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LETTER

A framework for metacommunity analysis of phylogenetic structure

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Abstract

It is well known that species evolutionary history plays a crucial role in community assembly. Here, we offer a formal analytical framework to integrate in metacommunity analysis the species' phylogeny with their functional traits and abundances. We define phylogenetic structure of a community as phylogenetically weighted species composition. This is used to reveal patterns of phylogenetic community variation and to measure and test by specified null models the phylogenetic signal at the metacommunity level, which we distinguish from phylogenetic signal at the species pool level. The former indicates that communities more similar in their phylogenetic structure are also similar in their average trait values, which may indicate species' niche conservation for the given traits. We apply this framework to an example from grassland communities and find that traits with significant phylogenetic signal at the metacommunity level exhibit ecological filtering along the resource gradient, but since both mechanisms act independently on traits, niche conservatism is not supported.

Keywords

Assembly rules, disturbance, ecological gradient, functional traits, niche conservatism, phylogenetic signal, phylogeny, soil nitrogen, trait convergence, trait divergence.

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INTRODUCTION

The problem of how communities are structured over space and time is a central issue in ecology. In general terms, community assembly is driven by niche-based habitat, environmental filtering and biotic interactions (e.g. Cornwell & Ackerly 2009). From the niche perspective, community assembly involves two paradoxical trends: species colonizing a site with a particular set of environmental conditions will tend to exhibit similarity for certain phenotypic traits, leading to trait convergence (Keddy 1992; Weiher *et al.* 1998). Nonetheless, ecological similarity may limit species coexistence within a local community; in this case, limiting similarity (MacArthur & Levins 1967) is likely to lead to phenotypic trait divergence. A given set of traits may express both trends simultaneously (Pillar *et al.* 2009). Furthermore, community assembly is also likely to result from spatial dispersal limitation and evolutionary events involving species which make up the regional species pool (Hubbell 2001). Independently of which factor (niche vs. neutral) predominates as the major driver of community assembly process, a topic exhaustively debated by ecologists (Bell *et al.* 2006; Gravel *et al.* 2006; Clark 2008, among many others), the role of the evolutionary history of species in community assembly is crucial for understanding how communities are structured over different spatial and temporal scales, as the regional abundance of species is largely determined by historical factors related to biogeographical and macro-evolutionary processes (Webb *et al.* 2002; Cavender-Bares *et al.* 2009). Together, niche, neutral and historical factors are expected to determine which species will constitute the species pool over a geographical range (γ diversity), as well as the composition of local communities within it (α diversity), and the unevenness of species distribution between communities (β diversity). Disentangling the interplay among these multiple mechanisms in determining biological diversity constitutes a major challenge of ecology.

Studies addressing the spatial variation of species distribution have broadened their scope to encompass phylogenetic aspects of community assembly (Brooks 1985; Losos 1996), and have attracted special interest of ecologists over the last decade (Ackerly 2000; Webb 2000; Silvertown *et al.* 2001; Webb *et al.* 2002; Cavender-Bares *et al.* 2004;

Wiens & Graham 2005; Kembel & Hubbell 2006; Emerson & Gillespie 2008; Johnson & Stinchcombe 2007), including the ecological dimension of phylogenetic β diversity (Bryant *et al.* 2008; Graham & Fine 2008). Communities are composed of species that respond in a highly variable way to present environmental conditions. For this reason, phenotypic traits of species assembled into communities are simultaneously a result of their evolutionary history and their ecological responses to present environment. It is largely expected that phylogenetically close-related species share more similar phenotypic traits among them than with others, more distantly related species. This tendency for phylogenetic conservatism of phenotypic traits among related species (phylogenetic signal, Blomberg & Garland 2002) has been usually measured for a given species pool as the covariation between phylogeny and trait values (Böhning-Gaese & Oberrath 1999; Blomberg & Garland 2002; Blomberg *et al.* 2003; Böhning-Gaese *et al.* 2003; Wiens & Graham 2005; Losos 2008). While phylogenetic signal in phenotypic traits helps us to understand the evolutionary ecology of different species lineages, the major challenge of assessing the extent to which the phylogenetic signal in a set of phenotypic traits affects community assembly processes depends on appropriate scaling-up of the phylogenetic signal to the metacommunity level. A metacommunity refers to a set of local communities that are linked by dispersal of multiple potentially interacting species (Leibold *et al.* 2004).

By doing so, we can adequately evaluate the interplay between phylogenetic (**P**) and ecological trait convergence (**T**) in the assembly of communities subject to environmental gradients (**E**), as recently hypothesized (Webb *et al.* 2002; Silvertown *et al.* 2006a; Silvertown *et al.* 2006b). As (1) similar habitat types tend to select species with close ecological requirements and similar phenotypic traits (a high correlation between **T** and **E**), and (2) species with more similar ancestry tend to show similar phenotypic traits (a high correlation between **T** and **P**), the β niche differentiation among species (that is, variation in species distribution across contrasting habitat types within a geographical range) is expected to be determined mostly by phylogenetically conserved traits (high phylogenetic signal) (Webb *et al.* 2002; Silvertown *et al.* 2006a; Silvertown *et al.* 2006b). Thus, where habitat filtering leads to trait convergence among species along environmental gradients, phylogenetically conserved traits should be favoured. In this case, the correlation between **E** and **T** is mediated by **P**, which can be formally expressed by the following causal model: $\mathbf{E} \rightarrow \mathbf{P} \rightarrow \mathbf{T}$. However, this relationship may not always hold, for it will depend on the traits and gradient being considered. For instance, in plant communities, evolutionary responses to disturbances may involve labile traits, which will not show trait convergence along the gradient (Grime 2006). There may be cases also in which both **E** and **P** are correlated to **T**, but are independent from each other; in this case the causal model connecting phylogeny, traits and environment would be $\mathbf{P} \rightarrow \mathbf{T} \leftarrow \mathbf{E}$.

