A novel perspective on the meaning of nestedness with conceptual and methodological solutions

Pinheiro, R.B.P., Dormann, C.F., Felix, G.M.F., and Mello M.A.R.

Rafael Barros Pereira Pinheiro. Graduate School in Ecology, Conservation and Wildlife Management, Federal University of Minas Gerais, Brazil. Current address: Animal Biology Department, University of Campinas, Brazil. E-Mail: rafael-bpp@hotmail.com.

Carsten F. Dormann. Department of Biometry and Environmental System Analysis, University of Freiburg, Germany. E-mail: carsten.dormann@biom.unifreiburg.de.

Gabriel Moreira Félix Ferreira. Graduate School in Ecology, University of Campinas, Brazil. E-mail: gabrielfelixmf@gmail.com.

Marco Aurelio Ribeiro Mello. Department of Ecology, University of São Paulo, Brazil. E-mail: marmello@usp.br.

Corresponding author: Rafael Barros Pereira Pinheiro. Departamento de Biologia Animal, Instituto de Biologia, Universidade Estadual de Campinas. Rua Monteiro Lobato, 255, CEP 13.083-862, Campinas, SP, Brasil. E-mail: rafael-bpp@hotmail.com.

ABSTRACT

Aim Nestedness is a common pattern in metacommunities and interaction networks, whose causes are still discussed. Nestedness inference is challenging because, beyond calculating an index, we need to compare observed values with values generated with a null model. There are different null models and the choice between them affects test outcomes. Furthermore, there is no established theoretical basis to guide this choice. Here, we propose a different look at the meaning of nestedness that improves our understanding of its causes and unveils the link between null models and hypotheses.

Innovation Nestedness of a matrix is a combination of marginal sum inequality and high overlap. The higher the overlap, the more predictable the cell values by marginal sums. Here, we show that nestedness actually measures how better one can predict cell values by marginal sums than by matrix dimensions and total sum alone. From this, we propose that two null models can be used to test for different topological hypotheses. The equiprobable model excludes all nestedness-generating mechanisms and provides the distribution of expected values for nestedness significance tests. The proportional model conserves nestedness-generating mechanisms and excludes nestedness-disrupting mechanisms, and thus, produces highly nested matrices. The proportional model provides the distribution of expected nestedness for nested matrices. Additionally, we evaluate the efficiency of several indices within this new perspective and illustrate our approach using an empirical plant-pollinator network.

Main conclusions Through a shift of perspective, our approach reconciliates contradictions in null model analysis and delimits the range of possible explanations for nestedness. The only way a process can increase nestedness in a matrix is by promoting marginal sum inequalities, without concomitantly introducing preferences. Consequently, in a species interaction network, explanations for nestedness should explain why some species interact more frequently than others.

Keywords: network science; interaction matrices; nestedness; network topology; metacommunity; species interactions; null model analysis; community ecology.

INTRODUCTION

The concept of nestedness originated in the study of island biotas. Nestedness was first defined as a biogeographical pattern in which faunas of species-poorer islands within an archipelago constitute a subset of the faunas of species-richer islands (Patterson & Atmar, 1986). These patterns have been recognized earlier by biogeographers (Darlington, 1957). Nevertheless, it was only after the influential work of Diamond (1975) that the structure of metacommunities, using species *vs* sites matrices, turned into a major focus of ecological studies, boosting the search for nestedness. In the following decades nestedness in species occurrences has been found in islands (Patterson & Atmar, 1986; Wright & Reeves, 1992), gradients (Harrison *et al.*, 1992), mountain ranges (Cutler, 1991), and several other contexts (Wright *et al.*, 1997). The later observation of nestedness in species interaction networks (Bascompte *et al.*, 2003) exposed it as a very common pattern in ecological systems.

Despite the ubiquity of nestedness, its drivers in ecological systems remain disputed (Pinheiro *et al.*, 2019). Nestedness in species occurrences is traditionally explained as the result of ordered selective colonization (Darlington, 1957; Patterson & Atmar, 1986) or extinction (Cutler, 1991) in local communities. The resulting order could arise, for instance, as the combined result of differential colonization abilities (or extinction vulnerability) by species and differential probabilities of colonization (or extinction) on islands depending on island size and isolation (Cutler, 1994). Similarly, nestedness in species interactions is often related to unequal probabilities of species to interact with others (Ulrich & Almeida-Neto, 2012), resulting, for instance, from unequal abundances (Vázquez *et al.*, 2007). Moreover, some authors suggest that nested assemblages are more stable and, therefore, likely to be found in nature (Thebault & Fontaine, 2010). Currently, nestedness is recognized as a common network topology (Delmas *et al.*, 2018) and has even been detected in other networks beyond ecology, for instance social (Borge-Holthoefer *et al.*, 2017) and economic (Bustos *et al.*, 2012).

To investigate the drivers of nestedness, it is first necessary to operationalize the concept and measure how nested real-word networks are. To do so, networks are usually represented as interaction matrices (i.e., incidence matrices), where rows and columns represent the nodes (e.g., pollinators and plants) and cells represent the links between them (e.g., floral visits). There exist a variety of nestedness indices based on different mathematical procedures and nestedness definitions (Ulrich *et al.*, 2009). A notable advancement was the development of nestedness indices for weighted matrices (Almeida-Neto & Ulrich, 2011). In real-world networks, interactions between nodes are not all equivalent, but vary in frequency or intensity. For instance, links in a pollination network might be weighted by the frequency of pollination events observed for each plant-pollinator combination (Watts *et al.*, 2016). Weighted matrices are more informative and, often, reveal patterns that are hidden in their binary counterparts (Banašek-Richter *et al.*, 2004; Scotti *et al.*, 2007). Nevertheless, in the end, the choice between weighted and binary matrices depends on the question under investigation (Fründ *et al.*, 2016; Jordano, 2016).

However, before invoking causal mechanisms, it is important to test for the significance of the observed nestedness, since even random matrices will present some degree of nestedness. To infer significance in nestedness tests, beyond calculating an index, the observed values are usually compared with the distribution of values calculated for null models composed of randomized matrices (Ulrich *et al.*, 2009). Null models are randomization procedures that conserve more or less inclusive subsets of the original matrix properties on the randomized matrices (Ulrich *et al.*, 2009). Therefore, null models are null hypothesis about the mechanisms that are sufficient to produce an specific pattern (Gotelli & Graves, 1996). In nestedness

test, these models are generally used as the null expectation for index values, as the randomized matrices are assumed to be void of the effects of nestedness-generating mechanisms.

