

# Package ‘IFAA’

January 5, 2022

**Title** Robust Inference for Absolute Abundance in Microbiome Analysis

**Version** 1.0.4

**Description** A novel approach to make inference on the association of covariates with the absolute abundance (AA) of 'microbiome' in an ecosystem. It can be also directly applied to relative abundance (RA) data to make inference on AA (even if AA data is not available) because the ratio of two RA is equal ratio of their AA. This algorithm can estimate and test the associations of interest while adjusting for potential 'confounders'. The estimates of this method have easy interpretation like a typical regression analysis. High-dimensional covariates are handled with regularization and it is implemented by parallel computing. This algorithm finds optimal reference 'taxa/OTU (Operational Taxonomic Unit)/ASV (Amplicon Sequence Barient)' and uses permutation to control FDR (False Discovery Rate).

**License** GNU General Public License version 2

**Encoding** UTF-8

**URL** <https://github.com/gitlzg/IFAA>,  
<https://arxiv.org/abs/1909.10101v3>,  
<https://link.springer.com/article/10.1007/s12561-018-9219-2>

**LazyData** true

**RoxygenNote** 7.1.2

**Depends** R (>= 3.6.0),

**Imports** qtlMatrix (>= 0.9.7), mathjaxr (>= 1.0-1), methods (>= 3.3.0),  
picasso (>= 1.2.0), expm (>= 0.999-3), foreach (>= 1.4.3),  
rlecuyer (>= 0.3-3), Matrix (>= 1.4-0), HDCI (>= 1.0-2),  
parallel (>= 3.3.0), doParallel (>= 1.0.11), future (>= 1.12.0), glmnet, stats

**RdMacros** mathjaxr

**Suggests** knitr, rmarkdown

**VignetteBuilder** knitr

**NeedsCompilation** no

**Author** Quran Wu [aut],  
Zhigang Li [aut, cre]

**Maintainer** Zhigang Li <zhigang.li@ufl.edu>

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dataC	<i>Sample covariates data</i>
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Description

A dataset ontains 5 covariates.

Usage

dataC

Format

A data frame with 20 rows and 60 variables:

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dataM	<i>Sample microbiome data</i>
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Description

A dataset contains 60 taxa with absolute abundances and these are gut microbiome.

Usage

dataM

Format

A data frame with 20 rows and 60 variables:

IFAA

*Robust association identification and inference for absolute abundance in microbiome analyses***Description**

Make inference on the association of covariates of microbiome

**Usage**

```
IFAA(
  MicrobData,
  CovData,
  linkIDname,
  testCov = NULL,
  ctrlCov = NULL,
  testMany = TRUE,
  ctrlMany = FALSE,
  nRef = 40,
  nRefMaxForEsti = 2,
  refTaxa = NULL,
  adjust_method = "BY",
  fdrRate = 0.25,
  paraJobs = NULL,
  bootB = 500,
  standardize = FALSE,
  sequentialRun = FALSE,
  refReadsThresh = 0.2,
  taxkeepThresh = 0,
  SDThresh = 0.05,
  SDquantilThresh = 0,
  balanceCut = 0.2,
  seed = 1
)
```

**Arguments**

MicrobData	Microbiome data matrix containing microbiome abundance with each row per sample and each column per taxon/OTU/ASV. It should contain an "id" variable to correspond to the "id" variable in the covariates data: CovData. This argument can take directory path. For example, MicrobData="C://...//microbiomeData.tsv".
CovData	Covariates data matrix containing covariates and confounders with each row per sample and each column per variable. It should also contain an "id" variable to correspond to the "id" variable in the microbiome data: MicrobData. This argument can take directory path. For example, CovData = "C://...//covariatesData.tsv".
linkIDname	Variable name of the "id" variable in both MicrobData and CovData. The two data sets will be merged by this "id" variable.
testCov	Covariates that are of primary interest for testing and estimating the associations. It corresponds to $X_i$ in the equation. Default is NULL which means all covariates are testCov.

