Package 'LDM'

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Description The LDM package provides a single analysis path that includes distance-based ordination, global tests of any effect of the microbiome, and tests of the effects of individual OTUs (i.e., operational taxonomic units) with false discovery rate (FDR)-based correction for multiple testing. It accommodates both continuous and discrete variables (e.g., clinical outcomes, environmental factors, treatment groups) as well as interaction terms to be tested either singly or in combination, allows for adjustment of confounding covariates, and uses permutation-based p-values that can control for correlation (e.g., repeated measurements on the same individual). It can also be applied to transformed data, and an `omnibus' test can easily combine results from analyses conducted on different transformation scales
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```
adjust.data.by.covariates
```

Adjust data by covariates

Description

This function produces adjusted distance matrix and otu table (if provided) after removing the effects of covariates (e.g., confounders). Observations with any missing data are removed.

Usage

```
adjust.data.by.covariates(formula = NULL, data = NULL,
  otu.table = NULL, tree = NULL, dist.method = "bray", dist = NULL,
  square.dist = TRUE, center.dist = TRUE, scale.otu.table = TRUE,
  center.otu.table = TRUE, freq.scale.only = FALSE)
```

Arguments

formu	la a	a symbolic	description	of the	covariate 1	model	with the	form	~ mod	del,	where
-------	------	------------	-------------	--------	-------------	-------	----------	------	-------	------	-------

model is specified in the same way as for lm or glm. For example, \sim a + b specifies a model with the main effects of covariates a and b, and \sim a*b, equivalently \sim a + b + a:b, specifies a model with the main effects of a and b as

well as their interaction.

data an optional data frame, list or environment (or object coercible by as.data.frame

to a data frame) containing the covariates. If not found in data, the covariates are taken from environment(formula), typically the environment from which

adjust.data.by.covariates is called. The default is NULL.

otu.table the n.obs by n.otu matrix of read counts. If provided, the adjusted (and column-

centered) otu.table at both the frequency scale and arcsin-root-transformed frequency scale are outputted. If provided, it is also used for calculating the distance matrix unless the distance matrix is directly imported through dist. The

default is NULL.

tree a phylogenetic tree. Only used for calculating a phylogenetic-tree-based dis-

tance matrix. Not needed if the calculation of requested distance does not require a phylogenetic tree, or if the distance matrix is directly imported through

dist. The default is NULL.

dist.method method for calculating the distance measure, partial match to all methods sup-

ported by vegdist in the vegan package (i.e., "manhattan", "euclidean", "canberra", "bray", "kulczynski", "jaccard", "gower", "altGower", "morisita", "horn", "mountford", "raup" , "binomial", "chao", "cao", "mahalanobis") as well as "hellinger" and "wt-unifrac". The default is "bray". For more details, see the

dist.method argument in the 1dm function.

dist a distance matrix. It can be the original distance matrix and then squared/centered

by the default square.dist=TRUE/center.dist=TRUE; it can also be squared/centered a priori and used with square.dist=FALSE/center.dist=FALSE. If not provided, the distance matrix is calculated using otu.table, tree, and dist.method.

The default is NULL.

square.dist a logical variable indicating whether to square the distance matrix. The default

is TRUE.

center.dist a logical variable indicating whether to center the distance matrix as described by Gower (1966). The default is TRUE.

scale.otu.table

a logical variable indicating whether to scale (i.e., divide) the rows of the OTU table by the library size, so the read counts becomes relative frequencies. The default is TRUE.

center.otu.table

a logical variable indicating whether to center the columns of the OTU table. The OTU table should be centered if the distance matrix has been centered. The default is TRUE.

freq.scale.only

a logical variable indicating whether to provide adjusted frequency-scale data matrix only (not adjusted data on the arcsin-root transformed frequency scale). The default is FALSE.

Value

a list consisting of

adj.dist the (squared/centered) distance matrix after adjustment of covariates.

x.freq the (column-centered) frequency-scale data matrix after adjustment of covari-

ates.

x.tran the (column-centered) arcsin-root-transformed data matrix after adjustment of

covariates.

Author(s)

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Examples

1dm

Testing hypotheses using a linear decomposition model (LDM)

Description

This function allows you to simultaneously test the global association with the overall microbiome composition and individual OTU associations to give coherent results. It is capable of handling complex design features such as confounders, interactions, and clustered data.

Usage

