

Package ‘ANCOMBC’

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Type Package

Title Microbiome differential abundance and correlation analyses with bias correction

Version 2.4.0

Description ANCOMBC is a package containing differential abundance (DA) and correlation analyses for microbiome data. Specifically, the package includes Analysis of Compositions of Microbiomes with Bias Correction 2 (ANCOM-BC2), Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC), and Analysis of Composition of Microbiomes (ANCOM) for DA analysis, and Sparse Estimation of Correlations among Microbiomes (SECOM) for correlation analysis. Microbiome data are typically subject to two sources of biases: unequal sampling fractions (sample-specific biases) and differential sequencing efficiencies (taxon-specific biases). Methodologies included in the ANCOMBC package are designed to correct these biases and construct statistically consistent estimators.

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License Artistic-2.0

Imports mia ($\geq 1.6.0$), stats, CVXR, DescTools, Hmisc, MASS, Matrix, Rdpack, S4Vectors, SingleCellExperiment, SummarizedExperiment, TreeSummarizedExperiment, doParallel, doRNG, energy, foreach, gtools, lme4, lmerTest, multcomp, nloptr, parallel, utils

Suggests dplyr, knitr, rmarkdown, testthat, DT, tidyr, tidyverse, microbiome, magrittr

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BugReports <https://github.com/FrederickHuangLin/ANCOMBC/issues>

URL <https://github.com/FrederickHuangLin/ANCOMBC>

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ancom	<i>Analysis of Composition of Microbiomes (ANCOM)</i>
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Description

Determine taxa whose absolute abundances, per unit volume, of the ecosystem (e.g. gut) are significantly different with changes in the covariate of interest (e.g. group). The current version of ancom function implements ANCOM in cross-sectional and repeated measurements data while allowing for covariate adjustment.

Usage

```
ancom(
  data = NULL,
  assay.type = NULL,
  assay_name = "counts",
  rank = NULL,
  tax_level = NULL,
  phyloseq = NULL,
  p_adj_method = "holm",
  prv_cut = 0.1,
```

```

lib_cut = 0,
main_var,
adj_formula = NULL,
rand_formula = NULL,
lme_control = lme4::lmerControl(),
struc_zero = FALSE,
neg_lb = FALSE,
alpha = 0.05,
n_cl = 1
)

```

Arguments

data	the input data. The data parameter should be either a phyloseq or a TreeSummarizedExperiment object, which consists of a feature table (microbial count table), a sample metadata table, a taxonomy table (optional), and a phylogenetic tree (optional). Ensure that the row names of the metadata table match the sample names in the feature table, and the row names of the taxonomy table match the taxon (feature) names in the feature table. For detailed information, refer to <code>?phyloseq::phyloseq</code> or <code>?TreeSummarizedExperiment::TreeSummarizedExperiment</code> .
assay.type	alias for assay_name.
assay_name	character. Name of the count table in the data object (only applicable if data object is a (Tree)SummarizedExperiment). Default is "counts". See <code>?SummarizedExperiment::assay</code> for more details.
rank	alias for tax_level
tax_level	character. The taxonomic level of interest. The input data can be agglomerated at different taxonomic levels based on your research interest. Default is NULL, i.e., do not perform agglomeration, and the ANCOM analysis will be performed at the lowest taxonomic level of the input data.
phyloseq	a phyloseq object. Will be deprecated.
p_adj_method	character. method to adjust p-values. Default is "holm". Options include "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none". See <code>?stats::p.adjust</code> for more details.
prv_cut	a numerical fraction between 0 and 1. Taxa with prevalences (the proportion of samples in which the taxon is present) less than prv_cut will be excluded in the analysis. For example, if there are 100 samples, and a taxon has nonzero counts present in less than 100*prv_cut samples, it will not be considered in the analysis. Default is 0.10.
lib_cut	a numerical threshold for filtering samples based on library sizes. Samples with library sizes less than lib_cut will be excluded in the analysis. Default is 0, i.e. do not discard any sample.
main_var	character. The name of the main variable of interest.
adj_formula	character string representing the formula for covariate adjustment. Please note that you should NOT include the main_var in the formula. Default is NULL.

<code>rand_formula</code>	the character string expresses how the microbial absolute abundances for each taxon depend on the random effects in metadata. ANCOM follows the <code>lmerTest</code> package in formulating the random effects. See <code>?lmerTest::lmer</code> for more details. Default is <code>NULL</code> .
<code>lme_control</code>	a list of control parameters for mixed model fitting. See <code>?lme4::lmerControl</code> for details.
<code>struc_zero</code>	logical. whether to detect structural zeros based on <code>main_var</code> . <code>main_var</code> should be discrete. Default is <code>FALSE</code> .
<code>neg_lb</code>	logical. whether to classify a taxon as a structural zero using its asymptotic lower bound. Default is <code>FALSE</code> .
<code>alpha</code>	numeric. level of significance. Default is 0.05.
<code>n_cl</code>	numeric. The number of nodes to be forked. For details, see <code>?parallel::makeCluster</code> . Default is 1 (no parallel computing).

Details

A taxon is considered to have structural zeros in some (≥ 1) groups if it is completely (or nearly completely) missing in these groups. For instance, suppose there are three groups: `g1`, `g2`, and `g3`. If the counts of taxon A in `g1` are 0 but nonzero in `g2` and `g3`, then taxon A will be considered to contain structural zeros in `g1`. In this example, taxon A is declared to be differentially abundant between `g1` and `g2`, `g1` and `g3`, and consequently, it is globally differentially abundant with respect to this group variable. Such taxa are not further analyzed using ANCOM, but the results are summarized in the overall summary. For more details about the structural zeros, please go to the [ANCOM-II](#) paper. Setting `neg_lb = TRUE` indicates that you are using both criteria stated in section 3.2 of [ANCOM-II](#) to detect structural zeros; otherwise, the algorithm will only use the equation 1 in section 3.2 for declaring structural zeros. Generally, it is recommended to set `neg_lb = TRUE` when the sample size per group is relatively large (e.g. > 30).

