Is the use of unconstrained ordination appropriate for understanding plant ecological strategies and ecosystem functioning?

Linhai Zhu, Jonathan S. Lefcheck, Bojie Fu

Zhu, L. H. (lhzhu@rcees.ac.cn) [1]

Lefcheck, J. S. (jslefche@vims.edu) [2]

Fu, B. J. (Corresponding author, bfu@rcees.ac.cn) [1]

[1]State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

[2]Virginia Institute of Marine Science, College of William & Mary, PO Box 1346, Gloucester Point, Virginia 23062-1346, USA

Running Head: The functional roles of traits

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2631v2 | CC BY 4.0 Open Access | rec: 17 Apr 2017, publ: 17 Apr 2017

Abstract

The use of functional traits has increased exponentially in ecology, particularly in attempting to understand plant strategies and ecosystem functioning. This popularity has led to many proposed definitions of functional traits, which in turn has informed recommendations about how to gather, summarize, and analyze trait data. In this paper, we revisit the definition of the functional trait from the perspective of physiological, community and ecosystem ecology, and reason towards a broad, unrestrictive, and applicable definition. We then outline the conceptual mismatch between this definition and the popular practice of summarizing trait information using unconstrained ordination. We make specific suggestions about alternative methods to gain a mechanistic insight into how traits translate into functions. We hope this paper will improve our ability to move towards an ecological synthesis using a trait-based approach.

Keywords: Biodiversity and ecosystem functioning; Trait dimensionality; Functional trait diversity; Principal coordinates analysis; Economics spectrum; Functional diversity; Principal components analysis; Whole-plant perspective; Network approach; Context dependency; Structural equation modeling; Fourth-corner analysis

Functional traits have long been considered generalized indicators of plant ecological strategies, and thus how plants both respond to and affect their environment (Westoby et al. 2002; Hooper et al. 2005; Lepš et al. 2006; Petchey & Gaston 2006; Westoby & Wright 2006; Mouillot et al. 2013). The general utility of a functional trait approach is diminished, however, because ecologists must make a number of biological and statistical choices that are rarely tested, discussed, or challenged. One such choice involves the application of unconstrained ordination, such as principal coordinates or principal components analysis, to reduce the dimensionality of the species-by-trait matrix.

Unconstrained ordination is often used by ecologists in the research of plant strategies, where the goal is to identify the leading independent dimensions of functional variation among plants (Stahl et al. 2013; Laughlin 2014; Díaz et al. 2016). Unconstrained ordination is also among the most widely-used approaches to summarize trait information into a single functional diversity measure, especially in situations where the number of traits is greater than the number of species, or qualitative traits are present in the trait matrix (Laliberté & Legendre 2010; Maire et al. 2015). In fact, the most popular R package for computing functional diversity, *FD* (Laliberté & Legendre 2010), does not even permit the user to employ the raw trait matrix, instead allowing them to specify only the number of reduced principal coordinates axes to use.

By reducing real-world measurements into composite axes, however, ordination contradicts the whole-plant perspective that plant individuals coordinate different functional dimensions to complete their life history. Ordination also defies the primary benefit of functional traits, namely that they can be generalized across systems and taxa and provide a mechanistic insight into ecosystem functioning (McGill et al. 2006). For all intents, ordination axes have no real meaning, and thus cannot be usefully extrapolated beyond a single study. Oppositely, studies that apply a single ordination across multiple regions and taxa may lead to equally unreliable inferences, particularly at the global level. Environmental gradients are likely to drive context-dependent trade-offs or synergies, which in turn may vary the number and identity of ecologically relevant traits through space and time. This context-dependency is lost when all trait values are summarizing using global ordination, potentially obscuring the most relevant ecological mechanisms leading to local adaptation and coexistence (e.g., Spasojevic & Suding et al. 2012). Even exploration of individual trait loadings onto the axes—if possible—does not present a clean test of their individual and independent effects, since traits evolve simultaneously and are never completely uncorrelated.

Central to the misapplication of ordination are the numerous and increasingly convoluted definitions of a ‘trait.’ Although both biodiversity scientists and plant ecologists use traits, they offer slight variations on their definition, the former from the perspective of ‘fitness,’ and the latter of ‘ecosystem functioning’ (Mlambo 2014; Shipley et al. 2016). To achieve a consistent definition of traits, we must recognize the origin and development of traits and trait dimensionality, or the full range of traits that make up individual organisms. A definition that is both flexible and generalizable is pivotal to their meaningful application to understand plant strategies, and plant responses to – and effects on—their ecosystem.

In this piece, we first compare the current definitions for functional traits. We then discuss the context dependency of trait dimensionality, and detail the conceptual and methodological pitfalls of unconstrained ordination in understanding plant ecological strategies and in the quantification of functional diversity. We discuss some newly proposed alternative methods to understand how traits translate into functions at the individual, species and ecosystem scales. Ultimately, we propose an integrated framework to enhance the interpretive ability of functional diversity to ecosystem functioning. We believe these methods will improve our ability to gain a mechanistic understanding of biodiversity and ecosystem functioning using trait-based approach.

