

A temporal beta-diversity index to identify sites that have changed in  
exceptional ways in space-time surveys

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Running headline: Temporal beta diversity analysis

2 tables, 6 figures in main text. 5 Appendices.

## 15 Abstract

16 **Aim** This paper presents the statistical bases for Temporal Beta diversity analysis, a method to study  
17 changes in community composition through time from repeated surveys at several sites. Surveys of that  
18 type are presently done by ecologists around the world. A Temporal Beta-diversity Index (TBI) is  
19 computed for each site, measuring the change in species composition between the first (T1) and second  
20 surveys (T2). TBI indices can be decomposed into losses and gains; they can also be tested for  
21 significance, allowing one to identify the sites that have changed in composition in exceptional ways.  
22 This method will be of value to identify exceptional sites in space-time surveys carried out to study  
23 anthropogenic impacts, including climate change.

24 **Innovation** The null hypothesis of the TBI test is that a species assemblage is not exceptionally  
25 different between T1 and T2, compared to assemblages that could have been observed at this site at T1  
26 and T2 under conditions corresponding to  $H_0$ . Tests of significance of coefficients in a dissimilarity  
27 matrix are usually not possible because the values in the matrix are interrelated. Here, however, the  
28 dissimilarity between T1 and T2 for a site is computed with different data from the dissimilarities used  
29 for the T1–T2 comparison at other sites. It is thus possible to compute a valid test of significance in that  
30 case. In addition, the paper shows how TBI dissimilarities can be decomposed into loss and gain  
31 components (of species, or abundances-per-species) and how a B-C plot can be produced from these  
32 components, which informs users about the processes of biodiversity losses and gains through time in  
33 space-time survey data.

34 **Main conclusion** Three applications of the method to different ecological communities are presented.  
35 This method is applicable worldwide to all types of communities, marine and terrestrial. R software is  
36 available implementing the method.

37 **Keywords** Beta diversity, B-C plots, space-time analysis, statistical power, temporal beta diversity,  
38 temporal beta diversity index (TBI), type I error.

## 39 Introduction

40 Community ecology is the scientific study of the interactions among species in natural communities,  
 41 their distribution through space and their evolution through time, and of the relationships between the  
 42 species and their environment. Changes in community composition through time are at the centre of  
 43 community ecology research (Pickett et al., 1987; McEwan et al., 2011; Vellend, 2016), including the  
 44 nature of these changes (e.g. gains and losses of species) and their quantitative importance.

45 In the spatial context, the variation in community composition among sites in a region of interest  
 46 has been called beta diversity by Whittaker (1972), who defined the well-known concepts of alpha,  
 47 beta and gamma diversities. In recent years, interest of ecologists and managers has turned to the study  
 48 of the temporal variation in community composition, either at a single site or at a series of sites  
 49 repeatedly surveyed across time. This temporal variation was called *temporal beta diversity* by  
 50 Legendre & Gauthier (2014) and Shimadzu et al. (2015). Temporal variation can be the result of  
 51 gradual or abrupt changes in environmental conditions, including man-induced alterations such as the  
 52 present worldwide climate warming.

53 Statistical inference methods have been proposed for the analysis of temporal changes in  
 54 community composition. For example, (a) the temporal convergence or divergence in composition of a  
 55 set of communities can be studied by testing for differences in multivariate dispersion among surveys  
 56 (Anderson, 2006); (b) shifts in mean composition of monitored communities can be statistically tested  
 57 using multivariate analysis-of-variance procedures (Legendre & Anderson 1999, Anderson 2001),  
 58 including null models (Schaefer et al., 2005); (c) the interaction between the factors space and time and  
 59 other complex spatio-temporal structures can be studied and tested for significance (Angeler et al.,  
 60 2009; Legendre et al., 2010; Legendre & Gauthier, 2014).

61 In several application fields, researchers want to compare observations made at several sites and  
 62 at two different times. The question of interest is: are there sites where the difference between survey  
 63 times seems exceptionally large? These sites would be worth examining in more detail to identify and  
 64 compare the causes of the differences. Here are some examples. (a) In palaeoecology, comparison of  
 65 ancient and modern diatom communities preserved in lake sediment cores may indicate areas where  
 66 acute anthropogenic processes have singularly changed the surrounding land use (e.g., Winegardner et  
 67 al., 2017). (b) When a strong natural or man-made or environmental impact has taken place at a known  
 68 point in time and an ecological community had been surveyed ahead of the impact, ecologists may  
 69 survey that community again to determine how it was affected by the impact, and then how it may have  
 70 recovered in later surveys (e.g., Legendre & Salvat, 2015). (c) In community ecology, when studying a  
 71 permanent stem-mapped forest dynamics plot divided into quadrats, examining surveys made at two  
 72 different times may indicate sections of the forest that have been exceptionally affected by a  
 73 disturbance, e.g. a climatic or anthropogenic event (e.g., Legendre & Condit, 2019).

74 This paper describes a method to test, for several sampling units (objects), the differences  
 75 between data vectors corresponding to observations made at time 1 (abbreviated T1) and time 2 (T2). I  
 76 will refer to these objects as sites in this paper, although they may be of other natures, e.g. experimental  
 77 enclosures. A dissimilarity  $D$  computed between times T1 and T2 for a site, using community  
 78 composition (occurrences, frequencies or biomass) or gene frequency data, is called a *Temporal Beta-*  
 79 *diversity Index* (TBI); it measures the change in community composition (or temporal beta diversity)  
 80 from T1 to T2. A change through time is directional; species presences, species abundances or gene  
 81 frequencies may have been gained and/or lost between T1 and T2. So, it will be of interest to examine  
 82 the loss and gain components of the TBI indices, in addition to the TBI index values and their  
 83 significance.

The observed data, assembled in matrices **Mat.1** for time T1 and **Mat.2** for T2 (Fig. 1), may be of different kinds; in landscape ecology and genetics, the data are community composition or population gene frequencies observed at different sites, the same in the two surveys. (1) The null hypothesis ( $H_0$ ) to be tested in the statistical testing part of the method is that a site is not exceptionally different in community composition between T1 and T2, for presence-absence or abundance data, compared to assemblages that could have been observed at this site at T1 and T2 under conditions corresponding to  $H_0$ . An exceptionally different site is a site with an index of dissimilarity between T1 and T2 that has an extreme value in the upper tail of the distribution of TBI index values. This value may also have been produced by a different (e.g. ecological) process than the one having generated most other values in the distribution. To determine how extreme a value has to be in the reference distribution before it is considered extreme, we will rely on the usual significance levels (e.g. 5%, 1%, etc.) of statistical analysis. (2) In the data representation part of the method, we will use the species losses and gains between T1 and T2 (for presence-absence or abundance data) to uncover the main ecological changes that have taken place between T1 and T2 in the study area or in subsets (geographic or environmental) of that area.

The paper is organised into sections as follows: *Methods*, *Numerical simulations*, *TBI analysis of environmental or trait data*, *Ecological applications* where the method is illustrated by three case studies, and *Concluding remarks and prospective applications*.

## Methods

The proposed method consists basically in the following analyses of temporal variation, detailed in the following subsections. (1) *Computation and testing*: (1.1) a Temporal Beta-diversity Index ( $TBI_i = D_i$ ) is computed for each site  $i$  between the data vectors corresponding to T1 and T2 (Fig. 1), using an appropriate dissimilarity index ( $D$ ). Then (1.2), when it is pertinent to the problem at hand, the indices can be tested for significance using a permutational procedure. (2) *Partitioning dissimilarities into losses and gains*: four of the  $D$  indices that can be used in this type of analysis (two indices for abundance and the corresponding two indices for presence-absence data) also allow the computation of species losses and gains at each site between T1 and T2. These statistical elements provide users with detailed information, at the site level, about the response of the community to the event or change that occurred between T1 and T2. (3) The loss and gain statistics can be used together to draw *B–C plots*, which illustrate whether the temporal changes at the various sites are dominated by gains or by losses. (4) The *Software* subsection lists the R software available to carry out the analyses.

### 1. Temporal Beta-diversity Indices (TBI): computation and testing

Since Whittaker (1972), ecological dissimilarities have been used to measure beta diversity among sampling units. Koleff *et al.* (2003) reviewed 24 beta-diversity indices proposed in the literature while Legendre & De Cáceres (2013) described 14 properties of 11 dissimilarity indices that are appropriate for beta-diversity studies. The Ružička dissimilarity (1958) was identified by Podani *et al.* (2013) and by Legendre (2014) as another appropriate index for beta-diversity studies.

Some of these indices are used as TBI indices in this paper: the percentage difference (Odum, 1950; sometimes abbreviated to %diff in the present section of the paper; this index is also known as the Bray-Curtis dissimilarity; see Legendre & De Cáceres, 2013, Table 1, about the application of the anteriority rule for the name of this index) and Ružička (1958) indices, as well as corresponding forms for presence-absence data, known as the Sørensen and Jaccard indices. Indices in this group of four contain in their equations the quantities  $B$  and  $C$ , which represent the species loss and gain components of these dissimilarities. The chord, Hellinger, and log-chord distances, which are widely used by

community ecologists, are also applicable as TBI indices, although they cannot be decomposed into losses and gains. All these indices can be subjected to the test procedure described in subsection 1.2.

Tests of significance for dissimilarity coefficients ( $D$ ) are usually not possible because the  $D$  values in a dissimilarity matrix are obtained from the computation of an index between all pairs of objects, e.g. sites in ecology (their number is  $n$ ), and are thus interrelated, each site contributing to  $(n - 1)$  of the dissimilarities in the half-matrix of dissimilarities. In the T1–T2 comparisons for individual sites, however, the dissimilarity between T1 and T2 for a site is computed for the data from that site only, and these differ from the data involved in the T1–T2 comparisons of other sites. Hence, it would be possible to compute a valid test of significance in that case (Methods, subsection 1.2).

### 1.1. Computation of Temporal Beta-diversity Indices (TBI)

Consider two data matrices, **Mat.1** and **Mat.2**, about the same objects observed at times T1 and T2; each matrix has  $n$  sites (with indices  $i$ ) in rows and the same  $p$  variables (e.g. species, with indices  $j$ ) in columns (Fig. 1). Individual values may be noted  $y_{ij,1}$  for T1 and  $y_{ij,2}$  for T2. Compute the dissimilarity  $D(\mathbf{y}_{i,1}, \mathbf{y}_{i,2})$  between the row vectors of values,  $\mathbf{y}_{i,1}$  and  $\mathbf{y}_{i,2}$ , for each site  $i$ . These dissimilarities form a vector of length  $n$ , which is the number of sites.

The percentage difference dissimilarity ( $D_{\%diff}$ , method "%difference" in the R function TBI.R, also known as the Bray-Curtis index in other computer packages), and the Ružička dissimilarity ( $D_{Ruz}$ , method "ruzicka" in the R function; see *Software* in subsection 4 below) can be used for beta diversity assessment. They are obtained by computing a dissimilarity function (equations shown below). With presence-absence data, the percentage difference produces the  $(1 - S_{Sørensen})$  dissimilarity whereas the Ružička dissimilarity produces  $(1 - S_{Jaccard})$ , where  $S$  designates a similarity index.

The chord, Hellinger, and log-chord distances are members of the Box-Cox family of distances (Legendre & Borcard 2018). They are classical indices for beta diversity studies (Legendre & De Cáceres 2013). Their calculation involves two steps: first the calculation of a transformation of each data row (i.e. the chord transformation, the Hellinger transformation, or the transformation  $\log(y+1)$  followed by the chord transformation; Legendre & Borcard 2018), followed by calculation of the Euclidean distance. These indices, as well as the Euclidean distance itself, are also implemented in the TBI.R function and will be used in the simulations and in *Ecological application 1* below, although they are less interesting for the comparison of community composition matrices than the percentage difference and Ružička indices, which provide additional information about losses and gains of species. The indices in the Box-Cox family are available in the computer function for TBI analysis to ensure compatibility with other multivariate analyses that users may want to do using these popular indices.

When the percentage difference or the Ružička dissimilarity are used as TBI indices, one can compute two derived indices to study the directionality of the change through time at each site, as proposed by Legendre & Salvat (2015). Consider data vectors  $\mathbf{y}_1$  and  $\mathbf{y}_2$  corresponding to the multi-species observations at T1 and T2 for a focal site of interest. The following calculations can be done on these vectors:

- $A_j$  is the part of the abundance of species  $j$  that is common to the two survey vectors:  $A_j = \min(y_{1j}, y_{2j})$ .  $A$  is the sum of the  $A_j$  values for all species. It represents the unscaled *similarity* between two surveys.
- $B_j$  is the part of the abundance of species  $j$  that is higher in survey 1 than in survey 2:  $B_j = (y_{1j} - y_{2j})$  if  $y_{1j} > y_{2j}$ ;  $B_j = 0$  otherwise.  $B$  is the sum of the  $B_j$  values for all species. It is the unscaled sum of *species losses* between T1 and T2.

•  $C_j$  is the part of the abundance of species  $j$  that is higher in survey 2 than in survey 1:  $C_j = (y_{2j} - y_{1j})$  if  $y_{2j} > y_{1j}$ ;  $C_j = 0$  otherwise.  $C$  is the sum of the  $C_j$  values for all species. It is the unscaled sum of *species gains* between T1 and T2.

The values  $A$ ,  $B$  and  $C$  are the building elements of the percentage difference,  $D_{\%diff} = (B+C)/(2A+B+C)$ , and the Ružička dissimilarity,  $D_{Ruz} = (B+C)/(A+B+C)$  (Podani et al. 2013; Legendre 2014, Table S1.2). These two indices are interchangeable for TBI comparison although their values are not monotonically related.  $(B+C)$  represent the unscaled dissimilarity. The sign of  $(C - B)$  indicates the directionality of the process of losses and gains of individuals of the different species between the two surveys.  $B$  and  $C$  can be scaled by division by a denominator  $den$ , which is  $den_{\%diff} = (2A+B+C)$  for  $D_{\%diff}$  index and  $den_{Ruz} = (A+B+C)$  for the  $D_{Ruz}$  index. The  $D_{\%diff}$  and  $D_{Ruz}$  dissimilarities measure the temporal beta diversity, or temporal change in community composition, for a site. The scaled  $B$  and  $C$  statistics can be called  $D_{loss}$  and  $D_{gain}$ , where  $D_{loss} = B/den$  and  $D_{gain} = C/den$ . An interesting relationship is that  $D_{loss} + D_{gain} = D_{\%diff}$  or  $D_{Ruz}$ , depending on the denominator,  $den_{\%diff}$  or  $den_{Ruz}$ , that is used. In other words,  $D_{loss}$  and  $D_{gain}$  partition the  $D_{\%diff}$  or  $D_{Ruz}$  dissimilarities into *loss* and *gain* components. Values of these indices are in the  $[0,1]$  range and are thus directly comparable.

The loss and gain statistics can be computed for occurrence (i.e. presence-absence) data as well, because  $D_{\%diff}$  becomes the Sørensen dissimilarity with occurrence data and  $D_{Ruz}$  becomes the Jaccard dissimilarity, as mentioned above. The Sørensen and Jaccard dissimilarity equations for occurrence data are represented in book and paper equations with lower-case instead of upper-case letters (e.g. in Koleff *et al.* 2003, Legendre & Legendre 2012, Legendre & De Cáceres 2013); their equations, which exactly correspond to those of  $D_{\%diff}$  and  $D_{Ruz}$ , are  $D_{Sorensen} = (b+c)/(2a+b+c)$  and  $D_{Jaccard} = (b+c)/(a+b+c)$ .

What are the ecological applications of  $D_{loss}$  and  $D_{gain}$ ? For each site, one can explore which process, between  $D_{loss}$  and  $D_{gain}$ , shows the largest contribution to the temporal  $D_{\%diff}$  or  $D_{Ruz}$  dissimilarity; in other words, which process is dominant at each site. The means of the  $D_{loss}$  and  $D_{gain}$  components across the sites express the dynamics of the community over all sites. For observations across a large number of sites within a region, or (e.g.) in all quadrats of a stem-mapped dynamics forest plot, the  $B/den$  and  $C/den$  statistics can be mapped, plotted as species losses and gains in the new B-C plots described in subsection 3 (see example in Ecological application 3), or studied in other ways (e.g. in Ecological application 2).

## 1.2. Testing procedure

When it is pertinent to the problem at hand, TBI indices can be tested for significance. An example is found in Ecological application 1. The data are permuted at random in both matrices, as described below, and the index is recomputed. This procedure is repeated a large number of times and a p-value is calculated for the TBI difference between T1 and T2 at each site  $i$ . A detailed description of the permutation method follows.

1. The null hypothesis ( $H_0$ ) of the TBI test is that a species assemblage at a focal site  $i$  (i.e. a site under study) is not exceptionally different between T1 and T2, compared to assemblages that could have been observed at the same two times at this site. The test involves a comparison of the TBI index computed for site  $i$  with other TBI indices obtained by randomization of the observed data under conditions corresponding to  $H_0$ . The null hypothesis focuses on values of the TBI statistic, which is the dissimilarity between data vectors observed at times T1 and T2 for site  $i$ . The statistical decision (one-tailed test) is taken as in any other parametric or permutational test; see paragraph 7 below.

2. Under the general hypothesis that the site data are permutable, the values in each column of **Mat.1** are permutable within that matrix, and similarly for **Mat.2**. The variation of each species among sites, in a given matrix, is assumed to be due to random sampling of a statistical population. Permutations are done for each species separately, following the concept that different species in an assemblage are under the influence of a variety of processes and do not form a pseudo-organismic entity that would react as a unit to these varied processes. More about this in Note 1 at the end of the present subsection. Technical aspects of this permutation method are described in paragraph 3 underneath. Permutation of the species occurrence or abundance values in each column, independently of one another, was also the method used to assess the significance of the *Local Contributions to Beta Diversity* (LCBD indices, describing the contributions of individual sites) in the Legendre & De Cáceres (2013) paper. The same logic is followed here to test the significance of TBI indices, which are also indices about individual sites.

3. The data are permuted columnwise, species by species, and in exactly the same way in matrices T1 and T2 (Fig. 2). To accomplish that in a computer function, a given permutation of the two matrices is started with the same random seed in both, and that seed is changed at the beginning of each new permutation. With this method, the abundance values of a given species at site  $i$  (e.g. site 1) in matrices T1 and T2 of the original data will be shifted to another site position (e.g. site 9) in both matrices in the permuted data. What is permuted is then a series of *differences* between T1 and T2, for each species separately.

4. In the TBI test, we are looking for site vectors whose dissimilarities between T1 and T2 would be exceptionally large. We are not interested in a systematic difference that would affect all sites concerning the loss or gain of a subset of the species, or of all species. Differences of this type are preserved, through the permutations, by *not permuting* data between **Mat.1** and **Mat.2**. Simulations reported in Appendix A1 showed that the test performed adequately in this respect; see Figs. A1.2 and A1.4 and the associated tables of simulation results in that appendix.

