

LETTER

Testing the effects of diversity on ecosystem multifunctionality using a multivariate model

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Abstract

Most ecosystems provide multiple services, thus the impact of biodiversity losses on ecosystem functions may be considerably underestimated by studies that only address single functions. We propose a multivariate modelling framework for quantifying the relationship between biodiversity and multiple ecosystem functions (multifunctionality). Our framework consolidates the strengths of previous approaches to analysing ecosystem multifunctionality and contributes several advances. It simultaneously assesses the drivers of multifunctionality, such as species relative abundances, richness, evenness and other manipulated treatments. It also tests the relative importance of these drivers across functions, incorporates correlations among functions and identifies conditions where all functions perform well and where trade-offs occur among functions. We illustrate our framework using data from three ecosystem functions (sown biomass, weed suppression and nitrogen yield) in a four-species grassland experiment. We found high variability in performance across the functions in monocultures, but as community diversity increased, performance increased and variability across functions decreased.

Keywords

Biodiversity, Diversity-Interactions model, ecosystem function, evenness, multifunctionality, multivariate, species interaction, species richness, trade-offs.

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INTRODUCTION

The biodiversity–ecosystem function (BEF) relationship has been widely researched over the past few decades and ecosystem functions such as biomass production or resistance to weed invasion are generally reduced as biodiversity is lost (Hector *et al.* 1999; Cardinale *et al.* 2011; Finn *et al.* 2013). Since most investigations of the BEF relationship have focused on a single ecosystem function, the impact of biodiversity losses on the delivery of ecosystem services may be underestimated, however, several recent studies have explored the BEF relationship for multiple ecosystem functions (multifunctionality) (Hector & Bagchi 2007; Gamfeldt *et al.* 2008; Mouillot *et al.* 2011; Allan *et al.* 2013; Byrnes *et al.* 2014a). These studies have generally shown that the number of species required to maintain multifunctionality increases with the number of functions being considered, partly because different sets of species control different functions (Hector & Bagchi 2007; Isbell *et al.* 2011).

Statistical methods for analysing the multifunctional BEF relationship include (1) qualitatively combining univariate models for each function (Allan *et al.* 2013), (2) the averaging approach (Mouillot *et al.* 2011), (3) the overlap method (Hec-

tor & Bagchi 2007), (4) the single threshold method (Gamfeldt *et al.* 2008) and (5) the multiple threshold method (Byrnes *et al.* 2014a). These methods are summarised in Appendix S1 and have been reviewed and critiqued in Byrnes *et al.* (2014a). Although these previous methods provide useful insights, each suffers from loss of information through simplifying the multivariate nature of the data (Box 1). This information loss includes reduced information on individual functions, correlations among functions not being measured and being ignored, species abundance being summarised as presence or absence and continuous information being converted to categorical thresholds. While reducing the multivariate nature of data can be useful, it may lead to misconceptions at the individual ecosystem function level, particularly when functions differ markedly in their responses to changing diversity (Bradford *et al.* 2014a,b; Byrnes *et al.* 2014b). These previous methods also focus strongly on species richness as the main driver of multifunctionality, ignoring other potentially highly influential aspects of diversity, such as the relative abundances of species or the ability of species to interact (Wilsey & Potvin 2000; Wilsey & Polley 2004; Kirwan *et al.* 2007; Finn *et al.* 2013).

The Diversity-Interactions approach (Kirwan *et al.* 2009; Connolly *et al.* 2013) models the BEF relationship for a single

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Box 1 Summary of the information loss associated with previous multifunctionality approaches (each described in Appendix S1) and description of the consolidation of the strengths of those approaches and the added benefits that the Multivariate Diversity-Interactions modelling framework provides.