```
ldm(formula, data = .GlobalEnv, tree = NULL, dist.method = "bray",
 dist = NULL, cluster.id = NULL, strata = NULL, how = NULL,
 perm.within.type = "free", perm.between.type = "none",
 perm.within.ncol = 0, perm.within.nrow = 0, n.perm.max = NULL,
 n.rej.stop = 20, seed = NULL, test.global = TRUE,
 test.otu = TRUE, fdr.nominal = 0.1, square.dist = TRUE,
 center.dist = TRUE, scale.otu.table = TRUE,
 center.otu.table = TRUE, freq.scale.only = FALSE)
```

Arguments

formula a symbolic description of the model to be fitted. The details of model specifica-

tion are given under "Details".

data an optional data frame, list or environment (or object coercible by as.data.frame

> to a data frame) containing the covariates of interest and confounding covariates. If not found in data, the covariates are taken from environment(formula), typically the environment from which 1dm is called. The default is .GlobalEnv.

a phylogenetic tree. Only used for calculating a phylogenetic-tree-based distree tance matrix. Not needed if the calculation of the requested distance does not

involve a phylogenetic tree, or if the distance matrix is directly imported through

dist.

dist.method method for calculating the distance measure, partial match to all methods sup-

> ported by vegdist in the vegan package (i.e., "manhattan", "euclidean", "canberra", "bray", "kulczynski", "jaccard", "gower", "altGower", "morisita", "horn", "mountford", "raup", "binomial", "chao", "cao", "mahalanobis") as well as "hellinger" and "wt-unifrac". Note that the option binary is set to FALSE (i.e., not allowing calculation of presence-absence distances) when we internally call

vegdist for its supported distances. The Hellinger distance measure (dist.method="hellinger")

takes the form 0.5*E, where E is the Euclidean distance between the squareroot-transformed frequency data. The weighted UniFrac distance (dist.method="wt-unifrac")

is calculated by interally calling GUniFrac in the GUniFrac package. Not used when anything other than dist=NULL is specified for dist. The default is "bray".

a distance matrix. It can be the original distance matrix and then squared/centered by the default square.dist=TRUE/center.dist=TRUE; it can also be squared/centered

a priori and used with square.dist=FALSE/center.dist=FALSE. If not specified, the distance matrix is calculated using otu. table, tree, and dist.method.

The default is NULL.

cluster.id character or factor variable that identifies clusters. The default value cluster.id=NULL

if the observations are not clustered (i.e., are independent).

dist

strata a character or factor variable that defines strata (groups), within which to con-

strain permutations. The default is NULL.

how a permutation control list, for users who want to specify their own call to the

how function from the permute package. The default is NULL.

perm.within.type

a character string that takes values "free", "none", "series", or "grid". The default is "free" (for random permutations).

perm.between.type

a character string that takes values "free", "none", or "series". The default is "none"

perm.within.ncol

a positive integer, only used if perm.within.type="grid". The default is 0. See documentation for permute package for additional details

perm.within.nrow

a positive integer, only used if perm.within.type="grid". The default is 0. See documentation for permute package for additional details

n.perm.max the maximum number of permutations. The default is NULL, in which case

a maximum of 5000 permutations are used for the global test and a maximum of n.otu * n.rej.stop * (1/fdr.nominal) are used for the OTU test, where n.otu is the number of OTUs. If a numeric value for n.otu is specified, this

value is used for both global and OTU-level tests.

n.rej.stop the minimum number of rejections (i.e., the permutation statistic exceeds the

observed statistic) to obtain before stopping. The default is 20.

seed an integer seed for the random number generator in the permutation procedure.

The default is NULL; with the default value, an integer seed will be generated internally and randomly. In either case, the integer seed will be stored in the

output object in case the user wants to reproduce the permutations.

test.global a logical value indicating whether to perform the global test. The default is

TRUE.

test.otu a logical value indicating whether to perform the OTU-specific tests. The default

is TRUE.

fdr.nominal the nominal FDR value. The default is 0.1.

square.dist a logical variable indicating whether to square the distance matrix. The default

is TRUE.

center.dist a logical variable indicating whether to center the distance matrix as described

by Gower (1966). The default is TRUE.

scale.otu.table

a logical variable indicating whether to scale the rows of the OTU table for the freq scale. For count data, this corresponds to dividing by the library size to give relative frequencies. Does not affect the tran scale. The default is TRUE.

center.otu.table

a logical variable indicating whether to center the columns of the OTU table. The OTU table should be centered if the distance matrix has been centered. The default is TRUE.

freq.scale.only

a logical variable indicating whether to perform analysis of the frequency-scale data only (not the arcsin-root transformed frequency data and the omnibus test). The default is FALSE.

Details

The formula has the form

