







Removing subordinate species in a biodiversity experiment to mimic observational field studies

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Abstract

Background: Positive effects of plant species richness on community biomass in biodiversity experiments are often stronger than those from observational field studies. This may be because experiments are initiated with randomly assembled species compositions whereas field communities have experienced filtering.

Methods: We compared aboveground biomass production of randomly assembled communities of 2–16 species (controls) with experimentally filtered communities from which subordinate species were removed, resulting in removal communities of 1–8 species.

Results: Removal communities had (1) 12.6% higher biomass than control communities from which they were derived, that is, with double species richness and (2) 32.0% higher biomass than control communities of equal richness. These differences were maintained along the richness gradient. The increased productivity of removal communities was paralleled by increased species evenness and complementarity.

Conclusions: Result (1) indicates that subordinate species can reduce community biomass production, suggesting a possible explanation for why the most diverse field communities sometimes do not have the highest productivity. Result (2) suggests that if a community of *S* species has been derived by filtering from a pool of *2S* randomly chosen species it is more productive than a community derived from a pool of *S* randomly chosen species without filtering.

KEYWORDS

environmental filtering, Jena Experiment, plant community, species pool, species richness

INTRODUCTION

Biodiversity–ecosystem functioning (BEF) experiments generally reveal positive relationships between plant species richness and community biomass production (Balvanera et al., 2006; Tilman et al., 2014). Nevertheless,

it is common to find highly productive plant communities in the field that consist of few species, seemingly contradicting the experimental findings (Fraser et al., 2015; Loreau, 2000; Schmid, 2002). This discrepancy between experiments and field observations is not unexpected, as Hagan et al. (2021) point out because

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initial species compositions of experimental communities represent a random sample that has not been “filtered” out by local environmental conditions and species interactions (Cadotte & Tucker, 2017; Grime, 1998; Petermann et al., 2010; Pfisterer et al., 2004). In contrast, field communities have already passed these filters and therefore can be expected to be more productive than experimental communities of similar species richness. This is because environmental filtering will most likely exclude species with low performance that contribute little to community biomass, leaving a nonrandom set of species with higher performance.

Applying this logic, BEF experiments may be considered as experiments that manipulate species pools, from which the local environment then filters out the local community. This can be seen by the uneven species biomass distributions that develop in experiments from initially even sowing or planting proportions, often within a single growing season (Mulder et al., 2004; Schmitz et al., 2013). The analysis of a BEF relationship generally uses the initial species richness, that is, that of the manipulated species pool, as the explanatory variable, even though some species disappear completely or occur so infrequently in a plot that they are not present in subplots harvested for biomass determination (Hagan et al., 2021; Schmid et al., 2002; Vogel et al., 2019). This is appropriate, because species may have legacy effects, reappear or influence biomass in a neighboring harvested subplot from outside. For example, this has been found in a tree biodiversity experiment, where neighborhood and plot-scale species richness both affected the growth of focal trees (Fichtner et al., 2018). However, qualitative results are usually robust to substituting sown with realized species richness (Jochum et al., 2020).

Nevertheless, to bridge the gap between BEF experiments and observational field studies we used randomly assembled experimental grassland communities with initially even species distributions ranging in richness from 2 to 4, 8, and 16 species and allowed them to develop uneven rank abundance distributions over two growing seasons. We then completed this natural filtering process by reducing the biomass abundance of some species at the expense of others, which presumably was due to abiotic conditions and competitive interactions between species, by removing the subordinate half of the species to obtain so-called removal communities and compared their aboveground community biomass in the next growing season with (1) the original control communities of twice the species richness and with (2) other control communities of the same species richness (but differing species compositions). For (1), we hypothesized that if subordinate species do not contribute to ecosystem functioning (Grime, 2002), then removal and control communities should subsequently have the same productivity. For (2), we hypothesized that the “filtered” removal communities should be more productive than “unfiltered” control communities of the same richness, for the reasons given at the end of the first paragraph. Our removal experiment thus corresponds to an observational field study where the productivity of local communities could be compared

with the productivity of species pools of double (1) or equal (2) richness.

MATERIALS AND METHODS

Experimental design

Our study was part of the so-called Jena Experiment in Germany, a grassland biodiversity experiment in which communities of 1, 2, 4, 8, 16, or 60 species were assembled from a pool of 60 species (Roscher et al., 2004; Weisser et al., 2017). The species were classified into four functional groups, namely grasses, legumes, tall herbs, and short herbs (Table 1) and for each species richness level different functional groups or functional group compositions were randomly chosen. In May 2002, the experimental communities were planted on 20 m × 20 m main plots—still existing now in 2022 but with reduced size—and in 3.5 m × 3.5 m replicate plots containing factorial split-plot treatments where density and evenness were manipulated (Schmitz et al., 2013). We used the high-density, even subplots, each 1.75 m × 1.75 m, of these replicate plots for the present experiment. High density refers to twice the sowing density of the main plots, that is, 2000 versus 1000 sown plants per m², and even refers to all species initially having equal numbers of individuals per mixture. Additional plots of 3.5 m × 3.5 m were established with two monoculture replicates for each of the 60 species (Roscher et al., 2004). These were used to calculate biodiversity effects using additive partitioning (Loreau & Hector, 2001, see below). Although these monocultures were sown at 1000 plants per m² and thus overall effects of the partitioned biodiversity effects might thus have been slightly overestimated, this should not have affected the differences of partitioned biodiversity effects between treatments. Furthermore, in a previous study, we found that plots sown with 2000 versus 1000 plants per m² had identical biomass after the first growing season (Schmitz et al., 2013). All these plots of the Jena Experiment were distributed over four blocks to account for spatial variation across the field site. Here, we only used the mentioned subplots with 2, 4, 8, or 16 species plus the monoculture plots. For the mixtures, we thus had 59 plots representing a total species pool of 56 species (Table 1), namely fourteen 2-species, sixteen 4- and 8-species, and thirteen 16-species plots. For each of the 56 species, we additionally had two monoculture plots.

