# A Quick Introduction to iNEXT.3D via Examples

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iNEXT.3D (INterpolation and EXTrapolation for three dimensions of biodiversity) is a sequel to iNEXT (Hsieh et al., 2016). Here the three dimensions (3D) of diversity include taxonomic diversity (TD), phylogenetic diversity (PD) and functional diversity (FD). An online version "iNEXT.3D Online" (<u>https://chao.shinyapps.io/iNEXT\_3D/</u>) is also available for users without an R background.

A unified framework based on Hill numbers (for TD) and their generalizations (Hill-Chao numbers, for PD and FD) is adopted to quantify 3D. In this framework, TD quantifies the effective number of species, PD quantifies the effective total branch length, mean-PD (PD divided by tree depth) quantifies the effective number of lineages, and FD quantifies the effective number of virtual functional groups (or functional "species"). Thus, TD, mean-PD, and FD are all in the same units of species/lineage equivalents and can be meaningfully compared; see Chao et al. (2014) for the basic standardization theory for TD, and Chao et al. (2021) for a review of the unified theory for 3D.

For each of the three dimensions of biodiversity, iNEXT.3D features two statistical analyses (non-asymptotic and asymptotic):

1. A non-asymptotic approach based on interpolation and extrapolation for 3D diversity (i.e., Hill-Chao numbers)

**INEXT.3D** computes the estimated 3D diversity for standardized samples with a common sample size or sample completeness. This approach aims to compare diversity estimates for equally-large (with a common sample size) or equally-complete (with a common sample coverage) samples; it is based on the seamless rarefaction and extrapolation (R/E) sampling curves of Hill-Chao numbers for q = 0, 1 and 2. For each dimension of biodiversity, **INEXT.3D** offers three types of R/E sampling curves:

- Sample-size-based (or size-based) R/E sampling curves: This type of sampling curve plots the diversity estimates with respect to sample size.
- Coverage-based R/E sampling curves: This type of sampling curve plots the diversity estimates with respect to sample coverage.
- Sample completeness curve: This curve depicts how sample coverage varies with sample size. The sample completeness curve provides a bridge between the size- and coverage-based R/E sampling curves.
- 2. An asymptotic approach to infer asymptotic 3D diversity (i.e., Hill-Chao numbers)

iNEXT.3D computes the estimated asymptotic 3D diversity and also plots 3D diversity profiles (q-profiles) for q between 0 and 2, in comparison with the observed diversity. Typically, the asymptotic estimates for  $q \ge 1$  are reliable, but for q < 1 (especially for q = 0, species richness), the asymptotic estimates represent only lower bounds. iNEXT.3D also features a time-profile (which depicts the observed and asymptotic estimate of PD or mean PD with respect to reference times), and a tau-profile (which depicts the observed and asymptotic estimate of FD with respect to threshold level tau).

## How to cite

If you publish your work based on results from iNEXT.3D package, you should make references to the following methodology paper and the package:

- Chao, A., Henderson, P. A., Chiu, C.-H., Moyes, F., Hu, K-H., Dornelas, M and. Magurran, A. E. (2021). Measuring temporal change in alpha diversity: a framework integrating taxonomic, phylogenetic and functional diversity and the iNEXT.3D standardization. Methods in Ecology and Evolution, 12, 1926-1940.
- Chao, A. and Hu, K.-H. (2023). The iNEXT.3D package: interpolation and extrapolation for three dimensions of biodiversity. R package available from CRAN.

## SOFTWARE NEEDED TO RUN INEXT.3D IN R

- Required: R
- Suggested: RStudio IDE

## HOW TO RUN INEXT.3D:

The iNEXT.3D package can be downloaded from CRAN or Anne Chao's iNEXT.3D github using the commands below. For a first-time installation, some additional packages must be installed and loaded; see package manual.

## or install the latest version from github
install.packages('devtools')
library(devtools)
install\_github('AnneChao/iNEXT.3D')

## import packages
library(iNEXT.3D)

There are six main functions in this package:

Two functions for non-asymptotic analysis with graphical displays:

- **iNEXT3D** computes standardized 3D diversity estimates of order q = 0, 1 and 2 for rarefied and extrapolated samples at specified sample coverage values and sample sizes.
- ggiNEXT3D visualizes the output from the function iNEXT3D.

Two functions for point estimation and basic data information

- estimate3D computes 3D diversity of order q = 0, 1 and 2 with a particular set of user-specified level of sample sizes or sample coverage values.
- DataInfo3D provides basic data information based on the observed data.

Two functions for asymptotic analysis with graphical displays:

- ObsAsy3D computes observed and asymptotic diversity of order q between 0 and 2 (in increments of 0.2) for 3D diversity; it also computes observed and asymptotic PD for specified reference times, and observed and asymptotic FD for specified threshold levels.
- ggObsAsy3D visualizes the output from the function ObsAsy3D.

## DATA INPUT FORMAT

#### Species abundance/incidence data format

Although species identities/names are not required to assess TD or compare TD across individual assemblages (as in the iNEXT package), they are required for PD and FD. Thus, for iNEXT.3D package, information on species identity (or any unique identification code) and assemblage affiliation is required. Two types of species abundance/incidence data are supported:

- Individual-based abundance data (datatype = "abundance"): When there are multiple assemblages, in addition to the assemblage/site names (as column names) and the species names (as row names), species abundance data (reference sample) can be input as a species (in rows) by assemblage (in columns) matrix/data.frame or a list of species abundance vectors. In the special case that there is only one assemblage, all data should be read in one column.
- 2. Sampling-unit-based incidence data: Incidence-raw data (datatype = "incidence\_raw"): for each assemblage, input data for a reference sample consist of a species-by-sampling-unit matrix, in addition to the sampling-unit names (as column names) and the species names (as row names). When there are N assemblages, input data consist of N lists of matrices, and each matrix is a species-by-sampling-unit matrix. Each element in the incidence raw matrix is 1 for a detection, and 0 for a non-detection. Input a matrix which combines data for all assemblages is allowed, but the argument nT in the function iNEXT3D must be specified so that the number of sampling units in each assemblage is specified.

For example, the dataset Brazil\_rainforest\_abun\_data included in the iNEXT.3D package consists of species sample abundances of two assemblages/habitats: "Edge" and "Interior". Run the following code to view the first 15 rows of the abundance data.

data("Brazil\_rainforest\_abun\_data")
Brazil rainforest abun data

	Edge	Interior
Carpotroche_brasiliensis	11	21
Astronium_concinnum	110	11
Astronium_graveolens	36	7
Spondias_macrocarpa	12	1
Spondias_venulosa	2	0
Tapirira_guianensis	7	1
Thyrsodium_spruceanum	11	11
Anaxagorea_silvatica	1	13
Annona_acutiflora	1	1
Annona_cacans	0	2
Annona_dolabripetala	3	3
Annona_sp	0	1
Duguetia_chrysocarpa	1	1
Ephedranthus_spl	1	0
Ephedranthus_sp2	0	1

We use data (Fish\_incidence\_data) collected from two time periods, namely "2013-2015" and "2016-2018", as an example. Each time period is designated as an assemblage. The purpose was to compare 3D diversity of the two time periods. In each time period, species incidence/occurrence was recorded in 36 sampling units in each assemblage; each sampling unit represents a sampling date. Thus, there are 36 columns in each time period. Run the following code to view the first 6 rows and 6 columns for each matrix.

## data("Fish\_incidence\_data")

Fish\_incidence\_data

\$`2013-2015`						
	17/01/2013	18/02/2013	19/03/2013	17/04/2013	16/05/2013	14/06/2013
Agonus_cataphractus	0	1	1	1	0	0
Alosa_fallax	0	0	0	0	0	0
Ammodytes_tobianus	0	0	0	0	0	0
Anguilla_anguilla	0	1	1	0	0	0
Aphia_minuta	0	0	0	0	1	1
Arnoglossus_laterna	0	0	0	0	0	0
\$`2016-2018`						
	18/01/2016	15/02/2016	16/03/2016	14/04/2016	12/05/2016	10/06/2016
Agonus_cataphractus	1	1	1	1	1	0
Alosa_fallax	0	0	0	0	0	0
Ammodytes_tobianus	0	0	0	0	0	0
Anguilla_anguilla	0	0	0	0	0	0
Aphia_minuta	0	0	0	0	1	0
Arnoglossus_laterna	0	0	0	0	0	0

#### Phylogenetic tree format for PD

To perform PD analysis, the phylogenetic tree (in Newick format) spanned by species observed in the pooled data is required. For the dataset Fish\_incidence\_data, the phylogenetic tree for all observed species (including species in both time periods) is stored in the file fish\_phylo\_tree; for the dataset

Brazil\_rainforest\_abun\_data, the phylogenetic tree for all observed species (including species in both Edge and Interior habitats) is stored in the file Brazil\_rainforest\_phylo\_tree. A partial list of the tip labels and node labels are shown below.

```
data("Brazil_rainforest_phylo_tree")
Brazil_rainforest_phylo_tree
Phylogenetic tree with 425 tips and 205 internal nodes.
Tip labels:
   Carpotroche_brasiliensis, Casearia_ulmifolia, Casearia_sp4, Casearia_sylvestris,
        Casearia_sp2, Casearia_sp3, ...
Node labels:
   magnoliales_to_asterales, poales_to_asterales, , , , celastrales_to_malpighiales, ...
Rooted; includes branch lengths.
```

## Species pairwise distance matrix format for FD

To perform FD analysis, the species-pairwise distance matrix (Gower distance computed from species traits) for species observed in the pooled data is required in a matrix/data.frame format. For the dataset Fish\_incidence\_data, the distance matrix for all observed species (including species in both time periods) is stored in the file fish\_dist\_matrix; for the dataset Brazil\_rainforest\_abun\_data, the distance matrix for all species (including species in both Edge and Interior habitats) is stored in the file Brazil\_rainforest\_dist\_matrix. The distance matrix for the first 3 Brazil rainforest tree species is shown below.

```
data("Brazil_rainforest_distance_matrix")
Brazil rainforest distance matrix
```

	Carpotroche_brasiliensis	Astronium_concinnum	Astronium_graveolens
Carpotroche_brasiliensis	0.000	0.522	0.522
Astronium_concinnum	0.522	0.000	0.000
Astronium_graveolens	0.522	0.000	0.000

## MAIN FUNCTION iNEXT3D(): RAREFACTION/EXTRAPOLATION

iNEXT3D(data, diversity = 'TD', q = c(0,1,2), datatype = "abundance", size = NULL, endpoint = NULL, knots = 40, nboot = 50, conf = 0.95, nT = NULL, PDtree = NULL, PDreftime = NULL, PDtype = 'meanPD', FDdistM, FDtype = 'AUC', FDtau = NULL, FDcut\_number = 50)

The arguments of this function are briefly described below, and will be explained in more details by illustrative examples in later text. This main function computes standardized 3D diversity estimates of order q = 0, 1 and 2, the sample coverage estimates, and related statistics for K (if knots = K in the specified argument) evenly-spaced knots (sample sizes) between size 1 and the endpoint, where the endpoint is described below. Each knot represents a particular sample size for which 3D diversity estimates will be calculated. By default, endpoint = double the reference sample size for abundance data or double the total sampling units for incidence data. For example, if endpoint = 10, knot = 4 is specified, diversity estimates will be computed for a sequence of samples with sizes (1, 4, 7, 10).