Algorithms that markedly differ from one another may be used to generate null models. Although the choice of null model strongly affects the outcome (Ulrich & Gotelli, 2007), there is no established theoretical basis to guide this decision in nestedness analysis. Null models that only conserve matrix dimensions and total sum are considered less rigorous, but more sensitive, to nestedness tests, than null models that conserve matrix marginal sums. And, as algorithms are not linked to explicit hypotheses, this choice is often regarded as a trade-off between type I and type II statistical errors (Ulrich & Gotelli, 2007; Strona *et al.*, 2018). This is a questionable interpretation, as different null models likely exclude the effects of different ecological mechanisms, and hence might not be appropriate alternatives to test the same hypothesis. Solving this dilemma requires a better understanding of how the mechanisms excluded or conserved by each null model relate to the emergence of nestedness, so that we can link each null model to a specific topological hypothesis.

In the present study, we propose a novel perspective on nestedness in interaction matrices. We believe that both the way in which observed nestedness values are compared with null models and the interpretation of these comparisons can be improved, and some contradictions reconciled by taking a different look at what nestedness quantifies. In several steps, we firstly define nestedness in a matrix as a combination of marginal sum inequalities and overlap. Secondly, we show that overlap measures how well marginal sums predict cell values. Thirdly, we argue that two commonly used null model algorithms are neither alternative nor conflicting, but complementary, as they simulate the exclusion of different mechanisms and, thus, test different hypotheses. The equiprobable null model excludes all nestedness-generating mechanisms and is appropriate for significance tests. The proportional model conserves all nestedness-generating mechanisms and exclude nestedness-disrupting mechanisms and can be used to test whether the matrix significantly deviates from a fully nested topology. Fourthly, we compare the efficiency of several nestedness indices from our new perspective, using both binary and weighted matrices. Lastly, we illustrate these new procedures using a pollination network.

A NOVEL PERSPECTIVE ON THE MEANING OF NESTEDNESS

While we focus our examples on species interaction networks, our rationale may be extended to other bipartite networks, both ecological (e.g., species vs. sites) and non-ecological (e.g., actor vs. movies). Thus, for the sake of generality, we use common terms for the study of interaction matrices and networks.

Orders of information on an interaction matrix

Matrix information can be partitioned into three levels of complexity (Dormann *et al.*, 2017). First-order information comprises the most general features of the interaction matrix: its dimensions (number of rows and columns) and the sum of all cell values (hereafter, total sum). Second-order information represent the specific properties of each row and column: the marginal sums. Third-order information is the actual cell values. In Figure 1, we illustrate these orders of information using a plant-animal interaction matrix.

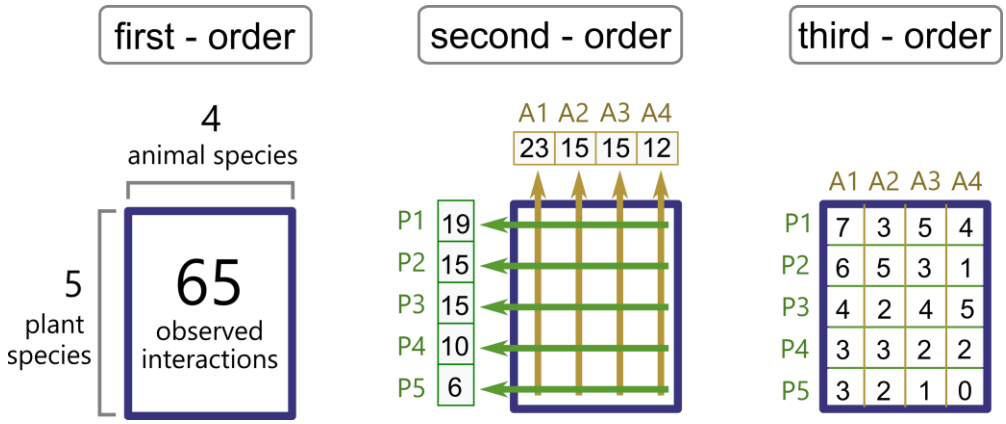

FIGURE 1 Orders of information in a weighted plant-animal interaction network. First-order refers to the general aspects of the matrix: the species richness on each side of the interaction (plants and animals) and the total frequency of interactions observed. Second-order refers to specific properties of the rows and columns: the frequency of interactions made by each plant and animal species. Third-order refers to the pairwise interactions between rows and columns: the frequency of interactions between each plant-animal pair.

Definition of nestedness

The concept of nestedness was first proposed as a pattern of species occurrence, latter generalized to binary bipartite networks, and only recently applied to weighted networks. Thus, it is not trivial to provide a general definition of nestedness for all kinds of matrices. Below, we provide a verbal definition, and additionally, a graphical definition, so it is easier to understand and operationalize the concepts.

Interaction networks are usually represented as incidence matrices, which have two classes of nodes (rows and columns). In incidence matrices, binary nestedness is the tendency of the nodes of one class (e.g., rows) with lower marginal sums to be linked (cell value = 1) with a subset of the nodes of the other class (e.g., columns), which are linked to the nodes with higher marginal sums of the same class (e.g., rows). So, to calculate binary nestedness, we always compare rows with rows, and columns with columns.

For weighted nestedness, beyond the identity of linked nodes, we may account for link weight (cell value) differences. Thus, weighted nestedness is the tendency of the nodes of one class (e.g., rows) with lower marginal sums to have weaker links (lower cell values) with the same set or a subset of nodes of the other class (e.g., columns), which are linked to the nodes with higher marginal sums of the same class (e.g., rows). To calculate weighted nestedness, we again compare rows with rows, and columns with columns.

To visualize nestedness we must organize rows and columns by decreasing marginal sums. In a binary matrix, nestedness is a tendency of 1s to be in the top-left, while 0s are in the bottom-right corner of the matrix. In a weighted matrix, nestedness is a tendency of decreasing cell values from the top-left to the bottom-right corner (Figure 2a).

There is, however, some controversy in the definition of weighted nestedness. Some authors argue that weighted nestedness should require binary marginal sum inequalities (decreasing degrees), so that weighted nestedness in a matrix is constrained by binary nestedness (Almeida-Neto & Ulrich, 2011). This definition aims at a better match between the concepts of nestedness and ecological specialization. Despite making sense for the study of ecological networks, this definition yields a mixed binary weighted rather than a weighted topology. Here, we opt to address separately the binary and the weighted structures of a matrix, which we believe leads to a clearer and more general interpretation.

Overlap is the tendency of different nodes of the same class (e.g., rows) to link to a similar set of nodes of the other class (e.g., columns). The core of any nestedness index is assessing the overlap between nodes with different marginal sums: measuring how often rows with lower marginal sums link to the same columns that rows with higher marginal sums; and how often columns with lower marginal sums link to the same rows that columns with higher marginal sums.