In fall 2003, the mixture subplots were diagonally divided into two triangles, each with an area of 1.53 m². At this time, that is, two growing seasons after sowing, experimental communities had developed uneven species biomass distributions due to differential growth and survival among the different species (Schmitz et al., 2013), a feature that is typically observed in grassland biodiversity experiments (see e.g., Hector et al., 2002; Mulder et al., 2004). One of the two triangles in each mixture subplot was randomly chosen for a removal treatment in which the 50% species from the tail of the biomass abundance–species rank curve obtained in the

TABLE 1 Ranking of plant species according to their removal probability p in the treatment triangles

Never removed		Rarely removed		Often removed		Always removed	
Species	p	Species	p	Species	p	Species	p
<i>Arrhenatherum elatius</i>	0	<i>Plantago lanceolata</i>	0.08	<i>Festuca pratensis</i>	0.56	<i>Ajuga reptans</i>	1
<i>Dactylis glomerata</i>	0	<i>Phleum pratense</i>	0.10	<i>Ranunculus repens</i>	0.56	<i>Priumula veris</i>	1
<i>Poa trivialis</i>	0	<i>Alopecurus pratensis</i>	0.11	<i>Ranunculus acris</i>	0.57	<i>Cardamine pratensis</i>	1
<i>Tarraxacum officinale</i>	0	<i>Leontodon autumnalis</i>	0.17	<i>Avenula pubescens</i>	0.60	<i>Carum carvi</i>	1
<i>Achillea millefolium</i>	0	<i>Trifolium repens</i>	0.17	<i>Leontodon hispidus</i>	0.67	<i>Heracleum sphondylium</i>	1
<i>Centaurea jacea</i>	0	<i>Fesuca rubra</i>	0.20	<i>Plantago media</i>	0.67	<i>Sanguisorba officinalis</i>	1
<i>Crepis biennis</i>	0	<i>Daucus carota</i>	0.20	<i>Pimpinella major</i>	0.67	<i>Lathyrus pratensis</i>	1
<i>Knautia arvensis</i>	0	<i>Galium mollugo</i>	0.20	<i>Prunella vulgaris</i>	0.71	<i>Trifolium campestre</i>	1
<i>Leucantheum vulgare</i>	0	<i>Bromus hordeaceus</i>	0.22	<i>Bromus erectus</i>	0.75	<i>Trifolium dubium</i>	1
<i>Rumex acetosa</i>	0	<i>Tragopogon pratensis</i>	0.25	<i>Geranium pratense</i>	0.78		
<i>Medicago varia</i>	0	<i>Trisetum flavescens</i>	0.37	<i>Trifolium fragiferum</i>	0.86		
<i>Onobrychis vicifolia</i>	0	<i>Holcus lanatus</i>	0.40	<i>Veronica chamaedris</i>	0.87		
<i>Trifolium hybridum</i>	0	<i>Glechoma hederacea</i>	0.40	<i>Campanula patula</i>	0.89		
<i>Trifolium pratense</i>	0	<i>Lotus corniculatus</i>	0.40	<i>Anthoxanthum odoratum</i>	0.90		
		<i>Poa pratensis</i>	0.43	<i>Medicago lupulina</i>	0.90		
		<i>Bellis perennis</i>	0.50	<i>Anthriscus sylvestris</i>	0.91		
		<i>Vicea craca</i>	0.50				

Note: p corresponds to the fraction of plots in which a particular species occurred and in which it was removed because it belonged to the 50% of species with the lowest biomass in the plot. For nomenclature see Jäger & Werner (2002).

previous biomass harvest were removed by pulling out individuals with roots (removal community or removal triangle). In the control triangle, a similar amount of aboveground biomass was randomly clipped without reducing species richness to avoid confounding species loss with loss of biomass (Díaz et al., 2003). To mimic the soil disturbance caused by pulling up roots in the removal triangles, a visually similar number of cuts into the soil were made with a hoe in the control triangle. The treatments were repeated at the beginning of the growing season in 2004. We note that the procedure applied to control triangles may not have fully mimicked the procedure applied to removal triangles because roots of plants clipped aboveground have remained in the control triangles and by decomposing might have caused a fertilizing effect. As consequence, the positive effects of the removal treatment on biomass production, which we will present in the Results section, may have been slightly underestimated. Between the end of May and the beginning of June 2004, the triangles were harvested. In a 20 m × 50 cm frame, plants were cut at 3 cm from the ground and the harvested material separated to species. The harvested biomass was then dried for 48 h at 70°C and weighed. The same harvest procedure was applied in the monocultures, but these were continued to be monitored as references for other experiments (see e.g., Marquard et al., 2013).

For the interpretation of results, control triangles were considered as communities whose species composition was based on random species loss according to the initial design of the Jena Experiment (Roscher et al.,

2004). These communities represented the species pool for the removal triangles. For these, the removal of the subordinates represented a nonrandom species loss obtained by a filtering process that first excluded the rarest species from a local community. In our experiment, these excluded species were not allowed to come back from the pool in removal triangles, even if they might have increased their abundance again in the control triangles.

Statistical analysis

We first visualized the average biomass abundance–species rank distributions for the different preremoval and post-removal species richness levels at the end of the experiment to indicate the results of the removal procedure (Figure 1). We then analyzed the effects of species richness (log₂-transformed), removal treatment and their interaction on the dependent variables community aboveground biomass, evenness of biomass distribution between species within communities, and biodiversity effects (net effect NE, complementarity effect CE, and selection effect SE calculated using the additive partitioning method or Loreau & Hector, 2001) using general linear models. The error model included block, subplot (i.e., given as plot in the analysis of variance tables), and triangle (i.e., residual). We analyzed the effects of species richness both before and after removal (preremoval and postremoval species richness), but present mostly results with the latter. We did not include functional group richness as an explanatory variable in our analyses

