Deciphering the enigma of undetected species, phylogenetic, and functional diversity based on Good-Turing theory

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Abstract. Estimating the species, phylogenetic, and functional diversity of a community is challenging because rare species are often undetected, even with intensive sampling. The Good-Turing frequency formula, originally developed for cryptography, estimates in an ecological context the true frequencies of rare species in a single assemblage based on an incomplete sample of individuals. Until now, this formula has never been used to estimate undetected species, phylogenetic, and functional diversity. Here, we first generalize the Good-Turing formula to incomplete sampling of two assemblages. The original formula and its two-assemblage generalization provide a novel and unified approach to notation, terminology, and estimation of undetected biological diversity. For species richness, the Good-Turing framework offers an intuitive way to derive the non-parametric estimators of the undetected species richness in a single assemblage, and of the undetected species shared between two assemblages. For phylogenetic diversity, the unified approach leads to an estimator of the undetected Faith’s phylogenetic diversity ($PD$, the total length of undetected branches of a phylogenetic tree connecting all species), as well as a new estimator of undetected $PD$ shared between two phylogenetic trees. For functional diversity based on species traits, the unified approach yields a new estimator of undetected Walker et al.’s functional attribute diversity ($FAD$, the total species-pairwise functional distance) in a single assemblage, as well as a new estimator of undetected $FAD$ shared between two assemblages. Although some of the resulting estimators have been previously published (but derived with traditional mathematical inequalities), all taxonomic, phylogenetic, and functional diversity estimators are now derived under the same framework. All the derived approaches reveal the sufficient conditions under which the estimators are nearly unbiased, thus offering new insights. Simulation results are reported to numerically verify the performance of the derived estimators. We illustrate all estimators and assess their sampling uncertainty with an empirical dataset for Brazilian rain forest trees. These estimators should be widely applicable to many current problems in ecology, such as the effects of climate change on spatial and temporal beta diversity and the contribution of trait diversity to ecosystem multi-functionality.

Key words: functional attribute diversity; functional diversity; phylogenetic diversity; shared diversity; species diversity; taxonomic diversity.

INTRODUCTION

Nearly all biodiversity studies and analyses are based on sampling data taken from focal assemblages. However, due to practical limitations, it is virtually impossible to detect all species, especially in hyper-diverse assemblages with many rare species. In almost every biodiversity survey and monitoring project, some proportion of the species that are present fail to be detected. Not only the presence, but also the functional traits of these species remain undetected. Moreover, the placement of undetected species on the phylogenetic tree of the observed species is unknown. Consequently, traditional measures of species, functional, and phylogenetic diversity from sample data typically underestimate the true diversities (observed plus undetected). The magnitude of this negative bias can be substantial.

For species diversity, the estimation of undetected richness based on incomplete samples from a single assemblage has been widely applied, not only in ecology and conservation biology, but also in many other disciplines; see Colwell and Coddington (1994), Chazdon et al. (1998), Magurran (2004), Chao (2005), Hortal
et al. (2006), Gotelli and Colwell (2011), Gotelli and Chao (2013) and Chao and Chiu (2016) for various applications. For two assemblages, shared species richness plays an important role in assessing assemblage overlap and forms a basis for constructing various types of beta diversity and (dis)similarity measures, such as the classic Sørensen and Jaccard indices (Colwell and Coddington 1994, Magurran 2004, Jost et al. 2011, Gotelli and Chao 2013). Compared with estimating species richness in a single assemblage, the estimation of shared species richness, taking undetected species into account, has received relatively little attention; see Chao and Chiu (2012) for a review.

In traditional measures of species diversity, all species (or taxa at some other rank) are considered to be equally distinct from one another. Species differences can be based directly on their evolutionary histories, either in the form of taxonomic classification or well-supported phylogenetic trees. A rapidly growing literature addresses phylogenetic diversity metrics and related (dis)similarity measures; see Cavender-Bares et al. (2012) for a review. A widely used phylogenetic metric is Faith’s (1992) PD (phylogenetic diversity), which is defined as the sum of the branch lengths of a phylogenetic tree connecting all species in the target assemblage. Throughout this paper, PD refers to Faith’s (1992) PD. For most data sets, PD is highly correlated with species richness (e.g., Matos et al. 2017). When a sample fails to detect all species present, the lineages/branches associated with these undetected species are also missing from the phylogenetic tree of the observed species. The undetected PD in an incomplete sample was not discussed until recent years (Cardoso et al. 2014, Chao et al. 2015a).

The phylogenetic version of the Jaccard dissimilarity index is referred to as the UniFrac measure, developed by Lozupone and Knight (2005). The phylogenetic version of the Sørensen similarity index is referred to as the PhyloSor (phylo-Sørensen) index, developed by Bryant et al. (2008) and Ferryer et al. (2007). All these phylogenetic (dis)similarity measures are based on the shared branch lengths between two phylogenetic trees. However, to our knowledge, the estimation of the undetected shared PD (i.e., the total length of undetected branches shared by two phylogenetic trees) has not previously been discussed in the literature.

When species are described by a set of traits that affect organismal and/or ecosystem functioning, pairwise species differences within an assemblage can also be measured by the dissimilarity or distances between their trait profiles, which can be weighted or unweighted by their abundances. Functional diversity or trait diversity quantifies the diversity of species’ traits among coexisting species in an assemblage (Tilman et al. 1997, Díaz and Cabido 2001, Swenson et al. 2012). Functional diversity is regarded as key to understanding ecosystem processes and their response to environmental stress or disturbance (Cadotte et al. 2009). A distance-based measure at the assemblage level for quantifying functional diversity is $FAD$ (functional attribute diversity, as defined by Walker et al. 1999), which is the sum of the species-pairwise functional distances. A modified version called $MFAD$ (modified $FAD$) was proposed by Schmeller et al. (2009), who replaced species with “functional units” (here the collection of all species with identical traits is regarded as a single functional unit). For simplicity, we focus on the estimation of $FAD$, but a similar approach can be applied if species are replaced by functional units or other clusters of species. For incomplete samples, the traits of undetected species are missing, and thus their pairwise distances are not recorded and cannot be considered in the observed $FAD$. As far as we are aware, there have been no estimators previously developed for undetected $FAD$ in a single assemblage or undetected $FAD$ shared by two assemblages.

In his famous cryptanalysis to crack German ciphers during World War II, Alan Turing, regarded as the founder of modern computer sciences, developed novel statistical methods to estimate the true frequencies of rare code elements (including still-undetected code elements), based on the observed frequencies in “samples” of intercepted Nazi code. According to Good (1953, 2000), Turing never published his wartime statistical work, but permitted Good to publish it after the war. The two influential papers by Good (1953) and Good and Toulmin (1956) presented Turing’s wartime statistical work on the frequency formula and related topics. The frequency formula is now referred to as the Good-Turing frequency formula, which has a wide range of applications in biological sciences, statistics, computer sciences, information sciences, and linguistics, among others (McGrayne 2011, p. 100).

In an ecological context, Turing’s statistical problem can be formulated as an estimation of the true frequencies of rare species when a random sample of individuals is drawn from an assemblage. In Turing’s case, the species abundances are highly heterogeneous, with many rare species, so that all samples have undetected species. The Good-Turing formula answers the following question: given a species that appears $r$ times ($r = 0, 1, 2, \ldots$) in an incomplete sample of $n$ individuals, what is its true relative frequency in the entire assemblage? As will be described below, Turing gave a surprising answer that is contrary to most people’s intuition.

Until now, the Good-Turing formula has never been applied to estimate undetected species, phylogenetic, and functional diversity. In this paper, we generalize the Good-Turing formula, originally developed for a single assemblage, to two assemblages. We also extend the Good-Turing formula to a phylogenetic version that incorporates evolutionary history among species as well as a functional version that takes into account functional traits associated with each species. We show in this paper that the Good-Turing formula and its generalizations can be used in a unified way to derive estimators of undetected species, phylogenetic, and functional diversities based on incomplete samples.
For species diversity, we apply the Good-Turing formula to intuitively derive an estimator of the number of undetected species in an assemblage. The resulting estimator turns out to be the Chao (1984) non-parametric lower bound. The two-assemblage generalized formula yields Pan et al.’s (2009) lower bound of the number of undetected shared species when a sample of individuals is taken from each of two assemblages. For phylogenetic diversity, the unified approach yields a recently published estimator of undetected biodiversity measures (including three previously published, derived from traditional mathematical inequalities, and three new ones) are theoretically lower bounds of the corresponding undetected diversities. Good-Turing’s perspectives also reveal the sufficient conditions under which the derived estimators are nearly unbiased. It would be impossible to reveal these conditions with the traditional derivations of the corresponding estimators from inequalities. Thus, Good-Turing perspectives further justify and provide new insights for the three established estimators.

An important advance beyond previous studies is that Good-Turing’s perspective here provides a novel and unified approach to the notation, terminology, and estimation of undetected diversity. All taxonomic, phylogenetic and functional diversity estimators are now derived and linked under the same framework. Simulation results are reported here to numerically validate Good-Turing theory and examine the performance of the derived estimators. An empirical dataset for tree species, collected in Brazilian rain forest, is used to illustrate all estimators.

Species Diversity: One-Assemblage Good-Turing Formula

The original Good-Turing formula

In the one-assemblage model formulation, we assume that there are \( S \) species and their true species relative abundances (or generally, species detection probabilities) are denoted by \( (p_1, p_2, \ldots, p_S) \). Assume a sample of \( n \) individuals is selected with replacement. Here we follow Good-Turing’s original model (Good 1953) in which the detection probability of each species is simply its relative abundance; all our derivations can be directly extended to a general model in which species detection probability is proportional to the product of its abundance and individual detectability; see Discussion. Let \( X_i \) denote the species frequency (abundance) of the \( i \)-th species in the sample, \( i = 1, 2, \ldots, S \), \( \sum_{X_i > 0} X_i = n \). Only species with frequency \( X > 0 \) in the sample are detected. Those species with frequency \( X = 0 \) in the sample are not detected (although they are present in the assemblage) and are therefore not included in the sample data.

We define the abundance frequency count \( f_r \) as the number of species each represented by exactly \( r \) individuals in the sample. Good (1953) referred to it as “the frequency of frequency \( r \).” Thus \( f_r \) is the number of “singletons” (those species that are represented by exactly 1 individual in the sample), and \( f_2 \) is the number of “doubletons” (those that are represented by exactly 2 individuals in the sample). Let \( S_{obs} \) denote the total number of those species observed in the sample; \( S_{obs} = \sum_{i > 0} f_i \equiv f_s \). Also, \( f_0 \) is the number of undetected species: species that are present in the assemblage of \( S \) species, but were not detected in the sample of \( n \) individuals and \( S_{obs} \) species. Therefore, we have \( S_{obs} + f_0 = S \).

Turing’s statistical problem can be formulated as follows. Given data, for those species that each appeared exactly \( r \) times \((r = 0, 1, 2, \ldots)\) in an incomplete sample of \( n \) individuals, the mean of their true relative abundances/frequencies in the assemblage, \( \alpha_r \), can be mathematically expressed as

\[
\alpha_r = \sum_{i=1}^{S} p_i I(X_i = r)/f_r, \quad r = 0, 1, 2, \ldots \tag{1a}
\]

where \( I(A) \) is the indicator function, i.e., \( I(A) = 1 \) if the event \( A \) occurs, and 0 otherwise. The numerator in Eq. (1a) represents the total true relative frequencies of those species that each appeared exactly \( r \) times in the sample. Dividing the total by \( f_r \), we obtain the mean (per species) of their relative frequencies. Turing and Good focused on the case of small \( r \), i.e., rare species (or rare code elements, in Turing’s case). Note that for the special case of \( r = 0 \), Eq. (1a) implies

\[
\alpha_{0f_0} = \sum_{i=1}^{S} p_i I(X_i = 0), \tag{1b}
\]

which is the “coverage deficit” (Chao and Jost 2012) or the complement of the “sample coverage” defined in Good (1953). The coverage deficit of the sample quantifies the proportion of the total individuals in the assemblage that belong to undetected species; it is also the probability that a new, previously-undetected species would be found if the sample were enlarged by one individual. This is a very important measure in diversity estimation (Chao and Jost 2012).

Turing and Good discovered a surprisingly simple and remarkably effective, although non-intuitive, estimator for \( \alpha_r \). The Good-Turing frequency formula states that \( \alpha_r, r = 0, 1, 2, \ldots \), is not estimated by its sample frequency \( rhn \), but rather by

\[
\bar{\alpha}_r = \frac{(r + 1)f_{r+1}}{n}, \quad r = 0, 1, 2, \ldots \tag{1c}
\]
In other words, \( \alpha_r \) should be estimated by \( \frac{r^* m}{n} \), where \( r^* = (r + 1)f_{r+1}/f_r \). The Good-Turing frequency formula is thus contrary to most people’s intuition because the estimator in (1c) depends not only on the sample frequency \( r \) of the focal species, but also on the frequency information derived from species in the next frequency class, \( r + 1 \).

Good (1953) used a fully Bayesian approach to theoretically justify the formula (1c), whereas Robbins (1968) derived it as an empirical Bayes estimator. Good (2000) wrote “when preparing my 1953 article, I had forgotten Turing’s somewhat informal proof in 1940 or 1941, which involved cards or urn models in some way, and I worked out a separate proof [Bayes estimator]. I still don’t recall Turing’s proof.” Nevertheless, Good (1983, p. 28) provided a very intuitive non-Bayesian justification of the Good-Turing frequency formula as follows: Given an original sample of size \( n \), consider the probability of the event that the next individual will be a species that had appeared \( r \) times in the original sample. (Mathematically, this probability is simply \( \sum_{x=r+1}^{\infty} P(X_i = r) = \alpha_r f_r \), as defined in Eq. 1a.) If this event occurs, then the species to which the additional individual belongs must appear \( r + 1 \) times in the enlarged sample of size \( n + 1 \). Because the order in which individuals were sampled is assumed to be irrelevant, the total number of individuals in the enlarged sample of size \( n + 1 \) for those species (that appeared in the additional individual and had appeared \( r \) times in the original sample) is \( (r + 1)f_{r+1} \). Thus, the probability of the aforementioned event in the enlarged sample of size \( n + 1 \) is \( (r + 1)f_{r+1} / (n + 1) \), which can be approximated by \( (r + 1)f_{r+1} / n \) if \( n \) is large enough.

Dividing by this number of such species, \( f_r \), we obtain the mean relative frequency of all undetected species, which is the classic Good-Turing frequency formula as given in Eq. (1c). Chiu et al. (2014b) proposed an improved formula \( \hat{\alpha}_r \) shown below for \( r = 0, 1, 2, \ldots \),

\[
\hat{\alpha}_r = \frac{(r + 1)f_{r+1}}{(n-r)f_r + (r + 1)f_{r+1}} \approx \frac{(r + 1)f_{r+1}}{(n-r)f_r}. \tag{1d}
\]

This improved estimator generally has smaller mean squared error than the original Good-Turing estimator. In our subsequent derivation, we adopt the rightmost term in Eq. (1d); a simple non-Bayesian proof is provided (in Appendix S1) to facilitate the generalization to the two-assemblage case.