5. If the selected TBI index is the chord, Hellinger or log-chord dissimilarity, a transformation is applied to the data before calculation of the Euclidean distance, as described in subsection 1.1 of the Methods. For correct calculation of these indices under permutation, the transformation is recomputed on the permuted untransformed data matrices. This is necessary to make sure that the permuted data are transformed in the same way as the initial data, with row sums (for Hellinger) or row norms (for chord) being 1. In this way, the  $D_i$  values of the permuted data remain comparable to the reference  $D_i$ .

6. After permutation, the TBI distances between **Mat.1** and **Mat.2** are recomputed for all sites  $i$  separately.

7. After a large number of permutations, a p-value is computed for each site  $i$ , as in any other permutation test. The test is one-tailed in the upper tail since we are looking for sites with large TBI statistic values. Thus, a site that changed very little while the other sites changed a lot will not be found exceptionally different by this test, which was designed to identify sites where the change is greater than at most pseudo-sites that can be produced by randomizing the data. If the one-tailed p-value is smaller than or equal to the significance level, e.g. 0.05,  $H_0$  is rejected. Rejection may be due to two different situations that are both of interest to ecologists: (1) a TBI value in the upper tail of the statistical distribution may correspond to a site subjected to the same process as the other sites in the study, that site having produced a high TBI value by chance. Although this technically corresponds to a type I error, these extreme sites in the statistical distribution may still be interesting to examine. (2) Else, rejection may indicate a site where some special event has taken place between T1 and T2, different from what happened at other sites, causing a large difference to appear in community

composition. Examples are climatic and geologic events, or the result of anthropogenic actions or processes. In both cases, the significant sites are of interest to ecologists who can concentrate work on them and investigate why community composition has changed in an exceptional way at these locations. Because the TBI indices for  $n$  sites are all tested at the same time, a correction for multiple testing must be applied to obtain a correct experiment-wise error rate.

Note 1: *Ecological interpretation of the permutation models* — The permutation procedure described in paragraphs 2 and 3 above, whereby the values are permuted separately for each species, follows a concept of species assemblages that considers the species in an assemblage to be under the influence of a variety of processes; they do not form a pseudo-organismic entity that would react as a unit to these processes. The first formulation of this concept is due to Gleason (1926) who argued that species responded individually to environmental conditions. He was opposing Clement's (1916) dominant view of the time that species assemblages formed an entity that reacted as a kind of pseudo-organism. A permutation method based on Clement's pseudo-organismic theory would permute at random entire rows of data in matrices **Mat.1** and **Mat.2**, not the values observed for each species separately. Most ecologists nowadays reject the pseudo-organismic view of Clement and favour an alternative view, which is an extension of Gleason's, involving different ecological processes. The most important are environmental filtering (as in Gleason's theory), neutral processes which include ecological drift and limited dispersal (Hubbell 2001), and interactions among species, all of which generate spatial structures in community data (Legendre 1993, Legendre & Legendre 2012). Permutation of values in individual species vectors, used in this paper and in the TBI.R function, follows this view about ecological communities.

Note 2 — A different permutation method is used in tests of significance in linear statistical modelling (regression, RDA, CCA). In canonical analysis, this method consists in permuting entire rows of data in either the response or the explanatory matrix. This method implements Clement's view described in the previous paragraph. That permutation method was used in additional simulations. Results of these simulations are not reported in detail in Appendix A1 but they are summarized in the second paragraph of section "b. Permutation methods" in that Appendix.

Note 3 — It is also possible to identify the species that have changed in a significant way between T1 and T2, either in the whole study area or in separate habitat types. This involves a different type of statistical test. The hypothesis ( $H_0$ ) that a species has not changed in abundances throughout the sites under study can be tested using a paired  $t$ -test. Because species abundances are not normally distributed, a permutation test must be used. The permutations involve the two facing values of the species observed at each site at T1 and T2. A correction for multiple testing must be applied to the  $p$ -values because several species are tested simultaneously. This method was used in the Legendre & Condit (2019) companion paper. An R function implementing that test is available; see subsection *Software* (below), first paragraph.

## 2. Partitioning dissimilarities into losses and gains

When the percentage difference or the Ružička dissimilarity are used as TBI indices,  $B$  is the unscaled sum of *species losses* and  $C$  is the unscaled sum of *species gains* between T1 and T2. These statistics are described in subsection 1.1 above. The unscaled statistics can be scaled to the  $[0,1]$  range by division by the percentage difference denominator  $den = (2A+B+C)$  or by the Ružička denominator  $den = (A+B+C)$ . The *dissimilarity*  $D$  is  $(B/den + C/den) = (B+C)/den$ . If the TBI dissimilarity is either the percentage difference or the Ružička dissimilarity, one can take advantage of this decomposition of  $D$  and list the  $B/den$  and  $C/den$  components of TBI indices for each site in the study. Examples are shown in Appendices A3 and A4, which complement Ecological applications 1 and 3. These basic statistics



303 can be used in two different ways:

304 2.1. We can compute summary statistics: the mean of  $(B/den)$ , the mean of  $(C/den)$  and the mean  
 305 of  $D = (B+C)/den$ . The following relationship holds:  $mean(B/den) + mean(C/den) = mean(D)$ . From  
 306 this decomposition of  $D$ , we can derive the contribution of the species losses to the total dissimilarity,  
 307  $B/(B+C)$ , and similarly the contribution of the species gains to the total dissimilarity,  $C/(B+C)$ . These  
 308 two ratios sum to 1, providing the relative importance of the species losses and gains phenomena. The  
 309 result is the same for calculation without a denominator  $den$ , or with either the percentage difference or  
 310 the Ružička denominator.

311 2.2. For each site, we can also obtain the sign of the difference (gains – losses), or  $(C - B)$ : if  $C >$   
 312  $B$  we note a plus (+) sign, and if  $C < B$  we note a minus sign (–) in the function output list. This  
 313 notation allows users to quickly identify the sites where gains or losses dominate. Similarly, the  
 314 difference  $mean(C/den) - mean(B/den)$  is computed; its sign tells us if gains (+ sign) or losses (– sign)  
 315 dominate across all sites. The significance of the difference between the two vectors of statistics  $B/den$   
 316 and  $C/den$  can be computed using a parametric or permutational paired  $t$ -test; the TBI function  
 317 mentioned in *Software* (subsection 4 below) computes both forms. These tests provide overall  
 318 indications of the direction of change in community composition over all sites. They help confirm the  
 319 asymmetry between abundance or occurrence losses ( $B/den$ ) and abundance or occurrence gains  
 320 ( $C/den$ ). In Ecological application 2 (Tikus Island coral communities), the two forms of calculation, on  
 321 abundance and occurrence data, provided complementary information.

### 322 3. Species losses and gains: the B-C plot

323 We can also use the  $B/den$  and  $C/den$  statistics as coordinates of points (representing sites) in bivariate  
 324 graphs with  $B/den$  in the abscissa and  $C/den$  in the ordinate. We call these graphs *B-C plots*. They  
 325 display visually the relative importance of the loss and gain processes across the study sites, informing  
 326 researchers about the detailed and global structure of the species losses and gains.

327 A B-C plot is presented in Ecological application 3 (Chesapeake Bay benthos data). In that B-C  
 328 plot, a diagonal green line, with slope of 1, was drawn through the origin; it represents the theoretical  
 329 positions of sites where  $D_{gain}$  would be equal to  $D_{loss}$ . A red line was also drawn parallel to the green  
 330 line, passing through the centroid of all points. When the red line is below the green line, it indicates  
 331 that the survey interval was dominated by species losses across the sites, and the opposite if the red line  
 332 is above the green line. Points found higher in the plot, in the diagonal direction towards the upper-  
 333 right corner, represent higher temporal beta diversity than points found lower in the direction of the  
 334 lower-left corner.

335 In B-C plots, the points representing sites can be labelled with colours or symbols representing  
 336 the types of environment, the geographic areas where they come from, or any other independent  
 337 classifier of interest. Separate B-C plots can be drawn for sites surveyed in different types of  
 338 environment, although all sites may have been analysed in the same TBI analysis. Comparison of these  
 339 separate plots will immediately show which types of environment have produced mostly losses or gains  
 340 in species occurrences or abundances.

### 341 4. Software

342 These calculations are implemented in the *TBI.R* function available in the R package *adespatial* (Dray  
 343 et al. 2019). Function *draw.BC.R* is also available in *adespatial* to draw B-C plots. Examples of output  
 344 files of the TBI function are shown in Appendices A3 and A4, which complement Ecological  
 345 applications 1 and 3. Function *paired.t.test.spec.R* can be used to identify the species whose

346 abundances have changed in a significant way between T1 and T2. That function is described in  
 347 Appendix A5; it is also available in the R package *adespatial* on CRAN.

348 Another R package, *codyn* (Hallett et al., 2016, 2018), computes a variety of metrics for temporal  
 349 diversity analysis. The percentage difference  $D$  is used as one of the metrics for temporal comparison  
 350 of communities observed at different times at a focal site. Function *turnover* of that package includes  
 351 an option to compute species losses and gains divided by the %difference denominator, as in the *TBI.R*  
 352 function; losses and gains are the indices called  $B$  and  $C$  in the present paper.

## 353 Numerical simulations

354 Numerical simulations were used to check the type I error rate and power of the permutation method  
 355 described in subsection 1.2 above. The data simulation methods and results are described in detail in  
 356 Appendix A1. A summary of these results is presented here, with recommendations to users.

### 357 Simulation to estimate type I error rates

358 The simulation results reported in Appendix A1 show that the TBI tests had correct rates of type I error  
 359 for the two community-like data generation methods (Poisson and lognormal) and all dissimilarity  
 360 indices available in the *TBI.R* function, and this for all significance levels ( $\alpha$ ) considered, from  $\alpha =$   
 361 0.01 to  $\alpha = 0.50$ . The testing method is thus valid in all these circumstances (Edgington, 1995).

### 362 Simulations to compare power of $D$ indices

363 For the analysis of community composition data, the percentage difference and Ružička indices  
 364 produced tests with the highest power, followed by the indices in the Box-Cox family: the chord,  
 365 Hellinger and log-chord distances. The Euclidean distance alone produced TBI tests with extremely  
 366 low power with community composition-like data. This distance should not be used for TBI tests of  
 367 community composition data (Figs. A1.5 and A1.6, Appendix A1). However, power was high enough  
 368 to recommend the Euclidean distance for test involving standardized environmental data.

369 In summary, the best combination to obtain TBI tests of community data with maximum power is  
 370 to use the percentage difference or the Ružička indices. These two dissimilarities can also be  
 371 decomposed into species losses ( $B/den$ ) and gains ( $C/den$ ), which can be used to examine the processes  
 372 of losses and gains at the site level and produce B-C plots.

373 Additional simulations involving different numbers of sites with an effect and different total  
 374 numbers of sites showed that power of the test was high as long as the proportion of sites with an effect  
 375 to be detected (i.e. sites made to be exceptional) was smaller than  $n/2$ , where  $n$  is the total number of  
 376 sites in the study (Fig. A1.7, Appendix A1).

## 377 TBI analysis of environmental or trait data

378 It could be interesting to identify the sites where the changes in environmental conditions (e.g. land  
 379 use) were the most important. The TBI method can be used to compare two matrices containing the  
 380 same environmental variables observed at T1 and T2 and determine, for example, if these sites are also  
 381 those where the community has changed the most. The analysis of environmental variables is a  
 382 situation where the Euclidean distance would be appropriate as a basis for computing a TBI index.

383 • If all environmental variables are quantitative, they should be standardized using the same parameters  
 384 (means, standard deviations) for matrices T1 and T2, before they are used as input in TBI analysis.  
 385 How to do that is described and implemented in a function provided in Appendix A2.

• If the data are factors or a mix of quantitative and factor variables, one should join matrices T1 and T2 one on top of the other, as described in the explanation paragraph of Appendix A2, then compute a Gower dissimilarity matrix **D**. Apply principal coordinate analysis (PCoA) to the square-rooted Gower dissimilarities because a Gower **D** matrix is non-Euclidean. Square rooting should make the Gower matrix Euclidean, which is necessary before PCoA in this case; we have to recuperate and use all PCoA axes for TBI analysis, hence the values should be real and not complex numbers. Then, separate the two transformed matrices and use them as input into TBI analysis.

• For community trait matrices with mixed precision levels (quantitative and qualitative traits), use the same method as in the previous paragraph: compute a Gower dissimilarity matrix, as recommended by Laliberté & Legendre (2010), then PCoA of the square-rooted dissimilarities; split the principal coordinates into two matrices and compute TBI analysis using the Euclidean distance. No application of TBI analysis to environmental or trait data is presented in this paper to save space.

## Ecological applications

The three ecological applications that follow use multivariate community data. They were chosen to illustrate different facets of TBI analysis, not to draw ecological conclusions about these three particular ecosystems. Application 1 illustrates the importance of carrying out TBI analysis using a dissimilarity index designed for the analysis of community composition data; the Euclidean distance produced non-significant and un-interpretable results. In application 2, The *B* and *C* components of the TBI dissimilarity are used to analyse the effects of a major disturbance (El Niño) on communities; the analysis is complemented with a standard canonical analysis of the community data. Application 3 illustrates the construction and interpretation of a B-C plot.

### Ecological application 1 – Insecticide treatments in mesocosms

Observations on the abundances of 178 invertebrate species (macroinvertebrates and zooplankton) subjected to insecticide treatments in aquatic mesocosms (called “ditches”) were used by van den Brink & ter Braak (1999) as an application example in their paper describing Principal Response Curves (PRC) analysis. The authors agreed to make the data available to researchers in the CANOCO program documentation and in the R package *vegan* (Oksanen et al., 2017).

The experiment involved twelve mesocosms, which were surveyed on eleven occasions. Four mesocosms served as controls (dose = 0) and the remaining eight were treated once with the insecticide chlorpyrifos, with dose levels of 0.1, 0.9, 6.0 and 44.0 µg/L in two mesocosms each. The data are log-transformed species abundances,  $y_{tr} = \log_e(10y + 1)$ . In their paper, the authors used the log-transformed invertebrate data in PRC analysis; PRC preserved the Euclidean distance among the observations.

The 12 mesocosms had been attributed at random to the treatments. However, to facilitate presentation of the results, they will be presented here in order of increased insecticide doses: {0, 0, 0, 0, 0.1, 0.1, 0.9, 0.9, 6.0, 6.0, 44.0, 44.0} µg/L.

Results of the calculations with the R function *TBI()* are presented for the species abundance and occurrence data of this ecological application. We will compare data of surveys #4 and #11. Survey #4 was done one week after the insecticide treatment in the mesocosms, and the fauna was considered to have fully recovered from treatment at the time of survey #11. To give examples, in the two mesocosms that had received the highest insecticide doses, species richness increased by 9 and 19 species from survey #4 to #11.

The TBI dissimilarities showed that in the two mesocosms with the highest insecticide doses, community compositions were the most different between T1 and T2 (Table 1, upper panel). The test found the changes in these two communities to be exceptional with reference to the T1-T2 difference found in communities simulated by permutations of the data obtained during in the experiment. The two mesocosms that had received the highest doses of the insecticide, M11 and M12, showed exceptional differences in community composition for the percentage difference and Ružička dissimilarities (Table 1, lower panel). The chord, Hellinger and log-chord distances led to the same conclusion. These five distances had been deemed appropriate for beta diversity study (Legendre & De Cáceres, 2013). On the contrary, the Euclidean distance is known to be inappropriate for such studies (Orlóci, 1978; Legendre & Legendre, 2012) and, indeed, TBI tests based on that distance did not produce significant differences in community composition between surveys #4 and #11 in any of the mesocosms, including M11 and M12.

Detailed analysis of the species losses (*B/den*) and gains (*C/den*), obtained from TBI analysis computed with the percentage difference (Appendix A3, first section), showed that in the 8 treated mesocosms (called Sites 5 to 12 in Appendix A3), the changes in community composition (abundance data) always consisted of species gains; that is, statistic *C/den* (gains) was always larger than *B/den* (losses). The mean values of *B/den* and *C/den* for these 8 mesocosms showed that gains (*C/den*) represented 56% of the dissimilarities, as expected in a study of recovery after an insecticide treatment. The permutational paired *t*-test showed a highly significant difference ( $p = 0.0066$ ) between losses and gains across the 8 treated mesocosms.

TBI calculations using the Sørensen *D* (Appendix A3, section 2) indicated that, in addition to mesocosms #11 and 12, mesocosm #10, treated with 6 µg/L of insecticide, also displayed a significant difference between T1 and T2 at significance level 0.05. This result indicates that in the insecticide experiment, the reappearance of species (positive change) gave a clearer signal of community recovery than the increase in species abundances (Appendix A3, section 1)

## Ecological application 2 – South Tikus Island coral communities

Brown & Suharsono (1990) surveyed coral communities (75 species) at 10 sites in the island of South Tikus, Indonesia, in the years 1981, 1983, 1984, 1985, 1987 and 1988. An El Niño event occurred in 1982–1983, which caused coral bleaching and death of coral colonies, and triggered changes in the composition of coral communities. They reported that “as many as 80-90% of corals died on the reef flats at the study sites, with the major casualties being branching species in the genera *Acropora* and *Pocillopora*”.

Coral forms colonies which occupy surfaces, so that the data are not in numbers of individuals but in areal cover of each species. The sum of the species areal covers at a site may exceed 100% because coral colonies may overlap one another vertically. The Brown & Suharsono (1990) data have been used in several papers to demonstrate the application of multivariate methods for the analysis of beta diversity and the comparison of surveys across time, e.g. by Warwick et al. (1990), Anderson et al. (2011) and Chao & Chiu (1996). Following these papers, the data in the present application were treated as if they were species abundances. They were obtained from Appendix S1 of the Anderson et al. (2011) article.

The data were analysed in two complementary ways. (1) Fig. 3 presents an analysis of the species loss (*B/den*) and gain (*C/den*) components of the dissimilarity *D* between the 1981 survey, before the El Niño event, and the five following surveys: 1983, 1984, 1985, 1987 and 1988, for abundance and presence-absence data. (2) This analysis is completed with a redundancy analysis (RDA) biplot shown

in Fig. 4, showing the changes in community composition with time. This biplot was produced as follows: first, a percentage difference matrix was computed among all years and sites; the dissimilarities were square-rooted to make the matrix Euclidean, and that matrix was subjected to principal coordinate analysis (Gower 1966). The entire matrix of principal coordinates was used as the response data in a RDA against a factor representing the 6 survey years of the study. This form of canonical ordination is called distance-based redundancy analysis (dbRDA, Legendre & Anderson 1999).

We will examine the changes in community composition between the 1981 survey, before the El Niño event, and the following five surveys: 1983, 1984, 1985, 1987 and 1988. This study is not meant to identify sites that were exceptionally different between two years or test specific hypotheses about them. Instead of testing the TBI statistics for sites, we will carry out a detailed study of the species loss ( $B/den$ ) and gain ( $C/den$ ) statistics described in the Methods. These statistics were computed with the denominator ( $den$ ) of the percentage difference index,  $D_{\%diff}$ ; they decompose the percentage difference  $D$  into additive components, losses ( $B/den$ ) and gains ( $C/den$ ).

