A Quick Introduction to iNEXT via Examples

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Latest Updates in July 2022: (1) We have modified (in the main function inext) the bootstrap method used to obtain confidence intervals for the coverage-based rarefaction and extrapolation curves. We have expanded the inext output (\$inextest) to include two lists (\$size_based and \$coverage_based). (2) In the function estimateD, for a given coverage value, we have refined our algorithm to find the corresponding sample size (not necessarily restricted to integers) to obtain more accurate diversity estimates. (3) We have changed some column names in the output in order to conform to our forthcoming iNEXT series (iNEXT.3D, iNEXT.4steps, iNEXT.link). Please download the latest version of inext available from CRAN or from Anne Chao's iNEXT_github, or use the latest version of iNEXT Online available from Shiny iNEXT-Online.

inext (iNterpolation and EXTrapolation) is an R package modified from the original version which was supplied in the Supplement of Chao et al. (2014). In the latest updated version, we have added more user-friendly features, improved some algorithms, and refined the graphic displays. In this document, we provide a quick introduction demonstrating how to run inext. Detailed information about inext functions is provided in the iNEXT Manual, also available in <u>CRAN</u>. See Chao & Jost (2012), Colwell et al. (2012) and Chao et al. (2014) for methodologies. A short review of the theoretical background and a brief description of methods are included in an application paper by Hsieh, Ma & Chao (2016). An online version of <u>iNEXT-online</u> is also available for users without an R background.

inext focuses on three measures of Hill numbers of order q: species richness (q = 0), Shannon diversity (q = 1, the exponential of Shannon entropy) and Simpson diversity (q = 2, the inverse of Simpson concentration). For each diversity measure, inext uses the observed sample of abundance or incidence data (called the "reference sample") to compute diversity estimates and the associated 95% confidence intervals for the following two types of rarefaction and extrapolation (R/E):

- Sample-size-based (or size-based) R/E sampling curves: inext computes diversity estimates for rarefied and extrapolated samples up to an appropriate size. This type of sampling curve plots the diversity estimates with respect to sample size.
- 2. Coverage-based R/E sampling curves: iNEXT computes diversity estimates for rarefied and extrapolated samples based on a standardized level of sample completeness (as measured by sample coverage) up to an appropriate coverage value. This type of sampling curve plots the diversity estimates with respect to sample coverage.

inext also plots the above two types of sampling curves and a sample completeness curve (which 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.

HOW TO CITE INEXT

If you publish your work based on the results from the inext package, you should make references to the following methodology paper (Chao et al. 2014) and the application paper (Hsieh, Ma & Chao, 2016):

Chao, A., Gotelli, N.J., Hsieh, T.C., Sander, E.L., Ma, K.H., Colwell, R.K. & Ellison, A.M. (2014)
 Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. Ecological Monographs, 84, 45–67.

 Hsieh, T.C., Ma, K.H. & Chao, A. (2016) iNEXT: An R package for interpolation and extrapolation of species diversity (Hill numbers). Methods in Ecology and Evolution, 7, 1451-1456.

SOFTWARE NEEDED TO RUN INEXT IN R

Required: R

Suggested: <u>RStudio IDE</u>

HOW TO RUN INEXT:

The inext package is available from <u>CRAN</u> and can be downloaded with a standard R installation procedure or can be downloaded from Anne Chao's <u>iNEXT_github</u> using the following commands. For a first-time installation, an additional visualization extension package (ggplot2) must be installed and loaded.

```
## install iNEXT package from CRAN
install.packages("iNEXT")
## install the latest version from github
install.packages('devtools')
library(devtools)
install_github('AnneChao/iNEXT')
## import packages
library(iNEXT)
library(ggplot2)
```

MAIN FUNCTION: iNEXT()

We first describe the main function inext() with default arguments:

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 diversity estimates of order q, the sample coverage estimates and related statistics for K (if knots = K) 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 diversity estimates will be calculated. By default, endpoint = double the reference sample size (total sample size for abundance data; total sampling units for incidence data). For an example, if endpoint = 10, knots = 4, diversity estimates will be computed for a sequence of samples with sizes (1, 4, 7, 10). In a later real-data example, we have endpoint = 336, knots = 40; diversity estimates will be computed for a sequence of samples with sizes (1, 10,19, 28, ..., 318, 327, 336).

| х | a matrix, data.frame, lists of species abundances, or lists of incidence frequencies (see data format/information below). |
|----------|---|
| q | a number or vector specifying the diversity order(s) of Hill numbers. |
| datatype | type of input data, "abundance", "incidence_raw" or "incidence_freq". |

| Argument | Description |
|----------|---|
| size | an integer vector of sample sizes for which diversity estimates will be computed. If NULL, then diversity estimates will be calculated for those sample sizes determined by the specified/default endpoint and knots. |
| endpoint | an integer specifying the sample size that is the endpoint for R/E calculation; If NULL, then endpoint=double the reference sample size. |
| knots | an integer specifying the number of equally-spaced knots between size 1 and the endpoint; default is 40. |
| se | a logical variable to calculate the bootstrap standard error and conf confidence interval. |
| conf | a positive number < 1 specifying the level of confidence interval; default is 0.95. |
| nboot | an integer specifying the number of bootstrap replications; default is 50. |

This function returns an "iNEXT" object which can be further used to make plots using the function ggiNEXT() to be described below.

DATA FORMAT/INFORMATION

Three types of data are supported:

- 1. Individual-based abundance data (datatype="abundance"): Input data for each assemblage/site include species abundances in an empirical sample of n individuals ("reference sample"). When there are N assemblages, input data consist of an S by N abundance matrix, or N lists of species abundances.
- 2. Sampling-unit-based incidence data: There are two kinds of input data.
- a. Incidence-raw data (datatype="incidence_raw"): for each assemblage, input data for a reference sample consisting of a species-by-sampling-unit matrix; each element in the raw matrix is 1 for a detection, and 0 otherwise. When there are N assemblages, input data consist of N lists of raw matrices, and each matrix is a species-by-sampling-unit matrix.
- b. Incidence-frequency data (datatype="incidence_freq"): input data for each assemblage consist of species sample incidence frequencies (i.e., row sums of the corresponding incidence raw matrix). When there are N assemblages, input data consist of an (S+1) by N matrix, or N lists of species incidence frequencies. The first entry of each column/list must be the total number of sampling units, followed by the species incidence frequencies.

RAREFACTION/EXTRAPOLATION VIA EXAMPLES

Four data sets are included in the inext package for illustration. There are two abundance data sets: spider (list of two vectors) and bird (in data.frame format), and two incidence data sets: ant (list of 5 vectors) and ciliates (list of 3 matrices). The input datatypes are the same for the two abundance data sets (datatype="abundance"), but the input datatypes are different for the ant data (datatype="incidence_freq") and the ciliates data (datatype="incidence_raw"). We first use the spider data for illustration; see Chao et al. (2014) for analysis details and data interpretations. The spider data consist of abundance data from two canopy manipulation treatments ("Girdled" and "Logged") of hemlock trees (Ellison et al. 2010). For these data, the following commands run the inext() function for q = 0.

```
iNEXT(spider, q=0, datatype="abundance")
```

The iNEXT() function returns the "iNEXT" object including three output lists: \$DataInfo for summarizing data information; \$iNextEst for showing size- and coverage-based diversity estimates along with related statistics for a series of rarefied and extrapolated samples; and \$AsyEst for showing asymptotic diversity estimates along with related statistics. \$DataInfo, as shown below, returns basic data information including the reference sample size (n), observed species richness (S.obs), sample coverage estimate for the reference sample (SC), and the first ten frequency counts (f1-f10). This part of output can also be computed by the function DataInfo()

```
$DataInfo: basic data information

Assemblage n S.obs SC f1 f2 f3 f4 f5 f6 f7 f8 f9 f10

Girdled 168 26 0.9289 12 4 0 1 0 2 0 1 1 0

Logged 252 37 0.9446 14 4 4 3 1 0 3 2 0 1
```

For incidence data, the list \$DataInfo includes the reference sample size (T), observed species richness (S.obs), total number of incidences (U), sample coverage estimate for the reference sample (SC), and the first ten incidence frequency counts (Q1-Q10).

