

## Rarefaction and nonrandom spatial dispersion patterns

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**Abstract** Rarefaction estimates how many species are expected in a random sample of individuals from a larger collection and allows meaningful comparisons among collections of different sizes. It assumes random spatial dispersion. However, two common dispersion patterns, within-species clumping and segregation among species, can cause rarefaction to overestimate the species richness of a smaller continuous area. We use field studies and computer simulations to determine (1) how robust rarefaction is to nonrandom spatial dispersion and (2) whether simple measures of spatial autocorrelation can predict the bias in rarefaction estimates. Rarefaction does not estimate species richness accurately for many communities, especially at small sample sizes. Measures of spatial autocorrelation of the more abundant species do not reliably predict amount of bias. Survey sites should be standardized to equal-sized areas before sampling. When sites are of equal area but differ in number of individuals sampled, rarefaction can standardize collections. When communities are sampled from

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different-sized areas, the mean and confidence intervals of species accumulation curves allow more meaningful comparisons among sites.

**Keywords** Sampling · Spatial autocorrelation · Species accumulation curves · Species diversity · Species richness

## 1 Introduction

Species diversity is a central theme in ecology (see [Preston 1962](#); [MacArthur and Wilson 1967](#); [Magurran 1988](#); [Ricklefs and Schluter 1993](#); [Rosenzweig 1995](#)), but species diversity indices are semantically, conceptually, and statistically problematic ([Hurlbert 1971](#)). The fundamental problem in quantifying community structure is that one variable does not adequately capture a complex phenomenon. Several factors determine community structure: the number of species, their relative abundances, the number of individuals, and the size of the area sampled ([James and Rathbun 1981](#)). To combine these variables into one statistic obscures their relative importance and discards much information ([James and Rathbun 1981](#); [Magurran 1988](#)).

Unlike species diversity indices, species richness does not confound the number of species with their abundance distribution, and some have argued that species richness better indicates community structure ([Magurran 1988](#); [Brewer and Williamson 1994](#)). However, because the number of species increases with sample size, a direct comparison of species richness between two samples may not be ecologically meaningful; differences in community structure may be confounded with differences in sampling intensity. One solution to this problem is rarefaction (Appendix A), a technique that attempts to remove the effect of sampling differences among collections of different sizes ([Simberloff 1979](#); [James and Rathbun 1981](#); [Magurran 1988](#)). Rarefaction uses a community's species abundance distribution to calculate a curve of the expected number of species vs. subsample size. Instead of comparing the number of species in a small collection of  $n_s$  individuals to the number in a larger collection of  $n_l$  individuals, one compares the number of species from the smaller collection to the number expected in a sample of  $n_s$  individuals from the larger one. After rarefaction, differences in species richness or species diversity can be ascribed to real differences in community structure, not sample size differences.

### 1.1 Assumption of spatial randomness

As do all commonly used measures of community structure, the rarefaction procedure (Appendix A) makes three assumptions: 1) The collection is a statistically adequate, representative sample of the community ([Tipper 1979](#)), 2) Conspecifics are uniformly dispersed, and 3) Species are dispersed independently. That is, the intra- and interspecific spatial dispersion patterns are both completely random. In a community with such a dispersion pattern (no spatial autocorrelation), rarefaction accurately estimates species richness at various sample sizes.

However, nonrandom spatial dispersion patterns typify ecology (Carpenter and Chaney 1983; Palmer 1988; Legendre and Fortin 1989; Meentemeyer 1989; Dutilleul and Legendre 1993; Legendre 1993). Two common nonrandom patterns, clumping within a species and segregation among species, can cause the rarefied estimate to exceed the actual values one would obtain in a sample from a smaller continuous area (Fager 1972; Heck et al. 1975; Simberloff 1979; Kobayashi 1981, 1982, 1983). The reasoning is simple: a sample from one location might well include many individuals from a few species (e.g., a grove of trees). In contrast, rarefaction selects individuals randomly from the whole sample so selecting entire clumps of conspecifics is unlikely. Thus, these common dispersion patterns cause rarefaction to overestimate the species richness of a smaller collection.

