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## Integrating spatial and phylogenetic information in the fourth-corner analysis to test trait–environment relationships

JOÃO BRAGA,<sup>1,4</sup> CAJO J. F. TER BRAAK,<sup>2</sup> WILFRIED THUILLER,<sup>1</sup> AND STÉPHANE DRAY<sup>3</sup>

<sup>1</sup>Laboratoire d'Écologie Alpine (LECA), Université Grenoble Alpes, CNRS, Université Savoie Mont Blanc, Grenoble F-38000 France

<sup>2</sup>Biometris, Wageningen University & Research, Wageningen, The Netherlands

<sup>3</sup>Laboratoire de Biométrie et Biologie Evolutive (LBBE), Université Claude Bernard (Lyon I), CNRS, UMR5558, Villeurbanne F-69100 France

**Abstract.** The fourth-corner analysis aims to quantify and test for relationships between species traits and site-specific environmental variables, mediated by site-specific species abundances. Since there is no common unit of observation, the significance of the relationships is tested using a double permutation procedure (site based and species based). This method implies that all species and sites are independent of each other. However, this fundamental hypothesis might be flawed because of phylogenetic relatedness between species and spatial autocorrelation in the environmental data. Here, using a simulation-based experiment, we demonstrate how the presence of spatial and phylogenetic autocorrelations can, in some circumstances, lead to inflated type I error rates, suggesting that significant associations can be misidentified. As an alternative, we propose a new randomization approach designed to avoid this issue, based on Moran's spectral randomization. In this approach, standard permutations are replaced by constrained randomizations so that the distribution of the statistic under the null hypothesis is built with additional constraints to preserve the phylogenetic and spatial structures of the observed data. The inclusion of this new randomization approach provides total control over type I error rates and should be used in real studies where spatial and phylogenetic autocorrelations often occur.

**Key words:** community ecology; fourth-corner analysis; functional ecology; Moran's spectral randomization; null models; type I error.

### INTRODUCTION

Analyzing how species with given functional traits respond to environmental gradients generally requires the simultaneous analysis of three types of information: a trait database, a species by site matrix, and an environment by site matrix. The first (table **T**) contains information on the morphological or physiological traits of each species (species as rows and traits as columns). The second (table **L**) contains species abundances (or presence and absence) for each site (sites as rows and species as columns). The third (table **E**, sites as rows and species as columns) contains a set of environmental variables for each site (e.g., soil properties, climate, or land use).

The simultaneous analysis of these three tables is a statistical challenge that has led to a wide range of developments. Regression-based approaches (e.g., Jamil et al. 2013, Brown et al. 2014) model species abundances (or species presence/absence) as a function of their respective traits, environmental variables and the interaction between both species traits and environment using generalized linear models (GLMs)

or generalized linear mixed effects models (GLMMs, with species and/or sites as random terms). In these models, the trait–environment relationship is measured by the interaction term. On the other hand, correlative approaches (for a review, see Kleyer et al. 2012) broadly include community-weighted mean approaches, where a single trait value for the each community is calculated and correlated against the environmental variable (Lavorel et al. 2008), ordination approaches, such as RLQ analysis (Dolédec et al. 1996) and the fourth-corner analysis, which measures and tests correlations between species traits and environmental variables (Legendre et al. 1997, Dray and Legendre 2008). The comparison between regression-based and correlative approaches is beyond the scope of this paper (some elements of comparison between correlative and regression-based methods are given in ter Braak et al. [2017]).

Among the correlative methods, the fourth-corner analysis can be used to measure and statistically test associations between a functional trait and an environment variable, while taking into account species' abundances. Depending on the nature (quantitative or qualitative) of the variables in tables **T** and **E**, different statistics have been proposed to quantify the link between traits and the environment (Legendre et al. 1997, Dray and Legendre 2008). For instance, when both the trait and the environment are continuous (quantitative variables), their association is

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<sup>4</sup>E-mail: joao.braga@univ-grenoble-alpes.fr

measured with a weighted cross-correlation coefficient (Legendre et al. 1997, Dray and Legendre 2008).

