

SPECIAL FEATURE

PLANT FUNCTIONAL EFFECTS ON ECOSYSTEM SERVICES

An experimental framework to identify community functional components driving ecosystem processes and services delivery

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Summary

1. There is a growing consensus that the distribution of species trait values in a community can greatly determine ecosystem processes and services delivery. Two distinct components of community trait composition are hypothesized to chiefly affect ecosystem processes: (i) the average trait value of the species, quantified by community-weighted mean trait values (CWM; related to the mass ratio hypothesis) and (ii) the degree to which trait values differ between species in a community, quantified by different indices of functional diversity (FD; related to non-additive community effects). The uncertainty on the relative effect of these two components is stimulating an increasing number of empirical studies testing their effects on ecosystem processes and services delivery.

2. We suggest, however, that the interdependence between CWM and FD poses a challenge on disentangling their relative importance. We present a framework that allows designing experiments to decouple and assess the effects of these two community functional components on ecosystem processes and services. To illustrate the framework, we focused on leaf litter decomposition, as this is an essential process related to important ecosystem services. Using simulations, we applied the framework for plant leaf litter traits (litter nitrogen and phenolic content) that are related to litter decomposition.

3. CWM and FD generally showed a hump-shaped relationship (i.e. at more extreme CWM values, communities can have only low FD values). Within this relationship, we showed that it is possible to select quasi-orthogonal combinations of CWM and FD that can be treated statistically. Within these orthogonal CWM and FD combinations, it is also possible to select species assemblages controlling for other community parameters, such as total biomass, total density and species richness.

4. Synthesis. The framework provides a novel approach for designing experiments to decouple the effects of CWM and FD of communities on ecosystem processes, which otherwise cannot be easily disentangled. To apply the framework and design proper experimental layouts, it is essential to have *a priori* knowledge of the key traits by which species affect ecosystem processes and service delivery.

Key-words: CWM, ecosystem functioning, ecosystem processes, ecosystem services, functional divergence, functional diversity, functional evenness, functional richness, mass ratio hypothesis, Rao index

Introduction

Understanding how ecological communities regulate ecosystem services and their underlying ecosystem processes is a pressing issue (Lavorel & Grigulis 2012; Mace, Norris &

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Fitter 2012), as over 60% of ecosystem services are deteriorating or are already overused in a global scale (Millennium Ecosystem Assessment 2005). There is a growing consensus that community trait composition, that is, the distribution of trait values of species in a community, greatly determines ecosystem processes and services (Garnier *et al.* 2004; Heemsbergen *et al.* 2004; Petchey, Hector & Gaston 2004; Díaz *et al.* 2007). Two main research hypotheses have emerged to explain how species traits within a community may influence ecosystem processes (de Bello *et al.* 2010). The mass ratio hypothesis (Grime 1998) proposes that the effect of a given species on ecosystem processes is proportional to its relative contribution to the total biomass of the community. Therefore, the mean trait value of the species present in the community, weighted by their relative abundance (the so-called community-weighted mean trait value – CWM), should be related to ecosystem processes and properties (Garnier *et al.* 2004; Quedstedt *et al.* 2007). Alternatively, it has been shown that functional diversity (the degree of dissimilarity in trait values between coexisting species – FD) promotes non-additive effects on ecosystem processes, that is, effects not predictable from single-species results due to antagonistic or synergistic interactions among species (Heemsbergen *et al.* 2004; Petchey, Hector & Gaston 2004; Mouillot *et al.* 2011). These non-additive effects have been advocated to be mainly caused by complementary use of resources and facilitation or interference (Petchey, Hector & Gaston 2004; Hooper *et al.* 2005; Spehn *et al.* 2005).

These hypotheses (mass ratio and non-additive effects) highlight the importance of distinct components of community trait composition (CWM and FD, respectively), but their effects on ecosystem processes are not necessarily mutually exclusive (Schumacher & Roscher 2009; Mouillot *et al.* 2011; Butterfield & Suding 2013; Conti & Díaz 2013). The relative importance of CWM and FD in driving ecosystem processes remains however debatable since only few studies evaluated both aspects of plant community trait composition simultaneously (Thompson *et al.* 2005; Díaz *et al.* 2007; Mokany, Ash & Roxburgh 2008; Schumacher & Roscher 2009; Laughlin 2011; Lavorel *et al.* 2011; Mouillot *et al.* 2011). In general, these few studies indicate a higher explanatory power of plant CWM on explaining variation of ecosystem processes as compared to indices of community FD. However, Schumacher & Roscher (2009) showed for grasslands that adding FD of plant height, leaf area and life cycle to models relating abiotic factors and CWM to above-ground primary productivity increased the amount of explained variation considerably. Similar findings were reported by Mouillot *et al.* (2011) for multiple ecosystem processes, that is, primary productivity and litter decomposition. These results suggest that considering CWM and FD separately is often insufficient to understand the mechanisms by which community trait composition affects ecosystem processes. Two questions that remain, therefore, are: what is the relative importance of each of these community functional components for ecosystem processes and service delivery, and under which circumstances may their relative importance change?

So far, most of the studies simultaneously testing the effect of CWM and FD on ecosystem processes were performed using an observational approach (Thompson *et al.* 2005; Díaz *et al.* 2007; Mokany, Ash & Roxburgh 2008; Schumacher & Roscher 2009; Laughlin 2011; Lavorel *et al.* 2011), which implies difficulties to infer cause–effect relationships. A problem, which we believe is even more serious, is that CWM and FD cannot be expected to be independent from each other. Ricotta & Moretti (2011) showed, for example, that Rao Q, one of the most used FD indices, and CWM describe complementary aspects of community trait composition, that is, dispersion and mean, respectively, and that these two aspects are mathematically related. Logically, when CWM approaches the upper and lower bounds of the trait range, FD will necessarily decrease because only species with similar trait values (high or low, respectively) will be present, leading to a hump-shaped relationship between CWM and FD. This will necessarily leave uncertainties if the community effects are due to CWM or FD as they vary together. In fact, in many cases, CWM and FD are highly correlated (Laughlin 2011; Mouillot *et al.* 2011; Ricotta & Moretti 2011), making it very difficult to disentangle the effects of these two community functional components. Furthermore, it has been proposed that FD cannot be summarized by one single index (Mason *et al.* 2005). Instead, FD can be described in terms of three independent metrics – functional divergence, functional evenness and functional richness (Villegger, Mason & Mouillot 2008) – which represent distinct aspects of the variation in trait values within a community. Although considering distinct FD metrics can help understanding the mechanisms linking biodiversity and ecosystem processes (Mason *et al.* 2008), the interdependence of each of these FD metrics with CWM has not yet been explored.

