

Interspecific trait integration increases with environmental harshness: A case study along a metal toxicity gradient

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Abstract

1. Functional traits are commonly used to calculate a wide array of functional diversity indices to infer different mechanisms of community assembly and species coexistence. Recently, the degree of interspecific covariation between multiple functional traits has been suggested as a mechanism influencing both species distributions and abundances in communities. However, empirical assessments of this theory along environmental gradients are still scarce due to the lack of an appropriate method and of sufficiently strong environmental gradients.
2. Here we compare interspecific trait integration (ITI) across plant communities along a marked gradient of copper toxicity in the soil, using new multivariate and bivariate indices. This was achieved using the range of the eigenvalues of a principal component analysis on the traits of the species in a local community (multivariate ITI index) and the correlations between traits in local communities (bivariate ITI index).
3. We show that the plant metal tolerance strategy (i.e. leaf metal content) is relatively independent from leaf economics, while negatively correlated to plant size. In addition, our results indicate a weak support for the expected general patterns of trait syndromes, such as the 'leaf economics spectrum' or the 'leaf-height-seed', at the whole-community scale. This arises from an increase in multivariate trait integration along the soil copper gradient. The strongest trait integration is caused by an increase in the degree of association between certain traits on metal-rich soils. The multivariate trait integration explains species richness better than other commonly used functional diversity indices.
4. Our study highlights the power of ITI, as well as its complementarity to other functional diversity indices, to investigate the variation in functional strategies and their drivers along environmental gradients. The increase in trait integration with soil metal toxicity in plant communities supports that highly constraining environments select increasingly coordinated sets of functional traits, in turn possibly driving the decrease in species richness. Further studies should assess the generality and underlying physiological mechanisms of such ecological patterns.

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KEYWORDS

community assembly, functional diversity, functional trait coordination, leaf economics spectrum, metal tolerance, species coexistence, toxicity gradient, trait multidimensionality

1 | INTRODUCTION

Plant functional traits (*sensu* Violle et al., 2007) are increasingly used to unravel assembly rules in plant communities. The functional structure of a plant community is generally described in terms of its species' trait mean values and trait dispersion (e.g. Bernard-Verdier et al., 2012). Both are driven by biotic and abiotic filters often linked to dramatic changes in community structure along environmental gradients (Kraft & Ackerly, 2013). However, traits do not vary independently from each other. Interspecific trait integration (ITI, the multivariate covariation between traits among species) might reflect functional trade-offs and constrain the space of functional variation within a community (Tilman, 1988). In a way, it relates to the concept of phenotypic integration, used in evolutionary biology to describe the covariation between phenotypic traits within species (Pigliucci & Preston, 2004).

Covariation patterns among functional traits have been extensively explored across species at large spatial scales. For example, the 'Leaf Economics Spectrum' (LES) has become a keystone concept of plant functional ecology (Wright et al., 2004). All plant species can be ordinated along a continuum of sets of traits: from long-lived organs with slow resource acquisition, to short-lived, fast acquisitive ones. The 'Whole Plant Economic Spectrum' (Reich, 2014) suggests that trait values for leaves, stems and roots should be highly correlated with each other and with relative growth rate, being reducible to a single axis of trait variation. Recently, Díaz et al. (2016) found that two orthogonal functional dimensions—namely size and resource use—could be used to characterize plant functional diversity. The interpretation of community assembly mechanisms often relies on traits of the 'Leaf-Height-Seed' (LHS) scheme (Westoby, 1998), based on the idea that these traits reflect different functional axes and are therefore weakly correlated (Laughlin, Leppert, Moore, & Sieg, 2010). The importance of the multidimensionality of the phenotype has already been emphasized (Laughlin, 2014) and is often considered when investigating community assembly and functioning (e.g. Blonder, 2018; Laliberté & Legendre, 2010).

Although trait covariations have been acknowledged to reflect fundamental functional trade-offs, constraining strategies at large spatial scales, it is still unclear to what extent these covariation patterns hold true along local environmental gradients (Messier, McGill, & Lechowicz, 2010; Wright & Sutton-Grier, 2012). At fine spatial scales, Messier, Lechowicz, McGill, Violle, and Enquist (2017) and Messier, Violle, Enquist, Lechowicz, and McGill (2018) showed that the classical relationships between traits were not conserved in tree saplings communities, either at the interspecific level or at the intrapopulation level. Instead, it was suggested that traits related to different functions can form a network of community-specific trait

interactions. Furthermore, in tropical forests communities, functions such as leaf economics and hydraulics spectrums can be decoupled (Li et al., 2015), hence suggesting many viable trait combinations and a low ITI. Nevertheless, the degree of trait covariation observed at the global scale has also been hypothesized to vary along environmental gradients at fine spatial scales. Trait integration was reported to increase along environmental stress at the community (Chapin III, Autumn, & Pugnaire, 1993), the intraspecific levels (Gianoli, 2004; Waitt & Levin, 1993) or at both (Read, Moorhead, Swenson, Bailey, & Sanders, 2014). As the environmental constraint increases, and because of the limited amount of energy to be allocated to growth, survival and reproduction, plant phenotypes are increasingly constrained by functional trade-offs. Therefore, the multidimensionality of functional traits *per se* could also influence species coexistence (Kraft, Godoy, & Levine, 2015; Laughlin & Messier, 2015).

Along local environmental gradients, ITI has been little investigated in comparison to trait mean values and other indices of functional diversity (see Mouchet, Villéger, Mason, & Mouillot, 2010). Recently, Dwyer and Laughlin (2017) measured the change in trait covariation along an aridity gradient to quantify the effect of environmental filters on functional trade-offs. They showed that the positive trait covariation between vegetative height and seed mass within communities was a better predictor of species richness than other univariate or multivariate indices of functional diversity. Consequently, trait covariation patterns may carry important information about the functional strategies selected along environmental gradient both within and between species and the relevance of ITI to understand the functional assembly of communities should be further investigated in a variety of environments.

In this paper, we explore the variation in ITI in metallophyte (i.e. species growing on metal-rich soils) communities along a metal toxicity gradient in the south of D.R. Congo. These savannas are unique from the floristic and functional points of view: a broad gradient of soil Cu and Co concentration drives a species and life forms turn-over in the communities (Boisson et al., 2020; Delhaye et al., 2016). This results in a decrease in size-related trait values, a change to fast resource acquisition strategies, and high leaf metal contents at the community level, while functional diversity decrease along with increasing soil Cu content (Delhaye et al., 2020). Furthermore, some species have a very narrow niche and are found strictly on soil with high or low Cu content, while others present a broad niche and occur along most of the gradient (Boisson et al., 2020). Yet, very little is known about global trait covariation patterns in the vegetation of metalliferous environments which could provide valuable information on plants' strategies to cope with the excess of metal (Faucon et al., 2016; Lange et al., 2017). Here, we analyse the covariation of functional traits, in the entire plant species pool of a metalliferous ecosystem and in local communities along a Cu content gradient.