In a recent paper (Pillar *et al.* 2009), we offered a method for discriminating trait-convergence and trait-divergence assembly patterns along ecological gradients. Analysing trait-based community assembly patterns along environmental gradients permit us to empirically find functional mechanisms explaining β and α niche differentiation. Here, we expand the analytical framework by incorporating the phylogenetic structure of ecological communities. We define phylogenetic structure not as synthetic measurements of phylogenetic clustering or overdispersion within a given community (e.g. Webb 2000; Kembel & Hubbell 2006), as some widely used indices do (Webb 2000; Webb *et al.* 2002). While these indices are useful for many purposes, they cannot be used to reveal complex phylogenetic patterns in metacommunity analysis; for instance, although two communities could show similar levels of phylogenetic clustering, species lineages in each community could be very different. Furthermore, methods suggested to measure community resemblance based on phylogeny often neglect species abundances (Ferrier *et al.* 2007; Bryant *et al.* 2008; Webb *et al.* 2008) or are restricted to a function derived from diversity partitioning as in Hardy & Senterre (2007), who employed Rao's quadratic entropy to compute β phylogenetic diversity of communities pairs, which were then taken as dissimilarities between communities. Mayfield *et al.* (2009) offered a method to evaluate the association between traits, phylogeny and habitat types, but it is restricted to binary traits and binary habitat types. Here, as we explain later, we adopt a more flexible approach in which phylogenetic structure of a community is defined by the species composition after fuzzy weighting (Pillar *et al.* 2009) of their presence or abundance by their phylogenetic similarities. Community resemblance is then computed on their phylogenetic-weighted species compositions.

In this paper, we propose a general analytical framework: (1) to compare communities by their phylogenetic-weighted species composition, (2) to measure phylogenetic signal at the metacommunity level, (3) to identify whether traits responsible for the phylogenetic signal at the metacommunity level are also expressing filtering or trait-divergence assembly patterns along ecological gradients, and (4) to test for phylogenetic niche conservatism in addition to phylogenetic signal. Phylogenetic-weighted species composition of communities allows applying customary analyses with composition data to reveal patterns of phylogenetic community variation. The phylogenetic signal at the metacommunity level is the correlation between the phylogenetic structure of the communities and trait-based assembly patterns, which is tested against appropriate null models. We then apply this framework to experimental data evaluating the effects of grazing and nitrogen addition on grassland communities of south Brazil.

METHODS

Input data

The analysis of phylogenetic assembly patterns and their links to traits and ecological processes (Pillar *et al.* 2009) requires data organized into the following matrices (Fig. 1a): (1) the presence or abundance of species in a set of communities (matrix **W**), (2) the phylogenetic pairwise similarities of these species (matrix $\mathbf{S}_\mathrm{F}$, in the range 0–1), (3) a set of functional traits describing the species (matrix **B**), which may be a mixture of binary and quantitative traits (ordinal, interval, ratio scales), but not nominal ones (these should be expanded into binary traits), and (4) the ecological gradient of interest, which may be one or more factors to which the communities respond or ecosystem effects of the communities (matrix **E**; see more explanation later in the example).

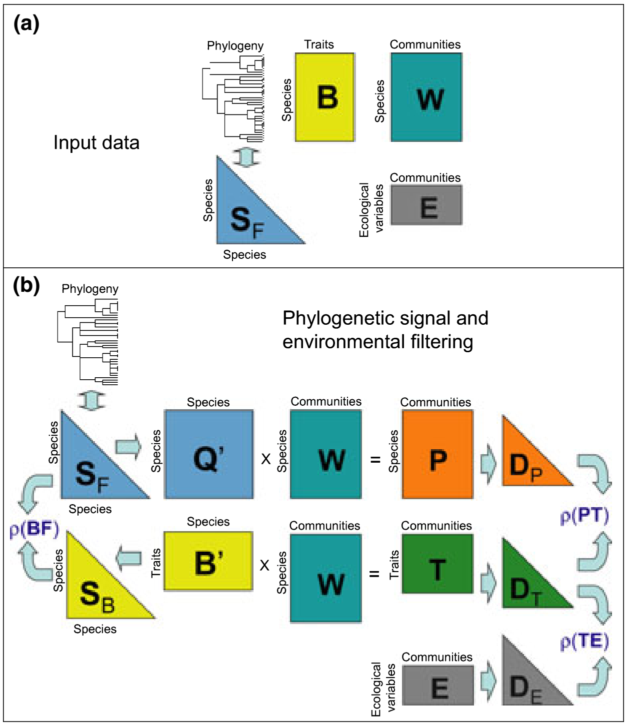

Figure 1 Scaling-up of phylogeny and trait-based data to the community level to reveal phylogenetic signal and trait-convergence assembly patterns related to an ecological filter. Input data matrices in (a) are: **W** with presence or abundance of species in communities, $\mathbf{S}_\mathrm{F}$ with phylogenetic pairwise similarities of species, **B** with traits describing the species, and **E** with the ecological gradient of interest. The outcome in (b) are matrix correlations measuring: ρ(**BF**) phylogenetic signal at the species level, ρ(**PT**) phylogenetic signal at the metacommunity level ($\mathrm{PSM_T}$), and ρ(**TE**) ecological filtering of the traits ($\mathrm{TCAP_E}$). See main text for details.

