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RESEARCH ARTICLE

'Equivalent numbers' for species, phylogenetic or functional diversity in a nested hierarchy of multiple scales

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Summary

1. Many recent studies have searched to integrate species' functions and phylogenies in the measurement of biodiversity. To obtain easily interpretable measures, some researchers recommended diversity indices expressed in terms of equivalent numbers of species: the number of equally likely and maximally dissimilar species needed to produce the given value of diversity. Then, biodiversity is often calculated at three scales: within communities (α diversity), among communities (β diversity) and in a region (γ diversity). These three scales are, however, insufficient to tackle the organization of biodiversity in space because, for most organisms, there is a nested hierarchy of multiple scales characterized by different patterns and processes, from the small neighbourhood to the biosphere.

2. We developed methodologies for analysing species, functional, taxonomic or phylogenetic diversity in a hierarchy of multiple scales using equivalent numbers of species. As an example, we analysed the taxonomic and functional diversity of macroinvertebrate assemblages in the Loire River, France, at four levels: within sites (α diversity), among sites within geological regions (β_1 diversity), among geological regions (β_2 diversity) and at the river scale (γ diversity). The new hierarchical approaches of biodiversity revealed very low differences among sites within regions and among regions in terms of taxonomy and functional traits (size and diet), despite moderate, significant species turnover among geological regions.

3. We compare our framework with those other authors have developed. We argue that different definitions of α, β, γ diversities are used in the literature reflecting different points of view on biodiversity. We make recommendations on how to normalize functional (or phylogenetic) dissimilarities among species to render sites and regions comparable, and discuss the pros and cons of our approach.

4. The hierarchical approaches of biodiversity in terms of 'equivalent numbers' respond to current demands to obtain intuitive, easily interpretable components of biodiversity. The approaches we propose go beyond current developments by considering a hierarchy of spatial scales and unbalanced sampling design. They will provide powerful tools to detect the ecological and evolutionary processes that act differently at different scales.

Key-words: alpha diversity, beta diversity, biodiversity, community ecology, community phylogenetics, diversity apportionment, gamma diversity, quadratic entropy

Introduction

Biodiversity, the variability of life on Earth, is a multifaceted concept, ranging from genes to ecosystems. One of these facets, the species diversity in a site increases with the number of species and with the evenness of species' abundances. Species diversity thus treats species as equivalent in the sense that replacing a species by another one with the same abundance will not modify the level of species diversity. Measuring biodiversity by including information on the species' phylogeny or functional traits provides a more realistic view of the amount of biodiversity present in a site. Rao (1982a,b) proposed an index of biodiversity, termed quadratic entropy (Q), that can be used to measure any of these aspects of biodiversity: species, phylogenetic and functional diversity. Consider S species and $p_{k|m}$ the relative abundance of species k in site m ($\sum_{k=1}^{S} p_{k|m} = 1$). We will use indices k and l for species and index m for sites. Let $\mathbf{p}_m = (p_{1|m}, \ldots, p_{k|m}, \ldots, p_{S|m})$ be the vector of species' relative abundances within site m. Let $\mathbf{D} = (d_{kl})$ be a matrix of (phylogenetic or functional) dissimilarities

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among species, where d_{kl} is the (phylogenetic or functional) dissimilarity between species k and l ($d_{kk} = 0$ for all $k = 1,\ldots, S$, $d_{kl} = d_{lk}$ and $d_{kl} \geq 0$ for all $k, l = 1, \ldots, S$). Here, we define Q in terms of both species' abundances and dissimilarities among species as follows:

$$Q(\mathbf{p}_m, \mathbf{D}) = \sum_{k,\, l=1}^{S} p_{k|m} p_{l|m} d_{kl}$$

Pavoine, Ollier & Pontier (2005) showed that the quadratic entropy can be maximized by reducing species richness when varying the species' proportions only (considering $\max_{\mathbf{p}_m}\{Q(\mathbf{p}_m, \mathbf{D})\}$) if the dissimilarities among species are not ultrametric. A matrix of distances (d_{ij}) is ultrametric if for any i, j, k, $d_{ij} \leq \max(d_{ik}, d_{jk})$. Here, we consider both ultrametric and non-ultrametric dissimilarities assuming that both the species' proportions and the dissimilarities among species can vary. If the dissimilarities are bounded between 0 and 1, then the maximum value of Q over all possible dissimilarity matrices $\mathbf{D}$ is obtained when $d_{kl} = 1$ for any $k \neq l$, i.e.

$$\max_{\mathbf{D}}\{Q(\mathbf{p}_m, \mathbf{D})\} = 1 - \sum_{k=1}^{S} p_{k|m}^2$$

This formula is the Gini–Simpson index of biodiversity (Gini 1912; Simpson 1949). Then, $\max_{\mathbf{p}_m, \mathbf{D}}\{Q(\mathbf{p}_m, \mathbf{D})\} = (S-1)/S$ is obtained when $d_{kl} = 1$ for any $k \neq l$ and the species' proportions are evenly distributed (i.e. in a given site m, $p_{k|m} = 1/S$ for all k).

Recent attempts have been made to transform Q into an equivalent number of species as 'the number of equally likely and maximally dissimilar species needed to produce the given value of Q' where maximally dissimilar means that two individuals either belong to the same species or always differ by the same, maximal amount (i.e. for which $d_{kl} = 1$ for all $k \neq l$) (Ricotta & Szeidl 2009). Equivalent numbers of species are easy to interpret: Their minimum value approaches 1 when all species are phylogenetically or functionally very similar to each other or when a site contains a single species only; in contrast, their maximum value is S, the total number of possible species, when all species are maximally dissimilar and have even abundances.

Ricotta & Szeidl (2009), de Bello *et al.* (2010), and Leinster & Cobbold (2012) all agreed on the following procedure: to translate Q into an equivalent number of species, the distances among species should first be bounded between 0 and 1. Then, writing Q as an equivalent number of species leads to index E defined as:

$$E(\mathbf{p}_m, \mathbf{D}) = \frac{1}{1 - \sum_{k,\, l=1}^{S} p_{k|m} p_{l|m} d_{kl}}$$

$E(\mathbf{p}_m, \mathbf{D})$ is bounded between 1 and S. Ricotta & Szeidl (2009), de Bello *et al.* (2010), and Leinster & Cobbold (2012) thus agreed that E measures the biodiversity of a site as an equivalent number of species.

To partition diversity into its classical α, β and γ components, Ricotta & Szeidl (2009) measured α diversity as the average Q within a site, γ diversity as the value of Q when sites are pooled, and β diversity as $E_\beta = (1 - \alpha)/(1 - \gamma)$. Then, de Bello *et al.* (2010, Eqn 14) suggested to normalize E_β between 0 and 1 by $E_\beta^* = (1 - 1/E_\beta)/(1 - 1/M)$, where M is the number of sites, and Villéger *et al.* (2012, $\hat{\beta}_{st}$ index) by $E_\beta^{**} = (E_\beta - 1)/(M - 1)$. These approaches all depend on the fact that the dissimilarities among species (the d_{kl}'s) are bounded between 0 and 1. There is also a constraint that should apply to the dissimilarities among species: for γ to be higher than or equal to α, the matrix of dissimilarities among species has to be squared Euclidean, as specified by Ricotta & Szeidl (2009, p. 301; see also Appendix S1, Supporting information and Champely & Chessel 2002). A matrix of dissimilarities (d_{kl}), for $k, l = 1, \ldots, S$, is squared Euclidean if one can find S points $M_1, \ldots, M_S$ in a Euclidean space, so that the Euclidean distance between any two points M_k, M_l is $\sqrt{d_{kl}}$ (i.e. matrix $[\sqrt{d_{kl}}]$ is Euclidean, Gower & Legendre 1986). Many distance coefficients are squared Euclidean, such as many indices reviewed in Gower & Legendre (1986), and the distance coefficient for mixed data (e.g. quantitative, nominal, ordinal, multiple choice and circular data) of Pavoine *et al.* (2009b) (see, e.g., Legendre & Legendre 1998 for a review and comparison of dissimilarity indices). If the dissimilarities d_{kl} are not squared Euclidean, transformations can be applied on the $\sqrt{d_{kl}}$'s to render them Euclidean [see, e.g., the functions cailliez, lingoes, quasieuclid in the R package ADE4 (Dray, Dufour & Chessel 2007; and references therein; R Core Team 2015)]. Let δ_{kl} be the dissimilarity between k and l obtained after having transformed the $\sqrt{d_{kl}}$'s; then, a squared Euclidean matrix of dissimilarities among species is $(\delta_{kl}^2)_{k,\, l}$. Alternatives for calculating functional and phylogenetic α, β and γ diversities have been introduced by Chao and colleagues. In Chiu & Chao's (2014) approach for functional diversity, α diversity is measured considering the dissimilarities among species from distinct sites. Their definition of α diversity, as defined by their ${}^qFD_\alpha$ index, is 'the effective total distance between species in a *pair* of local assemblages', which is more related to what we define here as β diversity.