What is a functional trait?

The functional trait has historically been defined from the perspective of 'fitness' or 'individual performance' (Violle et al. 2007; Mouillot et al. 2013; Shipley et al. 2016), or of 'ecosystem functioning' (Hooper et al. 2005; Mlambo 2014). Several intermediate or combined definitions are also given (de Bello et al. 2010; Díaz et al. 2013; Hortal et al. 2015; Appendix S1). Ecologists from the first group regard traits as functional when they influence the fitness (or growth, reproduction and survival, the three components of individual performance), and ignore any external factors which may mediate this relationship, preferring to instead call these 'response traits.' Ecologists holding the second perspective consider traits are functional when they are generally linked to the processes that underpin ecosystem functioning, such as primary production and nutrient cycling. This dichotomy seems counterproductive, since the two definitions are not mutually exclusive.

To bridge the gap, recent thinking about whole plants in combination with a hierarchical perspective might give us some hints (Stahl et al. 2013; Edwards et al. 2014; Kleyer & Minden 2015). The overarching aim of a plant individual is to complete the processes of establishment, growth and reproduction (not, for example, to provide fruit or oxygen for human beings, from the perspective of functioning). To achieve this aim, a plant individual must coordinate and modify the associated traits to use resources effectively while simultaneously coping with any limiting factors (Kleyer & Minden, 2015). The efficiency with which plants and other organisms acquire resources is not only critical to fitness, but is one of the most fundamental ecosystem functions recognized to date. Further, it is highly dependent on extrinsic factors that modify both the resources, the ecological community, and interactions among organisms (Hooper et al. 2005). As an alternate example of this false dichotomy, allelopathic defenses enhance individual fitness only to the extent that they reflect the plant's ability to respond to external stimuli, i.e. grazing. This trait also has relevance to resource acquisition of higher trophic levels, which is only indirectly relevant to fitness of primary producers, but can be highly informative.

Recently, Mlambo (2014) proposed to define traits based on whether they affect ecosystem processes, otherwise they are known as 'biological traits,' since they lack a demonstrated link to functioning. By dividing traits into binary camps depending on whether they do or do not affect ecosystem functioning, the definition of Mlambo (2014) ignores the incredibly important insights that might be gained from understanding when, where, and why functional traits are *not* useful in this context. For example, the study of Poorter et al. (2008) found that well-recognized traits such as seed volume, specific leaf area, wood density, and maximum adult height were not always significantly correlated with relative diameter growth rate and mortality rate, which would necessitate their exclusion as 'functional traits' despite their well-documented links to functioning in other contexts (e.g. Lavorel & Grigulis 2012; Butterfield & Suding 2013; Conti & Díaz 2013). Similarly, traits important under one set of circumstances may change or be modified through the expression of other traits under a different set of circumstances.

Most definitions of functional traits also ignore the importance of such context-dependency. In different ecosystems, the factors shaping multiple traits, hereafter referred to as 'trait dimensionality,' are often different, which include the intrinsic genetic feature of plants, biotic factors (e.g. prey, herbivory, competition, facilitation, mutualism, parasitism) (Bagousse-Pinguet et al. 2015), abiotic factors (e.g. shade, temperature, drought, nutrient, erosion), evolutionary (Cavender-Bares et al. 2009; Cornelissen & Cornwell 2014), biogeographic (Heberling & Fridley 2012) and historical factors (Zhang et al. 2016). Therefore, a trait, as an easily-measured proxy for true plant functions, may imply different functions in different contexts (Poorter et al. 2009).

We therefore favor a meaning of functional traits that is open and flexible, accounts for varied applications of traits, recognizes context-dependency, and yet still provides mechanistic insights into the ecosystem functioning. Diaz et al. (2013) provide such a definition:

> "Functional traits are morphological, biochemical, physiological, structural, phenological, or behavioral characteristics that are expressed in phenotypes of individual organisms and are considered relevant to the response of such organisms to the environment and/or their effects on ecosystem properties."

We clarify that 'relevant' can mean in only a particular set of circumstances, and not restricted to all circumstances. Note that there is no mention of 'individual fitness' since, as pointed out by Mlambo (2014), all traits theoretically link to fitness since they are expressed phenotypes of living creatures (with the exception of traits subject to neutral drift, which probably ought not to be considered as functional anyways; McGill et al. 2006). This flexible definition permits a variety of approaches, and the widest insight, while avoiding the pitfall of discouraging the broad application of various functional traits.

