Interspecific spatial associations as a facet of biodiversity, and a review of approaches to measure them

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1 Abstract

Interspecific spatial associations (ISA), which include co-occurrences, segregations, or attractions among two or more species, have been an under-represented topic in biodiversity science and in large-scale assessments of biodiversity change in the anthropocene. Also, ISA has not been perceived as a facet of biodiversity on par with beta diversity or species richness. This is likely because it is unclear what makes ISA useful in a biodiversity context, little is known about the theoretical connections between ISA and other biodiversity facets, and there is a confusing variety of approaches to measuring ISA. Here we address these three issues. First, we argue that ISA is an important facet of biological diversity since it not only reflects species interactions, but it also provides a benchmark for validation of ecological theories, and it can be explored for potentially predictive macroecological patterns. Second, we review links of ISA to other classical biodiversity facets, namely to alpha, beta, and gamma diversity, and species accumulation curves, showing that they mostly fail to reflect changes/variation in ISA, with the exception of average pair-wise beta diversity. Third, we review the metrics that are designed to reflect ISA. These are spatially implicit indices of association for both binary and abundance data, community variograms, and summary functions such as bivariate pair correlation functions for point patterns. We test and compare some of these approaches on empirical and simulated data. We provide specific recommendations for how to use these approaches in biodiversity science and macroecology. Our main conclusion is that measuring of ISA must evolve to embrace spatially explicit, i.e. distance dependent, approaches.

Keywords: C-score, point process, interspecific, intraspecific, conspecific, heterospecific, spatial scale, grain, semivariogram, geostatistics

“*... there is no absolutely general measure of the degree of dependence*”

(Cramér 1924, quoted by Goodman and Kruskal 1979, quoted by Hubálek 1982)

2 Introduction

Organisms are rarely distributed randomly in space. Instead, they aggregate or segregate as a result of spatial distribution of resources, barriers, environments, and/or by interactions with other organisms. Such non-random spatial aggregation and segregation occurs both among individuals within a single species and among species; we call the former the *conspecific spatial aggregation* (CSA), and the latter the *interspecific spatial association* (ISA) (Fig. 1, Appendix S1). Patterns of CSA are indicative of ecological niches, interactions among individuals of the same species, or dispersal limitations (Table 1). CSA also underpins concepts such as species endemism, beta diversity, and rarity (Table 1), and there is a substantial literature on these connections (Fig. S1). In contrast, ISA indicates overlap of niches among species, as well as interspecific interactions and interaction networks (Table 1), which underpins community assembly, coexistence theory, and ecosystem functioning. ISA is also foundational to classifications of communities to habitats, coenoses, or biomes (Hoekstra et al. 2004). Thus, both CSA and ISA can be seen as both useful and fundamental components or “facets” of biodiversity, if biodiversity is broadly defined as “variation of life at all levels of biological organization” (Gaston and Spicer 2009).

Perhaps surprisingly, ISA has been underrepresented relative to CSA in the study of macroecological and biogeographic patterns of biodiversity in space and time [with notable exceptions (Gotelli et al. 2010, Lyons et al. 2016, Tóth et al. 2019), and ISA has been missing in recent high-profile studies of the ongoing biodiversity change (Millenium Ecosystem Assessment 2005, WWF International 2012, Newbold et al. 2015, Blowes et al. 2019). To demonstrate this gap, we scanned Clarivate Web of Science (Fig. S1), finding that literature on biodiversity has most often focused on quantities at sites (i.e. species richness, functional and phylogenetic diversity, extinctions, invasions, relative abundances), or quantified similarity among sites (i.e. beta diversity, turnover, nestedness, species-area relationships; Fig. S1), which are also intimately connected with CSA (Storch et al. 2008). In contrast, terms associated specifically with ISA received an order of magnitude less attention measured by the total number of published papers per year (Fig. S1), although ISA analyses require exactly the same data as analysis of beta diversity. So the lack of attention cannot be explained by a lack of data.

Clearly, although ISA is an important component of biodiversity (Table 1, next section), it has not been perceived as such by biodiversity scientists. We propose three reasons for this: First, it may be unclear what makes ISA important or useful when studying biodiversity. Second, little is known about the theoretical connections, or their lack, between patterns of ISA and other biodiversity patterns (e.g. those of richness or beta diversity). Third, it is unclear how biodiversity scientists should actually measure ISA, given the plethora of approaches.

In this paper, we aim to address each of these three issues. In the first part, we summarize why ISA is an important facet of biodiversity that deserves to be quantified alongside the other facets such as beta diversity or species-area relationships. We then outline the theoretical links between ISA and some fundamental facets of biodiversity, focusing particularly on species richness, beta diversity and species-area relationships. Finally, we review the current methods to measure ISA. We sort the methods according to the types of data that ecologists are likely to encounter, and we compare the methods on empirical datasets and on simulated pairs of species with known ISA. Based on our review and the simulations, we offer guidelines to select the appropriate approach to analyzing ISA based on the question of interest and particular type of data at hand. We hope our results will stimulate the study of ISA and promote its use in biodiversity assessments alongside more traditional measures.

3 Why ISA matters for biodiversity

Why, in the context of biodiversity, should we consider ISA patterns in space and time? The best-known reason for analyzing patterns of inter-specific spatial associations and co-occurrences is that they may give hints on biotic interactions and interaction networks among species (Gotelli et al. 2010, Harris 2016, Morueta-Holme et al. 2016, Thurman et al. 2019), which is among the biggest topics of biodiversity science (Fig. S1, Proulx et al. 2005, Newman 2006, Bascompte and Jordano 2017). The traditional approach is to conduct null model randomizations of the presence-absence matrices (Connor and Simberloff 1979, Gotelli 2000), whereas more recent approaches statistically partition ISA due to shared environmental requirements and dispersal limitations from ISA caused by the interactions, for example by joint species distribution modelling (JSDM, Warton et al. 2015, Ovaskainen et al. 2017, Zurell et al. 2018), by looking at ISA within environmentally homogeneous environment with no dispersal barriers (e.g. Tóth et al. 2019), or by making an assumption about the spatial scale of biotic interactions vs. environmental variables, which enables to separate these two in a null model (e.g. Wiegand et al. 2012). The latter is practical since it requires no data on environmental conditions.

Other reasons for measuring ISA, which we cover below, are less appreciated. First, the problem of inferring biotic interactions from co-occurrences can also be meaningfully reversed. Instead of asking “How important are biotic interactions in a given system?” we can ask “What drives the observed patterns of ISA?” Or more specifically: “To what extent can we explain the observed ISA by environmental conditions, dispersal limitations, and/or biotic interactions?” This approach broadens the focus to all of the processes that potentially generate the observed ISA (Table 1). It is also a challenge for biogeography and macroecology, where explaining gradients of various facets of diversity has dominated the field for decades, yet patterns of co-occurrence have been largely overlooked (Fig. S1), in spite of some remarkable exceptions (Blois et al. 2014, Weinstein et al. 2017, D’Amen et al. 2018).

Second, ISA and its patterns are useful even without mechanistic underpinning, because patterns are useful as summary statistics for predictions and forecasts, based on inductive logic, as opposed to the causal one. Examples of such predictive biodiversity patterns that proved to be useful are species-area relationships with their utility to predict extinctions due to habitat loss (Keil et al. 2015) or the use of richness-environment correlations for making spatial interpolations and predictions of diversity patterns (Algar et al. 2009). There is a similar value in documenting generality of empirical patterns of ISA in both time and space, since they can enable us to predict ISA in data-deficient locations, or its change under anthropogenic environmental change.

We thus argue that ISA can be understood as a summary statistic capturing a unique state of nature, on a par with other popular quantities such as species richness or beta diversity. Simply reporting how these summary statistics vary geographically, or in time, repeatedly proved to be among the most powerful starting stimuli in the field, generating countless explanatory and testable hypotheses. An example is the very existence of latitudinal and altitudinal diversity patterns, which have fascinated ecologists for centuries. The recently uncovered temporal patterns of biodiversity proved to be similarly stimulating -- an example is the zero net change of local species richness coupled with decrease in beta diversity (Dornelas et al. 2014, Tóth et al. 2019, Blowes et al. 2019) and with decrease of global gamma diversity (Barnosky et al. 2011). Similar approach has recently gained traction in the co-occurrence field. For example, Lyons et al. (2016) hypothesize that observed changes in ISA patterns might be indicative of the impact of human expansion and agriculture on assembly of communities. Likewise, Tóth et al. (2019) isolate the footprint of biotic interactions via

ISA patterns, without direct interaction data.

Third, ISA can provide new explicanda for major ecological theories (McGill 2010, Currie 2019). This could be an addition to patterns which are correlated with CSA, but uncorrelated with ISA, for example the species-area relationships (Plotkin et al. 2000, Storch 2016) (see next section). For example, it has been demonstrated that neutral theory (Hubbell 2001) or the maximum entropy theory (Harte 2011) both reproduce realistic species-area relationships, but it is unknown if they predict patterns of ISA [but see Bell (2005)]. The ability to explain patterns of ISA can thus be a new panna cotta for validating theories.

The last reason for why biodiversity science should re-focus on ISA is that some of the simple measures of biodiversity, such as average species richness or rarefaction, are insensitive to ISA, as we will show in the next section. Any biodiversity assessment that relies only on these simple measures runs into a risk of missing variation, or temporal change, of ISA. This also emphasizes the need for metrics designed to specifically quantify ISA.

4 ISA vs other biodiversity metrics

In order to study ISA in the context of biodiversity, one should be aware of how it relates to other biodiversity metrics and patterns. As mentioned above, this may be critical in any comprehensive biodiversity assessment that aims to capture as many independent facets of biodiversity as possible. Apart from that, the other facets may interfere with our ability to capture ISA, or inversely, ISA may interfere with our ability to measure other facets, which may necessitate statistically accounting for the confounding effects. Below we focus on what we see as the simplest and most fundamental metrics, namely alpha, beta, and gamma diversity, species-area relationships, and species accumulation curves.

4.1 ISA vs alpha diversity

Here we show that local species richness at any single given site is potentially sensitive to changes in ISA, while mean local richness (alpha diversity) is insensitive to changes in ISA.

Let α_j be local species richness at a site j, where $j \in 1:n$, and where n is the total number of sites within a given spatial domain. Let O_i be the number of occupied sites (i.e. occupancy) by species i, where $i \in 1:\gamma$, and where γ is the total number of species (i.e. gamma diversity) present at all sites together. When we manipulate ISA in the system, values of α_j change as a result - for example, in Figure 2a, a change from interspecific segregation to aggregation results in corresponding changes in each site’s α_j (one site gains species and two sites loose species).

Let us now consider $\bar{\alpha}$, the alpha diversity averaged across all sites, which can also be calculated as the sum of prevalences O_i/n (Šizling and Storch 2004), i.e. as:

$$\bar{\alpha} = \frac{\sum_{j=1}^{n} \alpha_j}{n} = \sum_{i=1}^{\gamma} \frac{O_i}{n} \qquad (1)$$

Again consider the scenario in Figure 2a in which there was a shift in the ISA from segregated to aggregated, given that O_i, n and γ remained constant, $\bar{\alpha}$ will not be affected by the change in ISA. In other words, when species in a given spatial domain segregate or move close to each other, it has no effect on average local species richness. Hence, an empirical assessment of biodiversity which summarizes net change of $\bar{\alpha}$ over many locations may be blind to changes of ISA, unless it also assess patterns that are sensitive to ISA, for example pairwise beta diversity. Further, this insensitivity of $\bar{\alpha}$ propagates to species-area relationships and to species accumulation curves diversity, as we show later.

4.2 ISA vs gamma diversity

From a purely geometrical perspective, simply rearranging mutual positions (ISA) of species within a given spatial domain has no effect on total number of species γ in the domain (given that n is constant). A more interesting question is what happens when the effect is reversed, i.e. how ISA changes when γ increases or decreases. This has biological implications, since γ limits the magnitude of ISA within a given domain, which can also limit biotic interactions. It also has methodological implications, since variation in γ across spatial domains can hinder direct comparisons of ISA within these domains, which may require statistical treatment (Ulrich et al. 2018). Several propositions about the relationship between ISA and γ have been made and some of them have been empirically tested.

The first has been summarized by McGill (2010): the richer the metacommunities, the weaker the associations will appear on average, despite the presence of some strong associations (Paine 1988, Wootton 1997, McGill 2010). This can be seen by considering that the total number of possible pairwise associations in the domain is $\gamma(\gamma-1)/2$. If every species is significantly spatially associated with k species in a symmetric fashion, then the total number of significant spatial associations in the domain is $(\gamma k)/2$. Thus, for any given $k<(\gamma-1)$, the total number of all possible pairwise associations increases faster with γ than the number of actually significant associations.

The second proposition operates on relative abundances: If the total area and density of individuals are constant, but there is an increase of the total richness γ, then the average per-species abundance must decrease, which will reduce the likelihood of detecting inter-specific co-occurrences in communities (Hubbell and Foster 1986, Lieberman and Lieberman 2007, Wiegand et al. 2007b, Volkov et al. 2009, Wiegand et al. 2012, Rajala et al. 2019). This only concerns the observed co-occurrences, and the effect should disappear after the observed spatial associations are contrasted with those predicted from a null model that is constrained by the observed γ and n.

The third proposition exposes the mathematical constraint on the possible values of *negative* associations in a species-by-species association matrix (Brown et al. 2004). Simply put, if species A and B have strong negative association (represented by negative correlation, covariance, or other index), then it is mathematically impossible for a third species C to have strong negative association with both A and B. This is because the association matrix is positive semidefinite (Brown et al. 2004). Fox (2012) also showed that, under the assumption that all species have the same negative correlation with each other (e.g. due to competition-induced compensatory dynamics), the minimum possible value of the correlation approaches 0 as we increase species richness. Thus, on average, the more competing species are added to the metacommunity, the weaker their observed average negative association gets.

The fourth proposition, which is biological rather than statistical in nature, suggests that communities with more species may only be stable if the interactions get weaker on average, as found by May

(1972) in an analysis of the stability of Lotka-Voltera type multispecies models. Stone (2016) found in a generalization of the analysis that stability and feasibility under increasing species richness requires a strong reduction in the mean and standard deviation of the value of the interspecific competition coefficients.

Based on all of these arguments, we should expect the overall ISA to weaken as γ increases. Indeed, Wiegand et al. (2012) found that species associations were significantly weaker in rich forest communities compared to species poor ones, even after the null-expected associations were taken into account. However, it is unclear if this occurred for the purely statistical reasons provided in proposition 1-3, and thus it may be desirable to account for them if ISA is compared across systems with different γ. Ulrich et al. (2018) provide null models to account for the effect of varying γ in spatially implicit binary co-occurrence species-by-site matrices, suggesting a randomization procedure that preserves both column and row total of the species-by-site matrix. We are unaware of similar procedures that account for the statistical effect of γ on ISA in abundance-based matrices, or in spatially explicit data. Finally, we also note that there may be limits in the ability of the null models to control for these effects (Ulrich et al. 2017).

4.3 ISA vs beta diversity

Here we show how ISA is conceptually related to another important biodiversity facet: beta diversity. We also show how one particularly popular matrix-wise measure of beta diversity, Whittaker's index, is insensitive to ISA, while pairwise indices of beta diversity are sensitive to ISA (Fig. 2), although we still lack the exact mathematical theory for this. We show that spatially explicit point pattern analysis may offer the right toolbox to build such a theory.

There is a fundamental connection between ISA and beta diversity, i.e. the differentiation of species composition in space. The connection is best illustrated on a spatially implicit site-by-species community matrix Y. In short, ISA is the association among *species* (Simberloff and Connor 1979, Hubálek 1982, Bell 2005, Legendre and Legendre 2012), whereas beta diversity is the dissimilarity among *sites*. In this simplified case, both ISA and beta diversity are calculated using exactly the same data and indices (Tables 2, 3); the only difference between them is whether they are applied to the rows ("R-mode" of Legendre & Legendre 2012) or columns of Y ("Q-mode"). In other words, any index of beta diversity that is normally applied to sites can be applied to species and can be meaningfully interpreted as an index of ISA, and vice versa (Legendre and Legendre 2012, Arita 2017). For example, Araújo and Rozenfeld (2013) define a "co-occurrence score" as the "ratio of the number of geographical cells where species A and B co-occur to the total number of occupied cells". Although not stated, this is equivalent to the classic Jaccard index. Thus, the list of measures that have been typically used to measure ISA (Tables 2 and 3) can be expanded with e.g. Jaccard or Sørensen-type indices (Arita 2017). Inversely, the list of commonly used measures of beta diversity can be expanded by some typical ISA indices; for instance the C_{forbes} (Table 2) could find its niche in the beta diversity research, since it explicitly quantifies the deviation of the observed ISA (or beta diversity) from the ISA (or beta diversity) expected under random distribution of incidences (Forbes 1907).

However, little has been written about the actual relationship between beta diversity and ISA within a given spatial domain. There is insufficient theory and empirical evaluation showing exactly how ISA and beta diversity are related, and what the limits and constraints of the relationship are. The one simple exception is Whittaker's beta diversity when expressed as $\gamma/\bar{\alpha}$, which is insensitive to ISA (Fig. 2 and next section), given that the extent of the spatial domain remains constant. Some useful connections between ISA and beta diversity are given by Arita (2017), who showed that both ISA and beta diversity are linked to the fill of binary community matrix, from which it follows that pairwise metrics of beta diversity should somehow be correlated with ISA, although not perfectly (Fig. 2). Also, Šizling et al. (2011) and McGlinn and Hurlbert (2012) give a potential clue by showing the relationship between average pairwise Jaccard beta diversity and the Whittaker index is modulated by the occupancy frequency distribution [see also McGeoch and Gaston (2002)]. This reasoning could perhaps be extended to provide a link between pairwise beta diversity and ISA.

In contrast to the spatially implicit indices for binary and abundance data, the connection between ISA and beta diversity is well known in analyses of point patterns (Wiegand and Moloney 2014) which do not operate with discrete sites, but rather with continuously varying areas for which position and species identity of every single individual is known. The ISA-beta connection can be demonstrated in the spatially-explicit version of Simpson's index $\beta(r)$ (Shimatani 2001, Wiegand and Moloney 2014 section 3.1.5.1), and this is something that could potentially inspire development of theory for other data types. Unlike the traditional spatially implicit version of the Simpson's index (Simpson 1949) (i.e., the probability that two randomly selected individuals are heterospecifics), which is a measure of diversity, $\beta(r)$ is a measure of beta diversity, since it captures dissimilarity over a given distance (Shimatani 2001) (i.e., the probability that two randomly selected individuals distance *r* apart are heterospecifics). The index is defined as:

$$\beta(r)=\sum_{i=1}^{\gamma}\sum_{j=1}^{\gamma} f_i f_j \frac{g_{ij}(r)}{g(r)}\delta_{ij}=1-\sum_{m=1}^{\gamma}\frac{f_m^2 g_{mm}(r)}{g(r)} \qquad (2)$$

Note the two alternative but equivalent definitions. In the first definition in eq. 2, f_i and f_j are the relative abundances of species i and j, $g_{ij}(r)$ is the bivariate pair correlation function describing the spatially explicit ISA and $g(r)$ is the pair correlation function of all individuals together (see following sections). The $\delta_{ij}(r)=1$ if $i\neq j$ and $\delta_{ij}(r)=0$ if $i=j$. As expected, if there are no spatial patterns of ISA, i.e. when $g_{ij}(r)=1$, we obtain the non-spatial Simpson index, and depending on the abundances and ISA of the different species, beta diversity will be larger or smaller than this point of reference. The second definition in eq. 2 operates purely with con-specific aggregation (CSA), measured by within-species pair correlation function $g_{mm}(r)$. We can see that the spatially explicit $\beta(r)$ depends on the balance between the ISA and CSA, whose overall effect sums up to 1.

