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SPATIAL SCALE-MEASUREMENT, INFLUENCE, AND INTEGRATION (A MARTIN AND J HOLLAND, SECTION EDITORS)

A Review of Overlapping Landscapes: Pseudoreplication or a Red Herring in Landscape Ecology?

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Abstract

Purpose of Review Identifying the spatial scale at which a species or population most strongly responds to habitat composition and configuration is known as scale-of-effect and is a fundamental pursuit of landscape ecology. In conducting scale-of-effect studies, it is common to measure habitat in landscape buffers of varying sizes surrounding sample sites. When sample sites are in close spatial proximity to one another, these landscape buffers will overlap. Researchers commonly worry that data generated from these overlapping landscapes, and subsequently used as predictor variables in statistical modeling, represent a form of pseudoreplication that violates the assumption of independence.

Recent Findings Here, we review the concept of overlapping landscapes and their theoretical and practical implications in landscape ecology. We suggest that the perceived problem of overlapping landscapes is distinct from more important issues in landscape ecology, such as a robust sampling design complete with a discrete assessment of spatial autocorrelation. Through simulation, we demonstrate that changing the amount of landscape overlap does not alter the degree of spatial autocorrelation. Yet, in reviewing over 600 journal articles, we found that a third (29%) of the studies perceived overlapping landscapes as an issue requiring either changes in sampling design or statistical solutions. Researchers concerned with overlapping landscapes often go to great lengths to alter their sampling design by removing or aggregating sites. Overlapping landscapes remain a prevalent concern in landscape ecology despite previous studies that show that overlapping landscapes are not a violation of independence and represent an oversimplification of the statistical concerns of spatial autocorrelation.

Summary The concern over overlapping landscapes as a form of pseudoreplication persists in landscape ecology, but acts as a potential red herring detracting from more relevant concerns of proper sampling design and spatial autocorrelation in ecological studies.

Keywords Habitat fragmentation · Habitat loss · Landscape ecology · Macroecology · Sampling design · Spatial autocorrelation · Statistical independence

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Introduction

A fundamental pursuit of landscape ecology is quantifying how and at what scales species and populations respond to habitat composition and configuration [1]. One method to accomplish this is to model the relationship between a landscape predictor (e.g., forest patch density) and an ecological response (e.g., variation in abundance) across landscapes of varying spatial extents. The scale at which a landscape predictor best explains variation in an ecological response has been called the "scale-of-effect" [2]. Although the scale-of-effect is typically not known a priori, theory suggests that it should be

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strongly influenced by life-history characteristics such as body size, behavior, and dispersal limitations. For example, species with greater dispersal capabilities may respond to habitat availability at broader scales [3, 4], whereas behavioral traits, such as gap avoidance, result in smaller scales-of-effect [3]. Such information on the scale at which a species responds to their environment is critical for assessing the effects of habitat loss and fragmentation as well as informing management and conservation [2, 5, 6].

Quantifying the scale-of-effect in natural settings is difficult due to the need to evaluate species-environmental relationships across multiple spatial scales and landscapes of varying extents [7, 8]. As an example, an investigator may be interested in examining how forest cover explains variation in the abundance of a warbler at multiple sampling sites. For such a multi-scaled analysis, the investigator may conduct multiple bird surveys and then quantify the proportion of forest cover within landscape buffers of varying sizes (e.g., radii ranging from 1 to 5 km) surrounding each bird survey site. A practical issue when conducting such multi-scale analyses is that, when sampling sites are in close spatial proximity, these landscape buffers may overlap. A frequently cited concern of overlapping landscapes is that data generated from these landscapes violate the assumption of independence when used as predictors in statistical modeling [9, 10]. This concern has led researchers to address overlapping landscapes by altering their sampling design prior to data collection [11, 12] or filtering or aggregating data points after data collection [13, 14].

The concern that overlapping landscapes lead to a violation of statistical independence, however, is potentially misdirected and may distract from more important issues—thus acting as a "red herring" in landscape ecology. To evaluate whether overlapping landscapes violate statistical independence, Zuckerberg et al. [15] used data from two independent bird monitoring programs to quantify the relationship between forest cover and bird abundance and occurrence using multiple landscapes ranging from 100 m to 24 km in diameter with varying levels of overlap. They found no evidence that greater landscape overlap increased spatial autocorrelation in model residuals (a measure of statistical independence) and thus provided empirical evidence that the statistical concerns of overlapping landscapes are misdirected. Despite these findings, it remains unknown whether researchers continue to perceive overlapping landscapes as a contemporary problem in ecological investigation, and if so, what actions are taken to avoid overlapping landscapes in practice.

To explore the impacts of overlapping landscapes in landscape ecology, we (1) presented the statistical and sampling design issues of overlapping landscapes; (2) performed simulation of the effects of overlapping landscapes on species-environment relationships; (3) conducted a literature-based assessment of the perceived problem of overlapping landscapes; and (4) offered a synthesis of the issue and best practices for designing studies in landscape ecology.

