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Abstract:

Both inter- and intraspecific trait variation are critical to species distribution along environmental gradients, but our understanding of these patterns predominantly relies upon species-level trait means and variances. Trait integration, defined as how strongly multiple traits covary with one another, is a key indicator of the dimensionality of functional space for accommodating biodiversity in communities. As trait covariance can differ dramatically at the interspecific versus intraspecific levels, how intraspecific trait variability alters the strength of trait integration and eventually modulates biodiversity along environmental gradients has been rarely tested. Here, we measured nine traits (leaf area, specific leaf area, leaf and stem dry-matter content, leaf nitrogen and phosphorus contents, specific stem length, Huber value and maximum height) paired with site-specific soil fertility for 70 woody communities in subtropical Chinese forests. All species-by-site combinations were sampled to ensure a sufficient representation of intraspecific trait variation across sites. Community-level trait integration was quantified from the variance of eigenvalues of the trait correlation matrix. The direct and/or indirect effects of soil fertility and trait integration on species richness and trait diversity were assessed through path analyses. Trait integration quantified from both inter- and intraspecific variances was on average 21.7% weaker than that from only interspecific variance, indicating a crucial role of intraspecific trait variability in promoting niche dimensionality. Whether accounting for intraspecific variation or not, less fertile sites had stronger trait integration, which in turn depressed both taxonomic and functional diversity, supporting the assumption that higher environmental stress demanding stronger tradeoffs among multiple functions in viable strategies. Importantly, the negative association between trait integration and species richness became stronger when accounting for intraspecific variation, suggesting that species distribution and occurrence can be a consequence of intraspecific trait variability. This study highlights the importance of intraspecific trait variability in understanding functional tradeoffs underlying biodiversity patterns.

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Research

The importance of intraspecific trait variability in promoting functional niche dimensionality

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Introduction

A major goal of modern community ecology is to understand how niche occupancy and community assembly are governed by functional traits along environmental gradients (Weiher and Keddy 1995, McGill et al. 2006). To that end, community ecologists have largely focused on the mean and variance of traits at the species level to explore the relationship between traits and environment (Cavender-Bares et al. 2004, Šímová et al. 2015). This approach is somewhat insufficient because it ignores intraspecific trait variation (Lichstein et al. 2007, Albert et al. 2010) and covariance among traits (Laughlin 2014). Consideration of intraspecific trait variation to understand community structuring processes is particularly important because intraspecific trait variance accounts for a considerable proportion of total variance across communities (de Bello et al. 2011, Siefert et al. 2015) and moderates key ecological processes (Bolnick et al. 2011). Trait covariance (hereafter referred to as 'trait integration' to indicate the relationship between more than two traits) is important because it indicates how multiple traits vary in relation to each other (Pigliucci 2003), thereby providing useful insights into the functional tradeoffs underlying biodiversity patterns (Reich et al. 1997, Laughlin 2014, Díaz et al. 2016). Furthermore, some recent studies have suggested a possible influence of intraspecific trait variation on the strength of trait integration (Laughlin et al. 2017, Messier et al. 2017a). To date, it remains largely unclear how intraspecific trait variation moderates the pattern of community-level trait integration and, in turn, impacts biodiversity along environmental gradients. Such knowledge is necessary to establish a more complete picture of the architecture of niche space underlying community assembly and the distribution of biodiversity.

The concept of trait integration is rooted in the observation that traits of an individual or a species can vary in a coordinated fashion, optimizing some functions at the cost of others (Murren 2002, Armbruster et al. 2014, Messier et al. 2017b). In other words, there exists no 'Darwinian demon' that can maximize its fitness in all aspects and be able to occupy any position in a multidimensional niche space (Laughlin 2018). Precisely which function is optimized, or the extent to which traits are coordinated, is usually determined by the biophysical, evolutionary and/or prevailing environmental constraints in operation under a particular selection regime (Reich et al. 1997, Laughlin 2014, Díaz et al. 2016). Species occurring in a stressful environment may optimize the resource–conservation function at the cost of resource–acquisition function, while species occurring in a more benign environment may adopt the opposite strategy (Reich et al. 1997). Generally, trait integration tends to increase in more stressful environments (Waitt and Levin 1993, Dwyer and Laughlin 2017a) because species occupying a stressed site must coordinate multiple traits to optimize stress tolerance functions for their persistence. Under more benign environmental conditions, species may not need such strong trait coordination, allowing individual traits to vary relatively independently to accomplish more diverse functions (Dwyer and Laughlin 2017b).

If overall trait integration in a community is weak, this implies there are only weak constraints from functional tradeoffs. In this case, a variety of viable trait combinations (multivariate phenotypes) could be possible, allowing a large number of species with disparate functional designs to co-occur (Fig. 1a, variation in purple points). In contrast, if trait integration is strong, only a narrow range of trait combinations can occur, accommodating only a few species with specific functional designs (Fig. 1a, variation in red points). Indeed, higher degrees of trait integration – typically quantified from species-wise trait means – have been associated with lower species richness and trait diversity (Dwyer and Laughlin 2017b, Delhaye et al. 2020).

Trait integration is not just driven by evolutionarily preserved tradeoffs among species (Messier et al. 2017a). If there are hard tradeoffs between vital functions consistently operating across species and strong selection for coordination among multiple traits in any environment, the structure of intra- and interspecific trait covariance will be largely concordant (Umaña and Swenson 2019). In this case, the strength of trait integration should not influenced by how we aggregate trait information, interspecifically, intraspecifically or both. Alternatively, if intraspecific trait variation is caused by local adaption and/or phenotypic plasticity due to site-specific environmental stress (Albert et al. 2010, Messier et al. 2017a), trait integration quantified from both inter- and intraspecific variances should be weaker than that from only interspecific variance. This is because species can individualistically fulfill local environmental requirements through differential adjustment of their phenotypes (Fajardo and Siefert 2018, Umaña and Swenson 2019), and consequentially co-occurring species are represented by many alternative strategies with functional equivalency (Marks and Lechowicz 2006). Thus, viable strategies in a community representing good solutions to local environmental pressures might not necessarily be optimized around axes of interspecific tradeoffs, but rather stem largely from flexible functional designs within species. Intraspecific trait variability associated with individual spatial locations may thus increase the dimensionality of functional-trait space, in turn weakening the overall strength of trait integration (Fig. 1b–c).

