check.names = FALSE)

# Example using a data frame
```

```
beta_test_table(
    table = table,
    metadata = metadata,
    formula_str = "Location*Treatment",
    distance = "bray",
    test = "permanova",
    permutations = 999,
    strata_var = "Plot"
)

# Example using a distance matrix
dist_matrix <- vegan::vegdist(
    t(table[, setdiff(colnames(table), "taxonomy")]),
    method = "bray"
)
beta_test_table(
    table       = dist_matrix,
    metadata    = metadata,
    formula_str = "Location",
    test        = "betadisper"
)

# Compositional PERMANOVA (CLR via ALDEx2, then Euclidean)
beta_test_table(
    table = table,
    metadata = metadata,
    formula_str = "Location",
    distance = "compositional",
    test = "permanova",
    permutations = 999
)
```

---

`beta_turnover_plot` *Box plot of pairwise feature turnover (Hill numbers)*

---

## Description

Computes the pairwise beta diversity between samples with Hill numbers (q = 0, 1, 2; same values as `hillR::hill_taxa_parti_pairwise()`) and plots the proportion of feature turnover ($\beta - 1$, from 0 = identical to 1 = no shared features) as box plots, one facet row per Hill order. q = 0 weighs all features equally (presence/absence), q = 1 weighs them by their abundance and q = 2 gives more weight to dominant features.

## Usage

```
beta_turnover_plot(
  table,
  metadata,
  comparison_condition1,
```

```
  comparison_condition2 = NULL,
  condition1_col,
  condition2_col = NULL,
  facet_colors = NULL,
  group_colors = NULL,
  x_axis_title = "Section",
  y_axis_title = "Proportion of feature turnover",
  show_x_labels = FALSE,
  x_label_angle = 0,
  strip_text_bold = FALSE,
  strip_text_color = "white",
  aspect_ratio = NULL,
  stat = NULL,
  p_adjust_method = "holm",
  save_table = FALSE,
  table_filename = "betadiv_turnover.txt"
)
```

## Arguments

| | |
|---|---|
| `table` | A data frame with taxa in rows and samples in columns. The last column must be named `taxonomy`, containing full taxonomic strings. |
| `metadata` | A data frame containing sample metadata. Its first column must hold the sample IDs (the column names of `table`). |
| `comparison_condition1` | Character vector with the pairs of groups of `condition1_col` to show (e.g. `"Rhizosphere_vs_Roots"`, `"Rhizosphere_vs_Rhizosphere"`; the order of the two groups does not matter). Each pair is one box, labeled as written. |
| `comparison_condition2` | Optional character vector with the pairs of groups of `condition2_col` to keep (e.g. `"TC_vs_TC"`); the plot is faceted by them. Requires `condition2_col`. If `NULL` (default), no pairs are filtered by `condition2_col`. |
| `condition1_col` | Character. Name of the column in `metadata` whose groups are compared (the pairs of `comparison_condition1`). |
| `condition2_col` | Optional. Metadata column used to build `comparison_condition2`'s group pairs, and to facet the plot. Required when `comparison_condition2` is set. If given without `comparison_condition2`, the plot is faceted by it keeping only pairs of samples with the same value (e.g. `"TC_vs_TC"`, `"TD_vs_TD"`). |
| `facet_colors` | Optional character vector of background colors for the facet strips. If `NULL` (default), a neutral `"grey85"` is used. |
| `group_colors` | Optional character vector of colors, one per comparison on the x-axis, named after them or in their order. If `NULL` (default), the colorblind-friendly Okabe-Ito palette is used. |
| `x_axis_title` | Character. Title of the x-axis. Default `"Section"`. |
| `y_axis_title` | Character. Title of the y-axis. Default `"Proportion of feature turnover"`. |
| `show_x_labels` | Logical. If `TRUE`, the x-axis labels are shown. Default `FALSE`, since the groups are already named in the legend. |

x_label_angle Numeric. Rotation (degrees) of the x-axis labels (when show_x_labels = TRUE). Default 0 (horizontal); use 45 or 90 if they overlap.

strip_text_bold
Logical. If TRUE, the facet strip labels are bold. Default FALSE.

strip_text_color
Color of the top (x) facet strip labels, which sit on the facet_colors backgrounds. Default "white", unless facet_colors is left at its own light "grey85" default, in which case this defaults to "black" instead so it stays legible.

aspect_ratio Numeric. Aspect ratio (height/width) of each panel. Default NULL (automatic).

stat Character or NULL. Statistical test to compare the boxes within each panel. "wilcox.test" or "t.test" compare every pair of boxes, each with its own bracket and p-value (ggpubr::stat_pwc()); "kruskal.test" or "anova" give one global p-value per panel (ggpubr::stat_compare_means()). Default NULL (no test shown).

p_adjust_method
Character. Multiple-testing correction for the pairwise tests (stat = "wilcox.test" or "t.test"), applied within each panel, any method of stats::p.adjust(). Default "holm"; "none" uses the raw p-values.

save_table Logical. If TRUE, saves the turnover table as a tab-delimited file. Default FALSE.

table_filename Character. Name or path of the saved file (used when save_table = TRUE). Default "betadiv_turnover.txt".

## Details

Each box is one group pair from condition1_col, so a between-group comparison (e.g. "Rhizosphere_vs_Roots") can be contrasted with a within-group baseline (e.g. "Rhizosphere_vs_Rhizosphere"): if the between-group turnover is higher, the groups host distinct communities beyond the variability among replicates of the same group. An optional condition2_col keeps only pairs from the same level (e.g. the same treatment) and facets by it, so the comparison is not confounded by it.

## Value

A ggplot object.

## Examples