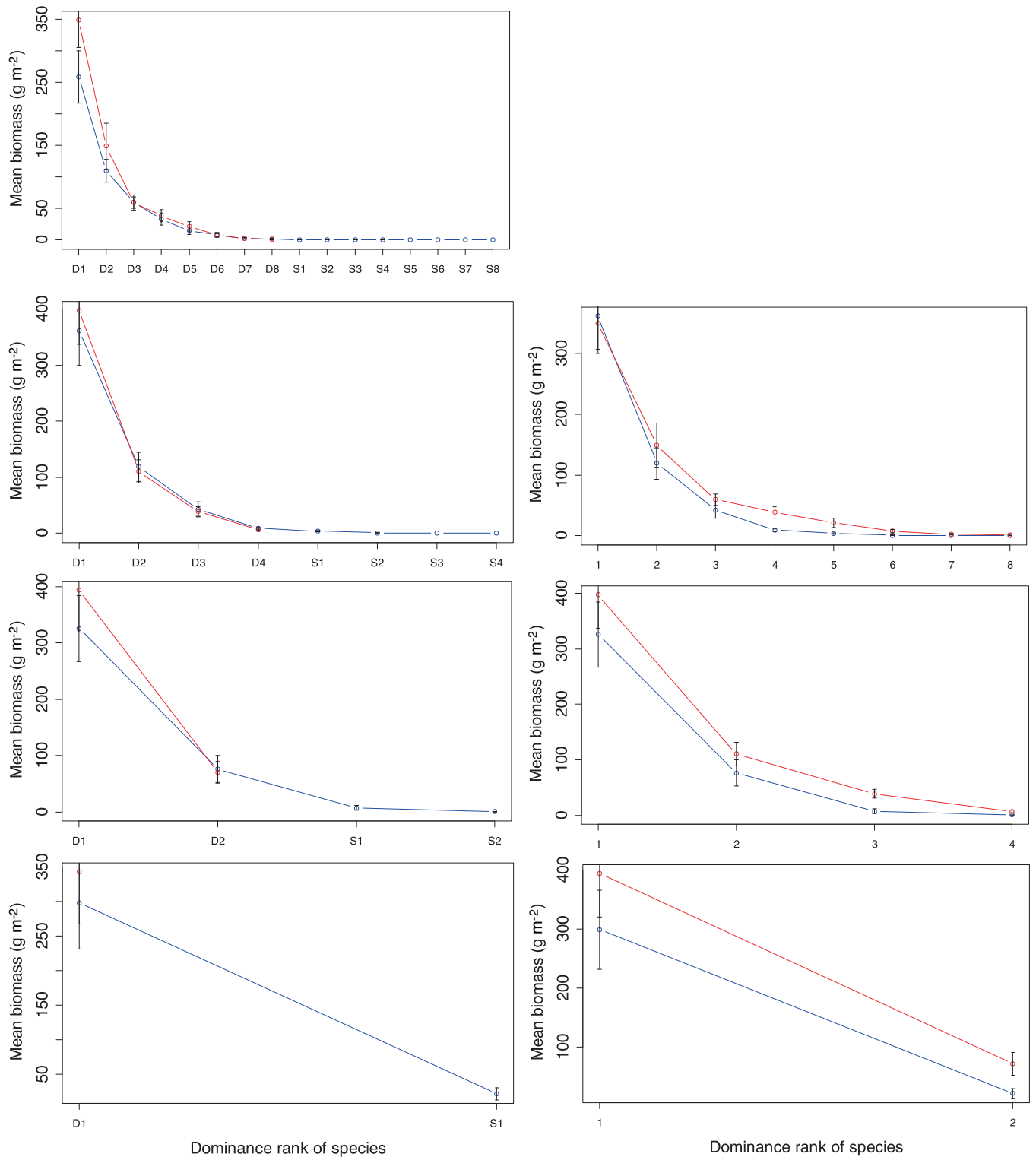


FIGURE 1 Average aboveground biomass of species as a function of dominance rank for communities of different species-richness levels: left column grouped according to preremoval richness, that is, comparing removal with control communities from which they were derived (from top-down 16, 8, 4, and 2 species), right column grouped according to postremoval richness, that is, comparing removal with control communities of the same richness (from top-down 8, 4, and 2 species). Blue circles and lines are for control communities, red circles and lines are for removal communities, and vertical black lines indicate ± 1 standard error of means. D stands for dominant species that had not been removed and S for subordinate species that had been removed in removal communities. Note that “dominant” is used in a relative sense for species ranking in the first half of the “dominance” hierarchy; obviously, there is still a large variation among dominants with regard to biomass.

because for the removal treatment functional group identity of species was not considered and thus postremoval functional group richness could not be considered as a design variable. Species richness effects were tested at the plot level as prescribed by the hierarchical error structure

(Schmid et al., 2002). Note that this analysis is equivalent to mixed-model analysis using restricted maximum likelihood (Schmid et al., 2017).

Evenness was calculated from Simpson's dominance index D as $(1/D)/S$ (Mulder et al., 2004), where S was the

TABLE 2 Analyses of variance for aboveground community biomass production, after the removal treatment, using (a) preremoval and (b) postremoval species richness as explanatory variables; (c) analysis of variance for evenness of aboveground biomass distribution among species, after the removal treatment, using postremoval species richness as an explanatory variable

Item	Df	MS	F value	p value
<i>(a) Biomass</i>				
Block	3	228 456	1.66	0.186
Preremoval species richness (log2)	1	1 006 509	7.32	0.009
Treatment	1	90 069	4.16	0.046
Species richness × treatment	1	28 440	1.31	0.257
Plot	54	137 590		
Residual	57	21 650		
<i>(b) Biomass</i>				
Block	3	228 456	1.66	0.186
Postremoval species richness (log2)	1	613 535	4.46	0.039
Treatment	1	483 043	22.31	<0.001
Species richness × treatment	1	28 440	1.31	0.257
Plot	54	137 590		
Residual	57	21 650		
<i>(c) Evenness</i>				
Block	3	0.0047	0.23	0.872
Postremoval species richness (log2)	1	2.7956	139.95	<0.001
Treatment	1	0.1257	14.88	<0.001
Species richness × treatment	1	0.0421	4.99	0.031
Plot	54	0.0200		
Residual	43	0.0084		

Note: Treatment refers to species removal versus control. Block and species richness were tested against between-plot variation (plot), while treatment and the interaction were tested against within-plot variation (residual). Significant *p* values ($p < 0.05$) in boldface.