As the fourth-corner involves two types of statistical units (species on which traits are measured and sites at which environment is measured), a statistically valid test requires two separate permutation tests, one site based and one species based. The significance of the trait–environment association is then given by the largest of the two  $P$ -values (Dray and Legendre 2008, ter Braak et al. 2012). The underlying assumption of this test, as with any permutation test, is that observations are independent under the null hypothesis. In the case of the fourth-corner, an important consequence of this assumption is that species and sites are considered as two sets of independent observations. However, observation dependence (i.e., autocorrelation) in ecological data is the rule rather than the exception for two main reasons. First, because spatial autocorrelation occurs when the values for a variable sampled at nearby sites are not independent from each other (Diniz-Filho et al. 2003). This is often the case with environmental variables (table E). However, it is also common in abundance data (table L), due to biotic interactions, limited dispersal, and environmental filtering (Dale and Fortin 2002, Dormann 2007). Second, species are not independent units as they share a common evolutionary history, which may not only create phylogenetic signal in traits (table T, i.e., phylogenetic autocorrelation; Blomberg et al. 2003), but also in abundance distributions (table L; Hardy 2008). These two patterns arise in cases of strong trait and niche conservatism (Münkemüller et al. 2012) and although they are not necessarily ubiquitous in ecological data (Pearman et al. 2014), they affect statistical performance when present.

How valid and powerful fourth-corner testing procedures are in the presence of spatial and phylogenetic autocorrelation is therefore a key question. It is indeed well known that the presence of autocorrelation produces a kind of pseudo-replication (Hurlbert 1984) leading to inflated type I error rates and biased and imprecise parameter estimates. Several approaches have therefore been proposed to solve this issue in the phylogenetic (e.g., Martins and Hansen 1997, Rohlf 2001) and spatial (e.g., Lichstein et al. 2002) context.

In the spatial context, Wagner and Dray (2015) recently proposed Moran spectral randomization (MSR) as an alternative strategy based on the use of spatially constrained randomization methods. The basic idea behind this method is to compare observed values for parameters to those obtained under a null model that preserves the spatial autocorrelation of the data. Although the idea of such null models is not a novelty per se (e.g., Fortin and Jacques 2000, Beale et al. 2008), the MSR method has the distinct advantage of dealing with irregular sampling with minimal parameterization while preserving the multiscale structure of spatial data (Wagner and Dray 2015). Although the MSR method was originally developed for correlation analyses in the presence of spatial autocorrelation, we argue that it is an appealing approach that can be extended to deal with both spatial and phylogenetic autocorrelations in a multivariate context. Combining this method with the fourth-corner analysis bridges the gap between spatial and phylogenetic analyses as it considers the information contained in the three tables E, T, and L and provides an original framework that adapts simultaneously to both spatial and phylogenetic

autocorrelation structure. Here, we perform a simulation study to demonstrate the impact from both phylogenetic and spatial autocorrelations on fourth-corner type I error rates. Then, we build MSR constrained randomizations for both traits and environmental variables in order to address these issues. In Data S1 we provide the code and tutorials required to implement the approach in R (R Development Core Team 2016).

## METHODS

### *Testing the trait–environment relationship with the fourth-corner*

The fourth-corner analysis relies on permutation models to measure the statistical significance of the observed statistic. After calculating the observed statistic (e.g., weighted correlation for quantitative traits and environmental variables) from the original data tables, table L is permuted  $n$  times and for each time a new value is computed for the fourth-corner statistic. The observed statistic is compared to this distribution under the null hypothesis to compute a  $P$ -value (Legendre et al. 1997).

Following the reasoning in Dray and Legendre (2008), ter Braak et al. (2012) demonstrated that two permutation tests are required to truly control for type I error. The first test randomizes species abundances across sites (i.e., permutes entire rows of table L or entire rows of table E). The second test randomizes abundances across species (i.e., permutes entire columns of table L or entire rows of table T). The larger of the two  $P$ -values resulting from these two tests are then taken as the final  $P$ -value to assess significance.

### *Spatially and phylogenetically constrained tests*

Random permutations are valid under the assumption that species and sites are independent statistical units. However, this assumption is violated when spatial and/or phylogenetic autocorrelation occur, requiring alternative randomization procedures. One solution is to generate a distribution of the fourth-corner statistic under a null hypothesis that also preserves the structural properties (i.e., autocorrelation) of the observed data. This changes the null hypothesis from “no link between traits and environment” to “no link between traits and environment *given* the presence of spatial and/or phylogenetic autocorrelations,” providing a testing procedure that controls for type I error rates in the presence of nonindependent data.