```
table_path <- system.file("extdata", "subset_table_bacteria.txt", package = "MicroBioMeta")
table <- read.delim(table_path, row.names = 1, check.names = FALSE)

metadata_path <- system.file("extdata", "subset_metadata_bacteria.txt", package = "MicroBioMeta")
metadata <- read.delim(metadata_path, check.names = FALSE)
colnames(metadata)[1] <- "OTUID"

# comparison_condition1/2 match group pairs regardless of order, so listing
# "Rhizosphere_vs_Roots" also catches pairs the self-join recorded the
# other way around ("Roots_vs_Rhizosphere") - no need to list both.
beta_turnover_plot(
    table = table,
    metadata = metadata,
```

```
  comparison_condition1 = c("Rhizosphere_vs_Roots", "Rhizosphere_vs_Rhizosphere"),
 comparison_condition2 = c("Control_vs_Control", "Moderate_drought_vs_Moderate_drought"),
  condition1_col = "Location",
  condition2_col = "Treatment",
  facet_colors = c("#5D478B", "#8B668B"),
  group_colors = c(
      "Rhizosphere_vs_Roots" = "#56B4E9",
      "Rhizosphere_vs_Rhizosphere" = "#E69F00"
  )
)
```

---

`cca_rda_biplot` *CCA/RDA biplot*

---

## Description

Performs a Canonical Correspondence Analysis (CCA) or a Redundancy Analysis (RDA) of the abundance table constrained by environmental variables, and plots the samples and the environmental vectors as a biplot.

## Usage

