

# A temporal beta-diversity index to identify exceptional sites in space-time surveys

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Running headline: Temporal beta diversity analysis

## Summary

1. The paper describes a method to test the differences between community data matrices corresponding to observations made at times T1 and T2 in space-time ecological surveys involving several sites. The objective is to identify the sites that have changed in an exceptional way in species composition between T1 and T2.

2. The null hypothesis is that a species assemblage is not exceptionally different between T1 and T2. The problem: testing the significance for dissimilarity coefficients is usually not possible because the values in a dissimilarity matrix are interrelated. However, the dissimilarity between T1 and T2 for a site is independent of the dissimilarities that concern T1–T2 data at other sites. The paper shows that it is possible to compute a valid test of significance in that case.

3. The method also allows users to examine the processes of biodiversity losses and gains through time at the different sites in space-time surveys. Three applications of the method to different ecological communities are presented.

4. This method will be of value to identify exceptional sites in space-time ecological surveys carried out to study anthropogenic impacts, including climate change. R software is available implementing the method.

**Key-words:** Beta diversity, B-C plots, power, space-time analysis, temporal beta diversity, type I error

## Introduction

In several application fields, researchers want to compare observations made at several sites and at two different times. The question of interest is: are there sites where the difference is so important that they do not seem to belong to the same statistical population as the other sites? If the difference is so exceptional as to lead to a statistically significant result, these sites are worth examining in more detail to identify the cause of the differences. The exceptional character of the difference indicates that some atypical process may be occurring there. Here are some examples. In palaeoecology, comparison of ancient and modern diatom communities preserved in lake sediment cores may indicate areas where acute anthropogenic processes have singularly changed the surrounding land use (e.g., Winegardner et al. 2017). When a strong environmental impact has taken place at a known point in time and an ecological community had been surveyed ahead of the impact, ecologists may survey that community again to determine how it was affected by the impact, and then how it may have recovered in later surveys (e.g., Legendre & Salvat 2015). In community ecology, when studying a permanent stem-mapped forest plot divided into regular quadrats, examining surveys made at two different times may indicate quadrats that have been exceptionally affected by a disturbance, e.g. a climatic or anthropogenic event (e.g., Legendre & Condit in prep.). In population genetics, comparing several local populations of a species observed at two different moments separated by an event of interest may indicate the locations where the event may have had exceptionally strong effects by changing the population genetic structure. Other examples can be found in other fields of biological and biomedical research.

This paper describes a method to test, for several sampling units (objects), the differences between data vectors corresponding to observations made at times T1 and T2. To fix ideas, I will

refer to these objects as sites in this paper, although they may be of different natures. The observed data, assembled in matrices **Mat.1** for time T1 and **Mat.2** for T2, may be of different kinds; in landscape ecology and genetics, the data are community composition or population gene frequencies observed at different sites. The hypothesis ( $H_0$ ) to be tested is that a site is not exceptionally different between T1 and T2, compared to other sites in the study that have been observed at the same two times, and belongs to the same statistical population as the other sites.

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 T1–T2 comparisons for individual sites, however, the dissimilarity between T1 and T2 for a site is independent of the dissimilarities computed for T1–T2 data at other sites. So it may be possible to work out a valid test of significance in that case. That test would be of value to identify exceptional sites, which may have a large dissimilarity for different reasons; these reasons may be worth investigating. If the number of sites is large, investigators may want to focus only onto the sites that produce exceptionally (in the statistical sense) large dissimilarity values in T1–T2 comparisons. A dissimilarity  $D$  computed between times T1 and T2 for a site, using community composition or gene frequency data for example, is called a *Temporal Beta-diversity Index* (TBI); it measures the change in community composition (or temporal beta diversity) from T1 to T2. A change through time is directional; something (e.g. species, species abundances, gene frequencies) has been gained and/or lost between T1 and T2, and these two components are both of interest to understand the change.

## Methods

### TBI computation and testing

The proposed method consists basically in the following steps: a dissimilarity index is computed for each site between the data vectors corresponding to T1 and T2, then the indices are tested for significance using a permutational procedure. Two of the dissimilarity indices that can be used in this type of analysis also allow the computation of species losses and gains at each site between T1 and T2. These data provide users with detailed information, at the site level, about the response of the community to the events that occurred between T1 and T2.

#### 1. Compute Temporal Beta-diversity Indices (TBI)

Consider two data matrices, **Mat.1** and **Mat.2**, about the same objects, each one with  $n$  sites as rows and the same  $p$  variables as columns (**Fig. 1**). Individual values may be noted  $y_{ij.1}$  and  $y_{ij.2}$ . Compute the dissimilarity  $D(\mathbf{y}_{i.1}, \mathbf{y}_{i.2})$  between the two vectors of values,  $\mathbf{y}_{i.1}$  and  $\mathbf{y}_{i.2}$ , for each site  $i$ . These  $n$  dissimilarities form a vector of length  $n$ .

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) 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  $(1 - S_{Sorensen})$  dissimilarity whereas the Ružička dissimilarity produces  $(1 - S_{Jaccard})$ , where  $S$  designates similarity.

The chord, Hellinger, and log-chord distances are members of the Box-Cox family of distances (Legendre & Borcard 2018). They are also classical indices for beta diversity studies (Legendre & De Cáceres 2013). These indices, as well as the Euclidean distance, are also implemented in the TBI.R function and will be used in the simulations and ecological applications below.

