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Combining the fourth-corner and the RLQ methods for assessing trait responses to environmental variation

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Abstract. Assessing trait responses to environmental gradients requires the simultaneous analysis of the information contained in three tables: **L** (species distribution across samples), **R** (environmental characteristics of samples), and **Q** (species traits). Among the available methods, the so-called fourth-corner and RLQ methods are two appealing alternatives that provide a direct way to test and estimate trait–environment relationships. Both methods are based on the analysis of the fourth-corner matrix, which crosses traits and environmental variables weighted by species abundances. However, they differ greatly in their outputs: RLQ is a multivariate technique that provides ordination scores to summarize the joint structure among the three tables, whereas the fourth-corner method mainly tests for individual trait–environment relationships (i.e., one trait and one environmental variable at a time). Here, we illustrate how the complementarity between these two methods can be exploited to promote new ecological knowledge and to improve the study of trait–environment relationships. After a short description of each method, we apply them to real ecological data to present their different outputs and provide hints about the gain resulting from their combined use.

Key words: Alps; fourth-corner matrix; functional ecology; permutation procedures; RLQ tables; trait–environment relationship.

Introduction

Recent increasing interest in trait-based approaches has renewed community ecology both on the theoretical (McGill et al. 2006) and the applied side (Vandewalle et al. 2010). By using species traits instead of their identities, these approaches improve the ability to understand the structure and dynamics of ecological communities and potentially predict their response to natural or human disturbances (Keddy 1992, Diaz and Cabido 1997). Functional traits are usually defined as any measurable features at the individual level that directly or indirectly affect overall fitness or performance (e.g., growth, fecundity, survival; Violle et al. 2007). Change in performance might affect demographic characteristics of populations (e.g., birth, death, immigration, emigration), which in turn might affect community structure and dynamics and ecosystem functioning. Among a set of traits, the identification of response traits, i.e., "which vary in response to changes in environmental conditions" (Violle et al. 2007) is a key issue for functional ecology (Bernhardt-Römermann et al. 2008). The methodological challenge associated to this goal relies on the analysis of the information contained in three tables: a table **Q** ($p \times$ s) describing s traits for p species, a table **R** ($n \times m$) with the measurements of m environmental variables in n samples (e.g., plot or site), and a third $n \times p$ table **L** with the abundances (or occurrences) of the p species within n samples. Several approaches have been developed to examine the link among these tables. Some authors (e.g., Pakeman and Marriott 2010) combined **Q** and **L** to build a sample-by-trait table that contains for each sample the (weighted by the species abundances) averages of numerical traits over all species present or the (weighted) frequencies of categorical traits. The link

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between the sample-by-trait and the **R** matrices can then be investigated using a two-table ordination method.

Legendre et al. (1997) and Dolédec et al. (1996) independently developed two methods that consider simultaneously the information contained in tables **R**, **L**, and **Q**: the fourth-corner approach and the RLQ analysis, respectively. Legendre et al. (1997) combined the three original tables into a matrix describing trait–environment associations (the so-called fourth-corner matrix) and proposed statistics and permutation procedures to evaluate the significance of these associations. RLQ analysis extends coinertia analysis (a two-table method, Dolédec and Chessel 1994) to produce a simultaneous ordination of three tables (Dray et al. 2003). Mathematically, it corresponds to the generalized singular value decomposition (e.g., Greenacre 1984) of the fourth-corner matrix.

Today, RLQ analysis and the fourth-corner approach represent the most integrated methods to analyze trait–environment relationships (Kleyer et al. 2012). Even if their mathematical principles are quite similar (both consider the fourth-corner matrix), their objectives (ordination vs. hypothesis testing) and their outputs are quite different. On the one hand, the ordination provided by RLQ analysis assigns scores to species, samples, traits, and environmental variables along orthogonal axes and yields graphical summary of the main structures. On the other hand, the fourth-corner approach measures and tests the multiple associations between one trait and one environmental variable at a time. These differences imply several drawbacks associated either to the RLQ (e.g., only a global test that does not allow identifying which environmental variables is acting on which combination of trait, complexity of the graphical outputs) or to the fourth-corner analyses (e.g., high number of tests, no consideration of the covariation among traits or among environmental variables, no information about samples and species). Here, we propose some methodological adjustments to overcome these drawbacks and to integrate the two approaches into a single framework. We adopt a data-driven presentation and use an ecological example to illustrate each method and show how their combined use improves the analysis of ecological data. We further provide a detailed tutorial (Supplement) guiding users through the new integrated framework conducted using the ade4 package (Dray and Dufour 2007) for the R software (R Core Team 2013).