Statistical Concerns of Overlapping Landscapes

The primary concerns of overlapping landscapes relate to concepts of statistical independence, pseudoreplication, and spatial autocorrelation. Statistical independence among sampling sites is a persistent concern in ecological studies, and in some cases, a source of both practical and theoretical disagreement [16–19]. This tension likely occurs for two reasons. First, adhering to statistical rigor with respect to independence is sometimes at odds with the realities of fieldwork and data collection [16]. Second, independence follows from careful study design and representative sampling [18] and is often not straightforward to assess. Evaluating pseudoreplication reflects the challenges of determining the appropriate scale of independent replication and occurs when we fail to account for temporal or spatial dependencies across observations that are assumed to be independent [20]. While problems of pseudoreplication are well-defined in traditional blocked and treatment-oriented designs [18], statistical independence and pseudoreplication are more difficult to evaluate in observational or mensurative studies of species-environment relationships [21].

Investigators often worry that overlapping landscapes are a form of pseudoreplication that leads to a violation of statistical independence due to similarity in values of a predictor variable across sites [9, 10, 22]. In other words, because portions of the same landscape may predict more than one response, it is suggested that these observations should be considered pseudoreplicates [10]. This implies that landscape overlap reduces the effective sample size of the data, inflating the risk of type I error. However, the concept of pseudoreplication articulated by Hurlbert et al. [18] is *not* that multiple replicates cannot be influenced by the same values of predictor variables. In fact, this is typical of nested or hierarchical data and regression makes no assumptions about the independence of predictors [23]. Rather, the assumption of independence relates strictly to the variation in the response *left unexplained by the predictors* (i.e., independence of residuals). Hurlbert et al. [18] were primarily concerned about lack of independence in study designs in which replicates are poorly interspersed or where sampling sites are influenced by a systematic, but unknown, predictor. In field settings, the clustering of sampling sites may lead to such a lack of independence where adjacent observations have similar data values, but there is a distinction between the poor interspersion of sampling sites and overlapping landscapes.

We offer the following scenario to better illustrate that issues of overlapping landscapes are distinct from the concerns of pseudoreplication. Suppose a researcher wishes to quantify the environmental predictors of species occurrences on a patchy landscape of forest and non-forest habitat (Fig. 1). Initially, the researcher implements a design with four randomly placed sampling sites and records species occurrence (Fig. 1a). Following sampling, the researcher measures habitat predictors within a fixed-radius landscape buffer, but fails to account for elevation in both the sampling design and the subsequent statistical analyses. As it happens, forest habitats were sampled in high-elevation areas, where the species is less likely to occur, leading to a biased estimate of occurrence in this habitat. This sampling design fails to adequately sample the landscape and reflects the concern of pseudoreplication because forest habitat replicates are not independent due to an unaccounted source of variation (elevation), as opposed to overlapping landscape buffers. Now suppose the researcher resamples using a systematic grid, improving both sampling site replication and coverage (Fig. 1b). Here, the pseudoreplication issue of the previous design is addressed (by sampling across the elevation gradient) and sampling bias is reduced, but many of the landscape buffers overlap. In response, the researcher reduces buffer sizes (Fig. 1c) or maintains the buffer size but reduces the number of sampling sites (Fig. 1d) to avoid overlapping landscapes. However, overlapping landscape buffers were not the source of non-independence; the sites potentially violated independence because they failed to sample an unknown and important environmental predictor. Importantly, this potential violation of independence would persist in both the presence and absence of overlapping landscapes. In fact, reducing buffer size (Fig. 1c) could artificially limit the scale of investigation or reduce sample size (Fig. 1d) and thereby sacrificing statistical power and inducing spatial autocorrelation.

Spatial autocorrelation describes the presence of systematic spatial variation in a variable and positive spatial autocorrelation, which is most often of concern in assessing statistical independence, is the tendency for sampling sites that are close together to have more similar values than sites that are far apart [24, 25]. Spatial autocorrelation is a common phenomenon in landscape ecology and often results from two distinct sources: *endogenous* factors relating to the life-history characteristics of a species (e.g., dispersal limitations or density dependence) or *exogenous* factors relating to the spatial clustering of environmental predictors [26–30]. Spatial autocorrelation, in of itself, is not a statistical artifact, but a natural manifestation of species' ecology and their relationship to environmental variation. However, spatial autocorrelation *is* a concern when it results from a flawed sampling design or a statistical model that does not adequately describe the spatial dependencies of the ecological phenomena of interest. A common diagnostic tool for assessing this problem is to test for spatial autocorrelation in the residuals of the fitted model; positive spatial autocorrelation