```
otu.table ~ (first set of covariates) + (second set of covariates) ... + (last set of covariates)
or
otu.table | confounders ~ (first set of covariates) + (second set of covariates)
... + (last set of covariates)
```

where otu. table is the OTU table with rows for samples and columns for OTUs and each set of covariates are enclosed in parentheses. The covariates in each submodel (set of covariates) are tested jointly, after projecting off terms in submodels that appear earlier in the model.

For example, given an OTU table y and a data frame metadata that contains 4 covariates, a, b, c and d, some valid formulas would be:

```
y ~ a + b + c + d ### no confounders, 4 submodels (i.e., sets of covariates)
y ~ (a+b) + (c+d) ### no confounders, 2 submodels each having 2 covariates;
y | b ~ (a+c) + d ### b is a confounder, submodel 1 is (a+c), and submodel 2 is d
y | b+c ~ a*d ### there are 2 confounders b and c; there is 1 submodel consisting of the three
```

terms a, d, and a:d (interaction). This example is equivalent to y | b+c ~ (a+d+a:d). y | as.factor(b) ~ (a+d) + a:d ### now confounder b will be treated as a factor variable,

submodel 1 will have the main effects a and d, and submodel 2 will have only the interaction between a and d

y | as.factor(b) \sim (a) + (d) + (a:d) ### there are 3 submodels a, d, and a:d. Putting paratheses around a single variable is allowed but not necessary.

Submodels that combine character and numeric values are allowed; character-valued variables are coerced into factor variables.

LDM uses two sequential stopping criteria. For the global test, LDM uses the stopping rule of Besag and Clifford (1991), which stops permutation when a pre-specified minimum number (default=20) of rejections (i.e., the permutation statistic exceeded the observed test statistic) has been reached. For the OTU-specific tests, LDM uses the stopping rule of Sandve et al. (2011), which stops permutation when every OTU test has either reached the pre-specified number (default=20) of rejections or yielded a q-value that is below the nominal FDR level (default=0.1). As a convention, we call a test "stopped" if the corresponding stopping criterion has been satisfied. Although all tests are always terminated if a pre-specified maximum number (see description of n.perm.max in Arguments list) of permutations have been generated, some tests may not have "stopped." This typically occurs when the relevant p-value is small; for global tests meeting the stopping criterion is not critical, but caution is advised when interpreting OTU-level tests that have not stopped as additional OTUs may be found with a larger number of permutations.

Value

a list consisting of

b	the matrix B as defined in Hu and Satten (2018)
dist	the (squared/centered) distance matrix
x.freq	the frequency-scale data matrix, scaled and centered if so specified
d.freq	a vector of the nonnegative diagonal elements of D that satisfies b^T $x.freq = D v^T$
v.freq	the v matrix with unit columns that satisfies $b^T x.freq = D v^T$
x.tran	the (column-centered) arcsin-root-transformed data matrix
d.tran	a vector of the nonnegative diagonal elements of D that satisfies b^T $x.tran = D v^T$

v.tran the v matrix with unit columns that satisfies b^T x.tran = D v^T a vector of lower indices for confounders (if there is any) and submodels low a vector of upper indices for confounders (if there is any) and submodels up VE.global.freq.confounders Variance explained (VE) by confounders, based on the frequency-scale data VE.global.freq.submodels VE by each submodel, based on the frequency-scale data VE.global.freq.residuals VE by each component in the residual distance, based on the frequency-scale data VE.otu.freq.confounders Contribution of each OTU to VE by confounders, based on the frequency-scale data VE.otu.freq.submodel Contribution of each OTU to VE by each submodel, based on the frequencyscale data VE.global.tran.confounders Variance explained (VE) by confounders, based on the arcsin-root-transformed frequency data VE.global.tran.submodels VE by each submodel, based on the arcsin-root-transformed frequency data VE.global.tran.residuals VE by each component in the residual distance, based on the arcsin-root-transformed frequency data VE.otu.tran.confounders Contribution of each OTU to VE by confounders, based on the arcsin-roottransformed frequency data VE.otu.tran.submodels Contribution of each OTU to VE by each submodel, based on the arcsin-roottransformed frequency data VE.df.confounders Degree of freedom (i.e., number of components) associated with the VE for confounders VE.df.submodels Degree of freedom (i.e., number of components) associated with the VE for each submodel F.global.freq F statistics for testing each submodel, based on the frequency-scale data F.global.tran F statistics for testing each submodel, based on the arcsin-root-transformed frequency data F.otu.freq F statistics for testing each OTU for each submodel, based on the frequencyscale data F statistics for testing each OTU for each submodel, based on the arcsin-root-F.otu.tran

p-values for the global test of each set of covariates based on the frequency-scale

p-values for the global test of each set of covariates based on the arcsin-root-

transformed data

transformed frequency data

data

p.global.freq

p.global.tran

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p.global.omni	p-values for the global test of each set of covariates based on the omnibus statistics, which are the minima of the p-values obtained from the frequency scale and the arcsin-root-transformed frequency data as the final test statistics, and use the corresponding minima from the permuted data to simulate the null distributions					
p.otu.freq	p-values for the OTU-specific tests based on the frequency scale data					
p.otu.tran	p-values for the OTU-specific tests based on the arcsin-root-transformed frequency data					
p.otu.omni	p-values for the OTU-specific tests based on the omnibus statistics					
q.otu.freq	q-values (i.e., FDR-adjusted p-values) for the OTU-specific tests based on the frequency scale data					
q.otu.tran	q-values for the OTU-specific tests based on the arcsin-root-transformed frequency data					
q.otu.omni	q-values for the OTU-specific tests based on the omnibus statistics					
n.perm.completed						
	number of permutations completed					
global.tests.stopped						
	a logical value indicating whether the stopping criterion has been met by all global tests					
otu.tests.stopped						
	a logical value indicating whether the stopping criterion has been met by all OTU-specific tests					
seed	a single-value integer seed that is user supplied or internally generated					

Author(s)

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References

Hu YJ, Satten GA. (2019) Testing hypotheses about microbiome using the linear decomposition model. bioRXiv:doi.org/10.1101/229831.

Examples

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permanovaFL

PERMANOVA test of association

Description

This function performs the PERMANOVA test that can allow adjustment of confounders and control of clustered data. As in ldm, permanovaFL allows multiple sets of covariates to be tested, in the way that the sets are entered sequentially and the variance explained by each set is that part that remains after the previous sets have been fit.

Usage