We use bivariate and multivariate indices of ITI to ask two specific questions:

1. How do the traits of metallophyte species compare in the general whole plant economics spectrum? We expect to find the well-known relationships observed between traits at the global scale, such as the LES. We also expect a trade-off between plant metal content and other functional axes such as resource acquisition or conservation.
2. Is there an increase in ITI along the gradient of increasing toxicity that could explain the decrease in species richness observed? We expect an increase in functional trade-offs with the increasing soil metal content. We also expect the strong negative and nonlinear relationship between trait integration and species richness predicted by theory (Dwyer & Laughlin, 2017).

2 | MATERIALS AND METHODS

2.1 | Sampling

The study site is located on the north part of Fungurume V hill (S 10°37'00" E 26°17'20"), a 3.3 ha site situated approximately 200 km WNW of Lubumbashi in the D.R. Congo (Séleck et al., 2013). The climate of the region is subtropical with a marked dry season (May–September) and with annual rainfall of 1,300 mm concentrated during the rainy season (November–April). The hill displays a strong gradient with soil Cu and Co concentrations increasing from the base to the top (92–6,736 mg/kg Cu and 10–927 mg/kg Co, respectively; acetate–EDTA extractible concentrations). Both concentrations are highly correlated ($r = 0.92$), representing a single, strong gradient of metal toxicity (Delhaye et al., 2020). Community composition and soil data were extracted from Séleck et al. (2013). Briefly, eighty-four 1-m² plots were installed, separated from each other by an elevation of 5 m, along six parallel transects from the base to the top of the hill. In each plot, the relative soil cover of each species was evaluated, and a composite soil sample was collected. In the present study, we use the acetate–EDTA extractible concentration of soil Cu as the gradient along which the ITI is studied. Reasons for this are that soil Cu contributed the most to the first axis of a PCA of all

soil variables, and it constitutes a steep gradient of metal toxicity and rockiness (Delhaye et al., 2020), which, in turn, drives the composition of communities (Séleck et al., 2013) and species turn-over (Boisson et al., 2020).

For the analyses, we ordered the 84 plots according to their soil Cu content and dissected the gradient into 21 groups of four plots each. The resulting 21 composite communities prevent mono-dominance of large-stature species or local extreme stochasticity in species composition due to the size of the plots. In all communities, all traits were available for at least 18 species (see further). Soil Cu content value of each composite community was calculated as the mean of the four plots. For functional trait measurements, we selected 65 abundant and representative species of the copper flora from the Katangan Copperbelt (see <http://www.copperflora.org>). This sample encompasses all dominant species, accounting for more than 90% of the total cover in each plot (Pakeman & Queded, 2007) but also some rare species, as these have been shown to present original and complementary functional trait combinations (Violle et al., 2017). Eight functional traits (vegetative height, leaf area, rooting depth, seed mass, specific leaf area, leaf dry matter, leaf Cu and Co contents) were measured for each species on 5–30 individuals distributed along the whole species-specific range of the Cu gradient (Boisson et al., 2020), following standardized protocols (Pérez-Harguindeguy et al., 2013 and see Appendix S1 for detailed trait measurement procedure). These traits (Table 1) encompassed the LHS strategy scheme (Westoby, 1998) as well as the LES (Wright et al., 2004), and leaf metal content related to the metal tolerance strategy (Baker, 1981). Traits values were averaged at the species level as intraspecific variation in this ecosystem has been showed to be low compared to interspecific differences (Delhaye et al., 2016).

2.2 | Statistical analyses

In order to achieve a normal distribution, LA, SLA, SM, Cu_{leaf} and Co_{leaf} were log transformed while VH and LDMC were square root transformed. All traits were standardized to a mean of 0 and a unit standard deviation prior to analysis. We investigated the relationships between traits using a principal component analysis (PCA) of the averaged trait values per species.

TABLE 1 Description of the functional traits averaged at the species level, with minimum (Min) and maximum (Max) values

Functional axis	Trait	Abbreviation	Unit	Min value	Max value
Size, dominance, resources acquisition	Vegetative height	VH	cm	0.4	81
	Leaf area	LA	mm ²	24	15,537
	Rooting depth	RD	cm	1	150
Leaf economic spectrum	Specific leaf area	SLA	m ² /kg	4	58
	Leaf dry matter content	LDMC	% mass	9	62
Reproduction	Seed mass	SM	g	2.10 ⁻⁵	0.11
Metal tolerance strategy	Leaf Cu content	Cu _{leaf}	mg/kg	2.2	1,918
	Leaf Co content	Co _{leaf}	mg/kg	0	1,693

To explore the variation in ITI along the gradient, we computed two multivariate ITI indices for each community. These indices represent the degree of association between traits. To investigate linear as well as nonlinear monotonous relationships between traits, traits values were replaced by their rank in each community (i.e. method analogous to the calculation of Spearman rank correlation). On these transformed values, we computed 21 separate PCAs using all traits for each community ($n = 21$). The two ITI indices were calculated as follows: (a) ITI_{range} —the range of the eigenvalues of each PCA (i.e. the difference between the eigenvalues of the first and the one of the last principal component axes) and (b) ITI_{sd} —the standard deviation of the eigenvalues of each PCA (Cheverud, Wagner, & Dow, 1989; Laughlin et al., 2017). Since ordination eigenvalues are proportional to the overall variation of the trait correlation matrix described by the corresponding axes, two contrasting situations can be expected: (a) if the first or first few axes capture much more variation than the others, hence indicating the presence of a strong trait integration, a high value of the ITI indices will be obtained and (b) in contrast, if the overall variation of the trait matrix is evenly distributed over the PCA axes, indicating little or no ITI, a low value of the index will be obtained. Consequently, the indices are expected to increase as the integration of traits increases. Adams and Collyer (2016) noted that the comparison of phenotypic integration between groups of samples is likely to be biased by the number of samples (i.e. species) used. To avoid this bias, we calculated the standardized effect size of the ITI indices. For each community, we calculated the observed value of both indices (ITI_{obs}). Then, we created a null distribution of the indices by sampling 1,000 random communities from the whole species pool, constraining species richness to be maintained at the same value than in the local community. Then, we calculated the mean ($\text{mean } ITI_{\text{null}}$) and standard deviation ($SD \text{ } ITI_{\text{null}}$) of the 1,000 null communities and calculated the standardized effect size as:

$$\text{sesITI} = \frac{ITI_{\text{obs}} - \text{mean } ITI_{\text{null}}}{SD \text{ } ITI_{\text{null}}}$$

This ensured avoiding any sample-size-related bias while keeping the structure of the community intact (Torices & Muñoz-Pajares, 2015). For comparison purposes, we also computed two other commonly measured multivariate functional diversity indices independent from species richness in each local community: the standardized effect sizes of functional richness (sesFRic) and functional dispersion (sesFDis ; Laliberté & Legendre, 2010; Mason, Bello, Mouillot, Pavoine, & Dray, 2013; Villéger, Mason, & Mouillot, 2008; Appendix S2 for detailed methods). The variation of sesITI , sesFRic and sesFDis along the soil Cu gradient was evaluated with Spearman's rank correlation coefficients. We also used Spearman's rank correlation coefficients to test the strength of the association between the different indices and local community species richness.

