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Components of beta diversity in hierarchical sampling designs: a new approach

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Abstract

Diversity partitioning has been generally used to estimate the contribution of different levels of sampling hierarchy to landscape diversity. However, beta diversity values derived by partitioning strongly depend on focus and sample size and the partitioning is inadequate to express the contribution of landscape elements to community variation. Pairwise dissimilarities are also frequently used to express community turnover, but related approaches capture only a limited aspects of it, especially for hierarchical sampling designs. To avoid these shortcomings, we suggest a procedure which quantifies the role of different levels of sampling hierarchy (relative beta diversity) and the share of landscape elements in the corresponding relative beta diversity (contribution value). Our novel method uses pairwise dissimilarities and is based on partitioning a dissimilarity matrix of sampling units. The new method is suitable to testing various null hypotheses via permutation techniques as demonstrated by artificial and actual data. Our novel method is a valuable tool in ecology because it complements existing approaches while providing a unique way to understand community diversity in space.

Highlights

- A method quantifying different aspects of community variation is proposed.
- We demonstrated its utility by examining artificial and actual data sets.
- Significance tests are possible via randomization models.
- It complements existing approaches to measure community variation.

Keywords

beta diversity, diversity partitioning, hierarchy theory, scale concept, turnover

1. Introduction

Studying and understanding the spatial aspect of biodiversity are the most challenging tasks of contemporary ecology (Beever et al., 2006; Bevilacqua et al., 2012; Rosenzweig, 1995; Villéger and Brosse, 2012; Whittaker et al., 2001). A wide range of conceptual and methodological approaches to this problem use the term beta diversity (Tuomisto, 2011) and include analyses of turnover along environmental gradients and variation in species composition among sites (Anderson et al., 2011). In the simplest case, turnover or variation are evaluated using sampling units without considering any *a priori* classification of them. In many situations, however, sampling units constitute an inclusive hierarchy: units are grouped according to habitat, similar habitats are merged into landscape elements, and so on. Such a sampling scheme, referred to as hierarchical sampling design (see Crist et al., 2003), allows a sophisticated evaluation of turnover within the community (Gering et al., 2003). In the present paper, we emphasize that community variation quantified using regional and local diversity values are confounded by differences in focus and sample size and consequently cannot be formally compared (Izsak and Price, 2001; Terlizzi et al., 2009). We also show that recently available approaches using pairwise dissimilarities capture only a limited aspect of community turnover for hierarchical sampling designs. Therefore, we suggest a procedure which quantifies the role of different levels of sampling hierarchy (relative beta diversity) and the share of landscape elements in the corresponding relative beta diversity (contribution value) such that differences in focus and sample size do not influence the estimates. From a practical point of view, our approach provides an invaluable tool for biodiversity monitoring because 1) it quantifies a standardized and therefore comparable aspect of community variation, and 2) it expresses the share of landscape elements in total diversity, an option not available in earlier methods. Thus, our method supplements the existing methodology of

diversity partitioning while providing a unique way to understand community diversity in space.

2. Terminology

2.1. The scale concept

In the scale concept, five terms: *sampling unit*, *grain*, *focus*, *sample size* and *extent*, are of central importance (see Kenkel et al., 1989; Palmer and White, 1994; Peterson and Parker, 1998; Scheiner et al., 2000, 2001; Wu, 2004). *Sampling unit* is the arbitrarily delimited tract of the community in the real space (synonyms are plots, quadrats). *Grain* is the standardised unit to which all data are adjusted, if necessary, before the analysis. This aspect of scale becomes particularly important in ecological research when data are obtained from different studies or from the same research research using sampling units of unequal size. For example, for eight sites we may have measures of species richness derived from 1 m^2 quadrats, whereas for another site we may have species richness derived from 2 m^2 quadrats. To use data from all sites, quadrats must be standardized to the same size, which becomes the grain of the study (Schneider et al., 2000). *Focus* is the scale at which the grains are aggregated and related grains form *focal units*. For example, when the species richness of a patch is estimated by aggregating the species inventories of three 1 m^2-quadrats, then the focal unit size is 3 m^2. Consequently, the size of *focal units* may be equal to or larger than the *grain* size. *Sample size* expresses the number of replicates of sampling units at the scale of grain or the number of focal units (at the scale of focus). Finally, *extent* is the geographical area within which the sampling units are arranged.

2.2. Hierarchy theory

In hierarchy theory, several *levels* of organisation are distinguished in a system, each involving a distinct set of attributes and problems (King, 1997). Consequently, the *level* does not indicate any physical dimension directly (contrary to scale) and is constrained by the level above it (Turner et al., 2001). A good example is the habitat hierarchy of streams (Frissell et al., 1986) which defines microhabitat, pool/riffle, reach, segment, and the stream system as different levels. These levels of habitat hierarchy are associated with unique geomorphological and hydrological features and events (see Fig. 2 in Frissell et al., 1986). Note that in this paper we consider only *discrete* hierarchical levels; if the levels themselves are continuous then a function may be invoked that describes that abstract continuum, and this function is also called the scale in hierarchy theory (Allen and Starr, 1982).

3. Quantifying community variation using diversity partitioning

Let us start with a simple example: we have a landscape with two habitat patches (A and B) and our aim is to quantify community variation (beta diversity) within and among patches. In this case, our habitat hierarchy consists of three levels: sample (level 1), patch (level 2) and landscape (level 3, see Fig. 1: Habitat hierarchy). Assume that we take 3 sampling units (sampling units 1, 2, and 3) from patch A and 3 sampling units (sampling units 4, 5, and 6) from patch B and that the grain size of the 6th sampling unit is larger than that of the others (Fig. 1: Sampling unit). Since the observed diversity depends on sampling unit size, to allow comparisons we have to standardise our sampling units to the same grain size. After this, grain size will be the same for all sample units (Fig. 1: Grain). In the next steps, sampling units at grain size are regarded as focal units (Fig. 1: Focal units, bottom row), or sampling units at grain size are aggregated to get focal units (Fig. 1: Focal units, 2 middle and top quadrats). Following this terminology, Whittaker (1960) put forward a Greek lettering scheme

121 referring to diversity observed within (α and γ diversities) and among (β diversity) *focal units*.
122 In our example, within sample focal unit alpha diversity is calculated as the mean diversity in
123 the lowest six focal units (Fig. 1, Focal units, below), within patch focal unit alpha diversity
124 as the mean diversity in the two focal units (Fig. 1, Focal units, middle), and total diversity of
125 the landscape as the diversity of the top focal unit (Fig. 1, Focal units, top). Given the above
126 scheme, beta diversity can be expressed in many different ways, including methods based on
127 additive and multiplicative partitioning (Anderson et al., 2011; Jurasinski et al., 2009; Koleff
128 et al., 2003; Ricotta, 2010; Tuomisto, 2010; Veech and Crist, 2010a, b; Whittaker, 1960).
129 Among sampling units variation is calculated as the relationship between within-patch focal
130 unit alpha diversity and within-sample focal unit alpha diversity. Among-patches variation is
131 quantified as the relationship between total gamma diversity and within-patch focal unit alpha
132 diversity. Here we should emphasize again that among-patches variation includes only that
133 part of community variation, which exists among patches but not within patches. In case of
134 additive partitioning, the relationship is measured via subtraction and thus beta diversity is
135 expressed in units of numbers of species, whereas in case of multiplicative partitioning it is
136 achieved via division and thus beta diversity is expressed as an unitless ratio. In addition,
137 diversity can be partitioned with respect to a two-level or a multi-level sampling hierarchy
138 (Chiarucci et al., 2008; Erős, 2007; Gering et al., 2003; Wagner et al., 2000) and thus the
139 focal scale concept is a generalization of two-level (regional and local) comparisons. In sum,
140 diversity partitioning described above has become one of the most influential approaches for
141 *assessing the contribution of the different levels of habitat hierarchy to the overall biological*
142 *diversity of a landscape*, thereby linking patterns in biological diversity to landscape level
143 environmental heterogeneity (Gering et al., 2003).

