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Environmental dependency of biodiversity- ecosystem functioning relationships

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Applied Biological Sciences

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'When the going gets tough, it pays to cooperate'

Eugene P. Odum

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Omgevingsafhankelijkheid van biodiversiteit-ecosysteem functie relaties.

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'It is fascinating to study, and rich in wonderful biology. But by studying it, do not expect any universal laws, even simple contingent general rules, to emerge. If and when they do, treasure them.'

John H. Lawton

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Dankwoord

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List of abbreviations

ANOVA	Analysis of variance
BEF	Biodiversity ecosystem functioning
EC ₅₀	50% effect concentration
EU	European Union
F/2	Half the concentration of the 'F-medium' formulation by Guillard and Ryther
GC-MS	Gas chromatography-mass spectrometry
HC ₅	5% hazardous concentration
MAPE	Mean absolute percentage error
NOEC	No observed effect concentration
SCOPE	Scientific committee on problems of the environment
SE	Standard error
SSD	Species sensitivity distribution

1

General introduction

1.1. The anthropogenic mass extinction

The history of life on Earth is characterised by a continuous turnover in species through evolution and natural selection^{1,2}. Driven by biotic and abiotic processes such as competition and climatological changes, background extinction rates are generally slow, typically under 1 extinction per million species-years³⁻⁶. Spikes in extinction rates can however be found throughout the fossil record, most remarkably at the end of the Ordovician, Devonian, Permian, Triassic and Cretaceous period when sudden, severe climate changes caused over 75% of the taxa to disappear.

Today, a sixth mass extinction event seems under way as anthropogenic activities are pushing extinction rates far above the background value³⁻⁷. After the industrial revolution, the world population has increased by a tenfold to over 7 billion today, and is projected to reach 10 billion by the middle of this century⁸. Human activities have thereby progressively interfered with Earth's biogeochemical cycles to meet society's growing demands for space and resources. The period following the industrial revolution is hence commonly referred to as the Anthropocene, the era of man⁹⁻¹¹. As a result, we are now increasingly breaching the barriers of a sustainable management of our '*spaceship Earth*'¹². Habitat destruction, eutrophication, chemical pollution and emission of greenhouse gasses have thereby become the main drivers of the ongoing global biodiversity decline^{13,14}, and their effects are expected to increase even further in the next century^{7,15}. Recent biodiversity assessments estimate current extinctions rates at values of up to 1000 times the background value based on the extinctions documented in the past century³. However, human activities continue to expand at such pace that many species are yet to face extinction, creating an extinction debt that is likely to push future extinction rates to even higher values^{16,17}. Climate change alone, for example, is estimated to destine a staggering 15-37% of the world's current taxa to extinction before the end of the century¹⁸. Understanding, estimating and potentially mediating the consequences of this rapid and unprecedented global biodiversity decline for the many ecosystem functions on which society currently depends has therefore become a pressing matter to science and society^{3-6,19-21}.

1.2. Biodiversity: cause or effect of ecosystem functioning?

Biodiversity in its broadest sense encompasses all variety in life forms, both between and within species²². Following the work of Darwin and Wallace, biodiversity has historically often been regarded as the passive consequence of the opportunities presented by the ecosystem. In the 19th and early 20th century, explorers and naturalists set out to document

Earth's biodiversity. Hence, most of the early notions on global biodiversity patterns originated from field observations, such as the latitudinal increase in species richness from the arctic to the tropics. This increase in biodiversity coincides with an increased productivity from the poles to the Equator, suggesting a positive interrelationship between biodiversity and productivity at a global scale (Figure 1.1A). Biodiversity is undoubtedly to some extent linked to the increase in productivity as a consequence of the increased energy input from the Arctic to the tropics²³. Since speciation is driven by selection for '*individuals best adapted to the place they fill in nature*'², highly productive systems such as tropical rainforests or coral reefs can therefore support more species than boreal forests, simply by having more niches available.

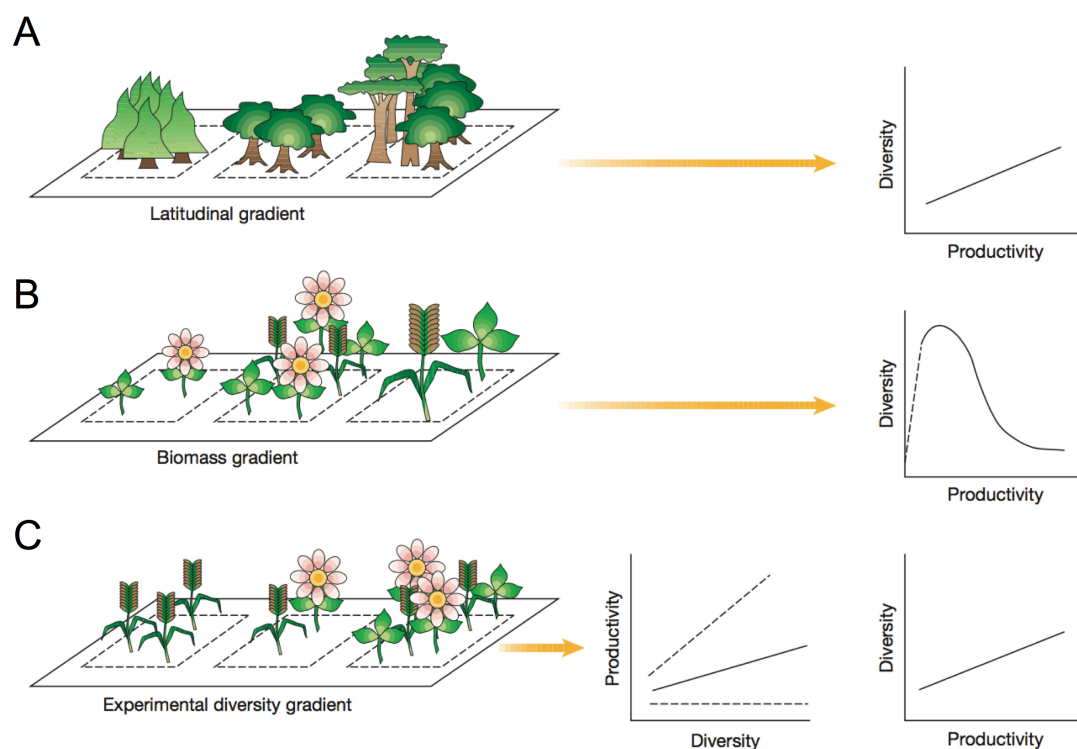


Figure 1.1: Scale-dependency of biodiversity-productivity relationships. (A) At global scales, biodiversity correlates positively to the system's productivity. Along a latitudinal gradient, for example, biodiversity and productivity increases from the Arctic toward the tropics. (B) At regional scales, biodiversity is expected to respond unimodally to the system productivity with low diversity in low-productive systems because of resource limitation and low diversity in highly productive systems because of strong resource competition (middle panel). (C) Experimental studies manipulate diversity to assess the effect on productivity (lower panel). Biodiversity is thus treated as a response variable in observational studies, and as an explanatory variable in experimental studies. Although generally positive, the strength of experimental diversity-productivity relationships can strongly differ between studies (solid and dashed lines). Redrawn from Purvis and Hector 2000²².

Over time, several theories have been proposed that use the properties of the systems as predictor variables for the number of species in the system^{24,25}. Ecologists thereby have tried to link local biodiversity to total amount of resources^{26,27}, the ratio by which resources are available^{28,29}, patch sizes³⁰, productivity³¹, disturbance regimes³² and the type of species interactions in the system^{33,34}.

At local scales, resource competition and resource constraints are predicted to be the main drivers of biodiversity. In low productive systems, resource limitation is predicted to reduce species richness, whereas strong resource competition, leading to a few dominant species, is predicted to reduce species richness levels in highly productive systems (Figure 1.1B)^{35,36}. Although theoretically appealingly simple, such unimodal relationship between productivity and biodiversity has been poorly supported by field studies. Instead, various types of biodiversity-productivity relationships have been reported, rendering productivity a poor predictor of the system's diversity^{37–39}. Moreover, even when occurring, such unimodal relationships are little informative as they cannot be uniquely attributed to resource limitation and competition as theoretically proposed, but can instead arise from a variety of mechanisms^{39,40}.

Understanding the consequences of biodiversity changes for ecosystem functioning therefore requires turning the question around. In *“the origin of species”* Darwin already raised the question what consequences it would have for a system if biodiversity changes would result in vacant niches. Based on an experiment in the gardens of Woburn Abbey where plots were sown with different mixtures of grasses and herbs, he states that *‘it has been experimentally proved that if a plot of ground be sown with one species of grass, and a similar plot be sown with several distinct genera of grasses, a greater number of plants and a greater weight of dry herbage can thus be raised’*^{1,41}. He attributed this increased yield to niche differentiation between species, allowing more diverse mixtures to make most of the available resources. As such, he provides one of the earliest accounts on the mechanisms underlying positive biodiversity effects on ecosystem functioning. Despite this early notion, studies on biodiversity effects on ecosystem functioning would remain mainly confined to intercropping experiments, which aimed at finding plant mixtures that maximised agricultural yields and not a general relationship between biodiversity and ecosystem functioning^{42,43}. Only by the late 1980s, ecologists would regain interest in the occurrence of biodiversity-ecosystem functioning relationships, due to the increased concerns of a global biodiversity decline^{1,22,41}.

During the first half of the 20th century, Odum⁴⁴ and Elton⁴⁵ strongly influenced the development of community and ecosystem ecology. They thereby focussed on another

aspect of biodiversity: its effect on ecosystem function stability. Based on observations across different terrestrial systems and the analysis of simple theoretical models, they argued that simplified systems, such as agricultural fields, are much more prone to disturbances and large fluctuations in population densities, so that '*nature tends toward stability in its mature stages*'⁴⁴. The occurrence of a positive biodiversity-ecosystem function stability relationship was challenged by the work of May in the 1970s⁴⁶. Turning to mathematical models, May presented rigorous theoretical evidence that biodiversity should instead increase population fluctuations^{33,47}. Despite May's suggestion that ecosystem functions could still be more stable at higher diversity levels in spite of lower populations stability, the opposing empirical and theoretical results casted doubts on the existence of biodiversity-ecosystem function stability relationship for the consecutive decade^{48,49}.

It was only by the 1990s that ample new empirical evidence was brought forward to firmly establish the occurrence of biodiversity effects on both ecosystem functioning and ecosystem function stability^{50,51}. By the late 1980s the awareness of an ongoing global biodiversity decline had made it to the political agenda⁵². Successively, the Scientific Committee on Problems of the Environment (SCOPE) launched a programme entitled '*Ecosystem Functioning of Biodiversity*' to assess the state of knowledge on the consequences of biodiversity changes for ecosystem functioning in 1991, which provided a new incentive for biodiversity research⁵³. Experimental studies thereby replaced field observations. Identical to the 19th century experiments in Woburn Abbey and Rothamsted^{41,42}, biodiversity effects on ecosystem functioning were studied by directly manipulating species richness (Figure 1.1C). Over the past two decades, hundreds of these studies have been conducted in both terrestrial and aquatic systems, studying the effects of biodiversity on both ecosystem functioning and ecosystem function stability. These experiments have now provided compelling empirical evidence that biodiversity affects both ecosystem functioning and ecosystem function stability^{54–63}.

1.3. Biodiversity-ecosystem functioning (BEF) relationships

The shape of the biodiversity-ecosystem functioning determines how much species can be lost from a system without jeopardizing its functions. Much of the early biodiversity research consequently revolved around whether universal biodiversity-ecosystem functioning relationship existed from which the consequences of global biodiversity declines could be estimated^{52,53,64,65}.

In 1981, Ehrlich and Ehrlich proposed a general biodiversity-ecosystem functioning relationship based on the dramatic, yet appealing comparison between removing species from an ecosystem and popping rivets from an airplane's wing⁶⁶. Central therein is the concept of functional redundancy^{67,68}. They proposed that, as several species often fulfil similar roles within a system, a number of species could be lost without affecting ecosystem functioning. However, at some point ecosystem functioning can no longer be sustained by the remaining species, and the system (partly) collapses (Figure 1.2A). Several alternative biodiversity-ecosystem functioning relationships have since been proposed in which the degree of functional redundancy between species plays an important part⁶⁸. When functional redundancy between species is high, many species can be lost and significant changes in ecosystem functioning only occur at very low levels of species richness (Figure 1.2B)^{67,69}. Opposite to functional redundancy is the occurrence of keystone species, which perform a unique role in the system. The loss of these unique species consequently results in a disproportionately large decrease in ecosystem functioning⁷⁰. Similarly, adding species to the system, for example by restoration, will not result in regaining the systems' original function as long as a keystone species are not included (Figure 1.2C). However, species generally make – to some extent – unique contributions to ecosystem functions (i.e. idiosyncrasy). As such, changes in ecosystem functioning can be highly variable and strongly depend what species are lost, and the consequences this has for the remaining species (Figure 1.2D)⁷¹.

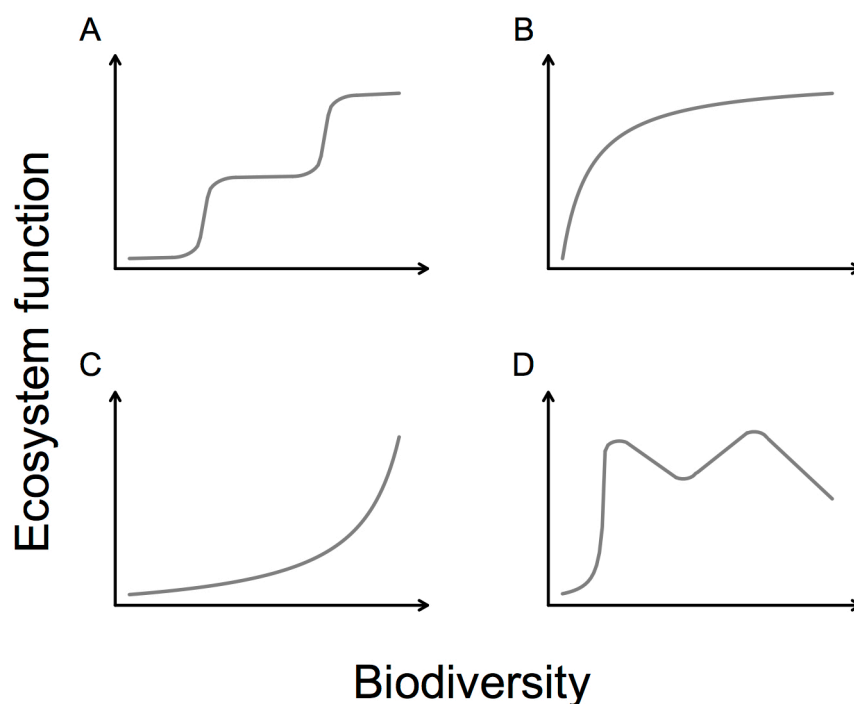


Figure 1.2: Examples of proposed biodiversity-ecosystem functioning relationships. (A) Rivet redundancy, (B) functional redundancy, (C) keystone species and (D) idiosyncrasy. Redrawn from Naeem 1998⁶⁸.

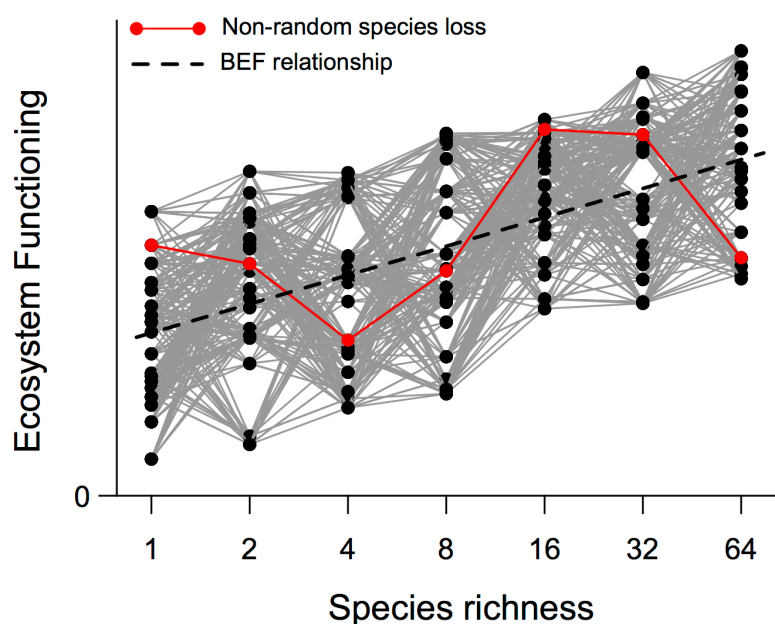


Figure 1.3: Effects of random and non-random species loss on ecosystem functioning.

Biodiversity effects typically consist of multiple systems that are randomly composed at each diversity levels, representing multiple trajectories of species loss (grey lines). The biodiversity-ecosystem functioning relationship (dashed black line) hence describes the average change in ecosystem functioning between diversity levels. Consequently, effects of non-random species loss (red line), that is a single trajectory, can deviate from this average BEF relationship because of idiosyncratic effects.

Due to idiosyncrasy, consequences of species loss strongly depend on the order in which species are lost^{72–80}. Moreover, the main drivers of biodiversity loss such as climate changes or habitat loss do not remove species at random from the system^{81–83}. Instead species get lost based on their sensitivity to environmental changes, as well as the sensitivity other species with which they interact^{3,7,15,84,85}. When species are to some extent unique in their functions, the sequence in which species are lost is therefore essential for estimating the consequences of biodiversity changes for a particular system. However, in search for a general biodiversity- ecosystem functioning relationship, disentangling these species identity effects from the net biodiversity effect posed a major challenge for the interpretation of biodiversity experiments in the 1990s^{78,86–90}. A richness gradient is generally constructed in these experiments by randomly assembling systems of different diversity levels from a common species pool. Experiments therefore need to include high replication and randomisation of species composition within each richness level to avoid biases by the traits of the selected species when statistically comparing ecosystem functioning between richness levels^{62,88}. Differences in species richness between systems are therefore analogous to random species loss in these experiments. Consequently, the biodiversity-ecosystem

functioning relationships observed in these studies represent the average of all possible trajectories of species loss included in this study (Figure 1.3). Effects of non-random biodiversity changes, as occurring in natural ecosystems, can therefore easily deviate from this average relationship^{86,87}. In addition, environmental drivers of species loss, such as global warming or pollutants, do not only eliminate species from the system but can also affect the functioning of the remaining species. This can augment the total effect on ecosystem functioning, causing the true biodiversity-ecosystem functioning relationship to deviate even more from that estimated from the direct manipulation of species richness⁸³. Observations have however been remarkably consistent across studies, trophic levels and ecosystem functions, and the different levels of biodiversity (genetic, species and functional group diversity) with the vast majority of studies reporting a positive, often saturating, relationship between biodiversity and both the extent and stability of ecosystem functions^{59,91–93}.

1.4. Biodiversity effects on ecosystem functions

Despite the consistent positive effect reported by early biodiversity experiments, the validity of these empirical results has been vividly debated in the 1990s due to concerns about potential confounding factors and hidden treatments in the experimental design^{86,87,89,94,95}. This debate was only resolved by the 2000s because of the development of a theoretical framework that identified the mechanisms by which observed biodiversity-ecosystem functioning relationships could arise^{96–100}.

Although numerous types of ecosystem functions can be defined, several functions such as recreational or aesthetic value are hard to quantify¹⁰¹. Biodiversity experiments have therefore generally been limited to easily physically measurable ecosystem functions, which generally consist of the aggregate of the individual species functional contributions (e.g. total biomass, nutrient retention or primary production). Two main biodiversity effects emerged from these experiments: Biodiversity increased the performance^{102,103} and temporal stability^{50,51} of these aggregated ecosystem functions. The magnitude of both effects, however, appears to be uncorrelated⁶⁰ and biodiversity effects on ecosystem functioning and functional stability have generally been treated separately, with their separate theoretical frameworks mechanisms^{56,57,59}.

