

# Package ‘MMUPHin’

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**Type** Package

**Title** Meta-analysis Methods with Uniform Pipeline for Heterogeneity in Microbiome Studies

**Version** 1.20.0

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**Description** MMUPHin is an R package for meta-analysis tasks of microbiome cohorts. It has function interfaces for:

- a) covariate-controlled batch- and cohort effect adjustment,
- b) meta-analysis differential abundance testing,
- c) meta-analysis unsupervised discrete structure (clustering) discovery, and
- d) meta-analysis unsupervised continuous structure discovery.

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---

*add\_back\_covariates*     *Add back covariate effects to batch-corrected feature abundance data*

---

**Description**

Add back covariate effects to batch-corrected feature abundance data

**Usage**

```
add_back_covariates(adj_data, l_stand_feature, l_ind)
```

**Arguments**

*adj\_data*             feature-by-sample matrix of batch-adjusted feature abundances (but without covariate effects), as returned by *relocate\_scale*.  
*l\_stand\_feature*       list of per-feature standardization fits, as returned by *fit\_stand\_feature*.  
*l\_ind*                 list of indicator matrices, as returned by *construct\_ind*.

**Value**

feature-by-sample matrix of batch-adjusted feature abundances with covariate effects retained.

---

*adjust\_batch*             *Zero-inflated empirical Bayes adjustment of batch effect in compositional feature abundance data*

---

**Description**

*adjust\_batch* takes as input a feature-by-sample matrix of microbial abundances, and performs batch effect adjustment given provided batch and optional covariate variables. It returns the batch-adjusted abundance matrix. Additional options and parameters can be passed through the *control* parameter as a list (see details).

**Usage**

```
adjust_batch(feature_abd, batch, covariates = NULL, data, control)
```

**Arguments**

*feature\_abd*         feature-by-sample matrix of abundances (proportions or counts).  
*batch*                name of the batch variable. This variable in data should be a factor variable and will be converted to so with a warning if otherwise.  
*covariates*          name(s) of covariates to adjust for in the batch correction model.  
*data*                 data frame of metadata, columns must include batch and covariates (if specified).  
*control*              a named list of additional control parameters. See details.

**Details**

control should be provided as a named list of the following components (can be a subset).

**zero\_inflation** logical. Indicates whether or not a zero-inflated model should be run. Default to TRUE (zero-inflated model). If set to FALSE then the correction will be similar to ComBat as provided in the sva package.

**pseudo\_count** numeric. Pseudo count to add feature\_abd before the methods' log transformation. Default to NULL, in which case adjust\_batch will set the pseudo count automatically to half of minimal non-zero values in feature\_abd.

**diagnostic\_plot** character. Name for the generated diagnostic figure file. Default to "adjust\_batch\_diagnostic.pdf". Can be set to NULL in which case no output will be generated.

**conv** numeric. Convergence threshold for the method's iterative algorithm for shrinking batch effect parameters. Default to 1e-4.

**maxit** integer. Maximum number of iterations allowed for the method's iterative algorithm. Default to 1000.

**verbose** logical. Indicates whether or not verbose information will be printed.

**Value**

a list, with the following components:

**feature\_abd\_adj** feature-by-sample matrix of batch-adjusted abundances, normalized to the same per-sample total abundance as feature\_abd.

**control** list of additional control parameters used in the function call.

**Author(s)**

Siyuan Ma, <siyuanma@g.harvard.edu>

**Examples**

```
data("CRC_abd", "CRC_meta")
CRC_abd_adj <- adjust_batch(feature_abd = CRC_abd,
                           batch = "studyID",
                           covariates = "study_condition",
                           data = CRC_meta)$feature_abd_adj
```

---

adjust\_EB

*Perform batch adjustment on standardized feature abundances, based on EB shrunked per-batch location and scale parameters*

---

**Description**

Perform batch adjustment on standardized feature abundances, based on EB shrunked per-batch location and scale parameters

**Usage**

```
adjust_EB(s_data, l_params_shrink, l_stand_feature, batchmod, n_batch,
          l_ind)
```

**Arguments**

s_data	feature-by-sample matrix of standardized abundances.
l_params_shrink	list of shrunk parameters, as returned by fit_shrink.
l_stand_feature	list of per-feature standardization fits, as returned by fit_stand_feature.
batchmod	design matrix for batch variables.
n_batch	number of batches in the data.
l_ind	list of indicator matrices, as returned by construct_ind.

**Value**

feature-by-sample matrix of batch-adjusted feature abundances.

---

aprior	<i>EB prior estimation for scale parameters</i>
--------	---

---

**Description**

EB prior estimation for scale parameters

**Usage**

```
aprior(delta_hat, na.rm = FALSE)
```

**Arguments**

delta_hat	frequentist per-batch scale estimations.
na.rm	whether or not missing values should be removed.

**Value**

shape hyper parameter

---

AST	<i>AST transformation (modified from Maaslin2 and is different)</i>
-----	---

---

**Description**

AST transformation (modified from Maaslin2 and is different)

**Usage**

```
AST(x)
```

**Arguments**

x	vector of abundance to be transformed.
---	--

**Value**

transformed vector of abundance.

---

back_transform_abd	<i>Transform batch adjusted feature abundances back to the original scale in feature_abd</i>
--------------------	--

---

**Description**

Transform batch adjusted feature abundances back to the original scale in feature\_abd

**Usage**

```
back_transform_abd(adj_data, feature_abd, type_feature_abd)
```

**Arguments**

adj_data	feature-by-sample matrix of batch-adjusted feature abundances with covariate effects retained.
feature_abd	original feature-by-sample matrix of abundances (proportions or counts).
type_feature_abd	type of feature abundance table (counts or proportions). If counts, the final output will be rounded into counts as well.

