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Biodiversity—functioning studies in grasslands: their design, analysis and the importance of realized diversity

Ph.D. Thesis

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Annotation

In this thesis, relationships between two facets of biodiversity, the sown species diversity and realized species diversity, and biomass production were studied using both theoretical approach and experimental data analysis. A pot biodiversity—functioning experiment was conducted to reveal the effect of initial sowing density on biodiversity effects. A comparison between several methods for biodiversity—functioning experiments analysis was made and methods requirements, results and ecological interpretations implied by their results discussed.

Declaration [in Czech]

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List of papers and manuscripts with author's contribution

(Terezie Rychtecká, née Stachová)

(***Paper 1***) Stachová T, Lepš J (2010) Species pool size and realized species richness affect productivity differently: a modelling study. *Acta Oecologica* 36: 578–586. IF (2012) = 1.62, IF (5 years) = 1.82
TS and JL designed the model and analyzed the results. TS wrote major part of the paper.
(***Manuscript 1***) Rychtecká, T., Lanta, V., Weiterová, I., Lepš, J. Sown species richness and realized diversity can influence functioning of plant communities differently. (Submitted after revision)
TR, VL and JL made concept of the study, IW provided part of the data, TS analyzed the data and wrote major part of the manuscript, with help of VL and JL.
(***Paper 2***) Stachová, T, Fibich, P., Lepš, J. (2013) Plant density affects measures of biodiversity effects. Journal of plant ecology 6: 1-11. IF (2012) = 1.36,
IF (5 years) = 2.33
TS, PF and JL designed the experiment. TS and PF together established the experiment, analyzed the data and wrote the major part of paper.
(***Manuscript 2***) Fibich, P., Rychtecká, T., Lepš, J. Analysis of biodiversity experiments: a comparison of traditional and linear-model-based methods. (Submitted)
PF and JL designed the individual based model (IBM). PF and TR together analyzed the data and wrote the major part of paper.

Contents

Chapter 1: General Introduction **1**

Chapter 2: (Paper 1) Species pool size and realized species richness affect productivity differently: A modeling study **15**

Chapter 3: (Manuscript 1) Sown species richness and realized diversity can influence functioning of plant communities differently **19**

Chapter 4: (Paper 2) Plant density affects measures of biodiversity effects **41**

Chapter 5: (Manuscript 2) Analysis of biodiversity experiments: a comparison of traditional and linear-model-based methods **45**

Chapter 6: General Discussion **93**

Chapter I

General Introduction

1.1 Biodiversity—Ecosystem Functioning Experiments: a brief history

Throughout their history, humans have always been altering their environment. However, with human population increasing, the demand for ecosystem services (e.g. food, fuel, fibre, medicinal substances…) and energy also increased. Human activities are causing changes in both biotic and abiotic components of ecosystems and this pressure triggered a series of global environmental changes, among them the loss of biodiversity (Hooper *et al*. 2012), sometimes being called "the sixth mass extinction crisis" (Barnosky *et al*. 2011). This decline of biodiversity has aroused both scientific and public concern about sustaining the quality of ecosystem services for mankind (Chapin *et al*. 2000). Will the anthropogenic changes to ecosystems including massive species extinctions alter ecosystem properties such as productivity, hydrology, nutrient cycling etc.? Will changes in these properties adversely influence human wellbeing?

These and similar questions lead to a whole new branch of ecological research, called by an acronym BEF (biodiversity and ecosystem functioning research), which started booming in the beginning of nineteen nineties (Hooper *et al*. 2005). However, the belief, that species rich systems function better, e.g. are more stable, than the species poor ones (the "diversity begets stability" statement) goes back to nineteen sixties. The mechanism of increasing stability with species richness is usually explained by what is now referred to as "insurance hypothesis": in a fluctuating environment, multiple species are needed to maintain functioning of a system, even if some species fail due to changed conditions, there are others which can take over (Grime 1998, Yachi and Loreau 1999). One of the common diversity indices, Shannon index, was originally proposed as an index of stability (Mac Arthur 1955), clearly on the basis of the "diversity begets stability" belief.

BEF studies traditionally involved grassland communities or artificially created plant assemblages which mimic such type of a community, mainly due to an easily measurable function: primary production. The second reason is that primary productivity is also one of those ecosystem services essentially important to humans and its potential reaction to biodiversity loss would be of a crucial importance. BEF experiments studying biomass production were preceded by agronomical studies of intercropping (Trenbath 1974, Vandermeer 1992) which were designed to answer similar question as BEF: will multi-crop plantations yield more and be more pathogen-resistant than monoculture stands? Despite encouraging application results and obvious link with BEF and BES (biodiversity and ecosystem service) research, the experience gained in intercropping research has not been fully realized and more or less remained out of the main scientific focus. The BEF researchers paved their own way and results from the first BEF experiment provoked debates over their interpretation. While some researchers explained the positive relationship between sown diversity and aboveground biomass production found in early projects as a direct causal influence of diversity on function (Naeem *et al*. 2000) the others suggested that the main drivers of ecosystem functioning may not be the diversity per se but more likely the functional traits of single species and functional composition of a community (Wardle *et al*. 2000). Also, design of the first experiments has been subjected to serious criticism, which, however, lead to an improvement of the following projects and finally the two schools of thought originally disagreeing strongly with each other seem to reach a type of consensus (Hooper *et al*. 2005).

During the past two decades, BEF experiments have undergone a fast evolution towards extension of their scopes: many experiments increased temporal and spatial scales, included a multitude of species, their traits and phylogenetic distances, incorporated also different trophic levels as well as multiple measures of community functioning (for review see e.g. Cardinale *et al*. 2012). Some of the BEF experiments are among the largest field experiments ever, the so called Jena experiment (Roscher *et al*. 2005) in Thuringia, Germany, makes a perfect example. This experiment consists of nearly 500 plots, some as large as 400 m^2 and has been running since 2003. Other important plant BEF projects include e.g. the BIODEPTH (Hector *et al*. 1999), CLUE (van der Putten *et al*. 2000), Cedar Creek which is also a LTER (long term ecological research) site etc. (Tilman *et al*. 1996). Apart from grassland BEF studies, reports from tropical forests, phytoplankton, fish, soil or algal benthos communities are reported (e.g. Bunker *et al*. 2005, McIntyre 2007, Vila *et al*. 2007, Bracken *et al*. 2008, Ptacnik *et al*. 2008, Eisenhauer *et al*. 2011).

After two decades of BEF research, the main challenge for scientist is to link directly the ecosystem function measured to the particular provisioning and regulating services of ecosystems, i.e. to link the BEF and BES (biodiversity and ecosystems service) research to provide relevant information to policy makers (Cardinale *et al*. 2012, Balvanera *et al*. 2013).

1.2 Biodiversity—productivity relationship in grasslands: potential mechanisms and the importance of sown vs. realized species richness

One of the focal topics of this thesis is the relationship between primary productivity and diversity in grassland communities. In the majority of BEF experiments which used above-ground biomass production as a measure of functioning and number of sown species as a measure of community diversity, a positive biodiversity—productivity relationship was found (Cardinale *et al*. 2007, Cardinale *et al*. 2012). In their meta-analysis, Cardinale *et al*. (2012) even listed the positive relationship between diversity and biomass production as a "consensus statement" which shows that convincing evidence has been gathered since the beginning of the BEF research.

Two mechanisms have been suggested to explain this phenomenon: the complementarity and selection effects (Loreau and Hector 2001). The selection or sampling effect is more or less a probability phenomenon of including species that does the „job"(exhibits high biomass production etc.) with rising number of species in a community (Lepš *et al*. 2001). The sampling effect creates a positive relationship between species diversity and productivity by increasing probability of presence of highly productive species in the community resulting from a larger species pool. Some authors (Huston 1997, Wardle 1999, Aarsen 1997) considered sampling effect as a statistical artifact or hidden treatment of BEF studies while Tilman with colleagues (Tilman *et al*. 1997) interpreted it as a valid biodiversity effect, which is now a widely accepted thesis.

On the other hand, complementarity is based on the theory of species co-existence, namely on the prediction of better use of limiting resources by multiple species differing in their niches. Complementarity can operate both belowground and aboveground though in three-dimensional belowground space there is more potential for niche differentiation, e.g. in catching soil water and nutrients than in two-dimensional above-ground competition which is predominantly for light. Also, complementarity occurs at many levels: between single species traits, single species and functional groups of species. Similar positive effects as those resulting from complementarity in resource use are created by facilitation. Some species (which can be considered as keystone species or ecosystem engineers, e.g. legumes in plant communities) alter the environment in a favorable way which is beneficiary for some other species. The keystones thus "facilitate" the survival of their counterparts in a community.

While addressing the question of diversity—productivity, one should keep in mind that the definition of "diversity" matters. In BEF experiments, diversity is usually considered as sown species richness which in fact corresponds to a regional species pool size in natural conditions. According to the species pool theory (Butaye *et al.* 2001), the observed species richness of a target community is given as local species pool (i.e. the total regional species pool minus species filtered out by dispersal limitation) without the species excluded by environmental filter and biotic interactions within a community.

This explains the apparently contradicting results between experimental and observational studies of diversity—productivity relationship. Observational studies have reported various shapes of observed diversity (often called realized diversity) and biomass production, including hump-backed and negative ones (Thompson *et al.* 2005, Lepš 2013, Mittlebach *et al.* 2001). Manipulative biodiversity experiments, which allowed for natural colonization (after the cessation of weeding or never weeded experiments), reported that the initially positive relationship between diversity and roductivity disappeared very quickly (among others Pfisterer *et al.* 2004, Lepš *et al.* 2007, Roscher *et al.* 2009).

The shape of diversity—productivity relationship is also influenced by the diversity metric we use. Many studies have proven that the simple number of species may not be sufficient for explaining observed patterns and suggesting a potential mechanisms behind them and metrics including also species relative proportions within a community or their functional traits or phylogenetic distances should be employed (Diaz and Cabido 2001, Garnier *et al*. 2004, Cadotte *et al*. 2008, Flynn *et al*. 2011). Time and spatial scale has also been recognized as a factor which can alter the diversity—productivity relationships, e.g. Chalcraft *et al*. (2004), Weiss *et al*. (2007).

1.3 Analysis of BEF experiments

In BEF experiments, sown species richness is manipulated and experimental communities (mixtures) are usually established from seeds grown in pots/plots. The experimental diversity gradient is then composed of several levels of sown species richness (mixture sizes) and various species compositions at each species richness level. Ideally, the experimental species should be equally represented at each species richness level (mixture level) and should be sown in equal proportions (Lepš 2013). Many experiments applied regular weeding to prevent immigrations of species that were not originally sown. However, as the primary purpose of BEF research was to understand the role of biological diversity in nature, the results from communities where natural processes as immigration and/or BEF which were not weeded see e.g. Rixen *et al*. 2008, Petermann *et al*. 2010).

For analyzing the results of BEF experiments, Loreau and Hector (2001) suggested calculation of so called biodiversity effects: selection and complementarity effect. Their calculation is based net biodiversity effect partitioning. The net biodiversity effect (NE) is the difference between actual mixture yield and its expected yield. Expected yield of a mixture is calculated from monoculture performance of species present in a given mixture which are corrected for the sowing proportion of individual species. Positive complementarity values arise when all constituent species have on average higher yield than expected while positive selection effect values arise when species have positive covariance between their observed and expected yields (i.e. a mixture is dominated by species having high monoculture yields).

Majority of BEF studies used the above mentioned net biodiversity effect partitioning, however, it is important to notice its limitations. First, in order to quantify the biodiversity effects, all monocultures of all experimental species are needed, as well as biomass contributions of all species to observed yield of a mixture. These pieces of information may be difficult (and for some measures of ecosystem functioning, so called "emergent" properties, even impossible) to obtain and imposes a serious limitations for experimental setup. The second problem concerns subsequent statistical analysis of biodiversity effects. Statistically, these biodiversity effect values (calculated for each mixture in each pot/plot) are not independent data points, as a limited number of monocultures repeatedly occur in all calculations. This may inflate the significance of statistical tests; we should use in fact fewer degrees of freedom in calculations. In spite of this, a majority of studies seem to neglect this problem and use standard statistical methods (linear models, generalized linear models etc.).

It is also important to keep in mind that the net effect partitioning method is a generalization of the relative yield total (RYT), which was originally designed for short-term plant competition experiments in which total density is kept constant (Jolliffe 2010 and references therein). Chosen experimental density may affect the values of resulting biodiversity effects through possibly differentional shapes of density—productivity relationship. Generally accepted shape of the density—productivity is called "the law of constant final yield" ("CFY", Weiner and Freckleton 2010), which is a positively saturated one. Initial increase of density results in a proportional increase of productivity. However, with further increase of density, strong in inter- or intraspecific competition arises and causes mortality of some individuals. This process is known as self-thinning (Yoda *et al*. 1963). Due to this process, the initially linear increase of productivity saturates at an asymptotic value. Despite the assumption that species should follow the constant final yield density—productivity relationship, it does not have to be always the case (Fibich *et al*. 2014).

To overcome the limitations of additive net effect partitioning, several alternative methods of BEF experiment analysis have been proposed recently (Kirwan *et al*. 2009, Bell *et al*. 2009). These methods are using classical statistical methods: linear models and are thus sometimes called "linear-model-based methods". Linear-model-based methods fit a linear model on the whole dataset and asses the diversity effects as linear model coefficients (these are called identity effects and interactions in the linear-model-based methods terminology). The main advantage of the linear-model-based methods is that neither the monocultures nor the species contributions to productivity are required.

1.4 Outline of the thesis

In this thesis, the role of both the sown species richness and realized diversity in BEF experiments (which correspond to species pool size and observed species richness in natural communities, respectively) was in focus. To address their potentially different relationship with productivity, a mathematical model using Lotka-Volterra competition equation was developed (***Paper 1***). Using this model, experimental communities differing in species pool size were generated and their "biomass production" and number of species surviving in equilibrium (realized diversity) were calculated. Also, parameters characterizing realized community were recorded to provide background for potential mechanisms. We tested the hypotheses generated by our model using experimental data from field and pot BEF experiments: Jena experiment and experiment of Špaekova and Lepš (***Manuscript 1***). The question of the influence of initial sowing density in BEF experiments was investigated in a pot BEF experiment using mixtures of four grassland species sown in all possible combinations and five initial densities (***Paper 2***). Finally, methods for analysis of BEF experiment proposed so far, including additive partitioning and two linear-model-based methods, were compared using three data sets and their both their advantages and limitations were discussed (***Manuscript 2***).

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Chapter II

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Species pool size and realized species richness affect productivity differently: A modeling study

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ABSTRACT

Current and expected decline in biodiversity have motivated a number of experiments studying how biodiversity affects ecosystem functioning. The positive relationship is usually found in experiments where species pool is manipulated; the relationship between productivity and realized species richness does not show any single trend.

We constructed a simple competition model for a plant community based on the classical Lotka–Volterra equations, with randomly generated parameters. We varied the species pool size and intensity of competition (range of competition coefficients). Then, we compared two measures of diversity used as predictors of productivity: (1) the size of the species pool and (2) the realized species richness, i.e. the number of species that remained in the system after competitive exclusion.

Simulation results showed that productivity was always positively affected by the size of the species pool. With increasing species pool, both the selection effect and complementarity increase. The relationship between realized species richness and productivity was extremely weak within a set of simulations with a fixed species pool (i.e. where the diversity gradient was caused only by differences in the randomly generated parameters). The relationship between realized species richness and productivity was slightly positive for small species pool sizes and slightly negative for larger species pools. A species with high carrying capacity within the generated set of species usually decreases the chance of other species to survive but increases the productivity of the mixture, leading to negative diversity productivity relationship. On the contrary, presence of highly complementary species (i.e. species with low mutual competition coefficients) increases both, the realized species richness and productivity, leading to positive diversity productivity relationship. These two effects mostly counterbalance each other. These trends are not affected by the competition intensity.

1. Introduction

Both scientific and public concerns about the global decline of biological diversity inspired many theoretical and experimental studies during the past two decades. Many studies were focused on so called biodiversity effects, i.e. the way in which diversity affects ecosystem function and services (e.g. Johnson et al., 1996; Schlapfer et al., 1999; Hector et al., 2007, for reviews see Chapin et al., 2000; Loreau et al., 2001; Hooper et al., 2005). It has traditionally been believed that diversity positively affects ecosystem functions (Hooper et al., 2005) and the idea has been proved by many studies (Cardinale et al., 2007). Ecosystem functioning is an umbrella term for various processes operating in an ecosystem, mainly flow of energy and matter within and between ecosystems (e.g. primary productivity, nutrient cycling etc.). In addition to often studied functions, such as primary productivity (e.g. Naeem et al., 1996; Tilman, 1999; Dukes, 2001) and chemical compound fluxes, other aspects of ecosystem function have been examined as well, including nutrient retention, soil microbial activity (e.g. in Wardle and Nicolson, 1996; Wardle et al., 1997; Donnison et al., 2000), temporal stability and resistance to invasions (e.g. Knops et al., 1999; Van Ruijven et al., 2003).

Published biodiversity and ecosystems functioning (BEF) studies number in the hundreds and include some of the largest experiments in field ecology (the Jena experiment in Germany consists of nearly 500 plots, some as large as 400 m^2 – Roscher et al., 2005) and large international projects (BIODEPTH – Hector et al., 1999, TERI-CLUE – Van der Putten et al., 2000, T-Links – Lepš et al., 2007, etc.). BEF studies traditionally involved grassland communities or artificially created assemblages of herbs which mimic a grassland

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Chapter III

Sown species richness and realized diversity can influence functioning of plant communities differently

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ABSTRACT

Biodiversity—ecosystem functioning experiments (BEF) typically manipulate sown species richness and composition of experimental communities to study ecosystem functioning as a response to changes in diversity. If sown species richness is taken as a measure of diversity and aboveground biomass production as a measure of community functioning, then this relationship is usually found to be positive. The sown species richness can be considered the equivalent of a local species pool in natural communities. However, in addition to species richness, realized diversity is also an important community diversity component. Realized diversity is affected by environmental filtering and biotic interactions operating within a community. As both sown species richness and the realized diversity in BEF studies (as well as local species pool vs. observed realized richness in natural communities) can differ markedly, so can their effects on the community functioning. We tested this assumption using two datasets: data from a short-term pot experiment and data from the long-term Jena biodiversity plot experiment. We considered three possible predictors of community functioning (aboveground biomass production): sown species richness, realized diversity (defined as inverse of Simpson dominance index) and survivor species richness.

Sown species richness affected biomass production positively in all cases. Realized diversity as well as survivor species richness had positive effects on biomass in approximately half of cases. When realized diversity or survivor species richness were tested together with sown species richness, their partial effects were negative. Our results suggest that we can expect positive diversity—productivity relationship when the local species pool size is the decisive factor determining realized observed diversity; in other cases the shape of the diversity—functioning relationship may be quite opposite.