First, we will plot the mean values of the  $B/den$ ,  $C/den$  and  $D$  statistics computed across the sites, in comparisons of the 1981 survey (before the El Niño event) with all successive surveys in turn (1983, 1984, 1985, 1987 and 1988, after El Niño) (Fig. 3), in order to study the effect of the El Niño event on the communities. This method of analysis had been used by Legendre & Salvat (2015, Fig. 3), who described the effects of a nuclear test on the mollusc communities of an atoll in the Pacific.

Fig. 3a shows the changes in  $D$  between years, and its components  $B/den$  and  $C/den$ . We observe that after El Niño, species losses ( $B/den$ ) dominated the changes, accounting for 96% of the mean dissimilarity ( $D$ ) between 1981 and 1983; species gains ( $C/den$ ) represented only 4% of mean  $D$ . In later years, the species losses decreased whereas gains increased till the 1981-1985 comparison. The TBI function was run over all year pairs over the 10 study sites. Results showed that dominance of  $B/den$  (losses) over  $C/den$  (gains) was significant for all year pairs, as shown by the overall paired  $t$ -tests of the asymmetry, described in the Methods.

Does that mean that some of the species that had disappeared had recovered, or that only the species that remained had increased their abundances-per-species? The answer is found in Fig. 3b, which displays the same statistics computed for species occurrence data. In that graph, the  $B/den$  and  $C/den$  lines would be horizontal at value 0 if all species had remained present and the change after El Niño was only in the abundances. Instead, that graph shows that many species disappeared at first from the surveyed sites after El Niño ( $B/den$  was 0.77 for the 1981-1983 comparison). Then some of the original species recovered on the reefs ( $B/den$  decreased to 0.62 for 1981-1984 and to 0.51 for 1981-1985), possibly by budding from colonies that had survived at nearby sites or by dispersion of larvae from elsewhere. Species losses stabilized around 0.60 in later years compared to the 1981 community composition. During that time, new species that were not present in 1981 occupied the depleted reefs, starting in the 1981-1983 comparison ( $C/den = 0.06$ ) and increasing in the following years (0.17 for 1981-1984 and 0.19 for 1981-1985). Gains of new species, compared to 1981, stabilized around 0.10-0.15 in later years.

Whereas the dissimilarity values  $D$  remained large for species abundance and occurrence data, the changes in  $D$  became small in the later-year comparisons and were possibly caused by sampling variation. The large values of  $D$  between 1981 and the post-El Niño years showed that the coral communities had settled to a new composition that was very different from what it was in 1981: from 1981-84 to 1981-88,  $D$  remained around 0.75 for the abundance data (Fig. 3a) and 0.74 for the presence-absence data (Fig. 3b).

The overall similarity in community composition between years can be appreciated in a RDA biplot, where the centroid of each year is shown surrounded by the 10 site observations of that year (Fig. 4). Computation of the biplot is described in the third paragraph of the present section. The figure shows that the sites in 1981 had quite different species composition than in surveys after El Niño. In 1983, the communities moved to a position in the ordination very distant from 1981 after heavy species losses; then it moved to a new position in 1984 after it recuperated some of its former species, plus some new species that were not present in 1981 and 1983. It moved again in 1985. From then on, the changes observed in 1987 and 1988 seem to represent random variation due to observed random losses and gains of species, which may be due in part to sampling variation and in part to random species losses and gains.

The communities found in South Tikus Island after the natural El Niño event strongly differed in species composition from the structure they had in 1981 and they kept changing, apparently randomly, in later years. A similar phenomenon was shown in the Legendre & Salvat (2015) study, where the disturbance of marine mollusc communities was due to a strong man-made disturbance. In both cases, the observed changes are compatible with the neutral theory of generation of biodiversity and changes in communities (Hubbell 2001).

### Ecological application 3 – Chesapeake Bay data

The data set used in this example was extracted from the Maryland Data Sets of the Chesapeake Bay Benthic Monitoring Program (<http://www.baybenthos.versar.com/data.htm>), which is a portion of the Chesapeake Bay Program (<http://www.chesapeakebay.net/>). Detailed information about the sampling protocol is found on that web page. The data, available online, come in the form of numerous text files, one per group of variables and per year. Legendre & Gauthier (2014) compiled and formatted the separate data files in a *Rdata* file for immediate analysis in R. The <ChesapeakeBay.Maryland.RData> data are available in a zipped file found in Appendix S5 of their paper. The file contains macrofaunal data (203 invertebrates and 2 chordates) collected in the sediment of 27 sites of the bay, spring and fall, during 13 years, i.e. from 1996 to 2008, for a total of 702 data rows.

Table 2 shows how the species are split between seasons and salinity groups. The spring survey data contain 181 species and the fall data 142 species. Two freshwater sites (#36 and #79) were present in the database; they contained 105 species. These two sites were excluded from the present example, which focuses on the 25 brackish sites where 155 species were identified. During the fall surveys in 2005 and 2008, 52 species (abundance data) were observed: 38 in 2005 and 45 in 2008, with an intersection of 31 species found in both years.

This example offers the opportunity to build a B-C plot described in subsection 3 of the Methods. The percentage difference index was used; the Ružička index would have produced similar results. These data will be used to demonstrate how to draw a B-C plot and how to interpret it. For the year pair 2005 and 2008, the B-C plot is shown in Fig. 5. In the plot, the red line is *above* the green line. This indicates that *gains* in benthic abundances-per-species dominated *losses* in the Chesapeake brackish sites (fall surveys) from 2005 to 2008.

A simple classification of the sites by an environmental factor, water temperature during the 2005 fall survey, was used to separate the sites in two groups, providing an example of the kind of information that can be derived from displaying different habitat groups as symbols or colours in B-C plots. These two groups of sites could also be drawn in separate B-C plots. These separate plots would show that species losses dominated in the warmer sites whereas species gains dominated in the colder sites. The B-C plot is an appropriate tool to display this ecological relationship.

In addition to the computation of the *B/den* and *C/den* components at each site, the R function also computed TBI indices (Appendix A4). A map of the 25 brackish sites on the Chesapeake Bay, plotted with the RgoogleMaps package, is shown in Fig. 6. On the map, symbol sizes are proportional to the TBI indices and signs on the symbols indicate the sites dominated by abundance-per-species gains (+) and losses (–) between 2005 and 2008.

## Concluding remarks and prospective applications

TBI analysis and B-C plots are useful to identify exceptional sites in space-time ecological surveys carried out to study the effects of natural and anthropogenic changes to ecosystems. Such studies are presently carried out by teams of ecologists around the world. They are collecting data over land, in lakes and in the oceans to assess the effects of climate change on natural communities and other types of biodiversity data. Researchers would like to identify the sites where important changes have taken place. They can then focus their attention onto these sites and seek what has been going on there, why community composition has changed in an exceptional way at these sites. The TBI method was designed for this type of research.

The present paper is the first description of the ecological theory and statistical developments behind TBI analysis, describing the method in its present state of development. It should provide opportunities to researchers to apply this new method of analysis to a broad palette of ecological and genetic questions.

The paper has shown that it is possible to compute a valid test of significance for dissimilarity indices, which are used to compare data about sites collected at different times. Additionally, the paper has shown how four of the TBI indices can be decomposed into loss and gain components (of species, or abundances-per-species) and how these components can be used to produce B-C plot, a new type of plot that informs users about the processes of biodiversity losses and gains through time found in space-time survey data.

The following indices were found to be appropriate for computation and testing of TBI indices for community composition data: the percentage difference, Ružička, Sørensen, Jaccard, chord, Hellinger and log-chord indices. The first four present the advantage that they can be decomposed into species losses and gains at each site, which makes them the preferred choices in most studies. In studies where community data must be projected into Euclidean space, for instance before PCA, RDA, or other forms of linear analysis, data can be transformed using the chord, Hellinger, and log-chord transformations, and the TBI analysis can be performed using the corresponding *D* indices, i.e. the chord, Hellinger, or log-chord distances. The simulation study has also shown that the Euclidean distance is inappropriate for TBI analysis of community composition data. It can be used, however, for TBI analysis of environmental or species trait data.

The *B* (species losses) and *C* (gains) components are also the building blocks of the numerators of the replacement and nestedness or richness/abundance difference indices of Andrés Baselga (Baselga 2010, 2012) and János Podani (Podani & Schmera 2011, Podani et al. 2013). These indices were reviewed by Legendre (2014). These authors have shown that the Jaccard/Sørensen and Ružička/percentage difference *D* indices can be decomposed into replacement (or spatial turnover),  $2\min(B,C)$  and richness/abundance difference,  $|B-C|$ , for either presence-absence or abundance data. Because they developed their indices for spatial analysis, Baselga and Podani did not emphasize the asymmetry implicit in a temporal comparison between T1 and T2. In temporal studies, where processes are directional, comparison of the loss (*B*) and gain (*C*) components, which are central to the TBI method described in the present paper, is informative.

Analysis of the *B* and *C* components brings us to the heart of the mechanisms by which communities change through time: losses (*b*) and gains (*c*) of species, losses (*B*) and gains (*C*) of individuals of the various species. B-C analysis is especially interesting in species-rich communities where researchers cannot examine the changes in each species individually.

B-C analysis can also be applied to subgroups of sites, e.g. habitat types. In addition, it can be used to compare the changes that occurred in specific groups of species that are known to react differently to environmental stressors, e.g. different age or size classes, or species of different origins, for example: the temperate, transitional and boreal trees found together in the forested southern portion of Canada.

Different ecological applications were worked out with co-authors during the development of the TBI method. Some of them have already been published. Working on these papers provided opportunities to develop the TBI theory and software, through the analysis of pertinent application questions, hypotheses and data. These applications provide examples that ecologists may find useful as guides for the analysis of their own data, in addition to the Ecological applications summarized in the previous section of the paper:

- Impact of a field experiment – The loss (*B/den*) and gain (*C/den*) statistics were first analysed by Legendre & Salvat (2015) to compare community composition data (marine molluscs) during 30 years, before and after a man-made disturbance on an atoll in the Pacific. This disturbance to the mollusc community was the atmospheric test of a Hydrogen bomb in 1968.
- A palaeoecological study – Winegardner et al. (2017) compared diatom communities in lake sediment surveyed 150 years apart across the USA. Temporal beta diversity varied significantly as a function of forest cover, with higher temporal beta in watersheds with contemporary lower forest cover.
- Space-time freshwater ecology – Kuczynski et al. (2018) compared freshwater fish surveys 20 years apart in rivers throughout France. They observed biotic homogenization over time in fish communities. Changes in community composition mainly resulted from population declines and were favoured by an increase in temperature seasonality and in non-native species density.
- Forest ecology – Legendre & Condit (2019) analysed B-C plots for six habitat types, comparing tree community composition (abundance data) from the surveys conducted 30 years apart, in 1985 and in 2015, in the Barro Colorado Island Forest Dynamics Plot in Panama (50 ha) divided into 1250 (20 m × 20 m) quadrats.

In a particular study, researchers may be mostly interested in identifying the sites with high and significant TBI indices. In other studies, interest may be in a fine analysis of the changes in the loss and gain components of the dissimilarity in community composition, compared to a pre-disturbance situation. One can look at these components in graphs that allow researchers to compare, for example, different subsets of the data. Ecological examples have been shown in the paper for these different situations.

## Data accessibility

The data used in the Ecological applications are publicly available in the references provided.

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## 742 **Supporting Information**

743 Additional supporting information:

744 **Appendix A1.** Simulations involving artificial survey data at times T1 and T2.

745 **Appendix A2.** R function to standardize environmental data prior to TBI analysis.

746 **Appendix A3.** Results of calculations with function TBI(), insecticide experiment data.

747 **Appendix A4.** Results of calculations with function TBI(), Chesapeake Bay data.

748 **Appendix A5.** Test function paired.t.test.spec.R.

## 749 **Author contributions**

750 P. Legendre designed the methods described in this paper, programmed the software, conducted the  
751 analyses of the numerical examples and the simulation study, and wrote the manuscript.

## 752 **Acknowledgements**

753 I am thankful to Amanda Winegardner and Lucie Kuczynski who experimented with this testing  
754 procedure and computed it on real data during the development phase of this paper, and to Daniel  
755 Borcard and Marie-Hélène Brice who provided useful comments on the manuscript before submission.  
756 Lucie Kuczynski suggested the acronym TBI for the new method. I am also thankful to Daniel Borcard  
757 who revised a preliminary version of the manuscript and to Joseph R. Bennet and three anonymous  
758 reviewers who provided helpful comments on the submitted manuscript. This research was supported  
759 by Natural Sciences and Engineering Research Council of Canada (NSERC) research grant no. 7738 to  
760 P. Legendre.

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791 Table 2. Number of species in subsets of the Chesapeake Bay fauna data surveyed during 13 years,  
 792 spring and fall. In total, 205 benthic species were found at the 27 survey sites.

793

	Spring	Fall	Spring and fall
Freshwater (2 sites)	93	58	105
Brackish (25 sites)	128	121	155
All survey sites (27 sites)	181	142	205

794

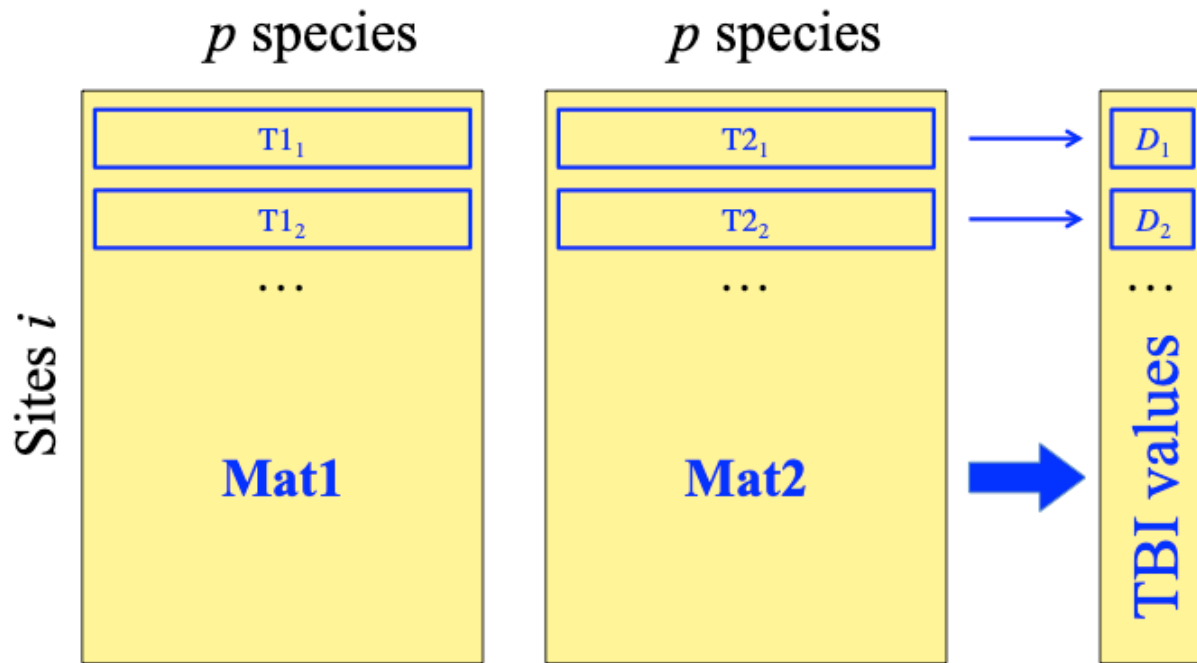


Fig. 1. Schematic representation of the first step of the method. Data in matrices **Mat.1** (for Time 1) and **Mat.2** (for Time 2) are used to compute a vector of TBI dissimilarities  $D_i$  for all sites  $i$  (data rows). For example, for site  $i = 1$ , vectors  $T1_1$  and  $T2_1$  are compared to compute  $D_1$ , the dissimilarity between data at time 1 (T1) and time 2 (T2).

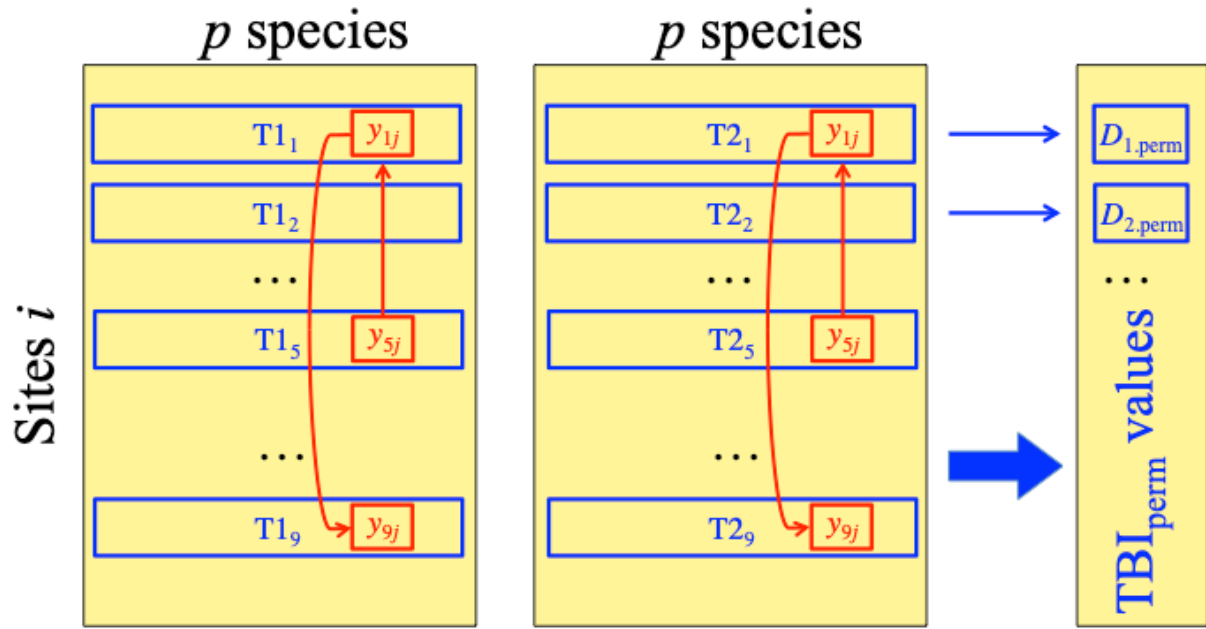
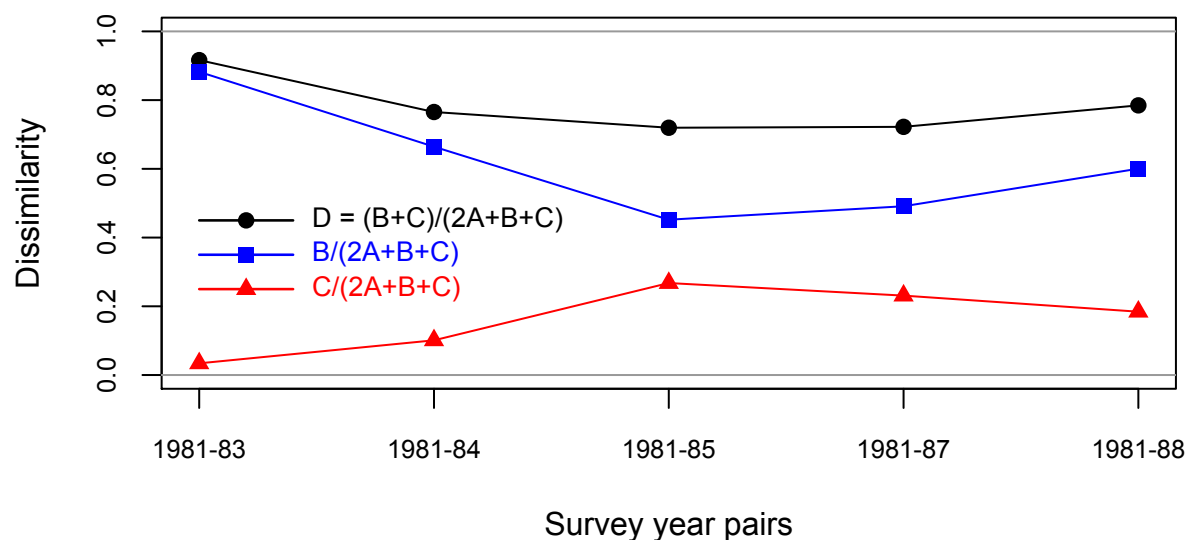


Fig. 2. Random permutation of the community composition data is done separately for each species (column), in matrices T1 (left) and T2 (right). This figure shows an example where a permutation of species  $j$  brings value  $y_{1j}$  to position  $y_{9j}$  and value  $y_{5j}$  to position  $y_{1j}$ . The exact same permutation, involving all values in column  $j$ , is done in matrices T1 (left) and T2 (right). Following similar permutations of all  $p$  species, dissimilarities are computed between the two vectors representing each site  $i$ , producing the values  $D_i$  under permutation.