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 and Salvat (2015). Consider data vectors  $y_1$  and  $y_2$  corresponding to the multi-species observations at T1 and T2 for a site. The following calculations can be done:

- $a_j$  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:  $b_j = y_{1j} - y_{2j}$ .  $B$  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:  $c_j = y_{2j} - y_{1j}$ .  $C$  is the sum of the  $c_j$  values for all species. It is the unscaled sum of *species gains* between T1 and T2.

$(B+C)$  represent the unscaled dissimilarity. 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,  $D_{Ruz} = (B+C)/(A+B+C)$  (Legendre 2014).  $(B - C)$  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  $(2A+B+C)$  for  $D_{\%diff}$  and  $(A+B+C)$  for  $D_{Ruz}$  case. The  $D_{\%diff}$  and  $D_{Ruz}$  dissimilarities measure the temporal beta diversity 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  $den$  that is used. In other words,  $D_{loss}$  and  $D_{gain}$  partition the  $D_{\%diff}$  and  $D_{Ruz}$  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.

What are the ecological applications of  $D_{loss}$  and  $D_{gain}$ ? For each site, one can explore which process, between  $D_{loss}$  and  $D_{gain}$ , presents the largest contribution to the temporal  $D_{\%diff}$  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 in all quadrats of a stem-mapped dynamics forest plot, the  $B/den$  and  $C/den$  statistics can be mapped, subjected to canonical analysis (see Ecological application 2), plotted as B-C plots (see subsection 4 below and Ecological application 3), or studied in other ways to understand the differences among the study sites.

## 2. Testing procedure

To test the significance of TBI indices, the data are permuted at random in both matrices and the indices are recomputed; this procedure is repeated a large number of times and a p-value is computed for the difference between T1 and T2 at each site. Permutations can be done in several ways. A simulation study will compare three permutation methods.

Permutation of species independently of one another is the method used to assess the significance of *Local Contributions to Beta Diversity* (LCBD indices) in the Legendre & De Cáceres (2013) paper. The same logic is followed here in permutation methods 1 and 2. Simulation results (below) will show that this method produces tests with higher power than method 3, the permutation of entire data rows.

### 2.1. Permutation method 1 –

Permute the raw abundance data at random within each column separately, but in the same way in the two matrices corresponding to T1 and T2.

2.1.1. In each matrix, the original values (e.g. species abundances) are permuted at random, independently in each column. Permutation of the two matrices is started with the same random seed, so that the values in each column (e.g. species) are permuted in the same way in **Mat1.perm** and **Mat2.perm**. With this method, it is the *differences* in values between T1 and T2, for each species, that are permuted at random among the sites. The justification is that we are testing dissimilarities, obtained by combining the species differences between T1 and T2.

2.1.2. The transformation, if any (in the case of chord, Hellinger or log-chord dissimilarities), is recomputed on the permuted 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 or row norms of 1. In this way, the  $D_i$  of the permuted data will remain comparable to the reference  $D_i$ .

2.1.3. The TBI distances between T1 and T2 are recomputed, for each site separately.

2.1.4. After a large number of permutations, a p-value is computed for site  $i$  (hence for each  $D(\mathbf{y}_{i-1}, \mathbf{y}_{i-2})$  index), in the same way as in any permutation test. A correction for multiple testing is applied to obtain a correct experimentwise error rate.

Some technical aspects of permutation method 1 are discussed in [Appendix S2](#).

### 2.2. Permutation method 2 –

A variant over method 1 is to permute each species independently, as in method 1, without worrying about using the same permutation for species  $j$  in matrices **Mat.1** and **Mat.2**. If that method has the same power as method 1, or better, it would lead to simpler code.

### 2.3. Permutation method 3 –

Another possible method is to permute entire rows of **Mat.1** and **Mat.2**, independently in these two matrices, as it is done in several permutational statistical procedures. The statistical hypothesis under test and the permutation set differ from those in methods 1 and 2 where each species is permuted independently. It will be included in the simulation study only because this method is widely used in multivariate data analysis.

## 2.4. Permutation method 4 –

• If the sites are part of a geographic broad-scale gradient on a map and spatial autocorrelation is considered to be a salient property of the data, each species could be permuted in a toroidal manner to preserve the spatial autocorrelation of the data. This option is not implemented at the moment in the TBI.R calculation function.

## 3. BCD computation

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 the *species gains* between T1 and T2. The unscaled statistics can be scaled to values in 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 that decomposition of  $D$  by listing the  $B/den$  and  $C/den$  components of TBI indices for each site in the study. These basic statistics can be used in two different ways:

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

3.2. For each site, we can also obtain the sign of the difference (gains – losses), or  $(C - B)$ : if  $B > C$ , we note a minus sign ( $-$ ), and if  $B < C$  we note a plus ( $+$ ) sign. This notation allows users to quickly identify the sites where losses or gains dominate. Similarly, the difference  $mean(C/den) - mean(B/den)$  is computed; its sign tells us if gains ( $+$  sign) or losses ( $-$  sign) dominate across all sites. The significance of the difference between the two vectors of statistics  $B/den$  and  $C/den$  can be computed using a parametric or permutational paired  $t$ -test; the R function mentioned in subsection 5 below computes both forms. These tests provide overall indications of the direction of change in community composition over all sites. They help confirm the asymmetry between abundance or occurrence losses ( $B/den$ ) and abundance or occurrence gains ( $C/den$ ). In Ecological application 2 (Tikus Island coral communities), the two forms of calculation provided complementary information.