Argument	Description
data	<ul> <li>a. For datatype = "abundance", data can be input as a vector of species abundances (for a single assemblage), matrix/data.frame (species by assemblages), or a list of species abundance vectors.</li> <li>b. For datatype = "incidence_raw", data can be input as a list of matrices/data.frames (species by sampling units); data can also be input as a single matrix/data.frame by merging all sampling units across assemblages based on species identity; in this case, the number of sampling units (nT, see below) must be specified.</li> </ul>
diversity	selection of diversity type: $\mathbf{v}_{\mathbb{TD}'}$ = Taxonomic diversity, $\mathbf{v}_{\mathbb{PD}'}$ = Phylogenetic diversity, and $\mathbf{v}_{\mathbb{FD}'}$ = Functional diversity.
q	a numerical vector specifying the diversity orders. Default is $c(0, 1, 2)$ .
datatype	<pre>data type of input data: individual-based abundance data (datatype = ``abundance''), or species by sampling-units incidence/occurrence matrix (datatype = ``incidence_raw'') with all entries being 0 (non-detection) or 1 (detection).</pre>
size	an integer vector of sample sizes (number of individuals or sampling units) for which diversity estimates will be computed. If NULL, then diversity estimates will be computed for those sample sizes determined by the specified/default endpoint and knots.
endpoint	an integer specifying the sample size that is the <code>endpoint</code> for rarefaction/extrapolation. If NULL, then <code>endpoint</code> = double the reference sample size.
knots	an integer specifying the number of equally-spaced knots (say K, default is 40) between size 1 and the endpoint; each knot represents a particular sample size for which diversity estimate will be calculated. If the endpoint is smaller than the reference sample size, then iNEXT3D() computes only the rarefaction estimates for approximately K evenly spaced knots. If the endpoint is larger than the reference sample size, then iNEXT3D() computes for approximately K/2 evenly spaced knots between sample size 1 and the reference sample size, and computes extrapolation estimates for approximately K/2 evenly spaced knots between the reference sample size and the endpoint.
nboot	a positive integer specifying the number of bootstrap replications when assessing sampling uncertainty and constructing confidence intervals. Enter 0 to skip the bootstrap procedures. Default is 50.
conf	a positive number < 1 specifying the level of confidence interval. Default is 0.95.
nT	(required only when datatype = "incidence_raw" and input data in a single matrix/data.frame) a vector of nonnegative integers specifying the number of sampling units in each assemblage. If assemblage names are not specified(i.e., names(nT) = NULL), then assemblages are automatically named as "assemblage1", "assemblage2",, etc.
PDtree	(required argument for diversity = "PD"), a phylogenetic tree in Newick format for all observed species in the pooled assemblage.
PDreftime	(argument only for <code>diversity = "PD"</code> ), a vector of numerical values specifying reference times for PD. Default is <code>NULL</code> (i.e., the age of the root of PDtree).
PDtype	(argument only for diversity = "PD"), select PD type: PDtype = "PD" (effective total branch length) or PDtype = "meanPD" (effective number of equally divergent lineages). Default is "meanPD", where meanPD = PD/tree depth.
FDdistM	(required argument for ${\tt diversity}$ = "FD"), a species pairwise distance matrix for all species in the pooled assemblage.
	(argument only for diversity = "FD"), select FD type: FDtype = "tau_values" for FD

(argument only for diversity = "FD"), select FD type: <code>FDtype</code> = "tau\_values" for FD

FDtype	under specified threshold values, or FDtype = "AUC" (area under the curve of tau-profile) for an overall FD which integrates all threshold values between zero and one. Default is "AUC".
FDtau	(argument only for diversity = "FD" and FDtype = "tau_values"), a numerical vector between 0 and 1 specifying tau values (threshold levels). If NULL (default), then threshold is set to be the mean distance between any two individuals randomly selected from the pooled assemblage (i.e., quadratic entropy).
FDcut_number	(argument only for diversity = "FD" and FDtype = "AUC"), a numeric number to cut [0, 1] interval into equal-spaced sub-intervals to obtain the AUC value by integrating the tau-profile. Equivalently, the number of tau values that will be considered to compute the integrated AUC value. Default is FDcut_number = 50. A larger value can be set to obtain more accurate AUC value.

For each dimension of diversity (TD, PD, FD), the main function iNEXT3D() returns the iNEXT3D object, which can be further used to make plots using the function ggiNEXT3D() to be described below. The "iNEXT3D" object includes three lists:

- 1. \$TDInfo (\$PDInfo, or \$FDInfo) for summarizing data information.
- \$TDINextEst (\$PDINextEst, or \$FDINextEst) for showing diversity estimates along with related statistics for a series of rarefied and extrapolated samples; there are two data frames (\$size\_based and \$coverage based) conditioning on standardized sample size or sample coverage, respectively.
- \$TDAsyEst (\$PDAsyEst, or \$FDAsyEst) for showing asymptotic diversity estimates along with related statistics.

## FUNCTION ggiNEXT3D(): GRAPHIC DISPLAYS

The function ggiNEXT3D(), which extends ggplot2 with default arguments, is described as follows:

ggiNEXT3D(output, type = 1:3, facet.var = "Assemblage", color.var = "Order.q")

Here output is the iNEXT3D() object. Three types of curves are allowed for 3D diversity:

- 1. Sample-size-based R/E curve (type = 1): This curve plots diversity estimates with confidence intervals as a function of sample size.
- Sample completeness curve (type = 2): This curve plots the sample coverage with respect to sample size.
- 3. Coverage-based R/E curve (type = 3): This curve plots the diversity estimates with confidence intervals as a function of sample coverage.

The argument facet.var = "Order.q", facet.var = "Assemblage", facet.var = "Both", or facet.var = "None" is used to create a separate plot for each value of the specified variable.

The ggiNEXT3D() function is a wrapper with the package ggplot2 to create a rarefaction/extrapolation sampling curve in a single line of code. The figure object is of class "ggplot", so it can be manipulated by using the ggplot2 tools.

## TAXONOMIC DIVERSITY (TD): RAREFACTION/EXTRAPOLATION VIA EXAMPLES

#### EXAMPLE 1: TD rarefaction/extrapolation for abundance data

Based on the dataset (Brazil\_rainforest\_abun\_data) included in the package, the following commands return all numerical results for TD. The first list of the output (\$TDInfo) returns basic data information including the name of the Assemblage, sample size (n), observed species richness (\$.obs), sample coverage estimate of the reference sample with size n (\$C(n)), sample coverage estimate of the extrapolated sample with size 2n (\$C(2n)) as well as the first five species abundance frequency counts in the reference sample (f1-f5). The output is identical to that based on the function DataInfo3D() by specifying diversity = 'TD' and datatype = "abundance"; see later text). Thus, if only data information is required, the simpler function DataInfo3D() (see later text) can be used to obtain the same output. More information about the observed diversity (for any order q between 0 and 2) can be obtained by function ObsAsy3D(), which will be introduced later.

 STDInfo

 Assemblage
 n S.obs SC(n) SC(2n)
 f1 f2 f3 f4 f5

 1
 Edge 1794
 319 0.939
 0.974 110 48 38 28 13

The second list of the output ( $\timestEst$ ) includes size- and coverage-based standardized diversity estimates and related statistics computed for 40 knots by default (for example in the "Edge" assemblage, corresponding to the target sample size m = 1, 95, 189, ..., 1699, 1794, 1795, 1899, ..., 3588), which locates the reference sample size at the mid-point of the selected knots. There are two data frames (size based and scoverage based).

The first data frame (<code>\$size\_based</code>) includes the name of the Assemblage, diversity order (<code>Order.q</code>), the target sample size (<code>m</code>), the Method (Rarefaction, Observed, or Extrapolation, depending on whether the size <code>m</code> is less than, equal to, or greater than the reference sample size), the diversity estimate of order q (<code>qTD</code>), the lower and upper confidence limits of diversity (<code>qTD.LCL</code> and <code>qTD.UCL</code>) conditioning on the sample size, and the corresponding sample coverage estimate (<code>sc</code>) along with the lower and upper confidence limits of sample coverage estimates with confidence intervals are used for plotting the sample completeness curve. If the argument <code>nboot</code> is greater than zero, then a bootstrap method is applied to obtain the confidence intervals for the diversity and sample coverage estimates. Otherwise, all confidence intervals will not be computed. Here only the first six rows of the <code>\$size\_based</code> output are displayed:

output\_TD\_abun\$TDiNextEst\$size\_based

	Assemblage	Order.q	m	Method	qTD	qTD.LCL	qTD.UCL	SC	SC.LCL	SC.UCL
1	Edge	0	1	Rarefaction	1.000	1.000	1.000	0.012	0.010	0.013
2	Edge	0	95	Rarefaction	66.306	65.043	67.569	0.484	0.468	0.500
3	Edge	0	189	Rarefaction	106.743	104.052	109.434	0.638	0.622	0.653
4	Edge	0	284	Rarefaction	137.029	133.025	141.033	0.718	0.704	0.733
5	Edge	0	378	Rarefaction	161.010	155.820	166.200	0.768	0.755	0.782
6	Edge	0	472	Rarefaction	181.073	174.781	187.366	0.803	0.790	0.816

The second data frame (<code>\$coverage\_based</code>) includes the name of assemblage, the diversity order (<code>Order.q</code>), the target sample coverage value (<code>sc</code>), the corresponding sample size (<code>m</code>), the <code>Method</code> (<code>Rarefaction</code>, <code>Observed</code>, or <code>Extrapolation</code>, depending on whether the coverage <code>sc</code> is less than, equal to, or greater than the reference sample coverage), the diversity estimate of order q (<code>qTD</code>), the lower and upper confidence limits of diversity (<code>qTD.LCL</code> and <code>qTD.UCL</code>) conditioning on the target sample coverage value. Here only the first six rows of the <code>\$coverage\_based</code> output are displayed below: (Note for a fixed coverage value, the confidence interval in the <code>\$coverage\_based</code> table is wider than the corresponding interval in the <code>\$size\_based</code> table. This is because, for a given coverage value, the sample size needed to attain a fixed coverage value varies with bootstrap replication, leading to higher uncertainty on the resulting diversity estimate.)

output\_TD\_abun\$TDiNextEst\$coverage\_based

	Assemblage	Order.q	SC	m	Method	qTD	qTD.LCL	qTD.UCL
1	Edge	0	0.012	1	Rarefaction	1.000	0.970	1.030
2	Edge	0	0.484	95	Rarefaction	66.306	61.976	70.636
3	Edge	0	0.638	189	Rarefaction	106.743	99.830	113.657
4	Edge	0	0.718	284	Rarefaction	137.029	127.987	146.072
5	Edge	0	0.768	378	Rarefaction	161.010	150.075	171.946
6	Edge	0	0.803	472	Rarefaction	181.073	168.376	193.771

The third list of the output ( $\tilde{TDAsyEst}$ ) includes the name of the Assemblage, diversity label ( $\tilde{TD}$ , species richness for q = 0, Shannon diversity for q = 1, and Simpson diversity for q = 2), the observed diversity ( $\tilde{TD}_{obs}$ ), asymptotic diversity estimate ( $\tilde{TD}_{asy}$ ) and its estimated bootstrap standard error (s.e.) as well as the confidence intervals for asymptotic diversity ( $\tilde{TD}_{absy}$ ) and its estimated bootstrap standard error (s.e.) as well as the confidence intervals for asymptotic diversity ( $\tilde{TD}_{lcL}$  and  $\tilde{TD}_{tCL}$ ). These statistics are computed only for q = 0, 1 and 2. More detailed information about asymptotic and observed diversity estimates for any order q between 0 and 2 can be obtained from function  $\tilde{Obs}_{absy3D}$ (). The output for  $\tilde{TD}_{absyEst}$  is shown below:

output\_TD\_abun\$TDAsyEst

 Assemblage
 qTD
 TD\_obs
 TD\_asy
 s.e.
 qTD.LCL
 qTD.UCL

 1
 Edge
 Species richness
 319.000
 444.971
 28.910
 388.309
 501.634

 2
 Edge
 Shannon diversity
 155.386
 178.000
 4.920
 168.357
 187.642

 3
 Edge
 Simpson diversity
 82.023
 85.905
 3.753
 78.550
 93.261

 4
 Interior
 Species richness
 356.000
 513.518
 28.411
 457.834
 569.202

 5
 Interior
 Shannon diversity
 163.514
 186.983
 6.553
 174.139
 199.827

 6
 Interior
 Simpson diversity
 72.153
 74.718
 4.713
 65.481
 83.955

The ggiNEXT3D function can be used to make graphical displays for rarefaction and extrapolation sampling curves. When facet.var = "Assemblage" is specified in the ggiNEXT3D function, it creates a separate plot for each assemblage; within each assemblage, different color curves represent diversity of different orders. An example for showing sample-size-based rarefaction/extrapolation curves (type = 1) is given below:



When facet.var = "Order.q" is specified in the ggiNEXT3D function, it creates a separate plot for each diversity order; within each plot, different color curves represent different assemblages. An example is shown below:



The following commands return the sample completeness (sample coverage) curve (type = 2) in which different colors represent different assemblages.

# Sample completeness curves for abundance data, separating by "Assemblage"
ggiNEXT3D(output\_TD\_abun, type = 2, color.var = "Assemblage")



The following commands return the coverage-based rarefaction/extrapolation sampling curves in which different color curves represent three diversity orders within each assemblage (facet.var = "Assemblage"), or represent two assemblages within each diversity order (facet.var = "Order.q"), respectively.

