

# Species richness of limestone grasslands increases with trait overlap: evidence from within- and between-species functional diversity partitioning

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## Summary

1. Considering both within- and between-species functional diversities in plant communities has been recently suggested as a way to understand potential assembly mechanisms that control species coexistence. In particular, relating variations in species richness to within- and between-species functional diversities can provide a useful framework for evaluating the importance of alternative community assembly theories. In addition, little is known about whether the relationship between species richness and functional diversity components arises from direct or indirect effects of the abiotic environment.

2. We tested the relationship between functional diversity and species richness by disentangling functional diversity components into within-species, between-species and total functional diversities and by considering potential direct and indirect effects of the abiotic conditions. Multi- and single-trait approaches were applied using three key plant functional traits (height, specific leaf area (SLA), LDMC). Traits were measured on species coexisting across sixteen species-rich limestone grasslands. Direct and indirect effects of the abiotic conditions were evaluated using multiple soil properties including heterogeneity in soil depths.

3. The within-species functional diversity ranged between 13.5% and 33.6% of the total functional diversity. Within-species diversity was the main functional component linked to variations in species richness, despite the within-species functional diversity being lower than between-species functional diversity. Environmental soil properties had a direct effect on species richness but did not affect functional diversity components.

4. *Synthesis:* Our results provide evidence that increasing the trait overlap between species, due to an increase in within-species diversity, may relate to greater species coexistence. Disentangling multiple functional diversity components indicated that there may be equalizing mechanisms that act as potential drivers of species coexistence. In addition, it suggests the possibility that this approach may provide a better understanding of the processes involved in the structure of plant communities.

**Key-words:** abiotic environment, assembly rules, determinants of plant community diversity and structure, functional diversity, single- and multi-trait approaches, species richness, trait overlap

## Introduction

Trait-based approaches have emerged as a promising way to understand the mechanisms structuring plant communities (e.g. Stubbs & Wilson 2004; McGill *et al.* 2006; Ackerly & Cornwell 2007; Schamp, Chau & Aarssen 2008; Suding *et al.* 2008). These approaches mainly focus on functional trait

differences *between-species* and are used to infer the effects of biotic and abiotic processes on species coexistence (McGill *et al.* 2006; Kattge *et al.* 2011). Focussing on between-species differences usually implies considering differences between species in terms of mean trait values (McGill *et al.* 2006; Weiher *et al.* 2011; Violle *et al.* 2012; but see Mason *et al.* 2011), which assumes that within-species trait variability is negligible compared with interspecific variability (Kraft, Valencia & Ackerly 2008).

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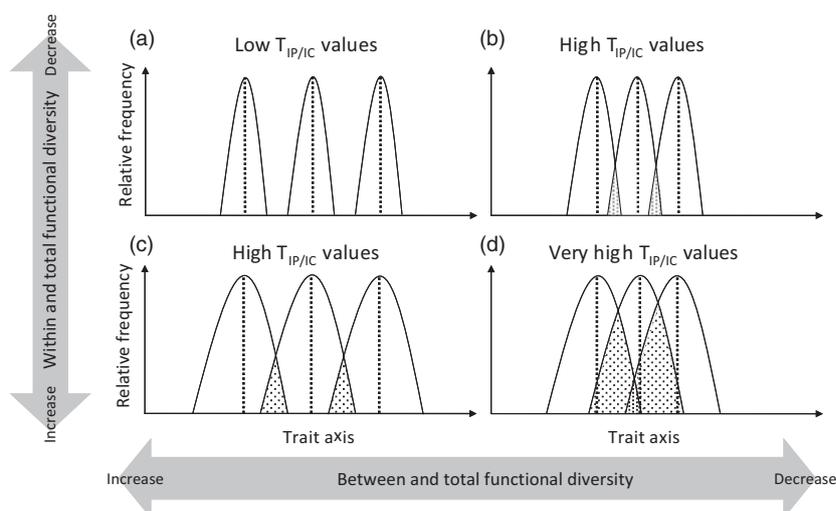
Recently, ecologists have reconsidered the importance of *within-species* trait variability. Plant species can display large within-species trait variability arising from phenotypic plasticity or genetic diversity (Schlichting 1986, 1989; Schlichting & Levin 1986). Within-species functional variability estimates the differences in trait values observed for a given species (Violle & Jiang 2009; Violle *et al.* 2012). Empirical studies have recently attempted to test the contribution of within-species diversity to the overall variability in plant functional traits within a community (e.g. Hulshof & Swenson 2010; de Bello *et al.* 2011; Auger & Shipley 2013). Within-species diversity can represent a non-negligible contribution to the overall functional trait variability, being as important as inter-specific trait variability (Valladares *et al.* 2000; Albert *et al.* 2010; Messier, McGill & Lechowicz 2010), and can have significant effects on community assembly (Fridley, Grime & Bilton 2007; Jung *et al.* 2010; Bolnick *et al.* 2011). Testing how changes in the balance between within-species, between-species and total functional diversities relate to species richness may therefore constitute a promising approach in functional community ecology (Violle *et al.* 2012; Hulshof *et al.* 2013).