**Value**

feature-by-sample matrix of batch-adjusted feature abundances, with covariate effects retained and scales consistent with original abundance matrix.

---

bprior	<i>EB prior estimation for scale parameters</i>
--------	---

---

**Description**

EB prior estimation for scale parameters

**Usage**

```
bprior(delta_hat, na.rm = FALSE)
```

**Arguments**

delta_hat	frequentist per-batch location estimations.
na.rm	whether or not missing values should be removed.

**Value**

scale hyper parameter

---

catchToList	<i>Utility for catching warning/error messages</i>
-------------	--

---

**Description**

Utility for catching warning/error messages

**Usage**

```
catchToList(expr)
```

**Arguments**

expr                    an expression to run that can generate potential errors/warnings

**Value**

a list, capturing both the return value of the expression, as well as generated errors/warnings (NULL if no errors/warnings)

---

check_batch	<i>Check batch variable</i>
-------------	-----------------------------

---

**Description**

Check batch variable

**Usage**

```
check_batch(x, min_n_batch = 2)
```

**Arguments**

x                        batch variable.  
min\_n\_batch            min. number of batches (for MMUPHin functions to run).

**Value**

if no errors then the batch variables (factorized if not already)

---

check_covariates	<i>Check covariates</i>
------------------	-------------------------

---

**Description**

Check covariates

**Usage**

```
check_covariates(data_covariates, batch)
```

**Arguments**

data_covariates	data frame of covariates.
batch	batch variable.

**Value**

vector of indicators per batch for if/which covariates can be fitted within the batches

---

check_covariates_random	<i>Check random covariates</i>
-------------------------	--------------------------------

---

**Description**

Check random covariates

**Usage**

```
check_covariates_random(data_covariates, batch)
```

**Arguments**

data_covariates	data frame of random covariates.
batch	batch variable.

**Value**

vector of indicators per batch for if/which random covariates can be fitted within the batches



---

check_D	<i>Check dissimilarity object</i>
---------	-----------------------------------

---

**Description**

Make sure that the input is a dissimilarity object

**Usage**

```
check_D(D)
```

**Arguments**

D                   dissimilarity object.

**Value**

returns an error if D is not a dissimilarity. Otherwise D as a matrix.

---

check_exposure	<i>Check exposure variable</i>
----------------	--------------------------------

---

**Description**

Check exposure variable

**Usage**

```
check_exposure(exposure, batch)
```

**Arguments**

exposure           exposure variable.

batch               batch variable.

**Value**

vector of indicators per batch for whether or not the exposure can be fitted within the batches

---

check_feature_abd	<i>Check feature abundance table</i>
-------------------	--------------------------------------

---

**Description**

Given a feature abundance table, make sure that a) it has no missing values, b) all values are non-negative, c) it is either proportions (all no greater than 1) or counts (all integers).

**Usage**

```
check_feature_abd(feature_abd)
```

**Arguments**

feature\_abd      feature-by-sample matrix of abundances (proportions or counts).

**Value**

returns an error if any of the check fails. Otherwise either "counts" or "proportions"

---

check_metadata	<i>Check that metadata data frame has all the variables and not missing</i>
----------------	---

---

**Description**

Check that metadata data frame has all the variables and not missing

**Usage**

```
check_metadata(data, variables, no_missing = TRUE)
```

**Arguments**

data              data frame of metadata.  
variables        name of variables (batch, covariates, etc.) to check

**Value**

data reduced to include only those specified in variables

---

check_options	<i>Utility for checking options</i>
---------------	-------------------------------------

---

**Description**

Utility for checking options

**Usage**

```
check_options(x, x_name, options)
```

**Arguments**

x	the specified value
x_name	name of the specified value
options	allowed options

**Value**

error if x is not in options. Otherwise returns x.

---

check_options_continuous	<i>Utility for checking continuous options</i>
--------------------------	--

---

**Description**

Utility for checking continuous options

**Usage**

```
check_options_continuous(x, x_name, range)
```

**Arguments**

x	the specified numeric value
x_name	name of the specified value
range	allowed range

**Value**

error if x is not within range (boundaries excluded). Otherwise returns x.

---

check_pseudo_count	<i>Utility for checking pseudo count</i>
--------------------	--

---

**Description**

Utility for checking pseudo count

**Usage**

```
check_pseudo_count(x)
```

**Arguments**

x                    the specified pseudo count

**Value**

error if pseudo count is smaller than zero. Otherwise returns x.

---

check_rank	<i>Check if a design matrix is full rank</i>
------------	--

---

**Description**

Check if a design matrix is full rank

**Usage**

```
check_rank(design)
```

**Arguments**

design                design matrix.

**Value**

TRUE/FALSE for whether or not the design matrix is full rank.

---

check_samples	<i>Check that sample numbers and names match between a feature table and a metadata data frame</i>
---------------	--

---

**Description**

Sample names (column names of the feature table, row names of the metadata data frame) must be matching exactly. Note that this dictates that they cannot be NULL because by design data (a data frame) should have non-empty row names.

**Usage**

```
check_samples(feature_abd, data)
```

**Arguments**

feature_abd	feature-by-sample matrix of abundances (proportions or counts).
data	data frame of metadata.

**Value**

matched sample names

---

check_samples_D	<i>Check that sample numbers and names match between a dissimilarity matrix and a metadata data frame</i>
-----------------	---

---

**Description**

Sample names (row/column names of the D matrix, row names of the metadata data frame) must be matching exactly. Note that this dictates that they cannot be NULL because by design data (a data frame) should have non-empty row names.