**(a) Changes in B, C, D along the years, abundance data**



**(b) Changes in B, C, D along the years, occurrence data**

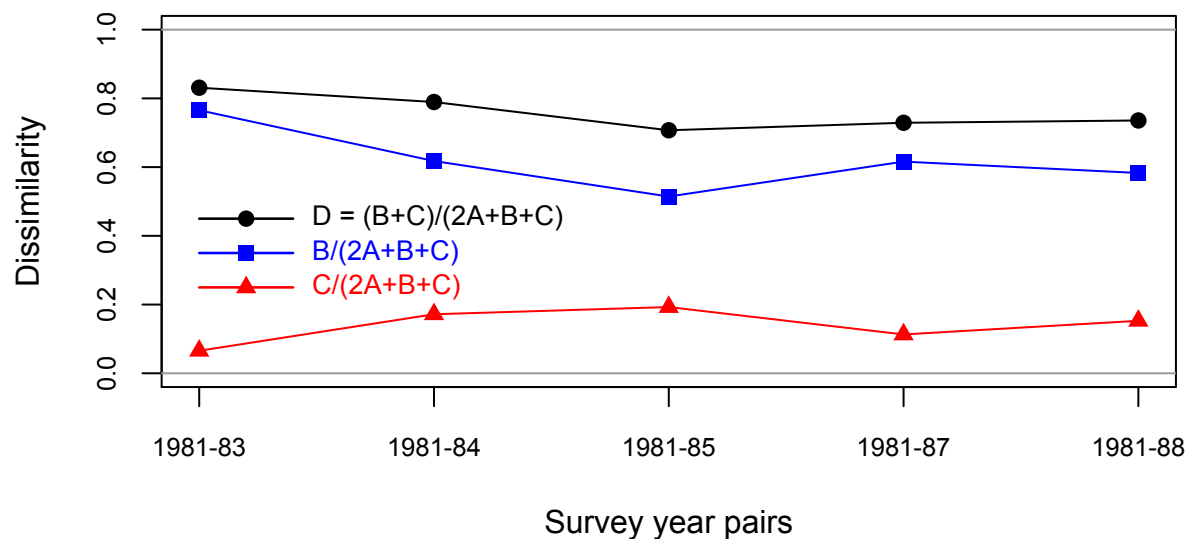


Fig. 3. Tikus Island coral data. (a) Changes in dissimilarity  $D$  computed from the quantitative coral community compositions between years, and its components  $B/den$  (losses) and  $C/den$  (gains);  $den$  is the denominator of the dissimilarity index  $D$ ,  $(2A+B+C)$  in this figure. The 1981 survey, before the El Niño event, is compared in turn to the 1983, 1984, 1985, 1987 and 1988 surveys. (b) Same for the species occurrence (i.e. presence-absence) data.

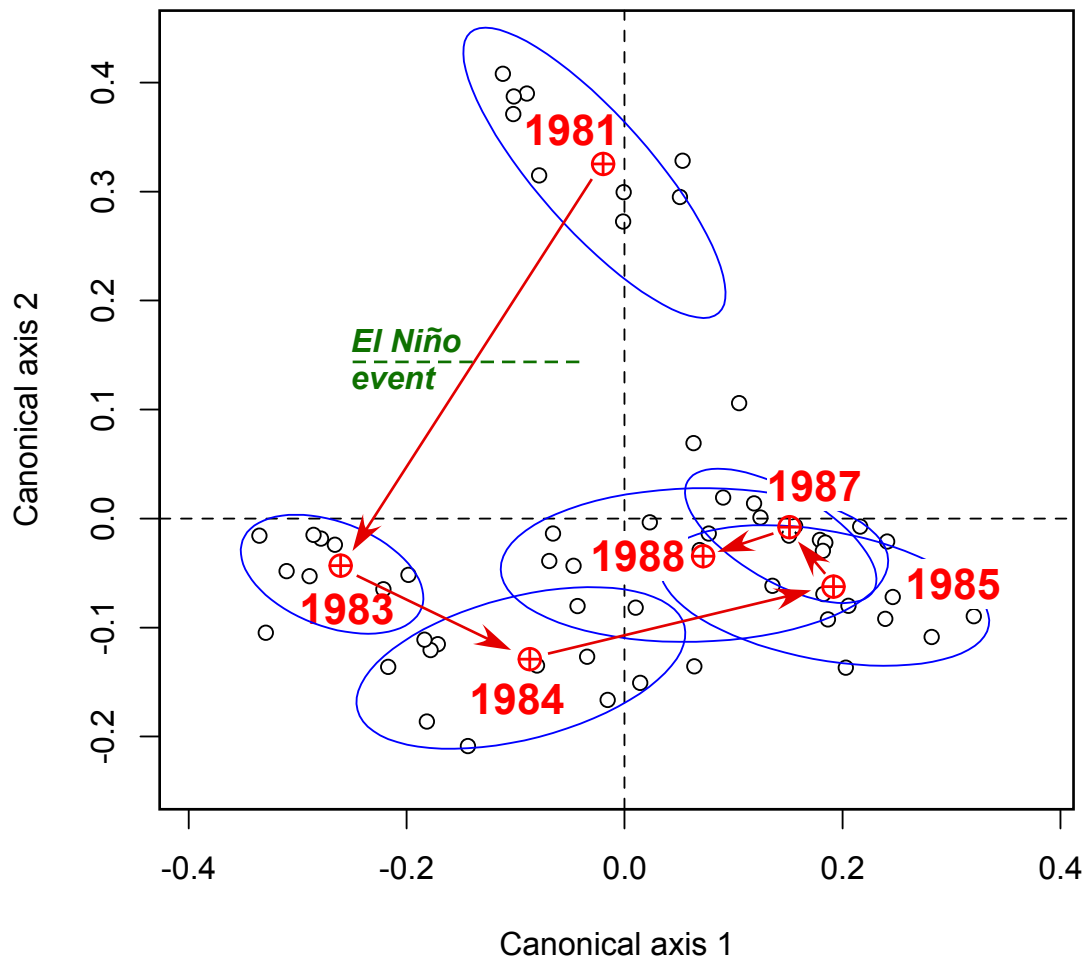


Fig. 4. Tikus Island coral data. Canonical ordination plot obtained by dbRDA for the quantitative coral community compositions data for the 6 years and 10 sites, constrained by a factor representing the 6 survey years. The years are marked by red symbols and the sites (open circles) for each year are incompletely surrounded by 60% coverage ellipses. Arrows materialize the sequence of years.

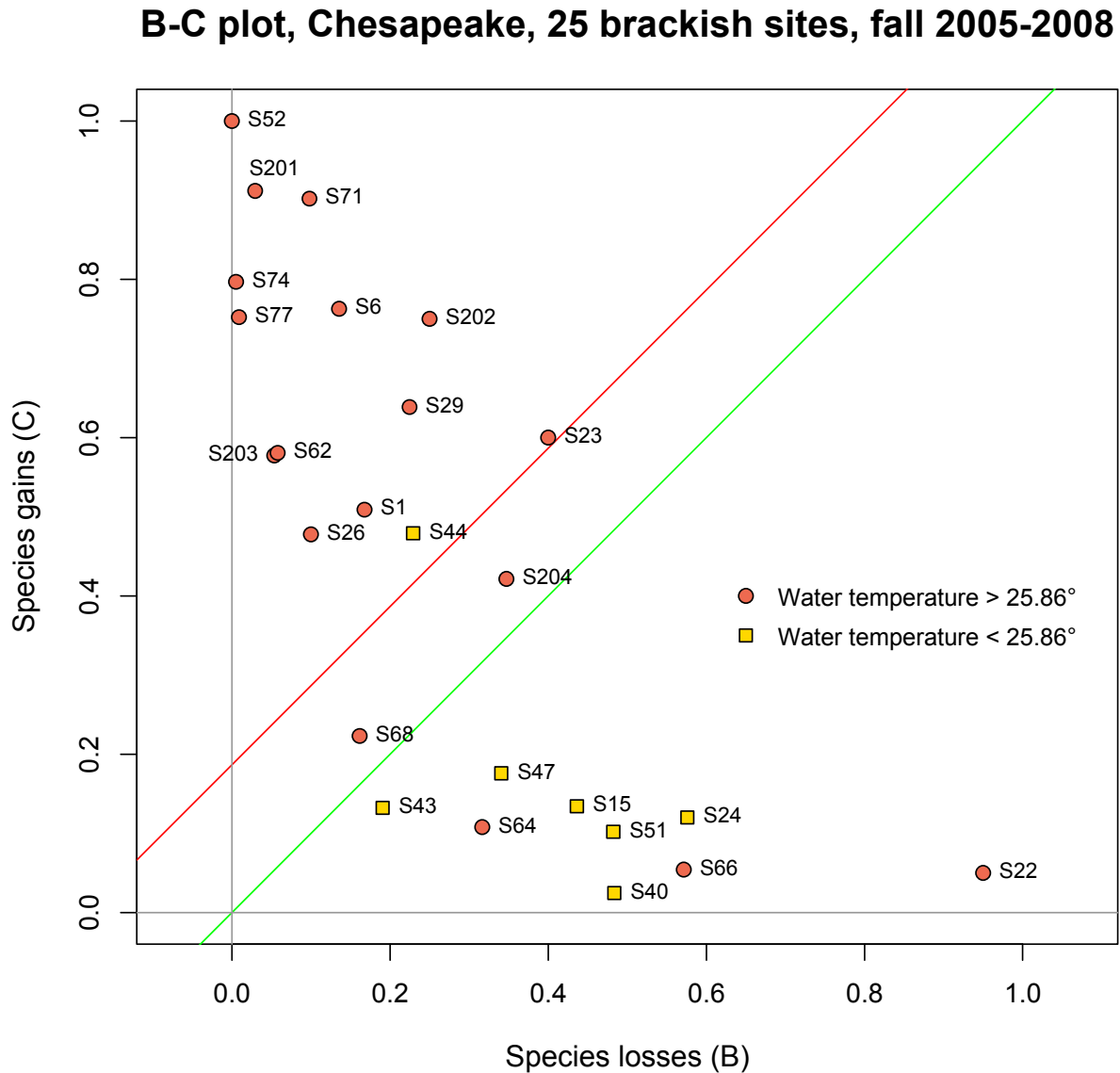


Fig. 5. Chesapeake Bay benthos data. B-C plot comparing the fall surveys of 2005 and 2008, where the 25 brackish sites are plotted using the losses ( $B/den$  statistics) and gains ( $C/den$  statistics) computed from the species abundance data. Sites are identified by their code of the Chesapeake Bay Benthic Monitoring Program. The sites are represented by symbols corresponding to two water temperature groups observed during the 2005 fall survey. Green line with slope of 1: line where gains equal losses. The red line was drawn parallel to the green line (i.e. with slope = 1) and passing through the centroid of the points. Its position above the green line indicates that, on average, species gains dominated losses from 2005 to 2008.

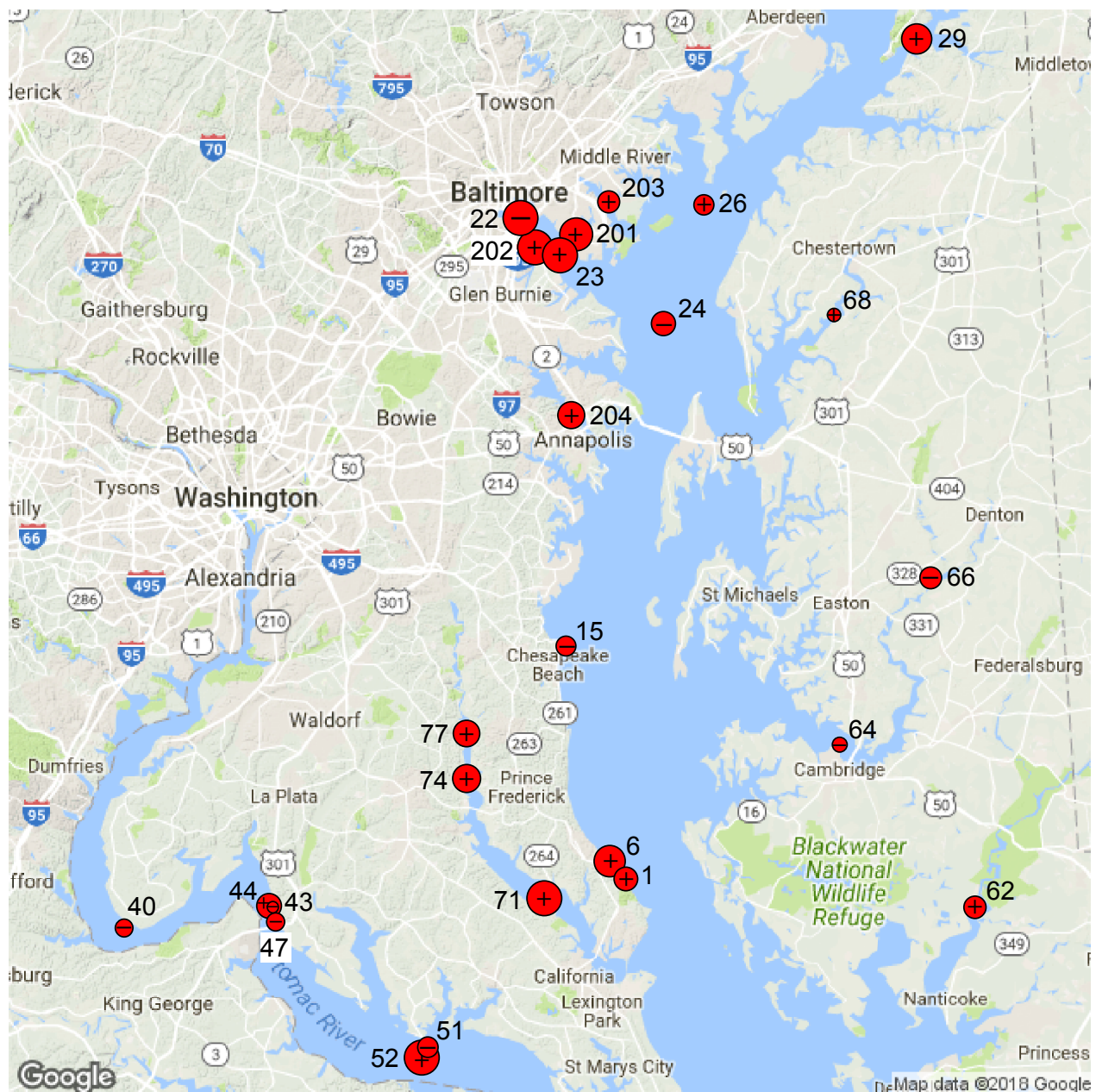


Fig. 6. Map of the 25 brackish sites (red symbols) of the Chesapeake Bay ecological survey, produced with the RgoogleMaps package in R. Comparison of survey in years 2005 and 2008, abundance data: point sizes are proportional to the TBI indices (percentage difference  $D$ ). + signs indicate the 17 sites where *gains* in abundances-per-species dominated; – signs, the 8 sites where *losses* dominated. The site identification numbers are those found in the Chesapeake Bay data base.

*Supporting information to:*

Legendre P. (2019) A temporal beta-diversity index to identify sites that have changed in exceptional ways in space-time surveys. *Ecology and Evolution* (in press).

## Appendix A1

### SIMULATIONS INVOLVING ARTIFICIAL SURVEY DATA AT TIMES T1 AND T2

## Introduction

This appendix reports the results of a simulation study carried out to determine if the new test of significance of the temporal beta change at individual sites (TBI) has correct type I error rates and is able to detect sites for which the response data had exceptionally high dissimilarities between time 1 (T1) and time 2 (T2). Simulations were done with random data generated in three different ways; six dissimilarity coefficients were used as TBI indices.

## Data generation methods

Two methods were used to generate random community-like data in matrices **Mat.1** and **Mat.2**. The first one (gen.method=1) was to draw values at random from the Poisson distribution. The second method (gen.method=2) was to use random lognormal data. On the one hand, Poisson error regression is often recommended in GLM software to analyse count data such as species abundances. On the other hand, artificial data with lognormal distributions, rounded to integers, are often used to represent community composition data in simulation studies because their skewness is comparable to that often encountered in real community data (Preston 1948).

The random Poisson deviates were generated with a probability of occurrence (lambda parameter of the distribution) of 0.8. The generated data were skewed and contained approximately 45% zeros. For the lognormal data, the normal distribution generating the deviates that are exponentiated to produce random lognormal data had mean = 0 and standard deviation = 2.0. The values were rounded to the nearest integer. The generated data were highly skewed and contained approximately 36.5% zeros. Random lognormal data are much more highly skewed than random Poisson data.