In the absence of more precise species phylogenetic similarities obtained by sequencing proper DNA regions (Hebert *et al.* 2003; Fazekas *et al.* 2009), phylogenetic relationships between species (matrix $\mathbf{S}_\mathrm{F}$) may be established according to the topology of phylogenetic trees (e.g. APG 2003 for Angiosperms). While such a matrix may not express the real evolutionary distances among species, similarities between taxon pairs are proportional to the number of tree nodes linking them and provide a useful measure expressing the phylogenetic relatedness between plant species (Webb 2000; Webb *et al.* 2002; Swenson 2009).

Scaling-up of phylogeny to the community level

The phylogenetic pairwise similarities in $\mathbf{S}_\mathrm{F}$ are used to define degrees of belonging q_{ij} to fuzzy sets. This is analogous to the definition of functional fuzzy sets (Pillar & Orlóci 1991; Pillar *et al.* 2009, see also Roberts 1986). Based on the phylogenetic similarities, every species i among s species in the metacommunity specifies a fuzzy set to which every species j ($j = 1$ to s species, including species i) belongs with a certain degree of belonging in the interval [0, 1]. In our definition, each row in matrix **Q** with the degrees of belonging must add to unit, i.e., the degrees of belonging of a given species across the fuzzy sets are standardized to unit total by

$$q_{ij} = \frac{s_{ij}}{\sum_{k=1}^{s} s_{ik}}$$

The method is illustrated in Fig. 1b. By matrix multiplication, $\mathbf{P} = \mathbf{Q}'\mathbf{W}$ will contain the composition of the communities after fuzzy weighting of species presence or abundance by the species' phylogenetic similarities. Each column in matrix **P** holds the phylogenetic structure of a community. The standardization of **Q** is essential for the community totals in each column in **W** remaining the same in **P**. Further, matrix **W** may be adjusted to unit column totals prior to multiplication, in which case the total richness or abundance within each community in **W** will be standardized. Matrix **P** can be explored for phylogenetic patterns at the metacommunity level by using, e.g. ordination techniques.

Trait-convergence and trait-divergence assembly patterns

The scaling-up of the trait data in **B** to the community level, so far described by **W**, is needed to relate the traits (or a subset of them) to the phylogenetic structure of communities. Here, we adopt the methods described in Pillar et al. (2009), which allow the identification of trait-convergence and trait-divergence assembly patterns. Trait-convergence assembly patterns (TCAP) may result from community assembly processes related to environmental filtering (Keddy 1992; Weiher *et al.* 1998). For TCAP, by matrix multiplication (see Fig. 1b), we define $\mathbf{T} = \mathbf{B}'\mathbf{W}$, which with previous standardization of $\mathbf{W}$ to unit column totals will contain the trait averages in each community. The elements in $\mathbf{T}$ are community weighted mean values or community functional parameters (Violle *et al.* 2007). Standardization of the traits (rows) in $\mathbf{T}$ is needed, if the trait set contains traits measured with different scales.

Trait-divergence assembly patterns (TDAP Pillar *et al.* 2009) may result from community assembly processes related to biotic interactions (Stubbs & Wilson 2004; Wilson 2007). For the identification of TDAP (see Appendix S1), in a first step the species pairwise similarities (in the range 0–1) in matrix $\mathbf{S}_\mathrm{B}$ based on traits in $\mathbf{B}$ are used to define matrix $\mathbf{U}$ with degrees of belonging of species to fuzzy sets, in a way analogous to the method already described for the scaling-up of phylogeny to the community level. By matrix multiplication, $\mathbf{X} = \mathbf{U}'\mathbf{W}$ will contain the species composition of the communities after fuzzy weighting by their trait similarities (each row in $\mathbf{X}$ will refer to a species). Matrix $\mathbf{X}$ expresses both TCAP and TDAP (Pillar *et al.* 2009).