From traits to ordination: all axes are not created equal

The context dependency of functional traits implies that bipartite trait relationships that reflect trait synergy or trade-off in one context do not always hold in other contexts (Grady et al. 2013; Edwards et al. 2014; Niinemets et al. 2015; Mason et al. 2016; Messier et al. 2016; see Appendix S2 for a more detailed description; see

Appendix S3-S5 for the three trait relationships collected from the literature). This context dependency also exists in the trait dimensionality captured by ordination analysis, i.e. a principal axis of the same rank obtained in different ecosystems might select for different traits and imply different ecological mechanisms (Albert et al. 2010; Stahl et al. 2013). Driven by different suites of drivers, individuals in different ecosystems cultivate different traits to develop an overall strategy that may not be evident from cross-system evaluations, or by focused investigators who fail to recognize this phenomenon. For example, a recent critique raised the critical point that comparisons could not be made among bird communities described using different numbers of ordination axes (Villéger et al. 2017).

In extreme circumstances of context dependency, ordination axes might not be easily or usefully summarized, particularly as the size of the species pool or the environmental gradient increases. The principal component analysis of six traits of 2214 species, which is the most comprehensive species trait matrix to date, revealed a two-dimensional global spectrum of plant form and function (Díaz et al. 2016). The first dimension represented the size of whole plants (adult plant height) and their parts (diaspore mass), but was strongly associated with stem specific density. The second dimension reflects the leaf economics spectrum, but closely associated with leaf area besides leaf nitrogen content and leaf mass per area (Díaz et al. 2016).

Such synthetic studies have tremendous value in describing the viable trait combinations for different taxonomic groups, and set a benchmark for comparing and understanding the variation of trait space and dimensionality across species, communities, and ecosystems (Díaz et al. 2016). Yet, they risk prioritizing a core set of traits at the expense of others. Numerous studies have found additional traits are key for explaining the structure and functioning of local systems. For example, root traits are important drivers of carbon and nitrogen cycling, and the formation and stability of soil, while most research of biodiversity and ecosystem functioning focus only on aboveground plant traits (Bardgett et al. 2014; Zhu et al. 2015). The upshot is that investigators may design experiments relying on these general findings or a limited number of traits (e.g., leaf traits) at the expense of traits that are uniquely important in their system or set of environmental conditions (e.g., root traits) (see also Paganelli et al. 2012; Milcu 2013).

Are functional dimensions completely independent and equally important?

The whole-plant perspective implies that the functional dimensions may not independent from each other, as an individual must coordinate different functions to develop an overall strategy and complete its life history. While the ecological dimensions may not be orthogonal, the ordination axes are mathematically orthogonal to each other. The analysis of Díaz et al. (2016), for example, found that the leaf area, diaspore size, height and stem density, which previously were considered as being perform different ecological functions (Westoby et al. 2002; Westoby & Wright 2006), are not independent from each other. In other words, traits in nature are correlated due to ecological and evolutionary pressures (McGill et al. 2006). These correlations make it difficult to extract the independent effect of any given trait from ordination axes. Practically, axes are probably informed to some degree or another by all or most traits, given the inherent correlations among most commonly-used plant traits in nature (Wright et al. 2005; Appendix S2-S5), confounding their interpretation and generalization.

This result also has important implications for functional diversity measures. In investigations of functional diversity, traits are almost always collapsed into a single univariate metric, often through ordination (e.g., Villéger et al. 2008). While there may be utility in consolidating highly correlated traits so the resulting indices maintain desirable statistical properties, again, the resulting values are still just abstractions that cannot be easily translated to other communities of other organisms (Laughlin 2014).

Like overly restrictive definitions of traits, the use of ordination axes may also eliminate potentially useful information. Usually, the ordination axes in the first several ranks can capture major variation of multiple traits, and therefore are given higher priority in understanding the responses of functional diversity to environmental variation, or their effects on ecosystem functioning. This behavior, for example, is the default for the *FD* package (Laliberté & Legendre 2010). However, examples exist where the last axes actually *better* explained response to environment variation or ecosystem functioning (e.g., Graham 2003).

The degree of the variance of functional dimensions do not equal to their importance in the plant life history. The rank of ordination axes depends on the

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2631v2 | CC BY 4.0 Open Access | rec: 17 Apr 2017, publ: 17 Apr 2017

number and identity of traits. The anatomical and biochemical properties of leaf are often measured and included in the ordination analysis, consequently the leaf economic spectrum are often identified as the first axes. However, we cannot defy the value of other trait dimensions. At this point, we think that a systematic and mechanistic thinking would be more appropriate to understand the trait relationships and dimensionality, and the relationships between traits and functions.

The advantage of ordination analysis lies in its handling of categorical traits, and in the cases that trait number are more than the species richness, which some multiple-trait functional diversity indices do not tolerate. However, including intraspecific variability in these indices may loosen the restriction that trait number must be more than species richness, and increase ecological reality of these indices (Fontana et al. 2016).

In lieu of ordination, we suggest multiple alternatives that address many of the shortcomings of ordination analysis and provide deeper and more mechanistic insight into the relationship between traits, plant strategies, and ecosystem functioning. This framework draws on emerging statistical tools and provides a prescribed routine for identifying important linkages across a range of scenarios.