1.4.1. Biodiversity effects on ecosystem functioning

Grasslands have played an important role as a model system in biodiversity-ecosystem functioning research, shaping the ideas on the underlying mechanisms^{93,102,103}. Two classes

of biodiversity effects are thereby discerned: complementarity and selection effects^{96,99,104,105}. Species complementarity has widely been accepted as a mechanism by which biodiversity can affect ecosystem functioning, being deeply rooted in the practice of intercropping⁴³. This agricultural practice aims at increasing yields by combining crops within a field. When plants differ in their niches (e.g. the preference for light or shade), this reduces the competition between heterospecifics compared to the competition between conspecifics. Species complementarity thus reduces the strength of competition an individual experiences, and consequently increases the number of individuals and/or the biomass that can be sustained by the system. Indeed, the first modern type biodiversity experiments, conducted at the beginning of the 19th century, manipulating the diversity of herbs and grasses was to increase herbage production, invariably observed an increased average herbage production in more diverse plots^{41,42}. Driven by increased species complementarity, more diverse plots could thereby sustain a greater number of individuals by making most of the available resources. Facilitative interactions can have similar effects on ecosystem functions. Facilitative interactions refer to increases in the number or functioning of other species through direct positive interactions (e.g. flower-pollinator interactions), whereas niche differentiation increases functioning by the avoidance of negative, competitive interactions. However, as niche differentiation and facilitation have similar effects, their relative contribution to changes in species functioning is often difficult to discern in experiments. Both are hence generally grouped under the term complementarity effects⁹⁹.

The validity of selection effects, in contrast, has been strongly debated^{86,87,89}. Selection effects refer to competitive processes that drive ecosystem functions by high functional contributions of species with particular traits. When ecosystem functioning is principally driven by species with particular traits, biodiversity may also increase productivity as a result of the increased probability of sampling species with these traits. However, if these species subsequently dominate the system to such extent that other species are lost, selection effects will merely reflect the effect of initial biodiversity, rather than a true biodiversity effect. This effect of initial diversity, increasing the chance of including dominant species, can therefore act as a hidden treatment in the experimental design in what is referred to as a sampling effect. In addition, sampling effects do not only affect selection effects, but can also affect complementarity effects by increasing the chance of including complementarity species or facilitative interactions.

The possibility that these sampling effects were the main driver of the observed positive biodiversity-ecosystem functioning relationships, spurred a vivid discussion in the 1990s on the validity of the design used for biodiversity experiments^{86,87,89,95,106–108}. As experiments

typically consists of communities of different diversity levels that were randomly assembled from a common species pool, concerns were not only raised about the increased probability of including species with high functional traits in more diverse systems, but also the consequences of the randomness of species loss. The *ECOTRON* study, for example, was one of the first studies to demonstrate positive effects of biodiversity on ecosystem functioning⁵¹. However, it consisted of 3 communities of a different diversity level, representing 3 stages of a depauperate ecosystem. Hence, the concluded positive biodiversity was disputed as it could not be ruled out that changes in ecosystem functioning also depending on the traits of the deleted species⁸⁶. High replication within richness levels has therefore become a standard practice in biodiversity research. Comparing the average level of ecosystem functioning between richness levels thereby allows to separating the net effect of changes in species richness from the effects of species identities (Figure 1.3)^{62,78,88}.

The next step in deepening our understanding of biodiversity-ecosystem functioning relationships required quantifying the contribution of selection and complementarity effects to BEF relationships^{97,98}. Additive partitioning methods have thereby resulted in a major advancement. First introduced by Loreau and Hector⁹⁹, additive partitioning methods (Box 1) compare species functional contributions to that expected from a null model under which no biodiversity effects occur. As such, they allow to factor out the sampling effect by making species-specific predictions for the system^{99,100,109}. Under the null-hypothesis that inter- and intraspecific interactions are equal, the amount of competition an individual experience is independent of the identity of the interacting species. The system consequently behaves neutrally as species densities and functional contributions undergo a random walk. For ecosystem functions that consist of the aggregated functional contribution of the individual species (e.g. nutrient retention, total biomass or primary production), the expected functional contribution therefore equals the initially planted or seeded fraction^{99,110}. Observed deviations in species functional contributions from that expected under the null hypothesis (i.e. the species' initial functional contribution) are then partitioned between terms that are interpreted as reflecting the various mechanisms through which biodiversity can affect ecosystem functioning.

Loreau and Hector⁹⁹ presented a bi-partite method partitioning biodiversity effects between two terms, that are interpreted as reflecting the complementarity effect and selection effect (Box 1). The selection effect was originally introduced as representing the analogous of natural selection in the Price equation¹¹¹, which partitions changes in allele frequencies over generations between selection, mutation and drift. Fox¹⁰⁰, however, revealed this analogy to be only partially true. Instead, he demonstrated how the selection effect *sensu* Loreau and

Hector⁹⁹ can be split into two effects: the dominance effect and the trait-dependent complementarity effect (Box 1). The selection effect *sensu* Loreau and Hector⁹⁹ refers to changes in ecosystem function by high abundance of species with particular traits, causing them to have a disproportional high functional contribution to ecosystem functioning. Dominance effects refer to the part of the selection effect that is caused by competitive replacement between species. The dominance effect thus quantifies to what extent species increase their functional contribution to ecosystem functioning directly at the expense of others based on their functional traits. Consequently, the dominance effect is the true analogue of natural selection *sensu* Price¹¹¹. The trait-dependent complementarity effect then comprises the part of the selection effect by which changes in functional contributions are related to their traits, but without replacing other species. Hence, the trait-dependent complementarity is interpreted as reflecting the effect of species complementarity or facilitation experienced by species with particular functional traits, for example from one-way facilitative interactions. This in contrast to the complementarity effect *sensu* Loreau and Hector⁹⁹, that comprises the average deviation in species functional contributions in the system from the null hypothesis. As such, it represents the average effect of species complementarity, irrespective of functional traits, and is therefore termed the trait-independent complementarity effect by Fox¹⁰⁰.

Box 1: Additive partitioning methods

Define for a mixture of n species:

M_i : the yield of species i in monoculture

$Y_{o,i}$: the observed yield of species i in a mixture

$RY_{e,i}$: the expected relative yield of species i in a mixture, which equals its initial proportion

$RY_{o,i} = Y_{o,i}/M_i$: the observed relative yield of species i in a mixture

$Y_{e,i} = RY_{e,i} M_i$: the expected yield of species i in a mixture

$Y_e = \sum Y_{e,i}$: expected total mixture yield

$Y_o = \sum Y_{o,i}$: observed total mixture yield

Under the null-hypothesis each species is expected to realise a mixture yield equal to its initial functional contribution. Note that the term yield used because the historical important role grassland experiments, but refers to any measurable ecosystem function. The total deviation in mixture yield can therefore be expressed as the sum of the individual species' deviations from the null hypothesis.

$$\begin{aligned}\Delta Y &= Y_o - Y_e = \sum_{i=1}^n Y_{o,i} - Y_{e,i} = \sum_{i=1}^n (RY_{o,i} - RY_{e,i})M_i = \sum_{i=1}^n \Delta RY_i M_i \\ &= n \text{cov}(\Delta RY_i, M_i) + n E(\Delta RY)E(M)\end{aligned}$$

This is the additive partitioning method by Loreau and Hector⁹⁹, where cov and E denote the covariance and expected value, unweighted for the initial proportion of the species in the mixture. The first term is the selection effect, quantifying the extent by which species deviations from the null hypothesis depend on their functional traits, that is their monoculture yield. Positive selection effects increase ecosystem functioning because of the high functional contribution of high-functioning species. The second term is the complementarity effects of which the sign depends on the average species deviation from the null model, $E(M)$. Positive complementarity effects thereby increase ecosystem functioning as species perform on average better in mixtures compared to their monoculture.

Fox¹⁰⁰ proposed an alternative partitioning method, splitting the species deviation from the null hypothesis, ΔRY_i , in a part quantifying the change in species frequency in the mixture compared to the null hypothesis, and a part quantifying changes in yield that does not result from changes in frequency:

$$\begin{aligned}\Delta Y &= \sum_{i=1}^n (RY_{o,i} - RY_{e,i})M_i = \sum_{i=1}^n \left(\frac{RY_{o,i}}{RYT} - RY_{e,i} + RY_{o,i} - \frac{RY_{o,i}}{RYT} \right) M_i \\ &= n \text{cov} \left(\frac{RY_{o,i}}{RYT} - RY_{e,i}, M_i \right) + \text{cov} \left(RY_{o,i} - \frac{RY_{o,i}}{RYT}, M_i \right) + n E(\Delta RY)E(M)\end{aligned}$$

Whereby RYT is the relative yield total, $\sum RY_{o,i}$. The first term hence represents the dominance effect. Quantifying to what extent changes in species relative frequencies in the mixture depend on their functional traits. Note that by dividing the relative yield by the relative yield total, a fraction is obtained. The difference between this normalised relative yield and the expected relative yield is the change in a species frequency in the mixture, which can only change as a result of replacement between species. The second term is the trait-dependent complementarity effect and quantifies the extent by which changes in species yield that do not result from competitive replacement depend on species functional traits. The third term, the trait-independent complementarity effect, is identical to the complementarity effect sensu Loreau and Hector⁹⁹ and depends on the average species deviation from the null hypothesis, irrespective of species functional traits.

1.4.2. Biodiversity effects on ecosystem function stability

The occurrence of biodiversity effects on ecosystem functioning stability has been the subject of a long standing debated, fuelled by apparent opposing empirical and theoretical results and confusion on the different levels at which ecosystem stability can be defined^{46,48,49}. The first notions of positive biodiversity effects on ecosystem functioning stability during the first half of the 20th century were mainly based upon field observation and simple theoretical models. Complex systems such as tropical rainforests appeared much more stable compared to simple boreal systems or the deliberately simplified agricultural systems, which were readily pest-infested. Moreover, simple models of one-predator-one-prey systems already showed rather complex dynamics, often lacking a stable equilibrium^{44,45,48,112}. It was therefore argued that biodiversity should increase stability at both the population and ecosystem level¹¹³. This view was challenged in the 1970s by the work of Robert May. His rigorous analysis of both low and highly complex systems provided mathematical evidence that biodiversity should instead result in more violent fluctuations in population densities^{33,47}. These opposing empirical and theoretical results on biodiversity effects on population-level stability resulted in a highly confused debate 1970s⁴⁸. However, May already suggested in the second of his book '*Stability and complexity in model ecosystems*' that larger population fluctuations could in fact drive the stability of ecosystem functions⁶². Similarly, Pimm⁴⁹ tried to resolve the debate by pointing out that the different scales at which stability could be defined should not necessarily yield the same biodiversity effects. Still, this scale-dependency was largely overlooked until the end of the 1990s when Doak et al. revived the idea of statistical averaging^{33,62,114}. Under statistical averaging, ecosystem function stability arises from population-level fluctuations¹¹⁴. When species respond differently to an environmental change, the decrease in function of some species can be (partially) compensated by an increase in function of other species, averaging out the net effect on their aggregated ecosystem function. The higher the number of species, the more likely that asynchronous fluctuations will occur (Figure 1.4). As such, statistical averaging revealed that negative effects on population stability, as predicted by May^{33,47}, does not contradict observed positive biodiversity effects on ecosystem function stability, but instead can drive the stability of aggregated ecosystem functions. Resembling the common banking practice of spreading investments over large number of stocks to avoid risks of violent fluctuation in the portfolio, this statistical averaging is also commonly referred to as the portfolio effect.

Several statistical and biological mechanisms can influence the effect of statistical averaging¹¹⁵. The degree by which the variance in species densities scale to their mean value is an important additional statistical determinant of the portfolio effect. Species

generally reach lower densities in multispecies systems compared to monocultures because of interspecific competition. When the variance in species density (σ_i^2) scales to the mean value (m_i) by a power z , $\sigma_i^2 = c m_i^z$, the decrease in species densities in multispecies systems results in an even larger decrease in the variance when $z > 1$. Increasing biodiversity thereby stabilizes ecosystem functions by reducing (the variability in) species densities, and consequently the variance in their aggregated ecosystem function^{94,114}. Larger values of z increase statistical averaging, whereas z values less than 1 result in a destabilization of ecosystem functions⁹⁴. In real systems, z values indeed often exceed 1 resulting in statistical averaging effect. In grasslands, for example, z values are estimated between 1.2 and 1.4^{94,116}.

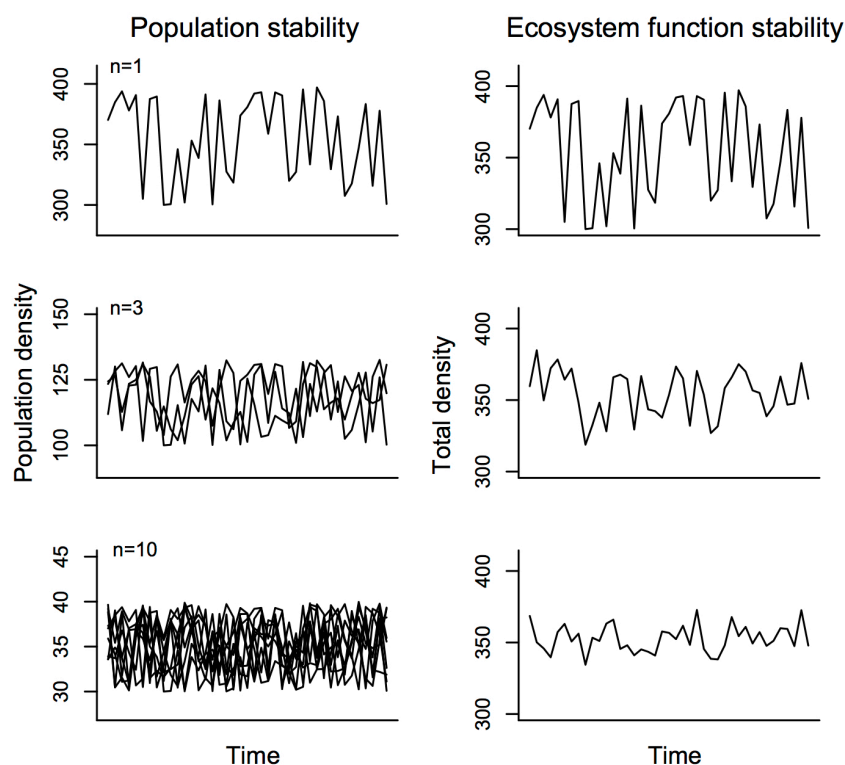


Figure 1.4: Statistical averaging. Biodiversity increase the probability that species fluctuations in individual species densities are average out at the level of their joint ecosystem function. Redrawn from Cottingham et al. 2001¹¹⁵.

Although interspecific differences in environmental response suffice to generate statistical averaging, the effect is promoted a greater asynchrony in species fluctuations^{94,114,117}. Perfectly asynchronous changes in species functional contributions fluctuate result after all in no effect on their aggregated ecosystem function. Hence, negative covariances between the functional contributions of species pairs increase the extent of functional compensation,

stabilizing ecosystem functions. Species interactions are therefore an important determinant of statistical averaging by determining the systems temporal dynamic in composition. As species interactions determine how changes in one species will affect other species, competitive interactions are consequently expected to stabilize ecosystem functions, by promoting asynchronous interactions by competitive release. Positive species interactions, in contrast, are expected to decrease stability by inducing positive covariance⁹⁴. However, the importance of species interactions on ecosystem stability has been debated. Although species interactions undoubtedly affect the degree of functional compensation, theoretical studies predict that biodiversity primarily increases ecosystem function stability by increasing the interspecific variability in environmental responses, whereas the type of species interactions only has a minor effect^{118,119}. In addition, the increased number of species interactions is expected to slow down population fluctuations when species interactions are similar between species, stabilizing ecosystem functions by reducing the effect of environmental changes on the system's composition¹²⁰. The long-term dynamics in the system's composition can also affect stability. Increased evenness prevents that species with favourable traits have to bounce back from low abundances, resulting in a faster compensation of functional loss in other species^{114,121} and increases effect of mean-variance scaling¹¹⁶.

1.5. The environmental dependency of biodiversity effects

The search for a general biodiversity-ecosystem functioning relationship has dominated biodiversity research for the past two decades. In an attempt to estimate the overall consequences of global biodiversity declines from such general relationship, the main objective was thereby to quantify the empirical support for positive, neutral or negative biodiversity effects^{57,59,65,93,122}. As the vast majority of studies has supported positive biodiversity effects on ecosystem functioning and ecosystem function stability, this has now lead to the consensus that ecosystems functions are globally at risk by ongoing biodiversity losses⁵⁹.

More recently, the focus of biodiversity research has however shifted towards a mechanistic understanding of the biotic and abiotic factors that can influence the strength biodiversity effects and explain differences between systems^{58,63,81,123}. The hundreds of empirical studies that have been conducted revealed a large degree of variability in biodiversity effects between study systems. Negative biodiversity-ecosystem functioning relationships are thereby frequently reported⁹³. Identifying selection effects and complementarity effects as main drivers of BEF-relationships provided a major breakthrough in reconciling these

opposing empirical results. Based on species interactions and interspecific differences in functional traits, selection effects and complementarity effects provided a mechanistic underpinning for BEF relationships^{97–99}. Consequently, this framework allowed to identify the conditions under which positive or negative relationships should arise, demonstrating how the wide range of observed BEF relationships could arise from the traits of the species in the system^{99,100,124}.

Now, an increasing amount of studies have established that also abiotic conditions can strongly affect the strength of biodiversity effects. Changes in temperature^{125,126}, nutrient availability^{81,127–130}, drought^{131–133}, shade¹³⁴, osmotic stress^{134,135}, toxic chemicals^{13,136} or flooding¹³⁷ have all been demonstrated to alter the strength of biodiversity effects within systems. However, how these changes in abiotic conditions alter biodiversity-ecosystem functioning relationships remains unclear as both increases and decreases in the slope of the BEF relationship by environmental changes have been reported^{133–136}. Differences in the BEF relationship can thereby, to some extent, be related to differences in environmental favourability, altering species fitness, and thus their ability to contribute to ecosystem functions^{123,131,133}. However, an integrated mechanistic framework that explains how system-specific differences in biodiversity effects on ecosystem functioning and ecosystem function stability depend on the biotic (species interactions and functional traits) and the abiotic (environmental conditions) properties of the system is currently lacking^{58,63,135,138}.

1.6. Rationale, research objectives and thesis outline

The empirical search for general relationships, which were initially based on correlations rather than theoretical frameworks, has caused biodiversity research to diverge from classical community and ecosystem ecology over time⁵⁸. Moreover, biodiversity effects on ecosystem functioning and ecosystem function stability have mainly been treated separately^{57,59,60,65}. This has led to a different focus in the mechanistic frameworks underpinning both biodiversity effects^{99,114,116}: While biodiversity effects on ecosystem functioning are mostly focussing on species interactions^{99,100}, effects on ecosystem function stability mainly related to interspecific differences in environmental response^{94,114,118}. However, both biodiversity effects are intrinsically linked. Changes in the BEF relationship between environmental conditions requires diversity-dependent changes in ecosystem functioning. Hence changes in biodiversity effects on ecosystem functioning must result from biodiversity effects on ecosystem function stability.