Here, we examine how robust rarefaction is to violation of the assumptions that conspecifics are uniform-randomly and independently dispersed and species are dispersed independently. For ten field studies, we measure the bias in rarefaction estimates for different size samples. We then simulate communities with different degrees and types of spatial autocorrelation. Using multiple linear regression, on both the field studies and the simulated communities, we determine whether simple spatial autocorrelation measures that can be estimated in the field (e.g., nearest neighbor distances among the more common species) can predict bias in rarefaction estimates.

## 2 Methods

### 2.1 Empirical analyses

The 10 data sets differed markedly in number of individuals collected, number of species present, and survey area (Appendix B; appendices B-E are located online at <http://invasions.bio.utk.edu/rarefaction/>). Each data set identified the species and spatial location of each individual in a continuous area. For each collection, we compared the rarefaction curve to corresponding species accumulation curves, which plot the cumulative number of species discovered as a function of the number of individuals collected (Colwell and Coddington 1994). An accumulation curve differs from a rarefaction curve because the latter is formed by random subsampling of a collection whereas a species accumulation curve is created by adding individuals and species in the order in which they are observed. Thus, a species accumulation curve is sensitive to a community's spatial dispersion pattern, and a rarefaction curve is not.

Because species accumulation curves are likely to vary depending on where the collecting begins, we compared each rarefaction curve to the mean of 1,000 species accumulation curves. If a community's spatial dispersion pattern is random, the average species accumulation curve should match a rarefaction curve. With clumping within species or segregation among species, an average species accumulation curve should be lower than the rarefaction curve.

For each data set, we constructed two types of species accumulation curves. In the first, we added individuals to the curve by sequentially adding the nearest individual to a randomly selected point until all individuals were included. Until a boundary is reached, this procedure is identical to adding individuals in an expanding circle. Condit et al. (1996) postulated that the difference between sampling from circular

and square areas should be small. In the second method, we tested this hypothesis by adding individuals in increasing squares.

## 2.2 Calculation of rarefaction bias

We measured rarefaction bias caused by nonrandom spatial patterns as the percent by which rarefaction overestimates species richness obtained in species accumulation curves, calculated by dividing the difference in area between the rarefaction curve and the mean species accumulation curve by the area beneath the latter, numerically integrated over  $n$ , and multiplied by 100:

$$bias = 100 * \sum_{p=1}^{subsamples} (((E(S_p) - \bar{x}_p)/(\bar{x}_p)) * ((n_{p+1} - n_{p-1})/2)) \quad (1)$$

where  $E(S_p)$  is the expected mean number of species in subsample  $p$  from rarefaction,  $\bar{x}_p$  is the mean number of species in subsample  $p$  from the species accumulation curves, and  $n_p$  is the number of individuals in subsample  $p$ .

We constructed 1,000 species accumulation curves and calculated actual species richness at a given sample size as the mean value of the species accumulation curves at that size. We used Monte Carlo simulations to determine whether the mean species accumulation curve differs from the corresponding rarefaction curve (Manly 1997; Appendix C). For a given number of individuals, if 95% of the species accumulation curves had fewer species than the rarefaction estimate, we considered the two curves to differ.

## 2.3 Generation of simulated communities

We simulated communities with two-dimensional spatial dispersion patterns by computer. We generated four data sets, each of 60 simulated communities. Each census plot was represented by a unit square. For each community, we used species abundances from Sanders' (1968) hypothetical community of 40 species with Simberloff's (1972) corrected total number of individuals equaling 1,002, not 1,000. Other researchers (e.g., Fager 1972; Simberloff 1972) have used this community to study rarefaction. In every simulated community, species A was most abundant (365 individuals), B was second (112 individuals), and C was third (81 individuals). We created nonrandom community dispersion patterns using two processes: the double Poisson process to create clumping within species and the nonhomogeneous Poisson process to generate positive and negative associations among species (Appendix C).