In the case of spatial autocorrelation, the original Moran spectral randomization (MSR) method (Wagner and Dray 2015) relies on Moran’s eigenvectors maps (MEM; Dray et al. 2006) to produce random variables that preserve the multi-scale spatial structure and the global autocorrelation of the original data. In the fourth-corner context, we suggest implementing MSR to generate random environmental descriptors under the new null hypothesis, i.e., random variables that preserve the spatial autocorrelation of the original variables (table E) while being generated independently of species distribution (i.e., not linked to L). These MSR replicates will then be used to build a distribution of the fourth-corner statistic (see below) under the null hypothesis and

preserve the level of spatial autocorrelation in environmental variables.

Although MSR was initially developed for spatial variables, we propose extending it to deal with phylogenetically structured data. In its original algorithm, MSR decomposes the variance of a spatial variable onto  $n - 1$  MEMs (i.e., eigenvectors of a spatial weights matrix,  $n$  being the number of sites), which are all used to reproduce the underlying structure of the data (Wagner and Dray 2015). Similarly, it is possible to compute a matrix of phylogenetic proximities (Pavoine et al. 2008) and then use the eigenvectors from this matrix in the MSR algorithm. These eigenvectors can be seen as a generalization of the phylogenetic eigenvectors proposed by Diniz-Filho et al. (1998): whereas the latter are the principal coordinates of the patristic distance matrix, the former can be generated for any kind of phylogenetic proximities (including model based; see Guénard et al. [2013] for more details). The resulting MSR method generates random traits that preserve the multiscale phylogenetic autocorrelation structure of the original one (table T) while being independent of species distribution (i.e., not linked to L). These MSR replicates will then be used to build a distribution of the fourth-corner statistic under the null hypothesis and preserve the level of phylogenetic signal in species traits.

We thus propose incorporating MSR into the standard fourth-corner analysis as follows:

- 1) Calculate the reference fourth-corner statistic value using original data tables (for more details, see Dray and Legendre [2008]).
- 2) Generate random replicates (e.g., 999) of the environmental variable using the MSR algorithm with a given spatial weights matrix. For each replicate, recalculate the fourth-corner statistics using the original trait and species abundance in order to obtain a distribution of the fourth-corner statistic under the null hypothesis that there is no traits–environment link for a given spatial autocorrelation level.
- 3) Compute the associated  $P$ -value by comparing the observed value of the fourth-corner statistic (step 1) with the null distribution obtained in step 2.
- 4) Generate random replicates of the trait (e.g., 999) using the MSR algorithm with a given phylogenetic proximity matrix. For each replicate, recalculate the fourth-corner statistics using the original environmental variable and species abundance in order to obtain a distribution of the fourth-corner statistic under the null hypothesis that there is no traits–environment link for a given phylogenetic signal.
- 5) Compute the associated  $P$ -value by comparing the observed value of the fourth-corner statistic (step 1) with the null distribution obtained in step 4.
- 6) Combine the results of both tests by taking the largest  $P$ -value from steps 3 and 5. This produces the final  $P$ -value for the test.

#### Simulation study

In our simulation design, adapted from Dray and Legendre (2008), we considered scenarios with varying levels of

spatial and phylogenetic autocorrelations. Community data consisted of 50 species across 100 sites, where each species was characterized by two traits, one active and one passive ( $\mathbf{t}_a$  and  $\mathbf{t}_p$ ) and each site was described by two environmental variables, one active and one passive ( $\mathbf{e}_a$  and  $\mathbf{e}_p$ ). The variables  $\mathbf{e}_a$  and  $\mathbf{t}_a$  were considered active in the sense that they drove species abundances using the following Gaussian response model:

$$L_{ij} = h_j \exp \left[ \frac{-(e_{ai} - t_{aj})^2}{2\sigma_j^2} \right]$$

where  $L_{ij}$  represented the abundance of species  $j$  in the  $i$ th site (varying between 0 and  $h_j$ );  $h_j$  was the maximum abundance for species  $j$  (drawn randomly from a uniform distribution between 0.5 to 1);  $e_{ai}$  was the environmental value for site  $i$ ;  $t_{aj}$  was the trait value for the  $j$ th species and corresponded to the species' optimum position along the environmental gradient;  $\sigma_j$  was the tolerance or niche breadth (randomly drawn from a normal distribution of mean of 5 and a standard deviation of 10) for the  $j$ th species. The variables  $\mathbf{e}_p$  and  $\mathbf{t}_p$  were passive in the sense that they had no influence on species abundances.