Therefore, we argue that the relative effect of CWM and FD on ecosystem processes cannot be easily disentangled. Using traditional biodiversity–ecosystem functioning (BEF) experiments does not solve this problem, because these experiments are usually designed to form a gradient of species richness, irrespective of the trait values of the selected species in the assemblages (Petchey, Hector & Gaston 2004; Meier & Bowman 2008; Mouillot *et al.* 2011). Some experiments have varied species richness and functional group composition (Lanta & Leps 2006; Marquard *et al.* 2009), but again this approach does not allow to distinguish between the two community components by which functional traits are expected to influence ecosystem processes. We argue that to properly disentangle the effects of CWM and FD, it is necessary to design experiments with species assemblages comprising orthogonal values of CWM and FD. However, as we will show later, it is not trivial to select species combinations providing such desirable values of CWM and FD, because these community components will vary with the number of species, the attributes of the species and the relative abundance of each species added to the assemblage. Even using a small pool of species to create assemblages would lead to a huge number of possibilities. As far as we know, there is no available framework to assist with constructing assemblages when

controlling for the values of CWM and FD that could be used to design such experiments.

Here, we propose a framework describing the necessary steps to create an experimental design to test the relative importance of CWM and FD for ecosystem processes. By examining the relationship between CWM and FD of simulated assemblages, the framework assists on the choice of species and assemblages providing the best species combinations to tease apart the effect of these two community functional components. To illustrate the framework, we primarily focused on leaf litter decomposition as this is an essential process related to important ecosystem services such as soil fertility and primary productivity. Studies have long recognized the non-additive effects of plant species diversity on litter decomposition, but have failed to generalize the magnitude and direction of such effects (Gartner & Cardon 2004; Hättenschwiler, Tiunov & Scheu 2005; Vos *et al.* 2011). Recently, chemical and physical leaf litter traits were proposed as better parameters explaining the effects of litter mixtures on decomposition (Epps *et al.* 2007; Meier & Bowman 2008; Barantal *et al.* 2011). When CWM and FD of these leaf litter traits were considered, the results of these works suggested that CWM plays a major role compared with FD on driving litter decomposition (Grigulis *et al.* 2013). However, these experiments were designed to form plant species richness gradients, and the relationship between CWM and FD was not evaluated or taken into account when designing the experiments and interpreting the results (but see Laughlin 2011; Mouillot *et al.* 2011; Grigulis *et al.* 2013).

Potential mechanisms of leaf litter diversity effects on decomposition include transfer of nutrients among litter types both by leaching and by microbial active transport (Schimel & Hättenschwiler 2007; Tiunov 2009), transfer of deterrent compounds by leaching (Gartner & Cardon 2004), decomposers' complementary use of resources (Hättenschwiler, Tiunov & Scheu 2005) and changes in stimulation and suppression of decomposers' activity (Gessner *et al.* 2010). Therefore, in our simulations, we used leaf litter traits that have been shown to be involved in these mechanisms: leaf nitrogen content and total phenolic content (Gartner & Cardon 2004; Hättenschwiler, Tiunov & Scheu 2005).

We used our framework to produce experimental designs with orthogonal values of CWM and FD for the above-mentioned leaf litter traits that relate to leaf litter decomposition. This approach could provide better causal inference on the relative importance of these two community functional components for decomposition process. We specifically asked if indices reflecting the three distinct aspects of FD show hump-shaped relationship with CWM and if there is enough variation of simulated assemblages to choose orthogonal values on FD and CWM.

Framework

Traditional BEF experiments consist of a series of assemblages comprising monocultures and species combinations to form a gradient of species richness (Hooper *et al.* 2005;

Spehn *et al.* 2005). To disentangle the effects of CWM and FD, we propose a similar approach including both monoculture and species mixtures, but changing the main focus from species richness to the trait composition of the species assemblages. The flow diagram in Fig. 1 describes the necessary steps to select species creating assemblages with quasi-orthogonal gradients of CWM and FD. This would make it possible to evaluate the relative importance of these two community functional components and their possible interactions. To follow the steps, it is necessary to consider (i) a list of species with values of demonstrated effect traits (i.e. a trait a species possesses and that has an effect on its environment, be it the next trophic level or an ecosystem processes or service; Lavelle & Garnier 2002) and (ii) biomass for each individual species. Therefore, we also include the necessary steps to collect this information. Below, we present in detail each of the steps as enumerated in Fig. 1:

1 Selecting effect traits: Select the most important effect traits that drives the ecosystem process of interest using information available in the literature (e.g. Cornelissen 1996), data bases (such as the plant trait data bases Leda and TRY) or through direct testing by means of an experimental comparative approach (Wardle *et al.* 1998). It should be noted that more than a single trait could be involved in the effect of species on ecosystems (Díaz *et al.* 2007; de Bello *et al.* 2010). While FD indices can be calculated with single or multiple