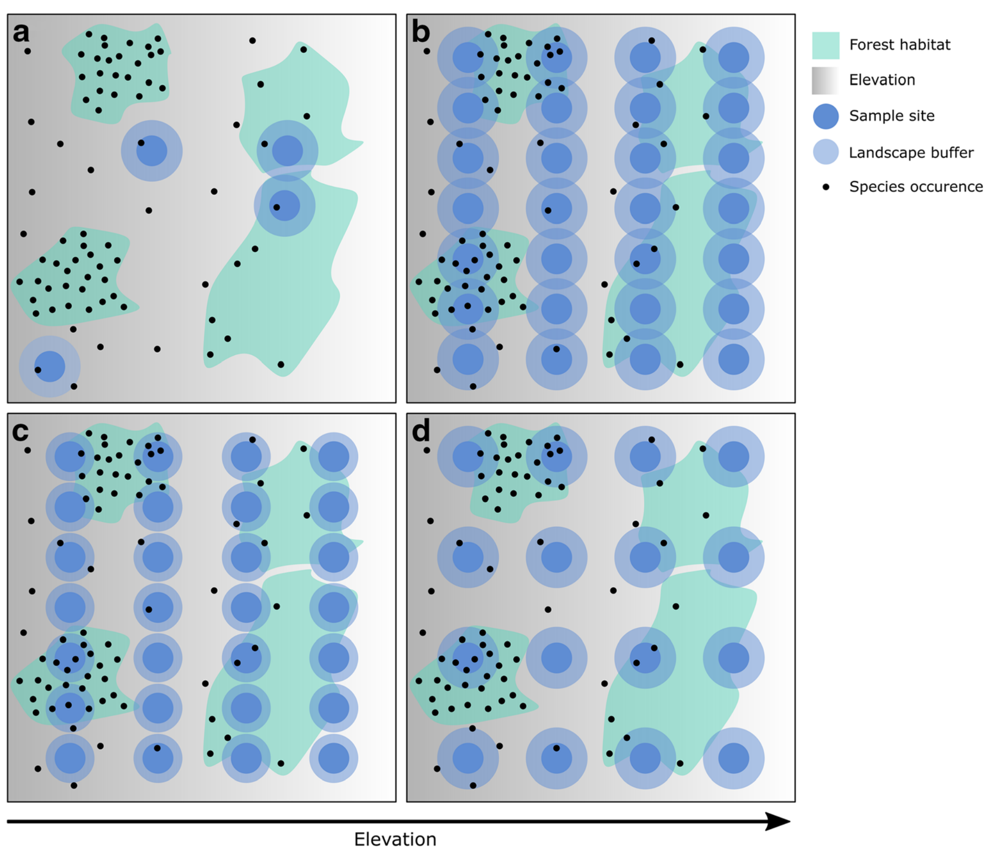

Fig. 1 Four experimental designs sampling species occupancy on a theoretical landscape. **a** A simple random sampling scheme demonstrating classical pseudoreplication by failing to adequately sample an important environmental predictor (elevation) operating across the study area. **b** A systematic sampling design with strong replication and landscape coverage, but with significant overlapping of landscape buffers. **c** A systematic design that attempts to avoid overlap by reducing buffering extent. **d** A systematic design that attempts to avoid overlap by sacrificing sample size

in model residuals suggests a mismatch between the statistical model and the ecological pattern. Positive spatial autocorrelation in model residuals is indicative of a violation of independence, but is distinct from pseudoreplication by which dependence among sampling replicates results from a flawed sampling design (described above) [18]. Consequently, it is important to ask: If overlapping landscapes do not themselves represent a violation of independence, is there any evidence that the presence of overlapping landscapes increases spatial autocorrelation in model residuals?

As discussed above, when residual spatial autocorrelation is present, but not explicitly accounted for in models, it will produce elevated type I error rates [26]. However, landscape overlap does not necessarily induce spatial autocorrelation [10]. To the contrary, reducing sample size or altering sampling design specifically to reduce landscape overlap may increase spatial autocorrelation in model residuals [15]. Thus, removing sampling sites to avoid landscape overlap could increase spatial autocorrelation. Consequently, altering sampling designs to avoid overlapping landscapes may unintentionally limit scales of analysis and ecological inference, provide a false sense of security that statistical independence was achieved, and even contribute to unexplained spatial dependencies in ecological models [16, 19].