By using matrix correlation, we evaluate how the trait patterns in $\mathbf{T}$ and $\mathbf{X}$ (TCAP and TDAP) are associated to ecological gradients in $\mathbf{E}$ (see Appendix S1). For relating $\mathbf{T}$ to $\mathbf{E}$, we define a distance matrix of the communities ($\mathbf{D}_\mathrm{T}$) using $\mathbf{T}$, and another distance matrix of the community sites ($\mathbf{D}_\mathrm{E}$) using $\mathbf{E}$. The matrix correlation $\rho(\mathbf{TE}) = \rho(\mathbf{D}_\mathrm{T};\mathbf{D}_\mathrm{E})$ measures the level of congruence between TCAP and $\mathbf{E}$ (Fig. 1b, TCAP$_\mathrm{E}$). A strong correlation $\rho(\mathbf{TE})$ indicates the factors directly or indirectly represented in $\mathbf{E}$ are involved in ecological filtering of species that, at least for the traits considered in the analysis, consistently produce TCAP along the gradient. Adopting a similar procedure, matrix correlation $\rho(\mathbf{XE}) = \rho(\mathbf{D}_\mathrm{X};\mathbf{D}_\mathrm{E})$ between $\mathbf{X}$ and $\mathbf{E}$ is defined. We then remove the trait convergence component $\rho(\mathbf{TE})$ from $\rho(\mathbf{XE})$ by computing the partial matrix (Mantel) correlation $\rho(\mathbf{XE.T})$, which measures the level of congruence between TDAP and $\mathbf{E}$ (TDAP$_\mathrm{E}$, see Appendix S1 and details in Pillar *et al.* 2009). A strong correlation $\rho(\mathbf{XE.T})$ indicates the factors directly or indirectly represented in $\mathbf{E}$ may be important in community assembly rules (Wilson 2007) so that at some point in the ecological gradient limiting similarity arises from species interactions related to the traits considered in the analysis. It is important to clarify that it follows from this definition that the patterns detected by TDAP$_\mathrm{E}$ refer to the metacommunity, while limiting similarity may be restricted to some communities along the gradient. Table 1 summarizes terms and acronyms used in this paper.

Phylogenetic signal at the species pool and metacommunity levels

We define the measure of phylogenetic signal at the species pool level (PSS) as the matrix correlation $\rho(\mathbf{FB}) = \rho(\mathbf{S}_\mathrm{F};\mathbf{S}_\mathrm{B})$ between species phylogenetic similarities (already defined as matrix $\mathbf{S}_\mathrm{F}$) and species trait similarities (already defined as matrix $\mathbf{S}_\mathrm{B}$) computed on any number of traits from matrix $\mathbf{B}$. The species pool refers to the species present in the metacommunity.

We define *phylogenetic signal at the metacommunity level* related to TCAP (PSM$_\mathrm{T}$) as the correlation between the phyloge-

Table 1 Definition of acronyms used in this paper

Acronym	Definition
PSS	*Phylogenetic Signal at the Species pool level* revealed by matrix correlation $\rho(\mathbf{FB}) = \rho(\mathbf{S}_\mathrm{F};\mathbf{S}_\mathrm{B})$ between species phylogenetic similarities ($\mathbf{S}_\mathrm{F}$) and species trait similarities ($\mathbf{S}_\mathrm{B}$).
TCAP	*Trait-Convergence Assembly Patterns* based on community trait averages in matrix $\mathbf{T}$ of traits by communities.
TCAP$_\mathrm{E}$	Environmental filtering revealed by matrix correlation $\rho(\mathbf{TE}) = \rho(\mathbf{D}_\mathrm{T};\mathbf{D}_\mathrm{E})$ between community distances $\mathbf{D}_\mathrm{T}$ based on $\mathbf{T}$ (TCAP) and community distances $\mathbf{D}_\mathrm{E}$ based on environmental variables.
TDAP$_\mathrm{E}$	*Trait-Divergence Assembly Patterns* related to environmental gradient, indicated by partial matrix correlation $\rho(\mathbf{XE.T}) = \rho(\mathbf{D}_\mathrm{X};\mathbf{D}_\mathrm{E}.\mathbf{D}_\mathrm{T})$ between community distances $\mathbf{D}_\mathrm{X}$ computed on species composition after fuzzy weighting by the species' trait similarities, and community distances $\mathbf{D}_\mathrm{E}$ computed on environmental variables, removing the effect of TCAP ($\mathbf{D}_\mathrm{T}$).
PSM$_\mathrm{T}$	*Phylogenetic Signal at the Metacommunity level* related to TCAP, revealed by matrix correlation $\rho(\mathbf{PT}) = \rho(\mathbf{D}_\mathrm{P};\mathbf{D}_\mathrm{T})$ between community distances ($\mathbf{D}_\mathrm{P}$) computed on phylogenetic structure (species composition after fuzzy weighting by the species' phylogenetic similarities) and community distances $\mathbf{D}_\mathrm{T}$ on $\mathbf{T}$ (TCAP).
PSM$_\mathrm{X.T}$	PSM related to trait divergence (TDAP) revealed by partial matrix correlation $\rho(\mathbf{PX.T}) = \rho(\mathbf{D}_\mathrm{P};\mathbf{D}_\mathrm{X}.\mathbf{D}_\mathrm{T})$ between community distances $\mathbf{D}_\mathrm{P}$ computed on phylogenetic structure and community distances $\mathbf{D}_\mathrm{X}$ computed on species composition after fuzzy weighting by the species' trait similarities, removing the effect of TCAP ($\mathbf{D}_\mathrm{T}$).

netic structure described in matrix **P** and the trait convergence structure described in matrix **T**. For this, a proper distance matrix (e.g. Euclidean distances) of communities ($\mathbf{D}_P$) is computed using **P** and another distance matrix of the same communities ($\mathbf{D}_T$) is computed using **T**. Then matrix correlation $\rho(\mathbf{PT}) = \rho(\mathbf{D}_P;\mathbf{D}_T)$ will measure the level of congruence between variation in **P** and **T**, which is a measure of PSM_T. A strong phylogenetic signal at the metacommunity level is expected when communities that are more similar in terms of phylogenetic structure are also similar regarding their average trait values.