144 Assume that we have a landscape with discrete patches of vegetation and we would
145 like to quantify community variation within (β_1) and between (β_2) patches. For simplicity,

146 sampling unit size is held constant, consequently grain equals to the sampling unit. Assume
147 further that only a single species is present in each sampling unit and sampling units share no
148 species. We sample the same landscape by four different sampling designs (A, B, C, and D):
149 in case A, two patches were sampled, each by 2 sampling units; in case B, 2 patches were
150 sampled, each by 4 sampling units; in case C, 4 patches were sampled, each by 2 sampling
151 units; and finally in case D, 4 patches were sampled, each by 4 sampling units (Table 1).
152 Additive diversity partitioning based on species richness shows that there are scale-
153 related differences in quantifying beta diversity within the same design. For instance, sample
154 sizes for calculating β diversities among sampling units (β_1) and among patches (β_2) differ
155 with sampling strategy (4, 8, 8, and 16 versus 2, 2, 4, and 4). This is critical when the different
156 β diversities are evaluated and interpreted because sample size has a strong effect on β
157 diversity (often called as the relationship between additive diversity partitions and sample-
158 based rarefaction, Crist and Veech, 2006; Gotelli and Colwell, 2001; but reference to this
159 phenomenon appears in other papers as well, e.g., Gering et al., 2003; Veech et al., 2002).
160 However, in comparing the β diversities one must consider that focal unit size also changes
161 (1, 1, 1, and 1 versus 2, 4, 2, and 4 in Table 2). This is critical again because the effect of
162 focal scale on species richness can be characterized by the well-known species-area
163 relationship (Crist and Veech, 2006; He and Legendre, 2002; Pielou, 1975; Schmera et al.,
164 2009): the larger the focus, the higher is the number of species. Crist and Veech (2006)
165 already realized this problem (i.e. within the same level, not only sample size but also
166 differences in focal unit sizes influence beta diversity) and suggested a methodology for
167 separating the effects of different focal unit sizes and sample size. However, this suggestion
168 does not solve the methodological problem associated with diversity partitioning, namely that
169 beta diversities are calculated based on different focal unit and sample sizes *from different*
170 *levels*. This is critical because focal unit sizes differ across levels. It is easy to see that focal

unit size depends on the grain size in general, and upper-level (≥2) focal unit sizes also on the sample sizes observed at the level below (Fig. 1). It follows that differences in sample size representing landscape elements and the handling of sampling units (aggregation into focal units) may strongly influence the result of diversity partitioning.

The output table shows that even small changes in sample size may affect substantially the results of diversity partitioning (Table 1). For instance, increasing sample size (no. of sampling units) from 4 to 8 raised among patches β_2 diversity from 2 to 4, while the number of patches examined (2) was unchanged (A to B). Similar change in sample size increased among patches β_2 diversity from 2 to 6 if the number of patches increased from 2 to 4 (A to C). Moreover, if both sample size and the number of patches changed (A to D), then among patches β_2 diversity increased from 2 to 12!

We do not say that small changes in sample size always have strong impact on the output of diversity partitioning for actual data (because in most cases community variation is smaller than in our artificial data), but our example calls attention to the inherent ecological weakness associated with diversity partitioning methodology. Moreover, habitat types in actual data sets often differ regarding the number of sampling units taken (Chiarucci et al., 2008; Erős, 2007; Müller and Großner, 2010). In these cases, community variation within habitats represented by large sample is overestimated in the calculations if compared to habitats sampled by fewer units. Furthermore, the focal unit size of habitats with large sample size will be greater than that for habitats with low sample sizes. This influences the output of beta diversity at upper levels.

Another problem associated with diversity partitioning is that whereas it estimates the contribution of a given level to total diversity, no information is provided on the possible difference between the contributions of focal units within the same level. In other words, diversity partitioning "facilitates the comparison of diversity components between habitat

196 types (...), but does not tell us which landscape elements (i.e. which habitat type) contribute
197 most to landscape species diversity" (Wagner et al., 2000). We argue that this information
198 might be essential in any management decision or conservation planning.
199 The above observations suggest that (1) comparison of different beta diversity values
200 originating from the same diversity partitioning is theoretically less meaningful because
201 sample size-dependence and the way sampling units are handled (aggregated) may be strongly
202 responsible for the results; and (2) diversity partitioning is uninformative about the
203 contribution of landscape elements. We do not say that the currently used method of diversity
204 partitioning should be disregarded or its use is absolutely meaningless, but rather we call
205 attention to some shortcomings of the approach.
206
207 **4. Quantifying turnover using pairwise dissimilarities**
208
209 Pairwise dissimilarity indices are commonly used in expressing beta diversity both in
210 basic research (Anderson, 2001; Anderson et al., 2006, 2011; Koleff et al., 2003; Vellend,
211 2001) and conservation practice (Cingolani et al., 2010; La Sorte et al., 2008). If sampling
212 scheme follows a hierarchical sampling design (i.e. sampling units can be grouped
213 successively at different levels), then pairwise dissimilarity matrices can be partitioned into
214 groups of dissimilarities (see Fig. 1 in Bacaro et al., 2012). Partitioning of dissimilarity
215 matrices is frequently used in molecular genetics (Analysis of Molecular Variance, AMOVA,
216 Excoffier et al., 1992) and community ecology (Analysis of Similarities, ANOSIM, Clarke
217 1993; Mean Similarity Approach, MSA, Van Sickle, 1997; Permutational Multivariate
218 Analysis of Variance using Distance Matrices, PERMANOVA, Anderson, 2001; Multiple
219 Response Permutation Procedure, MRPP, McCune and Grace, 2002).

Most of these tests aim to indicate the coherence of groups or the differences between groups by a comparison of (squared/rank of) dissimilarities within and between groups (AMOVA, ANOSIM, MSA and PERMANOVA) or by the comparison of dissimilarities among groups (MRPP), but are not necessarily designed for expressing turnover in well interpretable way. Capturing turnover values from the output files of these analyses is rather challenging, because these tests are based on squared dissimilarities (AMOVA, PERMANOVA), ranked dissimilarities (ANOSIM) and raw dissimilarities (MRPP, MSA) and because overall test statistics or group-related partial results are often standardized by the number of observations within the group (AMOVA, PERMANOVA), by the relative group size (MRPP), by the number of dissimilarity values within the group (MSA), or in such a way that the test statistic varies between -1 and +1 (ANOSIM). Consequently, even if the quantification of turnover by pairwise dissimilarities is not influenced by scale issues (because all methods express community turnover from one sampling unit to another) no methodology is available to express turnover of different levels of hierarchically collected samples.

5. Innovation

Here we suggest a procedure which quantifies the role of different levels of sampling hierarchy (relative beta diversity) and the share of landscape elements to the corresponding relative beta diversity (contribution value), such that differences in focus and in sample size do not influence the estimates.

