

Community trait response to environment: disentangling species turnover vs intraspecific trait variability effects

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Ecological communities and their response to environmental gradients are increasingly being described by various measures of trait composition. Aggregated trait averages (i.e. averages of trait values of constituent species, weighted by species proportions) are popular indices reflecting the functional characteristics of locally dominant species. Because the variation of these indices along environmental gradients can be caused by both species turnover and intraspecific trait variability, it is necessary to disentangle the role of both components to community variability. For quantitative traits, trait averages can be calculated from ‘fixed’ trait values (i.e. a single mean trait value for individual species used for all habitats where the species is found) or trait values for individual species specific to each plot, or habitat, where the species is found. Changes in fixed averages across environments reflect species turnover, while changes in specific traits reflect both species turnover and within-species variability in traits. Here we suggest a practical method (accompanied by a set of R functions) that, by combining ‘fixed’ and ‘specific averages’, disentangles the effect of species turnover, intraspecific trait variability, and their covariation. These effects can be further decomposed into parts ascribed to individual explanatory variables (i.e. treatments or environmental gradients considered). The method is illustrated with a case study from a factorial mowing and fertilization experiment in a meadow in South Bohemia. Results show that the variability decomposition differs markedly among traits studied (height, Specific Leaf Area, Leaf N, P, C concentrations, leaf and stem dry matter content), both according to the relative importance of species turnover and intraspecific variability, and also according to their response to experimental factors. Both the effect of intraspecific trait variability and species turnover must be taken into account when assessing the functional role of community trait structure. Neglecting intraspecific trait variability across habitats often results in underestimating the response of communities to environmental changes.

In the last few years, an increasing body of evidence has highlighted the importance of functional traits as reliable predictors of the ecological functions of communities and their response to environmental gradients (Díaz et al. 2004, Garnier et al. 2007, Lavorel et al. 2008, Fortunel et al. 2009, Pakeman et al. 2009, Shipley 2009). In particular, the traits of dominant species play key roles in the response of communities to the environment (Lepš et al. 1982, Ackerly et al. 2002) and on a wide array of underlying ecosystem processes and services (Garnier et al. 2004, Díaz et al. 2007, Gross et al. 2008). The mass ratio hypothesis (Grime 1998), in particular, states that the traits of dominant species in a community (those which represent most of the biomass in their trophic level) exert a key effect on many ecosystem processes. The dominant traits in a community can be characterized by different measures, such as the weighted trait mean value in a community

(or ‘aggregated trait average’, the average trait value in a community weighted by the relative abundance of the species, or population, carrying each value; Garnier et al. 2004, 2007, Violle et al. 2007, Shipley 2010) or, simply, as the relative abundance of given functional groups (e.g. the abundance of nitrogen-fixing species).

There have been many attempts to analyze the relationships between community trait composition and environmental conditions (Díaz et al. 1999, McInthyre and Lavorel 2001, Ackerly et al. 2002, Dray and Legendre 2008, Moretti et al. 2009 among others). Usually, three data matrices are used. The first is the species \times sample matrix, characterizing species composition in individual samples (the word ‘sample’ will be used here as usual in community ecology, i.e. a [quantified] list of species in an observational unit; statisticians would use the term ‘sampling unit’). The second is a samples \times environmental variables matrix.

The term ‘environmental variables’ denotes here both real environmental characteristics (such as pH), but also type of management, time since last major disturbance etc. (as in CANOCO; ter Braak and Šmilauer 2002). The data can be based on an observational study, but also a manipulative experiment. In the latter case, ‘environmental variables’ reflect more generally any experimental design treatment.

The third matrix includes trait information for each individual species. Most often for single traits, only a single fixed trait value per species is used (i.e. independent from the habitat conditions where the species is found). In this way, community weighted averages are most frequently calculated combining this matrix with the species \times sample matrix. For simplicity, we will use from now onwards the term Fixed average for averages calculated from the fixed, site-independent trait values; similarly, Specific average will be used for averages calculated from site-specific trait values.

$$\text{Fixed average} = \sum_{i=1}^S p_i x_i$$

where p_i is the proportion of the i -th species (e.g. based on biomass, number of individuals, cover etc.) in a given community, S is the number of species in a community, and x_i is the fixed mean trait value of the i -th species for all communities where the species is found.