**Undetected species richness**

Statistically, species richness (observed species plus the number of undetected species) is difficult to estimate accurately if there are many almost undetectable species in a hyper-diverse community. Practically, an accurate lower bound for species richness is preferable to an inaccurate point estimator. We now demonstrate that the improved Good-Turing formula can be intuitively used to provide a lower bound for the number of undetected species and to clearly reveal, for the first time, the conditions under which the lower bound is a nearly unbiased point estimator.

For \( r = 0 \), both Eq. (1c) and Eq. (1d) imply that the mean population relative frequency for those undetected species is approximated by \( \alpha_0 = f_1/(nf_0) \), which is not obtainable from observed data because \( f_0 \) is unknown. However, this relation implies that the product of \( \alpha_0 \) and \( f_0 \), the estimated proportion of the total number of individuals that is due to undetected species, can be well estimated by the proportion of singletons, \( f_1/n \). For notational simplicity, let \( \alpha_0 f_0 \) denote the estimator of the product of \( \alpha_0 \) and \( f_0 \). Then we have

\[
\hat{\alpha}_0 f_0 = \frac{f_1}{n}. \tag{2a}
\]

Equation (1d) also implies that, for those species that appeared as singletons (\( r = 1 \) in a sample), their mean relative frequency is estimated by

\[
\hat{\alpha}_1 = \frac{2f_2}{(n-1)f_1}. \tag{2b}
\]

Intuitively, we expect that the mean relative frequency of all undetected species should be less than the mean relative frequency of all singletons, i.e., \( \alpha_0 \leq \hat{\alpha}_1 \), and this ordering should be preserved by the corresponding estimates. Combining (2a) and (2b), we readily obtain a lower bound for the number of undetected species:

\[
\hat{f}_0 = \frac{\alpha_0 f_0}{\alpha_0} \geq \frac{\alpha_0 f_0}{\hat{\alpha}_1} = \frac{2f_2}{(n-1)f_1} = \frac{(n-1)f_1^2}{2f_2}. \tag{2c}
\]

This lower bound for \( f_0 \) is identical to that proved rigorously by Chao (1984, 1987) by means of a Cauchy-Schwarz inequality: \( E(f_0) \times 2E(f_2) \geq (1 - 1/n)E(f_1)^2 \), which may not be intuitively understood by most ecologists. Here Good-Turing’s approach is intuitive and provides a sufficient condition for the resulting estimator being unbiased, as elaborated later. Based on Eq. (2c), the estimated number of undetected species is based exclusively on the information on the rarest observed species (the number of singletons and doubletons). The idea behind this lower bound is that detected abundant species carry negligible information about the undetected species; detected rare species carry nearly all such information. From Eq. (2c), the Good-Turing formula leads to the following Chao1 species richness estimator, with a slight modification when \( f_2 = 0 \). (Colwell and Coddington 1994 gave the name Chao1 to this estimator):

\[
\hat{S}_{Chao1} = \left\{ \begin{array}{ll}
S_{obs} + \frac{(n-1)f_1^2}{2f_2}, & \text{if } f_2 > 0, \\
S_{obs} + \frac{(n-1)f_1(f_1 - 1)}{2}, & \text{if } f_2 = 0.
\end{array} \right. \tag{3a}
\]
Notice that, in the above derivation, if $\hat{\alpha}_0 \approx \hat{\alpha}_1$ (i.e., undetected species and singletons have identical mean relative abundances), then the inequality sign in Eq. (2c) becomes an equality sign, implying that the lower bound becomes an unbiased lower bound being nearly unbiased is that

$$\pi_i = \sum_{r=1}^{S_{\text{shared}}} I(X_{i1} = r, X_{i2} = v), r, v = 0, 1, 2, \ldots$$

That is, $f_{rv}$ denotes the number of shared species that are observed $r$ times in Sample I and $v$ times in Sample II. In particular, $f_{11}$ denotes the number of shared species that are singletons in both samples, and $f_{00}$ denotes the number of shared species that are undetected in both samples. Also, let $f_{rv}$ denote the number of shared species that are observed $r$ times in Sample I and are observed at least once (using a “+” sign to replace the index $v$) in Sample II, with a similar symmetric definition for $f_{rv}$. Thus, $f_{rv}$ becomes the number of observed species shared between the two samples. Mathematically, we have the following expressions:

$$f_{+v} = \sum_{r=1}^{S_{\text{shared}}} I(X_{i1} > 0, X_{i2} = v) = \sum_{r, v > 0} f_{rv}$$

$$f_{r+} = \sum_{v=1}^{S_{\text{shared}}} I(X_{i1} = r, X_{i2} > 0) = \sum_{r, v > 0} f_{rv}$$

$$f_{++} = \sum_{r=1}^{S_{\text{shared}}} I(X_{i1} > 0, X_{i2} > 0) = \sum_{r, v > 0} f_{rv} = S_{\text{shared obs.}}$$

Here we generalize the original Good-Turing formula to two assemblages. There are two parts to the generalization. The first part, below, is a direct generalization of the original formula; the second part is proved in Appendix S1 by an argument parallel to that applied in the developing the original formula.

(1) Given two-sample data, let $\alpha_{rv} = \sum_{i=1}^{S_{\text{shared}}} p_i I(X_{i1} = r, X_{i2} > 0)/f_{rv}$ be the mean of the true relative frequencies in Assemblage I for those shared species that each appeared exactly $r$ times in Sample I and appeared at least once in Sample II. A direct generalization of the original Good-Turing formula in Eq. (1d) leads to

$$\hat{\alpha}_{rv} = \frac{(r+1)f_{rv+1}}{(n_1 - r)f_{r+}}$$

Similarly, we have a symmetric formula for the mean of the true relative frequencies in Assemblage II for those shared species that appeared at least once in Sample I and appeared $v$ times in Sample II.

$$\hat{\alpha}_{rv} = \frac{(v+1)f_{r+1}}{(n_2 - v)f_{r+}}$$

(2) For any shared species that appeared exactly $r$ times in each sample, consider calculating the product of its true relative abundances in the two assemblages; the
mean of the products among all such shared species
(there are \( f_{rr} \) such shared species) can be expressed as
\[ \alpha_{rr} = \sum_{i=1}^{n_1} p_{ri} p_{r1} I(X_1 = r, X_2 = r)/f_{rr}, \quad r = 0, 1, 2, \ldots \]
The following generalized two-assemblage Good-Turing formula provides an estimator for \( \alpha_{rr} \) (see Appendix S1 for a proof):
\[
\hat{\alpha}_{rr} = \frac{(r + 1)^2 f_{r+1,r+1}}{(n_1 - r)(n_2 - r)f_{rr}}, \quad r = 0, 1, 2, \ldots
\]  

(5c)
The generalized formulas in Eqs. (5a)–(5c) lead elegantly to an estimator of undetected shared species richness between two assemblages, as shown below.

Undetected shared species richness between two assemblages

Under the two-assemblage model formulation and data framework, the true number of shared species can be expressed as the sum of four terms:
\[
S_{\text{shared}} = S_{\text{shared obs}} + f_{0+} + f_{+0} + f_{00}.
\]  

(6)
The four terms in the right hand side of Eq. (6), as defined earlier, represent, respectively, the number of shared species observed in both samples \( f_{++} \), the number of shared species observed only in Sample II \( f_{+0} \), the number of shared species observed only in Sample I \( f_{0+} \), and the number of shared species undetected in both samples \( f_{00} \), but present in both assemblages. Only the first term is observable. The sum of the last three terms represents the total undetected shared species richness. Applying Eqs. (5a) and (5b) and using notation and derivation similar to Eq. (2c), we have
\[
f_{+0} = \frac{\alpha_{0+} f_{+0}}{\alpha_{+0}} \geq \frac{\alpha_{0+} f_{0+}}{\alpha_{+0}}\frac{f_{+1}}{f_{+0}} = \frac{(n_1 - 1)}{n_1} f_{+1} \frac{2}{f_{+2}}.
\]  

(7a)
and a symmetric expression
\[
f_{+0} = \frac{\alpha_{0+} f_{0+}}{\alpha_{+0}} \geq \frac{\alpha_{0+} f_{0+}}{\alpha_{+0}}\frac{f_{+1}}{f_{+0}} = \frac{(n_2 - 1)}{n_2} f_{+1} \frac{2}{f_{+2}}.
\]  

(7b)
Furthermore, we expect that \( \alpha_{00} \leq \alpha_{11} \), and this ordering is preserved by the corresponding estimates, implying the following inequality, from Eq. (5c):
\[
f_{00} = \frac{\alpha_{00} f_{00}}{\alpha_{00}} \geq \frac{\alpha_{00} f_{00}}{\alpha_{11}}\frac{f_{11}}{f_{00}} = \frac{(n_1 - 1)(n_2 - 1)}{n_1 n_2} f_{11} \frac{2}{f_{+2}}.
\]  

(7c)
Combining (6) and (7a) – (7c), we obtain the following estimator of shared species richness:
\[
\hat{S}_{\text{Chao1 shared}} = S_{\text{shared obs}} + k_1 f_{+1}^2 \frac{2}{f_{+2}} + k_2 f_{+1}^2 \frac{2}{f_{+2}} + k_1 k_2 f_{+1}^2 \frac{2}{f_{+2}}
\]  

(7d)
where \( k_i = (n_i - 1)/n_i, \quad i = 1, 2 \). A modification similar to that in Eq. (3a) can be applied to each term to avoid a zero divisor. The sum of the last three terms estimates the undetected shared species richness. The resulting estimator in Eq. (7d) is identical to that derived by Pan et al. (2009), who derived it by means of complicated mathematical inequalities. Here we show that Good-Turing’s framework provides a simple, unified approach to inferring undetected diversity, not only for one assemblage but also for two assemblages. The estimator in Eq. (7d) is referred to as the Chao1-shared estimator because it can be regarded as an extension of the single-assemblage Chao1 estimator (Eq. 3a) to the case of two assemblages. Pan et al. (2009) also derived a variance estimator by using a standard approximation theory, allowing construction of a confidence interval for true shared species richness. From the above derivation, the Chao1-shared lower bound becomes a nearly unbiased point estimator provided \( \hat{\alpha}_{00} \approx \hat{\alpha}_{11}, \hat{\alpha}_{+0} \approx \hat{\alpha}_{+1}, \) and \( \hat{\alpha}_{00} \approx \hat{\alpha}_{11} \) in the derivations. Considering only shared species, we see that a simple sufficient condition is that the undetected species and the detected singletons have approximately the same abundances in each of the two assemblages.

Phylogenetic Diversity

Undetected Faith’s PD in a single assemblage

To formulate phylogenetic diversity, we assume that all \( S \) species in an assemblage are connected by a rooted ultrametric or non-ultrametric phylogenetic tree, with all species, observed and unobserved, as tip nodes. In this paper, all phylogenetic diversity measures and estimators are computed from a given fixed reference point that is ancestral to all taxa considered in the study. The choice of the reference point is thus independent of the sampling data. Assume that there are \( B \) branch segments and \( B \) corresponding nodes, \( B \geq S \). Let \( a_i \) denote the total relative abundance of the species descended from the \( i \)th node/branch, \( i = 1, 2, \ldots, B \), and \( L_i \) denote the length of branch \( i \). Therefore, the set of species relative abundances \( \{p_1, p_2, \ldots, p_B\} \) is expanded to a larger relative abundance set \( \{a_i, i = 1, 2, \ldots, B\} \) with \( \{p_1, p_2, \ldots, p_B\} \) as its first \( S \) elements. For simplicity, we refer to \( a_i \) as the node/branch relative abundance of the \( i \)th node/branch, although \( \sum a_i = 1 \) is not necessarily equal to unity; see Fig. 1 of Chao et al. (2015a) for an illustrative example. Faith’s (1992) PD is expressed as
\[
PD = \sum_{i=1}^{B} L_i a_i
\]
We assume an empirical sample of \( n \) individuals with sample species abundances \( \{X_1, X_2, \ldots, X_S\} \) is taken from the assemblage. Define \( X_i^* \) as the sum of the observed species abundances for those species in the
sample that are descended from branch $i$. Then we can expand the set of observed species abundances to a larger branch abundance set $\{X'_i, i = 1, 2, \ldots, B\}$ with $(X_1, X_2, \ldots, X_S)$ as its first $S$ elements. We refer to $X'_i$, $i = 1, 2, \ldots, B$, as the sample node/branch abundance of node/branch $i$. Let

$$g_r = \sum_{i=1}^{B} L_i I(X'_i = r), r = 0, 1, 2, \ldots$$  

be the total length of those branches with sample abundance $r$ in the set $\{X'_i, i = 1, 2, \ldots, B\}$, where the indicator function $I(\cdot)$ is defined in Eq. (1a). The undetected PD in the sample is $g_0$, which is the total length of undetected branches; $g_0$ is unknown but $\{g_1, g_2, \ldots\}$ can be computed from the sample and the observed tree (the tree spanned by the observed species). For $r > 0$, $g_r$ is identical to the total length of the branches with sample abundance $r$ in the observed tree. For example, $g_1$ denotes the total length of those nodes/branches with sample abundance $= 1$ in the observed tree; $g_2$ denotes the total length of those branches with sample abundance $= 2$ in the observed tree. Let $PD_{obs}$ denote the observed PD. Then we have $PD_{obs} = \sum_{r>0} g_r$ and $PD = PD_{obs} + g_0$. A simple example is provided in Appendix S2: Fig. S1 to illustrate these measures.