Value

a list with components:

- `res`, a data.frame containing ANCOM result for the variable specified in `main_var`, each column is:
 - `W`, test statistics.
 - `detected_0.9`, `detected_0.8`, `detected_0.7`, `detected_0.6`, logical vectors representing whether a taxon is differentially abundant under a series of cutoffs. For example, `TRUE` in `detected_0.7` means the number of ALR transformed models where the taxon is differentially abundant with regard to the main variable outnumbers $0.7 * (n_{tax} - 1)$. `detected_0.7` is commonly used. Choose `detected_0.8` or `detected_0.9` for more conservative results, or choose `detected_0.6` for more liberal results.
- `zero_ind`, a logical data.frame with `TRUE` indicating the taxon is detected to contain structural zeros in some specific groups.
- `beta_data`, a numeric matrix containing pairwise coefficients for the main variable of interest in ALR transformed regression models.
- `p_data`, a numeric matrix containing pairwise p-values for the main variable of interest in ALR transformed regression models.

- `q_data`, a numeric matrix containing adjusted p-values by applying the `p_adj_method` to the `p_data` matrix.

Author(s)

Huang Lin

References

- Mandal S, Van Treuren W, White RA, Eggesbo M, Knight R, Peddada SD (2015). "Analysis of composition of microbiomes: a novel method for studying microbial composition." *Microbial ecology in health and disease*, **26**(1), 27663.
- Kaul A, Mandal S, Davidov O, Peddada SD (2017). "Analysis of microbiome data in the presence of excess zeros." *Frontiers in microbiology*, **8**, 2114.

See Also

[ancombc](#) [ancombc2](#)

Examples

```
library(ANCOMBC)
library(mia)
data(atlas1006, package = "microbiome")
tse = mia::makeTreeSummarizedExperimentFromPhyloseq(atlas1006)

# subset to baseline
tse = tse[, tse$time == 0]

# run ancom function
set.seed(123)
out = ancom(data = tse, assay_name = "counts",
            tax_level = "Family", phyloseq = NULL,
            p_adj_method = "holm", prv_cut = 0.10, lib_cut = 1000,
            main_var = "bmi_group", adj_formula = "age + nationality",
            rand_formula = NULL, lme_control = NULL,
            struc_zero = TRUE, neg_lb = TRUE, alpha = 0.05, n_cl = 1)

res = out$res

# to run ancom using the phyloseq object
tse_alt = agglomerateByRank(tse, "Family")
pseq = makePhyloseqFromTreeSummarizedExperiment(tse_alt)
set.seed(123)
out = ancom(data = NULL, assay_name = NULL,
            tax_level = "Family", phyloseq = pseq,
            p_adj_method = "holm", prv_cut = 0.10, lib_cut = 1000,
            main_var = "bmi_group", adj_formula = "age + nationality",
            rand_formula = NULL, lme_control = NULL,
            struc_zero = TRUE, neg_lb = TRUE, alpha = 0.05, n_cl = 1)
```

ancombc	<i>Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC)</i>
---------	--

Description

Determine taxa whose absolute abundances, per unit volume, of the ecosystem (e.g., gut) are significantly different with changes in the covariate of interest (e.g., group). The current version of ancombc function implements Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC) in cross-sectional data while allowing for covariate adjustment.

Usage

```
ancombc(
  data = NULL,
  assay.type = NULL,
  assay_name = "counts",
  rank = NULL,
  tax_level = NULL,
  phyloseq = NULL,
  formula,
  p_adj_method = "holm",
  prv_cut = 0.1,
  lib_cut = 0,
  group = NULL,
  struc_zero = FALSE,
  neg_lb = FALSE,
  tol = 1e-05,
  max_iter = 100,
  conserve = FALSE,
  alpha = 0.05,
  global = FALSE,
  n_cl = 1,
  verbose = FALSE
)
```

Arguments

data	the input data. The data parameter should be either a phyloseq or a TreeSummarizedExperiment object, which consists of a feature table (microbial count table), a sample metadata table, a taxonomy table (optional), and a phylogenetic tree (optional). Ensure that the row names of the metadata table match the sample names in the feature table, and the row names of the taxonomy table match the taxon (feature) names in the feature table. For detailed information, refer to <code>?phyloseq::phyloseq</code> or <code>?TreeSummarizedExperiment::TreeSummarizedExperiment</code> .
assay.type	alias for assay_name.

assay_name	character. Name of the count table in the data object (only applicable if data object is a (Tree)SummarizedExperiment). Default is "counts". See ?SummarizedExperiment::assay for more details.
rank	alias for tax_level.
tax_level	character. The taxonomic level of interest. The input data can be agglomerated at different taxonomic levels based on your research interest. Default is NULL, i.e., do not perform agglomeration, and the ANCOM-BC analysis will be performed at the lowest taxonomic level of the input data.
phyloseq	a phyloseq object. Will be deprecated.
formula	the character string expresses how microbial absolute abundances for each taxon depend on the variables in metadata. When specifying the formula, make sure to include the group variable in the formula if it is not NULL.
p_adj_method	character. method to adjust p-values. Default is "holm". Options include "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none". See ?stats::p.adjust for more details.
prv_cut	a numerical fraction between 0 and 1. Taxa with prevalences (the proportion of samples in which the taxon is present) less than prv_cut will be excluded in the analysis. For example, if there are 100 samples, and a taxon has nonzero counts present in less than 100*prv_cut samples, it will not be considered in the analysis. Default is 0.10.
lib_cut	a numerical threshold for filtering samples based on library sizes. Samples with library sizes less than lib_cut will be excluded in the analysis. Default is 0, i.e. do not discard any sample.
group	character. the name of the group variable in metadata. The group parameter should be a character string representing the name of the group variable in the metadata. The group variable should be discrete, meaning it consists of categorical values. Specifying the group variable is required if you are interested in detecting structural zeros and performing global tests. However, if these analyses are not of interest to you, you can leave the group parameter as NULL. If the group variable of interest contains only two categories, you can also leave the group parameter as NULL. Default is NULL.
struc_zero	logical. whether to detect structural zeros based on group. Default is FALSE.
neg_lb	logical. whether to classify a taxon as a structural zero using its asymptotic lower bound. Default is FALSE.
tol	numeric. the iteration convergence tolerance for the E-M algorithm. Default is 1e-05.
max_iter	numeric. the maximum number of iterations for the E-M algorithm. Default is 100.
conserve	logical. whether to use a conservative variance estimator for the test statistic. It is recommended if the sample size is small and/or the number of differentially abundant taxa is believed to be large. Default is FALSE.
alpha	numeric. level of significance. Default is 0.05.
global	logical. whether to perform the global test. Default is FALSE.

n_cl	numeric. The number of nodes to be forked. For details, see <code>?parallel::makeCluster</code> . Default is 1 (no parallel computing).
verbose	logical. Whether to generate verbose output during the ANCOM-BC fitting process. Default is FALSE.