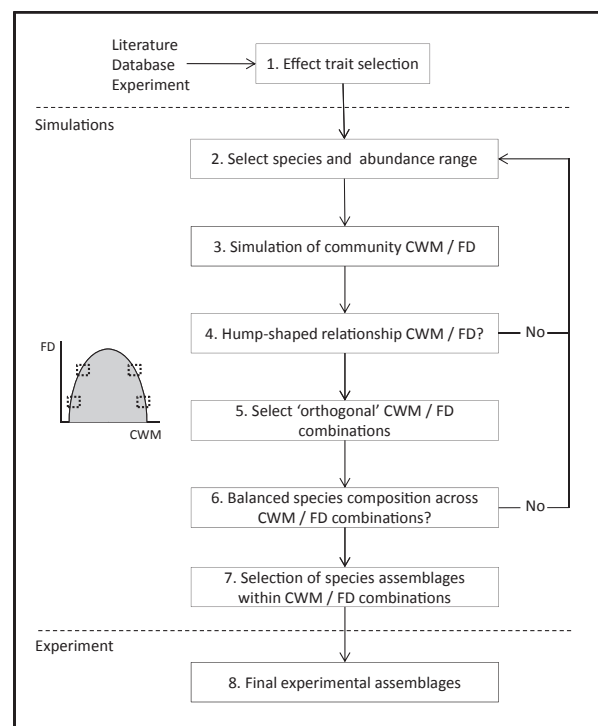


Fig. 1. Methodological framework showing the steps to design experiments with constructed assemblages to disentangle the effects of CWM and FD on ecosystem processes. Graph next to the flow diagram shows a schematic representation of the hump-shaped relationship between CWM and FD, including the orthogonal CWM–FD combinations (small squares).

traits, CWM can only be calculated for a single trait. If more than one trait is important for the ecosystem process of interest, we envisage two possible scenarios. First, when the effect traits considered are correlated to each other (Díaz *et al.* 2004; Wright *et al.* 2004), reduction of dimensionality, for example, via PCA axis scores, can be used to calculate CWM (Laughlin 2011; Mouillot *et al.* 2011). Alternatively, it is possible to choose one single trait that best correlates with the ecosystem process or that has better documented mechanisms explaining such effects. In the second scenario, when two uncorrelated traits are equally important on determining the effect of species on an ecosystem process, two experimental designs need to be applied, one for each trait while selecting species assemblages that can be used for both designs in order to reduce the size of the experiment. This scenario is presented and discussed in the case study below.

2 Selecting species and abundance range: From the total pool of species, for which data on effect traits are available, the researcher should select the species that will be used in the experiment. An increase in the number of species will enlarge the size of the experiment considerably so that the final number of species to be included will depend on the resources available to run the experiment. Selected species should cover a substantial range of trait values. Additionally, to make sure that species effects are not due to differences in species abundance, the total biomass per species should be comparable within assemblages. For this, the smaller species should necessarily have a higher number of individuals in the assemblages.

3 Running simulations: By using the trait and biomass values of the selected species (steps 1 and 2), we recommend simulating many assemblages (>5000) to be sure that a representative set of all possible species combinations will be present in the outcome. For the simulated assemblages, calculate FD indices (see Introduction), CWM, species richness and additional descriptors (i.e. total number of individuals and total biomass).

4 Evaluating the relationship between CWM and FD: Depending on the species selected in step 2, a complete hump-shaped relationship between assemblage CWM and FD might not appear. This happens, for instance, when one species' trait value is very dissimilar from the others'. In this case, most of the assemblages will be constrained to a very restricted part of the CWM and FD space, ruling out the possibility to disentangle their relative effects. If that happens, the species selection should be refined (back to step 2) by removing species with extremely dissimilar trait values. However, the effect of species with such extreme trait values, which have been documented in natural communities, can also be tested by designing the experiment without it and including a treatment with and without the addition of such species.

5 Selecting orthogonal CWM and FD combinations: If a hump-shaped relationship between CWM and FD is found, select orthogonal combinations of the two community functional components, that is, both high CWM and FD (HH), both low CWM and FD (LL), high CWM and low FD (HL)

and low CWM and high FD (LH). The selected combinations of CWM and FD should be as distant as possible while still containing a considerable number of simulated assemblages to be selected.

6 Evaluating species composition within the four CWM–FD combinations: Evaluate whether species compositions are balanced across the four selected CWM–FD combinations. If one of the four CWM–FD combinations is comprised of only assemblages with a very similar species composition, it will not be possible to disentangle the effect of the presence of a particular species (i.e. selection effect *sensu* Huston 1997) from those of assembly CWM or FD. If an unbalanced species composition is present, a new selection of species will be needed (back to step 2). Adding more species will probably solve this problem.

7 Selecting assemblages within CWM–FD combinations: Within each CWM–FD combination, different assemblages can be selected for the experiment. This should be constructed as a random stratified selection, where assemblages are randomly selected but not allowing assemblages with the same or very similar species composition to be selected. It is also important to have the same levels of species richness for each CWM–FD combination and a similar number of assemblages for each level of species richness. At least four to five different assemblages in each CWM–FD combination should be selected as real replicates for each combination of CWM and FD.

Materials and methods

CASE STUDY

We measured the selected leaf litter traits (leaf nitrogen content and total phenolic content, step 1) for seven common tree species, representing a gradient of traits that relate to decomposability: *Acer campestre*, *Alnus glutinosa*, *Corylus avellana*, *Crataegus monogyna*, *Fraxinus excelsior*, *Quercus petraea* and *Sambucus nigra*. These species commonly co-occur in natural communities in central and western Europe (Ellenberg & Strutt 2009) and form an important part of the diet of macrodetritivores. We collected recently fallen leaves of these species in the vicinity of the VU University, Amsterdam (52°33'N, 4°86'E), during time of leaf abscission (October 2010). Leaf biomass and two other leaf litter traits of interest, leaf N and leaf phenolic content, were measured for each species with standard protocols (following Cornelissen *et al.* 2003). The two litter traits were correlated (Pearson, $r = 0.70$), so that, in theory, choosing only one of the two traits (or a single condensed PCA axis) would have been sufficiently informative. To show that the framework can also be applied to multiple uncorrelated traits, we artificially disrupted the correlation between the two leaf litter traits by shuffling the leaf phenolic content values across species (Pearson, $r = 0.33$).