We now extend the Good-Turing frequency formula to its phylogenetic version. For those nodes/branches that each is with a sample abundance/frequency of $r$, the branch-length-weighted mean (per unit length) of their true abundances in the entire assemblage, $\lambda_r$, can be mathematically expressed as

$$\lambda_r = \sum_{i=1}^{B} L_i d_i I(X'_i = r)/g_r, r = 0, 1, 2, \ldots$$

Derivation steps parallel to those used for the species diversity lead to the following phylogenetic version of the single-assemblage Good-Turing frequency formula (Appendix S2):

$$\hat{\lambda}_r = \frac{(r+1)g_{r+1}}{(n-r)g_r}, r = 0, 1, 2 \ldots$$  

(8b)

Intuitively, we expect that $\hat{\lambda}_0 \leq \lambda_1$, and this ordering is preserved by the corresponding estimates. From the above formula, a lower bound for the undetected PD is obtained:

$$g_0 = \frac{\hat{\lambda}_0 g_0}{\hat{\lambda}_1} \geq \frac{\hat{\lambda}_0 g_0}{\lambda_1} = \frac{2g_2}{n} g_1 = \frac{(n-1)}{n} g_1^2/2g_2.$$  

(8c)

The lower bound in Eq. (8c) is identical to that proposed in Chao et al. (2015a) via the Cauchy-Schwarz inequality. Here, instead, we prove it under a unified framework by means of a phylogenetic version of the original Good-Turing formula. Cardoso et al. (2014) adapted the Chao1 estimator (Eq. 3a) to obtain the same estimator. When $g_2$ is relatively small, including the case of $g_2 = 0$, the above estimator may yield an extremely large value and thus exhibit a large variance. To cope with such cases, Chao et al. (2015a) and Hsieh and Chao (2017) proposed the following modified Chao1-PD estimator:

$$PD_{Chao1} = \begin{cases} 
PD_{obs} + \frac{(n-1)g_2^2}{2g_2}, & \text{if } g_2 > \frac{g_1^2}{2f_1} \\
PD_{obs} + \frac{(n-1)g_1(f_1 - 1)}{2(f_2 + 1)}, & \text{if } g_2 \leq \frac{g_1^2}{2f_1},
\end{cases}$$  

(8d)

where $f_1$ and $f_2$ denote, respectively, the number of nodes/branches with abundance $= 1$ and abundance $= 2$ in the observed tree. Since any node/branch with abundance $= 1$ can occur only at the tip nodes, we have $f_1 = f_1$ i.e., $f_1$ is identical to the number of singletons at the tip nodes. However, $f_2$ is not necessarily equal to $f_2$; see Appendix S2: Fig. S1 for an example.

Based on Eq. (8c), the Chao1-PD estimator is nearly unbiased when $\hat{\lambda}_1 \approx \lambda_0$, which means that the singletons (which occur only at the tip nodes) and the undetected nodes (which can be tip or interior nodes) have the same length-weighted mean abundances. A sufficient simple condition is that all rare nodes (including the singletons and the undetected) have approximately the same abundances. The variance of the Chao1-PD estimator can be obtained using Eq. (3b) with $\{f_1, f_2\}$ being replaced by $\{g_1, g_2\}$. The construction of the confidence interval for Faith’s PD based on the Chao1-PD estimator can be similarly obtained.

From the derivations above, we see that all estimation procedures and the derivation steps for developing the phylogenetic version of the Good-Turing formula and PD estimators are parallel to those for the original formula and species richness estimators. A summary of formulas and descriptions for estimating species richness and Faith’s PD is provided in Appendix S2: Table S1, where the analogy between the two estimation frameworks is transparently displayed. The analogy was first proposed by Faith (1992). From Faith’s perspective, each unit-length branch is regarded as a “feature” in phylogenetic diversity (like a “species” in species diversity). Chao et al. (2014a) subsequently referred to each unit-length branch segment as a phylogenetic entity. For example, a branch of 8-unit length is counted as 8 phylogenetic entities. All entities are phylogenetically equally distinct, just as all species are assumed taxonomically equally distinct in computing species richness. Instead of species, for PD we are measuring the total number of phylogenetic entities, or equivalently, the total branch length (because each entity has length of unity). Using this perspective, the measures of branch lengths $\{f_k, k = 0, 1, \ldots\}$ in estimating PD play the same role as the frequency counts $\{f_k, k = 0, 1, \ldots\}$ in estimating species richness. This analogy to counting-up species means that most ecological indices defined at the species level can be converted to PD equivalents (by counting phylogenetic entities rather than species).
Undetected shared PD between two assemblages

Following the approach to the two-assemblage model formulation and the data framework described in the section Two-assemblage Good-Turing Formulas, we assume that all S species of the pooled assemblage are indexed by 1, 2, ..., S. Assume these S species are connected by a rooted ultrametric or non-ultrametric phylogenetic tree, with the S species as tip nodes. Given a fixed reference point that is ancestral to all taxa considered in the study, we assume that there are B branch segments and B corresponding nodes in the pooled tree, and let L_i denote the length of branch i. Because the phylogenetic tree of each individual assemblage is a subtree of the pooled tree, the diversity for each individual assemblage can be computed from the pooled tree structure with only the node (or branch) abundances varying between assemblages, as illustrated by Chiu et al. (2014a, Figure 2). Assume that there are B_12 branches shared by the two assemblages for the given reference point, and without losing generality, these shared branches are indexed from 1, 2, ..., B_12 for notational simplicity. Denote the true shared PD (the total length of branch segments shared by the two individual sub-trees) by PD_{shared} and the shared PD between two observed trees by PD_{shared,obs}.

As in the preceding section, the two sets of relative species abundances in Assemblies I and II are, respectively, expanded to the nodalbranch relative abundance (or frequency) sets \{a_{i1}, i = 1, 2, ..., B\} and \{a_{i2}, i = 1, 2, ..., B\}. We also extend the set of observed species frequencies \(X_{11}, X_{21}, \ldots, X_{51}\) in Assemblage I to a larger sample node/branch frequency set \(\{X'_{i1}, i = 1, 2, \ldots, B\}\). Similarly, we extend the set of observed species frequencies \(X_{12}, X_{22}, \ldots, X_{52}\) in Assemblage II to a larger set \(\{X'_{i2}, i = 1, 2, \ldots, B\}\). The frequency counts of shared species, \(f_{r, v}\), (Eq. (4a)), are also extended to their phylogenetic versions:

\[
g_{rv} = \sum_{i=1}^{B_{12}} L_i I(X'_i = r, X'_i = v), r, v = 0, 1, 2, \ldots \tag{9}
\]

Here \(g_{rv}\) measures the total length of those shared branches with node/branch abundance \(r\) in Sample I and node/branch abundance \(v\) in Sample II. We can similarly define \(g_{r+}, g_{v+}, g_{++}\), as we did in Eqs. (4b)–(4d). Here \(g_{++}\) denotes the total length of those shared branches that have abundance \(r\) in Sample I and non-zero node/branch abundance in Sample II, with a similar interpretation for \(g_{++}\). Now \(g_{++}\) becomes the observed PD shared by the two samples, i.e., \(g_{++} = PD_{shared,obs}\). Thus \(\{g_{rv}, g_{r+}, g_{v+}, g_{++}; r, v = 0, 1, 2, \ldots\}\) are analogous to \(\{f_{rv}, f_{r+}, f_{v+}, f_{++}; r, v = 0, 1, 2, \ldots\}\) as defined in Eqs. (4a) – (4d).

The only difference is that each of the former set measures the lengths of shared branches, whereas each of the later set counts shared species.

The true shared PD can be expressed as the sum of four terms:

\[
PD_{shared} = PD_{shared,obs} + g_{0+} + g_{+0} + g_{00}. \tag{10a}
\]

The four terms in the right hand side of the above equation represent, respectively, the observed shared PD, the shared PD that is missed only in Sample I, the shared PD that is missed only in Sample II, and the shared PD that is missed by both samples. For any shared branch segment with node/branch abundance \(r\) in each observed branch set, consider calculating the product of its true branch relative abundances in the two assemblages. The branch-length-weighted mean (per unit-length) of the products among all such shared branches (their total length is \(g_{rr}\)) can be expressed as

\[
\lambda_{rr} = \sum_{i=1}^{B_{12}} L_i a_{i1}a_{i2} I(X'_i = r, X'_i = r)/g_{rr}, r = 0, 1, 2, \ldots \tag{10b}
\]

Under the intuitive expectation that \(\lambda_{00} \leq \lambda_{11}\) (and this ordering is preserved by the corresponding estimates), we obtain the following lower bound of \(g_{00}\):

\[
g_{00} = \frac{\lambda_{00}}{\lambda_{11}} g_{00} \geq \frac{\lambda_{00}}{\lambda_{11}} = \frac{g_{11}}{n_1} \frac{4g_{22}}{(n_1 - 1)(n_2 - 1)g_{rr}} = \frac{(n_1 - 1)(n_2 - 1)}{n_1 n_2} \frac{g_{11}^2}{g_{22}}. \tag{10c}
\]

Then the same estimation procedures we used to develop the Chao1-shared estimator in Eq. (7d) lead to the following Chao1-PD-shared estimator (Appendix S2):

\[
\bar{PD}_{Chao1} = PD_{shared,obs} + k_1 \frac{g_{11}^2}{2g_{22}} + k_2 \frac{g_{22}^2}{2g_{11}} + k_1 k_2 \frac{g_{11}^2}{4g_{22}}. \tag{10d}
\]

where \(k_i = (n_i - 1)/n_i, i = 1, 2, \) The sum of the last three terms estimates the total length of undetected branches shared by two individual sub-trees. A modification similar to that proposed in Eq. (8d) can be applied to each of the three terms. The sufficient conditions for the Chao1-PD-shared estimator being nearly unbiased can be similarly formulated as those for the Chao1-shared estimator, simply by replacing “shared species” with “shared nodes/branches.”

The variance and confidence interval associated with this estimator follow directly from those for the Chao1-
shared estimator, by replacing the counts \{f_{r}, f_{r+}, f_{++}, f_{++}; r, v = 0, 1, 2, \ldots \} with \{g_{r}, g_{r+}, g_{+}, g_{++}; r, v = 0, 1, 2, \ldots \} in the formulas. This correspondence also reflects the analogy between the estimation of shared species richness and in estimating shared \( PD \) between two assembles. A comparison of estimation formulas used in estimating shared species richness and in estimating shared \( PD \) is provided in Appendix S2: Table S2, where any unit-length branch shared by two assembles plays the same role as a “shared species” in the estimation of shared species richness. The measures of shared branch lengths \{g_{r}; r, v = 0, 1, 2, \ldots \} used in estimating shared \( PD \) play a role corresponding to the frequency counts \{f_{r}; r, v = 0, 1, 2, \ldots \} in estimating shared species diversity. Hence, not only within-assemble but also between-assemble measures defined at the species level can be converted to \( PD \) equivalents.

**Functional Diversity**

**Undetected \( FAD \) in a single assemblage**

Consider an assemblage in which all species are characterized by a set of functional traits, which can be categorical or continuous variables. The pairwise distances are calculated by some distance metric (e.g., Euclidean distance or Gower distance) based on the values of species traits. Let \( d_{ij} \) be the functional distance between the \( i \)th and \( j \)th species, with \( d_{ij} = d_{ji} > 0 \). Walker et al.’s (1999) \( FAD \) in the entire assemblage is expressed as

\[
FAD = \sum_{i,j=1}^{S} d_{ij}.
\]

Out of all the species which appeared exactly \( r \) times in the sample, consider choosing any two to form a pair and calculating the product of their true relative abundances in the assemblage. The distance-weighted mean (per unit-distance) of the products among all such pairs (their total pairwise distance is \( F_{rr} \)) can be expressed as

\[
\theta_{rr} = \sum_{i,j=1}^{S} d_{ij} p_{i} p_{j} I(X_{i} = r, X_{j} = r)/F_{rr}, r = 0, 1, 2, \ldots
\]

The functional version of the Good-Turing frequency formula provides the following estimator of \( \theta_{rr} \) (see Appendix S3 for a proof):

\[
\hat{\theta}_{rr} = \frac{(r + 1)^{2} F_{rr+1,r+1}}{(n - 2r)(n - 2r - 1)F_{rr}}, r = 0, 1, 2, \ldots
\]

The above formula is slightly different from the two-sample Good-Turing formulas for estimating shared species (Eq. 5c) and shared \( PD \) (Eq. 10b), because, here, we only have one-sample data taken from a single assemblage, whereas the data for the previous formulas are taken from two different assembles. Under the intuitive expectation that \( \theta_{00} \leq \theta_{11} \) (and this ordering is preserved by the corresponding estimates), we obtain the following lower bound for \( F_{00} \):

\[
F_{00} = \frac{\theta_{00} F_{00}}{\theta_{00}} \geq \frac{\theta_{00} F_{00}}{\theta_{11}} = \frac{F_{11}}{n^{2}} = \frac{(n - 2)(n - 3) F_{11}^{2}}{n(n - 1)4 F_{22}}.
\]

Then the same estimation procedures as we obtained for Chao1-shared in Eq. (7d) leads to the following Chao1-\( FAD \) estimator:

\[
\hat{FAD}_{\text{Chao1}} = \text{FAD}_{\text{obs}} + k \frac{F_{1+}^{2}}{2 F_{2+}} + k \frac{F_{++}^{2}}{2 F_{++}} + \frac{(n - 2)(n - 3) F_{11}^{2}}{n(n - 1)4 F_{22}},
\]

where \( k = (n - 1)/n \). The sum of the last three terms estimates the undetected \( FAD \). A modification similar to
that proposed in Eq. (8d) can be applied to each of the three terms. The variance and confidence interval associated with this estimator follow directly from those for the Chao1-shared estimator. Under the condition that undetected species and singletons have approximately homogenous abundances, the Chao1-FAD estimator is nearly unbiased for any given species-pairwise distance matrix.

A summary of formulas and descriptions for estimating shared species richness and FAD is given in Appendix S3: Table S1, where the analogy between the estimation procedures of the two measures can be seen. Chao et al. (2014a) define a “functional entity” as a species pair with one unit of distance between the two species. In FAD, a functional entity plays the same role as a “shared species” between two assemblages. For example, a species pair with distance \( d_{ij} = 5 \) is counted as 5 “shared species” (i.e., 5 functional entities). Thus the measures of total distances of species pairs, \( \{F_{rr}, F_{rv}, F_{sv}, F_{sv+s}; r, v = 0, 1, 2, \ldots\} \), play the same roles as the counts of shared species richness \( \{f_{rr}, f_{rv}, f_{sv}, f_{sv+s}; r, v = 0, 1, 2, \ldots\} \) (defined in Eqs. 4a – 4d).

Undetected shared FAD between two assemblages

Under the two-assemble model formulation and data framework described in the section “Two-assemble Good-Turing Formulas,” we further assume that the functional distance between the \( i \)th and \( j \)th species for all \( S \) species of the pooled assemblage is denoted as \( d_{ij} \), with \( d_{ij} = d_{ji} \geq 0 \). The FAD of each individual assemblage can be computed from a sub-matrix of the distance matrix of all \( S \) species in the pooled assemblage. Denote the number of shared species by \( S_{12} \), shared FAD (i.e., the total species-pairwise distance of two species each is shared by two assemblages) by \( FAD_{\text{shared}} \), and the FAD based on the observed shared species by \( FAD_{\text{shared,obs}} \).