## 4. B-C plot

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

A B-C plot is presented in Ecological application 3 (Chesapeake Bay benthos data). In that B-C plot, a diagonal green line, with slope of 1, was drawn through the origin; it represents the theoretical positions of sites where  $D_{gain}$  would be equal to  $D_{loss}$ . A red line was also drawn parallel to the green line, passing through the centroid of all points. When the red line is below

the green line, it indicates that the survey interval was dominated by species losses across the sites, and the opposite if the red line is above the green line. Points found higher in the plot towards the upper-right corner represent higher temporal beta diversity than points found lower in the direction of the lower-left corner.

Sites found at the highest diagonal margin of the distribution of points, in the direction of the upper-right corner of the plot, have high  $D$  values (beta differentiation). In most cases, this happens because communities have undergone great changes from T1 to T2. High  $D$  values may also be found at sites that contain very few species and individuals. This situation is discussed in Ecological application 3, where four such sites are found. Users should check the number of species and individuals involved in the dissimilarity calculation of these sites before drawing ecological conclusions.

In B-C plots, the points representing sites can be labelled with colours or symbols representing the types of environment, the geographic areas where they come from, or any other independent classifier of interest. Separate B-C plots can be drawn for sites surveyed in different types of environment, although all sites have been analysed in the same TBI analysis. Comparison of these plots will immediately show which types of environment have produced mostly losses or gains in species occurrences or abundances. Ecological application 3 shows a B-C plot with the sites separated in two classes of a temperature classifier.

## 5. Software

These calculations are implemented in the TBI() function in R, presently available on the Web page <http://adn.biol.umontreal.ca/~numeralecology/FonctionsR/>. Examples of output files of the TBI function are shown in Appendices S3 and S4.

## Numerical simulations

Numerical simulations were used to check the type I error rate and power of the permutation methods described in subsection 2 above. The data simulation methods and results are described in detail in [Appendix S1](#). A summary of these results is presented here, with recommendations to users of the method.

### Simulation to estimate type I error rates

The simulation results reported in Appendix S1 show that the TBI tests had correct rates of type I error with the three permutational testing methods, 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  $\alpha = 0.50$ .

### Simulations to compare power of $D$ indices and permutation methods

For the analysis of community composition data, permutation methods 1 and 2 are equally appropriate (Figs. S1.5 and S1.6, Appendix S1). The percentage difference and Ružička indices produced tests with the highest power, followed by the indices in the Box-Cox family: the chord, Hellinger and log-chord distances. The Euclidean distance alone produced TBI tests with extremely low power. It should not be used for TBI tests of community composition data.

The best combination to obtain TBI tests of community data with maximum power is to use the percentage difference or the Ružička indices with permutation methods 1 or 2. 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.

For standardized environmental variables, only the Euclidean distance was tested in the simulation study because this is the only one that makes sense with this type of data. The simulation results clearly showed that permutation method 1 produced the highest power with simulated quantitative environmental data. It would be the testing method of choice for this type of data.

Additional simulations involving different numbers of sites with an effect and different total numbers of sites showed that power of the test with permutation method 1 was high as long as the proportion of sites with an effect was smaller than  $n/2$ , independently of the total number of sites  $n$  in the study (Fig. S1.7, Appendix S1).

*Warning* – In real ecological studies, when the TBI test is applied to data where some sites are highly impoverished due to pollution or other extreme environmental situations, whereas other sites have higher species richness, this may result in sites with very few species and no species in common in the T1–T2 comparisons due to sampling variation at these impoverished sites. The TBI test will indicate a significant difference between T1 and T2 for these sites and this is a legitimate statistical outcome. When users of the method identify sites showing significant TBI tests in real data, they should check the species composition of these sites at T1 and T2. Interpretation of the test results should be done with caution when high and significant TBI indices are associated with community composition vectors with low richness and no species in common between T1 and T2. Examples are found in Ecological application 3, Chesapeake Bay data.

## **Application to physical environmental or community trait data**

It could be interesting to determine in what sites the changes in environmental data (e.g. land use) were the most important. One could then determine if these sites are also those for which the community has changed the most. Functional trait matrices could also be analysed in that way (Laliberté & Legendre 2010) in order to determine at which sites the trait composition of the community has been altered the most.

Use the TBI method to compare two matrices containing the same environmental variables observed at T1 and T2. This is a situation where the Euclidean distance would be appropriate as a basis for computing a TBI index. Data preparation:

- If all environmental variables are quantitative, they should be standardized before they are used in TBI analyses to make sure that all variables have the same weight (i.e. the same variance) in the calculation of TBI indices. The correct way of standardizing the variables is to put them in a single data table,  $Y = \text{rbind}(Y.T1, Y.T2)$ ; standardize  $Y$  by columns [ $Y.\text{stand} = \text{scale}(Y)$ ]; then separate the two tables before TBI analysis. In that way, the differences in values of each variable for all pairs of sites in the two tables will remain comparable to the original differences in unstandardized values and the distances computed between sites in T1 and T2 will be meaningful. Appendix S6 contains an R function to carry out this special standardization.



• If the environmental data contain a mixture of quantitative and qualitative data, one could put the two data tables together as above, then compute the Gower dissimilarity using the `gowdis(Y)` function of package `FD`, which can handle mixtures of quantitative and qualitative variables, and finally apply principal coordinate analysis (PCoA) to the square-rooted Gower dissimilarities. Square-rooting should make a Gower **D** matrix Euclidean before PCoA; see Legendre & Legendre (2012, Table 7.2). These operations will produce a table of principal coordinates, which can be split in two matrices and used as input into TBI analysis. No standardization of these data matrices will be required.

• For community trait matrices, use the same method: Gower dissimilarity, PCoA of the square-rooted dissimilarities, split the principal coordinates in two matrices, compute TBI using the Euclidean distance.