ASSEMBLAGE SIMULATIONS

We simulated assemblages for plant litter using the criteria described in the framework (steps 2 and 3). We built a simple R function (called 'Funziona', Appendix S1 in Supporting Information) to calcu-

late assembly CWM, number of species, total community biomass and different FD indices for the simulated assemblages that can be used to apply the framework. It should be noted that FD can be optimally summarized mainly by three families of metrics: functional richness (FRic), reflecting the amount (or range) of functional trait variability in a given species assemblage; functional evenness (FEve), representing the evenness of abundance distribution across species trait values; and functional divergence (FDiv), capturing the degree of divergence in the abundance distribution of species functional traits (Villegger, Mason & Mouillot 2008). These metrics are expected to express different mechanisms by which the community affects ecosystem processes (Mouchet *et al.* 2010). FDiv was computed here with the Rao quadratic entropy index of diversity (Rao Q). This index expresses the sum of the dissimilarities in the trait space among all possible pairs of species weighted by the product of relative species abundance and underpins a family of related functional divergence indices (Pavoine & Bonsall 2011).

As CWM can be computed only for a single trait (or a single multivariate axis reflecting multiple correlated traits, see step 1 of our framework), we first analysed the simulated assemblages for each trait separately. Consecutively, FD based on one and two traits was computed according to standard procedures (Laliberté & Shipley 2011) and included into our R function (Appendix S1). Among the seven plant species, we first selected five species (i.e. *Alnus*, *Corylus*, *Fraxinus*, *Quercus* and *Sambucus*) which were more evenly distributed among the range in leaf litter N and phenolic content values. We simulated communities ranging from 2 to 5 species, and species maximum abundance was negatively related to species average leaf mass, varying from 5 (*Corylus*) to 18 (*Fraxinus*) leaves. These differences in maximal abundance between species were chosen to ensure that total biomass per species had comparable values within assemblages. For a second test, we selected the four species (i.e. *Acer*, *Corylus*, *Crataegus* and *Quercus*) with more similar values for leaf N and one (*Alnus*) with a considerably higher value for leaf litter N content. Again, species richness of simulated communities ranged from 2 to 5 and species maximum abundance from 5 (*Corylus*) to 35 (*Crataegus*) leaves.

Results

RELATIONSHIP BETWEEN ASSEMBLAGE CWM AND FD

Following our expectations, we found hump-shaped relationships between CWM and FD, although the relationships showed some differences across the three metrics of FD, that is, FRic, FEve and FDiv (Fig. 2). For instance, FEve showed higher variation within intermediate levels of CWM, followed by FDiv and FRic. The relationships were very similar for all studied traits; all were hump-shaped. The shape of CWM–FD relationships was, however, dependent on the species included in the simulations (see step 4 in our framework). Hump-shaped relationships were obtained using leaf litter species evenly spaced along the whole range in leaf N and phenolic content values. However, when using four plant species with low leaf litter N content (0.71–0.97% N) and only one species (*Alnus*) with much higher leaf litter N content (2.89% N), the majority of the assemblages were strongly skewed towards low CWM values, covering very restricted parts of the CWM–FD space (Fig. 3). This result was obtained for all three FD metrics. Most of the assemblages fell into a strong positive

relationship between CWM and FDiv (Rao Q). This happened because if one species with an extremely high trait value is selected, assemblages with a high CWM will always have a high FD as those assemblages will necessarily contain at least two very dissimilar species. Therefore, it will not be possible to generate assemblages with high CWM and low FD, ruling out the possibility to disentangle their effects on ecosystem processes. This skewness was also observed for FRic, where an increase in CWM leads to a sharp increase in the range of N content due to the inclusion of *Alnus* with extreme high N content as compared to the other litter species. Contrastingly, most assemblages with a high FEve value had a low CWM value. This is because to increase assemblage CWM, a species with an extreme high N content must be included, minimizing the regularity of distances between species trait values. This indicates that without selecting an appropriate set of species, it is impossible to select orthogonal combinations of CWM and FD and consequently to decouple their effects on ecosystem processes.

SELECTION OF ASSEMBLAGES WITHIN CWM–FD COMBINATIONS

The boxes in Fig. 2 represent the quasi-orthogonal combinations of CWM and FD values (whose definition is necessary for step 5). The assemblages within these combinations are comprised of distinct species compositions and species richness varying from 2 to 5 (step 6). Due to the high number of assemblages that fall within these combinations and their variation in species richness and composition, it is possible to select assemblages to control for a diverse range of species richness, number of individuals and total biomass while testing the effects of CWM and FD (step 7).

USING MULTIPLE UNCORRELATED TRAITS

To demonstrate how to construct assemblages that consider the effect of multiple unrelated traits on ecosystem functions, we used leaf litter N and phenolic content to calculate FD indices based on both traits (Fig. 4). First, we followed all the steps in the framework for each of these traits separately. Assemblages falling in the quasi-orthogonal combinations of CWM and FD based on leaf N are widespread in the relationship between CWM and FD based on leaf phenols (Fig. 4, left and central panels). This means that producing testable assemblages of CWM and FD for litter N content does not guarantee that these assemblages can also be used for testing CWM and FD for phenolic content. Many assemblages that could be selected for leaf N, however, also fall in the quasi-orthogonal combinations for leaf phenolics and could therefore be used to evaluate both traits simultaneously. This would help reducing the number of assemblages necessary to test the effects of CWM and FD, that is, it is not necessary to run two complete sets of assemblages for each trait, but some assemblages can be applied for both. Finally, when using the two traits to calculate multivariate FD, it is also possible to select the same assemblages as those used for the traits separately (Fig. 4, right panel).