To investigate the bivariate relationships between traits within each community, we computed a bivariate ITI index. For each pair of traits and in each community, a Spearman rank correlation coefficient was calculated. To avoid any potential bias related to the

different numbers of species among communities, each coefficient was calculated on 1,000 subsamples of 18 species (i.e. lowest species richness among the local communities), weighting the probability to select a species by its relative abundance in the local community, and was then averaged for each community.

We tested for monotonous trends of the bivariate ITI index along the soil Cu content using Spearman rank correlation. A Šidák correction was used to adjust p values for multiple tests (Šidák, 1967), changing the significance threshold from 0.05 to 0.002. All analyses were carried out using the R software (R Core Team, 2019).

3 | RESULTS

3.1 | Interspecific traits integration

For the PCA including all traits and species, the first three principal components (PC) accounted for 38%, 20% and 12% of the dataset total variance (Figure 1; Table S1 for loadings on axes and eigenvalues). The first PC was highly positively correlated with Cu_{leaf} and negatively correlated with SM and VH. The second PC was highly positively correlated with LDMC and the third PC was positively correlated to SLA and Co_{leaf} .

Correlations between traits were generally weak (see Table S2). However, a few traits were consistently correlated within the whole species pool. Plant height was negatively correlated to Cu_{leaf} , Co_{leaf} and SLA ($\rho = -0.58$, -0.5 and -0.41 , respectively), confirming that an integrated set of traits reflects a trade-off between size, and metal accumulation coupled with a fast growth. Leaf area and

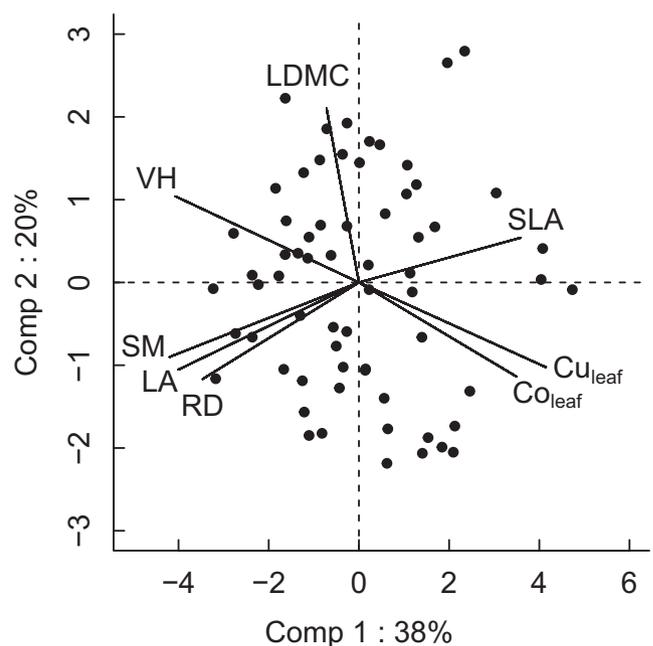


FIGURE 1 Principal component analysis of eight functional traits in the 65 species of the study site. Traits values are averaged at the species level. See Table 1 for abbreviations and Table S1 for loadings

rooting depth were positively correlated to one another ($\rho = 0.41$) and to seed mass ($\rho = 0.54$ and 0.46) while negatively correlated to SLA ($\rho = -0.4$). Cu_{leaf} and Co_{leaf} were also significantly correlated ($\rho = 0.52$), reflecting the simultaneous tolerance of high foliar levels for both metals. Interestingly, we found no significant correlations between traits expected to be related to a same function such as plant size (plant height and leaf area or rooting depth) or LES (SLA and LDMC: $\rho = -0.04$).

3.2 | Variation of traits integration along the Cu gradient

Both multivariate ITI indices highlighted a sharp increase in phenotypic integration along the Cu gradient (sesITI_{range} $\rho = 0.88$, $p < 0.001$, Figure 2a; sesITI_{sd} $\rho = 0.78$, $p < 0.001$, Figure S1). In comparison, both functional diversity indices decreased moderately along the gradient (sesFRic $\rho = -0.49$, $p = 0.03$; Figure 2c, sesFDIs $\rho = -0.45$, $p = 0.04$; Figure 2e). Both ITI were strongly negatively correlated with species richness (sesITI_{range} $\rho = -0.72$, $p < 0.001$, Figure 2b; sesITI_{sd} $\rho = -0.60$, $p = 0.004$; Figure S1), unlike the two functional diversity indices that showed a much weaker correlation

(sesFRic: $\rho = 0.30$, $p = 0.19$; Figure 2d, sesFDIs: $\rho = 0.47$, $p = 0.03$; Figure 2f).

Variation in the 28 pairwise trait correlations along the Cu gradient revealed the trait correlations driving the shifts in trait integration (Figure 3). After adjustment of the p values for multiple tests, six pairs of traits showed a significant increase in their association—both positive and negative—along the soil Cu content gradient. Notably, there was an increase in the positive association between vegetative height with rooting depth and leaf area, and between SLA and Cu_{leaf} . There was an increasingly negative association between vegetative height and SLA and between leaf area and SLA and Cu_{leaf} .

Two traits associations (seed mass with leaf area and LDMC) decreased as soil Cu content increased (correlation coefficient getting closer to zero as soil Cu content increases). The correlation coefficient between Cu_{leaf} and rooting depth changes from positive to negative, but with small, non-significant values.

Many trait associations were constant along the whole gradient, confirming the correlations observed within the whole species pool. Among the traits showing the strongest association, Cu_{leaf} and Co_{leaf} were highly positively correlated, and both negatively correlated with vegetative height. Rooting depth was positively correlated with leaf area and seed mass and negatively with SLA. Finally, some trait