Weighted indices of overlap account for, not only the identity of linked nodes, but whether nodes of the same class distribute weights among their links similarly. Several nestedness indices are indeed purely measures of overlap (e.g., temperature: Rodriguez-Girones & Santamaria, 2006). The opposite of overlap: a tendency of nodes of the same class to interact with different nodes of the other class is often called *turnover*, *specialization*, or *preferences*. Here, we opt for *preferences*, but notice that this term here refers to a matrix pattern (as Blüthgen *et al.*, 2006 refer to *specialization*), not necessarily matching the homonymous ecological concept.

However, as pointed out by previous studies (Baselga, 2012; Ulrich & Almeida-Neto, 2012), the original concept of nestedness also assumes a gradient in marginal sums. This realization prompted the development of nestedness indices explicitly demanding marginal sum inequalities, such as NODF (Nestedness based on Overlap and Decreasing Fill) (Almeida-Neto *et al.*, 2008). Nestedness is, thus, a combination of these two features: high overlap and unequal marginal sums (Figure 2a).

The meaning of overlap

Here, we propose an alternative perspective on the meaning of overlap with implications for the understanding of nestedness. Imagine that the nested weighted matrix (I) in Figure 2a represents the observed frequency of interactions between five pollinator species (columns: C1, C2, C3, C4, and C5) and five plant species (rows: R1, R2, R3, R4, and R5). A high overlap of interactions between plant species means that the frequency of interactions made by a given pollinator with any plant partially predicts its interactions with the other plants. For instance, as the pollinator C1 interacts more frequently with the plant R4 than does pollinator C4, we would expect it to also interact more frequently with other plant species than does C4.

The interactions of a given pollinator with each plant (cell values within a column) sum up to its total frequency of interactions (column sum). Thus, the total frequency of interactions of a pollinator is also a predictor of its interactions with each plant in a nested matrix. Applying the same logic to the plants (rows), we conclude that the frequency of interactions between each pollinator-plant pair in a nested matrix can be well predicted by the total frequency of interactions of each pollinator and each plant species (marginal sums) (Figure 2b). The same logics applies to binary nested matrices: cell values (0 or 1) can be predicted by the marginal sums. Ryti & Gilpin (1987), for example, used the R^2 of a logistic regression of cell values on marginal sums, as a measure of orderedness (there a synonym for nestedness) in binary matrices.

(a)

high nestedness

I	75	60	45	30	15
75	25	20	15	10	5
60	20	16	12	8	4
45	15	12	9	6	3
30	10	8	6	4	2
15	5	4	3	2	1

II	75	60	45	30	15
75	0	15	40	20	0
60	55	0	2	3	0
45	5	35	0	3	2
30	15	5	2	3	5
15	0	5	1	1	8

unequal marginal sums

III	45	45	45	45	45
45	9	9	9	9	9
45	9	9	9	9	9
45	9	9	9	9	9
45	9	9	9	9	9
45	9	9	9	9	9

high overlap

IV	45	45	45	45	45
45	30	0	13	1	1
45	0	40	2	0	3
45	10	5	0	6	24
45	5	0	20	8	12
45	0	0	10	30	5

low overlap

equal marginal sums

(b)

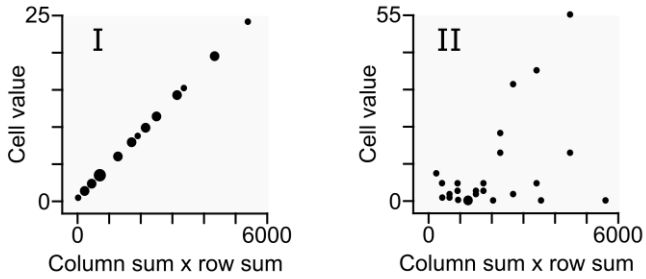

FIGURE 2 (a) Nestedness is a combination of high overlap and marginal sums inequality. Weighted overlap is the tendency of rows to establish links with the same columns and to distribute weights among these columns in similar proportions, and the tendency of columns to establish links with the same rows and to distribute weights among these rows in similar proportions. Let us imagine four matrices: two with unequal marginal sums (I and II, see the marginal sums in the grey cells), two with equal marginal sums (III and IV); two with high link overlap (I and III), two with low link overlap (II and IV). In a matrix with unequal marginal sums, high overlap results in nestedness. Here, we focus on weighted matrices, as similar explanations for binary matrices were already provided by other studies (Almeida-Neto *et al.*, 2008; Ulrich & Almeida-Neto, 2012). (b) Overlap in a matrix may alternatively be understood as a measure of how much each cell value is predictable by the outer product of the marginal sums of its respective row and column (second-order information). Here, cell values increase linearly with the outer product of their marginal sums in the nested matrix (I) but not in the matrix with low overlap (II).

This is the first key insight of our study: the higher the overlap in a matrix, the higher the predictive power of the second-order information (marginal sums) for cell values. However, when all marginal sums are equal, they provide no further information compared to a prediction based only on the first-order information. For instance, in a fully homogeneous matrix (Figure 2a III), by knowing the marginal sums, we can perfectly predict all cell values, but we achieve the same result by just averaging cell values. Thus, the second key insight of our study: for a given overlap, the higher the inequalities in the marginal sums, the higher the differences between a prediction informed by second-order information and a prediction only based on first-order information.

Putting these two insights together, we find that nestedness is directly related to how much information about cell values we gain by knowing, not only the first-order, but also the second-order properties of a network. In a matrix with high nestedness, it is a much better prediction to assume that each cell value is

proportional to the outer product of row and column sums than to guess that all cell values are the same. As nestedness decreases, this superiority of prediction decreases too.

Nestedness-generating and nestedness-disrupting mechanisms

It follows from the above discussion that the only way a mechanism can generate nestedness in a matrix is by promoting marginal sum inequalities without decreasing overlap. Therefore, nestedness-generating mechanisms must drive the second-order information of a matrix. On the other hand, mechanisms that introduce preferences (i.e., decreases overlap) on the matrix cause nestedness disruption. Thus, nestedness-disrupting mechanisms causes departures in cell values (third-order information) from the expected given the marginal sums (second-order information).

LINKING NULL MODELS TO HYPOTHESES ABOUT NESTEDNESS

Nestedness significance

Testing nestedness is challenging, first because orders of information in a matrix are not independent from one another. The first-order properties of a matrix delimit the universe of possibilities for second-order properties and to their effect on cell values. In binary matrices, for instance, degree distribution and, hence, nestedness is strongly conditional on connectance (Poisot & Gravel, 2014). Also, even in a network formed by random interactions between similar nodes, we would expect to find some degree of overlap and marginal sum inequalities in the matrix, thus, some degree of nestedness.