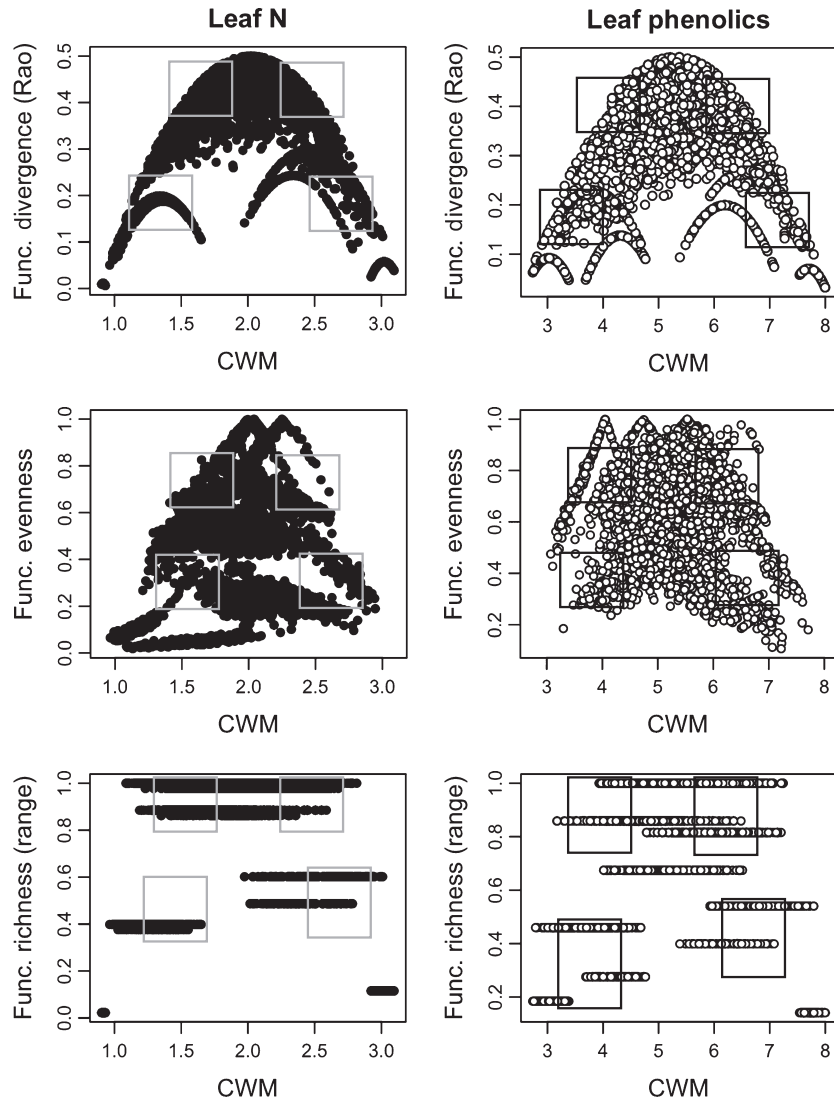


Fig. 2. Hump-shaped relationships between CWM and FD on simulated plant assemblages based on leaf litter N content (%) and phenolic content (%). Three indices corresponding to different aspects of functional diversity are shown: FDiv, FEve and FRic. Boxes indicate orthogonal combinations of CWM and FD.

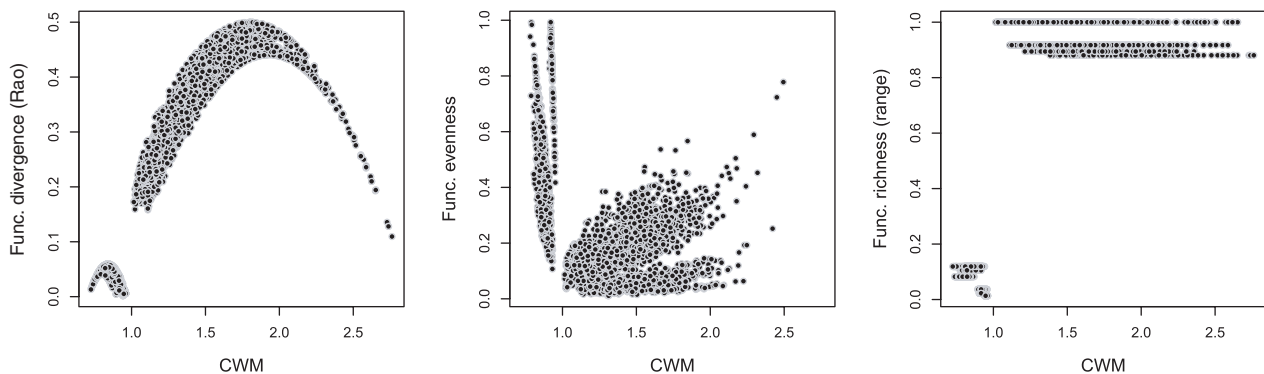


Fig. 3. Example of a strong coupling between CWM and FD components on simulated plant assemblages based on leaf N content (%). This relationship was obtained by selecting one species with much higher values of leaf N content (*Alnus*) compared to the other species (*Acer*, *Corylus*, *Crataegus* and *Quercus*).