# TD coverage-based R/E curves, separating by "Assemblage"
ggiNEXT3D(output\_TD\_abun, type = 3, facet.var = "Assemblage")



# TD coverage-based R/E curves, separating by "Order.q"
ggiNEXT3D(output\_TD\_abun, type = 3, facet.var = "Order.q")



#### **EXAMPLE 2: TD rarefaction/extrapolation for incidence data**

Based on the dataset (Fish\_incidence\_data) included in the package, the following commands return all numerical results for TD. The first list of the output (\$TDInfo) returns basic data information including the name of the Assemblage, number of sampling units (T), total number of incidences (U), observed species richness (S.obs), sample coverage estimate of the reference sample with size T (SC(T)), sample coverage estimate of the reference sample with size T (SC(T)), sample coverage estimate of the extrapolated sample with size 2T (SC(2T)) as well as the first five species incidence frequency counts in the reference sample (Q1-Q5). The output is identical to that based on the function DataInfo3D() by specifying diversity = 'TD' and datatype = "incidence\_raw"; see later text). Thus, if only data information is required, the simpler function DataInfo3D() (see later text) can be used to obtain the same output. More information about the observed diversity (for any order q between 0 and 2) can be obtained by function ObsAsy3D(), which will be introduced later.

\$TDInfo

 Assemblage
 T
 U
 S.obs
 SC(T)
 SC(2T)
 Q1
 Q2
 Q3
 Q4
 Q5

 1
 2013-2015
 36
 532
 50
 0.980
 0.993
 11
 6
 4
 1
 3

 2
 2016-2018
 36
 522
 53
 0.976
 0.989
 13
 5
 5
 2
 3

The second list of the output (\$TDINextEst) includes size- and coverage-based standardized diversity estimates and related statistics computed for 40 knots by default (for example in the "2013-2015" time period, corresponding to the target number of sample units mT = 1, 2, 4, ..., 34, 36, 37, 38, ..., 72), which locates the reference sampling units at the mid-point of the selected knots. There are two data frames (\$size\_based and \$coverage\_based).

The first data frame (<code>\$size\_based</code>) includes the name of the Assemblage, diversity order (<code>Order.q</code>), the target number of sampling units (<code>mT</code>), the <code>Method</code> (<code>Rarefaction</code>, <code>Observed</code>, or <code>Extrapolation</code>, depending on whether the target number of sample units <code>mT</code> is less than, equal to, or greater than the number of sampling units in the reference sample), the diversity estimate of order q (<code>qTD</code>), the lower and upper confidence limits of diversity (<code>qTD.LCL</code> and <code>qTD.UCL</code>) conditioning on the sample size, and the corresponding sample coverage estimate (<code>SC</code>) along with the lower and upper confidence limits of sample coverage (<code>SC.LCL</code> and <code>SC.UCL</code>). These sample coverage estimates with confidence intervals are used for plotting the sample completeness curve. If the argument <code>nboot</code> is greater than zero, then a bootstrap method is applied to obtain the confidence intervals for the diversity and sample coverage estimates. Otherwise, all confidence intervals will not be computed. Here only the first six rows of the <code>\$size\_based</code> output are displayed:

output\_TD\_inci\$TDiNextEst\$size\_based

	Assemblage	Order.q	mΤ	Method	qTD	qTD.LCL	qTD.UCL	SC	SC.LCL	SC.UCL
1	2013-2015	0	1	Rarefaction	14.778	13.921	15.635	0.606	0.575	0.636
2	2013-2015	0	2	Rarefaction	20.603	19.460	21.746	0.749	0.724	0.773
3	2013-2015	0	4	Rarefaction	27.079	25.501	28.658	0.851	0.833	0.868
4	2013-2015	0	6	Rarefaction	31.121	29.209	33.034	0.894	0.880	0.909
5	2013-2015	0	8	Rarefaction	34.042	31.847	36.237	0.919	0.906	0.931
6	2013-2015	0	10	Rarefaction	36.319	33.873	38.765	0.934	0.923	0.945

The second data frame (<code>scoverage\_based</code>) includes the name of assemblage, the diversity order (<code>order.q</code>), the target sample coverage value (<code>sc</code>), the corresponding number of sampling units (<code>mT</code>), the <code>Method</code> (<code>Rarefaction</code>, <code>Observed</code>, or <code>Extrapolation</code>, depending on whether the coverage <code>sc</code> is less than, equal to, or greater than the reference sample coverage), the diversity estimate of order q (<code>qTD</code>), the lower and upper confidence limits of diversity (<code>qTD.LCL</code> and <code>qTD.UCL</code>) conditioning on the target sample coverage value. Here only the first six rows of the <code>\$coverage\_based</code> output are displayed below: (Note for a fixed coverage value, the confidence interval in the <code>\$coverage\_based</code> table is wider than the corresponding interval in the <code>\$size\_based</code> table. This is because, for a given coverage value, the sample size needed to attain a fixed coverage value varies with bootstrap replication, leading to higher uncertainty on the resulting diversity estimate.)

output\_TD\_inci\$TDiNextEst\$coverage\_based

```
        Assemblage
        Order.q
        SC mT
        Method
        qTD qTD.LCL
        qTD.UCL
        qTD.Q
        qQ.Q
        qQ.Q
        qQ.Q
```

The third list of the output ( $\Delta SyEst$ ) includes the name of the Assemblage, diversity label ( $\Delta D$ , species richness for q = 0, Shannon diversity for q = 1, and Simpson diversity for q = 2), the observed diversity ( $\Delta D$ , asymptotic diversity estimate ( $\Delta D$ , and its estimated bootstrap standard error (s.e.) as well as the confidence intervals for asymptotic diversity ( $\Delta D$ , LCL and  $\Delta D$ , DCL). These statistics are computed only for q = 0, 1 and 2. More detailed information about asymptotic and observed diversity estimates for any order q between 0 and 2 can be obtained from function  $\Delta Sy3D()$ . The output is shown below:

output\_TD\_inci\$TDAsyEst

 Assemblage
 qTD TD\_obs
 TD\_asy
 s.e.
 qTD.LCL
 qTD.UCL

 1
 2013-2015
 Species richness
 50.000
 59.803
 18.179
 24.173
 95.433

 2
 2013-2015
 Shannon diversity
 30.089
 31.542
 1.173
 29.243
 33.840

 3
 2013-2015
 Simpson diversity
 23.961
 24.394
 0.885
 22.659
 26.128

 4
 2016-2018
 Species richness
 53.000
 69.431
 9.946
 49.937
 88.924

 5
 2016-2018
 Shannon diversity
 31.534
 33.393
 1.388
 30.674
 36.113

 6
 2016-2018
 Simpson diversity
 24.889
 25.409
 0.848
 23.746
 27.072

The ggiNEXT3D function can be used to make graphical displays for rarefaction and extrapolation sampling curves. When facet.var = "Assemblage" is specified in the ggiNEXT3D function, it creates a separate plot for each assemblage; within each assemblage, different color curves represent diversity of different orders. An example for showing sample-size-based rarefaction/extrapolation curves (type = 1) for incidence data is given below:

# TD sample-size-based R/E curves for incidence data, separating by "Assemblage"
ggiNEXT3D(output\_TD\_inci, type = 1, facet.var = "Assemblage")



When facet.var = "Order.q" is specified in the ggiNEXT3D function, it creates a separate plot for each diversity order; within each plot, different color curves represent different assemblages. An example is shown below:





The following commands return the sample completeness (sample coverage) curve  $(t_{ype} = 2)$  in which different colors are used for different assemblages.

# Sample completeness curves for incidence data, separating by "Assemblage"
ggiNEXT3D(output\_TD\_inci, type = 2, color.var = "Assemblage")



The following commands return the coverage-based rarefaction/extrapolation sampling curves in which different color curves represent three diversity orders within each assemblage (facet.var = "Assemblage"), or represent two assemblages within each diversity order (facet.var = "Order.q"), respectively.





# TD coverage-based R/E curves for incidence data, separating by "Order.q"
ggiNEXT3D(output\_TD\_inci, type = 3, facet.var = "Order.q")



## PHYLOGENETIC DIVERSITY (PD): RAREFACTION/EXTRAPOLATION VIA EXAMPLES

#### **EXAMPLE 3: PD rarefaction/extrapolation for abundance data**

Based on the dataset  $(Brazil_rainforest_abun_data)$  and the phylogentic tree  $(Brazil_rainforest_phylo_tree)$  included in the package, the following commands return all numerical results for PD. The first list of the output (\$PDInfo) returns basic data information including the name of the Assemblage, sample size (n), observed species richness (S.obs), sample coverage estimate of the reference sample with size n (SC (n)), sample coverage estimate of the extrapolated sample with size 2n (SC (2n)), the observed total branch length in the phylogenetic tree spanned by all observed species (PD.obs), the number of singletons and doubletons in the node/branch abundance set (f1\*, f2\*), the total branch length of those singletons and doubletons in the node/branch abundance set (g1,g2), and the reference time (Reftime). The output is identical to that based on the function DataInfo3D() by specifying diversity = 'PD' and datatype = "abundance"; see later text). Thus, if only data information is required, the simpler function DataInfo3D() (see later text) can be used to obtain the same output. More information about the observed diversity (for any order q between 0 and 2) can be obtained by function ObsAsy3D(), which will be introduced later.

The required argument for performing PD analysis is PDtree. For example, the phylogenetic tree for all observed species (including species in both Edge and Interior habitats) is stored in Brazil\_rainforest\_phylo\_tree. Then we enter the argument PDtree = Brazil\_rainforest\_phylo\_tree. Two optional arguments are: PDtype and PDreftime. There are two options for PDtype: "PD" (effective total branch length) or "meanPD" (effective number of equally divergent lineages, meanPD = PD/tree depth). Default is PDtype = "meanPD". PDreftime is a numerical value specifying a reference time for computing phylogenetic diversity. By default (PDreftime = NULL), the reference time is set to the tree depth, i.e., age of the root of the phylogenetic tree. Run the following code to perform PD analysis.

The second list of the output (pDiNextEst) includes size- and coverage-based standardized diversity estimates and related statistics computed for 40 knots by default (for example in the "Edge" assemblage, corresponding to the target sample size m = 1, 95, 189, ..., 1699, 1794, 1795, 1899, ..., 3588), which locates the reference sample size at the mid-point of the selected knots. There are two data frames ( $size_based$  and  $scoverage_based$ ).

The first data frame (<code>\$size\_based</code>) includes the name of the Assemblage, diversity order (<code>order.q</code>), the target sample size (<code>m</code>), the <code>Method</code> (<code>Rarefaction</code>, <code>Observed</code>, or <code>Extrapolation</code>, depending on whether the size <code>m</code> is less than, equal to, or greater than the reference sample size), the diversity estimate of order q (<code>qPD</code>), the lower and upper confidence limits of diversity (<code>qPD.LCL</code> and <code>qPD.UCL</code>) conditioning on the sample size, the corresponding sample coverage estimate (<code>sc</code>) along with the lower and upper confidence limits of sample coverage (<code>sc.lcL</code> and <code>sc.UcL</code>), the reference time (<code>Reftime</code>) and the type of PD (<code>Type</code>). These sample coverage estimates with confidence intervals are used for plotting the sample completeness curve. If the argument <code>nboot</code> is greater than zero, then a bootstrap method is applied to obtain the confidence intervals for the diversity and sample coverage estimates. Otherwise, all confidence intervals will not be computed. Here only the first six rows of the <code>\$size based</code> output are displayed:

output\_PD\_abun\$PDiNextEst\$size\_based

	Assemblage	Order.q	m	Method	qPD	qPD.LCL	qPD.UCL	SC	SC.LCL	SC.UCL	Reftime	Type
1	Edge	0	1	Rarefaction	1.000	0.984	1.016	0.012	0.011	0.013	400	meanPD
2	Edge	0	95	Rarefaction	18.547	17.956	19.137	0.484	0.469	0.499	400	meanPD
3	Edge	0	189	Rarefaction	26.723	25.867	27.579	0.638	0.624	0.652	400	meanPD
4	Edge	0	284	Rarefaction	32.305	31.275	33.336	0.718	0.706	0.731	400	meanPD
5	Edge	0	378	Rarefaction	36.498	35.336	37.661	0.768	0.757	0.780	400	meanPD
6	Edge	0	472	Rarefaction	39.882	38.610	41.153	0.803	0.792	0.814	400	meanPD

The second data frame (\$coverage\_based) includes the name of assemblage, the diversity order (Order.q), the target sample coverage value (sc), the corresponding sample size (m), the Method (Rarefaction, Observed, or Extrapolation, depending on whether the coverage sc is less than, equal to, or greater than the reference sample coverage), the diversity estimate of order q (qPD), the lower and upper confidence limits of diversity (qPD.LCL and qPD.UCL) conditioning on the target sample coverage value, the reference times (Reftime) and the

type of PD (Type). Here only the first six rows of the \$coverage\_based output are displayed below: (Note for a fixed coverage value, the confidence interval in the \$coverage\_based table is wider than the corresponding interval in the \$size\_based table. This is because, for a given coverage value, the sample size needed to attain a fixed coverage value varies with bootstrap replication, leading to higher uncertainty on the resulting diversity estimate.)