We thus conclude that point pattern analysis, through $\beta(r)$, offers a comprehensive framework that can link abundances, CSA, ISA, gamma diversity and alpha diversity, each with an exactly defined and mathematically tractable metrics. Not only does it stress the importance of making all of the diversity facets spatially explicit, but it also potentially offers a roadmap for future unification of the concepts in the field of macroecology that deals with plot-, grid-, or sample-based spatially implicit data on abundances or incidences.

4.4 ISA vs species-area relationships and species-accumulation curves

Here we demonstrate that species-area relationships and rarefaction curves are insensitive to ISA. We have stated that $\bar{\alpha}$ and γ in a given domain are insensitive to ISA, given that spatial extent or a study remains constant. These are the two components of Whittaker's index of beta diversity (Whittaker 1960), which is $\gamma/\bar{\alpha}$, and so Whittaker's index is insensitive to ISA. It means that nested species-area relationships (SAR), which are a generalization of the Whittaker index over a continuous range of $\bar{\alpha}$

and γ (Crist and Veech 2006) must also be insensitive to ISA (Fig. 2). However, this only holds when $\bar{\alpha}$ is considered in the nested SAR (Storch 2016); we are unaware of any study that investigates this for other types of SAR [e.g. island SAR (Scheiner 2003)].

When every individual's spatial position and identity is known, then the theory of point pattern analysis also makes it clear that there is no direct link between ISA and SAR. The relevant equation is (Shimatani and Kubota 2004):

$$S(r)=\sum_{i=1}^{\gamma} H_i(r), \qquad (3)$$

where $S(r)$ is number of species present within r from an arbitrarily chosen "test" location, H_i is the spherical contact distribution function for species i, which is the probability that the first neighbor of species i is distance r away from the test location. $S(r)$ becomes a species-area curve when r is converted to πr^2. Importantly, the H_i is insensitive to ISA, since it is only based on the locations of species i. We note that point pattern analysis also has a scaling curve that is sensitive to ISA: the individual species-area relationship [ISAR (Wiegand et al. 2007a)] which always focuses on a focal species f:

$$IS(r)=\sum_{i=1}^{\gamma} D_{fi}(r), \qquad (4)$$

where $D_{fi}(r)$ is the bivariate nearest neighbor distribution function, and $IS(r)$ is the probability that a point of species i is distance r away from an average point of focal species f. Again, r can be converted to area as πr^2.

Finally, we turn to species-accumulation curves, from which the classical examples are the spatially implicit individual-based and sample-based rarefaction curves (Gotelli and Colwell 2001), and their spatially-explicit versions (McGlinn et al. 2019). In the former, individuals or samples are accumulated randomly, irrespectively to their spatial position, which effectively breaks any pattern of both CSA or ISA, making the spatially implicit curves indeed insensitive to ISA. In the latter spatially explicit case, samples (plots) are accumulated by nearest neighbors, which makes these curves closely related to SARs, which we have shown to be sensitive to CSA, but insensitive to ISA. This is in line with the core idea of partitioning of rarefaction curves to their components (McGlinn et al. 2019), which are the regional species-abundance distribution, density of individuals, and con-specific aggregation, but not ISA.

4.5 ISA vs biodiversity - summary

To summarize this section, we have shown that although there are biodiversity facets that reflect ISA, such as pairwise between-site beta diversity, there are also many which ignore ISA. Specifically, these are the average local species richness $\bar{\alpha}$, the regional gamma diversity γ, their ratio $\gamma/\bar{\alpha}$, and species accumulation curves including species-area relationships and rarefaction curves. The insensitivity of these measures to ISA has two important practical implications: First, when the research focus is only species richness, con-specific aggregation, and their spatial scaling, then there is no need to worry that ISA confounds these facets. Second, for the same reason, studies that aim to describe the multi-faceted nature of biodiversity need to consider additional approaches designed specifically to capture ISA. Below we offer an overview of these approaches.

5 Approaches to measuring ISA

Methods capturing aspects of ISA have emerged from several schools of thought based on different data types (Fig. 3). The first school uses non-spatial information on presence/absence (or abundance) of species in different sites and has focused on tests of hypotheses related to assembly of ecological communities (Cody and Diamond 1979) by comparing the observed patterns of ISA with simulations of null models (Gotelli and Graves 1996, Ulrich and Gotelli 2010, Sanderson and Pimm 2015). The second school has been more descriptive, identifying typical groups of species that can be observed together, a typical goal in phytocoenology (Braun-Blanquet 1964). This has seen much of its development in continental Europe (Komárek 2017), particularly in geobotany, and it relies on methods such as ordinations and cluster analysis (ter Braak 1987, Šmilauer and Lepš 2014). The third school comes from the field of geostatistics (Cressie 2010) and uses additionally the spatial position of the sites, offering extensions of variograms that show how covariance between two species changes with spatial distance (Wagner 2003). The fourth school, requiring the most detailed data on exact spatial position of every individual, and offering the richest inference, is the analysis of point patterns (Wiegand and Moloney 2014). While each of these schools of thought have generated numerous conceptually diverse measures of ISA, there has been little cross-fertilization. Below we provide an overview of the main approaches.

5.1 Spatially implicit approaches to ISA

We begin with measures of ISA designed for one of the most common types of data in biodiversity science, the *community matrix* Y, which describes the distribution of γ species (rows) over n sites (columns). Each element y_{ij} of matrix Y contains either binary incidence or some measure of abundance, and $i \in 1:\gamma$ and $j \in 1:n$. We follow (Gotelli 2000) by setting rows as species and columns as sites, but others may prefer to reverse these. Since a community matrix Y contains no information on the spatial location of the sites, we use the term *spatially implicit* for all measures of ISA that only use Y.

To date, nearly 100 indices applicable to Y have been proposed to capture ISA in binary co-occurrence data (Hubálek 1982, Rajagopalan and Robb 2005, Legendre and Legendre 2012, Ulrich and Gotelli 2013, Arita 2017) and for abundance data (Legendre and Legendre 2012, Legendre and De Cáceres 2013), although only a dozen have been widely used in ecology. Some of the pairwise indices, such as C-score or togetherness (Table 2) were developed specifically to capture ISA, others were adopted from the literature on beta diversity (Hubálek 1982, Legendre and Legendre 2012, Arita 2017). Guidelines on the selection of appropriate indices exist based on theoretical properties and performance on empirical data (Hubálek 1982, Koleff et al. 2003, Legendre and Legendre 2012, Legendre and De Cáceres 2013), as well as on arguments of comparability between current and historical analyses (Hubálek 1982). In Tables 2 and 3, we give examples of some widely used and/or sufficiently unique indices. Most of the indices are available through R packages vegan (Oksanen et al. 2019), EcoSimR (Gotelli et al. 2015), bipartite (Dormann et al. 2008), and betapart (Baselga and Orme 2012).

5.1.1 Pairwise associations in binary data

The conceptually simplest approach is to look at associations between binary occurrences of two species, either focusing at a specific pair of interest, or as a species-by-species association matrix (Z)

of all possible pairwise associations. Table 2 lists some of the typical pairwise binary examples that have been widely used, or that represent a unique approach to capturing ISA. Pairwise indices for binary data (Table 2) are based on four different quantities that result from the community matrix: the number of sites occupied uniquely by species 1 (*c*) and species 2 (*b*), the number of sites where both species co-occur (*a*), the number of sites where none occurs (*d*), and $n=a+b+c+d$. The classical and widely used C-score ($C_{seg} = bc$), for example, gives the number of pairs of sites with a “checkerboard” (i.e., one hosts species 1, the other species 2). Inversely, togetherness ($C_{tog} = ad$), gives the number of pairs of sites with no species and those with both species. The Jaccard index then gives the proportion of sites occupied by both species among all occupied sites ($C_{jacc}=a/(a+b+c)$.

5.1.2 Pairwise associations in abundance data

Table 3 lists some commonly used indices for pair-wise ISA in abundance data. The first family includes indices of covariance and correlation, both parametric and non-parametric (i.e. CA_{cor} and CA_{rho}), applied on either raw or transformed abundance data. The typical transformations are logarithmic, Hellinger (avoids the double zero problem), or square root transformation; are all aimed at removing the strong leverage of extremely common or rare species on correlations (Legendre and Legendre 2012). Another family includes indices that are extensions of the incidence-based similarity measures, and example is Růžička similarity CA_{ruz}, which is one of the abundance-based versions of Jaccard’s index for binary data (Legendre and De Cáceres 2013).

5.1.3 Higher-order and matrix-wise associations

When the aim is to quantify the overall magnitude of ISA in the entire matrix Y in a single number, one option is to simply use the mean or median of the species-by-species association matrix Z. However, focusing only on pairwise comparisons ignores interactions of higher order [i.e. interactions between 3 or more species (Harris 2016)]. Although not widely deployed, methods have been proposed that capture the magnitude of species associations across multiple orders. For example, Baselga (2017) and Arita (2017) both present their variants of the classical similarity measures for incidence data and abundance data that, in theory, capture N-wise species associations when applied to the rows of Y. The problem with these indices is their sensitivity to double zeroes and it is still unclear how common are the higher-order associations [a.k.a. motifs (Milo 2002)] in the real world; accordingly, this approach to similarity measures has already attracted criticism (Marion et al. 2017). Tables 2 and 3 list some matrix-wise metrics that have been popular.

5.1.4 Single species’ contribution to overall ISA

Legendre and De Cáceres (2013) proposed an index of contribution of a single locality to the overall beta diversity in Y. The same index can be calculated for rows instead of columns of Y, thus becoming a measure of species contribution to overall association; however, we are unaware of a study that employs such an approach to species-wise ISA.

5.1.5 Statistical significance, null models, Z-scores

When measuring ISA, one may need to test statistical significance of the observed ISA metric. That is, to calculate the probability that the metric has been produced by chance only, given that there is no ISA -- this is the so called ‘null expectation’. The simplest test of significance of association in a two-species binary matrix is Fisher’s exact test (Arita 2016), which is identical to the randomization procedure of Veech (2013). However, when more than two species are involved, or when the data are abundances rather than incidences, one needs to use a randomization procedure that aims to produce the expected distribution of a given ISA metric under the null expectation. For binary data these null models are covered by Gotelli (2000), and available through R package EcoSimR (Gotelli et al. 2015), while Ulrich and Gotelli (2010) provide an extensive evaluation of the models for abundance data.

Apart from the calculation of statistical significance, one may also quantify the deviation of the observed metric from the null expectation, and use this deviation as a new metric of ISA. This idea underpins the C_{forbes} and C_{FETmP} metrics (Table 2), and the so-called Z-score (Gotelli and McCabe 2002, Ulrich et al. 2009, Ulrich and Gotelli 2013), where the latter is defined as $(E_{raw} - E_{\exp})/SD_{\exp}$, and where E_{raw} is the ISA metric (e.g. from Table 2) calculated on observed data, $E_{\exp}$ is the null expectation of the index, and $SD_{\exp}$ is the standard deviation of the null expectation. The Z-score approach is also useful when comparing between systems which differ in the number of samples and species which can influence a raw estimate of ISA.

5.2 Spatially explicit approaches to ISA

Thus far, we have only considered spatially implicit metrics of ISA that ignore the spatial distances (lags) between samples, individuals, or species' geographic ranges. Yet this spatial structure can be of key significance, particularly when attempting to detect nonrandom associations. Consider the simple example in Figure 4 in which the same community matrix can be underlain by either between-species aggregation or segregation at fine spatial scales. Spatially implicit metrics of ISA reviewed thus far, conflate these two spatial community patterns, and thus miss part of the ISA.

In contrast, spatially explicit techniques directly acknowledge that ISA is intimately connected to spatial distance between sites or individuals. When spatial coordinates of sites or individuals are available, the general way to make the ISA spatially explicit is to only consider pairs of sites that are within a given distance interval when using any of the metrics from the previous section (Tables 2 and 3), and then to examine a range of distances. This is also the idea behind community variograms (Wagner 2003) described below. A point pattern-based version of this, when the location and identity of each individual is known, is to use bivariate pair correlation functions which describe positive and negative ISA between individuals over continuous distance (Wiegand and Moloney 2014). In the following section, we describe principles of these two approaches, and refer to software implementations.

5.2.1 Community variograms

Community variograms, borrowed from geostatistics, use the species-by-site community matrix Y together with spatial coordinates of each site (Wagner 2003). A community variogram then expresses the species-by-species variance-covariance matrix $Z(r)$ as a function of spatial distance or lag r between two samples. Spatial lags can be binned to avoid interpreting distance lags with low sample size and thus high uncertainty in the estimate of covariance, and covariances should not be interpreted at lags larger than half the maximum extent (Wagner 2003, Wagner and Fortin 2005). The community variogram approach allows the following analyses:

- At each distance, one can examine the diagonal elements of $Z(r)$ for within-species variances (i.e. CSA), or the off diagonal elements which are the between-species covariances (an Euclidean measure of ISA, sensitive to double zeroes), allowing examination of how both of these change with distance, and also the relative magnitude of CSA and ISA at a given distance.

- At each distnace, negative and positive between-species covariances can be summarized separately, as a sum or average. This allows to asses the relative magnitude of positive (attraction) and negative (repulsion) ISA at a given distance.

We note that covariance calculated on binary data is closely algebraically tied to the C_{seg} or C_{tog} metrics and their scaled versions (Table 2), which summarize only negative or positive covariances respectively. Another closely related metric is the ratio of total community variance to within-species variance (i.e. C_{ratio}, Table 2), which should be 1 when species are independent. Similarly to covariance, the C_{seg}, C_{tog} and C_{ratio}, as well as many of the other metrics from Tables 2 and 3, can be made distance-dependent and used in the community variogram framework. We also note that spatially implicit metrics are simply the weighted sum of spatially explicit metrics across all possible spatial lags in which the weights are equivalent to the number of samples separated by a given lag. Finally, the approach can easily be integrated into the joint species distribution models, as also demonstrated by Ovaskainen et al. (2017).

To test for non-random patterns of spatial species covariance an appropriate null model is required. The distnce-dependent C_{ratio} is typically used with a trivial null model of $C_{ratio} = 1$ under species independence which can be biased in a number of different ways (Palmer and van der Maarel 1995). In particular, it can be biased by patterns of within-species clumping. Even if species are arranged independently on a landscape if they have strong patterns of within-species clumping they will appear to be spatially segregated at least up to the scale of the within-species clump size. Therefore, the most common type of null model is one in which the within-species spatial clumping is held constant but otherwise species are shuffled randomly (Palmer and van der Maarel 1995, Roxburgh and Chesson 1998). Two R packages that allow calculation of community variograms are spacemakeR (Dray 2019) with the function variomultiv, and package vario (https://github.com/dmcglinn/vario) with the function vario.

Even though the idea of community variograms has been featured in prominent methodological reviews (Dray et al. 2012), and elements of it occasionally appear in empirical analyses (Wagner et al. 2005, Seabloom et al. 2005, Kikvidze et al. 2005, Ovaskainen et al. 2017), we are unaware of its direct use specifically for the purpose of estimating distance-dependent ISA. At the same time the method is the closest thing to the truly spatially-explicit description of ISA for community matrices with additional spatial coordinates, and we thus see potentially important applications.

5.2.2 Point pattern analysis

There is a suite of methods that capture various facets of ISA in data where exact position and species identity of every single individual within a spatial domain is known. These data, known as point patterns, are rich in detail, offering the most accurate and spatially explicit quantification of ISA, but are also costly to get and thus rare, and they have limits when applied to mobile organisms. Consequently, analyses of ISA based on point patterns are less common than analyses based on community matrices. Here we focus on bivariate pair correlation functions and P-M classification scheme (Wiegand et al. 2007b, Wiegand and Moloney 2014).

The bivariate pair correlation function $g_{12}(r)$ measures the association between two species 1 and 2 at different spatial scales (Stoyan and Stoyan 1994, Wiegand et al. 2007b); it is the density of a second species at distance r of the typical individual of a first species, normalized with the overall density of the second species in the plot. Thus, positive association (attraction) occurs for $g_{12}(r) > 1$ (larger than expected neighborhood density) and negative association (segregation) for $g_{12}(r) < 1$ (smaller than expected neighborhood density). The $g_{12}(r)$ is particularly useful since it quantifies repulsion or attraction at each distance independently on other distances. Example studies that employ $g_{12}(r)$ are (Wiegand et al. 2007b, 2012, Wang et al. 2010). The popular K-function $K_{12}(r)$ is the cumulative version of $g_{12}(r)$. The Z-scores of the point pattern summary functions are also sometimes used (Velázquez et al. 2016); popular software to calculate $g_{12}(r)$ and $K_{12}(r)$ be are Programita (Wiegand and Moloney 2004, 2014), and R package spatstat (pcfcross function) (Baddeley et al. 2016).

The P-M classification scheme visualizes pairwise spatial association between species at a given spatial scale r, using the Z-score of the cumulative neighbourhood density $K_{12}(r)/\pi r^2$ as x-axis (M-axis) and the Z-score of the nearest neighbor distribution function $D_{12}(r)$ as y-axis (P-axis) (Getzin et al. 2014). Because $K_{12}(r)$ and $D_{12}(r)$ capture fundamentally different aspects of ISA, the scheme allows for four ISA association categories: independence, segregation, partial overlap, and mixing. The scheme is best used to compare the overall ISA structure e.g., among tropical forests at a given spatial scale. It can be calculated using the Programita software (Wiegand and Moloney 2004, 2014), and we also offer an R implementation at https://github.com/petrkeil/spasm that relies heavily on the spatstat package (Baddeley et al. 2016).

Null models for point patterns. A well developed toolbox, summarized by Wiegand and Moloney (2014), is available to those seeking to compare observed summary statistics [e.g. $g_{12}(r)$, $K_{12}(r)$, $D_{12}(r)$] with those expected under the null expectations of independence. These are based on breaking the association between species, while keeping all of the other properties of single-species point patterns intact. The simplest null model for independence is the toroidal shift where one entire pattern is displaced against the other by a random vector (and the points falling outside the plot are wrapped using torus geometry), with a more refined version based on pattern reconstruction (Wiegand et al. 2013, Getzin et al. 2014). Depending on the question, other null models can be used as well. For example, a heterogeneous point process where the individuals of pattern 2 are displaced only to random location within distance r of their original location. Here we note that particularly the heterogeneous point process null models (also combined with pattern reconstruction) should be appealing to all those seeking to estimate biotic interaction from mere occurrence data; notably, this can be done without actually having the environmental data at hand, but by assuming that the spatial distance of biotic interactions is shorter than the distance of environmental autocorrelation (Wiegand et al. 2007b). Because in this null model points are displaced only within distance r of their original location they mey land in a similar environments. Null models for point patterns are implemented in Programita software (Wiegand and Moloney 2004, 2014) and in the spatstat R package (Baddeley et al. 2016).

6 Comparison of the approaches

Here, we compare the performance of the approaches reviewed above when they are used on a common set of empirical and simulated communities. Our aim is to assess redundancy among the approaches, as well as their sensitivity to the common variables such as number of sites, number of species, magnitude of con-specific aggregation, or spatial grain. We are also interested in how well the different measures capture negative associations (i.e. segregation) as opposed to the positive associations (attraction) of ISA. Code and data for these analyses are at https://github.com/petrkeil/spasm.