Ecological Example: Response of Plant Traits to a Snow-Melting Gradient

Choler (2005) examined the functional diversity patterns of alpine plants and tested for a significant relationship between plant functional traits and habitat heterogeneity along a snow melting gradient. Snow cover duration may impact the structure and the dynamics of alpine grasslands through a variety of factors including the length of the favorable period for carbon uptake (Baptist and Choler 2008), the annual variation of soil temperature and soil water content (Campbell et al. 2005), or the disturbance regime by rodents (Choler 2005). The study site was located in the South Western Alps (Lieu-dit Aravo, Commune de Valloire, France; 45.067° N, 6.394° E; see Plate 1). It covers 2 ha between 2700 m and 2750 m elevation. Community composition of vascular plants was determined in 75 5×5 m plots. Each site was described by six environmental variables: mean snowmelt date over the period 1997–1999, slope inclination, aspect, index of microscale landform, index of physical disturbance due to cryoturbation and solifluction, and an index of zoogenic disturbance due to trampling and burrowing activities of the Alpine marmot. All variables are quantitative except the landform and zoogenic disturbance indices that are categorical variables with five and three categories, respectively. Eight quantitative functional traits (i.e., vegetative height, lateral spread, leaf elevation angle, leaf area, leaf thickness, specific leaf area, mass-based leaf nitrogen content, and seed mass) were measured on the 82 most abundant plant species (out of a total of 132 recorded species). See Appendix A for species and variables codes and Choler (2005) for further details on data collection.

Identifying Main Patterns of Variation

Separate ordinations on each table allow characterizing the main environmental gradients (**R**), understanding how species communities are organized (**L**), or identifying trait syndromes (**Q**). Correspondence analysis (CA), which provides a joint ordination of species and samples, is routinely applied to the table **L**. According to the type of variables, **R** and **Q** can be treated by different methods: principal component analysis for quantitative variables, multiple correspondence analysis (Tenenhaus and Young 1985) for qualitative variables or Hill-Smith analysis (Hill and Smith 1976) for a mix of qualitative and quantitative variables. Missing values and other types of variables (e.g., ordinal, circular) can also be considered if the original variables are first transformed into a distance matrix (Pavoine et al. 2009) and then analyzed by a principal coordinate analysis (Vallet et al. 2010).

RLQ combines the three separate analyses of **R**, **L**, and **Q** and aims at identifying the main relationships between environmental gradients and trait syndromes mediated by species abundances. The analysis computes an $s \times m$ matrix $\mathbf{\Omega}$ (see Appendix B) containing measures of the intensity of the link between species traits and environmental variables (Dray and Legendre 2008). The further eigendecomposition of $\mathbf{\Omega}^\top \mathbf{\Omega}$ allows identifying the main associations between traits and environmental variables (see Appendix B, Dray et al. [2002], and Dolédec et al. [1996] for mathematical details). For the first dimension, this analysis finds a vector $\mathbf{u}_1$ containing coefficients for the environmental variables and a vector $\mathbf{v}_1$ of coefficients for the species

