

# Biodiversity–multifunctionality relationships depend on identity and number of measured functions

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**Biodiversity ensures ecosystem functioning and provisioning of ecosystem services, but it remains unclear how biodiversity–ecosystem multifunctionality relationships depend on the identity and number of functions considered. Here, we demonstrate that ecosystem multifunctionality, based on 82 indicator variables of ecosystem functions in a grassland biodiversity experiment, increases strongly with increasing biodiversity. Analysing subsets of functions showed that the effects of biodiversity on multifunctionality were stronger when more functions were included and that the strength of the biodiversity effects depended on the identity of the functions included. Limits to multifunctionality arose from negative correlations among functions and functions that were not correlated with biodiversity. Our findings underline that the management of ecosystems for the protection of biodiversity cannot be replaced by managing for particular ecosystem functions or services and emphasize the need for specific management to protect biodiversity. More plant species from the experimental pool of 60 species contributed to functioning when more functions were considered. An individual contribution to multifunctionality could be demonstrated for only a fraction of the species.**

Ecosystem services, such as the supply of clean water, soil erosion control or pollination, depend on ecosystem functions that are controlled by the species living in an ecosystem<sup>1</sup>. Provisioning of these services is thought to be threatened by an ongoing loss of species worldwide<sup>2</sup>, driven largely by land-use change and overexploitation of natural populations<sup>3</sup>. Ecosystem functioning has been shown to decrease with decreasing biodiversity in experiments that manipulate plant species richness<sup>4</sup>. However, when individual functions are considered, species richness–ecosystem function relationships frequently saturate at low levels of species richness; for example, when three to six species are present in the system<sup>5,6</sup>. Such saturating relationships have been taken as support for the redundancy hypothesis<sup>7–9</sup>, which proposes that high functioning can be

achieved with only a few species. However, redundant species may contribute to maintaining ecosystem functions when other species are lost or under changing environmental conditions<sup>10</sup>, referred to as the insurance effect<sup>10,11</sup>. Also, a turnover in the identity of species contributing to a particular function may increase the cumulative number of species sustaining functioning over multiple years<sup>12</sup>. However, even with such mechanisms accounted for, a large proportion of species still seem to be redundant<sup>13</sup>. This limit to the number of species needed for ecosystem functioning raises questions if provisioning of ecosystem services can be a major argument for species conservation<sup>14</sup>.

Humans rely on ecosystems for their ability to maintain multiple functions and services simultaneously, as expressed in the concept

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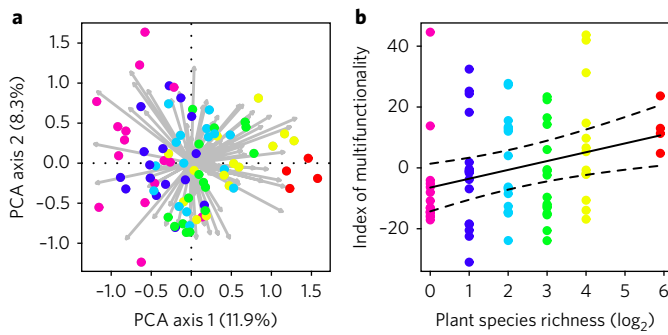
of ecosystem multifunctionality<sup>15,16</sup>. The number of species contributing to ecosystem multifunctionality is in general higher than the number of species needed for single functions<sup>17–22</sup>. Also, rare species have been shown to contribute to multifunctionality<sup>23</sup>. Thus, the importance of biodiversity for multifunctionality is higher than for individual functions. When multiple years, places and environmental change scenarios (so-called contexts) were considered simultaneously by synthesizing 12 functions across 17 biodiversity experiments, 84% of the 147 grassland plant species studied promoted ecosystem functioning in at least one context<sup>20</sup>. In addition to the question of whether all species are needed to maintain ecosystem multifunctionality, an equally important question is whether (and how) the effect of biodiversity on ecosystem multifunctionality depends on the identity and number of functions considered<sup>24</sup>. While a recent simulation study found no relationship between the number of functions included and the relationship between biodiversity and ecosystem multifunctionality<sup>24</sup>, this question remains untested with empirical data. This question is important as different ecosystem functions not only differ in how strongly they depend on biodiversity<sup>25</sup>, but biodiversity and ecosystem functions may also be positively or negatively correlated with each other. If ecosystem functions are positively correlated and depend on biodiversity, increasing biodiversity should increase many functions simultaneously. In this case, maximizing ecosystem multifunctionality would require high biodiversity. Consequently, maximizing multifunctionality would be equivalent to maximizing biodiversity. However, if functions are negatively correlated, maximizing one function should result in decreasing the other function, and the relationship between ecosystem multifunctionality and biodiversity would depend on the particular functions considered and how they are correlated with biodiversity. The same holds true if functions are uncorrelated, in which case they can be independently maximized. Thus, for the question of whether managing for ecosystem multifunctionality is equivalent to managing for a protection of biodiversity, both the relationships among the different functions considered and the dependence on biodiversity are important.