It may be of interest to ecologists to identify sites that are exceptional in the changes to their community structure on the one hand, and sites that are exceptional in the changes to their environmental conditions on the other hand. So, in a third series of simulations, quantitative environmental data will be generated through random normal deviates; they will represent environmental variables in simulations. Real quantitative environmental data are often not normally distributed, but in many cases then can be normalized using data transformations. Qualitative variables (factors) will not be used in the simulations. How to handle them is described in section “Application to physical environmental or community trait data” of the main paper.

## a. Dissimilarity coefficients

Six dissimilarity coefficients were used in the simulation study. Among the coefficients that are often used to analyse community composition data, we used the chord, Hellinger and log-chord distances, which belong to the Box-Cox family of distances (Legendre & Borcard 2018) and have the Euclidean property (a useful property for principal coordinate ordination, PCoA), and the percentage difference (Odum 1950) and Ružička (1958) dissimilarities, which are non-Euclidean dissimilarities and can

produce negative eigenvalues and complex axes in PCoA. The Euclidean distance was also included because it is the most widely used coefficient to analyse environmental data matrices transformed by standardization or ranging. Its behaviour for the analysis of community data will be assessed against the coefficients specialized for this type of data.

## b. Permutation methods

The permutation method implemented in the TBI.R function and used in the simulations reported in the following sections of this Appendix is described in the main paper. Briefly, the data are permuted separately in each column, in the same way in matrices **Mat.1** (for T1) and **Mat.2** (for T2).

Permutation of entire rows of data is the method used in tests of significance in canonical analysis (RDA, CCA), widely used by ecologists. That permutation method was used in additional simulations for the sake of the comparison; results of these additional simulations are summarized here. In the TBI test, entire rows were permuted in matrices **Mat.1** and **Mat.2** separately. The tests had correct type I error rates, so they were valid, but they had much lower power than the permutation method summarized in the previous paragraph. Hence permutation of entire rows of data should not be used to test the significance of TBI indices and that method is not included in the distributed version of the TBI.R function.

## c. Simulations to estimate type I error rates

Type I error simulations will provide an assessment of the validity of the testing method. “A statistical testing procedure is valid if the probability of a type I error (rejecting  $H_0$  when true) is no greater than  $\alpha$ , the level of significance, for any  $\alpha$ .” (Edgington 1995, p. 37).

### c.1. Simulation methods

#### c.1.1. *Simulation series 1*, community composition data, random Poisson deviates

Two subseries of simulations were carried out:

1.1. In the first subseries, the two matrices (**Mat.1** and **Mat.2**, of size  $n \times p$ ) contained random Poisson deviates, as described in the introduction of this Appendix; Fig. A1.1. The permutation method was used with 999 random permutations. See results in Table A1.1.

1.2. In the second subseries of simulations, in addition to the random data in mat.1a and mat.2a (which are called mat.1 and mat.2 in Fig. A1.1), a submatrix mat1.d containing  $p/3$  new species, with frequencies zero, was added to mat.1a and a matrix mat2.d of the same size, containing random Poisson deviates, was added to mat.2a; see Fig. A1.2. Results with  $p/3 = 6$  are shown in Table A1.2.

The objective of the second subseries was to show the effect on the tests of significance of having extra species in the data matrices showing strong difference between T1 and T2 (absent in **Mat.1** and present in **Mat.2**) but with only random differences among the sites. These extra species should have no effect on the results of TBI tests.

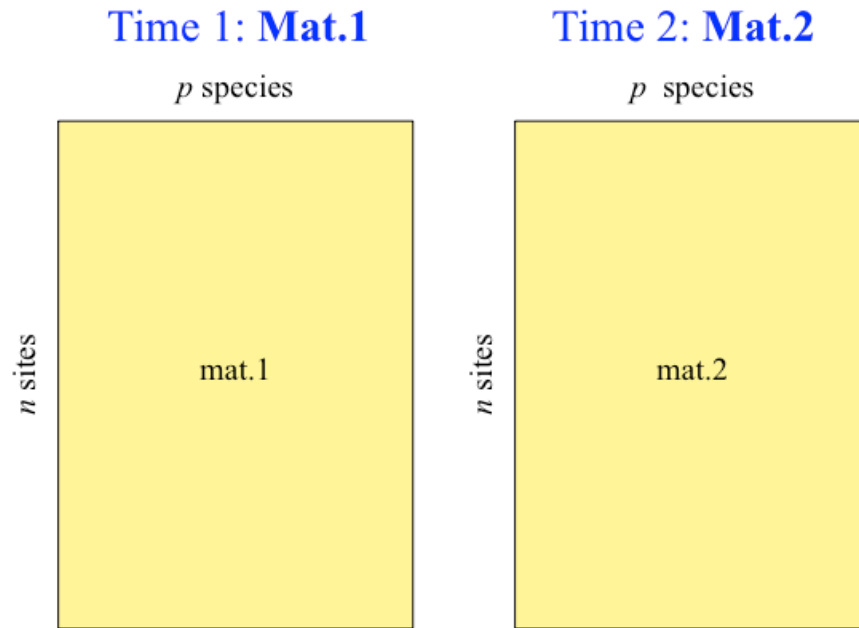


Fig. A1.1. Two data matrices (**Mat.1** and **Mat.2**) used in the type I error simulations. Sections mat.1 and mat.2 were filled with random numbers, so that  $H_0$  was true. Note: **Mat.1** and **Mat.2** contain only one section each here; they will contain more sections in the next figures.

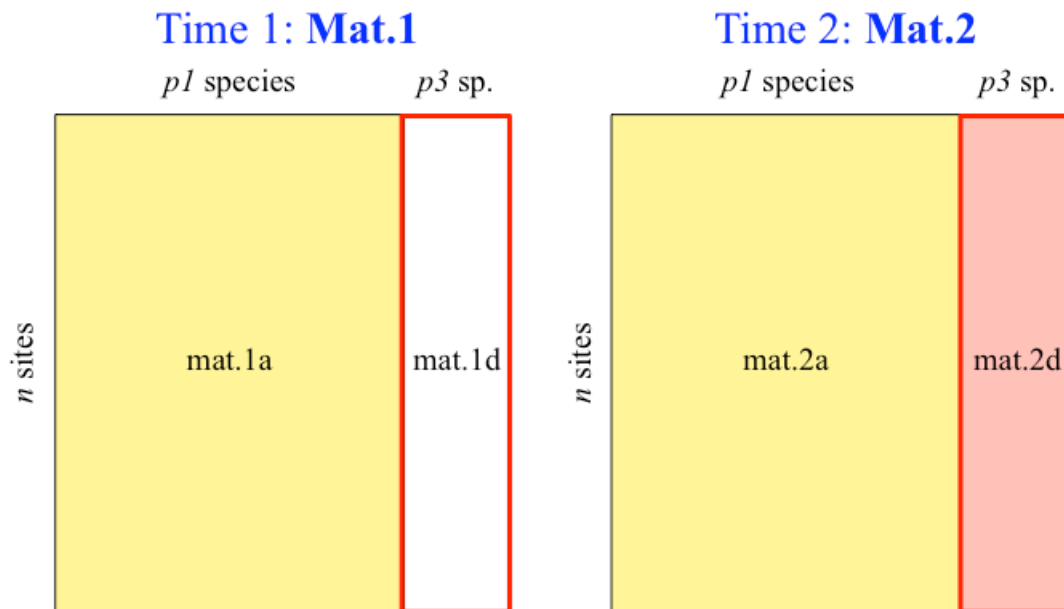


Fig. A1.2. Two data matrices (**Mat.1** and **Mat.2**) used in the type I error simulations; mat.1a and mat.2a contained random numbers, as in Fig. A1.1. In the simulations of subseries 2, mat.1d (white, containing zeros for  $p3$  new species) and mat.2d (pink, containing random numbers for the same number of species,  $p3$ ) were joined to mat.1a and mat.2a.



### c.1.2. *Simulation series 2*, community composition data, random lognormal deviates

Two subseries of simulations were carried out:

2.1. In the first subseries, the two matrices (mat.1 and mat.2, of size  $n \times p$ ) contained random lognormal deviates, as described in the introduction of this Appendix; Fig. A1.1. The permutation method was used with 999 random permutations. See results in Table A1.3.

2.2. In the second subseries of simulations, in addition to the random data in mat.1a and mat.2a (called mat.1 and mat.2 in Fig. A1.1), a submatrix mat.1.d containing  $p3$  new species, with frequencies zero, was added to mat.1a and a matrix mat.2.d of the same size, containing random lognormal deviates, was added to mat.2a; see Fig. A1.2. Results with  $p3 = 6$  are shown in Table A1.4.

The objective of the second subseries was the same as for subseries 1.2, using a different way of generating community composition-like data.

### c.1.3. *Simulation series 3*, environmental data, random normal deviates

In this last series, quantitative environmental data were simulated using random normal deviates instead of species-like data. There were  $n = 20$  sites and  $p = 20$  variables in matrices **Mat.1** and **Mat.2**, as in Fig. A1.1. TBI tests were only computed with the Euclidean distance. The other distances investigated in the previous simulation series only make sense for community composition and other frequency-like data (Legendre & Legendre 2012, Chapter 7). The permutation method was used with 999 random permutations.

The data vectors were standardized as described in Appendix A2. Explanation: (a) the two data matrices are joined into a single data matrix,  $\mathbf{Y} = \text{rbind}(\mathbf{Mat.1}, \mathbf{Mat.2})$ , before standardization. In this way, the differences in values of each variable for a given pair of sites in the two matrices will remain comparable to the differences computed from the original unstandardized values; in this way, the distances computed between sites in T1 and T2 will be meaningful. This precaution is important when there are differences in means between T1 and T2. (b) Standardizing the variables insures that all variables will contribute the same variance to the calculation of the TBI indices; the variances will not depend on the physical units of the variables or other contingencies that make the variances unequal.

## c.2. Results, type I error study

Results are presented in Tables A1.1 and A1.2 for random Poisson deviates, in Tables A1.3 and A1.4 for random lognormal deviates, and in Table A1.5 for normal deviates.

The simulations produced the expected result that type I error was always correct. The testing method is thus valid according to Edgington's (1995) definition shown above.

1. Examination of the tables of rejection rates of the null hypothesis (Table A1.1-A1.4) showed that the TBI tests had correct rates of type I error for the two community-like data generation methods (Poisson and lognormal) and all dissimilarity indices used, and this for all significance levels ( $\alpha$ ) considered, from  $\alpha = 0.01$  to  $\alpha = 0.50$ . Deviations from the nominal significance levels, shown at the top of each table, were very small. Simulations involving random environmental-like quantitative data (Table A1.5) also showed correct levels of type I error with the Euclidean distance used in the computation of TBI indices.

2. The tables of rejection rates were divided into separate matrices per data generation and permutation methods, and transformed into squared differences (or squared errors) between the rejection rates and



the nominal significance levels  $\alpha$ . The sum of the squared differences was computed for each matrix. Examination of the results (not shown in detail in this Appendix) showed no significant difference (Friedman's test) in type I error rejection rates between the data generation methods (Poisson or lognormal).

3. The additional species that were present in **Mat.2** but not in **Mat.1** (see Fig. A1.2) did not affect the type I error rates of the TBI tests. The rejection rates in Tables A1.1 and A1.2 (random Poisson deviates), and those in Tables A1.3 and A1.4 (random lognormal deviates), were very similar.

Note — The percentage difference and Ružička dissimilarity indices differ only by their denominators. The tests of significance of these two indices produce the same p-values if they are run with the same series of permuted vectors. Since the random number generator was started at the same value at the beginning of all simulation runs, it is normal that the rejection rates found for these two dissimilarities in TBI simulations be the same in the report tables (Tables A1.1 to A1.4).

## d. Simulations to compare power of $D$ indices

### d.1. Simulation method

For the power simulations, some of the sites were generated with a strong difference between T1 and T2 whereas other sites only had random differences. The objective of these simulations was to determine if some dissimilarity functions were better suited to identify sites with strong differences in community composition data between T1 and T2 than other dissimilarity indices.

Data generation proceeded as follows for community composition data.  $n1$  sites were assigned to a first group that differed in composition between T1 and T2, whereas  $n2$  sites only had random variation between T1 and T2.

1. The  $n1$  sites received strong differences between T1 and T2, as follows (Fig. A1.3):

- A first group of  $p1$  species received random abundance values in mat.1a.
- A second group of  $p2$  species received random abundance values in mat.2b.
- In **Mat.1**, the  $p2$  species received the values of mat.2b times a contribution constant (parameter *contr*) with values between 0 and 1. With *contr* = 0, the differences between **Mat.1** and **Mat.2** for the first 5 sites were so strong that  $H_0$  was rejected with very high rates so that the first 5 sites were nearly always identified as exceptional, except when the Euclidean  $D$  was used as TBI index. The value *contr* = 0 was too strong to produce useful simulation results. Hence the value *contr* = 0.2 was used in the power simulations with random Poisson deviates reported below. For the power simulations with random lognormal deviates, *contr* was either 0.01 or 0.02, as specified below. A higher value of *contr* reduces the test power.
- In **Mat.2**, the  $p1$  species received the values of mat.1a times the same contribution constant (*contr*) as in the previous paragraph.

The generated abundance-like values were either random Poisson or random lognormal deviates, as described at the beginning (section “Data generation methods”) of this Appendix.

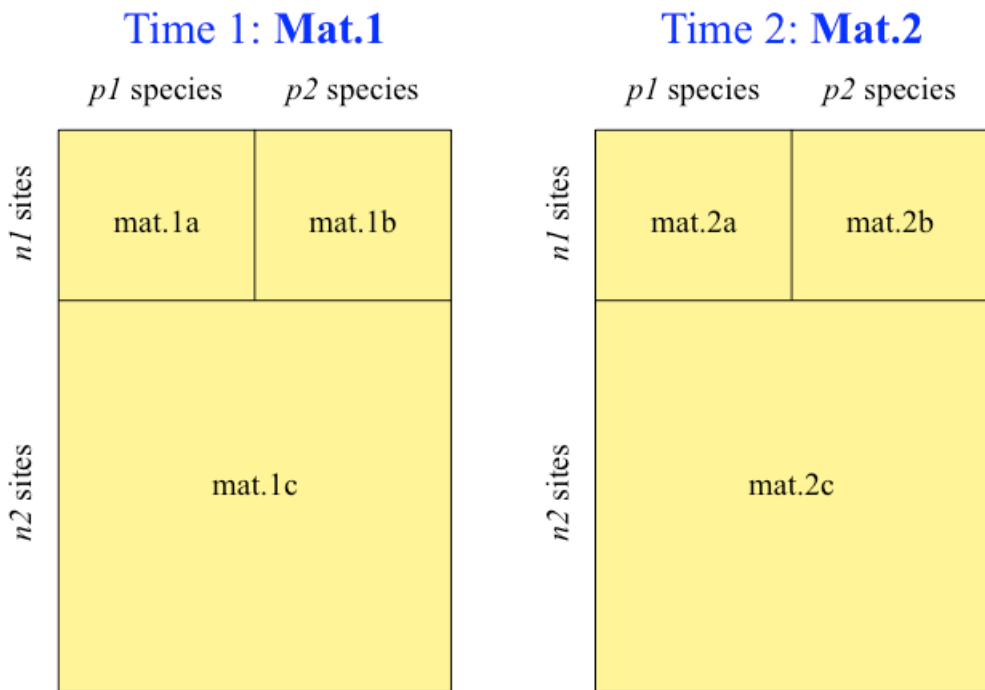


Fig. A1.3. Two matrices (**Mat.1** and **Mat.2**) used in the power simulations. Abundances in mat.1a and mat.2b were generated independently using either random Poisson or random lognormal deviates. Submatrix mat.1b received a fraction of the abundances in mat.2b and mat.2a received a contribution of the abundances in mat.1a. The values of these contributions are described in the text and depend of the random data generator used. Submatrices mat.1c and mat.2c received random deviates drawn from the same statistical population.

2. The  $n2$  sites received random abundance-like values, either Poisson or lognormal. Hence the differences between T1 and T2 were random for these sites (Fig. A1.3).

In the results reported below,  $n1 = 5$ ,  $n2 = 15$ ,  $p1 = p2 = 10$ . 1000 data sets were independently generated and analysed for each reported rejection rate (0.01, 0.05, 0.10) in Tables A1.6 and A1.7 reporting the results.

#### d.1.1. Power differences among D indices, community composition data

##### d.1.1.1. Simulations using random Poisson deviates

Two subseries of simulations were carried out:

1. In the first subseries, all matrices contained random Poisson deviates, structured following Fig. A1.3.
2. In addition, a submatrix mat1.d containing  $p3$  new species, with frequencies zero, was added to **Mat.1** and a matrix mat2.d of the same size, containing random Poisson deviates, was added to **Mat.2**, as in the subseries 2 simulations for type I error. See Fig. A1.4.

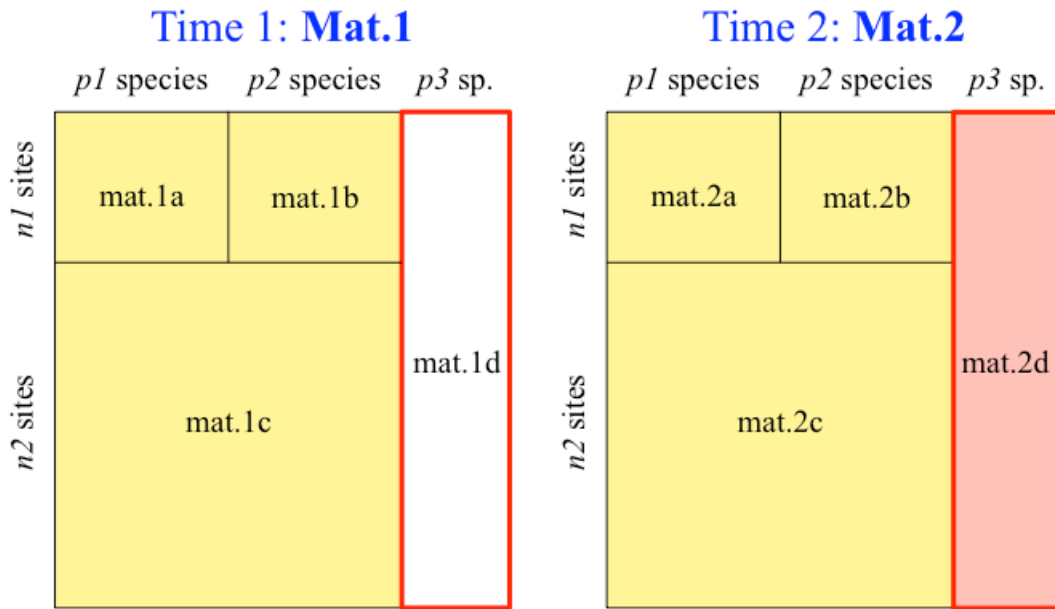


Fig. A1.4. Two matrices (**Mat.1** and **Mat.2**) used in additional power simulations with random deviates. In these simulations, mat.1d (white, containing zeros for  $p3$  new species) and mat.2d (pink, containing random lognormal or Poisson deviates for the same number of species,  $p3$ ) were joined to mat.1a-b-c and mat.2a-b-c.  $H_0$  was true for the  $p3$  species found in mat.1d and mat.2d.