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traits. These loadings measure the contributions of individual variables and are used to compute sample ($\mathbf{a}_1 = \mathbf{RD}_m\mathbf{u}_1$) and species scores ($\mathbf{b}_1 = \mathbf{QD}_s\mathbf{v}_1$) where $\mathbf{D}_m$ and $\mathbf{D}_s$ are diagonal matrices of variable weights (see Appendix B). RLQ chooses the coefficient vectors $\mathbf{u}_1$ and $\mathbf{v}_1$ in such a way that the derived sample and species scores have maximum squared cross-covariance $\mathrm{cov}_\mathbf{P}(\mathbf{a}_1, \mathbf{b}_1)^2 = (\mathbf{a}_1^\top \mathbf{P}\mathbf{b}_1)^2 = \lambda_1$ where λ_1 is the first RLQ eigenvalue. In other words, RLQ finds linear combinations of environmental variables (i.e., environmental gradient) and of traits (i.e., trait syndrome) such that their squared cross-covariance is maximum. The same quantity is maximized for the k dimensions with the additional constraints of orthogonality ($\mathbf{u}_i^\top \mathbf{D}_m\mathbf{u}_j = \mathbf{v}_i^\top \mathbf{D}_s\mathbf{v}_j = 0$ for $i \neq j$). Results are stored in matrices $\mathbf{U} = [\mathbf{u}_1 | \cdots | \mathbf{u}_k]$, $\mathbf{V} = [\mathbf{v}_1 | \cdots | \mathbf{v}_k]$, $\mathbf{A} = \mathbf{RD}_m\mathbf{U} = [\mathbf{a}_1 | \cdots | \mathbf{a}_k]$ and $\mathbf{B} = \mathbf{QD}_s\mathbf{V} = [\mathbf{b}_1 | \cdots | \mathbf{b}_k]$.

Ecological application

In our example (response of plant traits to a snow melting gradient), the relationships between traits and environmental variables can be summarized by the first two RLQ axes (86.7% and 9.8% of the cross-covariance between traits and environment for axis 1 and 2, respectively). The left (negative) part of the first RLQ axis identifies species (*Poa supina*, *Alchemilla pentaphyllea*, *or Taraxacum alpinum*; Fig. 1a) with higher specific leaf area (SLA) and mass-based leaf nitrogen content (NMass), lower height, and a reduced seed mass (Fig. 1c). These species were mostly found in late-melting habitats (Fig. 1b). The right part of the axis highlights trait attributes (upright and thick leaves) associated with convex landforms, physically disturbed and mostly early-melting sites. Corresponding species are *Sempervivum*, *montanum*, *Androsace adfinis*, or *Lloydia serotina*. The second RLQ axis outlined zoogenic disturbed sites located in concave slopes. These habitats were characterized by large-leaved species (*Cirsium acaule*, *Geum montanum*, *Alchemilla vulgaris*).

Testing Bivariate Associations

Similarly to RLQ analysis, the fourth-corner method computes an $s \times m$ matrix $\mathbf{\Omega}$ containing measures of trait–environment associations (see details in Legendre et al. [1997] and Dray and Legendre [2008]). While RLQ analysis provides a summary of the multivariate associations, the fourth-corner method allows evaluating the significance of bivariate associations (i.e., one single trait and one single environmental variable at a time) corresponding to cells of $\mathbf{\Omega}$. In other terms, if we consider a table of quantitative variables for which a correlation matrix can be computed, RLQ analysis would be similar to the PCA performed on this table whereas the fourth-corner method could be related to the correlation tests computed for each pair of variables. Since the fourth-corner method considers variables measured on different statistical units (species and samples), appropriate randomization procedures should be used to obtain an adequate testing procedure. Dray and Legendre (2008) showed that none of the procedures proposed by Dolédec et al. (1996) and Legendre et al. (1997) truly controlled the type I error and they proposed an alternative combining two permutation models (see Appendix B for a description of the different models):

1) Model 2: Permute the n samples (i.e., rows of $\mathbf{R}$ or $\mathbf{L}$) to test the null hypothesis that the distribution of species *with fixed traits* is not influenced by the environmental conditions. In other words, the null hypothesis assumes no relationship between $\mathbf{R}$ and $\mathbf{L}$ (given that the $\mathbf{L}$-$\mathbf{Q}$ link is preserved). The alternative hypothesis considers that the environment influences the distribution of species with fixed traits.
2) Model 4: Permute the p species (i.e., rows of $\mathbf{Q}$ or columns of $\mathbf{L}$) to test the null hypothesis that the species composition of samples *with fixed environmental conditions* is not influenced by the species characteristics. In other words, the null hypothesis assumes no relationship between $\mathbf{L}$ and $\mathbf{Q}$ (given that the $\mathbf{R}$-$\mathbf{L}$ link is preserved). The alternative hypothesis considers that the traits influence the composition of species assemblages found in samples with given environmental conditions.