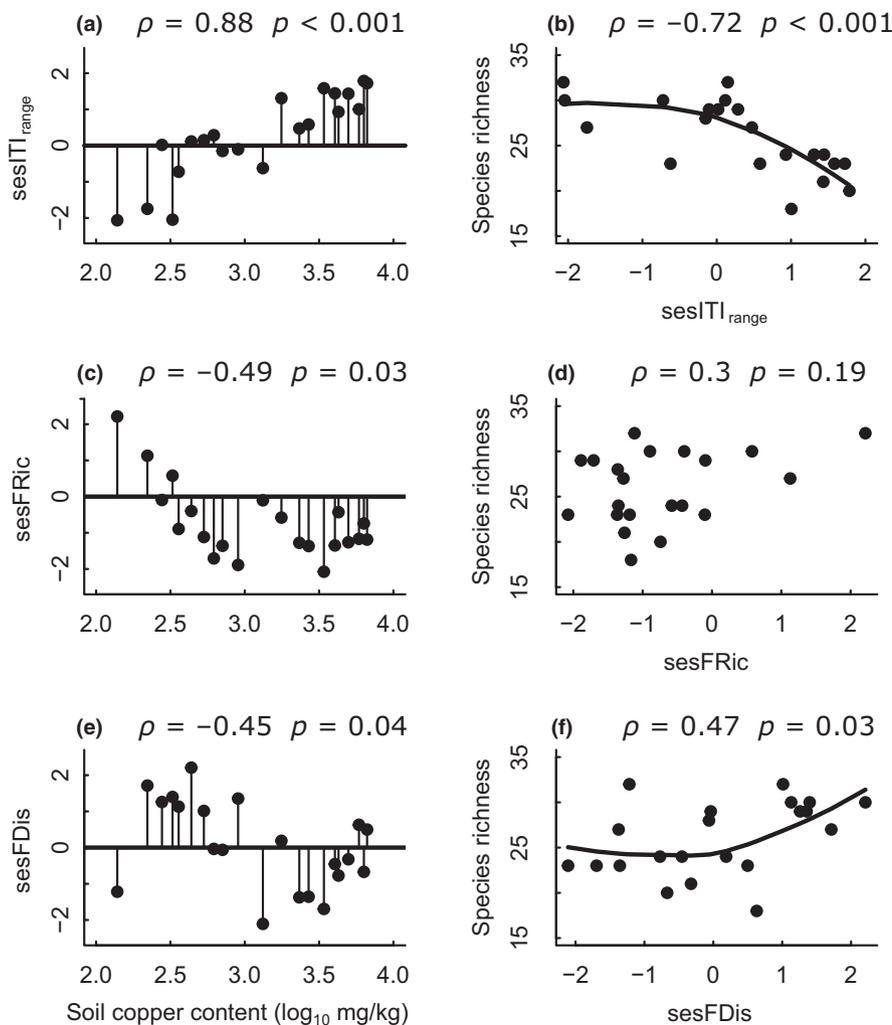


FIGURE 2 (a, c, e) Variation of the three multivariate indices (sesITI_{range}, sesFRic and sesFDIs) along the Cu gradient. (b, d, f) Association between species richness and the same indices. Each point represents one local community. The sesITI_{range} index is the standardized effect size of the multivariate ITI calculated as the range of the eigenvalues of the PCA on eight traits in each community. sesFRic and sesFDIs calculations are detailed in Appendix S2. The traits used are vegetative height, leaf area, rooting depth, leaf dry matter content, specific leaf area, seed mass, leaf Cu and Co content; see Table 1 and Section 2

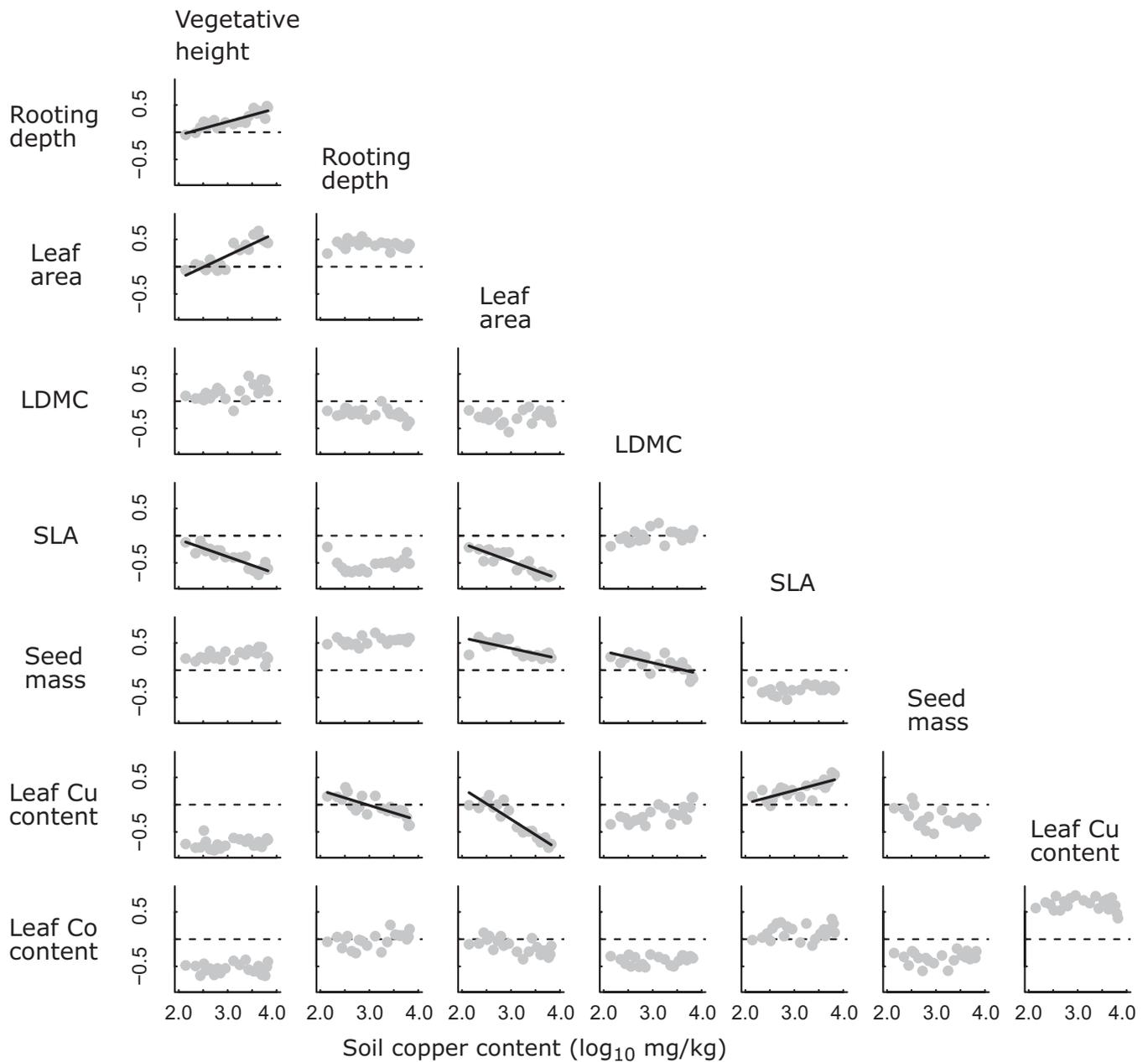


FIGURE 3 Variation of the degree of association (Spearman correlation index) between the trait pairs along the Cu gradient. Each dot is the correlation coefficient of the two corresponding traits among all species of one community. The variation of the degree of association between traits along the Cu gradient is tested with Spearman correlation coefficient. Significant linear trends are illustrated by a black plain line (adjusted p value for multiple tests: $p < 0.002$)

associations were close to zero along the whole gradient (e.g. LDMC and SLA or Co_{leaf} and rooting depth, see Figure 3).

