Comments

ANALYZING OR EXPLAINING BETA DIVERSITY? COMMENT

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In a recent paper, Tuomisto and Ruokolainen (2006, hereafter referred to as TR) discussed the domains of application of the so-called "raw-data approach" compared to the "distance (Mantel) approach" for studying and testing hypotheses about patterns and determinants of beta diversity. Following Legendre et al. (2005, hereafter referred to as LBP), they addressed the dilemma of an either/or approach in reference to a conceptual framework made of three different "levels of abstraction" depending upon the ecological question to be addressed and thus on the response variable to be studied: (1) community composition data; (2) variation in community composition data or beta diversity; or (3) variation in beta diversity, i.e., variation in variation in community composition data.

TR contradicted LBP, however, by claiming that only the third level of abstraction is relevant to address ecological hypotheses involving geographic distance such as the dispersal limitation underlying the neutral theory of biodiversity (Hubbell 2001). More specifically, they considered that submitting matrices of distances/dissimilarities in community composition to multiple regression along with Mantel tests is the only way to test such hypotheses (TR: p. 2700).

We disagree with such a restrictive vision and the main purpose of our comment is to show that spatially explicit, distance-based analyses of beta diversity do not necessarily belong to the so-called third level of abstraction, let alone to multiple regression on distance matrices and can, moreover, be viewed as a prolongation of the raw-data approach, in accordance with the overall concept of variance partition. This emerges from

a general definition of alpha and beta diversity components as functions of variance in species identity among individuals within and among communities, which is homologous to the definitions of diversity adopted in various other domains and especially in population genetics (e.g., Lewontin 1972, Nei, 1973, Rao 1982). In the sequel, we will show that such a definition leads naturally to an additive relationship between the portions of species diversity explained and unexplained by external environmental variables. We believe, just like Lande (1996), that this partitioning model is fully consistent with the well-rooted ecological notions of alpha, beta, and gamma diversity, even though Whittaker (1960, 1972), who introduced them, initially referred to an analytical multiplicative relationship (see Veech et al. 2002 for a recent review of the two approaches). We will then demonstrate that this additive framework based on well-mastered techniques of variance/covariance decomposition is also encompassing the double variance-partitioning scheme with respect to explanatory variables and principal components of the canonical analysis advocated by both LBP and TR in their second-level raw-data approach. Finally, we will show how, based on a rewriting of the beta component as a measure of dissimilarity, the concept of variogram leads to a direct distance-based additive apportionment of beta diversity, which doesn't require the invocation of a third level of abstraction nor of a Mantel tests approach. We shall finally discuss the goals of potential methods for third-level analyses of the variance among intersite dissimilarities, which is a variance of a variance as correctly presented by LBP. Throughout the text, our arguments are supported by specific references to and commentaries of LBP and TR.

First level, within-community diversity

On the argumentation that basically in ecology "the raw-data tables [. . .] consist of the observations of the abundances of one or more species [. . .] in more than one study site [. . .]", TR (p. 2698) defined community composition as first-level data. By that, they diverged from the proposition of LBP (p. 436) that a first-level analysis consists in "studying variation in species identity of individuals at a given site [which] is studying alpha diversity," an idea brought from genetics to ecology by Lande (1996). This opposition is all the more surprising given that TR say a little farther (p. 2702) "it is important to notice that species composition is not an entity that has ecological behavior of its own, but it is a result of how individuals belonging to different species behave." Indeed, from this last statement, which has our full support, the basic response variable of diversity analyses appears unambiguously to be the taxonomic

identities of the n individual organisms recorded according to a reference nomenclature during a given field survey, i.e., a taxonomic relevé, considered as representative of a given study area or region. For the purpose of data analysis, a straightforward translation of such a list is a binary random variable, say Y_{ij}, which indicates whether an arbitrary individual ($1 \leq i \leq n$) belongs to a particular species ($1 \leq j \leq s$). This can be written in matrix form as follows:

$$\mathbf{Y}[y_{ij}] = \begin{cases} 1 & \text{if the } i\text{th observation belongs to species } j \\ 0 & \text{otherwise} \end{cases}.$$

Matrix $\mathbf{Y}$ ($n \times s$) is called an individual $\times$ species-occurrence table, from Gimaret-Carpentier et al. (1998). When the list is comprised of individuals encountered in a set of m sampling sites (but this is, from our perspective, already and blatantly a second-level problem; see next section), summing per site the individual rows of $\mathbf{Y}$ yields a usual site $\times$ species abundance matrix, say $\mathbf{A}[a_{kj}]$ with size ($m \times s$), which is a "shrunken" version of $\mathbf{Y}$ ($n \times s$). From either $\mathbf{Y}$ or $\mathbf{A}$ table, the relative frequency of species j in the taxonomic relevé is given as

$$p_{+j} = \frac{1}{n} \sum_{i=1}^{n} y_{ij} = \frac{1}{n} \sum_{k=1}^{m} a_{kj} \quad (1)$$

and its (uncorrected) variance (Lande 1996) as

$$\text{SV}_j = \frac{1}{n} \sum_{i=1}^{n} (y_{ij} - p_{+j})^2 = p_{+j}(1 - p_{+j}). \quad (2)$$

Furthermore, the most popular diversity indices can be directly computed as a weighted sum of the SV_j values over the s observed number of species:

$$\text{TD} = \sum_{j=1}^{s} w_j \text{SV}_j. \quad (3)$$

Indeed, taking the weighting function w_j equal to one, whatever the species, means quantifying the total diversity of the taxonomic relevé via the Simpson index, whereas taking $w_j = 1/p_{+j}$ or $w_j = \log(1/p_{+j})/(1 - p_{+j})$ means relying on total species richness (minus 1, i.e., $s - 1$) or on the Shannon index, respectively (Pélissier et al. 2003). In the following discussion we refer to these three measures of species diversity as the usual diversity metrics, while additional metrics are thinkable from other definitions of w_j.

Lessons learned.—(1) Contrary to TR, but in accordance with LBP, we believe that a first-level analysis consists in characterizing the within-site/community diversity, regardless at this point whether there is or not overlap in species composition (i.e., shared species) between different sites or communities. (2) A simple general expression of the within-community diversity, which encompasses the most popular diversity indices, is

the generalized, multivariate variance given by TD (Eq. 3). (3) When the taxonomic relevé is limited to a single sampling site, TD measures alpha diversity of that site; when the taxonomic relevé is comprised of individuals encountered in a set of sampling sites distributed over an ecological region, TD measures gamma diversity.

Second level, explaining among-communities variation

Can the spatial variation in the abundance of a given species or the variation in community composition, i.e., in the abundances of all the species that form a community at a time, be explained by variation in environmental characteristics and/or geographical location? These ecological questions raised by TR (pp. 2698–2699) are, with respect to both theirs and LBP nomenclature, level-two questions to be addressed via the raw-data approach, i.e., using canonical analysis sensu Legendre and Legendre (1998). While we fully agree with this idea, we have to remember that canonical analysis is a two-step process, which involves a multiple linear regression, followed by principal component decomposition (Legendre and Legendre 1998). Hence, the above ecological questions are first and foremost specified as a general multivariate linear model equation (the first step of the canonical analysis [Pélissier et al. 2003, Pélissier and Couteron 2007]), for which we believe that our first-level individual $\times$ species-occurrence matrix, $\mathbf{Y}$, introduced in the previous section, is a much more appropriate "response variable" than the classical site $\times$ species abundance matrix, $\mathbf{A}$, as in TR and LBP. Associated to any form of linear model is of course an additive scheme of variance partitioning (Lebart et al. 1997:228), advocated in a spatially explicit context by LBP (pp. 440–441).

Taking matrix $\mathbf{Y}$ ($n \times s$) as the response variable and introducing $\mathbf{X}$ ($n \times q$) a matrix of dummy variables coding for habitat types as the "explanatory variable," it can be demonstrated that TD, the total variance in species identity among the n individuals of the community (Eq. 3) partitions into an explained or among-habitat component (TDA) and an unexplained residual or within-habitat component (TDW) (Couteron and Pélissier 2004), so that our first-step linear model enters within the additive diversity partitioning framework proposed by Lande (1996). This establishes a clear analytical relationship between our first and second levels of abstractions, which is holding for any usual diversity metric provided that the appropriate choice of the weighting function w_j is made:

$$\text{TD} = \text{TDW} + \text{TDA} = \sum_{j=1}^{s} w_j \text{SVW}_j + \sum_{j=1}^{s} w_j \text{SVA}_j. \quad (4)$$

To be more specific, we can denote by n_k the number of observations in habitat k, with

$$n = \sum_{k=1}^{m} n_k$$

and by $p_{kj} = a_{kj}/n_k$ the relative frequency of species j in habitat k, a_{kj} being as above the abundance of species j in habitat k. The approximation of $\mathbf{Y}$ by multiple linear regression on the variables contained in $\mathbf{X}$ is $\hat{y}_{ij}(k) = p_{kj}$ (Pélissier et al. 2003), from which we can derive explicit formulas for SVW_j, the contribution of a given species j to TDW, the mean within-habitat diversity (or alpha diversity as defined in the previous section), and for SVA_j, which is the contribution of j to TDA, the among-habitat diversity (or "between-habitat" diversity, an expression used as a synonymous for beta diversity by Whittaker [1972:230]). Namely

$$SVW_j = \sum_{k=1}^{m} \frac{n_k}{n} \cdot p_{kj}(1 - p_{kj}) \qquad (5)$$

$$SVA_j = \sum_{k=1}^{m} \frac{n_k}{n} \cdot (p_{kj} - p_{+j})^2. \qquad (6)$$

Couteron and Pélissier (2004) also provided explicit formulas for SVW_j and SVA_j for nested partitions (i.e., for subsequent partitions of SVA_j among habitat types and sampling locations), as well as guidelines on the nonparametric testing of statistical significance based on randomization procedures. The decomposition framework lends itself to tests based on random shifting procedures (as introduced by Harms et al. 2001), which upon availability of fully mapped or regularly sampled data are preferable for distinguishing between habitat effects and clumping effects unrelated to habitat. Moreover, in this well-established framework, which is encompassed by multivariate analysis of variance (MANOVA sensu Anderson 2001), multilevel hierarchical analyses are more straightforward than the approach proposed by Crist et al. (2003). MANOVA is indeed very general and applies either to questions about individual species' habitat preferences (testable via SV_j) or to variation in community composition (analyzable through TD).

Lessons learned.—(1) Contrary to TR statement, a direct relationship between alpha and beta diversity can be expressed through a simple general linear model that leads from the first to the second level of abstraction; consequently, it is only when gamma diversity is ignored that alpha diversity tells nothing about beta diversity. (2) The complementary nature of alpha and beta components of diversity established by Whittaker's work has long been hidden because authors have quantified alpha diversity by indices (e.g., Fisher's alpha, Shannon and Simpson indices) that have no direct connection with dissimilarity measures used to quantify beta diversity (e.g., Jaccard, Sorensen, and Steinhaus indices). (3) Our model is closely related to the linear model that underlies

classical canonical analysis; but using as the response variable, the individual species-occurrence matrix, $\mathbf{Y}$, in lieu of the site species abundance matrix, $\mathbf{A}$, is the only way to relate the raw-data approach to gamma diversity, via its natural, additive apportionment into a part explained (beta diversity) and a part unexplained (alpha diversity) by variation in environmental conditions (second level of abstraction). (3) Standard routines derived from MANOVA as well as nonparametric tests of statistical significance, which can be based either on randomization or random shifting procedures, are available to conduct these analyses.

Second level, the "raw-data" approach

In the previous section, we focused on the variance (diversity) partitioning scheme associated to the generalized linear models. We now turn to the one associated to the principal component decomposition, which is the core subject of multivariate analysis, including the canonical raw-data approach as referred to by LBP and TR. One can indeed recognize in

$$TDA = \sum_{j=1}^{s} w_j \cdot \sum_{k=1}^{m} \frac{n_k}{n} \cdot (p_{kj} - p_{+j})^2$$

(Eq. 4 and 6) an expression of the total inertia or total variance (i.e., the sum of all eigenvalues) of the correspondence analysis (CA; Legendre and Legendre 1998) of the site × species abundance matrix $\mathbf{A}$ when $w_j = 1/p_{+j}$, and non-symmetric correspondence analysis (NSCA; Gimaret-Carpentier et al. 1998) of $\mathbf{A}$ when $w_j = 1$. Taking $w_j = \log(1/p_{+j})/(1 - p_{+j})$ also leads to a form of column weighted correspondence analysis whose inertia is consistent with Shannon diversity (see the proofs in Pélissier et al. 2003). Total inertia demonstrated by the site × species abundance matrix, $\mathbf{A}$, is therefore the part of total community diversity (TD) explained by the dummy variables that partition the individual × species-occurrence matrix, $\mathbf{Y}$, into sampling sites. This quantifies between-site beta diversity, expressed consistently with any of the three usual diversity metrics using the species weighting function, w_j.

It follows that two-table variants of ordination methods such as CCA (canonical correspondence analysis) or RDA (redundancy analysis), whose particular forms can be made compatible with the usual diversity metrics (see Couteron and Ollier 2005), realize a first-stage additive partition of TDA into "explained" and residual "unexplained" portions by a set of environmental descriptors, before permitting a subsequent additive decomposition of either the explained or the residual fraction into canonical ordination axes (constrained vs. unconstrained ordinations, respectively).

Lessons learned.—(1) The so-called "raw-data approach" is directly related to the additive partitioning

framework of species diversity proposed by Lande (1996), a fact which is completely absent in LBP and TR. (2) It follows that ordination techniques provide subsequent apportionment of *TDA* according to principal axes, in a way that can be made consistent with the usual diversity metrics. (3) Thus, canonical partitioning in the classical sense of Legendre and Legendre (1998) refers to total inertia (or variance) of the site × species abundance table, **A**, i.e., to the among-site beta diversity (TDA), which is the only part of community total diversity, TD, accounted for by the sampling design. (4) Explained or unexplained portions of TDA relative to a set of environmental descriptors can subsequently be additively partitioned into canonical vs. partial canonical ordination axes.

From the "raw-data" approach to distance-based analyses

According to TR (p. 2697, 2703, 2705), the fact that beta diversity can be viewed as a distance (or more generally a dissimilarity) is the main justification for using the Mantel approach and to move from second to third level of abstraction, where the response variable is a dissimilarity matrix between pairs of sites. That beta diversity is usually quantified via dissimilarity indices, is used however to instill the misleading idea that it is not conceivable to consider "the variation in community composition, i.e. beta diversity" in the light of the geographic locations of the sites or of the inter-site distances. Though it is not blatantly stated as such, this idea is conveyed in many places of the paper, by rhetoric tricks or omissions. For instance, in Fig. 2, it is as if an analysis of inter-community or inter-site geographic distance, could not be used to explain variation in community composition. Why should using the inter-site geographic distance as an explanatory variable automatically mean skipping to an analysis of the variation in variation in community composition, i.e., to a third-level question? There is absolutely no compelling reason to do so, since several alternatives are possible.