Therefore, to infer the significance of nestedness in an interaction matrix, we must first state our expectations using a null model. The defining characteristic of a null model is that *it excludes the mechanism being tested* (Gotelli, 2001). Considering our definitions, to exclude all nestedness-generating mechanisms, such a null model should discard second- and third-order information of the matrix, so that the two defining elements of nestedness - inequality of marginal sums and overlap - derive from randomness. Thus, this null model must only conserves first-order information: matrix dimensions and total sum, hereafter called “equiprobable null model” (Ulrich *et al.*, 2009). An equiprobable null model provides the nestedness distribution expected for matrices with random topologies, excluding all nestedness-generating mechanisms, and yields a benchmark for significance tests on nestedness.

The nested topology

After inferring that a given network presents significant nestedness compared to a random topology, a following appropriate question is whether it significantly deviates from a fully nested network. In this case, we must state the expectations through a null model that conserve all nestedness-generating but discard the nestedness-disrupting mechanisms. Accounting for our definitions, such a null model must conserve first- and second-order information on the randomized matrices, so that, marginal sum inequalities are kept. Then, it must randomize third-order information, so that preferences are discarded.

An appropriate algorithm for this task is one in which the probability of each cell in the randomized matrix to receive a unit of weight is proportional to the respective marginal sums in the observed matrix, hereafter, called proportional null model (Gotelli, 2000). Proportional null models, however, do not strictly conserve marginal sums, they do that only probabilistically. Another group of null models that conserve the second-order information are the “swap models”, in which marginal sums are kept constant, and randomization occurs by interaction swaps (Gotelli, 2000). Swap models, on the other hand, are subject to complex mathematical constrains that may decrease interpretability. Here, we favor interpretability and opted to use

proportional models. Nevertheless, in specific situations, the distortion of marginal sums might justify the use of swap models. In these cases, swap models should be interpreted similarly to proportional models.

In a proportional model, cell values are fully driven by second-order information. Therefore, it provides the expected nestedness distribution for matrices whose topology is only shaped by nestedness-generating mechanisms. This understanding conflicts with the common use of proportional models as a null distribution to test nestedness significance. This model should be considered the upper distribution of expectation for nestedness, rather than the lower distribution. Otherwise, as earlier suggested by Wright and Reeves (1992), the null model itself hides the pattern that we attempt to detect.

Notice that the proportional model we discuss here does not have additional further constraints, such as maintaining connectance (Vázquez *et al.*, 2007), as this is not a first- neither a second-order property of weighted matrices. This mixed use of binary and weighted information is undesirable for our approach, as it cannot be linked to any specific hypothesis about nestedness.

Nestedness is a quantitative network property that, even when significant, might vary broadly in intensity. Although nestedness can and should be understood as a continuum, there are often pragmatic and philosophical reasons to classify networks into topological categories (e.g., nested, modular, and compound, Lewinsohn *et al.*, 2006). A topology, by definition, is a description of how the entire network is arranged (its general shape). Contrary to previous studies, we discourage the use of nestedness significance to delimit a topological category. Nestedness significance compared to an equiprobable null model just means that the observed pattern is unlike to emerge without some mechanism that affects marginal distributions, but does not rule out the action of other network shaping mechanisms. When significance is used as the unique criterium, a variety of structures that are not sufficiently coherent to be called a topology is like to be merged into a single category. On the other hand, a proportional null model is composed of matrices only shaped by nestedness-generating mechanisms. In a mechanistic perspective, matrices of a proportional null model are as nested as a nested matrix could be, and, thus, appropriate archetypes to delimit a coherent nested topology (Figure 3).

New procedure for testing nestedness

Accounting for this new interpretation of null models in nestedness analysis, we suggest a new procedure to nestedness testing. The steps of this procedure are: (1) calculate nestedness for the observed matrix (see section *Comparisons of indices*); (2) build an equiprobable null model; and (3) infer nestedness significance using values generated with the equiprobable model as the null distribution. If nestedness is significant, (4) build a proportional null model. (5) Use the proportional null model to generate the expected distribution of nestedness for matrices with a nested topology. Thereby, check whether the observed nestedness is coherent with the distribution of values generated with the proportional model. If the observed nestedness does not significantly differ from the proportional null model, the matrix topology can be classified as nested. (6) We recommend plotting the observed value and null model distributions of nestedness, prompting a more continuous and less classifying analysis of where a given matrix situates within the possibility space illustrated in Figure 3. R functions for using this procedure are available at GitHub (https://github.com/pinheirorbp/nestedness) and Zenodo (http://doi.org/10.5281/zenodo.4662871).

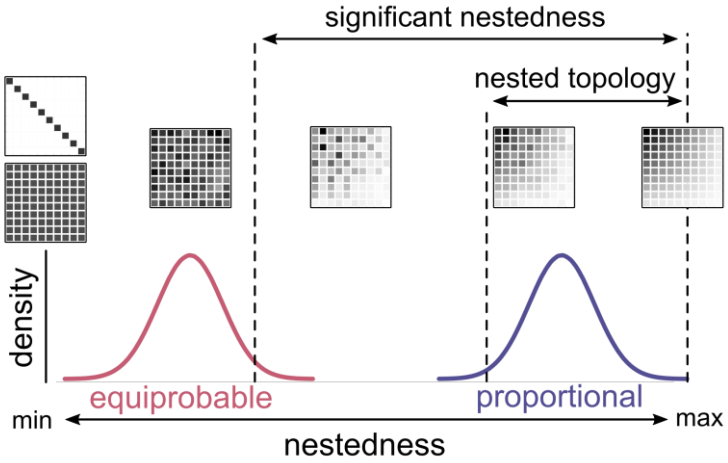

FIGURE 3 A new approach for the use of null models in tests of nestedness. First, we define nestedness as a combination of marginal sums inequality and high overlap. Second, we demonstrate that overlap indicates how well marginal sums predict cell values. Through this novel perspective we link two widely used null models to two explicit hypotheses about nestedness. The equiprobable null model conserves only the first-order properties of the matrix (dimensions and total sum), thus, excluding all nestedness-generating mechanisms and showing a random topology. Nestedness significance is checked by the comparison of the observed value with the distribution generated by the equiprobable null model. The proportional null model keeps the second-order properties (marginal sums) and maximize the overlap (by defining cell probabilities based on marginal sums). Consequently, it conserves the effects of nestedness-generating mechanisms and excludes the effects of nestedness-disrupting mechanisms, showing a nested topology. Observed nestedness values should be interpretated in comparison to the distributions generated by these null models, considering what mechanisms each of them conserves or discards, and what topology each of them shows. Nevertheless, when the aim is to classify networks into topological categories, we recommend two criteria to delimit a nested topology: first, nestedness must be significantly higher than the equiprobable model; second, nestedness must not be significantly lower than the proportional model.