Keywords

biodiversity; realized diversity; species pool; Jena experiment

INTRODUCTION

Species diversity is often considered to be a driving factor of ecosystem functioning. This idea is mostly based on “biodiversity—ecosystem functioning experiments” (BEF), which have demonstrated that in sown mixtures of randomly selected sets of species from a common pool, the ecosystem functioning (often represented by aboveground biomass production) increases with the number of sown species (Balvanera *et al.* 2006, Hooper *et al.* 2012, Naeem *et al.* 2012, but see Kenkel *et al.* 2000). To explain the mechanisms driving this positive relationship, two mutually non-exclusive hypotheses were suggested: the complementarity and selection (sampling) effects (Loreau and Hector 2001). The complementarity effect hypothesis states that multiple functionally different species should be able to use the available niche space better and thus increase their biomass production. The selection effect is more or less a probabilistic phenomenon produced by the BEF studies experimental setting: with increasing number of species in mixture, the chance that a species with a dominant role in community functioning will be included also increases.

It is important to note that a variety of community biodiversity measures have been proposed in addition to species richness; species evenness and functional trait composition have been recognized as important factors shaping community functioning (Diaz and Cabido 2001, Garnier *et al.* 2004, Sanderson 2010, Sasaki and Lauernroth 2011, Schmitz *et al.* 2013). In BEF studies the sown species richness (mixture size) is in fact equivalent to a local species pool in natural communities, as defined by Butaye *et al.* (2001). Following their concept, the local species pool can be defined as a pool of species which have reached a given locality and are able to grow under given environmental conditions. The observed local community is then composed of the local species pool minus species filtered out by several possible factors. Among the most important factors are interspecific interactions, mainly competition, but facilitation and multi-trophic interactions cannot be excluded either. Another factor which may influence the observed diversity in natural grasslands, as well as in field experiments using permanent plots, is a small scale environmental heterogeneity. This has been shown to increase the number of locally coexisting species and also to change the magnitude and relative importance of the biodiversity effects operating within a community (Wacker *et al.* 2008, Richardson *et al.* 2012). All these factors influence not only the presence of individual species, but also their relative proportions of biomass, and ultimately the diversity. The observed species richness and observed species proportions characterizing realized diversity are thus important characteristics of a community.

The diversity—productivity relationship is not only dependent on the diversity measure used but also on time and spatial scale studied (Chalcraft *et al.* 2004, Weiss *et al.* 2007). Long-term BEF experiments allow for species interactions to occur and possible competitive exclusion of some species or immigration of others, which affects the observed species richness. This can then be higher/lower than the sown one. The phenomenon of possible species extinctions is of great interest because the main aim of BEF studies has always been to predict consequences of such events in nature (Chapin *et al.* 2000). Some multi-seasonal BEF experiments were maintained by weeding while other let colonizers invade experimental plots. It has already been demonstrated that the positive relationship between observed species richness and biomass disappears quickly if the plots are open to new invasions (Pfisterer *et al.* 2004, Lepš *et al.* 2007, Rixen *et al.* 2008, Roscher *et al.* 2009, Petermann *et al.* 2010).

The shapes of diversity—productivity relationship differing from the positive one widely found in BEF experiments are often reported from observational studies located in temperate managed grasslands. These are the most similar communities to those in plant BEF studies and the relationship between observed local species richness and biomass production is often negative in these places, or not monotonous (Thompson *et al.* 2005, Lepš 2013, Mittlebach *et al.* 2001). In such communities, the species richness is limited mostly by environmental filtering and competitive exclusion of weak competitors.

In our study, we addressed the possibility of a differential relationship between sown species richness, two measures of realized diversity and biomass production. The sown species richness is the only manipulated variable in BEF studies, and therefore seems to be the natural explanatory variable. However, the species already excluded from a community can hardly participate in niche partitioning, resulting in the complementarity effect. Similarly, the potential for complementarity will decrease with decreasing evenness of species (Nijs and Roy 2000). This justifies using realized diversity as an explanatory variable in the study of the diversity—productivity relationship. Concerning the mechanisms behind the diversity—productivity relationship, there is also a question of causality. In BEF experiments the causal direction is clear: sown richness affects biomass production. In contrast, in natural conditions the community diversity is affected by both species pool and by processes within the community, making direction of causality unclear (Lepš 2013, Stachová and Lepš 2010). It is known that the positive biodiversity—productivity relationship is seldom found in nature (Schmid 2002). In natural conditions, we are usually only able to observe the realized species richness and diversity and not the sown diversity, which corresponds to the community local species pool (i.e. to the species that were able to arrive to the site). And so the community species pool size is generally unknown and although the estimation methods do exist, they are far from being precise enough to enable any comparison with BEF experiments. For this reason, the only way to compare the diversity—productivity relationships found in experiments with those in nature is using the realized diversity. Consequently we have asked which of the three possible predictors (sown species richness, survivor species richness, and realized diversity, defined as inverse of Simpson dominance index) predicts best the community productivity as a sole predictor (what are their marginal effects). We have also asked how the use of characteristics of realized diversity changes the predictions based on the sown diversity (what are their partial effects after accounting for the sown species richness).

We based our hypotheses on the mechanisms operating within the community. It is clear, that number of sown species is a determinant of selection effect – more species available (i.e. sown) leads to a higher chance of getting highly productive species in the mixture. Following this reasoning there should be an explicit positive relationship between sown species richness and biomass production; and as the sown species richness is a defined set of species at the beginning and does not change during an experiment, this positive relationship should be constant over time.

On the other hand complementarity should be mainly dependent on the species actually present in the community and on their proportions. So, there should be a tight positive relationship between the observed species richness (survivor species richness and realized diversity) and biomass production. We can expect this relationship to strengthen with the length of an experiment, as it has been shown in multi-seasonal BEF experiments that the complementarity effect size increases with time. This is not surprising because in all the theories explaining maintenance of species diversity, niche differentiation (and so the resource use complementarity) is one of the basic prerequisites of species coexistence (Wilson 2011). Therefore we can expect that species surviving in the experimental plots are those able to use the resources in a complementary way.

In our analyses, we also focus on the effect of realized diversity and survivor species richness in a constant local species pool (fixed mixture size in BEF studies). Our model (details in Stachová and Lepš 2010) predicted a non-existant or negative relationship between survivor species richness and biomass production in communities resulting from a species pool of constant size.

We examined two datasets: data from a seasonal greenhouse pot experiment using six plant species (Špaekova and Lepš 2001) and a publicly available dataset from a six-year period of the Jena experiment, one of the largest and longest BEF experiment ever (Weigelt *et al.* 2010). The short term experiment is a typical example of a widespread BEF experiment lasting only one season. In such experiments, time for competitive exclusion to occur is relatively short and thus the realized and sown species richness are usually quite similar. The communities were established in pots where the potential for even minor environmental heterogeneity is limited. In the long-term experiment the diversities, both realized and sown, had enough time to diverge allowing us to test the above hypotheses and theoretical predictions.

MATERIAL AND METHODS

Glasshouse experiment

For this experiment six naturally co-occurring meadow species from two functional groups were planted in pots (16 cm in diameter, 14 cm high). All possible species combinations within all species-richness level were used. After five months aboveground biomass was assessed and sorted into individual species. For details on this experiment see Špaekova and Lepš 2001.

Jena experiment

The dataset retrieved from Weigelt *et al.* (2010) was based on sampling of permanent plots 20 x 20 m. The species pool of this experiment consists of sixty species and the following species richness levels: monocultures, two, four, eight, 10 and 60-species mixtures. The plots were harvested twice a year before mowing (usually in May and August). We used data from six consecutive years (2003-2008), from the May harvest as well as the August harvest. Every plot consisted of three or four subplots 0.2 × 0.5 m and the exact coordinates of each subplot were randomly generated within the main plot before every harvest. This is exactly the spatial scale, at which the individuals are expected to interact with each other, thus we analyzed the subplots as separate observations. For details and publications based previous analyses of this dataset see Weigelt *et al.* 2010.

Data analysis

The diversity of plant communities of each pot/subplot was described by reciprocal of Simpson's index of dominance (Lepš 2013): $D = \sum_i \left(\frac{Ni}{N}\right)^2$, where *Ni* is biomass of *i*-th species and *N* is total biomass of a community. Note that in this form, 1/*D* is scaled as the number of species, and sometimes is called the equivalent number of species, because it is the number of species reaching the same diversity being equally represented in the community. We refer to the sown number of species "sown species richness" and *1/D* as "realized diversity". The "survivor species richness" was calculated as the number of species yielding more than five percent of the total pot/subsample biomass. This approach was used because in the pot experiment, there was not sufficient time for a species to become extinct, and we wanted to keep a unified methodology for both compared data sets. For the Jena data set, we have also used as survivor species richness number of all species present in a subsample (total survivor species richness); results are presented in the Supplementary material. In our linear regressions, aboveground biomass was considered as a response; with realized diversity, sown species richness and survivor species richness used as predictors. Sown species richness and the survivor species richness were log-transformed in all analyses of the Jena experiment dataset. First, the marginal effects of all predictors were evaluated (all predictors were used in three separate linear regressions for all years in the Jena experiment) and in the second step we used sown species richness as a first predictor and realized diversity or survivor species richness as a second predictor to get their partial effect on biomass production, again for all years in/of the Jena experiment. The partial effects show the effect of each of the predictors **in addition** to the first predictor, i.e., to the sown species richness. It can be also understood as the effects of the predictor if the sown species richness is kept constant. In the Jena experiment (which has enough various combinations of species composition at the same sown richness), we visualized this effect by inspecting the relationship between realized diversity and biomass for subsets of the same sown richness.

RESULTS

In the Jena experiment, marginal effects of sown species richness were significantly positive in all harvests and all seasons while marginal effects of both realized diversity and survivor species richness were significantly positive in around half of cases (six and seven out of eleven for realized diversity and survivor species richness, respectively) (Table 1). In a linear model using the predictors sown species richness and realized diversity, the partial effect of realized diversity was significantly negative in eight cases. Similar results (seven significantly negative cases) were detected for the partial effects of survivor species richness, again with sown species richness as the first predictor (for summarized statistical results for partial effects see Table 2). The same analyses (marginal and partial effects) performed using total survivor species richness from the Jena experiment again yielded rather similar results (see Supplementary material). The negative effects of realized species richness and survivor species richness at a fixed sown species richness level are displayed in Figure 1.

In the glasshouse experiment, marginal effects of all predictors were significantly positive and partial effects of realized diversity or survivor species richness were non-significant (Tables 1 and 2).

DISCUSSION

The diversity of a natural community is determined by the available species pool, abiotic filtering and biotic interactions within the community (Reitalu *et al.* 2008, Myers and Harms 2009, Götzenberger *et al.*, 2012, Lepš 2013). Our data support the frequently found positive dependence of productivity on the sown species richness (Cardinale *et al.* 2011, Naeem *et al.* 2012); which can be translated into a positive relationship between the local species pool and productivity in nature. In our view, all the species used in a BEF experiment can be understood as a regional ('total' *sensu* Butaye *et al.* 2001) species pool, i.e. all the species available in the geographical area. The set of sown species in individual plots corresponds to the local species pool, i.e. set of the species able to reach the site, and all the other species are artificially prevented from entering the community (either by not sowing, or by weeding if they were able to reach the site naturally). This in fact corresponds to the dispersal limitation of these species in nature. Sown species richness showed positive marginal effects on biomass production in all seasons and harvests studied in the Jena experiment and also in the glasshouse experiment. Realized diversity and survivor species richness are derived from and highly correlated with (r ranging between 0.45 and 0.7 in the Jena experiment) sown species richness, so their marginal effects were positive in half of cases in the Jena experiment and in the glasshouse experiment. Sown species richness had the strongest explanatory power of all the predictors. Nevertheless, the R^2 of all the positive relationships were rather low (see Table 1). Observation of a temporally stable positive relationship between sown species richness and biomass production is in line with our hypothesis concerning the strong mechanism of selection effect. The actual values of selection effect for the Jena experiment have been assessed by Marquard *et al.* (2009) and reported as decreasing over time. However, it has been debated whether or not the phenomenon of increasing importance of (statistically determined) complementarity effect and the corresponding decreasing importance of selection effect over time might be caused solely by deteriorating performance of monocultures. The monoculture values enter the formulas for biodiversity effects as a "reference" and it is very possible that the monoculture production diminished due to accumulation of pathogens and imbalanced depletion of resources (e.g. Marquard *et al.* 2013).

There seemed to be no directed temporal pattern for the relationship between biomass production and realized diversity measures in the Jena experiment. In the first year, there was a positive relationship between both measures of realized diversity and biomass production, which was not present in two subsequent years. However, between the fourth and sixth year, these relationships were positive again. The experiments which have found no relationship between realized diversity and community functioning usually allowed for immigration of new species resulting in a survivor species richness higher than the sown one (at least in smaller mixture sizes). Plots in the Jena experiment were regularly weeded, and so the survivor species richness was always lower than the sown one in the dataset we used. In our model (Stachová and Lepš 2010) which used different sown species richness and allowed for competitive exclusion only, the relationship between survivor species richness and biomass production was positively saturated since the positive slope was mainly caused by the lowest richness level. Our results on realized diversity and survivor species richness affecting biomass production in either positive or no way partially contradicts our hypothesis of increasing positive relationship with time due to increasing importance of complementarity effect. The analysis of the Jena experiment by Marquard *et al.* (2009) showed that the net and complementarity effects slightly decreased in the first years and after reaching a minimum in the third year began increasing in subsequent years. Correspondingly, a similar temporal trend of the average survivor species richness and average realized diversity was pronounced in our analyses. This means that the average complementarity and net effects were higher when more species survived and/or average species evenness in mixtures was higher.

The fact that the relationship between measures of realized diversity and biomass production was not always positive and was generally rather weak is caused by a mechanism counteracting the positive complementarity effect. This mechanism is linked with the nature of the commonly used measure of community functioning, biomass production. If a community contains highly productive species, their biomass production will suppress the other species, which can manifest in decreased evenness or even survival, and thus shape the realized diversity of the community. As we can see, the causality here is quite opposite: the functioning affects realized diversity.

The two mechanisms (the complementarity and a negative effect of dominant species on community diversity) are operating simultaneously and can either balance out each other or one of them prevails. If we screen out the positive effects of species pool, the effects of realized diversity /survivor species richness is negative or none (partial effects). This was also apparent in constant sown species richness (mixture levels) conditions (Fig.1), very similarly to the predictions of Stachová and Lepš (2010). We may also generalize this mechanism for the whole dataset: plants compete mainly for light, and as this competition is asymmetric, the presence of a highly productive species in a mixture increases its productivity, but also increases the suppression (or even extinction) of other species. Absence of such a species leads to lower productivity, but also increases the chance that none of the species will be outcompeted, particularly when the species use the resources in a complementary way.

Another possible control over the expression of sown richness and realized diversity would have been environmental filtering affecting species sorting. However, it is obvious that the species for the Jena experiment were selected on the basis of a deep field experience to fit the habitat conditions; the fact that the monocultures perform fairly well over long-term in the site (Marquard *et al.* 2013) confirms this view.

To conclude, if the diversity in diversity—productivity relationship in natural conditions is mostly determined by variation in species pool, then this relationship will be positive and the causality clear: the more species present, the more biomass they produce. However, we should be aware that in nature the species pool seldom differs among geographically close communities, as it is varied in BEF studies. If the size of species pool does not differ among communities, both the realized diversity and the productivity depend mostly on the traits of constituent species (and environmental characteristics which are usually kept constant in BEF). The realized diversity is then often negatively correlated with (or independent of) biomass production. This hypothesis was supported by a model (Stachová and Lepš 2010) which simulated composition of communities from a common pool: the more species a simulated community in equilibrium had, the more they were complementary (had lower competition coefficients). On the other hand, species poor communities consisted of highly productive species gaining dominance and increasing total community productivity. As a result, the model predicted no or negative diversity—productivity relationships depending on the species pool size. There is a direct causal relationship between the size of available species pool and community productivity. Both the realized diversity and the productivity are affected by a series of factors (including species pool size and composition, environmental factors, also determining biotic interactions). If the size of species pool is the decisive factor determining realized diversity, then we can expect positive diversity—productivity relationship. In other cases, the shape of the relationship will be highly variable.

The loss of species in Central European grasslands is most often connected with increased dominance of highly productive species (Lepš 2013), meaning it does not lead to decreased productivity. However, our results suggest that multiple local extinctions of various species will lead to a considerable decrease of regional and local species pool size, which might result in negative effects on ecosystem functioning in future.

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Table 1. Marginal effects of sown species richness (Nsp), realized diversity (D) and survivor species richness (S) on aboveground biomass production (B) of experimental communities for the Jena and glasshouse experiments. Sown and survivor species richness were log-transformed.

	Sown species richness	**Realized diversity**	**Survivor species richness**
2003 May	B = 344.32 **+ 84.05** Nsp (R^2 = 0.13, **p = 10^{-11}**)	B = 394.10 + **37.54** D (R^2 = 0.04, **p = 10^{-4}**)	B = 385.35 **+123.63** S (R^2 = 0.07, **p = 10^{-7}**)
2003 August	B = 145.99 **+ 52.43** Nsp (R^2 = 0.11, **p = 10^{-10}**)	B = 157.46 **+ 37.92** D (R^2 = 0.05, **p = 10^{-5}**)	B = 184.35 + **67.56** S (R^2 = 0.04, **p = 10^{-5}**)
2004 May	B = 373.67 **+ 53.39** Nsp (R^2 = 0.04, **p = 10^{-4}**)	B = 483.28 - 15.59 D (R^2= 0.0, p = 0.24)	B = 449.97 + 5.22 S (R^2 = 0.0, p = 0.85)
2005 May	B = 186.00 **+ 69.03** Nsp (R^2 = 0.09, **p = 10^{-7}**)	B = 250.83 + 21.40 D (R^2 = 0.0, p = 0.19)	B = 259.70 + 47.16 S (R^2 = 0.0, p = 0.10)
2005 August	B = 68.01 **+ 34.90** Nsp (R^2 = 0.09, **p = 10^{-8}**)	B = 107.42 + 7.64 D (R^2 = 0.0, p = 0.25)	B = 113.01 + 13.34 S (R^2 = 0.0, p = 0.30)
2006 May	B = 172.55 **+ 119.38** Nsp (R^2 = 0.13, **p = 10^{-11}**)	B = 311.31 + 21.18 D (R^2 = 0.0, p = 0.23)	B = 280.9 **+ 105.15** S (R^2 = 0.02, **p = 0.003**)
2006 August	B = 69.28 **+ 47.64** Nsp (R^2 = 0.21, **p = 10^{-16}**)	B = 113.36 **+ 15.35** D (R^2 = 0.02, **p = 0.02**)	B = 115.29 + **39.61** S (R^2 = 0.03, **p = 0.001**)
2007 May	B = 196.32 **+ 142.23** Nsp (R^2 = 0.17, **p = 10^{-14}**)	B = 340.05 + 33.61 D (R^2 = 0.0, p = 0.07)	B = 363.32 + 61.23 S (R^2 = 0.0, p = 0.1)
2007 August	B = 93.93 **+ 56.6** Nsp (R^2 = 0.23, **p = 10^{-15}**)	B = 126.51 + **27.45** (R^2 = 0.05, **p = 10^{-4}**)	B = 137.03 **+ 60.47** S (R^2 = 0.06, **p = 10^{-5}**)
2008 May	B = 75.56 **+ 105.2** Nsp (R^2 = 0.36, **p = 10^{-16}**)	B = 99.64 **+ 66.99** D (R^2 = 0.20, **p = 10^{-13}**)	B = 139.77 + **132.82** S (R^2 = 0.18, **p = 10^{-12}**)
2008 August	B = 49.68 **+ 28.06** Nsp (R^2 = 0.10, **p = 10^{-7}**)	B = 60.8 **+ 17.09** D (R^2 = 0.03, **p = 0.005**)	B = 71.57 **+ 31.45** S (R^2=0.03, **p = 0.004**)
Glasshouse experiment	B = 2.17 **+ 0.25** Nsp (R^2 = 0.11, **p = 10^{-10}**)	B = 2.09 + **0.59** D (R^2 = 0.05, **p = 10^{-5}**)	B = 2.42 **+ 0.29** S (R^2=0.03, **p = 10^{-4}**)

Table 2. Effect of sown species richness (Nsp), realized diversity (D) and survivor species richness (S) on aboveground biomass production (B) of experimental communities in the Jena and glasshouse experiments.
In 2004, only May harvest data were available. Sown species richness and survivor species richness were log-transformed in all analyses in the Jena experiment. Model coefficients in bold are significant (p<0.05).