We used these two processes to manipulate the relationship between measures of clumping across communities. We varied these relationships so clumping measures were correlated across communities in some data sets but not in others (Appendix C). In the first simulated data set, the spatial dispersion patterns of the three most abundant species are uncorrelated. In the second data set, the spatial dispersion patterns of these species are correlated across communities as in the

empirical data set: as one species becomes more clumped, the other two become more clumped and segregation between species increases. In the third data set, the eight most abundant species have random dispersion patterns in each community, and less abundant species vary in intensity of clumping among communities. In the fourth simulated data set, each of these eight species maintains one random point pattern in all communities, and less abundant species vary in intensity of clumping among communities.

## 2.4 Measuring spatial autocorrelation

To apply simulation results to collections for which little is known about dispersion, we used mean nearest neighbor distances to measure within-species clumping, and we analyzed spatial autocorrelation only for the three most common species. We standardized all spatial autocorrelation measures by the distance expected for a uniform random spatial dispersion pattern (Appendix C). That nearest neighbor distances do not characterize spatial dispersion patterns completely accurately need not mean they cannot predict rarefaction bias: this is an empirical matter.

## 2.5 Statistical analyses

For the empirical data set with 10 communities and four simulated data sets with 60 communities each, we used multiple linear regression to determine how well several simple measures of spatial autocorrelation predict rarefaction bias. For each community, we calculated percent rarefaction bias and 12 measures of spatial autocorrelation: mean nearest neighbor distance for each of the three most abundant species (nnAA, nnBB, nnCC), segregation among these three species (nnAB, nnAC, nnBA, nnBC, nnCA, and nnCB), and average (not nearest) neighbor distance for conspecifics of the three most common species (avgA, avgB, avgC). When necessary, we transformed variables to meet assumptions of linear regression.

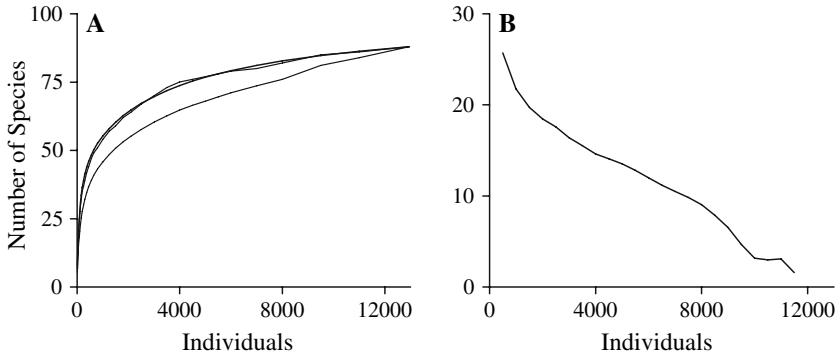
# 3 Results

## 3.1 Difference between square and circular species accumulation curves

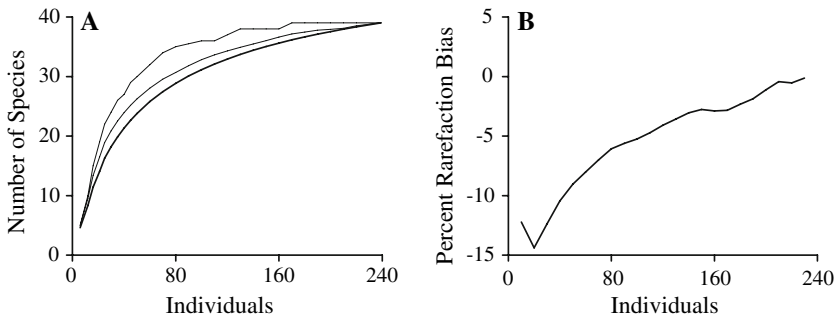
For the ten field data sets, there are negligible differences between circular and square species accumulation curves. When a difference does exist, square plots contain slightly more species on average. For the rest of the analyses, we compare rarefaction curves to species accumulation curves that add individuals in an increasing square, a conservative choice for examining rarefaction bias caused by nonrandom spatial patterns.