Although some traits are generally not variable within species (typically, in plants, the life form, ability to fix nitrogen etc.), most quantitative species’ traits are highly variable within species (Westoby et al. 2002, Cornelissen et al. 2003, Albert et al. 2010, Thuiller et al. 2010). Therefore, using fixed trait values neglects completely the extent of intraspecific trait variability across habitats. The variation of trait averages across environments using only ‘fixed’ trait values is then affected solely by changes in species composition. For this reason, it has been proposed to use several values of a trait per species measured in different environmental conditions, i.e. in order to better reflect the properties of community trait averages and community structure in general (Violle et al. 2007, Lavorel et al. 2008). For example, ‘specific averages’ can be calculated using trait values measured under different environmental conditions, as:

$$\text{Specific average} = \sum_{i=1}^S p_i x_{i_habitat}$$

where $x_{i_habitat}$ is the specific mean trait value of the i -th species, which is valid just for a given habitat sampled (different for different habitat types). However, with this last approach, the response of community trait averages can be caused by both changes in species composition and intraspecific trait variability, resulting in increased uncertainty to the real effect of each in the response of communities to environmental changes.

More generally, when studying the response of community traits composition to environmental conditions, we should take into account that changes in trait averages can be caused either by intraspecific trait variability (e.g. species composition remains unchanged, but the individuals of the species are taller in fertile habitats), by a change in species composition (e.g. the height of individuals is constant within a species, but taller species dominate the

fertile habitat), or, most often, by a combination of these two effects. Decomposing the effect of these two sources of community trait composition, therefore, can prove essential to understanding how communities react to environmental changes and what is the specific role of intraspecific trait variability and species turnover on the dynamics of ecosystems. The aim of this paper, therefore, is to propose a new practical method that quantifies and disentangles these effects and demonstrate its simple use with a case study from a semi-natural meadow (Table 1). To the best of our knowledge, we are not aware of any other method that allows the assessment and decomposition of the relevance of both intraspecific trait variability and species turnover on community aggregated averages.

Method description

The method proposed is based on the following principles. If we found differences among habitats in trait averages based on fixed trait values (i.e. ‘fixed averages’), the difference can only be caused by difference in species composition (i.e. species turnover). The difference in averages based on habitat specific values (i.e. ‘specific averages’) can be caused either by differences in species composition or intraspecific trait variation (or by both). The difference between specific and fixed averages can be caused solely by intraspecific trait variation. Our method hence combines together the analyses of the fixed and specific trait values and their difference.

First, a new community parameter is computed to estimate the effects of the intraspecific trait variability, i.e. the differences between ‘fixed’ and ‘specific averages’. Hence, we can define for each individual plot:

$$\begin{aligned} &\text{Intraspecific variability effect} \\ &= \text{Specific average} - \text{Fixed average} \end{aligned}$$

This step makes available three community parameters (fixed and specific averages and their difference). We can use each of them as a single response variable in separate analyses and explain them by the considered environmental factors. For example, in a factorial experiment (as in the case study below), we can run three parallel ANOVAs, one on each of the three parameters. By rejection of null-hypothesis, an analysis demonstrates that environmental factors have an effect on the analyzed parameters. In our case study, the distributional assumptions for the use of parametric tests in ANOVA were fulfilled and we generally expect that the averaging of traits over a community will lead to a close approximation of a normal

Table 1. List of traits used in the analyses and their abbreviations in the figures.

Traits	Abbreviation	Unit
Reproductive plant height	height	cm
Leaf nitrogen concentration	N	mg g ⁻¹
Leaf phosphorus concentration	P	mg g ⁻¹
Leaf carbon concentration	C	mg g ⁻¹
Specific leaf area	SLA	m ² kg ⁻¹
Leaf dry matter content	LDMC	mg g ⁻¹
Stem dry matter content	SDMC	mg g ⁻¹

distribution by the three community-level parameters, but non parametric tests, such as PERMANOVA (Anderson 2001) could also be applied when needed.

Nevertheless, the rejection of null hypotheses is only one (often expected) outcome of the analyses, and the present method can also quantify how much variability can be accounted for by individual components (e.g. species turnover or intraspecific variability alone) and also how much is explained by individual environmental factors. This is based on a Sum of Squares decomposition. The total Sum of Squares (SS) within each of the three ANOVAs corresponds to the total variability in each corresponding component (i.e. within fixed averages, intraspecific trait variability or specific averages). The SS within each ANOVA can be decomposed into the amount of variability explained by individual terms of the model (treatments and their interactions) and the unexplained variability (error), for example, for a factorial design with two factors, $SS_{\text{total}} = SS_{\text{factor1}} + SS_{\text{factor2}} + SS_{\text{factor1} \times \text{factor2}} + SS_{\text{error}}$. It should be noted that quantitative explanatory variables could also be included in the method, as with the decomposition of SS in general linear models (see Discussion). This decomposition of SS_{total} , i.e. within each ANOVA, answers the question of 'how much' each environmental factor accounts for the variation of fixed averages, intraspecific trait variability and specific averages.