4 | DISCUSSION

The variation in covariation patterns between functional traits within communities and along abiotic gradients was recently suggested to be an important mechanism explaining species' distribution and taxonomic richness in communities. Here, this hypothesis was addressed using a tropical savanna developed on a steep gradient of

Cu toxicity. We showed that, for a set of highly specialized species, some traits related to different functions are correlated. Further, the environmental filtering effect of the increasing Cu toxicity resulted in a strong increase in the ITI, increasing the association between some groups of traits that are not especially related to the same function. The results also supported the theoretical expectation that an increased trait covariation would drive a decrease in species richness (Dwyer & Laughlin, 2017). It is also worth mentioning that, regarding the bivariate ITI, while some trait associations were conserved along the whole gradient; other traits appeared to be consistently unrelated.

4.1 | Functional traits integration in metallophytes

Our results did not support the idea of a tight covariation of all trait dimensions (Reich, 2014) nor the two orthogonal axes related to size and resources use (Díaz et al., 2016) observed at global scale. We did not find strong support for the LHS strategy scheme, as smaller plants had larger SLA values. Furthermore, seed mass was highly correlated to leaf area and rooting depth, the latter traits reflecting plant size and resource capture axes. Instead, at the scale of a local plant community, we showed that traits related to different functions, such as size, resource use and metal tolerance, were moderately correlated, as suggested recently by Messier, McGill, Enquist, and Lechowicz (2017). Specific leaf area and LDMC were not consistently related along the whole gradient suggesting that they were not related to the same function in our community, contrary to expectations (Laughlin et al., 2010; Wilson, Thompson, & Hodgson, 1999). This could be explained by the fact that LES traits are considered to reflect differences in leaf life span (Funk & Cornwell, 2013; Messier, McGill, et al., 2017), while most species in this study presented a similar leaf life span, due to the strong constraints imposed by the marked seasonality. This might also reflect the fact that LDMC is more strongly related to a leaf hydraulics axis, which was suggested to be decoupled from a leaf photosynthesis axis, here represented by SLA (Li et al., 2015). We did not find a strong positive correlation between seed mass and plant height as previously suggested (Cornelissen, 1999; Hodgson et al., 2017). Larger-seed species, however, did have deeper roots and larger leaf areas, suggesting cohesive variation in some resource acquisition abilities across species. In these annually burned savannas, where biomass removal reduces competition for light (Zimmermann, Higgins, Grimm, Hoffmann, & Linstädter, 2010), competition could be more related to leaf area and rooting depth, advocating for the measure of several traits expected to be related to a same function (e.g. Aguirre-Gutiérrez et al., 2019).

Leaf metal concentrations are related to the strategy of metal tolerance (Baker, 1981). They can also be involved in the defence against herbivory (Behmer et al., 2005; Pollard & Baker, 1997) and could therefore be subject to trade-offs with other functions (Ernst, Verkleij, & Schat, 1992). When considering the entire species pool, smaller plants had a larger leaf Cu content. This suggests a trade-off between interspecific competitive ability and metal accumulation (Ernst et al., 1992); the sequestration of metal in the plant could incur a cost that would not allow larger species to reach reproductive maturity in a short growing season. However, this result could also support the idea that leaf metal accumulation is a mechanism of biotic interaction (allelopathy) that would benefit poorly competitive, small species (Mohiley, Tielbörger, Seifan, & Gruntman, 2020). There were only moderate interspecific correlations between leaf metal concentrations and LES traits, potentially indicating that the physiological mechanisms required for metal tolerance within the leaves do not interact with resources use. However, these relationships should be investigated more thoroughly in other communities developing on soils with high levels of metal toxicity, which would benefit from

the development of databases of traits (e.g. TRY) and of plant metal concentrations (Reeves et al., 2018). This should also be clarified by investigating experimentally physiological costs and effect on key plant functions of the different metal tolerance strategies.

4.2 | Variation in trait integration along the gradient

The central result of our study is that the strength of the relationships between some traits depends upon the abiotic environment and results in higher functional integration in harsher environments. This supports the idea that environmental harshness reduces the quantity of viable trait combinations in the community. The environmental filter would therefore not only act on mean trait values but also on ITI. In turn, as suggested by Dwyer and Laughlin (2017), increasingly stronger filtering effects on ITI could account for the gradual decrease in species richness observed along the soil Cu content gradient (Delhaye et al., 2020; Séleck et al., 2013). This filter effect would explain the decreasing proportion of species displaying a viable combination of traits with increasing Cu toxicity. Physical factors have been previously recognized to drive functional trade-offs within communities by imposing energetic constraints between physiological functions, resulting in a more integrated phenotype (Boucher, Thuiller, Arnoldi, Albert, & Lavergne, 2013; Webb, Hoeting, Ames, Pyne, & LeRoy Poff, 2010). This also possibly provides a mechanism explaining the lack of trait coordination observed within certain communities (e.g. Messier, McGill, et al., 2017; Silva, Souza, Caliman, Voigt, & Lichston, 2018).

This variation in multivariate ITI is the result of an increase in the bivariate trait correlations along the gradient, although this is not true for all trait pairs. This further supports the hypothesis that multivariate analyses can hide subtle patterns in trait-environment associations (Butterfield & Suding, 2013). The bivariate trait association analyses also bring some complementary insights into the trait covariation patterns. For example, the positive correlation between SLA and leaf Cu content observed among the species is not constant along the whole gradient: the association between the two traits increases with soil metal concentration. This reflects an increase in the proportion of metal rich, fast growing annual species on Cu-rich soils (Delhaye et al., 2020). We could hypothesize that accumulating and storing metals in the leaves is the most efficient strategy to ensure a rapid growth of annuals, while perennial species—with slower growth and flowering only after several years—might invest in long term, exclusion strategies. In this sense, it was demonstrated that metallicolous population of *Noccaea caerulea*, a hyperaccumulator species, present a shorter life cycle than non-metallicolous populations containing low leaf metal content (Dechamps et al., 2011). Furthermore, the associations between traits related to different functions in the LHS scheme (e.g. SLA and size) are also dependent on the environmental harshness, suggesting the necessity to reassess large patterns of traits covariation (e.g. Díaz et al., 2016) in relation to local environmental conditions.

Interestingly, contrary to Dwyer and Laughlin (2017), we did not find any increase in the association between seed mass and vegetative height with the environmental harshness, but only a weak, non-significant, positive correlation along the whole gradient. This expected association was hypothesized to be due to trade-off between fecundity, germination and survival (Dwyer & Laughlin, 2017). This result is likely due to the potentially weak contribution of competition for light in the interspecific interactions in these savannas. The absence of this biotic filter in the communities may have relaxed the constraints on the association between these two traits related to competition for light. Further analyses could include the relationship between the reproductive output and the vegetative biomass as this ratio could be more informative than vegetative parameters to assess plant tolerance to metals (Dechamps, Lefèbvre, Noret, & Meerts, 2007).