## 3.2 Rarefaction bias in empirical communities

Rarefaction overestimates actual species richness for eight of ten collections (mean bias=14.1%, range 4.4–34.2%; Appendix D). Figure 1a shows that the



**Fig. 1** A comparison between the mean of 1,000 species accumulation curves, the upper 95th percentile species accumulation curve, and the corresponding rarefaction curve for Luquillo Experimental Forest, a representative plant community (a). The rarefaction curve is in bold; the mean and 95th percentile species accumulation curve are not. Rarefaction bias for Luquillo Experimental Forest (b)



**Fig. 2** A comparison between the mean of 1,000 species accumulation curves, the upper 95th percentile species accumulation curve, and the corresponding rarefaction curve for summer birds in Wade Tract (a). The rarefaction curve is in bold; the mean and 95th percentile species accumulation curve are not. Rarefaction bias for summer birds in Wade Tract (b)

rarefaction curve is near the upper 95th percentile species accumulation curve; this curve is representative of the other seven collections (Appendix D). In these communities, rarefaction bias generally decreases as subsample size increases (Fig. 1b; Appendix D).

In the remaining two communities (summer bird communities) rarefaction slightly *underestimates* actual species richness (−1.7 and −4.3%; Fig. 2a; Appendix D). Rarefaction bias approaches zero as subsample size increases (Fig. 2b; Appendix D).

### 3.3 Rarefaction bias and nonrandom spatial patterns: empirical data set

The 12 measures of spatial autocorrelation vary widely among the 10 empirical communities (Appendix E). The 10 communities fall into two groups based on nearest neighbor measures. Most communities exhibit clumping within species and segregation among species. However, the two summer bird communities show the opposite

pattern; the three most abundant species exhibiting overdispersed intraspecific patterns and positive interspecific associations (aggregation).

The 12 measures of spatial autocorrelation are highly correlated. To reduce multicollinearity, we collapsed the 12 measures of spatial autocorrelation into three variables. We combined the three nearest neighbor distances into one measure, mean clumping. We also combined the six segregation measures into one statistic, average segregation distance. We combined the three average neighbor distances into one statistic, mean average neighbor distance. These three measures are still strongly correlated: mean clumping and mean average neighbor distance are positively correlated (0.92), and average segregation is negatively correlated with mean clumping ( $-0.68$ ) and with mean average neighbor distance ( $-0.77$ ). When one of these three measures changes to increase rarefaction bias, correlated changes in the other variables should also increase rarefaction bias. For example, when the nearest neighbor distances fall, average neighbor distances also fall, and segregation between species increases. These correlations should increase the ability of one measure to predict the amount of rarefaction bias. A regression of rarefaction bias on mean clumping explains 37% of the variation but is only marginally significant ( $R^2 = 0.373$ ;  $F_{1,8} = 4.75$ ;  $p = 0.061$ ). A regression of rarefaction bias on nearest neighbor distance for the most abundant species (nnAA) explains only 17% of the variation in rarefaction bias and is not significant ( $R^2 = 0.174$ ;  $p = 0.23$ ).

### 3.4 Rarefaction bias and nonrandom spatial patterns: simulated data sets

The intra- and inter-specific spatial dispersion patterns of the three most abundant species are uncorrelated across communities in the first data set (Appendix E). These communities exhibit a wide range of rarefaction bias (3.1–27.6%, mean 13.3%). We first regressed rarefaction bias on all 12 measures of spatial autocorrelation. A best subsets regression and both backward and forward regression produce no significant predictors. A regression of rarefaction bias on nearest neighbor distance of the most abundant species (nnAA) explains only 1% of the variation ( $R^2 = 0.013$ ;  $p = 0.38$ ).