Given two-sample data with species sample abundances \((X_{11}, X_{21}, \ldots, X_{S1})\) and \((X_{12}, X_{22}, \ldots, X_{S2})\), as defined earlier, we extend all frequency counts \( F_{r,v} \) defined in Eq. (11) to the two-assemble case. That is, we define for \( r, v, k, m = 0, 1, 2, \ldots \)

\[
F_{(r,v)(km)} = \sum_{i,j=1}^{S_{12}} d_{ij} I(X_{1i} = r, X_{j1} = v) \times I(X_{2k} = k, X_{2m} = m). 
\]  

(13)

Here \( F_{(r,v)(km)} \) measures the total functional distance of those pairs of shared species with abundances \((r, v)\) in Sample I and abundances \((k, m)\) in Sample II for the two species in each pair. Where any index is replaced by a “+” sign, it means that those species occur at least once in the corresponding sample. Thus, we can define \( F_{(r+v)(km)}, F_{(r+v)(km)}, F_{(r+s)(km)}, \) and \( F_{(r+k)(m+n)} \), etc. Then shared FAD can be expressed as the sum of 16 terms (see Appendix S3: Table S2 for illustrative details)

\[
FAD_{\text{shared}} = \sum_{r,v=0}^{+} \sum_{k,m=0}^{+} F_{(r,v)(km)} 
\]

\[= F_{(++)(++)} + F_{(++)(+)0} + F_{(++)(0+)} + \ldots + F_{(00)(00)}.
\]  

(14a)

Here \( F_{(++)(++)} = FAD_{\text{shared,obs}} \) denotes the observed shared FAD; the other 15 terms are unknown. For each term, we can apply similar-type Good-Turing formulas to obtain an estimator as given in Appendix S3: Table S3. Then we have the following Chao1-FAD-shared estimator:

\[
FAD_{\text{Chao1,shared}} = F_{(++)(++)} + \tilde{F}_{(++)(+)0} + \tilde{F}_{(++)(0+)} + \ldots + \tilde{F}_{(00)(00)}.
\]  

(14b)

A bootstrap method can be used to assess the sampling variance of the above estimator and to obtain the associated confidence interval.

Example

We apply all the estimators derived from the Good-Turing frequency formula and its generalizations to the rain-forest tree data described and discussed in Magnago et al. (2014). The tree species abundance data were collected between January 2011 and January 2012 from 11 forest fragments in Espírito Santo State, in southeastern Brazil. Sampling data for 10 fragments included one Edge and one Interior transect, whereas in one fragment sampling data included two Edge and two Interior transects (Magnago et al. 2014), with a distance of 5.7 ± 2.4 km between transects. In total, the study comprised 11 fragments and 24 transects. Each Edge transect was placed about 5 m inside the fragment and parallel to the forest edge, and each Interior transect was located at least 300 m from the nearest edge. One of the goals of the original study was to compare functional diversity between Edge habitat and Interior habitat. Within each transect, every living tree with a diameter at breast height (DBH) > 4.8 cm and 1.3 m height was recorded.

In the original data (Table S2 of Magnago et al. 2014), pooled from all 24 transect samples in 11 fragments, there were 443 species among 4140 individual trees. However, in order to construct the phylogenetic tree for the observed species, using Phylomatic (http://www.phylodiversity.net/phylomatic; Webb and Donoghue 2005), we had to exclude 18 species, including 5 species not identified in the species list of Magnago et al. (2014) and 13 species not included in Phylomatic. Among the 18 species excluded, 12 species were found in both habitats, 3 species were unique to the Edge habitat and 3 species were unique to the Interior habitat. The data considered in our analysis thus include 425 species from a total of 3868 individuals; species abundance data are available in Github (https://github.com/AnneChao). The species abundance frequency counts of these 425 species are summarized in Table 1. There were 319
species (including 110 singletons and 48 doubletons) among 1794 individuals in the data from the Edge habitat, and 356 species (including 123 singletons and 48 doubletons) among 2074 individuals in the data from the Interior habitat. There were 250 species in common between the two habitats in the data. The sample coverage estimates for these two habitats are nearly equal (93.9% for the Edge habitat and 94.1% for the Interior habitat), in spite of different sample sizes.

To illustrate our estimators, we focus only on the diversity analysis for the pooled samples over all fragments. That is, we regard the pooled tree records as sampling data from the entire study area represented by the 11 fragments and aim to infer and compare the estimated true or asymptotic diversity (observed plus undetected) of the two habitats for the whole study area. We could also apply our estimation to the Edge and Interior transect data within each fragment to compare the diversity of the two habitats; in this case, the target becomes the estimated true diversity of each fragment. Comparison of compositional, phylogenetic, and functional differentiation among the 11 fragments, i.e., beta diversity with adjustment for under-sampling bias will be reported elsewhere.

Estimates for species richness and shared species richness between the two habitats appear in Table 2. The undetected species richness estimates (Eq. 2c) for the Edge and Interior habitats are, respectively, 126 and 158 species, implying that the total richness estimate (Eq. 3a) for the Edge habitat is 445 species, with a 95% confidence interval of (396, 525), and for the Interior habitat 514 species, with a 95% confidence interval of (455, 609). These results show that the Interior habitat has higher estimated species richness; however, the difference is not statistically significant at a level of 5%. The undetected shared species richness between the two habitats is estimated to be 139, leading to an estimate of shared richness (Eq. 7d) of 389 species, with a 95% confidence interval of (347, 450). Although the true species richness and shared species richness between the two habitats are unknown and our estimates theoretically represent lower bounds, the data collector among us (L. F. S. Magnago) believes these estimates provide reasonable adjustments based on his experiences in the fields, CVRD (Herbarium of Natural Vale Reserve) herbarium collection, and other floristic studies for the region (Rolim and Nascimento 1997, Jesus and Rolim 2005, Paula and Soares 2011). We also assume that this conclusion can be extended to the following estimates of phylogenetic diversity and functional diversity. The biological processes that may give rise detection failure are elaborated in the Discussion section.

The phylogenetic tree (in Newick format) of the 425 observed species which was constructed using the software Phylomatic (Webb and Donoghue 2005) is given in Github (https://github.com/AnneChao). The observed Faith’s PD values are, respectively, 24516 and 27727 Myr in the Edge and Interior habitat, with a shared PD 20680 Myr. Table 3 shows the estimates for Faith’s PD in each habitat and shared PD between the two habitats. The PD estimates (Eq. 8d) for the Edge and Interior habitats are, respectively, 32011 with a 95% confidence interval of (31542, 32511), and 34550 with a 95% confidence interval of (34143, 34983). The two intervals do not overlap, implying that the Interior habitat has significantly higher PD than the Edge habitat. The shared PD between the two habitats is estimated (Eq. 10d) to be 29360 Myr, with a 95% confidence interval of (28976, 29761).
The functional traits for these tree data were based primarily on morphological and physical characteristics of trees, their roles as trophic resources, their dispersal modes, and their roles in carbon storage and forest structure. All observed species were described by a set of six functional traits, including five categorical variables: fruit size (size categories), seed size (size categories), fruit type, fruit dispersal syndrome, and successional group, together with one quantitative variable: wood density. Based on these six traits, the species pairwise distance matrix for the data (pooled across replicates) was calculated by a Gower mixed-variables coefficient of distance matrix for the data (pooled across replicates) was calculated by a Gower mixed-variables coefficient of distance with equal weights for all traits; the Gower distance reflects large sampling variance due to the estimation of 15 parameters and each estimate is subject to some degree of sampling uncertainty.

Our analysis suggests that the pooled Interior habitat has significantly higher phylogenetic diversity and significantly higher functional diversity than the pooled Edge habitat, but no such significance can be concluded for species diversity. These results are generally consistent with previous findings (Magnago et al. 2014, Matos et al. 2017) based on empirical data and model expectations. The previous authors found that the Edge habitat characteristics (microclimate, light, etc.) do not change with increasing fragment size whereas the Interior habitat characteristics change with fragment size. Consequently, fragment size usually does not produce effects on species richness and composition at fragment edges, but strongly influences the diversity in the interior of fragments. Because all fragment sizes are considered together in this paper, we thus expect greater diversity values for Interior than for Edge habitats.

Table 3. Summary of phylogenetic data and diversity estimates for the Edge and Interior habitats in forest fragments of south-eastern Brazil (Magnago et al. 2014), showing (a) undetected PD and Chao1-PD point and interval estimates for each habitat (see Eq. 10d), and (b) undetected shared PD between the two habitats and the corresponding Chao1-shared point and interval estimates (see Eq. 10d).

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Sample size</th>
<th>$g_1$</th>
<th>$g_2$</th>
<th>Observed PD</th>
<th>Undetected PD</th>
<th>Chao1-PD</th>
<th>95% conf. interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Edge</td>
<td>1794</td>
<td>6578</td>
<td>2885</td>
<td>24516</td>
<td>7495</td>
<td>32011</td>
<td>31542, 32511</td>
</tr>
<tr>
<td>Interior</td>
<td>2074</td>
<td>7065</td>
<td>3656</td>
<td>27727</td>
<td>6823</td>
<td>34550</td>
<td>34143, 34983</td>
</tr>
<tr>
<td>(b)</td>
<td>20680</td>
<td>3888</td>
<td>2177</td>
<td>3929</td>
<td>2125</td>
<td>1711</td>
<td>1579, 1729</td>
</tr>
<tr>
<td>Observed</td>
<td>$g_{+1}$</td>
<td>$g_{+2}$</td>
<td>$g_{+1}$</td>
<td>$g_{+2}$</td>
<td>$g_{+1}$</td>
<td>$g_{+2}$</td>
<td>$g_{+1}$</td>
</tr>
<tr>
<td>shared PD</td>
<td>Undetected PD</td>
<td>Chao1-PD</td>
<td>shared 95% conf. interval</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20680</td>
<td>3888</td>
<td>2177</td>
<td>3929</td>
<td>2125</td>
<td>1711</td>
<td>1579</td>
<td>8680, 29360</td>
</tr>
</tbody>
</table>

Note: The phylogenetic tree for all observed species is based on Phylomatic (Webb and Donoghue 2005).
TABLE 4. Summary of functional data and diversity estimates based on tree species abundance data, as well as species traits collected from the Edge and Interior habitats of forest fragments in south-eastern Brazil (Magnago et al. 2014), showing (a) undetected and Chao1-FAD point and interval estimates for each habitat (see Eq. 12d), and (b) undetected shared between the two habitats and the corresponding Chao1-FAD-shared point and interval estimates (see Eq. 14b and Appendix S3).

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Observed FAD</th>
<th>Undetected FAD</th>
<th>Observed shared FAD</th>
<th>Undetected shared FAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edge</td>
<td>36,693</td>
<td>1,245</td>
<td>5,572</td>
<td>130,064</td>
</tr>
<tr>
<td>Interior</td>
<td>43,438</td>
<td>1,479</td>
<td>6,059</td>
<td>179,921</td>
</tr>
</tbody>
</table>

Note: The functional distance matrix between any two observed species in the pooled assemblage is obtained by Gower distance.

CONCLUSION AND DISCUSSION

We have generalized the original one-assemblage Good-Turing frequency formula (Eqs. 1c and 1d) to the case of two assemblages (Eq. 5c), and also extended it to a phylogenetic version (Eqs. 8b and 10b) as well as a functional trait version (Eq. 12b and Appendix S3: Table S3). We have also applied the original and generalized formulas to obtain various estimators of undetected species, phylogenetic, and functional diversity, as summarized below.

1. For species diversity, the estimator of species richness derived from the Good-Turing frequency formula is identical to the Chao1 estimator (Chao 1984, 1987) in Eq. (3a). The estimator of shared species between two assemblages in Eq. (7d) is identical to the Chao1-shared species estimator proposed in Pan et al. (2009).

2. For phylogenetic diversity, the resulting estimator (Eq. 8d) of Faith’s PD is identical to the Chao1-PD estimator proposed recently by Chao et al. (2015a), but the estimator of the shared Faith’s PD (Eq. 10d) is new.

3. For functional trait diversity, the estimator of FAD in a single assemblage (Eq. 12d) and shared FAD between two assemblages (Eq. 14b) are both new; see Appendix S3: Tables S1–S3 for a summary.

The R code “Good-Turing” for computing all the estimators discussed in this paper is available in Github (https://github.com/AnneChao) along with a description of the running procedures. As an alternative, readers without a background in R, can utilize the online software “GoodTuring”, made available from https://chao.shinyapps.io/GoodTuring/ to facilitate all computations.

We have proved that each of the derived estimators is theoretically a lower bound of the corresponding diversity. Good-Turing’s perspectives reveal the sufficient conditions under which the resulting estimator is nearly unbiased. For example, a simple sufficient condition for the Chao1 species richness estimator being nearly unbiased is that rare species (specifically, undetected species and singletons in sample) have approximately homogeneous abundances. Similar conditions for other estimators are also clearly specified in each subsection. See the next paragraph for more relaxed conditions.

Although, in our derivations, we follow the Good-Turing original model by assuming that the detection probability of each species is simply its relative abundance, all our derivations can be directly extended to a general model as discussed in Chao and Chiu (2016). The general model assumes that detection probability is proportional to the product of abundance and individual detectability, which may vary among species. Based on samples of individuals, individual detectability for mobile organisms is determined by many possible factors such as individual movement patterns, color, size, habitat, life cycle and vocalizations; for assemblages of sessile organisms, such as trees, that are surveyed from selected sampling units (e.g., transects, plots or quadrats), individual
detectability may depend on the area and topography of selected sampling units, species spatial/temporal aggregation or clustering, life history stage, as well as other factors. Consequently, our estimators are actually valid in more relaxed conditions. For example, the Chao1 species richness estimator is nearly unbiased when hard-to-detect species (specifically, undetected species and species detected by one individual) have approximately homogeneous detection probabilities. Similar relaxed conditions can be formulated for other estimators. Under the special case that all individuals have the same detectability, the detection probability of each species reduces to its relative abundance. In other applications in which species detection probability may vary with time, our method can also be applied when the probability of detecting a species is modeled as the average detection probability over the sampling-time interval.

When rare or hard-to-detect species are highly heterogeneous in detection probabilities, such as in microbial assemblages or DNA sequencing data, all estimators derived in this paper provide non-parametric lower bounds which are valid for species, phylogenetic and functional diversities. In such assemblages, sample data do not provide sufficient information to accurately estimate asymptotic diversities due to heterogeneity of rare species; no statistical methods can produce reliable estimates unless strong assumptions are made. In such cases, from our perspective, an accurate lower bound is more practically useful than an imprecise point estimate. In Appendix S4, some representative simulation results are reported to validate the estimators derived from Good-Turing theory and also to demonstrate that our estimators provide useful and informative lower bounds when accurate point estimators are not attainable.

For cases that fail to meet or may not be assured to meet the criteria for nearly-unbiased point estimation, how can we make fair comparison of diversities across studies? We suggest using a non-asymptotic approach via sample-size- and coverage-based rarefaction and extrapolation on the basis of standardized sample size or sample completeness (as measured by sample coverage). This non-asymptotic approach facilitates fair comparison of diversities for equally-large or equally-complete samples across multiple assemblages. For species richness, sample-size-based rarefaction and extrapolation methods were developed by Colwell et al. (2012), and corresponding coverage-based methods were proposed by Chao and Jost (2012). For Faith’s \( PD \), a similar non-asymptotic method was recently presented by Chao et al. (2015b) and Hsieh and Chao (2017). Rarefaction and extrapolation methods for functional diversity are still under development by the authors.