In fact, TDA, which is a variance according to our definition, can be rewritten as a sum of intersite dissimilarities, and directly broken down into additive portions relating to classes of inter-site distance. Indeed, a classical result of variance decomposition (in its broader meaning) is that averaging squared departures around a mean value is equivalent to averaging squared differences (i.e., distances) between individual observations (see for instance Anderson 2001). It follows that the contribution of species j to the among-site beta diversity, SVA_j (Eq. 6), can be rewritten as

$$SVA_j = \sum_{k=1}^{m}\sum_{k'=1}^{m}SVA_j(k,k') = \frac{1}{2n^2}\sum_{k=1}^{m}\sum_{k'=1}^{m}n_k n_{k'}(p_{kj}-p_{k'j})^2.$$

(7)

At the multispecies level,

$$SVA(k,k') = \sum_{j=1}^{s} w_j SVA_j(k,k')$$

is a measure of dissimilarity between composition in sites k and k' (it is in fact a mathematical distance), which may be made fully consistent, through w_j, with any of the three usual diversity indices. Summing $SVA(k,k')$ values for all (k,k') pairs of sites yields the among-site beta diversity:

$$TDA = \sum_{k=1}^{m}\sum_{k'=1}^{m}SVA(k,k').$$

A distance-dependent partition of TDA follows from the dissimilarity measure defined above. As soon as a set $\{H_h\}$ of nonoverlapping distance classes is defined, the portion of the total among-site beta diversity relating to a given distance class centered on h is

$$TDA(h) = \sum_{d(k,k')\in H_h} SVA(k,k')$$

$$= \sum_{d(k,k')\in H_h}\sum_{j=1}^{s} w_j SVA_j(k,k'). \quad (8)$$

When the union of H_h contains the range of intersample distances, we logically derive the following from the above expression:

$$TDA = \sum_h TDA(h).$$

Alternatively, standardizing TDA(h) for the number of plots and individuals (see Couteron and Pélissier [2004] for details) in each distance class provides a dissimilarity variogram or, equivalently, a generalized, multivariate variogram, which can be plotted as a function of the intersite geographical distance and tested against the null hypothesis of an absence of spatial structure by randomly reallocating the taxonomic compositions among the sampling sites (Wagner 2004).

We note that TR (p. 2701) mention the variogram in a way that seems to involve the computation of a variance of intersite dissimilarity, i.e., in this case a variance of variance, and thus a third-level object. But according to the usual definition, the variogram expresses how intersite dissimilarity changes with distance by directly apportioning the overall variance of the response variable with respect to distance classes and dividing by the number of pairs of sites in each class. This does not involve the computation of a variance of intersite dissimilarity, and it is therefore not at all congruent with their definition of third-level analysis.

The principle of multivariate variography (sensu Wackernagel 1998) can be applied not only to TDA, but also to any partition of TDA into principal/canonical axes yielded by a given single- or two-table

ordination method (see Couteron and Ollier 2005 for an illustration). Hence, combining these different principles constitutes a very rich and flexible yet largely unexplored framework for comparing the effect of environmental variables on the observed relationship between beta diversity and intersite distance. Moreover, in our model, the part of total community diversity explained by external explanatory variables, ordination axes, or distance classes is quantified in the same measurement unit (one of the three usual diversity metrics, albeit this is not an absolute restriction), so that their relative proportions of explained total community diversity can be directly compared. This ultimately allows one to measure the respective influence of environment and space on diversity variation.

Lessons learned.—(1) TR seems to have missed that the among-site beta diversity can be seen as a sum of dissimilarities between all pairs of sites; such dissimilarities can be expressed in any of the three usual diversity metrics. (2) TDA is therefore amenable to an additive apportionment with respect to classes of intersite geographical distance, which can be combined in many ways using the potential of ordination methods, thereby reviving the concept of multiscale ordination (MSO; sensu Ver Hoef and Glenn-Lewin 1989, Wagner 2003, 2004); MSO is a straightforward extension of the raw-data approach. (3) Statistical tests of significance for the existence of nonrandom spatial structure exist and can also be applied to residual patterns after factoring out the effect of environmental descriptors; for instance, the absence of any significant residual spatial pattern would mean that dispersal limitation is probably not a pervasive factor in the communities under study.

Third-level analyses: for what and how?

In the previous points, we have gradually shifted from a raw-data to a distance-based analysis framework. The transition has been smooth because both frameworks rely on additive partitions of variance and covariance (used here in their generalized meaning; see Couteron and Ollier [2005]). Our method of carrying out distance-based analyses nevertheless does not pertain to the third level of abstraction as defined by TR.

We have not considered the variance among intersite beta-diversity values, which is a variance of variance, whereas TDA is simply a generalized variance that allows weighting of either sites or species. Nor have we tried to model individual intersite beta-diversity values from either environmental or spatial variables, which is the goal of multiple regression on distance matrices as presented by Duivenvoorden et al. (2002) and Tuomisto et al. (2003). This clearly demonstrates that reference to third level analyses is not a prerequisite for investigations into how beta diversity may be influenced by environment discrepancies and/or intersite distance.

There is thus no reason to share the opinion of TR that distance-dependent ecological hypotheses, such as the neutral theory of biodiversity, can only be tested using the Mantel approach, although we agree that canonical partitioning, which is the core of what is usually meant by the raw-data approach, is by itself not sufficient to address such hypotheses. In fact, the framework for distance-based analyses, which we have briefly summarized above, permits one to investigate and test any distance-based ecological hypothesis, including the neutral ones. For instance, based on theoretical results borrowed from population genetics, Chave and Leigh (2002) and Etienne (2005) featured explicit predictions under neutrality with respect to Simpson's intersite beta diversity (or to the closely related intersite similarity function), which may be used in the near future to derive analytical expectations of the multivariate variogram under neutral assumptions. It is hence not yet established that reference to the third level of abstraction will, by itself, permit more efficient testing of distance-based ecological hypotheses than the framework described in *From the "raw-data" approach to distance-based analyses.*

The first level of abstraction basically computes alpha diversity from a vector of species abundances. The second level deals with variation in abundances observed by a particular survey, which means comparing the abundance vectors making the site × species table or, equivalently, submitting this table to eigenanalysis and/or canonical partitioning (the raw-data approach). The objective of the third level should not be defined only from comparison of distance matrices via a Mantel test, since its broader objective is ultimately to compare diversity patterns found in distinct surveys, hence to compare the structures present in several site × species tables. Indeed, if surveys share either sites (e.g., diachronic relevés) or species (at least most of them), there are appealing alternatives to distance-matrix comparisons (Dray et al. 2003, Thioulouse et al. 2004), which probably use more of the available initial information of the set of site × species tables. It is only when surveys share neither sites nor species (e.g., different ecological regions) that comparisons of distance matrices and Mantel tests may justify themselves. Compared to the broad panel of aims and data types characterizing ecological investigations, these are in fact far more restrictive circumstances for using the Mantel approach than it may appear from reading TR.

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ANALYZING OR EXPLAINING BETA DIVERSITY? COMMENT

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Tuomisto and Ruokolainen (2006; hereafter referred to as TR) have recently argued that there has been confusion about what statistical approaches, "raw data" or "distance," are more appropriate when testing hypotheses about the origin and maintenance of beta diversity. They also argued that "inconsistencies and errors in [the] recommendations" of Legendre et al. (2005; hereafter referred to as LBP) gave way to more confusion on this issue. Essentially, TR stated that both the raw-data and distance approaches were appropriate, but targeted different predictions and should therefore be seen as complementary. However, TR's method of variation partitioning on distance matrices is based on an inaccurate definition of spatial autocorrelation, which makes the "spatial" fraction meaningless. Consequently, that method is unable to quantify the relative contribution of neutral processes to beta diversity. In any case, TR have provided no answer to the doubts expressed by LBP over the mathematical validity of variation partitioning on distance matrices, and simply claimed that as their method targeted a "different

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response variable" than the raw-data approach, its use was justified. Finally, the recommendation of TR that the distance approach is the only appropriate approach for testing Hubbell's (2001) neutral theory is incorrect. Here I will discuss these issues in more detail.

What is spatial autocorrelation?

In their *Ecological vs. statistical hypotheses* section (p. 2703), TR describe the predictions of the neutral model as follows:

Community composition is heterogeneous over the landscape at all spatial scales as a result of the cumulative effects of spatially autocorrelated random walk in species abundances. This spatial structure is entirely due to autocorrelation, and spatial dependence on underlying environmental variables is not present.

While their description of the neutral model is accurate, the statistical prediction they derive from it is that (p. 2703):

From [the neutral] hypothesis (…) it follows that two nearby sites should share more species in more similar abundances than two sites further apart.

That statistical prediction is inaccurate because spatial autocorrelation is not defined as the tendency of two nearby sites to be more similar than faraway sites (which would imply a simple monotonic decrease of similarity with increasing geographic distance), but is instead defined as "the property of random variables taking values, at pairs of locations a certain distance apart, that are more similar (positive autocorrelation) or less similar (negative autocorrelation) *than expected for randomly associated pairs of observations*" (Legendre 1993). A more formal and mathematically satisfying definition of spatial autocorrelation is "the lack of independence […] among the error components of field data, due to geographic proximity" (Legendre and Legendre 1998:9).

This distinction may appear trivial, yet it has important implications in the present debate. Even though random neutral processes may create spatial autocorrelation in the vegetation data and lead to a monotonic decrease in similarity (or conversely, to an increase in dissimilarity) with increasing geographic distance when there is species turnover (i.e., replacement) across a sampled transect or surface, this is not necessarily so, for instance, in the case of ubiquitous species. Simply put, there is no clear and unambiguous link between spatial autocorrelation and similarity decay with distance. Consequently, regressing community composition dissimilarity on geographical distances (log-transformed or not) to *quantify the contribution* (using R^2) of neutral processes to variation of beta diversity between pairs of sites, which is the goal of TR's method of variation partitioning on distance matrices, is fundamentally incorrect. While such a regression is often

used in similarity decay plots to fit a particular model (Nekola and White 1999), the coefficient of determination (R^2) should be interpreted as nothing more than a simple measure of the adjustment of that model to the data.

It is important here to distinguish two research questions: The objective of variation partitioning (either in the raw-data approach or in TR's variation partitioning on distance matrices) is not to quantify the *strength* of spatial autocorrelation in the data (which can be calculated through Mantel correlograms), but to quantify its *relative contribution* to the overall pattern. There is no link between the strength of spatial autocorrelation and its relative contribution to beta diversity. For example, spatial autocorrelation can be small (i.e., low Mantel r in an autocorrelogram) yet explain most of the variation of community composition if all of this variation is spatially structured and there is no dependence on environmental variables. In any case, the R^2 of a logarithmic similarity-decay curve is neither related to the strength or relative contribution of spatial autocorrelation to beta diversity.

Neutral theory and similarity decay plots

TR's statistical prediction to test the neutral model in the context of variation partitioning on distance matrices appears to stem from a direct, yet unfounded, extension of Hubbell's (2001) use of similarity decay plots (Nekola and White 1999) to test neutral theory. In chapter seven of his seminal monograph, Hubbell predicted that under neutral ecological drift community composition similarity *across the landscape* will decrease logarithmically with geographical distance, because at such scales dispersal limitation leads to clumped species distributions, and therefore to high species turnover.

The similarity decay with distance is greatly influenced by grain size (i.e., resolution) and spatial extent (i.e., area), with the best relationships observed with large grain sizes and spatial extents (Nekola and White 1999). Indeed, a decay of similarity will be detected only if the variation due to grain size is smaller than the variation due to spatial extent (Nekola and White 1999), a condition rarely met from censuses conducted at local scales. As such, Hubbell used similarity decay plots to make predictions about the importance of neutrality on beta diversity only at broad spatial scales (i.e., biogeographical scales), and these predictions are based on *the functional form of the decay curve*, not through variation partitioning between geographical and environmental distances. Hubbell argued that, since neutral theory predicts that similarity decay happens on environmentally homogeneous landscapes, the decay curve should be smooth (i.e., logarithmic) and only depend on the fundamental biodiversity number θ and dispersal rate m (Hubbell 2001). On the other hand, under niche-assembly theory, similarity decay results from species
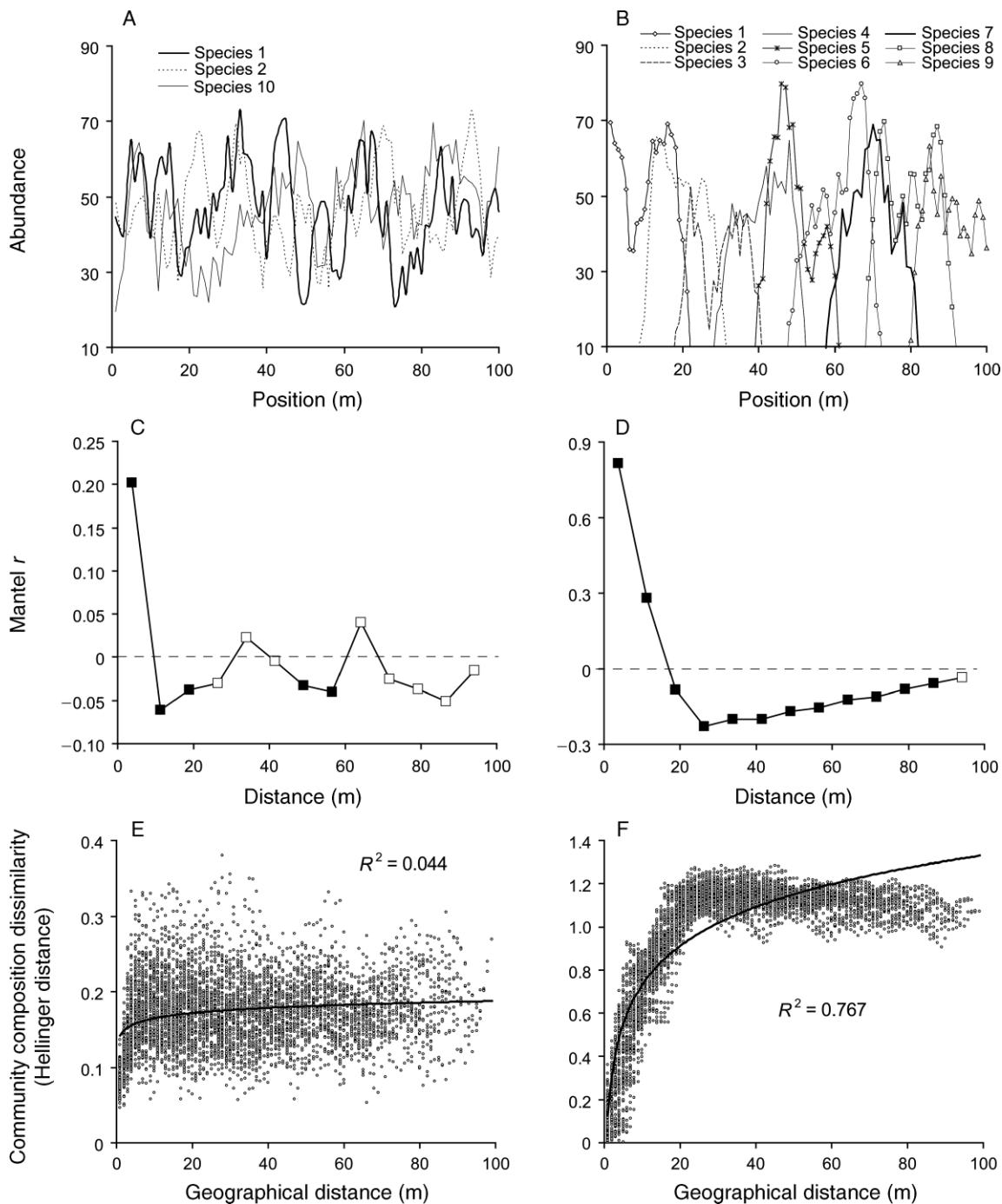
Fig. 1. Two cases of neutral communities. (A) Abundances of 10 species along a 100-m transect; only three species are shown on the graph for clarity. Data for each species were generated from a series of random numbers (one every meter) between 0 and 100 taken from a standard normal distribution, to which spatial autocorrelation was added by computing moving averages (window width = 5, i.e., the value plus the two neighbors on either side). (B) Abundances of 10 species along a 100-m transect; only the first nine species are shown. Data for each species were generated the same way as in panel (A), with the exception that species turnover along the transect was added by restricting the first nine species to limited but overlapping parts of the transect. (C) Mantel correlogram associated with panel (A). Hellinger distance was used for calculating community composition dissimilarity. Black squares indicate significant spatial autocorrelation after progressive Bonferroni correction ($\alpha = 0.05$, 999 permutations). Positive Mantel r values express positive spatial autocorrelation. (D) Mantel correlogram associated with panel (B); see description of panel (C) for explanation. (E) Relationship between community composition dissimilarity (Hellinger distance) and geographical

turnover along environmental gradients or habitats; the associated similarity decay will not be smooth because habitats are typically patchy, recurrent, and have sharp boundaries (Hubbell 2001).