In the Girdled treatment assemblage, by default, 40 equally spaced knots (samples sizes) between 1 and 336 (= 2 x 168, double the reference sample size, Chao et al. 2014) are selected. Diversity estimates and related statistics are computed for these 40 knots (corresponding to sample sizes m = 1, 10, 19, ..., 327, 336), which locates the reference sample at the mid-point of the selected knots. If the argument se=TRUE, then the bootstrap method is applied to obtain the 95% confidence intervals for each diversity and sample coverage estimate.

The list \$iNextEst output includes two data frames: \$size_based and \$coverage_based. (Note the output in the list \$iNextEst is different from that obtained from earlier iNEXT versions < 3.0.0, due to a modification in the bootstrap method.) For the sample size corresponding to each knot, the first data frame (as shown under \$size_based) includes the name of Assemblage, the sample size (m, i.e., each of the 40 knots), 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 order (order.q), the diversity estimate of order q (qD), the 95% lower and upper confidence limits of diversity (qD.LCL, qD.UCL), and the sample coverage estimate (SC) along with the 95% lower and upper confidence limits of sample coverage (SC.LCL, SC.UCL). These sample coverage estimates with confidence intervals are used for plotting the sample completeness curve.

```
$iNextEst: diversity estimates with rarefied and extrapolated samples.
$size based (LCL and UCL are obtained for fixed size.)
```

```
Assemblage m
                       Method Order.q
                                        qD qD.LCL qD.UCL
                                                           SC SC.LCL SC.UCL
1 Girdled
             1 Rarefaction
                                   0 1.000 1.000 1.000 0.122 0.089 0.156
10 Girdled
             84 Rarefaction
                                 0 18.912 15.902 21.923 0.900 0.872 0.927
20 Girdled
                     Observed
                                 0 26.000 21.492 30.508 0.929 0.904 0.954
          168
30 Girdled
            248 Extrapolation
                                  0 30.883 25.149 36.618 0.948 0.918 0.979
40 Girdled
            336 Extrapolation
                                  0 34.731 27.187 42.275 0.964 0.931 0.996
              1 Rarefaction
                                   0 1.000 1.000 1.000 0.145 0.109 0.180
41 Logged
50 Logged
            126 Rarefaction
                                   0 28.268 24.935 31.600 0.908 0.886 0.930
                                   0 37.000 31.789 42.211 0.945 0.925 0.964
60 Logged
            252
                     Observed
70 Logged
            371 Extrapolation
                                   0 42.786 35.844 49.727 0.958 0.935 0.980
80 Logged
            504 Extrapolation
                                   0 47.644 38.485 56.804 0.969 0.946 0.991
```

NOTE: The above output only shows five estimates for each assemblage; call iNEXT.object\$iNextEst\$size_based to view complete output.

The second data frame (as shown under \$coverage_based) includes the name of Assemblage, the standardized sample coverage (SC), the corresponding sample size for the standardized coverage (m, i.e.,

each of the 40 knots), the method (Rarefaction, Observed, or Extrapolation, depending on whether the sample coverage SC is less than, equal to, or greater than the reference sample coverage), the diversity order (order.q), the diversity estimate of order q (qD), and the 95% lower and upper confidence limits of diversity (qD.LCL, qD.UCL). These diversity estimates and confidence intervals are used for plotting the coverage-based R/E curves.

\$coverage_based (LCL and UCL are obtained for fixed coverage; interval length is wider due to
varying size in bootstraps.)

```
Method order.q qD qD.LCL qD.UCL
   Assemblage
                    SC m
                0.122 1 Rarefaction 0 1.000 0.857 1.143
1 Girdled
                0.900 84 Rarefaction
10 Girdled
                                                   0 18.912 10.761 27.064

      20 Girdled
      0.929 168
      Observed
      0 26.000 13.239 38.761

      30 Girdled
      0.948 248 Extrapolation
      0 30.883 12.129 49.638

40 Girdled 0.964 336 Extrapolation
                                                  0 34.731 9.788 59.673
                0.145 1 Rarefaction
41 Logged
                                                   0 1.000 0.796 1.204
                0.908 126 Rarefaction 0 1.000 0.796 1.204 0.908 126 Rarefaction 0 28.268 20.192 36.343 0.945 252 Observed 0 37.000 20.209 53.791
50 Logged
60 Logged
70 Logged
                0.958 371 Extrapolation
                                                  0 42.786 21.977 63.594
80 Logged
                0.969 504 Extrapolation
                                                  0 47.644 23.357 71.932
```

NOTE: The above output only shows five estimates for each assemblage; call iNEXT.object\$iNextEst\$coverage_based to view complete output.

In the above output (\$size_based and \$coverage_based), the confidence intervals of any standardized diversity are obtained by a bootstrap method. In the size-based standardization, the sample size is fixed in each regenerated bootstrap sample. In the coverage-based standardization, for a given standardized coverage value, the corresponding size needed to attain the same level of coverage may vary with regenerated bootstrap samples. Thus, the sampling uncertainty is greater in the coverage-based standardization and the resulting confidence interval is wider than that in the corresponding size-based standardization. For example, if the size for a future survey will be fixed at a sample size of 84, we can obtain a 95% CI of (15.9, 21.9) for the expected diversity (q = 0) based on the first data frame (\$size_based output). However, if the coverage of a survey is fixed at the level of 0.9, the size needed for the current data is 84, but the size needed for a regenerated bootstrap sample may be different from 84; the second data frame (\$coverage_based output) shows a CI of (10.8, 27.1), which is wider than the former one based on a size of 84. Because we use a random bootstrapping/regeneration process, with 50 replications (default), to obtain each CI, the output for qp.LCL and qp.UCL may vary slightly each time you enter the same data.

\$AsyEst lists the name of Assemblage, the Diversity (species richness for q = 0, Shannon diversity for q = 1, and Simpson diversity for q = 2), the observed diversity (Observed), the asymptotic diversity estimate (Estimator), the s.e. of the asymptotic estimator (s.e.) and the associated 95% lower and upper confidence limits (LCL, UCL). The estimated asymptotes are calculated via the functions ChaoRichness() for q = 0, ChaoShannon() for q = 1 and ChaoSimpson() for q = 2; see Chao et al. (2014) for the formulas of all asymptotic estimators. The output for the spider data is shown below.

\$AsyEst: asymptotic diversity estimates along with related statistics.

| | Assemblage | Diversity | Observed | Estimator | s.e. | LCL | UCL |
|---|------------|-------------------|----------|-----------|--------|--------|--------|
| 1 | Girdled | Species richness | 26.000 | 43.893 | 17.219 | 26.000 | 77.642 |
| 2 | Girdled | Shannon diversity | 12.060 | 13.826 | 1.339 | 11.201 | 16.451 |
| 3 | Girdled | Simpson diversity | 7.840 | 8.175 | 0.934 | 6.344 | 10.006 |
| 4 | Logged | Species richness | 37.000 | 61.403 | 19.692 | 37.000 | 99.998 |
| 5 | Logged | Shannon diversity | 14.421 | 16.337 | 1.864 | 12.684 | 19.990 |
| 6 | Logged | Simpson diversity | 6.761 | 6.920 | 0.926 | 5.106 | 8.734 |

The user may specify an integer sample size for the argument endpoint to designate the maximum sample size of R/E calculation. For species richness, the extrapolation method is reliable up to double the reference sample size; beyond that, the prediction bias may be large. However, for measures of q = 1 and 2, the extrapolation can usually be safely extended to the asymptote if data are not sparse; thus there is no limit for the value of the endpoint for these two measures.