Combining outputs produced by these two models allows testing the null hypothesis that *at least* one table ($\mathbf{R}$ or $\mathbf{Q}$) is not linked to $\mathbf{L}$ against the alternative hypothesis that both traits and environment influence species distributions (i.e., the links $\mathbf{L}$-$\mathbf{Q}$ *and* $\mathbf{R}$-$\mathbf{L}$ are significant). Dray and Legendre (2008) proposed to perform separate tests using Models 2 and 4 with a significance level equal to $\sqrt{\alpha}$ to obtain a global combined test with a significance level α (product of separate significance levels). This combined approach clearly improves the type I error compared to simple permutation models. However, the simulation study carried out by Dray and Legendre (2008) showed that this procedure is slightly liberal when $\mathbf{R}$, $\mathbf{L}$, and $\mathbf{Q}$ are not linked and that the type I error varies between 0.198 and 0.258 (with $\alpha = 0.05$) when $\mathbf{L}$ is only linked to one other table ($\mathbf{R}$ or $\mathbf{Q}$). As an alternative, ter Braak et al. (2012) suggested a sequential test that controls the type I error in all cases. This new test also consists of two steps, but differs conceptually from Dray and Legendre (2008) proposal in that the second test is only performed if the first test rejects the null hypothesis. In practice, both approaches are very similar: the only difference is that separate tests are performed using a significance level α instead of $\sqrt{\alpha}$. Hence, an association between a trait and an environmental variable is considered significant with the sequential approach if the largest of the two P values (obtained from Models 2 and 4) is lower than α. As the sequential test (or equivalently Model 6) fixes the level of type I error, we strongly advocate its use in future applications of the fourth-corner method and use it as the default in the ade4 package.