This neutral prediction should be restricted to the interpretation of broad-scale patterns in similarity decay plots, yet TR erroneously extend it to all spatial scales and use a matrix of log-transformed geographical distances to quantify the contribution of neutral processes in variation partitioning on distance matrices from any sampling design, regardless of grain size and spatial extent. Such a method, on top of being mathematically doubtful, can greatly underestimate the importance of neutral processes when many or all species are ubiquitous, which can often happen at local spatial scales or in species-poor systems. Again, this is because there is no clear link between spatial autocorrelation and distance decay of similarity. I will show this through the simple following numerical example.

A simple numerical example

Let us imagine a transect where environmental conditions are completely homogeneous throughout, so that no variation in community composition can be attributed to environmental control. Ten species are found along the 100-m transect (note that it could very well be 100 mm, cm, or km), but in two different arrangements (Fig. 1A, B; for clarity, not all species are shown on the graphs). In Fig. 1A, abundance data were generated from a series a 100 random numbers between 0 and 100 taken from a standard normal distribution, from which spatial autocorrelation was added by computing moving averages (window width = 5, i.e., the value plus the two neighbors on either side). In Fig. 1B, the exact same procedure was followed, with the exception that species turnover was added by restricting the first nine species to limited but overlapping parts of the transect. Thus, in both cases, variation of community composition is entirely due to random but spatially autocorrelated walks in species abundances, a purely neutral process. As it can be seen from Mantel correlograms (Fig. 1C, D), there is significant spatial autocorrelation in community composition at several distance classes in both cases.

This simple numerical example shows that when spatial autocorrelation leads to gradual species turnover (Fig. 1B), which is frequently observed at broader spatial scales, a good relationship between community composition dissimilarity and geographical distance (the inverse of a similarity decay plot) can be found (Fig. 1F).

Conversely, when species are ubiquitous (Fig. 1A), such a relationship is very much weaker (Fig. 1E).

By extending this to the context of variation partitioning, as TR suggest, one would partition the variation of beta diversity between pairs of sites (i.e., the response matrix) between a matrix of environmental distances (representing the environmental control model) and a matrix of log-transformed geographical distances (representing the neutral model). I must stress that I do not support the use of this method given that serious doubts have been expressed over its mathematical validity. Indeed, perhaps the main problem with variation partitioning on distances matrices is that the isolated fractions are not additive. Surprisingly, TR appear to be aware of this fact, as they mention (p. 2707) that in this method, "R^2 values will change depending on [...] whether all environmental variables are combined into a single distance matrix or used in separate matrices." This seriously undermines the credibility of the method itself, as it is very much unclear how the R^2 coefficients should be interpreted if the fractions themselves are not additive. Another problem concerns the potential, albeit unknown, effects of the lack of independence among the distances on the coefficients themselves (Legendre et al. 2005:442). Still, I will assume here that the method is valid (which clearly remains to be shown) and use it nonetheless to illustrate that on top of being doubtful, this method also greatly underestimates the contribution of neutral processes.

In that numerical example, environmental conditions are identical throughout the transect, so the environmental matrix would be filled with constant values and would explain none of the variation of beta diversity. Therefore, the contribution of neutral processes to variation of beta diversity, as suggested by TR, would then simply be expressed by the coefficients of determination of the logarithmic relationships shown in Fig. 1E, F. This would lead one to conclude that in Fig. 1A, <5% (taken from the R^2 of the logarithmic model) of the observed pattern was due to neutrality, whereas in Fig. 1B, ~77% of the pattern would be attributed to neutral processes. Such conclusions are obviously inaccurate given that, in both cases, patterns were entirely due to random, spatially autocorrelated walks in abundances, a purely neutral process. Here it is clear that using a matrix of log-transformed geographical distances to quantify the contribution of neutral processes can greatly underestimate their actual importance, particularly when most or all species are ubiquitous (e.g., Fig. 1A). Again, this is because spatial autocorrelation does not necessarily imply, for multi-species data, that two

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← distance (i.e., inverse of a similarity-decay plot) from the data of panel (A). The curve shows the logarithmic relationship with its R^2 value. (F) Relationship between community composition dissimilarity (Hellinger distance) and geographical distance from the data of panel (B); see description of panel (E) for explanation.

nearby sites will be more similar (multivariate similarity) than two faraway sites, as suggested by TR's statistical prediction. In other words, TR use an inaccurate statistical prediction to quantify the contribution of neutral processes to variation of beta diversity, and to test that prediction they propose and use a doubtful and unvalidated statistical method.

Variation of beta diversity between pairs of sites vs. between regions

TR argued that the distance approach targeted different kinds of questions about beta diversity than the raw-data approach, which they referred to as different "levels of abstraction." LBP had also used this "level of abstraction" concept and mentioned that one could either be interested in studying the variation of community composition among sites within a given region (i.e., beta diversity), or in studying the variation of beta diversity among groups of sites or regions. However, variation partitioning on distance matrices, as proposed by TR, focuses strictly on the variation of dissimilarities *between pairs of sites*. Individual pairs of dissimilarities taken alone do not give a measure of beta diversity of a large area (Anderson et al. 2006). Therefore, this method could not answer such questions as: "Does beta diversity differ between different groups of sites (i.e., that contain more than two sites) or regions?" And, more importantly, "Why does beta diversity vary between these groups of sites or regions?" Hence, even if variation partitioning on distance matrices could accurately quantify the contribution of spatial autocorrelation to the variation of beta diversity among pairs of sites (which, as I have shown earlier, is not the case), and even if its mathematical validity were demonstrated (which remains to be done), it would still be of limited practical use: Indeed, most ecologists that are truly interested in studying the variation of beta diversity among groups of sites or regions would likely want to compare regions in which more than two sites have been observed. A more appropriate way of answering questions related to the variation of beta diversity among groups of sites or regions would be to use multivariate dispersion on distance matrices, a method described by Anderson et al. (2006) to specifically answer such questions.

Testing neutral theory: raw data or distances?

One of the main conclusions of TR was that Hubbell's (2001) neutral theory can only be tested using the distance approach since its testable predictions are stated in terms of distances and not raw data. Their main argument against the use of the raw-data approach to test neutral theory was that the detection of a particular spatial pattern in community composition through spatial modeling techniques such as principal coordinate analysis of neighbor matrices (PCNM;

Borcard and Legendre 2002) does not support neutral theory because neutral theory does not predict that this was the expected spatial pattern, and that any specific spatial pattern is just as much in accordance with the neutral model as long as the degree of spatial autocorrelation is similar. I see no contradiction here. I argue that the detection of a significant *residual spatial structure* (i.e., after controlling for variation due to the environmental variables) provides support for the theory. This detection is quite easy using the raw-data approach, unless TR can demonstrate that the neutral model specifies a type of spatial autocorrelation that cannot be modelled by PCNM analysis. Given that previous simulation work has shown that PCNM analysis could accurately model a wide range of spatial structures, including spatially autocorrelated data (Borcard and Legendre 2002), such a demonstration appears unlikely.

The PCNM approach is closely related to spatial autocorrelation structure functions, and essentially consists in extracting from a predetermined spatial matrix the eigenvectors that maximize Moran's index of spatial autocorrelation (I); the resulting eigenvectors describe global to local spatial structures and can thus be used in regression to model spatial structures at all spatial scales (Dray et al. 2006). Therefore, the raw-data approach with PCNM uses explanatory variables that can model spatially autocorrelated patterns across a range of scales, and thus allows an accurate quantitative assessment of the contribution of spatial autocorrelation to variation in community composition. This is the exact opposite conclusion of TR, who argued that "the raw-data approach fails to address the neutral model in a relevant way, and is unable either to falsify the neutral hypothesis or to quantify its relative contribution to the observed spatial pattern" (p. 2704).

Although both the raw-data and the distance approach can be used to test neutral theory, they both have their respective domains of application. The raw-data approach with PCNM has promising applications, since it allows one to dissect the spatial structures of community composition at different scales (Borcard and Legendre 2002) and estimate the relative influence of niche and neutral processes at each of these scales. Such tests offer great opportunities for future tests of neutral theory (McGill et al. 2006), especially considering that spatial scale has been suggested as a way to reconcile empirical ecology with neutral models (Holyoak and Loreau 2006).

Still, as TR pointed out, a drawback of the raw-data approach is that it can sometimes be hard to distinguish between the relative importance of niche and neutral processes on community patterns because spatial and environmental variables often covary. The resulting "space-environment" fraction can either be interpreted as a spatially structured environmental influence con-

trolling abundance patterns or as neutral processes acting within a heterogeneous environment (Bell et al. 2006). One way of minimizing that problem is to use a sampling design that decouples the environmental and geographical distances (Gilbert and Lechowicz 2004). The "pure spatial" fraction may often be due to some spatially structured unmeasured environmental variables, which can then lead to an overestimation of the contribution of neutral processes. Hence, to use the raw-data approach to test neutral theory, one must have access to relevant, extensive, and accurately quantified environmental data.

As for the distance approach, the use of similarity decay plots is appropriate for identifying the best functional form of a decay curve in a similarity-decay plot: A smooth similarity-decay curve provides greater support for neutral theory, whereas a "bumpy" curve suggests that the environment exerts stronger control over beta diversity (Hubbell 2001). A drawback of this approach is that it only allows a qualitative assessment of the dominant process (i.e., niche or neutrality), yet does not allow one to quantify their relative importance. Still, as this method does not require environmental data, it can be particularly interesting when these are not available.

Conclusion

Research on the origin and maintenance of beta diversity has regained great attention since the publication of Hubbell's theory. Much theoretical and empirical work is currently under way to assess the relative importance of niche and neutral processes on community patterns. On the applied side, understanding the origin and maintenance of beta diversity has important implications for ecosystem management, such as the design of nature reserves. Therefore, it is crucial that researchers master the concepts and methods required for testing hypotheses about how beta diversity is maintained in ecosystems.

Throughout this comment I have stressed that an abusive interpretation of the relationship between the decay of similarity and spatial autocorrelation, as well as an unfounded use of Mantel R^2 values in the context of variation partitioning, both proposed by TR, should be avoided. Variation partitioning on distance matrices, in addition to being mathematically doubtful and yet unvalidated, is based on an inaccurate statistical prediction to quantify the contribution of neutral processes to variation of beta diversity.

The distance approach is appropriate for identifying the best functional form of the similarity decay curve in similarity decay plots. The raw-data approach, on the other hand, is appropriate to partition the variation of community composition between environmental and spatial factors and can accurately quantify the contribution of spatial autocorrelation to variation of community composition among sites. In summary, contrary to TR, who argued that only the distance approach could be used to test neutral theory, both the raw-data and the distance approaches are useful in testing different neutral predictions about the origin and maintenance of beta diversity. Yet, they both have their domains of application and can thus be seen as complementary.

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ANALYZING OR EXPLAINING BETA DIVERSITY? COMMENT

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In 2005, we published a paper (Legendre et al. 2005; hereafter referred to as LBP) explaining the ecological and statistical basis for the analysis of the variation in species composition among sites (this is one operational definition of beta diversity), a subject of great importance for the understanding of the generation and maintenance of beta diversity and the establishment of ecologically sound ecosystem conservation policies. Tuomisto and Ruokolainen (2006) (hereafter referred to as TR) pointed out several issues in our 2005 paper. As a response, this note seeks to clarify the issues behind this contention.

Our major point of disagreement concerns the links between (1) the ecological predictions derived from neutral theories of beta diversity, (2) the statistical hypotheses derived from these predictions, and (3) the statistical methods used to test these hypotheses. In LBP, TR, and the present *Comment*, distance-based methods refer to statistical methods where the geographic relationships among sites are represented by a distance matrix. These methods include the Mantel test and the derived method of regression on distance matrices. The raw-data approach refers to multiple regression and canonical analysis, where the spatial relationships among sites are represented by a rectangular table containing geographic coordinates, a polynomial of the geographic coordinates, principal coordinates of a neighbor matrix (PCNM) (Borcard and Legendre 2002), or derived forms.

The purpose of this *Comment* is to show that (1) some predictions of Hubbell's neutral theory, especially the presence of positive autocorrelation, can be stated and tested using the raw-data approach, (2) the distance approach as proposed by TR (partitioning on distance matrices) is statistically flawed, and (3) when a raw-data hypothesis is translated into distances, the corresponding statistical test lacks power; therefore, whenever a hypothesis can be formulated in terms of raw data

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instead of distances, it should be tested using the raw-data approach.