The user may also specify the number of knots in the sample size range between 1 and the endpoint. If you choose a large number of knots, then it may take a long time to obtain the output due to the time-consuming nature of the bootstrap method. Alternatively, the user may specify a series of sample sizes for R/E computation, as in the following example:

```
# set a series of sample sizes (m) for R/E computation
m <- c(1, 5, 20, 50, 100, 200, 400)
iNEXT(spider, q=0, datatype="abundance", size=m)</pre>
```

Further, inext can simultaneously run R/E computation for Hill numbers with q = 0, 1, and 2 by specifying a vector for the argument q as follows:

```
out <- iNEXT(spider, q=c(0,1,2), datatype="abundance", size=m)</pre>
```

A data frame input format for abundance-based analysis is also supported:

```
data(bird)
str(bird) # 41 obs. of 2 variables
iNEXT(bird, q=0, datatype="abundance")
```

GRAPHIC DISPLAYS: FUNCTION ggiNEXT()

The function ggiNEXT(), which extends ggplot2 to the "iNEXT" object with default arguments, is described as follows:

```
ggiNEXT(x, type=1, se=TRUE, facet.var="None", color.var="Assemblage", grey=FALSE)
```

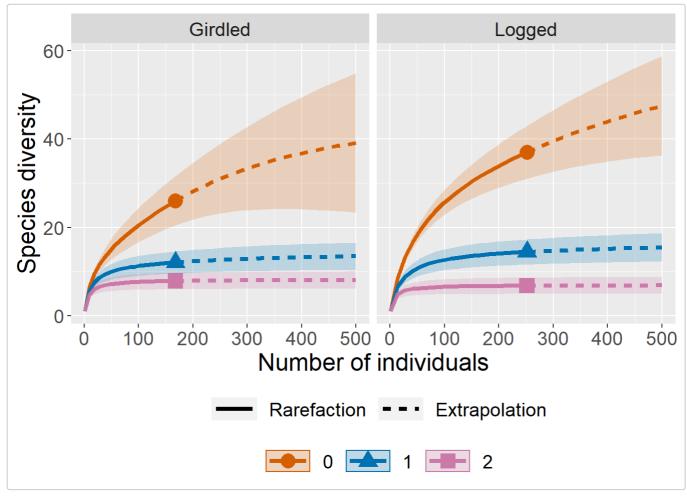
Here x is an "inext" object. Three types of curves are allowed:

- 1. Sample-size-based R/E curve (type=1): see Figs. 1a and 2a in Hsieh et al. (2016). This curve plots diversity estimates with confidence intervals (if se=TRUE) as a function of sample size up to double the reference sample size, by default, or a user-specified endpoint.
- 2. Sample completeness curve (type=2) with confidence intervals (if se=TRUE): see Figs. 1b and 2b in Hsieh et al. (2016). This curve plots the sample coverage with respect to sample size for the same range described in (1).
- 3. Coverage-based R/E curve (type=3): see Figs. 1c and 2c in Hsieh et al. (2016). This curve plots the diversity estimates with confidence intervals (if se=TRUE) as a function of sample coverage up to the maximum coverage obtained from the maximum size described in (1).

The <code>ggiNEXT()</code> function is a wrapper around the <code>ggplot2</code> package to create a R/E curve using a single line of code. The resulting object is of class <code>"ggplot"</code>, so it can be manipulated using the <code>ggplot2</code> tools. The argument <code>facet.var=("None", "Order.q", "Assemblage"</code> or <code>"Both"</code>) can be used to create a separate plot for each value of the specified variable. See the following examples.

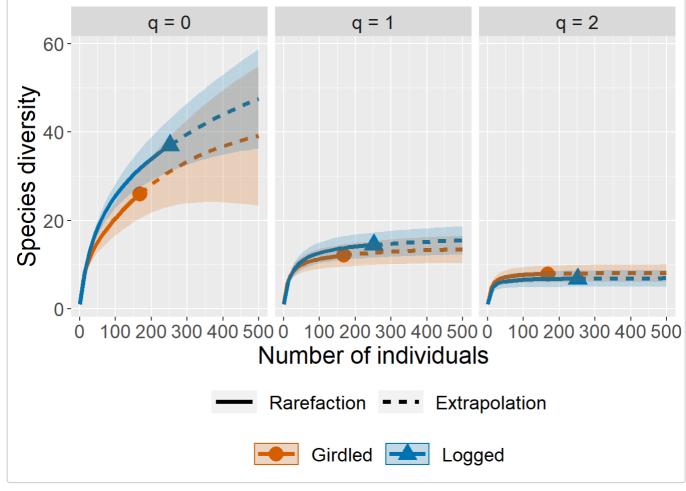
The argument facet.var="Assemblage" in the ggiNEXT function creates a separate plot for each assemblage as shown below:

```
# Sample-size-based R/E curves, separating by "Assemblage""
out <- iNEXT(spider, q=c(0, 1, 2), datatype="abundance", endpoint=500)
ggiNEXT(out, type=1, facet.var="Assemblage")</pre>
```



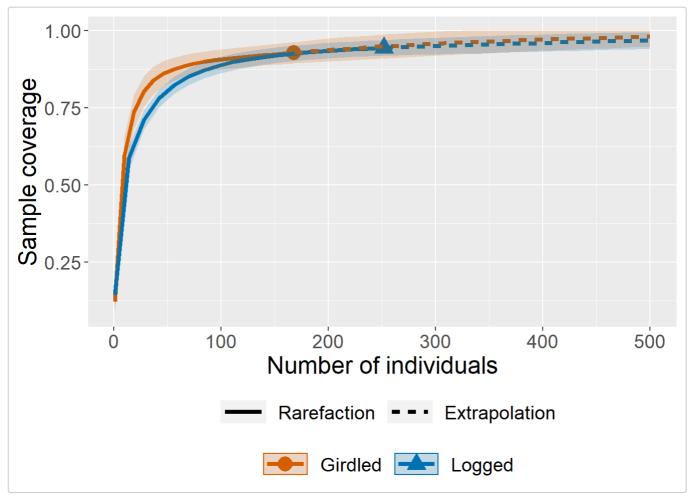
The argument facet.var="Order.q" and color.var="Assemblage" creates a separate plot for each diversity order assemblage, and within each plot, different colors are used for the two assemblages.

```
ggiNEXT(out, type=1, facet.var="Order.q", color.var="Assemblage")
```



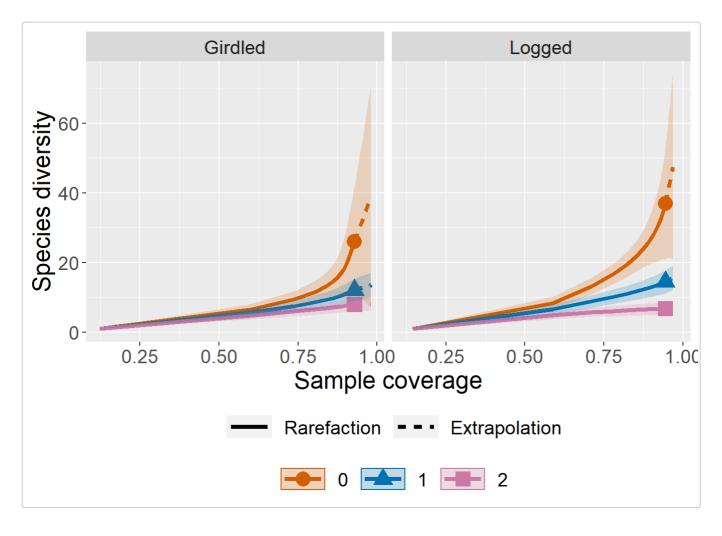
The following commands return the sample completeness curve in which different colors are used for the two assemblages:

```
ggiNEXT(out, type=2, facet.var="None", color.var="Assemblage")
```