```
cca_rda_biplot(
  table,
  env_data = NULL,
  env_vars,
  method = "hell",
  metadata,
  group_col = NULL,
  group_colors = NULL,
  legend_title = NULL,
  scale_env = TRUE,
  pval_threshold = 0.05,
  p_adjust_method = "none",
  show_all_env_vectors = FALSE,
  analysis = "CCA",
  scale_arrows = 1,
  title = "auto",
  save_table = FALSE,
  table_filename = "cca_rda_scores.txt"
)
```

## Arguments

| | |
|---|---|
| `table` | A data frame with taxa in rows and samples in columns. The last column must be named `taxonomy`, containing full taxonomic strings. |

env_data Optional data frame of environmental variables, with row names matching the sample names in table. Default NULL: the variables named in env_vars are taken from metadata. Use it only when the variables are in a separate table.

env_vars A character vector with the names of environmental variables (columns of metadata, or of env_data if given) to include in the analysis.

method Character. Transformation of the abundances, passed to vegan::decostand(). Default "hell" (Hellinger).

metadata A data frame containing sample metadata. Its first column must hold the sample IDs (the column names of table). The environmental variables can be columns of metadata (see env_vars).

group_col Character. Name of the column in metadata that defines the groups. Used to color the samples. Optional; NULL (default) for no groups.

group_colors Optional character vector of colors, one per group, named after the groups or in their order. If NULL (default), the colorblind-friendly Okabe-Ito palette is used.

legend_title Character. Title of the legend. If NULL (default), the name of group_col is used.

scale_env Logical. If TRUE (default), the environmental variables are scaled (without centering) before the analysis.

pval_threshold Numeric. P-value cutoff (after p_adjust_method) to draw only the significant environmental variables. Default 0.05.

p_adjust_method
Character. Multiple-testing correction for the vegan::envfit() p-values of the environmental variables, any method of stats::p.adjust(). Default "none" (raw p-values), the usual choice with few variables.

show_all_env_vectors
Logical. If TRUE, all the environmental variables are drawn, significant or not. Default FALSE.

analysis Constrained ordination method: "CCA" (default, Canonical Correspondence Analysis) or "RDA" (Redundancy Analysis). Case-insensitive.

scale_arrows Numeric. Multiplies the length of the environmental arrows; it only changes how they are drawn. Default 1.

title Character. Plot title. "auto" (default) shows "CCA Biplot" or "RDA Biplot"; NULL shows no title; any other text is used as the title.

save_table Logical. If TRUE, saves the sample scores and environmental vector loadings (one table, with a type column: "site" or "vector") as a tab-delimited file. Default FALSE.

table_filename Character. Name or path of the saved file (used when save_table = TRUE). Default "cca_rda_scores.txt".

## Details

The p-values of the environmental vectors come from vegan::envfit() permutations; call set.seed() before the function to make them reproducible.

## Value

A ggplot object with the biplot. The axis titles show the percentage of the variance (inertia) explained by each constrained axis.

## Examples

```
table_path <- system.file("extdata", "subset_table_bacteria.txt", package = "MicroBioMeta")
table <- read.delim(table_path, row.names = 1, check.names = FALSE)

metadata_path <- system.file("extdata", "subset_metadata_bacteria.txt", package = "MicroBioMeta")
metadata <- read.delim(metadata_path, check.names = FALSE)

# The environmental variables are columns of metadata, chosen with env_vars
cca_rda_biplot(
    table               = table,
    metadata            = metadata,
    env_vars            = c("pH", "TOC", "FW", "Root_FW", "DW"),
    analysis            = "RDA",
    show_all_env_vectors = TRUE,
    group_col           = "Location"
)
```

---

# collapse_table *Collapse table*

---

## Description

Collapses an abundance table to a taxonomic level (e.g. genus, family, phylum), summing the counts of the features that share the same taxonomy. Features not resolved to that level keep their deepest assigned rank and are also summed when they share the same taxonomy. Optionally converts the counts to relative abundance and saves the collapsed table.

## Usage

```
collapse_table(
  table,
  level = "genus",
  rel_abun = FALSE,
  save_table = FALSE,
  table_filename = "collapsed_table.txt"
)
```

## Arguments

| | |
|---|---|
| table | A data frame with taxa in rows and samples in columns. The last column must be named `taxonomy`, containing full taxonomic strings. All the other columns are taken as samples, in the order of the table. |

| | |
|---|---|
| level | Character. Taxonomic level: "kingdom", "phylum", "class", "order", "family", "genus" (default) or "species". Case-insensitive. |
| rel_abun | Logical. If TRUE, converts the counts to relative abundance (%) per sample. Default FALSE. |
| save_table | Logical. If TRUE, saves the collapsed table as a tab-delimited file. Default FALSE. |
| table_filename | Character. Name or path of the saved file (used when save_table = TRUE). Default "collapsed_table.txt". |

## Value

A list with two elements: collapsed_table (wide format, one row per collapsed taxon) and long_format (the same data pivoted to one row per taxon/sample combination).

## Examples

```
table_path <- system.file("extdata", "subset_table_bacteria.txt", package = "MicroBioMeta")
table <- read.delim(table_path, row.names = 1, check.names = FALSE)

collapse_table(
    table      = table,
    level      = "genus",
    rel_abun   = FALSE,
    save_table = FALSE
)
```

---

# corr_env_abund_plot *Correlation between environmental variables and taxa*

---

## Description

Computes the relative abundance of the taxa at a taxonomic level, correlates it with environmental variables, and shows the correlations as a heatmap (tile) or a bubble plot (circle).

## Usage