**Usage**

```
check_samples_D(D, data)
```

**Arguments**

D	sample-by-sample matrix of dissimilarities (proportions or counts).
data	data frame of metadata.

**Value**

matched sample names

---

construct_design	<i>Construct a design model matrix given a metadata data frame, with the option to exclude the intercept.</i>
------------------	---

---

### Description

Construct a design model matrix given a metadata data frame, with the option to exclude the intercept.

### Usage

```
construct_design(data, with_intercept = TRUE)
```

### Arguments

data                    metadata data frame.  
with\_intercept    should intercept terms be included in the model

### Value

design matrix.

---

construct_ind	<i>Create indicator matrices for which feature/batch/samples to adjust. This is relevant for zero_inflation is TRUE and only non-zero values are adjusted.</i>
---------------	--

---

### Description

Create indicator matrices for which feature/batch/samples to adjust. This is relevant for zero\_inflation is TRUE and only non-zero values are adjusted.

### Usage

```
construct_ind(feature_abd, n_batch, design, zero_inflation)
```

### Arguments

feature\_abd    feature-by-sample matrix of abundances (proportions or counts).  
n\_batch        number of batches in the data.  
design          design matrix.  
zero\_inflation zero inflation flag.

### Value

list of indicator matrices needed by fitting in adjust\_batch.

---

continuous_discover	<i>Unsupervised meta-analytical discovery and validation of continuous structures in microbial abundance data</i>
---------------------	---

---

## Description

continuous\_discover takes as input a feature-by-sample matrix of microbial abundances. It first performs unsupervised continuous structure discovery (PCA) within each batch. Loadings of top PCs from each batch are then mapped against each other to identify "consensus" loadings that are reproducible across batches with a network community discovery approach with **igraph**. The identified consensus loadings/scores can be viewed as continuous structures in microbial profiles that are recurrent across batches and valid in a meta-analytical sense. continuous\_discover returns, among other output, the identified consensus scores for continuous structures in the provided microbial abundance profiles, as well as the consensus PC loadings which can be used to assign continuous scores to any sample with the same set of microbial features.

## Usage

```
continuous_discover(feature_abd, batch, data, control)
```

## Arguments

feature_abd	feature-by-sample matrix of abundances (proportions or counts).
batch	name of the batch variable. This variable in data should be a factor variable and will be converted to so with a warning if otherwise.
data	data frame of metadata, columns must include batch.
control	a named list of additional control parameters. See details.

## Details

control should be provided as a named list of the following components (can be a subset).

**normalization** character. Similar to the normalization parameter in [Maaslin2](#) but only "TSS" and "NONE" are allowed. Default to "TSS" (total sum scaling).

**transform** character. Similar to the transform parameter in [Maaslin2](#) but only "AST" and "LOG" are allowed. Default to "AST" (arcsine square root transformation).

**pseudo\_count** numeric. Pseudo count to add feature\_abd before the transformation. Default to NULL, in which case pseudo count will be set automatically to 0 if transform="AST", and half of minimal non-zero values in feature\_abd if transform="LOG".

**var\_perc\_cutoff** numeric. A value between 0 and 1 that indicates the percentage variability explained to cut off at for selecting top PCs in each batch. Across batches, the top PCs that in total explain more than var\_perc\_cutoff of the total variability will be selected for meta-analytical continuous structure discovery. Default to 0.8 (PCs included need to explain at least 80 total variability).

**cos\_cutoff** numeric. A value between 0 and 1 that indicates cutoff for absolute cosine coefficients between PC loadings to construct the method's network with. Once the top PC loadings from each batch are selected, cosine coefficients between each loading pair are calculated which indicate their similarity. Loading pairs with absolute cosine coefficients surpassing cos\_cutoff are then considered as associated with each other, and represented as an edge between the

pair in a PC loading network. Network community discovery can then be performed on this network to identified densely connected "clusters" of PC loadings, which represent meta-analytically recurrent continuous structures.

**cluster\_function** function. `cluster_function` is used to perform community structure discovery in the constructed PC loading network. This can be any of the network cluster functions provided in **igraph**. Default to `cluster_optimal`. Note that this option can be slow for larger datasets, in which case `cluster_fast_greedy` is recommended.

**network\_plot** character. Name for the generated network figure file. Default to "clustered\_network.pdf". Can be set to NULL in which case no output will be generated.

**plot\_size\_cutoff** integer. Clusters with sizes smaller than or equal to `plot_size_cutoff` will be excluded in the visualized network. Default to 2 - visualized clusters must have at least three nodes (PC loadings).

**diagnostic\_plot** character. Name for the generated diagnostic figure file. Default to "continuous\_diagnostic.pdf". Can be set to NULL in which case no output will be generated.

**verbose** logical. Indicates whether or not verbose information will be printed.

## Value

a list, with the following components:

**consensus\_scores** matrix of identified consensus continuous scores. Columns are the identified consensus scores and rows correspond to samples in `feature_abd`.

**consensus\_loadings** matrix of identified consensus loadings. Columns are the identified consensus scores and rows correspond to features in `feature_abd`.

**mat\_vali** matrix of validation cosine coefficients of the identified consensus loadings. Columns correspond to the identified consensus scores and rows correspond to batches.

**network, communities, mat\_cos** components for the constructed PC loading network and community discovery results. `network` is a **igraph** graph object for the constructed network of associated PC loadings. `communities` is a `communities` object for the identified consensus loading clusters in `network` (output from `control$cluster_function`). `mat_cos` is the matrix of cosine coefficients between all selected top PCs from all batches.