Violle *et al.* (2012) specifically proposed to use a method of trait variance decomposition (de Bello *et al.* 2011) for this purpose. The method is based on the use of the  $T_{IP/IC}$  metric, that is, the ratio of trait variance within single species belonging to a community relative to the total trait variance of that community.  $T_{IP/IC}$  metric represents a good estimator of the trait overlap between species (Violle *et al.* 2012; de Bello *et al.* 2013). For a given level of between-species functional diversity, an increase in  $T_{IP/IC}$  values indicates an increasing probability for species to share similar trait values (Fig. 1). Low  $T_{IP/IC}$  values reflect therefore low trait similarities between species (Fig. 1a), whereas high  $T_{IP/IC}$  values indicate an increase in the trait overlap between species (Fig. 1b,c,d). Violle *et al.* (2012) also specifically suggested that the  $T_{IP/IC}$  metric might provide a useful insight for inferring the processes controlling species richness and assembly under different classical ecological theories. A negative species

richness– $T_{IP/IC}$  relationship scenario (i.e. an increase in the trait overlap accompanied by a decrease in species richness) would support the niche-based theory, that is, species coexist by being functionally different and by exploiting different niches (Hutchinson 1957; MacArthur & Wilson 1967). A scenario where an increase in the trait overlap does not relate to species richness either supports the neutral theory, where individuals and species are assumed to be ecologically equivalent (Hubbell 2001). Such a scenario may also arise from a complete balance between opposite forces driving community assembly, although it is unlikely to occur frequently (Gotzenberger *et al.* 2012). Finally, a last scenario would consider a positive relationship between  $T_{IP/IC}$  values and species richness. Under this scenario, the maintenance of diversity may actually either support the ‘individual variation’ theory (Clark *et al.* 2007; Clark 2010) or the ‘equalizing mechanism’ theory (Chesson 2000; Adler, HilleRisLambers & Levine 2007; Mayfield & Levine 2010). The ‘individual variation theory’ suggests that all individuals in a community, irrespective to the species they belong to, have different trait values to avoid competition from other individuals. Therefore, higher species richness should be accompanied by higher total functional diversity. In contrast, the ‘equalizing mechanism’ theory predicts that individuals from different species would tend to have similar trait values (i.e. an increase in the trait overlap between species) that minimize average fitness differences.

From the above, it follows that the functional components beside the  $T_{IP/IC}$  ratio (within-, between-species and total functional diversities; Fig. 1.) may be taken into account to allow inferring potential underlying mechanisms affecting the relationship between  $T_{IP/IC}$  and species richness (Hulshof *et al.* 2013). Furthermore, the abiotic environment may also play an important role when assessing the potential relationship between  $T_{IP/IC}$  and species richness. The abiotic environment can have direct effects on species richness by selecting for a given set of trait values (Grime 1973, 2006), but may also have indirect effects by affecting within- and between-species functional diversities (Albert *et al.* 2010; Jung *et al.* 2010; Fajardo & Piper 2011; Mason *et al.* 2011). Thus, it is also important to

**Fig. 1.** Effects of within-species and between-species functional diversities on  $T_{IP/IC}$  ratio. We represent three potential species as trait frequencies (see also Violle *et al.* 2012 for a similar representation). Under low within-species and between-species functional diversities (a), trait similarity is low (no trait overlap). Either the decrease in between-species functional diversity (b) or the increase in within-species functional diversity (c) increases the trait overlap between species (high  $T_{IP/IC}$  values, light grey areas). Changes in both within- and between-species functional diversities (d) lead to strong trait overlap reflected by very high  $T_{IP/IC}$  values.



consider separately the direct effects of abiotic factors on species richness from the indirect effects through changes in  $T_{IP/C}$  to disentangle possible interactions between biotic and abiotic forces driving community assembly.