Numerous pairwise dissimilarity measures are used to express beta diversity (e.g., Koleff et al., 2003). Although our method works with any of these measures, here we

calculate pairwise beta diversity values (β_{PAIR}) for all possible sampling unit pairs as follows (see Lande, 1996):

$$\beta_{PAIR} = \frac{b+c}{2}, \quad \text{(Eq1)}$$

where b is the number of species present only in the first sampling unit and c is the number of species present only in the second sampling unit.

In hierarchical sampling designs, pairwise beta diversities quantify turnover within and/or among landscape elements. Let us define $A_{x,j}$ as a set of pairwise beta diversities, which quantify the community turnover within a landscape element j (defined at level x) but not the community turnover within landscape elements defined at any levels lower than x. We quantify the role of different levels of sampling hierarchy as relative beta diversity (β_{REL})

$$\beta_{REL(x-1)} = \overline{\beta_{PAIR}} \mid \beta_{PAIR} \in \bigcup_{j} A_{x,j} \quad \text{(Eq 2)}$$

and the share of landscape element as contribution value (CV) given by

$$CV_{x,j} = \overline{\beta_{PAIR}} \mid \beta_{PAIR} \in A_{x,j} \quad \text{(Eq 3)}$$

In order to illustrate calculations of the novel method, consider a hierarchical sampling design with two patches and 4 sampling units (2 sampling units per patch) and the following data matrix in which columns represent sampling units and rows are species:

```
          patch 1|patch 2

          1   1   1   1
          1   0   0   0
D=        1   0   1   1
          1   1   1   0
          0   1   1   0
          0   0   1   1
```

The pairwise comparison of sampling units resulted in 6 pairwise beta diversities (Table 2). Two pairwise beta diversities (pairs 1-2 and 3-4) express within patch/among sampling units turnover, whereas the other four (pairs 1-3, 1-4, 2-3, and 2-4) within landscape/among patches community turnover. The results show that pairwise beta diversities as defined above vary

between 1 and 2 (Table 2). The relative beta diversity among sampling units (level-1) is 1.25 and among patches (level-2) is 1.5. Their difference shows that the second sampling level has a higher relative contribution to diversity than the first. In other words, diversity among sampling units from different patches is larger than among sampling units from the same patch. The contribution value of a patch expresses how the patch contributes to the relative beta diversity among sampling units. The contribution values of patches 1 and 2 differ (Table 2), suggesting that patches can be ranked based on their contribution to the between sampling unit relative beta diversity: from this point of view patch 1 is more "valuable" than patch 2, because community turnover in patch 1 is higher (1.5) than in patch 2 (1). It should be noted that from additive diversity partitioning we would conclude that among sampling unit beta diversity is larger (1.25) than among patches beta diversity (1).

6. Analyses of actual data sets

6.1. Stream dwelling caddisflies

Caddisflies were collected from the Kemence stream (Hungary) using a hierarchical sampling design (Schmera and Erős, 2012). Within the stream system, 3 segments (coded from 1 to 3); within each segment, 3 reaches (altogether 9, coded from 1 to 9), within each reach, 3 riffles (altogether 27, coded from 1 to 27) were randomly selected. Within each riffle, 12 (altogether 324) Surber sampling units (area: 0.09 m^2, mesh size: 0.5 mm) were taken to represent microhabitat level of the stream habitat hierarchy. Consequently, our stream habitat hierarchy includes the following levels: sampling unit/microhabitat, riffle, reach, segment and stream system (see figure and definition of levels in Schmera and Erős 2012).

Additive diversity partitioning applied to the species richness of caddisflies showed that among sampling units beta diversity had the strongest contribution to the total diversity of

297 the stream system (29 species) followed by among segments beta diversity (Fig. 2A). In
298 contrast, the novel methodology showed that among segments relative beta diversity ($\beta_{REL(4)}$)
299 has the strongest sample size-independent contribution to the caddisfly diversity of the
300 stream, followed by among reaches ($\beta_{REL(3)}$), among riffles ($\beta_{REL(2)}$) and among sampling units
301 ($\beta_{REL(1)}$) relative beta diversities (Fig. 2B).

302 Moreover, contribution values identified that 1) segment 3 has the strongest
303 contribution to the among reaches beta diversity followed by segments 2 and 1; 2) reaches 5
304 and 7 have the strongest contribution to among riffles beta diversity, whereas reaches 1 and 3
305 have the weakest; and 3) riffles 19 and 21 have the strongest contribution to among sampling
306 units beta diversity and riffles 3 and 17 have the weakest (Fig. 2C). Here we should
307 emphasise again that the contribution value of a landscape element (defined at level x)
308 quantifies the contribution of the landscape element to the relative beta diversity at level x
309 ($\beta_{REL(x)}$), and it is not a summary statistic of pairwise beta diversities within the landscape
310 element.

311 One of the advantages of the novel methodology is that corresponding measures from
312 different studies can easily be compared by traditional statistical approaches if the grain of
313 sampling units is the same. Such comparisons with traditional diversity partitioning are rather
314 complicated because both among focal-unit diversities and within focal-unit diversities at
315 higher level ($x > 1$) are strongly influenced by sample size and focus.

316 Testing the significance of relative beta diversities and contribution values within the
317 same study is not possible with traditional statistical approaches because these measures
318 originate from non-independent observations (i.e. the same sampling unit is used for
319 calculating many pairwise beta diversities). Therefore, we suggest using randomization-based
320 null models for statistical testing following Crist et al. (2003). The null-model approach is a
321 framework for comparing observed measures with expected ones, where expected ones are

derived from randomising the observed data (Gotelli and Graves, 1996). As the combination of null hypothesis and randomization technique provides a wide variety of null models, here we can only demonstrate test performance for a single null-hypothesis with the note that careful formulation of ecological hypotheses is a prerequisite to statistical tests.

Our test examines whether the observed relative beta diversities and contribution values are a consequence of sampling design. This corresponds to the second hypothesis (H_2) of Crist et al. (2003). Testing this hypothesis requires separate randomization for each level. In the first step, sampling units are randomly relocated into any other position as determined by the sampling design. Using this randomization, hereafter called as randomization #1, we can test whether among segments relative beta diversity is different from that expected by chance ($\beta_{REL(4)}$, Fig. 2B). In the second step, we constrain the randomization in such a way that sampling units remain in the same segment in which they were taken (randomization #2). Using this strategy, we can test whether among reaches relative beta diversity ($\beta_{REL(3)}$, Fig. 2B) and contribution values of segments (Fig. 2C) are different from that expected by chance, by keeping segment constrains. Finally, we constrain the randomization in such a way that sampling units should remain in the same segment and reach from which they are originally derived (randomization #3). Randomization #3 allows testing whether among riffles and among sampling units relative beta diversities ($\beta_{REL(2)}$ and $\beta_{REL(1)}$, Fig. 2A) and the contribution values of reaches and riffles (Fig. 2C) are different from that expected by chance, with segment and reach constraints unchanged. The analyses showed that among segments ($\beta_{REL(4)}$), among reaches ($\beta_{REL(3)}$,) and among riffles ($\beta_{REL(2)}$) relative beta diversities are significantly higher than expected by chance, whereas among sampling units beta diversities ($\beta_{REL(1)}$) are significantly lower (Fig. 2B) at $p=0.05$. Moreover, we tested the contribution values of different landscape elements (Fig. 2C).