TR (p. 2698) devoted a large section to three levels of abstraction that were proposed and defined in LBP (pp. 437–438). Level-1 questions concern alpha diversity, or the variation in the species identity of organisms at individual sites. For example: What are the soil conditions associated with the presence and identity of 10 tree species in a 1-ha plot of temperate mixed-wood forest? Level-1 questions are irrelevant for the present *Comment*. For levels 2 and 3, which are the concern of LBP and TR, the questions asked are the same in the two papers. Level 2 concerns the variation in community composition among sites in a region of interest, which is beta diversity (community composition encompasses species composition and species abundances); canonical variation partitioning is adequate to address questions pertaining to that level (LBP, p. 438; and TR, p. 2705). For example: Is the variation in community composition among sites due to variation in environmental conditions or to neutral community dynamics? Level-3 questions concern the variation in beta diversity among groups of sites; questions related to that level may be addressed by the distance-based approach (LBP, p. 438; and TR, p. 2705), provided that the technical and statistical uncertainties raised below are settled; see also point 2 in the section *Other points*. Note that TR restrict the level-3 questions to pairs of sites, whereas LBP use level-3 questions to investigate the differences among larger groups of sites; for example, adjacent geographic regions with several sites in each. For example: The public garden in town A is surrounded by identical flower beds, each one containing seven species. The beta diversity (variation in species composition among beds) in that garden is zero. In town B, the public garden was designed with flower beds that are all different in species compositions. Beta diversity is high among the flower beds. One may wonder why there is such a big difference in flower bed composition between the two towns. Is it cultural? Financial? Or is it due to the limited availability of suitable flowers in town A due to soil or climate? This question does not focus on the identities of the flower species, but on the variance in community composition between the two towns: Are the multispecies dispersion matrices homogeneous when comparing the two towns, and if not, why? This would be a level-3 question.

Spatial autocorrelation in community composition data

"We argue that S. P. Hubbell's neutral theory can only be tested using the distance approach, because its testable predictions are stated in terms of distances, not in terms of raw data" (TR, *Abstract*: details on p. 2703). The section *Can we test Hubbell's neutral theory using the raw-data approach?* will show that spatial autocorrelation can and should be tested using the raw-data

approach. Several other aspects and predictions of neutral theory can also be tested without recourse to distance matrices; see McGill et al. (2006) for a recent review. Here are a few examples. First, spatial variance can be used to test neutrality: Under random walk in species abundances ("ecological drift" sensu Hubbell 2001), variance is expected to increase with time (Clark and McLachlan 2003). Second, the compensatory dynamics hypothesis of neutral theory can be tested by assessing the statistical hypothesis that species covary negatively within communities; Houlahan et al. (2007) tested this hypothesis on 41 natural communities. Third, neutrality can be tested by an evolutionary approach, by studying extinction rates and the ages of species (Ricklefs 2006). Finally, the relative importance of environmental control and neutrality can be tested using variation partitioning by canonical analysis, as shown in LBP.

Tests of autocorrelation in the distance world.—Hubbell's neutral theory predicts the presence of positive autocorrelation in community composition due to dispersal limitation. In this section and the next, we will show that spatial autocorrelation can be tested both in the distance and raw-data worlds, but that the raw-data approach is more powerful and has better-known statistical properties. Let us review the distance approach first.

The procedure proposed by Nekola and White (1999), and used by Hubbell (2001) to detect spatial autocorrelation, is to plot ecological similarity as a function of geographic distance. Tuomisto et al. (2003) developed this idea further by log-transforming the geographic distances to make the relationship linear, and measured the fit using a squared matrix correlation (R_M^2), which is the square of the Mantel correlation between distance matrices. We agree with them that this is one way of describing the relationship in this simplified form of correlogram, and we mentioned it in LBP (*Abstract* and p. 442). The squared matrix correlation is then interpreted as an overall coefficient of spatial autocorrelation.

A Mantel correlogram is another form of analysis based on distance matrices (Legendre and Legendre 1998: section 13.1.5). It provides a more detailed analysis than an overall Mantel correlation. A critical point of logic is that a significant value for a distance class in a Mantel correlogram does not mean that *spatial autocorrelation* is present in the response data; it only means that there is a significant *spatial structure* in the data. Users of both raw-data and distance methods should be aware of that and be cautious about the interpretation of the results of correlogram analysis. A spatial structure can have different origins (Fortin and Dale 2005: chapter 5): It may indicate spatial dependence induced by the environmental factors, or spatial autocorrelation resulting from the stochastic demographic processes described by Hubbell (2001). The algebraic equation describing the spatial variation of a variable y at sampling locations i is: $y_i = f(\mathbf{X}_i) + SA_i + \varepsilon_i$ (Legendre et al. 2002). Because of their spatial structures, the environmental variables in table $\mathbf{X}$ may induce spatial dependence in the response y; that effect is separate from spatial autocorrelation proper (SA) which results from the spatial dynamics of y; ε_i is the "local innovation," or error term, at location i. For example, if there is an environmental effect in the species data and the forcing environmental variable has a broad-scale spatial structure, this will cause similarity decay plots to show monotonically decreasing similarity as geographic distance increases. A significant negative relationship (R_M) in a similarity decay plot or a significant value in a Mantel correlogram does not allow one to conclude about neutrality because it may be due to unmeasured environmental variables that are spatially autocorrelated and that influence the species distributions; it can be interpreted in terms of neutrality only if other factors were logically excluded or partialed out.

Can we test Hubbell's neutral theory using the raw-data approach?—In this section, we will show by simulations that the raw-data approach is the method that should be used to test hypotheses about positive spatial autocorrelation in neutral communities, one of Hubbell's predictions. We conducted a new Monte Carlo study akin to the one in our original publication (LBP). In that paper, canonical variation partitioning had proved to be the most powerful method to detect spatial autocorrelation in simulated community composition data, when compared to Mantel tests, whatever the method used to represent the spatial relationships in the two forms of analysis (see LBP: Table 1, columns with headings [b + c]). This was especially true when the spatial structure was modeled using PCNM variables, which are directly related to spatial autocorrelation functions (Dray et al. 2006). In the new study reported here, we simulated spatially patterned communities along a transect following Hubbell's neutral model with migration. Our Monte Carlo study was based on the following steps: (1) Generate a neutral metacommunity distribution of relative species abundances using Hubbell's algorithm (Hubbell 2001: Fig. 9.1, p. 291). The algorithm is based on two parameters: J_m is the number of individuals in the metacommunity, and θ is the fundamental biodiversity number. The metacommunity starts with a single individual ($j = 1$) of a single species and individuals are added until the metacommunity reaches J_m individuals. As each individual is added to the metacommunity, the individual is assigned to either an already existent species or to a new species (which is then added to the metacommunity). The probability of the jth individual being assigned to a new species is $\theta/(\theta + j - 1)$. If the jth individual was not assigned to a new species, then it was added to a previously existent species with a probability

TABLE 1. Rates of rejection of H_0 at significance level $\alpha = 0.05$ for the raw-data and distance approaches, each based on 1000 simulations, for detecting spatial structures due to migration in simulated neutral communities.

			$J = 1000$		$J = 10\,000$	
θ	m	lc	Raw data	Distance	Raw data	Distance
10	0.1	20	0.940	0.215	0.137	0.069
10	0.1	30	0.992	0.201	0.200	0.043
10	0.1	50	1.000	0.170	0.194	0.053
10	0.2	20	1.000	0.450	0.277	0.077
10	0.2	30	1.000	0.430	0.401	0.077
10	0.2	50	1.000	0.397	0.545	0.066
20	0.1	20	0.998	0.286	0.165	0.055
20	0.1	30	0.999	0.276	0.228	0.066
20	0.1	50	1.000	0.221	0.296	0.047
20	0.2	20	1.000	0.652	0.424	0.101
20	0.2	30	1.000	0.620	0.571	0.094
20	0.2	50	1.000	0.544	0.776	0.088
30	0.1	20	1.000	0.385	0.196	0.075
30	0.1	30	1.000	0.332	0.306	0.065
30	0.1	50	1.000	0.311	0.412	0.057
30	0.2	20	1.000	0.759	0.541	0.088
30	0.2	30	1.000	0.759	0.725	0.101
30	0.2	50	1.000	0.674	0.902	0.099

Note: Here, θ is the fundamental biodiversity number; m is the migration rate; lc is the number of local communities; and J is the number of individuals in the local community.

equal to the species' proportion of individuals in the metacommunity. (2) Once the neutral metacommunity was created, we randomly populated lc local communities with individuals from the metacommunity. Each local community was populated with the exact same number of individuals J (i.e., $J = J_m/\text{lc}$), randomly sampled with replacement from the metacommunity. Again, each species was sampled according to the species' proportion of its individuals in the metacommunity. (3) We then set out a death and immigration process, following McGill (2003), in a simulation based on 10 000 time steps as follows: First, local communities in step 2 were spatially distributed along a transect. Then, the simulation started, and at each time step, a randomly chosen individual from each local community was killed (deleted). Next, a random uniform number was generated: If smaller than m (migration rate), a randomly chosen individual was copied (i.e., birth with migration) from one of the two nearest-neighbor local communities along the transect, with equal chances, to replace the deleted individual; if larger than m, an individual from the local community was randomly chosen and duplicated (i.e., birth without migration). The probability of an individual belonging to a particular species was equal to the species' proportion of individuals in the local community (without migration) or in the chosen adjacent local community (with migration). (4) When the migration dynamics was completed after 10 000 time steps, we tested whether

the community distribution was spatially structured using both the raw-data and distance approaches.

The two analytical approaches were identical to those followed in LBP. The raw-data analyses used canonical redundancy analysis (RDA) of the Hellinger-transformed response species table by a table of principal coordinates of neighbor matrices (PCNM). For simplicity, $n/2$ PCNM variables with positive eigenvalues were used (i.e., no selection of the best PCNMs), providing a conservative test since a large number of degrees of freedoms are lost to the PCNMs. The PCNMs used in the tests only modeled patterns related to positive spatial autocorrelation, which was the kind predicted by Hubbell's theory. The distance approach was based on a Mantel test of the correlation between a Hellinger distance matrix among sites, representing the species data, and a geographic distance matrix $\mathbf{D}(XY)$ computed from the X and Y geographic coordinates (i.e., positions along the transect); this is the way spatial relationships are represented in most instances in Mantel tests. Significance tests were based on 999 random permutations. Using combinations of θ, J, lc, and m, we generated 1000 metacommunities for each combination, and each was tested using the raw-data and distance approaches. Results are presented in Table 1; they clearly show that the raw-data approach is a far more powerful method than the distance approach for detecting spatial autocorrelation in neutral communities.

In the last paragraph of page 2703, TR argued that

An existing spatial pattern in community composition can be described a posteriori, especially by such powerful methods as PCNM [...]. However, doing so does not test the neutral model, because the neutral model did not predict that this was the particular spatial pattern that was expected to emerge in this particular case. Any specific spatial pattern in community composition is just as much in accordance with the neutral model as any other, as long as the degree of spatial autocorrelation is similar.

The last statement is true for distance as well as raw-data methods and does by no means invalidate the latter in favor of the former. This is why we issued a warning about the various origins of spatial structure in the section *Tests of autocorrelation in the distance world* above. This being said, *if* other sources of variation have been logically or technically excluded, PCNM analysis (which is closely related to autocorrelation functions; Dray et al. 2006) is far more powerful at detecting raw-data structures emerging as a consequence of autocorrelation, than the distance approach is at detecting distance patterns emerging from autocorrelation. Therefore, we advocate the raw distance (PCNM) approach.

TR argued on page 2705 that, because Hubbell's neutral theory's "testable predictions are stated in terms

of distances, not in terms of raw data," only a method involving distances would be valid for testing the theory. They went on and wrote that "attempting to test this ecological hypothesis using the raw-data approach may give quite misleading results." Contrary to the simulations reported by LBP, autocorrelation in all communities analyzed in the present simulations was strictly generated under Hubbell's neutral model. Table 1 shows that the distance decay method was not nearly as powerful as the raw-data approach in detecting spatial autocorrelation in the data. Our new simulations show, therefore, that the raw-data approach is actually the most appropriate for testing this aspect of the theory.

Partitioning on distance matrices

Tuomisto et al. (2003) proposed to partition the variation of the ecological resemblance matrix using multiple regression on distance matrices: "More recently, variation partitioning has been extended to the distance approach by using multiple regression on distance matrices (Duivenvoorden et al. 2002, Tuomisto et al. 2003)" (TR, p. 2698). We developed that regression method for phylogenetic analysis (Legendre et al. 1994) and implemented it in the computer program (Casgrain 2001) that was used by Duivenvoorden et al. (2002), Tuomisto et al. (2003), and other authors who followed in their footsteps. A technical problem arises with the extension that they proposed. They combined by subtraction the coefficients of determination of three matrix regressions (R_M^2) to compute linearly independent fractions of variation allegedly corresponding to: (a) the nonspatially structured variation explained by the environmental distance matrix, (b) the spatially structured species variation explained by the environmental distance matrix, and (c) the spatially structured species variation not explained by the environmental distance matrix. We argue that variation partitioning cannot be computed in that way from distance matrices even if the ecological hypotheses are stated in the distance world. The problem here is not ecological in nature but mathematical (application of variation partitioning to distance matrices) and statistical (low power of the distance approach when compared to the raw-data approach). These points are discussed below.

TR have indeed proposed to carry out variation partitioning using distance-based R_M^2, but for this method to be demonstrated to be valid, one should first show (1) that it produces approximately correct estimates of the fractions of variation; (2) that the fractions of variation thus isolated are additive; (3) whether families of variables (e.g., environment) should be represented by a single synthetic distance matrix or by one matrix per variable, two procedures which lead to very different results; and (4) how the R_M^2 coefficients and the fractions of variation should be interpreted. The rationale presented by TR for their methodological

extension of variation partitioning solely relies on the Legendre et al. (1994) paper. This is insufficient: The validity of multiple regression on phylogenetic distance matrices does not warrant the extension to variation partitioning on ecological distance matrices. TR would provide an extremely constructive element to this debate by producing simulations giving clear answers to the four questions above.

Assuming that TR could answer the four questions about their partitioning method, the question of power remains acutely important. It motivated our comparative simulation study of the two partitioning methods. In both LBP and the present paper (previous section), we showed that the Mantel test had extremely low power in spatially explicit simulations. This means that it was unlikely to detect a species–environment relationship or a spatial structure when such an effect was present in the data. Considering the high cost of good ecological data, we felt it was our responsibility to explain to ecologists, who sought variation partitioning results to support their theories, what the most appropriate statistical method was to achieve their aims.

There are several statistical reasons for the low power of the Mantel test. The reasons revolve around (1) the fact that the R_M^2 statistic is inappropriate for questions about variation of community composition among sites in a region of interest (level-2 questions in LBP, TR, and in the section *Other points* below); (2) the difficulty of computing an adjusted form of R_M^2; and (3) the lack of additivity of R_M^2, which is crucial for variation partitioning.

Incorrect statistic.—The recent literature shows that many researchers still use the distance approach to solve level-2 questions, which is incorrect. LBP and TR agree on this point (TR: Fig. 2). Some examples are listed in LBP (pp. 438–439). It is thus urgent to warn researchers against this confusion. The technical reason is that the quantity which is partitioned in partitioning on distance matrices is the sum-of-squares of the distances, SS(**D**), and this quantity is not equivalent and cannot be reduced to the total sum-of-squares of the response data matrix SS(**Y**) (LBP: Eq. 2). Hence, the Mantel statistic R_M^2 is not equivalent to the canonical R^2. The simulation results reported in LBP were crucial in showing that this makes a big difference for interpretation of the results.