In order to simulate community data, we generated trait and environmental variables with varying degrees of phylogenetic and spatial autocorrelations, respectively. For each community data set, we simulated a pure-birth stochastic phylogenetic tree (branching rate of 0.05; sim.bdtree function from the *R* package geiger; Harmon et al. 2008). To introduce phylogenetic signal in traits (active and passive), we generated, for each community data set, the evolution of two traits  $\mathbf{t}_{Ba}$  and  $\mathbf{t}_{Bp}$  along the phylogenetic tree under a Brownian motion model using the rTraitCont function from the *R* package ape (Paradis et al. 2004). Both trait values ranged from 0 to 100, by applying  $\mathbf{t}_B = \frac{t_B + \min(t_B)}{\max(t_B)} \times 100$ . Then, the final active trait was computed by  $\mathbf{t}_a = (1 - w)\text{rand}(t_{Ba}) + w t_{Ba}$ , where  $\text{rand}(t_{Ba})$  corresponds to a random permutation of the vector  $\mathbf{t}_{Ba}$  and  $w$  is a coefficient ranging from 0 to 1. Coefficient  $w$  controlled the intensity of phylogenetic signal in the final active trait ( $w = 1$  corresponds to a pure Brownian model whereas  $w = 0$  corresponds to the absence of phylogenetic signal). The formula was also used for  $\mathbf{t}_p$  (replacing  $\mathbf{t}_{Ba}$  by  $\mathbf{t}_{Bp}$ ). In the scenarios described below, we set  $w = 1$  for  $\mathbf{t}_p$  and  $w$  ranged from 0 to 1 (by 0.1 intervals) for  $\mathbf{t}_a$ . For each phylogenetic tree we defined a phylogenetic proximity matrix. Given that our traits were simulated based on a pure Brownian model, we defined the phylogenetic proximity matrix as  $1 - \frac{d_{ij}}{d_{\max}}$ , where  $d_{ij}$  is the patristic distance between species  $i$  and  $j$  and  $d_{\max}$  is the maximum patristic distance between any two tips of the phylogenetic tree. This equation relates intimately to the hypothesis of a Brownian motion model, where proximity linearly decreases with time of convergence (Diniz-Filho et al. 2012).

For the sites, we considered a design where 100 sites are located on a 10 by 10 regular grid and computed the associated spatial weights matrix using row standardization weights specification in the nb2listw function from the spdep package (Bivand et al. 2005). A protocol similar to the traits protocol was used to introduce spatial autocorrelation in environmental variables  $\mathbf{e}_a$  and  $\mathbf{e}_p$ . We simulated two simultaneous

autoregressive random variables  $e_{Aa}$  and  $e_{Ap}$  (see Dray [2011] for more details) with an autoregressive parameter  $\rho = 0.9$  using the function `invIrM` in the `spdep` R package (Bivand et al. 2005). Both environmental variables ranged from 0 to 100, by applying  $e_A = \frac{e_A + |\min(e_A)|}{\max(e_A)} \times 100$ . Then, the final active environmental variable was computed by  $e_a = (1 - w)\text{rand}(e_{Aa}) + we_{Aa}$ , where  $\text{rand}(e_{Aa})$  corresponds to a random permutation of vector  $e_{Aa}$  and  $w$  is a coefficient ranging from 0 to 1. The formula was also used for  $e_p$  (replacing  $e_{Aa}$  by  $e_{Ap}$ ). Coefficient  $w$  controlled for the amount of spatial autocorrelation in the final environmental variables ( $w = 1$  corresponds to a pure simultaneous autoregressive model whereas  $w = 0$  corresponds to the absence of spatial structure). In the scenarios described below, we set  $w = 1$  for  $e_p$ , and  $w$  ranged from 0 to 1 (by 0.1 intervals) for  $e_a$ .