Irrespective of how diversity is partitioned into its α, β and γ components, the reality of ecological data sets is often more complex as they might include a variety of embedded spatial levels (Loreau 2000; Carmona *et al.* 2012, 2016; Marcon *et al.* 2012). Indeed, for most organisms, there is a nested hierarchy of multiple scales characterized by different patterns and processes, from a small neighbourhood to the biosphere, through the patch, community, landscape, regional and continental scales (Loreau 2000). Accordingly, Pavoine & Dolédec (2005) showed how Rao's apportionment of quadratic entropy (APQE, Rao 1982b, 1984) can be used to decompose Q into nested factors. However, the components of the decomposition were not expressed in terms of equivalent numbers of species or sites. As a result, they were difficult to interpret in terms of equivalent numbers, and it was difficult to compare them among data sets. Starting with Ricotta & Szeidl (2009) framework, we propose here alternative hierarchical approaches for Q in terms of any number of nested factors. For example,

imagine that several quadrats have been sampled in several sites within several regions. Then, the proposed approaches will measure α diversity as the diversity within quadrats, γ diversity as the diversity of the pooled regions and three components of β diversity: the diversity due to differences among quadrats within sites (in terms of species, phylogeny or functioning), the diversity due to differences among sites within regions and the diversity due to differences among regions. Part of the developed approaches can handle incomplete sampling designs, for example if regions have been analysed with different sampling efforts in terms of number of sites and quadrats per site. In these hierarchical approaches, each component of diversity can be expressed in terms of equivalent number of species, quadrats, sites, regions, etc. In addition, each component can be normalized into the range 0–1 to be comparable among data sets and sampling designs.

Materials and methods

For simplicity, we treat here the case of sites sampled within regions, but the methods are general and can be easily extended from one single level (e.g. sites) to any number of nested levels, for example quadrats, sites, regions, continents (see Appendix S2). When sites and regions are both considered, four components of diversity can be determined: α (the diversity within sites), β_1 (the diversity among sites within regions), β_2 (the diversity among regions) and γ diversity (the diversity of all sites and regions combined together). Our target components are β_1 and β_2 in order to evaluate whether there are differences among sites within regions and differences among regions. Our goal is first to express each component in terms of equivalent number of sites and regions, respectively, and then to rescale the components between 0 and 1, so that they are expressed independently of the number of sites and regions. Indeed, these numbers are dependent on the sampling design and most often independent of ecological or evolutionary processes. The same procedure is applied to α and γ diversity in order to have a complete framework for a hierarchical approach of diversity.

Consider that there are N regions, M_i sites in any region i and S species over all sites and regions. Let $n_{k|im}$ be the abundance (e.g. number of individuals, biomass or percentage cover) of species k in site m from region i and $n_{+im} = \sum_{k=1}^{S} n_{kim}$ the total abundance of all species in site m from region i. Then, the relative abundance of species k in site m of region i is $p_{k|im} = n_{kim}/n_{+im}$. If w_{im} is the weight attributed to site m in region i so that $\sum_{i=1}^{N}\sum_{m=1}^{M_i} w_{im} = 1$, then the weight attributed to region i is $w_{i+} = \sum_{m=1}^{M_i} w_{im}$, the relative abundance of species k in region i as a whole is $p_{k|i+} = \sum_{m=1}^{M_i} w_{im}p_{k|im}/w_{i+}$ and the relative abundance of species k over all sites and regions is $p_{k|++} = \sum_{i=1}^{N} w_{i+}p_{k|i+} = \sum_{i=1}^{N}\sum_{m=1}^{M_i} w_{im}p_{k|im}$ (Nayak 1986). This weighting scheme is consistent throughout the scales ensuring that all components of diversity are non-negative. The weights attributed to sites can be freely chosen, provided they sum to 1 as specified above. Even weights (i.e. $w_{ij} = 1/(NM_i)$) and weights proportional to size (sum of species' abundance) are traditionally used in the literature (e.g. Hardy & Jost 2008; Villéger & Mouillot 2008).

Chao, Chiu & Jost (2014) suggested that different methodologies should be developed when working on phylogenetic diversity, with indices relying on the tree structure of a rooted phylogeny, vs. functional diversity, with indices relying on a matrix of functional distances among species. We agree that distinct facets of diversity can be measured with distinct indices that best reflect their characteristics, such as the hierarchical structure of a rooted phylogeny with nodes representing ancestors and branches shared evolution. However, many recent ecological studies have compared phylogenetic and functional diversities (e.g. Cadotte *et al.* 2009; Devictor *et al.* 2010; Flynn *et al.* 2011). We recommend that, in this particular case, the same indices are used for measuring phylogenetic and functional diversities as the choice of an index may influence correlations between phylogenetic diversity and functional diversity just because different indices mean different points of view on diversity. Using distinct indices for phylogenetic vs. functional diversity, levels of correlation between phylogenetic and functional diversity patterns will be influenced by mathematical differences between indices rather than by the phylogenetic signal in functional traits (Pavoine *et al.* 2013). We thus consider here that d_{kl} is a measure of the dissimilarity between two species k and l, varying between 0 and 1, whatever the criterion used, be it phylogenetic or functional (trait based).

APPORTIONMENT OF QUADRATIC ENTROPY

With the above notations, in Rao's (1982b, 1984) APQE (see also Nayak 1986), the diversity would be decomposed as follows:

$$Q_\alpha = \sum_{i=1}^{N}\sum_{m=1}^{M_i} w_{im}\sum_{k,l=1}^{S} p_{k|im}p_{l|im}d_{kl}$$

$$Q_{\beta_1} = \sum_{i=1}^{N} w_{i+}\sum_{m,n=1}^{M_i}\frac{w_{im}}{w_{i+}}\frac{w_{in}}{w_{i+}} \left(\sum_{k,l=1}^{S} p_{k|im}p_{l|in}d_{kl} - \frac{1}{2}\sum_{k,l=1}^{S} p_{k|im}p_{l|im}d_{kl} - \frac{1}{2}\sum_{k,l=1}^{S} p_{k|in}p_{l|in}d_{kl}\right)$$

$$Q_{\beta_2} = \sum_{i,j=1}^{N} w_{i+}w_{j+} \left(\sum_{k,l=1}^{S} p_{k|i+}p_{l|j+}d_{kl} - \frac{1}{2}\sum_{k,l=1}^{S} p_{k|i+}p_{l|i+}d_{kl} - \frac{1}{2}\sum_{k,l=1}^{S} p_{k|j+}p_{l|j+}d_{kl}\right)$$

$$Q_\gamma = \sum_{k,l=1}^{S} p_{k|++}p_{l|++}d_{kl}$$

All components always are non-negative if and only if the matrix of dissimilarities among species (d_{kl}) is squared Euclidean (Lau 1985; Rao 1986; Pavoine 2012). This property is also required for the alternative approaches introduced below.

We develop below three alternatives to APQE. These alternatives should be preferred to APQE when the aim of a study is to evaluate the amount of β diversity among sites (or regions, etc.) and to compare this level to an extreme scenario where sites (or regions) are maximally dissimilar. By maximally dissimilar, we mean that they do not share species and any species in a site is maximally dissimilar to any species in any other site. Contrary to APQE, these alternatives are thus dependent on the definition of a maximum possible dissimilarity between species.