In the second data set, the intra- and inter-specific spatial dispersion patterns of the three most abundant species are correlated across communities to match the spatial patterns in empirical communities: as one species becomes more clumped, the other two become more clumped and segregation between species increases. The mean rarefaction bias is 19.4% (range  $-2.1$ –38.3; Appendix E). We regressed rarefaction bias on all 12 variables. Forward regression produces one significant predictor (avgA), but it explains little variation ( $R^2 = 0.068$ ). Backward regression produces no significant predictor. A best subsets regression shows that no regression with 5 or fewer variables accounts for more than 25% of the variation in rarefaction bias (maximum  $R^2 = 0.242$ , adj.  $R^2 = 0.172$ ). A regression of rarefaction bias on nearest neighbor distance of the most abundant species (nnAA) explains only 0.4% of the variation ( $p = 0.63$ ). Four communities show almost no rarefaction bias whereas the others all show a bias of about 10% or more (Appendix E). These four communities were generated from a random algorithm for the less common species, and all other communities were created with clumped patterns for the less common species.

In the third data set, the spatial dispersion patterns of the eight most abundant species are random, and the 12 measures of spatial autocorrelation from the three most abundant species do not predict rarefaction bias ( $p > 0.09$  for all variables). More importantly, these communities exhibit a wide range of rarefaction bias, from  $-0.36$  to  $20.2\%$  (Appendix E). Although mean rarefaction bias is lower in the third data set ( $6.83\%$ ) than in the second one ( $19.4\%$ ), some communities show as much rarefaction bias as those from the second data set.

When the point patterns of the spatial dispersion patterns of the eight most abundant species are fixed in all communities for the fourth data set, variation in rarefaction bias still exists; mean bias is  $7.0\%$ , (range  $-0.32$ – $20.4\%$ ). Because 500 species accumulation curves were generated for each community, sampling error in calculating the rarefaction bias is negligible. Variation in rarefaction bias across communities must be due to differences in spatial dispersion patterns of the less common species.

## 4 Discussion

### 4.1 Rarefaction bias in real communities

Nonrandom spatial dispersion usually causes rarefaction to overestimate actual species richness. In apparently homogeneous areas, rarefaction bias can be substantial, especially at smaller sample sizes. In fact, these areas are not homogeneous and contain patches, environmental gradients, or both. For the plant communities used here, the rarefaction curve is similar to the upper 95% species accumulation curve (Fig. 1a; Appendix D). Though interesting empirically, this finding does not predict amount of rarefaction bias. For some data sets, the rarefaction curve is above the upper 95% species accumulation curve. Because species accumulation curves are so variable within a collection, the rarefaction curve of some communities is lower than the upper 95% species accumulation curve, even with large rarefaction bias.

Nonrandom spatial patterns sometimes cause rarefaction to produce unreliable estimates. The bird communities show less rarefaction bias than the plant communities (Fig. 2a, Appendix D). In fact, rarefaction sometimes underestimates species richness for summer bird communities. Because both summer bird communities exhibit overdispersion within species and positive associations among species (Appendix E), this finding is unsurprising. This overdispersion could be created by summer territoriality, observer biases in mapping territories, or both. Because nonrandom spatial patterns exist even in areas that appear homogeneous, we recommend using the mean and confidence intervals of species accumulation curves rather than rarefaction to compare richnesses among collections of different size.

We have analyzed few collections, and these vary in several ways: area surveyed, number of individuals, and number of species collected (Appendix B). For example, all four bird collections contain far fewer individuals than the six plant collections. Taxonomic and geographic differences among collections may also be important in rarefaction bias. Adult conspecific tropical trees, for example, may be more regularly dispersed than temperate trees (Janzen 1970).