Species interactions and species environmental responses are both essential determinants

of the system's composition, species coexistence and consequently species' functional contributions to ecosystem functions. Integrating theory on compositional dynamics and species coexistence into contemporary biodiversity theories will therefore be an essential step for incorporating biotic and abiotic factors that can explain differences in biodiversity effects between systems and environmental conditions. Despite the recent plea to take on this holistic approach in biodiversity research^{58,63,138}, an integrated mechanistic framework is to date still lacking and attempts to separate environmental and biodiversity effects have been limited to the use of multivariate techniques¹²³.

Environmental changes and biodiversity changes are predicted to be the main drivers of changes in ecosystem functioning in the 21st century^{3,15,82,139}. The absence of a mechanistic framework explaining the environmental dependency of biodiversity effects is therefore a major knowledge gap for understanding, estimating and mediating their combined effects on the many ecosystem functions society depends^{82,139}. This thesis therefore aims to deepen our mechanistic understanding of the environmental dependency of biodiversity effects on ecosystem functioning. To this end, I explore how biodiversity theory on ecosystem functioning and ecosystem function stability can be linked through classic theory on compositional dynamics.

In **Chapters 2 and 3** I demonstrate how changes in BEF relationships between environmental conditions can be explained. Many empirical studies so far have demonstrated these environment-induced changes in BEF relationships. However, a mechanistic underpinning of the shifts is currently lacking. This is particularly important, as apparent contradictory results have been reported, with both increases and decreases in the slope of the BEF relationship by environmental changes (see also 1.5. The environmental dependency of biodiversity effects). In **chapter 2**, I first explain changes in the BEF relationship for a specific system. In a microcosm experiment, I exposed North Sea diatom communities, spanning multiple levels of species richness, to three concentrations of the herbicide atrazine. Diatom communities were thereby chosen as a study system since they are important primary producers in the North Sea ecosystem^{140,141}, and allow to study biodiversity effects over multiple generation in a short timespan. Next, changes in the BEF relationship between environmental conditions, and the corresponding changes of the underlying biodiversity effects as defined by Fox's¹⁰⁰ additive partition (Box 1) were quantified. These empirical observations were subsequently confronted with two theoretical frameworks that make opposing predictions on the contributions of species interactions on environment-induced changes in BEF relationships and the underlying biodiversity effects. While the stress gradient hypothesis predicts shifts in the strength of per-capita interactions

by environmental changes, coexistence theory does not. The microcosm experiment did not provide any empirical support for shifts in the strength of per capita interactions. Instead, changes in the BEF-relationship, as well as difference in changes in the underlying biodiversity effects between different communities, were predictable from the individual species responses to environmental changes and a constant strength of per-capita interactions. In **chapter 3** I generalise the findings of chapter 2. First, a comprehensive model is used to develop a general theory on how BEF relationships and the biodiversity effects defined by Fox¹⁰⁰ (Box 1) change over environmental gradients. I demonstrate how species environmental responses are expected to cause a general, unimodal response of the slope of the BEF relationship along an environmental gradient, driven by changes in the dominance effect. Biodiversity effects on ecosystem functioning are thus expected to be larger at intermediate levels of environmental stress. The exact shape of the unimodal response, and thus the stress level at which biodiversity peaks, however, is predicted to depend on the type of per-capita interactions of the system, which determine changes in the trait-dependent and trait-independent complementarity effect. Hence, the model provides a mechanistic underpinning of changes in BEF relationships, explaining why changes in the slope of BEF relationships can be both positive and negative, as well as what causes differences between studies. Next, I demonstrate that these theoretical predictions strikingly coincide with the observed changes in BEFs relationship in 52 studies that manipulated species richness under different environmental conditions.

In **chapter 4** a non-linear extension for the additive partitioning methods of both Loreau and Hector⁹⁹, and Fox¹⁰⁰ is presented. In their classic formulation, additive partitioning methods quantify biodiversity effects by assuming that species interactions cause species to deviate from the null hypothesis in a way that linearly depends on their functional traits (Box 1). However, other relationships between species deviations from the null hypothesis and their functional traits are equally likely. Such nonlinear deviations are, for example, likely when differences in the strength of species interactions (e.g. when one species is particularly dominant) or functional traits (e.g. when some species are very sensitive to environmental changes) are strong. I therefore first develop an n^{th} order extension of additive partitioning methods. Next, it is discussed how these higher order terms can be interpreted. Finally, it is demonstrated how a second order extension can help to increase our understanding of complex biodiversity effects by applying second order additive partitioning methods to the Cedar Creek Biodiversity II experiment, one of the longest running biodiversity experiments.

In **Chapters 5 and 6** it is demonstrate how compositional dynamics and biodiversity effects on ecosystem function stability are linked in changing environments. In **chapter 5** it revealed

how biodiversity increases functional stability through compositional stability. Although statistical averaging focuses on strong compositional turnover, ecological theory also predicts that in systems of similar species, such as primary producer systems, biodiversity should increase ecosystem function stability by increasing resistance, rather than resilience. Apart from theoretical models, the link between compositional and functional stability has rarely been assessed. Here, the predicted simultaneous effects on functional and compositional stability are tested using the same microcosm setup as in chapter 2. However, diatom communities were after 4 weeks of exposure to the herbicide atrazine transferred to atrazine-free medium for 3 more weeks. Confirming theoretical predictions, biodiversity indeed increased ecosystem function stability by increasing the system's compositional resistance, empirically establishing the tight link between the systems functional and compositional stability in primary producer systems. In **chapter 6** the consequences of the link between compositional and functional changes for environmental risk assessment procedures are assessed. These currently still assume a certain degree of functional redundancy between species, such that protecting the ecosystem's composition also protects its functions. However, ecosystem-level effects are thereby generally inferred from species-level effects without taking species interactions into account. This can hence lead to an over- or underestimation of effects on ecosystem structure and functioning through functional compensation or cascades, respectively. I explore how, in the absence of information on species interaction, estimations can be made on how well species-level effects of environmental changes correspond to ecosystem level effects based on species tolerances. More precisely, it is revealed how the correlations between species tolerance to environmental changes and their functional abilities under unstressed conditions affect ecosystem level effects. To this end, I confront a diatom microcosm experiment with a theoretical model. I demonstrate that the extent of functional compensation, and thus ecosystem-level effects indeed relates to the correlation between species tolerance and their functional abilities under unstressed conditions. Positive correlations thereby increase the probability of functional compensation, and reduce the effect on ecosystem functioning of environmental changes.

In the final chapter, **chapter 7**, I discuss how the results presented in this thesis fill several key gaps in biodiversity research and set the stage for future research.

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2

Environment-induced changes in biodiversity effects on ecosystem functioning

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Abstract

Environmental stress changes the relationship between biodiversity and ecosystem functions, but the underlying mechanisms are poorly understood. Because species interactions shape biodiversity-ecosystem functioning relationships, changes in per capita interactions under stress (as predicted by the stress gradient hypothesis) can be an important driver of stress-induced changes in these relationships. To test this hypothesis, productivity was measured in microalgae communities along a diversity and herbicide gradient. Based on additive partitioning and a mechanistic community model, it is demonstrated that changes in per capita interactions did not explain effects of herbicide stress on the biodiversity-productivity relationship. Instead, assuming that per the capita interactions remained unaffected by stress, causing species densities to only change through differences in stress tolerance, suffice to predict the stress-induced changes in the biodiversity-productivity relationship and community composition. Finally, it is discussed how these findings set the stage for developing theory on how environmental stress changes biodiversity effects on ecosystem functions.

2.1. Introduction

Since the 1990s, hundreds of empirical studies established that biodiversity affects ecosystem functions^{1–6}. Today, there is mounting empirical evidence that stress caused by changes in environmental conditions alters the biodiversity-ecosystem function relationship. However, observations have been inconsistent among studies. While the majority of studies reported a decreased effect of biodiversity on ecosystem functions with increasing stress^{7–16}, others reported no change^{17,18} or even an increase¹⁹. The mechanisms underlying these stress-induced changes in biodiversity effects – and possibly explaining the observed differences among studies – remain virtually unexplored. This lack of mechanistic understanding hampers our ability to predict the value of biodiversity in the many ecosystems that are currently challenged by environmental stress^{20,21}.

Biodiversity effects on ecosystem functions can be understood from species interactions. When inter- and intraspecific interactions differ in strength, biodiversity affects ecosystem functions as species will function differently in the presence of other species compared to their monocultures^{22–24}. Ecological theory distinguishes two classes of biodiversity effects. First, interspecific interactions can change species contributions to ecosystem functions because of competitive replacement. This dominance effect alters ecosystem functions because of the increased functional contribution of superior competitors²³. Second, interspecific interactions can also change species functional contributions without resulting in competitive replacement. Such effects are referred to as complementarity effects as they are mainly attributed to niche complementarity or facilitative interactions between species²³.

Species interactions are not only important determinants of biodiversity effects. They also regulate how stress will alter the contributions of species to ecosystem functions^{25–27}. Stress has a direct effect on species densities through effects on species fitness (reproduction and/or survival)^{28,29}. Species interactions thereby determine the extent by which these direct effects will affect other species^{26,27}. Species interactions thus take up a central position in both theory on biodiversity-ecosystem function relationships and stress ecology^{22,23,25–27}. Understanding whether, and to what extent stress affects species interactions is therefore crucial for the development of theory on stress-induced changes in biodiversity effects. Existing theories make conflicting predictions on the effect of environmental changes on the per capita strength of species interactions³⁰. The stress gradient hypothesis proposes that per capita interaction strengths are likely to shift from competitive to facilitative interactions under environmental stress^{31,32}. Coexistence theory, in contrast, does not make assumptions on the direct effect of stress on per capita species interactions. Hence, stress is assumed to alter the effect of species interactions principally through species-specific effects on

fitness^{33,34}. Both theories, by consequence, make different predictions on how stress can modulate biodiversity effects. If the per capita strength of species interactions is unaffected by stress, as assumed by coexistence theory, changes in biodiversity effects only result from the direct effects on species fitness and the same per capita interactions occurring in unstressed conditions. Hence, stress should principally change biodiversity effects on ecosystem functions through changes in dominance because of the replacement of sensitive by stress-tolerant species, as the latter by definition grow better when stressed. If, however, per capita interactions become more positive under stress, as predicted by the stress gradient hypothesis, also complementarity is expected to increase with stress.

Understanding how stress changes the effects of biodiversity on ecosystem functions is essential for ecosystem management but remains as yet virtually unexplored^{7,9,16}. In this chapter, it is examined how stress caused by the herbicide atrazine affects dominance and complementarity effects on productivity in marine diatom microcosms. Community composition and biovolume production in marine diatom communities was therefore measured along a diversity and herbicide (Atrazine) gradient in microcosms. It is tested what changes in biodiversity effects drive stress effects on the biodiversity-ecosystem function relationship, and if stress effects on the per capita strength of species interactions contribute to these changes. Two different approaches are used: (1) a partitioning method to quantify dominance and complementarity effects and (2) a mechanistic community model. Both approaches strongly support the absence of stress effects on per capita species interaction strengths. Instead, interspecific variability in stress tolerance and the strength of per capita species interactions in unstressed conditions could explain how stress alters biodiversity effects on ecosystem functions. Finally, it is discussed how these results are the first step towards a mechanistic theory explaining how environmental stress can change biodiversity effects on ecosystem functions in a variety of study systems.

2.2. Materials and methods

2.2.1. Algal strains

Diatoms were isolated from a single phytoplankton sample collected near the Thorntonbank (Southern bight of the North Sea) during the spring bloom in March 2013. Single cells were isolated from the sample using a micropipette. Next, cells were rinsed three times with growth medium and cultured as monoclonal stock cultures³⁵. F/2 medium³⁶ based on artificial seawater (salinity $33\pm 1\text{‰}$; Instant Ocean®) and supplemented with $30\text{ }\mu\text{g L}^{-1}$ Si as growth medium. Stock cultures were maintained in an acclimatized room ($20\pm 1^\circ\text{C}$) at a 12-hour photoperiod and a $35\pm 5\text{ }\mu\text{mol photons m}^{-2}\text{ s}^{-1}$ light intensity (Lumilux® 18W cool white

Osram). New cultures were inoculated weekly to sustain exponential growth. The photoperiod was prolonged to 16 hours weeks prior to the start of the experiment.

2.2.2. Microcosm experiment

Eight strains belonging to different species (*Bacillaria sp.*, *Coscinodiscus sp.*, *Ditylum sp.*, *Guinardia sp.*, *Gyrosigma sp.*, *Odontella sp.* and 2 species *Thalassiosira sp.*) differing in size, division rate and stress tolerance were randomly selected from the stock cultures (Appendix A Table S2). Communities of five levels of species richness were represented at each of the three levels of atrazine (i.e. a full-factorial design). To separate species-identity from diversity effects³⁷, 10 different random assemblages were made at each richness level, except at levels 1 and 8 where only 8 and 1 assemblages were possible (Appendix A Table S3). Atrazine concentrations (0, 25 and 250 $\mu\text{g L}^{-1}$) that represented a control, low stress and high stress treatment were selected from preliminary tests. Microcosms were established in three replicates at each concentration (351 microcosms in total).

Communities were inoculated in sterilised 100ml glass Erlenmeyer flasks filled with 35ml F/2 medium containing the required atrazine (Sigma Aldrich) concentration, and fitted with cellulose plugs. Species were inoculated at an equal proportion of the total initial biovolume ($10^7 \text{ mm}^3 \text{ L}^{-1}$). Note that species equilibrium densities differed by several orders of magnitude (Appendix A Table S2). Biovolume, rather than density was hence selected on the basis on which to define species functional abilities. To minimize variability between replicates and assemblages, species were inoculated from single stock cultures. Microcosms were cultured for 4 weeks at $20 \pm 1^\circ\text{C}$ and a $35 \pm 5 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ 16-hour photoperiod. Weekly, 80% of the growth medium was renewed to avoid nutrient limitation or stress reduction through the atrazine photolysis. To determine species densities, 1mL samples were taken, fixed with formaldehyde at a 6% final concentration, and stored at 4°C in 24-well plates until analysis. Cell densities were determined using an inverse microscope and Whipple grid. Only living cells were counted. Mortality could easily be assessed on the basis of empty frustules (i.e. the empty siliceous diatom cell walls that remain after the cells have died, see Appendix A Figure S3). Mortality rates were very low, independent of the diversity treatment. In nearly all communities, dead cells accounted for less than 1% of the total cells. Species that were completely inhibited by atrazine, however, showed an increased relative proportion of dead cells in the high stress treatment because population dynamics were only driven by mortality (see Appendix A Table S2). Biovolumes were calculated on the basis of the average cell volume of each species, calculated from a sample of 50 cells³⁸. A single cell volume was used per species for all treatments, as preliminary results did not reveal atrazine effects on cell volume. To verify constant stress levels and the absence of nutrient limitation, nitrate,

phosphate, and silicate concentrations were weekly spectrophotometrically determined (Aquamate®, Thermo Electron Corporation + Spectroquant® test kits, Merck Millipore). Atrazine concentrations were determined GC-MS (Thermo Quest Finnigan Trace DSQ coupled to Thermo Quest Trace 2000 series).

2.2.3. Calculation of biodiversity effects

Biodiversity effects were calculated using an additive tri-partite partitioning method²³. This method is based on the comparison of the observed yield of a species in mixture to that expected under the null-hypothesis that inter- and intraspecific competition are equal. Under this null hypothesis, species performance is independent of diversity. Hence, species are expected to realise a proportion of their monoculture yield (i.e. 'observed relative yield', RY_o) equal to their initial proportion in the mixture (i.e. 'expected relative yield', RY_e). This species-specific expected yield allows to factor out potential confounding effects related to differences in species composition effects (e.g. sampling effects)³⁹. The partitioning splits the deviation of the total mixture yield from that expected under the null-hypothesis (ΔY) in dominance, trait-dependent complementarity and trait-independent complementarity effects:

$$\Delta Y = \sum_i \Delta Y_i = N \text{cov} \left(M, \frac{RY_o}{RY_{T_o}} - RY_e \right) + N \text{cov} \left(M, RY_o - \frac{RY_o}{RY_{T_o}} \right) + N E(M) E(\Delta RY) \quad (1)$$

These three biodiversity effects reflect how the individual species yields (ΔY_i) deviate from the null hypothesis, and whether deviations depend on species functional abilities (i.e. the monoculture yield M). The first term, the dominance effect, quantifies the extent by which species deviate from the null hypothesis by replacing other. This is measured by the unweighted covariance (i.e. not accounting for the species' initial proportion in the mixture) between a species monoculture yield and the deviation of its realised fraction of the relative yield total, RY_{T_o} (i.e. as if the species were competing within a zero-sum game) from that expected under the null-hypothesis (i.e. RY_e). The second term, the trait-dependent complementarity effect, quantifies the extent by which species' deviations from null hypothesis that do not result in competitive replacement (i.e. deviates from a zero-sum game) correlate to the monoculture yield. The third term, the trait-independent complementarity effect, is the product of the average monoculture yield and the average species deviation from the null hypothesis, and quantifies to what extent species deviate on average from the null hypothesis, irrespective of their monoculture yield.

2.2.4. Data analysis

Linear mixed effects models were used to assess the effects of \log_{10} diversity ($LDiv$), atrazine concentration (C) and time (Day) on the \log_{10} biovolume, and of \log_{10} diversity and time on

stress-induced changes in biodiversity effects (i.e. dominance, trait-dependent complementarity and trait-independent complementarity effects) were evaluated using linear mixed effects models⁴⁰. Full models included all possible predictor interactions:

$$\log_{10} \text{biovolume}_i = \alpha + \beta_1 C_i + \beta_2 LDiv_i + \beta_3 Day_i + \beta_4 C_i \times LDiv_i + \beta_5 C_i \times Day_i + \beta_6 LDiv_i \times Day_i + \beta_7 C_i \times LDiv_i \times Day_i + \varepsilon_i \quad (2)$$

and

$$\Delta \text{Biodiversity effect}_i = \alpha + \beta_1 LDiv_i + \beta_2 Day_i + \beta_3 LDiv_i \times Day_i + \varepsilon_i \quad (3)$$

Models were optimized through a backward selection procedure. Interactions were only retained when main effects were significant or when removing them did no longer result in normal distributions of model residuals. Because of the temporal dependence of the data, full models were fitted with a continuous first order autocorrelation structure. Community assemblage was included as a random effect to account for species identity effects. Models that incorporated community assemblage as a random effect (i.e. a random intercept model) were significantly better than those without (ANOVA: $F_{14,13}=628$, $p<0.001$). Temporal autocorrelation structures, in contrast, were only required for models predicting changes in biodiversity effects (ANOVA: $F_{7,6}=5.3$, $P<0.05$). Validity of the optimal models was assessed based on the normality of model residuals (Appendix A Figure S4-11).

Next, it was tested to what extent stress-induced changes in biodiversity effects depended on direct stress effects on species growth, the strength of per capita interaction in unstressed conditions, and stress effects on these interactions. These predictors were respectively quantified as the mean weighted atrazine effects on monoculture growth (M_{250}/M_0), the per capita interaction coefficients in unstressed conditions (A_0) and the atrazine effects on the per capita interaction coefficients ($A_{250}-A_0$), which were estimated by the community model (see next section). All estimates were weighted for the relative species abundance in the control treatment. Initial full models included all pairwise interaction effects:

$$\Delta \text{Biodiversity effect}_i = \alpha + \beta_1 E\left(\frac{M_{250}}{M_0}\right) + \beta_2 E_w(A_0) + \beta_3 E_w(A_{250} - A_0) + \beta_4 E\left(\frac{M_{250}}{M_0}\right) \times E_w(A_0) + \beta_5 E\left(\frac{M_{250}}{M_0}\right) \times E_w(A_{250} - A_0) + \beta_6 E_w(A_0) \times E_w(A_{250} - A_0) + \varepsilon_i \quad (4)$$

Where E_w represents the weighted mean and ε_i the model residuals. Community composition was included as a random effect (ANOVA: $F_{9,8}=22.1$, $P<0.0001$). Model residuals were not temporally correlated. Optimal models were obtained from a backward selection procedure and normality of model residuals was assessed (Appendix A Figure S12-14). Analyses were conducted in R 3.1.1.⁴¹ using the lme4 package⁴². Only changes day 21 and 28 were

included since strong biodiversity-ecosystem function relationships only developed after 14 days (Figure 2.1). Estimates of species monoculture growth in unstressed (M_0) and high-stress conditions (M_{250}) and absolute interspecific competition coefficients ($\log A_{i,j}$) were obtained from the community model (see next section). Model estimates under scenario 4 were used for the fixed strength of per capita interactions, whereas estimated under scenario 5 were used for the change in per capita interaction strength (see parameter estimation).