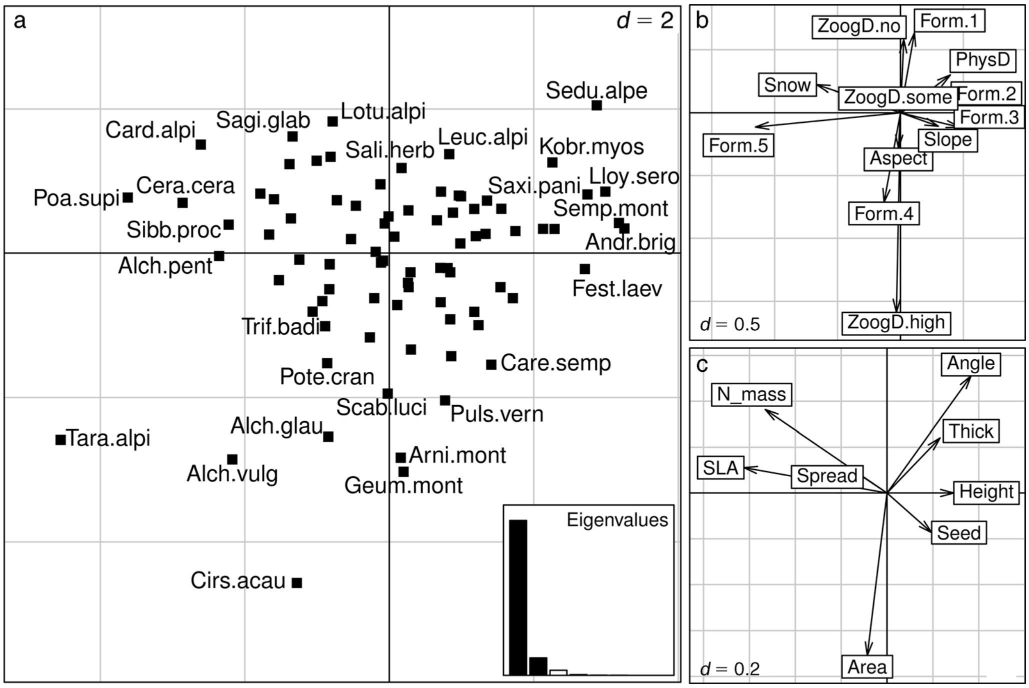

FIG. 1. Results of the first two axes of RLQ analysis: (a) eigenvalues and scores of species (the insert shows eigenvalues, with the first two axes in black), (b) coefficients for environmental variables, and (c) traits. The values of *d* give the grid size. Codes for species and variables are available in Appendix A.

The fourth-corner method only deals with bivariate associations (one trait and one environmental variable at a time) implying that $s \times m$ statistical tests are performed simultaneously. Hence, when more traits (s) and environmental variables (m) are considered, the number of tests increases and it becomes more likely to find "significant" associations. This multiple testing issue was not discussed by Legendre et al. (1997) nor by Dray and Legendre (2008) but testing procedures clearly require an adjustment of P values to control for the overall error rate. In practice, adjusting P values necessarily implies that randomization tests should be performed with a very high number of permutations to detect significant associations. For instance, if we use a Bonferroni correction and test the associations between 10 traits and 10 environmental variables, each individual hypothesis should be tested at a significance level $\alpha/100$ because 100 tests are performed simultaneously. If $\alpha = 0.05$, then we should use a significance level of 0.0005 and 2000 permutations at least are required to obtain a P value of this level.

Ecological application

The fourth-corner method has been used to test the significance of bivariate associations (see Supplement). In this paper, we used 49 999 permutations in all randomization procedures and the false discovery rate method (FDR; Benjamini and Hochberg 1995) to adjust P values for multiple testing. Among the 96 possible associations, 51 were found significant with the original combined (Dray and Legendre 2008) approach. Using the sequential approach (significance level $\alpha = 0.05$), 26 significant associations remained significant. When $\alpha = 0.05$ (sequential approach) and P values are adjusted for multiple testing, 18 significant associations remained significant (Fig. 2). SLA and NMass showed the same trend (positive

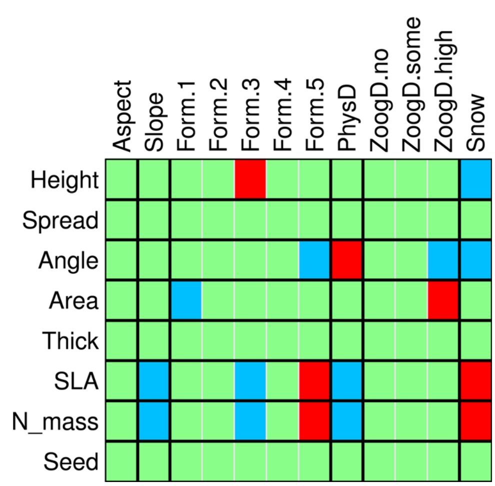

FIG. 2. Results of the fourth-corner tests. Significant ($P < 0.05$) positive associations are represented by red cells, and significant negative associations correspond to blue cells. Nonsignificant associations are in green. Black lines separate different variables; white lines separate different modalities for categorical variables. P values were adjusted for multiple comparisons using the FDR (false discovery rate) procedure. Codes for traits and variables are explained in Appendix A.

correlation with snow (Snow) and landform concavity (Form.5), negative correlation with right slope (Form.3) and physical disturbance (PhysD). This high number of significant tests is linked to the strong snow-melting gradient (also depicted by RLQ axis 1). Other significant bivariate tests could be identified, e.g., the associations between plant height (Height) and right slopes (Form.3), and between leaf area (Area) and zoogenic disturbance (ZoogD.high). This last relationship was indeed described by the RLQ axis 2.