Details

A taxon is considered to have structural zeros in some (≥ 1) groups if it is completely (or nearly completely) missing in these groups. For instance, suppose there are three groups: g1, g2, and g3. If the counts of taxon A in g1 are 0 but nonzero in g2 and g3, then taxon A will be considered to contain structural zeros in g1. In this example, taxon A is declared to be differentially abundant between g1 and g2, g1 and g3, and consequently, it is globally differentially abundant with respect to this group variable. Such taxa are not further analyzed using ANCOM-BC, but the results are summarized in the overall summary. For more details about the structural zeros, please go to the [ANCOM-II](#) paper. Setting `neg_lb = TRUE` indicates that you are using both criteria stated in section 3.2 of [ANCOM-II](#) to detect structural zeros; otherwise, the algorithm will only use the equation 1 in section 3.2 for declaring structural zeros. Generally, it is recommended to set `neg_lb = TRUE` when the sample size per group is relatively large (e.g. > 30).

Value

a list with components:

- `feature_table`, a data.frame of pre-processed (based on `prv_cut` and `lib_cut`) microbial count table.
- `zero_ind`, a logical data.frame with TRUE indicating the taxon is detected to contain structural zeros in some specific groups.
- `samp_frac`, a numeric vector of estimated sampling fractions in log scale (natural log).
- `delta_em`, estimated sample-specific biases through E-M algorithm.
- `delta_wls`, estimated sample-specific biases through weighted least squares (WLS) algorithm.
- `res`, a list containing ANCOM-BC primary result, which consists of:
 - `lfc`, a data.frame of log fold changes obtained from the ANCOM-BC log-linear (natural log) model.
 - `se`, a data.frame of standard errors (SEs) of `lfc`.
 - `W`, a data.frame of test statistics. $W = lfc/se$.
 - `p_val`, a data.frame of p-values. P-values are obtained from two-sided Z-test using the test statistic `W`.
 - `q_val`, a data.frame of adjusted p-values. Adjusted p-values are obtained by applying `p_adj_method` to `p_val`.
 - `diff_abn`, a logical data.frame. TRUE if the taxon has `q_val` less than alpha.
- `res_global`, a data.frame containing ANCOM-BC global test result for the variable specified in group, each column is:
 - `W`, test statistics.
 - `p_val`, p-values, which are obtained from two-sided Chi-square test using `W`.

- q_val, adjusted p-values. Adjusted p-values are obtained by applying p_adj_method to p_val.
- diff_abn, A logical vector. TRUE if the taxon has q_val less than alpha.

Author(s)

Huang Lin

References

Kaul A, Mandal S, Davidov O, Peddada SD (2017). "Analysis of microbiome data in the presence of excess zeros." *Frontiers in microbiology*, **8**, 2114.

Lin H, Peddada SD (2020). "Analysis of compositions of microbiomes with bias correction." *Nature communications*, **11**(1), 1–11.

See Also

[ancom ancombc2](#)

Examples

```
#####Build a TreeSummarizedExperiment Object from Scratch#####
library(mia)

# microbial count table
otu_mat = matrix(sample(1:100, 100, replace = TRUE), nrow = 10, ncol = 10)
rownames(otu_mat) = paste0("taxon", 1:nrow(otu_mat))
colnames(otu_mat) = paste0("sample", 1:ncol(otu_mat))
assays = SimpleList(counts = otu_mat)

# sample metadata
smd = data.frame(group = sample(LETTERS[1:4], size = 10, replace = TRUE),
                 row.names = paste0("sample", 1:ncol(otu_mat)),
                 stringsAsFactors = FALSE)
smd = DataFrame(smd)

# taxonomy table
tax_tab = matrix(sample(letters, 70, replace = TRUE),
                 nrow = nrow(otu_mat), ncol = 7)
rownames(tax_tab) = rownames(otu_mat)
colnames(tax_tab) = c("Kingdom", "Phylum", "Class", "Order",
                    "Family", "Genus", "Species")
tax_tab = DataFrame(tax_tab)

# create TSE
tse = TreeSummarizedExperiment(assays = assays,
                              colData = smd,
                              rowData = tax_tab)

# convert TSE to phyloseq
pseq = makePhyloseqFromTreeSummarizedExperiment(tse)
```

```

#=====Run ANCOMBC Using a Real Data=====
library(ANCOMBC)
data(atlas1006, package = "microbiome")
tse = mia::makeTreeSummarizedExperimentFromPhyloseq(atlas1006)

# subset to baseline
tse = tse[, tse$time == 0]

# run ancombc function
set.seed(123)
out = ancombc(data = tse, assay_name = "counts",
              tax_level = "Family", phyloseq = NULL,
              formula = "age + nationality + bmi_group",
              p_adj_method = "holm", prv_cut = 0.10, lib_cut = 1000,
              group = "bmi_group", struc_zero = TRUE, neg_lb = FALSE,
              tol = 1e-5, max_iter = 100, conserve = TRUE,
              alpha = 0.05, global = TRUE, n_cl = 1, verbose = TRUE)

res_prim = out$res
res_global = out$res_global

# to run ancombc using the phyloseq object
tse_alt = agglomerateByRank(tse, "Family")
pseq = makePhyloseqFromTreeSummarizedExperiment(tse_alt)
set.seed(123)
out = ancombc(data = NULL, assay_name = NULL,
              tax_level = "Family", phyloseq = pseq,
              formula = "age + nationality + bmi_group",
              p_adj_method = "holm", prv_cut = 0.10, lib_cut = 1000,
              group = "bmi_group", struc_zero = TRUE, neg_lb = FALSE,
              tol = 1e-5, max_iter = 100, conserve = TRUE,
              alpha = 0.05, global = TRUE, n_cl = 1, verbose = TRUE)

```

ancombc2

*Analysis of Compositions of Microbiomes with Bias Correction 2
(ANCOM-BC2)*

Description

Determine taxa whose absolute abundances, per unit volume, of the ecosystem (e.g., gut) are significantly different with changes in the covariate of interest (e.g., group). The current version of ancombc2 function implements Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC2) in cross-sectional and repeated measurements data. In addition to the two-group comparison, ANCOM-BC2 also supports testing for continuous covariates and multi-group comparisons, including the global test, pairwise directional test, Dunnett's type of test, and trend test.