2.2.5. Community model

A Lotka-Volterra model with a stress-dependent intrinsic growth rate and carrying capacity was used to simulate community dynamics:

$$\frac{dN_i}{dt} = \mu_i(c)N_i \left(1 - \sum_{j=1}^n \frac{a_{i,j}(c)N_j}{K_i(c)} \right) \quad (5)$$

Where N_i ($\mu\text{m}^3 \text{ L}^{-1}$) is the biovolume density, μ_i (d^{-1}) is intrinsic growth rate and K_i ($\mu\text{m}^3 \text{ L}^{-1}$) is the carrying capacity of species i , $\alpha_{i,j}$ (-) is the interaction coefficient between species i and j , n is the total number of species and c is the atrazine concentration ($\mu\text{g L}^{-1}$). Intraspecific interaction coefficients ($\alpha_{i,i}$) were set to 1. This equation can also be rewritten in terms of absolute competition coefficients $A_{i,j}(c) = \alpha_{i,j}(c) K_i(c)^{-1}$:

$$\frac{dN_i}{dt} = \mu_i(c)N_i \left(1 - \sum_{j=1}^n A_{i,j}(c)N_j \right) \quad (6)$$

2.2.6. Community model simulations and evaluation

Model parameters were optimized (see next section) under the restrictions of five different scenarios to test for stress-induced changes in per capita interaction strength (Table 2.1). The first scenario is a baseline scenario without interspecific interactions (i.e. $\alpha_{i,j}(c)=0$ or $A_{i,j}(c)=0$). Species densities thus only depend on the stress-effect on their demographic rates. In the second scenario, per capita inter- and intraspecific interaction strength are assumed to be equal (i.e. $\alpha_{i,i}(c)=\alpha_{i,j}(c)=1$ or $A_{i,i}(c)=A_{i,j}(c)$). Hence, community dynamics still only result from interspecific variability of stress effects on growth. In the third scenario, the ratio between the strength of inter- and intraspecific interaction is constant (i.e. $\alpha_{i,i}(c)/\alpha_{i,j}(c)^{-1} = \text{constant}$ or $A_{i,i}(c)/A_{i,j}(c) = \text{constant}$). The strength of per capita interactions, however, increases when stress decreases the species' maximum function $K_i(c)$. In the fourth scenario absolute the strength of per capita interactions are assumed to be constant (i.e.

$A_{i,j}(c)=constant$). In the fifth scenario, species interactions are allowed differ between stress levels without any assumptions.

In each scenario the upper and lower limits of $\mu_i(c)$ and $K_i(c)$ were constrained within 10% of the value estimated for the monocultures. When growth rates were lower than 0.1 d^{-1} , the upper limit were set to 0.15 d^{-1} to avoid too stringent conditions when parameters values are underestimated from the monoculture data. Despite this correction, estimated values never exceeded the monoculture value by more than 30%. Relative interaction coefficients were limited between 0 and 200 based on the estimated values from diversity level 2. Because the control and $25\text{ }\mu\text{g L}^{-1}$ treatment were not significantly different (Figure 2.1, Table 1), parameters were only estimated for the control and $250\text{ }\mu\text{g L}^{-1}$ atrazine treatment. Parameters were estimated 100 times for each scenario to estimate parameter uncertainty. Next, 1000 Monte Carlo simulations were run for each scenario sampling. For each run, parameters were randomly from a uniform distribution constrained by the 2.5 and 97.5 percentile of parameter estimates. The average species density of each community at the start of the experiment was used as initial densities for model simulations. Community densities were simulated for 28 days, analogous to the experiment. Scenarios were compared using the likelihood of the proportion functional lost (i.e. $\sum_{i=1}^n N_i(250) / \sum_{i=1}^n N_i(0)$) and the average Bray-Curtis similarity between observed and predicted community compositions (i.e. $E[\sum_{i=1}^n |N_{i,obs}(c) - N_{i,pred}(c)| / \sum_{i=1}^n N_{i,obs}(c) + N_{i,pred}(c)]$) for each Monte Carlo run. The likelihood based on species densities evaluates how well the model predicts stress-induced changes in ecosystem function; the average Bray-Curtis similarity evaluates stress-induced changes in community composition. Comparisons were made by a signed rank test with Bonferroni correction. All simulations were performed in R 3.1.1.⁴¹ using the GenSA package⁴³.

2.2.7. Community model parameter estimation

Optimal parameter values were estimated using a simulated annealing optimization algorithm and the time and density weighted mean absolute percentage error (MAPE) as objective function. The MAPE was selected as objective function because biovolumes could differ by 8 orders of magnitude between species in a community. Therefore an objective function that scaled model deviations was required to ensure a comparative goodness of fit for all species (i.e. a good prediction of community composition). The MAPE was weighted for the relative species abundance to ensure a good prediction of the total community biovolume in (highly) uneven communities and was weighted for the sampling day to deal with the larger uncertainty on the low densities at day 7 and 14 biovolumes which could exceed the values

expected from the per capita growth rate in some species. The final objective function S can thus be written as:

$$S = \sum_{t \in [7,14,21,28]} \sum_{i=1}^n w_t p_{i,t} \left| \frac{N_{i,t,o} - N_{i,t,e}}{N_{i,t,o}} \right| \quad (7)$$

$$p_{i,t} = \frac{N_{i,t,o}}{\sum_{i=1}^n N_{i,t,o}} \quad \text{and} \quad \sum_{i=1}^n p_{i,t} = 1$$

$$w_t = 0.1 \frac{t}{7} \quad \text{and} \quad \sum_{t \in [7,14,21,28]} w_t = 1$$

$N_{i,t,o}$ is the observed biovolume of species i at time t , $N_{i,t,e}$ is the expected biovolume, $p_{i,t}$ is the relative species abundance at time t and n is the number of species in the community.

To ensure an efficient exploration of the parameter space, parameter sets that resulted in species densities reaching infinity, extinction of more than one species or the MAPE exceeding 100% was penalized by setting the objective function to:

$$S = 10^{10} \sum_{i=1}^z (\beta_{0,i} - \beta_i)^2 \quad (8)$$

Where $\sum_{i=1}^z (\beta_{0,i} - \beta_i)^2$ is the Euclidean distance of the parameter set $(\beta_1, \dots, \beta_z)$ from the initial parameter values $(\beta_{01}, \dots, \beta_{0z})$ of the optimization algorithm. This ensures that the algorithm returns to the initial parameters when it runs into a series of irrelevant solutions. In addition, to avoid over fitting of the model by unrealistically high interaction coefficients, the mean value of the interaction effect of each species was assumed not to exceed 1000 times the average species abundance. When the mean value exceeded this cut-off value, the excess was added to the objective function. This favours a reduction of species density either by a reduction in carrying capacity or by competition with abundant species rather than by competition with rare species.

2.3. Results

2.3.1. Microcosm experiment

Atrazine application changed the shape of the biodiversity-ecosystem function relationship (Figure 2.1). Biodiversity decreased biovolume production in control and low stress ($25 \mu\text{g L}^{-1}$

atrazine) conditions, but increased biovolume production at elevated stress (250 $\mu\text{g L}^{-1}$ atrazine) conditions. Throughout the whole experiment there was no significant difference between the no stress and low stress treatment (Table 2.1). Atrazine had larger effects on biovolume production at lower richness (Table 2.1). The biodiversity-ecosystem function relationship thus shifted from negative to positive under stress because of reduced stress effects on productivity in more species-rich communities (Figure 2.1). This effect of stress on the slope of the biodiversity-ecosystem function relationship was entirely driven by corresponding changes in dominance (Figure 2.2, Appendix A Figure S1). Only atrazine-induced changes in the dominance effect increased with species richness, and increased over time (Table 2.2). Atrazine effects on complementarity effects, in contrast, occurred independent of species richness for both trait-dependent and trait-independent complementarity effects (Figure 2.2, Table 2.2).

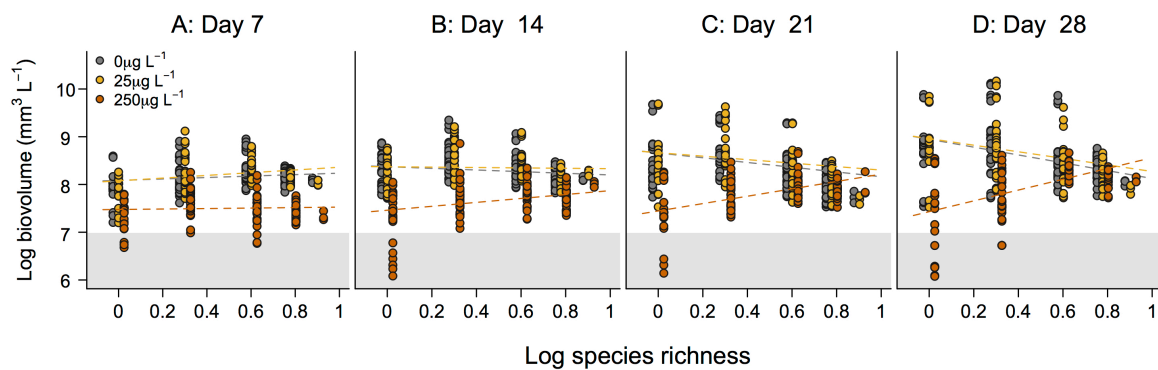


Figure 2.1: Stress-induced changes in BEF relationships. \log_{10} biovolume of the diatom communities at (A) day 7, (B) 14, (C) 21 and (D) 28 of the experiment for control, low stress and high stress conditions. Regression lines represent the predicted biodiversity-productivity relationship by the linear mixed effects model (Table 2.1). Grey areas indicate a negative net production.

Table 2.1: Biodiversity and stress effects on \log_{10} biovolume Mixed effects model estimates of the effect of \log_{10} diversity (LDiv), 25 $\mu\text{g L}^{-1}$ (LS) and 250 $\mu\text{g L}^{-1}$ (HS) atrazine stress on the \log_{10} biovolume over the course of the experiment (Day). SE is the standard error of the estimates

	DF	t-value	Estimate (SE)	P-value
Intercept	1355	77.8	7.799 (0.10)	<0.0001
LDiv	37	2.56	0.475 (0.19)	0.0144
Day	1355	12.4	0.041 (0.003)	<0.0001
LS	1355	-0.2	-0.016 (0.09)	0.8560
HS	1355	-3.5	-0.307 (0.09)	0.0006
LDiv × Day	1355	-7.6	-0.046 (0.006)	<0.0001
LDiv × LS	1355	0.8	0.140 (0.17)	0.3968
LDiv × HS	1355	-4.5	-0.785 (0.17)	<0.0001
LDiv × LS × Day	1355	-0.9	-0.008 (0.008)	0.3672
LDiv × HS × Day	1355	10.9	0.098 (0.008)	<0.0001

Table 2.2: Stress-induced changes in biodiversity effects Model 1: Mixed effects model estimating the effect of \log_{10} diversity (LDiv) on stress-induced changes in biodiversity effects over the course of the experiment (Day). Model 2: Mixed effects model estimating the effect of the weighted mean species stress tolerance (Tol) and per capita strength of interspecific interactions on changes in biodiversity effects at day 21 and 28. Means were weighted for the relative species abundance. Tolerance was calculated as the ratio between the species monoculture yield at $250 \mu\text{g L}^{-1}$ atrazine and in control conditions. The strength of interspecific interactions was based on the parameter estimates of the community model (see Materials and methods). SE is the standard error of the estimated fixed effects.

Dominance effect				
Model 1				
	DF	t-value	Estimate (SE)	p-value
Intercept	91	0.94	0.0014 (0.002)	0.35
LDiv	29	-1.47	- 0.0037 (0.003)	0.15
Day	91	-2.54	- 0.0002 (0.0001)	0.013
LDiv×Day	91	4.28	0.0005 (0.0001)	<0.0001
Model 2				
	DF	t-value	Estimate (SE)	p-value
Intercept	29	2.40	0.022 (0.009)	0.02
Tol	27	-2.41	-0.097 (0.04)	0.02
Inter	27	2.34	0.003 (0.001)	0.03
Tol×Inter	27	-2.48	-0.012 (0.005)	0.02
Trait-dependent complementarity effect				
Model 1				
	DF	t-value	Estimate (SE)	
Intercept	92	-0.91	-0.0008 (0.0009)	
LDiv				
Day	92	2.07	0.0001 (0.0001)	
LDiv×Day				
Model 2				
	DF	t-value	Estimate (SE)	p-value
Intercept	29	0.94	0.011 (0.01)	0.35
Tol	27	-3.02	-0.164 (0.05)	0.006
Inter	27	0.94	0.001 (0.001)	0.36
Tol×Inter	27	-3.06	-0.020 (0.006)	0.005
Trait-independent complementarity effect				
Model 1				
	DF	t-value	Estimate (SE)	p-value
Intercept	93	-2.078	-0.00127 (0.0006)	0.04
LDiv				
Day				
LDiv×Day				
Model 2				
	DF	t-value	Estimate (SE)	p-value
Intercept	29	3.46	0.044 (0.01)	0.002
Tol	29	3.60	0.005 (0.001)	0.001
Inter				
Tol×Inter				

2.3.2. Community model

In the microcosm study, the biodiversity-ecosystem function relationship changed because ecosystem functions were better buffered in more diverse systems (Figure 2.1). This result was driven by increased dominance by stress-tolerant species under stress (Figure 2.2, Appendix A Figure S1). A community model (Figure 2.3) was used to test to what extent atrazine effects on the per capita species interactions are needed to reproduce these two main patterns observed in the data: diversity-dependent buffering of atrazine effects and dominance shifts. An extensive set of model simulations, representing five scenarios making different assumptions on stress effects on per capita interactions, was thereby compared to these two patterns. This analysis indicated that there is no conclusive support for stress effects on per capita species interactions. Allowing for effects on per capita interactions did not significantly improve the model's fit to the observed stress effects on ecosystem functions (Wilcoxon signed-rank test: $W_{999}=533537$, $P=0.09$, Figure 2.3A). The predicted effects of atrazine on composition were highly similar between scenarios that assumed fixed (scenario 3 and 4) and changing per capita interaction strengths (scenario 5). Allowing for atrazine effects on interaction strengths improved the model fit by only 3% (Wilcoxon signed-rank test: $W_{999}=808299$, $P<0.001$, Figure 2.3B). The direct effects of atrazine on species fitness by reducing growth (i.e. as observed in monocultures) combined with the per capita species interactions for unstressed conditions (scenario 4) sufficed to predict the function and composition in stressed microcosms (Figure 2.3, Appendix A Figure S2).

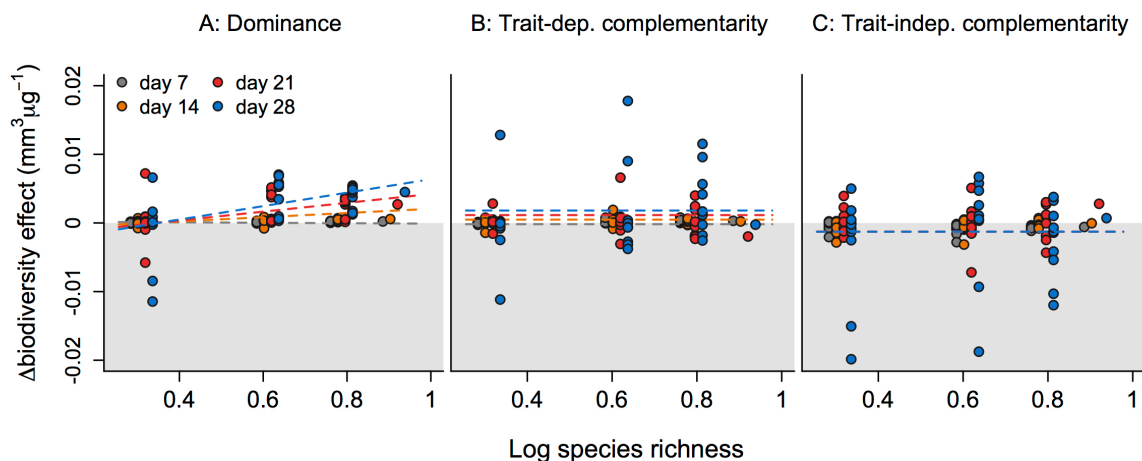


Figure 2.2: Stress-induced changes in biodiversity effects Changes in (A) dominance, (B) trait-dependent and (C) trait-independent complementarity effect at day 8, 14, 21 and 28 of the experiment. Regression lines correspond to the predicted stress-induced changes biodiversity effects by the linear mixed effects models using species richness, atrazine concentration and day as predictor variables (Table 2.2, model 1). Note that regression lines overlap for the trait-independent complementarity effect.

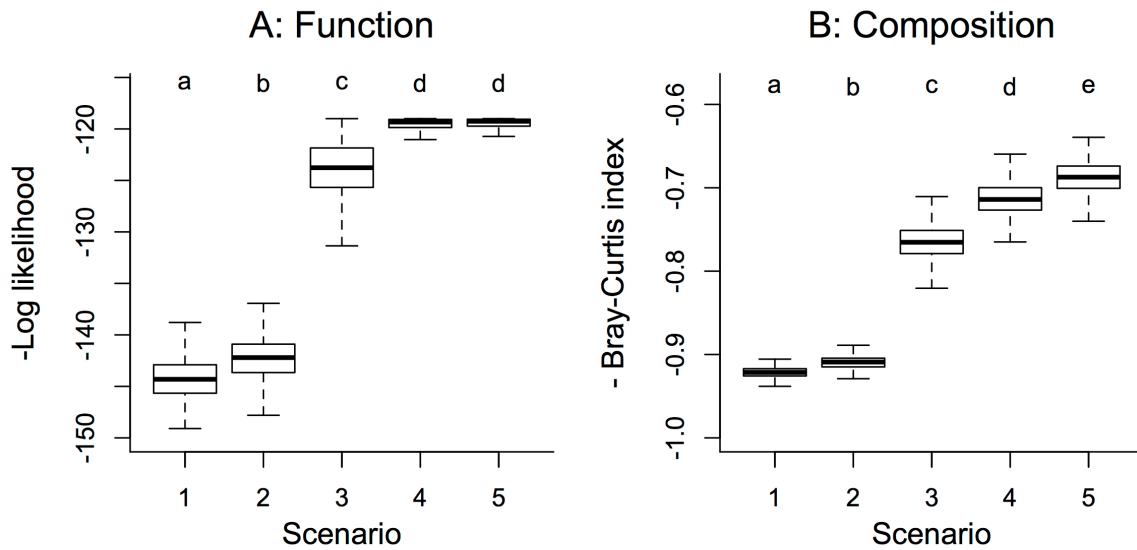


Figure 2.3: Community model predictions under different scenarios of stress effects

Boxplots of the negative log likelihood of the change in community productivity (A) and average negative Bray-Curtis dissimilarity index (B) for five scenarios of stress-induced effects in the per capita strength of species interactions. Scenario 1 is the baseline scenario without interspecific interaction ($A_{ij}=0$). Scenario 2 corresponds to equal inter- and intraspecific interaction strength ($A_{ii}=A_{ij}$). Scenario 3 corresponds to a constant ratio of inter- to intraspecific competition ($A_{ii,0}/A_{ij0}=A_{ii250}/A_{ij250}$). Scenario 4 corresponds to a constant per capita strength of interspecific interactions ($A_{ij0}=A_{ij250}$). Scenario 5 did not impose any limitations to changes in per capita interaction strength. Higher values indicate a better model fit and scenarios that do not share a common letter are significantly different (Bonferroni-corrected Wilcoxon signed rank test: $p<0.05$). Boxplot whiskers correspond to maximal 1.5 times the interquartile range.