For the analysis of multiple-assemble phylogenetic structures, Webb et al. (2002) and Webb et al. (2008) proposed using a null model randomization test based on \( PD \) (or other metrics) to assess whether a specific assemblage has a higher \( PD \) (more even or uniform) or a lower \( PD \) (phylogenetic aggregation or clustering) than expected from an assembly randomly sampled from the observed species pool. That is, the observed species pool is the composite list of species from all assemblages; \( PD \) is calculated from the phylogenetic tree spanned by these detected species. In Webb et al.’s approach and in most null model or randomization tests (Gotelli et al. 2010 is an exception), it is assumed that (1) sampling is complete for all assemblages, and (2) the aggregation of observed species for all assemblages constitutes the “complete” species pool. However, for the Brazilian rain forest data analyzed in our analysis, if we treat each fragment as an assemblage, then neither of these assumptions is satisfied. Within each fragment, some species were undetected by the transect data, and some species remained undetected even in the pooled transect data for the entire study area represented by the 11 fragments. In the following, we use the Brazilian data to illustrate our suggested modifications.

1. Based on the pooled transect data from all 11 fragments, the Chao1 richness estimator yields the estimated number of species for the complete pool, which comprises all species recorded in the fragment transects plus undetected species in the study area represented by these fragments. Following the approach of Chao et al. (2015b), we can construct a complete species-rank abundance distribution (RAD) by separately adjusting the sample relative abundances for the set of species detected in the pooled, observed data and estimating the relative abundances for the set of species undetected in the pooled data, but inferred to be present in the area represented by all fragments. The combined RAD then fully characterizes the taxonomic assemblage structure. Thus, not only species richness but also species relative abundances can be estimated for all species in the estimated complete pool. For this analysis, it is not necessary to know the identities of the undetected species, but we do need to estimate their number and relative abundances in the complete pool.

2. Next, a random assemblage of the same size as each specific assemblage is sampled from the complete pool. If the sampled assemblage includes species that belong to the group of undetected species in the pooled data, then we treat those species as unresolved in their phylogenetic placement and locate those undetected species on the observed tree in some random manner (e.g., Rangel et al. 2015). How to optimally locate undetected species is still under investigation. The Chao1-\( PD \) estimate (Eq. 8d) can thus be calculated not only for the data of the specific assemblage but also for each random assemblage sampled from the complete pool.

3. After many assemblages of the same size have been randomly selected from the complete species pool as in Step (2), the mean and standard error of the resulting \( PD \) values can be computed to obtain the standardized effect size (Gotelli and McCabe 2002), adjusted for the under-sampling biases for both the specific assemblage and the pooled assemblage.
A similar procedure can be applied to FAD and other metrics, although this procedure should first be benchmarked with simulated and empirical data sets to assess its performance and to see if this procedure is an effective remedy for the under-sampling problem that is pervasive in the analysis of community structure based on standardized biodiversity sampling of multiple samples.

This paper is restricted to the estimation of diversity in one assemblage and the estimation of shared diversity between two assemblages. When there are more than two assemblages, our approach can be further extended to such cases. All the derivations are nearly parallel. For example, we can directly obtain an estimator of the species shared by multiple assemblages based on sampling data from each assemblage, and the resulting estimator is identical to the one proposed by Pan et al. (2009).

Our derivation in this paper is limited to individual-based abundance data for individuals sampled randomly from assemblages. In many ecological field studies, the sampling unit is not an individual, but a trap, net, quadrat, plot, or timed survey. For such studies, the sampling units, not the individuals, are sampled randomly and independently. In these cases, estimation is usually based on a set of sampling units in which only the incidence (detection or non-detection) of each species is recorded.

This type of data is referred to as (multiple) incidence data. All estimators can be computed from the online software “GoodTuring”. We expect that Good-Turing’s theory will find wide applications in biodiversity studies; see Chao et al. (2017) for a recent application.

Acknowledgments

The authors thank a Subject Matter Editor (Tom Miller), Joaquín Hortal, and an anonymous reviewer for very thoughtful and helpful comments and suggestions. This work was supported by the Taiwan Ministry of Science and Technology under Contracts 104-2628-M-007-003 and 105-2628-M-007-001 (for AC) and 104-2118-M-002-008-MY3 (for CHC). RKC and RLC were supported by CAPES Ciência sem Fronteiras (Brazil). LFSM was supported by CAPES/PNPD. NJG was supported by U. S. NSF DEB 1257625, NSF DEB 1144055, and NSF DEB 1136644. The fieldwork survey was supported by Reserva Natural Vale, Fibria Celulose S.A., Marcos Daniel Institute, Pro-Tapir project and Reserva Biológica de Sooretama.

Literature Cited


Appendix S1

Deciphering the Enigma of Undetected Species, Phylogenetic, and Functional Diversity Based on Good-Turing Theory

Anne Chao, Chun-Huo Chiu, Robert K. Colwell, Luiz Fernando S. Magnago, Robin L. Chazdon, and Nicholas J. Gotelli

To make the contents of this appendix self-contained, some definitions introduced in the main text are repeated here.

**APPENDIX S1: Species diversity (mathematical details)**

**Proof of Eq. (1d) of the main text**

Assume that a random sample of $n$ individuals is taken from an assemblage with $S$ species and relative abundance set $(p_1, p_2, \ldots, p_S)$. Let $X_i$ denote the frequency (abundance) of the $i$-th species in the sample, $i = 1, 2, \ldots, S$, $\sum_{i=1}^{S} X_i = n$, and let $f_r$ denote the number of species each represented by exactly $r$ individuals in the sample, i.e.,

$$f_r = \sum_{i=1}^{S} I(X_i = r), \quad r = 0, 1, 2, \ldots,$$

where $I(A)$ is the indicator function, i.e., $I(A) = 1$ if the event $A$ occurs, and 0 otherwise. Given data, for those species that each appeared exactly $r$ times in an incomplete sample of $n$ individuals, the mean of their true relative abundances/frequencies in the assemblage, $\alpha_r$, can be mathematically expressed as

$$\alpha_r = \sum_{i=1}^{S} p_i I(X_i = r) / f_r, \quad r = 0, 1, 2, \ldots$$

In the main text, we reviewed a very intuitive justification by Good (1983, p. 28) for the Good-Turing frequency formula. Here present another simple proof for the following
improved Good-Turing formula that we adopted in our derivations:

$$\hat{\alpha}_r = \frac{(r+1)f_{r+1}}{(n-r)f_r}, \quad r = 0, 1, 2, \ldots$$

Assume that the sample frequencies ($X_1, X_2, \ldots, X_S$) follow a multinomial distribution with cell total $n$ and cell probabilities $(p_1, p_2, \ldots, p_S)$. Then each $X_i$, $i = 1, 2, \ldots, S$, is a binomial distribution $B(n, p_i)$, leading to the following formula for the expected value of $f_r$:

$$E(f_r) = E\left(\sum_{i=1}^{S} I(X_i = r)\right) = \sum_{i=1}^{S} \binom{n}{r} p_i^r (1-p_i)^{n-r}, \quad r = 0, 1, 2, \ldots$$

Based on the above formula, we can derive an approximation formula for the expected value of $\sum_{i=1}^{S} p_i I(X_i = r)$ (the numerator of $\alpha_r$) as follows.

$$E\sum_{i=1}^{S} p_i I(X_i = r) = \sum_{i=1}^{S} \binom{n}{r} p_i^r (1-p_i)^{n-r} \approx \sum_{i=1}^{S} \binom{n}{r} p_i^{r+1} (1-p_i)^{n-(r+1)}$$

$$= \binom{n}{r} \left[ \sum_{i=1}^{S} \binom{n}{r+1} p_i^{r+1} (1-p_i)^{n-(r+1)} \right] = \frac{(r+1)}{(n-r)} E(f_{r+1}).$$

This implies that the numerator of $\alpha_r$ can be estimated by $(r+1)f_{r+1}/(n-r)$, and thus Eq. (1d) is proved. For the special cases of $r = 0$ and $r = 1$, we have

$$\hat{\alpha}_0 \approx f_1/n; \quad \hat{\alpha}_1 \approx \frac{2f_2}{(n-1)f_1},$$

where $\hat{\alpha}_0 \approx f_0$ denote the estimator of the product of $\alpha_0$ and $f_0$. Then we obtain a lower bound (Eq. 2c in the main text) for the number of undetected species by the following inequality:

$$\hat{f}_0 = \frac{\hat{\alpha}_0 \hat{f}_0}{\hat{\alpha}_0} \geq \frac{\hat{\alpha}_0 f_0}{\hat{\alpha}_1} = \frac{f_1}{\frac{n}{2f_2} (n-1)f_1} = \frac{(n-1)}{n} \frac{f_2^2}{2f_2}.$$

**Proof of Eq. (5c) of the main text**

Consider the case of two assemblages (I and II), which can differ not only in their species richness, but also in their species composition. Assume that there are $S$ species in the pooled
assemblage. Following the two-assemblage model formulation and data framework discussed in the main text, we assume that the species sample frequencies \((X_{11}, X_{21}, \ldots, X_{s1})\) of Sample I taken from Assemblage I follow a multinomial distribution with cell total \(n_1\) and cell probabilities \((p_{11}, p_{21}, \ldots, p_{s1})\), and the species sample frequencies \((X_{12}, X_{22}, \ldots, X_{s2})\) of Sample II taken from Assemblage II follow a multinomial distribution with cell total \(n_2\) and cell probabilities and \((p_{12}, p_{22}, \ldots, p_{s2})\). As defined in the main text, let \(f_{rv}\) denote the number of shared species that are observed \(r\) times in Sample I and \(v\) times in Sample II. That is,

\[ f_{rv} = \sum_{i=1}^{S_{12}} I(X_{i1} = r, X_{i2} = v), \quad r, v = 0, 1, 2, \ldots \]

where \(S_{12}\) denotes the number of species shared by the two focal assemblages. Assuming, further, that the two samples are statistically independent, we have the following general formula for the expected value of \(f_{rv}\) for \(r, v = 0, 1, 2, \ldots\)

\[
E(f_{rv}) = \sum_{i=1}^{S_{12}} P(X_{i1} = r, X_{i2} = v),
\]

\[
= \sum_{i=1}^{S_{12}} \binom{n_1}{r} p_{i1}^r (1 - p_{i1})^{n_1-r} \binom{n_2}{v} p_{i2}^v (1 - p_{i2})^{n_2-v}.
\]

For any shared species that appeared exactly \(r\) times in each sample (there are \(f_{rr}\) such shared species in the two-sample data), consider calculating the product of its true relative abundances in the two assemblages; the mean of the products among all such shared species can be mathematically expressed as

\[
\alpha_{rr} = \sum_{i=1}^{S_{12}} p_{i1} p_{i2} I(X_{i1} = r, X_{i2} = r) / f_{rr}, \quad r = 0, 1, 2, \ldots
\]

As with the one-sample Good-Turing formula, we first approximate the expected value of the numerator of \(\alpha_{rr}\). Note that we can write

\[
E \sum_{i=1}^{S_{12}} p_{i1} p_{i2} I(X_{i1} = r, X_{i2} = r)
\]

\[
= \sum_{i=1}^{S_{12}} p_{i1} p_{i2} \binom{n_1}{r} p_{i1}^r (1 - p_{i1})^{n_1-r} \binom{n_2}{v} p_{i2}^v (1 - p_{i2})^{n_2-v}
\]
\[
\approx \sum_{j=1}^{n} \binom{n_1}{r} \binom{n_2}{r} p_{ri}^{r+1} (1 - p_{ri})^{n_i - (r+1)} \times p_{j2}^{r+1} (1 - p_{j2})^{n_j - (r+1)}
\]

\[
= \frac{\binom{n_1}{r} \binom{n_2}{r}}{\binom{n_1}{r+1} \binom{n_2}{r+1}} E(f_{r+1,r+1}) = \frac{(r+1)^2 E(f_{r+1,r+1})}{(n_1 - r)(n_2 - r)}.
\]

This implies that the numerator of \( \alpha_{rr} \) can be estimated by \((r+1)^2 f_{r+1,r+1} f((n_1 - r)(n_2 - r)].\)

We then obtain the generalized two-assemblage Good-Turing formula:

\[
\hat{\alpha}_{rr} = \frac{(r+1)^2 f_{r+1,r+1}}{(n_1 - r)(n_2 - r) f_{rr}}, \quad r = 0, 1, 2, \ldots
\]

In particular, we have

\[
\overline{a_{00}} f_{00} = \frac{f_{11}}{n_1 n_2}; \quad \hat{\alpha}_{11} = \frac{4 f_{22}}{(n_1-1)(n_2-1)f_{11}}.
\]

Thus, we have a lower bound for \( f_{00} \) (Eq. 7c in the main text) by the following inequality:

\[
f^2_{00} = \frac{a_{00}}{\overline{a_{00}}} \geq \frac{a_{00}}{\overline{a_{11}}} = \frac{f_{11}}{(n_1-1)(n_2-1)f_{11}} = \frac{(n_1-1)(n_2-1)}{n_1 n_2} \frac{f_{11}}{4 f_{22}}.
\]

This extends the original Good-Turing one-assemblage formula to the two-assemblage case.

**Reference for Appendix S1**


University of Minnesota Press, Minneapolis, USA.
Appendix S2

Deciphering the Enigma of Undetected Species, Phylogenetic, and Functional Diversity Based on Good-Turing Theory

Anne Chao, Chun-Huo Chiu, Robert K. Colwell, Luiz Fernando S. Magnago, Robin L. Chazdon, and Nicholas J. Gotelli

To make the contents of this appendix self-contained, some definitions introduced in the main text are repeated here.

APPENDIX S2: Phylogenetic diversity (mathematical details)

Proof of Eq. (8b) of the main text

As defined in the main text, let $a_i$ denote the node/branch relative abundance, i.e., $a_i$ represents the total relative abundance of the species descended from the $i$th node/branch, $i = 1, 2, \ldots, B$. The set of species relative abundances $(p_1, p_2, \ldots, p_S)$ for tip nodes is expanded to a larger relative abundance set $\{a_i, i = 1, 2, \ldots, B\}$ with $(p_1, p_2, \ldots, p_S)$ as its first $S$ elements.

For a sample of $n$ individuals with observed species abundances $(X_1, X_2, \ldots, X_S)$, let $X_i^*$ be the sum of the sample species abundances for those species descended from branch $i$. Then we can expand the set of observed species abundances or frequencies $(X_1, X_2, \ldots, X_S)$ to a larger branch abundance set $\{X_i^*, i = 1, 2, \ldots, B\}$ with $(X_1, X_2, \ldots, X_S)$ as its first $S$ elements. As introduced in the main text, we refer to $X_i^*$, $i = 1, 2, \ldots, B$, as the sample node/branch abundance of node/branch $i$. Let

$$ g_r = \sum_{i=1}^{B} \sum_{r=0}^{L_i} I(X_i^* = r), \quad r = 0, 1, 2, \ldots $$

be the total length of the branches with sample branch abundance $r$. A simple example is
provided in Appendix S2: Fig. 1 to illustrate these measures.