Year	**Harvest**	**Model 1: B ~ Nsp + R**	R^2	**Model 2: B ~ Nsp + S**	R^2
2003	May	B = 334.74 **+ 128.19 Nsp - 106.04 D**	0.14	B = 345.75 **+ 107.85 Nsp** - 54.16 S	0.13
	Aug	B = 141.9 **+ 88.61 Nsp** - 45.41 D	0.11	B = 144.71 **+ 73.33 Nsp** - 50.10 S	0.11
2004	May	B = 365.82 **+ 172.43 Nsp - 334.23 D**	0.15	B = 385.54 **+ 152.69 Nsp - 243.46 S**	0.11
2005	May	B = 180.51 **+ 130.43 Nsp - 185.71 D**	0.14	B = 193.93 **+ 122.96 Nsp - 141.10 S**	0.13
	Aug	B = 68.80 **+ 56.07 Nsp - 70.66 D**	0.13	B = 74.17 **+ 59.98 Nsp - 71.43 S**	0.14
2006	May	B = 170.21 **+ 235.86 Nsp - 336.81 D**	0.22	B = 187.71 **+ 192.37 Nsp - 184.58 S**	0.16
	Aug	B = 72.59 **+ 72.40 Nsp - 81.68 D**	0.26	B = 79.02 **+ 74.56 Nsp - 75.62 S**	0.26
2007	May	B = 224.90 **+ 338.38 Nsp - 538.83 D**	0.34	B = 255.86 **+ 326.43 Nsp - 442.57 S**	0.33
	Aug	B = 95.96 **+ 79.72 Nsp - 69.38 D**	0.25	B = 100.83 **+ 78.67 Nsp - 58.32 S**	0.25
2008	May	B = 75.01 **+ 110.03 Nsp** - 12.20 D	0.35	B = 76.90 **+ 125.44 Nsp** - 44.20 S	0.36
	Aug	B = 48.63 **+ 38.93 Nsp** - 32.70 D	0.11	B = 51.24 **+ 40.34 Nsp** - 31.25 S	0.11
Glasshouse experiment		B = 2.13 **+ 0.25 Nsp** + 0.04 D	0.11	B = 2.25 **+ 0.28 Nsp** - 0.1 S	0.11

This is online supplementary material to *"Sown species richness and realized diversity can influence functioning of plant communities differently"* **by Terezie Rychtecká[a,c,*], Vojtěch Lanta[b], Iva Weiterová[a], Jan Lepš[a,c]**

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Table S1. Marginal and partial effects of total survivor species richness (Sall) on aboveground biomass production (B) of experimental communities in the Jena experiment.

In 2004, only May harvest data were available. Total survivor species richness was log-transformed in all analyses. Model coefficients in bold are significant ($p<0.05$).

Year **Harvest**		**Model 1: B ~ Sall (marginal effect)**	R^2	**Model 2: B ~ Nsp + Sall (partial effect)**	R^2
2003	May	B = 343.97 **+ 109.15 Sall (p=10^{-12})**	0.14	B = 343.82 + 1.37 Nsp **+ 107.51 Sall**	0.13
	Aug	B = 149.91 **+ 73.97 Sall (p=10^{-11})**	0.13	B = 159.76 - 3.18 Nsp **+ 77.86 Sall**	0.12
2004	May	B = 381.48 **+ 61.85 Sall (p=10^{-4})**	0.03	B = 375.15 + 80.91 Nsp - 36.63 Sall	0.04
2005	May	B = 209.71 **+ 73.82 Sall (p=10^{-5})**	0.06	B = 186.70 **+ 149.68 Nsp - 112.57 Sall**	0.10
	Aug	B = 77.11 **+ 42.93 Sall (p=10^{-7})**	0.08	B = 68.02 **+ 34.84 Nsp** + 0.08 Sall	0.09
2006	May	B = 208.81 **+ 128.51 Sall (p=10^{-8})**	0.09	B = 171.76 **+ 218.87 Nsp - 133.49 Sall**	0.14
	Aug	B = 81.58 **+ 54.91 Sall (p=10^{-13})**	0.15	B = 70.10 **+ 84.53 Nsp - 51.95 Sall**	0.22
2007	May	B = 244.50 **+ 143.57 Sall (p=10^{-9})**	0.10	B = 223.59 **+ 515.13 Nsp - 509.74 Sall**	0.26
	Aug	B = 102.22 **+ 70.75 Sall (p=10^{-12})**	0.19	B = 94.88 **+ 69.18 Nsp** - 18.28 Sall	0.22
2008	May	B = 97.14 **+ 129.34 Sall (p=10^{-16})**	0.30	B = 75.40 **+ 102.42 Nsp** + 4.07 Sall	0.35
	Aug	B = 60.12 **+ 32.49 Sall (p=10^{-5})**	0.07	B = 49.95 **+ 39.53 Nsp** - 17.97 Sall	0.10

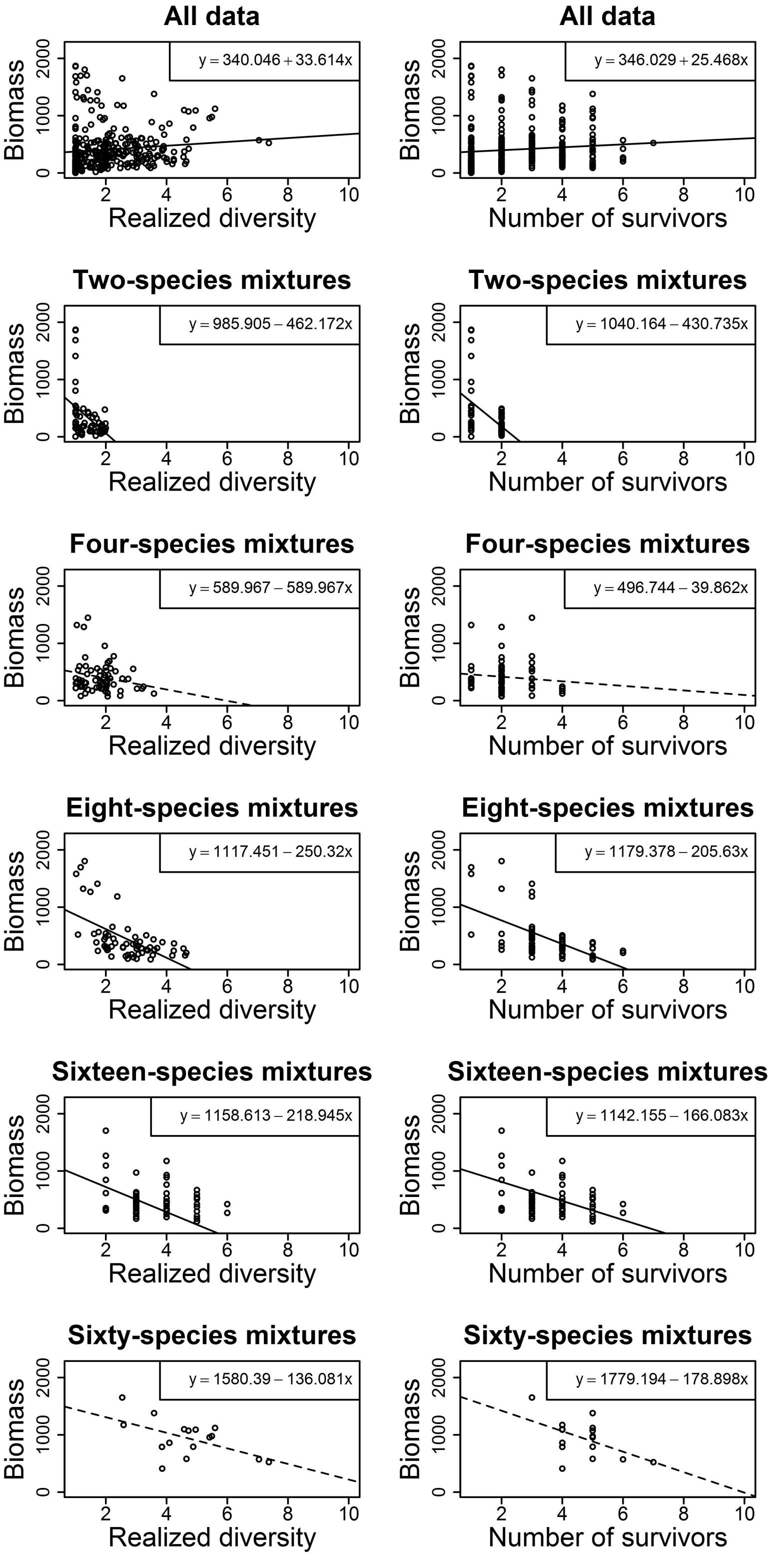

Fig. 1. Jena experiment: relationships between biomass production in 2007 and realized diversity or survivor species richness for mixtures. Regression lines in bold denote significant relationships, dashed lines non-significant ones.

Chapter IV

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Plant density affects measures of biodiversity effects

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Abstract

Aims:
We tested for the effect of final sowing plant density (i.e. density of established seedlings) on the values of biodiversity effects [transgressive overyielding, net effect, complementarity effect (CE) and selection effect (SE), trait-dependent complementarity and dominance effect] in a glasshouse pot experiment.

Methods:
We conducted a single-season (4 months) glasshouse experiment. Species monocultures and mixtures containing up to four common meadow species from different functional groups were sown and subsequently thinned to five density levels (8–128 individuals per pot, i.e. 200–3200 individuals m^{-2}). Community functioning was characterized by yield (both living and dead biomass) of all constituent species.

Important Findings:
Our results show that plant density (final sowing density in our case, but this finding can be generalized) affects the yields of both monocultures and mixtures. As these and their relationships are the basis for calculation of biodiversity effects, these effects also varied along the density gradient. Net biodiversity effect, CE and SE all increased with density. The net biodiversity effect and the CE switched from negative to quite positive in the four-species mixture. Using Fox's tripartite partitioning, trait-dependent complementarity was minor in comparison to the dominance effect. One of our experimental species did not follow the density-productivity relationship, called constant final yield (CFY), which was reflected in the biodiversity measures. The shape of the density-productivity relationship for experimental species affects also the values of biodiversity indices, particularly when species do not follow the CFY relationship. According to our data and recent simulation experiments, the values of commonly used biodiversity effects can be rather misleading if a species has, e.g. a unimodal dependence of yield for the density gradient and the density level used in the experiment is higher than the peak density.

Keywords: biodiversity effects • plant density • constant final yield

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INTRODUCTION

Global biodiversity loss during the past few decades has stimulated numerous scientific studies about the importance of species richness for ecosystem functioning (Hooper *et al.* 2005; Loreau *et al.* 2001). Many studies were focused on so-called biodiversity effects, i.e. the way in which diversity affects ecosystem function and services (for reviews see Cardinale *et al.* 2006; Chapin *et al.* 2000; Hooper *et al.* 2005; Loreau *et al.* 2001). Ecosystem functioning is an umbrella term for various processes operating in an ecosystem, mainly flow of energy and matter within and between ecosystems (e.g. primary productivity, nutrient cycling, etc.). Yield is most often considered as a measure of functioning because it can be measured relatively easily and it would be detrimental for humans if this function would deteriorate with decreasing diversity (Sala *et al.* 2000). Recent meta-analyses have shown that, on average, species-rich communities 'function' better than poor ones (Balvanera *et al.* 2006; Cardinale *et al.* 2006). Increased functioning with species richness is usually explained by two mechanisms: complementarity effect (CE) and selection effect (SE). Complementarity results from better resource use due to differences in traits among species in a community. This means that the niche space is better filled when numerous species are present. The SE creates a positive relationship between species

Chapter V

Analysis of biodiversity experiments: a comparison of traditional and linear-model-based methods

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ABSTRACT: The relationship between diversity and ecosystem functioning is often studied by biodiversity experiments. The traditional method used for evaluation of these experiments is the additive partitioning of net biodiversity effect by Loreau and Hector. Recently suggested methods of Kirwan (diversity—interaction model) and Bell (random partition model) are referred to as the linear-model-based methods. We compared data required and results given by both traditional and linear-model-based methods using three data sets: a simulated data set and two pot biodiversity experiments. For the simulated data, we also compared expected outputs based on defined ecological species traits with actual outcomes of the methods. BEF experiments were designed to answer five fundamental questions:

Q1) How does sown species richness change ecosystem functioning?
Q2) What ecological mechanisms cause this change?
Q3) How does the importance of ecological mechanisms change with sown species richness?
Q4) Which species are responsible for given ecological mechanisms?
Q5) How do other possible treatments change answers to all questions above?

We have shown that all methods were perfectly capable of answering Q1 and Q5 although different methods use different procedures to reach the answer. Concerning Q2-Q4, traditional methods provide more detailed insight than the linear model-based-methods which leave us just with brief information. A direct comparison between traditional biodiversity effects and effects from linear-model-based methods showed that interactions from the diversity—interaction model were significantly positively correlated with the net effect while species identity effects from diversity—interaction model were related to the species relative yield. The selection of an appropriate method for BEF experiment analysis thus depend on the questions we ask which in turn also affect the design of our BEF experiment. BEF experiments using short sown species richness gradient are capable of answering all the suite of questions presented above and we suggest using traditional methods and benefitting from the detailed information they provide at the species level and from their ability to address potential ecological mechanisms. Such results can be used to better understand mechanisms operating in larger communities. In BEF experiments with longer sown species richness gradient and/or larger pool of experimental species, usage of the traditional methods is often restricted by our ability to collect all the data needed; in this case, the linear-model-based methods provide a proper way to analyze the data and enable a general understanding of the studied system.

KEYWORDS: biodiversity effects, diversity—interaction models, species identity, species interactions, sown density

INTRODUCTION

Increasing species loss due to human impact and related concerns about deterioration of ecosystem services for humanity triggered the development of biodiversity—ecosystem functioning (BEF) experiments as a new branch of ecological research (Hector 1998, Diaz *et al.* 2006, Yesson *et al.* 2007, Dickson and Wilsey 2009, Mora *et al.* 2011). Their main purpose is to evaluate the relationship between species richness and various "ecosystem functions". In these experiments, the species richness is manipulated and subsequently treated as an independent variable and the measure of functioning (in plant ecology usually aboveground biomass) is considered as a response variable. The traditional belief that the relationship should be positive was supported by a majority of plant ecology BEF studies which used aboveground biomass as a measure of functioning (e.g. Hooper *et al.* 2005, Spehn *et al.* 2005, Cardinale *et al.* 2006).

To explain the positive diversity—functioning relationship, two mechanisms were suggested: the selection effect and the complementarity effect (Loreau 2000). The selection effect is a probabilistic phenomenon: with increasing sown species richness the probability that a species "doing the job" (i.e. having positive impact on the function) will be included increases as well. The complementarity effect builds on ecological mechanisms: niche differences among species, such as interspecific differences in resource use, enable more efficient acquisition and utilization of resources which in turn increase the functioning. The BEF experiments have become a standard part of plant community ecology and so has the traditional method of their analysis – the additive partitioning (Loreau and Hector 2001).

However, the comparison of performance of mixtures of various species diversity and of mixtures and monocultures is not a trivial task and has been discussed from the early days of biodiversity experiments (Aarssen 1997, Garnier *et al.* 1997). As a matter of fact, various methods of analysis were used much earlier in the intercropping research (Trenbath 1974). The fairly most often used is the (already mentioned) net effect additive partitioning (Loreau and Hector 2001), together with a simple comparison of the mixture function with the function of the best monoculture: the measure of transgressive overyielding (Garnier *et al.* 1997). We will call these methods "traditional" in further text. Recently, methods based on linear models have been suggested (Bell *et al.* 2009, Kirwan *et al.* 2009, Connoly *et al.* 2013), we will refer to them as „linear-model-based"methods.

These two groups of methods were classified as semi-mechanistic or mechanistic respectively (Hector *et al.* 2009). The terms "mechanistic" and "semi-mechanistic" should, however, be understood with great caution – all the mechanisms are inferred just from the changes in functioning; to prevent any misunderstanding, we will not use this terminology. Our aim in this contribution is to compare the traditional and the linear-model-based methods for biodiversity experiments analysis in general: their specific requirements, their advantages and disadvantages and the ecological interpretations of their outputs. To illustrate the differences among the methods and compare their results, we analyzed data from three BEF experiments. The first data set is a simulated biodiversity experiment (SE in further text) using four species with defined traits providing expected outcomes. The second data set originates from a glasshouse biodiversity experiment in which one to four species were planted in mixtures ranging from low to high initial sown density (GE1). The last data set is a seasonal glasshouse pot experiment using six plant species grown in all possible combinations (GE2).

As the individual methods present their results in a rather different way, we aim mainly to compare the ecological interpretations of the numerical results. Though, we also match the outputs of different methods directly and seek for correlations. We stick to the species level interpretation as it is the species level information that primarily enters all the analyses. In our opinion this provides the best insight into how different methods work and deal with the challenge to evaluate ecological mechanisms operating in a community. To our knowledge, there has been no such comparison of methods for biodiversity experiment evaluation (based on real and simulated data, using both traditional and linear-model-based methods) done to date.

METHODS

We will first mention some general points concerning the data required for and procedures applied in all methods. We will also present their outputs then provide details on each method used in this contribution. Finally, we briefly describe the datasets studied. In the following text, we use terms biomass and yield, as they are used in “classical” BEF experiments in plant ecology, nevertheless, there might be also different measures of “ecosystem functioning”.