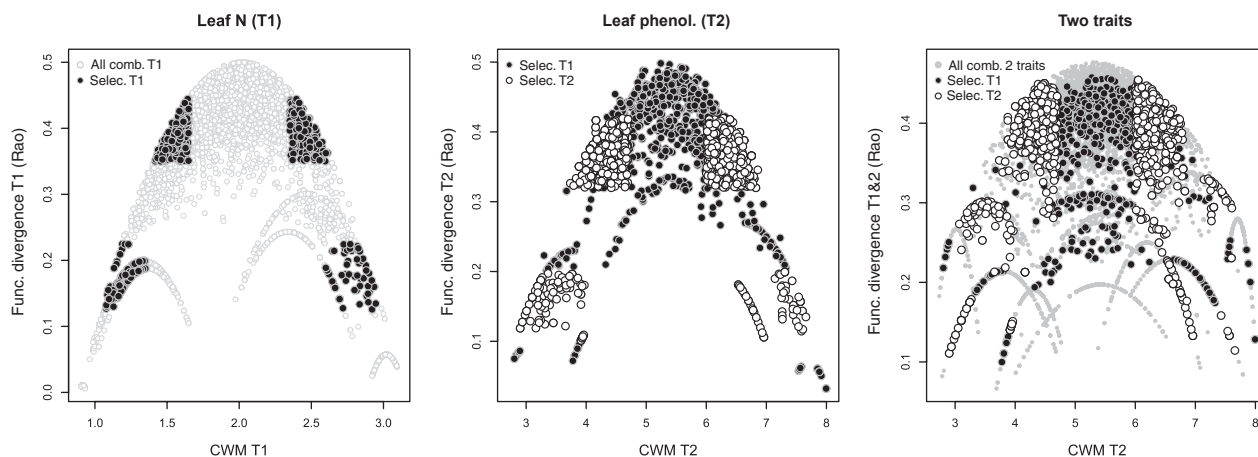


Fig. 4. Hump-shaped relationships between CWM and FDiv (Rao) on simulated assemblages based on two uncorrelated traits: leaf litter N content (%) and phenolic content (%). The assemblages within orthogonal combinations of CWM and FDiv based on leaf N (black circles, left panel) are widespread along the hump-shaped relationship between CWM and FDiv based on leaf phenolic content (middle panel). Some of the assemblages within orthogonal combinations based on leaf phenolic content (white circles, middle panel) coincide with those for the first trait and could, therefore, be selected to design an experiment testing both traits. Also, some assemblages within orthogonal combinations based on multivariate FDiv coincide with those for individual traits (right panel).

Discussion

It is now accepted that community trait composition has strong effects on ecosystem processes and services (Garnier *et al.* 2004; Heemsbergen *et al.* 2004; Petchey, Hector & Gaston 2004; Mouillot *et al.* 2011), but only recently a conceptual and methodological framework was proposed to incorporate both CWM and FD in ecosystem service assessments in the field (Díaz *et al.* 2007; Lavorel *et al.* 2011). Although this represents a great advance in using community trait composition to reduce uncertainty in the prediction of ecosystem processes and services, formal experimental tests on the relative importance of these two community functional components are still lacking. Here, we showed for the first time that these two key functional components of the community trait composition cannot be considered independent of each other and that the relationship between CWM and FD greatly depends on the trait values of the species considered. Our assemblage simulation framework makes it possible to analyse the interdependence of these two functional components of community trait composition and to select assemblages within orthogonal combinations of CWM and FD. These assemblages in turn can be used in experiments testing the relative importance of CWM and FD in driving ecosystem processes and services.

Selecting assemblages within the orthogonal combinations of CWM and FD is a crucial step that, in light of our results, deserves attention. For each CWM–FD combination, it is possible to find assemblages with distinct species richness and composition and, ideally, the assemblages selected for the experiment should be representative of this variation. This can be done by constrained random selection, where selecting an assemblage with a certain species composition twice is not allowed. Importantly, not necessarily all levels of species richness will be present within the four CWM–FD combinations.

Still, possible effects of species richness on ecosystem processes could be tested by including it as a variable in the statistical analyses. As in classical BEF experiments, another important decision is whether to replicate specific assemblages or not. Because only unique assemblages can be considered as true replicates for a given diversity level, many researchers have decided to use constrained random selection of the assemblages and construct as many distinct assemblages as possible for each level of diversity without replicating them (Tilman, Wedin & Knops 1996; van Ruijven & Berendse 2003; Marquard *et al.* 2009). The same rationale applies for our experimental framework with different levels of CWM and FD. However, replicating assemblages can be important, for instance, when large variability among experimental units (e.g. plots, microcosms or litterbags) is expected or when testing the effect of given species assemblages is desired (Bardgett & Shine 1999; Hector *et al.* 1999; Heemsbergen *et al.* 2004). In fact, monocultures should always be replicated as they are used to estimate the effects of each individual species, which is necessary to test for non-additive effects.

In our study, we also applied the proposed framework to effect traits of terrestrial isopods (data not shown), which are important members of macrodetritivore communities. We used leaf litter consumption rate as an effect trait because it is directly related to the amount of litter processed by isopods, and the results of the simulations showed very similar patterns as those presented here for litter traits. This suggests that our framework is a flexible tool that can be used to design experiments across a wide range of organisms belonging to different trophic groups (e.g. plants, decomposers, herbivores and pollinators), functional diversity metrics (e.g. FRic, FDiv and FEve) and ecosystem processes and services (e.g. productivity, decomposition and pollination). This is particularly relevant as the relative importance of CWM and FD might

differ depending on the ecosystem service of interest (Mouillot *et al.* 2011). While some studies found a preponderant effect of CWM of plant traits on ecosystem processes, such as litter decomposition and productivity (Meier & Bowman 2008; Mokany, Ash & Roxburgh 2008; Laughlin 2011; Grigulis *et al.* 2013), there is evidence that higher FD of pollinators can increase pollination success from single flowers to whole plant communities (Hoehn *et al.* 2008; Bluethgen & Klein 2011). Moreover, studies showed that both CWM and FD of plant traits explain an important part of variation of distinct ecosystem processes and services, such as primary productivity and decomposition (Mouillot *et al.* 2011), carbon sequestration (Butterfield & Suding 2013; Conti & Díaz 2013) and fodder production (Butterfield & Suding 2013), as well as ecosystem multifunctionality (i.e. mean ecosystem performance averaging different processes). This indicates that to reach high levels of predictability when modelling distinct or multiple ecosystem processes, CWM and FD have to be included in a common conceptual and analytical framework. The framework presented here is an important step in this direction, assisting the design of manipulative experiments, which will add a more comprehensive understanding of the mechanisms behind the relationships between community trait composition and ecosystem processes and service deliveries.