#### Assessing method performance

To compare the performance of the standard fourth-corner and fourth-corner with MSR, we applied both analyses to the same simulated data in order to quantify and compare their respective power and type I error rates. Each scenario corresponded to the analysis of the simulated abundance table with a combination of a trait ( $t_a$  or  $t_p$ ) and an environmental variable ( $e_a$  or  $e_p$ ). As table **L** was generated as a function of  $t_a$  and  $e_a$ , community data ranged from a phylogenetically random and spatially random structure ( $w = 0$ ) to a pure phylogenetically and spatially autocorrelated structure ( $w = 1$ ). For  $w = 1$ , if two sites were spatially close, they shared similarities in their environmental values, therefore they shared similarities in terms of species compositions. Likewise, if two species shared similar trait values due to phylogenetic relatedness, they had similar abundances, regardless of the site considered. Therefore, as the  $w$  increased the variables that generated table **L**, and table **L** itself, will be more phylogenetically and spatially structured. Five scenarios were thereby considered and in total we generated 1000 community data sets per value of  $w$ .

*Scenario  $e_a \leftrightarrow t_a$* —Since species abundances were driven by both variables  $t_a$  and  $e_a$ , we expected to find a significant association between them. This scenario evaluated the statistical power to detect the true association between trait and environmental variables.

*Scenario  $Ne_a \leftrightarrow Nt_a$* —After generating table **L**, we added random normal noise to  $e_a$  and  $t_a$  (mean of 5 and standard deviation of 2) and to table **L** (mean of 0 and standard deviation of 1). We replaced any negative values in table **L** by zeros. This scenario evaluated the statistical power in the presence of random noise to detect the true correlation between trait and environmental variables.

*Scenario  $e_a \leftrightarrow t_p$* —Since trait  $t_p$  did not influence species abundances, any significant association with  $e_a$  was considered as a false positive. This scenario evaluated type I error rates.

*Scenario  $e_p \leftrightarrow t_a$* —Since environmental variable  $e_p$  did not influence species abundances, any significant association with  $t_a$  was considered as a false positive. This scenario evaluated type I error.

*Scenario  $e_p \leftrightarrow t_p$* —Since neither environmental variable  $e_p$  nor trait  $t_p$  influenced species abundances, any significant association between  $e_p$  and  $t_p$  was considered as a false positive. This scenario evaluated type I error rates.

All tests used 999 repetitions for a 0.05 significance level. The fourth-corner analysis is available from the fourth-corner function in the R package `ade4` (Dray and Dufour 2007). We used the `me.phylo` function from the `ade4phylo` R package (Jombart et al. 2010) to calculate phylogenetic Moran's eigenvalues. For MSR, we used the singleton algorithm (Wagner and Dray 2015) as implemented in the `mnr` function in the `adespatial` package (Dray et al. 2016).

## RESULTS

When only active variables were considered, both the standard fourth-corner and the MSR version correctly identified the true associations (Fig. 1a). In contrast, in the presence of random noise on all tables, both methods decreased in power and this loss was higher for the fourth-corner analysis with the MSR algorithm (Fig. 1b). Despite this loss in power, both analyses identified more than 80% of true trait–environment associations.

When passive variables were among the tested variables, so that there was no real trait–environment association, the performance of the standard fourth-corner was strongly affected by spatial and/or phylogenetic autocorrelations. For  $w = 0$  (no spatial structure and no phylogenetic signal in table **L** as values in  $t_a$  and  $e_a$  are independent), rejection rates were always 5% or less for all tests involving  $t_p$  and/or  $e_p$ . However, as the strength of the spatial and phylogenetic autocorrelation increased in  $t_a$  and  $e_a$  (and thus in table **L**), the rejection rates increased (maximum was 42.1% for  $w = 1$  in scenario  $e_p \leftrightarrow t_a$ , Fig. 2a) for all scenarios involving  $t_p$  and/or  $e_p$  indicating inflated type I error (Fig. 2). This was not the case for MSR, as type I error rates were acceptable (around or below 5% threshold) for all scenarios involving at least one passive variable.