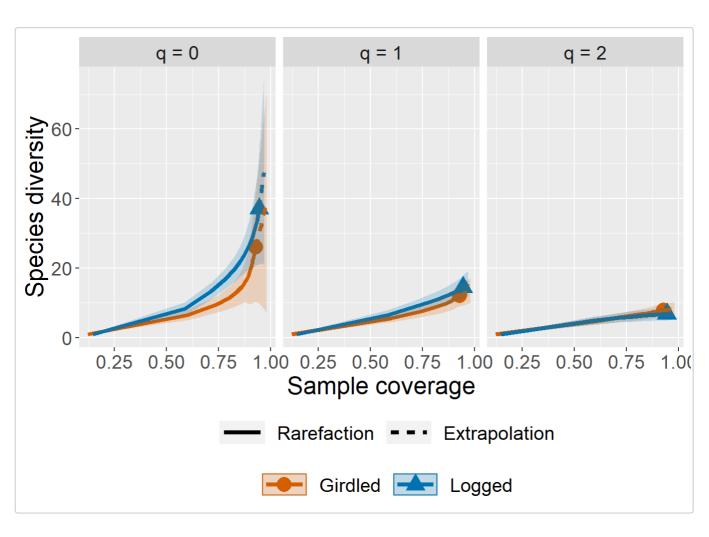


The following commands return the coverage-based R/E sampling curves in which different colors are used for the two assemblages (facet.var="Assemblage") and for three orders (facet.var="Order.q")

```
ggiNEXT(out, type=3, facet.var="Assemblage")
```



ggiNEXT(out, type=3, facet.var="Order.q", color.var="Assemblage")



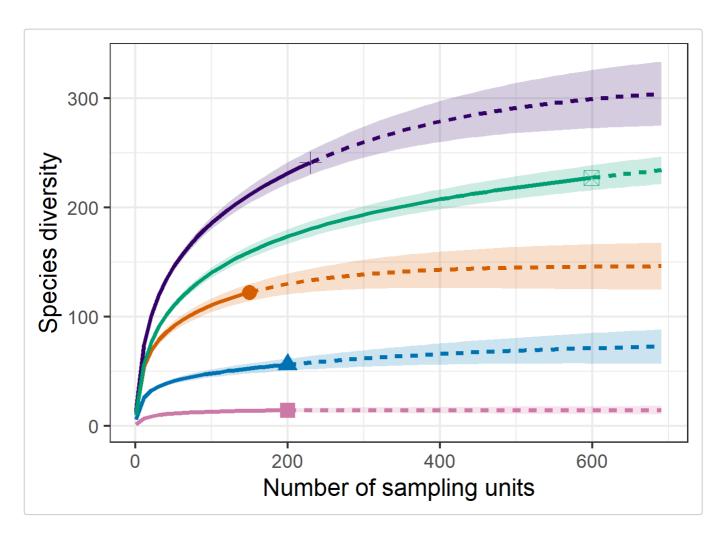
INCIDENCE DATA with datatype="incidence_freq"

For illustration, we use the tropical ant data (in the dataset ant included in the package) at five elevations (50m, 500m, 1070m, 1500m, and 2000m) collected by Longino & Colwell (2011) from Costa Rica. The 5 lists of incidence frequencies are shown below. The first entry of each list must be the total number of sampling units, followed by the species incidence frequencies.

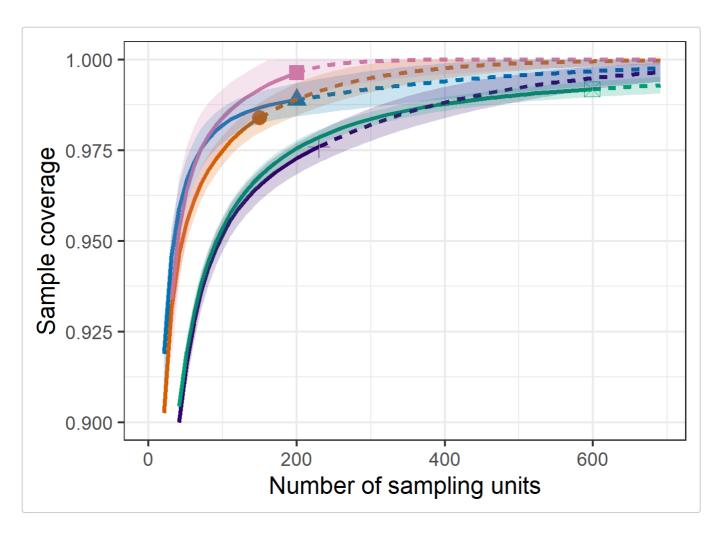
```
data(ant)
str(ant)
List of 5
$ h50m : num [1:228] 599 330 263 236 222 195 186 183 182 129 ...
$ h500m : num [1:242] 230 133 131 123 78 73 65 60 60 56 ...
$ h1070m: num [1:123] 150 99 96 80 74 68 60 54 46 45 ...
$ h1500m: num [1:57] 200 144 113 79 76 74 73 53 50 43 ...
$ h2000m: num [1:15] 200 80 59 34 23 19 15 13 8 8 ...
```

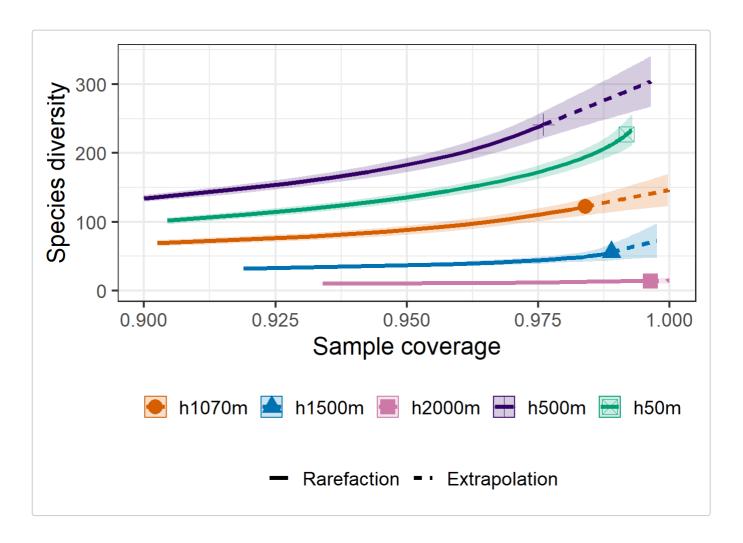
The argument color.var = ("None", "Order.q", "Assemblage" or "Both") is used to display curves in different colors for values of the specified variable. For example, the following code using the argument color.var="Assemblage" displays the sampling curves in different colors for the five assemblages. Note that theme_bw() is a ggplot2 function to modify the display setting from a grey to a white background with black gridlines. The following commands return three types of R/E sampling curves for the ant data.

```
t <- seq(1, 700, by=10)
out.inc <- iNEXT(ant, q=0, datatype="incidence_freq", size=t)
# Sample-size-based R/E curves
ggiNEXT(out.inc, type=1, color.var="Assemblage") +
    theme_bw(base_size = 18) +
    theme(legend.position="None")</pre>
```



```
# Sample completeness curves
ggiNEXT(out.inc, type=2, color.var="Assemblage") +
  ylim(c(0.9,1)) +
  theme_bw(base_size = 18) +
  theme(legend.position="None")
```





INCIDENCE DATA with datatype="incidence_raw"

We use the ciliates data collected from three coastal dune habitats to demostrate the use of the input datatype="incidence_raw". The data set (ciliates) included in the package is a list of three species-by-plots matrices. Run the following commands to get the output as shown below.

```
data(ciliates)
str(ciliates)
List of 3
 $ EtoshaPan
                      : int [1:365, 1:19] 0 0 0 0 0 0 0 0 0 0 ...
  ..- attr(*, "dimnames")=List of 2
  ....$: chr [1:365] "Acaryophrya.collaris" "Actinobolina.multinucleata.n..sp."
         "Afroamphisiella.multinucleata.n..sp." "Afrothrix.multinucleata.n..sp." ...
  .. ..$ : chr [1:19] "x53" "x54" "x55" "x56" ...
 $ CentralNamibDesert : int [1:365, 1:17] 0 0 0 0 0 1 0 0 0 0 ...
  ..- attr(*, "dimnames")=List of 2
  ....$: chr [1:365] "Acaryophrya.collaris" "Actinobolina.multinucleata.n..sp."
         "Afroamphisiella.multinucleata.n..sp." "Afrothrix.multinucleata.n..sp." ...
  .. ..$ : chr [1:17] "x31" "x32" "x34" "x35" ...
 $ SouthernNamibDesert: int [1:365, 1:15] 0 0 0 0 0 0 0 0 0 0 ...
  ... attr(*, "dimnames")=List of 2
  ....$: chr [1:365] "Acaryophrya.collaris" "Actinobolina.multinucleata.n..sp."
         "Afroamphisiella.multinucleata.n..sp." "Afrothrix.multinucleata.n..sp." ...
  .. ..$ : chr [1:15] "x9" "x17" "x19" "x20" ...
out.raw <- iNEXT(ciliates, q = 0, datatype="incidence_raw", endpoint=150)
out.raw
```

Compare 3 assemblages with Hill number order q = 0.