We also define *phylogenetic signal at the metacommunity level* related to TDAP ($PSM_{X.T}$) as the partial matrix correlation $\rho(\mathbf{PX.T}) = \rho(\mathbf{D}_P;\mathbf{D}_X.\mathbf{D}_T)$ between community distances $\mathbf{D}_P$ computed on phylogenetic structure and community distances $\mathbf{D}_X$ computed on species composition after fuzzy weighting by the species' trait similarities, removing the effect of TCAP ($\mathbf{D}_T$). This is analogous to $TDAP_E$. A strong $PSM_{X.T}$ indicates phylogenetic structure of the communities is important in community assembly rules associated to biotic interactions.

At last, matrix correlation $\rho(\mathbf{PE}) = \rho(\mathbf{D}_P;\mathbf{D}_E)$ measures the strength of the association between community distances based on their phylogenetic structure in $\mathbf{D}_P$ and environmental distances based on their environmental conditions ($\mathbf{D}_E$).

Null models

We tested by permutation against null models the statistical significance of PSS, PSM_T, $PSM_{X.T}$, $TCAP_E$ and $TDAP_E$. For testing the matrix correlations $\rho(\mathbf{TE})$ and $\rho(\mathbf{XE.T})$ of TCAP and TDAP to the ecological gradient, we followed the methods described in Pillar *et al.* (2009). By analogy, for testing partial matrix correlations $\rho(\mathbf{TE.P})$, needed for assessing phylogenetic niche conservatism (see next section), we adopted the same null model of Pillar *et al.* (2009) for $\rho(\mathbf{TE})$, which is based on permutations among species vectors in matrix **B**, in this case keeping **P** unchanged.

For PSS, the observed matrix correlation $\rho(\mathbf{S}_F;\mathbf{S}_B)$ is tested against a null model predicting that species similarities based on traits are independent from species phylogenetic similarities. The procedure is akin to a Mantel test, i.e., at each random permutation the species vectors are randomly permuted, then one of the similarity matrices involved in $\rho(\mathbf{S}_F;\mathbf{S}_B)$ is rearranged accordingly (say $\mathbf{S}_{F\text{-}RND}$), a new value of $\rho(\mathbf{S}_{F\text{-}RND};\mathbf{S}_B)$ is computed and compared to the observed $\rho(\mathbf{S}_F;\mathbf{S}_B)$, and the probability P for the test will be the proportion of $\rho(\mathbf{S}_{F\text{-}RND};\mathbf{S}_B)$ values not smaller than the observed $\rho(\mathbf{S}_F;\mathbf{S}_B)$ in a large number of permutations (10 000 permutations in our analyses, including the observed data as one permutation).

For testing PSM_T and $PSM_{X.T}$, the observed matrix correlations $\rho(\mathbf{PT})$ and $\rho(\mathbf{PX.T})$ are tested against a null model predicting that phylogenetic structure described in matrix **P** is independent from TCAP that may be present in matrix **T** or TDAP in matrix **X**. The null model retains most of the real data structures except the one that is to be tested. For this, the row vectors (species) of **Q** are randomly permuted among rows, keeping each row vector intact. By matrix multiplication, $\mathbf{P}_{RND} = \mathbf{Q'}_{RND}\mathbf{W}$ defines one possible community composition of the same species weighted by their permuted phylogeny under the null model. The null model preserves any TCAP in **T** and TDAP in **X**, and the intrinsic correlation structure between species in **Q**. As matrix **W** is unchanged, any temporal or spatial autocorrelation is preserved in the null model. At each random permutation, a new value of $\rho(\mathbf{P}_{RND}\mathbf{T})$ or $\rho(\mathbf{P}_{RND}\mathbf{X.T})$ is computed and compared to the observed $\rho(\mathbf{PT})$ or $\rho(\mathbf{PX.T})$. After a large number of permutations, the probability P is computed as already explained for PSS. Analogous procedure is adopted for testing the correlation $\rho(\mathbf{PE})$.

Testing for phylogenetic niche conservatism

Traits or trait combinations expressing both PSM and convergence/divergence patterns were further examined in relation to phylogenetic niche conservatism. For this, the validity of the two alternative models already mentioned in the Introduction section was tested by adopting the d-separation approach proposed by Shipley (2000). The logic is the following: given the path model $\mathbf{E} \rightarrow \mathbf{P} \rightarrow \mathbf{T}$, the causal relationship between **E** and **T** only exists indirectly by the effect of **E** on **P**, indicating phylogenetic niche conservatism. That is, the model being valid, **E** and **T** must be causally independent from each other, if the effect of **P** on that relationship is removed. By translating this statement into statistical language, the partial correlation between **E** and **T**, after controlling the effect of **P**, i.e., $\rho(\mathbf{TE.P})$ must be null (Sokal & Rohlf 1994) to validate the model. The same logic can be applied to the model $\mathbf{P} \rightarrow \mathbf{T} \leftarrow \mathbf{E}$, where both **P** and **E** cause **T**, but in an independent way; in this case, phylogenetic niche conservatism is unlikely to occur. To validate this model, the relationship between **P** and **E**, which can be translated in matrix correlation ($\rho\mathbf{PE}$), must be null. Based on these two matrix correlations, we considered valid the model with higher P-value (Shipley 2000).

Computer program

The analytical framework has been implemented in software SYNCSA, freely available at http://ecoqua.ecologia.ufrgs.br/ecoqua/software.html.