	Assemblage	Order.q	SC	m	Method	qPD	qPD.LCL	qPD.UCL	Reftime	Type
1	Edge	0	0.012	1	Rarefaction	1.000	0.983	1.017	400	meanPD
2	Edge	0	0.484	95	Rarefaction	18.547	17.553	19.541	400	meanPD
3	Edge	0	0.638	189	Rarefaction	26.723	25.350	28.097	400	meanPD
4	Edge	0	0.718	284	Rarefaction	32.305	30.674	33.936	400	meanPD
5	Edge	0	0.768	378	Rarefaction	36.498	34.671	38.325	400	meanPD
6	Edge	0	0.803	472	Rarefaction	39.882	37.898	41.866	400	meanPD

The third list of the output (pDasyEst) includes the name of the Assemblage, PD (or meanPD) for q = 0, 1, and 2 (qPD), the observed diversity ( $PD_obs$ ), asymptotic diversity estimates ( $PD_asy$ ), estimated asymptotic bootstrap standard error (s.e.) as well as the confidence intervals for asymptotic diversity with q = 0, 1, and 2 (qPD.LCL and qPD.UCL), the reference times (Reftime) and the type of PD (Type). These statistics are computed only for q = 0, 1 and 2. More detailed information about asymptotic and observed diversity estimates for any order q between 0 and 2 can be obtained from function ObsAsy3D(). The output is shown below:

output\_PD\_abun\$PDAsyEst

output\_PD\_abun\$PDiNextEst\$coverage based

qPD PD\_obs PD\_asy s.e. qPD.LCL qPD.UCL Reftime Type Assemblage 1 Edge q = 0 PD 61.290 80.027 5.580 69.091 90.964 400 meanPD 2 Edge q = 1 PD 5.246 5.372 0.095 5.184 5.559 400 meanPD Edge q = 2 PD 1.797 1.798 0.022 3 1.754 1.841 400 meanPD Interior q = 0 PD 69.318 86.375 4.457 77.640 95.110 4 400 meanPD Interior q = 1 PD 5.721 5.854 0.093 5 5.672 6.036 400 meanPD Interior q = 2 PD 1.914 1.915 0.023 1.869 1.961 400 meanPD 6

The ggiNEXT3D function can be used to make graphical displays for rarefaction and extrapolation sampling curves. When facet.var = "Assemblage" is specified in the ggiNEXT3D function, it creates a separate plot for each assemblage; within each assemblage, different color curves represent diversity of different orders. An example for showing sample-size-based rarefaction/extrapolation curves (type = 1) is given below:



# PD sample-size-based R/E curves, separating by "Assemblage"
ggiNEXT3D(output\_PD\_abun, type = 1, facet.var = "Assemblage")

When facet.var = "Order.q" is specified in the ggiNEXT3D function, it creates a separate plot for each diversity order; within each plot, different color curves represent different assemblages. An example is shown below:

# PD sample-size-based R/E curves, separating by "Order.q"
ggiNEXT3D(output PD abun, type = 1, facet.var = "Order.q")



The following commands return the sample completeness (sample coverage) curve (type = 2) in which different colors are used for different assemblages.



# Sample completeness curves for abundance data, separating by "Assemblage"
ggiNEXT3D(output\_PD\_abun, type = 2, color.var = "Assemblage")

The following commands return the coverage-based rarefaction/extrapolation sampling curves in which different color curves represent three diversity orders within each assemblage (facet.var = "Assemblage"), or represent two assemblages within each diversity order (facet.var = "Order.q"), respectively.

```
# PD coverage-based R/E curves, separating by "Assemblage"
ggiNEXT3D(output_PD_abun, type = 3, facet.var = "Assemblage")
```



# PD coverage-based R/E curves, separating by "Order.q"
ggiNEXT3D(output\_PD\_abun, type = 3, facet.var = "Order.q")



#### **EXAMPLE 4: PD rarefaction/extrapolation for incidence data**

Based on the dataset (Fish\_incidence\_data) included in the package and the phylogentic tree (Fish\_phylo\_tree), the following commands return all numerical results for PD. The first list of the output (pDInfo) returns basic data information including the name of the Assemblage, number of sampling units (T), total number of incidences (U), observed species richness (S.obs), sample coverage estimate of the reference sample with size T (SC(T)), sample coverage estimate of the extrapolated sample with size 2T (SC(2T)), the observed total branch length in the phylogenetic tree spanned by all observed species (PD.obs), the singletons/doubletons in the sample branch incidence (Q1\*,Q2\*), the total branch length of those singletons/doubletons in the sample branch incidence (R1,R2), and the reference time (Reftime). The output is identical to that based on the function DataInfo3D() by specifying diversity = 'PD' and datatype = "incidence\_raw"; see later text). Thus, if only data information is required, the simpler function DataInfo3D() (see later text) can be used to obtain the same output. More information about the observed diversity (for any order q between 0 and 2) can be obtained by function ObsAsy3D(), which will be introduced later.

The required argument for performing PD analysis is PDtree. For example, the phylogenetic tree for all observed species (including species in both "2013-2015" and "2016-2018" time periods) is stored in Fish\_phylo\_tree. Then we enter the argument PDtree = Fish\_phylo\_tree. Two optional arguments are: PDtype and PDreftime. There are two options for PDtype: "PD" (effective total branch length) or "meanPD" (effective number of equally divergent lineages, meanPD = PD/tree depth). Default is PDtype = "meanPD". PDreftime is a numerical value specifying a reference time for computing phylogenetic diversity. By default (PDreftime = NULL), the reference time is set to the tree depth, i.e., age of the root of the phylogenetic tree. Run the following code to perform PD analysis.

data(Fish\_incidence\_data)
data(Fish\_phylo\_tree)
data <- Fish\_incidence\_data
tree <- Fish\_phylo\_tree</pre>

output\_PD\_inci <- iNEXT3D(data, diversity = 'PD', q = c(0, 1, 2), datatype = "incidence\_raw", nboot = 20, PDtree = tree)

output\_PD\_inci\$PDInfo

#### \$PDTnfo

# A tibble: 2	x 12										
Assemblage	Т	U	S.obs	`SC(T)`	`SC(2T)`	PD.obs	`Q1*`	`Q2*`	R1	R2	Reftime
<chr></chr>	<int></int>	<int></int>	<int></int>	<dbl></dbl>							
1 2013-2015	36	532	50	0.98	0.993	9.62	11	7	0.69	1.23	0.977
2 2016-2018	36	522	53	0.976	0.989	9.44	13	6	0.368	0.345	0.977

The second list of the output (SPDINextEst) includes size- and coverage-based standardized diversity estimates and related statistics computed for 40 knots by default (for example in the "2013-2015" time period, corresponding to the target number of sample units mT = 1, 2, 4, ..., 34, 36, 37, 38, ..., 72), which locates the reference sampling units at the mid-point of the selected knots. There are two data frames (ssize\_based and \$coverage based).

The first data frame (*size\_based*) includes the name of the Assemblage, diversity order (*order.g*), the target number of sample units (mT), the Method (Rarefaction, Observed, Or Extrapolation, depending on whether the target number of sample units mT is less than, equal to, or greater than the number of sampling units in the reference sample), the diversity estimate of order q (gPD), the lower and upper confidence limits of diversity (qPD.LCL and qPD.UCL) conditioning on the sample size, the corresponding sample coverage estimate (sc) along with the lower and upper confidence limits of sample coverage (SC.LCL and SC.UCL), the reference time (Reftime) and the type of PD (Type). These sample coverage estimates with confidence intervals are used for plotting the sample completeness curve. If the argument nboot is greater than zero, then a bootstrap method is applied to obtain the confidence intervals for the diversity and sample coverage estimates. Otherwise, all confidence intervals will not be computed. Here only the first six rows of the *ssize* based output are displayed:

output PD inci\$PDiNextEst\$size based

	Assemblage	Order.q	mΤ	Method	qPD	qPD.LCL	qPD.UCL	SC	SC.LCL	SC.UCL	Reftime	Туре
1	2013-2015	0	1	Rarefaction	5.744	5.541	5.946	0.606	0.577	0.635	0.977	meanPD
2	2013-2015	0	2	Rarefaction	6.813	6.581	7.045	0.749	0.727	0.770	0.977	meanPD
3	2013-2015	0	4	Rarefaction	7.716	7.488	7.945	0.851	0.837	0.865	0.977	meanPD
4	2013-2015	0	6	Rarefaction	8.130	7.865	8.394	0.894	0.881	0.908	0.977	meanPD
5	2013-2015	0	8	Rarefaction	8.389	8.079	8.700	0.919	0.905	0.932	0.977	meanPD
6	2013-2015	0	10	Rarefaction	8.589	8.237	8.942	0.934	0.921	0.947	0.977	meanPD

The second data frame (scoverage based) includes the name of assemblage, the diversity order (Order.g), the target sample coverage value (sc), the corresponding number of sample units (mT), the Method (Rarefaction, Observed, or Extrapolation, depending on whether the coverage sc is less than, equal to, or greater than the reference sample coverage), the diversity estimate of order q (qPD), the lower and upper confidence limits of diversity (gPD.LCL and gPD.UCL) conditioning on the target sample coverage value, the reference time (Reftime) and the type of PD (Type). Here only the first six rows of the scoverage based output are displayed below: (Note for a fixed coverage value, the confidence interval in the scoverage\_based table is wider than the corresponding interval in the ssize based table. This is because, for a given coverage value, the sample size needed to attain a fixed coverage value varies with bootstrap replication, leading to higher uncertainty on the resulting diversity estimate.)

output PD inci\$PDiNextEst\$coverage based

Assemblage Order.g SC mT Method gPD gPD.LCL gPD.UCL Reftime Type 1 2013-2015 0 0.606 1 Rarefaction 5.744 5.542 5.946 0.977 meanPD 2 2013-2015 0 0.749 2 Rarefaction 6.813 6.598 7.028 0.977 meanPD 3 2013-2015 0 0.851 4 Rarefaction 7.716 7.492 7.941 0.977 meanPD 5 2013-2015 6 2013-2015 4 2013-2015 0 0.894 6 Rarefaction 8.130 7.852 8.407 0.977 meanPD 0 0.919 8 Rarefaction 8.389 8.055 8.724 0.977 meanPD 0 0.934 10 Rarefaction 8.589 8.204 8.975 0.977 meanPD

The third list of the output (\$PDAsyEst) includes the name of the Assemblage, PD (or meanPD) for q = 0, 1, and 2 (qPD), the observed diversity (PD obs), asymptotic diversity estimate (PD asy) and its estimated bootstrap standard error (s.e.), the confidence intervals for asymptotic diversity (gPD.LCL and gPD.UCL), the reference time (Reftime) and the type of PD ( $T_{YPe}$ ). These statistics are computed only for q = 0, 1 and 2. More detailed information about asymptotic and observed diversity estimates for any order q between 0 and 2 can be obtained from function ObsAsy3D(). The output is shown below:

output PD inci\$PDAsyEst

qPD PD obs PD asy s.e. qPD.LCL qPD.UCL Reftime Type Assemblage 1 2013-2015 q = 0 PD 9.847 10.039 0.702 8.663 11.416 0.977 meanPD 2 2013-2015 q = 1 PD 7.635 7.729 0.157 7.421 8.037 0.977 meanPD 3 2013-2015 q = 2 PD 7.013 7.057 0.152 6.760 7.355 0.977 meanPD 4 2016-2018 q = 0 PD 9.659 9.854 0.796 8.295 11.413 0.977 meanPD

```
      5
      2016-2018 q = 1 PD
      7.781
      7.859
      0.141
      7.583
      8.136
      0.977 meanPD

      6
      2016-2018 q = 2 PD
      7.202
      7.244
      0.116
      7.016
      7.471
      0.977 meanPD
```

The ggiNEXT3D function can be used to make graphical displays for rarefaction and extrapolation sampling curves. When facet.var = "Assemblage" is specified in the ggiNEXT3D function, it creates a separate plot for each assemblage; within each assemblage, different color curves represent diversity of different orders. An example for showing sample-size-based rarefaction/extrapolation curves (type = 1) is given below:

# PD sample-size-based R/E curves for incidence data, separating by "Assemblage"
ggiNEXT3D(output\_PD\_inci, type = 1, facet.var = "Assemblage")



When facet.var = "Order.q" is specified in the ggiNEXT3D function, it creates a separate plot for each diversity order; within each plot, different color curves represent different assemblages. An example is shown below:

# PD sample-size-based R/E curves for incidence data, separating by "Order.q"
ggiNEXT3D(output PD inci, type = 1, facet.var = "Order.q")



The following commands return the sample completeness (sample coverage) curve  $(t_{ype} = 2)$  in which different colors are used for different assemblages.