No application of TBI analysis to environmental or trait data is presented in this paper to save space.

## Ecological applications

### Ecological application 1 – Insecticide treatments in mesocosms

The invertebrate insecticide treatment data, from van den Brink & ter Braak (1999), are described in Appendix S3. We will compare data of surveys #4 and #11. Survey #4 was done one week after the insecticide treatment; then, the fauna of the mesocosms was considered by the authors to have fully recovered from treatment at the time of survey #11. For example, in the two mesocosms that had received the highest insecticide doses, species richness increased by 9 and 19 species from survey #4 to #11.

All TBI dissimilarities showed that in the mesocosms with the highest insecticide doses, community compositions was the most different between T1 and T2 (Table 1, upper panel). The p-values were identical for the percentage difference and Ružička dissimilarities (Table 1, lower panel); the two mesocosms that had received the highest doses of the insecticide, M11 and M12, showed significant differences in community composition between surveys #4 and 11. The chord, Hellinger and log-chord distances led to the same conclusion. These five distances are deemed appropriate for beta diversity study (Legendre & De Cáceres, 2013). On the contrary, the Euclidean distance is known to be inappropriate for such studies and, indeed, tests based on that distance did not show significant differences in community composition between surveys #4 and #11 in any of the mesocosms.

Detailed analysis of the species losses ( $B/den$ ) and gains ( $C/den$ ), obtained from TBI analysis computed with the percentage difference (Appendix S3), showed that in the 8 treated mesocosms, the changes in community composition always consisted of species gains; that is, statistic  $C/den$  (gains) was always larger than  $B/den$  (losses). Analysis of the mean values of  $B/den$  and  $C/den$  for these 8 mesocosms showed that  $C/den$  represented 58% of the dissimilarities, as expected in a study of recovery after an insecticide treatment. The permutational paired  $t$ -test showed a significant difference ( $p = 0.0074$ ) between T1 and T2 across the 8 mesocosms (additional calculations, not shown in Appendix S3).

TBI calculations using the Sørensen dissimilarity (Appendix S3) 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 in species occurrence data.

Further analyses were run with the three permutation methods proposed in this paper, using the percentage difference dissimilarity and 999 random permutations for the tests of significance. Only the results for mesocosms M10 to M12 are reported because all other p-values were 1.0 after correction for multiple testing.

- Permutation method 1 – The last three corrected p-values were 0.230, 0.012 and 0.012; M11 and M12 were significant at  $\alpha=0.05$ .

- Permutation method 2 – The last three corrected p-values were 0.470, 0.012 and 0.044; M11 and M12 were significant at  $\alpha=0.05$ .

- Permutation method 3 – The last three corrected p-values were 1.000, 0.120 and 0.759; M10, M11 and M12 were **not significant** at  $\alpha=0.05$ .

Permutation method 1 had the highest power to detect changes in species composition whereas method 3 lacked power; it did not detect the changes between T1 and T2 at any site. These observations are in agreement with the simulation results reported in a previous section and in Appendix S1 of this paper, which showed that method 3 had very low power with community composition data.

## Ecological application 2 – South Tikus Island coral communities

Data on the abundances of 75 coral species at 10 sites in the island of South Tikus, Indonesia, are described in Appendix S4. We will examine the changes in community composition between the 1981 survey and all five following 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 because specific environmental conditions at each site have not been reported for each year. Instead of testing the TBI statistics for individual sites, we will carry out a detailed study of the species loss ( $B/den$ ) and gain ( $C/den$ ) statistics, as described in the Methods. These statistics were computed with the denominator ( $den$ ) of the percentage difference index; they decompose the percentage difference into additive components.

First, we will plot the mean values of  $B/den$ ,  $C/den$  and  $D$  statistics across the sites, in comparisons of the 1981 survey with all successive surveys in turn (1983, 1984, 1985, 1987 and 1988) (Fig. 2). This method of analysis was used by Legendre and Salvat (2015, Fig. 3), who described the effects of a nuclear test on the mollusc communities of an atoll in the Pacific. Here, we are studying the effect of an El Niño event on coral communities.

Fig. 2a 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 dissimilarities ( $D$ ) between 1981 and 1983; species gains ( $C/den$ ) represented only 4% of  $D$ . In later years, the species losses decreased. The asymmetry between  $B/den$  and  $C/den$ , with dominance of  $B/den$  (losses) over  $C/den$  (gains), was significant for all year pairs in Figs. 2a and 2b, as shown by the overall paired  $t$ -tests of the asymmetry, described in the Methods, computed for each year pair over the 10 study sites, which were all significant.

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. 2b, which displays the same statistics, computed for species occurrence data. That second graph shows that many species disappeared at first from the surveyed sites after El Niño ( $B/den$  was 77% for the 1981-1983 comparison), then some of the original species recovered on the surveyed reefs ( $B/den$  decreased to 62% for 1981-1984 and to 45% for 1981-1985). Some of the coral species reestablished themselves at the surveyed sites during the following years, possibly by budding from colonies that had survived at nearby sites, or by dispersion of larvae from elsewhere. During that time, new species that were not present in 1981 occupied the depleted reefs, starting in the 1981-1983 comparison ( $C/den = 6\%$ ) and increasing in the following years (17% for 1981-1984 and 19% for 1981-1985). Observed changes in abundance-per-species and in occurrence became small, possibly caused by sampling variation.

The overall similarity in community composition between the 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. 3). This biplot was produced as follows: first, a percentage difference matrix was computed among all years and sites, square-rooted to make the dissimilarity matrix Euclidean, and 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 and Anderson 1999). The figure shows that the sites in 1981 had quite different species composition than in surveys after El Niño. The communities moved to a position in the ordination quite 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 to a new position 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.