The objective of the second subseries was to show the effect on the tests of significance of having extra species in the data matrices showing strong difference between T1 and T2 (absent in **Mat.1** and present in **Mat.2**) but with only random differences among the sites. These extra species should have little effect, if any, on the TBI tests of significance.

#### d.1.1.2. Simulations using random lognormal deviates

Two subseries of simulations were carried out with random lognormal deviates. Data were structured following Fig. A1.3.

1. In the first subseries, the contributions of the mat.2b data to the mat.1b data, and of the mat.1a data to the mat.2a data, was determined by  $contr = 0.01$ .
2. In the second subseries, the contribution parameter ( $contr$ ) had the value 0.02.

#### d.1.2. Power with simulated environmental data

For simulation involving data simulating pseudo-environmental variables, the construction of the data matrices followed the same method as in simulation subseries 1 above ( $n1 = 5$ ,  $n2 = 15$ ,  $p1 = p2 = 10$ ), except that the data were random normal deviates standardized as described in Appendix A2.

#### d.1.3. Differences in power for different values of $n1$ and $n2$

Power to detect an effect in statistical tests is well-known to be a function of three parameters: the importance of the effect to be detected, the significance level  $\alpha$ , and the number of observations  $n$ . Additional simulations were conducted to detect the effect on power of the number of exceptional sites

( $n1$ ) and the number of sites with random variation ( $n2$ ) in the study. Community composition was simulated using random lognormal deviates.

## d.2. Results, power study

Results are presented in Tables A1.6 and A1.7 for simulated community composition data and in Table A1.8 for simulated environmental data.

### d.2.1. Power differences among D indices, community composition data

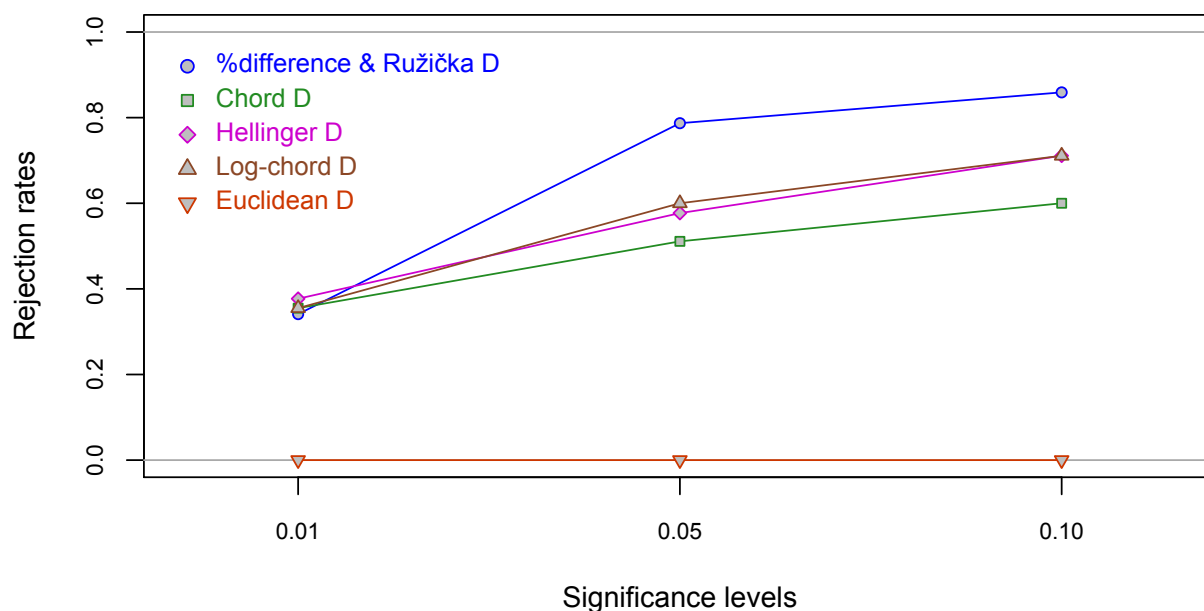
Power is the ability to detect an effect when one is present in the data. In the simulations reported here, we know that rather large effects were present in all data sets because the simulation function had generated it in the data.

From the simulation results for community composition data (simulation series 1: Table A1.6, Fig. A1.5; simulation series 2: Table A1.7, Fig. A1.6), we can make the following observations, working from the bottom of the graphs up:

1. All simulations involving the Euclidean distance for the computation of TBI indices had dismally low power (triangles pointing down). TBI indices computed with the Euclidean distance hardly ever detected the presence of exceptional sites in the species-like data files simulated with Poisson or lognormal deviates. The Euclidean distance should not be used for TBI tests of community composition data.
2. For data generated with Poisson or lognormal distributions, the most powerful TBI tests were computed with the percentage difference (aka Bray-Curtis) and Ružička dissimilarities, followed by the group {Hellinger, log-chord} distances which produced very similar results with species-like data simulated with Poisson random deviates, and log-chord distances for community data simulated with lognormal deviates. TBI tests based on the chord dissimilarity had the lowest power among the distances that produced usable tests. The log-chord distance was expected to be the most appropriate (and thus produce more powerful tests than the chord or Hellinger distances) with lognormally distributed data since the log transformation, which is the first transformation in the calculation of that distance, makes the random data normal before the chord transformation is computed.
3. When sites had entirely different species compositions between T1 and T2, the TBI test had maximum power: it always rejected  $H_0$  at significance levels of 0.05 and 0.10, and in 98.7% of the cases at level 0.01. In temporal studies, this situation may arise when a site has been subjected to an important environmental change between T1 and T2. The change may be natural or man-made, like a lake going from a prairie to an agricultural or urban environment. The situation was simulated by setting the contribution parameter to the value *contr*=0 for the generation of data in submatrices *mat1b* and *mat2b* (see Fig. A1.3). These rejection rates were obtained with all dissimilarity indices tested: percentage difference, Ruzicka, chord, Hellinger and log-chord distances.

The best combination for TBI tests of community composition data with maximum power is to use the percentage difference or the Ružička indices. These two dissimilarities can also be decomposed into species losses ( $B/den$ ) and gains ( $C/den$ ), which can be used to examine the processes of losses and gains at the site level and to produce B-C plots.

**(a) Power, random Poisson deviates**



**(b) Power, random Poisson with 6 extra species**

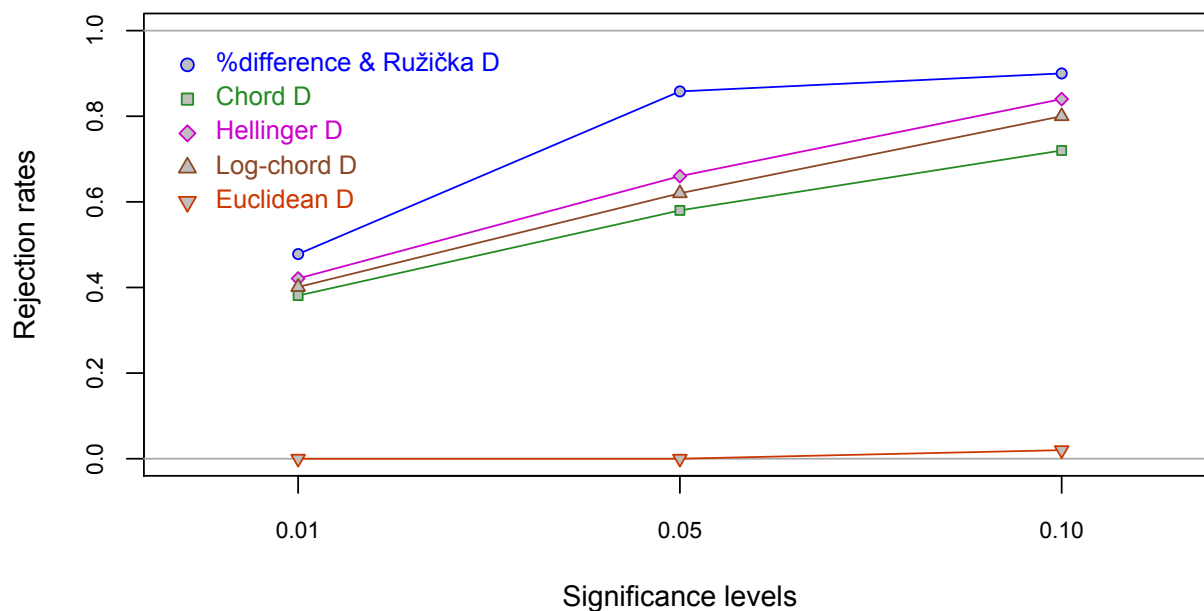


Fig. A1.5. Power study, **random Poisson deviates**. Rates of rejection of  $H_0$  through the simulations obtained with five dissimilarity coefficients, **(a)** without (Fig. A1.3) and **(b)** with extra species (Fig. A1.4). Rejection rates are reported for three significance levels alpha: 0.01, 0.05 and 0.10 (abscissa).

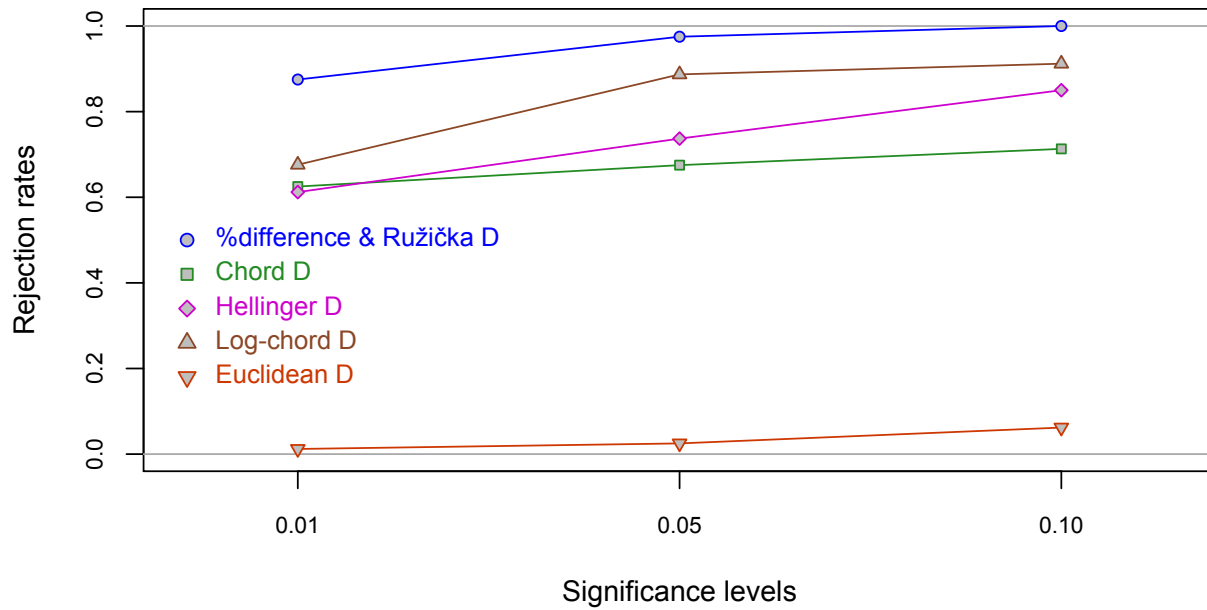
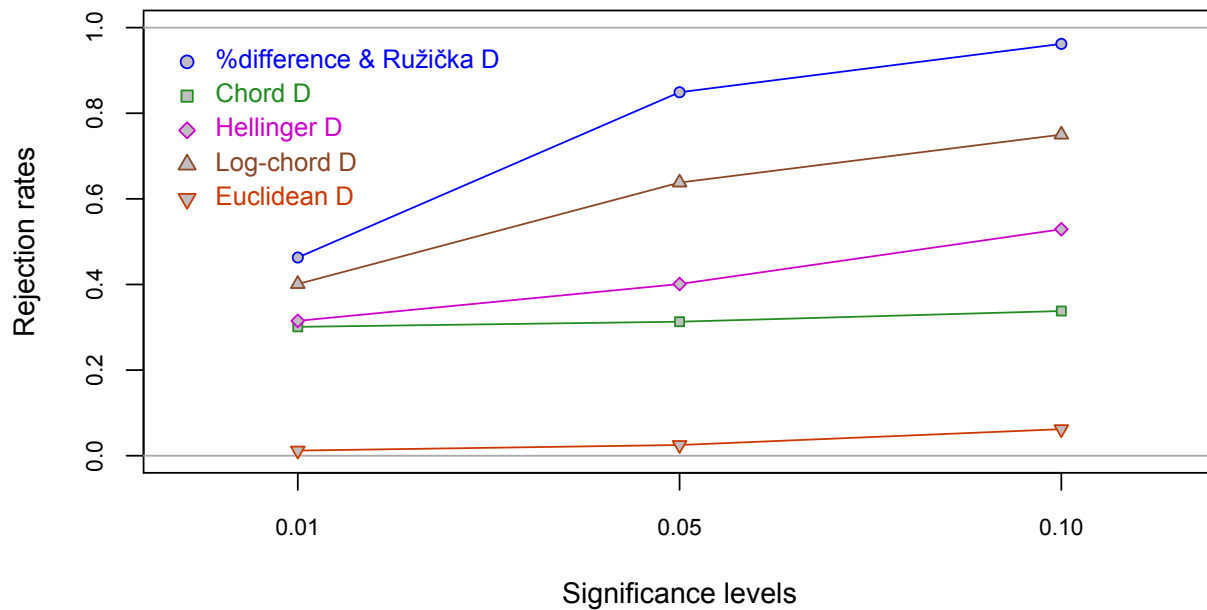
**(a) Power, random lognormal deviates,  $\text{contr} = 0.01$** **(b) Power, random lognormal deviates,  $\text{contr} = 0.02$** 

Fig. A1.6. Power study, **random lognormal deviates**. Rates of rejection of  $H_0$  through the simulations obtained with five dissimilarity coefficients, using two values of the contribution parameter: **(a)**  $\text{contr} = 0.01$ , **(b)**  $\text{contr} = 0.02$ . Rates of rejection of  $H_0$  are reported for three significance levels  $\alpha$ : 0.01, 0.05 and 0.10 (abscissa).

### d.2.2. Power with simulated environmental data

In the simulations representing environmental variables with simulated standardized random normal deviates, there were  $n1 = 5$  and  $n2 = 15$  sites,  $p1 = p2 = 10$  variables. The contribution parameter was  $contr = 0.05$ . The only distance tested in the simulation study was the Euclidean distance. 1000 data sets were independently generated and analysed for each reported rejection rate (0.01, 0.05, 0.10). The rates of rejection of  $H_0$  are presented in Table A1.8. Power was high enough to recommend the test for analysis of standardized environmental data.

### d.2.3. Differences in power for different values of $n1$ and $n2$

The simulations conducted to detect the effect on power of the number of exceptional sites ( $n1$ ) and the number of sites with random variation ( $n2$ ) in the study produced the following results.

1. The number of sites  $n$  was 20 in all simulations, with the number of affected sites  $n1 =$  varying from 1 to 19;  $n2 = (n - n1)$ . The simulations used the percentage difference index and  $contr = 0.02$ , 1000 independent simulations and 999 permutations per test.

The results show that for tests of significance at level  $\alpha = 0.05$ , optimal power was obtained with  $n1 = 1$  to 9 in simulations (Fig. A1.7a). The test can be recommended for data sets with  $n1$  smaller than  $n/2$ . Tests carried out with  $n1$  equal to or larger than  $n/2$  can still be used but they have lower power.

2. For  $n1 = n/4$ , simulations were repeated for different values, with  $n = \{8, 16, 24, 32, 40, 48, 56, 64\}$  and  $n1 = \{2, 4, 6, 8, 10, 12, 14, 16\}$ . Again, the simulations used random lognormal data, the percentage difference index,  $contr = 0.02$ , 1000 independent simulations and 999 permutations per test.

Rejection rates are reported in Fig. A1.7b for three significance levels  $\alpha$ : 0.01, 0.05 and 0.10. Power remained constant over all values of  $n$  investigated.

### d.2.4. Summary of the power study

Power of the test was high when the effect was strong, and as long as the proportion of sites with an effect was smaller than  $n/2$  (Fig. A1.7a). For a fixed proportion of affected sites, power did not increase when the total number of sites  $n$  in the study was larger (Fig. A1.7b).

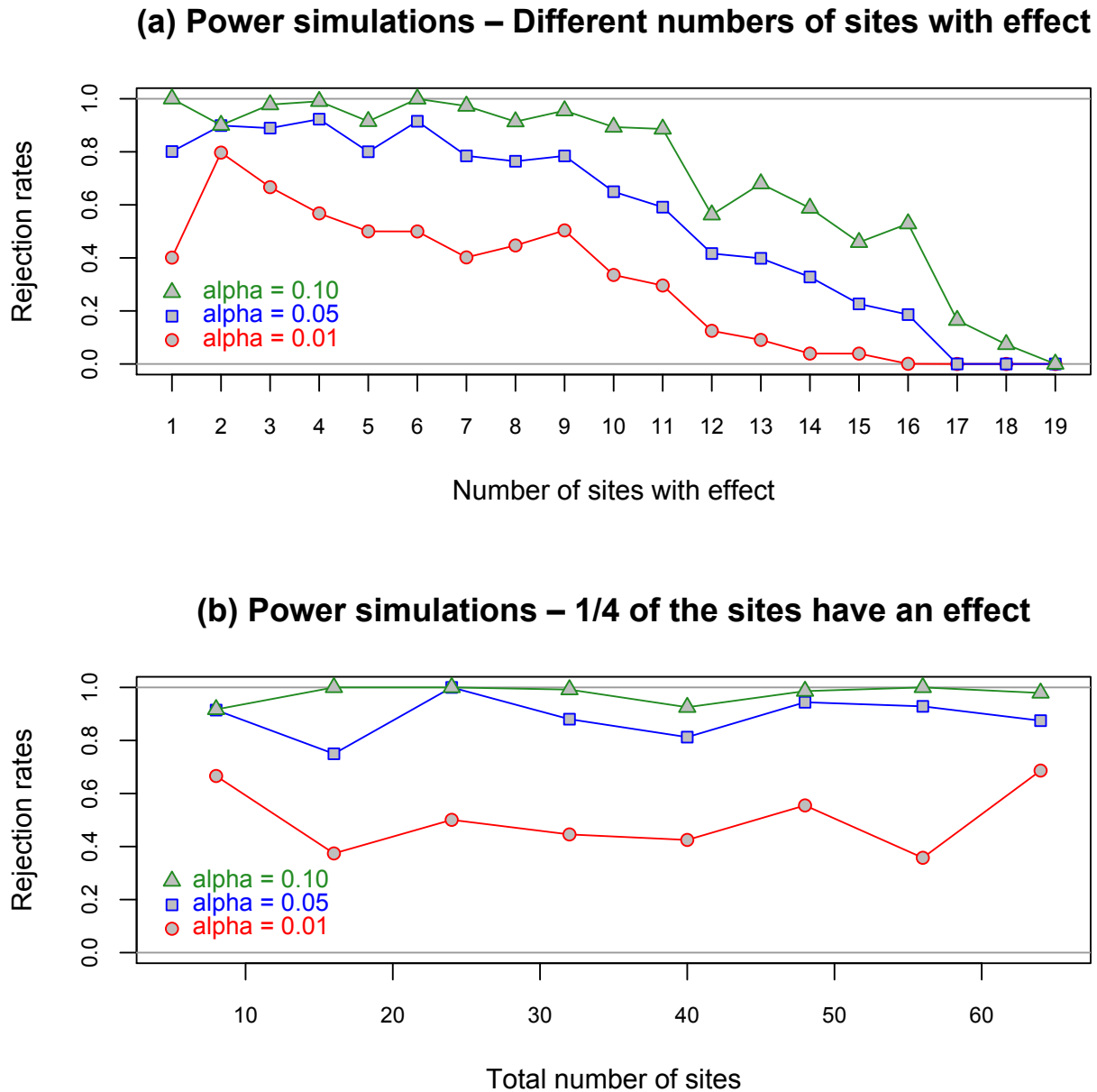


Fig. A1.7. Rejection rates of the TBI tests in power simulations. Rates of rejection of  $H_0$  through the simulations are reported for three significance levels  $\alpha$ : 0.01, 0.05 and 0.10. **(a)** Different numbers of sites received an effect (abscissa,  $nI = 1$  to 19), i.e. a difference in community composition between T1 and T2. There were 20 sites in total in each simulation. **(b)** The proportion of sites with an effect was kept constant, here  $nI = n/4$ , for different values of  $n$  (abscissa). The rejection rate is the mean proportion of the sites with an effect that were identified as significant at the stated  $\alpha$  level.