Data required

Various types of input data are required by various methods for BEF experiment analysis (Table 1). In particular, the additive partitioning (Loreau and Hector 2001) requires two information components which are often complicated and laborious to gain: monoculture yield of all constituent species and yield of each species in all mixtures. Practically, this means sorting all the harvested biomass into individual species, which is laborious, but still feasible to do; if some emergent property (e.g. nutrient leaching or gaseous emissions) is considered to be a response, determination of individual species contributions is even more problematic. Both these pieces of information are very useful for subsequent biological interpretation; however, they considerably restrict the experimental setup, limit the number and size of experimental/sampling units and the length of species richness gradient. The linear-model-based methods do require neither of these: this allows for larger species pool size or treatment/replicate range.

General differences among procedures and outcomes

One of the most important differences among the methods for evaluation of biodiversity experiments lies in the procedures we apply. The traditional methods, such as overyielding and additive partitioning (Garnier *et al.* 1997; Loreau 1998; Loreau and Hector 2001) use a two-step procedure: in the first step, the biodiversity effects for each mixture are calculated separately. Effects of all possible predictors (species richness, number of functional groups, sowing density etc.) on these effects are analyzed in the second step. Statistically, the biodiversity effect values for single replications are not independent data points: they are all based on a limited number of monoculture yields. This may inflate the significance of statistical tests. In spite of these facts, a majority of studies neglect this problem and use standard statistical methods.

Linear-model-based methods, such as the diversity—interactions model (Kirwan *et al.* 2009) or analysis of random partition design (Bell *et al.* 2009) apply classical statistical methods. The diversity effects are included in a single statistical model for the whole experiment (we have no information about biodiversity effects in individual plots). Species richness and/or other treatments can be easily included in a single model which is statistically credible: all biomass values entering it are independent data points.

The outcomes of traditional methods consist of one (e.g. overyielding) or several overall effects (e.g. selection, complementarity and net effects in additive partitioning) available for each sampling unit. In linear-model-based methods there are multiple species identities and interaction effects estimated for the whole experiment. However, this is more or less an arbitrary distinction. When calculating the overall effects in traditional methods, we use the individual species information (e.g. relative yields of single species used for calculation of the net biodiversity effect). Although it is usually not presented in papers, it can be potentially used for interpretation of mechanisms.

Species level effects have different interpretation in every method. In the traditional methods, species yield in mixtures is always related to monoculture yield and therefore correspond to species competitive strength. In the linear-model-based methods species identity effects cover species contribution to the overall yield and so these effects do not reflect any ecological mechanism directly.

There are notable differences even between the linear-model-based methods. The diversity—interaction model approach fits coefficients for each combination of species mixtures so we can examine or compare interactions between species. In the random partition design, we have no information on interactions between or among individual species, despite the fact that the coefficient associated with non-linear species richness is a proxy for species interactions. The only information we get from this coefficient value is whether there are interactions at all.

Methods used

Overyielding and relative yield measures

The measure of transgressive overyielding (OI) was calculated as $Y / \max(M_i)$, where Y is the total biomass of a given mixture and max(Mi) is the maximum of mean of monoculture biomass of all species constituting that mixture (I_1 of (Garnier *et al.* 1997) or so called transgressive OI). A value of OI greater than one is usually interpreted as evidence that resource partitioning and/or facilitation operates in the community (Beckage and Gross 2006; Schmid *et al.* 2008). If the yield of the mixture and the best monoculture is the same (i.e. comes from the same distribution), the OI has the median = 1, but the mean is higher and its distribution is positively skewed. Thus we log10 transformed OI for further analyses – under the same circumstances, the log OI is symmetrical around zero.

The relative (observed) yield (RYO, calculated for each species separately) tells us whether individual species gain more or less biomass in a mixture than expected from its performance in monoculture. RYO is defined as Y_O / Y_E, where Y_O is the observed yield in mixture and Y_E is the expected yield based on monoculture performance (corrected for proportion).

Additive partitioning

Additive partitioning (from now on referred to as the L&H method; Loreau and Hector (2001)) is based on relative yields and partitions the overall net biodiversity effect (difference between observed and expected yield) into two additive terms: the complementarity effect and the selection effect.

ΔY = Selection effect + Complementarity effect

Complementarity effect = $N\overline{\Delta\mathrm{RY}}\,\overline{M}$

Selection effect = $N\operatorname{cov}(\Delta\mathrm{RY}, M)$

where N is the number of species in the mixture and ΔRY is the deviation observed from the expected relative yield of species in a mixture. A positive complementarity means that, on average, the species have higher than expected yield in the mixture, which is interpreted as a consequence of niche partitioning (and thus complementary use of resources). Negative values are ascribed to inference competition. A positive selection effect indicates that species with greater than average monoculture yield dominates a mixture and *vice versa* for negative values. The tripartite partitioning method was introduced in order to improve the biological interpretability of net effect components (Fox 2005). This extension of additive partitioning further divided the complementarity effect into the dominance effect and the trait dependent complementarity (note, that despite the name, traits are NOT used in trait dependent complementarity). We decided not to include Fox's (2005), because this method is generally not used and in our detailed analysis GE1 experiment it did not seem to bring new insights (Stachová *et al.* 2013).

The diversity—interaction model

The diversity—interaction model approach (from now on referred to as Kirwan method; Sheehan *et al.* 2006; Kirwan *et al.* 2007; O'Hea *et al.* 2010; Connolly *et al.* 2011; Connolly *et al.* 2013) uses classical statistical fitting methods, where model coefficients reflect the effects of species identities, their interactions and treatments. Ecosystem response is explained by initial species abundances, sown proportions of each species, their (statistical) interactions and (possible) treatments. The sum of identity effects of species is called an identity effect (ID) and the sum of interaction terms is called a net biodiversity effect (DE). Two, three or more species interactions can be included into the DE. The sign of the interactions indicates whether the relationships between species are synergistic or antagonistic. Using such a model we can test the strength of ID/DE effects of/between species, average interaction effect, functional group or redundancy effect, community phylogenetic diversity effect and effect of environmental or other covariates. It is important to note, that statistical interactions (i.e. non-additivity of individual species effects) are interpreted as species interactions here.

We followed the Kirwan method and fitted a linear model with up to three species interactions

$$\textbf{(1)}\quad y=\Sigma_{i=1}^{s}\beta_i P_i+\alpha M+\Sigma_{i,j=1,i<j}^{s}\gamma_{ij} P_i P_j+\Sigma_{i,j,k=1,i<j<k}^{s}\gamma_{ijk} P_i P_j P_k$$

where P_i is the sown proportions of the *i*-th species, *M* is the initial abundance, *s* is the number of species and Greek symbols are the fitted coefficients. Here, α is the effect of overall initial abundance, β_i is the identity effect of the *i*-th species, γ_{ij} is the measure of the strength of inter-specific interaction between species *i* and *j.*

Random partition design

The method of random partition design (from now on referred to as the Bell method; Bell *et al.* 2005, Bell *et al.* 2009) is primarily targeted at analyzing data from communities with large species pools (e.g. Gravel *et al.* (2011)) and bears several similarities to the diversity—interaction interaction model. The experimental design takes a full species pool *N* and forms a diversity gradient by dividing by integer factors of *N* (i.e. the species pool is randomly divided in half, randomly divided into thirds etc.). This approach is then repeated using different random selections to produce different replicates, termed partitions. Each species is randomly drawn without replacement which ensures that within each replicate partition each species is present only once at every level of diversity. The method fits a model which includes terms for species richness (one as a continuous and the second as a categorical variable), the presence/absence (identity) of each species, and the composition of the community. There is also a possibility to include other treatments. In their latest contribution to the biodiversity experiment analysis methodology, Connolly *et al.* 2013 proposed the generalized diversity interaction model framework (GDI) which includes both Bell (2005, 2009) and Kirwan (2007, 2009) methods as special cases of the GDI, although experimental design suitable for these methods substantially differ.

The method fits a model

$$\textbf{(2)}\quad y=\beta_0+\beta_{LR}x_{LR}+\beta_{NLR}x_{NLR}+\Sigma_{i=1}^{s}(\beta_i x_i)+\beta_Q x_Q+\beta_M x_M+\beta_D$$

where β_0 is the intercept, β_{LR} is the coefficient associated with linear richness (richness treated as a continuous variable), β_{NLR} is the coefficient associated with species richness treated as a factor (proxy for species interactions), β_i is the coefficient associated with the presence/absence (1/0) of each species, β_Q is the coefficient associated with each partitioned species pool, β_M is the coefficient associated with each mixture and β_D is the coefficient associated with the final/initial sowing density when applicable. Sums of squares associated with terms are calculated sequentially according to their order in Eq. 2.

Methods comparison and statistical analyses

We directly compared species identity effects and interaction effects (product of identity effects) from diversity—interaction model with traditional biodiversity effects (net effect, selection and complementarity effects, species RYO and OI). All values were centered and standardized across all datasets and also within single experiments. To test for significant correlations, Pearson's product moment correlation coefficient and significance test for associations of paired samples were applied. Values for identity effects and pair wise species interactions were directly taken from the diversity—interaction model. For traditional effects mean value of all pots containing given species (for comparison with species identities) or pair of species (comparison with pair wise interaction) was used. Resulting from analogous methods, the identity effects from the Bell method were tightly correlated with identity effects from the Kirwan method. Comparison results were thus similar and we present results of the Kirwan method only.

Experimental datasets

Simulated experiment: (detailed model description in Fibich and Lepš 2011)

Our data were generated by a spatially explicit individual based model (Fibich & Lepš 2011) using a field of neighborhood (FON) approach to model competition (Berger *et al.* 2006). In our simulated experiment we used 4 species, all possible species combinations within all species richness levels, at a density of 400 individuals.

Species traits defining ecological expectations

The simulated species differ in two ecological traits: shade tolerance and maximal biomass (i.e. monoculture yield), each having two levels, high and low (see Table 2). Species 1 is highly shade tolerant and has low maximal biomass which enables it to withstand competition despite its small size. Species 2 is less shade tolerant and has low maximal biomass which forms the weakest species of all. Species 3 is highly shade tolerant and has high maximal biomass which defines the strongest species and species 4 is less shade tolerant with high maximal biomass which makes it highly productive in monoculture but not so efficient in mixtures.

With known species traits, we are able to define, which ecological mechanisms will be important, and so predict the values of diversity effects for two-species mixtures (Table 3). It is more complicated to predict the outcomes of linear-model-based methods. The term interaction for the effect from the Kirwan method intuitively leads us to conclusion, that it is similar to the complementarity effect. However, if we examine the linear model closely, it is obvious that these statistical interactions cover the deviation from additivity. This defines exactly the net biodiversity effect. The values of interaction effect thus suggest that species do interact without any further specification of the nature of these interactions (which is attempted by additive partitioning). The same analysis of the linear model show that the identity coefficients from diversity—interaction model correspond roughly to species monoculture yields, however, modified by their behavior in mixtures.

Glasshouse experiment 1 (GE1): (detailed experiment description in Stachová *et al.* 2013)

We used our data from a pot biodiversity experiment with four meadow plants sown in all possible combinations which also included five levels of sown densities. The experiment was terminated after four months. Aboveground living biomass was clipped 0.5 cm above ground and sorted to individual species. The response variable used in all following analyses is the weight of dry living biomass.

Glasshouse experiment 2 (GE2): (detailed experiment description in Špaeková and Lepš 2001)

For this experiment six naturally co-occurring species (three grasses and three herbs) were planted in pots in two initial densities. All possible species combinations within all species richness levels were used. After five months, aboveground biomass was assessed and sorted to individual species and was used as a response variable in our analyses.

RESULTS

Results are divided into four subsections (three data sets and an overall comparison). As the main aim of this paper is to compare the methods and interpretation of their outputs, we do not provide detailed results of single experiments (but see the OSM with additional figures and statistical tables – these start with S in further text).

Simulated Experiment

All methods identified the dominant and weak species in terms of yield. The analysis of overyielding and species RYO showed that species which were shade tolerant overyielded while the two less shade tolerant species underyielded (Fig. S1). Species identities coefficients from both linear-model-based methods identified the strongest (strong–big) and the weakest species (weak-small) concordantly with RYO analysis. However, there was a difference between the RYO analysis and the linear-model-based methods in the second most successful species: RYO identified the shade tolerant species with low maximal biomass (strong–small), whereas the linear-model-based methods the less shade tolerant species with high maximal biomass (weak–big). As already noted, RYO relates species mixture performance to its monoculture performance, while the linear model identity coefficients reflect the overall performance of a given species in all data. Thus, species success in mixtures (given by shade tolerance) was decisive for the RYO value in contrast to the value of linear model coefficient which is determined by species contribution to overall yield, which is naturally related to species performance in monoculture (defined as maximal biomass). We also tested our predictions about overyielding and diversity effects in two-species mixtures. The diversity effects (selection and complementarity effect) and overyielding index were in concordance with our predictions based on species traits (see Table 3).

Regarding the interactions from linear model-based-methods, almost all the mixtures of species with significant interaction had high complementarity effects determined by traditional methods, but some mixtures with high complementarity effects did not have a significant interaction in linear models. In both linear models the species identities were more important than species interactions. In traditional methods, the selection effect was stronger than the complementarity effect. The positive relationship between biomass production and number of species in the mixture was present in all methods (traditional and linear-model-based): in Bell method the coefficient associated with linear species richness was positive and the coefficients for non-linear species richness revealed that this relationship is mainly caused by the two-species mixtures. Also the Kirwan method predicted increase of biomass production with increasing number of species.

Glasshouse Experiment 1

As in SE, the dominance order of species differed between the traditional and linear-model-based methods (see OSM). The two most dominant species were reversed in the two groups of methods. In the analysis of RYO, the most dominant species performed poorly in monoculture, as it formed a dense canopy which resulted in high amount of litter at the time of the harvest (we used living biomass only in all analyses). This species highly overyielded in mixtures, where the strong intraspecific competition was alleviated. On the other hand, the linear-model-based methods identified as the most dominant species that was strong both in monoculture and in mixtures. The highest complementarity effect was found for the mixture of the two competitively weak species while the highest selection effect was found for the mixture of the strongest (according to RYO) with the weakest species. In this experiment the complementarity effect was stronger than the selection effect. Two out of five significant interactions from the Kirwan method were in concordance with high complementarity mixtures.

The Kirwan method species level results are often visualized in the ID and DE plot. Here, the ID curve (values of a given species identity coefficient) and DE (a sum of given species interaction coefficients) is plotted against increasing proportion of species in a mixture. Results for GE1 showed that IDs were always stronger than DEs (Fig. 1). Interestingly, the highest yield and DEs were predicted in mixtures without the species that was a dominant (Fig. 1C). Also, the predicted yield in mixtures containing the weakest species of all and its ID increased with an increasing number of species in the mixture. On the contrary, IDs of the two dominant species reached their maxima in monocultures and their IDs decreased with increasing species richness. (Fig. 1C, D).

Glasshouse Experiment 2

In GE2, the most and the least dominant species were identified concordantly, however, there were some slight differences (2 species reversed) in the dominance order of all species (see OSM). In this experiment with slightly larger species pool we did not perform mixture-level analyses of biodiversity effects, as the mixtures are too numerous. Instead, we focused on the sown species richness—diversity effect relationship. For this type of analyses, there is no counterpart in the linear-model-based methods. We found that the selection effect was increasing with number of sown species and the complementarity effect was irrespective of number of species.

In GE2, all the significant species interactions determined by the Kirwan method (two thirds of all possible interactions) were positive and the diversity effects of all species followed mostly unimodal shape which predicts the strongest species interactions in two-species mixtures. In addition, only one interaction out of fifteen was negative. It is important to note, that most pronounced interactions were between the strong grasses and the weakest species of all which was a herb. Here it is clear, that although the interaction coefficient indeed means some kind of interaction, it is a way different from what we usually have in mind when using this term in ecology. The herb was performing poorly whichever mixture it appeared in. Its presence allowed the dominant grasses to efficiently use more “space” which resulted in the increase of yield. However, this does not mean any “special” relationship operating between grasses and the herb and the fact, that a dominant species nearly drives a subordinate species to extinction is not what an ecologist imagines when speaking of “positive species interaction.”

Overall comparison of the effects

Species identity effects (ID) from diversity—interaction model and species relative observed yields (RYO) were positively correlated (significantly for all data and for GE2; Fig 2). No correlations of IDs with effects from traditional methods (net, complementarity and selection effects and OI) were observed. Species interaction effects from diversity—interaction model were positively correlated with overyielding and all additive partitioning effects (significantly for all data only; Fig 2). The strongest correlation was observed between the interactions and the net effect (significantly for all data and all experiments).

DISCUSSION

BEF experiments were designed to answer five fundamental questions:

Q1) How does sown species richness change ecosystem functioning?
Q2) What ecological mechanisms cause this change?
Q3) How does the importance of ecological mechanisms change with sown species richness?
Q4) Which species are responsible for given ecological mechanisms?
Q5) How do other possible treatments change answers to all questions above?

We used both traditional and linear-model-based methods, applied them to three data sets and compared their answers to the questions stated above. Q1 and Q5 can be answered adequately by both the traditional and the linear-mode-based methods. Concerning Q1: in all our datasets, the biomass production increased with mixture size (sown species richness). All methods used in this comparison came to this conclusion, although the procedures applied to reach it differed: in traditional methods, a linear regression is applied while in linear-model-based methods, the effect of sown species richness is either inherently present (random partition design) or can be readily included (diversity—interaction model). Now every method gives us an opportunity to explore this positive relationship into bigger detail. In the random partition model (Bell method), there is a coefficient associated with non-linear species richness which reveals which species richness level drove the overall relationship. For example, in two of the datasets we studied, GE1 and SE, the effects of non-linear species richness were positive for two-species mixtures only, showing that the majority of overyielding happened in these mixtures and overrode the negative effect of 4- species mixtures (again, this result was obtained by all methods used). In GE2, there were all mixtures associated with positive coefficients (only monocultures had a negative coefficient).

When answering Q5, the situation is similar as for Q1: other predictors than sown species richness are either analyzed separately (traditional methods) or included in the model right away (linear-model-based methods). In our datasets this additional treatment was final sowing density (GE1) and all of the models concordantly demonstrated that the ecological interpretation of results depends on the sowing density (also shown by Jolliffe 2000; Fibich and Lepš 2011; Stachová *et al.* 2013).