As in the Legendre and Salvat (2015) study, where the effect on communities was due to a man-made disturbance, the communities found in South Tikus after the natural El Niño event differed in species composition from the structure they had in 1981 and they kept changing, apparently randomly, in later years. These observations are compatible with the neutral theory of evolution of communities.

### Ecological application 3 – Chesapeake Bay data

The data of the Chesapeake Bay Benthic Monitoring Program are described in Appendix 5. They concern 205 benthic species caught at 27 sites in the Chesapeake Bay, sampled spring and fall during 13 years from 1996 to 2008. For the present example, we will concentrate on the faunal data of the 25 brackish sites observed during the fall surveys conducted in 2005 and 2008. 52 species were observed in these two years: 38 in 2005 and 45 in 2008, with an overlap of 31 species found in both years.

This example offers the opportunity to build a B-C plot described in section 4 of the Methods section. The percentage difference index was used; the Ružička index would have produced similar results. These data will be used to demonstrate how to draw B-C plots and how to interpret them.

For the pair of years 2005 and 2008, the B-C plot is shown in Fig. 4. 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 (fall surveys) from 2005 to 2008.

A simple classification of the sites by an environmental factor, water temperature, 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. In the present example, the dispersion of the sites shows a strong relationship between water temperature and the gains and losses of species. The analysis and B-C plots could have been repeated on species occurrence (presence-absence) data.

In addition to the computation of the *B/den* and *C/den* components at each site, the R function also computed TBI tests of significance of the difference between years at each site. Although this is not the prime purpose of this example, let us mention that four sites were significant at the 0.05 level after Holm correction for multiple testing (25 simultaneous tests). They are sites S22, S23, S52 and S71. These 4 sites, shown in Fig. 4, all had a TBI dissimilarity  $D = 1$ , no species in common between T1 and T2, and very few species present: 7 species at T1 and 1 species at T2 for site S22, 3 and 3 for S23, 0 and 3 for S52, 4 and 7 for S71. Due to the small numbers of species and individuals at these sites, the test results should not be taken to represent strong evidence of an important change in community composition. These  $D = 1$  results could be due to sampling variation.

A map of the 25 brackish sites on the Chesapeake Bay, plotted with the RgoogleMaps package, is shown in Fig. S5.1 (Appendix S5) of the present paper. In the map, signs on the symbols indicate the sites dominated by abundance-per-species gains and losses between 2005 and 2008. The site identification numbers are those found in the data base.

## Discussion

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 impacts, including the effects of climate change on natural communities and other types of biodiversity data.

The method was elaborated while different applications involving parts of the method were being developed. Some of these applications have already been published in papers that offered opportunities to develop the TBI theory and software, providing pertinent application questions and data.

- 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 community was the atmospheric test of a Hydrogen bomb in 1968.

- Palaeoecology – 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.

- Freshwater ecology – Kuczynski et al. (2017) 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 (to be submitted) computed and 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, divided into 1250 (20 m × 20 m) quadrats.

Other methods can be used to further our understanding of the difference between surveys conducted at T1 and T2.

- The space-time interaction (STI) can be tested in repeated surveys without replication, using the STI method (Legendre et al. 2010). When no significant interaction is found between space and time in multivariate community data, (a) we should not expect to identify sites that have exceptional values of TBI except for type I error cases. The test of space-time interaction can thus be considered as a global test of the STI indices in the comparison of two surveys carried out at times 1 and 2. (b) In that case, STI analysis allows us to test the overall difference between times, using MEM eigenfunctions as covariables. For two surveys only (T1, T2), testing the interaction with the STI method requires, however, that the coordinates of the sites be known. If they are not, the interaction cannot be tested.

- One can plot maps of the  $D_i$  dissimilarities, computed at all sites  $i$ , to assess their variation. Analysis of the spatial variation of the  $D_i$  values can be conducted, using for example a correlogram or variogram, kriging, or Moran's eigenvector map analysis described for example in Legendre & Legendre (2012, Chapter 14 and references therein).

- For field experiments with multiple treatments, as shown in Ecological application 1, TBI analysis is complementary to principal response curves (PRC), a method developed by van den Brink & ter Braak (1999) to analyse the results of experiments conducted over time involving multivariate response data (e.g. community composition data) and multiple treatments. The data in Ecological application 1 were those used by van den Brink & ter Braak (1999) to illustrate the PRC method.

- To determine what are the species responsible for the main changes in community composition, carry out RDA on chord, Hellinger, or log-chord-transformed community data on the two data matrices combined,  $\mathbf{Y} = \text{rbind}(\mathbf{Y.T1}, \mathbf{Y.T2})$ , with the binary factor "T1, T2" as the explanatory variable, and a factor representing the sites as covariable. The RDA plot will have a single canonical axis (abscissa) and the species more abundant at T1 and T2 will have long arrows pointing left or right.

- Other methods for space-time analysis have been described in the Legendre & Gauthier (2014) review paper.

The simulation study reported in this paper could be extended to include other aspects not covered in the simulations reported in Appendix S1; for example simulations of community composition data forming spatial gradients, which should be tested using torus permutations. Simulation ventures of that kind would represent nice projects for honour or M.Sc. students in statistical ecology or in statistics.