The first alternative directly extends Ricotta & Szeidl (2009) framework to multiple spatial scales but has components of β diversity whose range might depend on α diversity. If sites and regions are given uneven weights, then both the first two alternatives might have components of β diversity whose range depends on α diversity. The

third alternative has the advantage of allowing any weighting scheme for regions and for sites within regions. It has, however, the particularity of measuring β diversity using pairwise comparisons among sites and among regions, a strategy adopted in APQE so that α diversity is measured as an average (functional or phylogenetic) dissimilarity among species, and each level of β diversity as an average (functional or phylogenetic) dissimilarity among regions or an average (functional or phylogenetic) dissimilarity among sites within regions. Alternatively, the first two approaches have the advantage of considering multiple-site and multiple-region β diversity components, acknowledging, for example, that some species might be shared by more than two sites within each region and by more than two regions (e.g. Diserud & Ødegaard 2007). We recommend using the first alternative approach when the interest is in multiple-site and multiple-region β diversity, the sampling design is even (same number of sites within regions), sites within a region can be given equal weights and regions can be given equal weights; the second approach when the interest is in multiple-site (within regions) and multiple-region β diversity, sites within a region can be given equal weights and regions can be given equal weights but the sampling design is uneven (different numbers of sites within regions); the third approach when the interest is in β diversity expressed in terms of pairwise dissimilarities among sites (within regions) and among regions and/or sites within a region have different weights and regions have different weights (due, e.g., to uneven sampling pressures, uneven size of sites or regions).

FIRST ALTERNATIVE APPROACH

As a first step towards an alternative hierarchical approach of biodiversity using quadratic diversity, Q_α and Q_γ need to be transformed into their equivalent numbers of species as recommended by Ricotta & Szeidl (2009) and de Bello *et al.* (2010) (see also Carmona *et al.* 2012):

$$E_\gamma = \frac{1}{1 - Q_\gamma}$$

$$E_\alpha = \frac{1}{1 - Q_\alpha}$$

with $1 \leq E_\alpha \leq E_\gamma \leq S$. Ricotta & Szeidl (2009) measured E_β diversity as $E_\gamma/E_\alpha = (1 - Q_\alpha)/(1 - Q_\gamma)$, and, because of the generalized replication principle (as described in Ricotta & Szeidl 2009), this measure can be interpreted as an equivalent number of equally diverse, maximally distinct and evenly weighted sites (see also de Bello *et al.* 2010). An equivalent solution that can be used to measure E_{β_1} and E_{β_2} is thus

$$E_{\beta_1} = (1 - Q_\alpha)/(1 - Q_\alpha - Q_{\beta_1})$$

$$E_{\beta_2} = (1 - Q_\alpha - Q_{\beta_1})/(1 - Q_\gamma)$$

so that $E_\gamma = E_\alpha E_{\beta_1} E_{\beta_2}$. $Q_\alpha + Q_{\beta_1}$ is the total diversity within regions. E_{β_1} is expressed as an equivalent number of sites per region and E_{β_2} as an equivalent number of regions.

Jost (2006, 2007) advised the use of indices of β diversity whose range is unrelated to α diversity. It can be shown that the range of E_{β_2} might depend on the diversity within regions. However, if regions are given equal weights, that is $w_{i+} = 1/N$ for all i, then $1 \leq E_{\beta_2} \leq N$ (Appendix S2). If sites are given equal weights and if the sampling design is balanced so that $M_1 = M_2 = \ldots = M_N = M$ and $w_{im} = 1/MN$ for all i, m, then the following inequality also holds: $1 \leq E_{\beta_1} \leq M$ (see Appendix S2). Under this scenario, the range of E_{β_1} is thus constant whatever the diversity within sites and the range of E_{β_2} is constant whatever the diversity within regions. We thus restrict this approach to even weights for regions and for sites within regions. The β components can then be rescaled using one of the following methods:

$$E^*_{\beta_1} = (1 - 1/E_{\beta_1})/(1 - 1/M)$$

$$E^*_{\beta_2} = (1 - 1/E_{\beta_2})/(1 - 1/N)$$

or

$$E^{**}_{\beta_1} = (E_{\beta_1} - 1)/(M - 1)$$

$$E^{**}_{\beta_2} = (E_{\beta_2} - 1)/(N - 1)$$

If the sampling design is unbalanced so that at least two M_i's are different, even if sites within regions are given equal weights, that is $w_{im} = 1/(M_i N)$ for site m in region i (so that the weights attributed to regions are also even: $w_{i+} = \sum_{m=1}^{M_i} w_{im} = 1/N$ for any region i), the range of E_{β_1} might depend on the diversity within sites. Let $Q_{\alpha i}$ be the average diversity within sites in region i:

$$Q_{\alpha i} = \sum_{m=1}^{M_i} \frac{1}{M_i} \sum_{k,l=1}^{S} p_{k|im} p_{l|im} d_{kl}$$

It can be shown (Appendix S2) that:

$$1 \leq E_{\beta_1} \leq \frac{\sum_{i=1}^{N} (1 - Q_{\alpha i})}{\sum_{i=1}^{N} \frac{1}{M_i} (1 - Q_{\alpha i})} \leq \max_i(M_i)$$

$\sum_{i=1}^{N} (1 - Q_{\alpha i}) / \sum_{i=1}^{N} \frac{1}{M_i} (1 - Q_{\alpha i})$ is a weighted harmonic mean of the M_i's. E_{β_1} is expressed as the equivalent number of maximally dissimilar sites per region. In case of unbalanced sampling design, the range of E_{β_1} thus depends on the amount of diversity within sites if this amount is unbalanced among regions. E_{β_1} can then be normalized more generally by

$$E^*_{\beta_1} = \frac{1 - 1/E_{\beta_1}}{1 - \sum_{i=1}^{N} \frac{1}{M_i} (1 - Q_{\alpha i}) / \sum_{i=1}^{N} (1 - Q_{\alpha i})}$$

or

$$E^{**}_{\beta_1} = \frac{E_{\beta_1} - 1}{\sum_{i=1}^{N} (1 - Q_{\alpha i}) / \sum_{i=1}^{N} \frac{1}{M_i} (1 - Q_{\alpha i}) - 1}$$

That way, $E^*_{\beta_1}$ and $E^{**}_{\beta_1}$ equal 0 when sites are identical within regions. They reach the maximum value of 1 when sites within a region are maximally dissimilar whatever the level of diversity within sites: within any region, sites do not share species and any species from any site always is maximally dissimilar from all species in all other sites.

SECOND ALTERNATIVE APPROACH

As an alternative, to obtain a component of β_1 diversity whose maximum is unrelated to the diversity within sites when sites and regions are given equal weights ($w_{im} = 1/(M_i N)$ for site m in region i and $w_{i+} = 1/N$ for any region i), the equivalent number of sites in region i can be defined as

$$E_{\beta_{1,i}} = (1 - Q_{\alpha_i})/(1 - Q_{\alpha_i} - Q_{\beta_{1,i}})$$

where Q_{α_i} is the diversity within sites of region i defined above and

$$Q_{\beta_{1,i}} = \sum_{m,n=1}^{M_i} \frac{1}{M_i}\frac{1}{M_i} \left(\sum_{k,l=1}^{S} p_{k|im} p_{l|in} d_{kl} - \frac{1}{2}\sum_{k,l=1}^{S} p_{k|im} p_{l|im} d_{kl} - \frac{1}{2}\sum_{k,l=1}^{S} p_{k|in} p_{l|in} d_{kl} \right)$$

is the diversity among sites of region i. Then, the average equivalent number of sites per region can be measured as the harmonic mean:

$$\bar{E}_{\beta_1} = N \Big/ \left[\sum_{i=1}^{N} \frac{1}{E_{\beta_{1,i}}} \right]$$