Approach	Issues and information loss	Strengths that are included in the Multivariate Diversity-Interactions framework	Additional value of the Multivariate Diversity-Interactions framework
(1) Combining univariate models	No information on correlations among functions. Only qualitative information on multifunctionality.	Understanding the drivers of each individual function.	Tests the relative importance of the drivers across functions. Quantitative information on single functions <i>and</i> on multifunctionality. Incorporates correlations among functions into the assessment of drivers of multifunctionality
(2) The averaging approach	Loss of information at the individual ecosystem function level. Two communities with very different ecosystem functions can yield the same average metric value (e.g. with two functions, the two functions could be equal or one function could be very high and the other very low, but the two communities yield the same average) therefore it is an incomplete description of the underlying multivariate distribution.		Tests the drivers of individual functions. Tests the relative importance of the drivers across functions. Utilises correlations among functions in inference.
(3) The overlap method	Ignores how sets of species that positively influence some ecosystem functions might reduce other functions.	Quantifies the species that positively influence pairs of ecosystem functions.	Tests how all species and pairwise interactions positively or negatively affect all functions (not just pairs of functions), i.e. identifies conditions under which multiple functions all perform well, but will also identify trade-offs among functions.
(4) The single threshold method	Converts quantitative measurements to categorical thus there is loss of information on the amount by which a function exceeds or falls below a threshold. Subjective to the choice of threshold. Ignores effects of correlations among functions.	Identifies combinations of species that will achieve, e.g. 70% of the maximum performance.	Quantitative predictions on how each function performs under varying diversity characteristics. Identifies the combinations of species <i>and their relative abundances</i> that will attain, e.g. 70% of the maximum.
(5) The multiple threshold method	Requires carrying out the same tests repeatedly (at each threshold), but provides no statistical adjustment for the multiple tests. Ignores effects of correlations among functions.	Identifies combinations of species that will achieve a certain threshold of the maximum performance.	Quantitative predictions on how each function performs under varying diversity characteristics. Provides the combinations of species <i>and their relative abundances</i> that will attain a certain percentage of the maximum. Provides an adjustment for the multiple tests of comparison that are needed in any multifunctionality analysis giving statistical reassurance on the reliability of conclusions

ture communities were systematically varied at sowing; at each seed density level, there were four monocultures, a community where the four species were sown in equal abundance (0.25, 0.25, 0.25, 0.25), four communities which were each dominated by one species (e.g. 0.7, 0.1, 0.1, 0.1) and six communities which were co-dominated by two species (e.g. 0.4, 0.4, 0.1, 0.1). Each community can be described using an evenness metric (Kirwan *et al.* 2007), $E = [2s/(s-1)] * \sum_{i < j} P_i P_j = (8/3) * \sum_{i < j} P_i P_j$. The evenness values are $E = 0$ for monocultures, $E = 0.64$ for one species dominant, $E = 0.88$ for two species dominant and $E = 1$ for all species equally present. Inorganic nitrogen fertilizer was applied to all plots at a rate of 150 kg N ha⁻¹ annum⁻¹. Further details are available in Kirwan *et al.* (2014). Three ecosystem functions were recorded: (1) aboveground biomass of sown species (sown biomass) (t DM ha⁻¹), (2) aboveground biomass of weed species (weed biomass) (t DM ha⁻¹) and (3) the total annual yield of nitrogen in harvested aboveground biomass (N yield) (t DM ha⁻¹) for each plot and each harvest in 2003, the first year of the experiment following establishment. There were four harvests during the year that were summed for each plot and each ecosystem function to give the annual values. The experiment continued for a further 2 years, but only results from the first year are considered here.

Analysis

The three ecosystem functions were linearly transformed to a comparable scale allowing direct comparisons of the relative effects of the model terms (species relative abundances, species interactions and seed density) across the functions. High values of sown biomass and N yield, and low values of weed biomass are preferred in agronomic practice; to align the direction of desirability for all functions (i.e. make higher positive values desirable for each function), we first multiplied each weed biomass value by -1 and added the maximum (on the original scale) weed biomass value (Byrnes *et al.* 2014a) and called this new variable weed suppression. To linearly transform the data to a common scale, each ecosystem function (sown biomass, weed suppression and N yield) was then converted to a percentage of the average of the highest three values (top 10% of values from 30 plots) for that function (Appendix S2). From here on, these transformed variables are referred to as sown biomass, weed suppression and N yield. We did not apply any weighting to quantify differences in importance, which implicitly assumes that each function has equal importance (Appendix S2).

A range of Multivariate Diversity-Interactions models were fitted to the three transformed ecosystem functions to explore reductions in the dimensionality of the diversity effect explanation. The data rescaling ensured that model predictions for each ecosystem function were on the same scale, which enabled us to test specific predictions across functions to identify conditions (if they existed) under which all functions performed relatively well (e.g. when all ecosystem functions performed above an *a priori* specified level) and to determine if trade-offs occurred among functions under other conditions (e.g. when one or more functions performed above a

specified level but others fell below). These comparisons were made using *t*-tests.