Here, we analysed the correlation structure of a suite of ecosystem functions to test the hypothesis that strong effects of biodiversity on ecosystem multifunctionality depend on a large and diverse portfolio of functions to be included in the analysis that reflects the high complexity of ecosystem functioning in nature. We base our analyses on a selection of 82 different ecosystem variables identified from a larger collection<sup>25</sup> measured along a gradient of 1–60 plant species in a single biodiversity experiment (the Jena Experiment<sup>26</sup>; see Methods). Consistent with previous studies on ecosystem multifunctionality, we approximate ecosystem functions by ecosystem variables<sup>19,20,22,27</sup>. These variables quantify ecosystem functions either directly (“the changes in energy and matter over time and space through biological activity”; Reiss et al.<sup>28</sup>) or indirectly (“key ecosystem properties affected by ecosystem functions”; Jax<sup>29</sup>). Restricting analyses of multifunctionality to only directly measured functions would bias the portfolio of included functions considerably, as some types of functions—especially species interactions and belowground functions—are notoriously difficult to measure directly. The best way to include these functions in an analysis of multifunctionality is to quantify state changes or differences in the size of ecosystem compartments as indicators for the underlying changes in functions<sup>30</sup>. We chose a large number of 82 ecosystem variables to cover a range of different ecosystem functions, including above- and belowground plant productivity, nutrient and element cycling, antagonistic and mutualistic multitrophic interactions, and invasion resistance (detailed in Supplementary Table 1.1). Variables include measurements of the abiotic environment, plants and consumers. Information on consumers was separated into different functional groups when possible. Otherwise, different taxonomic groups were separated as these groups not

only indicate secondary productivity, but also mediate different ecosystem functions; for example, herbivory, predation, parasitism, decomposition, scavenging, pollination and seed predation/dispersal. To ensure that each ecosystem variable was included only once in the analysis, only the measurement in the last available year was selected, thus excluding repeated measures. Correlated ecosystem variables were not excluded a priori as investigating how the relationships among functions affect multifunctionality was an explicit goal of the present study. We also decided against combining different ecosystem variables that appeared to be related to the same ecosystem function in a single ‘true’ function, as what is considered an indicator of different functions is arbitrary and depends critically on the research question. For example, the above- and belowground biomass production of plants are both indicators of overall productivity, yet they are also individual indicators with particular impacts on the ecosystem; aboveground biomass mainly represents carbon fixation, transpiration and the potential for light interception, while belowground biomass indicates potential nutrient and water uptake, as well as respiration. Similar arguments hold for the different groups of herbivores. While all contribute to overall herbivory, they interact, depending on their feeding guild, with different parts of the food web. Herein, we refer to ‘ecosystem variables that indicate ecosystem functions’ as ‘functions’ for simplicity, as is common for studies of multifunctionality<sup>19,20,22,27</sup>.