Whatever the amount of diversity within the sites, $1 \leq \bar{E}_{\beta_1} \leq N \Big/ \left[\sum_{i=1}^{N} \frac{1}{M_i} \right]$, the harmonic mean of the number of sites per region. The harmonic mean is used because applying the same mean to α diversity leads to E_α. Indeed, let $E_{\alpha im} = 1/\left[1 - \sum_{k,l=1}^{S} p_{k|im} p_{l|im} d_{kl}\right]$, then the harmonic mean of the $E_{\alpha im}$ is E_α:

$$\frac{1}{\sum_{i=1}^{N}\sum_{m=1}^{M_i} \frac{1}{NM_i}\frac{1}{E_{\alpha im}}} = \frac{1}{\sum_{i=1}^{N}\sum_{m=1}^{M_i} \frac{1}{NM_i}\left(1 - \sum_{k,l=1}^{S} p_{k|im} p_{l|im} d_{kl}\right)} = \frac{1}{1 - \sum_{i=1}^{N}\sum_{m=1}^{M_i} \frac{1}{NM_i}\sum_{k,l=1}^{S} p_{k|im} p_{l|im} d_{kl}} = E_\alpha$$

γ diversity can then be redefined as $\bar{E}_\gamma = E_\alpha \bar{E}_{\beta_1} E_{\beta_2}$. $\bar{E}_\gamma$ is the product of the average equivalent number of species per site by the average equivalent number of sites per region and by the equivalent number of regions. The inconvenient of this approach is that $\bar{E}_\gamma \neq E_\gamma$. The advantage is that the maximum of E_{β_1} does not depend on the diversity within sites and it can be normalized considering all M_i's with one of the following formulas:

$$\bar{E}^{*}_{\beta_1} = \frac{N}{\sum_{i=1}^{N}\left(1/\bar{E}^{*}_{\beta_{1,i}}\right)} \text{ where } \bar{E}^{*}_{\beta_{1,i}} = \frac{1 - 1/\bar{E}_{\beta_{1,i}}}{1 - 1/M_i}$$

and

$$\bar{E}^{**}_{\beta_1} = \frac{N}{\sum_{i=1}^{N}\left(1/\bar{E}^{**}_{\beta_{1,i}}\right)} \text{ where } \bar{E}^{**}_{\beta_{1,i}} = \frac{\bar{E}_{\beta_{1,i}} - 1}{M_i - 1}$$

If a region is composed of one site only, we impose that $\bar{E}^{*}_{\beta_{1,i}} = \bar{E}^{**}_{\beta_{1,i}} = 0$. Although the null diversity within a site might be due to ecological factors, a region composed of one only site might be due to unbalanced sampling design. We thus advise to sample several sites per regions so that the (functional or phylogenetic) diversity among sites within regions can be calculated.

THIRD ALTERNATIVE APPROACH

This third approach was specifically developed for unbalanced sampling designs to obtain β diversity components whose ranges are unrelated to α diversity, while allowing for uneven weighting schemes for sites and regions. In the special case where only two evenly weighted sites are compared, Pavoine & Ricotta (2014) developed a family of indices of similarity and dissimilarity between sites, which included $D_{st} = E^{*}_{\beta}$ (between two evenly weighted sites) and $D_\beta = E^{**}_{\beta}$ (between two evenly weighted sites). Let $\mathbf{p} = (p_1, \ldots, p_k, \ldots, p_S)$ and $\mathbf{q} = (q_1, \ldots, q_k, \ldots, q_S)$ be two vectors of species' proportions,

$$D_{st}(\mathbf{p}, \mathbf{q}) = \frac{\sum_{k,l=1}^{S} p_k q_l d_{kl} - \frac{1}{2}\sum_{k,l=1}^{S} p_k p_l d_{kl} - \frac{1}{2}\sum_{k,l=1}^{S} q_k q_l d_{kl}}{1 - \frac{1}{2}\sum_{k,l=1}^{S} p_k p_l d_{kl} - \frac{1}{2}\sum_{k,l=1}^{S} q_k q_l d_{kl}}$$

$$D_\beta(\mathbf{p}, \mathbf{q}) = \frac{\frac{1}{2}\sum_{k,l=1}^{S} p_k q_l d_{kl} - \frac{1}{4}\sum_{k,l=1}^{S} p_k p_l d_{kl} - \frac{1}{4}\sum_{k,l=1}^{S} q_k q_l d_{kl}}{1 - \frac{1}{2}\sum_{k,l=1}^{S} p_k q_l d_{kl} - \frac{1}{4}\sum_{k,l=1}^{S} p_k p_l d_{kl} - \frac{1}{4}\sum_{k,l=1}^{S} q_k q_l d_{kl}}$$

We first modify the dissimilarities among species assemblages in β_1 and β_2, (i.e. the diversity among sites and regions, respectively) according to Pavoine & Ricotta (2014) D_{st} index (the D_β index could be used as an alternative). Let $\mathbf{p}_{im} = \left(p_{1|im}, \ldots, p_{k|im}, \ldots, p_{S|im}\right)$ be the vector of species' relative abundance within site m of region i, and $\mathbf{p}_{i+} = \left(p_{1|i+}, \ldots, p_{k|i+}, \ldots, p_{S|i+}\right)$ the vector of species' relative abundance within region i, then

$$\tilde{Q}_{\beta_{1,i}} = \sum_{m,n=1}^{M_i} \frac{w_{im}}{w_{i+}}\frac{w_{in}}{w_{i+}} D_{st}(\mathbf{p}_{im}, \mathbf{p}_{in})$$

$$\tilde{Q}_{\beta_2} = \sum_{i,j=1}^{N} w_{i+} w_{j+} D_{st}\left(\mathbf{p}_{i+}, \mathbf{p}_{j+}\right)$$

The formulas of $\tilde{Q}_{\beta_{1,i}}$ and $\tilde{Q}_{\beta_2}$ correspond to Q applied to the sites' weights and dissimilarities among sites but within region i and to the regions' weights and dissimilarities among regions, respectively. Because index D_{st} is bounded between 0 and 1, $\tilde{Q}_{\beta_{1,i}}$ and $\tilde{Q}_{\beta_2}$ are also bounded between 0 and 1 and they can thus be transformed into equivalent numbers of sites and regions, respectively, as follows:

$$\tilde{E}_{\beta_{1,i}} = \frac{1}{1 - \tilde{Q}_{\beta_{1,i}}}$$

$$\tilde{E}_{\beta_2} = \frac{1}{1 - \tilde{Q}_{\beta_2}}$$

Finally, the component $\tilde{E}_{\beta_1}$ measuring β_1 diversity can be defined as the average equivalent number of sites within regions as follows (harmonic mean of the $\tilde{E}_{\beta_{1,i}}$'s):

$$\tilde{E}_{\beta_1} = \frac{1}{\sum_{i=1}^{N} w_{i+}\left(1/\tilde{E}_{\beta_{1,i}}\right)} = \frac{1}{1 - \sum_{i=1}^{N} w_{i+}\tilde{Q}_{\beta_{1,i}}}$$

Measuring α diversity with index E_α, $\tilde{E}_{\beta_1}$ and E_α are computed with the same formula, although $\tilde{E}_{\beta_1}$ uses average distances among sites, whereas E_α uses average distances among species. A new component of γ diversity, $\tilde{E}_\gamma$, is defined as the product of the equivalent number of species per site and region by the equivalent number of sites per region and by the equivalent number of regions: $\tilde{E}_\gamma = E_\alpha \tilde{E}_{\beta_1} \tilde{E}_{\beta_2}$ ($\tilde{E}_\gamma \neq E_\gamma$). Uneven weights can be used for both sites and regions. $\tilde{E}_{\beta_2}$ can be normalized into 0 and 1 using one of the following formulas:

$$\tilde{E}^{*}_{\beta_2} = \frac{1 - 1/\tilde{E}_{\beta_2}}{1 - 1/N}$$

$$\tilde{E}^{**}_{\beta_2} = \frac{\tilde{E}_{\beta_2} - 1}{N - 1}$$

The maximum of $\tilde{E}_{\beta_1}$ does not depend on the diversity within sites. $\tilde{E}_{\beta_1}$ can then be normalized considering all M_i's with one of the following formulas:

$$\tilde{E}^{*}_{\beta_1} = \frac{1}{\sum_{i=1}^{N} w_{i+}\left(1/\tilde{E}^{*}_{\beta_{1,i}}\right)} \text{ where } \tilde{E}^{*}_{\beta_{1,i}} = \frac{1 - 1/\tilde{E}_{\beta_{1,i}}}{1 - 1/M_i}$$

and

$$\tilde{E}^{**}_{\beta_1} = \frac{1}{\sum_{i=1}^{N} w_{i+}\left(1/\tilde{E}^{**}_{\beta_{1,i}}\right)} \text{ where } \tilde{E}^{**}_{\beta_{1,i}} = \frac{\tilde{E}_{\beta_{1,i}} - 1}{M_i - 1}$$

As for $E^{*}_{\beta_{1,i}}$ and $E^{**}_{\beta_{1,i}}$, if a region is composed of one site only, we impose that $\tilde{E}^{*}_{\beta_{1,i}} = \tilde{E}^{**}_{\beta_{1,i}} = 0$.