REMAINING CHALLENGES

In real-world systems, interactions almost always have a weighted nature (e.g., frequencies and abundances). However, due to the way data are collected, networks are often studied using binary matrices (Dormann & Blüthgen, 2017). When based on binary data, marginal sum distributions are highly contingent on connectance (Poisot & Gravel, 2014), and so they are poor proxies for the respective weighted marginal sums, especially for matrices with high connectance (Figure S1a). As a result, proportional models, when applied to binary matrices often do not conserve the marginal sum distribution on the randomized matrix (Wright *et al.*, 1997). In addition, without weighted information, binary null models can only conserve connectance. Connectance, however, is not a direct proxy for the first-order information of the weighted matrices, as it is also highly affected by marginal sums and preferences (Figure S1b). Thus, when connectance is kept, null models cannot be fully distinguished by the orders of information conserved or discarded. Such distortions are likely to cause severe misestimation of nestedness in null models.

COMPARISONS OF INDICES

Here, we used simulated matrices to analyze the capacity of several nestedness indices to distinguish between matrices with random (equiprobable null model) and nested (proportional null model) topologies.

First, we produced probability matrices with dimensions: 5 x 5, 10 x 10, and 20 x 20, based on three different marginal probabilities: constant, linear decrease, and log-normal. These represent a gradient between an equiprobable null model and a proportional null model based on a more skewed marginal sum distribution. Then, following cell probabilities, we distributed interactions in simulated matrices with different sampling intensities (i.e., the total frequency of interactions distributed in each matrix): 50, 100,

200, 400, 800, 1600, 3200, and 6400 (Figure S2). For each unique setup we produced 10,000 matrices. For each matrix we calculated a set of nestedness indices, both binary and quantitative (Table 1), and graphically inspected the capacity of separating results between the equiprobable and the proportional (linear decrease and lognormal) models. Indices were calculated using the bipartite package for R (Dormann *et al.*, 2008). Commented scripts to reproduce all analyzes are available at GitHub (https://github.com/pinheirorbp/nestedness) and Zenodo (http://doi.org/10.5281/zenodo.4662871).

Table 1- Nestedness indices compared in this study.

Index	Reference
Binary	
Temperature	Rodriguez-Girones & Santamaria, 2006
Manhattan distance	Corso & Britton, 2012
NODF	Almeida-Neto *et al.*, 2008
β_{NES}	Baselga, 2010
Binary spectral radius	Staniczenko *et al.*, 2013
Weighted	
WNODF	Almeida-Neto & Ulrich, 2011
WNODA	Pinheiro *et al.*, 2019
Spectral radius	Staniczenko *et al.*, 2013

We found that weighted nestedness indices vary substantially in their capacities of distinguishing between random and nested matrices (Figure 4, Appendix S1). WNODF, due to mixing weighted (overlap) and binary (decreasing fill) information (Almeida-Neto & Ulrich, 2011), has a complex behavior and is not appropriate for using with highly filled matrices. WNODA and spectral radius show good separation and, thus, discriminate better between random matrices, matrices with significant nestedness, and matrices with a nested topology.

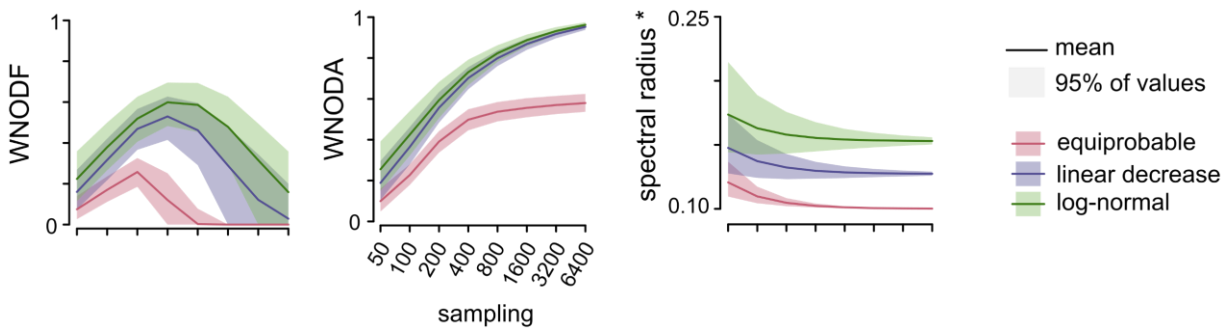

FIGURE 4 Ability of weighted indices to distinguish between nested matrices (cell values are determined by marginal sums: linear decrease and log-normal) and random matrices (cell values are randomly distributed: equiprobable), at different sampling intensities. The larger the separation between the distributions of random and nested matrices, the more efficient the index. Index values are shown in the y-axis and sampling (total sum of the matrix) on the x-axis (log scale). Only results from the 10 x 10 matrices are included in the figure, see Appendix S1 for other matrix dimensions and for binary indices. *Spectral radius values were divided by sampling intensity for better visualization.

As expected, matrices with a more skewed marginal sum distribution (log-normal) are more easily distinguished from the equiprobable null model than matrices with more similar marginal sums (linear-

decrease). The sampling required for the stabilization of the indices is higher in larger matrices (Appendix S1). WNODF, however, because of being contingent on connectance, is uninformative for the smallest matrices even with intermediary sampling (Appendix S1).

As binary matrices do not hold weighted information, we cannot fix the true total sum of weights on the null models, only connectance. For a more appropriate comparison of binary indices, we generated binary matrices from the probabilities with fixed connectance of 0.3, 0.5, 0.7, 0.9, 0.92, 0.94, 0.96, and 0.98. Moreover, in analysis of binary matrices, we usually do not know the original weighted marginal sums, only its binary respective. To inspect the distortion caused by this approximation, from each matrix produced with fixed connectance, we produced a randomized matrix with the same connectance using a proportional algorithm based on binary marginal sums (null model 2 of Bascompte *et al.*, 2003).

An issue with any randomizing algorithm based on probabilities is that it may produce matrices with empty rows or columns. In general, these are not likely to bias network analysis (Gotelli, 2000). Here, for the weighted matrices we kept all matrices. However, reducing matrix dimension modifies its connectance. Thus, when producing binary matrices, we discarded all the matrices with reduced dimensions.