#### 4.2 Predicting rarefaction bias: empirical data set

We used multiple linear regression to explore how well several measures of spatial autocorrelation of three abundant species can predict bias in rarefaction estimates. To find a practical solution that could be used by field researchers, we chose a small number of simple measures and focused on patterns of the more abundant species. Because few researchers collect many spatial data, field biologists cannot apply more sophisticated measures of spatial autocorrelation such as correlograms (Sokal and Oden 1978), spectral analyses (Renshaw and Ford 1984), and K-functions (Ripley 1981; Diggle 1983).

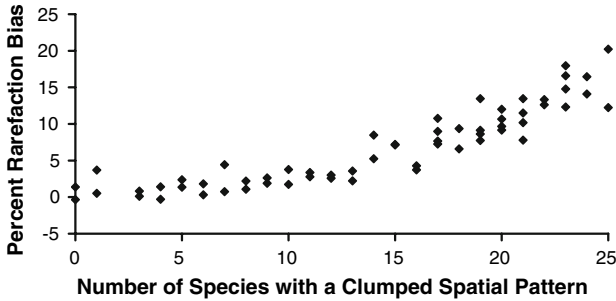
In the empirical data set, the nearest neighbor distance of the most abundant species explains only 17% of the variation in rarefaction bias among communities and is not significant. The 12 measures of spatial autocorrelation are very strongly correlated. When they are collapsed into three measures (mean clumping, average segregation, and mean average neighbor distance), these three are still highly correlated, so when one changes to increase rarefaction bias, correlated changes in the other variables should also increase rarefaction bias. Nevertheless, results from the empirical data set suggest that nearest neighbor measures cannot accurately predict amount of rarefaction bias across communities.

#### 4.3 Predicting rarefaction bias: simulated data sets

The first and second simulated data sets suggest that the negative result from the empirical data set is not due to low statistical power. In the first two simulated data sets, no simple measure of spatial autocorrelation predicts rarefaction bias. Nearest neighbor distance of the most abundant species explains less than 2% of the variation in rarefaction bias in both data sets. The relationship between the measures of spatial autocorrelation and rarefaction bias is surprisingly weak, even for the second simulated data set, where the three collapsed measures of spatial autocorrelation are correlated and act in concert to increase or decrease rarefaction bias, and where nearest neighbor measures of the three most abundant species are correlated.

In the second simulated data set, the fact that the four communities that showed almost no rarefaction bias were the only four communities with completely random spatial dispersion patterns for the less common species suggests that the dispersion of these species contributes importantly to rarefaction bias (Appendix E). It is possible that rarefaction produces highly biased results only when many species are clumped simultaneously (Fig. 3) and that strong clumping of a few species, even common ones, does not necessarily cause a large bias.

With the third and fourth simulated data sets, we determined that dispersion of the less common species is important for rarefaction bias. Therefore, to predict or to correct for spatial autocorrelation when rarefying collections, one must collect many spatial data. More sophisticated measures of spatial autocorrelation or further statistical analyses (e.g., examination of nonlinear patterns) are unlikely to predict rarefaction bias unless more species are examined.



**Fig. 3** Rarefaction Bias versus the Number of Clumped Species. In each community, the eight most common species and the seven least common species have a random spatial dispersion pattern. Rarefaction bias generally increases when more species have a clumped dispersion pattern. Even with random dispersion patterns for the eight most abundant species, many communities have considerable amounts of rarefaction bias. Data are from the fourth simulated data set

#### 4.4 Standardizing collections by area or by number of individuals

Communities can be compared based on a standardized number of individuals or a standardized area; these two measures of species diversity are termed “species richness” and “species density”, respectively. Though these measures are related, only species density is influenced by density of individuals. Not surprisingly, species richness and species density can yield qualitatively different results (James and Wamer 1982; McCabe and Gotelli 2000). Which measure is more appropriate depends on the question (Gotelli and Colwell 2001) and method of data collection (Gotelli and Graves 1996). McCabe and Gotelli (2000) and Gotelli and Colwell (2001) propose that species richness is more appropriate for most ecological hypotheses because many theoretical models in community ecology are based on per capita effects. Though this is true, many theoretical models also include abundance. Which measure is more appropriate depends on whether differences in density are important to the hypothesis; species density is a more appropriate measure of species diversity to test many hypotheses in ecology.