As recommended by Rao (1982a), our components of β diversity in this third approach are defined as the average dissimilarities among sites or regions. Baselga (2013) showed that this definition departs from that of multiple-site β diversity, as the average pairwise dissimilarities among sites do not consider how many species are shared by more than two sites. We agree that the two definitions are different and may respond to different objectives. However, the average pairwise dissimilarities among sites (or regions) and the associated effective number of sites (or regions) are indices of β diversity as soon the average dissimilarities among species and the associated effective numbers of species are admitted to be indices of α generalized entropy (Rao 1982a,b) and diversity (Ricotta & Szeidl 2009; de Bello *et al.* 2010), respectively. The relevance behind Rao's framework was to unify the way α diversity and β diversity are measured: α diversity as an average dissimilarity between species and β diversity as an average dissimilarity between sites. Consider, for example, that species are characterized by a qualitative trait with several levels. Then, the average pairwise dissimilarity between species does not consider how many trait levels are shared by more than two species. Consider three species: one has trait levels A and B, another has trait levels B and C, and the last one trait levels C and A. On average, species share one trait level, and each trait level is possessed by two species. Now consider the first species has trait levels A and B, the second has trait levels A and C and the last one A and D. On average in this second case, species also share a unique trait level, but trait level A is possessed by all species, whereas three trait levels (B, C and D) are unique to a species. The development of a diversity framework that is based on both multiple-species and multiple-site analyses could be the objective of further research and is beyond the scope of our paper that relies on Rao's framework.

CASE STUDY

We use the same data set as in our two previous studies that focused on decomposing biodiversity (Pavoine & Dolédec 2005) and measuring the biodissimilarities among sites (Pavoine & Ricotta 2014). A total of 40 macroinvertebrate species (here Coleoptera and Trichoptera) were sampled in 38 sites (stations) distributed in the Loire River (France) from the spring to 200 km upstream of the mouth. The river was divided into three regions according to its geology: granitic highlands, limestone lowlands and granitic lowlands. Sixteen sites were positioned in granitic highlands, 17 in limestone lowlands and five in granitic lowlands. Sites have been sampled in July 1989 and 1991 and in March and May 1993, in rubble riffle habitats with a hand-net for about 10 min per site. Individuals were identified at the species level and counted. Dissimilarities among species were considered using taxonomy, size and feeding habits. Taxonomic dissimilarities among species were defined as follows: 1/4 between species of same genus, 1/2 between species of different genera but same family, 3/4 between species of different families but same order and 1 between species of different orders. Size and feeding habits were defined using a fuzzy coding approach (Chevenet, Dolédec & Chessel 1994) where each species is attributed an affinity to each size category (four length categories ranging from ≤5 to 20–40 mm) and to each feeding category (engulfers, shredders, scrapers, deposit-feeders, active filter-feeders, passive filter-feeders and piercers). For each trait (either size or feeding habits), affinities for each category were estimated by expert opinion on an ordinal scale ranging from 0 (no affinity) to 3 (high affinity, Ivol *et al.* 1997). Then, the dissimilarity between two species was calculated as follows:

$$d_{kl} = 1 - \sum_{v=1}^{V} q_{kv} q_{lv} \Big/ \sqrt{\sum_{v=1}^{V} q_{kv}^2 \sum_{v=1}^{V} q_{lv}^2}$$

where d_{kl} is the dissimilarity between species k and l, V is the number of categories (four size categories and seven feeding categories), q_{kv} is the proportional affinity of species k for category v. This coefficient of dissimilarity was chosen to render the results comparable with those obtained with the APQE approach by Pavoine & Dolédec (2005). The taxonomy and biological traits are shown in Fig. 1, together with the sum of the abundances of each species within each geological region. We also considered averaged dissimilarities over size and feeding habits. Pavoine & Dolédec (2005) weighted sites and regions by their size (number of observed individuals). Instead, we attributed here even weights to regions and to sites within regions so that the range of $\bar{E}_{\beta_1}$ does not depend on the diversity within sites and the range of E_{β_2} does not depend on the diversity within regions.

We calculated all components of hierarchical approaches of biodiversity defined above to this data set and compared the results with those given by APQE. We provided the raw components and the normed $E^{**}_{\beta_1}$, $\bar{E}^{**}_{\beta_1}$, $\tilde{E}^{**}_{\beta_1}$, $E^{**}_{\beta_2}$ and $\tilde{E}^{**}_{\beta_2}$. We then applied permutation tests to the normed $E^{**}_{\beta_1}$, $\bar{E}^{**}_{\beta_1}$, $\tilde{E}^{**}_{\beta_1}$, $E^{**}_{\beta_2}$ and $\tilde{E}^{**}_{\beta_2}$ components, using the same permutation schemes as in Pavoine & Dolédec (2005). First, the differences among sites were tested by permuting the abundances of each species across sites but within regions calculating $E^{**}_{\beta_1}$, $\bar{E}^{**}_{\beta_1}$ and $\tilde{E}^{**}_{\beta_1}$ for each permutation. Secondly, the differences among regions were tested by permuting sites among regions, calculating $E^{**}_{\beta_2}$ and $\tilde{E}^{**}_{\beta_2}$ for each permutation. Developing and comparing permutation schemes was beyond the scope of our paper. We thus recommend that further studies focus on evaluating the type I error and power of a range of possible permutation schemes (see, e.g., Anderson 2001; Hardy 2008; Anderson & Walsh 2013).

Results

For α diversity, the maximum diversity would have been obtained if the 40 species were maximally dissimilar and were present within all sites with even abundances. In contrast, E_α revealed that the biodiversity within sites (α diversity) was very low whatever the aspect of biodiversity considered (species, taxonomic, size, diet and size & diet diversity) (Table 1b). This was not reflected by the APQE approach (Table 1a). For γ diversity, the maximum diversity would have been obtained if the 40 species were present with even abundances at the River level (pooling all sites and regions). In contrast, E_γ, $\bar{E}_\gamma$ and $\tilde{E}_\gamma$ revealed that, although the taxonomic and functional γ diversity over the Loire River is higher than the α diversity (within sites), it is also low, meaning that some taxonomic groups, with particular size and feed categories, dominate in abundance. As shown in Fig. 1, all dominant species belong to the genus *Hydropsyche* and are similar in size and diet habits: they are passive filter-feeders with size between 10 and 20 mm, except for the individuals of *Hydropsyche dinarica* that may have larger body sizes.