Simulation of Overlapping Landscapes

To explore whether landscape overlap influences spatial autocorrelation in statistical modeling, we simulated landscapes and species-environmental relationships under different sampling designs and varying degrees of landscape overlap. Our simulation adapted the methods of Graham et al. [29] for virtual landscapes (see Appendix 1 for full simulation details). In short, we built virtual landscapes representing patchy configurations of different land cover types (e.g., forest, non-forest) superimposed on a strong environmental gradient (e.g., elevation) at a 1-grid cell resolution (Fig. 2). Next, we generated a virtual species, modeling the spatial probability of its occurrence (0–1) influenced by both the distribution of forest and elevation mapped at a 1-grid cell resolution [26]. Next, we sampled grid cells within this 1-grid cell distribution to obtain our sampling sites. Then, we aggregated the 1-grid cell probability of occurrence to an average probability of occurrence that is based on either an 8- or 16-grid cell radius to generate probabilities of occurrence at the desired landscape level. We then superimposed the sampling sites to extract their corresponding aggregated probability of occurrence values and applied a random binomial distribution to generate a presence (1) or absence (0) for each sampling site. This results in the presence-absence data for each sampling site that is based on the environmental conditions at all cells within the landscape buffer of the sampling site.

We simulated four different sampling designs to estimate population presence-absence from landscape-level predictors (Fig. 3). In scenario A, we applied simple random sampling, stratified by only habitat type (18 sampling sites per habitat class for a total of 36 sampling sites), thus ignoring the environmental gradient, and using fixed 16-grid cell buffers. To simulate a biased sampling design, we constrained our

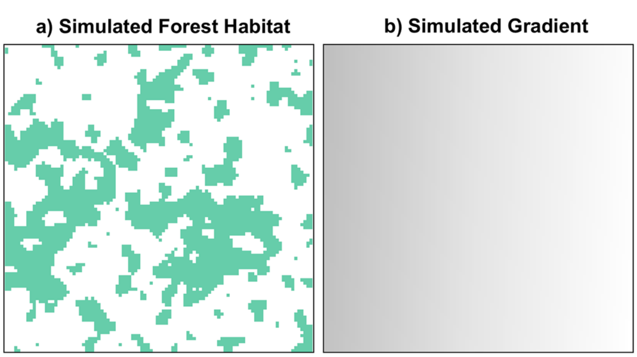

Fig. 2 Simulated landscapes consisting of forest habitat (green) and non-forest (white) habitats and a gradient of elevation were used for modeling virtual species occurrences

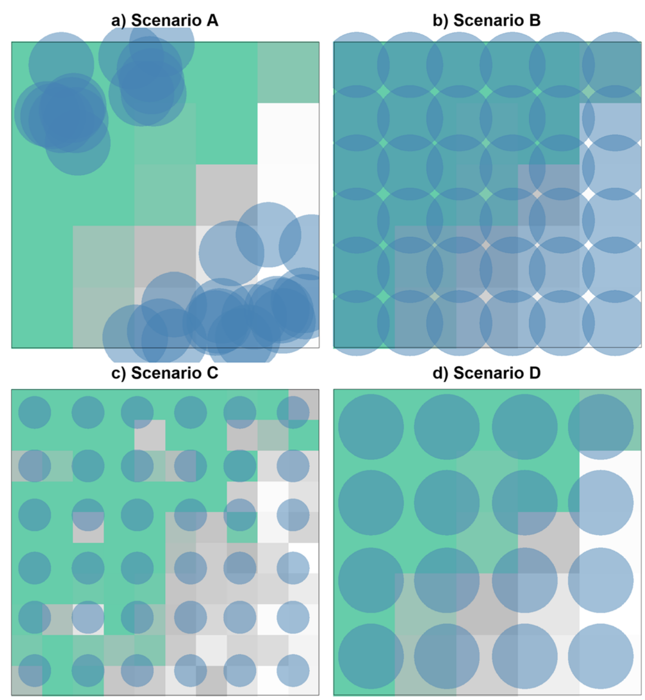

Fig. 3 Four different sampling scenarios superimposed on maps of probability of occurrence aggregated to different scales of resolution to match the corresponding landscape buffer. Scenario A implemented a biased sampling scheme with 18 sampling sites stratified by only habitat and ignored the environmental gradient. Scenario B used a regular sampling approach with overlapping landscape buffers. Scenario C used the same sampling sites as scenario B, but with a finer resolution (8-grid cell) to ensure non-overlapping buffers. Scenario D used the same buffer radius as scenarios A and B, but with fewer sampling sites to remove overlapping buffers. Overlapping landscapes were allowed to extend beyond the study region in order to avoid spatial bias towards the center of the landscapes (e.g., mid-domain effect)

sampling of forest and non-forest habitat to opposite extremes of the gradients. In scenarios B–D, we used systematic sampling with a total of 36 sampling sites covering the full range of the environmental predictors and allowing for overlapping landscapes (scenario B) or non-overlapping landscapes (scenarios C and D). We ensured non-overlapping landscapes by either reducing buffer size from 16 to 8-grid cell buffers while maintaining the sample size (scenario C) or maintaining the 16-grid cell buffers while reducing the number of sampling sites from 36 to 16 (scenario D). To match the scale-of-effect between the landscape-level predictors and simulated species occurrences, the presence-absence values for scenarios A, B, and D were based on the aggregate species probability of occurrence within a 16-grid cell resolution while in scenario C, these were based on 8-grid cell resolutions.