Combining Both RLQ and Fourth-Corner Methods

RLQ and fourth-corner methods have been already used jointly in some trait–environment studies (e.g., Lacourse 2009, Brind'amour et al. 2011). This joint use demonstrates the complementarity of the two approaches to describe multivariate patterns and to test the significance of bivariate associations. However, it also highlights the drawbacks of each method and suggests that using only one approach is not sufficient to interpret ecological results. On one hand, RLQ summarizes multivariate structures but it does not provide significance tests. Moreover, the produced factorial maps could be unreadable when a large number of variables is considered. On the other hand, the fourth-corner only tests the significance of bivariate associations and it does not consider the covariation among traits or among environmental variables. The resulting high number of statistical tests is also difficult to summarize. To take advantage of both methods that share the analysis of a matrix of trait–environment associations, it is important to consider a single framework that allows summarizing and simultaneously testing the main ecological structures. Three approaches can be envisaged to achieve this goal.

First, one can use a multivariate statistic that measures the global association among the three tables **R**, **L**, and **Q**. This statistic is equal to the sum of the slightly modified bivariate fourth-corner statistics over all possible pairs of traits and environmental variables (Dray and Legendre 2008). This statistic also equals the sum of eigenvalues of RLQ analysis as originally proposed by Dolédec et al. (1996). As for bivariate statistics, this multivariate measure should be tested with the sequential testing procedure to avoid inflation of type I error.

Second, an alternative approach consists in representing the results of the fourth-corner tests onto the factorial map produced by the RLQ analysis. In that case, RLQ scores are used to position traits and environmental variables on a biplot and significant associations detected by the fourth-corner tests are depicted by lines. This procedure results in a global representation of the significant links as edges of a correlation network. It has the main advantage of summarizing the results of the two analyses using a single biplot that facilitates the interpretation of ecological structures. However, the approach does not solve all the problems described above because the computation of each analysis is performed separately and their outputs are combined a posteriori.

Last, we propose a new approach that applies the fourth-corner tests directly on the outputs of RLQ analysis. The complete procedure associated to this approach consists of the following:

1) Perform RLQ analysis to summarize the main structures. Select k, the number of dimensions that should be kept for the interpretation, by a visual inspection of the bar plot of RLQ eigenvalues. Compute the sample scores $\mathbf{A} = \mathbf{R}\mathbf{D}_m\mathbf{U}$ (environmental gradients) and species scores $\mathbf{B} = \mathbf{Q}\mathbf{D}_s\mathbf{V}$ (trait syndromes).
2) Apply the fourth-corner tests to evaluate the statistical significance of the associations between traits and environmental gradients (**Q** and **A**) and/or trait syndromes and environmental variables (**B** and **R**). Here, RLQ scores (**A**, **B**) are treated as the variables in the fourth-corner instead of the original raw data tables and thus the testing procedure should be slightly modified. We describe the algorithm only for the case of the associations between traits and environmental gradients (**Q** and **A**) but the same logic is applied for the study of the link between **R** and **B**. The steps are:

   2.1) Compute observed values for the fourth-corner statistics (i.e., bivariate associations between the k RLQ environmental scores and the s traits).

   2.2) Repeat a large number of times (e.g., 999 times).

   2.2.a) Permute the n samples using Model 2, leading to the new table $\mathbf{R}^*$ and recompute scores by multiplying the permuted table and the coefficients matrices **U** ($\mathbf{A}^* = \mathrm{R}^*\mathbf{D}_m\mathbf{U}$). Compute the fourth-corner statistics using the permuted scores $\mathbf{A}^*$ and the original table **Q**.

   2.2.b) Permute the p species using Model 4 leading to the new table $\mathbf{Q}^*$. Compute the fourth-corner statistics using the permuted table $\mathbf{Q}^*$ and the original score **A**.

   2.3) Estimate P values by comparing observed values of the statistics to the distributions of the 999 values obtained under the null models in 2.2.a and 2.2.b. For the association between an environmental gradient and a trait, two P values, P_2 and P_4, are computed corresponding to Models 2 and 4.

   2.4) For a given bivariate association, combine P values of the two models by taking the maximum value between P_2 and P_4.