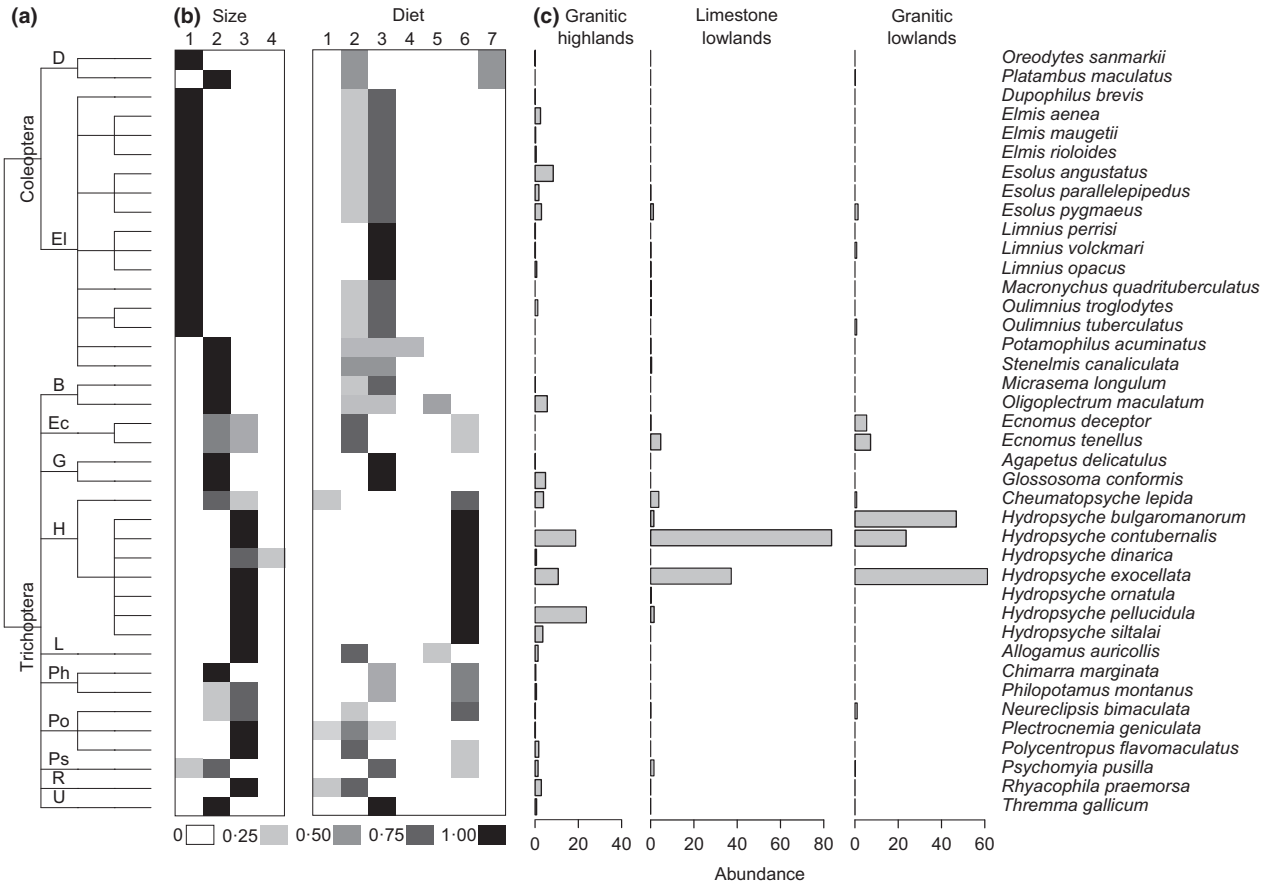

Fig. 1. Summary of the data set: (a) taxonomy, (b) biological traits, (c) abundance of each species within each geological region. The full data set is available in ADE4 package of R (Dray, Dufour & Chessel 2007). Levels in the taxonomy represent orders, families and genera. Codes for families are as follows: D = Dytiscidae, El = Elmidae, B = Brachycentridae, Ec = Ecnomidae, G = Glossosomatidae, H = Hydropsychidae, L = Limnephilidae, Ph = Philopotamidae, Po = Polycentropodidae, Ps = Psychomyiidae, R = Rhyacophilidae, U = Uenoidae. The categories of the two biological traits are as follows: for body size (mm), 1 =]0, 5], 2 =]5,10], 3 =]10,20], 4 =]20,40]; for diet habits, 1 = engulfers, 2 = shredders, 3 = scrapers, 4 = deposit-feeders, 5 = active filter-feeders, 6 = passive filter-feeders, 7 = piercers. A grey scale indicates the affinities of species for each category of a trait (either size or diet categories); affinities are expressed here as proportions per species. For example, *Oreodytes sanmarkii* has 50% affinities for the diet category 'shredders' and 50% for the diet category 'piercers'. Abundance data in (c) were summed per species and geological region.

The results of the permutation tests were globally unchanged compared with Pavoine & Dolédec (2005): only the differences among regions were significant except when taxonomy and diet habit were considered, in which case the differences among sites within regions were sometimes also significant (Table 1c). However, compared with APQE, the components E_{β_1}, $\bar{E}_{\beta_1}$, $\tilde{E}_{\beta_1}$, E_{β_2}, $\tilde{E}_{\beta_2}$ and their normed versions revealed the effect size of the differences among regions and sites. The normed $E^{**}_{\beta_1}$, $\bar{E}^{**}_{\beta_1}$ and $\tilde{E}^{**}_{\beta_1}$ that resulted from the dissimilarities in terms of species' taxonomic positions among sites within regions were low (<0·030) but higher than those that resulted from species' feeding habits. Normalized β_1 diversity varies between 0 (absence of dissimilarities among sites within regions) and 1 (when sites are maximally dissimilar). Although significant for some tests, the normalized β_1 diversity thus revealed very low dissimilarity in terms of both taxonomy and species' feeding habits among sites within regions. Whatever the aspect of biodiversity considered (in terms of species, size and feeding categories, or taxonomy), the β_2 diversity, which reflects dissimilarities among regions, was significantly higher than if sites were randomly distributed among regions. However, the $E^{**}_{\beta_2}$ and $\tilde{E}^{**}_{\beta_2}$ components revealed that, although significant, the dissimilarities among regions were low in terms of size, feeding categories and taxonomy (<0·070). In contrast, the dissimilarity was moderate (>0·300) in terms of species composition. Even in the presence of some species turnover or change in species' abundances among regions, the dominant species of all regions thus shared close size categories, feeding habits and taxonomic positions. Indeed, the dominant species, even if distinct across regions, were all *Hydropsyche* species with similar size categories and diet habits. The significance of the dissimilarities among regions in terms of size categories, feeding habits and taxonomic positions are thus due to subdominant species (mainly in the granitic highlands), which moderately, even if significantly, contribute to these dissimilarities (Fig. 1).

Discussion

Research on biodiversity has taken a new turn when Jost (2006, 2007) warned against the use of entropy indices for measuring biodiversity because their values were not directly

Table 1. Results of the hierarchical analyses of biodiversity according to the APQE framework (a) and the new approaches, with raw components (b), and with normed β components and tests (c). Even weights were given to regions and to sites within regions.

		Gini–Simpson	Taxonomy	Size	Diet	Size & Diet
(a) *APQE (components and tests)*						
Among regions	Q_{β_2}	0·110***	0·064***	0·035***	0·062***	0·048***
Among sites within regions	Q_{β_1}	0·189^{NS}	0·133*	0·087^{NS}	0·112***	0·099*
Within sites	Q_{α}	0·533	0·274	0·170	0·156	0·163
Total	Q_{γ}	0·832	0·471	0·292	0·329	0·310
(b) *New alternative approach (raw components)*						
Among regions	E_{β_2}	1·656	1·121	1·050	1·092	1·070
	$\bar{E}_{\beta_2}$	1·724	1·133	1·055	1·102	1·078
Among sites within regions	E_{β_1}	1·678	1·223	1·117	1·152	1·135
	$\bar{E}_{\beta_1}$	1·705	1·265	1·138	1·176	1·157
	$\tilde{E}_{\beta_1}$	1·705	1·260	1·137	1·174	1·154
Within sites	E_{α}	2·142	1·377	1·204	1·184	1·194
Total	E_{γ}	5·953	1·889	1·412	1·491	1·450
	$\bar{E}_{\gamma}$	6·049	1·953	1·438	1·521	1·478
	$\tilde{E}_{\gamma}$	6·299	1·965	1·444	1·533	1·486
(c) *New alternative approach (normed β components and tests)*						
Among regions	$E^{**}_{\beta_2}$	0·328***	0·061*	0·025*	0·046*	0·035*
	$\bar{E}^{**}_{\beta_2}$	0·362***	0·066*	0·027*	0·051*	0·039*
Among sites within regions	$E^{**}_{\beta_1}$	0·070^{NS}	0·027*	0·015^{NS}	0·019*	0·017^{NS}
	$\bar{E}^{**}_{\beta_1}$	0·043^{NS}	0·012^{NS}	0·005^{NS}	0·006^{NS}	0·006^{NS}
	$\tilde{E}^{**}_{\beta_1}$	0·045^{NS}	0·013^{NS}	0·006^{NS}	0·007*	0·006^{NS}