From traits to functions: an integrated framework

We propose an integrated framework (Figure 1) to enhance the interpretative power of functional diversity indices, especially ones incorporating multiple traits, to environmental change and ecosystem functioning. Before this, we must first highlight that selecting traits empirically-linked with ecosystem functioning and environmental variation is the overarching guarantee of sound understanding of responses and effects of ecological communities (Lepš et al. 2006; Petchey & Gaston 2006; Lefcheck et al. 2015). This causal understanding should ideally come from local experimental evidence and not extrapolation from synthetic analyses, evidence which is sorely needed in most local systems.

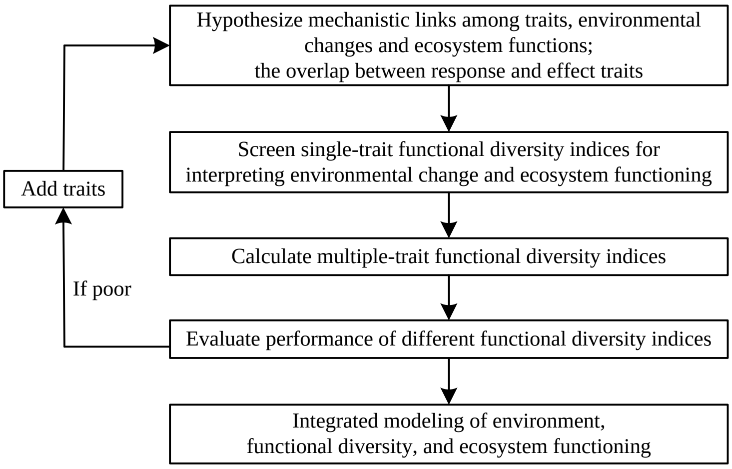

Figure 1. An integrated framework to enhance the interpretative ability of functional diversity indices, especially multi-trait ones, to environmental change and ecosystem functioning.

First, statistical methods that directly identify the mechanistic link between trait and ecosystem properties will provide valuable information for measuring functional diversity across various contexts (Dray & Legendre 2008; Jamil et al. 2013; Lefcheck et al. 2015). Such solutions include the fourth-corner analysis and a model-based approach (Dray & Legendre 2008; Jamil et al. 2013; Brown et al 2014). One key innovation that may provide a solution to the issues associated with ordination is the use of general(ized) linear mixed models (GLMMs), where both traits and environment are included as predictors in a multiple regression framework.

For example, Jamil et al. (2013) showed GLMMs were better able to parsimoniously select the trait and environmental variables predicting the presence of species in multiple datasets than alternate methods. The use of standardized regression coefficients would also permit the rigorous quantitative comparison of the relative importance of the predictors in a single application, including a mix of both categorical and continuous variables. The inclusion of random effects corresponding to species and other levels of organization (e.g. site) also relaxes assumptions pertaining to independence and homogeneity of variance (Jamil et al. 2013). However, we caution that collinearity among variables – such as between species or functional richness, or among individual traits and a composite index – can significantly affect the errors on parameter estimates. Even then, alternative methods, such as variable scaling or model selection using Akaike Information Criterion, exist to help identify important predictors in the presence of strong collinearity (Lefcheck & Duffy 2015).

After determining these mechanistic links, we can calculate each single-trait index to disentangle the confounded effects of individual traits, a notion that is completely ignored by ordination (Díaz et al. 2007; Spasojevic & Suding 2012; Butterfield & Suding 2013; Milcu et al. 2014). Then, if warranted, a multi-trait index can be constructed based on, or in addition to, the analyses of the single-trait indices. Such multi-trait indices can be useful. For example, Lefcheck & Duffy (2015) calculated individual trait indices to explain ecosystem functioning in experimental mesocosms, and found that their predicted effects did not differ from the integrated traits index. However, the standard errors on these predictions were significantly lower using the multi-trait index compared to the individual trait indices. Thus, it appears that when there are not trade-offs among traits, subtle variations among species across multiple traits may improve the accuracy of multi-trait indices.

The utilization of individual traits circumvents the growing problem of allowing statistical methods to make choices concerning trait selection, as opposed to innate biological knowledge of the organisms or even experimentation (Lefcheck et al. 2015). It also addresses the issue of context dependency by permitting evaluation of single traits, eliminating the potential for trade-offs to mask important ecological signals (e.g., Spasojevic & Suding 2012). Finally, careful *a priori* vetting of traits can reduce the influence of correlations, and many uni- and multi-trait indices have equally desirable mathematical properties (Lefcheck et al. 2015).

This is not to say that multiple traits should never be collapsed into a univariate index. Indeed, there may be some utility in doing so since it has been shown in some cases to greatly reduce prediction error (Lefcheck & Duffy 2015). Individual traits do, however, allow for the extrapolation of functional traits beyond the investigated organisms in a more straightforward way, in the truest application of a functional trait approach (McGill et al. 2006). The use of actual traits instead of ordination axes also reduces the risk of including unnecessary or redundant trait information (Villéger et al. 2008), especially since simulations have shown that several popular indices of functional trait diversity, including functional richness, are sensitive to redundant

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2631v2 | CC BY 4.0 Open Access | rec: 17 Apr 2017, publ: 17 Apr 2017

traits (Cornwell et al. 2006; Lefcheck et al. 2015).