Abbreviations: Df, degrees of freedom; MS, mean squares.

postremoval species richness. To calculate biodiversity effects we used the mean of the monoculture biomasses measured in monoculture plots in May/June 2003, 2004, and 2005 to obtain more stable reference values. Nevertheless, for two triangles (one 2- and one 8-species mixture) containing *Trifolium dubium*, which had the third-lowest monoculture biomass (0.21 g m^{-2}) but 500 times higher mixture biomass ($>100 \text{ g m}^{-2}$), we had to discard the partitioned biodiversity effects CE and SE because they reached extreme values more than 35 times larger or smaller, respectively, than the third most extreme value. The remaining partitioned biodiversity effects CE and SE were square-root transformed with sign reconstruction (e.g., $\sqrt{\text{abs(CE)}} \times \text{sign(CE)}$) before analysis (Loreau & Hector, 2001).

In addition to the above measurements, we also analyzed by logistic regression the probability of each species to be removed as less abundant from an

experimental community. This was used as an estimate of extinction probability related to the probability that a species would be removed by natural filtering in a real-world plant community. The data values were zero for each species in a removal triangle that was not removed and one for each species that was removed. All calculations and analyses were done with the statistical program GenStat 21 (VSN International, 2020).

RESULTS

In spring 2004, after removing the subordinate species, the community biomass varied between 37.1 and 1319.7 g m^{-2} in the control triangles and between 84.6 and 1326.3 g m^{-2} in the removal triangles. Removal probabilities did not differ between functional groups (ratio of mean deviance changes functional groups/species within functional groups: $F_{3,52} = 0.62$, $p = 0.608$) but did between species within functional groups (ratio of mean deviance change species within functional groups/mean residual deviance: $F_{52,351} = 8.35$, $p < 0.001$). The ranked observed removal probabilities are presented in Table 1. The sum of biomasses of only those species that occurred in both control and removal treatments of the same plot, that is, of the dominant species, was $416.7 \pm 36.8 \text{ g m}^{-2}$ in control communities and $495.9 \pm 40.6 \text{ g m}^{-2}$ in removal communities. That is, the removal of subordinates allowed the dominants to gain 19.0% in biomass. This is also reflected in the biomass abundance–species rank distributions observed at the end of the experiment (Figure 1).

Community aboveground biomass in spring 2004 increased linearly and in parallel for control and removal communities with both preremoval and postremoval species richness (Table 2a,b and Figure 2a). We then compared (1) removal communities of richness S with controls of richness 2S from which they were derived and (2) removal communities of richness S with controls that had the same richness S. This latter case (2) represents the comparison between communities in which S species were selected randomly from the overall experimental pool of 60 species and communities in which the same number of S species were selected as the dominants from a plot-scale experimental pool of 2S species. Removal of subordinates significantly increased community biomass in both cases, namely by 12.6% in (1) and by 32.0% in (2). Comparing the 19% increase of the dominants themselves after removal (see the previous paragraph), the subordinates in case (1) could therefore only make up for $19\% - 12.6\% = 6.4\%$, that is, about a third of the biomass that the dominants could gain without the subordinates.

As is typical in such experiments (e.g., Mulder et al., 2004), evenness declined with increasing species richness. However, for given postremoval richness levels, evenness was significantly higher in removal than in control triangles, in particular at low species richness (Table 2c, Figure 2b, see also Figure 1). This suggests that the higher productivity of removal-treatment communities as compared with control communities was partly caused by the increased evenness of the latter