Diversity was measured in terms of species (using the Gini–Simpson index), taxonomy, body length (size), diet habits, and both size and diet. The results of the permutation tests are given in terms of *P*-values with the following code: ***<0·005, *<0·05, $^{NS} \geq$ 0·05.

applicable for taking decisions of conservation actions. He generated vigorous debates (e.g. Ellison 2010; Whitlock 2011 and references therein). Hill (1973) early demonstrated how entropy indices can be modified in terms of equivalent numbers of species. Chao, Chiu & Jost (2010) showed how the framework of phylogenetic entropy indices (Pavoine, Love & Bonsall 2009a) could be modified in terms of equivalent numbers of species. The use of Hill's equivalent numbers of species has now been extended to both phylogenetic and functional diversity (e.g. Chao, Chiu & Jost 2010; Leinster & Cobbold 2012; Pavoine & Izsak 2014). Most solutions developed so far are thus based on the adaptations of traditional species diversity indices, mainly Hill numbers. However, contrasting definitions of equivalent number of species (or sites) have been developed in the literature. Our definition is similar whatever the entity considered (e.g. species or sites): the equivalent number of equally abundant and *maximally* dissimilar species (or sites) (Ricotta & Szeidl 2009).

We proposed here to adapt Rao's apportionment of the quadratic entropy (APQE) by allowing biodiversity to be considered in terms of equivalent number of species, sites, regions, etc., in a nested hierarchy of multiple scales. We applied the new approaches to the macroinvertebrate assemblages on Loire River in France, a data set previously analysed by Pavoine & Dolédec (2005) using Rao's APQE. Using the permutation schemes of Pavoine & Dolédec (2005; see also Excoffier, Smouse & Quattro 1992) to the new approaches, the results of the tests were mostly unchanged compared with those obtained by Pavoine & Dolédec (2005). However, the components of the new approaches are easier to interpret in terms of equivalent numbers of species, sites, regions, etc. They revealed low diversity within sites and low dissimilarities among sites in terms of species, taxonomy, size and feeding habits, but relatively high species dissimilarity among geological regions.

Here, we discuss on the pros and cons of our alternative approaches for a hierarchical analysis of diversity and compare these approaches with those of other authors. We show how different authors approach the characterization of the diversity of assemblages from different perspectives.

ON THE NORMALIZATION OF TRAIT DISTANCES

Our approaches depend on the existence of a maximum dissimilarity between two species. This maximum needs to be defined carefully. Normalizing the dissimilarities among species, d_{kl}'s, between 0 and 1 might hamper comparisons between data sets if done separately on each data set (Appendix S3; see also de Bello *et al.* 2013). The choice of the dissimilarities is thus important, keeping in mind that a close-to-0 value means very similar species, while a close-to-1 value means very different species. For example, if the objective is to compare the diversity of sites within a region, then the normalization of the d_{kl}'s between 0 and 1 should have been done at the regional level so that all sites are compared using the same matrix of dissimilarities among species but different vectors of species' abundances (see other examples in Appendix S3; see also Ricotta & Szeidl 2009 and R scripts in Appendix S2 of de Bello *et al.* 2010). Similarly, when different data sets are compared, the matrix of dissimilarities among species should have been measured with the same units for all data sets so that the data sets are comparable (an example is given in Fig. 2; other examples are available in

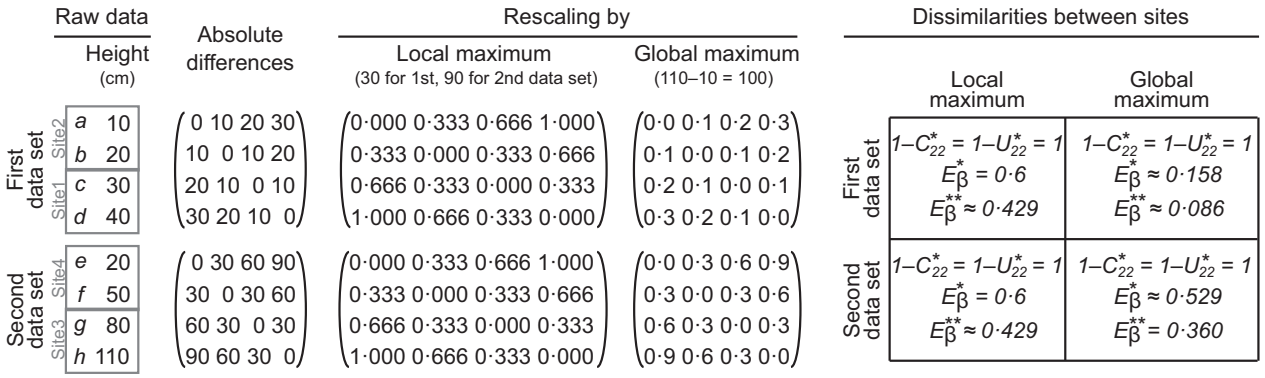

Fig. 2. Example of data sets where the way of normalizing the dissimilarities among species into the interval [0, 1] might affect the conclusions. Both data sets contain two sites. Each site has two species (named by letters from *a* to *h*), and the sites do not share species. Species are characterized by their height. Functional dissimilarities among species are first defined as the absolute difference in height. Then, these dissimilarities are rescaled into [0, 1] by two approaches. In the first approach, dissimilarities are divided by the local highest absolute difference in height, where 'local' means considering only the species of the data set (highest difference between species *a* and species *d* in the first data set; and between species *e* and species *h* in the second data set). In the second approach, dissimilarities are divided by the global highest absolute difference in height, where 'global' means considering the pooled species of the two data sets (highest difference between species *a* and species *h*). Using the local approach leads to the conclusion that the functional dissimilarity between the two sites is equivalent in both data sets. Using the global approach, indices E^{*}_{β}, and E^{**}_{β} (see main text for equations) correctly identify the higher functional dissimilarities among the two sites in the second data set. However, Chiu & Chao (2014) $1 - C^{*}_{22}(Q)$ and $1 - U^{*}_{22}(Q)$ indices are insensitive, in this example, to functional dissimilarities and reach their maximum because the sites, in each data set, do not share species. We advocate at least the global approach, or a reference to a larger species pool, each time data sets have to be compared.

Appendix S3). Flexibility in the normalization of the dissimilarities among species is thus important and was done in previous studies (see R scripts in Appendix S2 of de Bello *et al.* 2010; see also de Bello *et al.* 2013). Note also that some coefficients of dissimilarity include data normalization. For example in Gower (1971) coefficient, quantitative traits are standardized by their range, the definition of which is thus critical. We demonstrate in Appendix S1 that the spurious behaviour of indices E_{β}, E^{*}_{β}, E^{**}_{β} found by Chiu & Chao (2014) with non-ultrametric distances among species is not related to the ultrametric property and can be solved by normalizing data globally over data sets. Ricotta & Szeidl (2009), de Bello *et al.* (2010) and Villéger *et al.* (2012) approaches can all be applied to both ultrametric and non-ultrametric (but squared Euclidean) distances.