4.3 | Comparing ITI and functional diversity indices

We found that both FRic and FDis values decrease with increasing soil metal concentrations, which is commonly interpreted as the effect of an environmental filter (Mason et al., 2013). However, these indices seem to perform poorly in predicting species richness variation among communities, as reported previously for FDis (Dwyer & Laughlin, 2017). This poor predictive capacity appears to be particularly strong for FRic (Figure 2). The new approach presented here introduces the strength of multivariate functional trade-offs as a driver of species richness in communities. This approach is radically different from commonly used functional diversity indices such as those used in our comparison. Indeed, these functional diversity indices focus on the shape of the multivariate trait space: FRic is the hypervolume of trait space, as defined by Cornwell, Schilke, and Ackerly (2006), while FDis is the mean distance of each species to the centroid of the trait space, taking species abundances into account (Laliberté & Legendre, 2010; Villéger et al., 2008). In this regard, both indices are multivariate and contain a component of trait covariance. However, we expect ITI to be independent from these indices, as several patterns of trait covariation can produce similar hypervolumes and distances from the centroid. Multivariate ITI explains the decrease in species richness observed along our toxicity gradient better than functional diversity indices. This suggests that environmental filters do not select trait values independently but rather selects particular trait combinations, expressed at the community level by a stronger ITI between some traits. Analysing ITI therefore represents a complementary approach to investigate the community trait space and the ecological processes affecting it. We suggest that rigorous tests of the redundancy/complementarity of functional trait-related indices should be conducted in order to integrate functional trait integration in the larger framework aimed at characterizing the functional diversity of communities and predict community responses to rapidly changing environments towards physiologically stressing conditions (e.g. climate change).

5 | CONCLUSIONS

The analysis of ITI is a promising tool to investigate variation in multidimensional functional niche of species along gradients of environmental constraints. Since selection acts on multidimensional phenotypes, species traits are not independent from one another. Although trait values and ranges within communities can be expected to vary along gradients of environmental conditions, trait integration is likely to be a crucial element to uncover the ecological processes driving community assembly. We found that well-known relationships between traits at global scale may not be conserved in local communities, and more so in heterogeneous environments. These findings suggest that trait–trait relationships should be investigated in relation with environmental variables both at the community level and along larger environmental gradients. This could further provide some mechanistic explanations for the discrepancies observed between some interspecific and intraspecific trait covariation patterns (e.g. Rosas et al., 2019). ITI can convey important information about functional trade-offs within communities and highlight assembly mechanisms that could remain undetected through single-trait analyses.

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AUTHORS' CONTRIBUTIONS

G.D., D.B. and P.M. conceived the ideas and methods; G.D., M.S., E.l.w.l. and G.M. collected the data; G.D. analysed the data; G.D., D.B. and P.M. wrote the manuscript. All authors commented on the manuscript.

DATA AVAILABILITY STATEMENT

Data are available at <https://doi.org/10.5281/zenodo.3736538> (guilidelhaye, 2020).

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REFERENCES

- Adams, D. C., & Collyer, M. L. (2016). On the comparison of the strength of morphological integration across morphometric datasets. *Evolution*, 70(11), 2623–2631. <https://doi.org/10.1111/evo.13045>
- Aguirre-Gutiérrez, J., Oliveras, I., Rifai, S., Fauset, S., Adu-Bredu, S., Affum-Baffoe, K., ... Malhi, Y. (2019). Drier tropical forests are