## Results

To quantify the change in overall ecosystem functioning along the experimental gradient of plant species richness, we calculated a multifunctionality index based on all 82 functions. Different indices to quantify multifunctionality have been proposed<sup>18</sup>. Multifunctionality is a multifaceted ecosystem property, much like biodiversity<sup>31</sup>, and all of the proposed indices quantify slightly different facets of multifunctionality<sup>18</sup>. We extended the averaging approach proposed for individual functions<sup>18,19,32</sup> to a multivariate approach based on a principal component analysis (PCA) that analyses the correlation structure among the different functions. The main advantage of this new multivariate index of multifunctionality over the previous approaches<sup>18</sup> is that it accounts for both positive and negative correlations between ecosystem functions, which may otherwise bias the results of multifunctionality analyses<sup>33</sup>. We found that many of the functions analysed here were positively or negatively correlated with each other (Supplementary Fig. 2.4). The overall level of co-linearity was, however, limited, as indicated by the fact that 24 PCA axes were needed to explain 80% of the total variance in functional space (Supplementary Fig. 2.2). A visualization of the first two principal components showed that the most diverse 60-species plots form a distinct cluster at the right end of the first PCA axis (Fig. 1a). Plant species richness was strongly positively correlated with the first principal component axis ( $r=0.80$ ,  $t_{79}=12.0$ ,  $P<0.001$ ). Consequently, plant species richness was the most important single driver of ecosystem functioning in our experiment because the first principal component represents the maximum variance that can be summarized on one axis. Individual functions that correlated with the first PCA axis were also positively correlated with plant species richness; for example, the biomass and height of the plant community, bare ground cover (negatively correlated with plant species richness), microbial biomass and the abundance of earthworms and other animal groups. The PCA approach was robust against the inclusion of non-normally distributed data on ecosystem functions as demonstrated in a sensitivity analysis using principle coordinates analysis based on Gower distances (Supplementary Materials, section 3). Because principal components are uncorrelated, in contrast with the original functions, multifunctionality of a plant community can be related to the scores of the principal components without adding correlated information. We quantified total functioning of a particular plant community by

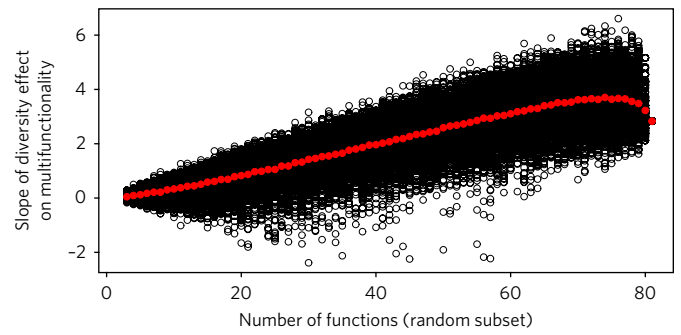


**Fig. 1 | Effects of biodiversity on multifunctionality.** **a**, Position of each plot (coloured dots; rainbow gradient from pink = monocultures to red = 60-species communities) in a multifunctional space spanned by the first two axes derived from a PCA based on 82 different ecosystem functions measured in the Jena Experiment. The red dots (60-species mixtures) form a distinct cluster on the right-hand side of the graph far from the centre of the plot. Each of the functions is shown as a grey arrow (vector) pointing in the direction of the ordination space in which it increases in value. The angles between the function vectors represent the degree of correlation between them. As the angles between function vectors in the ordination plane spanned by PCA axis 1 and 2 can be distorted by relationships with higher PCA axes, the precise correlation coefficients among all functions are given in Supplementary Fig. 2.4. Graphs with labelled arrows for the functions can be found in Supplementary Fig. 2.1. **b**, Effect of plant species richness on ecosystem multifunctionality, as found in the Jena Experiment. The multifunctionality index was calculated by summing the PCA axis scores for all experimental plots weighted by the eigenvalue of the respective PCA axis. The solid black line represents the effect of a linear model fit, while the dashed lines show the 95% confidence intervals around the fit.

adding the scores of all PCA axes, weighted by the eigenvector of the respective axis (see Methods). An increase in this multifunctionality index indicates increased functioning because the index is positively correlated with variables characterizing primary (for example, plant biomass, height, cover and LAI) and secondary productivity (for example, the number of many belowground fauna groups) as well as soil organic carbon and the biomass of microorganisms in the soil (Supplementary Table 4.1). The multifunctionality index was negatively correlated with disservices, such as the cover of bare ground and soil bulk density (Supplementary Table 4.1).

The multifunctionality index increased significantly with increasing plant species richness ( $F_{1,76} = 8.13$ ,  $P = 0.006$ ; Fig. 1b). Multifunctionality calculated using previously published, complementary approaches; that is, the 'averaging', 'threshold' and 'multiple thresholds' approaches<sup>18</sup>, confirmed this strong increase in multifunctionality with increasing plant species richness (Supplementary Fig. 5.1a–d). Furthermore, the effect of plant species richness on multifunctionality was robust when restricting the analysis to the 54 ecosystem functions measured in the same year—2004 (Supplementary Table 1.1 and Supplementary Materials, section 6). Thus, biodiversity also sustained multifunctionality when the previously demonstrated effects of temporal turnover<sup>12,20</sup> were excluded.