The distinct metrics of FD indicate different mechanisms by which FD can drive ecosystem processes and services (Mason *et al.* 2005; Villegger, Mason & Mouillot 2008). Therefore, a careful approach for formulating hypotheses about the relationships between FD and ecosystem processes is required, and the appropriate metric of FD to test those hypothesized relationships should be chosen (Hillebrand & Matthiessen 2009). Accordingly, when focusing on response traits (i.e. traits related to species response to changes in the environment, Lavorel & Garnier 2002), distinct components of FD can also indicate distinct mechanisms governing community assembly (i.e. competitive and environmental filters; Cornwell, Schwikl & Ackerly 2006; Villegger, Mason & Mouillot 2008). In this way, using response traits, our framework can be extended to assist designing experiments testing how communities respond to environmental change. For instance, experiments with factorial levels of CWM and FD based on response traits could be used to test whether communities with higher FD are more resilient to environmental changes, as predicted by the insurance hypothesis (Naeem & Li 1997), while controlling for CWM.

Conclusions

While experiments with artificial assemblages make up the methodological core of the species diversity–ecosystem functioning debate, the effect of community functional components has been mostly tested through observational studies. It is now time to fully merge these two research approaches and test the relative importance of these two community functional components on ecosystem processes and services delivery. Here, we showed that incorporating the trait-based approach into BEF experiments cannot be restricted to include

trait values when analysing experiments with species-richness gradients, but must start by using the trait values and community functional metrics to design next-generation BEF experiments. In this context, our simulation framework provides a novel approach for designing experiments to decouple the effects of CWM and FD on ecosystem processes that can be applied to different organisms and ecosystem processes underlying important ecosystem services.

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References

- Barantal, S., Roy, J., Fromin, N., Schimann, H. & Hättenschwiler, S. (2011) Long-term presence of tree species but not chemical diversity affect litter mixture effects on decomposition in a neotropical rainforest. *Oecologia*, **167**, 241–252.
- Bardgett, R.D. & Shine, A. (1999) Linkages between plant litter diversity, soil microbial biomass and ecosystem function in temperate grasslands. *Soil Biology & Biochemistry*, **31**, 317–321.
- de Bello, F., Lavorel, S., Díaz, S., Harrington, R., Cornelissen, J.H.C., Bardgett, R.D. *et al.* (2010) Towards an assessment of multiple ecosystem processes and services via functional traits. *Biodiversity and Conservation*, **19**, 2873–2893.
- Bluethgen, N. & Klein, A.-M. (2011) Functional complementarity and specialisation: the role of biodiversity in plant–pollinator interactions. *Basic and Applied Ecology*, **12**, 282–291.
- Butterfield, B.J. & Suding, K.N. (2013) Single-trait functional indices outperform multi-trait indices in linking environmental gradients and ecosystem services in complex landscape. *Journal of Ecology*, **101**, 9–17.
- Conti, G. & Díaz, S. (2013) Plant functional diversity and carbon storage – an empirical test in semi-arid ecosystems. *Journal of Ecology*, **101**, 18–28.
- Cornelissen, J.H.C. (1996) An experimental comparison of leaf decomposition rates in a wide range of temperate plant species and types. *Journal of Ecology*, **84**, 573–582.
- Cornelissen, J.H.C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D.E. *et al.* (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany*, **51**, 335–380.
- Cornwell, W.K., Schwikl, D.W. & Ackerly, D.D. (2006) A trait-based test for habitat filtering: convex hull volume. *Ecology*, **87**, 1465–1471.
- Díaz, S., Hodgson, J.G., Thompson, K., Cabido, M., Cornelissen, J.H.C., Jalili, A. *et al.* (2004) The plant traits that drive ecosystems: evidence from three continents. *Journal of Vegetation Science*, **15**, 295–304.
- Díaz, S., Lavorel, S., de Bello, F., Quetier, F., Grigulis, K. & Robson, 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**, 20684–20689.
- Ellenberg, H. & Strutt, G.K. (2009) *Vegetation Ecology of Central Europe*. Cambridge University Press, Cambridge.
- Epps, K.Y., Comerford, N.B., Reeves, J.B. III, Cropper, W.P. Jr & Araujo, Q. R. (2007) Chemical diversity – highlighting a species richness and ecosystem function disconnect. *Oikos*, **116**, 1831–1840.
- Garnier, E., Cortez, J., Billes, G., Navas, M.L., Roumet, C., Debussche, M. *et al.* (2004) Plant functional markers capture ecosystem properties during secondary succession. *Ecology*, **85**, 2630–2637.
- Gartner, T.B. & Cardon, Z.G. (2004) Decomposition dynamics in mixed-species leaf litter. *Oikos*, **104**, 230–246.
- Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H. & Hättenschwiler, S. (2010) Diversity meets decomposition. *Trends in Ecology & Evolution*, **25**, 372–380.
- Grigulis, K., Lavorel, S., Krainer, U., Legay, N., Baxendale, C., Dumont, M. *et al.* (2013) Relative contributions of plant traits and soil microbial proper-