Usage

```

ancombc2(
  data,
  assay.type = assay_name,
  assay_name = "counts",
  rank = tax_level,
  tax_level = NULL,
  fix_formula,
  rand_formula = NULL,
  p_adj_method = "holm",
  pseudo = 0,
  pseudo_sens = TRUE,
  prv_cut = 0.1,
  lib_cut = 0,
  s0_perc = 0.05,
  group = NULL,
  struc_zero = FALSE,
  neg_lb = FALSE,
  alpha = 0.05,
  n_cl = 1,
  verbose = FALSE,
  global = FALSE,
  pairwise = FALSE,
  dunnet = FALSE,
  trend = FALSE,
  iter_control = list(tol = 0.01, max_iter = 20, verbose = FALSE),
  em_control = list(tol = 1e-05, max_iter = 100),
  lme_control = lme4::lmerControl(),
  mdfdr_control = list(fwer_ctrl_method = "holm", B = 100),
  trend_control = list(contrast = NULL, node = NULL, solver = "ECOS", B = 100)
)

```

Arguments

data	the input data. The data parameter should be either a <code>phyloseq</code> or a <code>TreeSummarizedExperiment</code> object, which consists of a feature table (microbial count table), a sample metadata table, a taxonomy table (optional), and a phylogenetic tree (optional). Ensure that the row names of the metadata table match the sample names in the feature table, and the row names of the taxonomy table match the taxon (feature) names in the feature table. For detailed information, refer to <code>?phyloseq::phyloseq</code> or <code>?TreeSummarizedExperiment::TreeSummarizedExperiment</code> . It is recommended to use low taxonomic levels, such as OTU or species level, as the estimation of sampling fractions requires a large number of taxa.
assay.type	alias for <code>assay_name</code> .
assay_name	character. Name of the count table in the data object (only applicable if data object is a <code>(Tree)SummarizedExperiment</code>). Default is "counts". See <code>?SummarizedExperiment::assay</code> for more details.

rank	alias for tax_level.
tax_level	character. The taxonomic or non taxonomic(rowData) level of interest. The input data can be analyzed at any taxonomic or rowData level without prior agglomeration. Note that tax_level must be a value from taxonomyRanks or rowData, which includes "Kingdom", "Phylum", "Class", "Order", "Family", "Genus", "Species" etc. See ?mia::taxonomyRanks for more details. Default is NULL, i.e., do not perform agglomeration, and the ANCOM-BC2 analysis will be performed at the lowest taxonomic level of the input data.
fix_formula	the character string expresses how the microbial absolute abundances for each taxon depend on the fixed effects in metadata. When specifying the fix_formula, make sure to include the group variable in the formula if it is not NULL.
rand_formula	the character string expresses how the microbial absolute abundances for each taxon depend on the random effects in metadata. ANCOM-BC2 follows the lmerTest package in formulating the random effects. See ?lmerTest::lmer for more details. Default is NULL.
p_adj_method	character. method to adjust p-values. Default is "holm". Options include "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none". See ?stats::p.adjust for more details.
pseudo	numeric. Add pseudo-counts to the data. Please note that this option is now deprecated in ANCOM-BC2. The software will utilize the complete data (nonzero counts) as its default analysis input. Specifying a pseudo-count will not affect the analysis or its results.
pseudo_sens	logical. Whether to perform the sensitivity analysis to the pseudo-count addition. Default is TRUE. While ANCOM-BC2 utilizes complete data (nonzero counts) by default for its analysis, a comprehensive evaluation of result robustness is performed by assessing how pseudo-count addition to zeros may affect the outcomes. For a detailed discussion on this sensitivity analysis, refer to the Details section.
prv_cut	a numerical fraction between 0 and 1. Taxa with prevalences (the proportion of samples in which the taxon is present) less than prv_cut will be excluded in the analysis. For example, if there are 100 samples, and a taxon has nonzero counts present in less than 100*prv_cut samples, it will not be considered in the analysis. Default is 0.10.
lib_cut	a numerical threshold for filtering samples based on library sizes. Samples with library sizes less than lib_cut will be excluded in the analysis. Default is 0, i.e. do not discard any sample.
s0_perc	a numerical fraction between 0 and 1. Inspired by Significance Analysis of Microarrays (SAM) methodology, a small positive constant is added to the denominator of ANCOM-BC2 test statistic corresponding to each taxon to avoid the significance due to extremely small standard errors, especially for rare taxa. This small positive constant is chosen as s0_perc-th percentile of standard error values for each fixed effect. Default is 0.05 (5th percentile).
group	character. the name of the group variable in metadata. The group parameter should be a character string representing the name of the group variable in the metadata. The group variable should be discrete, meaning it consists of categorical values. Specifying the group variable is required if you are interested

in detecting structural zeros and performing performing multi-group comparisons (global test, pairwise directional test, Dunnett's type of test, and trend test). However, if these analyses are not of interest to you, you can leave the group parameter as NULL. If the group variable of interest contains only two categories, you can also leave the group parameter as NULL. Default is NULL.