Furthermore, the negative association between trait integration and trait diversity is expected to be more pronounced if accounting for intraspecific trait variation than if not (Fig. 1b). This is because accounting for intraspecific trait variation should accurately capture in situ constraints from local functional tradeoffs on trait combinations; whereas, assuming invariable species 'mean' phenotypes may average out local environmental effects on trait variation (Ames et al. 2016) and the exact constraints on trait combinations from local tradeoffs. To assume an invariable 'mean' phenotype across sites, as widely adopted in trait-based community ecology (Cavender-Bares et al. 2004, Delhaye et al. 2020), is in effect to release a Darwinian demon sensu lato that can occupy more niche space than what the phenotype allows for. In fact, intraspecific trait variability allows a species to adjust its phenotype to occur in an environment that

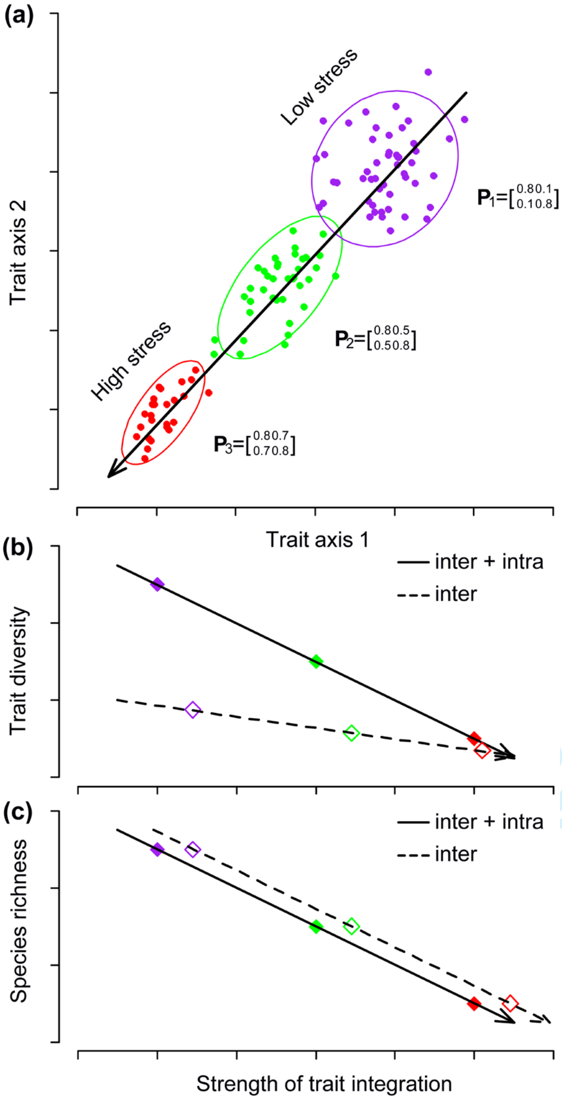

Figure 1. Conceptual representation of variation in trait diversity and species richness depending on trait integration and intraspecific trait variation along an environmental stress gradient (represented bold arrows). (a) Increasing stress imposes stronger tradeoffs as reflected by an increase in trait integration (flatness of ellipses), and in turn results in decreases in trait diversity (volume of ellipses) and species richness (number of dots). Different colors indicate different communities, and each constitute species is represented by a trait combination randomly generated from a bivariate normal distribution with a given variance–covariance structure (**P** matrix, shown next to each community). The covariance between trait and environment is kept constant. (b) Under relaxed constraints from tradeoffs, intraspecific trait variability is expected to weaken trait integration, and the negative relationship between trait integration and trait diversity is expected to be more pronounced with consideration of both inter- and intraspecific trait variation (—) than with consideration of only interspecific variation (---). (c) Likewise, the negative relationship between trait integration and species richness is also expected to be stronger with consideration of both inter- and intraspecific trait variation (—) than with consideration of only interspecific variation (---). Note that the slope is translocated horizontally because species richness does not change no matter how trait integration is quantified.

otherwise provides little opportunity, potentially promoting species richness (Crawford et al. 2019). Hence, the association between trait integration and species richness is likely to be stronger when accounting for ~~interspecific~~ trait variation (Fig. 1c).

In addition to intraspecific trait variation, environmental conditions can also influence species richness and trait diversity, either directly or indirectly via trait integration (Fig. 2). Species richness and trait diversity typically decrease with increasing environmental stress (Bello et al. 2006, Šímová et al. 2015), but increased trait integration with increasing environmental stress may also constrain species richness and trait diversity (Gianoli and Palacio-López 2009, Dwyer and Laughlin 2017b). In summary, intraspecific trait variation and site environmental conditions may have complex effects on the observed relationship between trait integration and biodiversity (Fig. 2). Assessing the direct and/or indirect effects of each of these factors is necessary to improve our ability to make inferences about the ecological processes underlying the distribution of biodiversity along environmental gradients.