Most important, the decomposition of SS can then also be applied across the three ANOVAs. As mentioned above, the variation in specific averages results from addition of the variation in fixed averages and intraspecific variability effects. The 'total' variation of community trait averages is therefore that in the specific averages. Consequently, we take the total variation in specific averages as 100% (i.e. species turnover plus intraspecific trait response). When the turnover and intraspecific effects vary independently, then $SS_{\text{specific}} = SS_{\text{fixed}} + SS_{\text{intraspec.variability}}$ (because variance of the sum of two independent variables is the sum of their variances; with a fixed DF, the same applies for SS). If the two effects are positively correlated (i.e. when high fixed average values are accompanied by positive intraspecific variability effect, and vice versa), then the SS_{specific} will be higher than when the two effects are independent. This is the case if, e.g. the same species will grow taller in sites dominated by genuinely tall species and lower in sites dominated by genuinely low species. When the two effects are negatively correlated the SS_{specific} will be lower than expected. In analogy with the covariance ($\text{var}_{A+B} = \text{var}_A + \text{var}_B + 2 \text{covar}_{A,B}$, i.e. $2 \text{covar}_{A,B} = \text{var}_{A+B} - \text{var}_A - \text{var}_B$), we can define the effect of 'covariation' (which corresponds to $2 \times \text{covariance} \times \text{DF}$) on the total variability (SS_{specific}) as $\text{covSS} = SS_{\text{specific}} - SS_{\text{fixed}} - SS_{\text{intraspec.variability}}$. As mentioned above, this part is negative when the two effects are negatively correlated (and, in this case, SS_{fixed} is bigger than SS_{specific} – so it exceeds 100% of total real variability). Not only the total variation ($SS_{\text{tot_specific}}$) can be decomposed into parts explained by species turnover ($SS_{\text{total_fixed}}$), intraspecific variability ($SS_{\text{total_intra}}$) and their covariation, but this reasoning can be applied also for each individual term of the model (e.g. in a two-way factorial design to SS_{factor1} , SS_{factor2} , $SS_{\text{factor1} \times \text{factor2}}$). The method produces a matrix of SS with four columns – where the SS in the first three columns (i.e. fixed average, intraspecific

variability, and covariation) sum to the fourth column, total variability (specific average) for each of the individual terms in the model (Table 2). Each column consists of rows, corresponding to individual terms of the ANOVA (or GLM) model, a row of unexplained variability (SS_{error}), and finally, a row for their total. If these terms are standardized by total variability (i.e. $SS_{\text{total_specific}}$), we directly obtain proportions of explained variability (Table 2). The complete analysis can be carried out (for any combination of categorical and quantitative predictors) using three S language functions for the R software (R Development Core Team 2008) provided in Supplementary material, together with the data on height used in the Case study. Nested designs (with multiple error levels) are not supported, however.

Case study

The case study used to illustrate this method comes from a long-term project studying the effect of mowing, fertilization, and dominant (*Molinia caerulea*) removal on vegetation structure in semi-natural meadows. The experiment was established in 1994 in a factorial design in a wet meadow in south Bohemia, Czech Republic, central Europe, (see Lepš 1999, 2004 for details). Here we use the part of the experiment without the removal treatment, as used in the VISTA project (Garnier et al. 2007). Three replications of each factorial combination of mowing and fertilization were used. The size of each plot was 2×2 m. In this paper, we use species composition characterized by dry biomass of individual species, estimated in June 2004 in each plot (each sample consists of biomass from two 20×20 cm quadrats pooled together). The traits considered are listed in Table 1 with measurements following the corresponding protocols by Cornelissen et al. (2003; see also Garnier et al. 2007). Trait measures were carried out on those species that represent most of the biomass in each plot (the species selected represented altogether more than 80% of the total biomass in each plot; see Pakemann and Quedstedt 2007) at the peak of the growing season (i.e. before mowing but after fertilizer application, which is added at the beginning of each growing season). Previous analyses from this experiment already showed that potential plant height responds positively to fertilization (Lepš 1999), but the effect of species turnover vs within species variability was not investigated yet.