## Acknowledgements

I am thankful to Amanda Winegardner and Lucie Kuczynski who experimented with this testing procedure and computed it on real data during the development phase of this paper, and to Daniel Borcard and Marie-Hélène Brice who provided useful comments on the manuscript before submission. Lucie Kuczynski suggested the acronym TBI for the method. This research was supported by Natural Sciences and Engineering Research Council of Canada (NSERC) research grant no. 7738 to P. Legendre.

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540 **Supporting Information**

541 Additional supporting information found on-line.

542 **Appendix S1.** Simulations involving artificial survey data at times T1 and T2.

543 **Appendix S2.** Discussion of some aspects of permutation method 1.

544 **Appendix S3.** Insecticide treatments in mesocosms.

545 **Appendix S4.** South Tikus Island coral communities.

546 **Appendix S5.** The Chesapeake Bay benthos data.

547 **Appendix S6.** An R function to standardize environmental data prior to TBI analysis (text file).

548



Table 1. The dissimilarities (top panel) and p-values (lower panel) associated with the tests of significance of the distances between T1 (survey #4) and T2 (survey #11), for 12 mesocosms (M1 to M12) shown in order of increased insecticide doses. The p-values were corrected for multiple testing (Holm correction); corrected significant values at the 0.05 level are marked with an asterisk (\*). Each test involved 9999 random permutations. The maximum possible value is 1 for the %difference and Ružička dissimilarities, and  $\sqrt{2} = 1.4142$  for the chord, Hellinger and log.chord distances. The Euclidean distance does not have an upper bound. Permutation method 1 was used in these tests.

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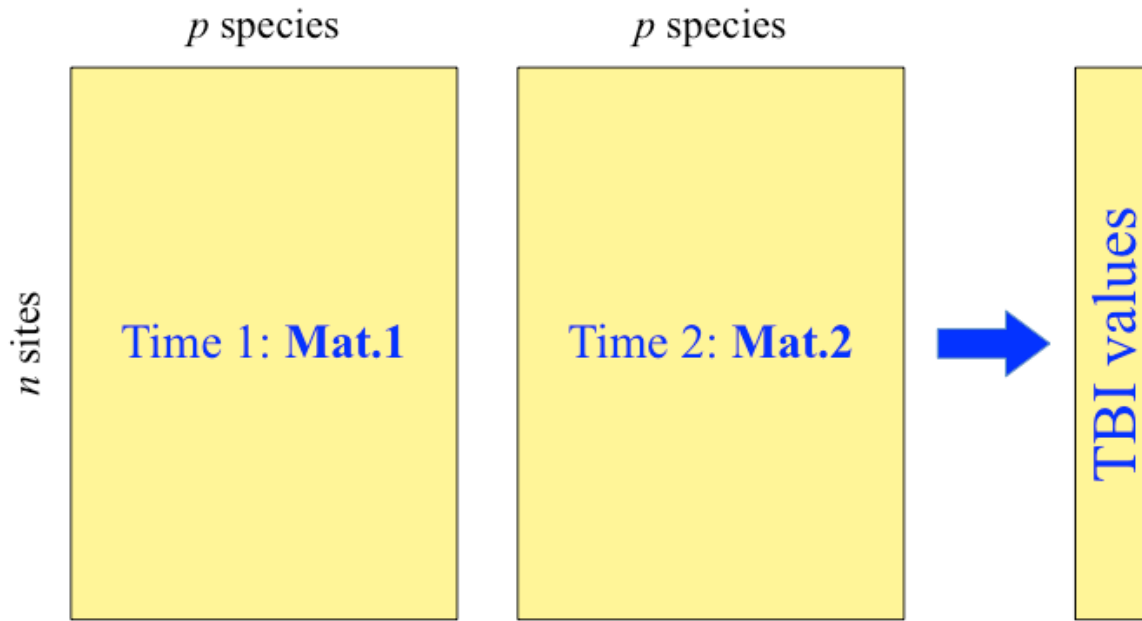
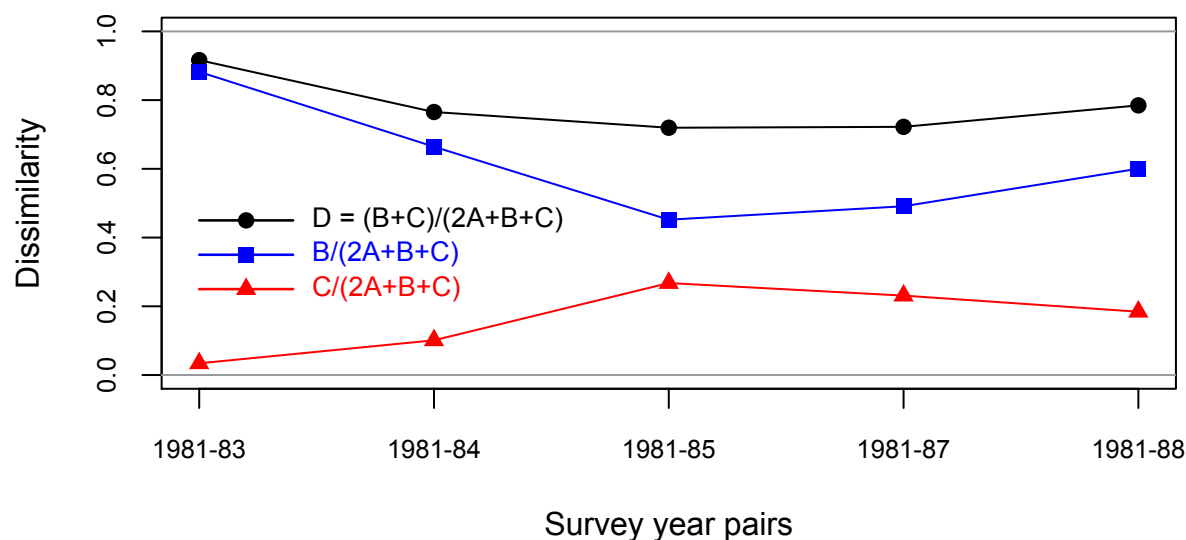


Fig. 1. Schematic representation of the first step of the method. Data in matrices **Mat1** and **Mat2** are used to compute a TBI dissimilarity  $D_i$  for each row (site)  $i$ , between **Mat1** and **Mat2**.