\$class: iNEXT

\$DataInfo: basic data information

```
Assemblage T U S.obs SC Q1 Q2 Q3 Q4 Q5 Q6 Q7 Q8 Q9 Q10 1 EtoshaPan 19 516 216 0.8017 107 44 26 14 6 5 4 3 2 2 2 CentralNamibDesert 17 379 130 0.8425 63 28 13 4 3 7 1 2 1 0 3 SouthernNamibDesert 15 358 150 0.7816 82 28 14 8 6 1 1 2 2 1
```

\$iNextEst: diversity estimates with rarefied and extrapolated samples. \$size_based (LCL and UCL are obtained for fixed size.)

| | Assemblage | t | Method | Order.q | qD | qD.LCL | qD.UCL | SC | SC.LCL | SC.UCL |
|-----|-----------------------------|-----|-----------------------|---------|---------|---------|---------|-------|--------|--------|
| 1 | EtoshaPan | 1 | Rarefaction | 0 | 27.158 | 24.893 | 29.423 | 0.190 | 0.164 | 0.216 |
| 10 | EtoshaPan | 10 | Rarefaction | 0 | 153.260 | 141.984 | 164.536 | 0.680 | 0.644 | 0.716 |
| 20 | EtoshaPan | 20 | ${\tt Extrapolation}$ | 0 | 221.386 | 203.508 | 239.263 | 0.810 | 0.778 | 0.843 |
| 30 | EtoshaPan | 88 | ${\tt Extrapolation}$ | 0 | 333.606 | 291.244 | 375.967 | 0.991 | 0.985 | 0.997 |
| 39 | EtoshaPan | 150 | ${\tt Extrapolation}$ | 0 | 338.901 | 293.346 | 384.456 | 0.999 | 0.999 | 1.000 |
| 40 | CentralNamibDesert | 1 | Rarefaction | 0 | 22.294 | 20.579 | 24.009 | 0.374 | 0.340 | 0.408 |
| 49 | CentralNamibDesert | 10 | Rarefaction | 0 | 98.993 | 90.442 | 107.545 | 0.764 | 0.736 | 0.791 |
| 58 | CentralNamibDesert | 24 | ${\tt Extrapolation}$ | 0 | 151.018 | 135.480 | 166.557 | 0.892 | 0.856 | 0.929 |
| 67 | CentralNamibDesert | 87 | Extrapolation | 0 | 195.191 | 156.456 | 233.925 | 0.996 | 0.990 | 1.000 |
| 76 | CentralNamibDesert | 150 | ${\tt Extrapolation}$ | 0 | 196.656 | 154.935 | 238.377 | 1.000 | 0.999 | 1.000 |
| 77 | ${\tt SouthernNamibDesert}$ | 1 | Rarefaction | 0 | 23.867 | 21.566 | 26.167 | 0.282 | 0.245 | 0.319 |
| 85 | ${\tt SouthernNamibDesert}$ | 9 | Rarefaction | 0 | 112.485 | 103.115 | 121.854 | 0.699 | 0.657 | 0.741 |
| 94 | ${\tt SouthernNamibDesert}$ | 30 | Extrapolation | 0 | 207.213 | 185.149 | 229.276 | 0.893 | 0.846 | 0.940 |
| 103 | ${\tt SouthernNamibDesert}$ | 93 | Extrapolation | 0 | 259.337 | 209.899 | 308.776 | 0.995 | 0.988 | 1.000 |
| 111 | SouthernNamibDesert | 150 | Extrapolation | 0 | 261.886 | 209.174 | 314.598 | 1.000 | 0.999 | 1.000 |

NOTE: The above output only shows five estimates for each assemblage; call iNEXT.object\$iNextEst\$size_based to view complete output.

\$coverage_based (LCL and UCL are obtained for fixed coverage; interval length is wider due to varying size in bootstraps.)

```
SC t
                                           Method Order.q
            Assemblage
                                                              qD qD.LCL qD.UCL
             EtoshaPan 0.1901402 1
                                      Rarefaction
1
                                                       0 27.158 24.894 29.422
             EtoshaPan 0.6799226 10
10
                                      Rarefaction
                                                       0 153.260 135.451 171.069
             EtoshaPan 0.8103610 20 Extrapolation
                                                       0 221.386 194.992 247.779
20
             EtoshaPan 0.9909111 88 Extrapolation
                                                       0 333.606 288.842 378.369
30
                                                       0 338.901 293.087 384.715
39
             EtoshaPan 0.9994305 150 Extrapolation
40
    CentralNamibDesert 0.3743404 1
                                      Rarefaction
                                                       0 22.294 20.572 24.016
    CentralNamibDesert 0.7635124 10
                                      Rarefaction
                                                       0 98.993 86.617 111.369
    CentralNamibDesert 0.8921419 24 Extrapolation
                                                       0 151.018 125.356 176.681
58
67
    CentralNamibDesert 0.9964228 87 Extrapolation
                                                       0 195.191 153.805 236.576
76
    CentralNamibDesert 0.9998814 150 Extrapolation
                                                       0 196.656 154.657 238.655
77 SouthernNamibDesert 0.2821229 1
                                      Rarefaction
                                                       0 23.867 21.581 26.152
                                                       0 112.484 97.518 127.451
85 SouthernNamibDesert 0.6993084 9
                                      Rarefaction
94 SouthernNamibDesert 0.8931001 30 Extrapolation
                                                       0 207.213 171.199 243.227
103 SouthernNamibDesert 0.9946808 93 Extrapolation
                                                       0 259.337 207.038 311.636
111 SouthernNamibDesert 0.9996478 150 Extrapolation
                                                       0 261.886 208.751 315.021
```

NOTE: The above output only shows five estimates for each assemblage; call iNEXT.object\$iNextEst\$coverage based to view complete output.

\$AsyEst: asymptotic diversity estimates along with related statistics.

```
Assemblage
                            Diversity Observed Estimator
                                                          s.e.
                                                                  LCL
                                                                          UCL
1 CentralNamibDesert Species richness 130.000
                                                196.706 19.523 158.441 234.971
  CentralNamibDesert Shannon diversity
                                       81.812 106.480 5.291 96.110 116.850
3
  CentralNamibDesert Simpson diversity
                                       54.225
                                                59.556 3.175 53.333 65.778
           EtoshaPan Species richness 216.000 339.255 23.121 293.938 384.571
5
           EtoshaPan Shannon diversity 158.367 222.936 11.125 201.130 244.741
           EtoshaPan Simpson diversity 116.677
                                                142.833 8.700 125.780 159.885
6
7 SouthernNamibDesert Species richness 150.000 262.067 30.638 202.018 322.115
8 SouthernNamibDesert Shannon diversity 103.705
                                                149.910 9.301 131.681 168.139
9 SouthernNamibDesert Simpson diversity
                                       72.327
                                                 84.597 5.276 74.255 94.938
```

POINT ESTIMATION FUNCTION: estimateD()

We also supply the following function

```
estimateD(x, datatype="abundance", base="size", level=NULL)
```

to compute diversity estimates with q = 0, 1, 2 for any particular level of sample size (base="size") or any specified level of sample coverage (base="coverage") for abundance data (datatype="abundance") or incidence data (datatype="incidence_freq" or "incidence_raw"). If base="size" and level=NULL, then this function computes the diversity estimates for the minimum among all doubled reference sample sizes. If base="coverage" and level=NULL, then this function computes the diversity estimates for the minimum among the coverage values for samples extrapolated to double the size of the reference sample.