**control** list of additional control parameters used in the function call.

## Author(s)

Siyuan Ma, <siyuanma@g.harvard.edu>

## Examples

```
data("CRC_abd", "CRC_meta")
fit_continuous <- continuous_discover(feature_abd = CRC_abd,
                                     batch = "studyID",
                                     data = CRC_meta)
```



CRC\_abd

*Species level feature abundance data of five public CRC studies***Description**

Species level relative abundance profiles of CRC and control patients in the five public studies used in Thomas et al. (2019). These were accessed through [curatedMetagenomicData](#).

**Usage**

```
data(CRC_abd)
```

**Format**

A feature-by-sample matrix of species-level profiles

**Source**

[curatedMetagenomicData](#)

**References**

Thomas, Andrew Maltez, Paolo Manghi, Francesco Asnicar, Edoardo Pasolli, Federica Armanini, Moreno Zolfo, Francesco Beghini et al. "Metagenomic analysis of colorectal cancer datasets identifies cross-cohort microbial diagnostic signatures and a link with choline degradation." *Nature medicine* 25, no. 4 (2019): 667.

**Examples**

```
data(CRC_abd)
# features included
rownames(CRC_abd)
# These are relative abundances
apply(CRC_abd, 2, sum)
# The following were used to generate the object
# library(curatedMetagenomicData)
# library(phyloseq)
# library(genefilter)
# datasets <- curatedMetagenomicData(
#   c("FengQ_2015.metaphlan_bugs_list.stool" ,
#     "HanniganGD_2017.metaphlan_bugs_list.stool",
#     "VogtmannE_2016.metaphlan_bugs_list.stool",
#     "YuJ_2015.metaphlan_bugs_list.stool",
#     "ZellerG_2014.metaphlan_bugs_list.stool"),
#   dryrun = FALSE)
# Construct phyloseq object from the five datasets
# physeq <-
#   # Aggregate the five studies into ExpressionSet
#   mergeData(datasets) %>%
#   # Convert to phyloseq object
#   ExpressionSet2phyloseq() %>%
#   # Subset samples to only CRC and controls
#   subset_samples(study_condition %in% c("CRC", "control")) %>%
#   # Subset features to species
```

```
# subset_taxa(!is.na(Species) & is.na(Strain)) %>%
# Normalize abundances to relative abundance scale
# transform_sample_counts(function(x) x / sum(x)) %>%
# Filter features to be of at least 1e-5 relative abundance in five
# samples
# filter_taxa(kOverA(5, 1e-5), prune = TRUE)
# CRC_abd <- otu_table(physeq)@.Data
```

---

CRC\_meta

*Sample metadata of five public CRC studies*

---

## Description

Metadata information of CRC and control patients in the five public studies used in Thomas et al. (2019). These were accessed through [curatedMetagenomicData](#).

## Usage

```
data(CRC_meta)
```

## Format

A data.frame of per-sample metadata information

## Source

[curatedMetagenomicData](#)

## References

Thomas, Andrew Maltez, Paolo Manghi, Francesco Asnicar, Edoardo Pasolli, Federica Armanini, Moreno Zolfo, Francesco Beghini et al. "Metagenomic analysis of colorectal cancer datasets identifies cross-cohort microbial diagnostic signatures and a link with choline degradation." *Nature medicine* 25, no. 4 (2019): 667.

## Examples

```
data(CRC_meta)
# has CRC and control samples across five studies
table(CRC_meta$studyID, CRC_meta$study_condition)
# The following were used to generate the object
# library(curatedMetagenomicData)
# library(phyloseq)
# library(genefilter)
# datasets <- curatedMetagenomicData(
#   c("FengQ_2015.metaphlan_bugs_list.stool" ,
#     "HanniganGD_2017.metaphlan_bugs_list.stool",
#     "VogtmannE_2016.metaphlan_bugs_list.stool",
#     "YuJ_2015.metaphlan_bugs_list.stool",
#     "ZellerG_2014.metaphlan_bugs_list.stool"),
#   dryrun = FALSE)
# Construct phyloseq object from the five datasets
# physeq <-
#   # Aggregate the five studies into ExpressionSet
```

```

# mergeData(datasets) %>%
#   Convert to phyloseq object
# ExpressionSet2phyloseq() %>%
#   Subset samples to only CRC and controls
# subset_samples(study_condition %in% c("CRC", "control")) %>%
#   Subset features to species
# subset_taxa(!is.na(Species) & is.na(Strain)) %>%
#   Normalize abundances to relative abundance scale
# transform_sample_counts(function(x) x / sum(x)) %>%
#   Filter features to be of at least 1e-5 relative abundance in five
#   samples
# filter_taxa(kOverA(5, 1e-5), prune = TRUE)
# CRC_meta <- data.frame(sample_data(physeq))
# CRC_meta$studyID <- factor(CRC_meta$studyID)

```

---

create\_table\_maaslin *Utility for generating empty Maaslin2 results table*

---

### Description

Utility for generating empty Maaslin2 results table

### Usage

```
create_table_maaslin(features, exposure, lvl_exposure)
```

### Arguments

features	name of the features fitted to Maaslin2.
exposure	the exposure variable.
lvl_exposure	levels of the exposure variable, if a factor.