Next, we constructed 4 binomial general linear models for each sampling scenario fitting species presence-absence data from the sampling sites as a response variable and used average forest amount calculated within each buffer as the predictor variables (including linear and quadratic effect forms) in each model [15]. We calculated the total percent overlap of the landscapes in the study area as the area of intersecting overlap/total area covered by landscapes [11] (Appendix 1), and quantified the spatial autocorrelation of model residuals using Moran's I [27]. We repeated this process with 1000 different virtual landscape patterns to account for potential variability in model outputs given differences in the spatial distribution of randomly sampled plots in scenario A and habitat configuration of random virtual landscapes.

Positive spatial autocorrelation in model residuals was strongest under a sampling method with non-overlapping landscapes, but with a low sample size that did not sufficiently account for spatial effects of the predictors (scenario D; Table 1; Fig. 4; Mood's median test $p < 0.001$). Scenario A also demonstrated residual positive spatial autocorrelation (Table 1; Fig. 4) and was characterized by a high likelihood of overlapping landscapes (Appendix S1) and a sampling design that did not sufficiently account for an important predictor (elevation). The spatial sampling biases of scenario A and D induced significantly higher spatial autocorrelation than scenarios B and C (Table 1; Fig. 4; Mood's median tests $p < 0.001$). Under a systematic sampling method (scenarios B–C), the levels of positive spatial autocorrelation in model residuals are significantly reduced regardless of whether the landscapes overlapped or not (Table 1; Fig. 4d). In scenarios B–C, the sampling sites captured the full breadth of environmental variation of both landscape predictors, thus reducing the likelihood of pseudoreplication. Scenario D, however, represents a design with fewer sampling points to avoid landscape overlap, and shows a significant increase in spatial autocorrelation (Table 1, Fig. 4c). This scenario has the highest potential for pseudoreplication among sampling units due to the dependencies imposed by a reduced sample size and under-sampling important spatial predictors. Importantly, our simulation demonstrates that the risk of insufficient sampling of spatial predictors in the sampling design is clearly more relevant for violating statistical independence than the risk of overlapping landscapes (Fig. 4).

Literature Review of Overlapping Landscapes: a Persistent Concern

We surveyed recent literature to evaluate how studies in landscape ecology assess and avoid potential issues of overlapping landscapes and spatial autocorrelation. Our primary goal was to compare the proportion of landscape ecology studies that addressed spatial autocorrelation in their data with those that investigated *only* the influence of overlapping landscapes. Our questions were the following: (1) are studies attempting to address overlapping landscapes, spatial autocorrelation, or neither?; (2) have studies shifted their approach over the last 25 years, following recent work suggesting that overlapping landscapes are not likely to bias landscape-scaled studies (e.g., 15)?; and (3) do studies vary in their approach depending on taxonomic focus, region, time-of-year, or ecological response (e.g., abundance, occurrence, species richness)? For this last question, we hypothesized that researchers may be more concerned regarding overlapping landscapes or spatial autocorrelation for more wide-ranging and vagile species (such as birds and mammals). We further hypothesized that consideration of overlapping landscapes varied by region and season because many species are likely to respond differently to habitat composition and configuration across their ranges and as a

Table 1 Median absolute Moran's I values (italicized) and Mood's median test results for statistical differences between the median of absolute Moran's I for each combination of scenarios. Values in bold refer to significant differences between medians

	Scenario A	Scenario B	Scenario C	Scenario D
Scenario A (stratified random sampling by habitat)	*0.090*			
Scenario B (systematic sampling; overlapping landscapes)	$\boldsymbol{p < 0.001}$	*0.072*		
Scenario C (systematic sampling; non-overlapping landscapes)	$\boldsymbol{p < 0.0.01}$	$p = 0.1$	*0.069*	
Scenario D (systematic sampling; non-overlapping landscapes; low sample size)	$\boldsymbol{p < 0.001}$	$\boldsymbol{p < 0.001}$	$\boldsymbol{p < 0.001}$	*0.147*