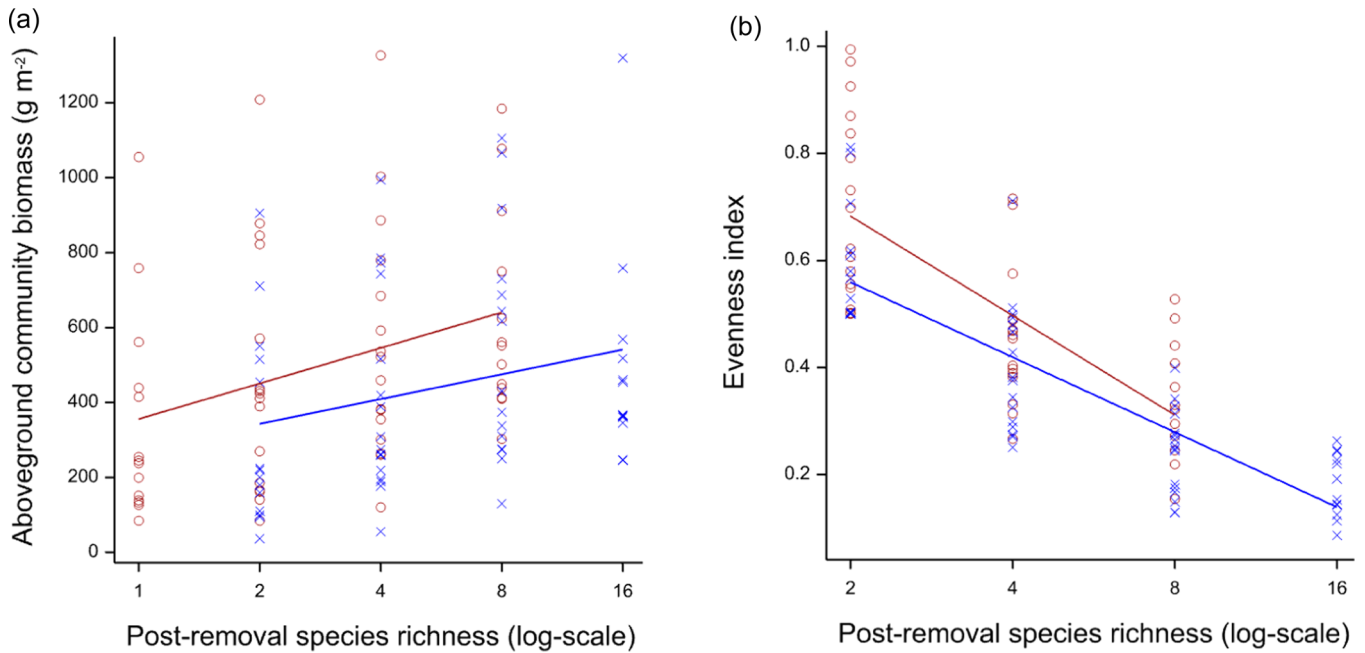


FIGURE 2 Aboveground biomass (a) and evenness (b) of control and removal communities as a function of postremoval species richness. Blue crosses and blue regression lines are for control communities, red circles and red regression lines are for removal communities. For significances see Table 1. For evenness, monocultures are excluded because of undefined values.

due to the removal of the subordinates (Figure 1). Removal communities also had increased complementarity effects (CEs) and marginally increased net biodiversity effects (NEs), whereas selection effects were nonsignificantly decreased (Table 3 and Figure 3). Furthermore, as also typically found in BEF experiments, NE and CE significantly increased with postremoval richness, but the interaction with removal treatment was not significant. However, evenness and CE were not correlated ($r = -0.151$, $p > 0.1$).

DISCUSSION

To bridge the gap between BEF experiments and observational field studies, we used a species removal treatment to complete the filtering started in randomly assembled experimental communities of 2, 4, 8, or 16 species as some species gained dominance and others became rare in terms of biomass. By removing the rare half of species, we simulated their extinctions as might happen by environmental filtering in observational field studies. We thus converted the already reduced “realized” species richness to a new—designed—postremoval richness of 1, 2, 4, or 8 species in removal communities, maintaining control communities at 2, 4, 8, or 16 species. Our results showed that simulated extinctions of subordinates increased community biomass relative to origin communities with twice the species number (1) and even more so relative to other communities where the same richness resulted from simulated random extinction (2).

Although, according to our first hypothesis in the Introduction, we had expected that removal would not change community biomass in case (1), the observed increase of 12.6% was significant and due to an increase

of 19% in the biomass of the dominant species remaining in the removal communities compared with their biomass in control communities. That removal communities had higher biomass than control communities with twice the richness from which they were derived suggested that subordinates in control communities were reducing the biomass of the dominants to a greater extent than the biomass contributed by these subordinates themselves. These subordinates may have had traits such as belowground biomass stores that allowed them to persist, but not to produce aboveground biomass at the same rate as the dominants. This demonstrates that completing the filtering by experimental removal of subordinates was necessary to assess the full effect of filtering. It is conceivable that in real-world ecosystems rare species may escape filtering because they occupy special microhabitats in a more heterogeneous environment, can survive as sink species, or are becoming extinct at a very slow rate (Hubbell, 2001; Petermann et al., 2010). If these species reduce the biomass of other species by a greater amount than the amount of biomass that they contribute themselves, a similar effect as observed in our experiment may occur. This offers a potential explanation for why in observational studies the most diverse field communities with a large number of rare species are often not the most productive ones (Fraser et al., 2015).

According to our second hypothesis (2) in the Introduction, a “filtered” removal community should have higher biomass than a control community with the same richness but an “un-filtered,” random species composition. This was clearly so, and the corresponding difference was as large as 32%. In other words, if a local community of species richness S is derived from a pool of $2S$ randomly chosen species by environmental filtering that only allows the more productive half of the species to

TABLE 3 Analyses of variance for (a) net, (b) complementarity, and (c) selection effects, after the removal treatment, using postremoval species richness as an explanatory variable

Item	Df	MS	F value	p value
<i>(a) Net effect</i>				
Block	3	203 906	2.03	0.1211
Postremoval species richness (log 2)	1	594 205	5.90	0.0185
Treatment	1	91 570	3.44	0.0707
Species richness × treatment	1	36 572	1.37	0.2479
Plot	54	100 649		
Residual	43	26 652		
<i>(b) Complementarity effect</i>				
Block	3	428	2.37	0.0811
Postremoval species richness (log 2)	1	1 149	6.36	0.0147
Treatment	1	397	4.37	0.0426
Species richness × treatment	1	139	1.53	0.2227
Plot	53	181		
Residual	42	91		
<i>(c) Selection effect</i>				
Block	3	30.1	0.19	0.9037
Postremoval species richness (log 2)	1	182.7	1.14	0.2896
Treatment	1	68.6	0.95	0.3342
Species richness × treatment	1	264.7	3.68	0.0618
Plot	53	159.7		
Residual	42	71.9		

Note: Block and species richness were tested against between-plot variation (plot), while treatment and the interaction were tested against within-plot variation (residual). Significant *p* values ($p < 0.05$) in boldface.

Abbreviations: Df, degrees of freedom; MS, mean squares.

persist, then this community is much more productive than a local community of the same species richness *S* but derived from a pool of *S* randomly chosen species, that is, without additional filtering. As suggested by Hagan et al. (2021), this may be the main reason why experimental results can differ from the results of observational field studies. BEF experiments thus hint at the importance of species pool sizes in real-world situations but may not well represent real-world local communities shaped by nonrandom filtering processes. In our experiment, filtering out rare, that is, low biomass-abundance species increased evenness and complementarity effects, possibly due to increased niche complementarity between species (Turnbull et al., 2016). Similar to the present study, Fargione et al. (2003) and Roscher et al. (2005) found a greater degree of niche complementarity among dominant species compared with subordinate ones. In real-world contexts, filtering by the local environment and species interactions, here mimicked by the removal treatment, may lead to increased community biomass via the same mechanisms.