The context of this study was deliberately placed into the Violle *et al.* (2012)'s framework to test the effects of  $T_{IP/C}$  ratio and its functional components on species richness. Specifically, within-species, between-species and total functional diversities were disentangled from the  $T_{IP/C}$  ratio to test the relationship between trait similarity and species richness and to infer to the underlying mechanisms of community assembly (Hulshof *et al.* 2013). Our study focussed on temperate dry limestone grasslands, being among the most diverse plant communities in Europe (Partel, Bruun & Sammull 2005). These habitats constitute an interesting study system to test the Violle *et al.* (2012)'s framework for different reasons. First, within-species diversity is expected to be an important driver of species coexistence and composition in dry grasslands (Booth & Grime 2003; Fridley, Grime & Bilton 2007). Secondly, species richness and functional diversity of dry grasslands are affected by stress factors such as water and nutrient limitations (e.g. Grime 1973), disturbance such as grazing (e.g. Grime 1973; Huston 1979) and light competition (e.g. Grime 1973; Hautier, Niklaus & Hector 2009). Grazing can strongly influence species diversity either directly by selecting for species with similar functional attributes (Grime 2006), or indirectly by preventing asymmetric light competition of tall, dominant species (Grime 1973; *i.e. release from competition*, Grubb 1977). Finally, environmental heterogeneity can also influence species diversity of limestone grasslands, for example, through variability in soil depth (Fridley *et al.* 2011). Environmental heterogeneity is supposed to increase within-species and between-species functional diversities by creating different niches in a community (Galloway 1995; Ackerly *et al.* 2000).

Overall, we explored how the environment (*i.e.* abiotic conditions and grazing) and the trait overlap, alone and in combination, relate to species richness. We acknowledge that there is likely to be no unidirectional cause–effect relationship between functional diversity and species richness (Vellend & Geber 2005), and we take this specifically into account in the data analyses. Three key functional traits generally involved in mechanisms of community assembly and particularly for these grasslands were measured on 1620 plant individuals. Both single- and multi-trait approaches were used to avoid potential masking effects in community assembly processes when traits are associated with opposing trait axes (Spasojevic & Suding 2012).

## Materials and methods

### STUDY AREA AND TRAIT MEASUREMENTS

Our study focussed on 16 limestone grasslands, within a 22 km<sup>2</sup> landscape in south-eastern Sweden. The 22 km<sup>2</sup> study area, in the central part of the Baltic island of Öland in south-eastern Sweden (56°40' N, 16°33' E), is characterized by a temperate climate with

low annual precipitation, (around 450 mm; see Reitalu *et al.* 2009 for details). The species richness and evenness of these grasslands are tightly related to the habitat age, land-use history, grazing, moisture status and eutrophication status (Reitalu *et al.* 2008, 2009). Abiotic filters, competition and grazing are also important drivers of the functional structure of these grasslands (de Bello *et al.* 2013). The management status of existing semi-natural grasslands in the area ranges from grasslands that are regularly grazed by domestic animals (mainly cows) to grasslands where grazing has been abandoned during the last one or two decades (Reitalu *et al.* 2009). Vegetation records were based on 16 grasslands widespread in the landscape.

Three key functional traits related to leaf morphology and plant size (height, leaf dry mass content (LDMC) and specific leaf area (SLA)) were assessed to quantify the within-species and between-species functional diversities of the 16 sampled grasslands. Size-related traits such as height are related to competitive ability (Westoby *et al.* 2002; Ackerly 2004; Violle *et al.* 2009). Specific leaf area (SLA) and leaf dry mass content are related to the leaf economic spectrum, reflecting plant species strategies for acquiring, using and conserving resources, including light, nutrients and water (Wright *et al.* 2004). These traits were selected because (i) they reflect different plant species strategies for acquiring, using and conserving resources (Westoby *et al.* 2002; Maire *et al.* 2009) and (ii) they are key plant traits for the functional structure of the studied grasslands (de Bello *et al.* 2013).

Five circular plots (50 cm diameter) were regularly placed within each of the 16 grasslands to sample trait variability. The five plots were always arranged in the same order: four plots were arranged as a square and the one remaining plot occurring in the centre. The distance to the central plot was approximately 2 m. Based on the species sampled, the species richness was estimated over the five plots. One non-damaged individual of each species found was selected in each plot. As such, up to five individuals per species and per grassland were collected, to ensure that most intraspecific variability was taken into account in our sampling (see Albert *et al.* 2011). All traits were measured following standard protocols (Pérez-Harguindeguy *et al.* 2013). Leaf trait measurements (SLA and LDMC) did not take petioles into account.