Next, we analysed how the number of functions used to calculate multifunctionality affected the relationship between biodiversity and multifunctionality by analysing random subsets of two to 82 ecosystem functions. We observed consistent positive effects of biodiversity on multifunctionality that, in contrast with simulated results<sup>24</sup>, became stronger when a larger number of functions was considered (Fig. 2). The increasingly strong effects of biodiversity on ecosystem multifunctionality with higher numbers of considered functions

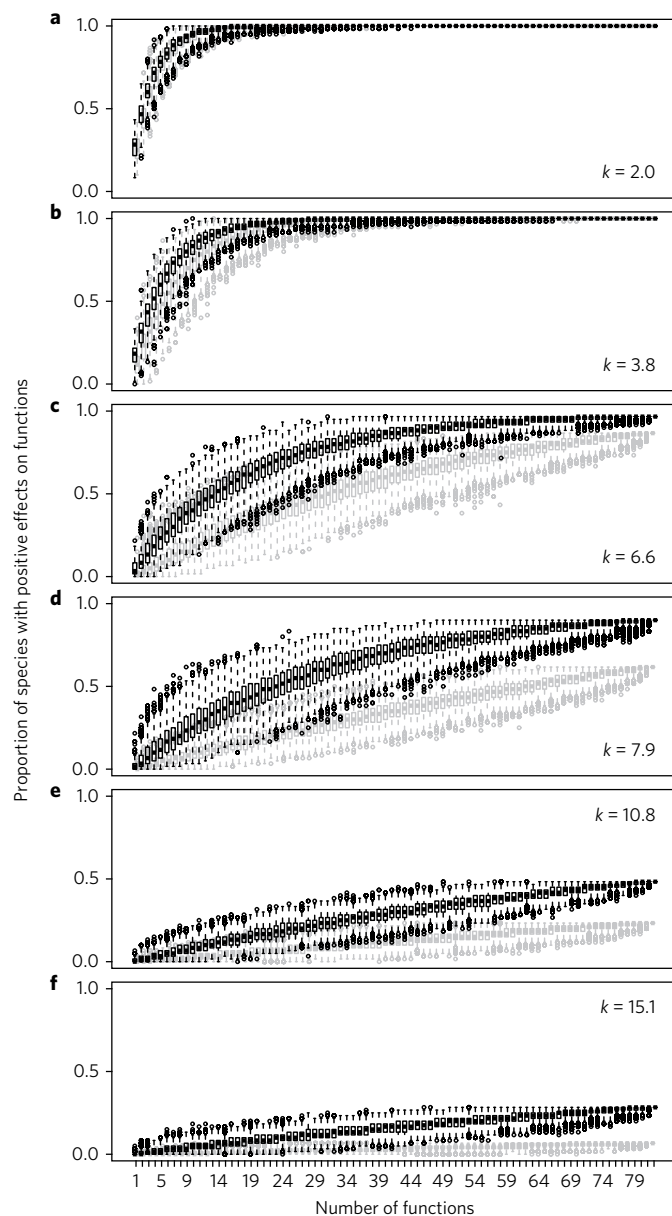


**Fig. 2 | Effects of the number and identity of ecosystem functions on the relationship between plant species richness and multifunctionality.** Each open circle shows the slope between the multifunctionality index and plant species richness for a particular random subset of the 82 ecosystem functions included in the analysis. The red filled circles show the mean slope for a particular number of functions, resulting from up to 500 random draws of this particular number of ecosystem functions.

documented in our experiment indicate that some properties of diverse plant communities were not included in the simulation study<sup>24</sup>. Thus, the expected effects of biodiversity on multifunctionality are largest when a high number of ecosystem functions are of interest. However, the strength of the relationship between biodiversity and ecosystem multifunctionality showed large variation around the mean slope for any given number of functions and critically depended on the identity of the ecosystem functions used for calculating multifunctionality. These identity effects imply that studies of multifunctionality based on different functions cannot be directly compared. Identity effects occurred because of trade-offs among ecosystem functions (Supplementary Figs. 2.1, 2.4 and 4.1a) and because of functions that were only weakly or even negatively related to multifunctionality (Supplementary Fig. 4.1b and Supplementary Table 4.1). It is likely that including such functions, or functions that are well sustained by low biodiversity, in the random draw explains the slight decrease in average multifunctionality that occurred when very high numbers of ecosystem functions were considered (Fig. 2). For particular subsets of functions, biodiversity had only minor effects on ecosystem multifunctionality (Fig. 2). This can explain why studies may also find weak effects of biodiversity on ecosystem multifunctionality<sup>18</sup>.