Adjusted coefficient of determination.—We now know that in regression, the R^2 statistic is a biased estimate of the true population R^2. Adjustments for this bias exist: Ohtani (2000) showed that, under certain assumptions, Ezekiel's (1930) adjusted coefficient of determination (R_a^2) is an unbiased estimator of the contribution of a set of explanatory variables **X** to the explanation of the variance of a single response variable **y**: $R_a^2 = 1 - a(1 - R^2)$, where $a = (n - 1)/(n - m - 1)$ for models with an intercept; n is the number of observations, and m is the number of explanatory

variables in the model. Peres-Neto et al. (2006) have shown that the adjusted canonical R_a^2, obtained by applying Ezekiel's correction to the canonical R^2 obtained in RDA, also produces unbiased estimates of the real contribution of the variables in $\mathbf{X}$ to the explanation of a response matrix $\mathbf{Y}$, and that it is these values of R_a^2 that must be used to obtain unbiased estimates of the fractions in variation partitioning. This conclusion is important for variation partitioning based upon distance matrices. No equation has been proposed to compute an adjusted R-square (R_{Ma}^2) in Mantel-type regression. In Ezekiel's equation, should we use $m = 1$ for a single explanatory matrix $\mathbf{X}$ in the regression, or should we make m equal to the number of original variables that were included in the calculation of the distances leading to $\mathbf{X}$? Should n be the number of original objects (sites) or the number of distances in half or the whole distance matrix? In any case, the very idea of an adjusted R^2 is suspicious in matrix regression because R_M^2 should be interpreted as a measure of fit of a model to pairwise distances and not in terms of the proportion of variation of a response matrix $\mathbf{D_1}$ explained by an explanatory matrix $\mathbf{D_2}$.

Additivity.—One final point concerns the additivity of the fractions resulting from variation partitioning. We now know how to partition the variation of a response matrix $\mathbf{Y}$ with respect to several explanatory matrices $\mathbf{X}$ using RDA. In raw-data partitioning, an identical total fraction of explained variation is obtained, whether all explanatory variables are put in a single table $\mathbf{X}$ or they are divided into any number of sub-tables (environmental, spatial, and so on). The effects of the explanatory variables are thus additive. This is not the case in partitioning on distance matrices: Different total amounts of explained variation for the response $\mathbf{Y}$ are obtained if one includes all explanatory variables in a single distance matrix or if separate distance matrices are computed for the various explanatory variables. This clearly shows that variation partitioning based on distances lacks the essential property of additivity, which is the basis for interpretation of variation partitioning results. TR are well aware of this fact, as they mention that in the distance approach "R^2 values will change depending on [. . .] whether all environmental variables are combined into a single distance matrix or used in separate matrices" (p. 2707).

The debate about the R^2 does not concern the statistical tests themselves (Mantel test for two matrices, or global test in a multiple regression on distance matrices). It only concerns the use of the squared Mantel coefficient, or the R^2 of the multiple regression on distance matrices R_M^2, as a measure of the fraction of explained variation, and following that as the basis for computation of the fractions in variation partitioning.

Question levels

We agree with TR that Mantel tests should be restricted to level-3 questions, described in the *Introduction*, whereas canonical variation partitioning addresses level-2 questions: We spelled that point out in LBP. However, TR's last paragraph of their section *Testing ecological hypothesis C* (p. 2704) claims that the hypothesis of environmental control of species distributions (a level-2 question) "is testable with the distance approach." They simply restate a level-2 question (the effect of environmental variables on species distributions) in level-3 terms (correlation between environmental distances and community distances). TR's recommendation is thus in blatant contradiction to our simulation results; distance-based methods must be avoided for level-2 questions. The simulations reported in Table 1 (sections B–E, column [a + b]) of LBP have clearly shown that Mantel tests were highly inefficient at detecting species–environment relationships when such relationships were present in data. It is therefore counterproductive to restate a level-2 question in distance terms.

Canonical variation partitioning is perfectly adequate to test predictions under TR's hypotheses A, B, and C (p. 2703). The low power of the Mantel approach to detect environmental relationships or spatial structures in community composition data where these relationships were present, as demonstrated by the simulations of LBP (Table 1, A–C), is a sufficient reason to avoid the distance approach to answer questions related to these hypotheses. Neutral processes generate spatial autocorrelation in community data, and PCNM analysis is very efficient at detecting it. This is shown by the simulation results reported in Borcard and Legendre (2002), in LBP, and in Table 1 of the present paper. These simulations also demonstrate that the Mantel test is very inefficient at detecting spatial autocorrelation in data. In all cases investigated here, the Mantel test is inadequate for level-2 questions reformulated in terms of distances, due to its extremely low statistical power; hence, the ecological hypotheses related to level-2 questions must be tested in the world of the raw data whenever that is possible.

Other points

1) In their section *The difference between "analyzing" and "explaining" beta diversity,"* TR (p. 2701) propose that any pairwise comparison of sites is a level-3 question. This is an incorrect statement: Pairwise comparisons can be made in the raw-data as well as in the distance world. It is therefore not surprising that, from this incorrect premise, they derived the incorrect conclusion that all the examples we cited as misuses of the Mantel test were in fact legitimate.

2) TR agreed with LBP that level-3 questions concern the variation in beta diversity among pairs or larger

groups of sites. Let us concentrate on larger groups of sites found in different sub-areas of the region under study: Establishing that there is variation among sub-areas can be translated into testing a hypothesis of homogeneity/heterogeneity of dispersion matrices among regions, each containing a group of sites. This can be done by the classical test of homogeneity of multivariate dispersion matrices (which compares the full within-group multivariate variance-covariance matrices; Kullback 1959), or the new test of homogeneity of within-group dispersions (ANOVA of distances of individual multivariate observations to their group centroid) described by Anderson (2006). More than two sites per group should be available for these analyses in order to obtain reliable estimates of the mean and variance parameters. The Mantel-based approach should be compared, by numerical simulations, to homogeneity analysis in order to determine which of the two approaches is the most powerful to detect heterogeneity in data.

Conclusion

The main argument presented by TR is that the predictions of Hubbell's model are formulated in terms of distances; hence, tests of significance should be conducted in the distance world. We have shown (1) that the presence of autocorrelation predicted by the neutral model is a level-2 question, which can and should be tested by canonical analysis using raw-data tables; (2) that the Mantel test should not be extended to variation partitioning on distance matrices until the very serious technical issues about that extension have been settled; and (3) that important level-2 hypotheses, for instance, those about environmental control determining community structure, should imperatively be tested in the world of raw data due to the extremely low power of the Mantel test.

The method of partitioning the variation of multivariate community composition data tables between environmental and spatial components (Borcard et al. 1992, Borcard and Legendre 1994), based on RDA, has now been generalized to several explanatory data matrices; a statistical function is freely available in the R language "vegan" library (Oksanen et al. 2007) to compute the results for up to four such matrices. Its domain of application as a method for spatial analysis was greatly improved by the development of PCNM analysis (Borcard and Legendre 2002, Borcard et al. 2004). We did the theoretical and the simulation work necessary to demonstrate the statistical correctness and usefulness of our method. Likewise, we developed regression on distance matrices for phylogenetic studies (Legendre et al. 1994), but we did not interpret R^2_M as anything but the adjustment of a model to data, for the reasons developed in the section *Partitioning on distance matrices* above. The proponents of partitioning on distance matrices are

invited to provide the same in-depth work to demonstrate the soundness of their approach.

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ANALYZING OR EXPLAINING BETA DIVERSITY? REPLY

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The background

Before going into details with the issues raised by Pélissier et al. (2008; hereafter referred to as PCD), Laliberté (2008; hereafter Laliberté), and Legendre et al. (2008; hereafter LBP*b*), we wish to restate the starting points of Tuomisto and Ruokolainen (2006; hereafter TR). Most importantly, TR focused on questions related to beta diversity, and beta diversity was defined as variation in community composition across sites, in agreement with Legendre et al. (2005; hereafter LBP*a*). With this focus, the obvious observation unit is a site, and the raw data (community composition) are logically presented as a sites × species table.

The raw-data table is a representation of the (within-site) data that describe the study sites (e.g., species abundances), so TR called it level-1 data (the first level of abstraction). The dissimilarity matrix is derived from the raw-data matrix and represents (among-site) variation in the raw data, so TR called it level-2 data (the second level of abstraction). The third level of abstraction is derived from the level-2 data and represents (among-site pair) variation in the dissimilarity data (see "The community composition path" in Fig. 1).

The most important topic in TR was to distinguish level-2 questions from level-3 questions. Level-2 questions were defined as those where the response variable is level-1 data, and the aim of the analysis is to explain level-2 data (variation in level-1 data). Level-3 questions were defined as those where the response variable is level-2 data, and the aim of the analysis is to explain level-3 data (variation in level-2 data). In either case, the purpose of the analysis is to quantify the proportion of the variation in the response variable that can be explained by variation in the available explanatory variables. Appropriate explanatory variables in beta diversity related studies are site descriptors such as environmental variables or site coordinates (in level-2 questions) or distance matrices derived from these (in level-3 questions).

Although TR concentrated on Mantel-based methods when discussing level-3 questions, this was simply because these methods are well known, and because we wanted to comment on some conclusions about the Mantel test drawn by LBP*a*. Our intention was not to claim that the dissimilarity matrix is the only possible form of level-2 data, nor that the Mantel test is the only possible analysis method for level-3 questions. The purpose of TR was also not to provide a review of all analysis methods that can be used in connection with studies that involve beta diversity, especially not of such methods that focus on different response variables than those mentioned in the previous paragraph. Similarly, TR limited the discussion on how to test Hubbell's neutral model (Hubbell 2001) to questions along the community composition path, but our intention was not to imply that other paths do not exist.

Questions related to beta diversity

As it happens, PCD wrote that they find the "individual × species-occurrence matrix [...] a much more appropriate 'response variable' than the classical site × species abundance matrix." With this statement, PCD revealed that the questions they were interested in were not the same as those TR were interested in. In the questions discussed by TR, the observation unit was the site; in the questions discussed by PCD, the observation unit was the individual. The individuals × species table is level-1 data just like the sites × species table, and either kind of raw data can be used as a starting point to derive level-2 data and level-3 data. When the raw data consist of the individuals × species table, the level-2 data quantify variation in species identity between individuals, i.e., gamma diversity (see "The species identity path" in Fig. 1).

The ecological questions targeted along the community composition path concern to what degree regional beta diversity (the overall variation in species composition among sites) can be explained by variation in site properties (level-2 question), and to what degree the
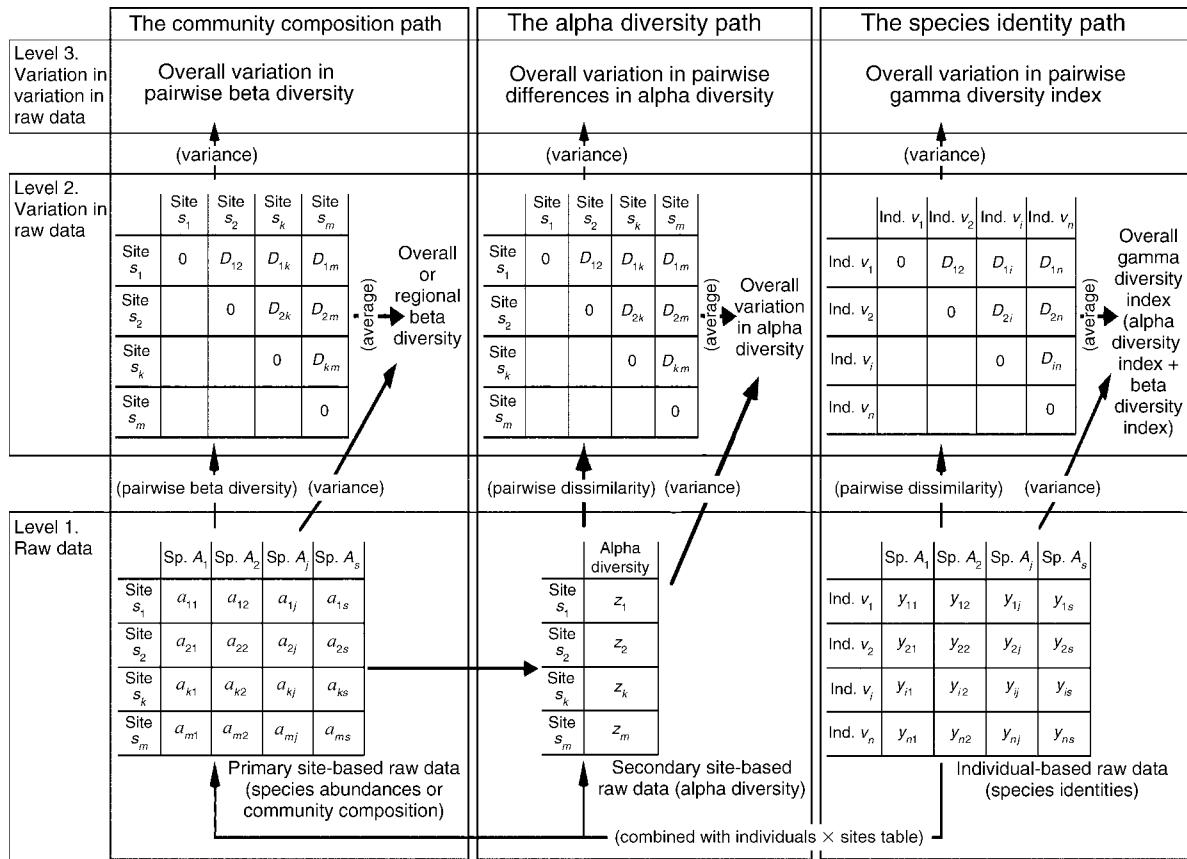
FIG. 1. Different paths and levels of abstraction relevant to diversity studies. The raw data at the first level of abstraction consist of observation units (sites s_1-s_m or individuals v_1-v_n) that are characterized by one or more descriptors (abundance a of species A_1-A_S or alpha diversity z for sites, binary information y on whether individual belongs to species A_1-A_S for individuals). In combination with an individuals × sites table, the individuals × species table can be used to derive the site-based raw-data tables. The primary site-based raw data can be used to derive the secondary site-based raw data. Variation in any raw data table can be represented at level 2 by a dissimilarity matrix, or summarized in a single value such as variance or the mean of the pairwise dissimilarities (D). In turn, the variation in the dissimilarity matrix forms the third level of abstraction. Each raw-data table forms the starting point for a different path, and the different kinds of diversity appear in different parts of these paths. Along the community composition path, beta diversity is level-2 data; alpha diversity and gamma diversity are not present along this path. Along the alpha diversity path, alpha diversity is level-1 data; beta diversity and gamma diversity are not present along this path. Along the species identity path, the index values of all three diversities are level-2 data.

variation in differences in species composition between sites can be explained by variation in differences in site properties (level-3 question). In contrast, the ecological questions targeted along the species identity path concern to what degree the total variation in species identity among individuals (index of gamma diversity) can be explained by variation in the identity of the sites (or habitat classes) in which the individuals occur (the beta diversity component) and to what degree it is unexplained by variation in site identity (the alpha diversity component). This is a level-2 question. The corresponding level-3 question would be: To what degree can the variation in whether individuals belong to the same species or not be explained by variation in whether the individuals grew in the same site (or habitat class) or not?