### QUANTIFICATION OF WITHIN-SPECIES, BETWEEN-SPECIES AND TOTAL FUNCTIONAL VARIANCES

The within-species, between-species and total functional diversities were quantified for each of the 16 sampled grasslands. We used the method of variance partitioning (de Bello *et al.* 2011), being tantamount to the decomposition of the quadratic entropy diversity (Pavone & Doledec 2005; Rao 2010). This method can be applied for both single- and multi-trait approaches (de Bello *et al.* 2011).

The total functional diversity is represented by the variance in trait values among all individuals collected in a given grassland (total variance). The within-species functional diversity is calculated, first, as the variance of trait values within each species (within-species variance). Then, a weighted average of all within-species trait variances is computed for each grassland, the weight being determined by the number of individuals per species (see de Bello *et al.* 2011 for details). The between-species functional diversity can be calculated, equally, both as the difference between total and within-species trait diversities and as the variance of species trait average values (between-species variance). Finally, the  $T_{IP/C}$  ratio was calculated using the ratio of within-species variance over the total community variance (Violle *et al.* 2012).

## ENVIRONMENTAL DATA

Direct and indirect environmental effects on species richness were evaluated by collecting data of various soil properties: soil depth, soil water content, organic matter content, pH, total nitrogen content, total phosphorus content and plant-available phosphorus. The mean soil depth was calculated for each of the 16 sampled grasslands using the five measures of soil depth (one for each plot within grassland). Soil samples were collected in each plot using the five top centimetres mixed together into a composite sample. Water content was determined as the difference between the weights of fresh sample soil and dried sample soil (dried during 12 h at 105 °C). Organic matter content was determined as the difference between the weights of the dried sample soil and burnt sample soil (2 h at 550 °C). Soil pH was measured in water. Total phosphorus and nitrogen were measured using flow injection analysis. Plant-available phosphorus was extracted according to Bray & Kurtz (1945) protocol.

Environmental heterogeneity was assessed in the studied grasslands using a coefficient of variation in soil depth ( $CV_{\text{soil}}$ ).  $CV_{\text{soil}}$  was calculated using the five measures of soil depth for each sampled grassland. Finally, the potential impact of grazing on species richness and functional diversity components was assessed using a grazing intensity index (see Reitalu *et al.* 2009 for details).

## DATA ANALYSES

### PCA on soil properties

A principal component analysis on correlation matrix (PCA) was performed using the 10 environmental soil properties measured on the sampled grasslands (Appendix S1 in Supporting Information). The PCA provided two main axes, explaining together 81.99% of the total variability. The first axis was mainly positively correlated with total phosphorus (Pearson correlation = 0.9) and nitrogen (0.89) and organic matter (0.87) and negatively with mean soil depth (0.73). The second axis is mainly negatively related to water availability (0.75) and soil depth (0.63). The first two PCA axes were used in further analyses, specifically to test the relationships between abiotic conditions and species richness and between abiotic conditions and the  $T_{\text{IP/IC}}$  ratio.

### Relationship between the environment, trait variances and species richness

Linear, partial and standardized major axis regression models were used to test the relationship between the environment,  $T_{\text{IP/IC}}$ , within-species, between-species, total functional variances and species richness. Linear regression models were used to test the relationship between environment (abiotic conditions including heterogeneity in soil depth and grazing) and functional variance components and species richness. To do so, linear regression models were performed with the  $T_{\text{IP/IC}}$  ratio as the response variable and using the first two PCA axes (Appendix S2), each of the soil properties including  $CV_{\text{soil}}$  (Appendix S2), or grazing as the predictors (Appendix S3). Linear regression models were also performed using species richness and functional variances as the response variables and the environment (soil properties and grazing) as predictors (Fig. 3a and Appendix S3, respectively).

Standardized major axis regression models were used to assess the relationship between functional variance components and species richness. Using standardized major axis regression models avoids

providing assumptions regarding unidirectional cause–effect relationship between functional diversity and species richness, as the residuals of the regression are computed for both variables in the model (Warton *et al.* 2006). We also tested indirect effects between soil properties and species richness through changes in functional diversity components using partial regression models (Fig. 3a and Appendix S4). To do so, a standardized major axis regression model was used to test the relationship between  $T_{\text{IP/IC}}$  as the predictor and the residuals of the regression model between species richness and soil properties as the response variable. Results of partial regression models were controlled using single models including all variables (abiotic and functional variance components), to avoid potential problems related to the adjustments in degrees of freedom (Garcia-Berthou 2001).