6.1 Spatially implicit measures vs empirical data

Our goal in this first exercise was to calculate the common metrics of ISA for a set of real-world and spatially implicit species-by-site matrices, and to see how the metrics correlate with each other. We evaluated the metrics listed in Tables 2 and 3 using 290 empirical binary community matrices collated in (Atmar and Patterson 1995), and 186 empirical abundance matrices collated in (Ulrich and Gotelli 2010). We excluded matrices with too little information to allow meaningful calculation of all of the indices, or which gave negative or positive infinity values of some of the metrics. For the pairwise metrics, we averaged them over the species-by-species association matrix Z to obtain a single number comparable with the matrix-wise measures. We subjected the metrics to principal components analysis (PCA), for which we transformed some of them to have an approximately normal distribution. We plotted the PCA as an ordination biplot, and we also plotted a graph representation of the correlation matrix of the measures (Fig. 5).

In both the incidence- and abundance-based spatially implicit indices we found clear differentiation along the PCA axes (Fig. 5a, b) and in the graph (Fig. 5c, d), reflecting the different aspects of ISA that they were designed to capture. In binary metrics, there is a clear cluster dissimilarity-based indices (C_{jacc}, C_{sor}), the Whittaker index (C_w), Alroy’s index (C_{alroy}), as well as its closely related connectance (C_{conn}) (Fig. 5 a, c, Fig. S1). Two measures that explicitly measure departure from Poisson randomness (C_{FETmP}, C_{forbes}) grouped together. The classical C-score (C_{segSc}) and its counterpart, togetherness (C_{togSc}), the matching coefficient (C_{match}), the checkerboard score ($C_{checker}$), and number of unique combinations (C_{combo}) are to some degree unrelated to the rest of the metrics. In the abundance-based metrics, we found similar clustering of the dissimilarity-based ISA metrics (CA_{ruz}, CA_{bray}, CA_{chi}, CA_{hell}), while the correlation-based metrics (CA_{cor}, CA_{cor_hell}, CA_{tau}) formed another group.

Notably, among the incidence-based metrics only C_{combo} is strongly correlated with the total number of sites in a matrix (n), while only $C_{checker}$ correlated strongly with the total number of species (γ) (Fig. S2). Unsurprisingly, covariance-based measures (CA_{cov}, CA_{cov_hell}) grouped together with the total number of sites n. Among all the metrics several metrics are correlated with γ, but none of them strongly (Figure S2, S3).

6.2 Spatially implicit measures vs spatially explicit simulations

In the second exercise, we devised simulations with known magnitude of ISA between two species (Fig. 6, S4, Appendix S2). We aimed to illustrate how the different approaches are able to recover this simulated ISA. In contrast to spatially implicit simulations performed in other studies (Gotelli 2000, Ulrich and Gotelli 2010), we simulated the position of every individual within a bounded domain. In each simulation, we generated point patterns of two species, with a given magnitude of ISA between them, and with varying con-specific aggregation and number of individuals per species (see Appendix S2 for details). See Keil (2019) for an example use of the same simulation procedure.

We modelled ISA as dependent on spatial distance (Fig. 6b), and it was controlled by a single parameter called simply ISA, with negative values for segregation (ISA < 0), zero for independence (ISA = 0), and positive for attraction (ISA > 0) (Fig. 6). In order to calculate measures based on incidence or abundance, we converted the point patterns to grids of varying resolutions (grains). Across all simulations, we then measured performance of the metrics from Tables 2 and 3 as their Spearman correlation with the ISA parameter. We also examined how the performance was affected by spatial grain and magnitude of conspecific spatial aggregation (CSA) (Fig. S6, S7). Detailed description of the simulations, and their analysis, is in Appendix S2, and complete code for the exercise is at https://github.com/petrkeil/spasm.

We found the best correlation with overall ISA, as well as correlation with both negative ISA (attraction) and negative ISA (repulsion), in Pearson's tetrachoric correlation (C_{pears}), Forbes index (C_{forbes}), and the mid-P variant of Fisher's Exact Test (C_{FETmP}) for binary data, and Spearman's correlation (CA_{rho}) and Hellinger distance (CA_{hell}) for abundance data (Fig. 7). The best overall Spearman correlations between the simulated ISA and its estimated measures were around 0.75 (Fig. 7), an imperfection that we attribute to the inability of the spatially implicit measures to capture the distance-dependent part of ISA (Fig. 4, Fig. 6b). We found no clear indication that either abundance or incidence-based metrics performed best. We also found that measures based on correlation (C_{pears}, CA_{rho}, CA_{cor}, CA_{cov}) , or on deviation from an expected null association (C_{forbes}, C_{FETmP}) performed better in capturing ISA, while dissimilarity measures (C_{sor}, C_{jacc}, CA_{bray}, CA_{ruz}) performed worse. This is also in line with our supplementary analysis of Z-scores (Figs. S6 and S7). We found that refining the spatial grain of the analysis, and increasing the magnitude of CSA, had both mostly negative or no effect on the performance of the metrics (Figs. S6 and S7), although there were exceptions from the trend.

Importantly, we found that the spatially implicit metrics tended to saturate at extreme negative ($ISA < 10$) association (Fig. S5), most likely because the community matrices are identical above these extreme ISA values (demonstrated in Fig. 4). Thus, most abundance-based metrics show weak overall correlation with repulsion (Fig. 7b), with the exception of the distance-based Ruzicka (CA_{ruz}) and percentage difference (CA_{bray}) indices. Finally, we found that when the metrics were averaged at a given spatial grain, they varied dramatically in their relationship with grain (Fig. S8), and this variation is difficult to interpret.

6.3 Spatially explicit measures vs spatially explicit simulations

Finally, we used the 2-species simulations to illustrate the idea of community variograms and pair correlation functions. Here, our goal was simply to show the potential of these methods, rather than to perform the same comprehensive evaluation as above, since these techniques do not measure ISA in a single number, but as a function of spatial distance r. Because of that, they have the potential to actually recover the shape of the entire probability density function $f_{sp2}(r)$ from Figure 6b. For the spatially explicit techniques, we performed 9 simulations that correspond exactly to the panels of Figure 6a (the only difference is that we used 200 individuals per species). For the purpose of fitting the community variograms, we used a single grid resolution with 20 cells along each side of the simulated domain.

Community variograms (Fig. 8a) estimated the relationship between distance r and negative covariance in a way that matched the shape of the original $f_{sp2}(r)$ from Figure 6b. However, they had problem with accurately capturing the spatial pattern of attraction when there was a confounding effect of strong con-specific dispersion ($CSA > 0.01$), where the pattern was indistinguishable from independence. In contrast to community variograms, pair correlation functions more accurately estimated the distance-dependent ISA (compare Fig. 6b with Fig. 8a), i.e. the estimated relationship between distance and pair correlation function closely matches the shape of the original $f_{sp2}(r)$ from Figure 6b. Similarly to community variograms, there was a somewhat limited performance for attraction and high CSA values, but the problem is less severe. Overall, the point pattern-based bivariate pair correlation functions provided the most complete picture of the “true” simulated ISA pattern.

7 Discussion and recommendations

Based on the existing literature and our comparisons of the methods, we make several concrete recommendations for capturing ISA in the context of biodiversity.

7.1 Best indices

There is a variety of indices that were designed to capture different aspects of ISA and some of them may even be uncorrelated [see our results, but also Hubálek (1982)]. Thus, in empirical assessments, we recommend not to rely on a single metric [as also recommended by Ulrich and Gotelli (2013)]. Overall, we suggest that the most promising indices for spatially implicit analyses are those that can capture both negative and positive ISA. They should also offer the option to be eventually integrated into, or compared with, spatially explicit analyses such as community variograms, it should be possible to contrast them with indices of con-specific aggregation, and they should be applicable in association matrices of parametric joint species distribution models. For abundance data these are covariance (CA_{cov}) and Pearson correlation (CA_{cor}), coupled with a data transformation (e.g. Hellinger or log) if appropriate. For incidence data these are the C-score (C_{seg}, C_{segSc}) and togetherness (C_{tog}, C_{togSc}) which capture the positive and negative ISA respectively and are mathematically linked to covariance. We caution that that low C-score values do not indicate high togetherness, and vice versa. Thus, both should be employed, unless only one direction of ISA is specifically of interest.

From the other indices for pairwise binary data, among the all-purpose metrics that capture both repulsion and attraction, we recommend the mid-P index (C_{FETmP}), Forbes’s coefficient of association (C_{forbes}), or Pearson’s tetrachoric correlation (C_{pears}); the latter is also (with reservations) recommended by Hubálek (1982). Jaccard’s index (C_{jacc}) is a good alternative, since it captures both negative and positive ISA, and it can easily interpreted as proportional overlap, although it may not reflect the overall ISA as accurately as the correlation-based indices. For pairwise abundance data, apart from covariance- and correlation-based parametric indices, we advocate for the Spearman rank-rank correlation (C_{rho}), since it requires no prior transformation, and it captures well both negative and positive ISA.

Concerning the community-wide measures that quantify the magnitude of ISA for all species in a single number, an obvious choice is to use summarized pairwise metrics, although one needs to beware that some summarizations (e.g. averaging) blend repulsions and attractions. Perhaps the best way to avoid this problem is to decompose the pairwise association matrix to positive vs negative ISA, by separately summarizing positive vs negative covariances in abundance data (this is also the approach used in community variograms), or by considering both togetherness and C-score in incidence data. Finally, we advise caution when using indices such as Whittaker’s (C_w) or the related connectance (C_{conn}) because of their problematic interpretation which conflates ISA with beta diversity.

7.2 ISA is better spatially explicit

Our results highlight a serious limitation of spatially implicit indices of ISA: they fail to consider spatial proximity of two species when they are already 100% spatially segregated (Fig. 4), a problem that will grow in severity as spatial resolution becomes finer. Ultimately, this problem will make the spatially implicit metrics meaningless at spatial grains that reach the size of an average individual, or when the data become point patterns. This partly explains why none of the examined spatially implicit metrics gave a perfect correlation with the simulated distance-dependent ISA, and why we observed the saturation of performance towards extreme values of ISA in our simulations (Fig. S5). This is a drawback of any analysis based purely on spatially implicit community matrices, even though the problem may be less severe at coarse spatial resolutions. We argue that there is only one way to address it systematically and conceptually: Whenever there is information on spatial position of the sites, indices of ISA are better considered as a function of spatial distance. Both the community variograms and point pattern analysis offer straightforward ways to do that.

Apart from being practical, going spatially explicit (when data permit) also makes ISA more biologically interpretable; after all, interspecific biotic interactions among individuals do happen over a certain distance. Thus, measuring ISA in a spatially explicit way can identify those distances over which the interactions matter. An added advantage of such an approach is that it also allows to separate ISA due to interactions vs environment, without the necessity of actually having the environmental data when it appears that fine-scale environmental heterogeneity is negligible (Wiegand et al. 2012).

One specific application in which ignoring spatial distance is likely to result in severe consequences is Joint Species Distribution Modeling, or JSDM (Warton et al. 2015, Ovaskainen et al. 2017, Zurell et al. 2018), where species-by-species covariances (or correlations) are estimated as model parameters, conditional on the species-environment relationships. We recommend that these covariance matrices also need to be conditional on spatial distance in order to avoid the saturation arising from strong ISA. One specific implementation of such an approach is offered through hierarchical parametric models of Ovaskainen et al. (2017).

7.3 Spatial scale

Most of the commonly studied facets of biodiversity depend on spatial scale. In the case of ISA, scale has been approached from two angles. The first focuses on ISA as a function of the average area of a site in a community matrix, which is equivalent to spatial resolution (grain) of a grid (Økland 1994, Hui 2009, Segurado et al. 2012, Araújo and Rozenfeld 2013, McNickle et al. 2018). These studies show that ISA is grain-dependent, but neither theory nor empirical observations predict a systematic direction of the grain dependence; ISA-area relationships can be increasing, decreasing, or hump-shaped. One thing that complicates consensus is the use of various metrics of ISA across studies. Our simulations (Fig. S8) show that selection of the ISA metric critically affects the grain dependency; for example, Z-scores of abundance-based metrics may have hump-shaped relationship with grain (e.g. Z-score of CA_{bray}), while raw binary dissimilarity may monotonically increase or decrease with grain (Fig. S8). This is something that the field needs to sort out before any empirical scaling of ISA is explored and interpreted.

The second approach to spatial scaling of ISA uses distance instead of grain in the form of community variograms or bivariate pair correlation functions (Wagner 2003, Wiegand and Moloney 2014). One advantage of this approach is its straightforward biological interpretation, particularly in the context of biotic interactions, since spatial distance is the ultimate condition for interactions (grain is only proximate). In other words, most ecologists will understand that species interact over a certain distance, but might be puzzled by the statement that they interact at a given grain.

Luckily, both the grain-based and distance-based approaches to the scaling of ISA can be combined in a single analysis [see Wiegand et al. (2012)]. Finally, point-pattern analysis has theory that links the area-based with the distance-based approaches to ISA (Wiegand and Moloney 2014). Future investigations of ISA should more explicitly embrace the issue of scale (both the grain and perhaps also extent) by focusing on the ISA-area relationships(Araújo and Rozenfeld 2013, McNickle et al. 2018), or by focusing on the spatially explicit approaches to ISA which handle scale more naturally than the spatially implicit ones.

7.4 Null models and Z-scores

Most of the literature on ISA emphasizes the importance of null models and null expectations, be it spatially implicit (Ulrich and Gotelli 2013) or explicit (Wiegand and Moloney 2014). The most common use of these is to get to statistical significance of the observed ISA, and to standardize the metrics so that they are comparable across different ecological situations or studies. In Figures S6 and S7, we show that transforming ISA indices to Z-scores makes them more robust against the confounding effects of conspecific aggregation or varying resolution, while (Ulrich et al. 2018) show that null models also account for the confounding effect of total species richness (γ). The same sentiment underlies Wiegand and Moloney's (2014) book: to analyze spatially explicit patterns of ISA, one has to contrast them against a null expectation of no ISA. However, one needs to bear in mind that when Z-scores and null models are used, they tend to converge to the same value, irrespective of the original meaning of the metric (Keil 2019); in a way, the meaning of the null model overrides the meaning of the index. Thus, when null models are deployed, we recommend that users know their ecological interpretation exactly.

Since null models often involve intense randomization algorithms, they may be computationally demanding, particularly when used in large-scale macroecological analyses of biodiversity. Luckily, there are analytical shortcuts for some of the approaches. For example, in spatially implicit methods, if computational efficiency is desirable, measures such as C_{FETmP} or C_{forbes} already have the deviation from the expected randomness implicit in their definition.

7.5 Measuring macroecological patterns of ISA

Finally, we return to the main point of this paper, which is the premise that patterns of ISA is an important standalone facet of biodiversity that is on par with macroecological classics such as species richness or beta diversity. We have demonstrated that ISA is at least partly independent of these other facets. Thus, in order to empirically document patterns of ISA, and their generality or variation over broad spatial and temporal scales, we need analytical approaches designed specifically to measure ISA with the kind of data available over broad scales. From all of the reviewed approaches, we see community variograms as the most useful, since:

(1) Community variograms are spatially explicit, thus they avoid the pitfalls of the spatially implicit indices mentioned above, offering rich biological interpretation.

(2) Community variograms are applicable to a wide range of data types, including systematic continental surveys such as the US North American Breeding Bird Survey, metacommunity data in databses such as PREDICTS, BioTime or CESTES (Newbold et al. 2015, Jeliazkov 2019, Blowes et al. 2019), regional gridded atlases such as the Atlas Florae Europaeae, and global grids such as the the IUCN red list data. In contrast, point pattern analysis has highly specific requirements on data format,

limiting its use to a small number of local plots with limited spatial extent.

(3) Calculation of community variograms is computationally feasible.

Given that there is an ever increasing availability of biodiversity data from large spatial extents (Jetz et al. 2012), we see an exciting opportunity here for exploration of empirical macroecological patterns of ISA, particularly through community variograms. Future work should examine how community variograms change along macroecological gradients.

8 Conclusions

We have based this paper on the premise that inter-specific spatial associations (ISA) are an underrepresented topic in biodiversity science and macroecology, and that this is an important knowledge gap. We argued that there are several reasons for why ISA, and its spatio-temporal patterns, are worth exploring. Apart from its connection to biotic interactions, ISA can also provide a benchmark for judging different types of ecological theories, and it can serve as a summary statistic capturing a unique property of nature. This is underscored by the fact that many of the biodiversity statistics that have been monitored are insensitive to ISA.

We provide an overview of the main approaches to measure ISA, which we sorted into three main schools of thought, based on the data that they use: spatially implicit indices, community variograms, and bivariate pair correlation functions. Our main conclusion is that considering space, and particularly spatial distance, is vital for the progress of the field, and for any broad-scale assessment of patterns of ISA in geographic space and in time. In all, we hope that our overview of ISA, its measures, and its utility provides a starting point for researchers interested in broadening the scope of biodiversity facets that they study, as well as a new inspiration for those who have already studied ISA. Among these, we turn to two kinds of researchers for whom our review seems particularly relevant: The first are those working in the emerging field of joint species distribution models (JSDM), where spatial distance has rarely been considered, and which still rely mostly on spatially implicit covariances and correlations. Second, we would like to appeal to those dealing with interaction networks and biodiversity-ecosystem function (BEF) issues. The critical role of spatial distance and scale in ISA is something that they need to consider, and our review offers specific ways how to approach that.

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10 References

Algar, A. C., H. M. Kharouba, E. R. Young, and J. T. Kerr. 2009. Predicting the future of species diversity: macroecological theory, climate change, and direct tests of alternative forecasting methods. Ecography 32:22–33.

Alroy, J. 2015. A new twist on a very old binary similarity coefficient. Ecology 96:575–586.

Araújo, M. B., and A. Rozenfeld. 2013. The geographic scaling of biotic interactions. Ecography 37:406–415.

Arita, H. T. 2016. Species co-occurrence analysis: pairwise versus matrix-level approaches: Correspondence. Global Ecology and Biogeography 25:1397–1400.

Arita, H. T. 2017. Multisite and multispecies measures of overlap, co-occurrence, and co-diversity. Ecography 40:709–718.

Atmar, W., and B. D. Patterson. 1995. Nestedness temperature calculator. AICS Research Inc, University Park, New Mexico, and The Field Museum, Chicago.

Baddeley, A., E. Rubak, and R. Turner. 2016. Spatial point patterns: methodology and applications with R. CRC Press, Taylor & Francis Group, Boca Raton, FL.

Barnosky, A. D., N. Matzke, S. Tomiya, G. O. U. Wogan, B. Swartz, T. B. Quental, C. Marshall, J. L. McGuire, E. L. Lindsey, K. C. Maguire, B. Mersey, and E. A. Ferrer. 2011. Has the Earth’s sixth mass extinction already arrived? Nature 471:51–57.

Bascompte, J., and P. Jordano. 2017. Mutualistic networks. Princeton University Press, Princeton, NJ.

Baselga, A. 2017. Partitioning abundance-based multiple-site dissimilarity into components: balanced variation in abundance and abundance gradients. Methods in Ecology and Evolution 8:799–808.

Baselga, A., and C. D. L. Orme. 2012. betapart : an R package for the study of beta diversity: *Betapart package*. Methods in Ecology and Evolution 3:808–812.

Bell, G. 2005. The co-distribution of species in relation to the neutral theory of community ecology. Ecology 86:1757–1770.