struc_zero	logical. Whether to detect structural zeros based on group. Default is FALSE. See <code>Details</code> for a more comprehensive discussion on structural zeros.
neg_lb	logical. Whether to classify a taxon as a structural zero using its asymptotic lower bound. Default is FALSE.
alpha	numeric. Level of significance. Default is 0.05.
n_cl	numeric. The number of nodes to be forked. For details, see <code>?parallel::makeCluster</code> . Default is 1 (no parallel computing).
verbose	logical. Whether to generate verbose output during the ANCOM-BC2 fitting process. Default is FALSE.
global	logical. Whether to perform the global test. Default is FALSE.
pairwise	logical. Whether to perform the pairwise directional test. Default is FALSE.
dunnet	logical. Whether to perform the Dunnett's type of test. Default is FALSE.
trend	logical. Whether to perform trend test. Default is FALSE.
iter_control	a named list of control parameters for the iterative MLE or RMEL algorithm, including 1) <code>tol</code> : the iteration convergence tolerance (default is 1e-02), 2) <code>max_iter</code> : the maximum number of iterations (default is 20), and 3) <code>verbose</code> : whether to show the verbose output (default is FALSE).
em_control	a named list of control parameters for the E-M algorithm, including 1) <code>tol</code> : the iteration convergence tolerance (default is 1e-05) and 2) <code>max_iter</code> : the maximum number of iterations (default is 100).
lme_control	a list of control parameters for mixed model fitting. See <code>?lme4::lmerControl</code> for details.
mdfdr_control	a named list of control parameters for mixed directional false discover rate (mdFDR), including 1) <code>fwcr_method</code> : family wise error (FWER) controlling procedure, such as "holm", "hochberg", "bonferroni", etc (default is "holm") and 2) <code>B</code> : the number of bootstrap samples (default is 100). Increase <code>B</code> will lead to a more accurate p-values. See <code>Details</code> for a more comprehensive discussion on mdFDR.
trend_control	a named list of control parameters for the trend test, including 1) <code>contrast</code> : the list of contrast matrices for constructing inequalities, 2) <code>node</code> : the list of positions for the nodal parameter, 3) <code>solver</code> : a string indicating the solver to use (default is "ECOS"), and 4) <code>B</code> : the number of bootstrap samples (default is 100). Increase <code>B</code> will lead to a more accurate p-values. See <code>vignette</code> for the corresponding trend test examples.

Details

A taxon is considered to have structural zeros in some (≥ 1) groups if it is completely (or nearly completely) missing in these groups. For instance, suppose there are three groups: `g1`, `g2`, and `g3`.

If the counts of taxon A in g1 are 0 but nonzero in g2 and g3, then taxon A will be considered to contain structural zeros in g1. In this example, taxon A is declared to be differentially abundant between g1 and g2, g1 and g3, and consequently, it is globally differentially abundant with respect to this group variable. Such taxa are not further analyzed using ANCOM-BC2, but the results are summarized in the overall summary. For more details about the structural zeros, please go to the [ANCOM-II](#) paper. Setting `neg_lb = TRUE` indicates that you are using both criteria stated in section 3.2 of [ANCOM-II](#) to detect structural zeros; otherwise, the algorithm will only use the equation 1 in section 3.2 for declaring structural zeros. Generally, it is recommended to set `neg_lb = TRUE` when the sample size per group is relatively large (e.g. > 30).

Like other differential abundance analysis methods, ANCOM-BC2 applies a log transformation to the observed counts. However, the presence of zero counts poses a challenge, and researchers often consider adding a pseudo-count before the log transformation. However, it has been shown that the choice of pseudo-count can impact the results and lead to an inflated false positive rate ([Costea et al. \(2014\)](#); [Paulson, Bravo, and Pop \(2014\)](#)). To address this issue, we conduct a sensitivity analysis to assess the impact of different pseudo-counts on zero counts for each taxon. This analysis involves adding a series of pseudo-counts (ranging from 0.01 to 0.5 in increments of 0.01) to the zero counts of each taxon. Linear regression models are then performed on the bias-corrected log abundance table using the different pseudo-counts. The sensitivity score for each taxon is calculated as the proportion of times that the p-value exceeds the specified significance level (α). If all p-values consistently show significance or nonsignificance across different pseudo-counts and are consistent with the results obtained without adding pseudo-counts to zero counts (using the default settings), then the taxon is considered not sensitive to the pseudo-count addition.

When performing pairwise directional (or Dunnett's type of) test, the mixed directional false discovery rate (mdFDR) should be taken into account. The mdFDR is the combination of false discovery rate due to multiple testing, multiple pairwise comparisons, and directional tests within each pairwise comparison. For example, suppose we have five taxa and three experimental groups: g1, g2, and g3. Thus, we are performing five tests corresponding to five taxa. For each taxon, we are also conducting three pairwise comparisons (g1 vs. g2, g2 vs. g3, and g1 vs. g3). Within each pairwise comparison, we wish to determine if the abundance has increased or decreased or did not change (direction of the effect size). Errors could occur in each step. The overall false discovery rate is controlled by the mdFDR methodology we adopted from [Guo, Sarkar, and Peddada \(2010\)](#) and [Grandhi, Guo, and Peddada \(2016\)](#).

Value

a list with components:

- `feature_table`, a data.frame of pre-processed (based on `prv_cut` and `lib_cut`) microbial count table.
- `bias_correct_log_table`, a data.frame of bias-corrected log abundance table.
- `ss_tab`, a data.frame of sensitivity scores for pseudo-count addition to 0s.
- `zero_ind`, a logical data.frame with TRUE indicating the taxon is detected to contain structural zeros in some specific groups.
- `samp_frac`, a numeric vector of estimated sampling fractions in log scale (natural log).
- `delta_em`, estimated sample-specific biases through E-M algorithm.
- `delta_wls`, estimated sample-specific biases through weighted least squares (WLS) algorithm.

- `res`, a `data.frame` containing ANCOM-BC2 primary result:
 - columns started with `lfc`: log fold changes obtained from the ANCOM-BC2 log-linear (natural log) model.
 - columns started with `se`: standard errors (SEs) of `lfc`.
 - columns started with `W`: test statistics. $W = lfc/se$.
 - columns started with `p`: p-values. P-values are obtained from two-sided Z-test using the test statistic `W`.
 - columns started with `q`: adjusted p-values. Adjusted p-values are obtained by applying `p_adj_method` to `p`.
 - columns started with `diff`: TRUE if the taxon is significant (has `q` less than `alpha`).
 - columns started with `passed_ss`: TRUE if the taxon passed the sensitivity analysis, i.e., adding different pseudo-counts to 0s would not change the results.
- `res_global`, a `data.frame` containing ANCOM-BC2 global test result for the variable specified in `group`, each column is:
 - `W`, test statistics.
 - `p_val`, p-values, which are obtained from two-sided Chi-square test using `W`.
 - `q_val`, adjusted p-values. Adjusted p-values are obtained by applying `p_adj_method` to `p_val`.
 - `diff_abn`, A logical vector. TRUE if the taxon has `q_val` less than `alpha`.
 - `passed_ss`, A logical vector. TRUE if the taxon has passed the sensitivity analysis.
- `res_pair`, a `data.frame` containing ANCOM-BC2 pairwise directional test result for the variable specified in `group`:
 - columns started with `lfc`: log fold changes.
 - columns started with `se`: standard errors (SEs).
 - columns started with `W`: test statistics.
 - columns started with `p`: p-values.
 - columns started with `q`: adjusted p-values.
 - columns started with `diff`: TRUE if the taxon is significant (has `q` less than `alpha`).
 - columns started with `passed_ss`: TRUE if the taxon has passed the sensitivity analysis.
- `res_dunn`, a `data.frame` containing ANCOM-BC2 Dunnett's type of test result for the variable specified in `group`:
 - columns started with `lfc`: log fold changes.
 - columns started with `se`: standard errors (SEs).
 - columns started with `W`: test statistics.
 - columns started with `p`: p-values.
 - columns started with `q`: adjusted p-values.
 - columns started with `diff`: TRUE if the taxon is significant (has `q` less than `alpha`).
 - columns started with `passed_ss`: TRUE if the taxon has passed the sensitivity analysis.
- `res_trend`, a `data.frame` containing ANCOM-BC2 trend test result for the variable specified in `group`:
 - columns started with `lfc`: log fold changes.
 - columns started with `se`: standard errors (SEs).