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



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

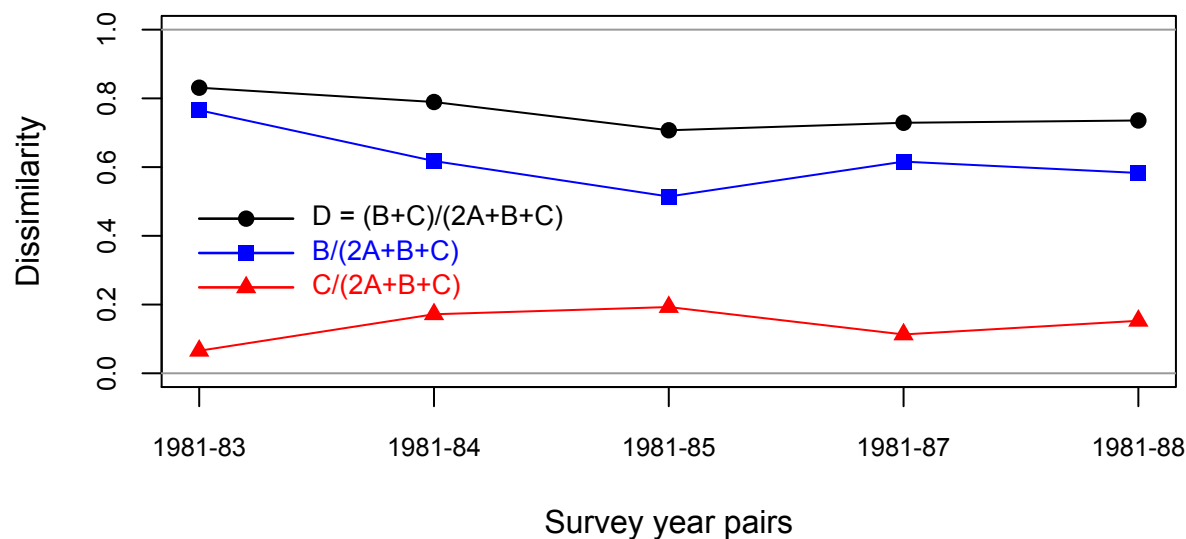


Fig. 2. Tikus Island coral data. (a) Changes in dissimilarity  $D$  computed from the quantitative coral community compositions between years, and its components  $B/den$  (losses) and  $C/den$  (gains). The 1981 survey is compared in turn to all successive surveys (1983, 1984, 1985, 1987, 1988). (b) Same for the species occurrence (i.e. presence-absence) data.