346 Calculations were performed by an Excel Macro developed by the first author. We
347 used 1000 randomizations.
348
349 *6.2. Grassland communities*
350 The second example comes from an extensive study of rock grasslands on the
351 dolomite bedrock of Sas-hill, within the city limits of Budapest, Hungary (Podani 1998).
352 Eighty sampling units were selected in the grasslands, representing three major vegetation
353 noda (or community types without sharp boundaries), namely open rock grassland (OG),
354 closed grassland (CG) and slope steppe (SS), and henceforth referred to as habitats. Each
355 sampling unit consisted of a series of 8 nested quadrats with a common corner, the smallest
356 being 0.5 m x 0.5 m, and the largest 4 m x 4 m, with 0.5 m side increments in between. For
357 the present study, we used 10, 8 and 7 sampling units from the above three habitats,
358 respectively, and in order to demonstrate sampling unit size-dependence of diversity studies,
359 we used four quadrat sizes: 1 m x 1 m, 2 m x 2 m, 3 m x 3 m, and 4 m x 4 m. Thus, we have
360 three levels of diversity to evaluate: within-quadrat alpha diversity, among quadrats and
361 among habitats beta diversity, plus gamma diversity of the total landscape.
362 Additive diversity partitioning applied to the grassland communities showed that
363 among sampling units beta diversity had the highest contribution to species richness
364 independently from the size of the sampling unit (Fig. 3A). Moreover, diversity values (α_1, β_1
365 and β_2) increased monotonically over increasing sampling unit size. In contrast, the novel
366 method showed that independently from the size of the sampling unit, among habitats relative
367 beta diversity ($\beta_{REL(2)}$) had stronger contribution to the diversity of the grassland of the hill
368 than among sampling units beta diversity ($\beta_{REL(1)}$). Both relative beta diversity values ($\beta_{REL(1)}$
369 and $\beta_{REL(2)}$) increased over sampling unit size (Fig. 3B). Contribution values showed that
370 independently from the sampling unit size, closed grassland had the highest contribution to

among sampling units beta diversity followed by slope steppe and open grassland habitats (Fig. 3C).

Considering relative beta diversity, we tested whether the observed relative beta diversities are different from that expected by chance. Our results showed that among sampling units beta diversities ($\beta_{REL(1)}$) were smaller than expected by chance whereas among habitat relative beta diversity ($\beta_{REL(2)}$) was higher than expected by chance (Fig. 3B). This suggests that turnover is larger among habitats than within habitats. The contribution values showed that closed grassland (CG) at 1 m ×1 m sampling unit size has higher contribution, whereas at other sampling unit sizes the contribution to the among sampling units beta diversity is lower than that expected by chance. That is, statistical significance is not independent of sampling unit size (or grain). Slope steppe (SS) and open grassland (OG) also had significantly low contribution to among sampling units beta diversity (Fig. 3C).

7. Bias, variation and error rates

We quantified the bias and the variation of relative beta diversities following widely-accepted directives adapted to our research questions. We created an artificial landscape with two, three and four patches, each with 20 sampling units and 20 possible species. We filled each sampling unit with 4, 10, or 16 species presence (20, 50, or 80% matrix fill). These matrices served as the starting landscape and we quantified its true relative beta diversities. We sampled each patch by 4, 8, 12, 16 and 20 sampling units to estimate relative beta diversity values. We repeated this procedure 100 times. To make the calculations independent from the configuration of the starting landscape, we produced altogether 100 random starting landscapes. We quantified bias as the difference between the true value and estimated values (Sokal and Rohlf, 1995). We found that bias is in general low (between –0.3 and +0.3) and

decreases with increasing sample size and, to a less extent, with increasing number of patches and with intermediate (50%) matrix fill (Fig. 4). We quantified variation as the dispersion of replicate estimates (Sokal and Rohlf, 1995). We found that mean variation of estimated beta diversities decreased with increasing sample size, that mean variation of estimated level-2 relative beta diversity ($Beta_{REL(2}$) was smaller than that of estimated level-1 beta diversity ($Beta_{REL(1)}$) and this difference increased over increasing patch sizes (Fig. 5). Matrix fill influenced the mean variation of estimated relative beta diversities: 50% matrix fill had the highest mean variation (Fig. 5).

We calculated the error rate of the relative diversity calculation combined with the randomization algorithm applied in the analysis of actual data sets. Similarly to the calculation of bias and variation, we produced starting landscapes (with different number of patches and with different matrix fill). We considered the true relative beta diversities independent from sampling design, if their actual values fell within the 95% confidence interval of randomly relocated samples. We tested this by a randomization test (n=200). Then we sampled the starting landscape by 4, 8, 12, 16 and 20 sampling units and calculated the estimated relative beta diversity values. We performed a randomization again (n=200) to test whether the estimated beta diversities predict independence from sampling design. To make the estimation of error rates independent from the configuration of the starting landscape, we produced altogether 200 starting landscapes. We quantified the type I error rates (the probability of rejecting the null hypothesis when it is true), and type II error rates (the probability of failing to reject the null hypothesis when the null hypothesis is false, Zar, 1999), of our null hypothesis with the assumption that the observed relative beta diversities are the consequence of sampling design. We found that the error rates are in general low and decrease with increasing sample sizes and that type I error rate is more sensitive to changes in sample size than type II error rate (Fig. 6).

8. Conclusions

Diversity partitioning has become one of the most common approaches for assessing the contribution of different levels of hierarchically collected samples to the overall biological diversity of a landscape (Gering et al., 2003). In the present paper, we showed that diversity partitioning suffers from dependence on sample size effects and aggregation of sampling units, and therefore it cannot quantify properly the contribution of landscape elements to the observed diversity patterns. To solve these problems, we suggested a methodology independent of sample size and demonstrated its usefulness with artificial and actual data sets.

Following the terminology of Tuomisto and Ruokolainen (2006), our approach explains variation in beta diversity (level-3 question): what is the contribution of different hierarchical levels of a sampling hierarchy to overall beta diversity (relative beta diversity), and what is the share of a landscape element to the corresponding relative beta diversity (contribution value). Our approach is clearly different from raw data-based methods of partitioning community composition variation among groups of explanatory variables (Legendre and Legendre, 1998; Legendre et al., 2005; Peres-Neto and Legendre, 2010) because our approach cannot provide information on shared variance fractions and cannot handle environmental.

The methodology proposed here allows easy comparison of different studies by traditional statistical approaches if the grain of sampling units is the same. Moreover, it can be expanded to testing various null hypotheses along the lines described by Crist et al. (2003). Since the number of potential null hypotheses is large, and there are many other factors that influence the tests (e.g., matrix size dependence, number of levels and so on), we suggest that both the null hypothesis and the corresponding randomization technique should be selected

carefully. We demonstrated by simulation studies that our approach has small bias, low variance (especially at larger sample sizes) and low error rates.

The indication of how biological diversity is distributed among different levels of a habitat hierarchy is a central question of biodiversity research. Additive diversity partitioning is a tool for answering this question and expresses the contribution of the levels of habitat hierarchy in units of numbers of species. Here we developed a novel method that quantifies the same concept also in units of numbers of species, and demonstrated its application using artificial and actual data sets. However, if one would express relative beta diversity as a unitless ratio (i.e. multiplicative diversity partitioning) or in any other way, then our approach can easily be extended into this direction because pairwise beta diversity can be expressed in different ways (multiplicative beta diversity, effective species turnover, Whittaker's species turnover, proportional species turnover, Jaccard similarity, see Koleff et al., 2003, Tuomisto, 2010).