- W: test statistics.
- p_val: p-values.
- q_val: adjusted p-values.
- diff_abn: TRUE if the taxon is significant (has q less than alpha).
- passed_ss, A logical vector. TRUE if the taxon has passed the sensitivity analysis.

Author(s)

Huang Lin

References

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See Also

[ancom](#) [ancombc](#)

Examples

```
#####Build a TreeSummarizedExperiment Object from Scratch#####
library(mia)

# microbial count table
otu_mat = matrix(sample(1:100, 100, replace = TRUE), nrow = 10, ncol = 10)
rownames(otu_mat) = paste0("taxon", 1:nrow(otu_mat))
colnames(otu_mat) = paste0("sample", 1:ncol(otu_mat))
assays = SimpleList(counts = otu_mat)

# sample metadata
smd = data.frame(group = sample(LETTERS[1:4], size = 10, replace = TRUE),
                 row.names = paste0("sample", 1:ncol(otu_mat)),
                 stringsAsFactors = FALSE)
smd = DataFrame(smd)
```



```

# taxonomy table
tax_tab = matrix(sample(letters, 70, replace = TRUE),
                 nrow = nrow(otu_mat), ncol = 7)
rownames(tax_tab) = rownames(otu_mat)
colnames(tax_tab) = c("Kingdom", "Phylum", "Class", "Order",
                    "Family", "Genus", "Species")
# Can also contain non-taxonomic information, for instance
# colnames(tax_tab) = c("G1", "G2", "G3", "G4", "G5", "G6", "G7")
tax_tab = DataFrame(tax_tab)

# create TSE
tse = TreeSummarizedExperiment(assays = assays,
                              colData = smd,
                              rowData = tax_tab)

# convert TSE to phyloseq
pseq = makePhyloseqFromTreeSummarizedExperiment(tse)

#####Run ANCOMBC2 Using a Real Data#####
library(ANCOMBC)
data(dietswap, package = "microbiome")
tse = mia::makeTreeSummarizedExperimentFromPhyloseq(dietswap)

colData(tse)$bmi_group = factor(colData(tse)$bmi_group,
                               levels = c("obese",
                                           "overweight",
                                           "lean"))

set.seed(123)
# Note that setting max_iter = 1 and B = 1 is only for the sake of speed
# Use default or larger values for max_iter and B for better performance
out = ancombc2(data = tse, assay_name = "counts", tax_level = "Phylum",
              fix_formula = "nationality + timepoint + bmi_group",
              rand_formula = NULL,
              p_adj_method = "holm", pseudo_sens = TRUE,
              prv_cut = 0.10, lib_cut = 1000, s0_perc = 0.05,
              group = "bmi_group", struc_zero = TRUE, neg_lb = TRUE,
              alpha = 0.05, n_cl = 1, verbose = TRUE,
              global = TRUE, pairwise = TRUE, dunnet = TRUE, trend = TRUE,
              iter_control = list(tol = 1e-2, max_iter = 1, verbose = TRUE),
              em_control = list(tol = 1e-5, max_iter = 1),
              lme_control = lme4::lmerControl(),
              mdfdr_control = list(fwer_ctrl_method = "holm", B = 1),
              trend_control = list(contrast =
                                   list(matrix(c(1, 0, -1, 1),
                                               nrow = 2,
                                               byrow = TRUE)),
                                   node = list(2),
                                   solver = "ECOS",
                                   B = 1))

res_prim = out$res
res_global = out$res_global

```

```
res_pair = out$res_pair  
res_dunn = out$res_dunn  
res_trend = out$res_trend
```

QMP

Quantitative Microbiome Project data

Description

The data containing quantitative microbiome count data of dimension 106 samples/subjects (in rows) and 91 OTUs (in columns). The raw dataset is pruned the taxa present less than 30 final dataset contains only healthy subjects from two cohorts: Study cohort and Disease cohort. For details, see <https://doi.org/10.1038/nature24460>.

Usage

```
data(QMP)
```

Format

The dataset in `matrix` format.

Details

The dataset is also available via the SPRING R package <https://github.com/GraceYoon/SPRING> in `matrix` format.

Value

Loads the dataset in R.

Author(s)

Huang Lin <huanglinfrederick@gmail.com>

References

Vanderputte et al. Nature. 551: 507-511, 2017. <https://doi.org/10.1038/nature24460>

secom_dist

*Sparse estimation of distance correlations among microbiomes***Description**

Obtain the sparse correlation matrix for distance correlations between taxa.