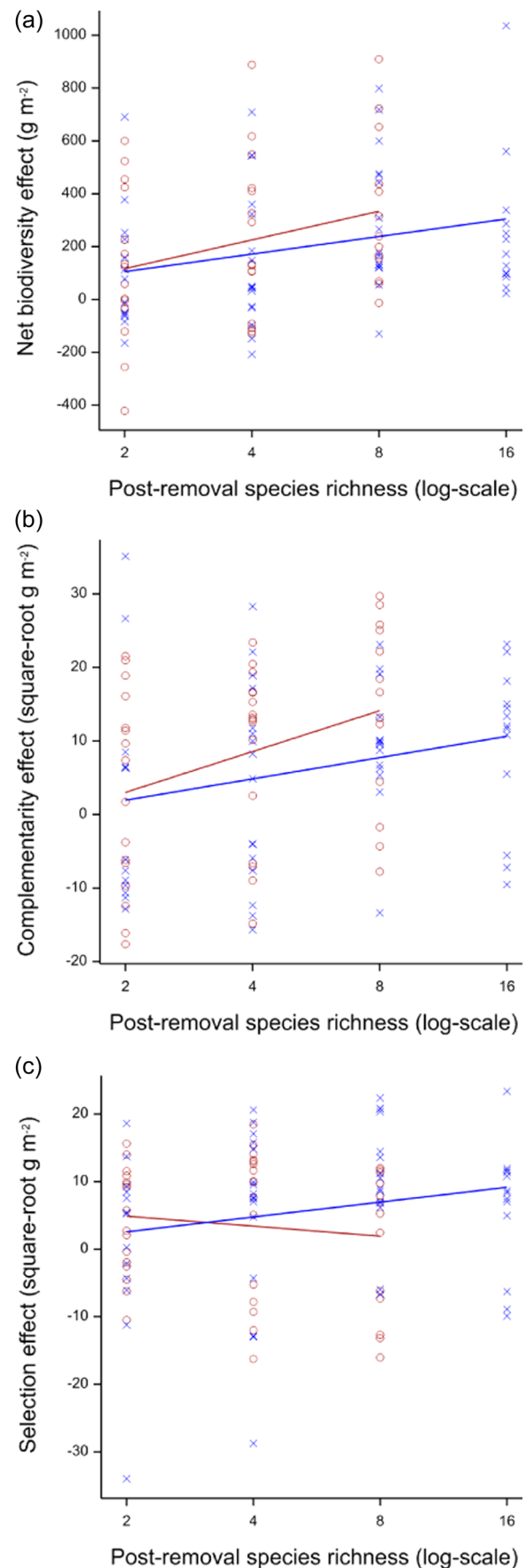


FIGURE 3 Net biodiversity effect (NE, a), complementarity effect (CE, b), and selection effect (SE, c) for control and removal communities as a function of postremoval species richness. Blue crosses and blue regression lines are for control communities, and red circles and regression lines are for removal communities. For significances see Table 2. Monocultures are excluded because of undefined values.

Our experiment is only a first step toward bridging the gap in observational field studies. First of all, the distinction between testing effects of species pool richness in experiments versus testing effects of local community richness in field studies is of course an extreme one. Experiments can also be designed to test how future species loss from an already filtered local field community would affect biomass production (Schmid & Hector, 2004). However, such experiments will not be able to explain existing patterns in observational field studies, because—by definition—future species loss has not yet occurred in the present. Second, we only applied one very specific type of filtering to obtain removal communities, namely selecting the dominant half of species from control species pools based on the biomass production of the individual species in the corresponding species mixture. From the species-pool side, experiments could be designed to select from a pool of given species number different numbers of species for local communities; and from the local-community side, experiments could be designed by filtering communities to given richness from pools with different numbers of species. For example, here we found, by analogy, that an S-species local community produced 32% more biomass when obtained from a 2S- instead of an S-species pool; it is easy to predict that even higher biomass could be obtained if an S-species local community would be derived from a 3S-species pool. Third, our results only explain a generally increased productivity of filtered communities but not a potentially reduced slope of the species richness–community biomass relationship in observational field studies due to filtering (cf. Figure 2a). It could have been expected that the effect of filtering from 2S to S species might decrease with increasing S, yet this was not the case in our experiment for the range of $S = 1$ to $S = 8$ and the relatively short duration. It is conceivable that with a greater range of species richness values or in the longer term slope differences would be more likely detected. Fourth, here we focused on aboveground community biomass as ecosystem-level and aboveground species biomass as population-level “performance” measures and their relations to species richness. However, there are many other aspects of performance that may influence ecosystem functioning and stability and whether a species can maintain non-negative population growth rates in real-world field contexts.

One possible explanation for the positive rather than neutral effect of species removal could be that removal experiments are fundamentally different from experiments that assemble synthetic communities (Symstad & Tilman, 2001). Although we removed similar amounts of biomass from control and removal communities and simulated a similar degree of soil disturbance, these two treatments might still have differed in undetected ways, such as the mentioned potential fertilizing effects of decaying roots in control communities, that might have influenced results. Therefore, for future experiments, we recommend a synthetic approach, where control and removal communities would be established by sowing or planting with the given species. Furthermore, our results are relevant for the short term over which the experiment was carried out. Despite the negative impact of the

subordinate species on community productivity in the short term, it is conceivable that the removed species might have become more important again with regard to ecosystem functioning in the longer term under varying environmental conditions or if the remaining dominant species would have decreased in performance due to intrinsic population dynamics or external influences (Isbell et al., 2011; Wardle et al., 2011), thus providing an insurance value of biodiversity (Yachi & Loreau, 1999). In this case, the “best” situation for biomass production would be if such species would disappear from local communities during times when they have negative effects on community biomass production and recolonize from the species pool when conditions change. However, beyond this insurance value, rare species can also have specific effects in excess of their proportion in the community, for example on ecosystem resistance to invasion (Lyons & Schwartz, 2001) or on soil fertility (Ives & Helmus, 2011).