All models were estimated with either maximum likelihood (ML) or restricted maximum likelihood (REML) using SAS software version 9.3 (SAS Institute Inc., Copyright © 2002–2010); model comparisons for testing fixed effects were made using likelihood ratio tests where the models were fitted using ML, while final models were estimated and comparisons among coefficients and predictions were performed using REML. Multivariate normality of the residuals from the final model was tested using Mardia's multivariate normality test in the MVN package (Korkmaz *et al.* 2014) in the software R version 3.1.2 (R Core Team 2014). When testing model terms across functions (e.g. the comparisons among the coefficients β_{11} , β_{12} and β_{13}), there were three pairwise *t*-tests of comparison (one comparison for each pair of functions), thus a Bonferroni correction was applied to each set of three tests to avoid the issues associated with multiple comparisons, giving the adjusted $\alpha^* = 0.05/3 = 0.017$. Note that the Multivariate Diversity-Interactions model could be fitted to the raw data and inference would be unchanged since only a linear transformation has been applied. However, the benefit of modelling the transformed ecosystem functions is the comparative ability across functions which would be meaningless with raw data modelling. Model predictions could be back-transformed to the original scale of each ecosystem function without affecting inference should this be desired. Note also that the ecosystem function that requires the most complex interaction structure may dictate the form of the final model since the same covariates are included for each ecosystem function; this is the case with any multivariate regression model. Further information on fitting and interpreting multivariate regression models is available (for example) in Johnson & Wichern (2007). Appendices S3, S4 and S5 provide the data, SAS and R code, and some interpretations of output to assist readers wishing to fit the framework themselves.

RESULTS

Fitting the Multivariate Diversity-Interactions models

Summary statistics for the three ecosystem functions are given in Table S1. After model comparisons (Table S2), the final parsimonious model selected for the *k*th transformed function was

$$y_k = \beta_{G1k} P_{G1} + \beta_{G2k} P_{G2} + \beta_{L1k} P_{L1} + \beta_{L2k} P_{L2} + \alpha_k \text{Dens} + \delta_{wfg1k} P_{G1} P_{G2} + \delta_{wfg2k} P_{L1} P_{L2} + \delta_{bfgk} (P_{G1} P_{L1} + P_{G1} P_{L2} + P_{G2} P_{L1} + P_{G2} P_{L2}) + \varepsilon_k \quad (4)$$

where P_{G1} , P_{G2} , P_{L1} and P_{L2} are the sown proportions of G1, G2, L1 and L2, respectively, and Dens is coded -1 and 1 for low and high seed density. The β_{G1k} coefficient (for example) is the expected performance of G1 in monoculture for ecosystem function *k* at average density. The 'within functional group' interaction coefficients for the two grasses and two legumes are δ_{wfg1k} and δ_{wfg2k} , respectively, for the *k*th function. The 'between functional group' interaction coefficient between any grass and legume is δ_{bfgk} for the *k*th function.

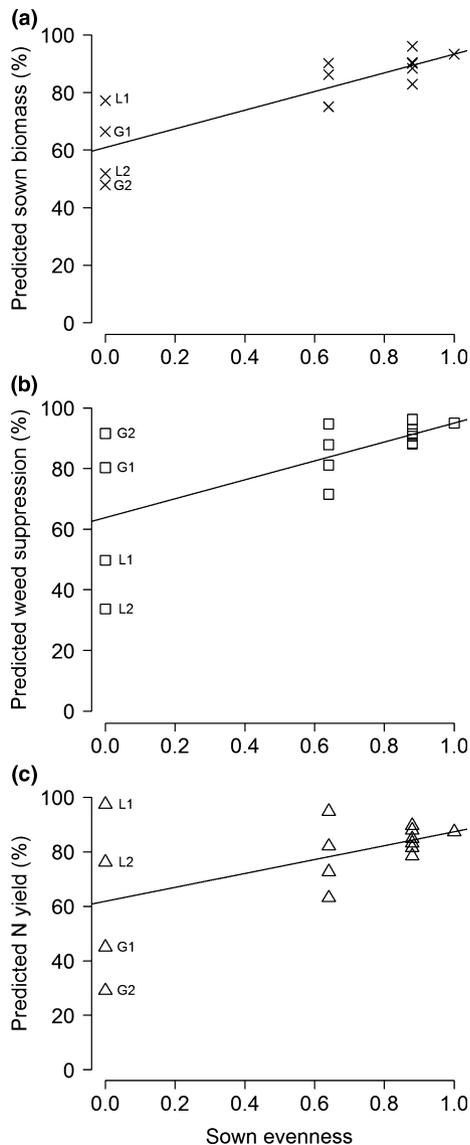


Figure 1 Predicted (a) sown biomass, (b) weed suppression and (c) N yield at average seed density for each community structure, monoculture ($E = 0$), one dominant species ($E = 0.64$), two dominant species ($E = 0.88$) and all species equally abundant ($E = 1$). The multiple points at each value of evenness represent the varying community types in the experimental design. Trend lines are added to indicate patterns as evenness increases and monocultures are labelled to indicate *Lolium perenne* (G1), *Phleum pratense* (G2), *Trifolium pratense* (L1) and *Trifolium repens* (L2).