Although the questions of interest change when a different raw-data table is used, the same levels of abstraction apply to the community composition path and the species identity path. Indeed, any observation units × descriptors table can be used as the level-1 data from which level-2 data and level-3 data are derived. This was not explicitly stated in TR, because there we were only interested in the distinction between level-2 questions and level-3 questions along the community composition path.

In contrast, PCD were interested in level-2 questions along the species identity path. This is an entirely valid focus, but it is important to notice that the species identity path explores different terrain than the community composition path (Fig. 1). Although beta diversity appears in some questions along both paths,

it is in different roles in them. In the questions of the community composition path, beta diversity is either the response variable (level-3 questions) or the variance to be explained (level-2 questions). In contrast, in the level-2 questions of the species identity path, the variance to be explained is an index of gamma diversity, and the beta diversity index is that fraction of the gamma diversity index that actually can be explained by variation in the explanatory variables.

Where does alpha diversity belong?

Both PCD and LBPb maintain that the first level of abstraction is alpha diversity, as in LBPa, rather than community composition, as in TR. In fact, alpha diversity is also level-1 data in the TR scheme. This is because alpha diversity can be presented in a sites × alpha diversity table, which is an observation units × descriptors table, which in turn is the basic format of level-1 data (Fig. 1). Alpha diversity can be considered secondary raw data because it summarizes the species abundance information from the primary raw data (sites × species table) through the computation of an alpha diversity index, but it is still level-1 data.

Both alpha diversity and beta diversity can be derived from the sites × species table, albeit via different computation routes (Fig. 1). When we discussed level-1 data in TR, we chose to concentrate on the sites × species table, rather than the sites × alpha diversity table, for two reasons.

First, clarifying the relationships between the levels of abstraction is easier when each higher level consists of the variation in the level below it. This made it possible to use an analogy from physics, where level-1 data consist of position, level-2 data of velocity, and level-3 data of acceleration. In mathematical terms, the level-2 data correspond to the first derivative and level-3 data to the second derivative of the original level-1 variable.

Second, our main interest was in beta diversity, and the sites × species table can be used to calculate beta diversity, whereas the sites × alpha diversity table cannot. Computing beta diversity requires knowledge on species identities, and this information is lost when the alpha diversity index is computed, so the sites × alpha diversity table cannot be used as a starting point when one is interested in questions along the community composition path (Fig. 1).

Just like one can derive level-2 data and level-3 data from the sites × species table and the individuals × species table, one can do the same with the sites × alpha diversity table (see "The alpha diversity path" in Fig. 1). Alpha diversity is at the same level of abstraction as community composition (level 1), and variation in alpha diversity is, hence, at the same level of abstraction as variation in community composition (level 2). Consequently, analyzing alpha diversity is a level-2 question

by our terminology, which contrasts with the view of PCD, LBPa, and LBPb, who call it a level-1 analysis.

Definitions of beta diversity

Over the years, beta diversity has been defined in different ways. Whittaker (1960) measured beta diversity with dissimilarity indices computed between site pairs, and later noted that the average of all such values for a data set can be considered an expression of beta differentiation (Whittaker 1972). Therefore, the individual cell values in the level-2 dissimilarity matrix along the community composition path can be termed "pairwise beta diversity," and the average of all off-diagonal values in the dissimilarity matrix is one expression of "regional beta diversity." Whittaker (1960) also defined the terms gamma diversity and alpha diversity, and related the three kinds of diversity to each other in a multiplicative way, i.e., $\gamma = \alpha \times \beta$, from which follows that $\beta = \gamma/\alpha$ (multiplicative beta diversity). The three diversity measures have since then also been related in an additive way, i.e., $\gamma = \alpha + \beta$, from which follows that $\beta = \gamma - \alpha$ (additive beta diversity; e.g., Lande 1996, Veech et al. 2002, PCD).

In both the multiplicative and the additive approach, alpha and gamma diversities can be measured as the number of species in a site and a larger region, respectively. Multiplicative beta diversity then measures how many times more species there are in an entire region than in an average site within that region. Additive beta diversity measures how many more species the entire region has than an average site within that region. Both are variants of "regional beta diversity."

For pairwise beta diversity, one can choose a dissimilarity metric from a wide variety of available indices, each one of which emphasizes different aspects of the raw data. Some of these indices are ratios whereas others are not. The classical beta diversity measures are ratios and therefore conform with the concept of multiplicative beta diversity. Examples include measures based on the Jaccard, Sørensen, or Bray-Curtis indices, which are often obtained by first subtracting two values and then dividing the result by a third value (to indicate, for example, what proportion of species found in two sites are not shared between the two sites). When $\beta = \gamma/\alpha$ is computed for site pairs, it equals 2 minus the Sørensen index.

In contrast, dissimilarity indices such as the Euclidean distance and the Manhattan distance are not ratios, but have the same unit as the input data (e.g., number of species) and therefore conform with the concept of additive beta diversity. The Manhattan distance, when computed using presence–absence data, indicates how many species are found in one of the sites to be compared but not both. This is obtained by subtraction, and the value thus obtained is used without relating it to the number of species that were found in both sites

together. When $\beta = \gamma - \alpha$ is computed for site pairs, it equals Manhattan distance divided by 2.

Even though the additive and multiplicative beta diversities measure conceptually different things, either approach can be applied to site pairs to obtain the pairwise beta diversity values in the dissimilarity matrix along the community composition path. However, the additive beta diversity indices have properties that are not desirable in this context (see Legendre and Legendre 1998 for details).

As discussed by PCD, alpha and gamma diversity can also be measured using an index that takes into account the relative abundances of species, such as the Shannon index H'. If this is done, some extra thought is necessary because beta diversity can no longer be interpreted in the terms outlined above. Already Whittaker (1972) noticed that the Shannon index should not be used in the multiplicative diversity equation, because H' involves logarithms, and therefore H'_α and H'_γ will necessarily converge when alpha diversity increases. Instead, diversities should be measured using $\exp H'$ which is the number equivalent of H' (the number of equally abundant species needed to obtain the observed value of H'). When this is done, the following happens:

$$\gamma = \alpha \times \beta \Leftrightarrow \exp H'_\gamma = \exp H'_\alpha \times \exp H'_\beta$$
$$= \exp(H'_\alpha + H'_\beta) \Leftrightarrow H'_\gamma = H'_\alpha + H'_\beta \Leftrightarrow \gamma' = \alpha' + \beta'.$$

With the Shannon index, applying the additive diversity partitioning to the index therefore leads to the same result as applying the multiplicative partitioning to its number equivalent, meaning that in both cases the same index values and the same number equivalents are obtained (see Jost 2007 for a more thorough discussion involving also other diversity indices).

When the Simpson index is used, the equation $\gamma' = \alpha' + \beta'$ can be interpreted in terms of variance in the species identity of individuals, as done by PCD. The beta diversity index derived in this way is incorporated in the level-2 questions of the species identity path, namely as the proportion of variation in the individuals × sites table that can be explained by the categorical explanatory variables (site or habitat class). This was discussed extensively by PCD. Unfortunately, when relating their arguments to those of TR, PCD failed to notice that they were discussing β', whereas TR were discussing β. The beta diversity index β' is not in the same role along the species identity path (the focus of PCD) as beta diversity β is along the community composition path (the focus of TR: Fig. 1).

Site groups in the distance approach

All three commentaries (PCD, Laliberté, and LBPb) erroneously claimed that TR restricted the use of the distance approach to site pairs rather than larger regions with more than two sites each. When giving examples of studies involving larger regions, PCD, Laliberté, and LBPb also demonstrated that their concept of the three levels of abstraction is not the same as that of TR. PCD wrote: "The objective of the third level [...] is ultimately to compare diversity patterns found in distinct surveys, hence to compare the structures present in several site × species tables." Subsequently, PCD made the claim: "It is only when surveys share neither sites nor species [...] that comparisons of distance matrices and Mantel tests may justify themselves." This is a perplexing statement, because a data set where none of the observation units (surveys) share any species cannot yield informative results in a Mantel test. This is because when observation units share no species, all compositional dissimilarities in the corresponding distance matrix are identical (if a dissimilarity index consistent with multiplicative beta diversity is used), and there is no variation that could be related to the variation in a second distance matrix.

Laliberté, in turn, wrote that the distance approach is of limited ecological use, because "most ecologists that are truly interested in studying the variation of beta diversity among groups of sites or regions would likely want to compare regions in which more than two sites have been observed." LBPb wrote along similar lines that LBPa "use level-3 questions to investigate the differences among larger groups of sites; for example, adjacent geographic regions with several sites in each" and specified: "This question does not focus on the identities of the flower species, but on the variance in community composition between the two towns. (Are the multispecies dispersion matrices homogeneous when comparing the two towns, and if not, why?). This would be a level-3 question."

In fact, all the above descriptions of proposed "level-3 questions" correspond to what TR consider level-2 questions. In addition, they differ from the questions discussed by TR in using a different raw-data table: The observation unit here is a region consisting of a group of sites rather than a single site, and the descriptor is within-region heterogeneity rather than community composition.

As we said in TR, beta diversity can be computed for any number of sites ≥ 2. We focused on the special case where each observation unit consists of a single site, and pairwise beta diversity is hence computed for pairs of sites, because this simplifies the interpretation of analysis results. The results from analyses where the observation unit is a group consisting of ≥ 2 sites are affected by many factors. If different groups contain different numbers of sites, it has to be taken into account that both the number of species and observed heterogeneity within a group tend to increase with the number of sites. If a fixed number of sites is used, how many sites should there be per group? The groups are the data points in the analyses, so if one allocates more sites to each, fewer

The regional beta diversity path				

Level 3.
Variation in
variation in
raw data

Overall variation in
pairwise differences in regional beta diversity

(variance)

Level 2.
Variation in
raw data

	Region r_1	Region r_2	Region r_p	Region r_q
Region r_1	0	D_{12}	D_{1p}	D_{1q}
Region r_2		0	D_{2p}	D_{2q}
Region r_p			0	D_{pq}
Region r_q				0

(average) → Overall variation in regional beta diversity

(pairwise difference)   (variance)

Level 1.
Raw data

	Regional beta diversity
Region r_1	B_1
Region r_2	B_2
Region r_p	B_p
Region r_q	B_q

Region-based raw data
(regional beta diversity, which is level-2 data
from the community composition path)

FIG. 2. The regional beta diversity path. The observation units along this path are regions (r_1–r_q) consisting of ≥ 2 sites, and the level-1 descriptor is within-region compositional heterogeneity (regional beta diversity B_1–B_q). For each region, the regional beta diversity value is obtained from the level-2 data of the community composition path (Fig. 1) where the sites × species table contains the sites belonging to the region in question.

data points can be obtained from the same amount of data, and the power of statistical tests decreases. Furthermore, it may not always be obvious which sites should belong to the same group, and changing the limits among groups may affect the results. All these problems are avoided if the analyses are run such that each group consists of a single site, and the beta diversity values are hence computed for pairs of sites. Of course, if the ecological question of interest is such that it can only be answered through comparisons among groups of several sites, then one has to compare groups of several sites.

If groups of ≥ 2 sites are used as the observation unit, at least two different analytical paths become available. Along the first path, the questions asked are similar to those along the community composition path of Fig. 1, except that the observation unit is now a region (or survey, as in PCD) rather than a single site. The level-1 data consist of a regions × species table, where species abundances for each region have been pooled over all sampling units that belong to the region in question. In the level-2 data, each region is compared with each other region in turn to obtain a matrix of pairwise beta diversity values between regions.

Along the second path, the observation unit is still the region, but the variable that is used to describe the region is its internal compositional heterogeneity, i.e., regional beta diversity. For each region, the regional beta diversity needs to be quantified from a sites × species table that contains those sites that belong to the region in question. Regional beta diversity can be derived in one of several alternative ways. For example, one can use the variance (or sum of squares) of the sites × species table (following LBPa), the average of the pairwise beta diversity values in the dissimilarity matrix derived from the sites × species table (following TR), or the mean distance to the regional centroid derived from an ordination of the sites within each region (following Anderson et al. 2006).

It is noteworthy that, although the variance of the sites × species table and the mean pairwise dissimilarity of the sites are level-2 data along the community composition path (Fig. 1), they become level-1 data along the regional beta diversity path (Fig. 2). The

regions × regional beta diversity table contains level-1 data just like any other observation units × descriptors table, and on the basis of this raw-data table, level-2 data and level-3 data can be derived. These, in turn, can be used to formulate level-2 questions and level-3 questions of the regional beta diversity path (Fig. 2). For example, a possible level-2 question would be: Is within-region compositional heterogeneity correlated with within-region environmental heterogeneity? A related level-3 question would be: Is the between-region difference in within-region compositional heterogeneity correlated with the between-region difference in within-region environmental heterogeneity?

Are we taking the same path?

All three commentaries (PCD, Laliberté, and LBP*b*) criticized TR's use of the Mantel test to address questions related to beta diversity, and suggested alternative analysis methods instead. PCD wrote that "the main purpose of our comment is to show that spatially explicit, distance-based analyses of beta diversity do not necessarily belong to the so-called third level of abstraction [...] and can, moreover, be viewed as a prolongation of the raw-data approach, in accordance with the overall concept of variance partition." Here it should be remembered that when TR discussed "analysis of beta diversity," they referred to an analysis of the community composition path where the response variable is pairwise beta diversity, and since pairwise beta diversity is level-2 data, this is a third-level question by definition. But the questions discussed by PCD were indeed level-2 questions, not level-3 questions; they concerned the analysis of the individuals × species table, which is level-1 data. In these analyses, the beta diversity index is not the response variable, but represents the explained part of the variance of the response variable (the response variable being the individuals × species table, and the variance of the response variable being a gamma diversity index).