Increasingly strong effects of biodiversity on multifunctionality when more functions are considered were also confirmed when we calculated the proportion of plant species that contributed to functioning using the 'turnover' approach<sup>17</sup>. For each ecosystem function, informative species effects were extracted from a full model, including the presence-absence data of all 60 plant species as explanatory variables, using a stepwise Akaike information criterion (AIC) approach. For each number of ecosystem functions between 1 and 82, up to 2,000 random subsets of functions were drawn, and the proportion of species (out of the total of 60) that had informative, positive effects on at least one of the functions in the subset was calculated. In the same way, negative effects were analysed in a separate analysis. The proportion of species that contributed positively to functioning increased strongly with the number of functions considered (Fig. 3). Using a critical  $\Delta AIC$  value of 2, as proposed by Hector and Bagchi<sup>17</sup> in the original description of the turnover approach, the proportion of species contributing to functioning was not statistically different from one (that is, all species contributed to functioning) when 11 or more functions were analysed (see also Supplementary Fig. 7.1a). These results exceed the proportions of species that were shown to contribute to functioning in earlier studies<sup>17,20</sup> also when only ecosystem functions measured in the same





**Fig. 3 | Proportion of the plant species pool that contributes positively to functioning when an increasing number of ecosystem functions is analysed simultaneously.** The plant species pool contained 60 species and the total number of functions considered was 82. Each plot shows the average proportion, together with quartiles, the 1.5 times interquartile range as whiskers and outliers beyond this range as dots, all of which were calculated for up to 2,000 random draws of each number of functions from the total of 82. The grey boxes show the results of a null model for which the measurements of each function were permuted over the plots of the experiment to disrupt associations between the presence of species and the level of functioning in a plot (see Methods). **a–f**, Each panel gives the results of an analysis with an increasingly strict criterion for the effect of a species to be considered informative. The  $k$  values stated are the minimum  $\Delta$ AIC values needed for the comparison of a model containing the presence of a species as an explanatory variable versus a simpler model without it to be considered informative.

at least one of the ecosystem functions investigated (Supplementary Fig. 7.1b). Yet, functioning increased with higher diversity because the effect sizes of positive contributions were higher than those of negative contributions (Supplementary Fig. 7.2). We calculated a null model based on data where the presence of species in plots had been permuted over the plots to disrupt the association between the presence of species and ecosystem functioning (see Methods). Comparison with the null model showed that, when using a critical  $\Delta$ AIC value of 2, a high number of false positive species effects inflates the proportion of species with informative effects. This inflation of the number of informative effects was confirmed in a simulation study (Supplementary Materials, section 8). To compensate for the statistical effect of fitting a large number of models estimating many parameters, we recalculated the analysis with a series of increasingly strict critical  $\Delta$ AIC values that needed to be surpassed for the effect of the presence of a species to be considered informative (see Methods). Using increasingly stricter  $\Delta$ AIC values, the proportion of species with informative effects continuously declined, as expected (Fig. 3a–f and Supplementary Materials, section 8). The proportion of species showing effects for the permuted data declined faster than for the measured data so that the asymptote for the proportion of species with effects was about 20 percent points higher for the measured data than for the permuted data (Fig. 3d–f). Thus, about 20% of the plants in the species pool showed effects on ecosystem functioning that could be unambiguously separated from potential statistical artefacts and are thus informative beyond doubt. It is important to note that this number is an extremely conservative estimate because (1) the turnover approach does not account for interactions between species (complementarity) and (2) with such strict criteria, the chances of rejecting true effects increases. The proportion stated here cannot be compared to previously published estimates of the proportion of the species pool affecting functioning<sup>17,20</sup> as these studies used much-less-strict criteria for the detection of effects. Given that in the design of the experiment every species occurs only in a minor fraction of the plots, and given that the proportion of species with effects increased with the number of functions considered and that the number of functions in real-world ecosystems probably exceeds even the 82 ecosystem functions considered in this study, we conclude that the proportion of species individually contributing to ecosystem functioning is likely to be much higher than can be shown here.