```
corr_env_abund_plot(
  table,
  env_data = NULL,
  metadata = NULL,
  env_vars = NULL,
  method = "spearman",
  hc.order = TRUE,
  geom = c("tile", "circle"),
  show_labels = TRUE,
  col_palette = NULL,
  diverging_palette = "BuOr",
  invert_axes = TRUE,
```

```
  taxonomy_db = "silva",
  level = "genus",
  pval_threshold = NULL,
  p_adjust_method = "BH",
  x_label_angle = 45,
  save_table = FALSE,
  table_filename = "corr.txt"
)
```

## Arguments

| | |
|---|---|
| table | A data frame with taxa in rows and samples in columns. The last column must be named taxonomy, containing full taxonomic strings. |
| env_data | Optional data frame of environmental variables, with row names matching the sample names in table. Default NULL: the variables named in env_vars are taken from metadata. Use it only when the variables are in a separate table. |
| metadata | A data frame containing sample metadata. Its first column must hold the sample IDs (the column names of table). The environmental variables can be columns of metadata (see env_vars). |
| env_vars | Character vector of environmental variables to include in the correlation analysis (columns of metadata, or of env_data if given). Required when env_data is NULL; with a separate env_data, NULL uses all its variables. |
| method | Character. Correlation method: "spearman" (default), "pearson" or "kendall". |
| hc.order | Logical. If TRUE (default), taxa and variables are reordered by hierarchical clustering. |
| geom | Character. Type of visualization to generate: "tile" (heatmap, default) or "circle" (bubble plot). Case-insensitive. |
| show_labels | Logical. If TRUE (default), the correlation values are shown. |
| col_palette | Optional character vector of colors for the correlation scale. If NULL (default), diverging_palette is used. |
| diverging_palette | Character. Colorblind-friendly diverging palette, used when col_palette is NULL: "BuOr" (blue-orange, default), "BuVm" (blue-vermillion), "BuPk" (blue-pink), "GnPk" (green-pink) or "PuYl" (purple-yellow); or "viridis", a sequential scale without a neutral midpoint (not recommended for correlations). |
| invert_axes | Logical. If TRUE (default), taxa and variables swap axes. |
| taxonomy_db | Character. Database the taxonomy strings come from: "silva" (default), "gg2" (Greengenes2, also "gg"), "unite" or "Kraken2" (also "kraken"). Case-insensitive. |
| level | Character. Taxonomic level: "kingdom", "phylum", "class", "order", "family", "genus" (default) or "species". Case-insensitive. |
| pval_threshold | Numeric or NULL. P-value cutoff (after p_adjust_method) to keep only taxa with a significant correlation with at least one variable. Default NULL (no filtering). |

p_adjust_method
: Character. Multiple-testing correction for the p-values of all taxon x variable correlations, any method of `stats::p.adjust()`. Default `"BH"`; `"none"` uses the raw p-values.

x_label_angle
: Numeric. Rotation (degrees) of the x-axis labels. Default `45`; `0` for horizontal labels.

save_table
: Logical. If `TRUE`, saves the plotted correlations (one row per taxon and variable, with the raw and adjusted p-values) as a tab-delimited file. Default `FALSE`.

table_filename
: Character. Name or path of the saved file (used when `save_table = TRUE`). Default `"corr.txt"`.

## Value

A ggplot object.

## Examples

```
table_path <- system.file("extdata", "subset_table_bacteria.txt", package = "MicroBioMeta")
table <- read.delim(table_path, row.names = 1, check.names = FALSE)

metadata_path <- system.file("extdata", "subset_metadata_bacteria.txt", package = "MicroBioMeta")
metadata <- read.delim(metadata_path, check.names = FALSE)

corr_env_abund_plot(
    table = table,
    metadata = metadata,
    env_vars = c("pH", "TOC", "FW", "Root_FW", "DW", "Root_L", "Stem_L"),
    method = "pearson",
    geom = "tile",
    hc.order = FALSE,
    invert_axes = TRUE,
    show_labels = FALSE,
    level = "phylum",
    taxonomy_db = "silva"
)
```

---

# from_phyloseq *Convert a phyloseq object to MicroBioMeta tables*

---

## Description

Extracts the count table, taxonomy and sample metadata of a `phyloseq` object into the plain data frames that every MicroBioMeta function takes.

## Usage