All models were performed using multi-trait (including height, LDMC and SLA: Fig. 3a, Appendix S2a and S3a) and single-trait approaches (Appendices S2b, S3b,c,d and S4). All statistical analyses were performed using R Core Team (2012). It is also noted that a structural equation modelling approach was provided to test for consistencies in results among statistical approaches. As the results were consistent among the two statistical approaches, only the results of linear/partial/standardized major axis regression models are presented.

## Results

### RELATIONSHIP BETWEEN SPECIES RICHNESS AND FUNCTIONAL DIVERSITY COMPONENTS

The within-species variance represented 20.19% of the total variance – ranging between 13.5% and 33.6% – when considering the multi-trait approach (Fig. 2). Within-species variance represented 22.6%, 24.22% and 17.06% of the total variance for height, SLA and LDMC, respectively.

The multi-trait approach indicated that the species richness was positively related to the increase in within-species trait variance (Fig. 3a,b, Table 1). In contrast, species richness was not related to between-species trait variance (Fig. 3a,c, Table 1) and total variance (Fig. 3a,d, Table 1). The within-species trait variance was the main variable linked to the observed increase in species richness with increasing  $T_{\text{IP/IC}}$  values across the 16 grasslands (Fig. 3a,e, Table 1). Single-trait approaches led to contrasted results. Results using height were the most consistent with the multi-trait approach (Appendix S4a), whereas no significant relationships were found for SLA and LDMC (Appendix S4b,c, respectively).

### ACCOUNTING FOR DIRECT AND INDIRECT EFFECTS OF THE ENVIRONMENT

The  $T_{\text{IP/IC}}$  ratio was not affected by the soil properties (Fig 3a), that is, using the two first axes from the PCA (Appendix S2a: Model 1). This pattern was also consistent using the single-trait approaches (Appendix S2b: Model 1). Neither the 10 soil variables, when tested independently (Appendices S2a,b: Models 2–9), nor the coefficient of variation in soil depth (Appendices S2a,b: Model 10) and nor grazing (Appendix S3) affected the  $T_{\text{IP/IC}}$  ratio. On the other hand, using the multi-environmental variables from the PCA,

our results indicated that soil properties had a direct significant effect on species richness (Fig 3a and Table 2: Model 1).

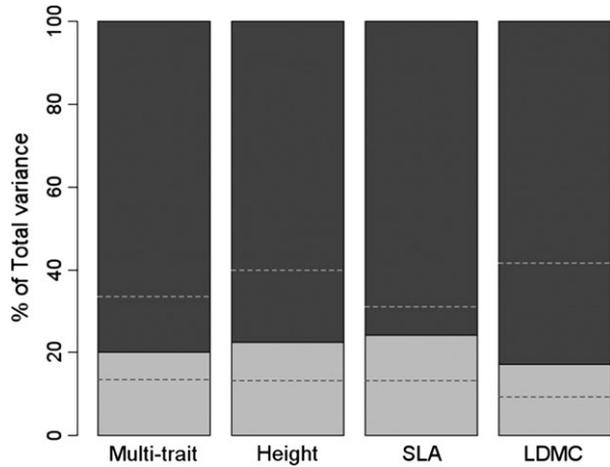
By removing the direct effects of the environmental variables on species richness, our results indicated stronger significant relationships between the  $T_{IP/IC}$  ratio and species richness for the multi-trait approach (Fig 3a, Table 2: Model

2) and for height (Appendix S4a). No significant patterns emerged when using the single-trait approaches for SLA (Appendix S4b) and LDMC (Appendix S4c).