Blois, J. L., N. J. Gotelli, A. K. Behrensmeyer, J. T. Faith, S. K. Lyons, J. W. Williams, K. L. Amatangelo, A. Bercovici, A. Du, J. T. Eronen, G. R. Graves, N. Jud, C. Labandeira, C. V. Looy, B. McGill, D. Patterson, R. Potts, B. Riddle, R. Terry, A. Tóth, A. Villaseñor, and S. Wing. 2014. A framework for evaluating the influence of climate, dispersal limitation, and biotic interactions using fossil pollen associations across the late Quaternary. Ecography 37:1095–1108.

Blowes, S. A., S. R. Supp, L. H. Antão, A. Bates, H. Bruelheide, J. M. Chase, F. Moyes, A. Magurran, B. McGill, I. H. Myers-Smith, M. Winter, A. D. Bjorkman, D. E. Bowler, J. E. K. Byrnes, A. Gonzalez, J. Hines, F. Isbell, H. P. Jones, L. M. Navarro, P. L. Thompson, M. Vellend, C. Waldock, and M. Dornelas. 2019. The geography of biodiversity change in marine and terrestrial assemblages. Science 366:339–345.

ter Braak, C. 1987. CANOCO - a FORTRAN program for canonical community ordination by partial detrended canonical correspondence analysis, principal components analysis and redundancy analysis (version 2.1). Agricultural Mathematics Group, Wageningen University, Wageningen.

Braun-Blanquet, J. 1964. Pflanzensoziologie: Grundzüge der Vegetationskunde. Springer, Wien.

Brown, J. H., E. J. Bedrick, S. K. M. Ernest, J.-L. E. Cartron, and J. F. Kelly. 2004. Constraints on negative relationships: mathematical causes and ecological consequences. Pages 298–323 *in* M. L. Taper and S. R. Lele, editors. The nature of scientific evidence: statistical, philosophical, and empirical considerations. University of Chicago Press, Chicago, IL.

Cody, M. L., and J. M. Diamond, editors. 1979. Ecology and evolution of communities. Belknap Press, Cambridge, MA.

Connor, E. F., and D. Simberloff. 1979. The assembly of species communities: chance or competition? Ecology 60:1132.

Cramér, H. 1924. Remarks on correlation. Skandinavisk Aktuarietidskrift 7:220–240.

Cressie, N. A. C. 2010. Statistics for spatial data. Wiley, New York, NY.

Crist, T. O., and J. A. Veech. 2006. Additive partitioning of rarefaction curves and species-area relationships: unifying alpha-, beta- and gamma-diversity with sample size and habitat area. Ecology Letters 9:923–932.

Currie, D. J. 2019. Where Newton might have taken ecology. Global Ecology and Biogeography 28:18–27.

D’Amen, M., H. K. Mod, N. J. Gotelli, and A. Guisan. 2018. Disentangling biotic interactions, environmental filters, and dispersal limitation as drivers of species co-occurrence. Ecography 41:1233–1244.

Dice, L. R. 1945. Measures of the amount of ecologic association between species. Ecology 26:297–302.

Dormann, C. F., J. Frund, N. Bluthgen, and B. Gruber. 2009. Indices, graphs and null models: analyzing bipartite ecological networks. The Open Ecology Journal 2:7–24.

Dormann, C. F., B. Gruber, and J. Frund. 2008. Introducing the bipartite package: Analysing ecological networks. R News 8:8–11.

Dornelas, M., N. J. Gotelli, B. McGill, H. Shimadzu, F. Moyes, C. Sievers, and A. E. Magurran. 2014. Assemblage time series reveal biodiversity change but not systematic loss. Science 344:296–299.

Dray, S. 2019. spacemakeR: Spatial modelling. R-Forge.

Dray, S., R. Pélissier, P. Couteron, M.-J. Fortin, P. Legendre, P. R. Peres-Neto, E. Bellier, R. Bivand, F. G. Blanchet, M. De Cáceres, A.-B. Dufour, E. Heegaard, T. Jombart, F. Munoz, J. Oksanen, J. Thioulouse, and H. H. Wagner. 2012. Community ecology in the age of multivariate multiscale spatial analysis. Ecological Monographs 82:257–275.

Forbes, S. A. 1907. On the local distribution of certain Illinois fishes: an essay in statistical ecology. Bulletin of the Illinois State Laboratory of Natural History 7:273–303.

Fox, J. W. 2012. An important but little known fact about compensatory dynamics. https://dynamicecology.wordpress.com.

Gaston, K. J., and J. I. Spicer. 2009. Biodiversity: an introduction. Blackwell, Malden, MA.

Getzin, S., T. Wiegand, and S. P. Hubbell. 2014. Stochastically driven adult-recruit associations of tree species on Barro Colorado Island. Proceedings of the Royal Society B: Biological Sciences 281:20140922–20140922.

Goodman, L. A., and W. H. Kruskal. 1979. Measures of association for cross classifications. Springer, New York, NY.

Gotelli, N. J. 2000. Null model analysis of species co-occurrence patterns. Ecology 81:2606–2621.

Gotelli, N. J., and R. K. Colwell. 2001. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. Ecology Letters 4:379–391.

Gotelli, N. J., and G. R. Graves. 1996. Null models in ecology. Smithsonian Press, Washington DC.

Gotelli, N. J., G. R. Graves, and C. Rahbek. 2010. Macroecological signals of species interactions in the Danish avifauna. Proceedings of the National Academy of Sciences 107:5030–5035.

Gotelli, N. J., E. M. Hart, and A. M. Ellison. 2015. EcoSimR: Null model analysis for ecological data. CRAN.

Gotelli, N. J., and D. J. McCabe. 2002. Species co-occurrence: A meta analysis of J. M. Diamond's assembly rules model. Ecology 83:2091–2096.

Harris, D. J. 2016. Inferring species interactions from co-occurrence data with Markov networks. Ecology 97:3308–3314.

Harte, J. 2011. Maximum entropy and ecology: A theory of abundance, distribution, and energetics. Oxford University Press, Oxford.

Hoekstra, J. M., T. M. Boucher, T. H. Ricketts, and C. Roberts. 2004. Confronting a biome crisis: global disparities of habitat loss and protection: Confronting a biome crisis. Ecology Letters 8:23–29.

Hubálek, Z. 1982. Coefficients of association and similarity, based on binary (presence-absence) data: an evaluation. Biological Reviews 57:669–689.

Hubbell, S. P. 2001. The unified theory of biodiversity and biogeography. Princeton University Press, Princeton, NJ.

Hubbell, S. P., and R. B. Foster. 1986. Biology, chance, and history and the structure of tropical rain forest tree communities. Pages 314–329 *in* J. M. Diamond and T. J. Case, editors. Community Ecology. Harper and Row Publishers, New York, NY.

Hui, C. 2009. On the scaling patterns of species spatial distribution and association. Journal of Theoretical Biology 261:481–487.

Jaccard, P. 1901. Étude comparative de la distribution florale dans une portion des Alpes et des Jura. Bulletin de la Société vaudoise des sciences naturelles 37:547–579.

Jeliazkov, A. 2019. A global database for metacommunity ecology, integrating species, traits, environment and space. Scientifc Data in press.

Jetz, W., J. M. McPherson, and R. P. Guralnick. 2012. Integrating biodiversity distribution knowledge: toward a global map of life. Trends in Ecology & Evolution 27:151–159.

Kallio, A., K. Puolamäki, M. Fortelius, and H. Mannila. 2011. Correlations and co-occurrences of taxa: The role of temporal, geographic, and taxonomic restrictions. Palaeontologia Electronica 14.

Keil, P. 2014. Limits of uncertainty about estimates of probability of ecological events. PeerJ PrePrints:2:e446v1.

Keil, P. 2019. Z scores unite pairwise indices of ecological similarity and association for binary data.

Ecosphere 10:e02933.

Keil, P., D. Storch, and W. Jetz. 2015. On the decline of biodiversity due to area loss. Nature Communications 6:8837.

Kikvidze, Z., F. I. Pugnaire, R. W. Brooker, P. Choler, C. J. Lortie, R. Michalet, and R. M. Callaway. 2005. Linking patterns and processes in Alpine plant communities: a global study. Ecology 86:1395–1400.

Koleff, P., K. J. Gaston, and J. J. Lennon. 2003. Measuring beta diversity for presence–absence data. Journal of Animal Ecology 72:367–382.

Komárek, S. 2017. Stručné dějiny biologie (Brief history of biology, in Czech with English summary). Academia, Praha.

Lebart, L., and J. P. Fénelon. 1971. Statistique et informatique appliqées. Dunod, Paris.

Legendre, P., and M. De Cáceres. 2013. Beta diversity as the variance of community data: dissimilarity coefficients and partitioning. Ecology Letters 16:951–963.

Legendre, P., and L. Legendre. 2012. Numerical Ecology. Elsevier, Amsterdam.

Lieberman, M., and D. Lieberman. 2007. Nearest-neighbor tree species combinations in tropical forest: the role of chance, and some consequences of high diversity. Oikos 116:377–386.

Lyons, S. K., K. L. Amatangelo, A. K. Behrensmeyer, A. Bercovici, J. L. Blois, M. Davis, W. A. DiMichele, A. Du, J. T. Eronen, J. Tyler Faith, G. R. Graves, N. Jud, C. Labandeira, C. V. Looy, B. McGill, J. H. Miller, D. Patterson, S. Pineda-Munoz, R. Potts, B. Riddle, R. Terry, A. Tóth, W. Ulrich, A. Villaseñor, S. Wing, H. Anderson, J. Anderson, D. Waller, and N. J. Gotelli. 2016. Holocene shifts in the assembly of plant and animal communities implicate human impacts. Nature 529:80–83.

Marion, Z. H., J. A. Fordyce, and B. M. Fitzpatrick. 2017. Pairwise beta diversity resolves an underappreciated source of confusion in calculating species turnover. Ecology 98:933–939.

May, R. M. 1972. Will a large complex system be stable? Nature 238:413–414.

McGeoch, M. A., and K. J. Gaston. 2002. Occupancy frequency distributions: patterns, artefacts and mechanisms. Biological Reviews 77:311–331.

McGill, B. J. 2010. Towards a unification of unified theories of biodiversity. Ecology Letters 13:627–642.

McGlinn, D. J., and A. H. Hurlbert. 2012. Scale dependence in species turnover reflects variance in species occupancy. Ecology 93:294–302.

McGlinn, D. J., X. Xiao, F. May, N. J. Gotelli, T. Engel, S. A. Blowes, T. M. Knight, O. Purschke, J. M. Chase, and B. J. McGill. 2019. Measurement of Biodiversity (MoB): A method to separate the scale dependent effects of species abundance distribution, density, and aggregation on diversity change. Methods in Ecology and Evolution 10:258–269.

McNickle, G. G., E. G. Lamb, M. Lavender, J. F. Cahill, B. S. Schamp, S. D. Siciliano, R. Condit, S. P. Hubbell, and J. L. Baltzer. 2018. Checkerboard score-area relationships reveal spatial scales of plant community structure. Oikos 127:415–426.

Millenium Ecosystem Assessment. 2005. Ecosystems and human well-being: synthesis. Island Press, Washington DC.

Milo, R. 2002. Network motifs: simple building blocks of complex networks. Science 298:824–827.

Morueta-Holme, N., B. Blonder, B. Sandel, B. J. McGill, R. K. Peet, J. E. Ott, C. Violle, B. J. Enquist, P. M. Jørgensen, and J.-C. Svenning. 2016. A network approach for inferring species associations from co-occurrence data. Ecography 39:1139–1150.

Newbold, T., L. N. Hudson, S. L. L. Hill, S. Contu, I. Lysenko, R. A. Senior, L. Börger, D. J. Bennett, A. Choimes, B. Collen, J. Day, A. De Palma, S. Díaz, S. Echeverria-Londoño, M. J. Edgar, A. Feldman, M. Garon, M. L. K. Harrison, T. Alhusseini, D. J. Ingram, Y. Itescu, J. Kattge, V. Kemp, L. Kirkpatrick, M. Kleyer, D. L. P. Correia, C. D. Martin, S. Meiri, M. Novosolov, Y. Pan, H. R. P. Phillips, D. W. Purves, A. Robinson, J. Simpson, S. L. Tuck, E. Weiher, H. J. White, R. M. Ewers, G. M. Mace, J. P. W. Scharlemann, and A. Purvis. 2015. Global effects of land use on local terrestrial biodiversity. Nature 520:45–50.

Newman, M. E. J. 2006. Modularity and community structure in networks. Proceedings of the National Academy of Sciences 103:8577–8582.

Odum, E. P. 1950. Bird populations of the highlands (North Carolina) plateau in relation to plant succession and avian invasion. Ecology 31:587–605.

Økland, R. H. 1994. Patterns of bryophyte associations at different scales in a Norwegian boreal spruce forest.

Journal of Vegetation Science 5:127–138.

Oksanen, J., R. Kindt, P. Legendre, B. O’Hara, M. H. H. Stevens, and M. J. Oksanen. 2019. vegan: Community Ecology Package. R package version 2.5-5, https://cran.r-project.org (accessed 28 Nov 2019).

Ovaskainen, O., G. Tikhonov, A. Norberg, F. Guillaume Blanchet, L. Duan, D. Dunson, T. Roslin, and N. Abrego. 2017. How to make more out of community data? A conceptual framework and its implementation as models and software. Ecology Letters 20:561–576.

Paine, R. T. 1988. Food webs: Road maps of interactions or grist for theoretical development? Ecology 69:1648–1654.

Palmer, M. W., and E. van der Maarel. 1995. Variance in species richness, species association, and niche limitation. Oikos 73:203.

Pearson, K., and D. Heron. 1913. On theories of association. Biometrika 9:159–315.

Plotkin, J. B., M. D. Potts, N. Leslie, N. Manokaran, J. Lafrankie, and P. S. Ashton. 2000. Species-area curves, spatial aggregation, and habitat specialization in tropical forests. Journal of Theoretical Biology 207:81–99.

Proulx, S., D. Promislow, and P. Phillips. 2005. Network thinking in ecology and evolution. Trends in Ecology & Evolution 20:345–353.

Rajagopalan, S., and R. Robb. 2005. Assessment of similarity indices to quantify segmentation accuracy of scaffold images for tissue engineering. Page 1636 *in* J. M. Fitzpatrick and J. M. Reinhardt, editors. Proc. SPIE. San Diego, CA.

Rajala, T., S. C. Olhede, and D. J. Murrell. 2019. When do we have the power to detect biological interactions in spatial point patterns? Journal of Ecology 107:711–721.

Rao, C. R. 1995. A review of canonical coordinates and an alternative to correspondence analysis using Hellinger distance. Qüestiió 19:23–63.

Roxburgh, S. H., and P. Chesson. 1998. A new method for detecting species associations with spatially autocorrelated data. Ecology 79:2180–2192.

Růžička, M. 1958. Anwendung mathematisch statisticher Methoden in der Geobotanik (synthetische Bearbeitung von Aufnahmen). Biologia, Bratislava 13:647–661.

Sanderson, J. G., and S. L. Pimm. 2015. Patterns in nature: the analysis of species co-occurrences. The University of Chicago Press, Chicago, IL.

Scheiner, S. M. 2003. Six types of species-area curves. Global Ecology and Biogeography 12:441–447.

Schluter, D. 1984. A variance test for detecting species associations, with some example applications. Ecology 65:998–1005.

Seabloom, E. W., O. N. Bjørnstad, B. M. Bolker, and O. J. Reichman. 2005. Spatial signature of environmental heterogeneity, dispersal, and compatition in successional grasslands. Ecological Monographs 75:199–214.

Segurado, P., W. E. Kunin, A. F. Filipe, and M. B. Araújo. 2012. Patterns of coexistence of two species of freshwater turtles are affected by spatial scale. Basic and Applied Ecology 13:371–379.

Shimatani, K. 2001. Multivariate point processes and spatial variation of species diversity. Forest Ecology and Management 142:215–229.

Shimatani, K., and Y. Kubota. 2004. Quantitative assessment of multispecies spatial pattern with high species diversity: Multispecies spatial pattern. Ecological Research 19:149–163.

Simberloff, D., and E. F. Connor. 1979. Q-mode and R-mode analyses of biogeographic distributions: null hypotheses based on random colonization. Pages 123–138 *in* P. P. Ganapati and M. L. Rosenzweig, editors. Contemporary quantitative ecology and related ecometrics. International Cooperative Pub. House, Fairland, MD.

Simpson, E. H. 1949. Measurement of diversity. Nature 163:688–688.

Šizling, A. L., W. E. Kunin, E. Šizlingová, J. Reif, and D. Storch. 2011. Between geometry and biology: the problem of universality of the species-area relationship. The American Naturalist 178:602–611.

Šizling, A. L., and D. Storch. 2004. Power-law species-area relationships and self similar species distributions within finite areas. Ecology Letters 7:60–68.

Šmilauer, P., and J. Lepš. 2014. Multivariate analysis of ecological data using Canoco 5. 2. ed. Cambridge University Press, Cambridge.

Sokal, R. R., and C. D. Michener. 1958. A statistical method for evaluating systematic relationships. The

University of Kansas Scientific Bulletin 38:1409–1438.

Sørensen, T. 1948. A method of establishing groups of equal amplitude in plant sociology based on similarity of species content and its application to analyses of the vegetation on danish commons. Biologiske Skrifter/Kongelige Danske Videnskabernes Selskab 5:1–34.

Stone, L. 2016. The Google matrix controls the stability of structured ecological and biological networks. Nature Communications 7:12857.

Stone, L., and A. Roberts. 1990. The checkerboard score and species distributions. Oecologia 85:74–79.

Stone, L., and A. Roberts. 1992. Competitive exclusion, or species aggregation?: An aid in deciding. Oecologia 91:419–424.

Storch, D. 2016. The theory of the nested species–area relationship: geometric foundations of biodiversity scaling. Journal of Vegetation Science 27:880–891.

Storch, D., A. L. Šizling, J. Reif, J. Polechová, E. Šizlingová, and K. J. Gaston. 2008. The quest for a null model for macroecological patterns: geometry of species distributions at multiple spatial scales. Ecology Letters 11:771–784.

Stoyan, D., and H. Stoyan. 1994. Fractals, random shapes and point fields. Methods of geometrical statistics. John Wiley & Sons, Chichester.

Thurman, L. L., A. K. Barner, T. S. Garcia, and T. Chestnut. 2019. Testing the link between species interactions and species co occurrence in a trophic network. Ecography 42:1658–1670.

Tóth, A. B., S. K. Lyons, W. A. Barr, A. K. Behrensmeyer, J. L. Blois, R. Bobe, M. Davis, A. Du, J. T. Eronen, J. T. Faith, D. Fraser, N. J. Gotelli, G. R. Graves, A. M. Jukar, J. H. Miller, S. Pineda-Munoz, L. C. Soul, A. Villaseñor, and J. Alroy. 2019. Reorganization of surviving mammal communities after the end-Pleistocene megafaunal extinction. Science 365:1305–1308.

Ulrich, W., M. Almeida-Neto, and N. J. Gotelli. 2009. A consumer’s guide to nestedness analysis. Oikos 118:3–17.

Ulrich, W., A. Baselga, B. Kusumoto, T. Shiono, H. Tuomisto, and Y. Kubota. 2017. The tangled link between β- and γ-diversity: a Narcissus effect weakens statistical inferences in null model analyses of diversity patterns. Global Ecology and Biogeography 26:1–5.

Ulrich, W., and N. J. Gotelli. 2010. Null model analysis of species associations using abundance data. Ecology 91:3384–3397.

Ulrich, W., and N. J. Gotelli. 2013. Pattern detection in null model analysis. Oikos 122:2–18.

Ulrich, W., Y. Kubota, B. Kusumoto, A. Baselga, H. Tuomisto, and N. J. Gotelli. 2018. Species richness correlates of raw and standardized co-occurrence metrics. Global Ecology and Biogeography 27:395–399.