## References

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-

**Table A1.1.** Type I error rates of the test of the TBI  $D$  indices shown in the first column: rejection rates (i.e. number of rejections of  $H_0$  divided by the number of simulations, which was 1000) of the TBI test when there were no exceptional sites in the simulated data. The data were drawn from a **random Poisson distribution**;  $n = 20$  sites,  $p = 20$  species (Fig. S1.1). All tests involved 999 random permutations. Simulation series 1: data at all sites and both times came from the same statistical population, hence  $H_0$  was true

	Nominal significance levels						
	0.01	0.05	0.10	0.20	0.30	0.40	0.50
% difference	0.000	0.034	0.108	0.208	0.275	0.335	0.491
Ružička	0.000	0.033	0.108	0.208	0.275	0.333	0.492
Chord	0.000	0.042	0.075	0.200	0.292	0.383	0.475
Hellinger	0.000	0.042	0.100	0.175	0.300	0.367	0.517
Log-chord	0.000	0.050	0.117	0.183	0.300	0.375	0.483
Euclidean	0.000	0.025	0.094	0.200	0.319	0.400	0.494

**Table A1.2.** Type I error rates of the test of the TBI  $D$  indices shown in the first column. See caption of Table A1.1. The data were drawn from a **random Poisson distribution**;  $n = 20$  sites. Simulation series 2: for the basic  $p1 = 20$  species, data at all sites and both times came from the same statistical population. In addition, T2 had  $p3 = 6$  species more than T1 (Fig. S1.2). All tests involved 999 random permutations. For these 6 species, there were no differences among the sites besides random variation; hence  $H_0$  was still true.

	Nominal significance levels						
	0.01	0.05	0.10	0.20	0.30	0.40	0.50
% difference	0.017	0.042	0.092	0.183	0.267	0.425	0.541
Ružička	0.017	0.042	0.091	0.183	0.267	0.425	0.541
Chord	0.000	0.062	0.112	0.212	0.312	0.450	0.525
Hellinger	0.000	0.075	0.112	0.213	0.312	0.375	0.550
Log-chord	0.000	0.075	0.100	0.238	0.312	0.400	0.550
Euclidean	0.008	0.050	0.100	0.208	0.300	0.383	0.508

**Table A1.3.** Type I error rates of the test of the TBI  $D$  indices shown in the first column: rejection rates (i.e. number of rejections of  $H_0$  divided by the number of simulations, which was 1000) of the TBI test when there were no exceptional sites in the simulated data. The data were drawn from a **random lognormal distribution**;  $n = 20$  sites,  $p = 20$  species (Fig. S1.1). All tests involved 999 random permutations. Simulation series 1: data at all sites and both times came from the same statistical population, hence  $H_0$  was true.

	Nominal significance levels						
	0.01	0.05	0.10	0.20	0.30	0.40	0.50
%difference $D$	0.014	0.064	0.121	0.193	0.293	0.386	0.486
Ružička $D$	0.014	0.064	0.121	0.193	0.293	0.386	0.486
Chord $D$	0.000	0.038	0.100	0.188	0.251	0.388	0.513
Hellinger $D$	0.000	0.050	0.100	0.175	0.263	0.375	0.562
Log-chord $D$	0.000	0.025	0.076	0.213	0.313	0.425	0.512
Euclidean $D$	0.000	0.050	0.112	0.188	0.312	0.413	0.500

**Table A1.4.** Type I error rates of the test of the TBI  $D$  indices shown in the first column. See caption of Table A1.3. The data were drawn from a **random lognormal distribution**;  $n = 20$  sites. All tests involved 999 random permutations. Simulation series 2: for the basic  $p1 = 20$  species, data at all sites and both times came from the same statistical population. In addition, T2 had  $p3 = 6$  species more than T1 (Fig. S1.2). For these 6 species, there were no differences among the sites besides random variation; hence  $H_0$  was still true.

	Nominal significance levels						
	0.01	0.05	0.10	0.20	0.30	0.40	0.50
%difference $D$	0.022	0.061	0.105	0.210	0.294	0.389	0.500
Ružička $D$	0.022	0.061	0.105	0.210	0.294	0.389	0.500
Chord $D$	0.017	0.061	0.100	0.222	0.300	0.411	0.517
Hellinger $D$	0.017	0.055	0.111	0.194	0.273	0.368	0.501
Log-chord $D$	0.022	0.072	0.116	0.194	0.273	0.401	0.506
Euclidean $D$	0.017	0.056	0.133	0.194	0.322	0.405	0.505

**Table A1.5.** Type I error rates of the test of TBI indices computed using the Euclidean distance. Simulation series 3: the data were drawn from a random normal distribution;  $n = 20$  sites,  $p = 20$  variables. All tests involved 999 random permutations. Data at all sites and both times came from the same statistical population, hence  $H_0$  was true.

	Nominal significance levels						
	0.01	0.05	0.10	0.20	0.30	0.40	0.50
Euclidean $D$	0.007	0.050	0.100	0.193	0.321	0.421	0.528

**Table A1.6.** Power analysis of TBI  $D$  indices shown in the first column, random Poisson deviates. There were  $n1 = 5$  exceptional sites in the simulated data. The results in the table are the mean rejection rates of the TBI test for these 5 sites, computed as the number of rejections of  $H_0$  divided by the number of simulations, which was 1000. (A) Data with  $p1$  and  $p2$  species only (Fig. S1.3). (B)  $p3 = 6$  extra species with values of 0 in Mat.1 and random Poisson deviates in all sites of Mat.2 (Fig. S1.4);  $H_0$  is true for these  $p3$  species.

		(A) $p1$ and $p2$ species			(B) $p1$ , $p2$ and $p3$ species		
		Significance levels			Significance levels		
		0.01	0.05	0.10	0.01	0.05	0.10
%difference $D$	1	0.341	0.787	0.859	0.478	0.858	0.900
Ružička $D$	1	0.341	0.787	0.859	0.478	0.858	0.900
Chord $D$	1	0.355	0.511	0.600	0.381	0.580	0.720
Hellinger $D$	1	0.377	0.577	0.711	0.421	0.660	0.840
Log-chord $D$	1	0.355	0.600	0.711	0.401	0.620	0.800
Euclidean $D$	1	0.000	0.000	0.000	0.000	0.000	0.020

**Table A1.7.** Power analysis of TBI  $D$  indices shown in the first column, random lognormal deviates. There were 5 exceptional sites in the simulated data. The results in the table are the mean rejection rates of the TBI test for these 5 sites, computed as the number of rejections of  $H_0$  divided by the number of simulations, which was 1000. Data with  $p1$  and  $p2$  species only (Fig. S1.3). There were no extra species in these simulations ( $p3 = 0$ ). The contribution parameter of these simulations varied: (A)  $contr = 0.01$ , (B)  $contr = 0.02$ .

		(A) $contr = 0.01$			(B) $contr = 0.02$		
		<u>Significance levels</u>			<u>Significance levels</u>		
		0.01	0.05	0.10	0.01	0.05	0.10
%difference $D$	1	0.875	0.975	1.000	0.463	0.849	0.962
Ružička $D$	1	0.875	0.975	1.000	0.463	0.849	0.962
Chord $D$	1	0.625	0.675	0.713	0.301	0.313	0.338
Hellinger $D$	1	0.612	0.737	0.850	0.315	0.401	0.529
Log-chord $D$	1	0.676	0.887	0.912	0.401	0.638	0.750
Euclidean $D$	1	0.012	0.025	0.062	0.012	0.025	0.062

**Table A1.8.** Power analysis of TBI indices computed using the Euclidean distance, random normal deviates. There were 5 exceptional sites in the simulated data. The results in the table are the mean rejection rates of the TBI test for these 5 sites, computed as the number of rejections of  $H_0$  divided by the number of simulations, which was 1000. Data with  $p1$  and  $p2$  species only. There were no extra variables in these simulations ( $p3 = 0$ );  $contr = 0.05$ .

		$contr = 0.05$		
		<u>Significance levels</u>		
		0.01	0.05	0.10
Euclidean $D$		0.627	0.772	0.843

## Appendix A2

### AN R FUNCTION TO STANDARDIZE ENVIRONMENTAL DATA PRIOR TO TBI ANALYSIS

```

3 #' Special standardization for environmental data prior to TBI analysis.
4 #'
5 #' After standardization, all variables will have the same weight (i.e. they will
6 #' all contribute the same variance) in the calculation of TBI indices.
7 #'
8 #' @param mat1 First data matrix, class matrix or data.frame.
9 #' @param mat2 Second data matrix, class matrix or data.frame.
10 #' @param non.neg=TRUE : make the data non-negative before scaling (recommended).
11 #' non.neg=FALSE: keep standardized data with signs (due to centring).
12 #'
13 #' @return A list with the two matrices standardized as described above.
14 #'
15 #' @details
16 #' The two data sets are joined into a single data matrix, Y = rbind(Y.T1, Y.T2).
17 #' Y is standardized [Y.stand = scale(Y)], then it is separated into two matrices
18 #' of the sizes of the original data matrices before analysis with function TBI().
19 #'
20 #' Explanation:
21 #' (a) the two data matrices are joined into a single data matrix, Y = rbind(Y.T1,
22 #' Y.T2), before standardization. In this way, the differences in values of each
23 #' variable for a given pair of sites in the two tables will remain comparable
24 #' to the differences computed from the original unstandardized values; in this
25 #' way, the distances computed between sites in T1 and T2 will be meaningful.
26 #' Important when there are differences in means and variances between T1 and T2.
27 #' (b) Standardizing the variables insures that all variables will contribute the
28 #' same variance to the calculation of the TBI indices; the variances will not
29 #' depend on the physical units of the variables or other contingencies that make
30 #' the variances unequal.
31 #'
32 #' Argument non.neg=TRUE makes all values  $\geq 0$ . Subtracting the matrix overall
33 #' minimum from all values does not change the Euclidean distances among the sites.
34 #'
35 #' @author Pierre Legendre \email{pierre.legendre@umontreal.ca}, 2018
36 'scale.for.TBI' <-
37   function(mat1,mat2,
38             non.neg=TRUE)
39   {
40     mat1 <- as.matrix(mat1)
41     mat2 <- as.matrix(mat2)
42     dim.1 <- dim(mat1)
43     dim.2 <- dim(mat2)
44     if(!is.numeric(mat1)) stop("First data matrix not numeric")
45     if(!is.numeric(mat2)) stop("Second data matrix not numeric")
46     if(dim.1[1] != dim.2[1]) stop("Data sets have different numbers of rows")
47     if(dim.1[2] != dim.2[2]) stop("Data sets have different numbers of columns")
48     n12 <- dim.1[1]
49     #
50     tmp <- scale(rbind(mat1,mat2))
51     if(non.neg) tmp <- tmp - min(tmp)
52     mat1 <- tmp[1:n12,]
53     mat2 <- tmp[(n12+1):(2*n12),]
54     list(mat1=mat1, mat2=mat2)
55   }

```

## Appendix A3

### RESULTS OF CALCULATIONS WITH R FUNCTION TBI(), INSECTICIDE EXPERIMENT

Pyrifos insect treatment data: compare survey #4 (one week after the insecticide treatment) to survey #11 (after full recovered from treatment). # indicate comments added to the output files.

```
library(vegan)
data(pyrifos)
survey4.order = c(38,39,41,47,37,44,40,46,43,48,42,45)
survey11.order = c(122,123,125,131,121,128,124,130,127,132,126,129)
```

#### 1. Comparison based upon species abundance data, percentage difference D

```
( res1 <- TBI(pyrifos[survey4.order,], pyrifos[survey11.order,], method="%diff",
nperm=9999, BCD=TRUE, test.t.perm=TRUE, clock=TRUE) )
# Computation time = 47.247000 sec
```

```
-----
$TBI
```

```
[1] 0.4332125 0.4490831 0.4048151 0.4593321 0.4958159 0.4392330 0.4884889 0.4851041
[9] 0.4740264 0.6205484 0.7345825 0.6721440
```

```
$p.TBI
```

```
[1] 0.8749 0.8082 0.9582 0.7518 0.5173 0.8588 0.5635 0.5938 0.6584 0.0192 0.0001 0.0018
```

```
$p.adj
```

```
[1] 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 0.1920 0.0012 0.0198
```

```
$BCD.mat
```

	B/(2A+B+C)	C/(2A+B+C)	D=(B+C)/(2A+B+C)	Change	
Site.1	0.1616465	0.2715660	0.4332125	+	# Untreated
Site.2	0.1973186	0.2517645	0.4490831	+	# Untreated
Site.3	0.2305092	0.1743059	0.4048151	-	# Untreated
Site.4	0.2643243	0.1950077	0.4593321	-	# Untreated
Site.5	0.2303800	0.2654359	0.4958159	+	# Treated, 0.1 microgram/L
Site.6	0.1980843	0.2411487	0.4392330	+	# Treated, 0.1 microgram/L
Site.7	0.2425404	0.2459484	0.4884889	+	# Treated, 0.9 microgram/L
Site.8	0.1854199	0.2996843	0.4851041	+	# Treated, 0.9 microgram/L
Site.9	0.1901665	0.2838599	0.4740264	+	# Treated, 6 micrograms/L
Site.10	0.3094316	0.3111168	0.6205484	+	# Treated, 6 micrograms/L
Site.11	0.3232546	0.4113279	0.7345825	+	# Treated, 44 micrograms/L
Site.12	0.1829121	0.4892319	0.6721440	+	# Treated, 44 micrograms/L

```
$BCD.summary # Here the BCD summary is computed for the 12 mesocosms, not the 8 treated
mean(B/den) mean(C/den) mean(D) B/(B+C) C/(B+C) Change
0.2263323 0.2866998 0.5130322 0.441166 0.558834 +
```

```
$t.test_B.C # Here the tests is computed for the 12 mesocosms, not for the 8 treated
mean(C-B) Stat p.param p.perm p<=0.05
Paired t.test 0.06036748 -2.132286 0.05635548 0.0383 # Permutation test signif.
```

```
$BC
```

```
[1] NA
```

```
-----
```

```
# Additional result -
# Paired t-test comparing the B and C stat. ($BCD.mat above) for the 8 treated mesocosms

$t.test_B.C
      mean(C-B)      Stat      p.param p.perm      p<=0.05
Paired t.test  0.08569554 -2.463362  0.04325165 0.0066      * # Both tests significant
```

---

## 2. Comparison based upon species occurrence (i.e. presence-absence) data, Sørensen D

```
( res2 <- TBI(pyrifos[survey4.order,], pyrifos[survey11.order,], method="sorensen",
nperm=9999, BCD=TRUE, test.t.perm=TRUE, clock=TRUE) )
# Computation time = 32.808000 sec
```

-----

```
$TBI
[1] 0.4390244 0.4324324 0.4457831 0.4705882 0.4666667 0.4358974 0.5000000 0.4153846
[9] 0.4545455 0.6800000 0.7551020 0.6595745
```

```
$p.TBI
[1] 0.8281 0.8660 0.8051 0.6656 0.6858 0.8487 0.4960 0.9181 0.7671 0.0016 0.0001 0.0042
```

```
$p.adj
[1] 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 1.0000 0.0176 0.0012 0.0420
```

```
$BCD.mat
      B/ (2A+B+C) C/ (2A+B+C) D= (B+C) / (2A+B+C) Change
Site.1  0.1463415 0.2926829      0.4390244      + # Untreated
Site.2  0.1891892 0.2432432      0.4324324      + # Untreated
Site.3  0.2048193 0.2409639      0.4457831      + # Untreated
Site.4  0.2205882 0.2500000      0.4705882      + # Untreated
Site.5  0.1733333 0.2933333      0.4666667      + # Treated, 0.1 microgram/L
Site.6  0.1666667 0.2692308      0.4358974      + # Treated, 0.1 microgram/L
Site.7  0.2105263 0.2894737      0.5000000      + # Treated, 0.9 microgram/L
Site.8  0.1384615 0.2769231      0.4153846      + # Treated, 0.9 microgram/L
Site.9  0.1363636 0.3181818      0.4545455      + # Treated, 6 micrograms/L
Site.10 0.2800000 0.4000000      0.6800000      + # Treated, 6 micrograms/L
Site.11 0.2857143 0.4693878      0.7551020      + # Treated, 44 micrograms/L
Site.12 0.1276596 0.5319149      0.6595745      + # Treated, 44 micrograms/L
```

```
$BCD.summary # Here the BCD summary is computed for the 12 mesocosms, not the 8 treated
mean(B/den) mean(C/den) mean(D) B/ (B+C) C/ (B+C) Change
0.189972 0.3229446 0.5129166 0.3703759 0.6296241      +
```

```
$t.test_B.C # Here the test is computed for the 12 mesocosms, not for the 8 treated
      mean(C-B)      Stat      p.param p.perm      p<=0.05
Paired t.test  0.1329727 -4.621706      0.000738 0.0008      * # Both tests signif.
```

```
$BC
[1] NA
```

---



## Appendix A4

### RESULTS OF CALCULATIONS WITH R FUNCTION TBI(), CHESAPEAKE BAY DATA

TBI tests of significance of the difference between years at each site and BCD.mat matrix containing the *B* and *C* statistics used to construct the B-C plot (Fig. 4); 25 brackish sites, years 2005 and 2008, fall survey data. Sites 4 and 8 had no species in common between 2005 (T1) and 2008 (T2). Significant adjusted p-values (Holm correction) are underscored in the results below.