Here we used site-specific trait and environmental data for 70 woody assemblages in subtropical China to assess the influence of intraspecific trait variation on trait integration–biodiversity associations along a soil fertility gradient. We particularly focus on two questions: 1) what are the consequences of intraspecific trait variation for the pattern of trait integration along the soil fertility gradient? and 2) whether accounting for intraspecific trait variation changes the trait integration–biodiversity association along the soil fertility gradient? We anticipated that trait integration quantified from both inter- and intraspecific variances should be weaker than that from only interspecific variance, and that the negative association between trait integration and species richness or functional (trait) diversity would be more pronounced when accounting for intraspecific variation.

Material and methods

Study area and natural history

This study was conducted in the Ningbo region of eastern China (28°51′–30°33′N, 120°55′–122°16′E, Supporting information). The landscape in this region is characterized by a mixture of plains, basins and low hills (4–900 m a.s.l.), covering a total area of 9816 km^2. The regional climate is subtropical, with hot and humid summers, and chilly and dry winters. Mean annual temperature is 16.5°C and mean annual rainfall is 1440 mm (Chen 2001). Regional topography is rugged and dominant soil types are ferralsols (i.e. red clay soils) (FAO 2014–2015). Due to strong oxidization and leaching in the wet and humid climate, ferralsols are typically rich in iron and aluminum oxides, but poor in organic matter and soluble minerals (Gong 2014). As cation exchange sites, soil organic matter is crucial for providing a reserve of nutrients for plant growth.

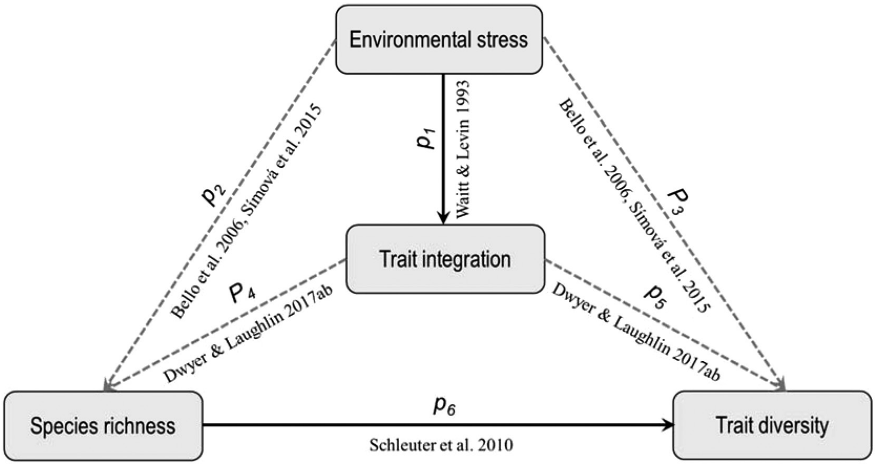

Figure 2. Schematic diagram of hypothesized causal relationships among environmental stress, trait integration, species richness and trait diversity. The direction of causality is indicated by arrows and marked with path coefficients. Solid and dashed arrows denote positive and negative effects, respectively. Previous theory and empirical results supporting the hypothesized paths are noted next to the arrows.

Historically, evergreen broadleaf forests (EBLFs) were the dominant vegetation type in the region. Extensive logging disturbed the regional vegetation until 1980s. Since then, the majority of the remnant EBLFs had been preserved; and these vegetation regenerated naturally. Currently, plenty of intact or semi-intact EBLFs are found in natural reserves, forest parks and hilly lands around water reservoirs, where human disturbances are minimum. These forests are usually dominated by *Cyclobalanopsis glauca* and *Lithocarpus glaber* (Fagaceae), *Machilus thunbergii* (Lauraceae), *Eurya muricata*, *Schima superba* and *Camellia fraterna* (Theaceae), *Symplocos sumuntia* (Symplocaceae), *Pinus massoniana* (Pinaceae), *Syzygium buxifolium* (Myrtaceae), *Loropetalum chinense* (Hamamelidaceae) and *Eurya rubiginosa* var. *attenuate* (Theaceae).

Data collection

Species composition

From July to September of 2015, we surveyed woody communities from 70 EBLF sites that differed visibly in terms of species composition and environmental conditions. In each site, we placed a 20 × 20 m plot for a detailed survey of woody vegetation. A minimum separation distance of 50 m between adjacent plots was maintained to reduce spatial autocorrelation and pseudo-replication. In each plot, all woody plants taller than 0.5 m were identified at the species level and their basal diameters were measured. Species richness per 20 × 20 m plot ranged from 11 to 43 (25 species on average), with a total of 131 species across all sites. Stem density ranged from 1350 to 15 350 stems ha^{-1}, and basal area ranged from 1.6 to 108.6 m^2 ha^{-1}.

Functional trait measurements

We considered nine functional traits, namely leaf area (LA), specific leaf area (SLA), leaf dry-matter content (LDMC), leaf nitrogen content (LNC), leaf phosphorus content (LNP), stem dry-matter content (SDMC), specific stem length (SSL), Huber value (HV) and maximum height (H_{max}) in this study. We choose these traits because they are known to represent important physiological, hydraulic and mechanical functions (Supporting information) that are assumed to link with species' fitness and have been shown to determine species distribution (Wright et al. 2006, 2007, Brenes-Arguedas et al. 2013).

To measure the site-specific leaf and stem traits, we collected species-specific leaf and twig samples from three individuals from each plot (n = 70 plots). For singleton and doubleton species within a plot, we sampled only one or two individuals. This sampling approach ensured that all species nested within plots have their trait measurements in situ and thus intraspecific trait variation across plots was accounted for.

To measure the leaf traits for each species in a plot, we cut one to three current-year twigs (ca 0.3 cm in diameter and 4–40 cm in length) from a plant at its canopy edge. We selected twigs with at least three fully-developed and healthy-looking leaves. Typically, there were 16 leaves per twig (range: 4–32 leaves). LA, SLA and LDMC were then measured typically from five leaves (four leaves in rare cases) per twig following standard protocols (Pérez-Harguindeguy et al. 2013). Leaf nitrogen and phosphorus concentrations per unit mass (LNC and LPC) were determined from the dried leaf samples, using a discrete autoanalyzer (Smartchem 200, Alliance, France).