Traditionally, there is an important decision to be made when computing trait averages, i.e. whether these averages should be weighted or not by species proportion (for discussion, Ackerly et al. 2002, Cingolani et al. 2007, de Bello et al. 2007, Pakeman et al. 2009). Here we used the averages weighted by species biomass as to reflect the role of dominant species on community structure. Otherwise, the same approach could be applied without considering species proportions. Because it is not feasible to take the trait measurement for each plot separately, habitat specific trait values were taken for up to ten individuals for each combination of mowing and fertilization where the species was present (see Discussion). The specific trait values were then calculated separately for each combination of mowing and fertilization where the species was present.

Table 2. Results of two-way ANOVAs for plant height. A) 'Fixed' and 'specific' averages and intraspecific variability effect analysed separately (two-way ANOVA). Note that SS (sum of squares) correspond to the amount of variability. Significant p-values ($p < 0.05$) are in bold while asterisks denote $p < 0.1$. B) Variability of individual components of height variation, and their parts explained by individual factors in the experiment. Note that Turnover, Interspecific variability and Total variation are identical to SS columns in the ANOVA for fixed traits Intraspecific variability and specific traits respectively. Covariation is obtained by subtracting the first two columns from the last. C) Proportions of variability of individual components, and their parts explained by individual factors in the experiment. Note that the matrix is obtained from matrix B by dividing all of its elements by Total SS for Total variation, i.e. for specific traits.

A)

	Fixed					Specific					Intraspecific variability				
	SS	DF	MS	F	p	SS	DF	MS	F	p	SS	DF	MS	F	p
Mowing	57.26	1	57.26	3.672	0.092*	434.67	1	434.67	16.388	0.004	176.42	1	176.41	72.256	<0.00
Fertilization	49.29	1	49.29	3.161	0.113	449.28	1	449.28	16.939	0.003	200.95	1	200.95	82.306	<0.00
Mowing × Fertil	0.16	1	0.16	0.01	0.922	447.63	1	447.63	16.876	0.003	464.69	1	464.69	190.329	<0.00
Error	124.73	8	15.59			212.19	8	26.52			19.53	8	2.44		

B)

	Turnover	Intraspecific variability	Covariation	Total = specific average
Mowing	57.26	176.41	201.00	434.67
Fertil	49.29	200.95	199.04	449.28
Mowing × Fertil	0.16	464.69	−17.22	447.63
Error	124.73	19.53	67.93	212.19
Total	231.43	861.59	450.76	1543.78

C)

	Turnover	Intraspecific variability	Covariation	Total = specific average
Mowing	0.037	0.114	0.130	0.282
Fertil	0.032	0.130	0.129	0.291
Mowing × Fertil	0.000	0.301	−0.011	0.290
Error	0.081	0.013	0.044	0.137
Total	0.150	0.558	0.292	1.000

Results

To better illustrate the method, we show first the detailed analysis for a single trait, i.e. plant height. Then, we illustrate the results for the other six traits.

Plant height

For plant height, the behaviour of specific and fixed averages differs considerably (Table 2). Whereas the effects of treatments on fixed averages are not significant (for main effect, we see just trends with $p \sim 0.1$), both the main effect and their interaction are highly significant for specific averages. Correspondingly, all the main treatment effects and their interaction are highly significant for intraspecific variability effect. In particular, the inclusion of intraspecific variability effect increases considerably the response of vegetation to mowing (Table 2, Fig. 1), for example by enhancing the differences between mown and unmown treatments in unfertilized conditions.

More detailed analyses of the results further help to disentangle the effect of intraspecific variability effect and species turnover on community trait response. With specific trait values, the average of vegetation height is roughly the same in both fertilized plots and unmown unfertilized plots, and is considerably lower in the mown unfertilized plots (i.e. in the separate factorial ANOVA of specific averages alone, both main effects and their interactions are significant [$p < 0.01$]). On the contrary, with fixed values, the trait averages differ much less ($p \sim 0.1$ for both main effects, but $p > 0.9$ for the interaction). The results suggest (if we are willing to interpret the differences at $p \sim 0.1$) slight changes in species composition so that potentially tall plants are supported by fertilization, and harmed by mowing, but a very pronounced effect of intraspecific variability.