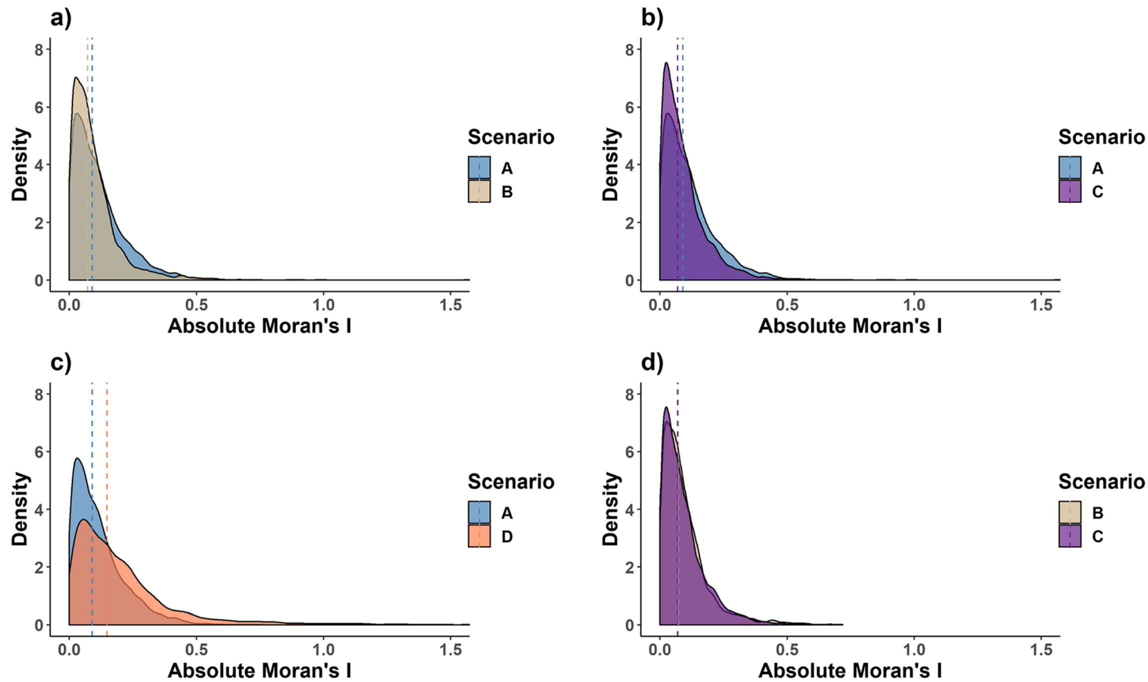

Fig. 4 Distribution of absolute Moran's I values across 1000 iterations for the four simulation scenarios. **a**, **b** Scenario A had higher residual autocorrelation compared with scenarios B and C ($p < 0.001$; Table 1). **c** Scenario D had the highest amount of residual spatial autocorrelation across all scenarios ($p < 0.001$; Table 1). **d** We do not find significant differences in spatial autocorrelation between scenarios B and C ($p = 0.10$), despite scenario B having overlapping landscapes. Medians for absolute Moran's I for each scenario (dashed lines)

function of varying life history stages (e.g., overlapping landscapes could be of greater concern during breeding seasons when many species are territorial and geographically clustered). Finally, we explored these relationships across a range of what we a priori considered to be the most common ecological responses collected in landscape-scale studies—abundance, occurrence, and species richness.

To answer our questions, we modified previous search terms for identifying multi-scale studies [31] and searched Web of Science in December 2019 for the following terms, aiming to find papers that studied the relationship between environmental heterogeneity and wildlife (and plant) populations or communities with some consideration of spatial scale: ((“spatial scale*” OR “spatial extent*”) AND (“landscape size” OR “multi-scale” OR “landscape area” OR “buffers” OR “focal patch*” OR “focal point*”) AND (“surrounding landscape*” OR “landscape context” OR “landscape structure” OR “landscape composition” OR “scale-of-effect”) AND (abundance OR occupancy OR incidence)). The selection of search terms was designed to identify a subset of the landscape ecology literature that explicitly analyzed relationships between land cover and an ecological response variable across varying spatial scales. Our search returned 1446 results, which we narrowed to 612 relevant studies after screening abstracts using the *metagear* package [28] (Appendix 2). We determined whether each paper either (1) did not attempt to address overlapping landscapes or spatial autocorrelation; (2) modified their sampling design or filtered/aggregated data post hoc to avoid overlapping landscapes, but ignored assessing spatial autocorrelation; or (3) assessed or accounted for spatial autocorrelation in their approach, regardless of whether overlapping landscapes were also considered. In addition, we recorded the taxonomic focus, continent, and season of each study. Finally, we recorded the response variable of the study as occurrence, abundance, richness, other response, or multiple responses.

The studies were evenly split across the three approaches, with 37% not addressing overlapping landscapes or spatial autocorrelation, 29% attempting to minimize the impact of overlapping landscapes despite not assessing spatial autocorrelation, and 33% of studies addressing spatial autocorrelation in some fashion (Fig. 5a). The proportion of studies attempting to address overlapping landscapes decreased slightly over time; 33% of studies before 2010 addressed the issue of overlapping landscapes ($n = 222$), but this declined to 27% after 2010 ($n = 390$) (Fig. 5b). Overlapping landscapes were considered an issue for studies across a diversity of ecological responses, including in 34% of studies that modeled multiple responses ($n = 185$), 30% of occurrence studies ($n = 177$), 23% of abundance studies ($n = 122$), and 28% of richness studies ($n = 96$) (Fig. 5c).