   2.5) Consider all the $k \times s$ bivariate associations and correct the combined P values using an adjustment method for multiple testing.
3) Represent significant associations between RLQ axes and traits and/or environmental variables on the RLQ factorial map or as a table.

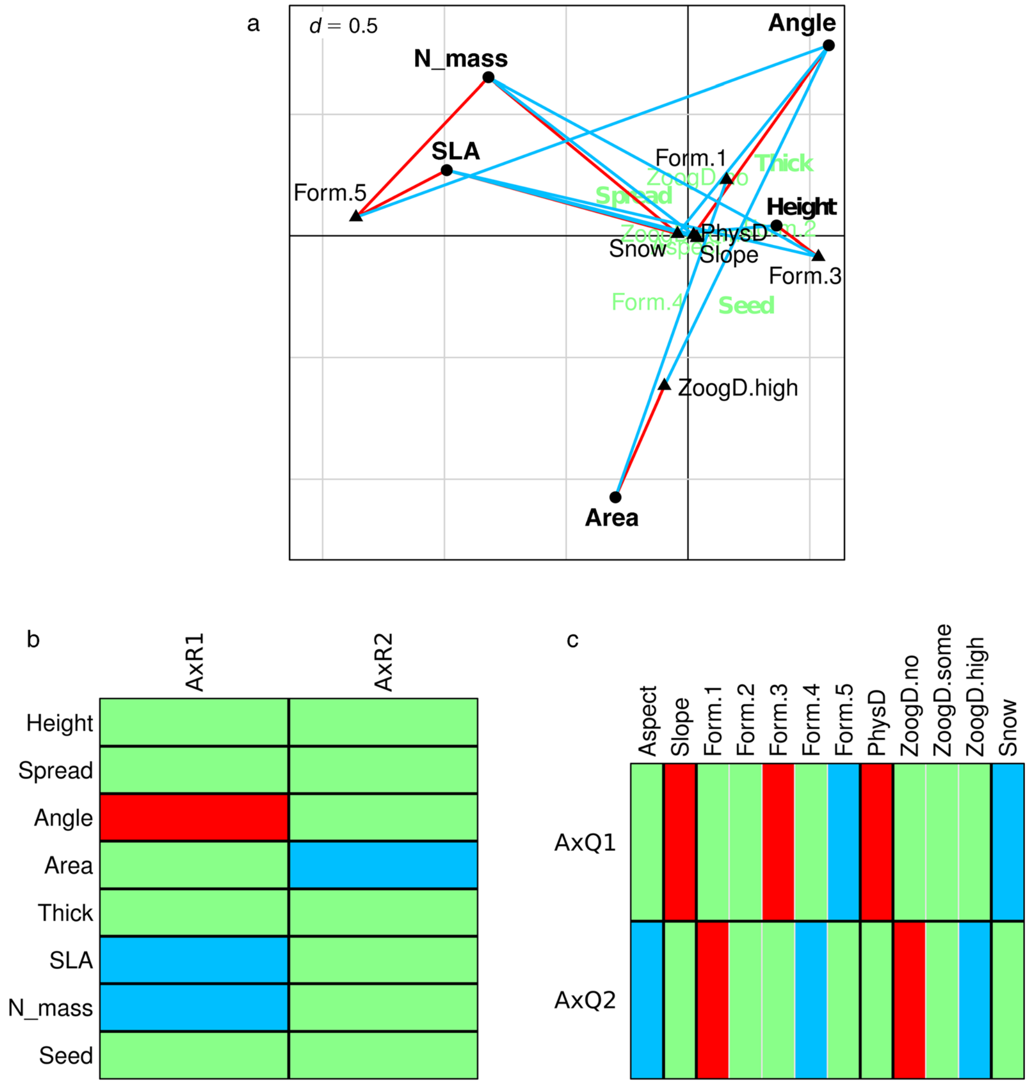

FIG. 3. Combination of fourth-corner and RLQ results. (a) Representation of significant ($P < 0.05$) associations identified by the fourth-corner method on the factorial map of RLQ analysis. The values of d give the grid size. (b) Fourth-corner tests between the first two RLQ axes for environmental gradients (AxR1/AxR2) and traits. (c) Fourth-corner tests between the first two RLQ axes for trait syndromes (AxQ1 and AxQ2) and environmental variables. Positive significant associations are represented by red lines and cells, and negative significant associations by blue lines and cells. In panel (a), traits are in boldface type and are represented by circles; environmental variables are in lightface type and are represented by triangles. In panels (b) and (c), black lines separate different variables; white lines separate different modalities for categorical variables. Variables with no significant associations are shown in green. P values were adjusted for multiple comparisons using the FDR procedure. Codes for traits and variables are explained in Appendix A.