## DISCUSSION

In this paper, we investigated the impact of phylogenetic and spatial autocorrelations on the type I error rates for fourth-corner analysis. When abundance data (table **L**) are not affected by spatial and phylogenetic autocorrelation (i.e.,  $w = 0$ ), both the fourth-corner and its MSR version have acceptable type I error rates. However, when abundance data are spatially and phylogenetically structured, but not linked to the environmental variable and/or trait considered in the analysis (scenarios considering a passive variable), the effects of spatial and phylogenetic autocorrelation can be dramatic. The type I error rates increased along with the intensity of autocorrelation. These results support Hurlbert's (1984) warning that the presence of autocorrelation in ecological data may lead to pseudo-replication and inflated type I error rates in statistical tests. Since spatial and phylogenetic autocorrelation commonly occur in ecological data sets, it is quite likely that some trait–environment relationships have been erroneously uncovered and discussed in the past literature.

Here, we proposed a new randomization procedure for the fourth-corner analysis using the MSR method. We

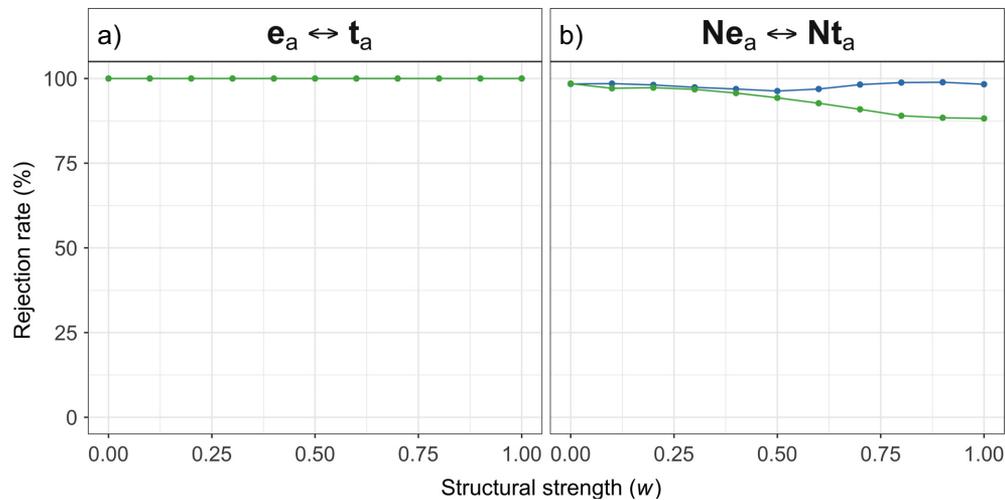


FIG. 1. Power study results for standard fourth-corner (blue) and its Moran spectral randomization (MSR) corrected version (green) according to structural strength ( $w$ ). Panel a shows results with absence of random noise (scenario  $e_a \leftrightarrow t_a$ ) and panel b shows results with presence of random noise (scenario  $Ne_a \leftrightarrow Nt_a$ ) on active variables and table L. Rejection rates were based on a 5% significance level. For each value of  $w$ , we simulated 1000 community data sets.

modified the machinery of the approach, rather than its statistics, such that spatial and phylogenetic structures are now accounted for in the hypotheses testing. In other words, the ecological interpretation remains as described in Dray and Legendre (2008), “the fourth-corner statistic is a measure of the link between the characteristics (traits) of species and their positions along the environmental gradient,” which is now tested while accounting for the spatial and phylogenetic structures of the data.

The fourth-corner analysis is not alone in solving the fourth-corner problem. Regression-based analyses have the potential advantage of not only describing correlations, but also predicting a community’s responses to environmental changes, given by its response to environment, traits and their interaction (Brown et al. 2014). Therefore, these approaches provide a more complete picture of community structure by decomposing the relative effect of each variable.

Nevertheless, regression-based approaches also share the autocorrelation problem highlighted in this paper (ter Braak 2017), and it is here that the fourth-corner analysis has its main methodological advantage. Regression-based approaches that account for spatial and phylogenetic autocorrelations require more advanced statistical models, such as linear mixed models and GLMMs. These require the estimation of random effects, which can be difficult as random effects are nonlinear parameters (Bolker et al. 2009). Recently, Li and Ives (2017) applied phylogenetic linear mixed effect models to account for phylogenetic dependencies between species into the fourth-corner problem, and showed that considering phylogenetic structure as random effects significantly improved type I error rates in comparison to traditional linear mixed effects models. However, their approach did not consider spatial autocorrelation and required the estimation of five variance components (corresponding to random effects for species and sites intercepts, species responses to the environment, phylogenetic relatedness between species intercepts and phylogenetic relatedness of species responses to the environment) for a model with a