- ties to mountain grassland ecosystem services. *Journal of Ecology*, **101**, 47–57.
- Grime, J.P. (1998) Benefits of plant diversity to ecosystems: immediate, filter and founder effects. *Journal of Ecology*, **86**, 902–910.
- Hättenschwiler, S., Tiunov, A.V. & Scheu, S. (2005) Biodiversity and litter decomposition in terrestrial ecosystems. *Annual Review of Ecology and Systematics*, **36**, 191–218.
- Hector, A., Schmid, B., Beierkuhnlein, C., Caldeira, M.C., Diemer, M., Dimitrakopoulos, P.G. *et al.* (1999) Plant diversity and productivity experiments in European grasslands. *Science*, **286**, 1123–1127.
- Heemsbergen, D.A., Berg, M.P., Loreau, M., van Haj, J.R., Faber, J.H. & Verhoef, H.A. (2004) Biodiversity effects on soil processes explained by inter-specific functional dissimilarity. *Science*, **306**, 1019–1020.
- Hillebrand, H. & Matthiessen, B. (2009) Biodiversity in a complex world: consolidation and progress in functional biodiversity research. *Ecology Letters*, **12**, 1405–1419.
- Hoehn, P., Tschamtker, T., Tylanakis, J.M. & Steffan-Dewenter, I. (2008) Functional group diversity of bee pollinators increases crop yield. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 2283–2291.
- Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S. *et al.* (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs*, **75**, 3–35.
- Huston, M.A. (1997) Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia*, **110**, 449–460.
- Laliberté, E. & Shipley, B. (2011) FD: measuring functional diversity from multiple traits, and other tools for functional ecology. R package version 1.0-11. Available at: <http://cran.r-project.org>
- Lanta, V. & Leps, J. (2006) Effect of functional group richness and species richness in manipulated productivity-diversity studies: a glasshouse pot experiment. *Acta Oecologica-International Journal of Ecology*, **29**, 85–96.
- Laughlin, D.C. (2011) Nitrification is linked to dominant leaf traits rather than functional diversity. *Journal of Ecology*, **99**, 1091–1099.
- Lavorel, S. & Garnier, E. (2002) Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional Ecology*, **16**, 545–556.
- 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.
- Lavorel, S., Grigulis, K., Lamarque, P., Colace, M.-P., Garden, D., Girel, J., Pellet, G. & Douzet, R. (2011) Using plant functional traits to understand the landscape distribution of multiple ecosystem services. *Journal of Ecology*, **99**, 135–147.
- Mace, G.M., Norris, K. & Fitter, A.H. (2012) Biodiversity and ecosystem services: a multilayered relationship. *Trends in Ecology & Evolution*, **27**, 19–26.
- Marquard, E., Weigelt, A., Temperton, V.M., Roscher, C., Schumacher, J., Buchmann, N., Fischer, M., Weisser, W.W. & Schmid, B. (2009) Plant species richness and functional composition drive overyielding in a six-year grassland experiment. *Ecology*, **90**, 3290–3302.
- Mason, N.W.H., Moullot, D., Lee, W.G. & Wilson, J.B. (2005) Functional richness, functional evenness and functional divergence: the primary components of functional diversity. *Oikos*, **111**, 112–118.
- Mason, N.W.H., Irz, P., Lanoiselee, C., Moullot, D. & Argillier, C. (2008) Evidence that niche specialization explains species-energy relationships in lake fish communities. *Journal of Animal Ecology*, **77**, 285–296.
- Meier, C.L. & Bowman, W.D. (2008) Links between plant litter chemistry, species diversity, and below-ground ecosystem function. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 19780–19785.
- Millennium Ecosystem Assessment (2005) *Ecosystems and Human Well-Being: Synthesis*. Island Press, Washington, DC, USA.
- 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.
- Mouchet, M.A., Vileger, S., Mason, N.W.H. & Moullot, D. (2010) Functional diversity measures: an overview of their redundancy and their ability to discriminate community assembly rules. *Functional Ecology*, **24**, 867–876.
- Moullot, D., Vileger, S., Scherer-Lorenzen, M. & Mason, N.W.H. (2011) Functional structure of biological communities predicts ecosystem multifunctionality. *PLoS ONE*, **6**, e17476.
- Naeem, S. & Li, S.B. (1997) Biodiversity enhances ecosystem reliability. *Nature*, **390**, 507–509.
- Pavoine, S. & Bonsall, M.B. (2011) Measuring biodiversity to explain community assembly: a unified approach. *Biological Reviews*, **86**, 792–812.
- Petchey, O.L., Hector, A. & Gaston, K.J. (2004) How do different measures of functional diversity perform? *Ecology*, **85**, 847–857.
- Quested, H., Eriksson, O., Fortunel, C. & Garnier, E. (2007) Plant traits relate to whole-community litter quality and decomposition following land use change. *Functional Ecology*, **21**, 1016–1026.
- Ricotta, C. & Moretti, M. (2011) CWM and Rao's quadratic diversity: a unified framework for functional ecology. *Oecologia*, **167**, 181–188.
- van Ruijven, J. & Berendse, F. (2003) Positive effects of plant species diversity on productivity in the absence of legumes. *Ecology Letters*, **6**, 170–175.
- Schimel, J.P. & Hättenschwiler, S. (2007) Nitrogen transfer between decomposing leaves of different N status. *Soil Biology & Biochemistry*, **39**, 1428–1436.
- Schumacher, J. & Roscher, C. (2009) Differential effects of functional traits on aboveground biomass in semi-natural grasslands. *Oikos*, **118**, 1659–1668.
- Spehn, E.M., Hector, A., Joshi, J., Scherer-Lorenzen, M., Schmid, B. *et al.* (2005) Ecosystem effects of biodiversity manipulations in European grasslands. *Ecological Monographs*, **75**, 37–63.
- Thompson, K., Askew, A.P., Grime, J.P., Dunnett, N.P. & Willis, A.J. (2005) Biodiversity, ecosystem function and plant traits in mature and immature plant communities. *Functional Ecology*, **19**, 355–358.
- Tilman, D., Wedin, D. & Knops, J. (1996) Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature*, **379**, 718–720.
- Tiunov, A.V. (2009) Particle size alters litter diversity effects on decomposition. *Soil Biology & Biochemistry*, **41**, 176–178.
- Vileger, S., Mason, N.W.H. & Moullot, D. (2008) New multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology*, **89**, 2290–2301.
- Vos, V.C.A., van Ruijven, J., Berg, M.P., Peeters, E.T.H.M. & Berendse, F. (2011) Macro-detritivore identity drives leaf litter diversity effects. *Oikos*, **120**, 1092–1098.
- Wardle, D.A., Barker, G.M., Bonner, K.I. & Nicholson, K.S. (1998) Can comparative approaches based on plant ecophysiological traits predict the nature of biotic interactions and individual plant species effects in ecosystems? *Journal of Ecology*, **86**, 405–420.
- Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F. *et al.* (2004) The worldwide leaf economics spectrum. *Nature*, **428**, 821–827.

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Supporting Information

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

Appendix S1. R function 'Funziona' for assemblage simulations.