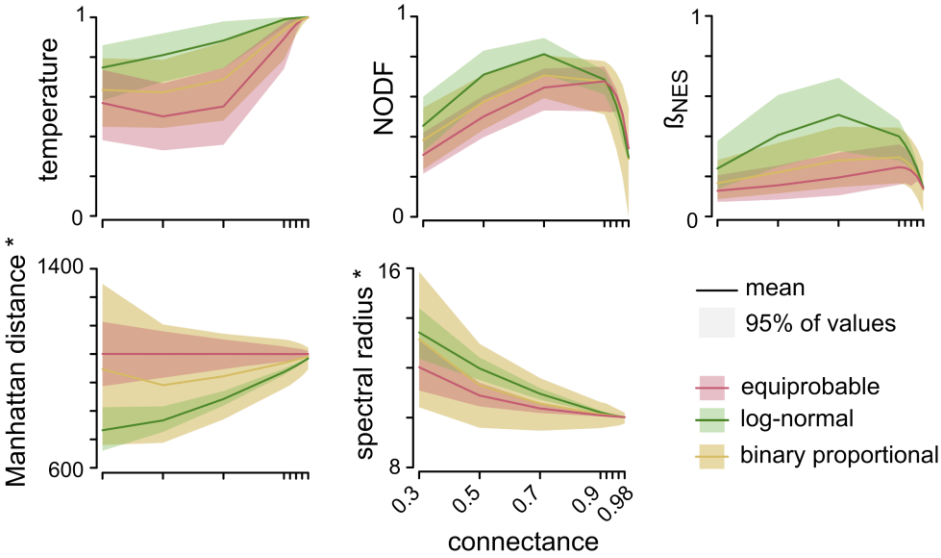

FIGURE 5 Ability of binary indices to distinguish between nested matrices (cell values are determined by marginal sums: log-normal) and random matrices (cell values are randomly distributed: equiprobable), at different levels of connectance. As binary matrices do not provide information on weighted marginal sums, we produced a proportional model based on the binary marginal sums of the matrices generated with the log-normal probabilities. Ideally, values for the binary proportional model (yellow) would match values for the matrices based on log-normal probabilities (green). They are, however, largely distorted. Index values in y-axis and connectance on the x-axis. Only results from the 10 x 10 matrices are presented here, see Appendix S2 for other matrix dimensions and details. Results with linear-decrease marginal probabilities were similar to log-normal probabilities and are not shown in the figure (see Appendix S2). *Binary spectral radius and Manhattan distance values were divided by the connectance, providing a better visualization.

When quantitative information was accounted for, binary indices varied in their capacity to distinguish between nested matrices formed by log-normal probabilities and random matrices with fixed connectance (Figure 5, Appendix S2). Manhattan distance had an outstanding ability over the entire range of connectance. NODF, by requiring inequalities in marginal sums, presented a more complex behavior, with strong decrease in efficiency for more connected matrices. Binary nestedness indices were more efficient in larger matrices and at intermediary connectance (Appendix S2).

Weighted information on the matrix marginal sums, however, is not available in binary matrices, and the use of binary information to estimate probabilities on the proportional model resulted in large distortions. Nestedness values for matrices of the proportional null model based on the binary probabilities do not match the range of values for the matrices produced from weighted information (Figure 5). For most of the indices, those values are highly underestimated, except for spectral radius and for Manhattan distance in which they presented a large variability, encompassing both groups.

CASE STUDY

To illustrate our novel perspective we used a weighted plant-pollinator network, originally described by Kaiser-Bunbury *et al.* (2010) and available in the Web of Life database (www.web-of-life.es, network: M_PL_060_17). The network includes 52 nodes, which represent 17 plant species and 35 pollinator species. Weights are the frequency of floral visits observed between each pollinator and each plant species (total of 444 visits) (Figure 6). This network was arbitrarily chosen from the database, without any preliminary analysis, following the only criterion of suitable dimensions for illustration.

We produced an equiprobable and a proportional null model, with 10,000 matrices each, and calculated WNODA for each matrix. Similarly, we analysed the binary structure of the network using NODF. Then, we produced a binary equiprobable null model in which connectance, rather than total sum of weights, was conserved in the randomized matrices. For last, we produced a proportional model based on binary information: binary marginal sums were used to calculate probabilities and connectance was conserved in the randomized matrices, to test whether it would result in a distortion of NODF values compared to the proportional model based on weighted data.

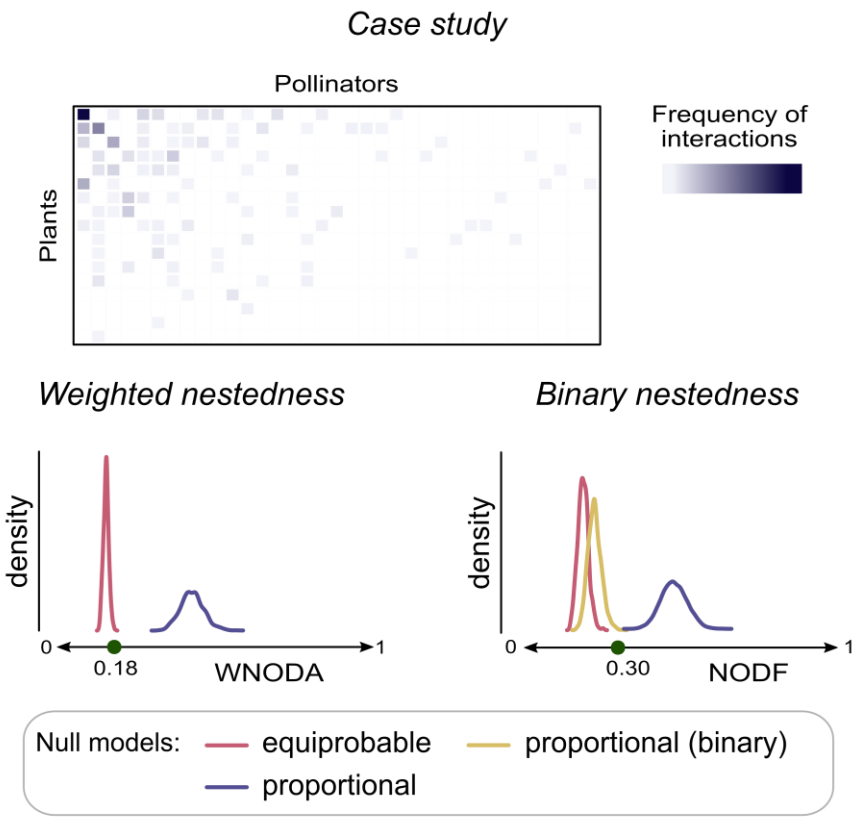

FIGURE 6 Case study using a weighted empirical plant-pollinator network (Kaiser-Bunbury et al., 2010) to illustrate the proposed procedures. *Weighted nestedness:* the observed weighted nestedness based on overlap and decreasing abundance

(WNODA) is high compared with the distribution generated with the equiprobable null model, meaning that there is significant nestedness in the network. Nestedness is, though, very low compared to the distribution generated with the proportional null model, meaning that the network's structure strongly deviates from a fully nested topology. We can, thus, conclude that there are important preferences in the pattern of observed interactions between plants and pollinators. *Binary nestedness:* we analyzed the binary topology of the network using the NODF index. When weighted information was used to generate the proportional model, conclusions were similar to the analysis with the weighted index. However, when we based the proportional model on node degrees (marginal sums of the binary matrix) and connectance instead of total weights were conserved, using the null model 2 of Bascompte *et al.* (2003), it resulted in much lower values. In comparison, the observed nestedness seemed higher than the expected given the degree distribution. This is, thus, a result of the distortion caused by using binary instead of weighted information. The distribution produced by the binary equiprobable null model was almost identical to the distribution produced by the weighted equiprobable null model and was not included in the figure.