```
permanovaFL(formula, data = .GlobalEnv, tree = NULL,
   dist.method = "bray", cluster.id = NULL, strata = NULL,
   how = NULL, perm.within.type = "free", perm.between.type = "none",
   perm.within.ncol = 0, perm.within.nrow = 0, n.perm.max = 5000,
   n.rej.stop = 20, seed = NULL, square.dist = TRUE,
   center.dist = TRUE, scale.otu.table = TRUE)
```

Arguments

formula

a symbolic description of the model to be fitted in the form of data.matrix ~ sets of covariates or data.matrix | confounders ~ sets of covariates. The details of model specification are given in "Details" of ldm. The only difference between the model here and that for ldm is that, the data.matrix here is either an OTU table or a distance matrix. If it is an OTU table, the distance matrix will be calculated internally using the OTU table, tree, and dist.method. If it is a distance matrix, it can be the original distance matrix and then squared/centered by the default square.dist=TRUE/center.dist=TRUE; it can also be squared/centered a priori and used with square.dist=FALSE/center.dist=FALSE.

data

an optional data frame, list or environment (or object coercible by as.data.frame to a data frame) containing the covariates of interest and confounding covariates. If not found in data, the covariates are taken from environment(formula), typically the environment from which permanovaFL is called. The default is .GlobalEnv.

tree

a phylogenetic tree. Used for calculating a phylogenetic-tree-based distance matrix. Not needed if the calculation of the requested distance does not involve a phylogenetic tree, or if a distance matrix is directly imported through formula. method for calculating the distance measure, partial match to all methods sup-

dist.method

ported by vegdist in the vegan package (i.e., "manhattan", "euclidean", "canberra", "bray", "kulczynski", "jaccard", "gower", "altGower", "morisita", "horn",

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"mountford", "raup", "binomial", "chao", "cao", "mahalanobis") as well as "hellinger" and "wt-unifrac". Not used if a distance matrix is specified in formula. The default is "bray". For more details, see the dist.method argument in the ldm function.

cluster.id cluster identifiers. The default is value of NULL should be used if the observa-

tions are not in clusters (i.e., independent).

strata a factor variable (or, character variable converted into a factor) to define strata

(groups), within which to constrain permutations. The default is NULL.

how a permutation control list, for users who want to specify their permutation con-

trol list using the how function from the permute R package. The default is

NULL.

perm.within.type

a character string that takes values "free", "none", "series", or "grid". The default is "free" (for random permutations).

perm.between.type

a character string that takes values "free", "none", or "series". The default is "none"

perm.within.ncol

a positive integer, only used if perm.within.type="grid". The default is 0. See the documentation for the R package permute for further details.

perm.within.nrow

a positive integer, only used if perm.within.type="grid". The default is 0. See the documentation for the R package permute for further details.

n.perm.max the maximum number of permutations. The default is 5000.

n.rej.stop the minimum number of rejections (i.e., the permutation statistic exceeds the

observed statistic) to obtain before stopping. The default is 20.

seed an integer seed for the random number generator in the permutation procedure.

The default is NULL; with the default value, an integer seed will be generated internally and randomly. In either case, the integer seed will be stored in the

output object.

square.dist a logical variable indicating whether to square the distance matrix. The default

is TRUE.

center.dist a logical variable indicating whether to center the distance matrix as described

by Gower (1966). The default is TRUE.

scale.otu.table

a logical variable indicating, for dist.method that are supported by vegdist in the vegan package, whether to scale the OTU table into frequency data before calling vegdist. The default is TRUE.

Value

a list consisting of

F. statistics F statistics for the global test of each set of covariates

p. permanova p-values for the global test of each set of covariates

n.perm.completed

number of permutations completed

permanova.stopped

a logical value indicating whether the stopping criterion has been met by all

global tests

seed a single-value integer seed that is user supplied or internally generated

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Examples