2.3.3. Stress-induced changes in biodiversity effects

Atrazine affected only the dominance effect in a way that depended on species richness (Figure 2.2). Atrazine also affected both complementarity effects, but not in a way that depended on richness. In fact, much of the among-community variation in the changes of the complementarity effects was left unexplained (Figure 2.2, Figure 2.4A). It is tested to what extent changes in biodiversity effects depended on direct stress effects on species growth (established in monoculture bioassays), the strength of per capita interaction in unstressed conditions (estimated under scenario 4), and stress effects on these interactions (estimated under scenario 5) could explain this variation. Estimated effects on per capita interaction strengths did not significantly explain the variation in any of the biodiversity effects (Appendix A Table S1). Instead, direct stress effects on species growth and the strength of per capita

interactions in unstressed conditions explained 46% of the observed variation in the observed changes in biodiversity effects (Figure 2.4B, Table 2.2).

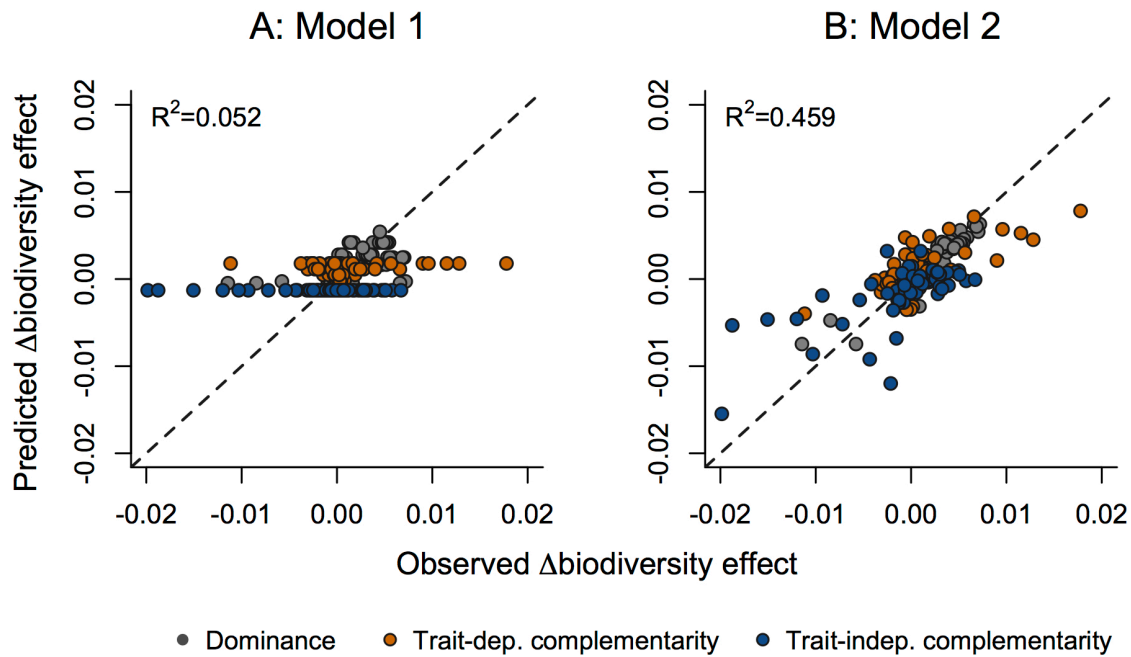


Figure 2.4: Predicted stress-induced changes in biodiversity effects Predicted plotted against observed changes in the dominance, trait-dependent complementarity and trait-independent complementarity effect for linear regression models (A) including diversity and day (Table 2.2, model 1) and (B) the weighted mean strength of per capita interactions and weighted mean species stress tolerance as predictor variables (Table 2.2, model 2).

2.4. Discussion

Confirming other studies^{7,9,13–16}, a relevant environmental stress factor changed the biodiversity-ecosystem function relationship (Figure 2.1, Table 2.1). In this chapter, it is demonstrated that stress effects on the per capita strength of interspecific interactions, if occurring at all, did not contribute in any ecologically meaningful way to such change. This conclusion is based on three lines of evidence. First, the change in the biodiversity-ecosystem function relationship was clearly not driven by stress effects on complementarity effects (Figure 2.2). Second, direct stress effects on species fitness, i.e. the growth reduction in monoculture bioassays, sufficed to predict the observed stress effects on ecosystem function and community composition with a mechanistic community model. This finding mechanistically demonstrates that stress effects on the biodiversity-ecosystem function relationship were mainly driven by direct effects on species growth (Figure 2.3). Allowing for stress effects on the per capita interaction strength did not significantly improve the model's

capacity to predict effects of stress on ecosystem functions (Figure 2.3A). While allowing for such effects improved model predictions of community composition, this improvement (3%) was smaller than the variability among replicates (5%). Hence, this improvement merely reflects a different number of free parameters between scenarios and the extremely high power when sample sizes are very large ($n=31000$). This improvement thus does not indicate an ecologically relevant improvement of model fit. Third, the direct effect of atrazine on species growth and the strength of species interactions estimated in unstressed conditions could explain the variability in the biodiversity effects among systems (Figure 2.4).

The positive effect of stress on the slope of the biodiversity-ecosystem function relationship can be expected in many different communities and is no specific feature of the study system. Indeed, the insurance hypothesis^{44,45} postulates that diverse communities are more likely to contain species that can thrive under stress and buffer ecosystem functions by replacing sensitive species^{44,46}. Therefore, functions that are merely the sum of individual species contributions should be affected less by stress in more diverse systems and the slope of the biodiversity-ecosystem function relationship should increase. This is exactly what was found: functional replacement and thus the dominance effect increased with diversity (Figure 2.2 and Appendix A Figure S1), and atrazine affected production less in more diverse communities (Figure 2.1).

The presented results demonstrate that stress can not only affect the slope of the biodiversity-ecosystem function relationship by changing dominance but also through shifts in complementarity (Figure 2.4). Because the sign and size of these shifts depend on the interspecific per capita interaction strengths in unstressed conditions (Table 2.2), these shifts are most likely system-specific. Depending on the strength of these interactions in a study system, complementarity shifts can counteract, offset, or add to the general effect stress has on dominance. Differences in interaction strengths among studies can thus potentially lead to different effects of stress on the biodiversity-ecosystem function relationships^{13,19}.

In this experiment planktonic microalgae were used, which generally experience strong interspecific competition because of limited spatial heterogeneity⁴⁷. Algal community performance is therefore often determined by the dominant species, and frequently leads to negative dominance^{48–50}, and even a negative biodiversity-ecosystem function relationship⁵¹. Such a negative relationship in unstressed conditions amplifies the positive effect of biodiversity on the buffering of ecosystem functions, shifting the relationship from negative to positive under stress. Studies with terrestrial systems, in contrast, often reported positive biodiversity-ecosystem function relationships that are driven by strong complementarity

effects^{9,12,14,22}. So, even though studies that quantified biodiversity effects reported an increased dominance effect through environmental changes, the overall slope decreased because the increasing dominance effect was outweighed by a decrease in complementarity effects^{9,14}.

These findings offer a first step towards a mechanistic understanding how environmental stress alters the biodiversity-ecosystem function relationship. The presented results suggest that dominance effects can generally be expected to increase under stress by changes in fitness through interspecific differences in stress tolerance. However, if per capita interactions remain unaffected, stress does not necessarily increase complementarity effects, as expected based on the stress gradient hypothesis. Therefore it is unlikely that stress affects biodiversity-ecosystem function relationships and the underlying biodiversity effects in a general way as previously suggested⁷. Instead, stress effects can strongly depend on the species interactions, specific to the study system. As such, system specific conservation efforts may therefore be required to preserve the services provided by the many ecosystems that currently suffer from environmental stress factors, including organic chemicals such as pesticides^{21,52}.

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3

Changes in BEF relationships over environmental gradients

Redrafted from: Baert J.M., Eisenhauer N., Janssen C.R. and De Laender F. Biodiversity effects on ecosystem functioning respond unimodally to environmental stress. *Under Review*

Abstract

Understanding how biodiversity and ecosystem functioning are interrelated is essential for estimating the consequences of ongoing global biodiversity changes. Empirical evidence is mounting that the slope (i.e. the direction and strength) of biodiversity-ecosystem functioning (BEF) relationships depends on the environmental conditions. Still, the mechanisms causing this environmental dependency of BEF relationships remain largely unstudied. This hampers our ability to predict how forecasted changes in environmental conditions and biodiversity can jointly affect ecosystem functioning. In this chapter, a comprehensive model is used to demonstrate that the slope of the BEF relationship is expected to change in a unimodal way along environmental stress gradients in single-trophic-level systems. It is revealed that this unimodal response, whereby maximal positive biodiversity effects occur at intermediate environmental stress, should invariably arise when species respond differently to environmental changes. The exact shape of the unimodal response, that is the environmental stress level at which the maximal slope is attained, thereby depends on the type and strength of species interactions in the system. Systems with more positive interactions are predicted to experience maximal biodiversity effects at lower stress levels compared to systems with more negative interactions. These theoretical predictions strikingly concur with a meta-analysis of a global dataset of 52 experiments that manipulated primary producer diversity (from unicellular algae to vascular plants) under different environmental conditions. The presented results therefore suggest that increases in stress from predicted environmental changes (e.g. climate change or chemical pollutants) are likely to increase the consequences of biodiversity changes compared to expectations based on the present-day conditions.

3.1. Introduction

Over the past 25 years, a compelling number of experiments has demonstrated that biodiversity affects ecosystem functioning^{1–5}. As the vast majority of these studies reported a positive relationship between biodiversity and ecosystem functioning (BEF), this has raised concerns that ecosystem function provisioning is currently at risk by the ongoing global biodiversity changes^{3,5,6}. However, evidence is also mounting that the consequences of biodiversity changes may not be estimated from a single, universal BEF relationship^{7–9}. Besides differences in the shape of BEF relationships between ecosystems⁴, an increasing number of studies now demonstrates that changes in environmental conditions can also alter the shape of BEF relationships within a system^{10–17}. To date, these changes in BEF relationships along environmental gradients, as well as the underlying mechanisms, remain largely unstudied^{11,13,16,18}. This is a major knowledge gap as observations and prognoses suggest rapid future changes in environmental conditions to go hand in hand with biodiversity change, which can cause the consequences of predicted biodiversity changes to deviate from current expectations that are based on the current-day environmental conditions^{6,19–21}.

BEF relationships result from differences in the relative strength of inter- and intraspecific interactions, which cause species to function differently in the presence of other species^{22,23}. Two classes of biodiversity effects are thereby discerned: dominance and complementarity effects^{22,23}. Dominance effects refer to diversity-dependent changes in species' functional contributions to ecosystem functions through competitive replacement²³. Dominance effects hence occur when species functional and competitive abilities are correlated. Positive dominance effects increase ecosystem functioning when high functioning species replace low functioning species. Complementarity effects, in contrast, refer to diversity-dependent changes in species functional contributions without species replacement. Niche complementarity and facilitation are major drivers of positive complementarity effects, increasing species and ecosystem functioning by reducing the amount of competition individuals experience^{22,23}. Accordingly, negative BEF relationships result from antagonistic interactions that increase competition, or dominance of species with low functional abilities^{22,23}.

Theoretically, the environmental dependency of BEF relationships should thus arise from changes in species functional contributions that alter dominance and complementarity effects on function¹⁶. Changes in species density, and consequently their functional contribution to ecosystem functioning, must thereby at least depend on interspecific differences in species responses to the environmental changes and on species interactions^{24–26}. The first determines the direct effect of environmental changes on species fitness, altering species

densities through their mortality or reproduction rates, whereas species interactions determine the indirect effect of density changes in other species.

In this chapter, a comprehensive model is introduced to explore how effects of environmental changes on species fitnesses and species interactions alter the BEF relationship and the underlying biodiversity effects. Environmental changes are thereby assumed to affect species fitness through their reproduction rates, while the per-capita strength of species interactions are assumed to be independent of the environmental conditions, as found in chapter 2^{16,27–29}. Using a Monte-Carlo approach, 1000 BEF relationships were simulated over a full environmental change gradient ranging from optimal conditions to complete functional inhibition of all species. Next, it is tested how changes in BEF relationships, and the underlying dominance, trait-dependent complementarity and trait-independent complementarity effects, depend on the strength of species interactions. Finally, predicted changes in BEF relationships are confronted with a meta-analysis of 52 studies that manipulated primary producer species richness under different environmental conditions in both aquatic and terrestrial systems.

3.2. Materials and methods

3.2.1. Model structure

A stochastic version of the Lotka-Volterra competition model was used to simulated stress-induced changes on ecosystem functioning³⁰:

$$r_i(t) = \log N_i(t + 1) - \log N_i(t) = [b_i(SI) - d_i] \left[1 - \sum_{j=1}^n \frac{N_j}{[b_i(SI) - d_i] a_{i,j}} \right] \quad (1)$$

The species per-capita growth rate (r_i) is expressed as a function of the species density (N_i), the strength of intra- ($\alpha_{i,i}$) and interspecific interactions ($\alpha_{i,j}$), and the probability of reproduction (b_i) and mortality (d_i). Environmental changes alter species fitness through a reduction in the per-capita growth rate, $b_i(SI)$ ²⁹. The species-specific functional response of the birth rate was modelled as a gamma distribution: $b_i(SI) = \Gamma(k, \theta)$. The per-capita strength of species interactions was considered independent of environmental conditions^{28,31}.

3.2.3. Model parameterisation and simulations

The transition probabilities for each species are governed by the per-capita birth rate (b_i), mortality rate (d_i), intraspecific ($a_{i,i}$) and interspecific interactions ($a_{i,j}$):

$$P[N_i(t + 1) = N_i(t) + 1] = b_i(SI) N(t) \quad (2)$$

$$P[N_i(t+1) = N_i(t) - 1] = d_i N(t) + N_i(t) \sum_{j=1}^n \frac{N_j(t)}{a_{i,j}} \quad (3)$$

For each simulation, parameter values for $b_i(0)$, k , θ , d_i , $\alpha_{i,i}$ and $\alpha_{i,j}$ were randomly drawn (Appendix B Figure S2). Parameter distributions were chosen to represent an exhaustive set of ecologically relevant BEF relationships (ranging from strongly negative to strongly positive, Appendix B Figure S3). Upper limits were set for all parameters to keep simulation times feasible without loss of generality. Increasing parameters beyond the set upper limits for the birth rate, death rate, or intraspecific interactions (which determines the carrying capacity) only increases the number of time steps required to obtain the stationary distribution, but did not affect model predictions (Appendix B Figure S3). The species-specific environmental responses of the birth rate, and thus the species' environmental niche and fitness, were modelled using gamma distributions (Figure 3.1). For each species, an optimal value, defining the mean value of the gamma distribution (i.e. $b_i(0) = k\theta$) was randomly sampled from the $[0,1]$ interval, and a variance (i.e. $\sigma_{b_i(SI)} = k\theta^2$) was randomly sampled between 1 and 10% of the optimal value. Analogously, mean values of the per capita mortality rate (d_i) were sampled from the $[0, b_i(0)]$ interval for each species. The strength of intraspecific interactions, $a_{i,i}$ which determine the species carrying capacities ($= a_{i,i}[b_i(SI) - d_i]$) are sampled from the $[0, 1000]$ interval. Interspecific interactions $a_{i,j}$ were drawn from a gamma distribution with mean values drawn from the $[0.1a_{i,i}, 25a_{i,i}]$ interval and standard deviation drawn from the $[0.01a_{i,i}, 125a_{i,i}]$ to encompass no, positive and negative species interactions.

The biodiversity-ecosystem relationship was simulated under environmental conditions that were drawn from the stress intensity distribution obtained from the empirical data (Appendix B Figure S5). For each simulation, first a pool of 20 species was generated by randomly drawing species parameters as described above. Next, 10 communities of 2, 4, 8, and 16 species were randomly assembled from this species pool. Community dynamics were then simulated under unstressed and stressed environmental conditions. Community dynamics were numerically simulated and reached equilibrium, the latest at $t=30$. Simulations were run till $t=50$. Stationary species distributions were calculated by randomly sampling species densities between $t=40$ and $t=50$. Each simulation was reiterated 12 times to ensure convergence of the stationary distribution (Appendix B Figure S3). A sensitivity analysis of the parameters distributions revealed that model predictions did not depend on the assumed parameter distributions. Using different, ecologically relevant, parameters distributions did not alter the results (Appendix B Figure S5). Biodiversity-ecosystem function relationships were calculated from the summed mean functional contribution of each species, obtained from species stationary distributions. A detailed flow chart of the modelling process is provided in Appendix B Figure S2.

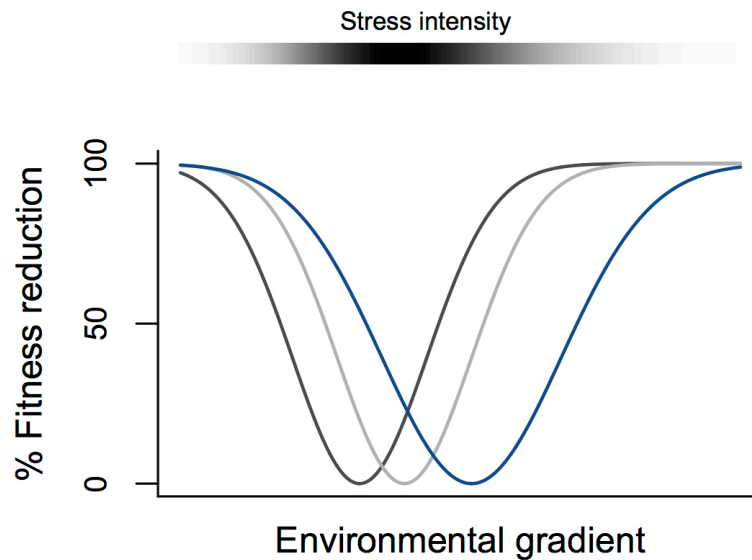


Figure 3.1. Definition of environmental stress intensity used in the model. Niches of 3 hypothetical are depicted as function of the % fitness reduction (the % reduction in per-capita birth rate in the model). The stress intensity of an environmental condition is then calculated as the average % reduction in species fitness (equation 4). The colour scale of stress intensity corresponds to that in Figure 3.2, ranging from maximal average fitness (black) to complete inhibition of all species (white).