<code>ctrlCov</code>	Potential confounders that will be adjusted in the model. It corresponds to $\$W_i\$$ in the equation. Default is NULL which means all covariates except those in <code>testCov</code> are adjusted as confounders.
<code>testMany</code>	This takes logical value TRUE or FALSE. If TRUE, the <code>testCov</code> will contain all the variables in <code>CovData</code> provided <code>testCov</code> is set to be NULL. The default value is TRUE which does not do anything if <code>testCov</code> is not NULL.
<code>ctrlMany</code>	This takes logical value TRUE or FALSE. If TRUE, all variables except <code>testCov</code> are considered as control covariates provided <code>ctrlCov</code> is set to be NULL. The default value is FALSE.
<code>nRef</code>	The number of randomly picked reference taxa used in phase 1. Default number is 40.
<code>nRefMaxForEsti</code>	The maximum number of reference taxa used in phase 2. The default is 1.
<code>refTaxa</code>	A vector of taxa or OTU or ASV names. These are reference taxa specified by the user to be used in phase 1. If the number of reference taxa is less than ' <code>nRef</code> ', the algorithm will randomly pick extra reference taxa to make up ' <code>nRef</code> '. The default is NULL since the algorithm will pick reference taxa randomly.
<code>adjust_method</code>	The adjusting method used for p value adjustment. Same as <code>p.adjust</code> function in R.
<code>fdrRate</code>	The false discovery rate for identifying taxa/OTU/ASV associated with <code>testCov</code> . Default is 0.25.
<code>paraJobs</code>	If <code>sequentialRun</code> is FALSE, this specifies the number of parallel jobs that will be registered to run the algorithm. If specified as NULL, it will automatically detect the cores to decide the number of parallel jobs. Default is NULL. It is safe to have 4gb memory per job. It may be needed to reduce the number of jobs if memory is limited.
<code>bootB</code>	Number of bootstrap samples for obtaining confidence interval of estimates in phase 2. The default is 500.
<code>standardize</code>	This takes a logical value TRUE or FALSE. If TRUE, all design matrix X in phase 1 and phase 2 will be standardized in the analyses. Default is FALSE.
<code>sequentialRun</code>	This takes a logical value TRUE or FALSE. Default is FALSE. This argument could be useful for debug.
<code>refReadsThresh</code>	The threshold of non-zero sequencing reads for choosing the reference taxon in phase 2. The default is 0.2 which means at least 20% non-zero sequencing reads.
<code>taxkeepThresh</code>	The threshold of number of non-zero sequencing reads for each taxon to be included into the analysis.
<code>SDThresh</code>	The threshold of standard deviations of sequencing reads for choosing the reference taxon in phase 2. The default is 0.5 which means the standard deviation of sequencing reads should be at least 0.5.
<code>SDquantilThresh</code>	Threshold for the quantile of standard deviation for selecting final reference taxon
<code>balanceCut</code>	The threshold of non-zero sequencing reads in each group of a binary variable for choosing the reference taxon in phase 2. The default number is 0.2 which means at least 20% sequencing reads are non-zero in each group.
<code>seed</code>	Random seed for reproducibility. Default is 1.

## Details

To model the association, the following equation is used:

$$\log(\mathcal{Y}_i^k) | \mathcal{Y}_i^k > 0 = \beta^{0k} + X_i^T \beta^k + W_i^T \gamma^k + Z_i^T b_i + \epsilon_i^k, \quad k = 1, \dots, K + 1$$

where

- $\mathcal{Y}_i^k$  is the AA of taxa  $k$  in subject  $i$  in the entire ecosystem.
- $X_i$  is the covariate matrix.
- $W_i$  is the confounder matrix.
- $Z_i$  is the design matrix for random effects.
- $\beta^k$  is the regression coefficients that will be estimated and tested with the IFAA() function.

The challenge in microbiome analysis is that  $\mathcal{Y}_i^k$  can not be observed. What is observed is its small proportion:  $Y_i^k = C_i \mathcal{Y}_i^k$ , where  $C_i$  is an unknown number between 0 and 1 that denote the observed proportion.

The IFAA method can handle this challenge by identifying and employing reference taxa. The IFAA() will estimate the parameter  $\beta^k$  and their 95% confidence intervals. High-dimensional  $X_i$  is handled by regularization.

## Value

A list containing the estimation results.

- analysisResults\$*sig\_results*: A list containing estimating results for all significant taxon for the variables in *testCov*.
- analysisResults\$*full\_results*: A list containing estimating results for taxon. NA denotes unestimable.
- covariatesData: A dataset containing covariates and confounders used in the analyses.

## References

- Li et al.(2021) IFAA: Robust association identification and Inference For Absolute Abundance in microbiome analyses. Journal of the American Statistical Association
- Zhang CH (2010) Nearly unbiased variable selection under minimax concave penalty. Annals of Statistics. 38(2):894-942.
- Liu et al.(2020) A bootstrap lasso + partial ridge method to construct confidence intervals for parameters in high-dimensional sparse linear models. Statistica Sinica

## Examples

```
data(dataM)
dim(dataM)
dataM[1:5, 1:8]
data(dataC)
dim(dataC)
dataC[1:5, ]

results <- IFAA(MicrobData = dataM,
                CovData = dataC,
                linkIDname = "id",
                testCov = c("v1", "v2"),
```

```
ctrlCov = c("v3"), nRef = 3,
paraJobs = 2,
fdrRate = 0.25,
bootB = 5)
```

MZILN

*Conditional regression for microbiome analysis based on multivariate zero-inflated logistic normal model*

## Description

Make inference on the associations of microbiome with covariates given a user-specified reference taxon/OTU/ASV.