The results of a simulation study (see Appendix C) demonstrate that this new approach has correct type I error rates.

Ecological application

In our example, the global testing procedure (i.e., multivariate statistic equal to the sum of eigenvalues of RLQ analysis) was highly significant ($P = 0.00002$ for both permutation Models 2 and 4 and thus their maximum), indicating a global relationship between species traits and environmental variables. The representation of the significant associations identified by the fourth-corner method onto the RLQ factorial map helps interpreting the main patterns of variation and correlation (Fig. 3a). Compared to the classical RLQ outputs (Fig. 1b and 1c), the interpretation focuses only on traits and environmental variables that are significantly related. Groups of significant positive associations can be identified (e.g., SLA, NMass with snow and concavity, leaf area with high zoogenic disturbance). However, it is much harder to summarize the high number of significant negative associations (blue lines in

PLATE 1. Autumn view of the studied Alpine meadows showing the contrasting mesotopographical situations that control snow cover duration. Flat areas in the foreground with reddish patches of *Salix herbacea* correspond to late snowmelting sites. Early snowmelting sites in the upper slopes and ridges are covered by turf meadows dominated by *Kobresia myosuroides*. The background shows the high summits of the Massif du Grand Galibier, France. Photo credit: P. Choler.

Fig. 3a). Testing directly the associations between RLQ axes and traits/environmental variables clearly improves the interpretation of RLQ and fourth-corner results (Fig. 3b and c). The first axis is significantly negatively correlated with snow cover and concavity (late melting) and positively with physical disturbance and slope (early melting). Associated traits are higher specific leaf area and nitrogen content for late-melting sites and higher angle and plant height for early melting sites. Choler (2005) hypothesized that high leaf angle in the physically disturbed, early-melting habitats limits nocturnal radiative loss of leaf surfaces and ensures a better structural photoprotection against low-temperature photoinhibition. The second axis opposes convex sites with no zoogenic disturbance and concave slopes where marmots are present. Communities found in these disturbed sites have higher leaf area and lower angle. Zoogenic disturbance and milder habitat conditions in the middle part of the mesotopographical gradient may explain the occurrence of large-leaved, light-demanding rosette forbs such as *Geum montanum*, *Alchemilla glaucescens*, or *Arnica montana* (Fig. 1a), a set of species that are more commonly found at lower elevation.

CONCLUSIONS

RLQ and fourth-corner analyses are complementary, and their combined use will allow ecologists to appropriately analyze the response of organism traits to environmental changes. These methods are quite flexible, and several developments can be foreseen. For instance, RLQ has been recently extended to introduce spatial (Brind'amour et al. 2011) and/or phylogenetic (Pavoine et al. 2011) information or to partial out the effects of covariables (Wesuls et al. 2012). Considering these aspects in the fourth-corner testing procedure would allow, among other things, to evaluate how a common evolutionary history (i.e., phylogenetic signal) influences trait–habitat relationships (Ernst et al. 2012).

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Supplemental Material

Appendix A

Description and codes for the variables and species of the plant data set (*Ecological Archives* E095-002-A1).

Appendix B

Detailed description of RLQ and fourth-corner methods (*Ecological Archives* E095-002-A2).

Appendix C

Results of the simulation study (estimation of Type I error) for the new approach that combines the fourth-corner and RLQ methods (*Ecological Archives* E095-002-A3).

Supplement

A tutorial to perform fourth-corner and RLQ analyses in R (*Ecological Archives* E095-002-S1).