# Sample completeness curves for incidence data, separating by "Assemblage"
ggiNEXT3D(output\_PD\_inci, type = 2, color.var = "Assemblage")



The following commands return the coverage-based rarefaction/extrapolation sampling curves in which different color curves represent three diversity orders within each assemblage (facet.var = "Assemblage"), or represent two assemblages within each diversity order (facet.var = "Order.q"), respectively.





# PD coverage-based R/E curves for incidence data, separating by "Order.q"
ggiNEXT3D(output\_PD\_inci, type = 3, facet.var = "Order.q")



## FUNCTIONAL DIVERSITY (FD): RAREFACTION/EXTRAPOLATION VIA **EXAMPLES**

#### EXAMPLE 5: FD rarefaction/extrapolation for abundance data

Based on the dataset (Brazil rainforest abun data) and the the distance matrix (Brazil\_rainforest\_distance\_matrix) included in the package, the following commands return all numerical results for FD. The first list of the output (\$FDInfo) returns basic data information including the name of the Assemblage, sample size (n), observed species richness (S.obs), sample coverage estimate of the reference sample with size n (SC(n)), sample coverage estimate of the extrapolated sample with size 2n (SC(2n)), and the minimum, mean, and maximum distance among all non-diagonal elements in the distance matrix(dmin, dmean, dmax). The output is identical to that based on the function DataInfo3D() by specifying diversity = 'FD' and datatype = "abundance"; see later text). Thus, if only data information is required, the simpler function DataInfo3D() (see later text) can be used to obtain the same output. More information about the observed diversity (for any order q between 0 and 2) can be obtained by function ObsAsy3D(), which will be introduced later.

The required argument for performing FD analysis is FDdistM. For example, the distance matrix for all species (including species in both Edge and Interior habitats) is stored in Brazil\_rainforest\_distance\_matrix. Then we enter the argument FDdistM = Brazil\_rainforest\_distance\_matrix Three optional arguments are (1) FDtype: FDtype = "AUC" means FD is computed from the area under the curve of a tau-profile by integrating all plausible threshold values between zero and one; FDtype = "tau\_values" means FD is computed under specific threshold values to be specified in the argument FD\_tau. (2) FD\_tau: a numerical value specifying the tau value (threshold level) that will be used to compute FD. If FDtype = "tau\_values" and FD\_tau = NULL, then the threshold level is set to be the mean distance between any two individuals randomly selected from the pooled data over all data (i.e., quadratic entropy).

```
data(Brazil_rainforest_abun_data)
data(Brazil_rainforest_distance_matrix)
data <- Brazil_rainforest_abun_data
distM <- Brazil_rainforest_distance_matrix</pre>
output_FD_abun <- iNEXT3D(data, diversity = 'FD', datatype = "abundance", nboot = 10,</pre>
                           FDdistM = distM, FDtype = 'AUC')
```

output\_FD\_abun\$FDInfo

```
ŚFDInfo
 Assemblage n S.obs SC(n) SC(2n) dmin dmean dmax
1
      Edge 1794 319 0.939 0.974 0 0.372 0.776
   Interior 2074 356 0.941 0.973 0 0.329 0.776
2
```

The second list of the output (SFDINextEst) includes size- and coverage-based standardized diversity estimates and related statistics computed for 40 knots by default (for example in the "Edge" assemblage, corresponding to the target sample size m = 1, 95, 189, ..., 1699, 1794, 1795, 1899, ..., 3588), which locates the reference sample size at the mid-point of the selected knots. There are two data frames (size based and scoverage based).

The first data frame (ssize based) includes the name of the Assemblage, diversity order (Order.g), the target sample size (m), the Method (Rarefaction, Observed, or Extrapolation, depending on whether the size m is less than, equal to, or greater than the reference sample size), the diversity estimate of order q (GFD), the lower and upper confidence limits of diversity (qFD.LCL and qFD.UCL) conditioning on the sample size, and the corresponding sample coverage estimate (sc) along with the lower and upper confidence limits of sample coverage (sc.lcl and sc.ucl). These sample coverage estimates with confidence intervals are used for plotting the sample completeness curve. If the argument nboot is greater than zero, then a bootstrap method is applied to obtain the confidence intervals for the diversity and sample coverage estimates. Otherwise, all confidence intervals will not be computed. Here only the first six rows of the <code>size\_based</code> output are displayed:

output\_FD\_abun\$FDiNextEst\$size\_based

	Assemblage	Order.q	m	Method	qFD	qFD.LCL	qFD.UCL	SC	SC.LCL	SC.UCL	
1	Edge	0	1	Rarefaction	1.000	1.000	1.000	0.012	0.010	0.013	
2	Edge	0	95	Rarefaction	10.900	10.442	11.358	0.484	0.466	0.502	
3	Edge	0	189	Rarefaction	12.993	12.117	13.868	0.638	0.619	0.657	
4	Edge	0	284	Rarefaction	14.129	12.888	15.371	0.718	0.702	0.735	
5	Edge	0	378	Rarefaction	14.860	13.304	16.416	0.768	0.755	0.782	
6	Edge	0	472	Rarefaction	15.383	13.549	17.216	0.803	0.792	0.814	

The second data frame (\$coverage based) includes the name of assemblage, the diversity order (Order.g), the target sample coverage value (sc), the corresponding sample size (m), the Method (Rarefaction, Observed, or Extrapolation, depending on whether the coverage sc is less than, equal to, or greater than the reference sample coverage), the diversity estimate of order q ( $_{QFD}$ ), and the lower and upper confidence limits of diversity (qFD.LCL and qFD.UCL) conditioning on the target sample coverage value. Here only the first six rows of the scoverage based output are displayed below: (Note for a fixed coverage value, the confidence interval in the scoverage based table is wider than the corresponding interval in the ssize based table. This is because, for a given coverage value, the sample size needed to attain a fixed coverage value varies with bootstrap replication, output\_FD\_abun\$FDiNextEst\$coverage\_based

	Assemblage	Order.q	SC	m	Method	qFD	qFD.LCL	qFD.UCL
1	Edge	0	0.012	1	Rarefaction	1.000	1.000	1.000
2	Edge	0	0.484	95	Rarefaction	10.900	10.472	11.328
3	Edge	0	0.638	189	Rarefaction	12.993	12.328	13.657
4	Edge	0	0.718	284	Rarefaction	14.129	13.209	15.049
5	Edge	0	0.768	378	Rarefaction	14.860	13.696	16.025
6	Edge	0	0.803	472	Rarefaction	15.383	13.991	16.775

The third list of the output ( $\product{SpDAsyEst}$ ) includes the name of the Assemblage, FD for q = 0, 1, and 2 ( $\product{qFD}$ ), the observed diversity ( $\product{FD_obs}$ ), asymptotic diversity estimate ( $\product{FD_asy}$ ) and its estimated bootstrap standard error (s.e.) as well as the confidence intervals for asymptotic diversity ( $\product{qFD_LCL}$  and  $\product{qFD_UCL}$ ). These statistics are computed only for q = 0, 1 and 2. More detailed information about asymptotic and observed diversity estimates for any order q between 0 and 2 can be obtained from function  $\product{ObsAsy3D}$ (). The output is shown below:

output\_FD\_abun\$FDAsyEst

	Assemblage			qFD	FD_obs	FD_asy	s.e.	qFD.LCL	qFD.UCL	
1	Edge	q	=	0	FD(AUC)	17.851	19.008	4.997	9.214	28.801
2	Edge	q	=	1	FD(AUC)	11.781	12.037	0.521	11.016	13.057
3	Edge	q	=	2	FD(AUC)	9.139	9.228	0.397	8.451	10.006
4	Interior	q	=	0	FD(AUC)	17.168	18.208	8.415	1.716	34.700
5	Interior	q	=	1	FD(AUC)	9.716	9.922	0.276	9.381	10.463
6	Interior	q	=	2	FD(AUC)	7.007	7.055	0.148	6.766	7.345

The ggiNEXT3D function can be used to make graphical displays for rarefaction and extrapolation sampling curves. When facet.var = "Assemblage" is specified in the ggiNEXT3D function, it creates a separate plot for each assemblage; within each assemblage, different color curves represent diversity of different orders. An example for showing sample-size-based rarefaction/extrapolation curves (type = 1) is given below:

```
# FD sample-size-based R/E curves, separating by "Assemblage"
ggiNEXT3D(output_FD_abun, type = 1, facet.var = "Assemblage")
```



When facet.var = "Order.q" is specified in the ggiNEXT3D function, it creates a separate plot for each diversity order; within each plot, different color curves represent different assemblages. An example is shown below:

# FD sample-size-based R/E curves, separating by "Order.q"
ggiNEXT3D(output\_FD\_abun, type = 1, facet.var = "Order.q")



The following commands return the sample completeness (sample coverage) curve (type = 2) in which different colors are used for different assemblages.



# Sample completeness curves for abundance data, separating by "Assemblage"



The following commands return the coverage-based rarefaction/extrapolation sampling curves in which different color curves represent three diversity orders within each assemblage (facet.var = "Assemblage"), or represent two assemblages within each diversity order (facet.var = "Order.q"), respectively.





# FD coverage-based R/E curves, separating by "Order.q"
ggiNEXT3D(output\_FD\_abun, type = 3, facet.var = "Order.q")



#### **EXAMPLE 6: FD rarefaction/extrapolation for incidence data**

Based on the dataset (Fish\_incidence\_data) and the the distance matrix (Fish\_distance\_matrix) included in the package, the following commands return all numerical results for FD. The first list of the output (FDInfo) returns basic data information including the name of the Assemblage, number of sampling units (T), total number of incidences (U), observed species richness (S.obs), sample coverage estimate of the reference sample with size T (SC(T)), sample coverage estimate of the reference sample with size T (SC(T)), sample coverage estimate of the reference sample with size 2T (SC(T)), and the minimum, mean, and maximum distance among all non-diagonal elements in the distance matrix(dmin, dmean, dmax). The output is identical to that based on the function DataInfo3D() by specifying diversity = 'FD' and datatype = "incidence\_raw"; see later text). Thus, if only data information is required, the simpler function DataInfo3D() (see later text) can be used to obtain the same output. More information about the observed diversity (for any order q between 0 and 2) can be obtained by function ObsAsy3D(), which will be introduced later.

The required argument for performing FD analysis is FDdistM. For example, the distance matrix for all species (including species in both "2013-2015" and "2016-2018" time periods) is stored in Fish\_distance\_matrix. Then we enter the argument FDdistM = Fish\_distance\_matrix Three optional arguments are (1) FDtype: FDtype = "AUC" means FD is computed from the area under the curve of a tau-profile by integrating all plausible threshold values between zero and one; FDtype = "tau\_values" means FD is computed under specific threshold values to be specified in the argument FD\_tau. (2) FD\_tau: a numerical value specifying the tau value (threshold level) that will be used to compute FD. If FDtype = "tau\_values" and FD\_tau = NULL, then the threshold level is set to be the mean distance between any two individuals randomly selected from the pooled data over all data (i.e., quadratic entropy).

```
$FDInfo
Assemblage T U S.obs SC(T) SC(2T) dmin dmean dmax
1 2013-2015 36 532 50 0.980 0.993 0.006 0.240 0.733
2 2016-2018 36 522 53 0.976 0.989 0.006 0.237 0.733
```

output FD inci\$FDInfo

The second list of the output (\$FDINextEst) includes size- and coverage-based standardized diversity estimates and related statistics computed for 40 knots by default (for example in the "2013-2015" time period, corresponding to the target number of sample units mT = 1, 2, 4, ..., 34, 36, 37, 38, ..., 72), which locates the reference sampling units at the mid-point of the selected knots. There are two data frames (\$size\_based and \$coverage\_based).