Usage

```
secom_dist(
  data,
  assay.type = assay_name,
  assay_name = "counts",
  rank = tax_level,
  tax_level = NULL,
  pseudo = 0,
  prv_cut = 0.5,
  lib_cut = 1000,
  corr_cut = 0.5,
  wins_quant = c(0.05, 0.95),
  R = 1000,
  thresh_hard = 0,
  max_p = 0.005,
  n_cl = 1
)
```

Arguments

data	a list of the input data. The data parameter should be a list containing input data objects, which can be either phyloseq or TreeSummarizedExperiment objects. Each object within the list consists of a feature table (microbial count table), a sample metadata table, a taxonomy table (optional), and a phylogenetic tree (optional). Ensure that the row names of the metadata table match the sample names in the feature table, and the row names of the taxonomy table match the taxon (feature) names in the feature table. For detailed information, refer to <code>?phyloseq::phyloseq</code> or <code>?TreeSummarizedExperiment::TreeSummarizedExperiment</code> . It is recommended to use low taxonomic levels, such as OTU or species level, as the estimation of sampling fractions requires a large number of taxa. If working with multiple ecosystems, such as gut and tongue, stack the data by specifying the list of input data as <code>data = list(gut = tse1, tongue = tse2)</code> .
assay.type	alias for assay_name.
assay_name	character. Name of the count table in the data object (only applicable if data object is a (Tree)SummarizedExperiment). Default is "counts". See <code>?SummarizedExperiment::assay</code> for more details.
rank	alias for tax_level.

tax_level	character. The taxonomic level of interest. The input data can be agglomerated at different taxonomic levels based on your research interest. Default is NULL, i.e., do not perform agglomeration, and the SECOM analysis will be performed at the lowest taxonomic level of the input data.
pseudo	numeric. Add pseudo-counts to the data. Default is 0 (no pseudo-counts).
prv_cut	a numerical fraction between 0 and 1. Taxa with prevalences (the proportion of samples in which the taxon is present) less than prv_cut will be excluded in the analysis. For example, if there are 100 samples, and a taxon has nonzero counts present in less than 100*prv_cut samples, it will not be considered in the analysis. Default is 0.50.
lib_cut	a numerical threshold for filtering samples based on library sizes. Samples with library sizes less than lib_cut will be excluded in the analysis. Default is 1000.
corr_cut	numeric. To avoid false positives caused by taxa with small variances, taxa with Pearson correlation coefficients greater than corr_cut with the estimated sample-specific bias will be flagged. When taxa are flagged, the pairwise correlation coefficient between them will be set to 0s. Default is 0.5.
wins_quant	a numeric vector of probabilities with values between 0 and 1. Replace extreme values in the abundance data with less extreme values. Default is c(0.05, 0.95). For details, see ?DescTools::Winsorize.
R	numeric. The number of replicates in calculating the p-value for distance correlation. For details, see ?energy::dcor.test. Default is 1000.
thresh_hard	Numeric. Pairwise correlation coefficients (in their absolute value) that are less than or equal to thresh_hard will be set to 0. Default is 0.3.
max_p	numeric. Obtain the sparse correlation matrix by p-value filtering. Pairwise correlation coefficients with p-value greater than max_p will be set to 0s. Default is 0.005.
n_cl	numeric. The number of nodes to be forked. For details, see ?parallel::makeCluster. Default is 1 (no parallel computing).

Details

The **distance correlation**, which is a measure of dependence between two random variables, can be used to quantify any dependence, whether linear, monotonic, non-monotonic or nonlinear relationships.

Value

a list with components:

- s_diff_hat, a numeric vector of estimated sample-specific biases.
- y_hat, a matrix of bias-corrected abundances
- mat_cooccur, a matrix of taxon-taxon co-occurrence pattern. The number in each cell represents the number of complete (nonzero) samples for the corresponding pair of taxa.
- dcorr, the sample distance correlation matrix computed using the bias-corrected abundances y_hat.

- dcorr_p, the p-value matrix corresponding to the sample distance correlation matrix dcorr.
- dcorr_fl, the sparse correlation matrix obtained by p-value filtering based on the cutoff specified in max_p.

Author(s)

Huang Lin

See Also

[secom_linear](#)

Examples

```
library(ANCOMBC)
data(dietswap, package = "microbiome")
tse = mia::makeTreeSummarizedExperimentFromPhyloseq(dietswap)

# subset to baseline
tse = tse[, tse$timepoint == 1]

set.seed(123)
res_dist = secom_dist(data = list(tse), assay_name = "counts",
                      tax_level = "Phylum", pseudo = 0,
                      prv_cut = 0.5, lib_cut = 1000, corr_cut = 0.5,
                      wins_quant = c(0.05, 0.95), R = 1000,
                      thresh_hard = 0.3, max_p = 0.005, n_cl = 2)

dcorr_fl = res_dist$dcorr_fl
```

secom_linear

Sparse estimation of linear correlations among microbiomes

Description

Obtain the sparse correlation matrix for linear correlations between taxa. The current version of secom_linear function supports either of the three correlation coefficients: Pearson, Spearman, and Kendall's τ .

Usage

```
secom_linear(
  data,
  assay.type = assay_name,
  assay_name = "counts",
  rank = tax_level,
  tax_level = NULL,
  pseudo = 0,
```

```

prv_cut = 0.5,
lib_cut = 1000,
corr_cut = 0.5,
wins_quant = c(0.05, 0.95),
method = c("pearson", "spearman"),
soft = FALSE,
thresh_len = 100,
n_cv = 10,
thresh_hard = 0,
max_p = 0.005,
n_cl = 1
)

```

Arguments

data	a list of the input data. The data parameter should be a list containing input data objects, which can be either phyloseq or TreeSummarizedExperiment objects. Each object within the list consists of a feature table (microbial count table), a sample metadata table, a taxonomy table (optional), and a phylogenetic tree (optional). Ensure that the row names of the metadata table match the sample names in the feature table, and the row names of the taxonomy table match the taxon (feature) names in the feature table. For detailed information, refer to <code>?phyloseq::phyloseq</code> or <code>?TreeSummarizedExperiment::TreeSummarizedExperiment</code> . It is recommended to use low taxonomic levels, such as OTU or species level, as the estimation of sampling fractions requires a large number of taxa. If working with multiple ecosystems, such as gut and tongue, stack the data by specifying the list of input data as <code>data = list(gut = tse1, tongue = tse2)</code> .
assay.type	alias for assay_name.
assay_name	character. Name of the feature table within the data object (only applicable if the data object is a (Tree)SummarizedExperiment). Default is "counts". See <code>?SummarizedExperiment::assay</code> for more details.
rank	alias for tax_level.
tax_level	character. The taxonomic level of interest. The input data can be agglomerated at different taxonomic levels based on your research interest. Default is NULL, i.e., do not perform agglomeration, and the SECOM analysis will be performed at the lowest taxonomic level of the input data.
pseudo	numeric. Add pseudo-counts to the data. Default is 0 (no pseudo-counts).
prv_cut	a numerical fraction between 0 and 1. Taxa with prevalences (the proportion of samples in which the taxon is present) less than prv_cut will be excluded in the analysis. For example, if there are 100 samples, and a taxon has nonzero counts present in less than 100*prv_cut samples, it will not be considered in the analysis. Default is 0.50.
lib_cut	a numerical threshold for filtering samples based on library sizes. Samples with library sizes less than lib_cut will be excluded in the analysis. Default is 1000.
corr_cut	numeric. To avoid false positives caused by taxa with small variances, taxa with Pearson correlation coefficients greater than corr_cut with the estimated