Veech, J. A. 2013. A probabilistic model for analysing species co-occurrence. Global Ecology and Biogeography 22:252–260.

Velázquez, E., I. Martínez, S. Getzin, K. A. Moloney, and T. Wiegand. 2016. An evaluation of the state of spatial point pattern analysis in ecology. Ecography 39:1042–1055.

Volkov, I., J. R. Banavar, S. P. Hubbell, and A. Maritan. 2009. Inferring species interactions in tropical forests. Proceedings of the National Academy of Sciences 106:13854–13859.

Wagner, H. H. 2003. Spatial covariance in plant communities: integrating ordination, geostatistics, and variance testing. Ecology 84:1045–1057.

Wagner, H. H., and M.-J. Fortin. 2005. Spatial analysis of landscapes: concepts and statistics. Ecology 86:1975–1987.

Wagner, H. H., R. Holderegger, S. Werth, F. Gugerli, S. E. Hoebee, and C. Scheidegger. 2005. Variogram analysis of the spatial genetic structure of continuous populations using multilocus microsatellite data. Genetics 169:1739–1752.

Wang, X., T. Wiegand, Z. Hao, B. Li, J. Ye, and F. Lin. 2010. Species associations in an old-growth temperate forest in north-eastern China. Journal of Ecology 98:674–686.

Warton, D. I., F. G. Blanchet, R. B. O’Hara, O. Ovaskainen, S. Taskinen, S. C. Walker, and F. K. C. Hui. 2015. So many variables: Joint modeling in community ecology. Trends in Ecology & Evolution 30:766–779.

Weinstein, B. G., C. H. Graham, and J. L. Parra. 2017. The role of environment, dispersal and competition in explaining reduced co-occurrence among related species. PLoS ONE 12:e0185493.

Whittaker, R. H. 1960. Vegetation of the Siskiyou Mountains, Oregon and California. Ecological Monographs

30:279–338.

Wiegand, T., C. V. S. Gunatilleke, I. A. U. N. Gunatilleke, and A. Huth. 2007a. How individual species structure diversity in tropical forests. Proceedings of the National Academy of Sciences 104:19029–19033.

Wiegand, T., S. Gunatilleke, and N. Gunatilleke. 2007b. Species associations in a heterogeneous Sri Lankan dipterocarp forest. The American Naturalist 170:E77–E95.

Wiegand, T., A. Huth, S. Getzin, X. Wang, Z. Hao, C. V. S. Gunatilleke, and I. A. U. N. Gunatilleke. 2012. Testing the independent species’ arrangement assertion made by theories of stochastic geometry of biodiversity. Proceedings of the Royal Society B: Biological Sciences 279:3312–3320.

Wiegand, T., and K. A. Moloney. 2004. Rings, circles, and null-models for point pattern analysis in ecology. Oikos 104:209–229.

Wiegand, T., and K. A. Moloney. 2014. Handbook of spatial point-pattern analysis in ecology. CRC Press, Boca Raton, FL.

Wiegand, T., J. Raventós, E. Mújica, E. González, and A. Bonet. 2013. Spatio-temporal analysis of the effects of hurricane Ivan on two contrasting epiphytic orchid species in Guanahacabibes, Cuba. Biotropica 45:441–449.

Wootton, J. T. 1997. Estimates and tests of per capita interaction strength: diet, abundance, and impact of intertidally foraging birds. Ecological Monographs 67:45–64.

WWF International. 2012. Living planet report 2012: Biodiversity, biocapacity and better choices. WWF International, Gland, Switzerland.

Zurell, D., L. J. Pollock, and W. Thuiller. 2018. Do joint species distribution models reliably detect interspecific interactions from co-occurrence data in homogenous environments? Ecography 41:1812–1819.

Table 1 Brief summary of processes that generate CSA and ISA, biodiversity patterns that are linked to CSA and ISA, and the approaches to measure them.

	Conspecific spatial aggregation (CSA)	**Interspecific spatial association (ISA)**
What generates it?	species-specific dispersal limitations, *conspecific interactions* among individuals, interplay between *niche width* and spatial aggregation of environmental conditions	differences in dispersal limitations among species, *interspecific interactions* among individuals, interplay between *niche overlap* and aggregation of environmental conditions
Which biodiversity facets and patterns are sensitive to it?	beta diversity and its distance decay, species-area relationships (SAR), endemics-area relationships (EAR), occupancy-area relationships (OAR), altitudinal and latitudinal gradients of range size and endemism, spatial gradients of diversity	still mostly unclear
Broad approaches to mesure it	beta diversity indices, local slope of SAR, EAR and OAR, species accumulation curves (MoB framework), mean range size, spatial Taylor's power law, spatial overdispersion	indices of association, community variograms, pair correlation functions, K-functions

Table 2 Measures of ISA for binary community data. From more than 70 measures (Keil 2019) we have included those that have either been popular, recommended based on suitable properties, or that represent a distinct approach to ISA. Notation for pairwise measures for the pairwise comparison of two species: *a* is the number of sites where both species co-occur, *c* and *b* are numbers of sites occupied uniquely by each species respectively, *d* is the number of sites where none of the species occur, and *n* is the total number of sites, i.e. $a + b + c + d$. Other notation is explained directly in the table.

Measure	**Reference**	**Symbol**	**Formula or description**	**R code in**
A) Pairwise				
C-score	(Stone and Roberts 1990)	C_{seg}	bc	bipartite::C.score EcoSimR::c_score vegan::designdist
Scaled C-score	(Ulrich and Gotelli 2013)	C_{segSc}	$\frac{bc}{n(n-1)/2}$	vegan::designdist
Togetherness	(Stone and Roberts 1992)	C_{tog}	ad	bipartite::togetherness vegan::designdist
Scaled togetherness	(Ulrich and Gotelli 2013)	C_{togSc}	$\frac{ad}{n(n-1)/2}$	vegan::designdist
Jaccard similarity	(Jaccard 1901)	C_{jacc}	$\frac{a}{a+b+c}$	betapart::betapart vegan::betadiver
Dice-Sorensen similarity	(Dice 1945, Sørensen 1948)	C_{sor}	$\frac{2a}{2a+b+c}$	betapart::beta.pair vegan::betadiver
Coefficient of association	(Forbes 1907)	C_{forbes}	$\frac{na}{(a+b)(a+c)}$	vegan::betadiver
Alroy's coefficient (a new take on C_{forbes})	(Alroy 2015)	C_{alroy}	$\frac{a(z+\sqrt{z})}{(a+b)(a+c)+a\sqrt{z}+\frac{bc}{2}}$ where $z=a+b+c$	vegan::designdist
Pearson tetrachoric correlation	(Pearson and Heron 1913)	C_{pears}	$\frac{ad-bc}{[(a+b)(c+d)(a+c)(b+d)]^{0.5}}$	vegan::designdist
Mid-P variant of Fisher's exact test	(Kallio et al. 2011, Tóth et al. 2019)	C_{FETmP}	$\left(\sum_{x_{min}\leq x\leq x_{max}} \frac{(i¦x)(n-i¦j-x)}{n¦j}\right)-\frac{(i¦a)(n-i¦b)}{2(n¦j)}$ Where $i = a+b$, $j = a+c$, and x is the possible number of overlaps, with $x_{min} = \max(a-d, 0)$ and $x_{max} = \min(i, j)$. The symbol ¦ denotes the 'choose' operation.	https://github.com/anikobtoth/FCW
Matching coefficient	(Sokal and Michener 1958)	C_{match}	$\frac{a+d}{n}$	vegan::designdist
B) Matrix-wise				
Whittaker index	(Whittaker 1960, Arita 2017)	C_w	This is the fill of the Y matrix, which is $\frac{\gamma}{\bar{\alpha}}=\frac{n}{\bar{n}}$ where $\bar{n}$ is the average number of occupied sites per species.	vegan::betadiver

Variance ratio	(Schluter 1984)	C_{ratio}	$S_T^2/\sum\sigma^2$, where S_T^2 is the variance of per-site species richness and σ^2 is the sum of per-species variances of incidences.	EcoSimR:v_ratio https://github.com/mcglinnlab/vario
Checker score	(Gotelli 2000)	$C_{checker}$	Number of species pairs forming perfect checkerboard distributions.	EcoSimR::checker
Number of unique species combinations	(Gotelli 2000)	C_{combo}	Number of species that always co-occur.	EcoSimR::species_combo
Network connectance	(Dormann et al. 2009)	C_{conn}	$F/\gamma n$, where F is the number of all co-occurrences	bipartite::networklevel

Table 3 Select measures of ISA for abundance data. From the phletora of existing measures have included those that have been popular, recommended, or that represent a distinct approach to ISA. Specifically, we selected three correlation-based indices, two distance-based indices, two abundance-based variants of the binary indices, and two matrix-wise indices. *x* and *y* are the vectors of abundance of two species, $\bar{x}$ and $\bar{y}$ are their means, x_+ and y_+ are their sums, σ_x and σ_y are their standard deviations, x_i and y_i are abundances at site *i*, and *n* is the total number of sites.

Measure	**Reference**	**Symbol**	**Formula or description**	**Notes**	**R code in**
A) Pairwise					
Covariance	(Legendre and Legendre 2012)	CA_{cov}, CA_{cov_hell}	$\frac{1}{n}\sum_{i=1}^{n}(x_i-\bar{x})(y_i-\bar{y})$	With optional transformation (Hellinger, log, sqrt) of raw abundances.	stats::cov
Pearson correlation (scaled covariance)	(Legendre and Legendre 2012)	CA_{cor}, CA_{cor_hell}	$\frac{CA_{cov}(xy)}{\sigma_x \sigma_y}$	With optional transformation (Hellinger, log, sqrt) of raw abundances.	stats::cor
Spearman’s Rho	(Legendre and Legendre 2012)	CA_{rho}	CA_{cor} between the rank values of *x* and *y*		stats::cor
Chi-squared distance	(Lebart and Fénelon 1971, Legendre and De Cáceres 2013)	CA_{chi}	$\sqrt{(x_+ + y_+)\sum_{i=1}^{n}\frac{1}{x_i+y_i}\left(\frac{x_i}{x_+}-\frac{y_i}{y_+}\right)^2}$		vegan::decostand with stats::dist
Hellinger distance	(Rao 1995, Legendre and De Cáceres 2013)	CA_{hell}	$\sqrt{\sum_{i=1}^{n}\left(\sqrt{\frac{x_i}{x_+}}-\sqrt{\frac{y_i}{y_+}}\right)^2}$		vegan::decostand with stats::dist
Percentage difference (former “Bray-Curtis” index)	(Odum 1950, Legendre and De Cáceres 2013)	CA_{bray}	$\frac{\sum_{i=1}^{n}\lvert x_i-y_i\rvert}{x_+ + y_+}$	One of the abundance-based variants of C_{sor}.	vegan::vegdist
Ruzicka similarity	(Růžička 1958, Oksanen et al. 2019)	CA_{ruz}	$\frac{2CA_{bray}}{1+CA_{bray}}$	One of the abundance-based variants of C_{jacc}.	vegan::vegdist
B) Matrix-wise					
N-wise Ruzicka	(Baselga 2017)	CA_{ruzN}	Long formula, see Baselga (2017).	Multi-species version of CA_{ruz}.	betapart::beta.multi .abund
Variance ratio	(Ulrich and Gotelli 2010)	CA_{ratio}	The same principle as the binomial C_{ratio}.		

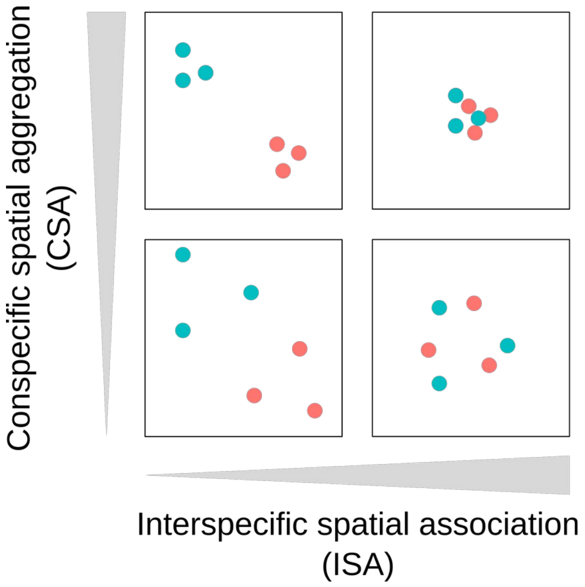


Figure 1 The difference between con-specific spatial association (CSA, vertical gradient) and inter-specific association (ISA, horizontal gradient), where the latter is the subject of this paper. Points are individuals, colours mark species.

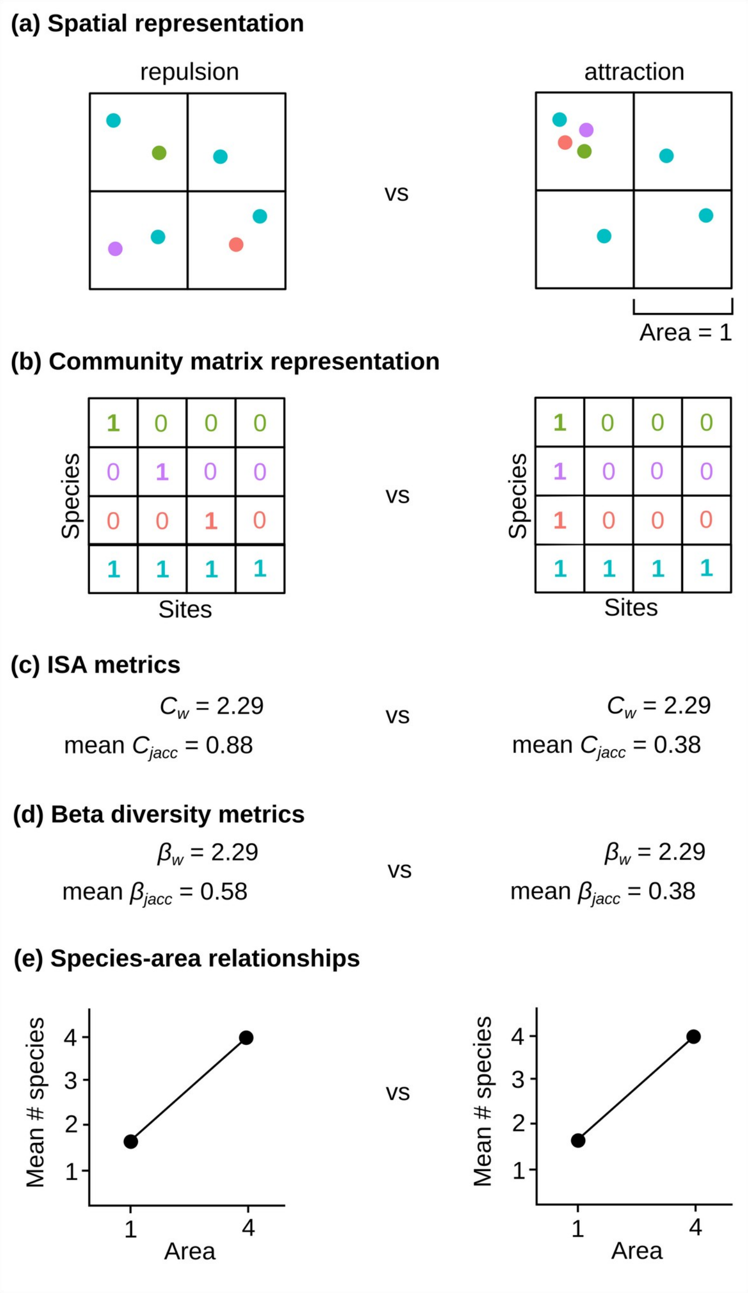


Figure 2 Insensitivity of species-area relationships and Whittaker's index to ISA. (a) Two communities consist of 4 square sites each, with 4 species (colours). The communities differ in the magnitude of pairwise ISA; species in the left one are segregated, those in the right one are attracted to each other. (b) These communities can be described by spatially implicit community matrices Y from which metrics of ISA (c) can be calculated. While the C_w metric remains constant in both communities, the mean pairwise C_{jacc} correctly reflects ISA. (d) The same logic applies when beta diversity measures such as β_w and β_{jacc}, where the former is simply a ratio of gamma diversity (total richness of a community) and mean alpha diversity (mean richness across sites), which is also the slope of slope of SAR, which remains constant even though ISA changes (e). Beware that β_w and C_w are the same thing (Arita 2017), which severely limits their interpretation as either beta diversity or ISA metric.

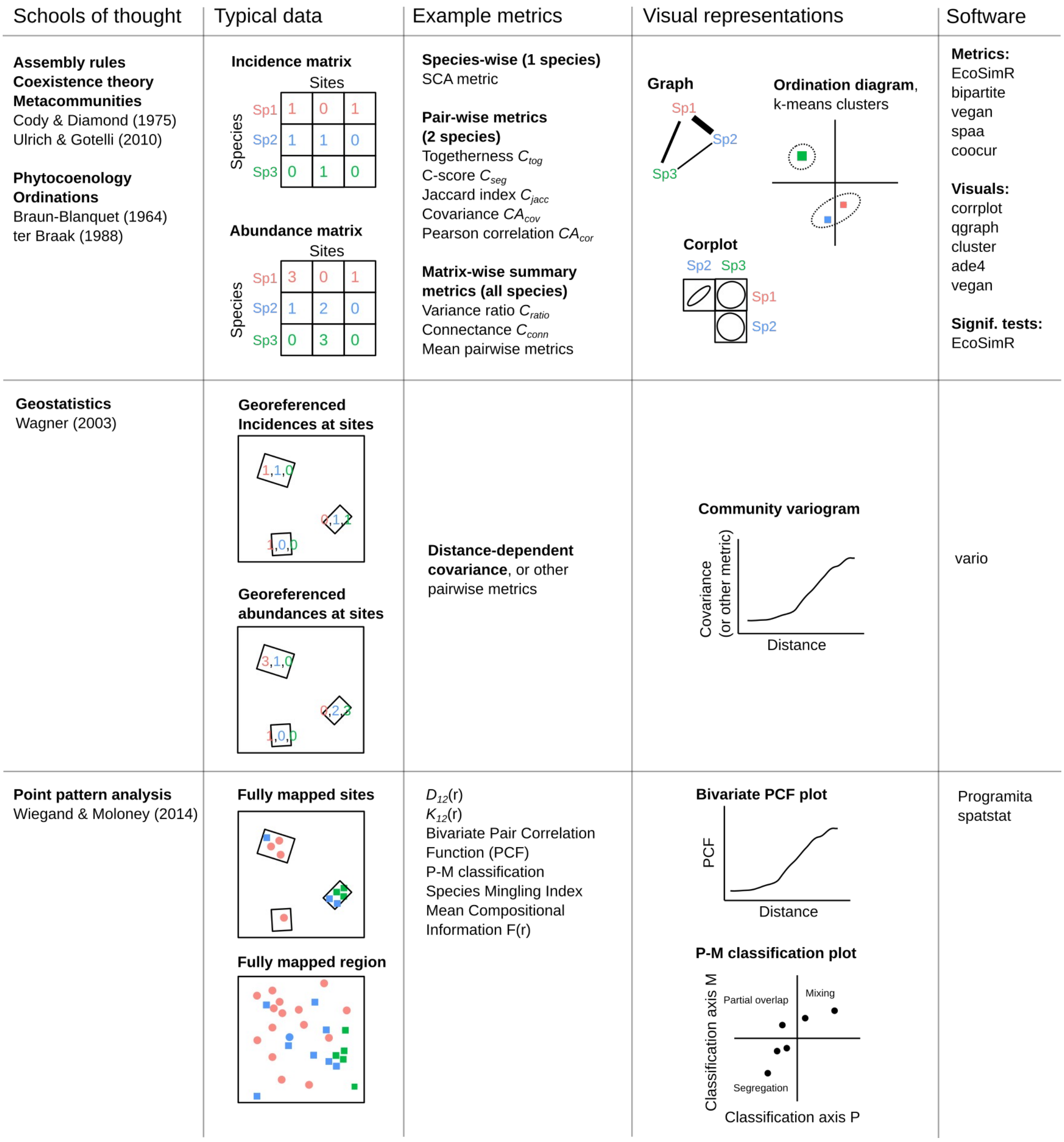


Figure 3 Approaches to capturing inter-specific spatial association (ISA) among species, classified by the broad ecological schools of thought, together with typical data that are used in the approaches. Note that there is a gradient of the amount of information/detail in the data. With the exception of Programita, all the listed software consists of R packages.