Decomposition of total variability in height (i.e. $SS_{\text{tot_specific}}$, Table 2) demonstrates that the among-plot

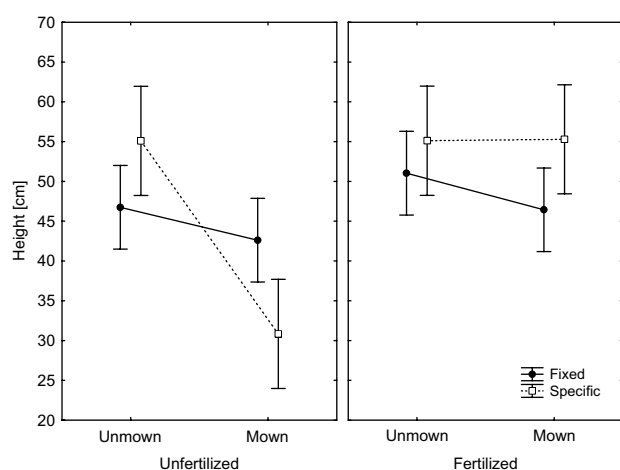


Figure 1. Weighted averages of plant height in the four combinations of mowing and fertilization. The fixed averages are based on species trait values that are constant over treatments, while the specific averages are based on values for a given combination of mowing and fertilization. Error bars denote confidence intervals. The results of the corresponding ANOVAs are given in Table 2.

variability caused by intraspecific variability is nearly four times higher than that caused by species turnover (0.558 vs 0.150). More importantly, however, the effect of total trait variability is well explained by fertilization, mowing, and their interaction (only 0.013 out of 0.558 remained unexplained), whereas the species composition effect is explained very poorly (0.081 out of 0.150 unexplained). The variability in specific averages is further increased by a positive covariation between turnover and intraspecific variability effects (0.291). The scatterplot of the dependence of the two effects (Fig. 2) shows that there is indeed a positive correlation between these two effects (but the relationship is far from linear), particularly on the level of the main effects: fertilization favours genuinely tall plants and also, plants of the same species grow taller in the fertilized plots. Similarly, mowing favours low plants and the same species grow lower in mown plots. The low correlation within the group is probably partially caused by the measurement design of individual traits (see Discussion). Also, the correlation is mainly due to mown unfertilized plots, which have the lowest fixed aggregated average, and also highly negative trait variability values. The strong interaction signifies that only mowing in combination with no fertilization limits plant height – in the mown fertilized plots, individual plants are rather tall – and is caused solely by intraspecific variability (which is in fact slightly decreased by negative covariation).

Comparison across traits

Although there are some common features, the response of trait averages differs considerably among individual traits (Fig. 3). In particular, the variability in height, SLA, and P can be well explained by the experimental treatments (i.e. the unexplained (error) variability is low), while the

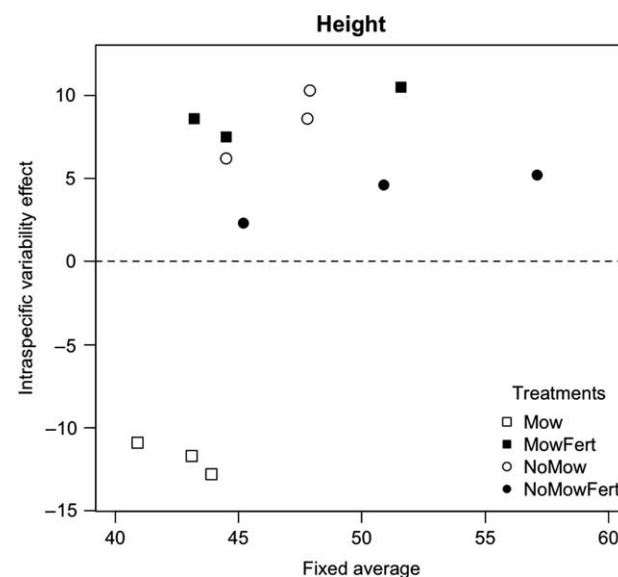


Figure 2. Covariation between the fixed average and intraspecific trait variability (expressed as the difference between specific and fixed average) for height [cm]. Different symbols denote different treatments (Mow = mowing and Fert = fertilization)