## Discussion

Our findings have two major implications for the management of ecosystems. First, our results demonstrate that not all ecosystem services can be maximized simultaneously when these services rely on functions that show trade-offs<sup>34,35</sup>. Thus, management to maximize a particular ecosystem service will probably decrease the provisioning of other ecosystem services<sup>36</sup> and may not maximize, but may even decrease multifunctionality. A similar result was found with respect to the effect of land use on the provisioning of ‘ecosystem service bundles’, where the magnitude and even the direction of effects depended on the composition and weighting of ecosystem services in a bundle<sup>37</sup>. However, our analysis also indicates that (1) adding species (if it is still possible to add a species) should be beneficial, as adding a species to an ecosystem would likely increase some ecosystem function (more than it would decrease others) and (2) it is possible to maximize particular functions, such as biomass production, and simultaneously favour other functions, such as increased water- or nutrient-use efficiency, resulting in reduced environmental impacts combined with potential economic benefits. Second, our results increase misgivings about the assumption that managing ecosystems to maximize ecosystem service provisioning guarantees the preservation of biodiversity, as would be expected if high biodiversity always underlies

year (2004; Supplementary Table 1.1) were used in a sensitivity analysis (Supplementary Fig. 6.1a). In addition to the positive effects of the presence of species on functioning, every species also decreased

high ecosystem service provisioning<sup>38</sup>. While biodiversity was the strongest driver of ecosystem functioning in our study, underlining its importance, our results show that the particular combination of ecosystem functions considered determines whether—and to what extent—multifunctionality is related to biodiversity (Fig. 2). Consequently, managing an ecosystem to maximize biodiversity will not necessarily maximize a particular subset of functions for which managers are aiming and management for services will not necessarily protect biodiversity. We thus emphasize that ecosystem service provisioning cannot replace high biodiversity as the aim of management interventions. The need for specific management to protect biodiversity is further underlined by the result that only a minor fraction of the species pool showed a significant functional importance of the individual species.

## Methods

**Data basis/Jena Experiment.** At a 10 ha former arable field near Jena (Germany), we established 82 plots in 2002, each 20 m × 20 m, with a controlled number of plant species, number of functional groups and plant functional group identity, in a randomized block design<sup>36</sup>. A single plot—the monoculture of *Bellis perennis*—was later given up due to the low establishment of the target species and therefore removed from this analysis, resulting in 81 plots. The plots were assigned to four blocks running parallel to the river Saale, accounting for differences in soil texture with increasing distance from the river. The plots were sown in May 2002 with 1, 2, 4, 8, 16 and 60 grassland plant species, and with 16, 16, 16, 16, 14 and 4 replicates, respectively. Plot compositions were randomly chosen from 60 plant species typical of local *Arrhenatherum* grasslands. Plots were maintained by mowing, weeding and herbicide applications. Between 2002 and 2007, data on a multitude of ecosystem functions were measured in the Jena Experiment, including measurements conducted above and below ground, measurements of pools and fluxes and measurements spanning different organizational levels: habitat, primary producers and higher trophic levels. We based this analysis on a total of 82 ecosystem variables that are indicative of different aspects of ecosystem functioning (Supplementary Table 1.1). For each ecosystem variable, the measurements in the last year available were selected and averaged within this year if the variable had been measured in multiple seasons or different soil depths, to ensure that every variable was included only once in the analysis. We selected different years for different functions because it was not possible to measure all functions in the same year. As the best possible dataset the last year in which a function was measured was used in the analysis to avoid potential transient effects in early years<sup>39</sup>. To test for the potential effects of including different years (temporal context<sup>40</sup>), we conducted a sensitivity analysis that was based on only 52 functions that had all been measured in the year 2004, which was the year with the most measured ecosystem functions available (Supplementary Table 1.1). Variables were excluded if measurements were missing in more than 10% of the plots. Missing values for the remaining variables were imputed with the mean of the variable over all plots. Variables were standardized to a common scale by scaling to zero mean and unit variance (z-transformation) using the R function `decostand` from the `vegan` package<sup>40</sup>. All statistics were calculated using R version 3.1.0 (ref. <sup>41</sup>).