After the functional diversity indices are calculated, the performance of specific trait combinations can also be evaluated by compared their results with these of all possible trait combinations (Petchey et al. 2004; Flynn et al. 2009; Lefcheck & Duffy 2015). For example, Mokany et al. (2008) identified traits likely to affect ecosystem functioning, and then selected four single traits that best predict investigated ecosystem processes using the coefficient of determination. Finally, they selected the trait (or trait combinations) with maximum explanatory power by calculating functional diversity indices using all the possible combinations of these four traits. This approach combines the best of both worlds, acknowledging both trade-offs among individual traits and the increased accuracy that may come with a multi-trait index. If the performance of the indices is poor, we can measure additional traits or environmental covariates and repeat the analysis.

From traits to functions: a network approach

While these techniques allow the direct quantification of responses and effects of traits, in reality, traits may have cascading or indirect effects through individuals, populations, or communities. Network analysis represents a potential tool which can describe trait, functions, and environment in a mechanistic and systematic way. For example, Structural Equation Modelling (SEM), an important tool of causal network analysis, has already been used to successfully explore the relationships among multiple traits (Shipley et al. 2006; Poorter et al. 2014; Mason et al. 2016). The third-generation of SEM also draws on GLMMs, allowing them to be combined to draw inferences across the entire network (Shipley 2009, Lefcheck 2016). No matter which method is used, we expect that the relationship between traits and functions can be further validated and tested to improve our understanding of plant ecological strategies. Although unconstrained analysis can implement this task, a network analysis will provide a fresh, causal, and holistic perspective.

To limit the complexity of these networks, correlation network analysis (CNA) can determine the degree of trait dimensionality, and identify the key traits in different dimensions (Proulx et al. 2005; Dale & Fortin 2010). CNA will straightforwardly describe each dimension as an irreplaceable one. Next, the SEM can take the trait, function and environmental variables as separate variables, and model them together, which will yield a more holistic understanding and better prediction of plant functions (Liu et al. 2016). Although SEM can elucidate the casual relationship between variables, evaluating many traits can become burdensome or even computationally inestimable (even using local estimation). CNA can include more variables, and simplify net structure by first describing its dimensionality and key variables (Proulx et al. 2005; Dale & Fortin 2010). After identifying key variables using CNA, SEM can incorporate different types of functions describing the relationships among these variables in a single causal network (Lefcheck 2016).

Finally, individual traits, functional trait diversity, environmental change, and ecosystem functions can be integrated and modelled simultaneously. Once again, SEM is useful for predicting complex, real-world ecosystems, which can evaluate

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2631v2 | CC BY 4.0 Open Access | rec: 17 Apr 2017, publ: 17 Apr 2017

multiple hypotheses simultaneously. For example, SEM has been used successfully in the exploration of the trait drivers of ecosystem functioning across trophic levels (Lefcheck & Duffy 2015). Recent advances in the methods to produce AIC and BIC scores (Shipley 2013) permits robust comparisons of causal networks derived from, for example, different regions or taxa.

An exciting frontier is incorporating context dependency into functional diversity metrics themselves. Faith (2015) proposed the EDf method, which infers environmental gradients based on allocation of species to different ‘demand points’ in bivariate trait space—essentially the environmental ‘centroids’ identified through the observed manifestation of community trait composition. Thus, EDf captures which suites of traits are most ‘attracted’ to which environmental conditions, providing a simultaneous solution for context dependency. The method can produce additional measures, including another popular metric of functional diversity, Rao’s quadratic entropy.

Conclusions

We suggest that a definition of functional traits should accommodate a wide variety of perspectives, and facilitates communication among both plant physiological ecologists and those in the field of biodiversity and ecosystem functioning. We suggest that trait dimensionality should be understood from a whole-plant perspective in specific ecosystems, and the dimensions may not completely independent and all essential to plant life history. We propose that multivariate techniques, such as CNA

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2631v2 | CC BY 4.0 Open Access | rec: 17 Apr 2017, publ: 17 Apr 2017

and SEM, can be used to understand trait relationships and whole-plant ecological strategies both within and across ecosystems. We also argue for a more nuanced statistical approach than is afforded by ordination, which omits potentially useful information, does not address ecological trade-offs and is a poor proxy for understanding ecological mechanism, with no utility outside of the study in which it was conducted. We suggest that trait selection not be limited by prior evidence, particularly those from large syntheses that have identified important traits using ordination, since such an approach does not recognize the context-dependency of natural systems and could be potentially misleading. Instead, evidence should be acquired through rigorous experimentation and observation under a variety of scenarios, where new statistical tools can help disentangle the contributions of individual and multiple traits.

Acknowledgements

This research was funded by the National Natural Science Foundation of China (grant no. 41230745, 41501285) and the China Postdoctoral Science Foundation (grant no. 2014M561077). We thank the two reviewers, including D. Faith, for their comments that greatly improved our manuscript. There is no conflict of interest.