Concerning Q2 and Q3, traditional methods seem to be giving more ecologically relevant answers than the linear-model-based. The complementarity and selection effects are suggestive of an ecological mechanism. We managed to show that these traditional effects indeed depict ecological mechanisms by inspecting their values at species level (Q4): by designing SE with known species traits we were able to generate expected outputs. All effects in traditional methods matched expected results: OI was the highest in mixtures of species the strongest and the weakest species and high complementarity was found for the mixtures of shade tolerant species (Table 3). On the other hand, the interaction coefficient from the diversity—interaction model is equivalent to the net effect, which simply means that species interact without any further specification. This interaction in a statistical sense does not necessarily mean a biological interaction. The Kirwan method applies interactions between the sown proportions of species; therefore the coefficients of interaction terms can also be compared between themselves to show differences between mixtures. The meaning of a significant interaction in the linear model differs between transformed and un-transformed data. In untransformed data, a significant interaction is a deviation from additivity (and corresponds to net effect) while for log-transformed data it is a deviation from multiplicativity and it is not clear what the deviation from multiplicativity means biologically (we used non-transformed data). However, neither deviation from additivity nor from multiplicativity can be considered as proof that species mechanistically interact in the field. For example, in GE2, there was a significantly positive interaction for a pair consisting of a dominant and an inferior species. In most mixtures, the dominant species nearly wiped out the inferior species which is not generally considered a positive interaction. If we want to study the biodiversity effects more closely, we inevitably end up at the species level (Q4). When using the traditional methods we may ask which species cause overyielding, which are complementary and which are driving the selection effect. In the linear-model-based methods the species-specific effects are their identity effects. We already mentioned their relation to monoculture yields of given species. Species which overyield also often have high monoculture yields, which is why all the methods used identified concordantly the weakest and the strongest species in all datasets studied. However, some species were placed differently at the gradient from competitively strong to weak species and the reasons were already presented in the Results section. The notion, that species IDs are related to the parameter maximal biomass (monoculture) was also shown in SE. There, ID coefficients were higher for species with high maximal biomass than for species with small maximal biomass. Even when using the Kirwan model, we are able to reconstruct (to some extent) what is happening within the community by inspecting the ID, DE and predicted yield plot. GE1 may serve as a good example. In this experiment, the resulting predicted yield was determined by the slope of ID. Species whose IDs increased with their proportion (highest ID in monoculture) were the successful ones while those with decreasing ID were the weak ones. The presence of the weakest species and, quite counter intuitively, of the second dominant decreased the predicted yield of mixtures (Fig. 1). This has a very different explanation for each species. In the case of the weakest species, the phenomenon is caused by its low yield (ID curve) given by its creeping life form. The same effect (total yield reduction) was caused just by the opposite properties of the second most dominant species. Its relatively high biomass production (ID curve) was not sufficient to compensate for its negative interactions with other species (diversity effect curve) which in turn decreased the overall production. The example of GE1 shows that from the information we obtain from the Kirwan method, we are not able to say anything more about the mechanisms operating among experimental species. Here we can make use of the “costly” information we collected for the L&H method of additive partitioning: single species contributions to mixture biomass. From these data, we knew that the dominant species was highly successful in competition, which led to substantial elimination of the other species co-occurring in the mixture and thus ending in a lower yield for the mixture. This is a nice example of the drawbacks of the linear-model-based methods. If we are interested in the exact nature of the relationships operating among species, these methods leave us with just a brief impression and many of our questions remain unanswered. It is only logical that there is a trade-off between costly collecting of data values for individual species performance in mixtures which also give us an invaluable piece of information, and the elegant, not so time consuming design analysis, which gives us more or less suggestions of what could be happening in the system.

Our attempt to compare biodiversity effects and coefficients from linear-model-based methods showed that species IDs are positively correlated only with RYO, but not with selection effects, as one might presume. The relationship between the selection effect and IDs suggest that species with either extremely low or extremely high ID have strong selection effects. This reflects that if mixture is supposed to have a strong selection effect, there must be a species which is either extremely strong (which suppress its partner in the mixtures) or extremely weak (which allows even moderately strong species to dominate).

Correlations between species interactions effects from diversity—interaction model and additive partitioning effects (net effect, selection and complementarity effects) or overyielding were positive in all cases. Both selection and complementarity effects directly contribute to the functioning of the communities (have additive effect on the final yield) and species interaction's effects from linear-model-based methods are defined in the same way. Moreover, the interactions are compound effects, because they cover all species interactions (positive and negative), and therefore we observed the best and tight correlation with the net effect (sum of the selection and complementarity effects; Fig. 1). Correlation with overyielding was not as strong as with the net effect, because transgressive overyielding we used divides mixture yield by the best monoculture and this equalizes values for many species mixtures.

Based on the analysis of our data sets, we conclude that the selection of an appropriate method for BEF experiment analysis thus depend on the questions we ask which in turn also affect the design of our BEF experiment. BEF experiments using short sown species richness gradient are capable of answering all the suite of questions presented above and we suggest using traditional methods and benefitting from the detailed information they provide at the species level and from their ability to address potential ecological mechanisms. Such results can be used to better understand mechanisms operating in larger communities. In BEF experiments with longer sown species richness gradient and/or larger pool of experimental species, usage of the traditional methods is often restricted by our ability to collect all the data needed; in this case, the linear-model-based methods provide a proper way to analyze the data and enable a general understanding of the studied system.

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Table 1. Requirements of the common biodiversity methods denote if monocultures (single species performance), final species contributions (how much each species contributed to the final mixture performance) or initial species proportions are necessary for the methods.

Data required	**Traditional methods**			**Linear-model-based mehods**	
	Overyielding	Relative yield	Loreau & Hector method*	Bell's method**	Kirwan's method***
Monocultures	✓	✓	✓	x	x
Final species contributions	x	✓	✓	x	x
Initial species proportions	x	✓	✓	x	✓

* Additive partitioning (Loreau and Hector 2001), ** The method of random partition design (Bell et al. 2009), *** The diversity—interaction model (Kirwan et al. 2009)

Table 2. Species parameters in the simulated experiment. Other parameters are in the (Fibich & Lepš 2011) except growth rate, which is lower in our simulated experiment (0.05).

Species (abbrev.)	**Maximal biomass**	**Shade tolerance**	**Description**
strong-small(ss)	100 (low)	1.1 (high)	Can withstand competition in mixture
weak-small (ws)	100 (low)	0.7 (low)	Weakest species
strong-big (sb)	800 (high)	1.1 (high)	Strongest species
weak-big (wb)	800 (high)	0.7 (low)	Efficient in monoculture, but weak in mixtures

Table 3. Expected additive selection and complementarity effects and overyielding for two species mixtures in the simulated experiment. More or less + denote stronger or weaker effect. For species characteristics see Table 2.

Two-species mixture	Selection effect	Complementarity effect	Overyielding
strong-small & weak-small	+	+	++
strong-small & strong-big	++	+++	+
strong-small & weak-big	+	++	+
weak-small & strong-big	+++	++	+
weak-small & weak-big	++	++	+
strong-big & weak-big	++	+	++

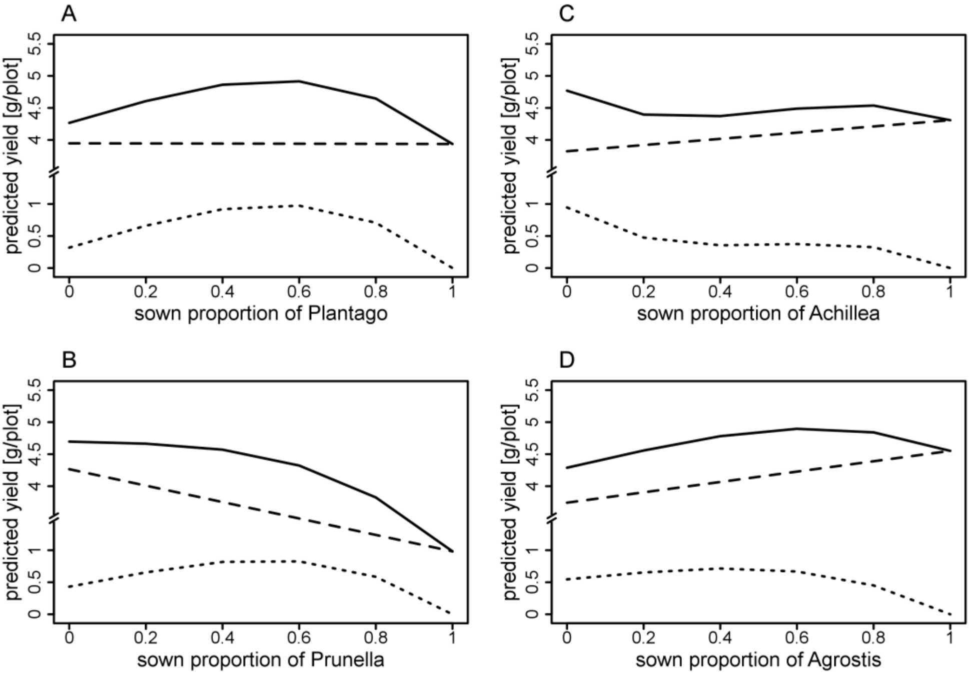

Fig.1. GE1: Mean yield predicted by the linear model (Table 4 in the main text) of the Kirwan method for average density. Total predicted yield (solid line) is the sum of the identity (ID, dashed line) and diversity effects (DE, dotted line). Only the proportion of one species is varied in one subplot.

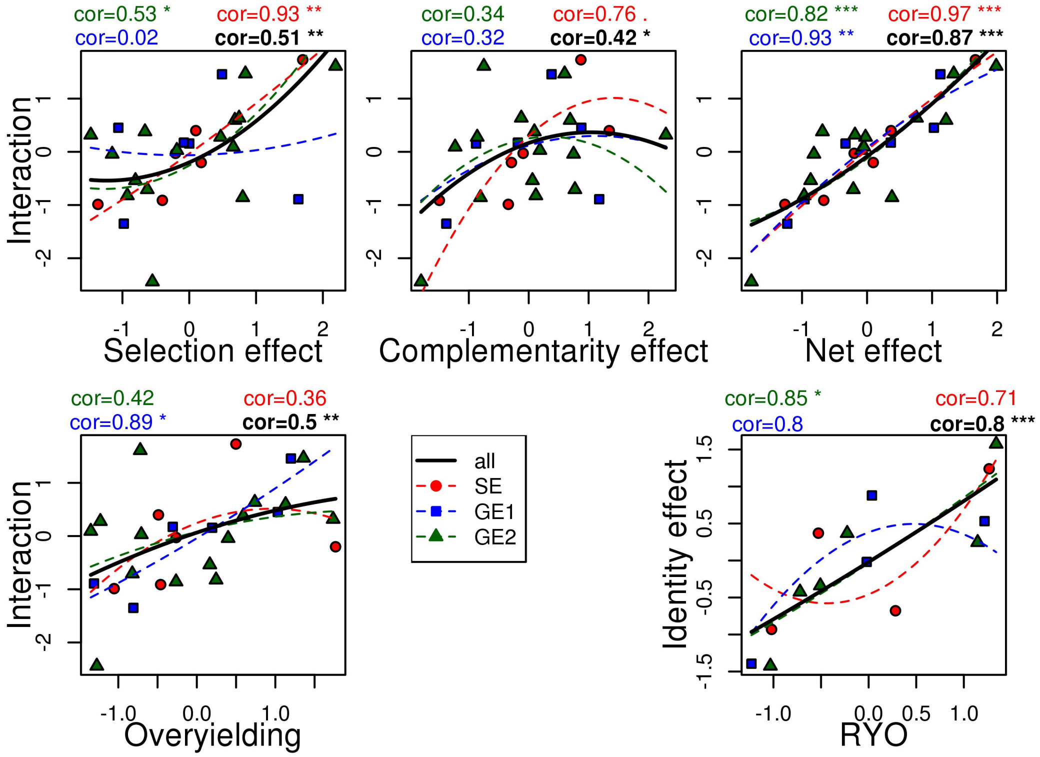

Fig 2. Scatter plots of species interactions and identity effects, relative yield total, additive partitioning effects and overyielding for all three experimental datasets. Data points were centered and standardized. Symbols and colors correspond to experiments. Lines correspond to the fitted second degree polynomial (dashed lines for single experiments and bold for all data pooled together). Cor is Pearson correlation coefficient with stars for P-values (***<0.001, ** <0.01, * <0.05, . < 0.1).

Supplementary material for Fibich et al. 2014: Analysis of biodiversity experiments: a comparison of traditional and linear-model-based methods

Supplementary material is divided according to studied data sets.

GE1 (Glasshouse experiment 1): for details see Stachová et al. (2013)
Species codes in GE1:
pla= *Plantago lanceolata*, pru = *Prunella vulgaris*, ach = *Achillea millefolium*, agr = *Agrostis capillaris.*

GE2 (Glasshouse experiment 2): for details see Špaeková and Lepš (2001)
Species codes in GE2:
Hol = *Holcus lanatus,* Fes= *Festuca rubra,* Bri = *Briza media,* Lych= *Lychnis flos-cuculi,* Pru *= Prunella vulgaris,* Lys *= Lysimachia vulgaris.*

SE (Simulated experiment): for details see main article and Fibich and Lepš (2011)
Species codes in SE:
ss is the “strong-small”, ws the “weak-small”, sb the “strong-big” and wb the “weak-big” species. For species traits see Table 2 in the main article.

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Simulated experiment (SE)

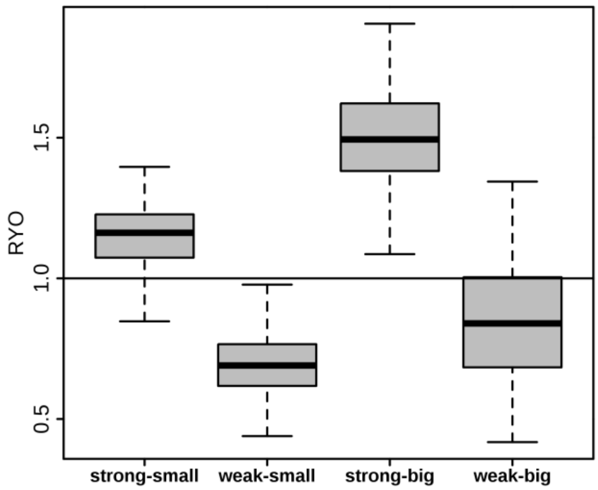

Figure S1. SE, RYO: Relative observed yields for all four species for simulated data.

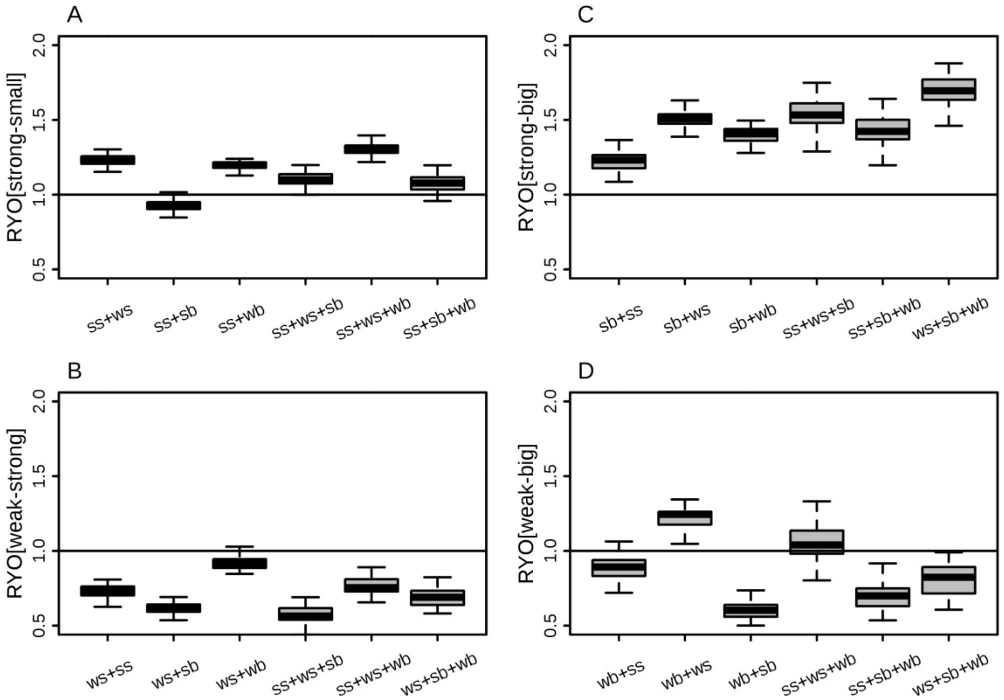

Figure S2. SE, RYO: Relative observed yields for all four species in all species compositions. ss, ws, sb and wb are abbreviations for strong-small, weak-small, strong-big and weak-big species, respectively. For predefined species traits see Table 2.

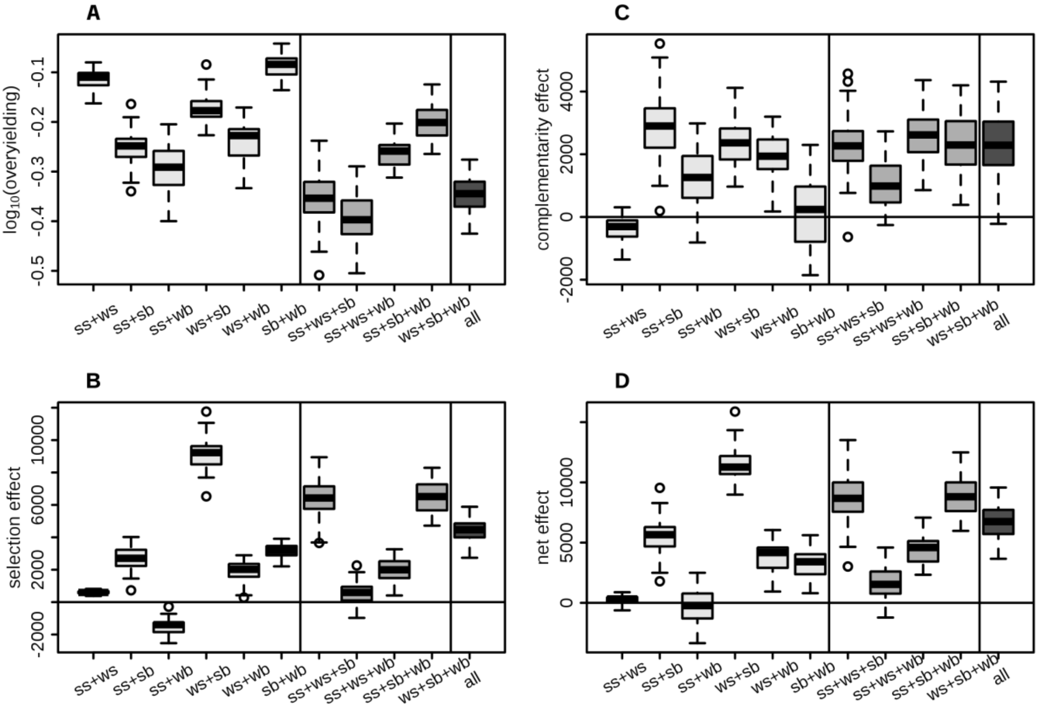

Figure S3. SE, L&H method: Overyielding index (A), selection effect (B), complementarity effect (C) and net effect (D) on the species richness gradient (all mixture compositions). ss, ws, sb and wb are abbreviations for strong-small, weak-small, strong-big and weak-big species, respectively. For predefined species traits see Table 2.

Table S1. SE, Kirwan method. Parameter estimates minus intercept from yield analysis. Larger estimated values indicate a higher influence on yield. ss, ws, sb and wb are shortcuts for strong-small, weak-small, strong-big and weak-big species. For species traits see Table 2. ID and DE, denote identity and diversity effects, respectively. Significant terms are in bold.