ON THE DEFINITION OF MAXIMALLY DISSIMILAR COMMUNITIES

Chiu & Chao's (2014) measures of functional dissimilarity among sites reach their maximum when the sites do not share species, irrespective of the functional similarities among species as shown in Fig. 3. Their definition of maximally dissimilar is the following: *N* assemblages are completely distinct if there are no shared species (and thus no shared species' pairwise distances). In contrast, our definition of maximally dissimilar sites is different: two sites are maximally dissimilar if any species of one site is maximally dissimilar of any species in the other site (Fig. 3). In Fig. 3a, although including the functional dissimilarities among species, Chiu & Chao (2014) indices provide maximum dissimilarity between the two sites (because they do not share species). In contrast, de Bello *et al.* (2010) and Villéger *et al.* (2012) indices provide low dissimilarity between the two sites because species *a*, *c*, *e* from the first site are very similar to species *b*, *d*, *f*, respectively, contained in the second site. In the extreme case where species *a*, *c*, *e* have identical functional traits as species *b*, *d*, *f*, respectively, Chiu & Chao (2014) indices still provide maximum dissimilarity between the two sites, whereas de Bello *et al.* (2010) and Villéger *et al.* (2012) indices acknowledge the similarity between the two communities (β diversity = 0). In Fig. 3b, all indices agree that the communities are maximally dissimilar, Chiu & Chao (2014) indices because the sites do not share species (or species-pairs), and de Bello *et al.* (2010) and Villéger *et al.* (2012) indices because any species in the first site is maximally dissimilar with any species from the other site. Different definitions of maximally dissimilar communities have thus been proposed in the literature and can respond to different questions on the structure of communities and regions and thus to different objectives (see also Appendix S3 for a discussion in the particular case of diversity measured by phylogenetic trees).

ARE β DIVERSITIES BOUND TO BE LOW IN APPLICATIONS?

A criticism raised towards Rao's APQE framework was that, when applied to real data sets, low values were observed for β diversity components. It was underlined (e.g. de Bello *et al.* 2009) that this was because Rao's quadratic entropy is a generalization of the Gini–Simpson index for which a link between α and β diversity was demonstrated with low values of β diversity as soon as α was high (even allowing γ diversity to vary, see also Appendix S4). However, this dependence of the range of Rao's β diversity (Q_{β}) on Rao's α diversity (Q_{α}) is true only if the dissimilarities among species are bounded between 0 and 1 and, in that case, it is solved by transforming entropy into

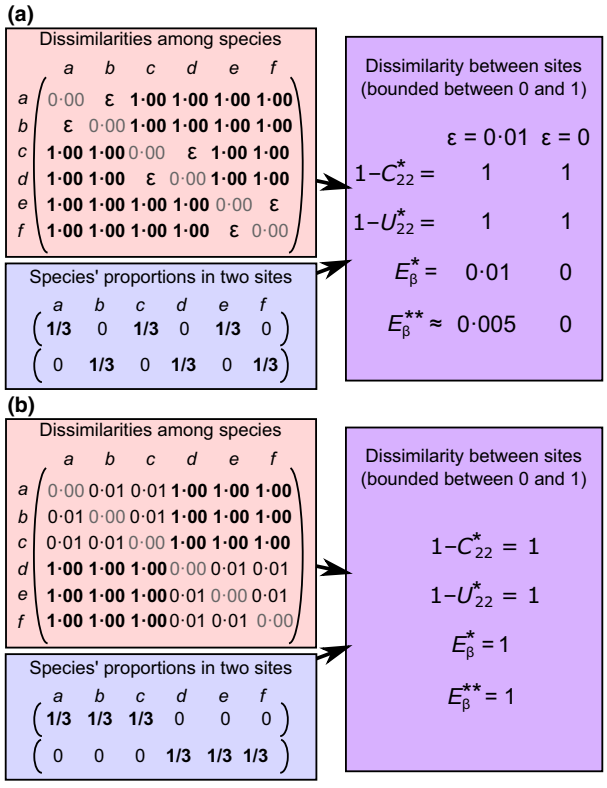

Fig. 3. Illustration of the different ways used to define maximally dissimilar sites: (a) Sites do not share species, but the species from one site are functionally similar to one of the species in the other site; (b) Sites do not share species and any species in one site is maximally functionally dissimilar to all species in the other site. In (a) and (b), the matrices of pairwise dissimilarities are given in the top-left panel (the dissimilarity between a species and itself always is 0; the dissimilarities between species *a*, *c*, *e* and *b*, *d*, *f*, respectively, in case a are given by a parameter ε that takes two values in our example: 0·01 and 0). We then consider two sites and provide their compositions in terms of species' proportions (bottom-left panel). The matrix of dissimilarities and the vectors of species' proportions within sites are used to calculate E^*_β, E^{**}_β, and Chiu & Chao (2014) $1 - C^*_{22}(Q)$ and $1 - U^*_{22}(Q)$ indices.

equivalent number of species (Jost 2007; Ricotta & Szeidl 2009; de Bello *et al.* 2010). If, on the contrary, both the dissimilarities among species and the compositions of the communities are allowed to change indefinitely, then for fixed values of diversity within communities, Q_β can always vary from 0 to infinity so that its range does not depend on Q_α (Appendix S4).

We developed β diversity measures in the case where the dissimilarities among species are bounded between 0 and 1 in order to express diversity in terms of equivalent numbers of species. These β diversity measures take maximum value when communities do not share species and species from a community are maximally dissimilar to all other species in any other community. This situation will not be possible; for example, if one considers more than two communities, species are characterized by a single mean value of a quantitative trait and the dissimilarity between the species is the absolute difference in their mean value. Indeed in that case only two mean trait values are maximally dissimilar: the minimum and the maximum possible values of the trait. If a region contains two communities, they can be maximally dissimilar if one contains similar species all characterized by the minimum trait value and the other contains similar species characterized by the maximum trait value. If a third community is added in the region, then it will necessarily share at least partial similarities with one of the communities (e.g. if its species have low values for the trait) or both (e.g. if its species have averaged values of the trait). If the same species are characterized by several values reflecting their intraspecific diversity and if the dissimilarity between two species is based on trait overlap (e.g. de Bello *et al.* 2013; Carmona *et al.* 2016), then more species can have dissimilarities close to 1 even with a single trait and β diversity can be high.

The observed range of values for our components of β diversity thus depends on how the dissimilarities among species are defined. At first, species are all considered maximally dissimilar, then for a sufficient number of species compared with the number of communities, the β components can reach their theoretical maximum (i.e. the total number of communities for β components expressed in terms of effective number of communities and 1 for their normed versions). Then, adding species' traits or phylogeny will necessarily add similarities among species including possible similarities among species from distinct communities, decreasing thus all components of diversity. It does not mean that the indices are insensitive to the differentiation among communities but instead that, given the data used to characterize species, there are similarities among communities. For example, if species are characterized by a qualitative trait with many levels and few species associated with the same level, the β components as defined in our paper will remain high compared with considering all species as maximally dissimilar. Null models can help to evaluate whether the level of β diversity could have been observed randomly given the way species have been (functionally or phylogenetically) characterized. Further research is thus needed on the relevance and performance of null models in functional and phylogenetic ecology (e.g. Hardy 2008).

Conclusion

To conclude, biodiversity is a multifaceted notion, and it would be detrimental to search for a single way of measuring it. On the other hand, the results provided by biodiversity indices must be unambiguously understandable by ecologists and conservationists. Jost (2006, 2007) thus claimed for intuitive indices that could be directly applicable to prioritize areas for conservation. We developed our approaches to satisfy this requirement. Therefore, we believe our approaches are possible solutions towards understandable ways for analysing nested levels of community organization integrating many aspects of biodiversity including species, functional or phylogenetic diversity through a variety of spatial scales.

Acknowledgements

We thank the associate editor and referees for their comments on our paper.

Data accessibility

Data are available in the R package ADE4 version 1.7-2 (Dray, Dufour & Chessel 2007; R Core Team 2015). R script and manual can be downloaded as online supporting information (Appendixes S5–S7).

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1. About ultrametric and non-ultrametric distances among species.

Appendix S2. Details on the new approaches for a hierarchical analysis of biodiversity.

Appendix S3. Details on how to normalize the (functional or phylogenetic) distances among species.

Appendix S4. β diversity and Rao's framework.

Appendix S5. Manual for R functions and examples of their use.

Appendix S6. R scripts for functions.

Appendix S7. R scripts for performing the examples given in Appendix S5.