Here we first extend the Good-Turing frequency formula to its phylogenetic version for a single assemblage. For those nodes/branches that each is with a sample abundance of \( r \), the branch-length-weighted mean (per unit length) of their true abundances in the entire assemblage, \( \lambda_r \), can be expressed as

\[
\lambda_r = \sum_{i=1}^{\beta} L_i a_i \; I(X_i^* = r) / g_r, \quad r = 0, 1, 2, \ldots
\]

We now prove the corresponding phylogenetic version of the Good-Turing one-assemblage frequency formula:

\[
\hat{\lambda}_r = \frac{(r+1) g_{r+1}}{(n-r) g_r}, \quad r = 0, 1, 2, \ldots
\]

All the following derivation steps are parallel to those in the original Good-Turing formula (Appendix S1) for species richness. Under the model that the sample branch frequency \( X_i^* \) follows a binomial distribution \( B(n, a_i) \), we have a general form for the expected value of \( g_r \):

\[
E(g_r) = \sum_{i=1}^{\beta} L_i \; I(X_i^* = r) = \sum_{i=1}^{\beta} L_i \binom{n}{r} a_i^r (1-a_i)^{n-r}, \quad r = 0, 1, 2, \ldots
\]

Then we can estimate the expected value of the numerator of \( \lambda_r \) by the following:

\[
E \sum_{i=1}^{\beta} L_i a_i \; I(X_i^* = r) = \sum_{i=1}^{\beta} L_i a_i \binom{n}{r} a_i^r (1-a_i)^{n-r} \approx \sum_{i=1}^{\beta} L_i \binom{n}{r} a_i^{r+1} (1-a_i)^{n-(r+1)}
\]

\[
= \binom{n}{r} \sum_{i=1}^{\beta} L_i \binom{n}{r+1} a_i^{r+1} (1-a_i)^{n-(r+1)} = \frac{(r+1)}{(n-r)} E(g_{r+1}).
\]

This implies that the numerator of \( \lambda_r \) can be estimated by \( (r+1) g_{r+1} / (n-r) \), and we obtain the phylogenetic version of the Good-Turing one-assemblage frequency formula. For the special cases of \( r = 0 \) and \( r = 1 \), we have

\[
\overline{\lambda_0} g_0 = \frac{g_1}{n}; \quad \hat{\lambda}_1 = \frac{2 g_2}{(n-1) g_1}.
\]

Therefore, a lower bound for the undetected PD is obtained:
\[ \hat{g}_0 = \frac{\lambda_0 g_0}{\lambda_0} \geq \frac{\lambda_0 g_0}{\lambda_1} = \frac{g_1}{\frac{n}{2g_2}} = \frac{(n-1)}{n} \frac{g_1^2}{2g_2}. \]

Appendix S2: Table S1 summarizes the analogy between species richness estimation and PD estimation. This analogy means that most ecological indices defined at the species level can be converted to PD equivalents; see Faith (1992) and Chao et al. (2014).
Fig. S1. (a) A hypothetical tree spanned by \( S = 9 \) species (tip nodes) indexed by 1, 2, \ldots, 9 in an assemblage. Here the root of the entire assemblage is selected as the reference point for illustration. (b) A sub-tree spanned by the observed 5 species (1, 2, 3, 5 and 6, black tip dots) in a sample from the assemblage; Species 4, 7, 8 and 9 are not detected in the sample (grey tip dots), so only a portion of the tree (solid branches in the left panels) is observed, as shown in Panel (b). The sample abundance/frequency vector for 9 species is \((X_1, X_2, \ldots, X_9) = (6, 1, 1, 0, 2, 1, 0, 0, 0)\), with \( f_1 = 3, f_2 = 1 \); only non-zero frequencies are observed in the sample. Black dots in Panel (a) are nodes with sample frequency > 0; grey dots are nodes with frequency = 0. The branch set in the assemblage includes \( B = 15 \) branches/nodes (indexed from 1 to 15) with branch lengths \((L_1, L_2, \ldots, L_{15})\). The corresponding node/branch frequencies are denoted by \((X_{i}^*, X_{i}^*, \ldots, X_{i}^*, X_{i}^*, X_{i}^*, X_{i}^*, X_{i}^*) = (6, 1, 1, 0, 2, 1, 0, 0, 0, 2, 8, 2, 1, 0, 1)\) with \((X_1, X_2, \ldots, X_9)\) as its first 9 elements. The dotted branches in Panel (a) are not detected in the sample, and the total length of the undetected branches is \( g_0 = L_4 + L_7 + L_8 + L_9 + L_{14} \). Black dots in Panel (b) are nodes in the observed tree; greys dots are not observed in the tree. The total length of those branches with sample node/branch frequency \( X_{i}^* = 1 \) in the observed tree is \( g_1 = \sum_{i=1}^{9} L_i I(X_{i}^* = 1) = L_2 + L_5 + L_6 + L_{13} + L_{15} \) (as shown by green lines in the observed tree in Panel (b)); the total length with sample node/branch frequency \( X_{i}^* = 2 \) in the observed tree is \( g_2 = \sum_{i=1}^{9} L_i I(X_{i}^* = 2) = L_5 + L_{10} + L_{12} \) (as shown by red lines in the observed tree in Panel (b)). The number of nodes/branches with abundance = 1 is \( f_1^* = 3 \); the number of nodes/branches with abundance = 2 is \( f_2^* = 2 \).
Table S1. A summary of the analogy between species richness estimation and PD estimation. A phylo-entity (= phylogenetic entity) is defined as a branch of unit length. A phylogenetic entity in estimating Faith’s PD plays exactly the same role as a species in estimating species richness. $f_i$: the number of species each represented by exactly $r$ individuals in the sample. $g_r$: the total length of the branches with sample branch abundance/frequency $r$.

<table>
<thead>
<tr>
<th>Species richness</th>
<th>Faith’s phylogenetic diversity (PD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description</strong></td>
<td><strong>Formula</strong></td>
</tr>
<tr>
<td>Species richness</td>
<td>$S$</td>
</tr>
<tr>
<td>Observed species richness</td>
<td>$S_{obs} = \sum_{i&gt;0} f_i \equiv f_+$</td>
</tr>
<tr>
<td>Undetected species richness</td>
<td>$f_0 = \sum_{i=1}^{S} I(X_i = 0)$</td>
</tr>
<tr>
<td>Good-Turing estimating target</td>
<td>$\alpha_r = \frac{\sum_{i=1}^{S} p_i I(X_i = r)}{f_r}$</td>
</tr>
<tr>
<td>Good-Turing frequency formula</td>
<td>$\hat{\alpha}<em>r = \frac{(r+1)f</em>{r+1}}{(n-r)f_r}$</td>
</tr>
<tr>
<td>Estimator of undetected species richness</td>
<td>$\hat{f}_0 = \frac{(n-1)}{n} \frac{f_1^2}{2f_2}$</td>
</tr>
<tr>
<td>Chao1 estimator</td>
<td>$\hat{S}<em>{Chao} = S</em>{obs} + \hat{f}_0$</td>
</tr>
</tbody>
</table>
Proof of Eq. (10b) of the main text

Under the two-assemblage model formulation and data framework discussed in the main text, the set of species relative abundances \((p_{11}, p_{21}, \ldots, p_{S1})\) of Assemblage I is expanded to a larger relative abundance set \(\{a_{i1}, i = 1, 2, ..., B\}\). Similarly, the set of species relative abundances \((p_{12}, p_{22}, \ldots, p_{S2})\) of Assemblage II is expanded to a larger relative abundance set \(\{a_{i2}, i = 1, 2, ..., B\}\). Assume that there are \(B_{12}\) branches shared by two assemblages, and without loss of generality, these shared branches are indexed from 1, 2, …, \(B_{12}\) for notational simplicity. Denote the true shared PD (the total length of branch segments shared by the two individual trees) by \(PD_{\text{shared}}\).

As described in the main text, the set of the observed species frequencies in Assemblage I, \((X_{11}, X_{21}, \ldots, X_{S1})\), is expanded to a larger sample node/branch frequency set \(\{X_{i1}, i = 1, 2, ..., B\}\). Similarly, we expand the set of observed species frequencies in Assemblage II, \((X_{12}, X_{22}, \ldots, X_{S2})\), to a larger sample branch abundance set \(\{X_{i2}, i = 1, 2, ..., B\}\). As in one-assemblage case, \(X_{i1}^*\) follows a binomial distribution \(B(n_1, a_{i1})\), and \(X_{i2}^*\) follows a binomial distribution \(B(n_2, a_{i2})\). Let \(g_{rv}\) be the total length of those shared branches with sample branch abundance \(r\) in Sample I and sample branch abundance \(v\) in Sample II. That is,

\[
g_{rv} = \sum_{i=1}^{B_{12}} L_i I(X_{i1}^* = r, X_{i2}^* = v), \quad r, v = 0, 1, 2, \ldots
\]

Assuming further that the two samples are statistically independent, we have a general formula for the expected value of \(g_{rv}\) for \(r, v = 0, 1, 2, \ldots\)

\[
E(g_{rv}) = \sum_{i=1}^{B_{12}} L_i P(X_{i1}^* = r, X_{i2}^* = v),
\]

\[
= \sum_{i=1}^{B_{12}} L_i \binom{n_1}{r} a_{i1}^r (1 - a_{i1})^{n_1 - r} \binom{n_2}{v} a_{i2}^v (1 - a_{i2})^{n_2 - v}.
\]
For any shared branch segment with node/branch abundance $r$ in each observed branch set, consider calculating the product of its true branch relative abundances in the two assemblages.

The branch-length-weighted mean (per unit-length) of the products among all such shared branches (their total length is $g_{rr}$) can be expressed as

$$\lambda_{rr} = \sum_{i=1}^{n_i} L_i a_{i1} a_{i2} I(X_{i1}^* = r, X_{i2}^* = r) / g_{rr}, \quad r = 0, 1, 2, \ldots$$

We first approximate the expected value of the numerator of $\lambda_{rr}$. Note that we can write

$$E \sum_{i=1}^{n_i} L_i a_{i1} a_{i2} I(X_{i1}^* = r, X_{i2}^* = r)$$

$$= \sum_{i=1}^{n_i} L_i a_{i1} a_{i2} \left( \frac{n_i}{r} a_{i1}^r (1 - a_{i1})^{n_i - r} \left( \frac{n_2}{v} a_{i2}^v (1 - a_{i2})^{n_2 - v} \right) \right)$$

$$\approx \sum_{i=1}^{n_i} L_i \left( \binom{n_i}{r} a_{i1}^r (1 - a_{i1})^{n_i - r} \right) \left( \frac{n_2}{v} a_{i2}^v (1 - a_{i2})^{n_2 - v} \right)$$

$$= \left( \binom{n_1}{r} \binom{n_2}{r} \right) E(g_{r+1, r+1}) = \frac{(r + 1)^2 E(g_{r+1, r+1})}{(n_1 - r)(n_2 - r)}.$$

Thus, the numerator of $\lambda_{rr}$ can be estimated by $(r + 1)^2 g_{r+1, r+1} / [(n_1 - r)(n_2 - r)]$. We then obtain the generalized two-assemble phylogenetic Good-Turing formula:

$$\hat{\lambda}_{rr} = \frac{(r + 1)^2 g_{r+1, r+1}}{(n_1 - r)(n_2 - r) g_{rr}}, \quad r = 0, 1, 2, \ldots$$

In particular, we have

$$\hat{\lambda}_{00} \hat{g}_{00} = \frac{g_{11}}{n_1 n_2}; \quad \hat{\lambda}_{11} = \frac{4g_{22}}{(n_1 - 1)(n_2 - 1) g_{11}}.$$

Then we obtain a lower bound of $g_{00}$:

$$\hat{g}_{00} = \frac{\hat{\lambda}_{00} \hat{g}_{00}}{\hat{\lambda}_{00}} \geq \frac{\hat{\lambda}_{00} \hat{g}_{00}}{\hat{\lambda}_{11}} = \frac{\frac{g_{11}}{n_1 n_2}}{\frac{4g_{22}}{(n_1 - 1)(n_2 - 1) g_{11}}} = \frac{(n_1 - 1) (n_2 - 1) g_{11}^2}{n_1 n_2 4g_{22}}.$$

All the above derivation steps are parallel to those in Appendix S1 for estimating shared species richness. Appendix S2: Table S2 summarizes the analogy between shared species
richness estimation and shared $PD$ estimation. The analogy lies in that any unit-length branch shared by two assemblages plays the same role as a “shared species” in the estimation of shared species richness. Thus, each unit-length branch segment can be regarded as a shared phylogenetic entity. For example, a shared branch of 8-unit length is counted as 8 shared phylogenetic entities. In the estimation of shred $PD$, we are measuring the total number of shared phylogenetic entities, equivalently, the total shared branch length (because each entity has length of unity). Using this perspective, the counts of shared branch lengths 
\[ \{g_{rv}, r, v = 0, 1, 2, \ldots \} \]
in estimating shared $PD$ play a role corresponding to the frequency counts 
\[ \{f_{rv}, r, v = 0, 1, 2, \ldots \} \]
in estimating shared species richness. Hence, not only within-assemblage but also between-assemblage measures defined at the species level can be directly converted to their $PD$ equivalents.
Table S2. A summary of the analogy between shared species richness estimation and shared PD estimation. \( B_{12} \): number of shared branches between two assemblages. A shared phylo-entity (= shared phylogenetic entity) is defined as a shared branch with a unit of length. A shared phylogenetic entity in estimating shared PD plays the same role as a shared species in estimating shared species richness. \( k_i = (n_i - 1)/n_i \), \( i = 1, 2 \).

<table>
<thead>
<tr>
<th>Shared species richness</th>
<th>Shared PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>Formula</td>
</tr>
<tr>
<td>Shared species richness</td>
<td>( S_{shared} ) or ( S_{12} )</td>
</tr>
<tr>
<td>Observed shared species richness</td>
<td>( S_{shared obs} = \sum_{i,j&gt;0} f_{ij} = f_{++} )</td>
</tr>
<tr>
<td>Undetected shared species richness</td>
<td>( f_{0+} + f_{-+} + f_{00} )</td>
</tr>
<tr>
<td>Two-assemblage Good-Turing estimating target</td>
<td>( \alpha_r = \frac{\sum_{i,j} p_i p_j I(X_{ij} = r, X_{ij} = r)}{f_{rr}} )</td>
</tr>
<tr>
<td>Two-assemblage Good-Turing frequency formula</td>
<td>( \hat{\alpha}<em>r = \frac{(r+1)^2 f</em>{r+1,r+1}}{(n_i - r)(n_j - r)f_{rr}} )</td>
</tr>
<tr>
<td>Estimator of undetected shared species richness</td>
<td>( k_1 \frac{f_{1+}^2}{2f_{2+}} + k_2 \frac{f_{+1}^2}{2f_{+2}} + k_1 k_2 \frac{f_{11}^2}{4f_{22}} )</td>
</tr>
<tr>
<td>Chao1 shared estimator</td>
<td>( \hat{S}<em>{Chao, shared} = S</em>{shared obs} + ) ( estimator of undetected )</td>
</tr>
</tbody>
</table>
References for Appendix S2


Appendix S3

Deciphering the Enigma of Undetected Species, Phylogenetic, and Functional Diversity Based on Good-Turing Theory

Anne Chao, Chun-Huo Chiu, Robert K. Colwell, Luiz Fernando S. Magnago, Robin L. Chazdon, and Nicholas J. Gotelli

To make the contents of this appendix self-contained, some definitions introduced in the main text are repeated here.