The following command returns the species diversity with a specified level of sample coverage of 98.5% for the ant data. For some assemblages, this coverage value corresponds to rarefaction (i.e., less than the coverage of the reference sample), while for the others it corresponds to extrapolation (i.e., greater than the coverage of the reference sample), as indicated under the method column of the output.

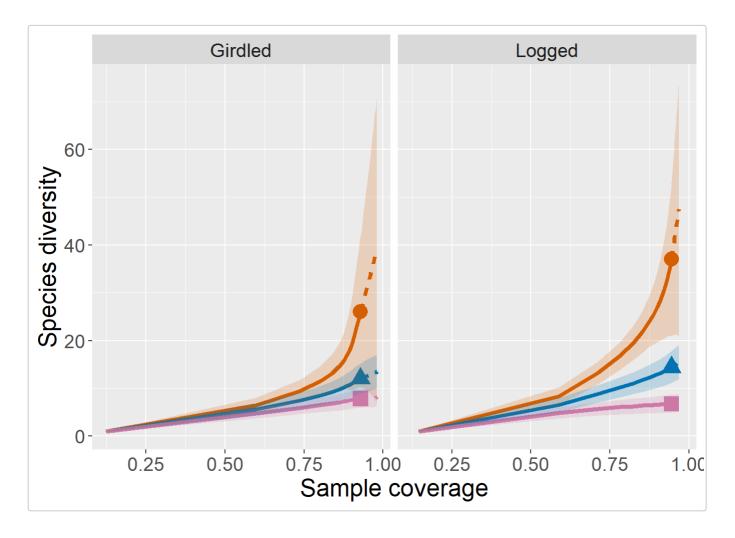
```
Assemblage
                            Method Order.q
                                              SC
                                                     qD qD.LCL qD.UCL
1
        h50m 327.165
                       Rarefaction
                                        0 0.985 197.488 186.058 208.918
2
        h50m 327.165
                       Rarefaction
                                         1 0.985 78.053 75.389 80.717
                       Rarefaction
                                        2 0.985 50.461 48.640 52.282
3
        h50m 327.165
4
       h500m 342.859 Extrapolation
                                        0 0.985 268.726 242.802 294.650
5
       h500m 342.859 Extrapolation
                                         1 0.985 103.847 100.256 107.438
       h500m 342.859 Extrapolation
                                         2 0.985 64.758 61.983 67.534
6
7
      h1070m 158.951 Extrapolation
                                         0 0.985 123.609 113.000 134.218
      h1070m 158.951 Extrapolation
                                        1 0.985 59.592 56.903 62.280
9
      h1070m 158.951 Extrapolation
                                         2 0.985 41.775 39.465 44.085
10
      h1500m 125.959
                       Rarefaction
                                        0 0.985 50.479 41.666 59.291
      h1500m 125.959
                       Rarefaction
11
                                        1 0.985 26.249
                                                         24.575 27.923
12
      h1500m 125.959
                       Rarefaction
                                         2 0.985 18.649
                                                         17.446 19.852
13
      h2000m 104.631
                       Rarefaction
                                         0 0.985 12.910 11.002 14.817
14
                       Rarefaction
      h2000m 104.631
                                         1 0.985 7.711
                                                          6.915
                                                                  8,506
      h2000m 104.631
15
                       Rarefaction
                                         2 0.985
                                                  5.795
                                                          5.079
                                                                  6.510
```

Hacking ggiNEXT()

The <code>ggiNEXT()</code> function is a wrapper around the <code>ggplot2</code> package to create a R/E curve using a single line of code. The resulting object is of class <code>"ggplot"</code>, so it can be manipulated using the <code>ggplot2</code> tools. The following are some useful examples for customizing graphs.

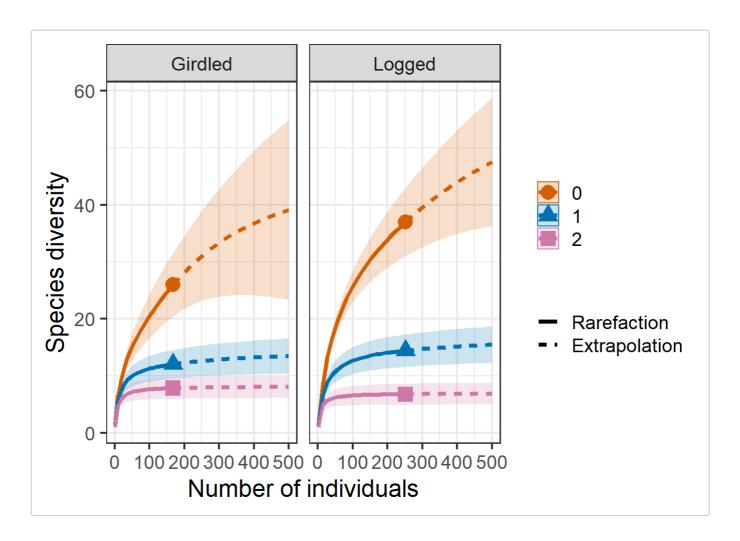
Remove legend

```
ggiNEXT(out, type=3, facet.var="Assemblage") +
  theme(legend.position="None")
```



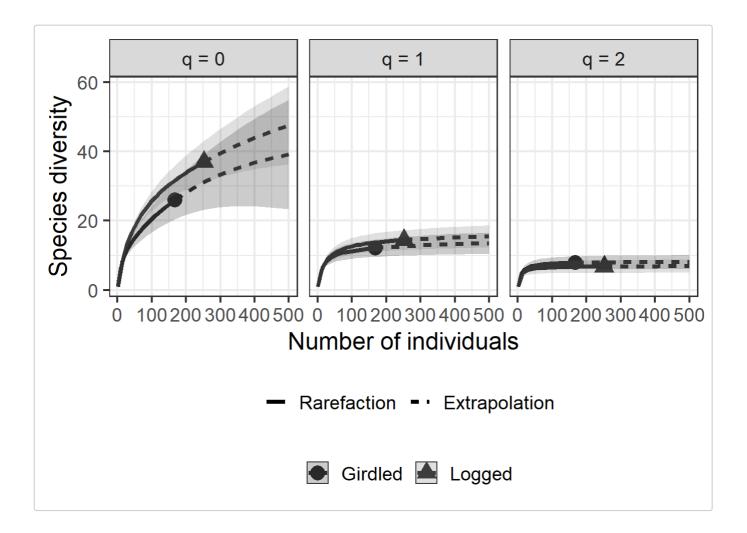
Change the theme and legend.position

```
ggiNEXT(out, type=1, facet.var="Assemblage") +
  theme_bw(base_size = 18) +
  theme(legend.position="right")
```



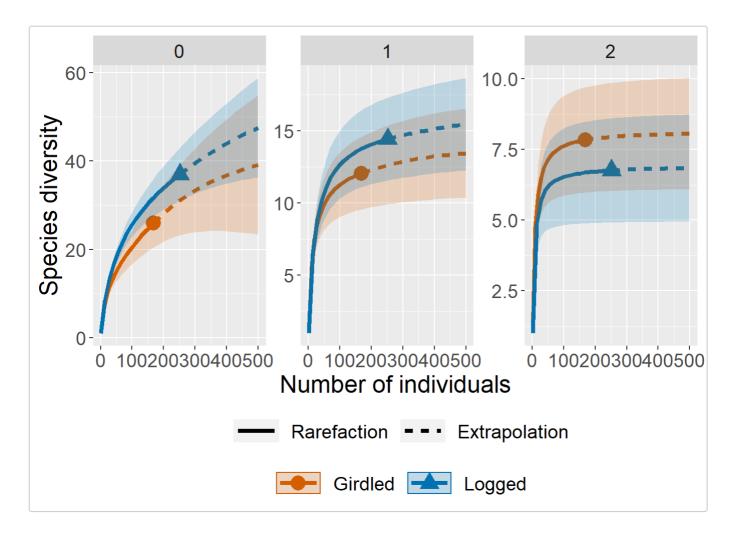
Display black-white figures

ggiNEXT(out, type=1, facet.var="Order.q", grey=TRUE)