### Value

a table for each feature-exposure value pair; reference level of exposure, if a factor, is taken out because is absorbed into the intercept term in Maaslin2 regression

---

diagnostic\_adjust\_batch

*Diagnostic visualization for adj\_batch function*

---

### Description

Diagnostic visualization for adj\_batch function

### Usage

```
diagnostic_adjust_batch(feature_abd, feature_abd_adj, var_batch, gamma_hat,
  gamma_star, output)
```

**Arguments**

feature_abd	original feature-by-sample matrix of abundances (proportions or counts).
feature_abd_adj	feature-by-sample matrix of batch-adjusted feature abundances, with covariate effects retained and scales consistent with original abundance matrix.
var_batch	the batch variable (should be a factor).
gamma_hat	estimated per feature-batch gamma parameters.
gamma_star	shrunk per feature-batch gamma parameters
output	output file name

**Value**

(invisibly) the ggplot2 plot object

---

diagnostic\_continuous\_discover

*Diagnostic visualization for continuous.discover function*

---

**Description**

Diagnostic visualization for continuous.discover function

**Usage**

```
diagnostic_continuous_discover(mat_vali, lvl_batch, cos_cutoff, output)
```

**Arguments**

mat_vali	matrix of maximum correlations between the cluster-specific consensus loadings and top PC loadings from each batch
lvl_batch	unique batches in the data
cos_cutoff	the specified cosine coefficient cutoff
output	output file name

**Value**

the invisible ggplot2 plot object

---

 diagnostic\_discrete\_discover

*Diagnostic visualization for discrete.discover function*


---

### Description

Diagnostic visualization for discrete.discover function

### Usage

```
diagnostic_discrete_discover(stats_internal, stats_external, lvl_batch,
  output)
```

### Arguments

stats\_internal list of internal evaluation summary statistics  
 stats\_external list of external validation summary statistics  
 lvl\_batch unique batches in the data  
 output

### Value

the invisible ggplot2 plot object

---

 discrete\_discover

*Unsupervised meta-analytical discovery and validation of discrete clustering structures in microbial abundance data*


---

### Description

discrete\_discover takes as input sample-by-sample dissimilarity measurements (generated from microbial abundance profiles), and performs unsupervised clustering within each batch across a range of cluster numbers. It then evaluates the support for each cluster number with both internal (i.e., samples within the batch) and external (i.e., samples in other batches) data. Internal evaluation is realized with [prediction.strength](#) and external evaluation is based on a generalized version of the same method. discrete\_discover generates as output the evaluation statistics for each cluster number. A cluster number with good support from both internal and external evaluations provides meta-analytical evidence for discrete structures in the microbial abundance profiles.

### Usage

```
discrete_discover(D, batch, data, control)
```

### Arguments

D sample-by-sample dissimilarity measurements. Should be provided as a [dist](#) object.  
 batch name of the batch variable. This variable in data should be a factor variable and will be converted to so with a warning if otherwise.  
 data data frame of metadata, columns must include batch.  
 control a named list of additional control parameters. See details.

## Details

`control` should be provided as a named list of the following components (can be a subset).

**k\_max** integer. Maximum number of clusters to evaluate. `discrete_discover` will evaluate clustering structures corresponding to cluster numbers ranging from 2 to `k_max`. Default to 10.

**cluster\_function** an interface function. This function will be used for unsupervised clustering for discrete structure evaluation. This corresponds to the `clustermethod` parameter in `prediction.strength`, and similarly, should also follow the specifications as detailed in `clusterboot`. Default to `claraCBI`

**classify\_method** character. Classification method used to assign observations in the method's internal and external evaluation stage. Corresponds to the `classification` parameter in `prediction.strength`, and can only be either "centroid" or "knn". Default to "centroid".

**M** integer. Number of random iterations to partition the batch during method's internal evaluation. Corresponds to the `M` parameter in `prediction.strength`. Default to 30.

**nnk** integer. Number of nearest neighbors if `classify_method="knn"`. Corresponds to the `nnk` parameter in `prediction.strength`. Default to 1.

**diagnostic\_plot** character. Name for the generated diagnostic figure file. Default to "discrete\_diagnostic.pdf". Can be set to NULL in which case no output will be generated.

**verbose** logical. Indicates whether or not verbose information will be printed.

## Value

a list, with the following components:

**internal\_mean, internal\_se** matrices of internal clustering structure evaluation measurements (prediction strengths). Columns and rows corresponds to different batches and different numbers of clusters, respectively. `internal_mean` and `internal_se`, as the names suggest, are the mean and standard error of prediction strengths for each batch/cluster number.

**external\_mean, external\_se** same structure as `internal_mean` and `internal_se`, but records external clustering structure evaluation measurements (generalized prediction strength).

**control** list of additional control parameters used in the function call.

## Author(s)

Siyuan Ma, <siyuanma@h.harvard.edu>

## Examples

```
data("CRC_abd", "CRC_meta")
# Calculate Bray-Curtis dissimilarity between the samples
library(vegan)
D <- vegdist(t(CRC_abd))
fit_discrete <- discrete_discover(D = D,
                                batch = "studyID",
                                data = CRC_meta)
```

---

fill_dimnames	<i>Fill in artificial row/column names to a matrix or data frame, if they are missing</i>
---------------	---

---

**Description**

Fill in artificial row/column names to a matrix or data frame, if they are missing

**Usage**

```
fill_dimnames(x, row_prefix, col_prefix)
```

**Arguments**

x	matrix or data frame
row_prefix	prefix for the artificial row names
col_prefix	prefix for the artificial column names

**Value**

x but with the missing dimension names filled in

---

fit_EB	<i>Parametric estimation of per-batch location and scale parameters, and Empirical Bayes estimation of their priors</i>
--------	---

---

**Description**

Parametric estimation of per-batch location and scale parameters, and Empirical Bayes estimation of their priors

**Usage**

```
fit_EB(s_data, l_stand_feature, batchmod, n_batch, l_ind)
```

**Arguments**

s_data	feature-by-sample matrix of standardized abundances.
l_stand_feature	list of per-feature standardization fits, as returned by fit_stand_feature.
batchmod	design matrix for batch variables.
n_batch	number of batches in the data.
l_ind	list of indicator matrices, as returned by construct_ind.