Parameter	Effect	Estimate	SE	t (751)	p-value
strong-small (ss)	ID	20327.8	171	118.864	<0.001
weak-small (ws)	ID	15605.7	171	91.252	<0.001
strong-big (sb)	ID	56237.3	171	328.839	<0.001
weak-big (wb)	ID	39957.6	171	233.646	<0.001
ss:ws	DE	1534.1	834.8	1.838	0.665
ss:sb	DE	23375.7	834.8	28.001	<0.001
ss:wb	DE	270.6	834.8	0.324	0.750
ws:sb	DE	46576.0	834.8	55.792	<0.001
ws:wb	DE	16267.2	834.8	19.486	<0.001
sb:wb	DE	13380.7	834.8	16.029	<0.001
ss:ws:sb	DE	20644.6	5541.4	3.726	<0.001
ss:ws:wb	DE	-7948.6	5541.4	-1.434	0.152
ss:sb:wb	DE	12569.0	5541.4	2.268	0.024
ws:sb:wb	DE	13331.4	5541.4	2.406	0.0164

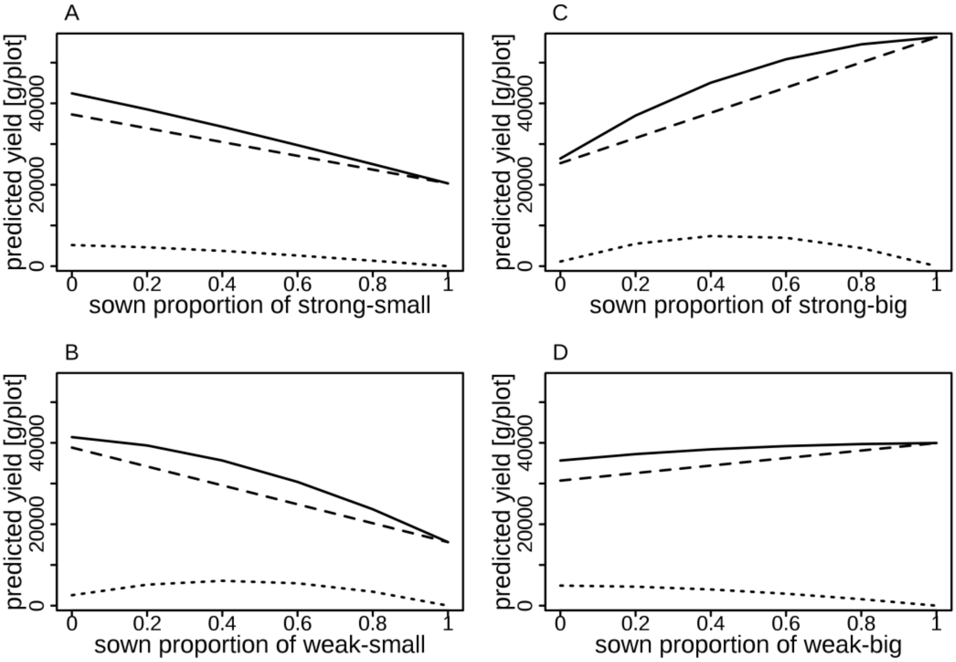

Figure S4. SE, Kirwan method: Mean yield predicted by the model in Table 3 in the main text. Total predicted yield (solid line) is the sum of the identity (ID, dashed line) and diversity effects (DE, dotted line). Only the proportion of one species is varied in one subplot. For species traits see Table 2 in the main text.

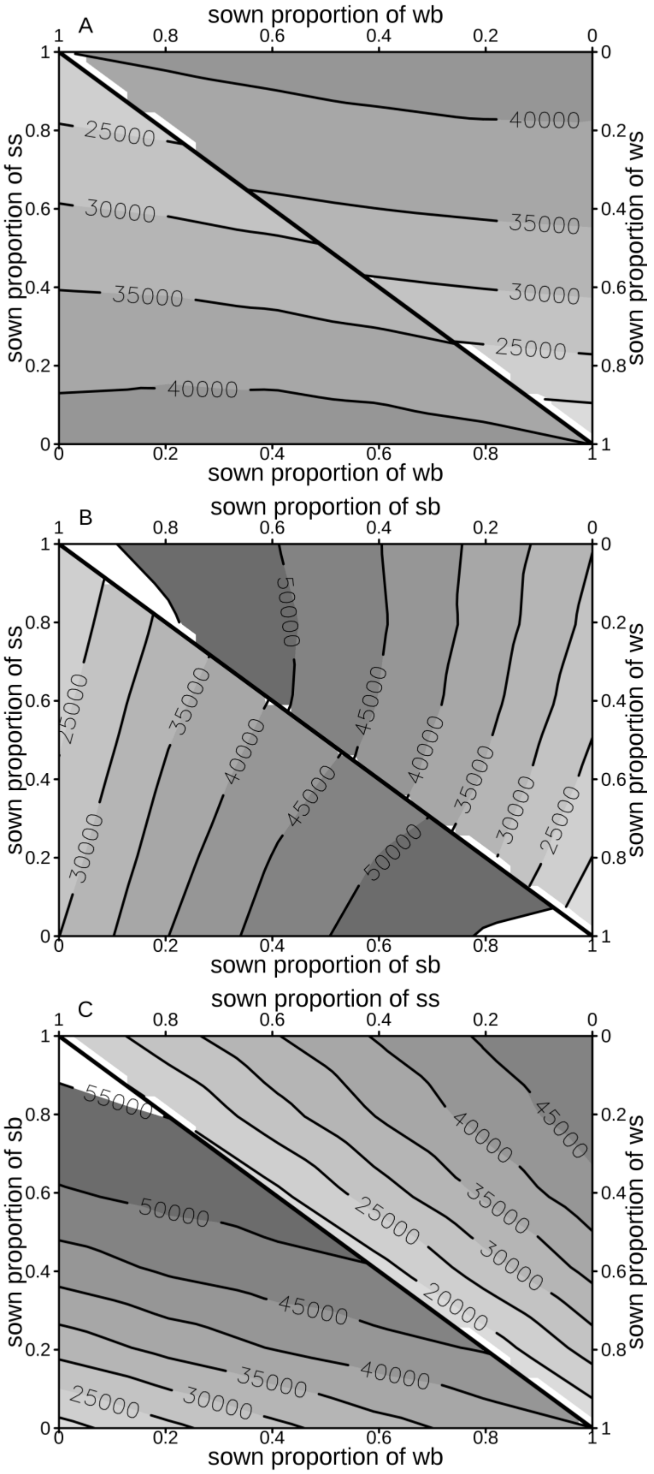

Figure S5. SE, Kirwan method: Mean predicted yield by the model in Table S1, based on varied proportions of two species. Darker color reflects higher yield. For species traits see Table 2 in the main text.

Table S2. SE, Kirwan method: Tested models and corresponding AIC statistics. P stands for species proportions, e is an error term.

Model no	**Description**	**Equation**	***AIC***
1	*Mean yield*	$y=1+e$	16548.1
2	*Model 1 Identities*	$y=\Sigma_{i=1}^{s}\beta_i P_i+e$	14598.6
3	*Model 2+ separate pairwise interactions*	$y=\Sigma_{i=1}^{s}\beta_i P_i+\Sigma_{i,j=1,i<j}^{s}\gamma_{ij} P_i P_j+e$	13075.4
4	*Model 3+ three-species interactions*	$y=\Sigma_{i=1}^{s}\beta_i P_i+\Sigma_{i,j=1,i<j}^{s}\gamma_{ij} P_i P_j+\Sigma_{i,j,k=1,i<j<k}^{s}\gamma_{ijk} P_i P_j P_k+e$	13062.4

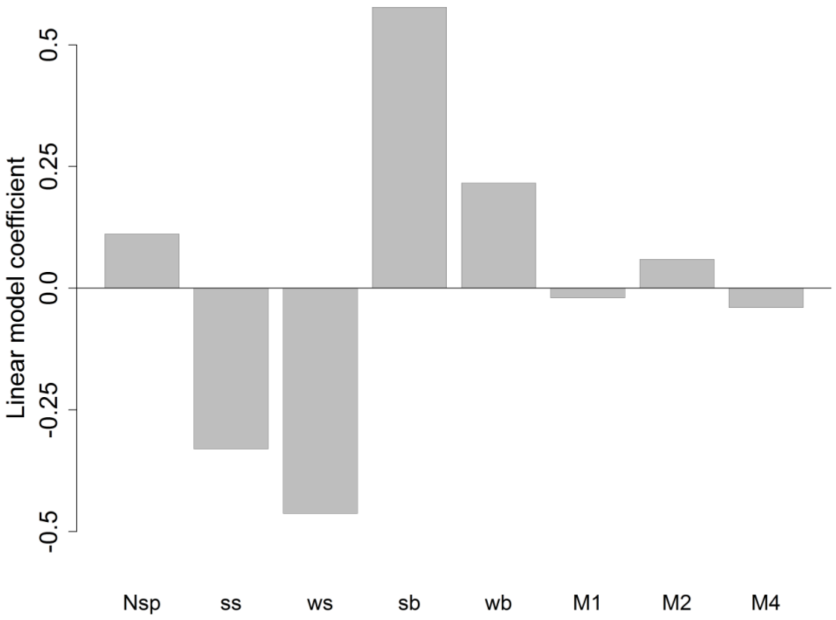

Figure S6. SE, Bell method: Linear model coefficients. Nsp is linear species richness, M1–M4 is non-linear species richness (summed coefficients are proxy for species interactions). ss, ws, sb and wb are abbreviations for strong-small, weak-small, strong-big and weak-big species. For species traits see Table 2 in main text.

Table S3. SE, Bell method: Summary, bold coefficients are significant. M1–M4 stand for non-linear species richness. ss, ws, sb and wb are abbreviations for strong-small, weak-small, strong-big and weak-big species. For species traits see Table 2 in main text. Stars for P-values (***<0.001, ** <0.01, * <0.05, . < 0.1).

Parameter	**Estimate**	**P-value**
(Intercept)	**10.1852**	**(***)**
Nsp	**0.1116**	**(***)**
ss	**-0.3302**	**(***)**
ws	**-0.4632**	**(***)**
sb	**0.5774**	**(***)**
wb	**0.2161**	**(***)**
M 1	**-0.0199**	**(***)**
M 2	**0.0597**	**(***)**
M 4	**-0.0398**	**(***)**

Glasshouse experiment 1 (GE1)

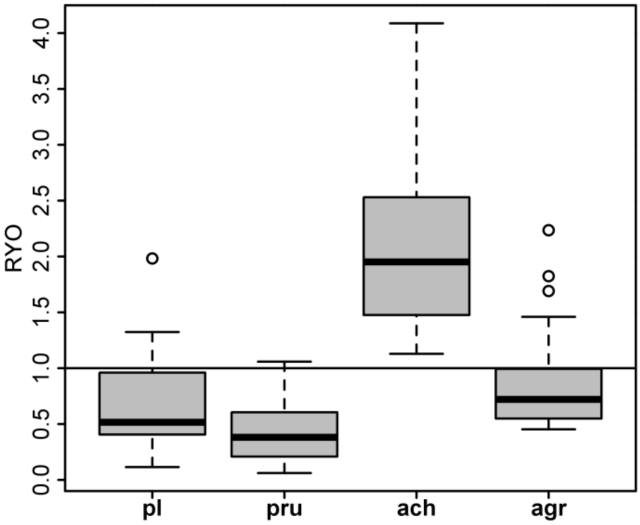

Figure S7. GE1, RYO: Detailed RYO of all experimental species averaged across densities for the 4-species mixture.

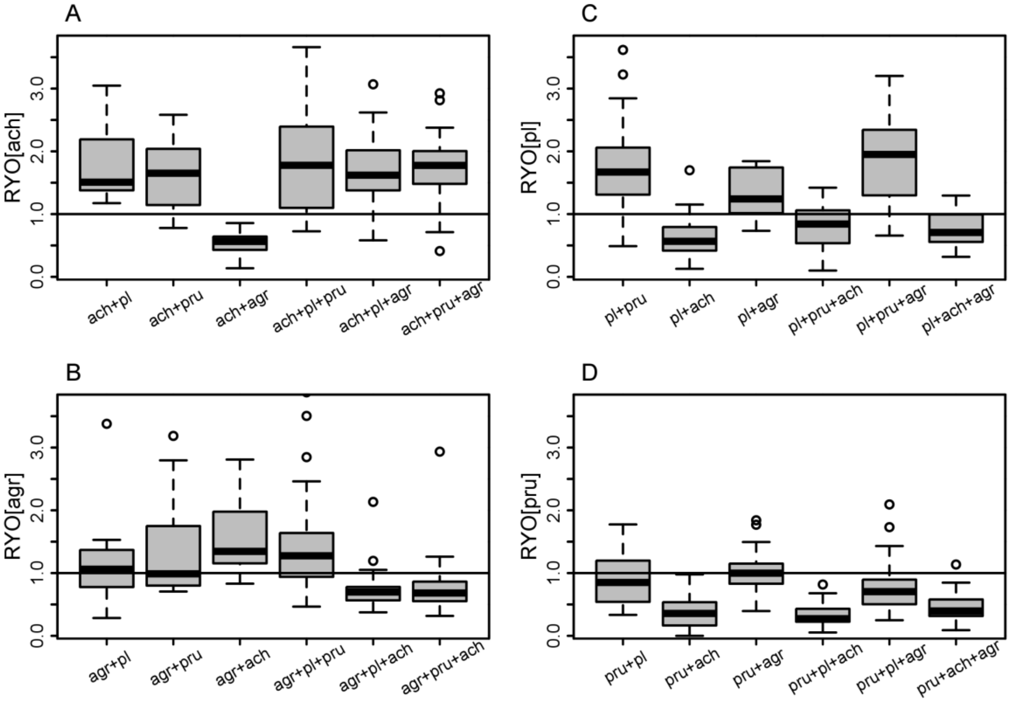

Figure S8. GE1: Detailed RYO of all experimental species averaged across densities for 2 and 3-species mixtures.

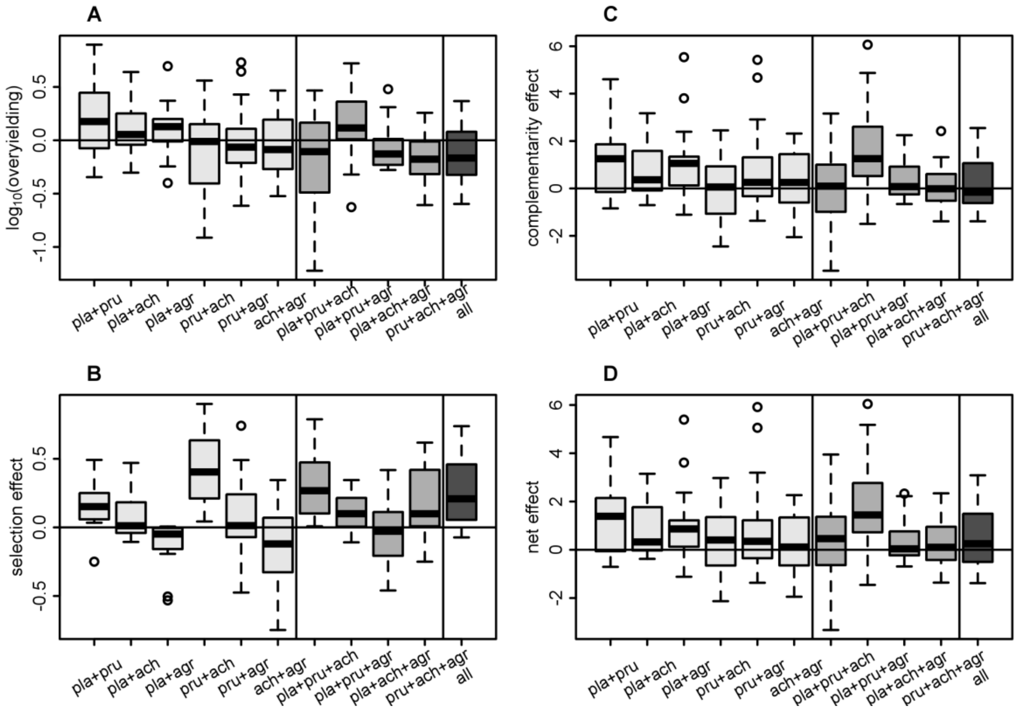

Figure S9. GE1, L&H method: Overyielding, complementarity, selection and net effects for species mixtures.

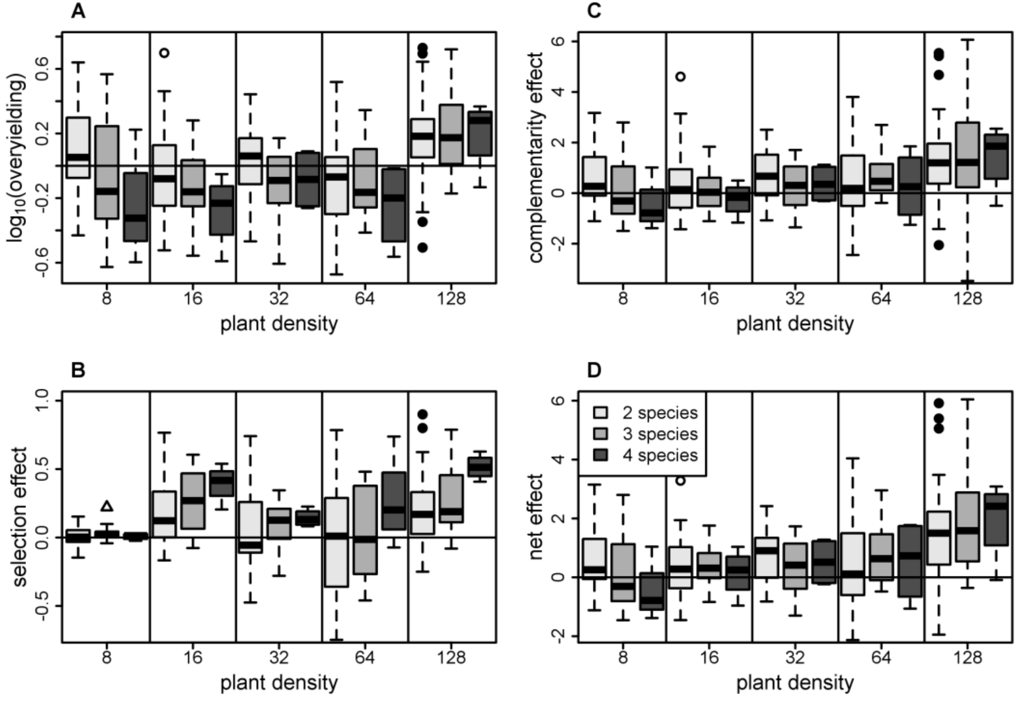

Figure S10. GE1, L&H method: Overyielding, complementarity, selection and net effects for different final sowing plant densities and number of species.

Table S4. GE1, Kirwan method: Parameter estimates minus intercept from yield analysis by model in Eq. 2. Larger estimated values indicate a higher influence on yield. ID and DE denote identity and diversity effects, respectively. Significant terms are in bold.