single trait and a single environmental variable. Adding more environmental and trait variables would then require more variance components (Jamil et al. 2013), which can be computationally intensive. While Li and Ives (2017) fitted linear mixed models by maximum likelihood methods, Ovaskainen et al. (2017) proposed GLMMs with phylogenetic and spatial covariance matrices using a Bayesian framework to solve the fourth-corner problem. This approach requires the user to specify an appropriate distribution for the abundance data, such as a negative binomial or Tweedie distribution with or without zero inflation. While this is seemingly easy in a Bayesian framework where Markov Chain Monte Carlo sampling replaces numerical integration of random effects, Bayesian approaches have their own challenges. While maximum likelihood methods may fail due to numerical issues (Bolker et al. 2009), Bayesian methods can have convergence issues such as burn-in and poor mixing (Lesaffre and Lawson 2012). Moreover, because any of these approaches need considerable computer time and may fail (Rue et al. 2009), it is difficult to explore variants of models. Also, there are no guarantees that the whole procedure has desirable statistical properties, such as control of false positives as investigated in this paper. As noted in the discussion of ter Braak (2017), even a simple model, such as the negative binomial GLM, requires resampling for statistical inference as the parametric version inference may be inaccurate, whereas GLMMs for binary response also present inferential problems (Engel 1998, Fong et al. 2010). Finally, both Li and Ives (2017) and Ovaskainen et al. (2017) used linear models without addition of (trait-dependent) quadratic (or other) terms to allow for basic ecological niche models. In contrast, Jamil et al. (2014) analyzed trait–environment relationships in the Bayesian framework starting from the Gaussian response model. Despite the great progress reported in these papers, the application of these methods to solve the fourth-corner problem while accounting for both phylogenetic and spatial autocorrelation is still far from being straightforward and easy. The fourth-corner analysis is at