With our removal experiment, we wanted to provide a proof-of-concept study of how BEF experiments might be expanded in such a way that they better mimic observational field studies about BEF relationships. While there is always the difference that in experiments we manipulate biodiversity, thus allowing us to study its causal effects on ecosystem functioning, whereas in observational studies this causality can be reversed (Grace et al., 2016), there are possibilities to mimic the latter as we did here by completing an environmental filtering process that was indicated by local communities themselves. Randomly assembled experimental communities of different species richness may be best compared with species pools in a field study. But even in such an experiment, it is possible in some cases to identify species compositions among the randomly assembled ones that more or less closely reflect particular environmental filtering processes; in this case, a corresponding analysis can make the link to observational field studies (Chen et al., 2020). The alternative we suggest here is that in a first step a BEF experiment is used as a species pool experiment. This will suggest which local communities result after environmental filtering and species interactions; these filtered communities can then be assembled in a second step.

AUTHOR CONTRIBUTIONS

Bernhard Schmid: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; software; supervision; validation; visualization; writing—original draft preparation; writing—review and editing. **Martin Schmitz:** Conceptualization; data curation; formal analysis; investigation; methodology; validation; writing—original draft preparation; writing—review and editing. **Michael Rzanny:** Investigation; methodology; writing—review and editing. **Michael Scherer-Lorenzen:** Conceptualization; supervision; writing—review and editing. **Peter N. Mwangi:** investigation; methodology; writing—review and editing. **Wolfgang W. Weisser:** Funding acquisition; project administration; supervision; writing—review and editing. **Andrew Hector:** Supervision; validation; writing—review and editing. **Roland Schmid:** Formal analysis; methodology; software. **Dan F. B. Flynn:** Formal analysis;

methodology; validation; writing—original draft preparation; writing—review and editing.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.


DATA AVAILABILITY STATEMENT

The raw data used in this study are available as supplementary online information, file “SchmidEtAlDataScriptAnovas 22.xlsx.”

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REFERENCES

- Balvanera, P., Pfisterer, A. B., Buchmann, N., He, J. S., Nakashizuka, T., Raffaelli, D., & Schmid, B. (2006). Quantifying the evidence for biodiversity effects on ecosystem functioning and services. *Ecology Letters*, *9*, 1146–1156.
- Cadotte, M. W., & Tucker, C. M. (2017). Should environmental filtering be abandoned? *Trends in Ecology and Evolution*, *32*, 429–437.
- Chen, Y., Huang, Y., Niklaus, P. A., Castro-Izagirre, N., Clark, A. T., Bruelheide, H., Ma, K., & Schmid, B. (2020). Directed species loss reduces community productivity in a subtropical forest biodiversity experiment. *Nature Ecology and Evolution*, *4*, 550–559.
- Diaz, S., Symstad, A. J., Chapin, F. S., Wardle, D. A., & Huenneke, L. F. (2003). Functional diversity revealed by removal experiments. *Trends in Ecology & Evolution*, *18*, 140–146.
- Fargione, J., Brown, C. S., & Tilman, D. (2003). Community assembly and invasion: An experimental test of neutral versus niche processes. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 8916–8920.
- Fichtner, A., Härdtle, W., Bruelheide, H., Kunz, M., Li, Y., & von Oheimb, G. (2018). Neighbourhood interactions drive overyielding in mixed-species tree communities. *Nature Communications*, *9*, 1144.
- Fraser, L. H., Pither, J., Jentsch, A., Sternberg, M., Zobel, M., Askarizadeh, D., Bartha, S., Beierkuhnlein, C., Bennett, J. A., Bittel, A., Boldgiv, B., Boldrini, I. I., Bork, E., Brown, L., Cabido, M., Cahill, J., Carlyle, C. N., Campetella, G., Chelli, S., ... Zupo, T. (2015). Worldwide evidence of a unimodal relationship between productivity and plant species richness. *Science*, *349*, 302–306.
- Grace, J. B., Anderson, T. M., Seabloom, E. W., Borer, E. T., Adler, P. B., Harpole, W. S., Hautier, Y., Hillebrand, H., Lind, E. M., Pärtel, M., Bakker, J. D., Buckley, Y. M., Crawley, M. J., Damschen, E. I., Davies, K. F., Fay, P. A., Firn, J., Gruner, D. S., Hector, A., ... Smith, M. D. (2016). Integrative modelling reveals mechanisms linking productivity and plant species richness. *Nature*, *529*, 390–393.
- Grime, J. P. (1998). Benefits of plant diversity to ecosystems: Immediate, filter and founder effects. *Journal of Ecology*, *86*, 902–910.
- Grime, J. P. (2002). Declining plant diversity: Empty niches or functional shifts? *Journal of Vegetation Science*, *13*, 457–460.
- Hagan, J. G., Vanschoenwinkel, B., & Gamfeldt, L. (2021). We should not necessarily expect positive relationships between biodiversity and ecosystem functioning in observational field data. *Ecology Letters*, *24*, 2537–2548.
- Hector, A., Loreau, M., & Schmid, B., BIODEPTH project. (2002). Biodiversity manipulation experiments: Studies replicated at multiple sites. In M. Loreau, S. Naeem, & P. Inchausti (Eds.), *Biodiversity and ecosystem functioning: Synthesis and perspectives* (pp. 36–46). Oxford University Press.
- Hubbell, S. P. (2001). The unified neutral theory of biodiversity and biogeography. In S. A. Levin & H. S. Horn (Eds.), *Monographs in population biology* (Vol. 32). Princeton University Press.
- Isbell, F., Calcagno, V., Hector, A., Connolly, J., Harpole, W. S., Reich, P. B., Scherer-Lorenzen, M., Schmid, B., Tilman, D., Ruijven, J. v., Weigelt, A., Wilsey, B. J., Zavaleta, E. S., & Loreau, M. (2011). High plant diversity is needed to maintain ecosystem services. *Nature*, *477*, 199–203.
- Ives, A. R., & Helmus, M. R. (2011). Generalized linear mixed models for phylogenetic analyses of community structure. *Ecological Monographs*, *81*, 511–525.
- Jäger, E. J., & Werner, K. (2002). *Rothmaler - Exkursionsflora von Deutschland*. Band 4. Gefäßpflanzen: Kritischer Band. Spektrum Akademischer Verlag.
- Jochum, M., Fischer, M., Isbell, F., Roscher, C., van der Plas, F., Boch, S., Boenisch, G., Buchmann, N., Catford, J. A., Cavender-Bares, J., Ebeling, A., Eisenhauer, N., Gleixner, G., Hölzel, N., Kattge, J., Klaus, V., Kleinebecker, T., Lange, M., Le Provost, G., ... Manning, P. (2020). The results of biodiversity–ecosystem functioning experiments are realistic. *Nature Ecology and Evolution*, *4*, 1485–1494.
- Loreau, M. (2000). Biodiversity and ecosystem functioning: Recent theoretical advances. *Oikos*, *91*, 3–17.
- Loreau, M., & Hector, A. (2001). Partitioning selection and complementarity in biodiversity experiments. *Nature*, *412*, 72–76.
- Lyons, K. G., & Schwartz, M. W. (2001). Rare species loss alters ecosystem function-invasion resistance. *Ecology Letters*, *4*, 358–365.
- Marquard, E., Schmid, B., Roscher, C., De Luca, E., Nadrowski, K., Weisser, W. W., & Weigelt, A. (2013). Changes in the abundance of grassland species in monocultures versus mixtures and their relation to biodiversity effects. *PLOS ONE*, *8*(9), e75599.
- Mulder, C. P. H., Bazely-White, E., Dimitrakopoulos, P. G., Hector, A., Scherer-Lorenzen, M., & Schmid, B. (2004). Species evenness and productivity in experimental plant communities. *Oikos*, *107*, 50–63.
- Petermann, J. S., Fergus, A. J., Roscher, C., Turnbull, L. A., Weigelt, A., & Schmid, B. (2010). Biology, chance or history? The predictable re-assembly of temperate grassland communities. *Ecology*, *91*, 408–421.
- Pfisterer, A. B., Joshi, J., Schmid, B., & Fischer, M. (2004). Rapid decay of diversity–productivity relationships after invasion of experimental plant communities. *Basic and Applied Ecology*, *5*, 5–14.
- Roscher, C., Schumacher, J., Baade, J., Wilcke, W., Gleixner, G., Weisser, W. W., Schmid, B., & Schulze, E. D. (2004). The role of biodiversity for element cycling and trophic interactions: An