Parameter	Effect	Estimate	SE	t (284)	p-value
***Plantago* (Pla)**	ID	3.93	0.30	13.026	<0.001
***Prunella* (Pru)**	ID	2.98	0.30	9.868	<0.001
***Achillea* (Ach)**	ID	4.31	0.30	14.255	<0.001
***Agrostis* (Agr)**	ID	4.55	0.30	15.059	<0.001
Density	ID	0.67	0.53	12.629	<0.001
Density^2	ID	0.11	0.04	2.476	0.014
Pla : Pru	DE	5.74	1.41	4.073	<0.001
Pla : Ach	DE	3.50	1.41	2.486	0.014
Pla : Agr	DE	4.01	1.41	2.848	0.005
Pru : Ach	DE	1.70	1.41	1.207	0.233
Pru : Agr	DE	3.53	1.41	2.505	0.0128
Ac : Agr	DE	0.91	1.41	0.642	0.5212
Pla : Pru : Ach	DE	-23.03	9.36	-2.462	0.0144
Pla : Pru : Agr	DE	7.440	9.36	0.796	0.426
Pla : Ach : Agr	DE	-16.34	9.36	-1.746	0.082
Pru : Ach : Agr	DE	-11.34	9.36	-1.212	0.226

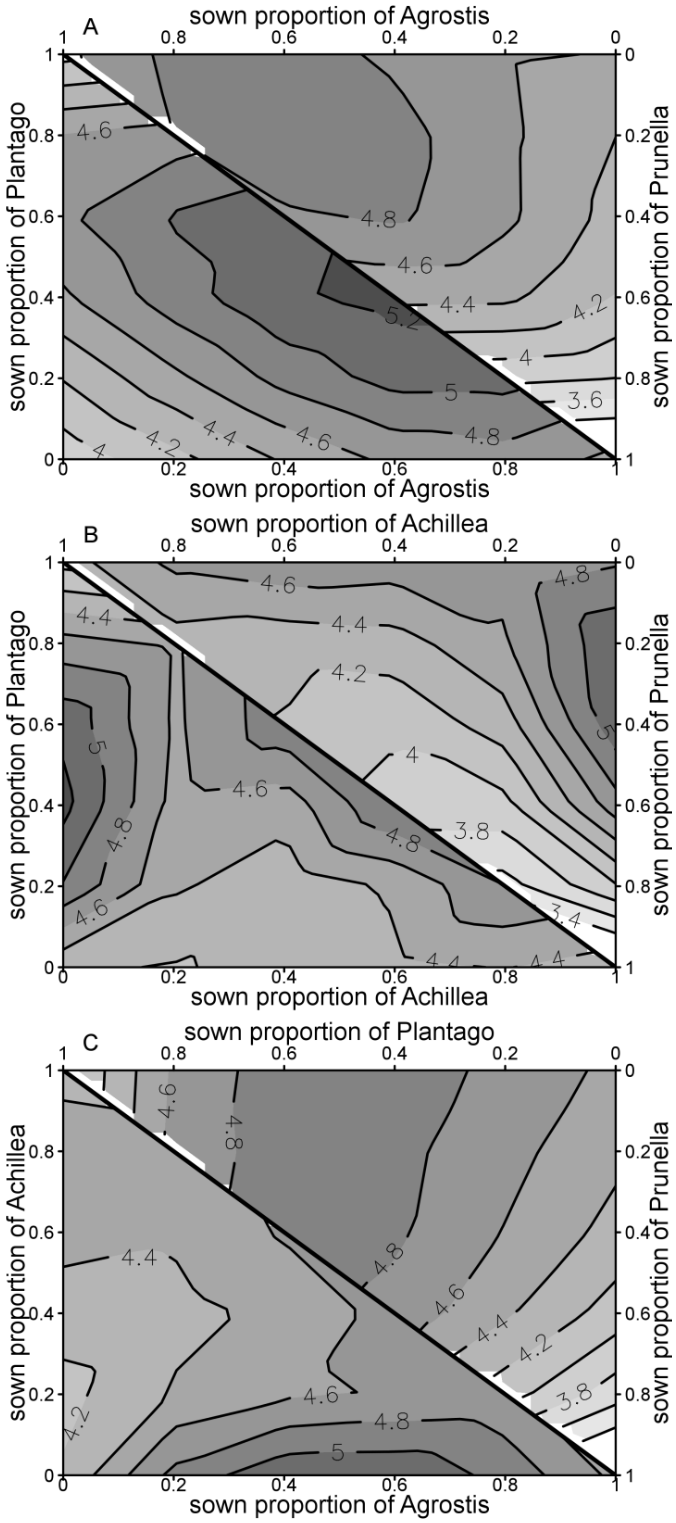

Figure S11. GE1, Kirwan method: Mean predicted yield by the model in Table S4 for average density based on varied proportions of two species. Darker color reflects higher yield.

Table S5. GE1, Kirwan method: Tested models and corresponding AIC statistics. M stands for final density, P for species proportions and e for error term.

Model no	**Description**	**Equation**	**AIC**
1	*Density only*	$y=\alpha M+e$	1059.7
2	*Model 1+Identities*	$y=\Sigma_{i=1}^{s}\beta_i P_i+\alpha M+e$	1048.0
3	*Model 2+quadratic density*	$y=\Sigma_{i=1}^{s}\beta_i P_i+\alpha M+\alpha M^2+e$	1044.4
4	*Model 3+ separate pairwise interactions*	$y=\Sigma_{i=1}^{s}\beta_i P_i+\alpha M+\alpha M^2+\Sigma_{i,j=1,i<j}^{s}\gamma_{ij} P_i P_j+e$	1026.6
5	*Model 4+ three-species interactions*	$y=\Sigma_{i=1}^{s}\beta_i P_i+\alpha M+\alpha M^2$ $+\Sigma_{i,j=1,i<j}^{s}\gamma_{ij} P_i P_j+\Sigma_{i,j,k=1,i<j<k}^{s}\gamma_{ijk} P_i P_j P_k+e$	1024.5

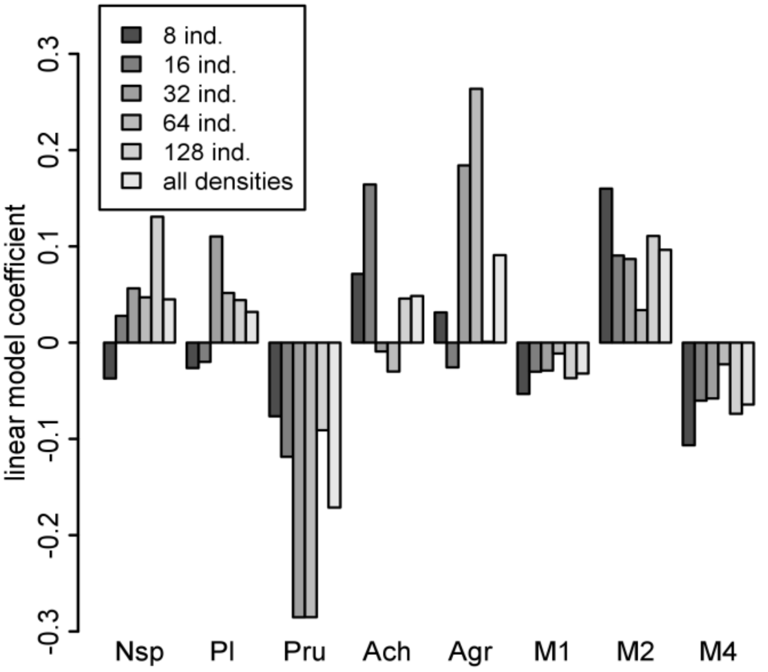

Figure S12. GE1: All linear model coefficients for single species in all experimental densities (D1-D5) and averaged across all densities (“all” in legend). Nsp stands for the effect of linear richness, M1–M4 stand for the non-linear (categorical) effect of species richness.

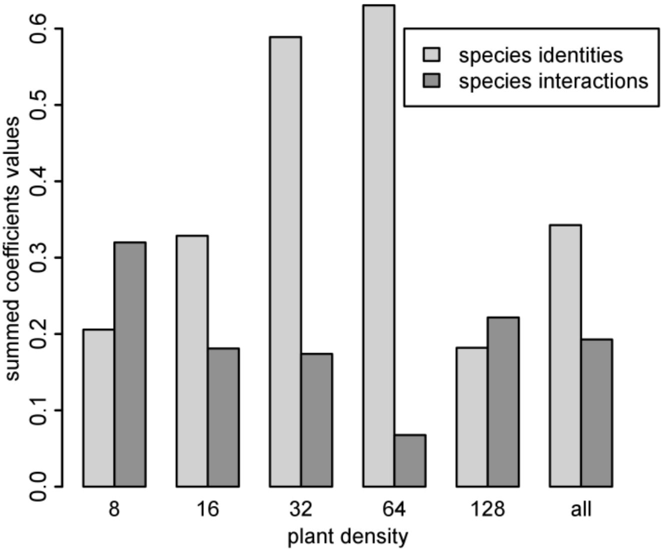

Figure S13. GE1, Bell method: Sums of absolute values of linear model coefficients for species identities and species interactions (NLR). D1-D5 stand for single densities, all means the average across all densities.

Table S6. GE1, Bell method: Coefficients from linear models according to the Bell method for every density setting, asterisks for significant results. D1–D5 stands for increasing initial sowing densities, Nsp for the effect of linear species richness of mixtures, M1–M4 for non-linear effect of species richness of mixtures. Stars for P-values (***<0.001, ** <0.01, * <0.05, . < 0.1).

Density/ Parameter	**8 ind.**	**16 ind.**	**32 ind.**	**64 ind.**	**128 ind.**	**All densities**
(Intercept)	1.242	1.292	1.288	1.513	1.471	1.362
Nsp	-0.037	0.028	**0.056***	0.047	**0.131*****	**0.045****
***Plantago* (Pla)**	-0.026	-0.02	**0.11***	0.052	0.0442	0.032
***Prunella* (Pru)**	-0.077	**-0.119.**	**-0.285*****	**-0.285*****	-0.091	**-0.171*****
***Achillea* (Ach)**	0.071	**0.164*****	-0.009	-0.03	0.0458	**0.049***
***Agrostis* (Agr)**	0.031	-0.026	**0.184*****	**0.264*****	0.001	**0.091****
M 1	**-0.053***	-0.03	**-0.029***	-0.011	-0.037	**-0.032****
M 2	0.16	0.091	0.087	0.034	0.111	0.096
M 4	-0.107	-0.06	-0.058	-0.023	-0.074	-0.064
Density	NA	NA	NA	NA	NA	**0.023*****

Glasshouse experiment 2 (GE2)

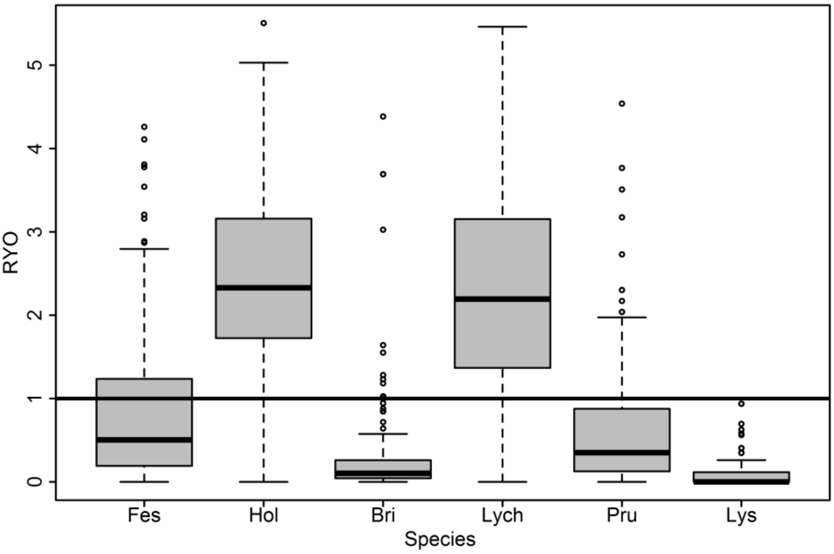

Figure S14. GE2, RYO: RYO of individual species averaged for the two density levels.

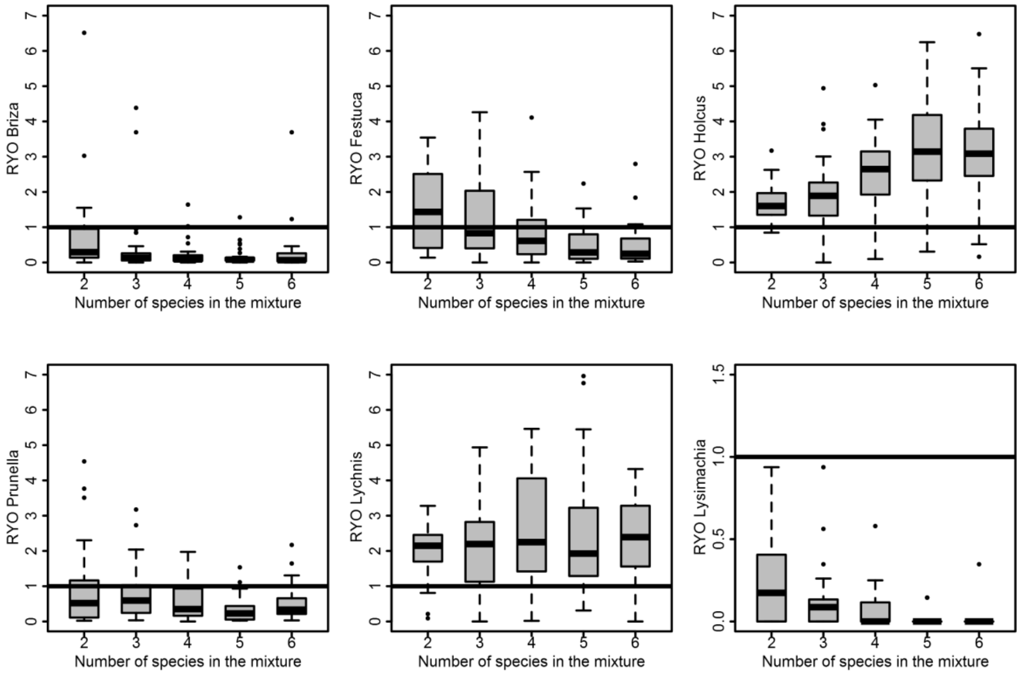

Figure S15. GE2, RYO: RYOs of all experimental species.

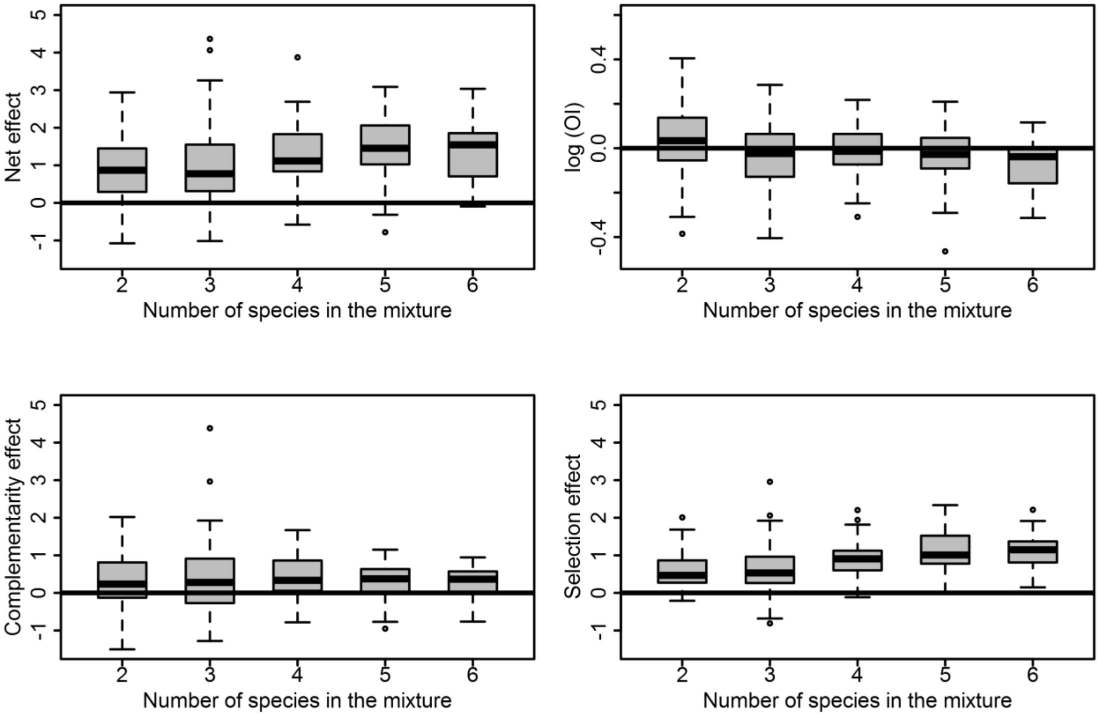

Figure S16. GE2, overyielding: OI, net effect,complementarity and selection effects averaged for the two density levels.

Table S7. GE2, Kirwan method: Parameter estimates from yield analysis by model Eq. 2. Larger estimated values indicate a higher influence on yield. Significant terms are in bold.

Parameter	Effect	Estimate	SE	t (322)	p-value
Holcus	ID	3.773	0.224	16.81	<0.001
Festuca	ID	2.357	0.233	10.11	<0.001
Briza	ID	1.427	0.284	5.02	<0.001
Lychnis	ID	2.213	0.243	9.01	<0.001
Prunella	ID	1.526	0.243	6.28	<0.001
Lysimachia	ID	0.252	0.285	0.89	0.38
Density	ID	0.129	0.047	2.71	0.007
Hol x Fes	DE	3.448	1.289	2.67	0.008
Hol x Bri	DE	3.580	1.319	2.72	0.007
Hol x Lych	DE	1.898	1.240	1.53	0.127
Hol x Pru	DE	3.969	1.200	3.31	0.001
Hol x Lys	DE	6.771	1.593	4.25	<0.001
Fes x Bri	DE	4.184	1.328	3.15	0.002
Fes xLych	DE	4.054	1.238	3.28	0.001
Fes x Pru	DE	1.656	1.257	1.32	0.189
Fes x Lys	DE	6.471	1.418	4.56	<0.001
Bri x Lych	DE	2.255	1.258	1.79	0.073
Bri x Pru	DE	-1.748	1.422	-1.23	0.220
Bri x Lys	DE	1.582	1.504	1.05	0.294
Lych x Pru	DE	3.298	1.271	2.59	0.001
Lych xLys	DE	4.637	1.388	3.34	0.001
Pru x Lys	DE	4.724	1.385	3.41	0.001

Table S8. GE2, Kirwan method: Tested models and corresponding AIC statistics.