Author contributions

BJF, LHZ and JSL developed the idea, LHZ collected the literature, LHZ, JSL and BJF wrote the manuscript.

References

Albert, C.H., Thuiller, W., Yoccoz, N.G., Douzet, R., Aubert, S. & Lavorel, S. 2010. A multi-trait approach reveals the structure and the relative importance of intra- vs. interspecific variability in plant traits. Functional Ecology 24: 1192–1201.

Bagousse-Pinguet, Y.L., Börger, L., Quero, J.L., García-Gómez, M., Soriano, S., Maestre, F.T. & Gross, N. 2015. Traits of neighbouring plants and space limitation determine intraspecific trait variability in semi-arid shrublands. Journal of Ecology 103: 1647–1657.

Bardgett, R.D., Mommer, L. & De Vries, F.T. 2014. Going underground: root traits as drivers of ecosystem processes. Trends in Ecology & Evolution 29(12): 692–699.

Brown, A.M., Warton, D.I., Andrew, N.R., Binns, M., Cassis, G. & Gibb, H. 2014. The fourth-corner solution – using predictive models to understand how species traits interact with the environment. Methods in Ecology and Evolution 5: 344–352.

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: 9–17.

Cavender-Bares, J., Kozak, K.H., Finel, P.V.A. & Kembel, S.W. 2009. The merging of community ecology and phylogenetic biology. Ecology Letters 12: 693–715.

Conti, G. & Díaz S. 2013. Plant functional diversity and carbon storage – an empirical test in semi-arid forest ecosystems. Journal of Ecology 101: 18–28.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2631v2 | CC BY 4.0 Open Access | rec: 17 Apr 2017, publ: 17 Apr 2017

Cornelissen, J.H.C. & Cornwell, W.K. 2014. The Tree of Life in ecosystems: evolution of plant effects on carbon and nutrient cycling. Journal of Ecology 102: 269–274.

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.

Dale, M.R.T. & Fortin, M.J. 2010. From graphs to spatial graphs. Annual Review of Ecology, Evolution, and Systematics, 41, 21–38.

de Bello, F., Lavorel, S., Díaz, S., Harrington, R., Cornelissen, J.H.C., Bardgett, R.D., Berg, M.P., Cipriotti, P., Feld, C.K., (..) & Harrison P.A. 2010. Towards an assessment of multiple ecosystem processes and services via functional traits. Biodiversity and Conservation 19: 2873–2893.

Díaz, S., Kattge, J., Cornelissen, J.H.C., Wright, I.J., Lavorel, S., Dray, S., Reu, B., Kleyer, M., Wirth, C., (…) & Gorné, L.D. 2016. The global spectrum of plant form and function. Nature 529: 167–171.

Díaz, S., Lavorel, S., de Bello, F., Quétier, F., Grigulis, K. & Robson, T.M. 2007. Incorporating plant functional diversity effects in ecosystem service assessments. Proceedings of the National Academy of Sciences of the United States of America 104 (52): 20684–20689.

Díaz, S., Purvis, A., Cornelissen, J.H.C., Mace, G.M., Donoghue, M.J., Ewers, R.M., Jordano, P. & Pearse W.D. 2013. Functional traits, the phylogeny of function, and ecosystem service vulnerability. Ecology and Evolution 3(9): 2958–2975.

Dray, S. & Legendre, P. 2008. Testing the species traits–environment relationships:

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2631v2 | CC BY 4.0 Open Access | rec: 17 Apr 2017, publ: 17 Apr 2017

the fourth-corner problem revisited. Ecology 89(12): 3400–3412.

Edwards, E.J., Chatelet, D.S., Sack, L. & Donoghue, M.J. 2014. Leaf life span and the leaf economic spectrum in the context of whole plant architecture. Journal of Ecology 102: 328–336.

Faith, D.P. 2015. The unimodal relationship between species' functional traits and habitat gradients provides a family of indices supporting the conservation of functional trait diversity. Plant Ecology 216: 725–740.

Flynn, D.F.B., Gogol-Prokurat, M., Nogeire, T., Molinari, N., Richers, B.T., Lin, B.B., Simpson, N., Mayfield, M.M. & DeClerck F. 2009. Loss of functional diversity under land use intensification across multiple taxa. Ecology Letters 12: 22–33.

Fontana, S., Petchey, O.L. & Pomati, F. 2016. Individual-level trait diversity concepts and indices to comprehensively describe community change in multidimensional trait space. Functional Ecology 30: 808–818.

Grady, K.C., Laughlin, D.C., Ferrier, S.M., Kolb, T.E., Hart, S.C., Gerard, J., Allan, G.J. & Whitham, T.G. 2013. Conservative leaf economic traits correlate with fast growth of genotypes of a foundation riparian species near the thermal maximum extent of its geographic range. Functional Ecology 27: 428–438.

Graham, M.H. 2003. Confronting multicollinearity in ecological multiple regression. Ecology 84(11): 2809–2815.