## Usage

```
MZILN(
  MicrobData,
  CovData,
  linkIDname,
  allCov = NULL,
  targetTaxa = NULL,
  refTaxa,
  adjust_method = "BY",
  fdrRate = 0.2,
  paraJobs = NULL,
  bootB = 500,
  bootLassoAlpha = 0.05,
  taxkeepThresh = 0,
  standardize = FALSE,
  sequentialRun = TRUE,
  seed = 1
)
```

## Arguments

MicrobData	Microbiome data matrix containing microbiome abundance with each row per sample and each column per taxon/OTU/ASV. It should contain an "id" variable to correspond to the "id" variable in the covariates data: CovData. This argument can take directory path. For example, MicrobData="C://...//microbiomeData.tsv".
CovData	Covariates data matrix containing covariates and confounders with each row per sample and each column per variable. It should also contain an "id" variable to correspond to the "id" variable in the microbiome data: MicrobData. This argument can take directory path. For example, CovData="C://...//covariatesData.tsv".
linkIDname	Variable name of the "id" variable in both MicrobData and CovData. The two data sets will be merged by this "id" variable.

allCov	All covariates of interest (including confounders) for estimating and testing their associations with microbiome. Default is 'NULL' meaning that all covariates in covData are of interest.
targetTaxa	The taxa that should be used as numerator. Default is NULL.
refTaxa	Reference taxa specified by the user and will be used as the reference taxa.
adjust_method	The adjusting method used for p value adjustment. Same as p.adjust function in R.
fdrRate	The false discovery rate for identifying taxa/OTU/ASV associated with allCov. Default is 0.25.
paraJobs	If sequentialRun is FALSE, this specifies the number of parallel jobs that will be registered to run the algorithm. If specified as NULL, it will automatically detect the cores to decide the number of parallel jobs. Default is NULL. It is safe to have 4gb memory per job. It may be needed to reduce the number of jobs if memory is limited.
bootB	Number of bootstrap samples for obtaining confidence interval of estimates in phase 2. The default is 500.
bootLassoAlpha	The significance level in phase 2. Default is 0.05.
taxkeepThresh	The threshold of number of non-zero sequencing reads for each taxon to be included into the analysis.
standardize	This takes a logical value TRUE or FALSE. If TRUE, all design matrix X in phase 1 and phase 2 will be standardized in the analyses. Default is FALSE.
sequentialRun	This takes a logical value TRUE or FALSE. Default is TRUE since there is only 1 reference taxon.
seed	Random seed for reproducibility. Default is 1.

## Details

The regression model for MZILN() can be expressed as follows:

$$\log\left(\frac{\mathcal{Y}_i^k}{\mathcal{Y}_i^{K+1}}\right) | \mathcal{Y}_i^k > 0, \mathcal{Y}_i^{K+1} > 0 = \alpha^{0k} + \mathcal{X}_i^T \alpha^k + \epsilon_i^k, \quad k = 1, \dots, K$$

where

- $\mathcal{Y}_i^k$  is the AA of taxa  $k$  in subject  $i$  in the entire ecosystem.
- $\mathcal{Y}_i^{K+1}$  is the reference taxon (specified by user).
- $\mathcal{X}_i$  is the covariate matrix for all covariates including confounders.
- $\alpha^k$  is the regression coefficients along with their 95% confidence intervals that will be estimated by the MZILN() function.

High-dimensional  $\mathcal{X}_i$  is handled by regularization.

## Value

A list containing the estimation results.

- analysisResults\$full\_results: A list containing estimating results for all significant taxa.
- analysisResults\$targettaxa\_result\_list: A list containing estimating results for targetTaxa. Only available when targetTaxa is non-empty.
- covariatesData: A dataset containing all covariates used in the analyses.

## References

Li et al.(2018) Conditional Regression Based on a Multivariate Zero-Inflated Logistic-Normal Model for Microbiome Relative Abundance Data. *Statistics in Biosciences* 10(3): 587-608

Zhang CH (2010) Nearly unbiased variable selection under minimax concave penalty. *Annals of Statistics*. 38(2):894-942.

Liu et al.(2020) A bootstrap lasso + partial ridge method to construct confidence intervals for parameters in high-dimensional sparse linear models. *Statistica Sinica*

## Examples

```
data(dataM)
dim(dataM)
dataM[1:5, 1:8]
data(dataC)
dim(dataC)
dataC[1:5, ]

results <- MZILN(MicrobData = dataM,
  CovData = dataC,
  linkIDname = "id",
  allCov=c("v1", "v2", "v3"),
  targetTaxa = "rawCount6",
  refTaxa=c("rawCount11"),
  paraJobs=2)
```



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