We measured the length and base diameter of each of the harvested twigs using a vernier caliper. Fresh mass and dry mass (oven-dried at 105°C for 72 h) of each twig sample were then determined. Stem dry-matter content (SDMC) was defined as the ratio of dry to fresh mass for a twig. Specific stem length (SSL), which indicates the capacity of axial growth and elongation for a given stem biomass (Poorter and Rose 2005), was defined as the ratio of twig length to dry mass. Huber value (HV) was approximated as the ratio of the base cross-sectional area of a twig to its total leaf area. This measure was proposed to indicate the hydraulic conductance of the branches or the whole plant (Huber 1928). For leaf and stem traits, all conspecific trait measurements in a plot were averaged to represent a site-specific trait value for a given species.

We estimated tree height visually using a Christen hypsometer based on trigonometric principles (Buckner et al. 1977). For maximum height, we calculated the 95th percentile of height estimates for each species in a plot. To assess the reliability of maximum height estimates, we correlated them with 95th percentile of basal diameters. The overall correlation coefficient regardless of species and plot was 0.86 ($p < 0.001$), and species-specific correlation coefficients for 58 species occupying more than 10 plots averaged at 0.78 ± 0.08. We believed that the estimation was reliable. Here, we did not assume maximum height is invariable for a species, but considered the upper limit that a species actually reached in a plot.

Environmental conditions

To characterize the environmental conditions of a plot, we measured soil moisture content, bulk density and soil total carbon. These edaphic factors are known to be relevant for species distribution and functional trait distribution in the study area (Yan et al. 2013). For soil moisture content and bulk density, five soil samples were collected using a foil sampler at a depth of 0–20 cm after removing litter layer from five random locations in each plot. All soil samples were collected on sunny days with no record of rain in the past three days prior to the date of sampling. These five soil samples were mixed to make a composite sample for a plot. After taking the fresh weight, soil samples were placed in an oven for about 48 h (or until a constant weight was achieved) at 105°C to determine dry weight. Soil moisture content (dry weight/fresh weight) and soil bulk density (dry weight/volume of foil sampler) were then calculated.

For soil total carbon, we took four soil samples with a metal corer from the 0 to 20 cm soil layer at four quadrant centers within each plot, for a total of 280 soil samples. These soil samples were first air-dried in the laboratory, then passed through a 0.15 mm sieve and oxygenized at a high temperature to determine total carbon concentration using a TOC analyzer (vario TOC cube, Elementar, Germany). Values obtained from the four samples within each plot were averaged to represent a plot-level measure.

We conducted a principal component analysis (PCA) to summarize the three plot-level edaphic factors into a composite environmental stress gradient. PC1 explained 45% of total variance and was loaded positively with soil bulk density (correlation with PC1 = 0.61; $p < 0.01$) and negatively with soil moisture contents (−0.68; $p < 0.01$) and soil total carbon (−0.71; $p < 0.01$) Hence, PC1 represents a gradient of decreasing soil fertility, with high PC1 scores indicating compacted (i.e. high bulk density), dry (low moisture content) and nutrient-poor (low C) soils (Supporting information).

Multivariate variance partitioning

To assess the proportion of total trait variance attributed to within-species, between-species, within-site and between-site variation, we followed a multivariate variance partitioning approach (Eslami et al. 2011). In this approach, total trait variance is represented in a matrix form, in which species-by-site combinations are presented in rows and trait values are presented in columns. Each of nine trait columns is first standardized to the mean of 0 and variance of 1. Total multivariate trait variance (variance in nine traits) is then calculated as the trace (sum of all diagonal elements) of the trait variance matrix (**P** matrix). We then partitioned the proportion of total variance attributed to interspecific variance as the trace of among-species **P** matrix. By subtracting species mean trait value from site-specific trait values for each species, we obtained a matrix of 'species-free' trait values. Species-free trait values were then averaged with respect to each plot. Based on the **P** matrix of plot-aggregated species-free trait values, we yield intraspecific variance from its trace. Note that intraspecific variance in this study occurred mostly among plots, so a plot-level aggregation of species-free trait values was needed. The rest variance in the species-free trait values was due to unknown sources (i.e. residual). A similar procedure was employed to yield among-plot variance and among-species within-plot variance. The variances attributed to different sources were converted into proportions by dividing the total variance.

Quantification of species richness, trait diversity and trait integration

Since sites with higher abundance tend to have higher species richness, we computed rarefied species richness to remove the potential effects of unequal species abundance on richness (Hurlbert 1971). Specifically, we rarefied the number of species through random subsamples of each plot with the same number of individuals. We implemented the rarefication using the 'rarefy' function from the R package 'vegan' and set the sample size as the smallest number (54 in our case) of individuals across the 70 plots. As we sought to examine the effects of intraspecific trait variation across plots rather than within plots (below in detail), each species within a plot was assumed functionally identical and therefore species abundance was less relevant here. Moreover, to explore the role of functional tradeoffs in constraining the variety of phenotypes, it was important to focus on phenotypically rather than numerically distinct species. Hence, rarefied richness was a more pertinent index than raw richness, as the latter tacitly integrates abundance information.