3.2.3. Review of literature data

The Thomas Reuters Web of Knowledge (www.webofknowledge.com) and Google Scholar (www.scholar.google.com) were searched for studies that manipulated species richness under at least two environmental conditions. The search terms ‘biodiversity’, ‘ecosystem’, ‘function’, ‘productivity’, ‘stress’, ‘temperature’, ‘nutrient’, ‘precipitation’, ‘chemical’, ‘salinity’, ‘environment’ were used in various combinations. Additionally the cited literature of studies obtained this way was checked for additional original studies. Data were available as text files, excel files or were digitized from the figures in the original publications. Digitized data did not differ by more than 1% among different applications (e.g. Engauge, WebPlot or ExtractGraph digitizer). Only studies that reported the species monoculture functions for all environmental conditions were considered, as this is a prerequisite to calculate the intensity of environmental stress^{11,12,14–16,38–46} (see 3.2.4. Data normalisation). No distinction was made between ecosystem functions. However, ‘productivity’ was included as a separate search term as most studies are based on primary producer systems, with primary production as the main ecosystem function of interest. An overview of the study system, ecosystem function under study en environmental stressor for all 52 studies is given in Appendix B Table S1.

3.2.4. Data normalisation

Literature and simulated data were normalized prior to analysis. The severity of the environmental stress was calculated as the proportional difference between the average monoculture function in the stressed condition (\bar{M}_{stress}) and the average unstressed monoculture function (\bar{M}_0)¹¹:

$$SI = 1 - \frac{\bar{M}_{stress}}{\bar{M}_0} \quad (4)$$

Unstressed conditions were defined as those environmental conditions under which species attained the highest mean monoculture functions. Hence, normalized stress intensity (SI) metric ranges from 0 for optimal conditions to 1 for severely stressed conditions. Slopes were normalized by dividing the linear regression coefficient of the biodiversity-ecosystem function relationship by the average monoculture function under unstressed conditions.

3.2.5. Comparison of empirical data and model predictions

To test if the slope and intercept of the relationship between the slope under unstressed conditions and the average change in slope over a concentration gradient (Figure 3.2E) differed between the model predictions and the assembled empirical datasets (see 3.2.1. Review of literature data), the following linear model was fitted to the data:

$$\Delta Slope \sim \beta_{0,dataset} + \beta_{1,dataset} Slope_0 \quad (5)$$

where $\Delta Slope$ is the normalized change in slope of the biodiversity-ecosystem function relationship between unstressed and stressed conditions, and $Slope_0$ is the slope of the biodiversity-ecosystem function relationship under unstressed conditions. β_0 and β_1 are the regression coefficients for the intercept and the effect of the change in slope. The dataset was included as factor variable in the model, resulting in an estimated intercept and slope for both the regression for the dataset obtained from the meta-analysis and model simulations.

Second order polynomials fitted the stress intensity and the slope of the BEF relationship for the 40 studies that included minimally 3 different environmental conditions (Appendix B Figure S6):

$$Slope(SI) = Slope(0) + \beta_1 SI + \beta_2 SI^2 \quad (6)$$

The intercept $Slope(0)$ was fixed at the slope value under unstressed conditions, whereas the coefficients β_1 and β_2 are the least square estimates. For each study, the estimated stress intensity at which the maximal slope was attained was derived from these polynomials. Next,

the relationship between the slope under unstressed conditions and the stress intensity at which the maximal slope is attained (SI_{max}), was normalized by \log_{10} -transforming the slope under unstressed conditions:

$$SI_{max} \sim \beta_{0,dataset} + \beta_{1,dataset} \log_{10}[Slope_0] \quad (7)$$

This however excluded 3 studies with negative slopes under unstressed conditions. Residual diagnostics of both regression models were assessed for deviations from normality and homoscedasticity (Appendix B Figures S7-8).

3.3. Results

3.3.1. Model predictions

Model predictions suggest a unimodal response of the slope of the BEF relationship along an environmental stress gradient, with biodiversity effects peaking at intermediate levels of environmental stress (Figure 3.2). This unimodal response is driven by environmental stress-induced changes in dominance effects that arise because of interspecific differences in responses to environmental changes. Increasing interspecific fitness differences caused by stress promote positive dominance effects because stress tolerant species will increasingly contribute to ecosystem functions by replacing species experiencing severe fitness reductions^{18,32,33}. Environmental stress thus inevitably induces a positive correlation between species functional and competitive abilities, resulting in a positive dominance effect at elevated environmental stress levels (Appendix B Figure S1). In addition, biodiversity acts as an insurance by increasing the likelihood that the system contains species that can compensate for the functional loss of other species³², which reduces the effect of environmental stress on ecosystem functioning. The strength of dominance effects and the slope of the BEF relationship, however, decrease again when levels of environmental stress become so high that fitness of most species is reduced, limiting the potential for functional replacement. Positive biodiversity effects thus peak at intermediate stress levels, where diversity maximally enhances the average ability for functional compensation. When environmental stress surpasses this threshold stress level, species' fitnesses are reduced to such an extent that the ability for functional compensation also starts to strongly decrease in more diverse systems^{10,11,34}. From this point onward, the slope of the BEF relationship decreases until it reaches a flat line at extreme levels of environmental stress, where the function of all species is inhibited (Figure 3.2A-D).

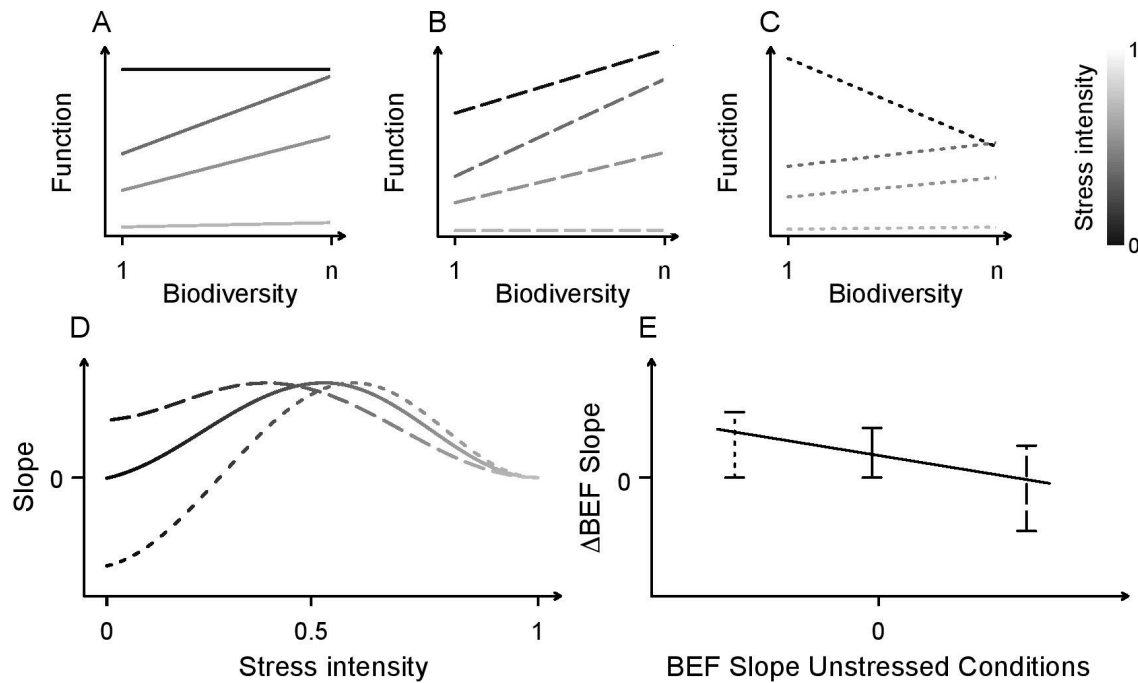


Figure 3.2. Simulated changes for a neutral (A), positive (B), or negative (C) BEF relationship under unstressed conditions. BEF relationships are represented at four levels of stress intensity (0, 0.5, 0.7 and 0.9), illustrating how the slope of BEF relationships changes because of the better preservation of ecosystem functions in more diverse systems under stress. The slope of BEF relationships consistently increase under stress levels, attain a maximum and become zero at high stress (D). Positive relationships under unstressed conditions (dashed line) peak at low stress, neutral relationships (solid line) at intermediate, and negative relationships (dotted line) at high stress. As a consequence, BEF relationships that are negative under unstressed conditions (dotted line) increase on average under stress, whereas relationships that are neutral (solid line) or positive (dashed line) under unstressed conditions decrease on average, causing a negative relationship between the average change in slope over a stress gradient, and the slope in unstressed conditions (E). Slopes were divided by the maximal slope in panel D.

The type and strength of species interactions determines the stress level at which the maximal positive slope of the BEF relationships is attained. Species interactions are the main determinants of the strength of the biodiversity effects under unstressed conditions, and thus how these will change by alterations in species functioning through environmental changes (Figure 3.2D). In case no biodiversity effects occur under unstressed conditions, biodiversity effects will emerge under increasing stress. The unimodal response is thereby principally driven by the occurrence of dominance effects under stress, as explained in the previous paragraph (Figure 3.2A, Appendix B Figure S1). When biodiversity effects occur under unstressed conditions, changes in species' functional contributions are driven by alterations in both complementarity and dominance effects. At increasing stress levels, the number of

species that can significantly contribute to ecosystem functions is progressively reduced. This decreases the ratio between inter- and intraspecific interactions experienced by the remaining species, which reduces complementarity effects, both positive and negative, at these elevated stress levels (Appendix B Figure S1). Dominance effects, in contrast, invariably become positive under stress through the increased functional contribution of species that have the highest fitness under stress (Appendix B Figure S1).

Table 3.1: Estimated theoretical and empirical effects. Estimates and standard deviations of the slope under unstressed conditions on the average change in slope over an environmental gradient, and the environmental conditions at which biodiversity effects peak.

Average change in BEF relationship	
Intercept meta analysis	0.0392 (0.013)**
ΔIntercept model predictions	-0.0120 (0.015)
Slope meta analysis in unstressed conditions	-0.5469 (0.062)***
ΔSlope model predictions in unstressed conditions	0.129 (0.064)*.....
Stress intensity at which maximal biodiversity effects are attained	
Intercept meta analysis	0.2624 (0.057)***
ΔIntercept model predictions	0.0353 (0.058)
Log ₁₀ slope meta analysis in unstressed conditions	-0.1068 (0.0346)**
ΔLog ₁₀ slope model predictions in unstressed conditions	-0.0206 (0.0427)

* p<0.05, **p<0.01, ***p<0.001

BEF relationships that are positive in unstressed conditions attain a maximal positive slope at lower stress levels than BEF relationships that are negative in unstressed conditions (Figure 3.2D). BEF relationships start to decline when dominance effects start to decrease, or when dominance effects can no longer compensate for a decrease in positive complementarity effects (Appendix B Figure S1). Strong positive complementarity effects under unstressed conditions are therefore predicted to decrease more when stress reduces the number of species that substantially contribute to ecosystem functions. Strong positive relationships are consequently already likely to decrease at low stress levels (Figure 3.2). Negative relationships under unstressed conditions, in contrast, first shift to positive under increasing stress, and hence are predicted to attain a maximal biodiversity effect at higher stress levels (Figure 3.2C). This shift from negative to positive biodiversity effects can be driven by a reduction in negative complementarity effects, a shift from negative to positive dominance

effects, or both. More negative complementarity and dominance effects under unstressed conditions require higher stress levels to be overcome. As environmental stress increases, this reduces the number of substantially contributing species. This reduces the strength of negative complementarity effects and induces positive dominance effects, which causes the BEF relationship to become positive. Consequently, the average change in the slope of the BEF relationship is predicted to negatively correlate with the slope the BEF-relationship under unstressed conditions (Figure 3.2E).

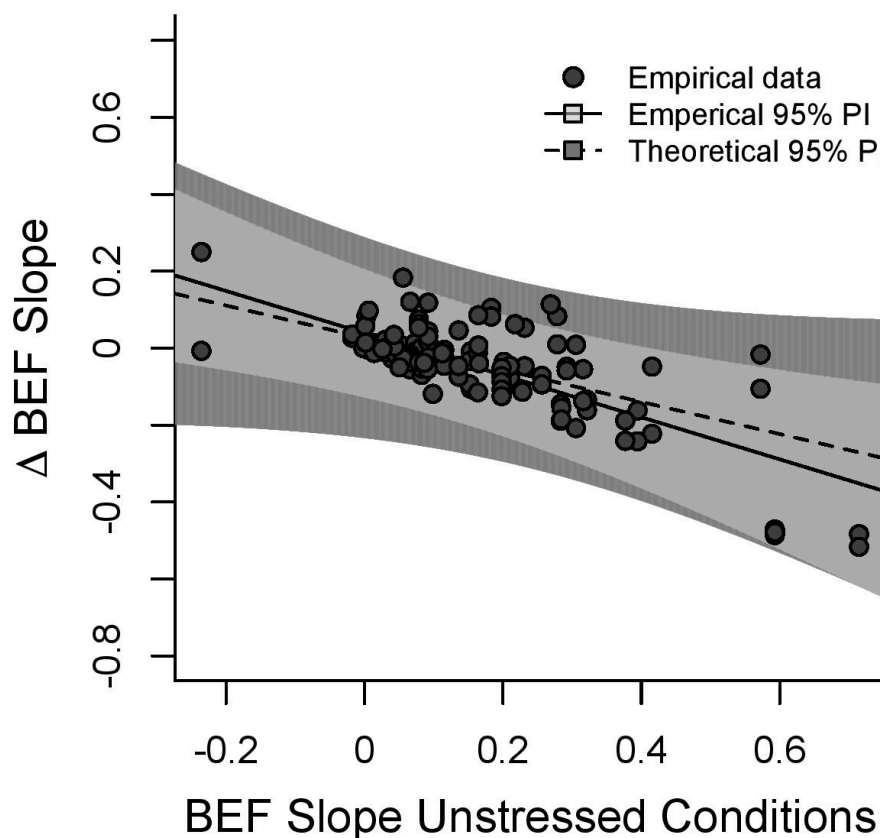


Figure 3.3 Effect of the slope under unstressed conditions on the average changes in slope (Δ BEF Slope). Dots represent empirical data. The empirical regression model and 95% prediction interval (PI) are represented by the dashed line and light shading, the regression model and 95% prediction interval obtained from model simulations are represented by the solid line and dark shading.

3.3.2. Meta-analysis of biodiversity experiments

Observed environmentally induced changes in the slope of BEF relationships reported in the 52 empirical studies correspond strikingly with the model predictions (Figure 3.3 and 3.4). These studies encompass a variety of terrestrial and aquatic primary producer systems in

which biodiversity was manipulated under at least two different environmental conditions. Confirming model predictions, the stress-induced average change in the slope of the BEF relationship in these studies indeed negatively correlated with the effect the slope of the BEF-relationship under unstressed conditions ($t_{129}=-13.15$, $p<0.001$; Figure 3.3). Studies that reported a negative, neutral, or a slightly positive BEF relationship under unstressed conditions on average reported an increase in the BEF slope in stressful environments, whereas the slope of strongly positive BEF relationships under unstressed conditions decreased on average under stress (Figure 3.3). The intercept of the empirical regression did not differ significantly from the theoretical regression ($t_{4334}=-0.825$, $p=0.41$; Table 3.1). The slope, in contrast, was significantly different but still very similar between the empirical and theoretical regression ($t_{4334}=2.019$, $p=0.044$; Table 3.1).

Fitting second order polynomials to the 40 studies that involved at least 3 environmental conditions supported the unimodal change in the slope of BEF relationships along environmental stress gradients predicted by the model (Figure 3.4). In the majority of these studies, fitted polynomials peaked at intermediate levels of environmental stress (Figure 3.4A). Monotonically decreasing polynomials were only supported for studies with highly positive biodiversity effects in unstressed conditions. Although the model did not predict such monotonic reductions, this deviation from the model can be due to the coarse resolution of most studies. By including only 3 or 4 environmental conditions, low environmental stress levels, at which maximal biodiversity effects are predicted to occur, can easily be excluded from the experimental design. Confirming model predictions (Figure 3.2D), the stress level at which maximal biodiversity effects were estimated increased as the strength of positive biodiversity effects in unstressed conditions decreased, and the estimated effect of the slope under unstressed conditions did not differ between theoretical predictions and empirical results ($t_{291}=-0.484$, $p=0.63$; Figure 4B, and Table 3.1).

So far, only a few studies have manipulated species richness under a broad range of environmental conditions, and these confirm the unimodal response of BEF relationships along environmental gradients predicted by the model (Figure 3.4 and Appendix B Figure S6). However, most studies have been confined to a limited number of environmental conditions (2 or 3), often covering only parts of an environmental gradient and often reporting different results, including increases, decreases, and no change of the BEF slope along environmental gradients (Appendix B Figure S6). The theory presented in the present study allows these results to be interpreted within a single generalised framework. Specifically, differences among studies reflect different system-specific realisations of a general pattern, the unimodal response of BEF relationships to environmental stress gradients, and not

mechanistic differences. These realisations can be understood from species interactions in the system, which leave a fingerprint in the BEF slope under unstressed conditions (Figure 3.2).

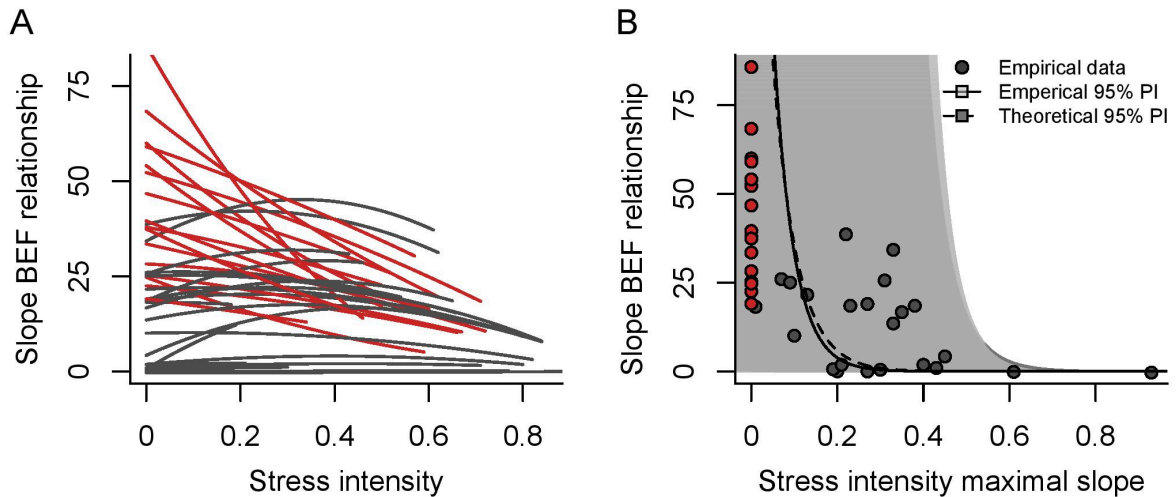


Figure 3.4 Empirical changes in slope over environmental gradients. (A) Second-order polynomials fitted to the changes in biodiversity–ecosystem function (BEF) relationships over a stress gradient for the 40 empirical studies containing at least 3 environmental conditions. Monotonically decreasing relationships are depicted in red ($n=13$), unimodal relationships in dark grey ($n=27$). (B) Theoretically predicted and empirically observed relationship between the BEF slope under unstressed conditions and the stress intensity at which maximal positive biodiversity effects are attained. The empirical regression model and 95% prediction interval (PI) are represented by the dashed line and light shading, the regression model and 95% prediction interval obtained from model simulations are represented by the solid line and dark shading. The colours of the points correspond to the polynomials fitted to the empirical studies depicted in panel A.

3.4. Discussion

In this chapter it is demonstrated that environmental stress alters the consequences of biodiversity loss, and that the joint effects of environmental and biodiversity changes for ecosystem functioning can strongly differ between systems. A model was presented, which based on a minimal set of mechanisms (Figure 3.1), disentangles a general unimodal response from system-specific effects (Figure 3.2), and allows making quantitative, system-specific predictions on changes in biodiversity–ecosystem functioning relationships over environmental stress gradients depending on the type of species interactions (Figure 3.3 and 3.4).