- susceptible to functional changes in response to a long-term drought. *Ecology Letters*, 22(5), 855–865. <https://doi.org/10.1111/ele.13243>
- Baker, A. J. (1981). Accumulators and excluders—strategies in the response of plants to heavy metals. *Journal of Plant Nutrition*, 3(1–4), 643–654. <https://doi.org/10.1080/01904168109362867>
- Behmer, S. T., Lloyd, C. M., Raubenheimer, D., Stewart-clark, J., Knight, J., Leighton, R. S., ... Smith, J. A. C. (2005). Metal hyperaccumulation in plants: Mechanisms of defence against insect herbivores. *Functional Ecology*, 19(1), 55–66. <https://doi.org/10.1111/j.0269-8463.2005.00943.x>
- Bernard-Verdier, M., Navas, M. L., Vellend, M., Violle, C., Fayolle, A., & Garnier, E. (2012). Community assembly along a soil depth gradient: Contrasting patterns of plant trait convergence and divergence in a Mediterranean rangeland. *Journal of Ecology*, 100(6), 1422–1433. <https://doi.org/10.1111/1365-2745.12003>
- Blonder, B. (2018). Hypervolume concepts in niche- and trait-based ecology. *Ecography*, 41(9), 1441–1455. <https://doi.org/10.1111/ecog.03187>
- Boisson, S., Monty, A., Séleck, M., Shutcha, M. N., Faucon, M. P., & Mahy, G. (2020). Ecological niche distribution along soil toxicity gradients: Bridging theoretical expectations and metallophyte conservation. *Ecological Modelling*, 415, 108861. <https://doi.org/10.1016/j.ecolmodel.2019.108861>
- Boucher, F. C., Thuiller, W., Arnoldi, C., Albert, C. H., & Lavergne, S. (2013). Unravelling the architecture of functional variability in wild populations of *Polygonum viviparum* L. *Functional Ecology*, 27(2), 382–391. <https://doi.org/10.1111/1365-2435.12034>
- Butterfield, B. J., & Suding, K. N. (2013). Single-trait functional indices outperform multi-trait indices in linking environmental gradients and ecosystem services in a complex landscape. *Journal of Ecology*, 101(1), 9–17. <https://doi.org/10.1111/1365-2745.12013>
- Chapin III, F. S., Autumn, K., & Pugnaire, F. (1993). Evolution of suites of traits in response to environmental stress. *The American Naturalist*, 142, 78–92. <https://doi.org/10.1086/285524>
- Cheverud, J. M., Wagner, G. P., & Dow, M. M. (1989). Methods for the comparative analysis of variation patterns. *Systematic Zoology*, 38(3), 201–213. <https://doi.org/10.2307/2992282>
- Cornelissen, J. H. C. (1999). A triangular relationship between leaf size and seed size among woody species: Allometry, ontogeny, ecology and taxonomy. *Oecologia*, 118(2), 248–255. <https://doi.org/10.1007/s004420050725>
- Cornwell, W. K., Schwilk, D. W., & Ackerly, D. D. (2006). A trait-based test for habitat filtering: Convex hull volume. *Ecology*, 87(6), 1465–1471. [https://doi.org/10.1890/0012-9658\(2006\)87\[1465:ATTFHF\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2006)87[1465:ATTFHF]2.0.CO;2)
- Dechamps, C., Elvinger, N., Meerts, P., Lefebvre, C., Escarré, J., Colling, G., & Noret, N. (2011). Life history traits of the pseudometallophyte *Thlaspi caerulescens* in natural populations from Northern Europe. *Plant Biology*, 13, 125–135. <https://doi.org/10.1111/j.1438-8677.2010.00387.x>
- Dechamps, C., Lefebvre, C., Noret, N., & Meerts, P. (2007). Reaction norms of life history traits in response to zinc in *Thlaspi caerulescens* from metalliferous and nonmetalliferous sites. *New Phytologist*, 173(1), 191–198. <https://doi.org/10.1111/j.1469-8137.2006.01884.x>
- Delhaye, G., Hardy, O. J., Séleck, M., Ilunga wa Ilunga, E., Mahy, G., & Meerts, P. (2020). Plant community assembly along a natural metal gradient in central Africa: Functional and phylogenetic approach. *Journal of Vegetation Science*, 31, 151–161. <https://doi.org/10.1111/jvs.12829>
- Delhaye, G., Violle, C., Séleck, M., Ilunga wa Ilunga, E., Daubie, I., Mahy, G., & Meerts, P. (2016). Community variation in plant traits along copper and cobalt gradients. *Journal of Vegetation Science*, 27(4), 854–864. <https://doi.org/10.1111/jvs.12394>
- Díaz, S., Kattge, J., Cornelissen, J. H. C., Wright, I. J., Lavorel, S., Dray, S., ... Gorné, L. D. (2016). The global spectrum of plant form and function. *Nature*, 529(7585), 167–171. <https://doi.org/10.1038/nature16489>
- Dwyer, J. M., & Laughlin, D. C. (2017). Constraints on trait combinations explain climatic drivers of biodiversity: The importance of trait covariance in community assembly. *Ecology Letters*, 20(7), 872–882. <https://doi.org/10.1111/ele.12781>
- Ernst, W. H. O., Verkleij, J. A. C., & Schat, H. (1992). Metal tolerance in plants. *Acta Botanica Neerlandica*, 41(3), 229–248. <https://doi.org/10.1111/j.1438-8677.1992.tb01332.x>
- Faucon, M.-P., Le Stradic, S., Boisson, S., wa Ilunga, E. I., Séleck, M., Lange, B., ... Mahy, G. (2016). Implication of plant-soil relationships for conservation and restoration of copper-cobalt ecosystems. *Plant and Soil*, 403(1–2), 153–165. <https://doi.org/10.1007/s11104-015-2745-5>
- Funk, J. L., & Cornwell, W. K. (2013). Leaf traits within communities: Context may affect the mapping of traits to function. *Ecology*, 94(9), 1893–1897. <https://doi.org/10.1890/12-1602.1>
- Gianoli, E. (2004). Plasticity of traits and correlations in two populations of *Convolvulus arvensis* (Convolvulaceae) differing in environmental heterogeneity. *International Journal of Plant Sciences*, 165(5), 825–832. <https://doi.org/10.1086/422050>
- guidelhaye. (2020). Data from: guidelhaye/FE-2020-00076: FE-2020-00076 (Version data). *Zenodo*, <https://doi.org/10.5281/zenodo.3736538>
- Hodgson, J. G., Santini, B. A., Montserrat Marti, G., Royo Pla, F., Jones, G., Bogaard, A., ... Warham, G. (2017). Trade-offs between seed and leaf size (seed–phytomer–leaf theory): Functional glue linking regenerative with life history strategies ... and taxonomy with ecology? *Annals of Botany*, 120(5), 633–652. <https://doi.org/10.1093/aob/mcx084>
- Kraft, N. J., & Ackerly, D. D. (2013). The assembly of plant communities. In R. Monson (Ed.), *Ecology and the environment* (pp. 1–19). New York, NY: Springer. https://doi.org/10.1007/978-1-4614-7612-2_1-5
- Kraft, N. J., Godoy, O., & Levine, J. M. (2015). Plant functional traits and the multidimensional nature of species coexistence. *Proceedings of the National Academy of Sciences of the United States of America*, 112(3), 797–802. <https://doi.org/10.1073/pnas.1413650112>
- Laliberté, E., & Legendre, P. (2010). A distance-based framework for measuring functional diversity from multiple traits. *Ecology*, 91, 1299–1305. <https://doi.org/10.1890/08-2244.1>
- Lange, B., van der Ent, A., Baker, A. J. M., Echevarria, G., Mahy, G., Malaisse, F., ... Faucon, M.-P. (2017). Copper and cobalt accumulation in plants: A critical assessment of the current state of knowledge. *New Phytologist*, 213(2), 537–551. <https://doi.org/10.1111/nph.14175>
- Laughlin, D. C. (2014). The intrinsic dimensionality of plant traits and its relevance to community assembly. *Journal of Ecology*, 102(1), 186–193. <https://doi.org/10.1111/1365-2745.12187>
- Laughlin, D. C., Leppert, J. J., Moore, M. M., & Sieg, C. H. (2010). A multi-trait test of the leaf-height-seed plant strategy scheme with 133 species from a pine forest flora. *Functional Ecology*, 24(3), 493–501. <https://doi.org/10.1111/j.1365-2435.2009.01672.x>
- Laughlin, D. C., Lusk, C. H., Bellingham, P. J., Burslem, D. F., Simpson, A. H., & Kramer-Walter, K. R. (2017). Intraspecific trait variation can weaken interspecific trait correlations when assessing the whole-plant economic spectrum. *Ecology and Evolution*, 7(21), 8936–8949. <https://doi.org/10.1002/ece3.3447>
- Laughlin, D. C., & Messier, J. (2015). Fitness of multidimensional phenotypes in dynamic adaptive landscapes. *Trends in Ecology & Evolution*, 30(8), 487–496. <https://doi.org/10.1016/j.tree.2015.06.003>
- Li, L. E., McCormack, M. L., Ma, C., Kong, D., Zhang, Q., Chen, X., ... Guo, D. (2015). Leaf economics and hydraulic traits are decoupled in five species-rich tropical-subtropical forests. *Ecology Letters*, 18(9), 899–906. <https://doi.org/10.1111/ele.12466>
- Mason, N. W., de Bello, F., Mouillot, D., Pavoine, S., & Dray, S. (2013). A guide for using functional diversity indices to reveal changes in