**Multivariate multifunctionality approach.** To calculate a multifunctionality index, we extended the averaging approach that sums up individual ecosystem functions<sup>19,32</sup> to a multivariate measure. We did so by calculating a PCA for the multifunctional space spanned by the ecosystem variables indicating functions and summing the PCA axis scores for each experimental plot. In this PCA-based approach, correlated functions do not contribute multiple times to the index of multifunctionality as they would in the averaging approach because all PCA axes are, by definition, orthogonal to each other. Ecosystem functions were not weighted. Therefore, we implicitly assume that all ecosystem functions are equally important. The PCA was calculated using the R function `rda` from the `vegan` package based on the standardized function data, which is equivalent to using the correlation matrix for calculating the PCA. A PCA does not define the orientation of axes. Thus, axis scores can be multiplied by  $-1$  without altering the results of the PCA. However, for the calculation of the index of multifunctionality, the orientation of the PCA axis is critical. As the orientation of the axis is not defined mathematically, we defined the orientation based on the biological meaning of the axis. To do so, we identified for each PCA axis the ecosystem function with the highest loading (absolute value) and checked whether this loading was positive or negative. Next, we checked for each of these functions if high or low values contribute to overall functioning (Supplementary Table 1.1). In cases where there was a mismatch (that is, functions with high values contributing to functioning that had negative loadings or functions with low values contributing to functioning that had positive loadings), the orientation of the axis was inverted by multiplying all scores for this axis by  $-1$ . By doing so, all PCA axes were oriented in a biologically meaningful direction. Next, the multifunctionality index was calculated by summing for each experimental plot the scores on all

biologically oriented PCA axes weighted by the eigenvalue of each axis. The eigenvalues quantify the proportion of variance in multifunctional space explained by the respective PCA axis. The resulting multifunctionality index was regressed against sown species richness using the R function `lm` with `block` as an additional explanatory variable fitted first.

**Alternative approaches to multifunctionality.** To check whether the results were sensitive to the index of multifunctionality used, all different approaches proposed in a recent review<sup>18</sup> were calculated using the respective R functions provided in the `multifunc` package<sup>42</sup>. For the averaging approach, all ecosystem functions were averaged per experimental plot after functions for which lower values indicate higher functioning (Supplementary Table 1.1) had been inverted<sup>19</sup>. In the threshold approach, for each plot, the number of functions that exceeded 75% of the maximum level of functioning was calculated. Following the recommendation by Byrnes, et al.<sup>18</sup>, the maximum level of functioning was calculated as the average of the five plots with the highest values measured for the respective function. In the multiple-thresholds approach, the same calculations were performed with all threshold values from 1 to 99%<sup>18</sup>.

**Including different numbers and identities of ecosystem functions.** We conducted a simulation study to test for the effects of including different numbers and identities of ecosystem functions on the resulting relationship between multifunctionality and biodiversity. To do so, we created random subsets (without replacement) of the 82 ecosystem variables indicating ecosystem functioning in our analysis. We did so for each number of ecosystem functions from 2 to 82, creating every time 500 random combinations of different ecosystem functions. For each of these subsets of ecosystem functions, we repeated all calculations of the multivariate multifunctionality approach, as described above, and regressed the resulting index of multifunctionality against plant species richness, as described for the main analysis. From these models, we extracted the slopes of the biodiversity effects which were plotted against the number of ecosystem functions included in the random subset.