An example from grassland communities

We apply the proposed analytical framework to available trait- and community data we have used previously (Pillar & Sosinski 2003; Pillar *et al.* 2009) from an experiment evaluating the effect of N-fertilizer and grazing levels on natural grassland, located in Eldorado do Sul, Brazil (30°05′52″ S, 51°41′14″ W, 31 m a.s.l.). Fourteen experimental plots were subjected during 5 years to limited combinations of N-fertilizer (0, 30, 100, 170 and 200 kg-N ha^{-1} $year^{-1}$) and grazing levels (4, 6, 9, 12 and 14 kg of forage dry matter per 100 kg of cattle live weight). Each experimental gradient will define matrix **E** in separate analysis. The species found (81 species) were estimated for cover abundance and locally described for traits in quadrats (Appendix S2). The data for matrix **B** refer here to trait averages for each species. All traits were treated as ordinal traits (Podani 1999). For the analysis, the quadrats in each experimental plot were pooled and therefore the columns in matrix **W** refer to the experimental plots.

Phylogenetic relationships between plant species (matrix $\mathbf{S}_F$) were established according to APG (2003). For this, we built up a categorical matrix of plant species by clades (matrix **F**, see Appendix S3), from higher monophyletic groups (e.g. Monocots, Eudicots) to families and genera, and computed species similarities by using the Gower index (Podani 1999), which for nominal variables is equivalent to the simple matching coefficient.

With these data we explored patterns in the phylogenetic structure of the communities by using an ordination technique; in this case we applied PCoA ordination based on Euclidean distances computed on matrix **P**. Then we measured PSS, PSM_T and $PSM_{X.T}$, measured the correlations $TCAP_E$ and $TDAP_E$ to the N and grazing gradients, and interpreted the results on the basis of the corresponding probabilities generated by permutation testing against the specified null models. Finally, we tested alternative causal models supporting or not supporting phylogenetic niche conservatism.

Since we tested phylogenetic signal considering different traits alone or trait combinations, an adjustment of the alpha threshold for multiple comparisons was required. A Bonferroni-type of correction would be too conservative given the traits are not independent. Thus, we adjusted for multiple comparisons using the method described by Manly *et al.* (1986) based on permutation distributions to control family wise error across the tests involved.

RESULTS

Patterns in the phylogenetic structure of the communities are depicted in the ordination diagrams in Appendix S4. The distribution, among the communities, of species belonging to different phylogenetic clades suggests communities under higher levels of nitrogen addition were characterized by Monocot species from Poales and Asparagales, while those under lower levels of N were characterized by species of Oxalidaceae, Fabaceae, Malvaceae and Myrtales. However, the correlation between phylogenetic structure of the communities and the N gradient was weak and not significant [$\rho(\mathbf{PE}) = 0.226$, $P = 0.2386$]. Regarding the grazing gradient, there was no clear phylogenetic pattern (Appendix S4).

The results in Table 2 indicated that four traits (leaf area, length, shape and texture) considered individually showed significant PSS. That is, the variation among species for these traits was significantly correlated to the species phylogeny. Among these traits, only two ones (leaf length and shape) expressed significant PSM_T, i.e., communities that were more similar based on the average traits were also more similar based on their phylogenetic structure. None of the traits taken alone expressed significant $PSM_{X.T}$. It is noteworthy that significant phylogenetic signal at the metacommunity level could only be expressed by traits that showed significant PSS.

Taking into account trait combinations, we found that leaf length and shape combined maximized PSM_T, resulting in a highly significant matrix correlation $\rho(\mathbf{PT}) = 0.830$. In this case also $\rho(\mathbf{PX.T}) = 0.295$, though weaker, was significant at $\alpha = 0.1$. Considering all traits combined, PSM_T was again significant [$\rho(\mathbf{PT}) = 0.586$]. Further, canopy height combined to leaf texture, length and shape maximized $PSM_{X.T}$ showing a significant $\rho(\mathbf{PX.T}) = 0.663$.

The results in Table 2 also allowed for the identification of possible ecological mechanisms associated to PSM_T and $PSM_{X.T}$. Among the traits that when considered individually had significant PSM_T, none showed significant environmental filtering effect for the N gradient, and none were significant for the grazing gradient. It is noteworthy that plant inclination maximized the correlation of trait-divergence assembly patterns ($TDAP_E$) to the N gradient [$\rho(\mathbf{XE.T}) = 0.647$] but did not show any phylogenetic signal.

Considering trait combinations, leaf length and shape combined, which maximized PSM_T, did not show significant environmental filtering effect [$\rho(\mathbf{TE}) = 0.349$]. Also, plant inclination, plant height and leaf length combined, that maximized the correlation of TCAP to the N gradient [$\rho(\mathbf{TE}) = 0.614$] did not show a significant PSM_T [$\rho(\mathbf{PT}) = 0.526$]. Plant height and leaf resistance to tension, which maximized $TCAP_E$ on the grazing gradient, did not show any significant PSM. Also, leaf texture, tension, length and area combined maximized the perception of $TDAP_E$ related to the grazing gradient, but also did not show any significant PSM. Among the traits that revealed significant PSM_T only the combination of plant inclination, canopy

Table 2 Phylogenetic signal at the species pool and metacommunity levels, and the correlations of trait-convergence and trait-divergence assembly patterns to the N and grazing experimental gradients in natural grasslands in south Brazil