We observed the greatest variation in concern for overlapping landscapes based on the taxonomic focus of the study. Studies focused on birds and mammals addressed overlapping landscapes 35% ($n = 122$) and 33% ($n = 179$) of the time, respectively, while studies modeling invertebrates (23%, $n =$

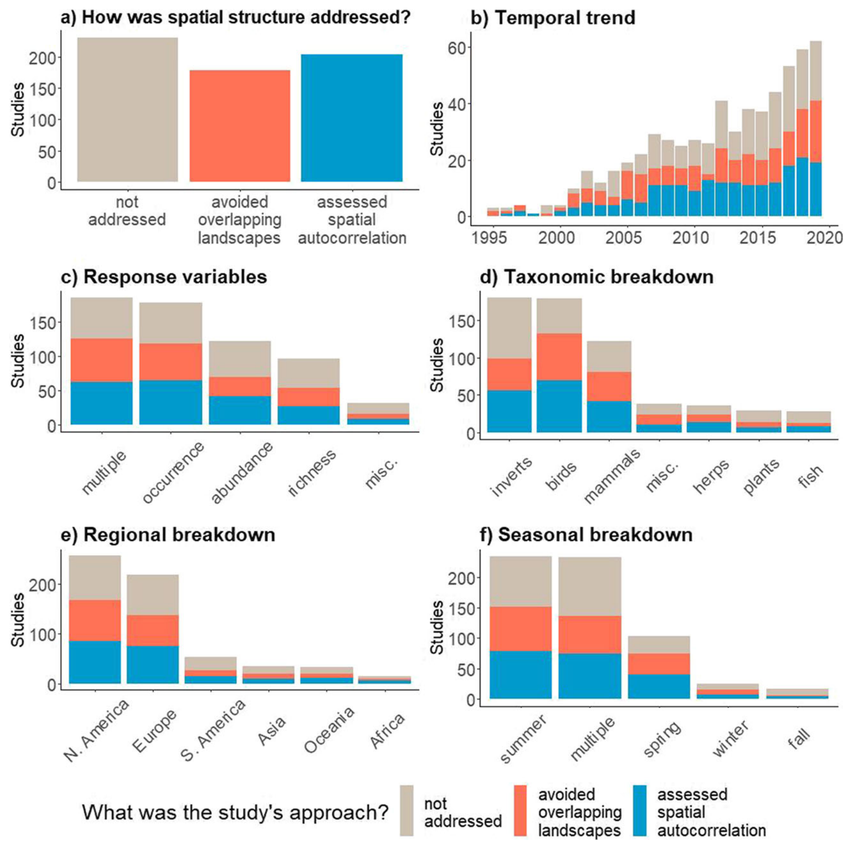

Fig. 5 How have studies (n = 612) addressed overlapping landscapes and spatial autocorrelation? Studies were split roughly evenly between three approaches: (1) no effort to address spatial effects (gray); (2) avoided overlapping landscapes through sampling design or data analysis, but did not test for spatial autocorrelation (red); (3) or assessed or accounted for spatial autocorrelation in their data, regardless of whether they addressed overlapping landscapes (blue). This split varied over time, by taxonomic focus, and by response variable of the study

180), reptiles or amphibians (28%, $n = 36$), fish (14%, $n = 28$), or plants (21%, $n = 29$) addressed them less often (Fig. 5d). A potential reason for this taxonomic focus could be that scale-of-effect studies on birds and mammals assume comparatively higher dispersal distances and home ranges (compared with more sessile organisms) and would be interested in larger landscapes with a higher likelihood of potential overlap. There was little variation in concern for overlapping landscapes and spatial autocorrelations among geographic regions and seasons (Fig. 5e–f). Overall, it is clear that overlapping landscapes remain a perceived concern across many ecological studies.

How Researchers Address Overlapping Landscapes

Based on our review of over 600 studies, researchers go to great lengths to avoid overlapping landscapes, despite the lack of any rigorous testing of spatial autocorrelation. In reviewing these studies, one common approach to address the perceived concern of overlapping landscapes, used in 25% of studies, is to deploy or adapt a sampling design in which landscape buffers do not overlap. Many of these studies either limited landscape buffer sizes following data collection or spaced sampling locations far enough apart to prevent overlap [32–36]. Other studies made no mention of landscape overlap per se but still employed sampling designs in which it was avoided [15]. In these cases, capturing sufficient variation in the landscape was usually cited as the primary factor used to determine landscape buffer sizes and distances between sample sites.