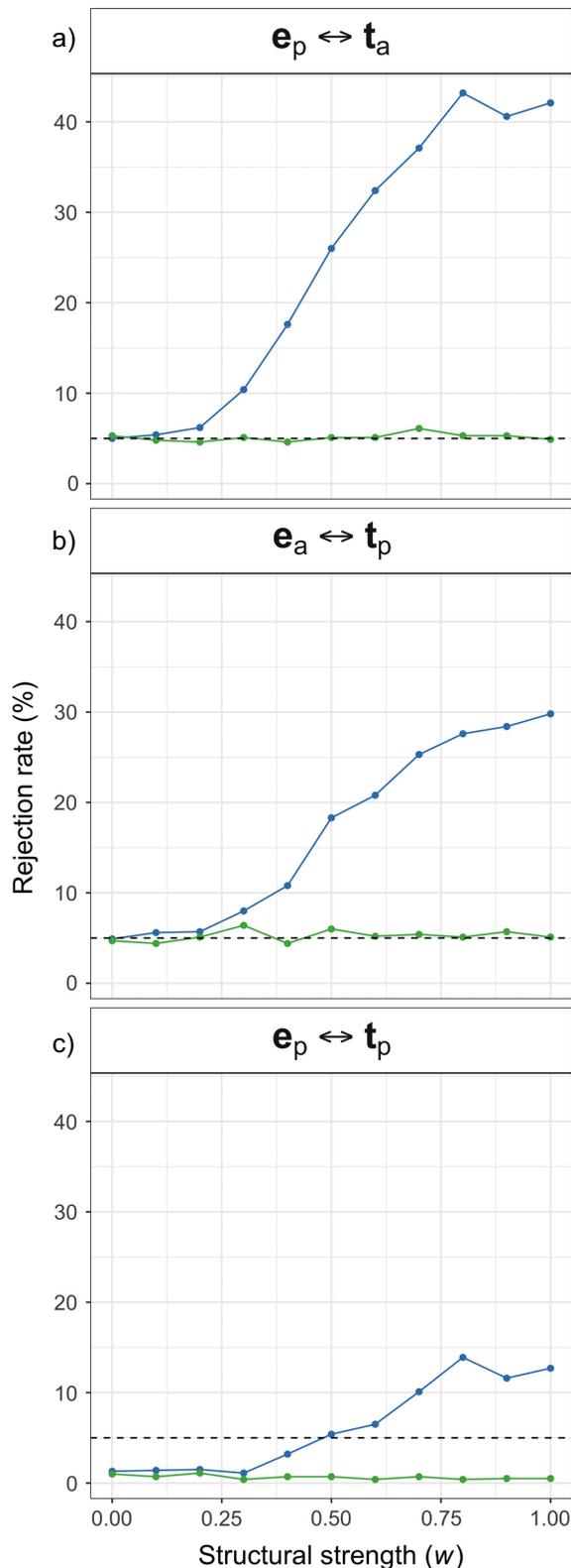


FIG. 2. Type I error rates for standard fourth-corner (blue) and its MSR corrected version (green) according to structural strength ( $w$ ) for scenarios (a)  $e_p \leftrightarrow t_a$ , (b)  $e_a \leftrightarrow t_p$  and (c)  $e_p \leftrightarrow t_p$ . Rejection rates were based on a 5% significance level (shown by the gray dotted line). For each value of  $w$ , we simulated 1000 community data sets.

the simple side of the spectrum of simple to complex methods. Its computational speed allows extensive randomization methods to be applied, as we show here. ter Braak et al. (2017) compared the computational speed of a GLM with Poisson and negative binomial response in the case of a single trait and a single environmental variable and showed that their performances (power and type I error rates) were similar to those of the fourth-corner analysis, but that the computation time was 140 and 1,400 times slower for the GLM. Inclusion of variance components, i.e., using GLMMs, would increase computation times even further. For these reasons, we believe that the fourth-corner is a valid and applicable solution that allows one to incorporate both spatial and phylogenetic structure into a comprehensive testing framework.

It should be noted that the fourth-corner analysis deals only with the case of a single correlation whereas regression-based approaches allow one to consider multiple traits and multiple environmental variables in a single analysis. Solutions for such limitation exist. For instance, the fourth-corner multivariate framework can be extended to multiple traits and multiple environmental variables giving double constrained correspondence analysis (ter Braak et al. 2018). This multivariate approach, which already exists for a long time (Lavelle et al. 1998, 1999), has been revived by ter Braak (2017), who linked the fourth-corner correlation and its multi-trait multi-environmental variable extension to a GLM framework. The randomization methods of this paper have also potential application in this extended context. In summary, our proposed randomization model resolves the issues related to autocorrelated data and the modified version of the fourth-corner analysis controls type I error rates in all situations, including when data are not spatially and phylogenetically structured. Therefore, we advocate the use of this method instead of the standard fourth-corner test in all situations.

The disadvantage of using MSR could be the loss of power in the statistical analyses and our simulation study did indeed identify a small decrease in power (Fig. 1b). As a consequence, this may produce more conservative conclusions. However, the power of the analysis was consistently above acceptable levels (above 80%, Fig. 1).

Our study is the first extension of the MSR method to the phylogenetic autocorrelation context and our results suggest that it is a very flexible way of dealing with issues relating to autocorrelation in various statistical methods. We were able to manage spatial and phylogenetic autocorrelation in a common statistical framework, with minimal parameterization and made it possible to define null distributions for all kinds of statistics (Wagner and Dray 2015). It is important to note that in our simulations we computed MSR using the phylogenetic proximities and spatial weights matrices that were used to generate the data. These results may therefore be overly optimistic compared to real data analysis in which these matrices are unknown. Further work is required to evaluate the influence of misspecifications of proximity matrices in the MSR algorithm, specifically when considering other trait evolutionary models, such as Ornstein-Uhlenbeck and Pagel's  $\lambda$  models. As the MSR approach is very general, it can be extended to other contexts. For instance,

the fourth-corner analysis has been used to understand how species interact in mutualistic and trophic networks (Dehling et al. 2014, Spitz et al. 2014). Our framework can help to properly study interactions taking into account the information contained in two phylogenies. However, one current limitation of MSR is its inability to deal with noncontinuous data, as the original algorithm is only capable of simulating quantitative variables (Wagner and Dray 2015). Further work is required to extend the method to deal with categorical variables, which often occur, especially in trait databases.

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