**Value**

list of parameter estimations.

---

fit_shrink	<i>A posteriori shrink per-batch location and scale parameters towards their EB priors</i>
------------	--

---

**Description**

A posteriori shrink per-batch location and scale parameters towards their EB priors

**Usage**

```
fit_shrink(s_data, l_params, batchmod, n_batch, l_ind, control)
```

**Arguments**

s_data	feature-by-sample matrix of standardized abundances.
l_params	list of parameter fits, as returned by fit_EB.
batchmod	design matrix for batch variables.
n_batch	number of batches in the data.
l_ind	list of indicator matrices, as returned by construct_ind.
control	list of control parameters (passed on to it_sol)

**Value**

list of shrunked per-batch location and scale parameters.

---

fit_stand_feature	<i>Fit lm and standardize all features</i>
-------------------	--

---

**Description**

Fit lm and standardize all features

**Usage**

```
fit_stand_feature(s_data, design, l_ind)
```

**Arguments**

s_data	feature-by-sample matrix of abundances (proportions or counts).
design	design matrix.
l_ind	list of indicator matrices, as returned by construct_ind.

**Value**

list of two componet: the standardized feature abundance matrix, and a list of per-feature standardization fits.



---

it_sol	<i>Iteratively solve for one feature's shrunked location and scale parameters</i>
--------	---

---

### Description

Iteratively solve for one feature's shrunked location and scale parameters

### Usage

```
it_sol(s_data, g_hat, d_hat, g_bar, t2, a, b, control)
```

### Arguments

s_data	the feature's standardized abundances.
g_hat	the feature's location parameter frequentist estimations.
d_hat	the feature's scale parameter frequentist estimations.
g_bar	EB estimation of location hyper parameters.
t2	EB estimation of location hyper parameters.
a	EB estimation of scale hyper parameters.
b	EB estimation of scale hyper parameters.
control	list of control parameters

### Value

matrix of shrunked location and scale parameters.

---

lm_meta	<i>Covariate adjusted meta-analytical differential abundance testing</i>
---------	--

---

### Description

lm\_meta runs differential abundance models on microbial profiles within individual studies/batches, and aggregates per-batch effect sizes with a meta-analysis fixed/random effects model. It takes as input a feature-by-sample microbial abundance table and the accompanying meta data data frame which should includes the batch indicator variable, the main exposure variable for differential abundance testing, and optional covariates and random covariates. The function first runs [Maaslin2](#) models on the exposure with optional covariates/random covariates in each batch. The per-batch effect sizes are then aggregated with [rma.uni](#) and reported as output. Additional parameters, including those for both [Maaslin2](#) and [rma.uni](#) can be provided through control (see details).

**Usage**

```
lm_meta(
  feature_abd,
  batch,
  exposure,
  covariates = NULL,
  covariates_random = NULL,
  data,
  control
)
```

**Arguments**

<code>feature_abd</code>	feature-by-sample matrix of abundances (proportions or counts).
<code>batch</code>	name of the batch variable. This variable in data should be a factor variable and will be converted to so with a warning if otherwise.
<code>exposure</code>	name of the exposure variable for differential abundance testing.
<code>covariates</code>	names of covariates to adjust for in Maaslin2 differential abundance testing models.
<code>covariates_random</code>	names of random effects grouping covariates to adjust for in Maaslin2 differential abundance testing models.
<code>data</code>	data frame of metadata, columns must include exposure, batch, and covariates and covariates_random (if specified).
<code>control</code>	a named list of additional control parameters. See details.

**Details**

`control` should be provided as a named list of the following components (can be a subset).

**normalization** character. normalization parameter for Maaslin2. See [Maaslin2](#) for details and allowed values. Default to "TSS" (total sum scaling).

**transform** character. transform parameter for Maaslin2. See [Maaslin2](#) for details and allowed values. Default to "AST" (arcsine square root transformation).

**analysis\_method** character. analysis\_method parameter for Maaslin2. See [Maaslin2](#) for details and allowed values. Default to "LM" (linear modeling).

**rma\_method** character. method parameter for rma.uni. See [rma.uni](#) for details and allowed values. Default to "REML" (restricted maximum-likelihood estimator).

**output** character. Output directory for intermediate Maaslin2 output and the optional forest plots. Default to "MMUPHIn\_lm\_meta".

**forest\_plot** character. Suffix in the name for the generated forest plots visualizing significant meta-analytical differential abundance effects. Default to "forest.pdf". Can be set to NULL in which case no output will be generated.

**rma\_conv** numeric. Convergence threshold for rma.uni (corresponds to `control$threshold`. See [rma.uni](#) for details. Default to 1e-4.

**rma\_maxit** integer. Maximum number of iterations allowed for rma.uni (corresponds to `control$maxiter`. See [rma.uni](#) for details. Default to 1000.

**verbose** logical. Indicates whether or not verbose information will be printed.

**Value**

a list, with the following components:

**meta\_fits** data frame of per-feature meta-analytical differential abundance results, including columns for effect sizes, p-values and q-values, heterogeneity statistics such as  $\tau^2$  and  $I^2$ , as well as weights for individual batches. Many of these statistics are explained in detail in [rma.uni](#).

**maaslin\_fits** list of data frames, each one corresponding to the fitted results of Maaslin2 in a individual batch. See [Maaslin2](#) on details of these output.