Heberling, J.M. & Fridley, J.D. 2012. Biogeographic constraints on the world-wide leaf economics spectrum. Global Ecology and Biogeography 21: 1137–1146.

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M. & Wardle, D.A. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs 75(1): 3–35.

Hortal, J., de Bello, F., Diniz-Filho, J.A.F., Lewinsohn, T.M., Lobo, J.M. & Ladle, R.J. 2015. Seven shortfalls that beset large-scale knowledge of biodiversity. Annual Review of Ecology, Evolution, and Systematics 46: 523–549.

Jamil, T., Ozinga, W.A., Kleyer, M. & ter Braak, C.J.F. 2013. Selecting traits that explain species–environment relationships: a generalized linear mixed model approach. Journal of Vegetation Science 24: 988–1000.

Kleyer, M. & Minden, V. 2015. Why functional ecology should consider all plant organs: an allocation-based perspective. Basic and Applied Ecology 16: 1–9.

Laliberté, E. & Legendre, P. 2010. A distance-based framework for measuring functional diversity from multiple traits. Ecology 91: 299–305.

Laughlin, D.C. 2014. The intrinsic dimensionality of plant traits and its relevance to community assembly. Journal of Ecology 102: 186–193.

Lavorel, S. & Grigulis K. 2012. How fundamental plant functional trait relationships scale-up to trade-offs and synergies in ecosystem services. Journal of Ecology, 100: 128–140.

Lefcheck, J.S. & Duffy, J.E. 2015. Multitrophic functional diversity predicts ecosystem functioning in experimental assemblages of estuarine consumers. Ecology 96(11): 2973–2983.

Lefcheck, J.S. 2016. PIECEWISESEM: Piecewise structural equation modelling in R for ecology, evolution, and systematics. Methods in Ecology and Evolution 7: 573–579.

Lefcheck, J.S., Bastazini, V.A.G. & Griffin, J.N. 2015. Choosing and using multiple traits in functional diversity research. Environmental Conservation 42 (2): 104–107.

Lepš, J., de Bello, F., Lavorel, S. & Berman, S. 2006. Quantifying and interpreting functional diversity of natural communities: practical considerations matter. Preslia 78: 481–501.

Liu, X.J., Swenson, N.G., Lin, D.M., Mi, X.C., Umaña, M.N., Schmid, B., Ma, K.P. 2016. Linking individual-level functional traits to tree growth in a subtropical forest. Ecology 97(9): 2396–2405.

Maire, E., Grenouillet, G., Brosse, S. & Villéger, S. 2015. How many dimensions are needed to accurately assess functional diversity? A pragmatic approach for assessing the quality of functional spaces. Global Ecology and Biogeography 24: 728–740.

Mason, C.M., Goolsby, E.W., Humphreysand, D.P. & Donovan, L.A. 2016. Phylogenetic structural equation modelling reveals no need for an ‘origin’ of the leaf economics spectrum. Ecology Letters 19: 54–61.

McGill, B.J., Enquist, B.J., Weiher, E. & Westoby, M. 2006. Rebuilding community ecology from functional traits. Trends in Ecology and Evolution 21 (4): 178–185.

Messier, J., McGill, B.J., Enquist, B.J. & Lechowicz, M.J. 2016. Trait variation and integration across scales: Is the leaf economic spectrum present at local scales?

Ecography DOI: 10.1111/ecog.02006.

Milcu, A., Allan, E., Roscher, C., Jenkins, T., Meyer, S.T., Flynn, D., Bessler, H., Buscot, F., Engels, C., (…) & Eisenhauer, N. 2013. Functionally and phylogenetically diverse plant communities key to soil biota. Ecology 94(8): 1878–1885.

Milcu, A., Roscher, C., Gessler, A., Bachmann, D., Gockele, A., Guderle, M., Landais, D., Piel, C., Escape, C., (…) & Roy, J. 2014. Functional diversity of leaf nitrogen concentrations drives grassland carbon fluxes. Ecology Letters 17: 435–444.

Mlambo, M.C. 2014. Not all traits are ‘functional’: insights from taxonomy and biodiversity-ecosystem functioning research. Biodiversity and Conservation 23: 781–790.

Mokany, K., Ash, J. & Roxburgh, S. 2008. Functional identity is more important than diversity in influencing ecosystem processes in a temperate native grassland. Journal of Ecology 96: 884–893.

Mouillot, D., Nicholas A.J, Graham, N.A.J., Villéger, S., Mason, N.W.H. & Bellwood, D.R. 2013. A functional approach reveals community responses to disturbances. Trends in Ecology & Evolution 28(3): 167–177.

Niinemets, U. 2015. Is there a species spectrum within the world-wide leaf economics spectrum? major variations in leaf functional traits in the Mediterranean sclerophyll *Quercus ilex*. New Phytologist 205: 79–96.

Paganelli, D., Marchini, A. & Occhipinti-Ambrogi, A. 2012. Functional structure of marine benthic assemblages using Biological Traits Analysis (BTA): A study along the Emilia-Romagna coastline (Italy, North-West Adriatic Sea). Estuarine, Coastal and Shelf Science 96: 245–256.