Model no	**Description**	**Equation**	**AIC**
1	*Density and mixture identity*	$y=\lambda_m+\alpha D+\varepsilon$	839.14
2	*Model 1+Identities*	$y=\sum_{i=1}^{6}\beta_i P_i+\alpha D+\varepsilon$	920.72
3	*Model 2+ separate pairwise interactions*	$y=\sum_{i=1}^{6}\beta_i P_i+\alpha D+\sum_{i<j}^{6}\delta_{ij} P_i P_j+\varepsilon$	826.74
4	*Model 2+ average interaction*	$y=\sum_{i=1}^{6}\beta_i P_i+\alpha D+\delta_{av}\sum_{i<j}^{6} P_i P_j+\varepsilon$	829.96
5	*Model 2+functional groups*	$y=\sum_{i=1}^{6}\beta_i P_i+\alpha D+\delta_{wfg1}\sum_{i<j}^{3} P_i P_j+\delta_{wfg2}\sum_{\substack{i<j\\ i=4}}^{6} P_i P_j+\delta_{bfg12}\sum_{\substack{i=1,\ldots,3\\ j=4..6}} P_i P_j+\varepsilon$	831.88

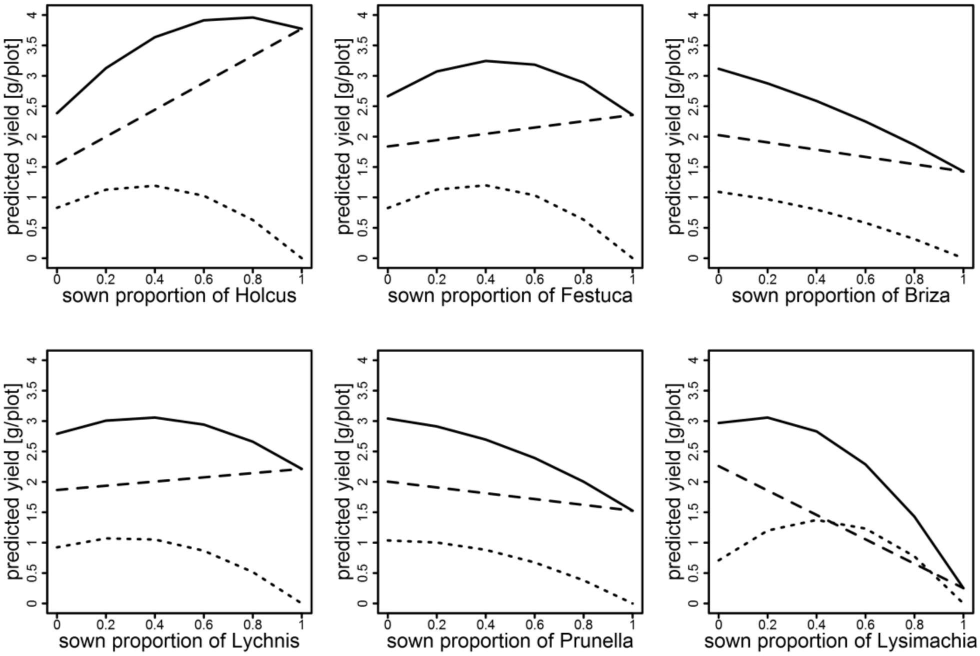

Figure S17. GE2, Kirwan method: Single species yields, diversity and identity effects in species mixtures (expressed as species proportions in the mixtures). Bold line is the yield, dashed line the identity effects and dotted line the diversity effect.

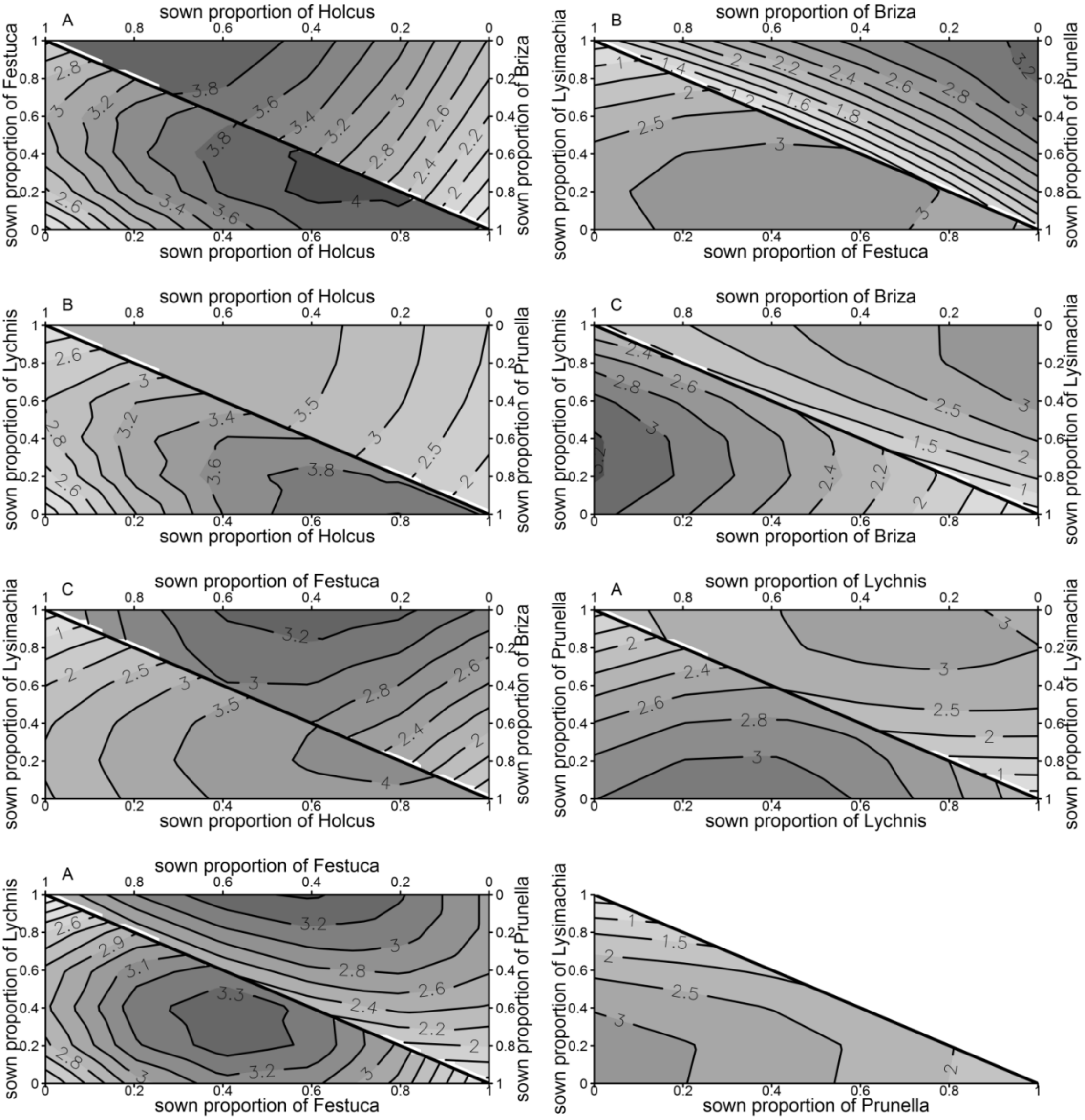

Figure S18. GE2, Kirwan method: Mean predicted yield by the Kirwan model (Table 5 in the main text) for average density based on varied proportions of two species. Darker color reflects higher yield.

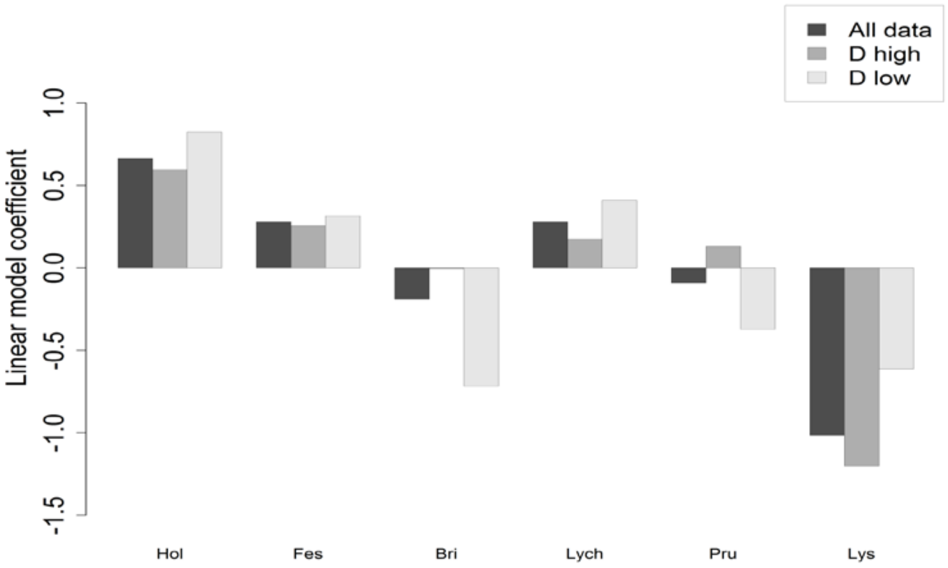

Figure S19. GE2, Bell method: Linear model coefficients for species for all dataset and two density settings.

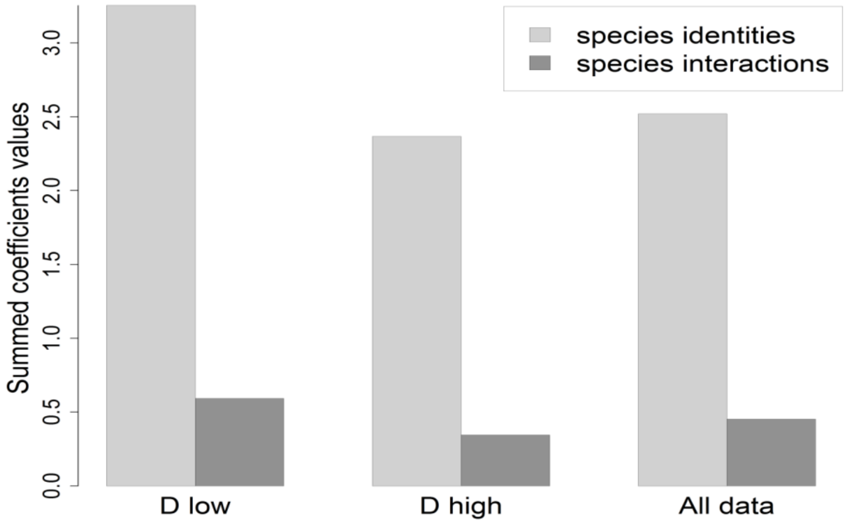

Figure S20. GE2, Bell method: Species identities and species interactions for all data and two density settings.

Table S9, GE2, Bell method: Summary, bold coefficients are significant.

Parameter/Density	low	high	All data
(Intercept)	**0.261**	**0.471**	**0.386**
Nsp	**0.155**	**0.130**	**0.140**
Hol	**0.826**	**0.596**	**0.667**
Fes	**0.318**	**0.258**	**0.280**
Bri	**-0.715**	-0.004	**-0.190**
Lych	**0.413**	**0.175**	**0.279**
Pru	**-0.370**	**0.134**	-0.089
Lys	**-0.612**	**-1.200**	**-1.015**
M 1	**-0.251**	**-0.070**	**-0.138**
M 2	**0.239**	**0.259**	**0.276**
M 3	0.040	0.006	0.030
M 6	0.063	-0.010	0.008
Density low	NA	NA	**-0.184**
Density high	NA	NA	**0.120**

Chapter VI

General Discussion

6.1 Biodiversity—Ecosystem Functioning Experiments: the role of sown vs. realized diversity

Biodiversity—functioning studies have repeatedly found increasing above-ground biomass production with increasing number of sown species in a community (Cardinale *et al.* 2007, Cardinale *et al.* 2012). On the other hand, observational studies or geobotanical experience often cannot confirm this positive relationship (Mittelbach *et al*. 2001, Thompson *et al*. 2005, Lepš 2013). To explore this obvious contrast, both theoretical approach (***Paper 1***) and analysis of experimental data (***Manuscript 1***) were used.

Our simple competition model based on Lotka-Volterra equations was able to mimic the results of sowing biodiversity experiments and also suggest mechanisms responsible for it. Our model proved that with increasing number of sown species, the average carrying capacities (which can be considered as species maximal biomass yield) of species surviving in a stable community in equilibrium increased. This phenomenon is usually called selection effect (Loreau and Hector 2001). At the same time, the probability, that species which are complementary (have mutually low competition coefficients) will be present increase with increasing sown species richness as well, which corresponds to the complementarity effect. Increasing selection and complementarity effects with sown species richness have been demonstrated in numerous BEF studies (Cardinale *et al*. 2007).

On the other hand, results using realized diversity (i.e. number of species in a community in equilibrium) of communities established from a fixed sown diversity were quite different. Within these communities, the relationship between realized diversity and biomass was usually non-significant (usually with negative slope) and closer analysis of species characteristics revealed the reason: the high-yielding species with high carrying capacities were present at low realized diversities only. We investigated publicly available dataset from the Jena biodiversity experiment (Weigelt *et al*. 2010) and also data from a glasshouse experiment (Špaeková and Lepš 2001) to find if the predictions of our model are supported by experimental data. Indeed, we found a positive relationship between sown species richness and biomass and also observed a negative relationship between realized diversity and biomass production at mixture (sown species richness) level.

In order to apply our results on natural communities, we argue that the sown species richness (mixture sizes) used in BEF are in fact equivalent to a local species pool size, as defined by Butaye *et al*. (2001). Local species pool is a set of species able to reach the community and grow in given physical conditions. As the whole experimental pool of species used in biodiversity experiments is composed of those which are generally able to grow in given conditions (they were selected this way, meaning that the environmental filter should not prevent any species from growing in the site), the selection of species in BEF corresponds to species that were able to overcome the dispersal limitation. This total species pool of a BEF experiment might be considered as a regional species pool. Realized diversity is then the local species pool minus species filtered out by biotic interactions.

Based on both our theoretical predictions and experimental data analysis, we conclude that: 1) In natural conditions, there is a direct causal relationship between the size of available species pool and community productivity. 2) Both the realized diversity and the biomass production are affected by various factors (including species pool size and composition, environmental factors, also determining biotic interactions). If the size of species pool is the decisive factor determining realized diversity, then we can expect positive diversity—productivity relationship. In other cases, the shape of the relationship will be highly variable and may be even negative.

6.2 Biodiversity experiments: their design and analysis

In our contribution (***Paper 2***), we were examining the role of initial sowing density on biodiversity effects (i.e. the complementarity and selection effect *sensu* Loreau and Hector 2001), which are usually assessed in BEF experiments. Since the beginning of BEF research, some scientists pointed out that sowing densities might influence biodiversity effects (Garnier *et al*. 1997, Huston *et al*. 2000, Polley *et al.* 2003) while others claimed this influence to be negligible (Loreau and Hector 2001).

Our results showed that all monocultures and mixtures were influenced by sowing density which in turn influenced all biodiversity effects. The biodiversity effects were not only affected in a quantitative way, but also qualitatively, i.e. changed signs (change in a sign mean a different ecological interpretation of a given effect).

We found that biodiversity effects values are particularly sensitive to the shape of the density—yield relationship exhibited by a given species in monoculture. If the monoculture follows the law of constant final yield (CFY, i.e. positively saturated relationship, Weiner and Freckleton 2010), the effects change in a quantitative way only. However, the most pronounced problem arises when there is a different shape between initial sowing density and yield, e.g. unimodal. The unimodal relationship is caused by high individual mortality induced at high densities. As a result, fewer individuals survive and these ultimately produce lower yield. Biodiversity effects are calculated using monocultures as “references” and thus the shape of monocultures yield on sowing density gradient affects all effects in all mixtures.

When using a species with unimodal density—yield relationship in experimental density higher than the optimal (optimal density has the highest yield), biodiversity effects provide a misleading indication of ecological mechanism, as was also suggested by simulation model of Fibich and Lepš (2011).

Moreover, mixtures are affected by sowing density as well. Sowing density determine at which stage of an individual development severe competition occurs which affects the relative success of individual species in competition. The importance of sowing density was confirmed in the studies by He *et al.* (2005), who found decreasing species evenness and altered functional group composition with increasing density, similarly to Flynn *et al*. (2008).

Our experimental results suggest that the belief that biodiversity effects are rather insensitive to sowing density need not to by fully warranted and we suggest testing the shape of density-yield relationship for the experimental species prior to choosing experimental density.

Apart from calculating the above mentioned biodiversity effects based on the net biodiversity effect partitioning (Loreau and Hector 2001), there are also other possibilities how to analyze data obtained from a BEF experiment. Several new methods have been proposed recently. Among them the methods applying classical linear models (Bell *et al*. 2009, Kirwan *et al*. 2009, Connoly *et al*. 2013) and we refer to them as “linear-model-based methods”. The fairly most often used is the (already mentioned) net effect additive partitioning, together with a simple comparison of the mixture function with the function of the best monoculture: the measure of overyielding (Garnier *et al*. 1997). We call these methods “traditional”.

Our comparison between the traditional and linear model based methods of BEF experiment analysis (***Manuscript 2***) revealed differences in requirements, results and interpretations given by these two main analytical approaches. We used three data sets: two glasshouse experiments and a simulation experiment, in which the ecological species traits (shade-tolerance and maximal biomass) were known. Thank to that knowledge, we were able to predict the ecological mechanisms operating within a given community (pot) and compare these with biodiversity effects (from traditional methods) and liner model coefficients (linear-model-based methods). Based on the analysis of our data sets, it seems that additive partitioning provided more insight into the mechanisms operating in biodiversity experiments than the linear-model-based methods. The linear-model-based methods are not able to distinguish, e.g. the negative effect of a species on mixture biomass due to suppression of other species when the species itself is not able to compensate with its own production, from the negative effect due to its low productivity. On the other hand, there are two important advantages of the linear-model-based methods: their statistical correctness (i.e. the values analyzed by the final statistical model are independent), and limited requirements for laborious data collection, which allows for experiments with large species pools.

6.3 Final conclusion

The loss of biodiversity is a serious problem for humanity (Cardinale *et al*. 2012, Balvanera *et al*. 2013). BEF experiments have been designed to identify the effects of various forms of species diversity on various measures of ecosystem functioning. In order to provide results with possible application, these experiments should reflect features of natural systems, and appropriate methods of their analysis should be applied. In our study, we have shown that initial sowing density plays an important role in monoculture stands and through the effect on monocultures influence the values of (statistically determined) biodiversity effects.

Our results also shed light on the differential shapes of diversity—biomass production relationships in manipulative BEF experiments and observational experiments or long-term BEF experiments without weeding. In majority of BEF experiments, the sown diversity is used as explanatory variable. Sown diversity is an equivalent to local species pool size in natural systems and the diversity we can observe and directly measure in field conditions is the realized diversity. Their effects on functioning might be quite different as shown in this study.

In central European grasslands, the decrease of species diversity often results in increased dominance of highly productive species (Lepš 2014). This means that functioning (primary productivity) does not decrease. However, our study suggests that multiple local extinctions of various species will lead to a considerable decrease of regional and local species pool size, which might result in negative effects on ecosystem functioning in the future.

6.4 Literature

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Butaye J., Jacquemyn H., Honnay, O., Hermy, M. (2001) The species pool concept applied to forests in a fragmented landscape: dispersal limitation versus habitat limitation. *Journal of Vegetation Science* 13: 27–34.

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