FDdistM = distM, FDtype = 'AUC')

The first data frame (<code>\$size\_based</code>) includes the name of the Assemblage, diversity order (<code>order.q</code>), the target number of sample units (<code>mT</code>), the <code>Method</code> (<code>Rarefaction</code>, <code>Observed</code>, or <code>Extrapolation</code>, depending on whether the target number of sample units <code>mT</code> is less than, equal to, or greater than the number of sampling units in the reference sample), the diversity estimate of order q (<code>qFD</code>), the lower and upper confidence limits of diversity (<code>qFD.LCL</code> and <code>qFD.UCL</code>) conditioning on the sample size, and the corresponding sample coverage estimate (<code>sc</code>) along with the lower and upper confidence limits of sample coverage (<code>sc.lcL</code> and <code>sc.ucL</code>). These sample coverage estimates with confidence intervals are used for plotting the sample completeness curve. If the argument <code>nboot</code> is greater than zero, then a bootstrap method is applied to obtain the confidence intervals for the diversity and sample coverage estimates. Otherwise, all confidence intervals will not be computed. Here only the first six rows of the <code>\$size\_based</code> output are displayed:

output\_FD\_inci\$FDiNextEst\$size\_based

	Assemblage	Order.q	mΤ	Method	qFD	qFD.LCL	qFD.UCL	SC	SC.LCL	SC.UCL
1	2013-2015	0	1	Rarefaction	14.778	13.862	15.694	0.606	0.575	0.637
2	2013-2015	0	2	Rarefaction	15.318	14.403	16.234	0.749	0.723	0.774
3	2013-2015	0	4	Rarefaction	15.888	14.972	16.803	0.851	0.832	0.869
4	2013-2015	0	6	Rarefaction	16.224	15.301	17.146	0.894	0.880	0.909
5	2013-2015	0	8	Rarefaction	16.463	15.530	17.396	0.919	0.906	0.931
6	2013-2015	0	10	Rarefaction	16.652	15.706	17.598	0.934	0.923	0.945

The second data frame (<code>\$coverage\_based</code>) includes the name of assemblage, the diversity order (<code>order.q</code>), the target sample coverage value (<code>sc</code>), the corresponding number of sample units (<code>mT</code>), the <code>Method</code> (<code>Rarefaction</code>, <code>Observed</code>, or <code>Extrapolation</code>, depending on whether the coverage <code>sc</code> is less than, equal to, or greater than the reference sample coverage), the diversity estimate of order q (<code>qFD</code>), and the lower and upper confidence limits of diversity (<code>qFD.LCL</code> and <code>qFD.UCL</code>) conditioning on the target sample coverage value. Here only the first six rows of the <code>\$coverage\_based</code> output are displayed below: (Note for a fixed coverage value, the confidence interval in the <code>\$coverage\_based</code> table is wider than the corresponding interval in the <code>\$size\_based</code> table. This is because, for a given coverage value, the sample size needed to attain a fixed coverage value varies with bootstrap replication, leading to higher uncertainty on the resulting diversity estimate.)

output\_FD\_inci\$FDiNextEst\$coverage\_based

 Assemblage
 Order.q
 SC mT
 Method
 qFD qFD.LCL
 qFD.UCL

 1
 2013-2015
 0
 0.606
 1
 Rarefaction
 14.778
 14.179
 15.376

 2
 2013-2015
 0
 0.749
 2
 Rarefaction
 15.318
 14.741
 15.896

 3
 2013-2015
 0
 0.851
 4
 Rarefaction
 15.288
 15.243
 16.533

 4
 2013-2015
 0
 0.894
 6
 Rarefaction
 16.224
 15.515
 16.932

 5
 2013-2015
 0
 0.919
 8
 Rarefaction
 16.463
 15.699
 17.226

 6
 2013-2015
 0
 0.934
 10
 Rarefaction
 16.652
 15.843
 17.461

The third list of the output (\$PDAsyEst) includes the name of the Assemblage, FD for q = 0, 1, and 2 (qFD), the observed diversity ( $FD_obs$ ), asymptotic diversity estimate ( $FD_asy$ ) and its estimated bootstrap standard error (s.e.), and the confidence intervals for asymptotic diversity ( $qFD_LCL$  and  $qFD_UCL$ ). These statistics are computed only for q = 0, 1 and 2. More detailed information about asymptotic and observed diversity estimates for any order q between 0 and 2 can be obtained from function obsAsy3D(). The output is shown below:

output\_FD\_inci\$FDAsyEst

```
AssemblageqFD FD_obsFD_asys.e.qFD.LCLqFD.UCL12013-2015q = 0FD(AUC)17.90418.9061.38616.18821.62322013-2015q = 1FD(AUC)15.94416.0430.46915.12416.96132013-2015q = 2FD(AUC)15.46315.4900.45514.59816.38342016-2018q = 0FD(AUC)17.73919.7704.93110.10629.43452016-2018q = 1FD(AUC)15.74915.8670.60714.67817.05662016-2018q = 2FD(AUC)15.27515.3050.53214.26216.348
```

The ggiNEXT3D function can be used to make graphical displays for rarefaction and extrapolation sampling curves. When facet.var = "Assemblage" is specified in the ggiNEXT3D function, it creates a separate plot for

each assemblage; within each assemblage, different color curves represent diversity of different orders. An example for showing sample-size-based rarefaction/extrapolation curves ( $t_{ype} = 1$ ) is given below:





When facet.var = "Order.q" is specified in the ggiNEXT3D function, it creates a separate plot for each diversity order; within each plot, different color curves represent different assemblages. An example is shown below:

```
# FD sample-size-based R/E curves for incidence data, separating by "Order.q"
ggiNEXT3D(output FD inci, type = 1, facet.var = "Order.q")
```



The following commands return the sample completeness (sample coverage) curve (type = 2) in which different colors are used for different assemblages.

# Sample completeness curves for incidence data, separating by "Assemblage"
ggiNEXT3D(output\_FD\_inci, type = 2, color.var = "Assemblage")



The following commands return the coverage-based rarefaction/extrapolation sampling curves in which different color curves represent three diversity orders within each assemblage (facet.var = "Assemblage"), or represent two assemblages within each diversity order (facet.var = "Order.q"), respectively.





# FD coverage-based R/E curves for incidence data, separating by "Order.q"
ggiNEXT3D(output\_FD\_inci, type = 3, facet.var = "Order.q")



## **FUNCTION DataInfo3D(): DATA INFORMATION**

The function DataInfo3D() provides basic data information for the reference sample in each individual assemblage. The function DataInfo3D() with default arguments is shown below:

```
DataInfo3D(data, diversity = "TD", datatype = "abundance",
    nT = NULL, PDtree, PDreftime = NULL,
    FDdistM, FDtype = "AUC", FDtau = NULL)
```

All arguments in the above function are the same as those for the main function iNEXT3D. Running the DataInfo3D() function returns basic data information including sample size, observed species richness, two sample coverage estimates (SC(n) and SC(2n)) as well as other relevant information in each of the three dimensions of diversity. We use Brazil\_rainforest\_abun\_data and Fish\_incidence\_data to demo the function for each dimension of diversity.

#### TAXONOMIC DIVERSITY (TD): Basic data information for abundance data

```
data(Brazil_rainforest_abun_data)
DataInfo3D(Brazil_rainforest_abun_data, diversity = 'TD', datatype = "abundance")
Assemblage n S.obs SC(n) SC(2n) f1 f2 f3 f4 f5
1 Edge 1794 319 0.939 0.974 110 48 38 28 13
2 Interior 2074 356 0.941 0.973 123 48 41 32 19
```

#### Output description:

- Assemblage = assemblage name.
- n = number of observed individuals in the reference sample (sample size).
- s.obs = number of observed species in the reference sample.
- SC(n) = sample coverage estimate of the reference sample with size n.
- sc(2n) = sample coverage estimate of the reference sample with size 2n.
- f1-f5 = the first five species abundance frequency counts in the reference sample.

#### TAXONOMIC DIVERSITY (TD): Basic data information for incidence data

```
data(Fish_incidence_data)
DataInfo3D(Fish_incidence_data, diversity = 'TD', datatype = "incidence_raw")

Assemblage T U S.obs SC(T) SC(2T) Q1 Q2 Q3 Q4 Q5
1 2013-2015 36 532 50 0.980 0.993 11 6 4 1 3
2 2016-2018 36 522 53 0.976 0.989 13 5 5 2 3
```

#### Output description:

- Assemblage = assemblage name.
- T = number of sampling units in the reference sample (sample size for incidence data).
- u = total number of incidences in the reference sample.
- s.obs = number of observed species in the reference sample.
- sc(T) = sample coverage estimate of the reference sample with size T.
- SC(2T) = sample coverage estimate of the reference sample with size 2T.
- Q1-Q5 = the first five species incidence frequency counts in the reference sample.

#### PHYLOGENETIC DIVERSITY (PD): Basic data information for abundance data

```
data(Brazil_rainforest_abun_data)
data(Brazil_rainforest_phylo_tree)
data <- Brazil_rainforest_abun_data
tree <- Brazil_rainforest_phylo_tree
DataInfo3D(data, diversity = 'PD', datatype = "abundance", PDtree = tree)</pre>
```

#### Output description:

- Assemblage, n, S.obs, SC(n) and SC(2n): definitions are the same as in the TD abundance output and thus are omitted.
- PD.obs = the observed total branch length in the phylogenetic tree spanned by all observed species.
- f1\*,f2\* = the number of singletons and doubletons in the node/branch abundance set.
- g1,g2 = the total branch length of those singletons/doubletons in the node/branch abundance set.
- Reftime = reference time for phylogenetic diversity (the age of the root of phylogenetic tree).

## PHYLOGENETIC DIVERSITY (PD): Basic data information for incidence data

```
data(Fish_incidence_data)
data(Fish_phylo_tree)
data <- Fish_incidence_data
tree <- Fish_phylo_tree
DataInfo3D(data, diversity = 'PD', datatype = "incidence_raw", PDtree = tree)</pre>
```

#### Output description:

- Assemblage, T, U, S.obs, SC(T) and SC(2T): definitions are the same as in the TD incidence output and thus are omitted.
- PD.obs = the observed total branch length in the phylogenetic tree spanned by all observed species.
- Q1\*,Q2\* = the singletons/doubletons in the sample branch incidence.
- R1,R2 = the total branch length of those singletons/doubletons in the sample branch incidence.
- Reftime = reference time.

#### FUNCTIONAL DIVERSITY (FD): Basic data information for abundance data

 Assemblage
 n S.obs
 SC(n)
 SC(2n)
 dmin
 dmean
 dmax

 1
 Edge
 1794
 319
 0.939
 0.974
 0
 0.372
 0.776

 2
 Interior
 2074
 356
 0.941
 0.973
 0
 0.329
 0.776

#### Output description:

- Assemblage, n, S.obs, SC(n) and SC(2n): definitions are the same as in TD abundance output and thus are omitted.
- dmin = the minimum distance among all non-diagonal elements in the distance matrix.
- dmean = the mean distance between any two individuals randomly selected from each assemblage.
- dmax = the maximum distance among all elements in the distance matrix.

#### FUNCTIONAL DIVERSITY (FD): Basic data information for incidence data

#### Output description:

• Assemblage, T, U, S.obs, SC(T) and SC(2T): definitions are the same as in the TD incidence output and

thus are omitted.

- dmin = the minimum distance among all non-diagonal elements in the distance matrix.
- dmean = the mean distance between any two individuals randomly selected from each assemblage.
- dmax = the maximum distance among all elements in the distance matrix.

### FUNCTION estimate3D(): POINT ESTIMATION

estimate3D is used to compute 3D diversity (TD, PD, FD) estimates with q = 0, 1, 2 under any specified levels of sample size (when base = "size") and sample coverage values (when base = "coverage") for abundance data (datatype = "abundance") or incidence data (datatype = "incidence\_raw"). When base = "size", level can be specified with a particular vector of sample sizes (greater than 0); if level = NULL, this function computes the diversity estimates for the minimum sample size among all samples extrapolated to the double reference sizes. When base = "coverage", level can be specified with a particular vector of sample coverage values (between 0 and 1); if level = NULL, this function computes the diversity estimates for the minimum sample coverage samong all samples extrapolated to the double reference sizes. When base = "coverage", level can be specified with a particular vector of sample coverage values (between 0 and 1); if level = NULL, this function computes the diversity estimates for the minimum sample coverage among all samples extrapolated to the double reference sizes. All arguments in the function are the same as those for the main function iNEXT3D.

estimate3D(data, diversity = "TD", q = c(0, 1, 2), datatype = "abundance", base = "coverage", level = NULL, nboot = 50, conf = 0.95, nT = NULL, PDtree, PDreftime = NULL, PDtype = "meanPD", FDdistM, FDtype = "AUC", FDtau = NULL, FDcut\_number = 50)

## **TAXONOMIC DIVERSITY (TD):** point estimation

#### Example 7a: TD for abundance data with two target coverage values (93% and 97%)

The following commands return the TD estimates with two specified levels of sample coverage (93% and 97%) based on the Brazil\_rainforest\_abun\_data.