Examining species diversity patterns with both metrics allows one to separate effects of density from per capita effects and might help distinguish among competing hypotheses. For example, one explanation for increased species diversity with increased productivity is that more productive areas support more individuals. This hypothesis predicts that a low productivity community will have fewer species when standardized by area (species density) but the same number when standardized by number of individuals (species richness). In contrast, both the hypothesis that the productivity-diversity pattern is caused by the loss of species that require higher resource levels or minimum population sizes (Grime 1973) and the hypothesis that higher productivity causes greater variation in resource availability (Tilman 1988) predict a decrease in both measures of species diversity in the low productivity community. Examining both species density and species richness might also be useful in studies of disturbance (see McCabe and Gotelli 2000), pollution, the paradox of enrichment (Rosenzweig and Abramsky 1993), and the role of tree-fall gaps in maintaining high species diver-

sity in tropical forests (Hubbell et al. 1999; Chazdon et al. 1999; Vandermeer et al. 2000).

#### 4.5 Sampling from spatially heterogeneous communities

The presence of spatial heterogeneity complicates decisions about sampling methods. Researchers should sample communities from equal-sized areas when possible to allow a direct comparison of species density; species richness can be compared using rarefaction. The size of the area sampled must be adjusted for the taxon and the question.

When communities are sampled from different-sized areas, we recommend using the mean and confidence intervals of species accumulation curves to compare species density or richness among sites. This approach requires Monte Carlo simulations on data that are subdivided spatially (into subplots) or temporally (into time periods). Using the mean and confidence intervals from species accumulation curves eliminates the bias inherent in rarefaction when spatial heterogeneity exists in the larger community.

#### 4.6 Sampling from communities that vary in the density of individuals

Even when research is designed to study communities from equal-sized areas, some plots might contain too many individuals to sample completely (McCabe and Gotelli 2000). If the area sampled cannot be reduced (because low-density areas then contain too few individuals), subsampling must be employed. Species density cannot be calculated for subsampled plots because all individuals were not censused. When subsampling is necessary, we recommend sampling all individuals from subplots in both high- and low-density plots and comparing species density and species richness as the number of subplots increases. Again, this approach requires Monte Carlo simulations and spatial data. If this requirement is too costly, one can sample individuals at random from the high-density plots and examine species richness only.

#### 4.7 Conclusions

Rarefaction is not robust to nonrandom spatial dispersion, and environmental heterogeneity often occurs in apparently homogeneous communities. Rarefaction often overestimates actual species richness, especially at small sample sizes.

Because rarefaction often does not accurately estimate richness, sampled survey sites should be standardized to equal-sized areas. This would reduce the need for rarefaction and lessen one problem that spatial patterns pose. If collections vary in size, we recommend using the mean and confidence intervals of species accumulation curves rather than rarefaction to correct for size differences. This approach requires that data be subdivided spatially (into subplots) or temporally (into time steps). To generate a species accumulation curve, one must select a starting point and sample individuals sequentially (by distance, if spatial coordinates are known) or sample adjacent

subplots (if coordinates are not known) until the entire collection is sampled. One should obtain a sample of ca. 1,000 curves, then compare the mean number of species and the confidence interval for the number of species obtained from the smaller collection. Although using species accumulation curves is time-consuming and requires spatial data, estimates of expected species richness are lower than those generated from rarefaction, often much lower. Whether one standardizes collections based on area or on numbers of individuals depends on the question, and some studies would benefit by examining how patterns change with each metric.

We have not determined circumstances under which rarefaction is likely to be reliable, nor have we provided a “correction” for spatially autocorrelated data. Simple measures of spatial autocorrelation, such as nearest neighbor distances of the more abundant species in a community, cannot predict the amount of rarefaction bias. Even with more sophisticated measures of spatial autocorrelation, the ability of data on the most common species to predict rarefaction bias is limited.