Residuals showed no evidence of a deviation from the multivariate normal distribution.

Figure 1 and Table 1a show how positive species interactions both within and between functional groups were strong drivers of a positive diversity effect for each individual ecosystem function [addressing question (1) as laid out in the Introduction]. There were no significant seed density effects for any function (Table 1a).

There was a positive correlation among the residuals from sown biomass with the other two functions (Table 1b). The estimated covariances feed directly into the tests of compar-

ison and allow for correct inference when comparing effects across functions [addressing question (2)].

Comparisons of multifunctionality across monocultures and multispecies communities

No one species in monoculture performed best across the three ecosystem functions (Fig. 2, the first set of clusters of bars). There was also no monoculture for which all three ecosystem functions performed poorly, rather there was considerable variability in performance across the functions for each monoculture. Comparisons of the estimated monoculture performances across ecosystem functions (Table 1a, comparison of each β coefficient across functions) showed that the performance of *Lolium perenne* (G1) was better for sown biomass and weed suppression than for N yield, and the performance of *Phleum pratense* (G2) was better for weed suppression than both sown biomass and N yield. Not surprisingly, given their nitrogen fixing abilities, the performances of *Trifolium pratense* and *Trifolium repens* (L1 and L2) in monoculture were far better for N yield than for either sown biomass or weed suppression [addressing question (3)]. The tests displayed in Fig. 2 show that choosing either of the grass monocultures (over other monocultures) to optimise weed suppression results in relatively poorer performances of sown biomass (G2 only) and N yield (both G1 and G2) while choosing either of the legume monocultures to optimise N yield results in lower relative performances of sown biomass and weed suppression [addressing question (4)]. Table S3 provides details of the tests illustrated in Fig. 2.

The predicted performance of ecosystem functions in community types with evenness equal to 0.64 (one species dominant) varied depending on which species was dominant (Fig. 2, the second set of clustered bars); the performance of N yield was better relative to the other two functions when *Trifolium pratense* (L1) was dominant, while the performance of weed suppression was better relative to the two other functions when *Phleum pratense* (G2) was dominant. When L1 was dominant, all three ecosystem functions were higher than 70% ($P < 0.05$ for each test). At evenness levels 0.88 (two species co-dominant) and 1 (centroid), each function performed at a high level; predictions for each ecosystem function and all community types at $E = 0.88$ or 1 were higher than 70% ($P < 0.05$ for each test). There were still some small (but significant) differences within each cluster at the higher levels of evenness with sown biomass generally outperforming N yield (Fig. 2). Note that 70% has been chosen arbitrarily for illustration here but should be chosen *a priori* in practice. If multiple thresholds are tested rather than an *a priori* choice of threshold, then adjustments for multiple tests should be included.

Despite there being significant differences among the three functions for 14 of the 15 community types presented in Fig. 2, the magnitude of the differences decreased as evenness increased. For example, the estimated difference between sown biomass and N yield was 21% for *Lolium perenne* (G1) monoculture ($E = 0$), 14% for a four-species community dominated by G1 ($E = 0.64$), 9% for a four-species community co-domi-

Table 1 Estimated model terms for the transformed ecosystem functions sown biomass, weed suppression and N yield, (a) fixed coefficients, (b) the variance covariance matrix (left) and correlations (right)

(a) Fixed coefficients		Ecosystem function								
		Sown biomass (%)		Weed suppression (%)		N yield (%)				
Term	Coefficient	Est	SE	Est	SE	Est	SE			
G1	β_{G1k}	66.48	4.50	a	80.29	8.47	a	45.02	4.60	b
G2	β_{G2k}	47.95	4.50	a	91.57	8.47	b	29.08	4.60	c
L1	β_{L1k}	77.22	4.50	a	49.75	8.47	b	97.43	4.60	c
L2	β_{L2k}	51.88	4.50	a	33.67	8.47	a	76.26	4.60	b
Dens	α_k	1.15	1.31	a	0.50	2.47	a	-0.63	1.34	a
G1*G2	δ_{wfg1k}	105.37	41.94	a	-31.99	78.92	a	150.46	42.82	a
L1*L2	δ_{wfg2k}	64.64	41.94	a	159.97	78.92	ab	-5.32	42.82	b
$\Sigma G*L$ (bfg)	δ_{bfgk}	87.24	18.81	a	92.95	35.39	a	65.24	19.21	a