```
from_phyloseq(physeq)
```

## Arguments

| | |
|---|---|
| physeq | A phyloseq object with an OTU table and a taxonomy table and sample data. |

## Value

A list with two data frames:

table Taxa in rows, samples in columns, and a last column taxonomy with the full taxonomic string (e.g. "k__Bacteria; p__Firmicutes; c__Bacilli").

metadata Sample metadata whose first column, SAMPLEID, holds the sample names of table.

## See Also

from_tse for (Tree)SummarizedExperiment objects.

## Examples

```
if (requireNamespace("phyloseq", quietly = TRUE)) {
    counts <- matrix(c(10, 0, 5, 3, 8, 1),
        nrow = 3,
        dimnames = list(c("ASV1", "ASV2", "ASV3"), c("S1", "S2"))
    )
    tax <- matrix(
        c(
            "Bacteria", "Bacteria", "Bacteria",
            "Firmicutes", "Proteobacteria", NA
        ),
        nrow = 3, dimnames = list(rownames(counts), c("Kingdom", "Phylum"))
    )
    samples <- data.frame(
        Soil = c("Rhizosphere", "Bulk soil"),
        row.names = c("S1", "S2")
    )
    ps <- phyloseq::phyloseq(
        phyloseq::otu_table(counts, taxa_are_rows = TRUE),
        phyloseq::tax_table(tax),
        phyloseq::sample_data(samples)
    )
    mbm <- from_phyloseq(ps)
    mbm$table
    mbm$metadata
}
```

---

# from_tse *Convert a (Tree)SummarizedExperiment object to MicroBioMeta tables*

---

## Description

Extracts one assay (the counts), the taxonomy (`rowData`) and the sample metadata (`colData`) of a `TreeSummarizedExperiment` or any other `SummarizedExperiment`, e.g. one built with the **mia** package, into the plain data frames that every MicroBioMeta function takes.

## Usage

```
from_tse(tse, assay_name = "counts")
```

## Arguments

| | |
|---|---|
| `tse` | A `TreeSummarizedExperiment` or `SummarizedExperiment` object with taxa in rows and samples in columns. |
| `assay_name` | Character. Name of the assay with the counts. Default `"counts"`. |

## Value

A list with two data frames:

`table` Taxa in rows, samples in columns, and a last column `taxonomy` with the full taxonomic string (e.g. `"k__Bacteria; p__Firmicutes; c__Bacilli"`).

`metadata` Sample metadata whose first column, `SAMPLEID`, holds the sample names of `table`.

The taxonomy is built from the `rowData` columns named after taxonomic ranks (Kingdom or Domain, Phylum, Class, Order, Family, Genus, Species; any case). If `rowData` already has a `taxonomy` column, it is used as is.

## See Also

`from_phyloseq` for phyloseq objects.

## Examples

```
if (requireNamespace("SummarizedExperiment", quietly = TRUE)) {
    counts <- matrix(c(10, 0, 5, 3, 8, 1),
        nrow = 3,
        dimnames = list(c("ASV1", "ASV2", "ASV3"), c("S1", "S2"))
    )
    tax <- data.frame(
        Kingdom = "Bacteria",
        Phylum = c("Firmicutes", "Proteobacteria", NA),
        row.names = rownames(counts)
    )
    samples <- data.frame(
        Soil = c("Rhizosphere", "Bulk soil"),
        row.names = c("S1", "S2")
    )
    se <- SummarizedExperiment::SummarizedExperiment(
        assays = list(counts = counts), rowData = tax, colData = samples
    )
    mbm <- from_tse(se)
```

```
    mbm$table
    mbm$metadata
}
```

---

`merge_feature_taxonomy` *Merge feature table and taxonomy*

---

## Description

This function joins a feature table (counts per sample) with the corresponding taxonomy data.

## Usage