The comparison of traditional diversity partitioning and the new methodology suggests that they are complementary (Table 3). The differences come from that traditional diversity partitioning uses *raw* beta diversities, whereas sample size-independent measurement of beta diversity adapts *relative* beta diversities. Although a consistent terminology of species diversity is a subject of ongoing debate (Jurasinski and Koch, 2011; Tuomisto, 2011), in our view relative beta diversity and contribution values are valuable tools for landscape ecologists because they complement existing approaches while providing a unique way to understand community diversity in space.

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611 Table 1: Effect of sample size (SS) and focus (F) on diversity (α_1, β_1, β_2, and γ diversity) at
612 three different levels based on traditional additive diversity partitioning in four artificial
613 sampling designs. Focus is expressed by the mean number of sampling units pooled.
614

Sampling design	Level 1			Level 1			Level 2			Level 3		
	SS	F	α_1	SS	F	β_1	SS	F	β_2	SS	F	γ
A	4	1	1	4	1	1	2	2	2	1	4	4
B	8	1	1	8	1	3	2	4	4	1	8	8
C	8	1	1	8	1	1	4	2	6	1	8	8
D	16	1	1	16	1	3	4	4	12	1	16	16

615

Table 2: Illustration of the new approach using data set **D** given in the text. Results include pairwise beta diversities (β_{PAIR}), among sampling units relative beta diversity ($\beta_{REL(1)}$) among patches relative beta diversity ($\beta_{REL(2)}$), contribution value of patch 1 ($CV_{2,1}$) and contribution value of patch 2 ($CV_{2,2}$). Subscript 2,1 means that landscape unit can be interpreted at patch [2] level and this is the first patch. × denotes pairs used in calculating the summary statistics $\beta_{REL(1)}$, $\beta_{REL(2)}$, $CV_{2,1}$ and $CV_{2,2}$

Pairs	β_{PAIR}	$\beta_{REL(1)}$	$\beta_{REL(2)}$	$CV_{2,1}$	$CV_{2,2}$
1-2	1.5	×		×	
1-3	1.5		×		
1-4	1.5		×		
2-3	1		×		
2-4	2		×		
3-4	1	×			×
		1.25	1.5	1.5	1

624 Table 3: Comparison of diversity partitioning and our sample size-independent methodology

	Diversity partitioning	Sample size-independent measurement
Interpretation of beta diversity	Expresses the *raw* contribution of sampling levels	Expresses the *relative* contribution of sampling levels (relative beta diversity)
Sensitiveness to the spatial scale of sampling	Comparisons within and between partitioning are rather problematic	Comparisons within and between partitioning are possible, if the grain of sampling units is the same
Partitioning (sum of alpha and beta diversities equals to gamma diversity)	TRUE	NOT TRUE
Able to express the contribution of landscape elements?	NO	YES, through contribution values

625

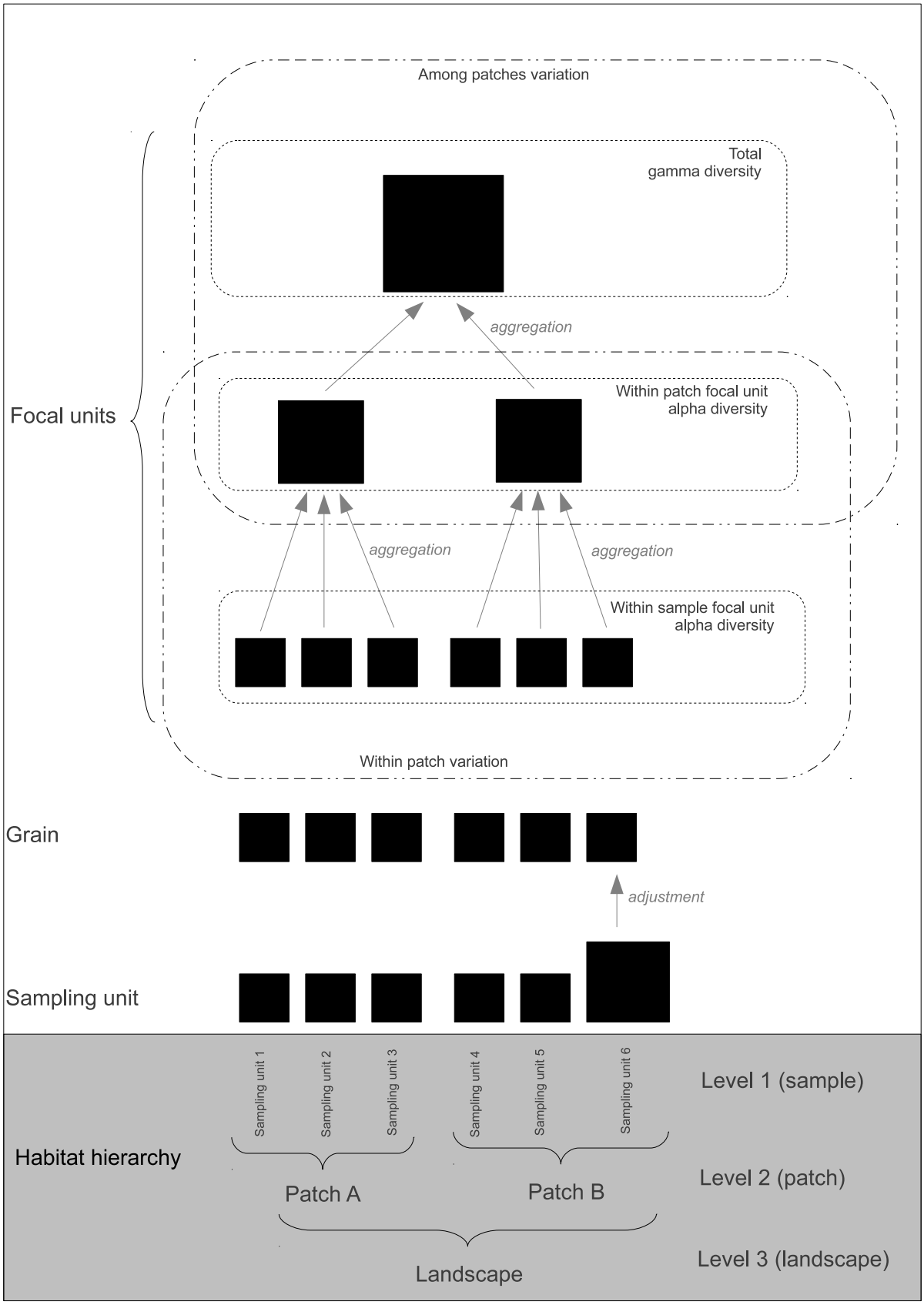
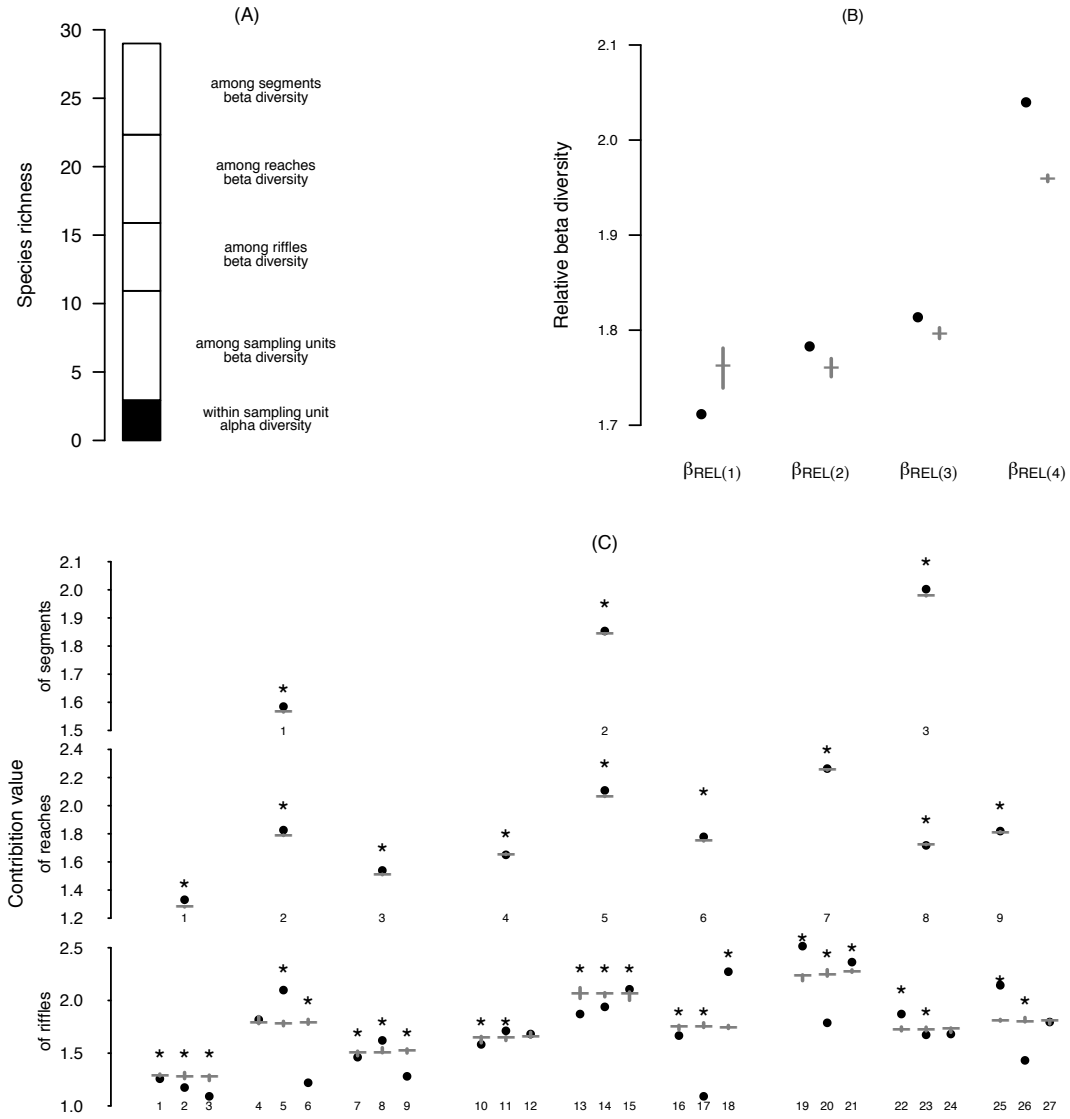