(b) The variance covariance matrix (left) and correlations (right)	Variances and covariances			Correlations	
	Sown biomass	Weed suppression	N yield	Weed suppression	N yield
Sown biomass	51.6				
Weed suppression	49.1	182.7		0.51	0.82
N yield	43.1	6.6	53.8		0.07

Significant ($\alpha < 0.05$) coefficients in (a) are highlighted in bold. Within each row (i.e. across ecosystem functions), coefficients that are not significantly different have a letter in common, where the level of significance determined by the Bonferroni correction is $\alpha^* = 0.05/3 = 0.017$.

[Correction added on 5 October 2015, after first online publication: Rendering of Table 1b columns has been corrected.]

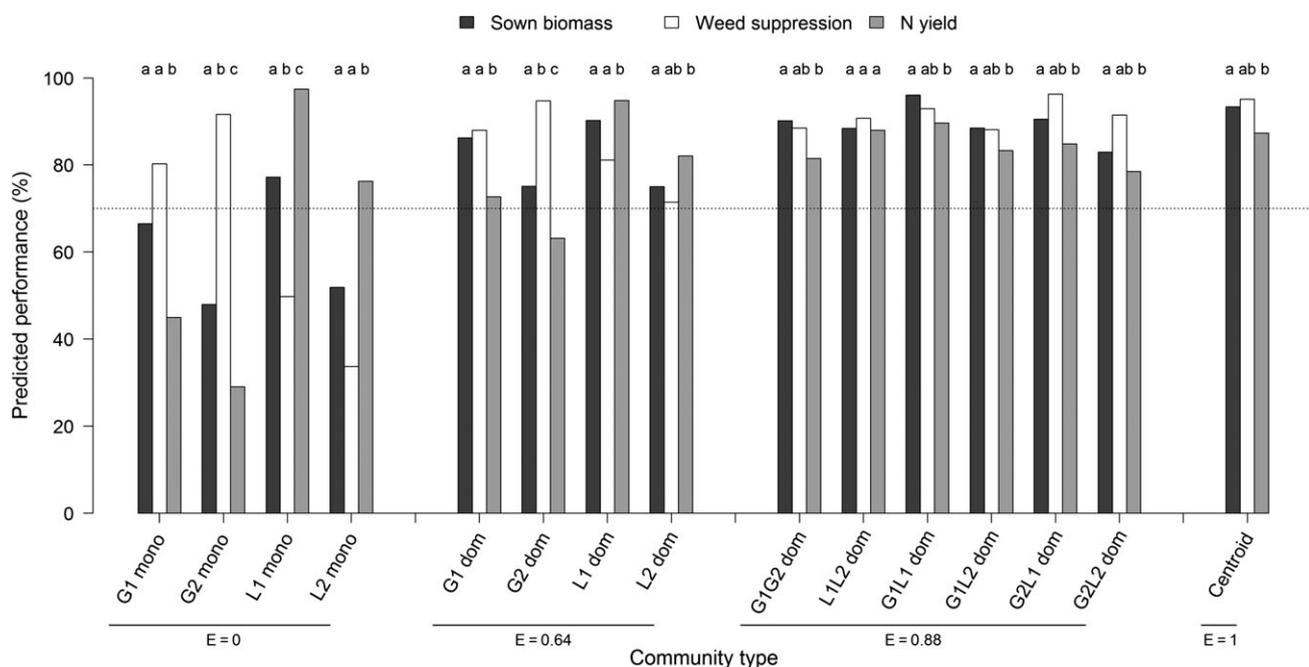


Figure 2 Predicted response for ecosystem functions sown biomass, weed suppression and N yield for each design community type (monocultures, one species dominant, two species co-dominant and all species equally abundant) at average seed density. Bars within a cluster that share a letter do not differ significantly. The level of significance for all tests of comparison is determined by the Bonferroni correction, $\alpha^* = 0.05/3 = 0.017$. Note that, e.g. G1 mono is a grass 1 monoculture, G1 dom is (0.7, 0.1, 0.1, 0.1), G1G2 dom is (0.4, 0.4, 0.1, 0.1) and the centroid is (0.25, 0.25, 0.25, 0.25). The species are *Lolium perenne* (G1), *Phleum pratense* (G2), *Trifolium pratense* (L1) and *Trifolium repens* (L2). A horizontal line is included at $y = 70\%$ to aid comparisons.