**control** list of additional control parameters used in the function call.

**Author(s)**

Siyuan Ma, <siyuanma@g.harvard.edu>

**Examples**

```
data("CRC_abd", "CRC_meta")
fit_meta <- lm_meta(feature_abd = CRC_abd,
  exposure = "study_condition",
  batch = "studyID",
  covariates = c("gender", "age"),
  data = CRC_meta)$meta_fits
```

---

LOG

*LOG transformation (modified from Maaslin2 and is different)*

---

**Description**

LOG transformation (modified from Maaslin2 and is different)

**Usage**

LOG(x)

**Arguments**

x                      vector of abundance to be transformed.

**Value**

transformed vector of abundance.

---

Maaslin2_wrapper	<i>Wrapper function for Maaslin2</i>
------------------	--------------------------------------

---

**Description**

Wrapper function for Maaslin2

**Usage**

```
Maaslin2_wrapper(feature_abd, data, exposure, covariates = NULL,
  covariates_random = NULL, output = tempdir(),
  normalization = "TSS", transform = "AST", analysis_method = "LM")
```

**Arguments**

feature_abd	feature*sample matrix of feature abundance.
data	data frame of metadata.
exposure	name of exposure variable.
covariates	name of covariates.
covariates_random	name of random covariates.
output	directory for Maaslin2.
normalization	normalization parameter for Maaslin2.
transform	transformation parameter for Maaslin2.
analysis_method	analysis method parameter for Maaslin2.

**Value**

a data frame recording per-feature coefficients, p-values, etc. from running Maaslin2.

---

match_control	<i>Match user-specified control parameters with default, and modify if needed</i>
---------------	---

---

**Description**

Match user-specified control parameters with default, and modify if needed

**Usage**

```
match_control(default, control)
```

**Arguments**

default	list of default control parameters
control	list of user-provided control parameters

**Value**

list of control parameters, set to user provided values if specified and default other wise

---

normalize_features	<i>Normalize feature abundance table (modified from Maaslin2)</i>
--------------------	---

---

**Description**

Normalize feature abundance table (modified from Maaslin2)

**Usage**

```
normalize_features(features, normalization = "NONE", pseudo_count = 0)
```

**Arguments**

features	feature-by-sample matrix of abundances (proportions or counts).
normalization	normalization method.
pseudo_count	pseudo count to be added to feature_abd.

**Value**

normalized abundance table.

---

relocate_scale	<i>Relocate and scale feature abundances to correct for batch effects, given shrinked per-batch location and scale parameters</i>
----------------	---

---

**Description**

Relocate and scale feature abundances to correct for batch effects, given shrinked per-batch location and scale parameters

**Usage**

```
relocate_scale(s_data, l_params_shrink, batchmod, n_batch, l_ind)
```

**Arguments**

s_data	feature-by-sample matrix of standardized abundances.
l_params_shrink	list of shrinked parameters, as returned by fit_shrink.
batchmod	design matrix for batch variables.
n_batch	number of batches in the data.
l_ind	list of indicator matrices, as returned by construct_ind.

**Value**

feature-by-sample matrix of batch-adjusted feature abundances (but without covariate effects).

---

rename_maaslin	<i>Utility for temporarily renaming samples/features for Maaslin2 run to bypass the rare cases where unconventional names can cause exceptions</i>
----------------	--

---

**Description**

Utility for temporarily renaming samples/features for Maaslin2 run to bypass the rare cases where unconventional names can cause exceptions

**Usage**

```
rename_maaslin(old_names, prefix)
```

**Arguments**

old_names	vector of names.
prefix	prefix for the replacement (new numbered names).

**Value**

vector of new names - numbered vector with same length as old names and with the specified prefix

---

rma_wrapper	<i>Wrapper for fitting fixed/random effects meta-analysis model using metafor</i>
-------------	---

---

**Description**

Wrapper for fitting fixed/random effects meta-analysis model using metafor

**Usage**

```
rma_wrapper(maaslin_fits, method = "REML", output = tempdir(),
  forest_plot = NULL, rma_conv = 1e-06, rma_maxit = 1000,
  verbose = TRUE)
```

**Arguments**

maaslin_fits	list of Maaslin2 result data frames, outputted from Maaslin2_wrapper.
method	meta-analysis model to run, options provided in metafor::rma.
output	directory for the output forest plots.
forest_plot	logical. should forest plots be generated for the significant associations.
rma_conv	rma threshold control.
rma_maxit	rma maximum iteration control.
verbose	should verbose information be printed.

**Value**

a data frame recording per-feature meta-analysis association results. (coefficients, p-values, etc.)

---

set_pseudo	<i>Set pseudo count for an abundance matrix. Pseudo count is currently set to half of minimum non-zero values</i>
------------	---

---

**Description**

Set pseudo count for an abundance matrix. Pseudo count is currently set to half of minimum non-zero values

**Usage**

```
set_pseudo(features)
```

**Arguments**

features            feature-by-sample matrix of abundances (proportions or counts).

**Value**

the pseudo count

---

shorten_name	<i>Utility for shorter names Useful when plotting per-feature figures where feature names could be cutoff</i>
--------------	---

---

**Description**

Utility for shorter names Useful when plotting per-feature figures where feature names could be cutoff

**Usage**

```
shorten_name(x, cutoff = 3, replacement = "..")
```

**Arguments**

x                    vector of names  
 cutoff              number of maximum string length before start cutting off the middle

**Value**

vector of new names with .. replacing the middle part if name is longer than cutoff

---

standardize_feature	<i>Centralize (by design matrix) and standardize (by pooled variance across all batches) feature abundances for empirical Bayes fit</i>
---------------------	---

---

**Description**

Centralize (by design matrix) and standardize (by pooled variance across all batches) feature abundances for empirical Bayes fit

**Usage**

```
standardize_feature(y, i_design, n_batch)
```

**Arguments**

y	vector of non-zero abundance of a single feature (if zero-inflated is true).
i_design	design matrix for the feature; samples with zeros are taken out (if zero-inflated is true).
n_batch	number of batches in the data.