Petchey, O.L. & Gaston, K.J. 2006. Functional diversity: back to basics and looking forward. Ecology Letters 9: 741–758.

Petchey, O.L., Hector, A. & Gaston, K.J. 2004. How do different measures of functional diversity perform? Ecology 85: 847–857.

Poorter, H., Lambers H. & Evans, J.R. 2014. Trait correlation networks: a whole-plant perspective on the recently criticized leaf economic spectrum. New Phytologist 201: 378-382.

Poorter, H., Niinemets, Ü., Poorter, L., Wright, I.J. & Villar, R. 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytologist 182: 565–588.

Poorter, L., Wright, S.J., Paz, H., Ackerly, D.D., Condit, R., Ibarra-Manríquez, G., Harms, K.E., Licona, J.C., Martínez-Ramos, M., (…) & Wright I.J. 2008. Are functional traits good predictors of demographic rates? Evidence from five neotropical forests. Ecology 89(7): 1908–1920.

Proulx, S.R., Promislow, D.E.L. & Phillips, P.C. 2005. Network thinking in ecology and evolution. Trends in Ecology and Evolution 20 (6): 345–353.

Shipley, B. 2009. Confirmatory path analysis in a generalized multilevel context. Ecology 90 (2): 363–368.

Shipley, B. 2013. The AIC model selection method applied to path analytic models compared using a d-separation test. Ecology 94(3): 560–564.

Shipley, B., de Bello, F., Cornelissen, J.H.C., Laliberté, E., Laughlin, D.C. & Reich, P.B. 2016. Reinforcing loose foundation stones in trait-based plant ecology. Oecologia 180: 923–931.

Shipley, B., Lechowicz, M.J., Wright, I. & Reich, P.B. 2006. Fundamental trade-offs generating the worldwide leaf economics spectrum. Ecology 87(3): 535–541.

Spasojevic, M.J. & Suding, K.N. 2012. Inferring community assembly mechanisms from functional diversity patterns: the importance of multiple assembly processes. Journal of Ecology 100(3): 652–661.

Stahl, U., Kattge, J., Reu, B., Voigt, W., Ogle, K., Dickie, J. & Wirth, C. 2013. Whole-plant trait spectra of North American woody plant species reflect fundamental ecological strategies. Ecosphere 4(10):128.

Villéger, S., Maire, E. & Leprieur, F. 2017. On the risks of using dendrograms to measure functional diversity and multidimensional spaces to measure phylogenetic diversity: a comment on Sobral et al. (2016). Ecology Letters 20(4): 554–557.

Villéger, S., Mason, N.W.H. & Mouillot, D. 2008. New multidimensional functional diversity indices for a multifaceted framework in functional ecology. Ecology 89: 2290–2301.

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: 882–892.

Westoby, M. & Wright, I.J. 2006. Land-plant ecology on the basis of functional traits. Trends in Ecology and Evolution 21: 261–268.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2631v2 | CC BY 4.0 Open Access | rec: 17 Apr 2017, publ: 17 Apr 2017

Westoby, M., Falster, D.S., Moles, A.T. Vesk, P.A. & Wright, I.J. 2002. Plant ecological strategies: some leading dimensions of variation between species. Annual Review of Ecology, Evolution, and Systematics, 33, 125–159.

Wright, I.J., Reich, P.B., Cornelissen, J.H.C, Falster, D.S., Groom, P.K., Hikosaka, K., Lee, W., Lusk, C.H., Niinemets, Ü., (…) & Westoby, M. 2005. Modulation of leaf economic traits and trait relationships by climate. Global Ecology and Biogeography 14: 411–421.

Zhang, J., Nielsen, S.E., Mao, L.F., Chen, S.B. & Svenning, J.C. 2016. Regional and historical factors supplement current climate in shaping global forest canopy height. Journal of Ecology 104: 469–478.

Zhu, H.X., Fu, B.J., Wang, S., Zhu, L.H., Zhang, L.W., Jiao, L. & Wang, C. 2015. Reducing soil erosion by improving community functional diversity in semi-arid grasslands. Journal of Applied Ecology 52(4): 1063–1072.

Supporting Information

Appendix S1. Different definitions for functional trait.

Appendix S2. The context dependency of trait trade-offs and synergies.

Appendix S3. The relationship between leaf photosynthetic rate (the response variable) and leaf nitrogen concentration (the explanatory variable) on a mass basis.

Appendix S4. The relationship between leaf mass per area (the response variable) and leaf nitrogen concentration per mass (the explanatory variable).

Appendix S5. The relationship between leaf nitrogen concentration (the response

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2631v2 | CC BY 4.0 Open Access | rec: 17 Apr 2017, publ: 17 Apr 2017

variable) and leaf phosphorus concentration (the explanatory variable) on a mass basis.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2631v2 | CC BY 4.0 Open Access | rec: 17 Apr 2017, publ: 17 Apr 2017