The empirical plant-pollinator network showed significant nestedness (WNODA = 0.18, comparison against the equiprobable model: $Z = 2.73$, $p = 0.006$), but cannot be classified as presenting a nested topology (comparison against the proportional model: $Z = -6.73$, $p < 0.001$; Figure 6: Weighted nestedness). The binary index (NODF) leads to similar conclusions (NODF: 0.30; comparison against the equiprobable model: $Z = 6.61$, $p < 0.001$; comparison against the proportional model: $Z = -4.87$, $p < 0.001$). The distribution produced by the binary equiprobable null model was almost identical to the distribution produced by the weighted equiprobable null model, resulting in similar results ($Z = 6.70$, $p < 0.001$). However, when the proportional model was based in binary information, there was a large underestimation of nestedness in the randomized matrices (Figure 6: Binary nestedness), and because of that, the observed nestedness is seemingly too high compared to the proportional model ($Z = 3.31$, $p < 0.001$).

DISCUSSION

Nestedness and null models

In the present study we demonstrate that nestedness in a matrix can only result from marginal sum inequalities. This conclusion, arriving from a deductive argument, corroborate previous studies that made similar claims based on comparisons between empirical networks and null models (Jonhson *et al.*, 2013; Payrató-Borràs *et al.*, 2019). We show that overlap, one of the two defining elements of nestedness, simply quantifies how well cell values are predictable by marginal sums. Preferences in the matrix, thus, reduce nestedness. In addition, for a matrix to present nestedness, it must have unequal marginal sums, otherwise marginal sums provide no information in addition to matrix dimensions and total sum.

This understanding delimits appropriate explanations for nestedness. The only way that a mechanism can generate nestedness is by increasing marginal sum inequalities, without adding preferences to the matrix. Thus, any explanation for nestedness in a species interaction network should address the question of why some species interact more frequently than others. Unequal abundances is an obvious first guess (Vázquez *et al.*, 2007), but does not always apply (Vizentin-Bugoni *et al.*, 2014). Other mechanisms may be involved, such as differences in fitness, activity, or detectability between species. Phylogenetic (Krasnov *et al.*, 2012) and trait (Minoarivelo & Hui, 2016) specialization, on the other hand, lead to preferences and reduce the predictive power of second-order information on cell values, they thus reduce nestedness.

Analogously, in an archipelago, differences in island isolation and area may result in different chances of receiving or supporting species, generating a nested pattern of species occurrences. Local adaptation or competitive exclusion between pair of species, on the other hand, may result in preferences in the matrix, which decrease nestedness. Indeed, factors producing a hierarchy among sites or among species, and consequently skewed marginal sums, have been traditionally pointed out as the main causes of nestedness in metacommunities (Patterson & Atmar, 1986; Cutler, 1994).

Null model analysis has been a controversial step on nestedness tests, as there were no strict rules on the choice of randomization algorithms, although this choice strongly affects inference (Ulrich *et al.*, 2009). The approach proposed here overcomes this controversy by linking null models to explicit hypotheses to be tested. The equiprobable null model is used to test whether a given matrix present significantly higher nestedness than expected in the absence of nestedness-generating mechanisms. Matrices of an equiprobable null model present a random topology. The proportional null model tests whether the matrix is as nested as expected when nestedness-generating mechanisms dominate. Matrices of a proportional null model are appropriate archetypes to delimit a *nested topology*. This viewpoint challenges the perception that null model choice is a decision between type I and type II statistical errors: there is no common hypothesis being tested, but rather different hypotheses. Those null models are, thus, neither alternative nor conflicting, but can be used complementarily in nestedness analysis.

Ecological networks tend to have significant nestedness (Wright *et al.*, 1997; Bascompte *et al.*, 2003) and several previous studies looked for explanations for this ubiquity (e.g., Thebault & Fontaine, 2010). In our perspective, ubiquity of nestedness is expected, as nestedness may easily arrive from many different ecological and evolutionary mechanisms (Valverde *et al.*, 2018; Pinheiro *et al.*, 2019). After all, unequal marginal sums promote nestedness, and real-world networks are never formed by random connections between species with equal abundances and fitness.

Nevertheless, for a network to be as nested as expected by the proportional null model, it cannot include preferences between nodes. This may be the case in homogeneous and small-scale systems, but much less probable in more diverse networks (Lewinsohn *et al.*, 2006). For instance, in a pollination network containing a few plants with similar flowers, we may expect no differential preferences of the pollinators for the plants. However, if the network comprises several plant families or plants with different pollination syndromes, it is more likely that different pollinators preferentially interact with each plant or group of plants (Cutler, 1994). Nested topologies are, thus, likely to occur in species interaction networks comprising low phylogenetic diversity (e.g., Flores *et al.*, 2011), but unlikely in diverse networks (e.g., Flores *et al.*, 2013). In diverse networks, topologies that include preferences, such as modular and compound, tend to predominate (Mello *et al.*, 2019).

Methodological issues

Our study sheds light on the efficiency of nestedness indices. In weighted matrices, we recommend the use of WNODA (Pinheiro *et al.*, 2019) and spectral radius (Staniczenko *et al.*, 2013), which were very efficient in distinguishing between nested and random matrices. Beyond the observed efficiency, WNODA has the advantage of explicitly accounting for marginal sum inequalities, which provides a better match between nestedness index and concept. In binary matrices, most of the indices were reasonably efficient at intermediate levels of connectance, but inappropriate for too sparse or too connected matrices.

The use of binary information to produce proportional null models caused large distortions, both in our simulated matrices and in the case study, and, for most of the indices, resulted in large underestimation of nestedness by the null model. This is not a trivial problem, as this kind of null model is the current standard methodology and has been widely applied for nestedness analysis in the last decades (Bascompte *et al.*, 2003). And it may be the cause of several studies finding higher nestedness in empirical binary ecological networks and metacommunities than in proportional null models (Patterson & Atmar, 1986; Bascompte *et al.*, 2003). This diagnostic is corroborated by the absence of such pattern when quantitative information is used (Dormann *et al.*, 2009) or when a more elaborate null model is applied (Payrató-Borràs *et al.*, 2019). We echo previous authors in their call for a move beyond binary towards weighted matrices in ecological

studies (Banašek-Richter *et al.*, 2004; Scotti *et al.*, 2007; Ings *et al.*, 2009). We agree that there might be reasons to study the binary instead of the weighted structure of a matrix (Fründ *et al.*, 2016). However, even in these cases, null models based on weighted information are more appropriate for nestedness analysis.