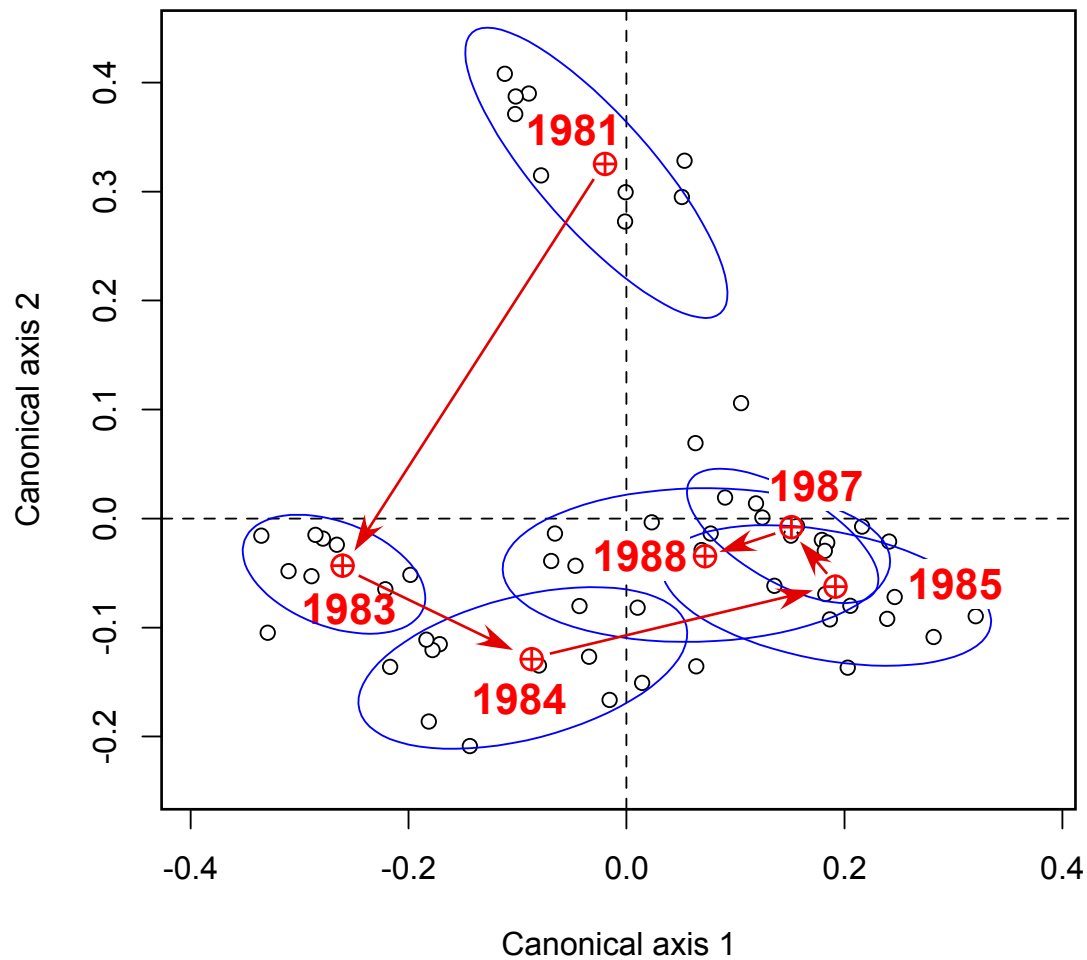


Fig. 3. Tikus Island coral data. Canonical ordination plot obtained by dbRDA for the quantitative coral community compositions data for the 6 years and 10 sites, constrained by a factor representing the 6 survey years. The years are marked by red symbols and the sites (open circles) for each year are incompletely surrounded by 60% coverage ellipses. Arrows materialize the sequence of years.

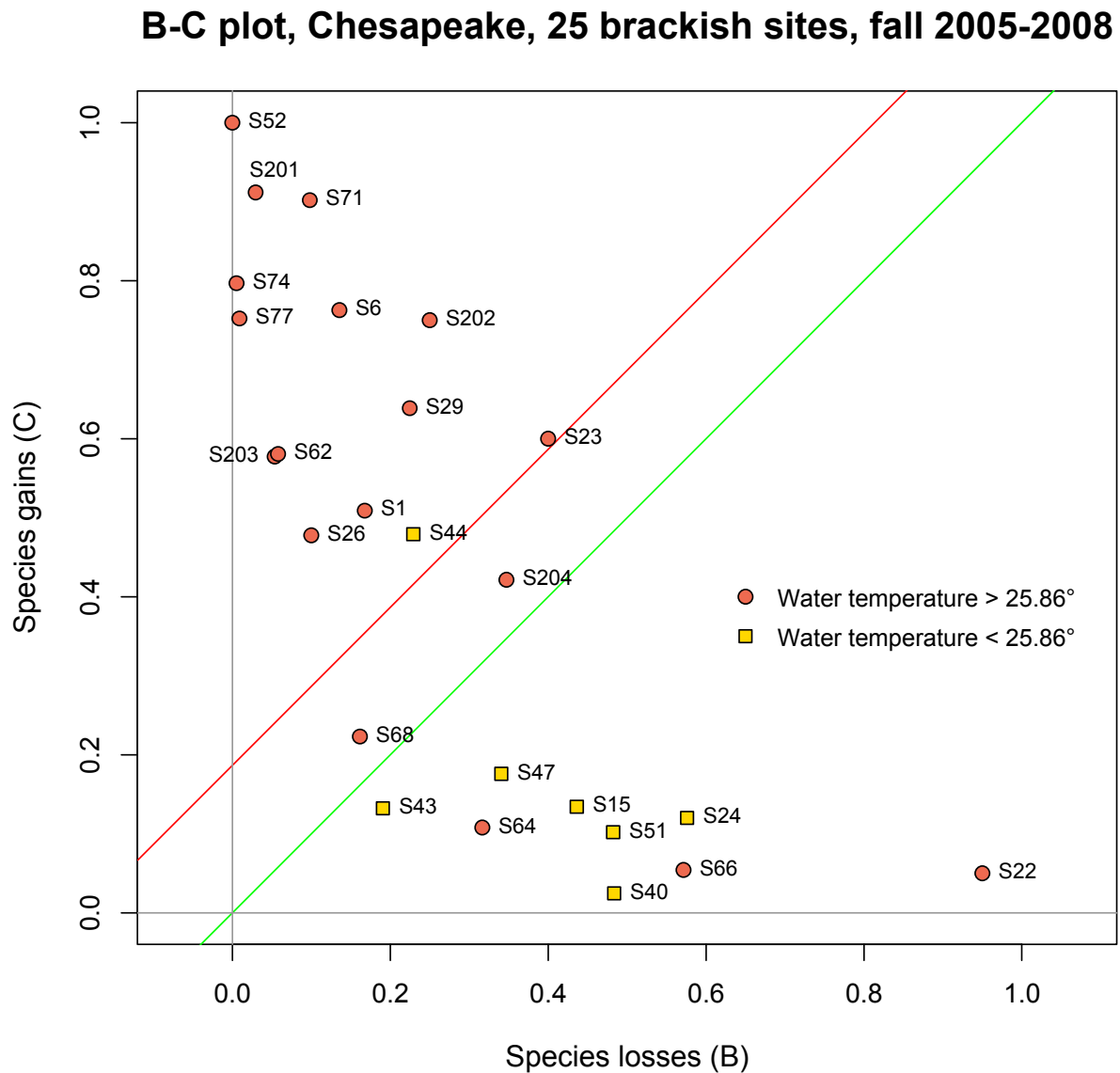


Fig. 4. Chesapeake Bay benthos data: B-C plot, 25 brackish sites, fall 2005–2008, species abundances. Sites are identified by their code of the Chesapeake Bay Benthic Monitoring Program. The sites are represented by symbols corresponding to two water temperature groups observed during the 2005 fall survey. Green and red lines: see Methods, section 4, B-C plots.