Fig. 1: The scheme of diversity partitioning. Upper part of the figure shows the handling of sampling units during the calculations whereas lower part of the figure (in grey) depicts the habitat hierarchy of sampling. Dotted line groups focal units used for calculating within focal

630 unit diversity (alpha and gamma) and 2 dots-3 dash line links focal units used for calculating
631 beta diversity.
632



633
634 Fig. 2: Diversity of caddisfly assemblages in the Kemence stream (Hungary). A: Results of
635 additive diversity partitioning. B: Relative beta diversities: full circles show observed relative
636 beta diversity values, horizontal grey lines expected relative beta diversity values (median of
637 randomized values) and grey vertical lines the 95% confidence intervals of the randomized
638 values. Note that the departure of $\beta_{REL(4)}$ was tested by randomization #1, $\beta_{REL(3)}$ by
639 randomization #2 and $\beta_{REL(2)}$ and $\beta_{REL(1)}$ by randomization #3 (see text). C: Contribution
640 values: full circles show observed conservation values, horizontal grey lines expected
641 contribution values (median of randomized values) and vertical grey lines the 95% confidence
642 intervals of the randomized values. Statistically significant departures ($P \leq 0.05$) of observed
643 and expected values are highlighted by asterisks. Note that the departure of the contribution
644 value of segments (top) was tested by randomization #2 and that of reaches and riffles by
645 randomization #3 (see text). Landscape elements are ordered from left to right (see numbers at
646 the bottom of the subfigures).
647