Topologies beyond nestedness

Matrices with random topologies are well defined by their first-order properties: dimensions and total weights, while nested matrices are explained by their unequal marginal sums. Nevertheless, interaction matrices are often shaped by preferences between row-column pairs. In these cases, matrices are neither random nor nested, and require more information for a better description of their topologies.

When preferences are clustered, for instance by phenology or phylogeny, the result is modularity (Cordeiro *et al.*, 2020). The relationship between nestedness and the other topologies still demands clarification, and our novel perspective may help to address this challenge more efficiently. A nested matrix, according to our definition, cannot be modular at the same time, because it presents no preferences. However, nestedness and modularity are also not the opposite extremes of a continuum (Valverde *et al.*, 2020) and, as in a modular network preferences are clustered, nestedness may still prevail within each module (Lewinsohn *et al.*, 2006; Felix *et al.*, 2017; Mello *et al.*, 2019).

Conclusion

Here we show that nestedness quantifies the effect of unequal marginal sums on the cell values of an interaction matrix. This change of perspective leads to a clearer understanding of the meaning of nestedness and of the range of potential processes generating it. Moreover, by linking two different null models to explicit hypotheses, it allows a more rational use of them for testing topologies. We hope that this perspective contributes to solving old contradictions and works towards a better understanding of the topology of species interactions, metacommunities, and other ecological and non-ecological bipartite networks.

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DATA ACCESSIBILITY

Our study is mostly based on simulated data. The source of the only dataset used as a case study is referenced in the text. All analyses were performed in R version 3.4.0 (R Core Team 2017). Commented scripts to reproduce the study and functions for applying the proposed procedures on alternative networks are available at GitHub (https://github.com/pinheirorbp/nestedness) and Zenodo (http://doi.org/10.5281/zenodo.4662871).

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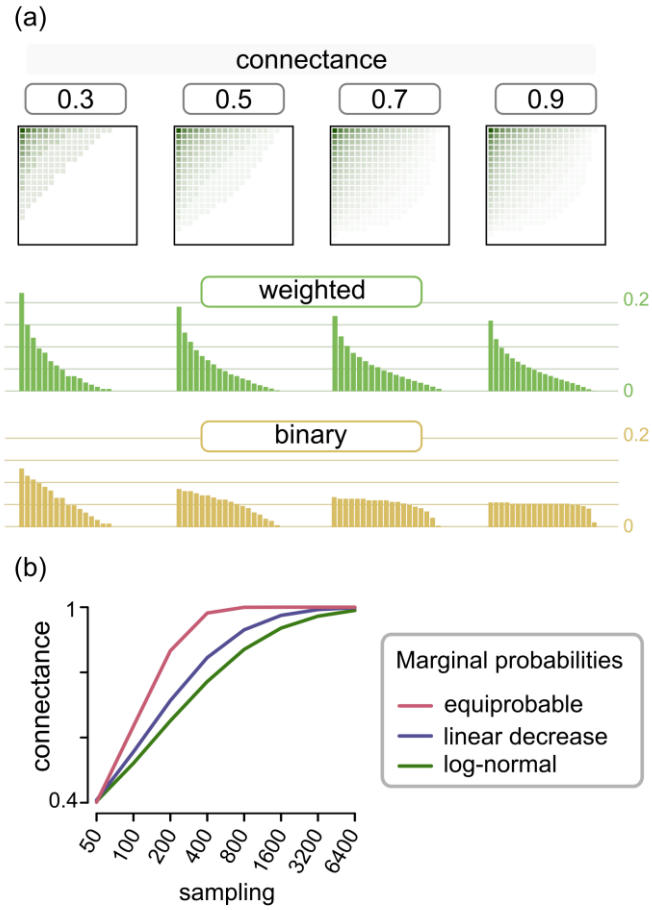

FIGURE S1 (a) Binary marginal sum distributions are contingent on matrix dimensions and connectance. They are, therefore, poor proxies for the weighted marginal sum distributions, especially in highly connected matrices. Matrices were produced following a log-normal distribution on marginal sums. Relative marginal sums on bars. For simplicity we did not remove the empty rows and columns on the matrices, which would modify the connectance (actual connectances are 0.47, 0.62, 0.78, and 0.9). (b) Connectance is not a direct proxy for the first-order information on the weighted matrix. We created probability matrices (dimensions: 10 x 10) based on marginal probability distributions and sampled interactions, to produce interaction matrices. For each marginal probability we created 10,000 matrices and measured their connectance at different sampling intensities. In the plot we see that the mean connectance (lines) is not only affected by the sampling, a first-order information, but also by the marginal probability distribution, a second-order information, of the weighted network.

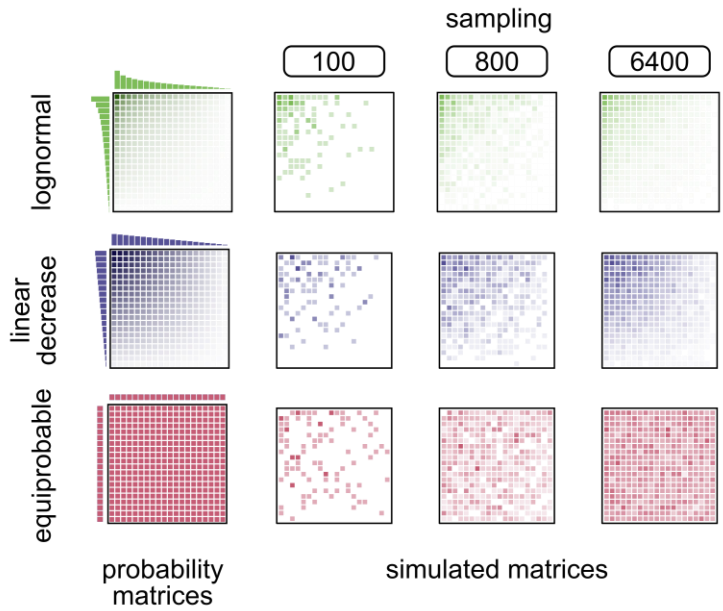

FIGURE S2 To check whether nestedness indices are efficient in distinguishing matrices with different topologies, we simulated matrices following different probability distributions. First, we produced probability matrices with three different distributions of node probabilities: log-normal, linear decrease, and equiprobable. Then, we generated randomized matrices with different total sums (sampling) following cell probabilities.