	Phylogenetic signal						
	Species pool level	Metacommunity level		Environmental filtering $\rho(\mathbf{TE})$		Trait-divergence assembly patterns $\rho(\mathbf{XE.T})$	
Traits	$\rho(\mathbf{BF})$	$\rho(\mathbf{PT})$	$\rho(\mathbf{PX.T})$	Nitrogen	Grazing	Nitrogen	Grazing
Canopy height (he)	0.044	0.195	−0.049	0.340	0.150	0.151	−0.048
Leaf area (la)	0.058*	−0.237	−0.067	−0.002	0.059	0.014	−0.172
Leaf length (ll)	0.323***	0.710**	−0.034	0.286	−0.218	0.022	0.092
Plant inclination (pi)†	0.030	0.048	−0.006	0.389	−0.061	0.647**	0.014
Leaf shape (sh)	0.436***	0.798***	−0.049	0.334	−0.196	0.254	−0.029
Leaf resistance to traction (ts)	0.033	−0.138	0.139	0.109	0.209	0.100	0.004
Leaf texture (tx)	0.068*	−0.145	0.600	−0.059	−0.127	0.087	−0.125
ll sh‡	0.467***	0.830***	0.295*	0.349	−0.228	0.064	−0.059
tx he ll sh§	0.378***	0.764**	0.663*	0.400	−0.176	−0.168	−0.184
pi he ll¶	0.186***	0.526	−0.002	0.614**		0.182	
pi he ll sh††	0.337***	0.764***	0.267	0.569*		0.176	
he ts‡‡	0.052	0.024	−0.023		0.305*		0.005
tx ts ll la§§	0.155***	0.212	−0.416		−0.079		0.347*
All traits	0.272***	0.586*	0.043	0.537*	−0.128	−0.324	0.033
Adjusted α thresholds for family wise error	0.0012	0.0010	0.0008	0.0013	0.0010	0.0012	0.0012
	0.0070	0.0052	0.0038	0.0070	0.0051	0.0061	0.0057
	0.0149	0.0116	0.0078	0.0155	0.0108	0.0129	0.0117

†Trait subset (in this case one trait) maximizing $\rho(\mathbf{XE.T})$ on the N gradient.
‡Trait subset maximizing $\rho(\mathbf{PT})$.
§Trait subset maximizing $\rho(\mathbf{PX.T})$.
¶Trait subset maximizing $\rho(\mathbf{TE})$ along the N gradient.
††Trait subset maximizing the product of $\rho(\mathbf{PT})$ and $\rho(\mathbf{TE})$ on the N gradient.
‡‡Trait subset maximizing ρ(TE) on the grazing gradient.
§§Trait subset maximizing ρ(XE.T) on the grazing gradient.
*α = 0.10, **α = 0.05, ***α = 0.01.

height, and leaf length and shape, or all traits, also presented significant TCAP_E related to the N gradient.

Since the combination of plant inclination, canopy height, and leaf length and shape revealed a more significant PSM_T, we evaluated causal models for testing niche conservatism only for this trait combination. The comparison of competing causal models (Fig. 2) indicates that the probability for model (b) $\mathbf{P} \rightarrow \mathbf{T} \leftarrow \mathbf{E}$ ($P = 0.2386$) is higher than for model (a) $\mathbf{E} \rightarrow \mathbf{P} \rightarrow \mathbf{T}$ ($P = 0.0017$) and thus we conclude that model (b) should be considered more tenable for the proposed causal relationships.

DISCUSSION

The phylogenetic aspects of community assembly have been intensively studied in the last decade (see e.g. Cavender-Bares *et al.* 2009), but to date a formal analytical framework was not available that could integrate in metacommunity analysis the species phylogeny with species functional traits and abundances. Indeed, our example results demonstrate the utility of the proposed analytical framework for disentangling the links between species' phylogeny and community assembly. The ordination analysis with the grassland data showed some phylogenetic pattern associated to the nitrogen gradient, though not statistically significant, but not to the grazing gradient. We have defined phylogenetic structure of a community as species composition that is fuzzy weighted by the species' phylogenetic similarities (matrix **P**), which may reveal complex phylogenetic patterns in metacommunity analysis. The application of fuzzy set theory in the analysis of ecological communities is not novel (Roberts 1986; Pillar & Orlóci 1991; Pillar *et al.* 2009, and references therein) and here is expanded for the definition of phylogenetic structure. Community structure in this sense is not a synthetic measurement of phylogenetic clustering or overdispersion within a given community as in Webb (2000) and Webb *et al.* (2002), but information that can be further analysed by the customary tools used by ecologists to explore patterns and test hypotheses. Though in this paper we do not examine options other than ordination, based on

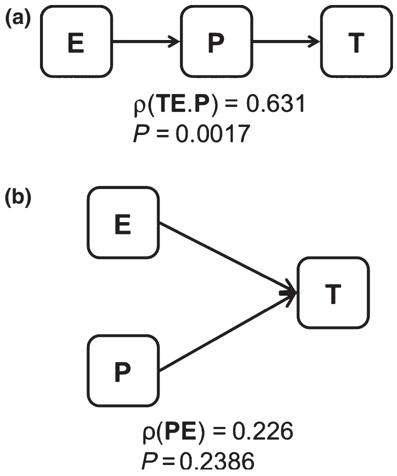

Figure 2 Two alternative models for causal relationships between N-fertilizer experimental levels (**E**), phylogenetic structure (**P**) and trait-convergence assembly patterns (**T**) in natural grasslands in south Brazil. The traits defining **T** are plant inclination, canopy height, and leaf length and shape. *P*-values were obtained by permutation tests (10 000 permutations) against a null model.

matrix **P** we can assess hypotheses about influencing factors on the phylogenetic structure in metacommunities using permutation tests against specifically designed null models, or other methods such as multivariate analysis of variance by permutation testing, Mantel test, and canonical ordination.