- assembly processes along ecological gradients. *Journal of Vegetation Science*, 24, 794–806. <https://doi.org/10.1111/jvs.12013>
- Messier, J., Lechowicz, M. J., McGill, B. J., Violle, C., & Enquist, B. J. (2017). Interspecific integration of trait dimensions at local scales: The plant phenotype as an integrated network. *Journal of Ecology*, 105(6), 1775–1790. <https://doi.org/10.1111/1365-2745.12755>
- Messier, J., McGill, B. J., Enquist, B. J., & Lechowicz, M. J. (2017). Trait variation and integration across scales: Is the leaf economic spectrum present at local scales? *Ecography*, 40(6), 685–697. <https://doi.org/10.1111/ecog.02006>
- Messier, J., McGill, B. J., & Lechowicz, M. J. (2010). How do traits vary across ecological scales? A case for trait-based ecology. *Ecology Letters*, 13(7), 838–848. <https://doi.org/10.1111/j.1461-0248.2010.01476.x>
- Messier, J., Violle, C., Enquist, B. J., Lechowicz, M. J., & McGill, B. J. (2018). Similarities and differences in intrapopulation trait correlations of co-occurring tree species: Consistent water-use relationships amid widely different correlation patterns. *American Journal of Botany*, 105(9), 1477–1490. <https://doi.org/10.1002/ajb2.1146>
- Mohilev, A., Tielbörger, K., Seifan, M., & Gruntman, M. (2020). The role of biotic interactions in determining metal hyperaccumulation in plants. *Functional Ecology*, 34(3), 658–668. <https://doi.org/10.1111/1365-2435.13502>
- Mouchet, M. A., Villéger, S., Mason, N. W., & Mouillot, D. (2010). Functional diversity measures: An overview of their redundancy and their ability to discriminate community assembly rules. *Functional Ecology*, 24(4), 867–876. <https://doi.org/10.1111/j.1365-2435.2010.01695.x>
- Pakeman, R. J., & Quested, H. M. (2007). Sampling plant functional traits: What proportion of the species need to be measured? *Applied Vegetation Science*, 10(1), 91–96. <https://doi.org/10.1111/j.1654-109X.2007.tb00507.x>
- Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., ... Cornelissen, J. H. C. (2013). New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany*, 61, 167–234. <https://doi.org/10.1071/BT12225>
- Pigliucci, M., & Preston, K. (Eds.). (2004). *Phenotypic integration: Studying the ecology and evolution of complex phenotypes*. Oxford, UK: Oxford University Press.
- Pollard, A. J., & Baker, A. J. (1997). Deterrence of herbivory by zinc hyperaccumulation in *Thlaspi caerulescens* (Brassicaceae). *New Phytologist*, 135(4), 655–658. <https://doi.org/10.1046/j.1469-8137.1997.00689.x>
- R Core Team. (2019). *R foundation for statistical computing*. Vienna, Austria. Retrieved from <http://www.R-project.org/>
- Read, Q. D., Moorhead, L. C., Swenson, N. G., Bailey, J. K., & Sanders, N. J. (2014). Convergent effects of elevation on functional leaf traits within and among species. *Functional Ecology*, 28(1), 37–45. <https://doi.org/10.1111/1365-2435.12162>
- Reeves, R. D., Baker, A. J., Jaffré, T., Erskine, P. D., Echevarria, G., & van der Ent, A. (2018). A global database for plants that hyperaccumulate metal and metalloids trace elements. *New Phytologist*, 218(2), 407–411. <https://doi.org/10.1111/nph.14907>
- Reich, P. B. (2014). The world-wide 'fast-slow' plant economics spectrum: A traits manifesto. *Journal of Ecology*, 102(2), 275–301. <https://doi.org/10.1111/1365-2745.12211>
- Rosas, T., Mencuccini, M., Barba, J., Cochard, H., Saura-Mas, S., & Martínez-Vilalta, J. (2019). Adjustments and coordination of hydraulic, leaf and stem traits along a water availability gradient. *New Phytologist*, 223(2), 632–646. <https://doi.org/10.1111/nph.15684>
- Séleck, M., Bizoux, J.-P., Colinet, G., Faucon, M.-P., Guillaume, A., Meerts, P., ... Mahy, G. (2013). Chemical soil factors influencing plant assemblages along copper-cobalt gradients: Implications for conservation and restoration. *Plant and Soil*, 373(1–2), 455–469. <https://doi.org/10.1007/s11104-013-1819-5>
- Šidák, Z. (1967). Rectangular confidence regions for the means of multivariate normal distributions. *Journal of the American Statistical Association*, 62(318), 626–633. <https://doi.org/10.1080/01621459.1967.10482935>
- Silva, J. L., Souza, A. F., Caliman, A., Voigt, E. L., & Lichston, J. E. (2018). Weak whole-plant trait coordination in a seasonally dry South American stressful environment. *Ecology and Evolution*, 8(1), 4–12. <https://doi.org/10.1002/ece3.3547>
- Tilman, D. (1988). *Plant strategies and the dynamics and structure of plant communities (Monograph in Population Biology No. 26)*. Princeton, NJ: Princeton University Press.
- Torices, R., & Muñoz-Pajares, A. J. (2015). PHENIX: An R package to estimate a size-controlled phenotypic integration index. *Applications in Plant Sciences*, 3(5), 1400104. <https://doi.org/10.3732/apps.1400104>
- Villéger, S., Mason, N. W., & Mouillot, D. (2008). New multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology*, 89, 2290–2301. <https://doi.org/10.1890/07-1206.1>
- Violle, C., Navas, M. L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., & Garnier, E. (2007). Let the concept of trait be functional! *Oikos*, 116(5), 882–892. <https://doi.org/10.1111/j.0030-1299.2007.15559.x>
- Violle, C., Thuiller, W., Mouquet, N., Munoz, F., Kraft, N. J. B., Cadotte, M. W., ... Mouillot, D. (2017). Functional rarity: The ecology of outliers. *Trends in Ecology & Evolution*, 32(5), 356–367. <https://doi.org/10.1016/j.tree.2017.02.002>
- Waite, D. E., & Levin, D. A. (1993). Phenotypic integration and plastic correlations in *Phlox drummondii* (Polemoniaceae). *American Journal of Botany*, 80(10), 1224–1233. <https://doi.org/10.1002/j.1537-2197.1993.tb15356.x>
- Webb, C. T., Hoeting, J. A., Ames, G. M., Pyne, M. I., & LeRoy Poff, N. (2010). A structured and dynamic framework to advance traits-based theory and prediction in ecology. *Ecology Letters*, 13(3), 267–283. <https://doi.org/10.1111/j.1461-0248.2010.01444.x>
- Westoby, M. (1998). A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant and Soil*, 199(2), 213–227. <https://doi.org/10.1023/A:1004327224729>
- Wilson, P. J., Thompson, K. E. N., & Hodgson, J. G. (1999). Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. *New Phytologist*, 143(1), 155–162. <https://doi.org/10.1046/j.1469-8137.1999.00427.x>
- Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., ... Villar, R. (2004). The worldwide leaf economics spectrum. *Nature*, 428(6985), 821–827. <https://doi.org/10.1038/nature02403>
- Wright, J. P., & Sutton-Grier, A. (2012). Does the leaf economic spectrum hold within local species pools across varying environmental conditions? *Functional Ecology*, 26(6), 1390–1398. <https://doi.org/10.1111/1365-2435.12001>
- Zimmermann, J., Higgins, S. I., Grimm, V., Hoffmann, J., & Linstädter, A. (2010). Grass mortality in semi-arid savanna: The role of fire, competition and self-shading. *Perspectives in Plant Ecology, Evolution and Systematics*, 12(1), 1–8. <https://doi.org/10.1016/j.ppees.2009.09.003>

SUPPORTING INFORMATION

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