nated by G1 and G2 ($E = 0.88$) and 6% for the centroid community ($E = 1$), a significant difference (t -tests, $P < 0.01$) in each case but the effect size (i.e. the differences 21, 14, 9 and

6%) decreased as evenness increased (tests not shown). On average, performance across the three functions was higher and more stable in the communities with evenness equal to

0.88 or 1 when compared to the lower and more variable responses in monoculture and at $E = 0.64$. Thus, we show that the ecosystem functions in this experiment showed trade-offs against one another at low levels of evenness but exhibited desired levels of performance ($> 70\%$) at higher levels of evenness [addressing question (4)].

DISCUSSION

The Multivariate Diversity-Interactions framework developed here provides quantitative tools to enhance our understanding of ecosystem multifunctionality. Our framework can test how multiple ecosystem functions are simultaneously driven by species abundances, species identities, species interactions, composition, richness and evenness. It can also test the relative importance of those drivers and identify key species and influential pairwise species interactions across multiple ecosystem functions. The framework provides quantitative information on individual as well as multiple functions and can aid decision making to support the management of ecosystems in which the high performance of several functions is desired, such as in the agronomic communities in our example.

Our framework integrates the analytical outputs and insights formerly obtained from several separate multifunctionality approaches, including species-level information provided by the overlap approach and community-level information provided by the averaging and multiple threshold approaches. By combining these types of information, our framework is uniquely able to identify combinations of species and relative abundances that produce desirable levels of multiple ecosystem functions. For example, we found that four-species mixtures that were co-dominated by *Lolium perenne* (G1) and *Trifolium pratense* (L1) provided nearly maximal levels of all three ecosystem functions (Fig. 2). As manipulated evenness increased, we also showed that ecosystem functions were higher on average and that the variability among the three ecosystem functions decreased (Fig. 2). Other studies have examined ecosystem multifunctionality over time (Isbell *et al.* 2011; Cardinale *et al.* 2013; Pasari *et al.* 2013), trophic levels and ecosystem types (Lefcheck *et al.* 2015) but not variability among the levels of multiple functions across a manipulated treatment. Our agronomic example provides further evidence of the benefits of increased diversity on ecosystem multifunctionality.

A key strength of the Multivariate Diversity-Interactions framework is its comparative ability whereby model coefficients and model predictions under varying diversity conditions can be tested for differences across functions. This ability is directly enabled by the estimation of the variance covariance matrix (Table 1b). Had three separate univariate Diversity-Interactions models been fitted instead of a multivariate model, the coefficient estimates and their standard errors (Table 1a) would be no different, but the variance covariance matrix (Table 1b) would not have been estimated and thus it would not have been possible to correctly make comparisons across functions. For example, the t -test statistic for comparing β_{G11} and β_{G13} [the expected *Lolium perenne* (G1) monoculture performance for sown biomass and N yield respectively] was 7.83 with $P < 0.0001$. This test and its infer-

ence are valid since the covariance between the two functions contributes to the test statistic calculation. If, however, a zero covariance between the estimates had been assumed, the test statistic would be calculated (incorrectly) as 3.33 with $P = 0.002$ resulting in approximately a halving of the test statistic and any inference from this incorrect test would not be valid. This comparative ability of the Multivariate Diversity-Interactions framework allows (1) the identification of compositions and relative abundances where all ecosystem functions perform well or (2) the identification of how functions may trade off against one another and (3) understanding of how optimisation of one function impacts other functions. In our example, the G2 monoculture attained 92% in weed suppression but only 48 and 29% in sown biomass and N yield, respectively, illustrating trade-offs among functions in this monoculture (and others). There were no significant differences among the ecosystem functions for the community co-dominated by L1 and L2 and each function was higher than 70%, illustrating conditions where all functions had similarly high levels of performance (Fig. 2).