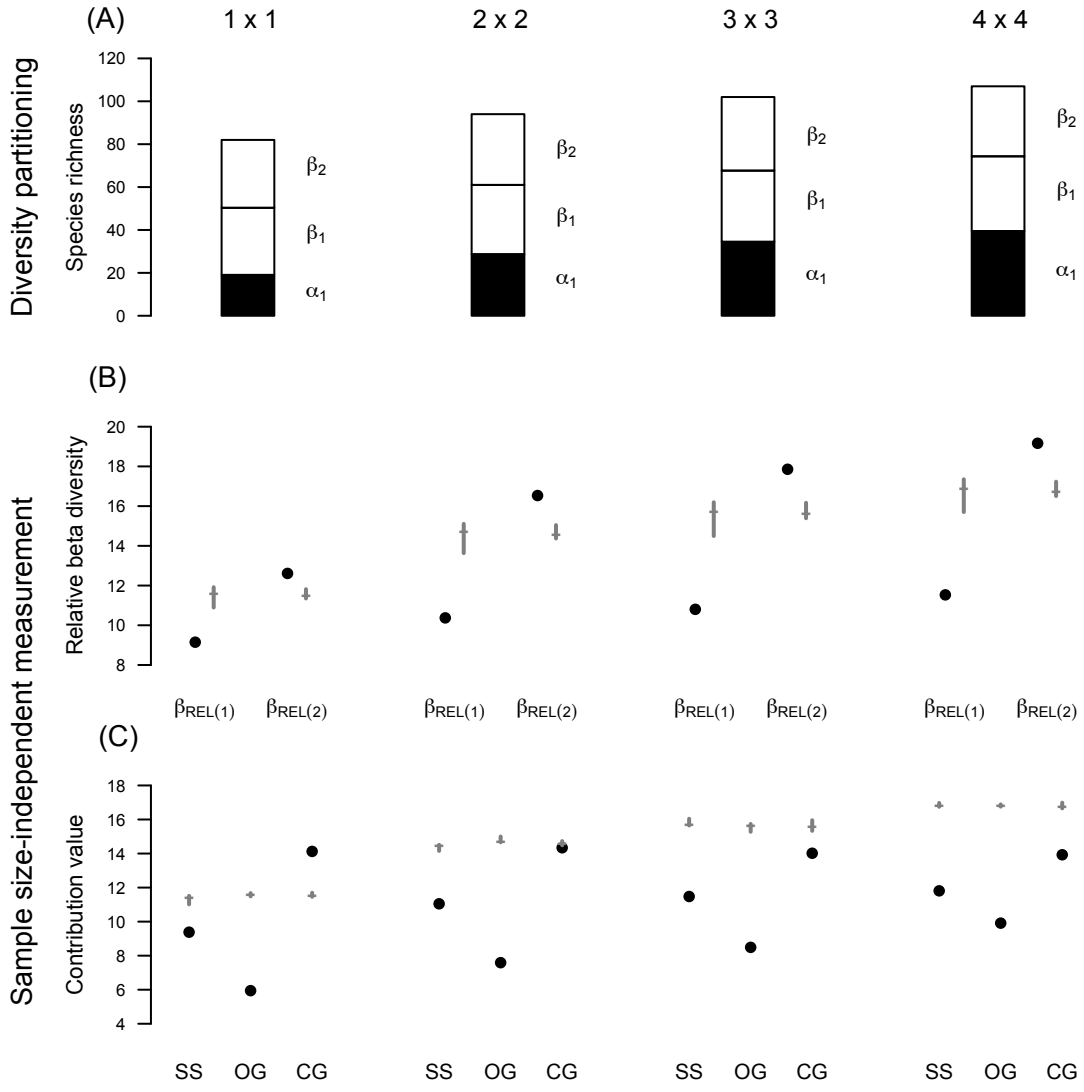


Fig. 3: Comparison of the output of diversity partitioning (A) and sample size-independent measurement (i.e. relative beta diversity [B] and contribution value [C]) of the grassland community of Sas-hill (Budapest, Hungary). Columns from left to right show outputs from samples containing sampling units of size 1×1, 2×2, 3×3 and 4×4 m^2. For diversity partitioning, black colour and α_1 show within sampling unit alpha diversity, whereas white shows beta diversities (β_1 is between sampling unit beta diversity and β_2 is between habitats beta diversity). In case of relative beta diversity, full circles show observed relative beta diversity values, horizontal grey lines expected relative beta diversity values (median of randomized values) and grey vertical lines the 95% confidence intervals of the randomized values. In case of contribution value, full circles show observed contribution values, horizontal grey lines expected contribution values (median of randomized values) and vertical grey lines the 95% confidence intervals of the randomized values. SS: Slope steppe, OG: Open grassland, CG: Closed grassland.

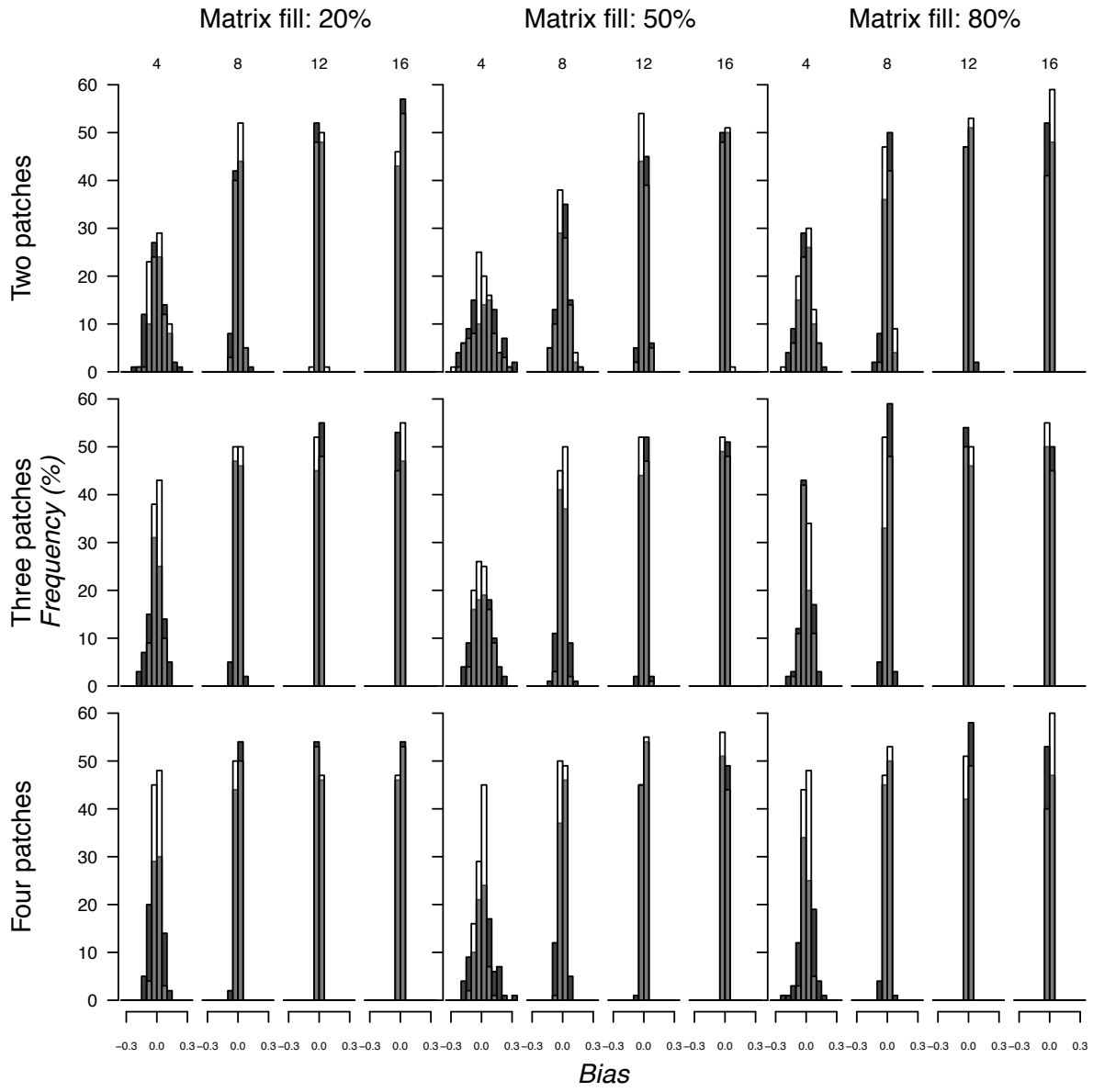


Fig. 4: The effect of sample size (4, 8, 12 and 16) on the frequency distribution of bias (horizontal values) in relation to increasing patch size (rows: two, three and four patches) and matrix fill (columns: 20%, 50% and 80% matrix fill). White columns show the distribution of bias of only $\beta_{REL(1)}$, dark grey columns show the distribution of bias of only $\beta_{REL(2)}$, whereas light grey columns show the overlapping distribution of bias of $\beta_{REL(1)}$ and $\beta_{REL(2)}$.