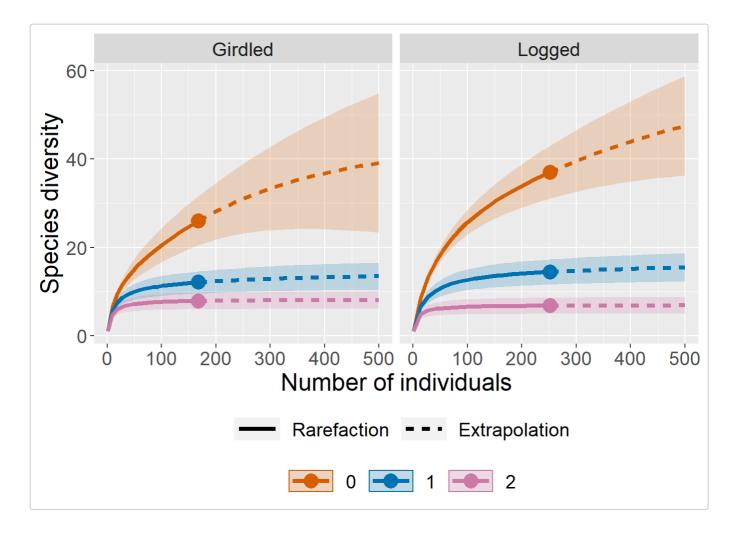
Free the scales of the axis

```
ggiNEXT(out, type=1, facet.var="Order.q") +
facet_wrap(~Order.q, scales="free")
```



change the shape of the reference sample point

```
ggiNEXT(out, type=1, facet.var="Assemblage") +
scale_shape_manual(values=c(19,19,19))
```



General customization

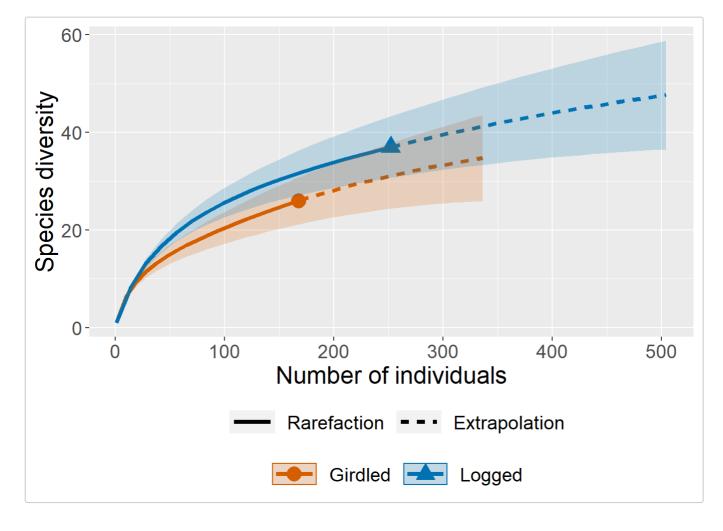
The data visualization package <code>ggplot2</code> provides the <code>scale_</code> function to customize data which is mapped into an aesthetic property of a <code>geom_</code>. The following functions would help user to customize <code>ggiNEXT</code> output.

- change point shape: scale_shape_manual
- change line type : scale_linetype_manual
- change line color: scale_colour_manual
- change band color: scale_fill_manual see <u>quick reference</u> for style setting.

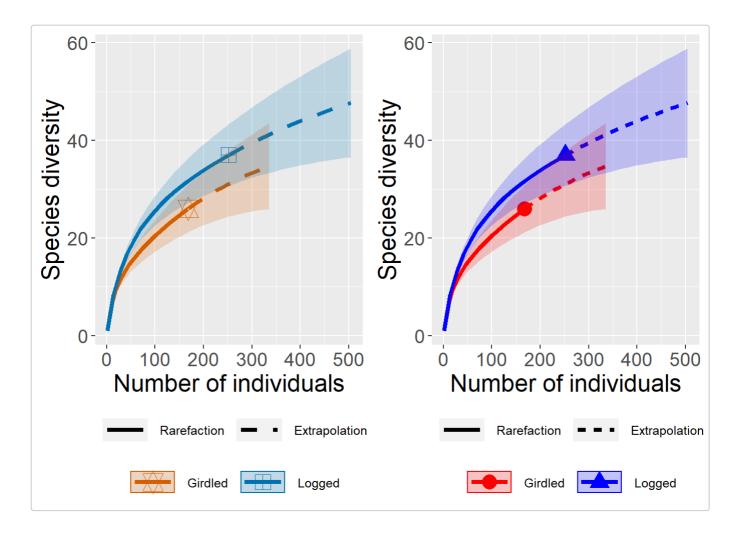
Example: spider data

To show how to custmize ggiNEXT output, we use abundance-based data spider as an example.

```
library(iNEXT)
library(ggplot2)
library(gridExtra)
library(grid)
data("spider")
out <- iNEXT(spider, q=0, datatype="abundance")
g <- ggiNEXT(out, type=1, color.var = "Assemblage")
g</pre>
```



Change shapes, line types and colors



Customize point/line size by hacking

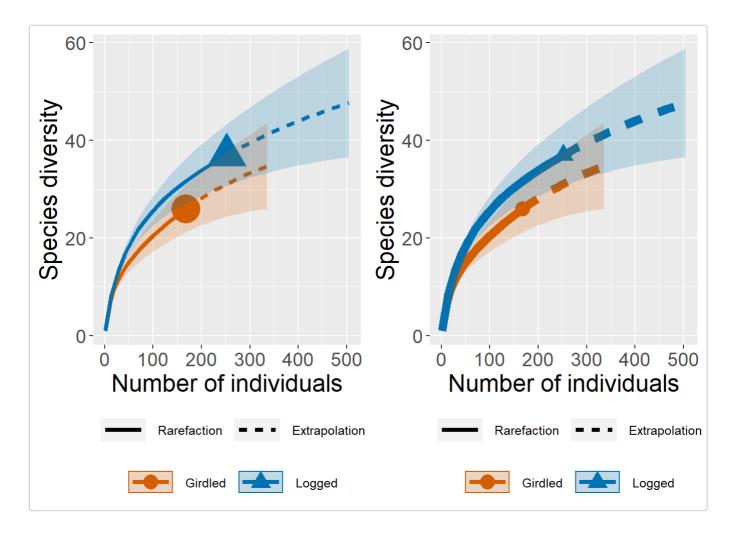
In order to change the size of the reference sample point or rarefaction/extrapolation curve, the user need to modify the <code>ggplot</code> object.

change point size:
 the reference sample size point is drawn on the first layer by ggiNEXT. Hack the point size by the following

```
# point is drawn on the 1st layer, default size is 5
gb3 <- ggplot_build(g + theme(legend.text = element_text(size = 9.5)))
gb3$data[[1]]$size <- 10
gt3 <- ggplot_gtable(gb3)
# use grid.draw to draw the graphical object
# Library(grid)
# grid.draw(gt3)</pre>
```

change line width (size):
 the reference sample size point is drawn on the second layer by ggiNEXT. Hack the point size by the following