	Assemblage	Order.q	SC	m	Method	qTD	s.e.	qTD.LCL	qTD.UCL
1	Edge	0	0.93	1547.562	Rarefaction	302.879	12.456	278.465	327.293
2	Edge	0	0.97	3261.971	Extrapolation	383.307	18.571	346.909	419.705
3	Edge	1	0.93	1547.562	Rarefaction	152.374	4.504	143.547	161.202
4	Edge	1	0.97	3261.971	Extrapolation	166.837	4.992	157.052	176.622
5	Edge	2	0.93	1547.562	Rarefaction	81.437	3.760	74.069	88.806
6	Edge	2	0.97	3261.971	Extrapolation	83.726	3.953	75.978	91.474
7	Interior	0	0.93	1699.021	Rarefaction	331.917	12.276	307.858	355.977
8	Interior	0	0.97	3883.447	Extrapolation	433.807	18.549	397.452	470.162
9	Interior	1	0.93	1699.021	Rarefaction	159.330	4.855	149.814	168.847
10	Interior	1	0.97	3883.447	Extrapolation	175.739	5.128	165.689	185.790
11	Interior	2	0.93	1699.021	Rarefaction	71.611	3.922	63.924	79.297
12	Interior	2	0.97	3883.447	Extrapolation	73.326	4.068	65.353	81.299

#### Example 7b: TD for incidence data with two target coverage values (97.5% and 99%)

The following commands return the TD estimates with two specified levels of sample coverage (97.5% and 99%) for the Fish incidence\_data.

```
data(Fish incidence data)
output est TD inci <- estimate3D(Fish incidence data, diversity = 'TD', q = c(0, 1, 2),
                                      datatype = "incidence raw", base = "coverage",
                                       level = c(0.975, 0.99))
output est TD inci
  Assemblage Order.q SC mT Method qTD s.e. qTD.LCL qTD.UCL

        2013-2015
        0
        0.975
        29.169
        Rarefaction
        47.703
        3.264
        41.306
        54.100

        2013-2015
        0
        0.990
        58.667
        Extrapolation
        54.914
        4.665
        45.771
        64.057

1
   2013-2015
2
3 2013-2015
                      1 0.975 29.169 Rarefaction 29.773 1.197 27.427 32.118
4 2013-2015
                     1 0.990 58.667 Extrapolation 30.751 1.214 28.372 33.130
                     2 0.975 29.169 Rarefaction 23.861 0.825 22.245 25.478
5 2013-2015
6 2013-2015
                     2 0.990 58.667 Extrapolation 24.126 0.840 22.479 25.773
    2016-2018
                       0 0.975 34.825 Rarefaction 52.574 6.997 38.860 66.288
7
```

0 0.990 76.971 Extrapolation 62.688 14.646

33.983

91.393

8

2016-2018

9	2016-2018	1	0.975	34.825	Rarefaction	31.479	1.223	29.082	33.875
10	2016-2018	1	0.990	76.971	Extrapolation	32.721	1.186	30.397	35.046
11	2016-2018	2	0.975	34.825	Rarefaction	24.872	0.755	23.392	26.352
12	2016-2018	2	0.990	76.971	Extrapolation	25.163	0.743	23.708	26.618

## **PHYLOGENETIC DIVERSITY (PD): point estimation**

#### Example 8a: PD for abundance data with two target sample sizes (1500 and 3500)

The following commands return the PD estimates with two specified levels of sample sizes (1500 and 3500) for the Brazil rainforest abun data.

output\_est\_PD\_abun

	Assemblage	Order.q	m	Method	SC	qPD	s.e.	qPD.LCL	qPD.UCL	Reftime	Туре
1	Edge	0	1500	Rarefaction	0.928	58.370	1.007	56.396	60.344	400	meanPD
2	Edge	0	3500	Extrapolation	0.973	71.893	2.233	67.516	76.270	400	meanPD
3	Edge	1	1500	Rarefaction	0.928	5.224	0.103	5.021	5.426	400	meanPD
4	Edge	1	3500	Extrapolation	0.973	5.320	0.105	5.115	5.526	400	meanPD
5	Edge	2	1500	Rarefaction	0.928	1.797	0.024	1.749	1.844	400	meanPD
6	Edge	2	3500	Extrapolation	0.973	1.797	0.024	1.749	1.845	400	meanPD
7	Interior	0	1500	Rarefaction	0.922	63.555	0.917	61.758	65.353	400	meanPD
8	Interior	0	3500	Extrapolation	0.965	78.004	1.749	74.576	81.431	400	meanPD
9	Interior	1	1500	Rarefaction	0.922	5.675	0.113	5.454	5.896	400	meanPD
10	Interior	1	3500	Extrapolation	0.965	5.784	0.114	5.560	6.008	400	meanPD
11	Interior	2	1500	Rarefaction	0.922	1.913	0.032	1.851	1.976	400	meanPD
12	Interior	2	3500	Extrapolation	0.965	1.914	0.032	1.852	1.977	400	meanPD

#### Example 8b: PD for incidence data with two target coverage values (97.5% and 99%)

The following commands return the PD estimates with two specified levels of sample coverage (97.5% and 99%) for the Fish\_incidence\_data.

Assemblage Order.q SC mT Method qPD s.e. qPD.LCL qPD.UCL Reftime Type 1 2013-2015 0 0.975 29.169 Rarefaction 9.672 0.381 8.926 10.419 0.9770115 meanPD 2 2013-2015 0 0.990 58.667 Extrapolation 10.018 0.616 8.810 11.226 0.9770115 meanPD 2013-2015 1 0.975 29.169 Rarefaction 7.612 0.149 7.320 1 0.990 58.667 Extrapolation 7.680 0.147 7.393 3 7.905 0.9770115 meanPD 7.967 0.9770115 meanPD 4 2013-2015 2 0.975 29.169 Rarefaction 7.003 0.147 6.715 7.290 0.9770115 meanPD 2013-2015 5 6 2013-2015 2 0.990 58.667 Extrapolation 7.030 0.146 6.745 7.315 0.9770115 meanPD 7 2016-2018 0 0.975 34.825 Rarefaction 9.646 0.464 8.737 10.556 0.9770115 meanPD 8 2016-2018 0 0.990 76.971 Extrapolation 9.831 0.896 8.075 11.587 0.9770115 meanPD 2016-2018 1 0.975 34.825 Rarefaction 7.779 0.130 7.524 8.033 0.9770115 meanPD 9 10 2016-2018 11 2016-2018 1 0.990 76.971 Extrapolation 7.835 0.140 7.561 8.109 0.9770115 meanPD 2 0.975 34.825 Rarefaction 7.201 0.121 6.963 7.439 0.9770115 meanPD 12 2016-2018 2 0.990 76.971 Extrapolation 7.224 0.124 6.982 7.466 0.9770115 meanPD

## FUNCTIONAL DIVERSITY (FD): point estimation

#### Example 9a: FD for abundance data with two target coverage values (93% and 97%)

The following commands return the FD estimates with two specified levels of sample coverage (93% and 97%) for the Brazil\_rainforest\_abun\_data.

output\_est\_FD\_abun

[FD.UCL
21.645
23.949
12.341
12.534
9.632
9.701
20.457
27.599
10.175
10.374
7.308
7.350
1 2 2 1

## Example 9b: FD for incidence data with two target number of sampling units (30 and 70)

The following commands return the FD estimates with two specified levels of sample sizes (30 and 70) for the Fish\_incidence\_data.

	Assemblage	Order.q	mΤ	Method	SC	qFD	s.e.	qFD.LCL	qFD.UCL	
1	2013-2015	0	30	Rarefaction	0.976	17.748	0.519	16.730	18.766	
2	2013-2015	0	70	Extrapolation	0.993	18.550	0.696	17.186	19.914	
3	2013-2015	1	30	Rarefaction	0.976	15.929	0.314	15.314	16.545	
4	2013-2015	1	70	Extrapolation	0.993	16.006	0.315	15.388	16.624	
5	2013-2015	2	30	Rarefaction	0.976	15.459	0.277	14.915	16.003	
6	2013-2015	2	70	Extrapolation	0.993	15.477	0.278	14.932	16.022	
7	2016-2018	0	30	Rarefaction	0.972	17.503	0.562	16.401	18.606	
8	2016-2018	0	70	Extrapolation	0.988	18.705	1.207	16.340	21.070	
9	2016-2018	1	30	Rarefaction	0.972	15.729	0.371	15.001	16.457	
10	2016-2018	1	70	Extrapolation	0.988	15.816	0.364	15.103	16.530	
11	2016-2018	2	30	Rarefaction	0.972	15.268	0.386	14.512	16.025	
12	2016-2018	2	70	Extrapolation	0.988	15.290	0.386	14.533	16.046	

# FUNCTION ObsAsy3D: ASYMPTOTIC AND OBSERVED DIVERSITY PROFILES

```
ObsAsy3D(data, diversity = "TD", q = seq(0, 2, 0.2), datatype = "abundance",
    nboot = 50, conf = 0.95, nT = NULL,
    method = c("Asymptotic", "Observed"),
    PDtree, PDreftime = NULL, PDtype = "meanPD",
    FDdistM, FDtype = "AUC", FDtau = NULL, FDcut_number = 50
    )
```

All arguments in the above function are the same as those for the main function iNEXT3D (except that the default of q here is seq(0, 2, 0.2)). The function ObsAsy3D() computes observed and asymptotic diversity of order q between 0 and 2 (in increments of 0.2) for 3D diversity; these 3D values with different order q can be used to depict a q-profile in the ggObsAsy3D function.

It also computes observed and asymptotic PD for various reference times by specifying the argument PDreftime; these PD values with different reference times can be used to depict a time-profile in the ggobsAsy3D function.

It also computes observed and asymptotic FD for various threshold tau levels by specifying the argument FDtau; these FD values with different threshold levels can be used to depict a tau-profile in the gg0bsAsy3D function.

For each dimension, by default, both the observed and asymptotic diversity estimates will be computed.

## FUNCTION ggObsAsy3D(): GRAPHIC DISPLAYS OF DIVERSITY **PROFILES**

ggObsAsy3D(output, profile = "q")

ggObsAsy3D is a ggplot2 extension for an ObsAsy3D object to plot 3D q-profile (which depicts the observed diversity and asymptotic diversity estimate with respect to order q) for q between 0 and 2 (in increments of 0.2).

It also plots time-profile (which depicts the observed and asymptotic estimate of PD or mean PD with respect to reference times when diversity = "PD" specified in the ObsAsy3D function), and tau-profile (which depicts the observed and asymptotic estimate of FD with respect to threshold level tau when diversity = "FD" and FDtype = "tau\_values" specified in the ObsAsy3D function) based on the output from the function ObsAsy3D.

In the plot of profiles, only confidence intervals of the asymptotic diversity will be shown when both the observed and asymptotic diversity estimates are computed.