**Value**

a list with component: y\_stand for vector of centralized and standardized feature abundance, and stand\_mean/varpooled for the location and scale factor (these are used later to back transform the batch-shrunked feature abundance).

---

transform_features	<i>Transform feature abundadnce table (modified from Maaslin2)</i>
--------------------	--

---

**Description**

Transform feature abundadnce table (modified from Maaslin2)

**Usage**

```
transform_features(features, transform = "NONE", pseudo_count = 0)
```

**Arguments**

features	feature-by-sample matrix of abundances (proportions or counts).
transform	transformation method.
pseudo_count	pseudo count to be added to feature_abd..

**Value**

transformed abundance table.



---

TSS	<i>TSS normalization (modified from Maaslin2)</i>
-----	---

---

**Description**

TSS normalization (modified from Maaslin2)

**Usage**

TSS(x)

**Arguments**

x                      vector of abundance to be normalized.

**Value**

normalized vector of abundance.

---

vaginal_abd	<i>Species level feature abundance data of two public vaginal studies</i>
-------------	---

---

**Description**

Species level relative abundance profiles of vaginal samples in the two public studies provided in [curatedMetagenomicData](#).

**Usage**

data(vaginal\_abd)

**Format**

A feature-by-sample matrix of species-level profiles

**Source**

[curatedMetagenomicData](#)

**References**

Pasolli, Edoardo, Lucas Schiffer, Paolo Manghi, Audrey Renson, Valerie Obenchain, Duy Tin Truong, Francesco Beghini et al. "Accessible, curated metagenomic data through ExperimentHub." Nature methods 14, no. 11 (2017): 1023.

**Examples**

```

data(vaginal_abd)
# features included
rownames(vaginal_abd)
# These are relative abundances
apply(vaginal_abd, 2, sum)
# The following were used to generate the object
# library(curatedMetagenomicData)
# library(phyloseq)
# datasets <- curatedMetagenomicData(
#   "*metaphlan_bugs_list.vagina*",
#   dryrun = FALSE)
# Construct phyloseq object from the five datasets
# physeq <-
#   # Aggregate the five studies into ExpressionSet
#   mergeData(datasets) %>%
#   # Convert to phyloseq object
#   ExpressionSet2phyloseq() %>%
#   # Subset features to species
#   subset_taxa(!is.na(Species) & is.na(Strain)) %>%
#   # Normalize abundances to relative abundance scale
#   transform_sample_counts(function(x) x / sum(x)) %>%
#   # Filter features to be of at least 1e-5 relative abundance in two samples
#   filter_taxa(kOverA(2, 1e-5), prune = TRUE)
# vaginal_abd <- otu_table(physeq)@.Data

```

---

vaginal\_meta

*Sample metadata of two public vaginal studies*


---

**Description**

Metadata information of vaginal samples in the two public studies provided in [curatedMetagenomicData](#).

**Usage**

```
data(vaginal_meta)
```

**Format**

A data.frame of per-sample metadata information

**Source**

[curatedMetagenomicData](#)

**References**

Pasolli, Edoardo, Lucas Schiffer, Paolo Manghi, Audrey Renson, Valerie Obenchain, Duy Tin Truong, Francesco Beghini et al. "Accessible, curated metagenomic data through ExperimentHub." Nature methods 14, no. 11 (2017): 1023.

**Examples**

```

data(vaginal_meta)
# has vaginal samples across two studies
table(vaginal_meta$studyID, vaginal_meta$body_site)
# The following were used to generate the object
# library(curatedMetagenomicData)
# library(phyloseq)
# datasets <- curatedMetagenomicData(
#   "*metaphlan_bugs_list.vagina*",
#   dryrun = FALSE)
# Construct phyloseq object from the five datasets
# physeq <-
#   # Aggregate the five studies into ExpressionSet
#   mergeData(datasets) %>%
#   # Convert to phyloseq object
#   ExpressionSet2phyloseq() %>%
#   # Subset features to species
#   subset_taxa(!is.na(Species) & is.na(Strain)) %>%
#   # Normalize abundances to relative abundance scale
#   transform_sample_counts(function(x) x / sum(x)) %>%
#   # Filter features to be of at least 1e-5 relative abundance in two samples
#   filter_taxa(kOverA(2, 1e-5), prune = TRUE)
# vaginal_meta <- data.frame(sample_data(physeq))
# vaginal_meta$studyID <- factor(vaginal_meta$studyID)

```

---

```
visualize_continuous_discover
```

*Visualization of the clustered network for the continuous.discover function*

---

**Description**

Visualization of the clustered network for the continuous.discover function

**Usage**

```
visualize_continuous_discover(graph_pc, membership_loading,
  size_communities, plot_size_cutoff, short_names, output)
```

**Arguments**

graph_pc	the full pc network constructed from correlated PCs
membership_loading	membership of PC loadings from community discovery
size_communities	ordered (largest to smallest) size of the identified communities
plot_size_cutoff	cluster size cutoff (for cluster to be included in the visualized PC network)
short_names	shorter names of the loadings
output	output file name

**Value**

an invisible list of the subsetting network and memberships (to reproduce the plot)

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