- experimental approach in a grassland community. *Basic and Applied Ecology*, 5, 107–121.
- Roscher, C., Temperton, V. M., Scherer-Lorenzen, M., Schmitz, M., Schumacher, J., Schmid, B., Buchmann, N., Weisser, W. W., & Schulze, E.-D. (2005). Overyielding in experimental grassland communities—Irrespective of species pool or spatial scale. *Ecology Letters*, 8, 419–429.
- Schmid, B. (2002). The species richness–productivity controversy. *Trends in Ecology and Evolution*, 17, 113–114.
- Schmid, B., Baruffol, M., Wang, Z., & Niklaus, P. A. (2017). A guide to analyzing biodiversity experiments. *Journal of Plant Ecology*, 10, 91–110.
- Schmid, B., & Hector, A. (2004). The value of biodiversity experiments. *Basic and Applied Ecology*, 5, 535–542.
- Schmid, B., Hector, A., Huston, M. A., Inchausti, P., Nijs, I., Leadley, P. W., & Tilman, D. (2002). The design and analysis of biodiversity experiments. In M. Loreau, S. Naeem, & P. Inchausti (Eds.), *Biodiversity and ecosystem functioning: Synthesis and perspectives* (pp. 61–75). Oxford University Press.
- Schmitz, M., Flynn, D. B. F., Mwangi, P. N., Schmid, R., Scherer-Lorenzen, M., Weisser, W. W., & Schmid, B. (2013). Consistent effects of biodiversity on ecosystem functioning under varying density and evenness. *Folia Geobotanica*, 48, 335–353.
- Symstad, A. J., & Tilman, D. (2001). Diversity loss, recruitment limitation, and ecosystem functioning: Lessons learned from a removal experiment. *Oikos*, 92, 424–435.
- Tilman, D., Isbell, F., & Cowles, J. M. (2014). Biodiversity and ecosystem functioning. *Annual Review of Ecology Evolution and Systematics*, 45, 471–493.
- Turnbull, L. A., Isbell, F., Purves, D. W., Loreau, M., & Hector, A. (2016). Understanding the value of plant diversity for ecosystem functioning through niche theory. *Proceedings of the Royal Society B*, 283, 20160536.
- Vogel, A., Ebeling, A., Gleixner, G., Roscher, C., Scheu, S., Ciobanu, M., Koller-France, E., Lange, M., Lochner, A., Meyer, S., Oelmann, Y., Wilke, W., Schmid, B., & Eisenhauer, N. (2019). A new experimental approach to test why biodiversity effects strengthen as ecosystems age. *Advances in Ecological Research*, 61, 221–264.
- VSN International. (2020). *Genstat reference manual (release 21), part 1 summary*. VSN International.
- Wardle, D. A., Bardgett, R. D., Callaway, R. M., & Van der Putten, W. H. (2011). Terrestrial ecosystem responses to species gains and losses. *Science*, 332, 1273–1277.
- Weisser, W. W., Roscher, C., Meyer, S., Ebeling, A., Luo, G., Allan, A., Beßler, H., Barnard, R., Buchmann, N., Buscot, F., Engels, C., Fischer, C., Fischer, M., Gessler, A., Gleixner, G., Halle, S., Hildebrandt, A., Hillebrand, H., de Kroon, H., ... Eisenhauer, N. (2017). Biodiversity effects on ecosystem functioning in a 15-year grassland experiment: Patterns, mechanisms, and open questions. *Basic and Applied Ecology*, 23, 1–73.
- Yachi, S., & Loreau, M. (1999). Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. *Proceedings of the National Academy of Sciences of the United States of America*, 96, 1463–1468.

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