We quantified trait diversity by using Rao's quadratic entropy, Q (Rao 1982, Botta-Dukát 2005). Q was calculated as, $\sum_{i}^{S}\sum_{j}^{S} d_{ij} p_i p_j$, where S is the number of species in a plot, d_{ij} is the trait difference between the ith and jth species and p_i and p_j are the relative importance values of the ith and jth species respectively. Based on species abundance and basal area, we obtained the relative importance value of each species (i.e. $p_{i,j\ldots n}$) in each plot. Note that the Q index is inherently entangled with the problem of abundance since raw species richness is used in its calculation. Nevertheless, this index is a useful summary of the extant of functional distinctness among dominant species in a community as it downweights the differences among rare species. We used Mahalanobis (1936) distance to estimate interspecific trait differences (d_{ij}), which accounts for trait covariance. We separately quantified Q in the following two scenarios: 1) when intraspecific trait variation was considered, site-specific trait measurements were used to compute d_{ij}; and 2) when intraspecific trait variation was not considered, species-wise mean trait values across all sites ($n = 70$) were used to compute d_{ij}. A Kolmogorov–Smirnov test was conducted to examine whether Q differed between the two scenarios.

We quantified the strength of trait integration among nine traits for each plot ($n = 70$) by following Cheverud et al. (1989). In this method, the strength of trait integration (D) is computed as the variance of the eigenvalues (λs) of the trait correlation matrix (**R**). The trait correlation matrix **R** summarizes the shape of trait distribution in a n-dimensional vector space. λ values satisfy the equation $|\mathbf{R} - \lambda\mathbf{I}| = 0$, where **I** is the identity matrix with all diagonal elements being one and elsewhere being zero. In geometrical terms, an eigenvector that is associated with a real nonzero eigenvalue points in a stretched direction in the newly created coordinate system after transformation, and an eigenvalue corresponds to the relative length of its associated vector. If the raw vectors are linearly uncorrelated, no single eigenvector can represent the overall shape of **R** and eigenvalues tend to be equal; but if the raw vectors are correlated, two or three dominant eigenvectors can represent the overall shape of **R** and their associated eigenvalues tend to be larger than the rest. Therefore, a higher value of D indicates a greater degree of trait integration. If D is applied to quantify a community, it reflects the dimensionality of a functional niche space available for constituent species.

In this study, we built a trait correlation matrix (**R**) for each plot from its species by trait matrix. When intraspecific trait variation was considered, trait values for species-by-site combinations were used to build the matrix **R** (incorporating both inter- and intraspecific covariances); and when intraspecific trait variation was ignored, species-wise trait means across all sites ($n = 70$) were used to build the matrix **R** (accounting for only interspecific covariance). Then, the eigenvalues of the plot-wise respective **R** matrix were extracted to calculate the strength of trait integration (D) separately in the two scenarios with and without intraspecific variation. We tested whether D values differed between the two scenarios, using two-sample Kolmogorov–Smirnov test.

Path analyses

To evaluate the hypothesized causal relationships among environmental stress (decreasing soil fertility approximated by PC1 scores), trait integration, species richness and trait diversity outlined in Fig. 2, we conducted two piecewise path analyses. In piecewise path analyses, the assumption of multivariate normal distribution is relaxed; and a series of linear (structured) equations are evaluated separately, but not solved simultaneously as in ordinary structural equation modelling (Shipley 2009). We estimated the standardized path coefficients (p_1–p_6) from a series of generalized least squares regressions, which allowed unequal variances and/or correlated errors. The regression models were defined as follows:

$$D = a + p_1E + \varepsilon \tag{1}$$

$$S = b + p_2E + p_4D + \varepsilon \tag{2}$$

$$Q = c + p_3E + p_5D + p_6S + \varepsilon \tag{3}$$

where D = the strength of trait integration, S = rarefied species richness, Q = trait diversity, E = the degrees of environmental stress, a, b and c are intercepts and ε = error. We assumed spherical covariance structures in ε to account for potential spatial autocorrelation in D, S and Q. Path models were run iteratively in the two scenarios of trait aggregation with and without intraspecific variation.

As the number of free parameters exactly equaled the number of known values, the full model was saturated. We therefore set free a path with least predictability and successively fitted the reduced models using directed separation (Shipley 2009). This procedure is used to test conditional independence among paring variables without a directed path connecting them in the hypothesized relationships. A chi-square test in terms of Fisher's C statistic (a combined statistic of raw p values of all independence claims) was implemented to examine whether the collection of hypothesized relationships in the reduced models occur by chance. Large p values associated with Fisher's C statistic indicate that there is strong support for the collection of the conditional independence claims and that no missing path exists among unconnected variables. In our case the set-free path (i.e. p_3 in Fig. 2) did not represent a missing path, and there was weak support for the collection of the conditional dependence claims associated with the hypothesized relationships ($p > 0.36$). Therefore, our reduced models fit the empirical data well. Path analyses were implemented using R package 'piecewiseSEM' (Lefcheck 2016).

Bivariate relationships

Our path analyses focused on direct and indirect effects of environmental stress and trait integration on species richness and trait diversity separately for the two scenarios with and without intraspecific trait variation. Therefore, we conducted a follow-up analysis to directly evaluate how intraspecific trait variation may impact the relationships for species richness versus trait integration and for trait diversity versus trait integration. For that, we first removed the broader linear trends associated with environmental stress from rarefied richness, trait integration and trait diversity (Flandrin et al. 2004), by modeling y (i.e. S, D or Q) as a function of E. Rarefied richness, trait integration and trait diversity after controlling for E are referred to as $S|E$, $D|E$ and $Q|E$, respectively. Then, using the scenario of trait aggregation (with or without intraspecific variation) as a dummy variable, we evaluated the effect of intraspecific trait variation on species richness versus trait integration relationship through ANCOVA by modeling $S|E = D|E + \text{scenario} + \text{scenario} \times D|E + \text{error}$; and on trait diversity versus trait integration relationship by modeling $Q|E = D|E + \text{scenario} + \text{scenario} \times D|E + \text{error}$. Note that, if the interaction term (i.e. scenario × $D|E$) was non-significant, we fitted a common slope and tested for the difference in intercepts between the two scenarios. All residuals met the assumptions of approximate normality and homogeneous variances across groups. All statistical analyses were conducted in R 3.5.1.