**Proportion of species pool contributing to ecosystem functioning.** The number of species that significantly contribute to an increasing number of functions was calculated with the turnover approach<sup>17</sup> using the respective R functions provided in the `multifunc` package<sup>42</sup>. A linear model for each ecosystem function using the presence or absence of all 60 plant species of the species pool as explanatory variables was estimated and reduced to informative predictors via a stepAIC approach. From these models, the species with significant positive or negative contributions to the observed level of functioning were extracted. In the last step, the number of species contributing to the combination of an increasing number of ecosystem functions was calculated for 2,000 random combinations for each number of ecosystem functions, separately for positive and negative effects. Because we fitted a large number of models and a large number of parameters within models by calculating the turnover approach for a species pool of 60 (that is, 60 parameters per model) and 82 ecosystem variables (that is, 82 models), we checked for a potential inflation of the number of detected species identity effects in a sensitivity analysis where the presence of species in plots had been permuted over the plots to disrupt the association between the presence of species and ecosystem functioning (see Supplementary Materials, section 8). This analysis confirmed too high a number of identified species identity effects. These results were confirmed using simulated data (see Supplementary Materials, section 8). To mitigate the apparent inflation of detected species identity effects, we repeated the turnover approach using stricter critical  $\Delta$ AIC values than the difference of two AIC points that is commonly used<sup>17,18</sup>. This difference of two AIC points is equivalent to a critical  $P$  value of 0.16 in model selection based on the significance of tested effects<sup>43</sup>. We adapted the functions provided by the `multifunc` package<sup>42</sup> to use a different critical  $\Delta$ AIC value to identify informative species effects. The R code for these modified functions is provided as a separate file in the Supplementary Materials. We performed a series of calculations of the turnover approach with critical  $\Delta$ AIC values of 2, 3.8, 6.6, 7.9, 18.8 and 15.1, which are equivalent to  $P$  values of 0.16, 0.05, 0.01, 0.005, 0.001 and 0.0001, respectively. For all runs of the turnover approach, the results of the measured data are provided, together with the results obtained after permutating the data for comparison.

**Life Sciences Reporting Summary.** Further information on experimental design is available in the Life Sciences Reporting Summary.

**Code availability.** R code to calculate the multivariate multifunctionality index and R code to update the turnover approach to multifunctionality to included more stringent criteria for considering an effect of an individual species informative is provided in the Supplementary Information files.

**Data availability.** All data on the measured ecosystem variables indicating ecosystem functions that support the findings of this study are included within this paper and its Supplementary Information files.

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## Author contributions

S.T.M., R.P., H.H. and W.W.W. conceived the study and developed the analytical procedure. S.T.M. and R.P. performed the analyses with contributions from W.W. All authors contributed measured data. S.T.M. and W.W.W. wrote the paper. All authors contributed to writing and editing the paper.

## Competing interests

The authors declare no competing financial interests.

## Additional information

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### ▶ Experimental design

#### 1. Sample size

Describe how sample size was determined.

All individual ecosystem variables measured in the Jena Experiment between 2002 and 2007 were used in this analysis. This selection resulted in 82 ecosystem variables which have been measured on 81 plots in the Main Experiment resulting in 6642 individual data points in the main analysis.

#### 2. Data exclusions

Describe any data exclusions.

A single plot – the monoculture of *Bellis perennis* – showed very low establishment of the target species and therefore was removed from this analysis. For this reason, there are 81 rather than the originally sown 82 plots.

#### 3. Replication

Describe whether the experimental findings were reliably reproduced.

Many of the relationships between individual ecosystem variables and plant species richness have been documented repeatedly over multiple years in the Jena Experiment and have been published in various peer-reviewed journals. Positive effects of plant species richness on ecosystem functions have been documented in a wide range of biodiversity experiments.

#### 4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Plant communities and diversity levels were allocated randomly to plots in the Jena Experiment in a blocked design.

#### 5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Researchers collecting data in the field were not blinded to sown diversity level on the plot but were unaware of the results for most other ecosystem variables.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.



## 6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a Confirmed

- The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- A statement indicating how many times each experiment was replicated
- The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
- A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- The test results (e.g.  $P$  values) given as exact values whenever possible and with confidence intervals noted
- A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- Clearly defined error bars

See the web collection on [statistics for biologists](#) for further resources and guidance.

## ► Software

Policy information about [availability of computer code](#)

## 7. Software

Describe the software used to analyze the data in this study.

All statistics were calculated in R with the functions and packages as indicated in the methods section. R-code to calculate the multivariate approach to multifunctionality is provided in the supporting materials as is R code to update the turnover approach to multifunctionality to included more stringent criteria for considering an effect of an individual species informative.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* [guidance for providing algorithms and software for publication](#) provides further information on this topic.

## ► Materials and reagents

Policy information about [availability of materials](#)

## 8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

No restrictions.

## 9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Not applicable

## 10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

Not applicable

b. Describe the method of cell line authentication used.

Not applicable

c. Report whether the cell lines were tested for mycoplasma contamination.

Not applicable

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by [ICLAC](#), provide a scientific rationale for their use.

Not applicable



## ► Animals and human research participants

Policy information about [studies involving animals](#); when reporting animal research, follow the [ARRIVE guidelines](#)

### 11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Not applicable

Policy information about [studies involving human research participants](#)

### 12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Not applicable

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