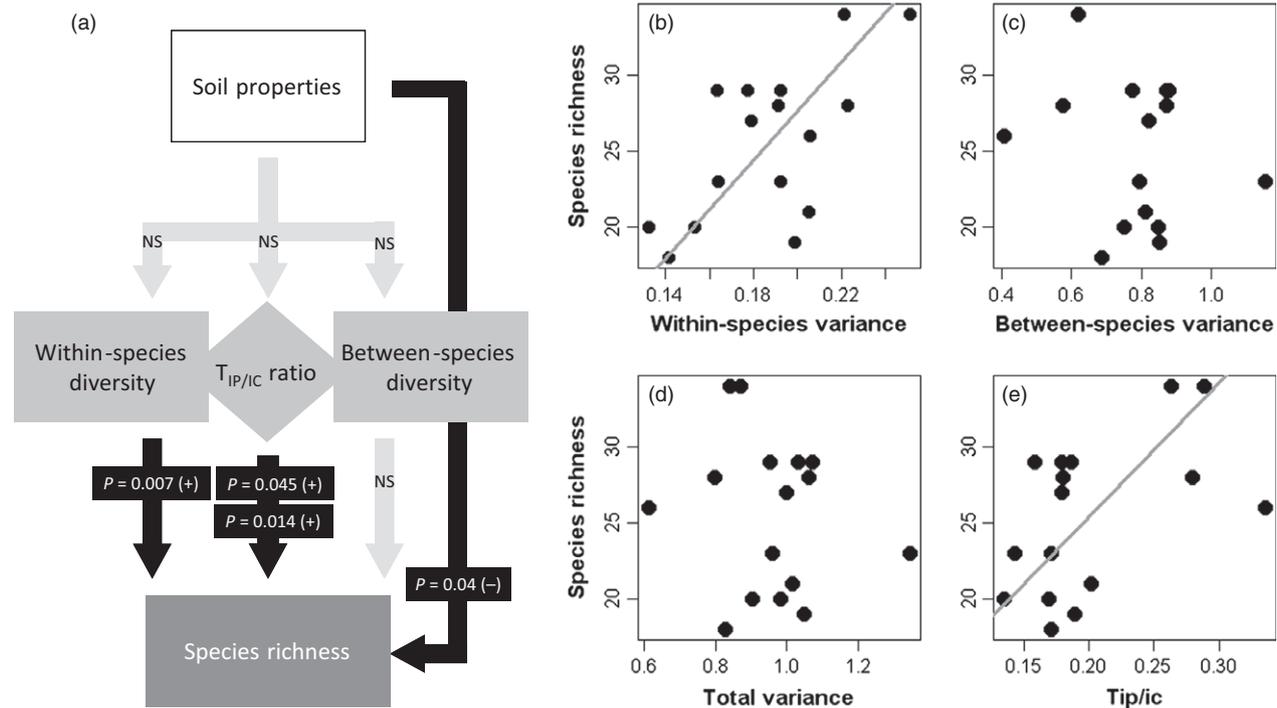
### Discussion

The aim of this study was to apply, and expand, Violle *et al.* (2012)'s framework relating species richness to the  $T_{IP/IC}$  metric, potentially providing a test for variations in species richness under various theories of community assembly. Expanding this framework, by disentangling the role of within-species, between-species and total functional diversities (de Bello *et al.* 2011; Hulshof *et al.* 2013) and taking into account direct and indirect effects of the abiotic environment, may help in refining our understanding on underlying processes driving community assembly.

Among the three functional traits measured in the 16 grasslands, variations in within-species functional diversity for height were the main component of functional diversity related to changes in species richness. The positive relationship between trait overlap for height and species richness suggests that asymmetric light competition might be an important driver structuring these dry calcareous grasslands (Grime 1973). Increasing within-species functional diversity in height might decrease the chance that individuals from tall species can out-compete those of the smaller species in size asymmetric



**Fig. 2.** Partitioning of the total variance in to within-species (light grey) and between-species variances (dark grey) for multi- and single-trait approaches. Dashed lines represent minimum and maximum values of within-species variance.



**Fig. 3.** Relationships between (a) soil properties, functional components and species richness, (b) within-species variance and species richness, (c) between-species variance and species richness, (d) total variance and species richness and (e)  $T_{IP/IC}$  ratio and species richness. All models are presented for the multi-trait approach including height, SLA and LDMC. For the relationship between soil properties, functional components and species richness, we indicate the significance and slopes of the statistical models. The  $P$  value in bold represents the impact of  $T_{IP/IC}$  on species richness after removing the confounding direct effects of environmental soil properties. All other parameters for each model (model adjusted  $R^2$ , slope, d.f.) are presented in Tables 1 and 2. For the standardized major axis regressions (b,c,d,e), lines are presented when significant. All other parameters for each model (model adjusted  $R^2$ , slope, d.f.) are presented in Table 1.