Results

Over the entire data set, about 7.8% of total trait variance was attributed to intraspecific trait variation, 51.2% to interspecific trait variation and 41% to unspecified sources. When we looked into site effects, we found that about 46% of total trait variance was attributed to within-site trait variation, 12% to between-site trait variation and 42% to unspecified sources.

As hypothesized, the strength of trait integration quantified from the combination of inter- and intraspecific variances was significantly lower than that quantified from only interspecific variance ($p < 0.001$), with the former being on average 21.7% lower than the latter (Fig. 3a). Trait diversity with intraspecific variation was significantly ($p < 0.001$) but only slightly (4.4%) higher than that without intraspecific variation (Fig. 3b).

Whether accounting for intraspecific variation or not, higher environmental stress (i.e. lower soil fertility) resulted in stronger trait integration ($p \leq 0.05$, Fig. 4a–b; Supporting information). Lower species richness was directly linked with higher environmental stress and stronger trait integration ($p \leq 0.02$, Fig. 4a–b; Supporting information). The immediate effect (i.e. path coefficient) of trait integration on species richness was significantly negative in both scenarios (−0.58 versus −0.50, $p < 0.001$, Fig. 4). The indirect effect of environmental stress on species richness via trait integration was also significantly negative, irrespective of the scenarios ($p \leq 0.05$, Supporting information).

The strength of trait integration had a significant negative effect on trait diversity, especially when intraspecific trait variation was considered ($p = 0.008$, Fig. 4a). This effect of trait integration on trait diversity was non-significant when disregarding intraspecific trait variation ($p = 0.54$, Fig. 4b). Interestingly, when trait diversity was quantified with intraspecific trait variation, species richness had no significant direct effect on trait diversity ($p = 0.07$, Fig. 4a, Supporting information), but when trait diversity was quantified without intraspecific trait variation, a significantly positive effect of species richness on trait diversity was detected ($p = 0.03$, Fig. 4b). Overall, environmental stress affected trait diversity mostly indirectly, with supported indirect effects dependent on the scenarios. When intraspecific trait variation was explicitly considered, environmental stress-dependent trait integration was a significant mediator of trait diversity (p <

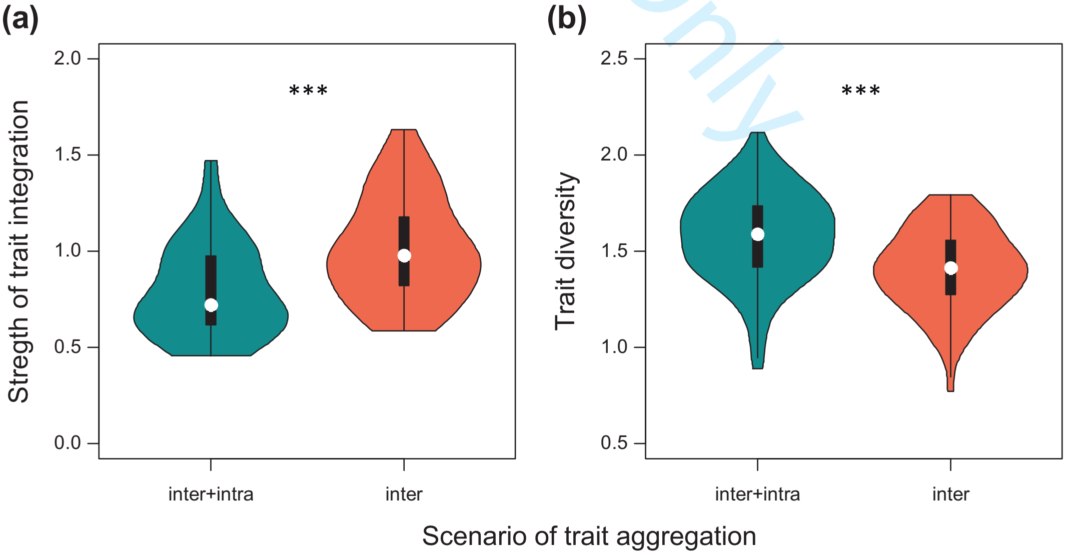

Figure 3. The frequency of the strength of trait integration (a) and trait diversity (b) across plots with (inter + intra) and without (inter) considering intraspefic trait variation. Open circles are medians, black boxplots are the ranges from lower to upper ~~quintiles~~, black solid lines are the ranges from 5th to 95th percentiles, and the width of violin indicate the kernel density of particular trait integration or trait diversity. *** denotes significant difference between the two scenarios ($p < 0.001$).