Separating a general from a system specific response over an environmental gradient allows reconciling the apparent contradictions among the results reported by experiments manipulating biodiversity under different environmental conditions. Most of these studies only considered a limited number of environmental conditions, and increases³⁵, decrease^{11,12,14,15,33,36–40} and no effect^{41,42} on the slope of the BEF relationship have been observed. The theory presented in this study thus allows interpreting these results within a single generalised framework. More precisely, model predictions demonstrate that differences among studies can be understood as reflecting different system-specific realisations of a general, unimodal response of the BEF relationship along environmental gradients (Figure 3.4, Appendix B Figure S6). To date only few studies have manipulated species richness under a broad range of environmental conditions. Nevertheless, these unequivocally confirm the unimodal response of BEF relationships along environmental gradients predicted by the model (Appendix B Figure S6)^{11,12,14}.

Model predictions indicate that differences in changes in the BEF relationship between studies can be explained based on the slope of BEF relationship under unstressed conditions (Figure 3.3 and 3.4B). As species interactions are the prime driver of BEF relationships, the BEF slope under unstressed conditions directly results from the type and strength of interactions in the system^{22,23}. Hence, systems with positive interactions and biodiversity effects were predicted to experience decreases in the slope of the BEF relationship at lower levels of environmental stress compared with systems with more negative interactions (Figure 3.2). Strong positive BEF relationships have been established on a wide variety of ecosystem functions, and are significant across trophic groups including terrestrial and benthic primary producer systems^{4,5,43}. These systems make up an interesting case, because the presented theory and data analysis show that lower levels of environmental stress are needed to reduce the BEF slope in these systems than in systems with negative BEF relationships under the current-day environmental conditions such as pelagic primary producer systems^{4,44}.

Although species interactions are known to be able to shift along an environmental gradient, the model was able to explain changes in BEF relationships across a range of systems and different types of environmental stressors, based on the assumption of a constant per capita strength of species interactions (Table 3.1). Changes in per-capita interactions, both increases in competition and shifts to facilitation, have been reported for various systems, including primary producer systems⁴⁵. In particular when environmental stress is caused by changes in resource availability, per-capita interactions are known to shift^{28,45,46}, for example by improving water retention⁴⁰. Such shifts in per capita interactions can results in more

complex changes in biodiversity effects than predicted by the model. For example, environmental stress inducing shifts towards facilitation at elevated stress levels can increase both trait-dependent and trait-independent complementarity effects at elevated levels of environmental stress. The importance of these potential shifts in per capita interactions for environmental induced changes in ecosystem functioning remains however subject of debate^{46,47}. Similarly, the results presented here demonstrate that, although shifts in per-capita interactions could affect how BEF relationships change over environmental gradients, their effect on the general response should be limited (Figure 3.3 and 3.4).

The general, unimodal response presented here suggests that the consequences of biodiversity effect are likely to increase at low to intermediate levels of environmental stress (Figure 3.2 and 3.4). Thus, at such levels of environmental change, effects of biodiversity loss will be more negative than at present-day environmental conditions. Environmental and biodiversity changes pose major threats to ecosystems worldwide⁶. Understanding how both processes are intertwined is therefore a major challenge to appropriately assess the consequences of ongoing and future biodiversity changes^{12,48,49}. Many ecosystems are currently subject to increasing environmental stress, such as global warming, acidification, drought, or chemical pollution^{6,50}. The presented results suggest that the consequences of future biodiversity loss are likely to exceed our current expectations based on the current-day environmental conditions. Preserving and restoring biodiversity is therefore essential to maintain ecosystem function provisioning^{5,12,13}. Increasing our understanding of biodiversity effects in current systems can, however, provide important insights to make system-specific predictions and prioritize conservation efforts.

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4

Non-linear biodiversity effects on ecosystem functioning

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Abstract

Assessing the consequences of biodiversity changes for ecosystem functioning requires separating the net effect of biodiversity from potential confounding effects such as the identity of the gained or lost species. Additive partitioning methods allow factoring out these species identity effects by comparing species' functional contributions against the predictions of a null model under which functional contributions are independent of biodiversity. Classic additive partitioning methods quantify biodiversity effects based on a linear relationship between species deviations from the null model and their functional traits. However, based on ecological theory, non-linear relationships are also possible. Here it is demonstrated how additive-partitioning methods can easily be extended to describe such non-linear relationships, and explain how non-linear biodiversity effects can be interpreted. Both linear and non-linear partitioning methods are applied to the Cedar Creek Biodiversity II experiment. Non-linear relationships were detected in the majority of plots, and increased with diversity. Non-linear partitioning thereby identified a convex relationship between species functional traits and their deviations from the null model, driven by strong positive effects of both species with low and high functional trait values on ecosystem functioning. The presented non-linear extension of additive partitioning methods is therefore essential for revealing more complex biodiversity effects on ecosystem functioning, which are likely to occur in biodiversity experiments.

4.1. Introduction

Anthropogenic activities are causing unprecedented global biodiversity changes with potential major consequences for ecosystem function provisioning^{1–3}. Understanding how biodiversity and ecosystem functioning are interrelated has therefore become a major objective in ecology. Since the 1990s, hundreds of studies have assessed the biodiversity-ecosystem-functioning relationship by randomly assembling communities of different levels of species richness from a common species pool^{4–8}. However, the increased likelihood of sampling species with favourable traits in the high diversity treatments can thereby bias regression analysis^{9–12}. Because both the number and identity of species can influence, estimating the biodiversity-ecosystem functioning relationship hence requires high replication to control for the effect of species identities¹³.

Additive partitioning methods allow factoring out the effect of species identities by using a null model^{14,15}. Under the null hypothesis that the strengths of inter- and intraspecific interactions are equal, species functioning is independent of the identity and number of species in the system. Observed deviations from this null model can then be partitioned between two¹⁴ or three¹⁵ terms that reflect various classes mechanisms through which biodiversity can affect ecosystem functioning. Contrary to classic regression analyses that estimate biodiversity effects by comparing ecosystem functioning across a species richness gradient, additive partitioning methods thus estimate biodiversity effects based on a system-specific prediction of ecosystem functioning in the absence of biodiversity effects.

Current additive partitioning methods assume a linear relationship between species deviations from the null model and their functional traits^{14,15}. However, based on ecological theory, there is a priori no reason to assume the relationship between species deviations from the null model and functional traits should be linear. For example, non-linear relationships have shown to arise over time when ecosystem functioning becomes increasingly driven by particular (groups of) species¹⁶. Although a linear relationship quantifies the general pattern, including higher order terms allows more accurately describing deviations from the null model, and consequently leads to a more comprehensive treatment of biodiversity effects on ecosystem functioning.

In this chapter, it is first demonstrated how current additive partitioning methods can easily be extended with higher order terms to include non-linear dependencies between species' deviations from the null model and their functional traits. Next, it is explained how these higher order terms can be ecologically interpreted. Finally, the non-linear additive partitioning methods is applied to data from the Cedar Creek biodiversity II experiment to illustrate the

occurrence of non-linear biodiversity effects and discuss how the non-linear partitioning results in a more detailed insight into the effect of biodiversity on ecosystem functioning.

4.2. Linear additive partitioning methods

Many ecosystem functions are the aggregate of the individual species' functional contributions (e.g. primary production, nutrient retention or total biomass). Under the null hypothesis that inter- and intraspecific interactions are equal, the functional contribution of an individual is independent of the identity of the individuals with which it interacts. As the system behaves neutrally under the null hypothesis, species functional contributions undergo a random walk. Species are hence expected to function equally well in monocultures as in mixed cultures. The expected value of a species' functional contribution to ecosystem functioning therefore equals its initial fraction in the mixed culture times its contribution in monoculture^{14,15}. For a system containing n species, the expected value of an aggregated ecosystem function or yield (Y_e) under the null hypothesis can thus be expressed as¹⁴:

$$Y_e = \sum_{i=1}^n Y_{e,i} = \sum_{i=1}^n \frac{Y_{e,i}}{M_i} M_i = \sum_{i=1}^n RY_{e,i} M_i \quad (1)$$

$Y_{e,i}$ is the individual species yield, which can also be expressed as the realised fraction of the species monoculture yield (M_i), the relative yield ($RY_{e,i}$). As the system behaves neutrally, the expected relative yield remains constant over time. Note that the term “yield” is used to refer to any measurable ecosystem function, or a species functional contribution thereto, to comply with common terminology^{14,15}. For any aggregated ecosystem function, the observed deviation in ecosystem functioning from the null model (ΔY) equals the sum of the deviations of the individual species observed yield (Y_o) or relative yield (RY_o) from the null model¹⁴:

$$\Delta Y = Y_o - Y_e = \sum_{i=1}^n Y_{o,i} - Y_{e,i} = \sum_{i=1}^n (RY_{o,i} - RY_{e,i}) M_i = \sum_{i=1}^n \Delta RY_i M_i \quad (2)$$

Loreau and Hector¹⁴ demonstrated that rewriting equation 2 based on the expected value of the product of two dependent variables, results in two terms that can be interpreted as reflecting the complementarity effect and selection effect:

$$\Delta Y = n E(\Delta RY) E(M) + n \text{cov}(\Delta RY, M) \quad (3)$$

The sign of the first term depends on the average species' deviation from the null hypothesis. The term becomes positive when species perform better on average in mixed cultures

compared to monocultures, which is interpreted as the consequence of niche complementarity and/or facilitative interactions between species. The second term quantifies the linear dependency between species' deviations from the null model and their monoculture yields. Hence, this term is positive when species with above average monoculture yields have on average a higher functional contribution to ecosystem functions, reflecting the effect of dominance of species with particular functional traits on ecosystem functioning. Note that throughout this paper the term 'functional traits' will be used to refer the species monoculture yields. Loreau and Hector¹⁴ therefore referred to the second term as the selection effect, mimicking the effect of natural selection in evolution as given by the Price equation¹⁷. Fox¹⁵ demonstrated that the selection effect is only partially analogous to natural selection *sensu* Price¹⁷. The latter reflects changes in frequencies whereas ΔRY_i is not limited to the [0;1] interval, nor needs $\sum \Delta RY_i$ to equal 1. Therefore, Fox¹⁵ proposed an alternative partitioning of species' deviations from the null-model:

$$\Delta Y = \sum_{i=1}^n (RY_{o,i} - RY_{e,i}) M_i = \sum_{i=1}^n \left(\frac{RY_{o,i}}{RYT} - RY_{e,i} \right) M_i + \sum_{i=1}^n \left(RY_{o,i} - \frac{RY_{o,i}}{RYT} \right) M_i \quad (4)$$

Under the assumption that the number of individuals in the system is independent of species richness, that is a substitutive design, the expected relative yield ($RY_{e,i}$) equals the species' initial proportion in the mixture. By dividing the observed relative yield by the relative yield total ($RYT = \sum RY_{o,i}$) the first term of equation 4 thus now does represent the changes from species' initial frequencies. Analogous to equation 2, equation 4 can be rewritten as¹⁵:

$$\Delta Y = n E(\Delta RY) E(M) + n \text{cov} \left(\frac{RY_{o,i}}{RYT} - RY_{e,i}, M \right) + n \text{cov} \left(RY_{o,i} - \frac{RY_{o,i}}{RYT}, M \right) \quad (5)$$

This additive partitioning presented by Fox¹⁵ now splits the selection effect into two covariance terms: the dominance effect and the trait-dependent complementarity effect. The first covariance term quantifies the extent by which deviations from the expected frequency linearly depend on their monoculture yield. Changes in species frequencies imply that the increase in one species necessarily results in the decrease of other species. Consequently, the first term reflects changes in ecosystem functioning by dominance of species with particular functional traits, analogous to natural selection *sensu* Price¹⁷. The second covariance term, the trait-dependent complementarity effect, quantifies the linear dependency between species' monoculture yields and the extent by which species relative yields do not result from replacement. Hence, this term is interpreted as reflecting the effect of asymmetrical species complementarity that hat occurs depending on species functional traits. This in contrast to the first term of equation 5, which is identical to the complementarity

effect by Loreau and Hector¹⁴, and depends on the average deviation from the null model, which occurs irrespective of species monoculture yields. This term is hence also referred to as the trait-independent complementarity effect¹⁵.

4.3. Non-linearly extending additive partitioning methods

Both the bi-¹⁴ and tripartite¹⁵ method are based on the expected value of the product of two dependent variables. These methods use covariances (equations 3 and 5), measuring the linear dependence between deviations from the null model and species traits (i.e. monoculture yields). To extend additive partitioning methods with higher order terms, a generic function $g(\tilde{M}_i, \theta)$ is introduced, describing the relationship between the deviation of species functional contribution from that expected under the null hypothesis that inter- and intraspecific interactions are equal, and their centred monoculture yields, $\tilde{M}_i = M_i - E(M)$:

$$\Delta RY_i - E(\Delta RY) = g(\tilde{M}_i, \theta) + \varepsilon_i \quad (6)$$

θ is a vector containing the unknown parameters of the function g and ε_i is the model error term for species i . Fitting this model to data with ordinary least squares leads to the identity:

$$\Delta RY_i - E_{uw}(\Delta RY) = g(\tilde{M}_i, \hat{\theta}) + e_i \quad (7)$$

to the least squares estimate $\hat{\theta}$ for θ and to the residuals:

$$e_i = [\Delta RY_i - E_{uw}(\Delta RY)] - g(\tilde{M}_i, \hat{\theta}), \quad i = 1, \dots, n \quad (8)$$

Note that the function g has a zero intercept as the mean deviation from the null hypothesis is subtracted from species deviation from the null hypothesis in equation 6. The function g is thus centred on the mean deviation from the null hypothesis and monoculture yield, so that all terms depend on \tilde{M}_i . Inserting identity 7, equation 2 can now be rewritten as:

$$\begin{aligned} \Delta Y &= \sum_{i=1}^n [\Delta RY_i - E(\Delta RY)] M_i + \sum_{i=1}^n E(\Delta RY) M_i \\ &= \sum_{i=1}^n g(\tilde{M}_i, \hat{\theta}) \tilde{M}_i + \sum_{i=1}^n e_i \tilde{M}_i + n E(\Delta RY) E(M) \end{aligned} \quad (9)$$

If the relationship between species deviations from the null model and the monoculture yield is linear, i.e. $g(\tilde{M}_i, \hat{\theta}) = \hat{\theta}_1 \tilde{M}_i$, it follows from ordinary least squares theory that $\sum_{i=1}^n e_i \tilde{M}_i = 0$, and consequently equation 9 then becomes:

$$\begin{aligned}\Delta Y &= \hat{\theta}_1 \sum_{i=1}^n \tilde{M}_i^2 + n E(\Delta RY) E(M) = n \hat{\theta}_1 s_M^2 + n E(\Delta RY) E(M) \\ &= n \text{cov}(\Delta RY, M) + n E(\Delta RY) E(M) \quad (10)\end{aligned}$$

This is the additive partitioning by Loreau and Hector¹⁴. Note that $\hat{\theta}_1$, the optimal least square estimate of θ_1 , equals $\frac{\text{cov}(\Delta RY, M)}{s_M^2}$ and that s_M^2 is the sample variance of the species monoculture yields. Analogous, equation 4 can be rewritten as:

$$\begin{aligned}\Delta Y &= \sum_{i=1}^n g^{(1)}(\tilde{M}_i, \hat{\Theta}^{(1)}) \tilde{M}_i + \sum_{i=1}^n \varepsilon_i^{(1)} \tilde{M}_i + \sum_{i=1}^n g^{(2)}(\tilde{M}_i, \hat{\Theta}^{(2)}) \tilde{M}_i + \sum_{i=1}^n \varepsilon_i^{(2)} \tilde{M}_i \\ &\quad + n E(\Delta RY) E(M) \quad (11)\end{aligned}$$

whereby, similar to identity (7):

$$\frac{RY_{o,i}}{RYT} - RY_{e,i} - E\left(\frac{RY_0}{RYT} - RY_e\right) = g^{(1)}(\tilde{M}_i, \hat{\Theta}^{(1)}) + e_i^{(1)} \quad (12)$$

and

$$RY_{o,i} - \frac{RY_{o,i}}{RYT} - E\left(RY_o - \frac{RY_0}{RYT}\right) = g^{(2)}(\tilde{M}_i, \hat{\Theta}^{(2)}) + e_i^{(2)} \quad (13)$$

Note that $g^{(1)}$ describes the deviation from the expected frequency (i.e. the dominance effect) and that $g^{(2)}$ describes deviations that are not associated with changes in frequency (i.e. the trait-dependent complementarity effect). Hence, when $g^{(1)}(\tilde{M}_i, \hat{\Theta}^{(1)}) = \hat{\theta}_1^{(1)} \tilde{M}_i$ and $g^{(2)}(\tilde{M}_i, \hat{\Theta}^{(2)}) = \hat{\theta}_1^{(2)} \tilde{M}_i$, equation 11 equals equation 5, which is the partitioning presented by Fox¹⁵. When $g(\tilde{M}_i, \hat{\Theta})$, $g^{(1)}(\tilde{M}_i, \hat{\Theta}^{(1)})$ and $g^{(2)}(\tilde{M}_i, \hat{\Theta}^{(2)})$ are m^{th} order polynomials equations 9 and 11 can be written as:

$$\Delta Y = \hat{\theta}_1 \sum_{i=1}^n \tilde{M}_i^2 + \hat{\theta}_2 \sum_{i=1}^n \tilde{M}_i^3 + \dots + \hat{\theta}_m \sum_{i=1}^n \tilde{M}_i^{m+1} + n E(\Delta RY) E(M) \quad (14)$$

and

$$\begin{aligned}\Delta Y &= \left\{ \hat{\theta}_1^{(1)} \sum_{i=1}^n \tilde{M}_i^2 + \hat{\theta}_2^{(1)} \sum_{i=1}^n \tilde{M}_i^3 + \dots + \hat{\theta}_{m^{(1)}}^{(1)} \sum_{i=1}^n \tilde{M}_i^{m^{(1)}+1} \right\} \\ &\quad + \left\{ \hat{\theta}_1^{(2)} \sum_{i=1}^n \tilde{M}_i^2 + \hat{\theta}_2^{(2)} \sum_{i=1}^n \tilde{M}_i^3 + \dots + \hat{\theta}_{m^{(2)}}^{(2)} \sum_{i=1}^n \tilde{M}_i^{m^{(2)}+1} \right\} \\ &\quad + n E(\Delta RY) E(M) \quad (15)\end{aligned}$$

These equations now partition the selection effect (equation 14) or dominance effect and trait-dependent complementarity effect (equation 15) in m terms, describing the linear up to the m^{th} order dependency of the deviation from the null hypothesis on species' monoculture yields. As equation 3, 5, 14 and 15 are decompositions of ΔY , the sum of the m terms in equations 14 and 15 still equals the covariance terms in the partitioning methods of Loreau and Hector¹⁴ and Fox¹⁵.