Laliberté, in turn, wrote: "A more appropriate way of answering questions related to the variation of beta diversity among groups of sites or regions would be to use multivariate dispersion on distance matrices, a method described by Anderson et al. (2006)." Laliberté also gave examples of such questions: "Does beta diversity differ between different groups of sites (i.e., that contain more than two sites) or regions?" and "Why does beta diversity vary between these groups of sites or regions?" If one is interested in finding out whether the regional beta diversity values observed within two regions are statistically different, then the distance-based test of homogeneity of multivariate dispersions (Anderson 2006, Anderson et al. 2006) is indeed appropriate, but the Mantel test is not. The Mantel test quantifies correlation between cell values in two distance matrices, and since correlation is not affected by scaling param-

eters, the Mantel test cannot be used to find out whether the average cell values in the two matrices differ. The second question of Laliberté is a typical example of a level-2 question of the regional beta diversity path, and as such, it cannot be addressed by the Mantel test either (this being a level-3 method). Along the regional beta diversity path, the observation unit is the region, and in level-2 questions of this path, regional beta diversity is in the role of the response variable. This contrasts with the situation along the community composition path (the focus of TR), where the observation unit is a single site, and in level-2 questions of this path, the regional beta diversity is in the role of the variance to be explained (the response variable being community composition).

LBP*b* wrote that "The Mantel-based approach should be compared, by numerical simulations, to homogeneity analysis in order to determine which of the two approaches is the most powerful to detect heterogeneity in data." As explained in the previous paragraph, the two methods do not target the same response variable, and their null hypotheses are not linked in any way. Simulations are neither necessary nor useful in deciding which of the two methods is most powerful to detect heterogeneity in data, because one of them answers this question and the other one does not.

We agree that the analytical approaches promoted by PCD are appropriate when one is interested in level-2 questions of the species identity path, and that multivariate dispersion can be used as the response variable when one is interested in level-2 questions of the regional beta diversity path, as promoted by Laliberté. However, this has no bearing on the validity of our conclusions in TR, because there we were not discussing the species identity path or the regional beta diversity path, and therefore none of our conclusions about beta diversity were meant to be interpreted in the context of these paths. All the questions discussed in TR were along the community composition path, where the response variable is either the sites × species table at the beginning of the path, or the dissimilarity matrix based on this table further up the path. If the observation unit is not the site (as in TR), but the individual (as in PCD) or the region (as in Laliberté), then the questions addressed are fundamentally different, and the analysis approaches may also be different.

Spatial autocorrelation and spatial dependence

Hubbell's neutral hypothesis posits that species abundances (and hence community composition) fluctuate randomly, but are spatially autocorrelated due to dispersal limitation (Hubbell 2001). In TR, we wrote that, from this, it follows that two nearby sites are expected to share more species in more similar abundances than two sites further apart. Laliberté claimed that this "statistical prediction is inaccurate because spatial autocorrelation is not defined as the

tendency of two nearby sites to be more similar than faraway sites," but as "the lack of independence [...] among the error components of field data, due to geographic proximity." Following Legendre and Legendre (1998:11), the value of the autocorrelated variable y at site j can be calculated using the following equation:

$$y_j = \mu_y + \sum f(y_i - \mu_y) + \varepsilon_j \qquad (1)$$

where μ_y is the overall mean of y. The rest of the equation describes the error component, in which $\Sigma f(y_i - \mu_y)$ is the spatial autocorrelation term. This is a weighted average over all points i of the deviation of y_i from the overall mean of y. The weights used in function f are inversely related to the geographical distance between points i and j. As a consequence, the closer to each other the points i and j are spatially, the stronger the influence of y_i on y_j, and the more similar, on average, these values will be. As a result, nearby sites tend to have more similar y values than faraway sites. Along the community composition path, y stands for community composition.

Laliberté claimed that spatial autocorrelation does not necessarily lead to a monotonic decrease in similarity with increasing geographic distance, but did not explain why. We offer two possibilities: random noise and restricted range. If the random error term ε_j in Eq. 1 is much larger than the autocorrelation term, then the effect of spatial autocorrelation is swamped by random noise. When similarity in y is then regressed against distance, a lot of scatter will be observed around the regression line, and its slope might be statistically indistinguishable from zero. In such a case, y would be considered weakly, or not at all, autocorrelated spatially. This happens under the neutral model when the probability of immigration from the metacommunity to the local community is given a large value (Hubbell 2001).

Even when the spatial autocorrelation term is large when compared with random noise, spatial autocorrelation may be of restricted range. Then y_i has an effect on y_j only when the distance between points i and j is smaller than the range, and at longer distances y_j becomes independent of y_i. In this case, similarity in y would decrease with distance at distances shorter than the range but not at longer distances. Laliberté did not use this argument, but instead wrote that only broad-scale patterns in distance decay plots should be interpreted in terms of the neutral model. However, Hubbell himself has argued that spatial autocorrelation is present at all spatial scales in neutral communities, and has shown examples of distance decay plots where the geographical distances range from a few meters to thousands of kilometers. At local scales, spatial autocorrelation is caused by random population dynamics and dispersal limitation, and at broad scales by the cumulative effects of such dynamics over long time periods, as well as random speciation events (Hubbell 2001).

Laliberté wrote further: "Consequently, regressing community composition dissimilarity on geographical distances [...] to *quantify the contribution* (using R^2) of neutral processes to variation of beta diversity between pairs of sites, which is the goal of TR's method of variation partitioning on distance matrices, is fundamentally incorrect" (Laliberté's italics). Here Laliberté misinterprets our analyses. TR did not claim that the spatial component of variation partitioning as such is a measure of the contribution of neutral processes. It cannot be, because the neutral model also predicts random noise, and because deterministic processes lead to a similar distance decay pattern when community composition is dependent on spatially autocorrelated environmental variables (as explicitly stated in TR; see also *Tests of autocorrelation in the distance world* in LBP*b*, and *Purpose of variation partitioning on distance matrices*, below).

When both spatial autocorrelation and dependence on environmental variables are operating, the value of y can be expressed using the following equation (note the similarity with the equation presented by LBP*b*):

$$y_j = \mu_y + f(\text{environmental variables})$$
$$+ \sum f(y_i - \mu_y) + \varepsilon_j. \qquad (2)$$

What happens when difference in y is quantified between two sites j and k? If we are only interested in the magnitude of the difference (and not in which site has the larger value of y), the relevant equation is

$$\Delta_{j,k} y = |y_j - y_k|$$
$$= \Delta_{j,k} f(\text{environmental variables})$$
$$+ \Delta_{j,k} \sum f(y_i - \mu_y) + \Delta_{j,k}\varepsilon. \qquad (3)$$

The term μ_y is a constant and therefore gets eliminated from Eq. 3. If environmental variables have an effect on y, and f(environmental variables) is a monotonic function (which is a reasonable assumption when y is community composition), then $\Delta_{j,k} f$(environmental variables) is smallest for sites where the values of the environmental variables are most similar, and $\Delta_{j,k} y$ should hence be positively correlated with difference in the environmental variables. The term $\Delta_{j,k} \Sigma f(y_i - \mu_y)$ is by definition smallest for sites that are surrounded by the same neighbors, and similarity in the neighborhoods around j and k logically decreases with increasing geographical distance between them. As a consequence, $\Delta_{j,k} y$ will be positively correlated with geographical distance. The term ε_j is just random noise, and $\Delta_{j,k}\varepsilon$ is therefore not related to either environmental or geographical distance.

Purpose of variation partitioning on distance matrices

Our interest in variation partitioning on distance matrices stems from its usefulness in quantifying the unique and shared statistical contributions of variation

in environmental vs. geographical distances to explaining variation in community differences, because this helps in separating environmental dependence from spatial autocorrelation. When variation partitioning is used in this framework, it partitions the variation in the pairwise beta diversity values in the response dissimilarity matrix to four fractions: purely environmental (explained by variation in environmental differences only), purely spatial (explained by variation in geographical distances only), joint spatial–environmental (explained jointly by variation in environmental and geographical distances), and unexplained (not explained by variation in any of the available explanatory distance matrices).

When f(environmental variables) is a monotonic function, it follows that the stronger the effect of environmental variables on y, the less scatter there is around the linear regression of Δy on differences in the environmental variables, and the larger the R^2 of this regression. Similarly, the stronger the spatial autocorrelation of y, the less scatter there is around the linear regression of Δy on geographical distances, and the larger the R^2 of this regression (unless the range of spatial autocorrelation is much less than the extent of the study). If all relevant environmental variables have been measured, then the purely environmental fraction corresponds to the effect of nonspatial environmental dependence, i.e., the part of $\Delta_{j,k} f$(environmental variables) that is not related with spatial autocorrelation in the environmental variables. The purely spatial fraction corresponds to the effect of spatial autocorrelation in y itself, i.e., $\Delta_{j,k} \Sigma f(y_i - \mu_y)$, and the unexplained fraction to the effect of random error $\Delta_{j,k}\varepsilon$. The joint spatial–environmental fraction may represent either environmental dependence of y on spatially autocorrelated environmental variables, spatial autocorrelation in y itself, or any combination of both, and the relative contributions of $\Delta_{j,k} f$(environmental variables) and $\Delta_{j,k} \Sigma f(y_i - \mu_y)$ to this fraction cannot be separated.

According to Hubbell's neutral model, there is no environmental dependence, so f(environmental variables), and therefore Δf(environmental variables), should equal zero for all values of the environmental variables. Consequently, community differences should not be explainable by environmental differences. If it is found that they are, an explicit prediction of the neutral model has been falsified. The purely environmental fraction therefore quantifies the proportion of variation in community differences that logically cannot be due to neutral processes. Whether the rest of the variation is caused by neutral processes or not remains an open question. It could be, because both spatial autocorrelation and random noise are compatible with the neutral model. However, because all relevant environmental variables can never be measured, both the pure spatial

and the unexplained fraction are probably overestimated in practical applications.

The neutral model also predicts that community compositional differences should be positively correlated with geographical distances, as explained above. If it is found that the correlation is negative, it can be assumed that other forces than spatially autocorrelated neutral dynamics determine community composition in the study region. A nonsignificant correlation is indecisive, because it could be due to high migration rate, which increases the effect of the random error $\Delta_{j,k}\varepsilon$.

Obviously, all ecological conclusions based on such analyses are dependent on the sampling being adequate for the purpose. Although Laliberté suggested otherwise, we certainly do not advocate that conclusions on the neutral theory are drawn from data with "any sampling design, regardless of grain size and spatial extent." For example, a sampling setup that covers such a small spatial extent that all species are ubiquitous (as in the numerical example shown in Fig. 1A of Laliberté) seems to us so restricted that it would be of limited interest in this context.

Raw-data vs. distance approach in testing the neutral model

Under *Testing neutral theory: raw data or distances?* Laliberté overlooked some general principles of statistical testing. Hubbell (2001) proposed the neutral model as a null hypothesis, which is logical, because there is no way to prove that observational data are the result of purely random processes. Indeed, this is what is generally assumed a priori, until analyses of the observational data provide evidence to the contrary. When testing a hypothesis, one needs to derive such predictions about the observations whose falsification serves as evidence against the hypothesis. The fact that the neutral model gives a role for spatial autocorrelation, but not for environmental dependence, is crucial in formulating such predictions. If a pattern observed in the data is in conflict with model predictions, it can be concluded that the pattern was caused by some other process than the one modeled. However, even when a pattern observed in the data is in agreement with model predictions, causality cannot automatically be assumed. Furthermore, failure to reject a null hypothesis does not prove that the null hypothesis is true. These general principles apply no matter whether analyses are done using the raw-data approach or the distance approach, contrary to what Laliberté seemed to suggest.

Under *Spatial autocorrelation and spatial dependence*, above, we explained how the neutral model leads to two explicit predictions about the values of $\Delta_{j,k} y$. Since $\Delta_{j,k} y$ is level-2 data, predictions concerning variation in $\Delta_{j,k} y$ need to be tested using a level-3 method, i.e., the distance approach. PCD, Laliberté, and LBPb advocated testing the neutral model using the raw-data
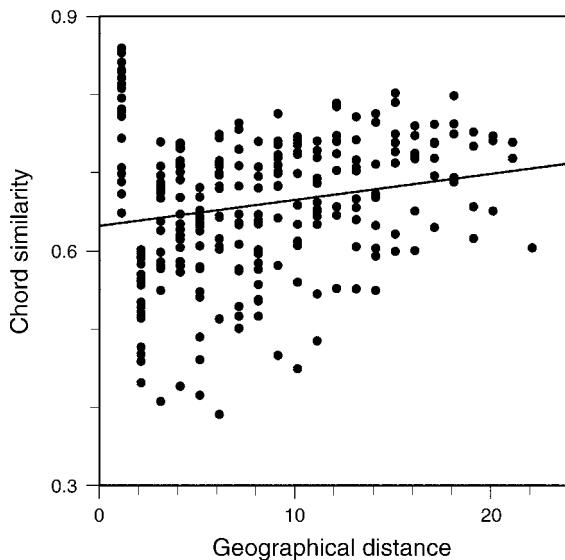
FIG. 3. Similarity in species composition between site pairs plotted against geographical distance, with linear regression shown. The data come from an artificial transect with 23 equidistant plots and a total of 14 species.

approach, which by definition concerns variation in y. However, in this case, it is difficult to see how the variables included in the regression models are related to the neutral model they are supposed to represent.

The least ambiguous component is f(environmental variables). Since the neutral model predicts f(environmental variables) to be zero, finding that environmental variables actually have an impact on y falsifies a prediction of the neutral model. However, spatial autocorrelation causes a problem here, both because it might inflate the apparent contribution of the environmental variables, and because it is capable of creating spatial pattern. Therefore, neither the existence nor the nonexistence of statistically significant spatial pattern (whether environmentally correlated or not) in the raw data serves as proof against the neutral model.

In the raw-data-based variation partitioning advocated by Laliberté and LBPb, the explanatory variables do not include a real autocorrelation term $\Sigma f(y_i - \mu_y)$, but a model of spatial structure as represented by principal coordinate analysis of neighbor matrices (PCNM) variables. The autocorrelation term models the value of y in a focal observation unit on the basis of the values of y in neighboring units (whatever their spatial coordinates), whereas the PCNM variables model the value of y in a focal observation unit on the basis of its spatial coordinates (whatever the values of y in neighboring units). As stated in TR (p. 2703), PCNM variables are very flexible, so they can efficiently model many types of spatial structure. Some of these structures may be compatible with the neutral model, whereas others are not. Since PCNM variables are actually wave

functions, they are especially good at modeling patchiness that recurs with a regular periodicity over the landscape. However, under neutral dynamics there is no reason why recurring patchiness should arise. Even though random fluctuations in species abundances easily lead to patchy distribution, there is no reason for the high-density patches of a given species to be of the same size or at regular intervals. Furthermore, under the neutral model there is no reason why the patches of different species should coincide, so community composition (which integrates the abundances of all species) is expected to vary smoothly over the landscape. Using the existence of regular spatial patchiness at the community level to support the neutral model is therefore unwarranted.