Furthermore, by integrating species phylogenies, species functional traits and species abundances in communities, we measure and test against a null model the significance of phylogenetic signal at the metacommunity level (PSM_T and $PSM_{X.T}$), which we distinguish from PSS. Our results with the grassland data indicate that among the seven plant traits examined individually, four of them (leaf area, length, shape and texture) showed significant PSS, but only two (leaf length and shape) had significant PSM_T. We also found that significant PSS is a precondition for significant PSM. A significant PSM_T indicates that communities that are more similar in their phylogenetic structure are also similar in their average trait values. If the assumption that the traits being considered represent functional niche dimensions (Stubbs & Wilson 2004) is true, i.e., they are functional, then the species are niche conserved and, therefore, significant PSM_T could be taken as evidence of phylogenetic niche conservatism (Wiens & Graham 2005) for the given phylogeny. However, as no environmental variable is involved in the analysis at this point, we cannot know what ecological processes are behind the pattern. An important point of discussion is the ambiguity in the distinction made by others between phylogenetic signal and phylogenetic niche conservatism (Wiens & Graham 2005; Losos 2008). Our operational definition of phylogenetic signal at these two levels of biological organization (species pool and metacommunity levels) clearly overcomes the ambiguity.

Although we adopt the term metacommunity to refer to the set of communities involved in the analysis, there is no specific upper limit for the regional scale of a metacommunity as defined by Leibold *et al.* (2004), since any communities can be compared by their species traits and phylogenetic similarities and they may have been connected by dispersal at some point in the past. Therefore, though we do not explore this possibility, the method could be applied to the joint analysis of communities that are located far apart in geographical terms.

The analytical framework we described helps finding possible ecological mechanisms associated to PSM_T and $PSM_{X.T}$. By analysing TCAP and TDAP along environmental gradients (Pillar *et al.* 2009), we can identify, for a given ecological gradient, traits that are involved in community assembly processes related to abiotic environmental filtering and traits that are involved in community assembly processes related to biotic interactions. Our results indicate that only the combination of plant inclination, canopy height, and leaf length and shape, or all traits, are candidates for our testing of phylogenetic niche conservatism, since these traits revealed significant PSM_T and also expressed TCAP (environmental filtering) along the resource availability (nitrogen) gradient. We also found that the same trait combinations did not show any significant TDAP along this gradient.

Some authors (Webb *et al.* 2002; Silvertown *et al.* 2006a; Silvertown *et al.* 2006b) have proposed that β-niches defined by traits expressing convergence along environmental gradients are expected to involve phylogenetically conserved traits. Here, we have an opportunity to objectively test this hypothesis, as significant PSM_T and environmental convergence for these traits could corroborate it. However, the test of the proposed causal models for plant inclination, canopy height, and leaf length and shape along the resource gradient does not corroborate the hypothesis of β-niche phylogenetic conservatism. Both **P** and **E** cause **T**, but in an independent way, in which case phylogenetic niche conservatism is unlikely to occur. Furthermore, the fact that none of the traits expressing TCAP along the disturbance (grazing) gradient presented significant PSM_T supports the hypothesis that evolutionary responses to disturbances involve labile traits, that are more likely to change among phylogenetically close-related plant species, than the traits associated with the ability of plants to exploit productive and unproductive habitats (Grime 2006).

The framework we propose makes possible the evaluation of phylogenetic signal at the metacommunity level, enabling us to assess the ecological effects of phylogenetic structure of communities distributed along ecological gradients, in contrast to other available tools, which deal mainly with within-community phylogenetic structure. Although it is not our aim in this paper to explicitly include historical factors or to consider spatial effects, which may affect phylogenetic structure, our proposed framework may be easily expanded to include these other sources of variation. For instance, well-known variation partitioning methods based on partial canonical ordinations or matrix correlations (Borcard *et al.* 1992; Legendre *et al.* 2005; Tuomisto & Ruokolainen 2006) and path analysis (Duarte *et al.* 2009) could be employed to discriminate multiple factors determining phylogenetic structure in metacommunities.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Scaling-up of trait-based data to the community level to reveal trait-divergence assembly patterns related to ecological variables (Pillar *et al.* 2009).

Appendix S2 Plant traits used for description of species populations in a natural grassland experiment in south Brazil. All traits were taken as ordinal. See Pillar *et al.* (2009) for details.

Appendix S3 (a) Categorical matrix of plant species by clades (matrix **F**) from higher monophyletic groups (e.g. Monocots, Eudicots) to families and genera. Phylogenetic relationships established according to APG (2003). (b) Categorical matrix of plant species by clades [matrix **F**, see (a)] after numerical coding.

Appendix S4 Principal Coordinates Analysis ordination based on the phylogenetic structure of grassland communities in south Brazil under experimental levels of nitrogen (0–200 kg-N ha^{-1} year^{-1}) and grazing intensity (4–14% of forage allowance).

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