**APPENDIX S3: Functional diversity (mathematical details)**

**Proof of Eq. (12c) of the main text**

Consider an assemblage in which all species are characterized by a set of functional traits, which can be categorical or continuous variables. The pairwise distances are calculated by some distance metric (e.g., Euclidean distance or Gower distance) based on the values of species traits. Let $d_{ij}$ be the functional distance between the $i$th and $j$th species, with $d_{ij} = d_{ji} > 0$. Walker et al.’s (1999) FAD in the entire assemblage is expressed as $FAD = \sum_{i,j=1}^{S} d_{ij}$.

Assume that a random sample of $n$ individuals is taken from an assemblage with $S$ species and relative abundance set $(p_1, p_2, \ldots, p_S)$. Let $X_i$ denote the sample frequency (abundance) of the $i$-th species in the sample, $i = 1, 2, \ldots, S$, $\sum_{i=1}^{S} X_i = n$. As defined in the main text, let $F_{rv}$ denote the total distance of those species pairs with sample abundances $r$ and $v$ respectively for the two species in each pair. That is,

$$F_{rv} = \sum_{i,j=1}^{S} d_{ij} I(X_i = r, X_j = v) .$$

Assume that the sample frequencies $(X_1, X_2, \ldots, X_S)$ follow a multinomial distribution with cell total $n$ and cell probabilities $(p_1, p_2, \ldots, p_S)$. Then $(X_i, X_j)$ follows a trinomial distribution.
with cell total \( n \) and cell probabilities \( (p_i, p_j, 1-p_i-p_j) \), leading to a general formula for
the expected value of \( F_{rv} \):

\[
E(F_{rv}) = E \sum_{i,j=1}^{S} d_{ij} I(X_i = r, X_j = v)
\]

\[
= \sum_{i,j=1}^{S} d_{ij} \frac{n!}{r!r!(n-r-v)!} p_i^{r+1} p_j^{r+1} (1-p_i-p_j)^{n-r-v}.
\]

Out of all the species which appeared exactly \( r \) times in the sample, consider choosing any
two to form a pair and calculating the product of their true relative abundances in the
assemblage. The distance-weighted mean (per unit-distance) of the products among all such
pairs (their total pairwise distance is \( F_{rv} \)) can be expressed as

\[
\theta_{rv} = \sum_{i,j=1}^{S} d_{ij} p_i p_j I(X_i = r, X_j = r) / F_{rv}, \quad r = 0, 1, 2, \ldots
\]

We first approximate the expected value of the numerator of \( \theta_{rv} \). Note that we can write

\[
E \sum_{i,j=1}^{S} d_{ij} p_i p_j I(X_i = r, X_j = r)
\]

\[
\approx \sum_{i,j=1}^{S} d_{ij} \frac{n!}{r!r!(n-2r)!} p_i^{r+1} p_j^{r+1} (1-p_i-p_j)^{n-2r}
\]

\[
= \frac{n!}{(r!)^2(n-2r)!} E(F_{r+1,r+1}) = \frac{(r+1)^2 E(F_{r+1,r+1})}{(n-2r)(n-2r-1)}.
\]

Then we have

\[
\hat{\theta}_{rv} = \frac{(r+1)^2 F_{r+1,r+1}}{(n-2r)(n-2r-1) F_{rv}}, \quad r = 0, 1, 2, \ldots
\]

In particular, we have

\[
\hat{\theta}_{00} F_{00} = \frac{F_{11}}{n(n-1)}; \quad \hat{\theta}_{11} = \frac{4F_{22}}{(n-2)(n-3) F_{11}}.
\]

Then we obtain a lower bound for \( F_{00} \):
\[ \hat{F}_{00} = \frac{\theta_{00} \hat{F}_{00}}{\theta_0} \geq \frac{\theta_{00} \hat{F}_{00}}{\theta_{11}} = \frac{F_{11}}{n(n-1)} \frac{4 F_{22}}{(n-2)(n-3) F_{11}} = \frac{(n-2)(n-3)}{n} \frac{F_{11}^2}{n-1} \frac{4 F_{22}}{n} \]

Appendix S3: Table S1, below, summarizes the analog between shared species richness estimation between two assemblages and FAD estimation in a single assemblage. A “functional entity” (defined as a species pair with one unit of distance) plays the same role as a “shared species” between two assemblages.
Table S1. A summary of the analogy between shared species richness estimation between two assemblages and FAD estimation in a single assemblage. $S_{1:2}$: number of shared species between two assemblages. In the table, a func-entity (= functional entity) is defined as a species pair with a unit of distance of dissimilarity. Functional entities play the same role as "shared species" between two assemblages; $k_i = (n_i - 1)/n_i$, $i = 1, 2$.

<table>
<thead>
<tr>
<th>Shared species richness</th>
<th>Formula</th>
<th>FAD (Functional Attribute Diversity)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Shared species richness</strong></td>
<td>$S_{\text{shared}}$ or $S_{1:2}$</td>
<td>$\hat{FAD} = \sum_{ij=1}^{S} d_{ij}$</td>
<td>Func-entity richness</td>
</tr>
<tr>
<td><strong>Observed shared species richness</strong></td>
<td>$S_{\text{shared,obs}} = \sum f_{rv} = f_{++}$</td>
<td>$\hat{FAD}<em>{\text{obs}} = \sum f</em>{rv} = f_{++}$</td>
<td>Observed func-entity richness</td>
</tr>
<tr>
<td></td>
<td>$= \sum_{ij=1}^{S} I(X_{i1} &gt; 0, X_{i2} &gt; 0)$</td>
<td>$= \sum_{i,j=1}^{S} d_{ij} I(X_{i} &gt; 0, X_{j} &gt; 0)$</td>
<td></td>
</tr>
<tr>
<td><strong>Undetected shared species richness</strong></td>
<td>$f_{0+} + f_{+0} + f_{00}$</td>
<td>$F_{0+} + F_{+0} + F_{00}$</td>
<td>Undetected func-entity richness</td>
</tr>
<tr>
<td><strong>Two-assemblelge</strong></td>
<td>$\alpha_{rr} = \frac{S_{1:2}}{\sum_{i,j=1}^{S} f_{ij} p_{i2} I(X_{i1} = r, X_{i2} = r)}$</td>
<td>$\hat{\theta}<em>{rr} = \frac{\sum</em>{i,j=1}^{S} d_{ij} p_{i} p_{j} I(X_{i} = r, X_{j} = r)}{F_{rr}}$</td>
<td>Functional Good-Turing estimating target</td>
</tr>
<tr>
<td><strong>Good-Turing estimating target</strong></td>
<td>$\hat{\alpha}<em>{rr} = \frac{(r+1)^2 f</em>{r+1,r+1}}{(n_1-r)(n_2-r)f_{rr}}$</td>
<td>$\hat{\theta}<em>{rr} = \frac{(r+1)^2 F</em>{r+1,r+1}}{(n-2r)(n-2r-1)F_{rr}}$</td>
<td></td>
</tr>
<tr>
<td><strong>Good-Turing frequency formula</strong></td>
<td>$\hat{\alpha}<em>{rr} = \frac{f</em>{rr}}{2f_{2r}} + k \frac{f_{r1}^2}{2f_{r1}} + k \frac{f_{12}^2}{4f_{22}}$</td>
<td>$\hat{\theta}<em>{rr} = \frac{F</em>{rr}^2}{2F_{2r}} + k \frac{F_{r1}^2}{2F_{r1}} + (n-2)(n-3) \frac{F_{12}^2}{n(n-1)}$</td>
<td>Estimator of undetected func-entity richness</td>
</tr>
<tr>
<td><strong>Estimator of undetected shared species richness</strong></td>
<td>$\hat{S}<em>{\text{Chao,shared}} = S</em>{\text{shared,obs}} + \text{estimator of the undetected}$</td>
<td>$\hat{FAD}<em>{\text{Chao1}} = \hat{FAD}</em>{\text{obs}} + \text{estimator of the undetected}$</td>
<td>Chao1-FAD estimator</td>
</tr>
</tbody>
</table>
Proof of Eq. (14b) of the main text

Under the two-assemblage model formulation and data framework discussed in the main text, the total distance of the species pairs shared by two assemblages can be expressed as the sum of 16 terms, which are shown, below, in a 4 x 4 matrix (Appendix S3: Table S2).

\[
FAD_{\text{shared}} = \sum_{r,v=+,0} \sum_{k,m=+,0} F_{(rv;km)}
\]

\[
= F_{(++;++)} + F_{(++;+0)} + F_{(++;0+)} + \ldots + F_{(00;00)}.
\]

Here \( F_{(rv;km)} \) measures the total functional distance of those pairs of shared species with abundances \((r, v)\) in Sample I and abundances \((k, m)\) in Sample II for the two species in each pair. Where any index is replaced by a “+” sign, it means that those species occur at least once in the corresponding sample. In the above equation, the first term \( F_{(++;++)} = FAD_{\text{shared,obs}} \) denotes the observed shared FAD; the other 15 terms are unknown. The matrix in Appendix S3: Table S2 is symmetric, so that there are only 10 unique terms. For each term, we can apply a similar type of Good-Turing formula to obtain an estimator (given in Appendix S3: Table S3), leading to the following Chao1-FAD-shared estimator:

\[
\overline{FAD}_{\text{Chao1,shared}} = F_{(++;++)} + \hat{F}_{(++;+0)} + \hat{F}_{(++;0+)} + \ldots + \hat{F}_{(00;00)}.
\]

A bootstrap method can be used to assess the sampling variance of the above estimator and to obtain the associated confidence interval.
**Table S2.** The FAD shared by two assemblages can be written as the sum of 16 terms (in colored cells). For any shared species pair \((i, j)\) with abundances \((X_{i1}, X_{j1})\) in Sample I and abundances \((X_{i2}, X_{j2})\) in Sample II for the two shared species in each pair, the green cell (Cell 1) denotes the case that two species \(i\) and \(j\) appear in both samples; the total distance of such pairs is \(F_{(+)(+)}\). Cell 3 denotes the case that the two species \(i\) and \(j\) both appear in Sample I, but only species \(i\) appears in Sample II; the total distance of such pairs is \(F_{(+)(0)}\). Cell 3* denotes the case that the two species \(i\) and \(j\) both appear in Sample I, but only species \(j\) appears in Sample II; the total distance of such pairs is \(F_{(+)(0)}\). Here we have \(F_{(+)(0)} = F_{(+)(0)}\) so that the matrix is symmetric. The blue cells (Cells 2, 3, 3*) plus the green cell denote the case that the two species \(i\) and \(j\) both appear in Sample I. The yellow cells (Cells 4, 4*, 8) plus the green cell denote the case that the two species \(i\) and \(j\) both appear in Sample II. Other cases are in grey cells.

<table>
<thead>
<tr>
<th>Shared species pair ((i, j))</th>
<th>(X_{j1} &gt; 0)</th>
<th>(X_{j1} = 0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(X_{i1} &gt; 0)</td>
<td>(X_{i2} &gt; 0)</td>
<td>(X_{i2} = 0)</td>
</tr>
<tr>
<td>(X_{i2} &gt; 0)</td>
<td>(F_{(+)(+)})</td>
<td>(F_{(+)(0)})</td>
</tr>
<tr>
<td>(F_{(+)(0)})</td>
<td><strong>Cell 1</strong></td>
<td><strong>Cell 3</strong></td>
</tr>
<tr>
<td>(X_{i2} = 0)</td>
<td>(F_{(+)(+)})</td>
<td>(F_{(+)(0)})</td>
</tr>
<tr>
<td>(F_{(+)(0)})</td>
<td><strong>Cell 3</strong></td>
<td><strong>Cell 2</strong></td>
</tr>
<tr>
<td>(X_{i1} = 0)</td>
<td>(X_{i2} &gt; 0)</td>
<td>(F_{(0)(+)})</td>
</tr>
<tr>
<td>(F_{(0)(+)})</td>
<td><strong>Cell 4</strong></td>
<td><strong>Cell 6</strong></td>
</tr>
<tr>
<td>(X_{i2} = 0)</td>
<td>(F_{(0)(+)})</td>
<td>(F_{(0)(0)})</td>
</tr>
<tr>
<td>(F_{(0)(+)})</td>
<td><strong>Cell 5</strong></td>
<td><strong>Cell 7</strong></td>
</tr>
</tbody>
</table>
Table S3. The observed shared FAD (Cell 1) and the estimator for each of the 15 unknown terms in the expression of the true FAD shared by two assemblages; see Appendix S3: Table S2 for cell definitions.