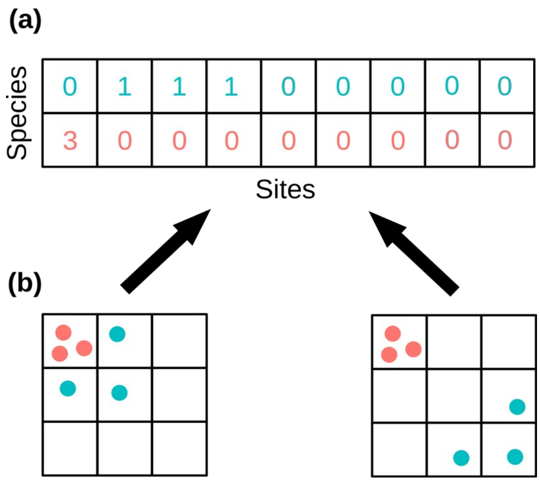
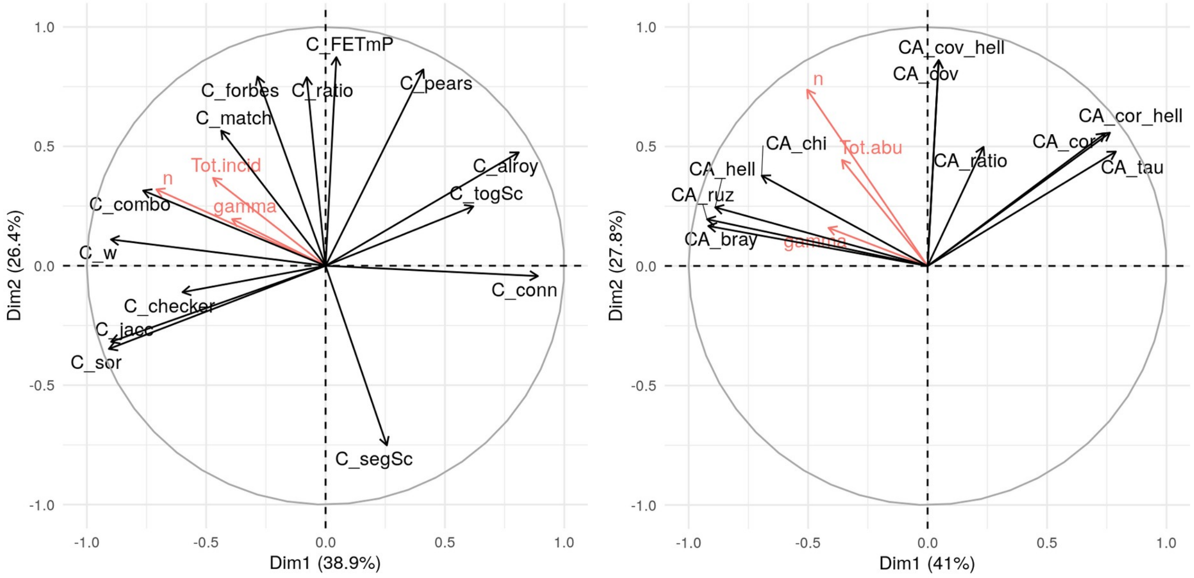
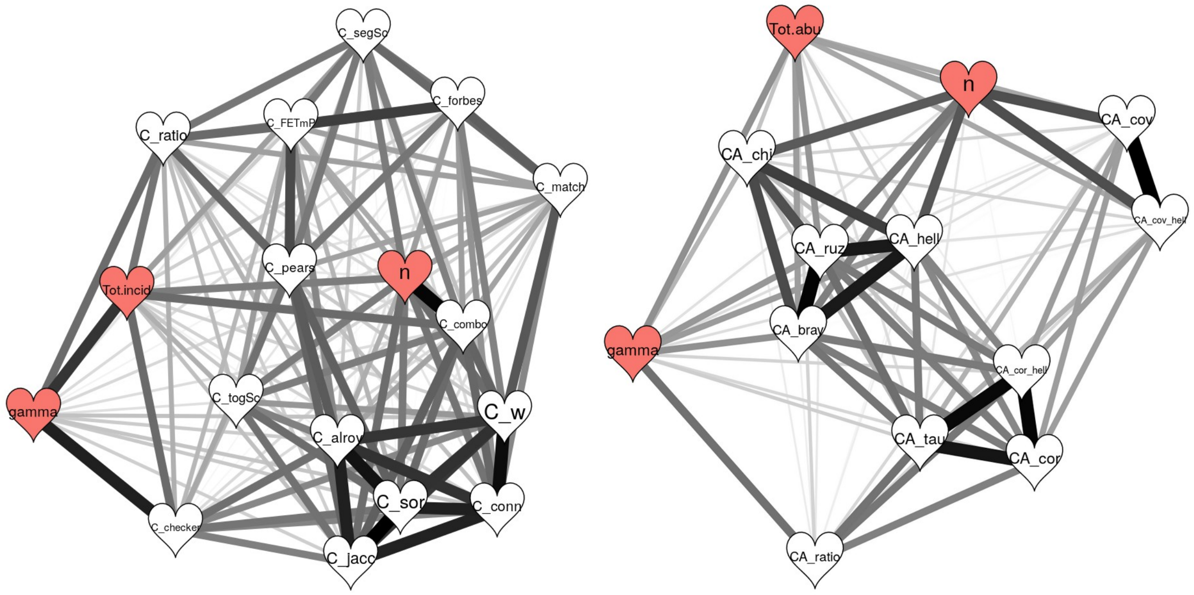


Figure 4 The fundamental importance of spatial distance in ISA. A single community matrix (a) reflects two different spatial arrangements of the community (b), each with a different magnitude of ISA - the two species in the left spatial matrix are more attracted, while on the right they are more segregated, yet this is not in any way reflected by the community matrix.



(a) Binary data - PCA

(b) Abundance data - PCA



(c) Binary data - graph

(d) Abundance data - graph

Figure 5 Comparison of ISA metrics calculated on empirical community matrices of Atmar and Patterson (1995) and Ulrich and Gotelli (2010). Panels (a-b) use the binary measures from Table 2, and are based on binary (presence/absence) version of all matrices. Panels (c-d) use the abundance-based measures of Table 3, and use only the abundance matrices of Ulrich and Gotelli (2010). Panels (a-b) show first two axes of principal components analysis (PCA), panels (c-d) show a graph representations of correlation matrices between the metrics. Red indicates variables that are not ISA metrics.

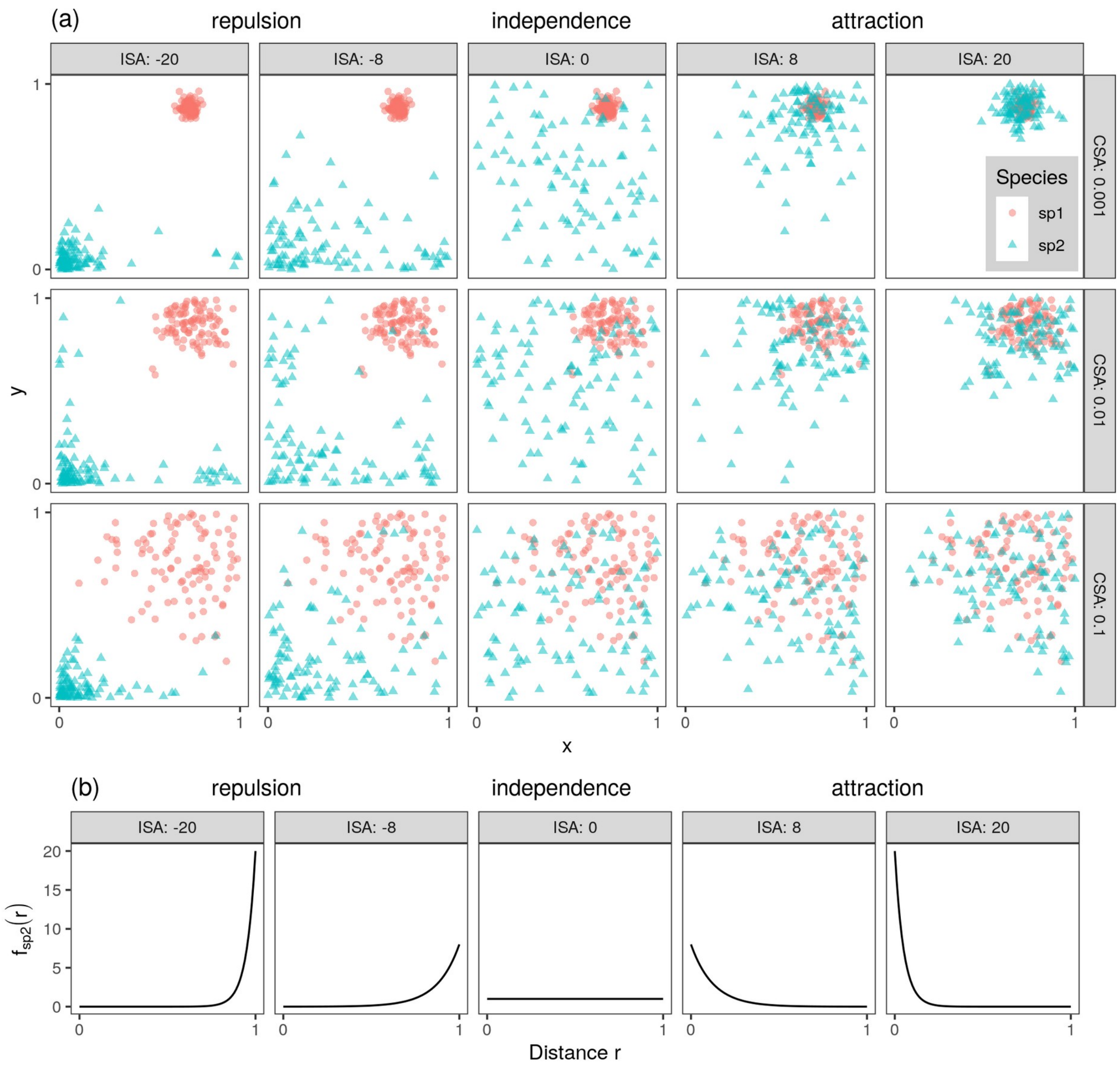


Figure 6 (a) Simulated spatial distributions of individuals (points) of two species (sp1 and sp2) in a square domain under 3 levels of con-specific aggregation (CSA) of sp1 and 5 levels of inter-specific aggregation (ISA). (b) Truncated exponential probability density function [$f_{sp2}(r)$ (Keil 2014)] describes how likely is to observe an individual of sp2 at a given distance from any individual of sp1. This $f_{sp2}(r)$ is convenient since its shape depends on a single parameter (here called ISA) which represents various magnitudes of inter-specific repulsion (left) and attraction (right) and their relationship with distance. Similar figure was also used in Keil (2019).

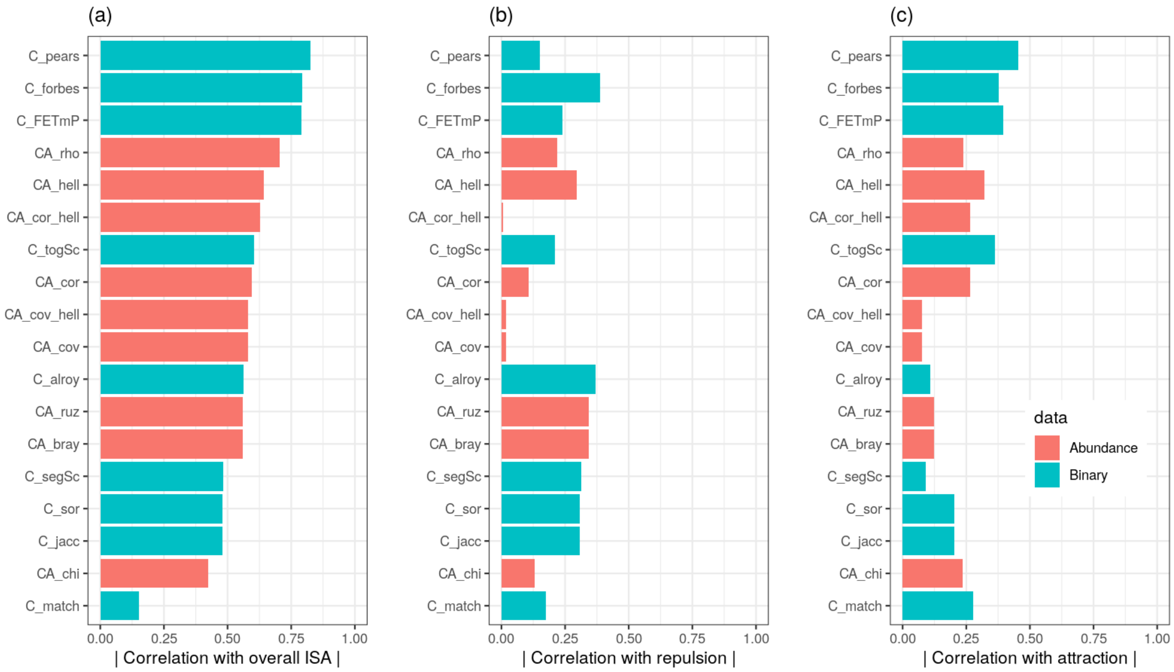
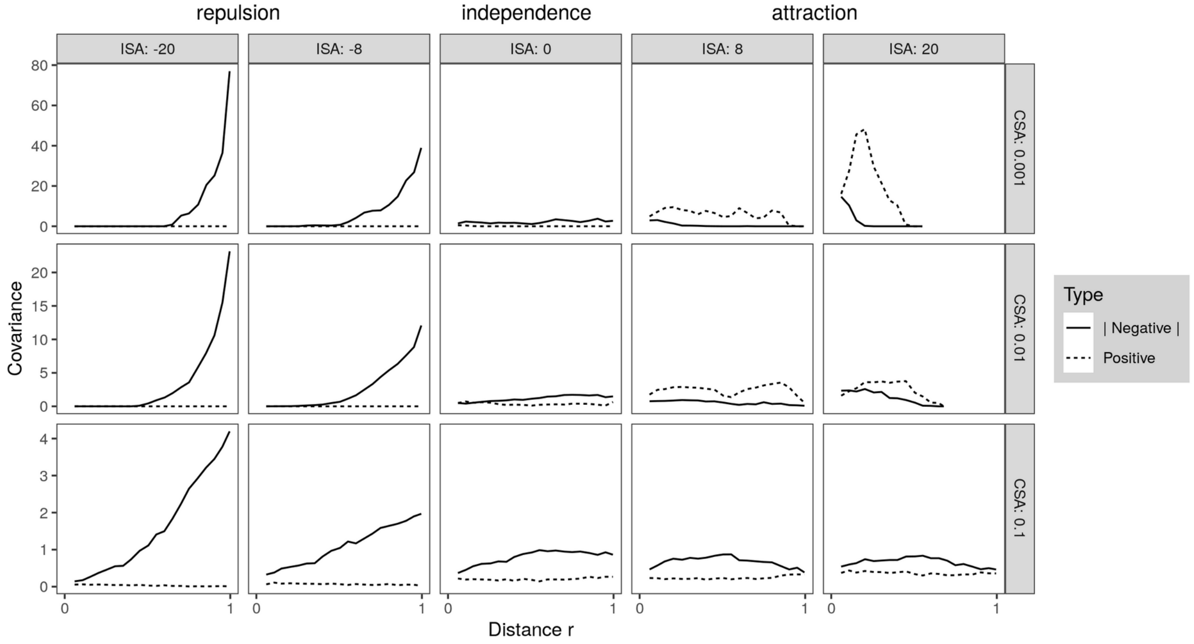


Figure 7 Ability of pairwise spatially implicit metrics from Table 1 and 2 to recover the magnitude of ISA from spatially explicit simulations. Panel (a) shows overall correlation between the true ISA and the metric. Panels (b) and (c) show the correlation when only inter-specific repulsion is considered (ISA < 0) or when only positive attraction is considered (ISA > 0). Given are absolute values of Spearman correlation coefficient. Note that some metrics very well separate negative from positive associations (i.e. they have good correlation with overall ISA), but within these two categories they have weak correlation with the underlaying ISA parameter, e.g. covariance-based measures (CA_{cov}, CA_{cov_hell}). See Fig. S5 for details of these relationships.

(a) Community variograms



(b) Bivariate pair correlation functions

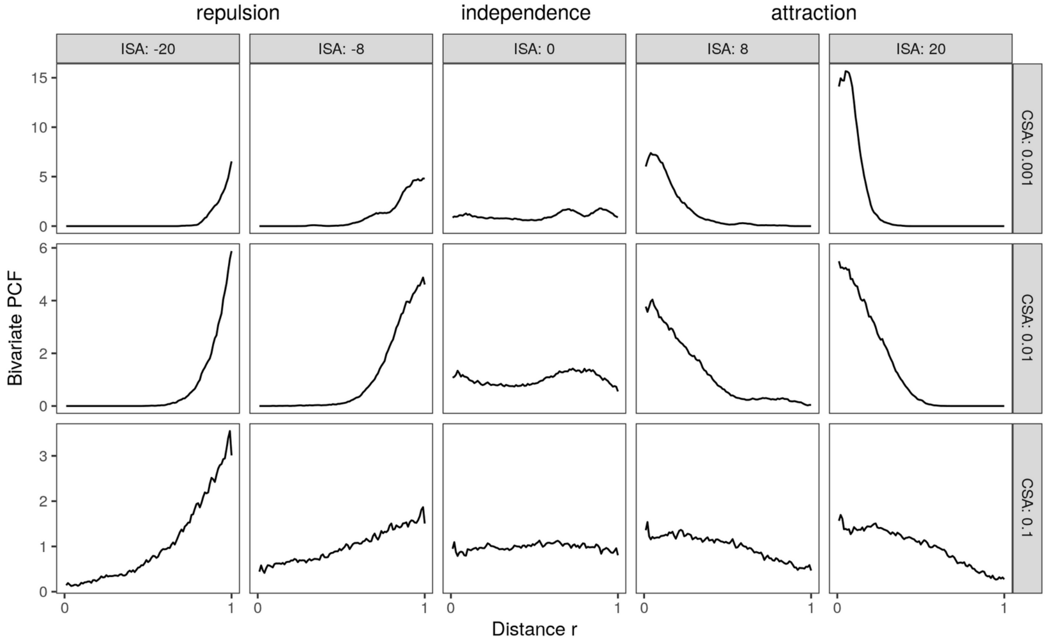


Figure 8. Ability of two spatially explicit approaches to recover ISA patterns of the two species from Figure 4. (a) Community variograms calculated on abundance data obtained by aggregation of the point pattern in a 20 x 20 pixel grid. Also, 200 points were simulated for each species, instead of the 100 points in Fig. 4. (b) Bivariate pair correlation function calculated directly from the point patterns.