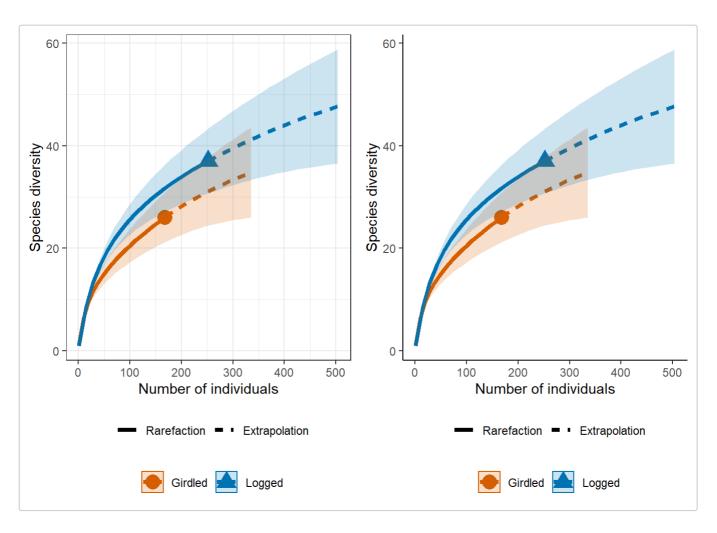
```
# Line is drawn on the 2nd Layer, default size is 1.5
gb4 <- ggplot_build(g + theme(legend.text = element_text(size = 9.5)))
gb4$data[[2]]$size <- 3
gt4 <- ggplot_gtable(gb4)
# grid.draw(gt4)</pre>
```

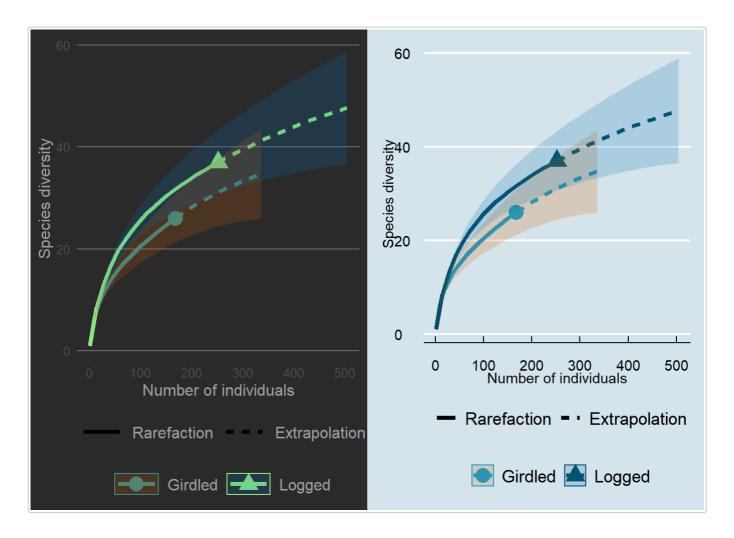


Customize theme

A ggplot object can be themed by adding a theme. The User could run help(theme_grey) to show the default themes in ggplot2. Further, some extra themes are provided by the ggthemes package. Examples are shown in the following:

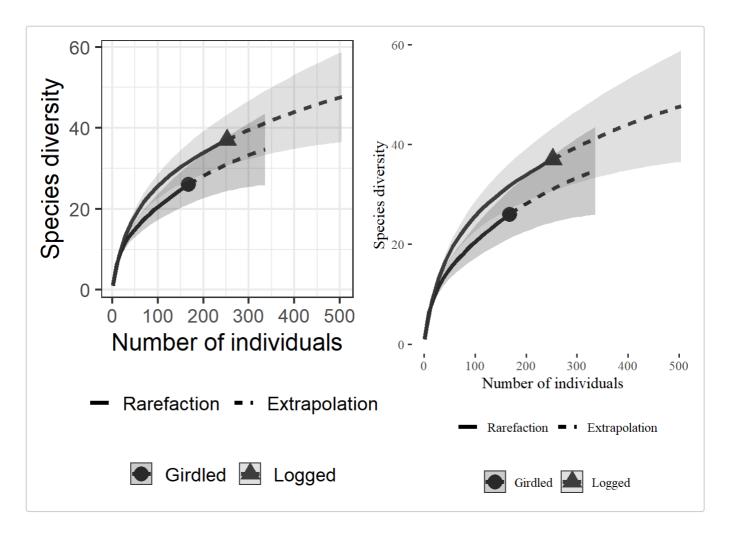
```
g5 <- g + theme_bw() + theme(legend.position = "bottom", legend.box = "vertical")
g6 <- g + theme_classic() + theme(legend.position = "bottom", legend.box = "vertical")
grid.arrange(g5, g6, ncol=2)</pre>
```





Black-White figures

The following are custmized themes for black-white figures. To modify the legend, see Cookbook for R for more details.

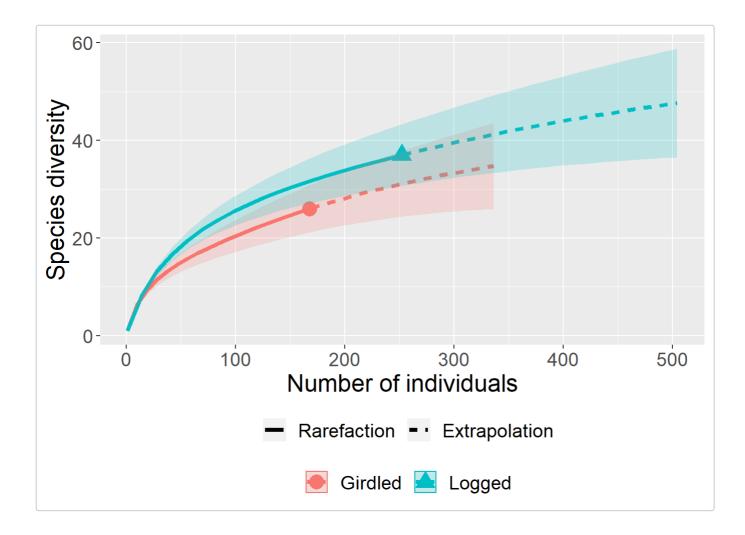


Draw R/E curves by yourself

In <u>inext</u>, we provide a S3 ggplot2::fortify method for class inext. The function fortify offers a single plotting interface for rarefaction/extrapolation curves. Set argument type = 1, 2, 3 to plot the corresponding rarefaction/extrapolation curves.

```
df <- fortify(out, type=1)</pre>
head(df)
   datatype plottype Assemblage
                                     Method Order.q x
                                                                      y.lwr
                                                                               y.upr
1 abundance
                   1
                        Girdled Rarefaction
                                                   0 1 1.000000 1.000000
                                                                             1.00000
2 abundance
                        Girdled Rarefaction
                                                   0 10 6.478617 5.983295 6.97394
3 abundance
                   1
                        Girdled Rarefaction
                                                   0 19 9.450323 8.530705 10.36994
                        Girdled Rarefaction
4 abundance
                   1
                                                   0 28 11.514220 10.253775 12.77466
5 abundance
                        Girdled Rarefaction
                                                   0 37 13.126817 11.575192 14.67844
6 abundance
                        Girdled Rarefaction
                                                   0 47 14.622424 12.778255 16.46659
df.point <- df[which(df$Method=="Observed"),]</pre>
df.line <- df[which(df$Method!="Observed"),]</pre>
df.line$Method <- factor(df.line$Method,</pre>
                         c("Rarefaction", "Extrapolation"),
                         c("Rarefaction", "Extrapolation"))
ggplot(df, aes(x=x, y=y, colour=Assemblage)) +
  geom_point(aes(shape=Assemblage), size=5, data=df.point) +
  geom_line(aes(linetype=Method), lwd=1.5, data=df.line) +
  geom_ribbon(aes(ymin=y.lwr, ymax=y.upr,
                  fill=Assemblage, colour=NULL), alpha=0.2) +
  labs(x="Number of individuals", y="Species diversity") +
```

```
theme(legend.position = "bottom",
    legend.title=element_blank(),
    text=element_text(size=18),
    legend.box = "vertical")
```



License

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

References

- Chao, A., Gotelli, N.J., Hsieh, T.C., Sander, E.L., Ma, K.H., Colwell, R.K. & Ellison, A.M. (2014)
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 Ecology and Evolution, 1, 168–179.
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| 0 | Longino, J.T. & Colwell, R.K. (2011) Density compensation, species composition, and richness of ants on a neotropical elevational gradient. Ecosphere, 2:art29. | | | | | | | |
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