<table>
<thead>
<tr>
<th>Cell</th>
<th>Functional distance</th>
<th>Statistics/Estimator</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$F_{(++)(++)}$</td>
<td>$F_{(++)(++)} = F_{AD_{shared}}$</td>
</tr>
<tr>
<td>2</td>
<td>$F_{(++)(00)}$</td>
<td>$(n_2 - 2)(n_2 - 3) \frac{F^2_{(++)(11)}}{n_2(n_2-1) , 4F_{(++)(22)}}$</td>
</tr>
<tr>
<td>3, 3*</td>
<td>$F_{(++)(00)} = F_{(++)(00)}$</td>
<td>$\frac{n_2 - 1}{n_2} \frac{F^2_{(++)(11)}}{2F_{(++)(22)}}$</td>
</tr>
<tr>
<td>4, 4*</td>
<td>$F_{(0+)(++)} = F_{(0+)(++)}$</td>
<td>$\frac{n_1 - 1}{n_1} \frac{F^2_{(0+)(11)}}{2F_{(0+)(22)}}$</td>
</tr>
<tr>
<td>5, 5*</td>
<td>$F_{(0+)(++)} = F_{(0+)(++)}$</td>
<td>$\frac{n_1 - 1}{n_1} \frac{(n_2 - 1)(n_2 - 1)}{n_2} \frac{F^2_{(1+)(++)}}{4F_{(0+)(22)}}$</td>
</tr>
<tr>
<td>6, 6*</td>
<td>$F_{(0+)(00)} = F_{(0+)(++)}$</td>
<td>$\frac{n_1 - 1}{n_1} \frac{(n_2 - 1)(n_2 - 1)}{n_2} \frac{F^2_{(1+)(++)}}{4F_{(0+)(22)}}$</td>
</tr>
<tr>
<td>7, 7*</td>
<td>$F_{(0+)(00)} = F_{(0+)(00)}$</td>
<td>$\frac{n_1 - 1}{n_1} \frac{(n_2 - 2)(n_2 - 3)}{n_2(n_2-1)} \frac{F^2_{(11)(++)}}{8F_{(0+)(22)}}$</td>
</tr>
<tr>
<td>8</td>
<td>$F_{(00)(++)}$</td>
<td>$\frac{n_1 - 2}{n_1} \frac{(n_1 - 3)}{n_1(n_1-1)} \frac{F^2_{(11)(++)}}{4F_{(22)(++)}}$</td>
</tr>
<tr>
<td>9, 9*</td>
<td>$F_{(00)(++)} = F_{(00)(++)}$</td>
<td>$\frac{n_1 - 2}{n_1} \frac{(n_1 - 3)}{n_1(n_1-1)} \frac{F^2_{(11)(++)}}{8F_{(22)(++)}}$</td>
</tr>
<tr>
<td>10</td>
<td>$F_{(00)(00)}$</td>
<td>$\frac{n_1 - 2}{n_1} \frac{(n_1 - 3)}{n_1(n_1-1)} \frac{F^2_{(11)(++)}}{16F_{(22)(22)}}$</td>
</tr>
</tbody>
</table>
Appendix S4

Deciphering the Enigma of Undetected Species, Phylogenetic, and Functional Diversity Based on Good-Turing Theory

Anne Chao, Chun-Huo Chiu, Robert K. Colwell, Luiz Fernando S. Magnago, Robin L. Chazdon, and Nicholas J. Gotelli

APPENDIX S4: Simulation Results
All estimators derived from Good-Turing theory are non-parametric lower bounds of the corresponding species, phylogenetic, and functional diversity. Here the lower bounds are nonparametric in the sense that they are universally valid for any species abundance distribution. Good-Turing theory reveals that each estimator is nearly unbiased if rare species/nodes in each assemblage have approximately homogenous abundances; the abundant species/nodes could be highly heterogeneous without affecting the estimator. We refer this condition as the condition for unbiasedness.

Here we report some representative simulation results to numerically validate Good-Turing theory and examine the performance of various estimators derived from the theory. In our simulation study, we generated data from several theoretical species abundance models, as specified below. In most natural assemblages, there are many rare species and only a few abundant species. Mixture models (Colwell et al. 2004, Mao and Colwell 2005) have been widely used to mimic such assemblages. We considered four mixture models. Each is a mixture of two distributions; one for modeling the abundances of the group of rare species and the other for modeling the abundances of the group of abundant species. Models 1A and 1B are mixtures of uniform distributions, whereas Models 2A and 2B are mixtures of lognormal distributions. Under these four mixture models, when a sample of individuals is
taken from this assemblage, the singletons and undetected species in the sample are almost surely from the rare group and thus do not greatly differ in their abundances, satisfying the condition of unbiasedness. By contrast, we also considered a single uniform distribution (Model 1C) and a single lognormal distribution (Model 2C). In these two models, the abundances of singletons and undetected species in any sample span a wider range and differ to some extent, implying the sufficient condition for unbiasedness is not satisfied. The theoretical value of the CV (which is the ratio of standard deviation and mean) for each model is also given to quantify the degree of heterogeneity of species relative abundances. When all abundances are equal, CV = 0. A larger value of CV signifies a higher degree of heterogeneity among species abundances.

The number of species in each assemblage was fixed to be 300. For each fixed model, we first generated 300 random variables from a designated model. Then their normalized values represent the “true” species relative abundances of the complete assemblage, $(p_1, p_2, ..., p_{300})$, which we regarded as fixed parameters in the simulation. Since the generated set of abundances varies among trials, two sets of abundances generated from the same model may differ to some extent, and the empirical CV of each generated abundance set may deviate slightly from the theoretical CV value. Here we report the simulation results for the following six models.

**Model 1A**: A mixture of two uniform distributions denoted by $p_i \sim c [0.9 \times U(0.1, 0.2) + 0.1 \times U(0.2, 1)]$, whereby $c$ is a normalizing constant in all cases, such that $\sum_{i=1}^{300} p_i = 1$. The theoretical CV for this model is 0.80. In this model, the assemblage comprises roughly 90% rare species, whose abundances were generated from a uniform distribution in the range $[0.1, 0.2]$, and 10% abundant species, whose abundances were generated from a uniform distribution in $[0.2, 1]$. 
Model 1B: Same as Model 1A, but the percentages for rare and abundant species are changed to 80% and 20% respectively. The theoretical CV for this model is 0.87.

Model 1C: A single, uniform distribution in the range [0, 1], denoted by $p_i \sim c \times U(0, 1)$, whereby $c$ is a normalizing constant in all cases, such that $\sum_{i=1}^{S} p_i = 1$. The theoretical CV for this model is 0.58.

Model 2A: A mixture of lognormal distributions denoted by $p_i \sim c \times [0.9 \times \text{lognormal (1, 0.2)} + 0.1 \times \text{lognormal (2, 1)}]$, where lognormal ($\mu$, $\sigma$) denotes a lognormal distribution with mean $\mu$ and standard error (s.e.) $\sigma$, and the constant $c$ is a normalizing constant. The theoretical CV for this model is 1.56. In this model, the assemblage comprises roughly 90% rare species, whose abundances were generated from a lognormal distribution with mean 1 and s.e. 0.2, and 10% abundant species, whose abundances were generated from a lognormal distribution with mean 2 and s.e. 1.

Model 2B: Same as Model 2A, but the percentages for rare and abundant species are changed to 80% and 20% respectively. The theoretical CV for this model is 1.74.

Model 2C: A single lognormal distribution with mean 1 and s.e., denoted by $p_i \sim c \times \text{lognormal (1, 1)}$, i.e., species abundances were generated from a lognormal distribution, where $c$ is a normalizing constant in all cases, such that $\sum_{i=1}^{S} p_i = 1$. The theoretical CV for this model is 1.31.

Species richness estimator (Appendix S4: Fig. S1; see Eq. 3a in the main text for the estimator)

For each of the above six models (with 300 species), we considered a range of sample sizes ($n = 400$ to 1000 in increments of 100). Then for each combination of abundance model and sample size, 200 simulated data sets were generated. The empirical species richness and the Chao1 estimator were computed based on each generated data set. The mean values of the
observed richness and the Chao1 estimates are plotted as a function of sample size in Appendix S4: Fig. S1. Because the simulation procedures for the following five measures (shared richness, \(PD\), shared \(PD\), \(FAD\) and shared \(RAD\)) were generally parallel, details are omitted.

**Shared species richness estimator (Appendix S4: Fig. S2; see Eq. 7d in the main text for the estimator)**

To examine the performance of the Chao1-shared species estimator, we simulated data from two assemblages; the species relative abundances for both assemblages were generated from one of the six models (Models 1A–1C and 2A–2C) described above. The number of species in each assemblage was fixed to be 300, out of which 250 species were shared species and 50 were non-shared. Thus, there were a total of 350 species in the pooled assemblage. Specifically, we first generated two sets of 300 species relative abundances from each model and then sorted the abundances in each set from the largest to smallest. We then assigned the first 250 species as the shared species between the two assemblages (i.e., shared species were more abundant ones in each assemblage) and the remaining 50 species as the non-shared species. The CV values for the two generated sets of species relative abundances are denoted as CV1 and CV2 in each panel of Appendix S4: Fig. S2.

**Phylogenetic diversity (\(PD\)) estimator (Appendix S4: Fig. S3; see Eq. 8d in the main text for the estimator)**

The one-assemblage species abundance structures were generated in the same manner as those for species richness estimation. The rooted phylogenetic tree for 300 species in each assemblage was generated by the R function “rtree” from the “ade4” package.

**Shared \(PD\) estimator (Appendix S4: Fig. S4; see Eq. 10d in the main text for the estimator)**
The two-assemblage abundance structures were generated in the same manner as those for shared species richness estimation. In each assemblage, the rooted phylogenetic tree for a total of 350 species was generated by the R function “rtree” from the “ade4” package.

Functional attribute (FAD) estimator (Appendix S4: Fig. S5; see Eq. 12d in the main text for the estimator)

The one-assemblage species abundance structures were generated in the same manner as those for species richness estimation. The non-diagonal elements of the symmetric species-pairwise distance matrix was generated from a uniform U(0, 1) distribution; the diagonal elements were fixed to be zero.

Shared FAD estimator (Appendix S4: Fig. S6; see Eq. 14b in the main text for the estimator)

The two-assemblage abundance structures were generated in the same manner as those for shared species richness estimation. The non-diagonal elements of the symmetric species-pairwise distance matrix was generated from a uniform U(0, 1) distribution; the diagonal elements were fixed to be zero.

Results and Conclusion

All figures (Appendix S4: Figs. S1–S6, below) reveal consistent and similar patterns. The empirical approach typically underestimates, as the curve for the observed diversity estimator (dashed line in each panel) lies below the true curve (dotted line in each panel). The magnitude of the negative bias is substantial, especially for small sample sizes. As expected, the mean curve for each estimator derived from the Good-Turing theory (red solid line) for Models 1A, 1B, 2A and 2B matches well with the true parameter line, implying that each estimator is nearly unbiased. For Models 1C and 2C, although our estimator provides only a lower bound, as predicted from theory, the lower bound always improves the estimate over
the empirical approach and greatly reduces under-sampling bias of the observed diversity. As discussed in the main text, when rare species are heterogeneous in abundances, data are not sufficient to accurately estimate the true diversity, and statistically we can, at best, provide only a lower bound. In this case, we recommend the use of our estimators as reliable and informative lower bounds. Chiu et al. (2014) further evaluated the magnitude of the bias for the Chao1 lower bound and derived an approximate formula for the first-order bias. They estimated the approximate bias using additional information (namely, the numbers of tripletons and quadrupletons) and proposed an improved lower bound; see Chiu et al. (2014) for details.
(Model 1A) $0.9 \, U(0.1, 0.2) + 0.1 \, U(0.2, 1)$, CV=0.767

(Model 2A) $0.9 \, \text{lognormal} (1, 0.2) + 0.1 \times \text{lognormal} (2, 1)$, CV=1.246

(Model 1B) $0.8 \, U(0.1, 0.2) + 0.2 \, U(0.2, 1)$, CV=0.863

(Model 2B) $0.8 \, \text{lognormal} (1, 0.2) + 0.2 \times \text{lognormal} (2, 1)$, CV=1.916

(Model 1C) $U(0, 1)$, CV=0.571

(Model 2C) $\text{lognormal} (1, 1)$, CV=1.431

**Fig. S1.** The performance of the Chao1 species richness estimator as a function of sample size under six abundance models (dotted line = true richness = 300, red solid line = Chao1 estimator, dashed line = observed species richness.)
Fig. S2. The performance of the Chao1-shared estimator as a function of sample size under six abundance models (dotted line = true shared richness = 250, red solid line = Chao1-shared estimator, and dashed line = observed shared species richness.)
Fig. S3. The performance of the Chao1-PD estimator as a function of sample size under six abundance models and a generated phylogenetic tree (dotted line = true PD = 297.5, red solid line = Chao1-PD estimator, dashed line = observed PD.)
(Model 1A) \(0.9 \text{U}(0.1, 0.2) + 0.1 \text{U}(0.2, 1)\), CV1=0.855, CV2=0.744

(Model 2A) \(0.9 \lognormal(1, 0.2) + 0.1 \times \lognormal(2, 1)\), CV1=1.322, CV2=1.283

(Model 1B) \(0.8 \text{U}(0.1, 0.2) + 0.2 \text{U}(0.2, 1)\), CV1=0.902, CV2=0.874

(Model 2B) \(0.8 \lognormal(1, 0.2) + 0.2 \times \lognormal(2, 1)\), CV1=1.611, CV2=1.416

(Model 1C) \(\text{U}(0, 1)\), CV1=0.563, CV2=0.557

(Model 2C) \(\lognormal(1, 1)\), CV1=1.569, CV2=1.204

**Fig. S4.** The performance of the Chao1-PD-shared estimator as a function of sample size under six abundance models and a generated phylogenetic tree (dotted line = true shared PD = 250.4, red solid line = Chao1-PD-shared estimator, and dashed line = observed shared PD.)
(Model 1A) $0.9 \text{U}(0.1, 0.2) + 0.1 \text{U}(0.2, 1)$, CV=0.78

(Model 2A) $0.9 \lognormal (1, 0.2) + 0.1 \times \lognormal (2, 1)$, CV=1.43

(Model 1B) $0.8 \text{U}(0.1, 0.2) + 0.2 \text{U}(0.2, 1)$, CV=0.87

(Model 2B) $0.8 \lognormal (1, 0.2) + 0.2 \times \lognormal (2, 1)$, CV=1.57

(Model 1C) $\text{U}(0, 1)$, CV=0.58

(Model 2C) $\lognormal (1, 1)$, CV=1.33

Fig. S5. The performance of the Chao1-$FAD$ estimator as a function of sample size under six abundance models and a generated distance matrix (dotted line = true $FAD = 44870.4$, red solid line = Chao1-$FAD$ estimator, and dashed line = observed $FAD$.)
(Model 1A) $0.9 \text{U}(0.1, 0.2) + 0.1 \text{U}(0.2, 1)$, $\text{CV}_1 = 0.769$, $\text{CV}_2 = 0.788$

(Model 2A) $0.9 \text{lognormal}(1, 0.2) + 0.1 \times \text{lognormal}(2, 1)$, $\text{CV}_1 = 1.629$, $\text{CV}_2 = 1.177$

(Model 1B) $0.8 \text{U}(0.1, 0.2) + 0.2 \text{U}(0.2, 1)$, $\text{CV}_1 = 0.859$, $\text{CV}_2 = 0.862$

(Model 2B) $0.8 \text{lognormal}(1, 0.2) + 0.2 \times \text{lognormal}(2, 1)$, $\text{CV}_1 = 1.239$, $\text{CV}_2 = 1.455$

(Model 1C) $\text{U}(0, 1)$, $\text{CV}_1 = 0.667$, $\text{CV}_2 = 0.601$

(Model 2C) Lognormal (1, 1), $\text{CV}_1 = 1.268$, $\text{CV}_2 = 1.333$

**Fig. S6.** The performance of the Chao1-$FAD$-shared estimator as a function of sample size under six abundance models and a generated distance matrix (dotted line = true shared $FAD = 31176.7$, red solid line = Chao1-$FAD$-shared estimator, and dashed line = observed shared $FAD$.)
References for Appendix S4