#### 1. Comparison based upon species abundance data, percentage difference *D*

```
( res.fauna.05.08.pcdiff = TBI(Y1, Y2, "%diff", pa.tr=FALSE, nperm=99999, BCD=TRUE,
test.BC=TRUE, test.t.perm=TRUE, clock=TRUE) )
# Computation time = 604.271000 sec
```

-----

\$TBI

```
[1] 0.6766467 0.5704698 0.9411765 1.0000000 0.6309524 0.7685950 1.0000000 1.0000000
[9] 0.6960000 0.5777778 0.8632812 0.5081967 0.3229572 0.7083333 0.5164835 0.5843137
[17] 1.0000000 0.8983051 0.6385965 0.4244604 0.6256158 0.3846154 1.0000000 0.8020833
[25] 0.7611940
```

\$p.TBI

```
[1] 0.38334 0.61550 0.00627 0.00062 0.48268 0.19719 0.00062 0.00056 0.34265 0.59802
[11] 0.06230 0.74991 0.97718 0.31635 0.72990 0.58333 0.00064 0.03035 0.46790 0.89258
[21] 0.49572 0.93799 0.00058 0.14253 0.20731
```

\$p.adj

```
[1] 1.00000 1.00000 0.12540 0.01426 1.00000 1.00000 0.01426 0.01400 1.00000 1.00000
[11] 1.00000 1.00000 1.00000 1.00000 1.00000 1.00000 0.01426 0.57665 1.00000 1.00000
[21] 1.00000 1.00000 0.01400 1.00000 1.00000
```

\$BCD.mat

	B/ (2A+B+C)	C/ (2A+B+C)	D= (B+C) / (2A+B+C)	Change	
Site.1	0.167664671	0.50898204	0.6766467	+	
Site.2	0.436241611	0.13422819	0.5704698	-	
Site.3	0.029411765	0.91176471	0.9411765	+	* Mostly abundances-per-sp. gains
Site.4	0.250000000	0.75000000	1.0000000	+	* Mostly abundances-per-sp. gains
Site.5	0.053571429	0.57738095	0.6309524	+	
Site.6	0.347107438	0.42148760	0.7685950	+	
Site.7	0.950000000	0.05000000	1.0000000	-	* Mostly abundances-per-sp. losses
Site.8	0.400000000	0.60000000	1.0000000	+	* 40% Ab.-per-sp. losses, 60% gains
Site.9	0.576000000	0.12000000	0.6960000	-	
Site.10	0.100000000	0.47777778	0.5777778	+	
Site.11	0.224609375	0.63867188	0.8632812	+	
Site.12	0.483606557	0.02459016	0.5081967	-	
Site.13	0.190661479	0.13229572	0.3229572	-	
Site.14	0.229166667	0.47916667	0.7083333	+	
Site.15	0.340659341	0.17582418	0.5164835	-	
Site.16	0.482352941	0.10196078	0.5843137	-	
Site.17	0.000000000	1.00000000	1.0000000	+	* Only abundances-per-sp. gains
Site.18	0.135593220	0.76271186	0.8983051	+	
Site.19	0.057894737	0.58070175	0.6385965	+	
Site.20	0.316546763	0.10791367	0.4244604	-	
Site.21	0.571428571	0.05418719	0.6256158	-	
Site.22	0.161538462	0.22307692	0.3846154	+	
Site.23	0.098039216	0.90196078	1.0000000	+	* Mostly abundances-per-sp. gains
Site.24	0.005208333	0.79687500	0.8020833	+	
Site.25	0.008955224	0.75223881	0.7611940	+	

```

$BCD.summary
  mean(B/den) mean(C/den)   mean(D)   B/(B+C)   C/(B+C) Change
    0.2646503    0.4513519 0.7160022 0.3696222 0.6303778    +

$t.test_B.C, nperm=99999
      mean(C-B)      Stat    p.param  p.perm  p<=0.05
Paired t.test    0.1867016    1.826046 0.08031088 0.07998

$BC
[1] NA
-----

```

**Note** – The site names, Site.1 to Site.25, found in the function output file, correspond to the following site names on the Chesapeake Bay map:

```

site.names
[1] "S1"   "S15"  "S201" "S202" "S203" "S204" "S22"  "S23"  "S24"  "S26"
[11] "S29"  "S40"  "S43"  "S44"  "S47"  "S51"  "S52"  "S6"   "S62"  "S64"
[21] "S66"  "S68"  "S71"  "S74"  "S77"

```

---

## 2. Comparison based upon species occurrence (i.e. presence-absence) data, Sørensen D

```
( res.fauna.05.08.sor = TBI(Y1, Y2, "sorensen", pa.tr=FALSE, nperm=9999, BCD=TRUE,
test.BC=TRUE, test.t.perm=TRUE, clock=TRUE) )
```

```
# Computation time = 45.459000 sec
```

```
-----
```

```
$TBI
```

```
[1] 0.4838710 0.4166667 0.6666667 1.0000000 0.2727273 0.1578947 1.0000000 1.0000000
[9] 0.3846154 0.1818182 0.3600000 0.3333333 0.2500000 0.4000000 0.2500000 0.2857143
[17] 1.0000000 0.5238095 0.2592593 0.2592593 0.3333333 0.1538462 1.0000000 0.1304348
[25] 0.2592593
```

```
$p.TBI
```

```
[1] 0.1607 0.2984 0.0217 0.0010 0.7423 0.9574 0.0005 0.0004 0.3902 0.9261 0.4807
[12] 0.5910 0.8108 0.3526 0.8042 0.7025 0.0009 0.1202 0.7714 0.7748 0.5940 0.9593
[23] 0.0006 0.9768 0.7727
```

```
$p.adj
```

```
[1] 1.0000 1.0000 0.4340 0.0210 1.0000 1.0000 0.0120 0.0100 1.0000 1.0000 1.0000
[12] 1.0000 1.0000 1.0000 1.0000 1.0000 0.0198 1.0000 1.0000 1.0000 1.0000 1.0000
[23] 0.0138 1.0000 1.0000
```

```
$BCD.mat
```

	B / (2A+B+C)	C / (2A+B+C)	D = (B+C) / (2A+B+C)	Change	
Site.1	0.06451613	0.41935484	0.4838710	+	
Site.2	0.25000000	0.16666667	0.4166667	-	
Site.3	0.16666667	0.50000000	0.6666667	+	
Site.4	0.50000000	0.50000000	1.0000000	0	* Equal numbers of losses and gains
Site.5	0.09090909	0.18181818	0.2727273	+	
Site.6	0.15789474	0.00000000	0.1578947	-	
Site.7	0.87500000	0.12500000	1.0000000	-	* Mostly species losses
Site.8	0.50000000	0.50000000	1.0000000	0	* Equal numbers of losses and gains
Site.9	0.15384615	0.23076923	0.3846154	+	
Site.10	0.09090909	0.09090909	0.1818182	0	
Site.11	0.16000000	0.20000000	0.3600000	+	
Site.12	0.22222222	0.11111111	0.3333333	-	
Site.13	0.08333333	0.16666667	0.2500000	+	
Site.14	0.20000000	0.20000000	0.4000000	0	
Site.15	0.12500000	0.12500000	0.2500000	0	
Site.16	0.14285714	0.14285714	0.2857143	0	
Site.17	0.00000000	1.00000000	1.0000000	+	* Only species gains
Site.18	0.38095238	0.14285714	0.5238095	-	
Site.19	0.11111111	0.14814815	0.2592593	+	
Site.20	0.11111111	0.14814815	0.2592593	+	
Site.21	0.28571429	0.04761905	0.3333333	-	
Site.22	0.03846154	0.11538462	0.1538462	+	
Site.23	0.36363636	0.63636364	1.0000000	+	* Mostly species gains
Site.24	0.04347826	0.08695652	0.1304348	+	
Site.25	0.11111111	0.14814815	0.2592593	+	

```
$BCD.summary
```

mean(B/den)	mean(C/den)	mean(D)	B / (B+C)	C / (B+C)	Change
0.2091492	0.2453511	0.4545004	0.460174	0.539826	+

```
$t.test_B.C
```

	mean(C-B)	Stat	p.param	p.perm	p<=0.05
Paired t.test	0.0362019	0.6218665	0.5398928	0.5560	

```
$BC
```

```
[1] NA
```

```

#                               Appendix A5

#                               Test function paired.t.test.spec.R

#' Paired t-tests of differences between T1 and T2 for each species
#'
#' This Function computes paired t-tests for each species, for abundances at T1 and T2.
#' The test is one-tailed in the direction of the sign (+ or -) of the t statistic.
#'
####' The required function "t.paired.perm.R" is listed in the lower part of this file.
####' It is also available in the adespatial R package on CRAN.
#'
#' Arguments -
#' mat1: site-by-species data at time T1 (data.frame or matrix).
#' mat2: site-by-species data at time T2 (data.frame or matrix).
#' nperm: Number of permutations.
#'       Use more than 999 permutations to allow correction of p-values for multiple tests.
#' list.all: if FALSE, output only lists t.test results for species with t.stat not 0;
#'           if TRUE, output lists t.test results for all species; when the t-statistic
#'           is 0, the p-values in the output table receive the code -999 and Sign(T1-T2) is 0.
#'
#' Details -
#' The species that do not vary in either data set are discarded before the calculations
#' of the paired t-tests begin.
#' p-values should be corrected for multiple testing. Use function p.adjust() of {stats}:
#'   p.adjust(res$t.test$p.param) or p.adjust(res$t.test$p.perm)
#' Correction methods "holm" (default) and "hochberg" are fine for this type of analysis.
#'
#' Value -
#' 1. A table with species in rows and 6 columns:
#'    "mean(T1-T2)", "t.stat", "p.param", "p.perm", "p<=0.05", "Sign(T1-T2)"
#'    The parametric and permutational p-values are not corrected for multiple tests.
#'    A star is shown in column "p<=0.05" if the parametric p-value is  $\leq 0.05$ .
#' 2. A list of names of the species tested; their t statistics were not 0.
#' 3. A list of names of the species not tested because their t-statistics were 0.
#'
#' References -
#'
#' Legendre, P. 2019. A temporal beta-diversity index to identify sites that have changed
#' in exceptional ways in space-time surveys. Ecology and Evolution (in press).
#'
#' Legendre, P. & R. Condit. 2019. Spatial and temporal analysis of beta diversity in the
#' Barro Colorado Island forest dynamics plot, Panama. Forest Ecosystems (in press).
#'
#' van den Brink, P. J. & C. J. F. ter Braak. 1999. Principal response curves: analysis of
#' time-dependent multivariate responses of biological community to stress. Environmental
#' Toxicology and Chemistry 18: 138-148.
#'
#' Author - Pierre Legendre, August 2018
#'
#' Example -
#'
####' Invertebrate communities subjected to insecticide treatment.
#'
## As an example in their paper on Principal Response Curves (PRC), van den Brink & ter
## Braak (1999) used observations on the abundances of 178 invertebrate species
## (macroinvertebrates and zooplankton) subjected to treatments in 12 mesocosms by the
## insecticide chlorpyrifos. The mesocosms were sampled at 11 occasions. The data,
## available in the {vegan} package, are log-transformed species abundances, ytransformed =
## loge(10*y+1).
#'
## The data of survey #4 will be compared to those of survey #11 in this example.
## Survey #4 was carried out one week after the insecticide treatment, whereas the
## fauna of the mesocosms was considered to have fully recovered from the treatment
## at the time of survey #11.
#'
require(vegan)

```

```

#' data(pyrifos)
#'
#' ## The mesocosms had originally been attributed at random to the treatments. However,
#' to facilitate presentation of the results, they will be listed here in order of
#' increased insecticide doses: {0, 0, 0, 0, 0.1, 0.1, 0.9, 0.9, 6, 6, 44, 44} µg/L.
#'
#' survey4.order = c(38,39,41,47,37,44,40,46,43,48,42,45)
#' survey11.order = c(122,123,125,131,121,128,124,130,127,132,126,129)
#'
#' ## Paired t-tests of differences between survey.4 and survey.11 for the p species
#'
#' res <- paired.t.test.spec(pyrifos[survey4.order,],pyrifos[survey11.order,])
#'
#' =====
#'
paired.t.test.spec <- function(mat1, mat2, nperm=999, list.all=FALSE)
{
  epsilon <- .Machine$double.eps
  n = nrow(mat1)
  p = ncol(mat1)
  if(nrow(mat2) != n) stop("Unequal number of rows")
  if(ncol(mat2) != p) stop("Unequal number of species")

  # Check species names in files mat1 and mat2
  sp.diff <- which(colnames(mat1) != colnames(mat2))
  if(length(sp.diff)>0)
    cat("Warning - The following species names differ between mat1 & mat2:\n",sp.diff,"\n\n")

  # Select the species that have variances > epsilon. Discard the other species.
  comm.gr = rbind(mat1, mat2)
  tmp = apply(comm.gr, 2, var)
  sel.sp = which(tmp > epsilon)
  pp <- length(sel.sp)
  cat((p-pp),"species were eliminated because they did not vary in the combined data set\n")
  cat(pp,"species retained:\n")
  mat1.sel = mat1[,sel.sp]
  mat2.sel = mat2[,sel.sp]

  #
  res = matrix(0,pp,4)
  sp.names <- rownames(res) <- colnames(mat1.sel)

  #
  Star <- NA
  Keep <- NA
  Reject <- NA
  for(j in 1:pp) {
    vec1 = mat1[,j]
    vec2 = mat2[,j]
    # if(any(abs(vec1-vec2) > 0)) Keep = c(Keep,j) else Reject = c(Reject,j)
    cat(j," ") # Print ID of species being processed
    tmp <- t.test(vec1, vec2, paired=TRUE)
    if(tmp$estimate == 0) {
      Reject = c(Reject,j)
      res[j,3:4] = c(-999,-999)
      Star <- c(Star, " ")
    } else {
      Keep = c(Keep,j)
      if(sign(tmp$estimate)<0) tail="less" else if(sign(tmp$estimate)>=0) tail="greater"
      t.res <- t.paired.perm(vec1, vec2, nperm=nperm, alternative=tail, silent=TRUE)
      res[j,1:4] <- c(t.res$estim, t.res$t.ref, t.res$p.param, t.res$p.perm)
      Star <- c(Star, ifelse(t.res$p.perm>0.05, " ", "*"))
    }
  }
  cat("\n\n")
  Keep <- Keep[-1]
  Reject <- Reject[-1]

```

```

tmp <- length(Reject)
if(tmp>0) cat(tmp,"species not tested because t.stat = 0. See 'No_test' output list\n\n")
res = data.frame(res,Star[-1],sign(res[,1]))
colnames(res) = c("mean(T1-T2)","t.stat","p.param","p.perm","p<=0.05","Sign(T1-T2)")
if(list.all)
  out <- list(t.tests=res, Tested=sp.names[Keep], No_test=sp.names[Reject])
else out <- list(t.tests=res[Keep,],Tested=sp.names[Keep],No_test=sp.names[Reject])
out
}

#' t.paired.perm for permutational paired t-test
#'
#' This function computes a permutation test of comparison of the means
#' of two paired vectors (related samples).
#' For each object, permutations are restricted to the two related observations.
#'
#' Parametres of the function
#'
#' vec1, vec2: the two vectors to be compared
#' nperm = number of permutations (default value: 999)
#' alternative = c("two.sided", "less", "greater"). Dafault value: "two.sided"
#' silent = FALSE: calculation results are printed to the R console.
#'           = TRUE : calculation results are not printed to R console (for simulations).
#'
#' Values returned
#'
#' t.ref : reference value of the t-statistic
#' p.param : parametric p-value
#' p.perm : permutational p-value
#' nperm : number of permutations
#'
#' Example: Deer leg length data from Zar (1999, p. 162)
#'
#' deer = matrix( c(142,140,144,144,142,146,149,150,142,148,138,136,147,139,143,141,143,
#' 145,136,146),10,2)
#' rownames(deer) = c('Deer.1','Deer.2','Deer.3','Deer.4','Deer.5','Deer.6','Deer.7',
#' 'Deer.8','Deer.9','Deer.10')
#' colnames(deer) = c('Hind.leg', 'Fore.leg')
#'
#' res = t.paired.perm(deer[,1], deer[,2]) # Two-tailed test by default
#'
#' Compare results to: res2 = t.test(deer[,1], deer[,2], paired=TRUE)
#'
#'
#' Pierre Legendre, November 2009
#' Guillaume Blanchet, September 2015 (faster permutation code)

t.paired.perm <- function(vec1, vec2, nperm=999, alternative="two.sided", silent=FALSE)
{
  n1 <- length(vec1)
  n2 <- length(vec2)
  if(n1 != n2) stop("The two vectors have different lengths. They cannot be paired.")

  alt <- match.arg(alternative, c("two.sided", "less", "greater"))

  res = t.test(vec1, vec2, paired=TRUE, alternative=alt)
  t.ref = res$statistic

  # Print these first results
  if(!silent) {
    cat('\nt-test comparing the means of two related samples','\n','\n')
    cat('Number of objects:',n1,'\n')
    cat('Mean of the differences:',res$estimate,'\n')
    cat('t statistic (paired observations):',t.ref,'\n')
    cat('95 percent confidence interval of t:',res$conf.int,'\n')
    cat('Degrees of freedom:',res$parameter,'\n')
    cat('Alternative hypothesis:',alt,'\n')
    cat('Prob (parametric):',res$p.value,'\n')
  }
}

```

```

    }

# Perform the permutation test
# Permutations are restricted to the two related observations for each object.
nPGE <- 1

for(i in 1:nperm)
{
## New permutation code, GB
  mat <- cbind(vec1,vec2)
  topermute <- rbinom(n1,1,0.5)
  mat[topermute==1,] <- mat[topermute==1,2:1]
## End new code, GB

  res.perm = t.test(mat[,1], mat[,2], paired=TRUE, alternative=alt)
  t.perm = res.perm$statistic

  if(alt == "two.sided") if( abs(t.perm) >= abs(t.ref) ) nPGE <- nPGE+1
  if(alt == "less")      if(t.perm <= t.ref) nPGE <- nPGE+1
  if(alt == "greater")   if(t.perm >= t.ref) nPGE <- nPGE+1
}

# Compute and print the permutational p-value
P <- nPGE/(nperm+1)
if(!silent) cat('Prob (',nperm,'permutations):',
formatC(P,digits=5,width=7,format="f"),'\n')
#
return(list(estim=res$estimate, t.ref=t.ref, p.param=res$p.value, p.perm=P, nperm=nperm))
}

```