```
\label{lem:continuous} res.permanova <- permanovaFL (formula=throat.otu.tab | (Sex+AntibioticUse) ~ SmokingStatus+PackYears, \\ data=throat.meta, dist.method="bray", seed=123)
```

throat.meta

Meta data of the throat microbiome samples

Description

This data set includes samples from the microbiome of the nasopharynx and oropharynx on each side of the body. It were generated to study the effect of smoking on the microbiota of the upper respiratory tract in 60 individuals, 28 smokers and 32 nonsmokers.

Usage

```
data("throat.meta")
```

Format

A data frame with 60 observations on 16 variables.

Source

Charlson ES, Chen J, Custers-Allen R, Bittinger K, Li H, et al. (2010) Disordered Microbial Communities in the Upper Respiratory Tract of Cigarette Smokers. PLoS ONE 5(12): e15216.

References

R package "GUniFrac"

Examples

```
data(throat.meta)
```

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throat.otu.tab OTU count table from 16S sequencing of the throat microbic ples	ome sam-
--	----------

Description

This data set contains 60 subjects with 28 smokers and 32 nonsmokers. Microbiome data were collected from right and left nasopharynx and oropharynx region to form an OTU table with 856 OTUs.

Usage

```
data("throat.otu.tab")
```

Format

A data frame with 60 observations on 856 variables.

Source

Charlson ES, Chen J, Custers-Allen R, Bittinger K, Li H, et al. (2010) Disordered Microbial Communities in the Upper Respiratory Tract of Cigarette Smokers. PLoS ONE 5(12): e15216.

References

R package "GUniFrac"

Examples

```
data(throat.otu.tab)
```

throat.tree

UPGMA tree of the OTUs from 16S sequencing of the throat microbiome samples

Description

The OTU tree is constructed using UPGMA on the K80 distance matrix of the OTUs. It is a rooted tree of class "phylo".

Usage

```
data("throat.tree")
```

Format

List of 4 data frames.

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Source

Charlson ES, Chen J, Custers-Allen R, Bittinger K, Li H, et al. (2010) Disordered Microbial Communities in the Upper Respiratory Tract of Cigarette Smokers. PLoS ONE 5(12): e15216.

References

R package "GUniFrac"

Examples

data(throat.tree)

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