```
merge_feature_taxonomy(
  table,
  taxonomy,
  save_table = FALSE,
  table_filename = "merged_feature_taxonomy.txt"
)
```

## Arguments

| | |
|---|---|
| `table` | A data frame with features in rows and samples in columns. Row names must hold the feature IDs (OTUs, ASVs, species...). If the table has no row names (e.g. read without `row.names = 1`), its first column is used as feature IDs. |
| `taxonomy` | A data frame or matrix with the taxonomy. Row names (or, if absent, the first column) must hold the feature IDs of `table`. Features are matched by ID, so both inputs can be in any order. |
| `save_table` | Logical. If `TRUE`, saves the merged table as a tab-delimited file. Default `FALSE`. |
| `table_filename` | Character. Name or path of the saved file (used when `save_table = TRUE`). Default `"merged_feature_taxonomy.txt"`. |

## Value

A data frame with the counts of `table` and the taxonomy columns. A single taxonomy column is renamed to `taxonomy`.

## Examples

```
table_path <- system.file("extdata", "subset_table_bacteria.txt", package = "MicroBioMeta")
full_table <- read.delim(table_path, row.names = 1, check.names = FALSE)

feature_table <- full_table[, setdiff(colnames(full_table), "taxonomy")]
taxonomy_df <- full_table[, "taxonomy", drop = FALSE]

merged <- merge_feature_taxonomy(
```

```
    table    = feature_table,
    taxonomy = taxonomy_df
)
```

---

`random_forest_lollipop_plot` *Random forest feature importance plot*

---

## Description

Fits a random forest (randomForest) that predicts a metadata variable from the abundances, and plots the most important features as a lollipop plot colored by phylum.

## Usage

```
random_forest_lollipop_plot(
  table,
  metadata,
  top_n = 15,
  size = 8,
  variable_to_predict,
  group_colors = NULL,
  title = NULL,
  save_table = FALSE,
  table_filename = "randomforest_importance.txt",
  x_axis_title = "Feature importance (MeanDecreaseGini)"
)
```

## Arguments

| | |
|---|---|
| `table` | A data frame with taxa in rows and samples in columns. The last column must be named `taxonomy`, containing full taxonomic strings. |
| `metadata` | A data frame containing sample metadata. Its first column must hold the sample IDs (the column names of `table`). |
| `top_n` | Integer. Number of most important features to show. Default `15`. |
| `size` | Numeric. Size of the lollipop points. Default `8`. |
| `variable_to_predict` | Character. Name of the column in `metadata` to predict (a categorical variable). |
| `group_colors` | Optional character vector of colors, one per phylum (recycled if shorter). If `NULL` (default), the package palette is used. |
| `title` | Character. Plot title. `NULL` (default) shows no title. |
| `save_table` | Logical. If `TRUE`, saves the feature-importance table as a tab-delimited file. Default `FALSE`. |
| `table_filename` | Character. Name or path of the saved file (used when `save_table = TRUE`). Default `"randomforest_importance.txt"`. |
| `x_axis_title` | Character. Title of the x-axis (feature importance). Default `"Feature importance (MeanDecreaseGini)"`. |

## Details

The random forest is random; call `set.seed()` before the function to make the result reproducible.

## Value

A ggplot object.

## Examples

```
table_path <- system.file("extdata", "subset_table_bacteria.txt", package = "MicroBioMeta")
table <- read.delim(table_path, row.names = 1, check.names = FALSE)

metadata_path <- system.file("extdata", "subset_metadata_bacteria.txt", package = "MicroBioMeta")
metadata <- read.delim(metadata_path, check.names = FALSE)

random_forest_lollipop_plot(
    table               = table,
    metadata            = metadata,
    variable_to_predict = "Location",
    top_n               = 20,
    size                = 6
)
```

---

# ratios_bubble_plot *Compare taxon abundance ratios between two conditions*

---

## Description

Computes relative abundances from a taxonomic abundance table and compares two experimental conditions by calculating a directional abundance ratio for each taxon.

## Usage