**Table 1.** Results of the standardized major axis regression models (SMA) between each of the functional diversity indices, that is, within-species functional diversity (With.div), between-species functional diversity (Betw.div), total functional diversity (Tot.div) and  $T_{IP/IC}$  as predictors and species richness as the response variable. It is noted that we present the results from the multi-trait approach including height, SLA and LDMC (see appendices for the single-trait approaches). We indicated regression parameters for each model and parameters (model adjusted  $R^2$ ,  $P$  values and direction when significant, d.f. = 1, 14 in all cases)

Predictors	Adjusted $R^2$	$P$
With.div	0.41	<b>0.007(+)</b>
Betw.div	0.07	0.29
Tot.div	-0.03	0.54
$T_{IP/IC}$	0.26	<b>0.045(+)</b>

Significant effects are indicated in bold.

**Table 2.** Results of the linear regression model using the two axes of the PCA for soil properties as predictors and species richness as the response variable (Model 1). We also indicate the  $P$  value for each predictor. We also present the results of the standardized major axis regression (SMA) between  $T_{IP/IC}$  as the predictor and the residuals of Model 1 as the response (Model 2). Using the residuals of Model 1 allows avoiding confounding effects of the environment on the species richness, that is, to test the pure effect of  $T_{IP/IC}$  on species richness. It is noted that we present the results from the multi-trait approach including height, SLA and LDMC (see appendices for the single-trait approaches). We indicated regression parameters for each model and parameters (model adjusted  $R^2$ ,  $P$  values and direction when significant, d.f. = 2, 13 for Model 1 and d.f. = 1, 14 for Model 2)

Model	Predictors	Adjusted $R^2$	$P$
Model 1	<b>PCA Axe 1 (0.02)</b> PCA Axe 2 (NS)	0.25	<b>0.04(-)</b>
Model 2	$T_{IP/IC}$	0.36	<b>0.014(+)</b>

Significant effects are indicated in bold.

competition for light, that is, to minimize fitness/competitive ability differences among species (Appendix S5). Thus, increasing within-species functional diversity can reduce the effects of above-ground size advantage among competing species limiting the occurrence of competitive exclusion (Semchenko *et al.* 2012).

The multi-trait approach was only consistent with the single-trait approach using height although it was reinforced by including SLA and LDMC. These results highlight that patterns and processes can be only evident by analysing functional diversity patterns of both individual and multiple traits (Spasojevic & Suding 2012). For instance, using only a multi-trait approach would have obscured the importance of height and asymmetric light competition to coexist in the studied communities. Studies focusing solely on multivariate functional diversity that do not decompose functional diversity into the components associated with each trait

(e.g. Weiher, Clarke & Keddy 1998), or only focus on phylogenetic diversity (e.g. Cadotte, Hamilton & Murray 2009), may suffer from integrating multiple niches axes into one variable (Spasojevic & Suding 2012).

Overall, our result highlights the occurrence of a positive relationship between within-species functional diversity and species richness (e.g. Clark *et al.* 2007; Clark 2010). The increase in within-species functional diversity was also accompanied by an increase in the  $T_{IP/IC}$  ratio for height and multiple traits, suggesting an increased trait overlap (Violle *et al.* 2012). As such, our results suggest that the increase in species diversity was associated with an increase in the trait overlap between species. Within-species functional diversity represented a small fraction of the total functional diversity ranging from 13% to 33%. Nonetheless, within-species diversity was the main component of functional diversity related to species richness in the studied grasslands. Within-species functional diversity has been increasingly recognized to significantly impact on community assembly (Fridley, Grime & Bilton 2007; Jung *et al.* 2010) and dynamics (Bolnick *et al.* 2011). Its meaningful relationship with species richness suggests that this component of functional diversity can be fundamental for species to coexist, particularly when between-species functional diversity alone does not relate to species richness. Thus, our results reinforce recent calls to recognize within-species diversity as an important element in functional community ecology (e.g. McGill *et al.* 2006; Jung *et al.* 2010; Bolnick *et al.* 2011; Violle *et al.* 2012).

High variations in within-species functional diversity can relate to species richness either because individuals in a given community tend to have different trait values from other individuals to avoid competition (Clark *et al.* 2007; Clark 2010) or because individuals from different species tend to have similar trait values that minimize average fitness differences (Chesson 2000; Grime 2006; Adler, HilleRisLambers & Levine 2007; Mayfield & Levine 2010). In our case, an increase in total functional diversity (i.e. a decrease in trait similarity among all individuals in each of the sixteen grasslands) was not related to species richness. Thus, our results do not seem to support the 'individual variation' theory as such (Clark *et al.* 2007; Clark 2010). Rather, the observed increase in the trait overlap between species ( $T_{IP/IC}$  ratio) was mainly driven by an increase in within-species functional diversity. Individuals from different species tended to have greater similarity in trait attributes relating to an increase in species coexistence, supporting the 'equalizing mechanism' theory (Chesson 2000; Grime 2006). We acknowledge that our study can only infer to the occurrence of equalizing mechanism as a potential underlying mechanism driving species richness in our grasslands. Nonetheless, our results reinforce recent calls stressing the overlooked impact of equalizing fitness processes in structuring plant communities (Mayfield & Levine 2010; Spasojevic & Suding 2012).