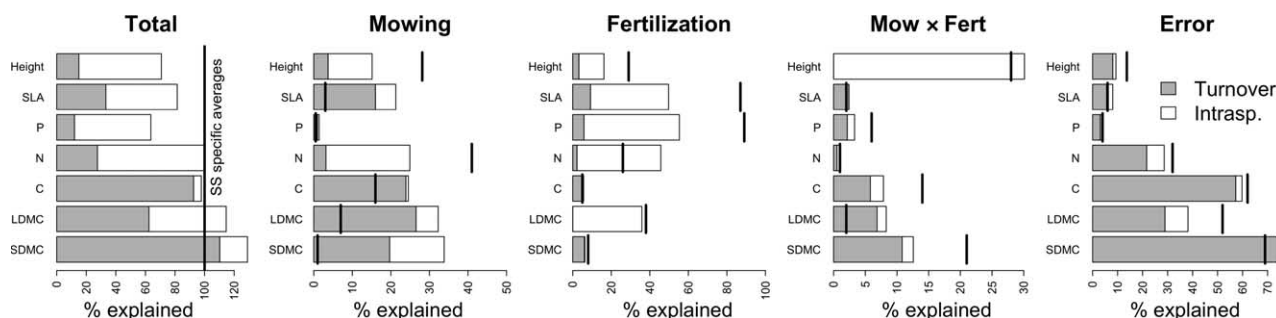


Figure 3. Decomposition of total variability in individual traits studied (for description of traits see Table 1). Grey part of the columns corresponds to species turnover, and open part to intraspecific variability effects. Black bars denote total variation (i.e. variation in specific averages). The space between the top of the column and the bar corresponds to the effect of covariation; if the bar is above the column, the covariation is positive, if the bar crosses the column, the covariation is negative. The values are standardized by total variation in specific averages.

variability in C and particularly SDMC is mostly independent of the treatments. The variability explained by mowing corresponds for most traits to species turnover (for SLA, C, LDMC and SDMC), intraspecific variability prevails only for height and N, while it is negligible for P. On the contrary, the variability explained by fertilization is mainly caused by intraspecific trait variability (with the exceptions of C and SDMC, where the amount of explained variability is negligible). Note that both fertilization and mowing use one df each, so that the variability explained by each of them are directly comparable. In four traits (height, SLA, P, N), intraspecific variability comprises the main part of variation. In three traits (height, SLA, P), the total variation is strongly increased by a positive correlation between fixed traits and intraspecific variability effects; on the contrary, in SDMC, the covariation is negative (Fig. 3).

Discussion

The results of the case study show that all intraspecific trait variability effects, species turnover effects and their covariation need to be assessed when considering the response of trait composition to environment. The effect of species turnover can be more marked along some environmental gradients (here, e.g. with mowing) and in some species traits, while in others the effect of intraspecific trait variability becomes predominant (here, e.g. with fertilization). Neglecting intraspecific trait variability results usually in great underestimation of community trait composition response to environmental variability as intraspecific variability comprises the main part of variation for several traits. This indicates the important risk in using only fixed trait values for species, as for example extracted from a very broad trait database (Cornelissen et al. 2003, Kleyer et al. 2008, Klimešová and de Bello 2009), in the calculation of community trait composition. It also indicates that combining the analyses of fixed and specific trait averages provides a more complete understanding of the response of trait averages to environment.

The combination of different community trait parameters can be also essential to understand in which extent the species turnover and intraspecific trait variability effects reinforce or compensate each other in the selection for dominant trait values in communities. In our case study, the

prevailing positive covariation between the effect of trait variability and species turnover (in the three traits where this covariation is pronounced, i.e. height, SLA, and P) suggests that species turnover and intraspecific trait variability effects select for similar dominant trait values. This implies that the abiotic and biotic factors selecting for dominant species in a community with certain trait values will also affect trait plasticity reinforcing such trait selection effect. For example, in more productive environment (such as fertilized conditions in the case study) the competition for light is expected to prevail leading to dominance of tall species. At the same time, individuals that are able to grow taller will be also selected. This way turnover and intraspecific trait variability effects reinforce each other and this seems to be also supported by the fact that the positive covariation between turnover and intraspecific trait variability effects was found in traits well explained by experimental factors. An opposite effect was found in SDMC, where turnover and intraspecific trait variability effects are likely to compensate each other (i.e. when species with higher SDMC are selected, individuals with lower SDMC are promoted). Therefore, even if some environmental factors act more on the selection of different dominant species (such as mowing here) and others on trait plasticity (as here fertilization), the response of communities can result from the reinforcement or compensation of their effects. These effects remain largely ignored in the literature, leaving uncertainty on the complex response of biological communities to environmental changes.