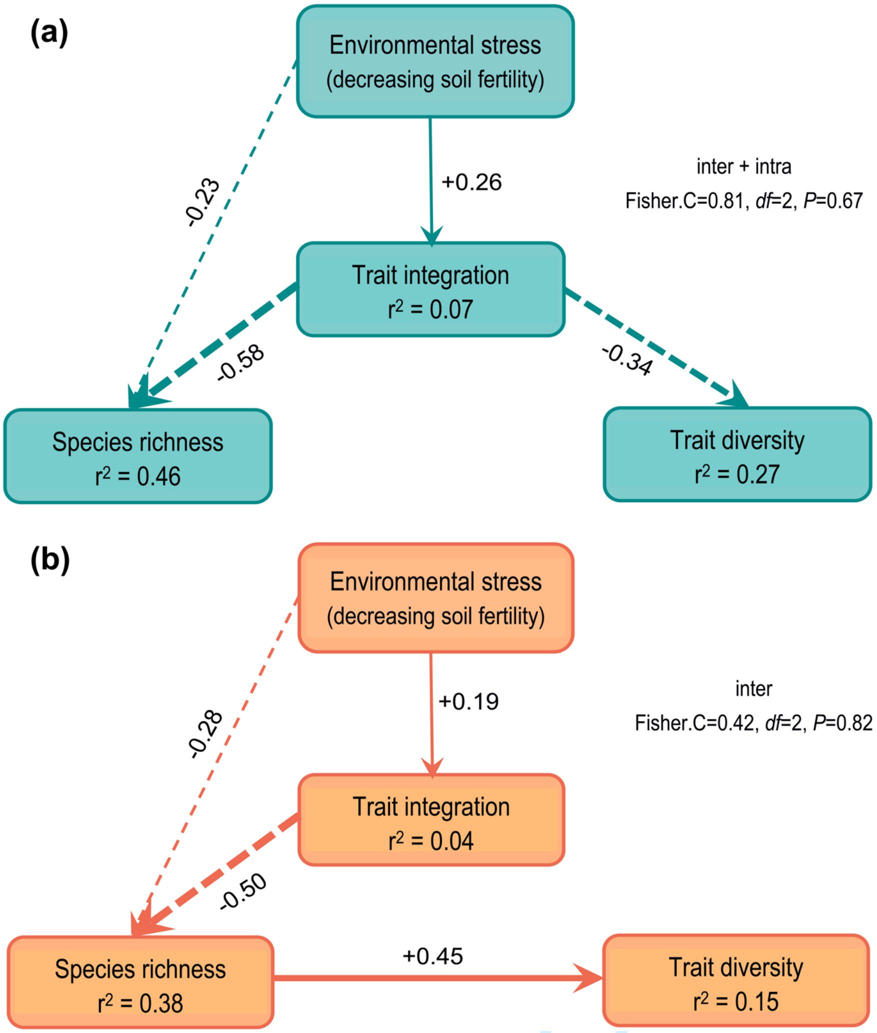

Figure 4. Path models showing the empirical relationships between environmental stress, rarefied richness, trait integration and trait diversity with (a) and without (b) accounting for intraspecific trait variation (inter + intra versus inter). Only significant ($\alpha = 0.05$) relationships are shown. Figure 2 for the hypothesized path model.

0.05). However, when only interspecific trait variation was considered, environmental stress-dependent species richness was the significant mediator of trait diversity ($p < 0.05$, Fig. 4a–b, Supporting information).

As predicted, the negative relationship between trait diversity and trait integration was more pronounced when accounting for intraspecific variation, with the slope of the negative relationship of being steeper than when disregarding intraspecific variation ($p = 0.03$, Fig. 5a). With respect to the relationship between species richness and trait integration, the slopes were equal between the two scenarios of trait aggregation ($p = 0.31$, Fig. 5b), but the negative correlation between species richness and trait integration was more pronounced when accounting for intraspecific variation (−0.60 versus −0.53, Supporting information). The intercept of the negative relationship between species richness and trait integration was significantly ~~higher~~ when accounting for intraspecific trait variation ($p < 0.01$, Fig. 5b).

Discussion

Our results indicate that 1) trait integration grows stronger along a gradient from more benign to stressful environment, 2) intraspecific trait variability contributes to weaken the overall trait integration in a community and 3) the negative association between trait integration and species richness or trait diversity is more pronounced when accounting for intraspecific trait variation. These results imply that measuring intraspecific, not merely interspecific, trait variation improves inference about functional tradeoffs underlying biodiversity patterns along environmental gradients, even though intraspecific trait variance accounted for a relatively low proportion of total trait variance across the dataset (< 8% in our case).

Our result of increasing strength of trait integration with decreasing soil fertility suggests that species occurring in stressed sites are subject to relatively strict multi-trait

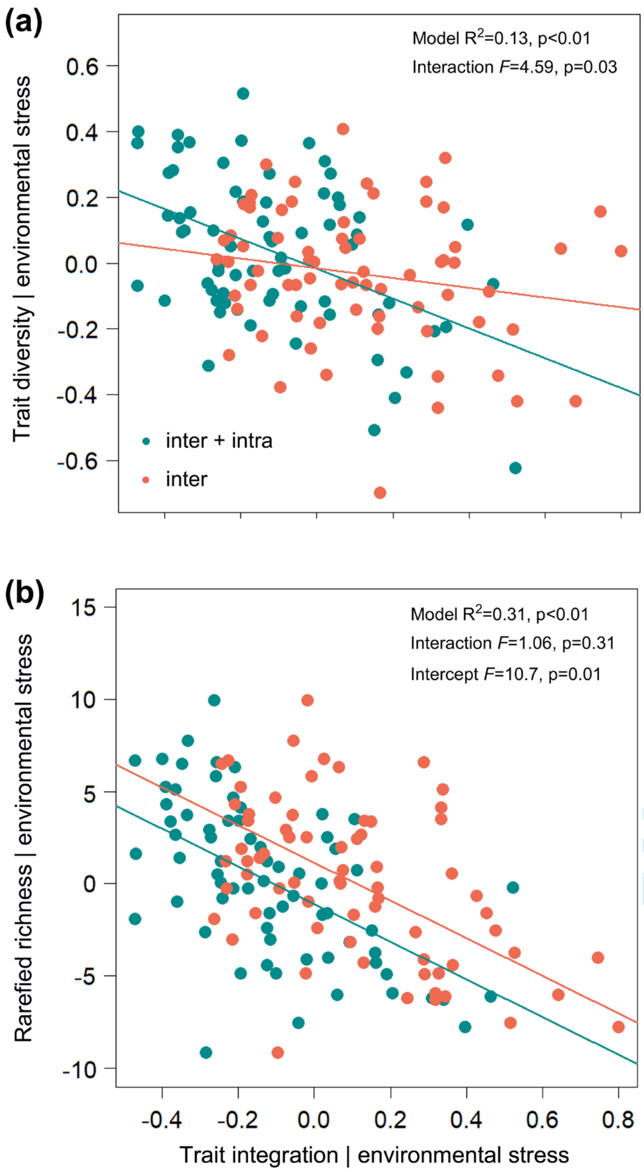

Figure 5. Bivariate relationships between trait integration versus trait diversity (a) and between trait integration versus species richness (b) and their dependence on the two scenarios of trait aggregation with and without intraspecific variation (inter + intra versus inter), after controlling for the effects of environmental stress (decreasing soil fertility as represented by PCA1 scores). A significant interaction term indicates that the slopes of response differ between scenarios, while a significant intercept term indicates that the difference in mean of response variable between scenarios.