The abiotic conditions had a direct effect on the species richness (using soil PCA axes and single soil variables as predictors) but did not affect the different components of functional diversity. Altogether, these results suggested that the

abiotic conditions and functional diversity components impact simultaneously, but independently, on species richness in our grasslands. Our results are in accordance with recent findings in other grasslands (Mason *et al.* 2011). Nonetheless, we do not exclude that a potential relationship between the abiotic conditions and functional diversity may occur using stronger abiotic gradients (Auger & Shipley 2013).

The coefficient of variation in soil depth ( $CV_{\text{soil}}$ , here used to evaluate environmental heterogeneity) did not impact on the functional diversity components and species richness. The absence of relationship between the  $CV_{\text{soil}}$  and functional diversity components may suggest that no strong environmental heterogeneity effects occurred in our studied system. Such a result is surprising, as environmental heterogeneity has been found to impact on community structure and composition through changes in soil depth (Fridley *et al.* 2011). In particular, environmental heterogeneity can largely influence within-species and between-species functional diversities (Galloway 1995; Ackerly *et al.* 2000) with higher environmental heterogeneity increasing magnitudes of within-species functional diversity (Bradshaw & Hardwick 1989). We acknowledge that environmental heterogeneity may occur in our studied grassland through unmeasured environmental soil variables or additional trait axes associated with below-ground resources. It is clear that using only above-ground functional traits may overlook the importance of below-ground competition in community assembly (Tilman 1982, 1988; Sammul, Oksanen & Mägi 2006). At the same time, the observed variations in within-species functional diversity may also arise from phenotypic plasticity in response to biotic environment such as plant interactions (Violle *et al.* 2012) or from genetic diversity, an important driver of species coexistence (Vellend & Geber 2005), particularly in these limestone grasslands (Booth & Grime 2003; Fridley, Grime & Bilton 2007).

Finally, grazing did not impact on species richness in the studied limestone grasslands. Spatial heterogeneity due to grazing pressure is known to be an important driver of species coexistence in grasslands (McNaughton 1983) and might explain the observed increase in within-species functional diversity for height. Thus, the spatial heterogeneity due to grazing may indirectly increase species richness by preventing the chance that individuals from tall species can out-compete those of the smaller species in size asymmetric competition. We acknowledge that our data set does not allow testing the impact of spatial heterogeneity within grasslands due to grazing pressure. However, our data set can test for the impact of grazing among studied grasslands, and we assume that grazing would cause a similar heterogeneity in all grasslands where it occurs (due to the presence of generalist herbivores such as cows). Thus, the absence of grazing effect on functional diversity components rather indicates that the relationship occurring between within-species functional diversity and species richness does not arise from spatial heterogeneity due to grazing.

To conclude, we placed the context of this study into the Violle *et al.* (2012)'s framework. However, it should be noted that species richness and functional diversity may be related

in three different and not mutually exclusive ways (Vellend & Geber 2005). Species richness and functional diversity could arise due to (i) parallel effects of local characteristics (environmental heterogeneity) on both species richness and functional diversity, (ii) direct causal effects of species richness on functional diversity or (iii) direct causal effects of functional diversity on species richness. In parallel to relating species richness to the  $T_{IP/IC}$  metric, we invite an exploration of the different directions of the relationship between species richness and functional diversity. It may represent an exciting new opportunity for furthering our understanding of how trait variation within- and between-species interact to determine the structure of communities.

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## Supporting Information

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

**Appendix S1.** Results of the principal component analyses (PCA) for the environmental soil properties.

**Appendix S2.** Relationships between the ten environmental soil properties and the  $T_{IP/C}$  ratio.

**Appendix S3.** Results of the linear regression models between grazing as predictor and each of the functional diversity indices, that is, within-species functional diversity (With.div), between-species functional diversity (Betw.div), total functional diversity (Tot.div) and  $T_{IP/C}$  as the response variables.

**Appendix S4.** Relationships between the components of the hypothesized 'complex world' using single-trait approaches.

**Appendix S5.** Representation of the positive effect of increasing within-species functional diversity on species richness.