Supplementary material

This is supplementary material for manuscript "*Interspecific spatial associations as a facet of biodiversity, and a review of approaches to measure them*" by Petr Keil, Thorsten Wiegand, Anikó B. Tóth, Daniel McGlinn, and Jonathan M. Chase.

Appendix S1 - Glossary

Several attempts have been made to clarify the terminology of spatial associations and resemblance in the sites vs. species context (Hubálek 1982, Legendre and Legendre 2012), but the consensus is not entirely clear and many of the offered definitions are author- or study-specific. Here we provide what we see as rough signal across the literature that we have reviewed:

- **Inter-specific** and **hetero-specific** are used synonymously for relationships among individuals of two or more species.
- **Intra-specific** and **con-specific** are used synonymously for relationships among individuals within a single species.
- **Association** is a general term that describes any of mutual arrangement of spatial positions of two or more species, irrespectively to its direction, range of values, or particular interpretation.
- **Resemblance** is more general than association. Legendre and Legendre (2012) use association for relationships among species, while resemblance is the more general term applicable to both beta diversity among sites and association among species.

- **Co-occurrence** can be used for an occurrence of two or more species at a given site, or it can be used for a magnitude of the observed inter-specific association. It is usually reserved for binary (presence/absence) data. Co-occurrences should not be confused with biotic interactions.
- **Similarity** (and its mathematical complement, **dissimilarity**) are measures of resemblance that always take values between 0 and 1, are not metric, but can be converted/re-scaled to metric distances.
- **Distances** are measures of resemblance that have a minimum at 0, but can have various upper bounds (including infinity), depending on the constraints of the space in which they are measured.
- **Correlation-based metrics of resemblance** are centered around 0 (no relationship), with values > 0 for positive relationship, and < 0 for negative relationship. They can be converted/re-scaled to take values between 0 and 1.
- **Repulsion or segregation vs attraction or aggregation**. These usually refer to negative vs positive spatial associations among species, but they are often also used for con-specific patterns. A typical example is aggregation, which is often used for spatial clumping among conspecific individuals.
- **Biotic interaction** or **inter-specific interaction** is an effect of individuals of one species on individuals of other species (it can also be mutual). Examples are predation, mutualism, parasitism, or competition. Interactions do influence interspecific spatial associations, but usually together with other factors.

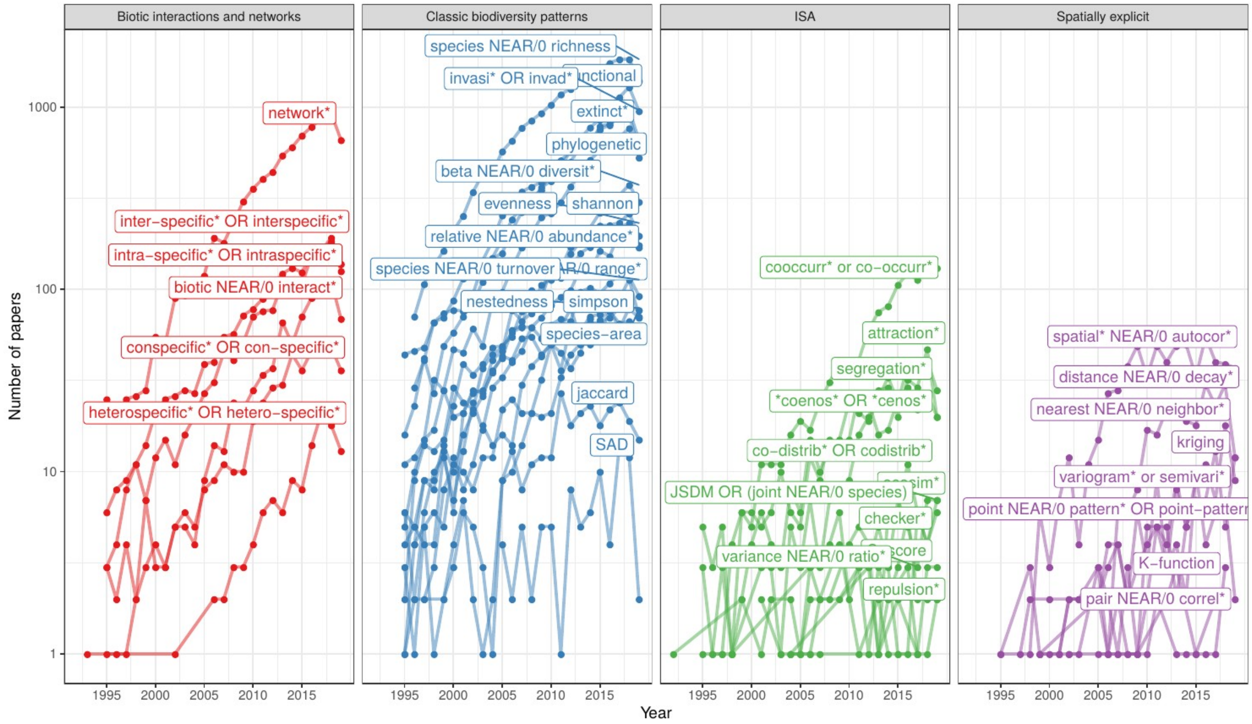


Figure S1 Temporal trends in sub-fields of biodiversity science. Y-axis shows the total number of papers published in a given year that are listed on Clarivate Web of Science, and that contain the search given term AND biodiversity. Note the $\log_{10}$ scale of the y-axis. Left plot (red lines) shows terms related to general notion of association and interaction among species. Second to the left plot (blue lines) shows terms associated with classical biodiversity patterns in space and time. Second to the right plot (green lines) shows terms specifically related to ISA. Right plot (purple lines) shows terms related to spatially explicit aspects of biodiversity. The search was done on 19 Sep 2019. The data and code for this plot are available from the online repository at https://github.com/petrkeil/spasm.

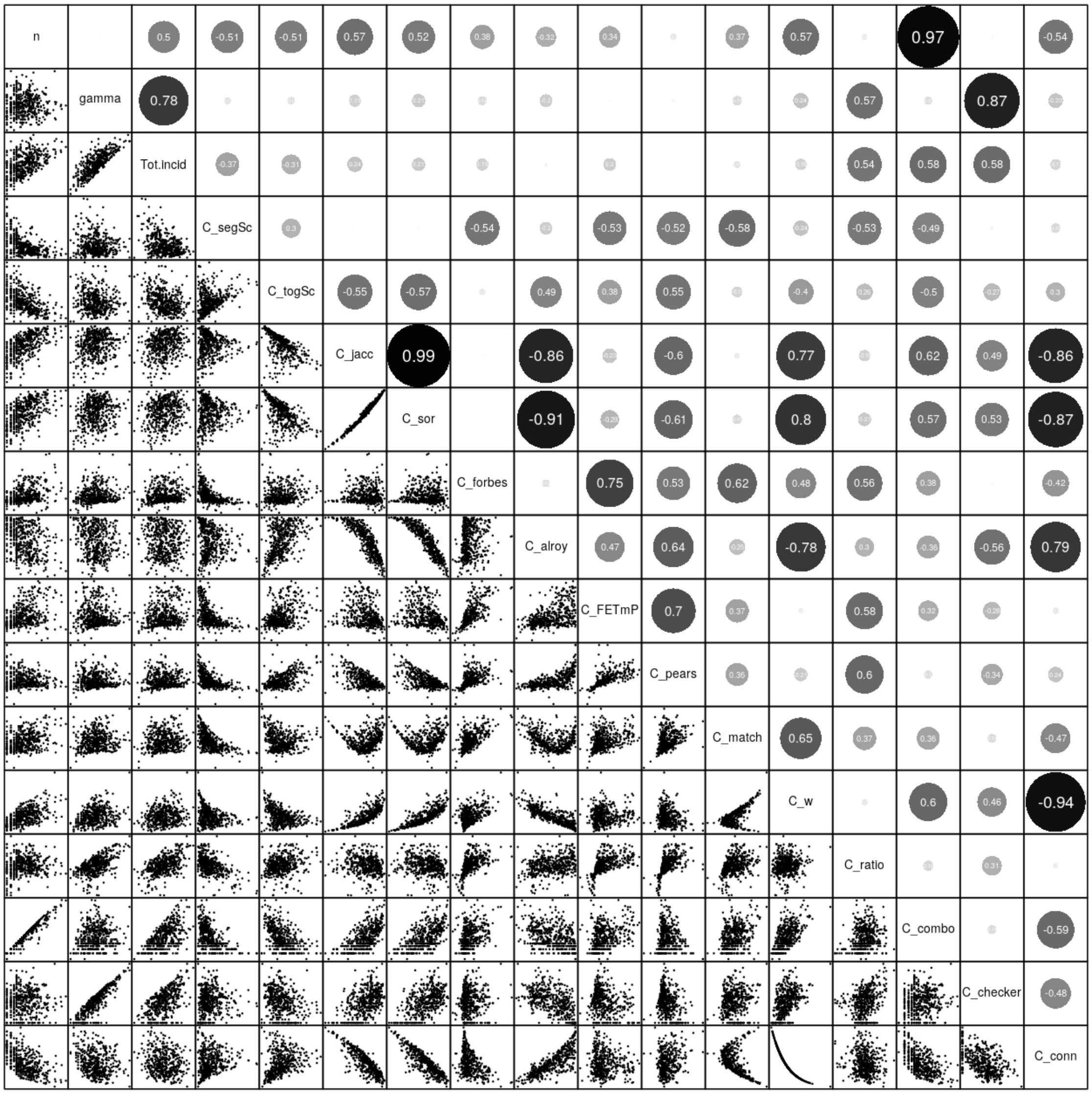


Figure S2 Correlations between ISA metrics for binary data from Table 2 applied to the empirical community matrices by Atmar and Patterson (1995) and Ulrich and Gotelli (2010). In case of the pairwise metrics, each point represents the mean value of the entire matrix. *N*, Gamma, and Tot.incid are numbers of sites, species, and incidences (i.e. matrix fill) respectively. C_{forbes}, C_{w}, C_{combo}, C_{ratio}, *n*, gamma, and Tot.incid were were log-transformed. C_{segSc}, C_{togSc}, $C_{checker}$, were log(x+1) transformed. Numbers in the upper triangular section are Pearson correlations, with size and grey intensity proportional to the magnitude of the correlation.

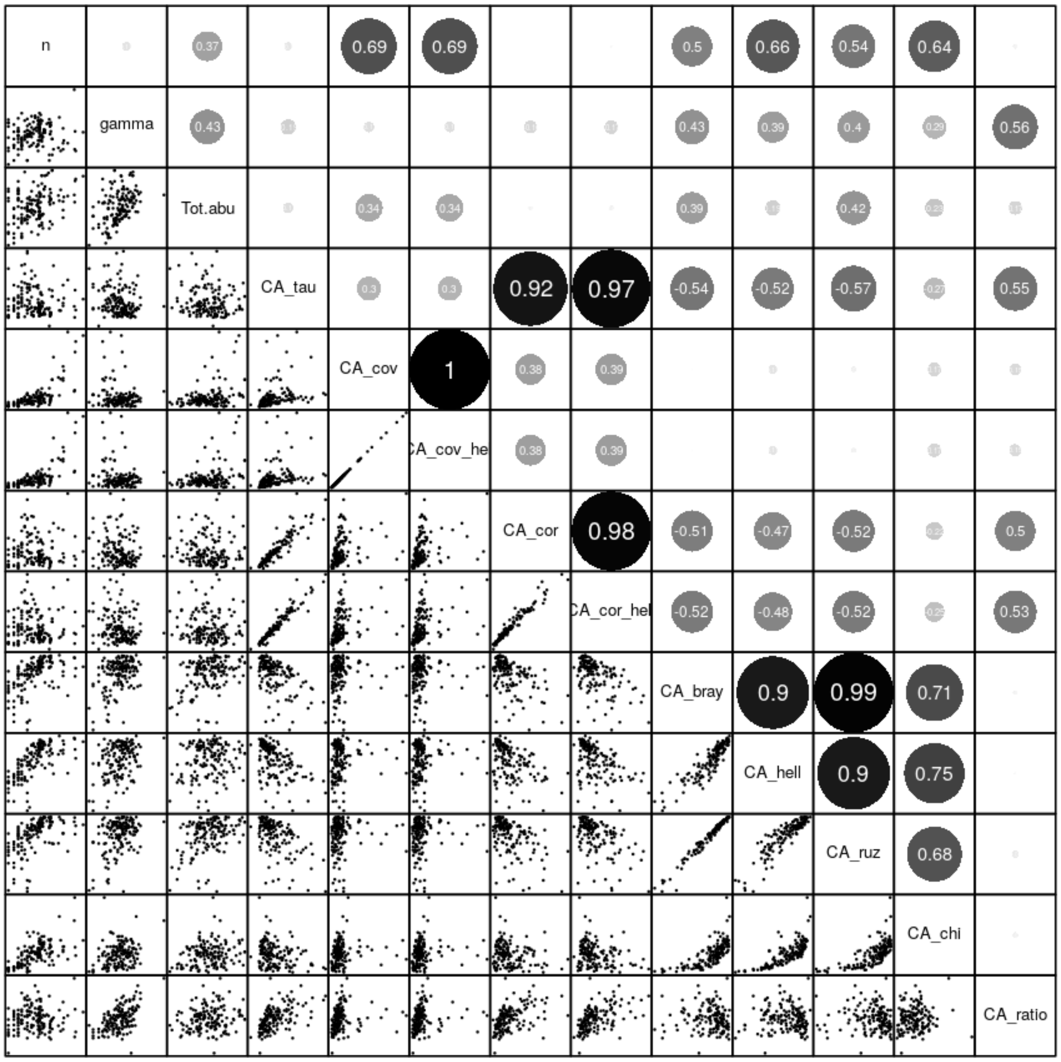


Figure S3 Correlations between ISA metrics for abundance data from Table 2 applied to the empirical community matrices by Ulrich and Gotelli (2010). In case of the pairwise metrics, each point represents a mean value of the entire matrix. *n*, gamma, and Tot.abu are numbers of sites, species, and the total number of individuals in the entire community matrix respectively. CA_{ratio}, CA_{cov}, CA_{cov_hell}, *n*, gamma, and Tot.abu were were log-transformed. Numbers in the upper triangular section are Pearson correlations, with size and grey intensity proportional to the magnitude of the correlation.

Appendix S2 - Exact simulation procedure

Why only pairs? We refrained from simulating spatially explicit spatial association between more than two species, since the simulated patterns would need to achieve specified spatial association between each pair of species simultaneously, as well as the decay (or increase) of each of these associations with distance. Although the most recent advances in multivariate geostatistics may enable this (Genton and Kleiber 2015), these methods are still young, not widely available, and are computationally challenging to simulate cross-covariance structures of more than 3 layers (species). Further, there is the limit on the magnitude of negative associations in multi-species matrices (Brown et al. 2004) which would prevent us from exploring scenarios with strong negative ISA. As a result, we only evaluated pairwise measures of ISA. For each ISA metric we measured its correlation with the ISA parameter across all simulation setting.

Simulations. We simulated spatially explicit distributions of two species, sp1 and sp2 with abundances N1 and N2 respectively, as two point patterns in a square domain with side of 1 (Fig. 6a in the main text). One simulation proceeded as follows:

- (Fig. S4a) We chose a random point with coordinates μ_x and μ_y within the domain, with uniform probability density across the domain; this point was the center of distribution of sp1.
- (Fig. S4b) We created 2-dimensional probability density of points of sp1 as a bivariate normal distribution $f_{sp1}(\mu, \Sigma)$, where Σ is the covariance matrix with marginal variances $\sigma_x = \sigma_y = CSA$ and with covariance $\sigma_{xy} = 0$. μ is the vector of coordinates μ_x and μ_y.
- (Fig. S4c) We drew N1 points from that probability density surface. These are individuals of species 1.
- (Fig. S4d) For every location in the domain we calculated its distance *r* from the nearest point of sp1.
- (Fig. S4e) We transformed *r* using a truncated exponential function (Keil 2014) $f_{sp2}(r) = \frac{\alpha e^{\alpha r}}{e^{\alpha b} - e^{\alpha a}}$ where $r \in [a, b]$, where $a = 0$ and $b = 1$, but these truncation points can be set to any value depending on the size of the domain. We use the negative value of parameter α as the strength of interspecific association ($ISA = -\alpha$), with $ISA < 0$ being segregation, $ISA = 0$ for independence, and $ISA > 0$ being positive attraction between species.
- (Fig. 4b) We drew N2 points from from the $f_{sp2}(r)$. These are individuals of species 2.

We repeated this procedure for each combination of the following parameter values: $CSA \in \{0.001, 0.01, 0.1\}$, $N1 \in \{10, 100, 1000, 10000\}$, $N2 \in \{10, 100, 1000, 10000\}$, and for $ISA \in \{-20, -17.5, -15, \ldots, 0, \ldots, 15, 17.5, 20\}$, which we then aggregated to square spatial grids with $\{32, 16, 8, 4\}$ grid cells along each side. Thus, the value of 32 represents the finest (smallest) grain, and 4 the coarsest (largest) grain. Altogether, this produced 3264 simulated pairs of species, each represented by either a point pattern or a grid, and we further converted to 3264 community matrices Y, with either abundance or incidence values, and with known exact spatial position of each grid cell.

Analysis of the simulations. For each of the simulated community matrices Y (each with 2 species) we calculated the pair-wise incidence-based ISA measures from Table 2, and the abundance-based measures from Table 3. In some of the measures we also tested their Z-score variant, calculated as s $(E_{raw} - E_{\exp})/SD_{\exp}$, where E_{raw} is the ISA metric (from Tables 2 and 3) calculated on observed data, $E_{\exp}$ is the null expectation of the index, and $SD_{\exp}$ is the standard deviation of the null expectation calculated over a set of community matrices obtained by 200 randomizations of Y. The randomizations were as follows: For the incidence matrices, we took the total number of incidences of each species, randomly assigned each of the incidence to a new site with uniform probabilities for each site, which corresponds to the “sim2” algorithm of EcoSimR package (Gotelli et al. 2015). For the abundance-based measures we randomly re-assigned each individual to a new site with uniform probability. Thus, in both algorithms, incidences or individuals are reshuffled only within each row of Y, irrespectively to the matrix column totals. In case of the abundance data, we also tried to run the simulations with the IT algorithm of Ulrich and Gotelli (2010), but the results were similar. To evaluate performance of each metric, we calculated its Spearman’s Rho (rank-rank correlation) with the ISA parameter across all 4,080 simulations. All functions and scripts for the simulations and their analysis are provided at https://github.com/petrkeil/spasm.