The Multivariate Diversity-Interactions framework includes the benefits and addresses the losses of information that are inherent in other methods for analysing multifunctionality. Our framework estimates the relationship between individual ecosystem functions and manipulated diversity or treatment variables (the univariate approach, Allan *et al.* 2013), quantifies which species positively influence ecosystem function (the overlap method, Hector & Bagchi 2007) and can identify what combination of species will yield a certain percentage of the maximum of ecosystem function performance (the single and multiple threshold methods, Gamfeldt *et al.* 2008 and Byrnes *et al.* 2014a). In addition, our framework also measures correlations among functions, provides a means for statistical tests of comparisons across multiple functions, provides quantitative estimates on multifunctionality across varying compositions and relative abundances, and identifies important species and species interactions for individual functions and tests their relative importance across functions, which other approaches cannot do. Analysing each ecosystem function individually allows only for qualitative inference on multifunctionality (Byrnes *et al.* 2014a), while dimension-reducing indices which quantify multifunctionality may omit important information at the individual ecosystem function level (Bradford *et al.* 2014a,b; Byrnes *et al.* 2014b); the ability of our framework to assess individual ecosystem functions in conjunction with multifunctionality is therefore highly desirable. We thus present our framework as a consolidation of the strengths of previous approaches that also provides several additional advances in the quantification of ecosystem multifunctionality (Box 1).

The rich information available from using our framework goes beyond what is achievable with other approaches used to analyse the biodiversity and ecosystem multifunctionality relationship. In our experiment, the four-dimensional simplex design space was well represented, therefore we can use our model to predict each ecosystem function for any set of relative abundances and compositions of these four species. For example, we can estimate each ecosystem function for the community compositions (0.5, 0.5, 0, 0) and (0.8, 0.1, 0.05, 0.05), even though these are not represented by any specific

design point; this predictive power reflects an important added advantage of the approach. Generally, when a traditional linear regression model with $\log(\text{richness})$ as a covariate is fitted, the model can predict at each level of richness but cannot distinguish between communities with differing relative abundances at the same level of richness; e.g. the two markedly different communities (0.25, 0.25, 0.25, 0.25) and (0.85, 0.05, 0.05, 0.05) would yield the same prediction in the traditional model but our framework would provide unique predictions. This distinctive trait is exclusive to our approach and is not provided by other ecosystem multifunctionality approaches. Some studies of single functions have included a measure of evenness as a factor along with richness (e.g. Wilsey & Polley 2004), but our framework can jointly test the continuous effects of evenness and richness through the diversity effect. Richness effects can be illustrated with our framework by predicting each ecosystem function for equi-proportional communities at each level of richness. We can also use the Multivariate Diversity-Interactions framework to identify zones in the simplex space when all or most functions perform well or at close to their maximum value.

We found that the most parsimonious model was one of intermediate complexity, which included functional group interactions, rather than unique interactions for all pairs of species (Table S2). The between grass-legume functional group interaction coefficients were strong and positive for each function highlighting the benefits of mixing these functional groups for multifunctionality in grassland systems (Table 1a). This benefit is well documented for individual functions (Ledgard & Steele 1992; Spehn *et al.* 2002; Nyfeler *et al.* 2011) but is shown here for the first time for ecosystem multifunctionality. The two grasses also interacted strongly and positively for both sown biomass and N yield perhaps reflecting the fast-establishing and temporally persistent traits of G1 and G2 respectively.

The intricacies involved in research questions about ecosystem multifunctionality are compounded when the ecosystem is more complex. It is therefore not surprising that difficulties can arise with our multivariate approach when the numbers of species or ecosystem functions increase. These difficulties are a natural consequence of the increasing complexity of the system; we summarise them and outline possible solutions in the following three points.

(1) When the number of species increases, the number of coefficients per ecosystem function also increases. Kirwan *et al.* (2009) suggested constraints among interaction coefficients to alleviate this problem and here we constrained interaction coefficients according to functional groupings. Kirwan *et al.* (2009) and Connolly *et al.* (2011, 2013) each provide alternative solutions to reduce the dimensionality of the diversity effect description which readily apply to our multivariate setting. In our experience with single ecosystem functions, it is frequently possible to model the diversity effect using a small number of coefficients even with high species richness, e.g. a 10-species grassland system (Connolly *et al.* 2011) and a 72-species bacterial system (Connolly *et al.* 2013) were both modelled with just two diversity coefficients. It is also possible to test for biologically meaningful patterns among the identity effect (β_i) coefficients.

(2) When the number of ecosystem functions increases, so too does the overall number of coefficients; our method maintains individual function information and if this is desirable then there is no option but to increase the number of equations and hence number of coefficients used to describe the system. If individual function information is not required, then alternative multifunctionality approaches (Appendix S1) may be more useful and we encourage their usage.