combinatorial constraints to earn community membership. In our study, less fertile sites (i.e. characterized by lower soil moisture, lower soil carbon and higher soil bulk density) were dominated by only a few species with integrated conservative strategies such as low specific leaf area, high stem dry-matter content and Huber value (Supporting information). More fertile sites, by contrast, supported both conservative and acquisitive strategies with small to large leaf area, low to high specific leaf area, stem dry-matter content and Huber value (Supporting information). Logically, the number of species with functional design under strict multi-trait combinatorial constraints in the stressed sites should be lower than that under relaxed constraints in benign sites. In other words, stressed sites necessitate strong tradeoffs in viable strategies, leaving low availability of niche space for potential colonizers. Low species richness and trait diversity were therefore associated with a high degree of trait integration in the relatively stressed sites, and vice versa (Gianoli and Palacio-López 2009, Dwyer and Laughlin 2017b, Delhaye et al. 2020).

While both environmental stress and trait integration constrain species richness and trait diversity, the direct effect of trait integration on species richness or trait diversity seems to be stronger than the direct effect of environmental stress (Supporting information; Fig. 4). These results suggest that niche dimensionality predicated by a particular environmental regime, but not necessarily the environmental regime itself, play important roles in shaping species and trait diversity of local communities (Dwyer and Laughlin 2017b). Hence, the pattern of trait integration is useful to explain species richness and trait diversity along environmental gradients (Dwyer and Laughlin 2017b).

Consistent with our hypothesis, we found that trait integration generally became weaker when accounting for intraspecific trait variation. For multiple traits, interspecific trait integration is usually generated by evolutionarily fixed genetic correlation and/or broad-scale habitat filtering, while intraspecific trait integration mainly stems from fine-scale environmentally induced plasticity, neighborhood competition and/or ontogeny (Messier et al. 2017a, Anderegg et al. 2018). Idiosyncratic local drivers of intraspecific trait variation result in highly varied architectures of trait covariance in different sites, and consequentially constrains the extent of trait integration (Albert et al. 2010, Anderegg et al. 2018). This means that intraspecific trait variability allows species to vary in their position in functional space, seizing more niche opportunities.

Interestingly, the negative relationship between trait diversity and the strength of trait integration was more pronounced if accounting for intraspecific variation. This result suggests that intraspecific trait variation captures important aspects of trait covariance in situ, reflecting a role of microscale site-specific trade-offs in constraining trait diversity beyond the macroevolutionary constraints. It has been suggested that individual-level trait variation is important for the maintenance of high diversity (Clark 2010, Clark et al. 2011). We thus extend Clark's hypothesis and suggest that intraspecific trait variance as well as intraspecific trait covariance (or trait integration) play important roles for explaining biodiversity patterns in natural communities (Laughlin and Messier 2015).

Our results provide novel insights into the dependence of species richness on intraspecific trait variability. At first glance, species richness should be more strongly associated with trait integration quantified with interspecific variance. However, our results showed a more negative correlation between trait integration and species richness when accounting for both inter- and intraspecific variation. The underlying key point is that intraspecific trait variability allows for more niche opportunities through relaxed constraints on trait combinations from functional tradeoffs. It has been demonstrated that intraspecific trait variability can promote the opportunity for some species to occur in a site, increasing species richness (Crawford et al. 2019).

At a given level of trait integration conditional on environmental stress, rarefied richness was lower (the lower intercept in Fig. 5b) when intraspecific variation was included. Accordingly, rarefied richness appeared higher when disregarding intraspecific variation. As trait variation across sites within a species was aggregated to represent the 'mean' phenotype of the species, the 'mean' phenotype was assumed to occupy more sites than it actually could, thereby representing a Darwinian demon sensu lato. In fact, each phenotype can only be favored in a limited environmental range where its corresponding trait combination is allowed by local functional tradeoffs (Laughlin and Messier 2015, Zirbel and Brudvig 2020). The community that we observed in any environment consists of phenotypically adjustable species that otherwise would be less likely to occur there (Crawford et al. 2019). Therefore, species richness at a given level of trait integration conditional on environmental stress is apparently amplified by the assumed presence of Darwinian demons sensu lato in the scenario without intraspecific variation. Collectively, species richness can partly be the consequence of intraspecific trait variability.

To conclude, species richness and trait diversity are negatively associated with the strength of trait integration, reflecting that stronger functional tradeoffs allow a reduced number of trait combinations to be incarnated in a niche space. These results imply the importance of multidimensional integrated phenotypes in shaping species' establishment success along environmental gradients (Murren 2002, Laughlin and Messier 2015) and, in turn, signify the multidimensional nature of community assembly processes (Clark et al. 2010, Kraft et al. 2015). Moreover, intraspecific trait variability promotes niche dimensionality and, in turn, species richness. It is thus crucial to account for intraspecific trait variation to understand the link between niche dimensionality and the distribution of biodiversity along environmental gradients.

Data availability statement

The dataset supporting the results is published as Supporting information.

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