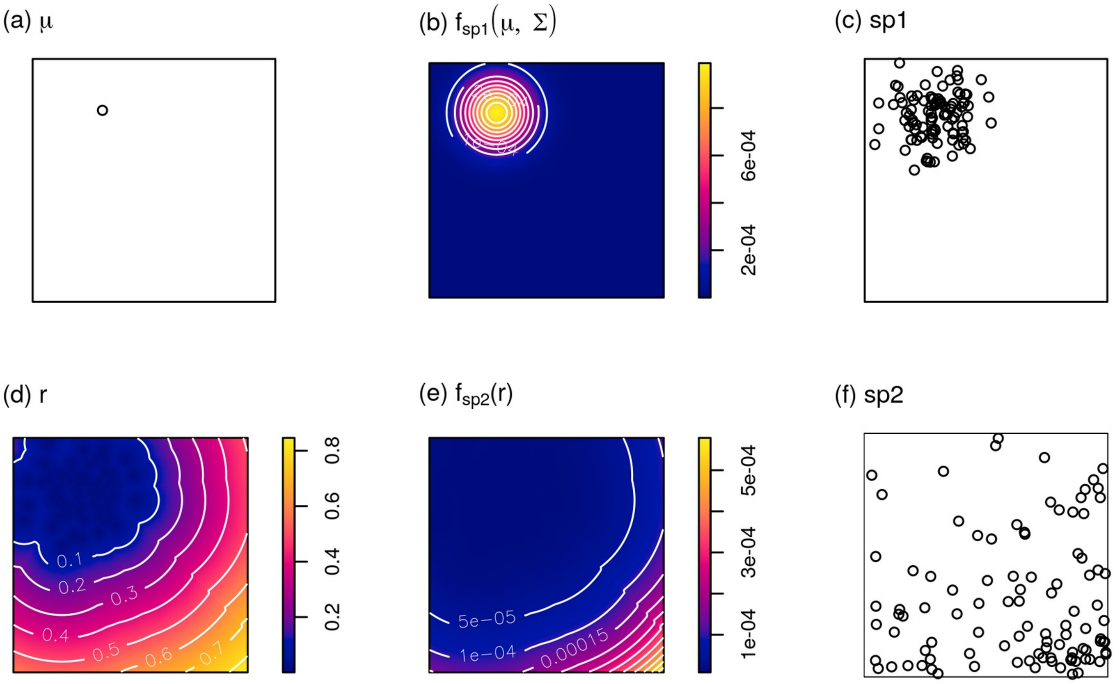


Figure S4 Simulation of two point patterns of two species, sp1 and sp2. The procedure is described in the main text. Briefly, point pattern of sp1 is simulated as a point process with bivariate normal probability density $f_{sp1}(\mu, \Sigma)$ with zero covariance and marginal variances describing the con-specific aggregation (CSA) of sp1. Point pattern of sp2 is then simulated as a point process with $f_{sp2}(r)$ describing the magnitude of ISA, where r is distance to the nearest point of sp1. This figure was also used in Keil (2019).

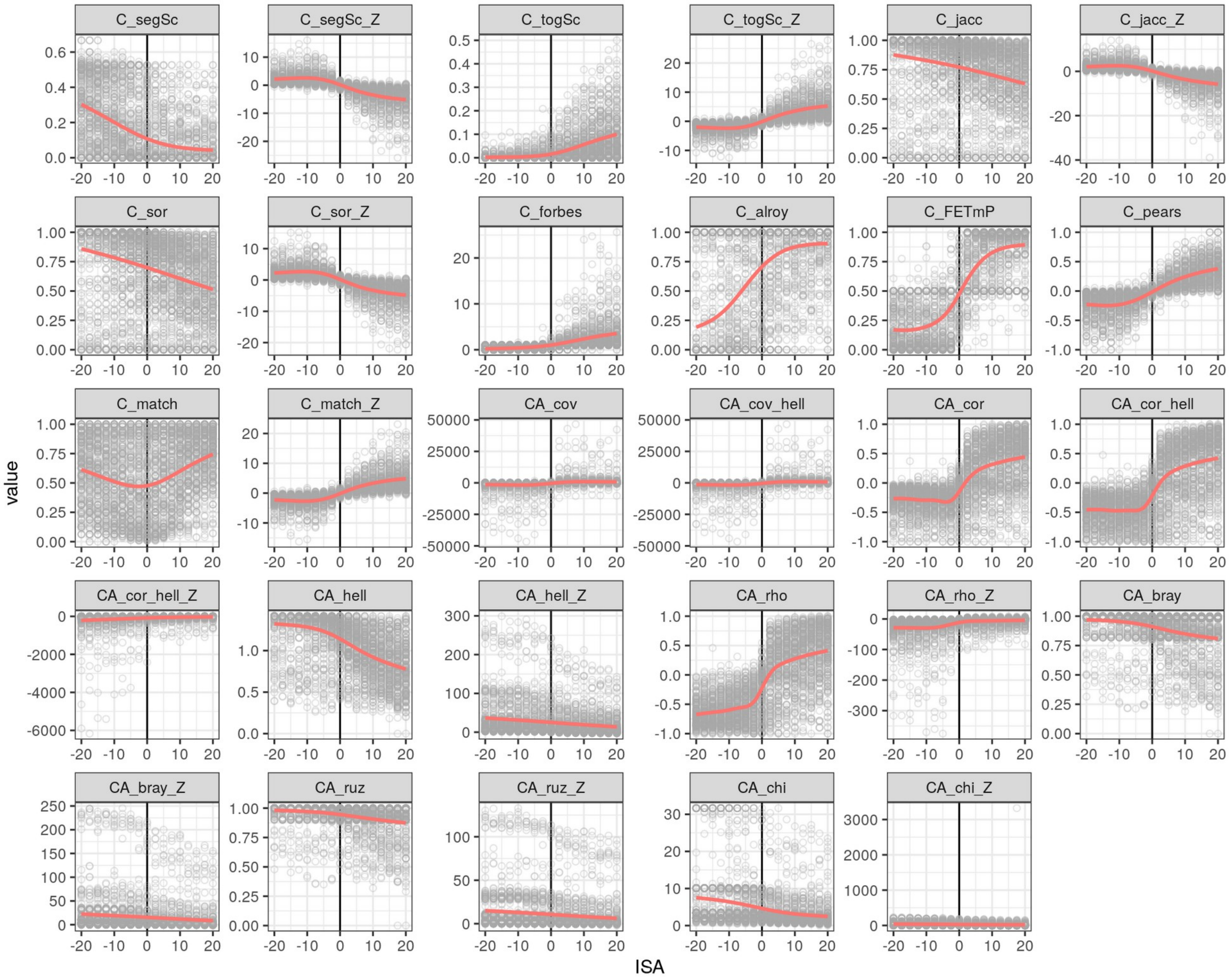


Figure S5 Relationships between the magnitude of ISA in artificial 2-species point pattern communities, and indices (measures) of ISA. These plots are the basis for Figure 7 in the main text.

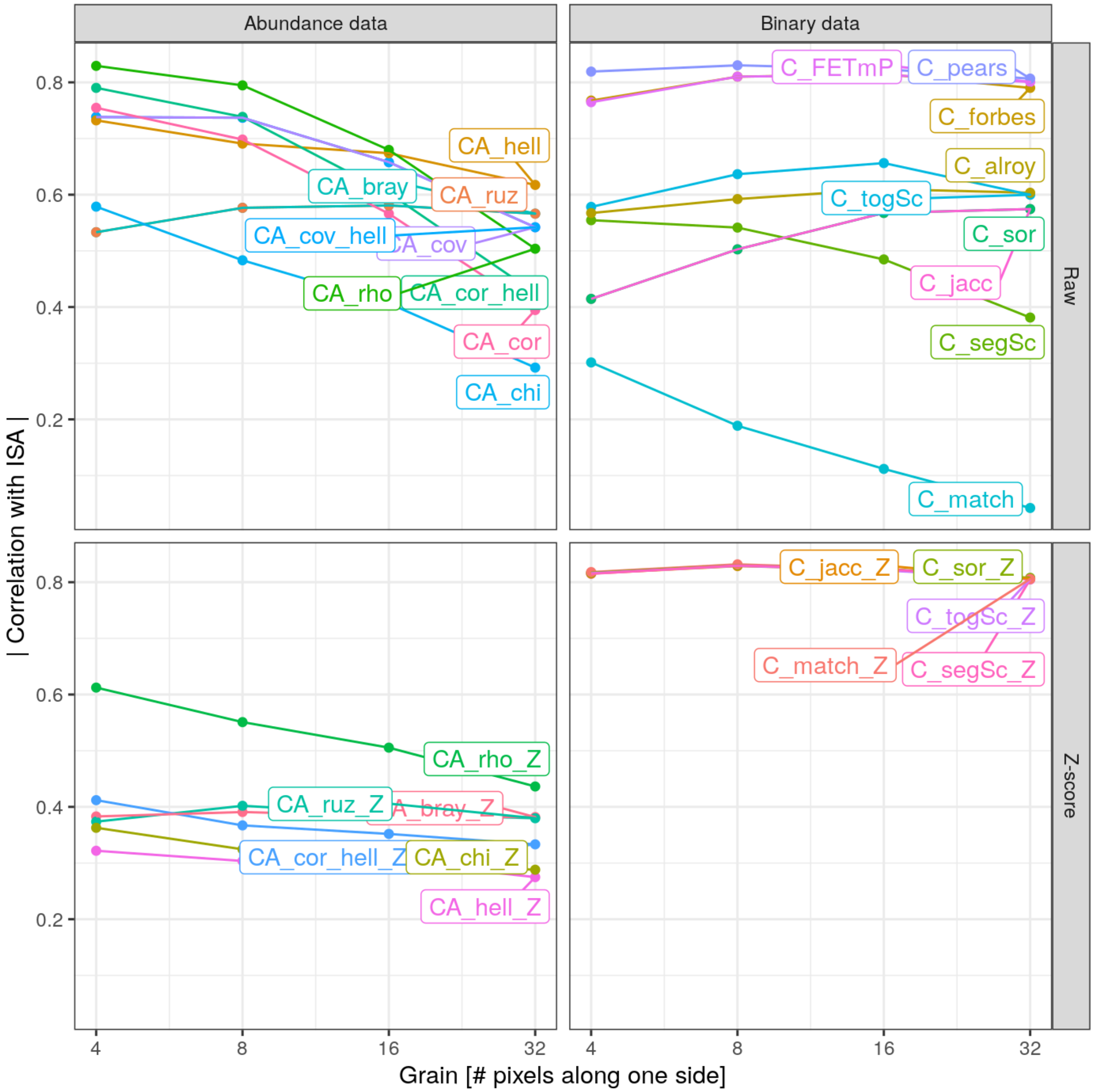


Figure S6 Relationship between the performance of ISA metrics and spatial grain that was used to aggregate point patterns. The performance is the absolute value of Spearman correlation between parameter ISA and the metric.

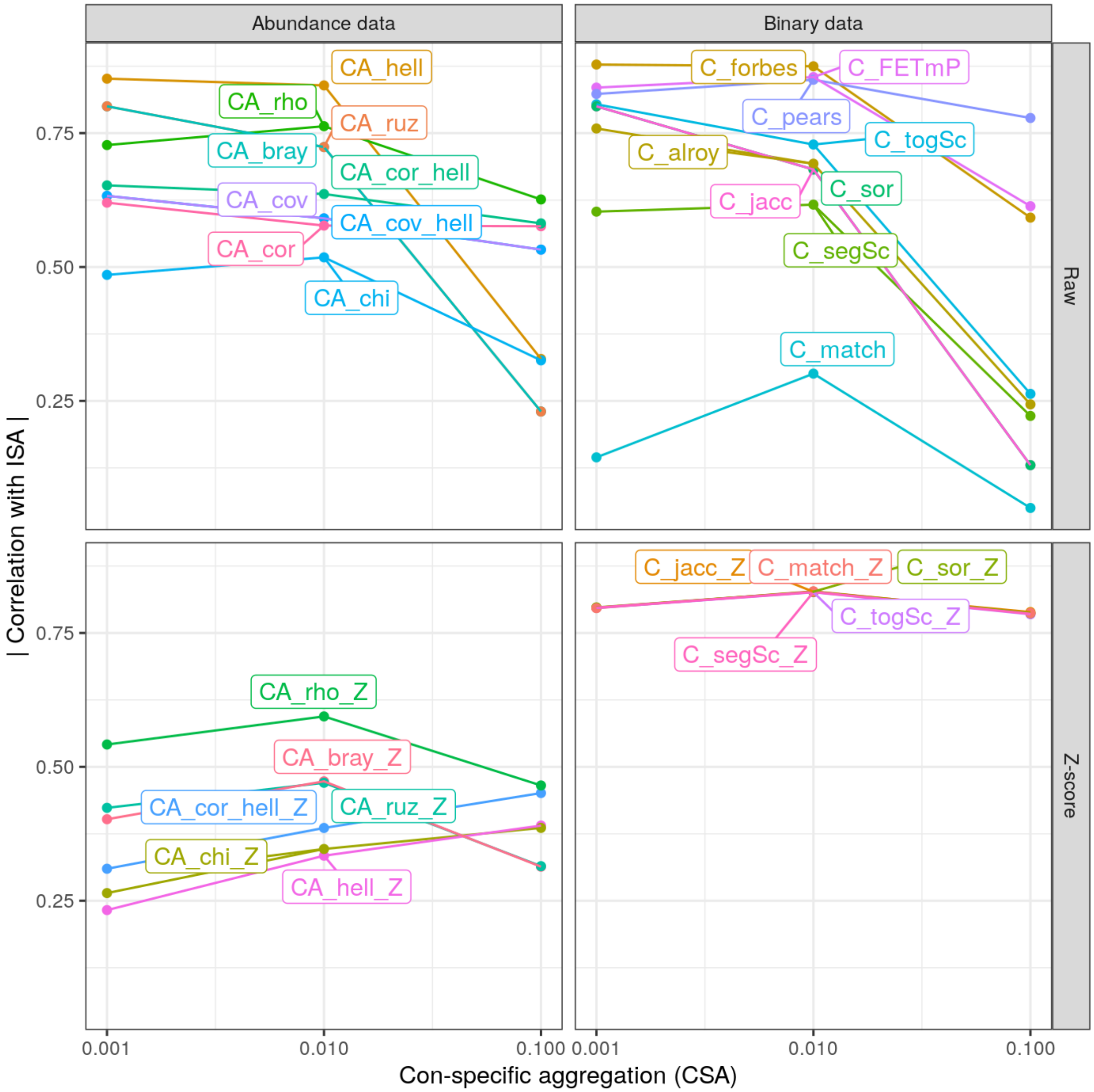


Figure S7 Relationship between the performance of ISA metrics and the magnitude of con-specific aggregation of species 1 in the simulations. The performance is the absolute value of Spearman correlation between parameter ISA and the metric.

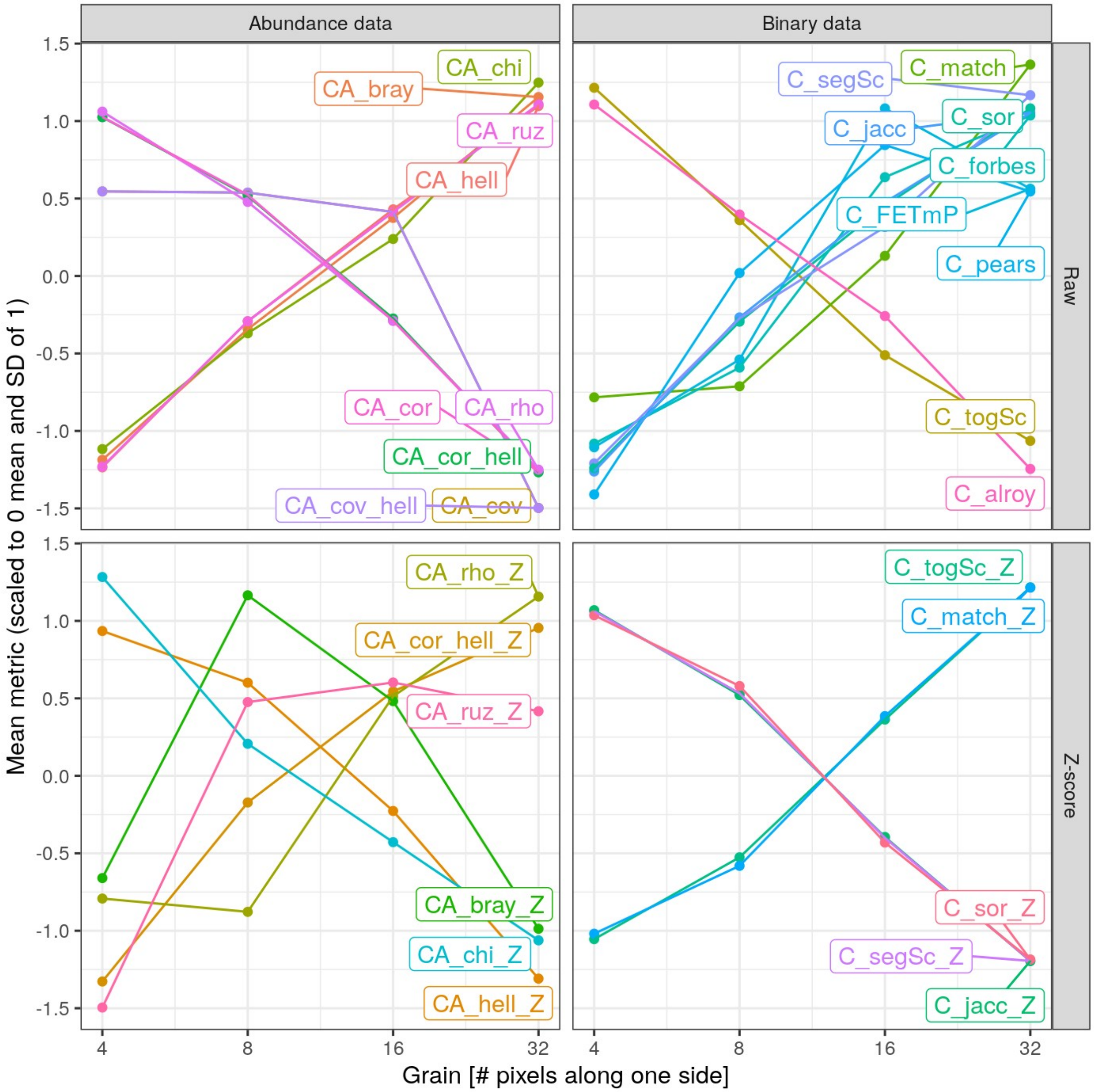


Figure S8 Relationships between mean metrics of ISA and spatial grain that was used to aggregate point patterns. The y-axis is the scaled (to 0 mean and SD of 1) value of the mean across all simulations at a given grain.

Supplementary references

Atmar, W., and B. D. Patterson. 1995. Nestedness temperature calculator. AICS Research Inc, University Park, New Mexico, and The Field Museum, Chicago.

Brown, J. H., E. J. Bedrick, S. K. M. Ernest, J.-L. E. Cartron, and J. F. Kelly. 2004. Constraints on negative relationships: mathematical causes and ecological consequences. Pages 298–323 *in* M. L. Taper and S. R. Lele, editors. The nature of scientific evidence: statistical, philosophical, and empirical considerations. University of Chicago Press, Chicago, IL.

Genton, M. G., and W. Kleiber. 2015. Cross-covariance functions for multivariate geostatistics. Statistical Science 30:147–163.

Gotelli, N. J., E. M. Hart, and A. M. Ellison. 2015. EcoSimR: Null model analysis for ecological data. CRAN.

Hubálek, Z. 1982. Coefficients of association and similarity, based on binary (presence-absence) data: an evaluation. Biological Reviews 57:669–689.

Keil, P. 2014. Limits of uncertainty about estimates of probability of ecological events. PeerJ PrePrints:2:e446v1.

Keil, P. 2019. Z scores unite pairwise indices of ecological similarity and association for binary data. Ecosphere 10:e02933.

Legendre, P., and L. Legendre. 2012. Numerical Ecology. Elsevier, Amsterdam.

Ulrich, W., and N. J. Gotelli. 2010. Null model analysis of species associations using abundance data. Ecology 91:3384–3397.