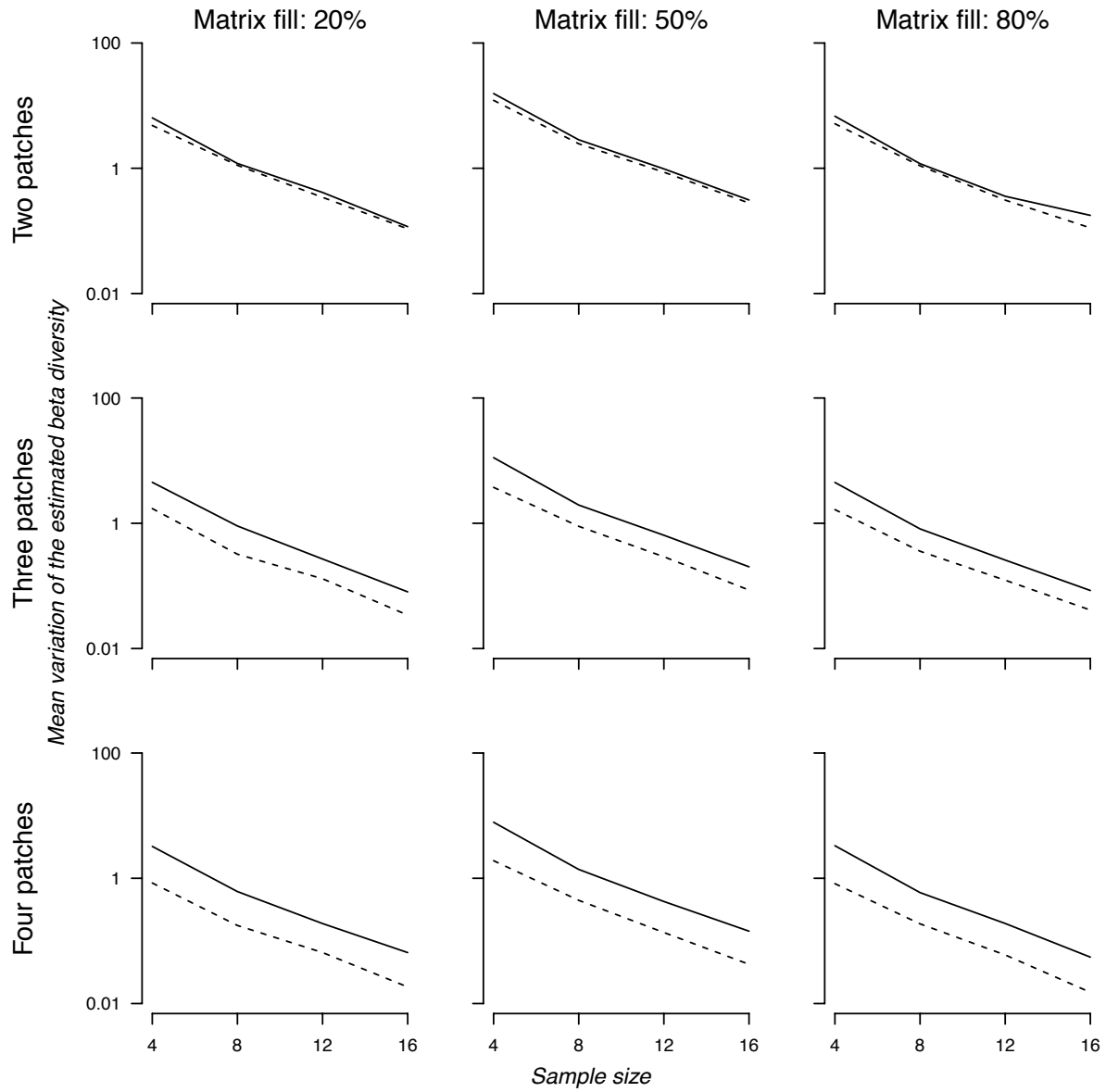
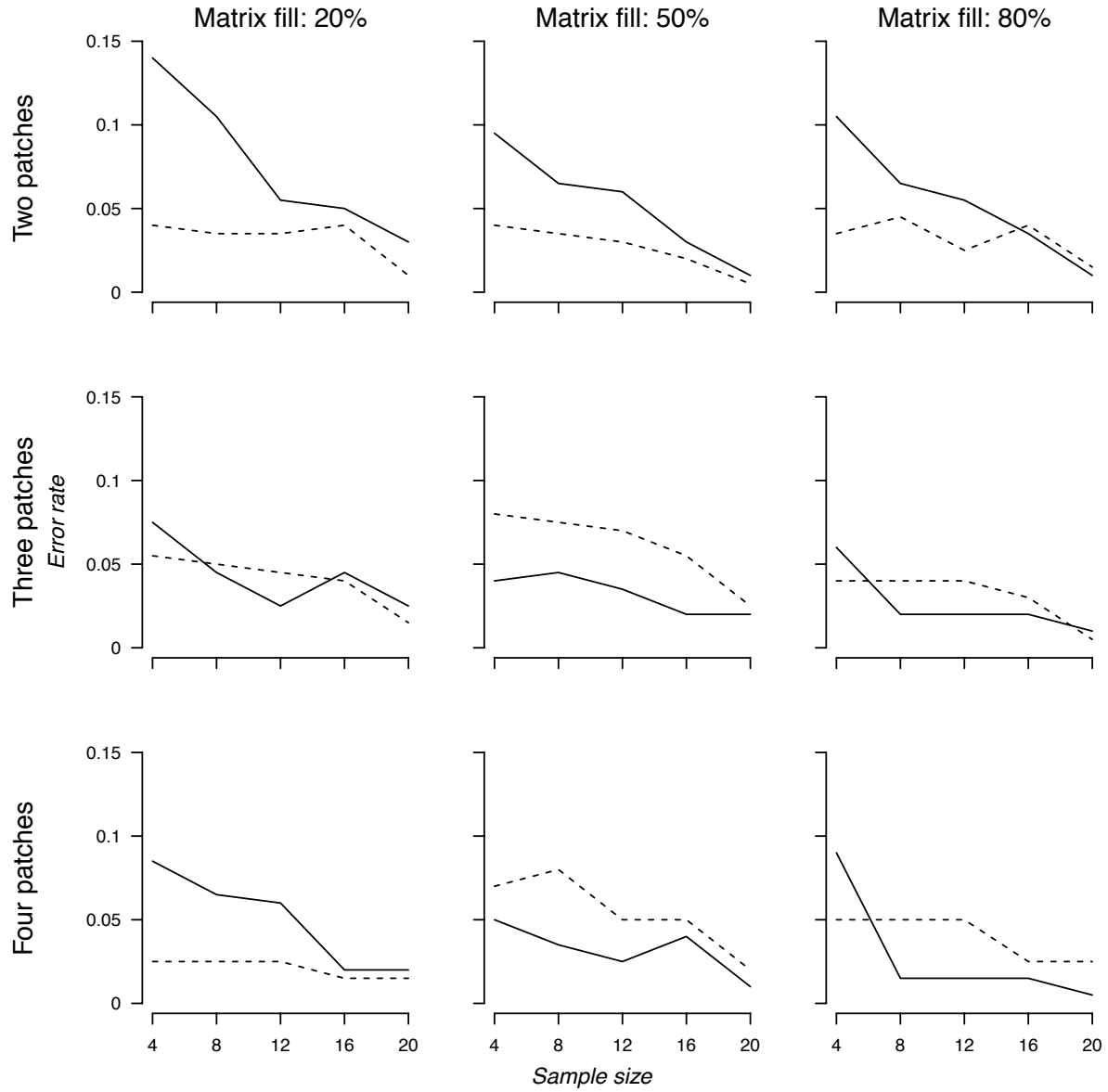


Fig. 5: Effect of sample size on the mean variation of estimated beta diversity in relation to increasing patch size (rows: two, three and four patches) and matrix fill (columns: 20%, 50% and 80% matrix fill). Solid lines show $\beta_{REL(1)}$, dashed lines show $\beta_{REL(2)}$ diversity.



675
676 Fig. 6: The effect of sample size on the type I (solid line) and type II (dashed line) error rates
677 in relation to increasing patch size (rows: two, three and four patches) and matrix fill
678 (columns: 20%, 50% and 80% matrix fill).