	sample-specific bias will be flagged. When taxa are flagged, the pairwise correlation coefficient between them will be set to 0s. Default is 0.5.
wins_quant	a numeric vector of probabilities with values between 0 and 1. Replace extreme values in the abundance data with less extreme values. Default is $c(0.05, 0.95)$. For details, see <code>?DescTools::Winsorize</code> .
method	character. It indicates which correlation coefficient is to be computed. It can be either "pearson" or "spearman".
soft	logical. TRUE indicates that soft thresholding is applied to achieve the sparsity of the correlation matrix. FALSE indicates that hard thresholding is applied to achieve the sparsity of the correlation matrix. Default is FALSE.
thresh_len	numeric. Grid-search is implemented to find the optimal values over thresh_len thresholds for the thresholding operator. Default is 100.
n_cv	numeric. The fold number in cross validation. Default is 10 (10-fold cross validation).
thresh_hard	Numeric. Pairwise correlation coefficients (in their absolute value) that are less than or equal to thresh_hard will be set to 0. Default is 0.3.
max_p	numeric. Obtain the sparse correlation matrix by p-value filtering. Pairwise correlation coefficients with p-value greater than max_p will be set to 0s. Default is 0.005.
n_cl	numeric. The number of nodes to be forked. For details, see <code>?parallel::makeCluster</code> . Default is 1 (no parallel computing).

Value

a list with components:

- `s_diff_hat`, a numeric vector of estimated sample-specific biases.
- `y_hat`, a matrix of bias-corrected abundances
- `cv_error`, a numeric vector of cross-validation error estimates, which are the Frobenius norm differences between correlation matrices using training set and validation set, respectively.
- `thresh_grid`, a numeric vector of thresholds in the cross-validation.
- `thresh_opt`, numeric. The optimal threshold through cross-validation.
- `mat_cooccur`, a matrix of taxon-taxon co-occurrence pattern. The number in each cell represents the number of complete (nonzero) samples for the corresponding pair of taxa.
- `corr`, the sample correlation matrix (using the measure specified in `method`) computed using the bias-corrected abundances `y_hat`.
- `corr_p`, the p-value matrix corresponding to the sample correlation matrix `corr`.
- `corr_th`, the sparse correlation matrix obtained by thresholding based on the method specified in `soft`.
- `corr_fl`, the sparse correlation matrix obtained by p-value filtering based on the cutoff specified in `max_p`.

Author(s)

Huang Lin

See Also[secom_dist](#)**Examples**

```

library(ANCOMBC)
data(dietswap, package = "microbiome")
tse = mia::makeTreeSummarizedExperimentFromPhyloseq(dietswap)

# subset to baseline
tse = tse[, tse$timepoint == 1]

set.seed(123)
res_linear = secom_linear(data = list(tse), assay_name = "counts",
                          tax_level = "Phylum", pseudo = 0,
                          prv_cut = 0.5, lib_cut = 1000, corr_cut = 0.5,
                          wins_quant = c(0.05, 0.95), method = "pearson",
                          soft = FALSE, thresh_len = 20, n_cv = 10,
                          thresh_hard = 0.3, max_p = 0.005, n_cl = 2)

corr_th = res_linear$corr_th
corr_fl = res_linear$corr_fl

```

sim_plnm

Simulate Microbial Absolute Abundance Data by Poisson lognormal (PLN) model Based on a Real Dataset

Description

Generate microbial absolute abundances using the Poisson lognormal (PLN) model based on the mechanism described in the [LDM](#) paper (supplementary text S2).

Usage

```
sim_plnm(abn_table, taxa_are_rows = TRUE, prv_cut = 0.1, n, lib_mean, disp)
```

Arguments

abn_table	the input microbial count table. It is used to obtain the estimated variance-covariance matrix, can be in either <code>matrix</code> or <code>data.frame</code> format.
taxa_are_rows	logical. TRUE if the input dataset has rows represent taxa. Default is TRUE.
prv_cut	a numerical fraction between 0 and 1. Taxa with prevalences less than <code>prv_cut</code> will be excluded in the analysis. For instance, suppose there are 100 samples, if a taxon has nonzero counts presented in less than 10 samples, it will not be further analyzed. Default is 0.10.
n	numeric. The desired sample size for the simulated data.

lib_mean	numeric. Mean of the library size. Library sizes are generated from the negative binomial distribution with parameters lib_mean and disp. For details, see ?rnbinom.
disp	numeric. The dispersion parameter for the library size. For details, see ?rnbinom.

Details

The PLN model relates the abundance vector with a Gaussian latent vector. Because of the presence of a latent layer, the PLN model displays a larger variance than the Poisson model (over-dispersion). Also, the covariance (correlation) between abundances has the same sign as the covariance (correlation) between the corresponding latent variables. This property gives enormous flexibility in modeling the variance-covariance structure of microbial abundances since it is easy to specify different variance-covariance matrices in the multivariate Gaussian distribution.

However, instead of manually specifying the variance-covariance matrix, we choose to estimate the variance-covariance matrix from a real dataset, which will make the simulated data more resemble real data.

Value

a matrix of microbial absolute abundances, where taxa are in rows and samples are in columns.

Author(s)

Huang Lin

References

Hu Y, Satten GA (2020). "Testing hypotheses about the microbiome using the linear decomposition model (LDM)." *Bioinformatics*, **36**(14), 4106–4115.

Examples

```
library(ANCOMBC)
data(QMP)
abn_data = sim_plnm(abn_table = QMP, taxa_are_rows = FALSE, prv_cut = 0.05,
                   n = 100, lib_mean = 1e8, disp = 0.5)
rownames(abn_data) = paste0("Taxon", seq_len(nrow(abn_data)))
colnames(abn_data) = paste0("Sample", seq_len(ncol(abn_data)))
```

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