Several studies demonstrated a desire to avoid overlapping landscapes based on recommendations from previous reviews on sampling designs [16, 37, 38]. For example, reviews on experimental approaches in landscape ecology recommend that studies evaluate response variables from within patches and landscape variables within a specified landscape radius, without overlap between the resulting landscape buffers [7, 39]. The argument is that avoiding landscape overlap would strengthen ecological inference by eliminating the confounding effects of spatial autocorrelation. Consequently, studies designed around these recommendations indirectly endorsed the idea that eliminating overlapping landscapes avoids pseudoreplication and reduces spatial autocorrelation.

Attempting to avoid overlapping landscapes is logistically challenging and may complicate experimental design. For example, Talaga et al. [40] stated a desire to minimize spatial overlap, but the buffers around each site, which ranged from 10 to 70 m, had considerable overlap with buffers greater than 50 m. Similarly, in their study of the scale-of-effect of land cover on the density of an agricultural pest, Horak et al. [12] attempted to use non-overlapping landscapes across six spatial scales to avoid violating site independence, but the two largest scales (32 and 64 km) still had substantial overlap between landscapes. This indicates that, despite the desire or intention to avoid overlapping landscapes, there could be logistical or other challenges associated with altering sampling design. Another response to overlapping landscapes involved removing or aggregating sampling sites when landscape buffers overlapped (i.e., data filtering) due to stated concerns of a lack of independence among sampling sites (e.g., [18]).

Many studies in our literature review explicitly stated that their sampling design was adapted to avoid landscape overlap, a practice that could be constraining research on the scale-of-effect. Determining the size of an appropriate landscape buffer is often done a priori and should reflect aspects of the species biology such as maximum dispersal distance, home range size, or sensitivity to habitat loss and fragmentation [31, 41]. However, Jackson and Fahrig [31] note that the characteristic scale-of-effect often appears to be larger (or smaller) than the range investigated by researchers, which suggests that truncating landscape size to avoid overlap can compromise ecological inference.

Conclusions

The concern that overlapping landscapes represent a violation of statistical independence and result in pseudoreplication persists in landscape ecology. Pseudoreplication is ultimately failing to account for temporal or spatial dependencies across observations [20]. With respect to scale-of-effect relationships, the primary concern relates to dependencies of spatial autocorrelation imposed by exogenous sources on an ecological response. However, both empirical results and simulation demonstrate that overlapping landscapes do not induce or protect from residual spatial autocorrelation. Despite these findings and statistical arguments, our literature review revealed that nearly one-third of studies exploring scale dependency in species-environment associations perceive overlapping landscapes as a problem. Studies that focus on birds and mammals appear to be disproportionately concerned about overlapping landscapes, perhaps due to a priori assumptions that these species respond to habitat composition and configuration at larger spatial scales. This concern of overlapping landscapes is not without costs, and researchers implement a number of "solutions" such as altering sampling design or data filtering.

The concern that overlapping landscapes are a violation of statistical independence continues to vex landscape ecologists. Importantly, evaluating and detecting patterns of spatial autocorrelation in model residuals should be standard practice, regardless of overlapping landscapes, and can be achieved using spatial correlograms [27]. If residual spatial autocorrelation is detected, researchers should first consider whether there are important environmental conditions that could have been overlooked when designing the study. If so, researchers should consider modifying future data collection to capture that missing environmental predictor or include a measurement of that variable in their statistical model and check to see if that reduces residual spatial autocorrelation to an acceptable level. Sampling designs that emphasize systematic sampling are more robust to spatial independencies induced by an unknown environmental predictor. However, in cases when such a sampling design is not possible due to logistical difficulties, researchers should not assume that avoiding overlapping landscapes is a safeguard from issues of spatial autocorrelation.

Spatial autocorrelation is a well-known issue in ecology and there are a plethora of statistical methods available to diagnose and accommodate spatial dependencies in ecological data [21, 26, 27, 41]. In terms of data analysis, approaches such as simple autoregressive or kernel-based models can account for spatial autocorrelation induced by exogenous factors [42, 43]. For example, Chandler and Hepinstall-Cymerman [42] developed an approach based on smoothing kernels to identify the scale-of-effect of primary productivity on the abundance of Canada warblers (*Cardellina canadensis*). Importantly, this approach does not rely on using pre-defined landscape buffers, but rather on a distance-weighted average of landscape features surrounding the sampling sites; such a framework can easily accommodate spatial dependencies without having to worry about overlapping landscapes. Investigators can also implement a diversity of mixed effects models and spatial random effects designed for nested or hierarchical data to address spatial dependencies [20, 44]. Overlapping landscapes and spatial autocorrelation are two separate issues in the modeling of scale-of-effect relationships: Non-overlapping landscapes do not ensure spatial independence and overlapping landscapes do not necessarily lead to greater spatial autocorrelation. The exploration and study of scale-of-effect have important implications for species conservation and management and ecological inference should not be limited by the perceived problem of overlapping landscapes.

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Compliance with Ethical Standards

Conflict of Interest Dr. Zuckerberg has no conflicts of interests to declare.

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