```
ratios_bubble_plot(
  table,
  metadata,
  group_col,
  condition_A,
  condition_B,
  taxonomy_db = "silva",
  top_n = 30,
  level = "genus",
  x_axis_title = "Taxon",
  legend_title = NULL,
  group_colors = NULL,
  save_table = FALSE,
  table_filename = "ratios_bubble_table.txt"
)
```

## Arguments

| | |
|---|---|
| table | A data frame with taxa in rows and samples in columns. The last column must be named taxonomy, containing full taxonomic strings. |
| metadata | A data frame containing sample metadata. Its first column must hold the sample IDs (the column names of table). |
| group_col | Character. Name of the column in metadata that defines the groups. condition_A and condition_B are two of its values. |
| condition_A | Character. Name of the first condition to compare. |
| condition_B | Character. Name of the second condition to compare. |
| taxonomy_db | Character. Database the taxonomy strings come from: "silva" (default), "gg2" (Greengenes2, also "gg"), "unite" or "Kraken2" (also "kraken"). Case-insensitive. |
| top_n | Integer. Number of taxa with the highest mean abundance to show. Default 30. |
| level | Character. Taxonomic level: "kingdom", "phylum", "class", "order", "family", "genus" (default) or "species". Case-insensitive. |
| x_axis_title | Character. Title of the x-axis (taxon names). Default "Taxon". |
| legend_title | Character. Title of the legend and of the axis showing the dominant condition. If NULL (default), the name of group_col is used. |
| group_colors | Optional character vector of two colors, one per condition. If NULL (default), the package's orange/blue pair is used. |
| save_table | Logical. If TRUE, saves the ratio table as a tab-delimited file. Default FALSE. |
| table_filename | Character. Name or path of the saved file (used when save_table = TRUE). Default "ratios_bubble_table.txt". |

## Value

A ggplot object.

## Examples

```
table_path <- system.file("extdata", "subset_table_bacteria.txt", package = "MicroBioMeta")
table <- read.delim(table_path, row.names = 1, check.names = FALSE)

metadata_path <- system.file("extdata", "subset_metadata_bacteria.txt", package = "MicroBioMeta")
metadata <- read.delim(metadata_path, check.names = FALSE)

ratios_bubble_plot(
    table = table,
    metadata = metadata,
    group_col = "Location",
    condition_A = "Rhizosphere",
    condition_B = "Roots",
    taxonomy_db = "silva",
    level = "genus",
    top_n = 20
)
```

venn_plot *Venn diagram of taxa shared between sample groups*

## Description

Creates a Venn diagram of the taxa shared between groups of samples, with ggvenn or ggVennDiagram.

## Usage

```
venn_plot(
  table,
  metadata,
  merge_by,
  selected_samples = NULL,
  min_prevalence = 0,
  title = NULL,
  method = "ggvenn",
  group_colors = NULL,
  save_table = FALSE,
  table_filename = "venn_taxa_sets.txt"
)
```

## Arguments

| | |
|---|---|
| table | A data frame with taxa in rows and samples in columns. The last column must be named taxonomy, containing full taxonomic strings. |
| metadata | A data frame containing sample metadata. Its first column must hold the sample IDs (the column names of table). |
| merge_by | Character. Name of the column in metadata that defines the groups (one set of taxa per group). |
| selected_samples | Optional character vector of the sample IDs to use. If NULL (default), all samples are used. |
| min_prevalence | Numeric (0-1). Minimum fraction of the samples of a group where a taxon must be present to count it in that group. Default 0 (present in at least one sample). |
| title | Character. Plot title. NULL (default) shows no title. |
| method | Character. Package used to draw the Venn diagram: "ggvenn" (default) or "ggVennDiagram". Case-insensitive. |
| group_colors | Optional character vector of colors, one per group, named after the groups or in their order. If NULL (default), the colorblind-friendly Okabe-Ito palette is used. |
| save_table | Logical. If TRUE, saves the taxa of each group (long format) as a tab-delimited file. Default FALSE. |
| table_filename | Character. Name or path of the saved file (used when save_table = TRUE). Default "venn_taxa_sets.txt". |

## Value

A ggplot object.

## Examples

```
table_path <- system.file("extdata", "subset_table_bacteria.txt", package = "MicroBioMeta")
table <- read.delim(table_path, row.names = 1, check.names = FALSE)

metadata_path <- system.file("extdata", "subset_metadata_bacteria.txt", package = "MicroBioMeta")
metadata <- read.delim(metadata_path, check.names = FALSE)

venn_plot(
    table          = table,
    metadata       = metadata,
    merge_by       = "Location",
    min_prevalence = 0
)

## Filtering taxa by prevalence
venn_plot(
    table          = table,
    metadata       = metadata,
    merge_by       = "Location",
    min_prevalence = 0.2
)

## Custom colors
venn_plot(
    table          = table,
    metadata       = metadata,
    merge_by       = "Location",
    min_prevalence = 0,
    group_colors   = c("#1B9E77", "#D95F02")
)
```

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