Conclusions of some studies that have used rarefaction should be viewed with caution. Studies that have shown a smaller collection containing fewer species than expected for a sample from a larger collection are in greatest jeopardy. Studies in which a large collection is rarefied to a very small sample size are also likely to exhibit high rarefaction bias and are suspect.

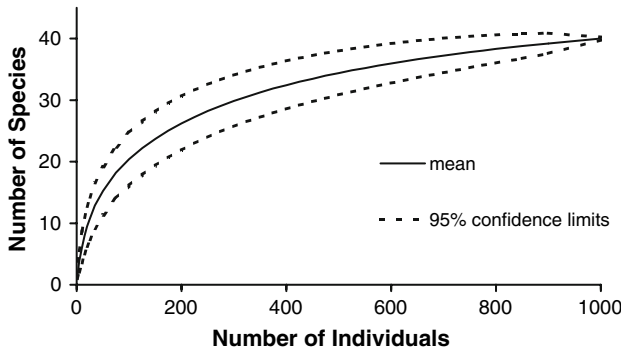
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## Appendix A: Description of rarefaction

Rarefaction estimates the number of species expected in a sample of individuals selected at random from a larger collection (Sanders 1968; Hurlbert 1971; Simberloff 1972). Gotelli and Graves (1996) discuss questions addressed using rarefaction. Rarefaction produces a hyperbolic curve of the expected number of species for a given sample size. Confidence limits can be calculated for each sample size (Fig. A.1). Hurlbert (1971) and Simberloff (1972) independently developed identical equations directly calculating the rarefaction curve using probability theory. Heck et al. (1975) provided an explicit means of calculating variance in estimates of the expected number of species.

If an unrarefied collection consists of  $N$  individuals and  $S$  species, and the abundance of each species is given by the rank-abundance array  $\mathbf{N} = \{N_1, N_2, N_3, \dots, N_S\}$ , the expected mean number of species,  $E(S_n)$ , in a random sample of  $n$  individuals is

$$E(S_n) = S - \binom{N}{n} \exp^{-1} \sum_{i=1}^S \binom{N - N_i}{n} \quad (\text{A1})$$



**Fig. A.1** Sample Rarefaction Curve. A rarefaction curve and the 95% confidence limits for Sanders’ (1968) hypothetical community of 1,002 individuals in 40 species. The rarefaction curve shows the number of species expected in a random sample. For example, the rarefaction curve shows that 30 species are expected in a random sample of 300 individuals

and the variance,  $\sigma^2(S_n)$ , is

$$\sigma^2(S_n) = \binom{N}{n} \exp^{-1} \left[ \sum_{i=1}^S \binom{N - N_i}{n} \left( 1 - \frac{\binom{N - N_i}{n}}{\binom{N}{n}} \right) + 2 \sum_{\substack{j=2 \\ i < j}}^S \left( \binom{N - N_i - N_j}{n} - \left( \frac{\binom{N - N_i}{n} \binom{N - N_j}{n}}{\binom{N}{n}} \right) \right) \right] \quad (A2)$$

In contrast to many other measures of community structure, rarefaction does not produce a single statistic. Rather, rarefaction produces a curve of number of species expected vs. area or number of sampled individuals. A rarefaction result is easily interpreted: the number of species expected in a random sample. Because the expected number of species in a sample depends on both species richness and relative abundances, rarefaction measures species diversity, not just species richness.

Although rarefaction loses information (Magurran 1988; Brewer and Williamson 1994; Rosenzweig 1995) and assumes that the spatial dispersion patterns are uniform random, these stipulations apply to all commonly used measures of community structure. Rarefaction is the only diversity measure that is independent of sample size and sensitive to rare species (Smith and Grassle 1977). Unlike other measures of species diversity, rarefaction does not subsume the components of community structure (number of species, their relative abundances, number of individuals, and area sampled) into a single statistic.

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