Not all kinds of spatial structure that can be modeled by PCNM variables involve monotonic decrease in community similarity with increasing geographical distance at all distances, which is what the neutral model predicts. Fig. 3 shows one such example, where community similarity of an artificial data set actually increases with increasing geographical distance ($R_M = 0.19$, $P = 0.015$). Since this is the opposite of what the neutral model predicts, both the graph and the Mantel test result show that the data are in conflict with a level-3 prediction of the neutral model. When the same data set is used in redundancy analysis (RDA; a level-2 method), six PCNM variables can explain 42% of the variation in community composition ($P < 0.05$). The neutral model makes no predictions about the expected relationship between species abundances and PCNM variables, so we do not know if this RDA result is in agreement with the neutral model or not. If significant spatial structure as detected by PCNM variables is interpreted as support to the neutral model (as advocated by Laliberté, LBPa, and LBPb), then the level-3 results and the level-2 results point to different conclusions about the agreement between data and theory. We prefer to rely on the level-3 results, because according to our logic, the Mantel test addresses an explicit prediction of the neutral model, but RDA fails to do so. If Laliberté and LBPb disagree with our reasoning above, we would like to see them specify which predictions of the neutral model the level-2 methods actually test, and how they do that.

Hubbell's neutral theory can be compared with a hypothesis that kites move at random in space and are unaffected by wind or other external forces, but that kites are not able to disapparate and instantly apparate in another location, so kite position is temporally autocorrelated. Two predictions can be derived from this hypothesis. First, the velocity of the kite should not be correlated with wind velocity. Second, the shorter the time interval between kite observations, the smaller the average difference in the corresponding kite positions should be. Both predictions can be tested with the

distance approach. In contrast, the random movement hypothesis does not predict any specific pattern in kite position that could be tested with the raw-data approach. Of course, it is possible to model the realized trajectory of the kite a posteriori. For example, raw-data-based variation partitioning can be used to quantify the proportions of the variation in kite position that are uniquely and jointly explainable by variation in the position of the air mass surrounding the kite and by a chosen mathematical function that models how the high and low coordinate values behave over time (e.g., PCNM variables). But how these tests are related to any predictions of the random movement hypothesis is not at all obvious, so in practice, the results shed no light on what the relative roles of spatially autocorrelated random movement and external forces have been in shaping the observed kite trajectory.

Simulations

Laliberté and LBP*b*, just like LBP*a*, justified their preference for the raw-data approach by referring to simulation results. However, when choosing between analysis methods, the selection criterion should not be how well a given simulated data set conforms with the method's null hypothesis, but how well the null hypothesis targets the ecological question at hand. TR argued that the analysis of spatial autocorrelation is a level-3 question by definition, and therefore it cannot be analyzed using RDA, which is a level-2 method. Neither LBP*b* nor Laliberté disproved this argument, because they did not explain in what way spatial autocorrelation is modeled in RDA.

LBP*b* wrote in reference to their simulation studies that "PCNM analysis [...] is far more powerful at detecting raw-data structures emerging as a consequence of autocorrelation, than the distance approach is at detecting distance patterns emerging from autocorrelation." This statement has no relation with the analysis results that LBP*b* actually presented. What they did is to subject simulated data sets to level-2 and level-3 analyses and compare the rejection rates of the corresponding null hypotheses. The simulation results can be interpreted as follows.

1) The simulations produced such spatial structure in the data that was well modeled by PCNM variables in RDA (high rejection rate of H_0: there is no spatial structure related to the PCNM variables in the raw data).

2) The simulations did not produce a monotonic decrease in community similarity with geographical distance in the data (rejection rate was low for H_0: Compositional dissimilarity between communities is not linearly correlated with the geographical distance between them). This is not entirely unexpected, given that migration to a community was only allowed from immediately neighboring communities, so the realized

range of spatial autocorrelation was probably rather small in the simulated data.

LBP*b* compared the rejection rates of the two analysis methods to draw conclusions about their relative statistical power. The problem is that since H_0(raw data) is not the same as H_0(distance), there is no reason to expect the same rejection rate even if the power of both analysis methods is identical. Differences in rejection rates simply indicate that the simulated data happened to conform better with one model than the other, and drawing conclusions on the statistical superiority of one analysis method over the other on this basis is unjustified.

LBP*b* wrote that the Mantel test "was unlikely to detect a species–environment relationship or a spatial structure when such an effect was present in the data" without acknowledging that the Mantel test is not meant to target such questions. The Mantel test is designed to address questions like: Is dissimilarity in community composition correlated with geographical distance? If one is interested in more specific species–environment relationships or in documenting such spatial structures that are not adequately modeled by a linear correlation between distance matrices, then one should use some other method than the Mantel test. RDA is a possibility here, but the reason for choosing RDA over the Mantel test is not the low power of the Mantel test, but the fact that one is interested in a question that the Mantel test does not answer.

The difference between RDA and the Mantel test is loosely comparable with the difference between a measuring tape and a speedometer, respectively. If one is interested in the geographical position of a kite but not in its speed, a measuring tape is a useful tool, whereas a speedometer is not, and vice versa. Even if the measuring tape would give more accurate readings than the speedometer, the measuring tape still does not measure speed, and using it for that purpose is not an option. Comparing the results of RDA and the Mantel test as if they had answered the same statistical question is just as wrong as comparing the readings of a measuring tape and a speedometer as if they had quantified the same physical variable.

Methodological issues with variation partitioning on distance matrices

Under *Partitioning on distance matrices*, LBP*b* wrote that before using multiple regression on distance matrices "one should first show (1) that it produces approximately correct estimates of the fractions of variation; (2) that the fractions of variation thus isolated are additive; (3) whether families of variables (e.g., environment) should be represented by a single synthetic distance matrix or by one matrix per variable [...]; and (4) how the R_M^2 coefficients and the fractions of variation should be interpreted."

The answer to points (1), (2), and (4) is already available in the statistical literature. Multiple regression on distance matrices was developed in the 1980s, and, e.g., Smouse et al. (1986) and Legendre et al. (1994) explicitly stated that it is mathematically similar to the usual linear multiple regression in all respects but one: The statistical significance of the partial regression coefficients and R^2 values have to be estimated using permutations, because cell values in a distance matrix are not independent of each other.

When Legendre et al. (1994) used multiple regression on distance matrices, they interpreted the R^2_M values just like they would interpret the R^2 values in any multiple regression, namely in terms of the proportion of the variation in the response variable that is explained by the multiple regression model. Legendre et al. (1994) were not concerned about possible biases in R^2_M at all, and the only methodological aspect they discussed at length was how to obtain an appropriate permutation test for different kinds of dissimilarity matrices.

Points (1), (2), and (4) were of no concern to Borcard et al. (1992) when they proposed to obtain a variation partitioning on the basis of multiple regression as implemented in RDA and CCA. The possibility to perform variation partitioning follows logically from how multiple regression works, and the fractions of variation thus obtained are additive by definition. Therefore, the theoretical answer to these questions was already known, and a computer program (Canoco) was already available that had solved the practical problem of running the computations.

When we started using multiple regression on distance matrices for variation partitioning as proposed by Duivenvoorden et al. (2002), we followed the same logic: The theory of multiple regression analysis was already known, and the computer program Permute! was available to run the computations. In their concluding paragraph, Legendre et al. (1994) had even written: "This method may also be useful for other problems, where several types of distance matrices are simultaneously considered: [...] explanation of the community, disease or behavior structure by different types of biotic, abiotic and spatial variables." We really cannot understand why LBP*b* now write "the validity of multiple regression on phylogenetic distance matrices does not warrant the extension to variation partitioning on ecological distance matrices."

As to point (3), of course one can combine all environmental variables into one "environment" dissimilarity matrix, just as one can combine all environmental variables into one "environment" index in RDA or any other (multiple) regression method. The reasons for doing so or not doing so depend on the details of the study no matter which method is used. In most cases, combining explanatory variables just reduces the flexibility of the regression model and leads to loss of information. However, sometimes the questions asked dictate that variables be combined. For example, if one is interested in the effect of the straight-line geographical distance between sites, a single distance matrix combining latitude and longitude needs to be used rather than separate distance matrices for the east–west and north–south directions. If there are many explanatory variables and few sites, one may also wish to reduce the number of explanatory variables included in the analysis by combining some of the available variables into a synthetic index. Obviously, results based on such an index have to be interpreted in terms of the index and not in terms of the original variables that were used to derive the index. This is the case with both level-1 data and level-2 data, so we cannot see why combined variables should be a problem in multiple regression on distance matrices but not in multiple regression on raw data.

Both Laliberté and LBP*b* questioned the additivity of the fractions resulting from variation partitioning on distance matrices on the basis of similar arguments, which LBP*b* formulated as follows: "different total amounts of explained variation for the response **Y** are obtained if one includes all explanatory variables in a single distance matrix or if separate distance matrices are computed for the various explanatory variables. This clearly shows that variation partitioning based on distances lacks the essential property of additivity." The argument is based on a misrepresentation of distance-based variation partitioning and has no relevance to the property of additivity. This is because the explanatory variables in distance-based variation partitioning are distance matrices, not the original variables on which the distance matrices are based.

For example, the explanatory variables in a variation partitioning analysis could be $D(A)$, $D(B)$, $D(C)$, and $D(G)$, where $D(A)$, $D(B)$, and $D(C)$ are distances based on the environmental variables A, B, and C, respectively, and $D(G)$ is geographical distance based on latitude and longitude. Then distance-based variation partitioning involves comparing the R^2 values from three multiple regression analyses: one including only $D(G)$; one including $D(A)$, $D(B)$, and $D(C)$; and one including all four distance matrices. The fractions of variation obtained on this basis are additive by definition.

Of course, one can also run a separate variation partitioning analysis that uses the distance matrix **D**(A,B,C), which combines information from all three environmental variables into a single distance matrix, or $D(A + B + C)$, where the distances are based on the sum of the original variables. The results of the three analyses can be expected to differ because they use different explanatory variables. The situation is similar in the raw-data approach. One can run one variation partitioning analysis using A, B, and C as three separate explanatory variables, and another variation partition-

ing analysis where the three environmental variables are combined into a single explanatory variable such as $A + B + C$. The results of the two analyses can be expected to differ because they use different explanatory variables. However, the fact that different analyses can combine the available environmental variables in different ways has no bearing on whether the fractions isolated in any one analysis are additive or not.

LBP*b* claimed further that since it is not known how to obtain adjusted coefficients of determination for multiple regression on distance matrices, variation partitioning cannot be based on this method. A formula for calculating adjusted R^2 values for multiple regression on raw data has been available for a long time, but was apparently only applied to RDA-based analyses in 2006 (Peres-Neto et al. 2006). CCA/RDA-based variation partitioning is still commonly done with the unadjusted R^2 values. We see no reason why the distance approach should be treated more stringently in this respect than the raw-data approach.

We do agree that it would be useful to know how to calculate adjusted R^2 values for the distance approach, and hope that someone does the necessary theoretical work soon. LBP*b* mentioned several problems in this, but these seem more imaginary than real. The purpose of the adjusted R^2 value is to take into account the number of degrees of freedom in the model, and only variables that are free to vary in relation to each other within the model affect its degrees of freedom. Therefore, when LBP*b* asked "Should we use $m = 1$ for a single explanatory matrix $\mathbf{X}$ in the regression, or should we make m equal to the number of original variables that were included in the calculation of the distances leading to $\mathbf{X}$?", they actually ignored their own answer to this question a few lines earlier: "m is the number of explanatory variables in the model." A synthetic index used in RDA is one explanatory variable even if it was originally derived from several kinds of measurements. The same principle must be applied in the distance case: Each distance matrix in the regression model counts as one explanatory variable. Likewise, "Should n be the number of original objects (sites) or the number of distances in half or the whole distance matrix?" seems to have the logical answer in what LBP*b* wrote a few lines earlier: "n is the number of observations," i.e., the number of observation units, as this is what ultimately constrains the number of degrees of freedom in the model.

A final comment on levels of abstraction

Under *Question levels*, LBP*b* confuse ecological hypotheses with statistical ones. The level-2 hypotheses and level-3 hypotheses are statistical hypotheses, and each of them is derived from the corresponding ecological hypothesis directly and independently. We tried to make this distinction clear in TR in the section

Ecological vs. statistical hypotheses, but apparently did not succeed. The important point is that to derive a statistical hypothesis of a certain level of abstraction, it is sufficient to know what the corresponding ecological hypothesis predicts for this level of abstraction, it is not necessary to first formulate any statistical hypothesis at another level of abstraction.

For example, the ecological hypothesis "soil N content affects the occurrences of plant species" allows deriving the prediction "sites with similar soil N contents should have more similar species compositions than sites with more different soil N contents." This prediction can be tested with the level-3 statistical hypothesis "similarity in plant community composition is positively and linearly correlated with similarity in soil N content." The same ecological prediction can also be tested with other level-3 statistical hypotheses, such as "similarity in plant community composition is positively and linearly correlated with similarity in the logarithm of soil N content." Both level-3 statistical hypotheses can be derived without reference to any level-2 statistical hypothesis. Possible level-2 statistical hypotheses derived from the same ecological hypothesis include "plant species abundances are linearly related with soil N content," "plant species abundances are linearly related with the logarithm of soil N content," "plant species abundances have a symmetric unimodal relationship with soil N content," and "plant species abundances have a symmetric unimodal relationship with the logarithm of soil N content." When deriving a level-3 statistical hypothesis, one does not need to worry about which of the four level-2 statistical hypotheses would be most appropriate, because the level-3 statistical hypotheses are not derived from any level-2 statistical hypothesis. Indeed, they cannot be, because the level-2 statistical hypotheses concern how species abundances vary in relation to soil N content, whereas the level-3 statistical hypotheses concern how dissimilarity in community composition varies with difference in soil N content.

We cannot understand why LBP*b* categorically advise researchers to avoid level-3 analyses. In our opinion, this unnecessarily limits the scope of ecological questions that can be addressed statistically. When researchers are interested in beta diversity, they may legitimately approach it using different paths and different levels of abstraction. In TR, we were only concerned with the community composition path. In that context, the level-2 questions treat regional beta diversity as the variation to be partitioned (or explained), and the response variable is community composition; the level-3 questions treat pairwise beta diversity as the response variable, and its variation is what gets partitioned (or explained). Although not discussed in TR, other paths are also available. In the context of the regional beta diversity path, the level-2 questions treat regional beta diversity as the response variable, and its variation is what gets

partitioned (or explained). In the context of the species identity path, level-2 questions treat a beta diversity index as that part of the regional diversity index that can be explained by categorical explanatory variables.

The most important questions that researchers should ask themselves when choosing a statistical analysis method are: (1) Does the analysis target the response variable of interest? (2) Does the statistical null hypothesis of the analysis relate the response and explanatory variables to each other in a way that is relevant to the ecological question at hand? Since both the response variables and the formulation of the possible null hypotheses differ between level-2 analyses and level-3 analyses, the answers to these two questions should be sufficient to justify a choice between the raw-data and distance approaches.

Conclusions

One of the main purposes of TR was to draw attention to the fact that analyses focusing on different response variables address different questions, and one may miss one's target question if one uses an analysis method that focuses on the wrong response variable. The commentaries of PCD, Laliberté, and LBP*b* highlight this even more: It is of utmost importance that researchers are aware of what the response variables in their analyses are. One can miss one's target in several ways, for example, by advancing too far or not far enough along the right path, or by taking the wrong path to start with.

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