(3) We used a Bonferroni correction to adjust for multiple comparisons but if the number of ecosystem functions were to increase so too would the number of comparisons resulting in the criterion for a significant result becoming stricter and Bonferroni adjustments would likely be unduly conservative (Gotelli & Ellison 2004). The multiple comparisons issue arises in other approaches developed for analysing multifunctionality (e.g. Hector & Bagchi 2007; Gamfeldt *et al.* 2008; Isbell *et al.* 2011; Byrnes *et al.* 2014a) but has not been dealt with in any of those methods. Here we show that adjusting for multiple comparisons can be relatively straightforward, at least for a small number of functions. For a larger number of functions, alternative more powerful large scale methods for adjusting for multiple comparisons to the Bonferroni correction should be used (e.g. Donoghue 2004; Verhoeven *et al.* 2005).

The Multivariate Diversity-Interactions framework is applicable to data from many types of designed experiments although sometimes it is not appropriate. For example, it is not recommended to fit a Diversity-Interactions model to an experiment with monocultures of each species and replicates of only one type of mixture that contains all species in equal relative abundances (e.g. Griffin *et al.* 2009). In such a design there is inadequate coverage of the simplex space and all mixtures are equal in respect of diversity manipulations (richness and evenness are constant across all mixtures), therefore it is not possible to estimate pairwise interactions. Many biodiversity experiments have equi-proportional mixtures across a manipulated gradient of richness (e.g. Hector *et al.* 1999; Roscher *et al.* 2004) and a smaller number of studies manipulated evenness at a single level of richness (e.g. Wilsey & Potvin 2000; Finn *et al.* 2013). Our framework is fully suited to the analysis of such data as has been shown in previous work in the univariate setting (e.g. Connolly *et al.* 2011) for richness manipulations and in our example here for evenness manipulations. A design with both evenness and richness manipulations combined with our modelling approach would provide even further predictive power but both manipulations are not a requirement. Note that the estimation of pairwise interaction terms does not specifically require two-species mixtures in the design. It is also possible to apply the Multivariate Diversity-Interactions framework to observational data although reliability would depend on the data in question as the usual regression model caveats apply; these include ensuring there is sufficient representation in the design space and that caution is exercised in inferring causation from observed correlations.

The Multivariate Diversity-Interactions framework is flexible and can be extended in several directions, four of which are highlighted here. (1) The model can analyse multiple ecosystem functions across a range of treatments or environments. Here

we presented data with two sown seed densities; however, other treatments, such as different levels of applied nitrogen, can easily be incorporated into the model (e.g. see Kirwan *et al.* 2009). (2) The framework can be extended for the analysis of multiple functions across temporal and spatial variables (Isbell *et al.* 2011), as has already been done for the univariate Diversity-Interactions modelling approach (Kirwan *et al.* 2007; Finn *et al.* 2013). (3) It is possible to allow for nonlinearity in the relationship between the ecosystem functions and the species interactions (see the Generalised Diversity-Interactions approach by Connolly *et al.* 2013). (4) The model here assumes a constant variance across plots for each ecosystem function but could easily be adjusted if this were not the case, e.g. the variance for an ecosystem function could differ between monoculture and mixture communities (Schmid *et al.* 2008). These potential extensions further illustrate the benefits of our framework. Structural equation models have been used to assess the biodiversity and ecosystem function relationship for single functions (e.g. Grace *et al.* 2007; Bowker *et al.* 2010). These models may also have a useful role in understanding ecosystem multifunctionality, however initial attempts to do so may not be valid due to the questionable model selection process used (see PloS ONE reader comments on Mouillot *et al.* 2011).

The Multivariate Diversity-Interactions framework examines the multifunctional BEF relationship through a multivariate model fit that does not suffer from the loss of information inherent in other approaches. The framework consolidates the strengths and improves on the weaknesses of previous approaches for analysing ecosystem multifunctionality. It can identify the drivers of multiple ecosystem functions and test the relative performances across functions. The Multivariate Diversity-Interactions framework can be adapted to suit varying experimental conditions and is a valuable tool to improve understanding of ecosystem multifunctionality.

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AUTHORSHIP

JC, LK, JF and CB conceived the idea, all authors contributed to the concept development. CB and AD performed the analyses. CB and AD wrote the paper with substantial contributions from FI, JF and JC.

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