Despite the apparent advantages of this method, one should also be aware of the possible limitations of the approach. If, for example, there is (nearly) no overlap in species composition across treatments (so that various treatments share a negligible number of species), it is obviously difficult to distinguish the effect of species turnover and intraspecific trait variability. This is typical in studies comparing long environmental gradients over large geographical areas (Garnier et al. 2007, Fortunel et al. 2009, Moretti et al. 2009). Logically, maybe trivially, considering the effect of intraspecific trait variability requires there to be at least some species that occur along a considerable part of the environmental gradient studied (Albert et al. 2010, Thuiller et al. 2010). If, for example, the unfertilized and fertilized plots in our experiment would not share any species, it would be impossible to say

anything about intraspecific trait variability with respect to fertilization, even if this would be occurring. In this case, the analyses would reveal only effects by species turnover although we cannot neglect that species could show different trait values under different environmental conditions. In fact, we can still obtain some results, by using measured trait values in the studied habitats as 'specific' trait values, and database values as 'fixed' values; interpretation of such results must be, however, done extremely carefully as we usually do not know exactly to which habitat the individual database values correspond.

In our example, we used only categorical explanatory variables (mowing and fertilization). The same method (and the same macro in R) could be used when having continuous explanatory variables (e.g. pH, elevation), including the effect of covariation. Analogously to the categorical explanatory variables, the situation when intraspecific trait variation will be affected in the same direction as species composition (e.g. higher elevations would select for genuinely smaller species, and the same species would grow smaller at higher elevations) will be reflected by a positive covariation; in this case, the slope of the specific trait will be steeper than for the fixed one.

Various practical considerations should also be taken into account when applying this method. Measuring traits for all of the species in each plot could prove to be prohibitively laborious (Cornelissen et al. 2003, Cianciaruso et al. 2009). For a moderately sized study, with 25 plots and an average of 30 species per plot, each characterized by ten measured individuals, we would need 7500 measurements for a single trait. The practical solution (used here) would be to consider the trait values as fixed (in fact less variable) under a set of environmental conditions (as for categorical treatments in different experimental approaches; de Bello et al. 2011). However, this might need some further approximations when the plots are set on continuous environmental gradients. As we cannot usually afford to measure values in all of the individual plots, one of the possibilities would be to construct regression models for the dependence of trait values on environmental variables on the basis of measured values in selected plots, and then use the fitted values from the regression models for the other plots. Similar approximations are necessary for all times when it is impossible to measure all individuals in all plots, which occurs in most cases (de Bello et al. 2011). Then, we have always to keep this constraint in mind when interpreting the results. For example, in our case study, the same specific trait values were used for all plots with the same combination of mowing and fertilization treatments. This naturally leads to an underestimation of the error (i.e. within group) interspecific trait variability and its covariation with the fixed effect (indeed, for the majority of traits these values were smaller than $\pm 3\%$). Similar restrictions apply if we estimate the specific trait values by a regression model.

To illustrate the present method we decided to present separate analyses for each trait. It should be noted that it is also possible to analyse all of the traits together using multivariate methods. We applied Redundancy Analysis (RDA) using CANOCO (ter Braak and Šmilauer 2002) for all traits together to specific averages, fixed averages and their difference (results not shown). Because trait behaviour

(response to individual treatments, proportion of species turnover and intraspecific variability) differs very much among traits, we believe that the trait by trait analysis (Fig. 3) provides ecologically more interesting information than would the multivariate total variability decomposition. Nevertheless, in our view, the RDA analyses still provides a useful tool for visualization of individual components of trait responses and particularly of their mutual correlation. As always, the selection of the method depends on the questions we are more interested in.

We believe that our method could be applied to answer different ecological questions (as highlighted in the method description). For example, separating the effect of intraspecific trait variability from those of species turnover alone could, in particular, allow for testing the role of these two components of community structure on different ecological processes (Grime 1998, Díaz et al. 2007). Also, intraspecific trait variability could play a more marked role in the resistance and stability of ecosystems to short-term and low intensity environmental changes, while species turnover should reflect processes linked to the effect of resilience and the marked response of communities to long-term and intense environmental changes.

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Supplementary material (Appendix E6904 at <www.oikos.office.lu.se/appendix>).