## TAXONOMIC DIVERSITY (TD): q-profiles

#### Example 10a: TD q-profiles for abundance data

The following commands returns the observed and asymptotic taxonomic diversity ('TD') for the Brazil rainforest abun data, along with its confidence interval for diversity order q between 0 to 2. Here only the first ten rows of the output are shown.

```
data(Brazil_rainforest_abun_data)
output ObsAsy_TD_abun <- ObsAsy3D(Brazil_rainforest_abun_data, diversity = 'TD',</pre>
                                         datatype = "abundance")
output ObsAsy TD abun
   Assemblage Order.q qTD s.e. qTD.LCL qTD.UCL
                                                                    Method
1
      Edge 0.0 444.971 25.175 395.629 494.314 Asymptotic
2
         Edge 0.2 375.270 16.678 342.582 407.958 Asymptotic
         Edge 0.4 312.452 10.496 291.880 333.024 Asymptotic
3

        Edge
        0.6 258.379
        6.878 244.900 271.859
        Asymptotic

        Edge
        0.8 213.730
        5.445 203.057 224.403
        Asymptotic

4
                     0.6 258.379 6.878 244.900 271.859 Asymptotic
```

1.8 96.948 5.137 86.880 107.016 Asymptotic

Edge 1.0 178.000 5.138 167.930 188.069 Asymptotic 6 Edge 1.2 149.914 5.123 139.874 159.955 Asymptotic 7 8 Edge 1.4 127.945 5.135 117.879 138.010 Asymptotic 9 Edge 1.6 110.672 5.139 100.599 120.745 Asymptotic Edge

The following commands plot the corresponding q-profiles, along with its confidence interval for q between 0 to 2.

```
# q-profile curves
ggObsAsy3D(output_ObsAsy_TD_abun)
```

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Example 10b: TD q-profiles for incidence data

The following commands return the observed and asymptotic taxonomic diversity ('TD') estimates for the

Fish\_incidence\_data, along with its confidence interval for diversity order q between 0 to 2. Here only the first ten rows of the output are shown.

```
data(Fish_incidence_data)
output_ObsAsy_TD_inci <- ObsAsy3D(Fish_incidence_data, diversity = 'TD',</pre>
                                       datatype = "incidence raw")
output_ObsAsy_TD_inci
   Assemblage Order.q
                            qTD s.e. qTD.LCL qTD.UCL
                                                                Method
1 2013-2015 0.0 59.803 9.908 40.384 79.223 Asymptotic
2 2013-2015 0.2 50.828 5.806 39.449 62.207 Asymptotic
3 2013-2015 0.4 43.790 3.281 37.359 50.221 Asymptotic
4
   2013-2015 0.6 38.458 1.911 34.713 42.204 Asymptotic

        2013-2015
        0.8
        34.490
        1.248
        32.044
        36.936
        Asymptotic

        2013-2015
        1.0
        31.542
        0.947
        29.685
        33.398
        Asymptotic

5
6
   2013-2015 1.2 29.328 0.803 27.754 30.902 Asymptotic
7
8 2013-2015 1.4 27.635 0.724 26.217 29.053 Asymptotic
9 2013-2015 1.6 26.312 0.673 24.992 27.632 Asymptotic
10 2013-2015 1.8 25.255 0.639 24.002 26.509 Asymptotic
```

```
The following commands plot the corresponding q-profiles, along with its confidence interval for q between 0 to 2.
```





## PHYLOGENETIC DIVERSITY (PD): time-profiles and q-profiles

#### Example 11a: PD time-profiles for abundance data

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The following commands return the observed and asymptotic phylogenetic diversity ('PD') estimates for the  $Brazil_rainforest_abun_data$ , along with its confidence interval for diversity order q = 0, 1, 2 under reference times from 0.01 to 400 (tree height). Here only the first ten rows of the output are shown.

```
data(Brazil_rainforest_abun_data)
data(Brazil_rainforest_phylo_tree)
data <- Brazil rainforest abun data
tree <- Brazil_rainforest_phylo_tree</pre>
output_ObsAsy_PD_abun <- ObsAsy3D(data, diversity = 'PD', q = c(0, 1, 2),</pre>
                                        PDreftime = seq(0.01, 400, length.out = 20),
                                         datatype = "abundance", nboot = 20, PDtree = tree)
output_ObsAsy_PD_abun
   Assemblage Order.q
                           qPD s.e. qPD.LCL qPD.UCL
                                                                  Method Reftime Type
      Edge 0 444.971 29.001 388.130 501.812 Asymptotic 0.100 meanPD
1
                       1 178.000 5.074 168.055 187.944 Asymptotic 0.100 meanPD
          Edge
2
        Edge
                     2 85.905 4.149 77.773 94.038 Asymptotic 0.100 meanPD
3
4
   Interior
                     0 513.518 29.215 456.256 570.779 Asymptotic 0.100 meanPD
5
   Interior
                     1 186.983 5.190 176.812 197.154 Asymptotic 0.100 meanPD

        Interior
        2
        74.718
        4.210
        66.466
        82.969
        Asymptotic
        0.100
        meanPD

        Edge
        0
        371.100
        25.520
        321.082
        421.117
        Asymptotic
        10.354
        meanPD

6
7
```

Edge 1 141.418 3.841 133.891 148.946 Asymptotic 10.354 meanPD

9	Edge	2	72.848	3.260	66.458	79.238	Asymptotic	10.354 meanPD
10	Interior	0	413.568	22.401	369.663	457.472	Asymptotic	10.354 meanPD

The argument profile = "time" in the ggObsAsy3D function creates a separate plot for each diversity order q = 0, 1, and 2 with x-axis being "Reference time". Different assemblages will be represented by different color lines.

```
# time-profile curves
ggObsAsy3D(output_ObsAsy_PD_abun, profile = "time")
```



#### Example 11b: PD q-profiles for incidence data

The following commands return the observed and asymptotic taxonomic diversity ('PD') estimates for the Fish\_incidence\_data, along with its confidence interval for diversity order q between 0 to 2. Here only the first ten rows of the output are shown.

```
data(Fish_incidence_data)
data(Fish_phylo_tree)
data <- Fish incidence data
tree <- Fish phylo tree
output ObsAsy PD inci <- ObsAsy3D(data, diversity = 'PD', q = seq(0, 2, 0.2),
                                datatype = "incidence_raw", nboot = 20, PDtree = tree,
                                  PDreftime = NULL)
```

output\_ObsAsy\_PD\_inci

```
Assemblage Order.q qPD s.e. qPD.LCL qPD.UCL Method Reftime Type

        2013-2015
        0.0
        10.039
        0.823
        8.426
        11.653
        Asymptotic
        0.977
        meanPD

        2013-2015
        0.2
        9.462
        0.656
        8.177
        10.748
        Asymptotic
        0.977
        meanPD

        2013-2015
        0.4
        8.802
        0.387
        8.043
        9.561
        Asymptotic
        0.977
        meanPD

1
2
3
4 2013-2015 0.6 8.329 0.257 7.825 8.833 Asymptotic 0.977 meanPD
5 2013-2015 0.8 7.985 0.192 7.608 8.362 Asymptotic 0.977 meanPD
6 2013-2015 1.0 7.729 0.158 7.419 8.039 Asymptotic 0.977 meanPD
7 2013-2015 1.2 7.533 0.139 7.260 7.805 Asymptotic 0.977 meanPD

        2013-2015
        1.4
        7.378
        0.128
        7.126
        7.629
        Asymptotic
        0.977
        meanPD

        2013-2015
        1.6
        7.252
        0.122
        7.012
        7.492
        Asymptotic
        0.977
        meanPD

8
9
10 2013-2015 1.8 7.147 0.119 6.913 7.381 Asymptotic 0.977 meanPD
```

The following commands plot the corresponding q-profiles, along with its confidence interval for q between 0 to 2, for the default reference time = 0.977 (the tree depth).

# q-profile curves ggObsAsy3D(output ObsAsy PD inci, profile = "q")



## FUNCTIONAL DIVERSITY (FD): tau-profiles and q-profiles

#### Example 12a: FD tau-profiles for abundance data

The following commands returns observed and asymptotic functional diversity ('FD') for  $Brazil_rainforest_abun_data$ , along with its confidence interval at diversity order q = 0, 1, 2 under tau values from 0 to 1. Here only the first ten rows of the output are shown.

```
data(Brazil rainforest abun data)
data(Brazil rainforest distance matrix)
data <- Brazil rainforest abun data
distM <- Brazil_rainforest_distance_matrix</pre>
output_ObsAsy_FD_abun_tau <- ObsAsy3D(data, diversity = 'FD', q = c(0, 1, 2),</pre>
                                     datatype = "abundance", nboot = 10, FDdistM = distM,
                                      FDtype = 'tau_values', FDtau = seq(0, 1, 0.05))
output_ObsAsy_FD_abun_tau
   Assemblage Order.q qFD s.e. qFD.LCL qFD.UCL
                                                       Method Tau
       Edge 0 444.971 22.481 400.909 489.034 Asymptotic 0.00
1
                   1 178.000 5.377 167.461 188.538 Asymptotic 0.00
2
        Edge
       Edge
                 2 85.905 4.471 77.143 94.668 Asymptotic 0.00
3
                 0 79.904 22.161 36.468 123.340 Asymptotic 0.05
4
       Edge
5
       Edge
                  1 45.187 1.216 42.804 47.569 Asymptotic 0.05
                   2 32.092 0.799 30.526 33.658 Asymptotic 0.05
       Edge
6
                   0 73.276 23.497 27.223 119.328 Asymptotic 0.10
1 42.200 1.137 39.972 44.427 Asymptotic 0.10
7
        Edge
8
        Edge
                 2 30.182 0.683 28.843 31.521 Asymptotic 0.10
9
        Edge
                 0 35.372 24.511 0.000 83.413 Asymptotic 0.15
10
       Edge
```

The following commands plot the corresponding tau-profiles, along with its confidence interval for diversity order q = 0, 1, 2.

# tau-profile curves
ggObsAsy3D(output\_ObsAsy\_FD\_abun\_tau, profile = "tau")



## Example 12b: FD q-profiles for abundance data

The following commands returns the observed and asymptotic taxonomic diversity ('FD') for the Brazil\_rainforest\_abun\_data, along with its confidence interval for diversity order q between 0 to 2 with FDtype = 'AUC'. Here only the first ten rows of the output are shown.

output\_ObsAsy\_FD\_abun

	Assemblage	Order.q	qFD	s.e.	qFD.LCL	qFD.UCL	Method
1	Edge	0.0	19.008	7.049	5.191	32.824	Asymptotic
2	Edge	0.5	14.698	1.144	12.456	16.941	Asymptotic
3	Edge	1.0	12.037	0.362	11.328	12.746	Asymptotic
4	Edge	1.5	10.345	0.233	9.889	10.802	Asymptotic
5	Edge	2.0	9.228	0.189	8.857	9.600	Asymptotic
6	Interior	0.0	18.208	8.615	1.322	35.094	Asymptotic
7	Interior	0.5	13.071	1.076	10.963	15.179	Asymptotic
8	Interior	1.0	9.922	0.249	9.434	10.410	Asymptotic
9	Interior	1.5	8.103	0.167	7.776	8.430	Asymptotic
10	Interior	2.0	7.055	0.143	6.776	7.335	Asymptotic

The following commands plot the corresponding q-profiles, along with its confidence interval for q between 0 to 2.

```
# q-profile curves
ggObsAsy3D(output_ObsAsy_FD_abun, profile = "q")
```



#### Example 12c: FD q-profiles for incidence data

The following commands returns observed and asymptotic functional diversity ('FD') for Fish incidence data, along with its confidence interval at diversity order q from 0 to 2. Here only the first ten rows of the output are shown.

```
data(Fish incidence data)
data(Fish_distance_matrix)
data <- Fish_incidence_data
distM <- Fish distance matrix
output_ObsAsy_FD_inci <- ObsAsy3D(data, diversity = 'FD', datatype = "incidence_raw",</pre>
                                nboot = 20, FDdistM = distM, FDtype = 'AUC')
output ObsAsy FD inci
  Assemblage Order.q qFD s.e. qFD.LCL qFD.UCL
                                                    Method
  2013-2015 0.0 18.906 2.329 14.341 23.470 Asymptotic
1
   2013-2015
                0.2 17.826 1.264 15.348 20.303 Asymptotic
2
3
   2013-2015
                0.4 17.115 0.736 15.673 18.557 Asymptotic
   2013-2015
                0.6 16.624 0.518 15.609 17.639 Asymptotic
4
   2013-2015 0.8 16.284 0.435 15.430 17.137 Asymptotic
5
6
  2013-2015 1.0 16.043 0.401 15.257 16.828 Asymptotic
7
   2013-2015 1.2 15.868 0.383 15.117 16.618 Asymptotic
```

The following commands plot the corresponding q-profiles, along with its confidence interval for q between 0 to 2.

```
# g-profile curves
ggObsAsy3D(output_ObsAsy_FD_inci, profile = "q")
```

2013-2015 1.4 15.736 0.372 15.007 16.466 Asymptotic 
 2013-2015
 1.6
 15.635
 0.365
 14.919
 16.351
 Asymptotic

 2013-2015
 1.8
 15.555
 0.360
 14.849
 16.262
 Asymptotic

1.8 15.555 0.360 14.849 16.262 Asymptotic



#### License

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The iNEXT.3D package is licensed under the GPLv3. To help refine iNEXT.3D, your comments or feedback would be welcome (please send them to Anne Chao or report an issue on the iNEXT.3D github iNEXT.3D github.

#### References

- Chao, A., Henderson, P. A., Chiu, C.-H., Moyes, F., Hu, K.-H., Dornelas, M. and Magurran, A. E. (2021). Measuring temporal change in alpha diversity: a framework integrating taxonomic, phylogenetic and functional diversity and the iNEXT.3D standardization. Methods in Ecology and Evolution, 12, 1926-1940.
- Hsieh, T. C., Ma, K-H, and Chao, A. (2016). iNEXT: An R package for rarefaction and extrapolation of species diversity (Hill numbers). Methods in Ecology and Evolution, 7, 1451-1456.