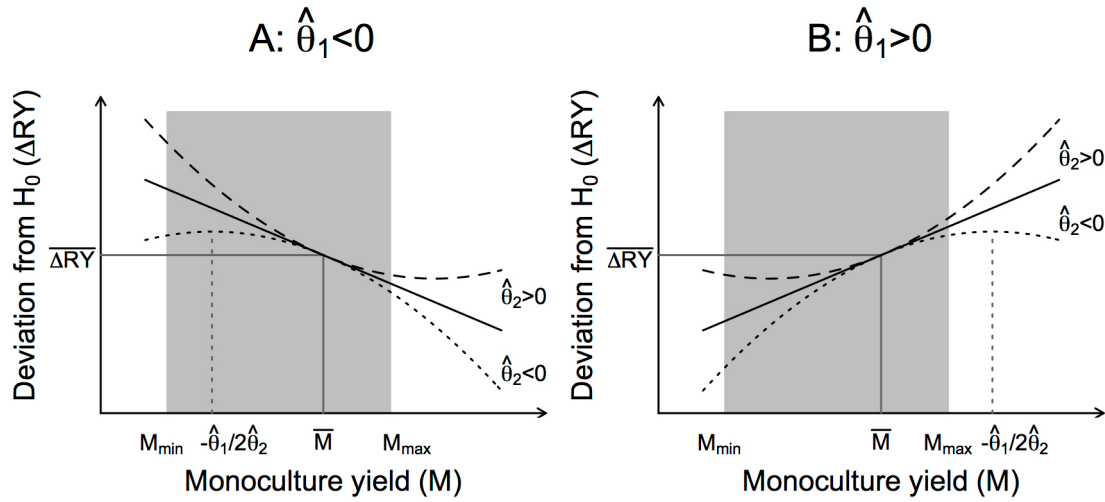


Figure 4.1: Linear and quadratic deviations from the null model Linear and quadratic deviations from the null model. The first order regression coefficient determines whether the general relationship between species deviations from the null model and the monoculture yield is negative (A) or positive (B). The second order regression coefficient determines whether the relationship is convex ($\hat{\theta}_2 > 0$) or concave ($\hat{\theta}_2 < 0$). Whether the relationship is unimodal or monotonic depends on whether the tip of the parabola ($-\hat{\theta}_1/2\hat{\theta}_2$) is located within the species monoculture range (indicated in grey) or not, respectively. M_{\min} and M_{\max} are respectively the minimum and maximum monoculture yield. Note that the linear relationship between species monoculture yields and deviations from the null model of linear additive partitioning methods is tangent to the parabola at the average deviation from the null model ($\overline{\Delta RY}$) and average monoculture yield (\bar{M}).

The use of m^{th} order polynomials to describe the relationship between species monoculture yields and deviations from the null model allow for a more versatile analysis of species deviations from the null model. When sufficient data is available, it allows distinguishing between the linear, quadratic and up to m^{th} order deviations from the null model. However, as most biodiversity experiments only include a limited number of species (rarely over 16 species), fitting third or higher order polynomials can result in over fitting the data. In addition, first and second order terms can more easily be ecologically interpreted than higher order terms. First order terms describe how species with above average monoculture yields differ in their deviation from the null model compared to species with below average monoculture

yields. When the first order regression coefficient ($\hat{\theta}_1$) is positive, species with higher monoculture yields deviate more from the null model than the average deviation, taken across all species in the system (Figure 4.1, right panel). Analogously, species with lower monoculture yields deviate more than average from the null model when the first order regression coefficient is negative (Figure 4.1, left panel). The second order regression coefficient describes to what extent the deviation from the null model is non-linearly related to monoculture yield. They therefore represent a parabolic relationship and the sign of the second order regression coefficient ($\hat{\theta}_2$) determines whether the parabola is convex ($\hat{\theta}_2 > 0$) or concave ($\hat{\theta}_2 < 0$). The straight line described by the first order regression coefficient is the tangent of this parabola in the point $(\bar{M}, \overline{\Delta RY})$. Both first and second order regression coefficients determine the position of the tip of the parabola $(-\hat{\theta}_1/2\hat{\theta}_2)$. When the tip of the parabola lies inside the monoculture range, the relationship is unimodal (Figure 4.1 concave relationship in the left panel, or convex relationship in the right panel). As the tip of the parabola shifts away from the monoculture range, the deviations from the linear relationship become more asymmetrical (Figure 4.1). In these extreme cases, the second order term describes to what extent with high or low monoculture yield deviate from the linear term. When the tip of the parabola lies closer to the average monoculture range, relationships become more symmetrical and so describe to what extent both species with low and high monoculture yields deviate from the linear relationship. When this tip lies outside the monoculture range (Figure 4.1 convex relationship in the left panel, or concave relationship in the right panel), the second order polynomial describes a monotonic relationship.

4.4. Application and occurrence of non-linear biodiversity effects

The Cedar Creek Biodiversity II experiment is a field experiment conducted to assess the effects of species diversity on grassland productivity by randomly assembling systems of 1, 2, 4, 8 and 16 species from a pool of 18 grassland perennials^{18,19}. This experiment is one of the most long-lasting biodiversity experiments, and thirteen years of data gathered between 2001 and 2013, were made publically available by the University of Minnesota²⁰. To avoid strong effects of species loss or gain on ecosystem functioning only plots for which a minimum of 75% of the original species were still present, and whereby colonisation by new species had limited effects on the total aboveground biomass were included to avoid strong effects of species loss or gain on ecosystem functioning^{21–23}. Both a partitioning based on linear relationships^{14,15} and an extension of these methods based on second order polynomials was applied to the data to test which relationship described the observed deviations from the null model best (F-ratio test using a 5% significance level, assuming

normality of model residuals, Appendix C Figure S1). To be able to fit second order polynomials, only plots with an initial species richness at least four species were considered. Higher order polynomials were not considered due to constraints in the maximal degrees of freedom.



Figure 4.2: Comparison between additive partitioning methods based on linear relationships and order polynomials. The number of years for which 2nd order polynomials fitted (A) selection effects¹⁴ and (B) dominance and (C) trait-dependent complementarity effects¹⁵ better than linear relationships (F-ratio test, $p < 0.05$).

Second order polynomials fitted deviations from the null model better in most plots. Partitioning deviations between the selection and complementarity effect¹⁴ revealed that in 69 of the 91 plots selection effects were better described by second order polynomials in at least one of the years. The number of plots in which second order polynomials fitted selection effects significantly better to the observed deviations from the null model increased with diversity (Kruskal-Wallis $\chi^2_2 = 177.3$, $p < 0.001$). In 17 out of the 35 plots containing 16 species, second order polynomials fitted selection effects better in at least half of the years (Figure 4.2). For systems of 4 and 8 species, 2nd order polynomials fitted selection effects better for at least half of the years in only 3 out of the 30 and 2 out of the 26 plots, respectively. The non-linearity in selection effects was caused by the non-linearity of the trait-dependent complementarity effects sensu Fox¹⁵ (Figure 4.2). The results found for trait-dependent complementarity effects were similar to those found for the selection effect: the number of plots in which deviations were better described by second order polynomials increased with diversity (Kruskal-Wallis $\chi^2_2 = 191.1$, $p < 0.001$).

In highly diverse systems, linear additive partitioning methods underestimate the deviations from the null model for species driving productivity (Figure 4.3). Diversity increases the

number, and thus potentially the complexity, of species interactions. Competition for nitrogen plays an important role in grasslands^{24–26}. The presence of nitrogen fixing legumes has therefore a significant impact on the system’s productivity by increasing the amount of nitrogen available, and thus the potential for species to overyield (i.e. a positive ΔRY). Hence, strong competitors for nitrogen are expected to benefit most from the presence of legumes²⁶.

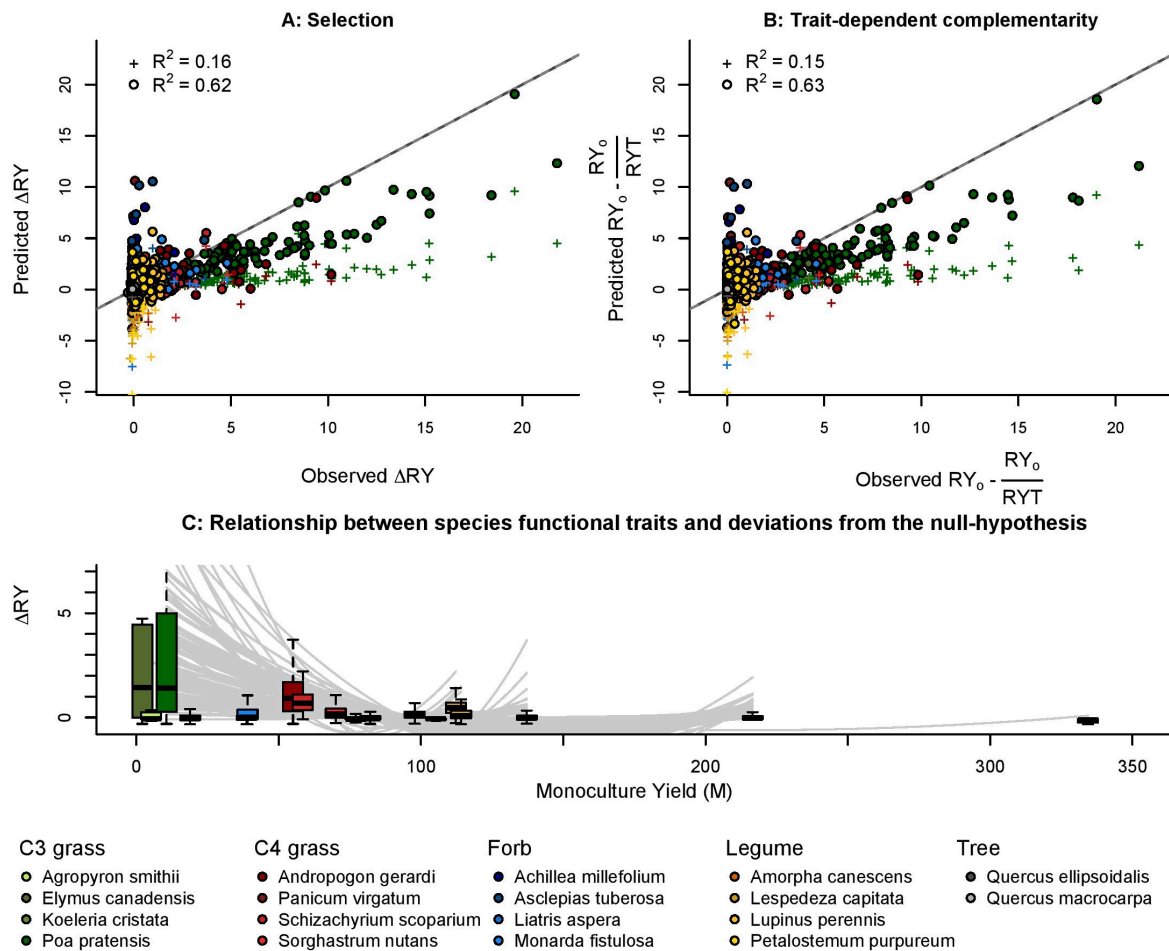


Figure 4.3: Comparison of model performance between partitioning methods based on linear and 2nd order relationships. Upper panels represent the predicted deviations from the null model according to linear partitioning (+) and second order partitioning methods (o), plotted against the observed deviations for the (A) selection and (B) trait-dependent complementarity (right) effect. The lower panel (C) represents the relationships between species deviation from the null model and the monoculture yield. Boxplot represent observed species deviations, and whiskers correspond to maximal 1.5 times the interquartile range. Grey lines represent the fitted second order polynomials that fitted deviations from the null model significantly better than linear relationships (F-ratio test,).

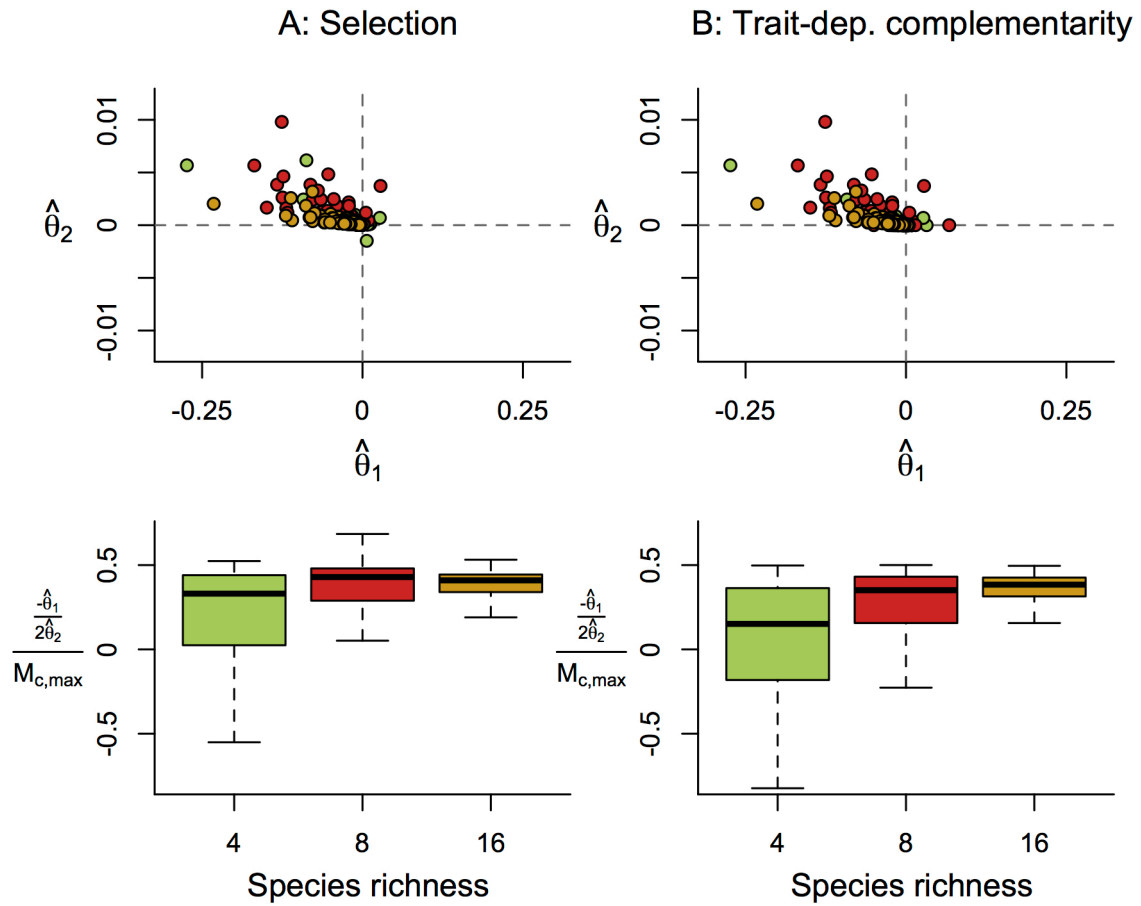


Figure 4.4: Regression coefficients for second order polynomials (upper panels) and relative position of the tip of the parabola (lower panels). Regression coefficients for the selection (A) and trait-dependent complementarity (B) effect. Positive values in the lower panels indicate a tip of the parabola to the right of the mean monoculture yield, negative values to the left. Note that all tips are within the monoculture range, as values do not exceed 1.

Indeed, strong positive deviations from the null model occurred for the dominant grass species such as *Poa pratensis*, *Andropogon gerardi* and *Schizachyrium scoparium*, but also legumes overyielded in mixtures due to their nitrogen fixing abilities, reducing competition for nitrogen (Figure 4.3)^{26,27}. Non-linear partitioning methods thus captured deviations from the null model better for these grasses with low monoculture yields, and legumes with high monoculture yields by fitting a convex relationship instead of a linear relationship between species deviations from the null model and their monoculture yields (Figure 4.3). In systems where second order polynomials described deviations from the null model best, first order regression coefficients were almost invariably negative, whereas second order regression coefficients were positive for both selection and trait-dependent complementarity effects (Figure 4.4, upper panels). Although the symmetry of the relationship differed among systems, all relationships are rather asymmetrical with the tip of the parabola near the upper

limit of the monoculture range, so that deviations from the null model were strongest for grass species with low monoculture yields (Figures 4.3 and 4.4).

4.5. Discussion

The non-linear extension presented here increases the flexibility of additive partitioning methods. The analysis of the Cedar Creek Biodiversity II experiment demonstrates that non-linear relationships between the deviation from the null model and functional traits are likely to occur in biodiversity experiments (Figure 4.2). A more accurate description of this relationship by non-linear additive partitioning methods therefore offers a more detailed insight in how biodiversity affects ecosystem functioning (Figures 4.3 and 4.4). In addition, the extension introduced here can resolve leverage problems that can occur when fitting a linear model to nonlinear deviations from the null model.

Biodiversity experiments are often designed with equal initial contribution to ecosystem functioning among species. This even initial condition does however not necessarily correspond to equilibrium conditions. Indeed, species interactions can change species functional contributions and biodiversity effects over time²⁸. The presented results reveal that such strong non-linear deviations from the initial conditions already occurred after a limited number of generations in the Biodiversity II grassland experiment (Figure 4.2), and could thus be widespread in biodiversity studies.

How deviations from the null model relate to species monoculture yields depend on the type of interactions. For example, legumes generally overyield in mixtures due to their nitrogen fixing ability, irrespective of community composition, whereas grasses overyield due to the positive effect of legumes²⁶. The convex relationships between deviations from the null model and monoculture yield therefore primarily occurred in high diverse treatments where legumes and grasses were both present. The non-linear partitioning method presented here thus offers the advantage of being able to directly splitting the contribution of species with intermediate functional traits from the contribution of species with extreme trait functional values. Splitting selection or dominance and trait-dependent effects in first and second order terms does not change the total strength of these effects as calculated by linear additive partitioning methods. However, it does allow for a more detailed understanding of how biodiversity affects ecosystem functioning in a single analysis. Depending on the symmetry of the parabola, the second order term quantifies the non-linear effect of species with low, high or both functional trait values (Figure 4.1). Moreover, as these linear and higher-order partitioning methods represent a nested set of models, it can easily be formally tested if

higher-order extensions, and thus the additional inclusion of parameters in the model, are warranted.

The limitation of the non-linear extension, however, is that the use of second order polynomials is limited to systems with at least three species, whereas linear additive partitioning methods can also be applied to systems with two species. Many biodiversity experiments use systems with two species as the lowest diversity level, and so the non-linear approach present here is not applicable to these low-diversity cases. In more diverse systems, however, non-linear partitioning methods, can help to avoid several statistical issues that can occur when fitting an inappropriate model structure to the data. When species deviations from the null model are non-linear, the estimated regression coefficients of a linear regression can be strongly affected by outliers²⁹. Outliers can therefore have severe effects on the magnitude, and particularly the sign (which alters their ecological interpretation) of selection, dominance or trait-dependent complementarity effects. Species with extremely low monoculture functions have therefore routinely been excluded from analyses with additive partitioning as their deviation from the null-hypothesis can easily approach infinity^{14,28}. In addition, the increase in measurement error when sampling for rare species may increase the uncertainty on the deviations from the null hypothesis for species with low monoculture yields³⁰. By splitting selection or dominance and trait-dependent complementarity effects in a linear and a quadratic term, non-linear partitioning can mitigate these leverage problems, capturing strong deviations from the linear relationship in the quadratic term. Such strong deviations are increasingly likely to occur in systems where species strongly differ in their competitive abilities. In particular, when species inferior competitors also have low monoculture yields, which can result in very high relative yields. The problem of outliers can (partially) be circumvented using robust regression. Alternatively, functional contributions to ecosystem function can be expressed as a linear combination of multiple functional traits³¹. The approach presented here, however, allows solving this problem by using a general function $g(\tilde{M}, \theta)$ that allows specifying an appropriate model structure. Here, the case of polynomials was considered, but other functions could be used if necessary. Second order polynomials thereby sufficed to eliminate strong potential leverage problems of species that strongly deviated from the null model (Figure 4.3). By splitting selection or dominance and trait-dependent complementarity effects in a linear and a quadratic term, non-linear partitioning can mitigate these leverage problems, capturing strong deviations of some species from the linear relationship in the quadratic term. Although higher order polynomials could be used, higher order terms are not only more difficult to interpret ecologically, but are also likely to over fit the data due to the low number of species generally used in biodiversity studies.

The use of null models by additive partitioning methods allows separating species-identity from biodiversity effects on function. The results presented here demonstrate that non-linear deviations from these null models might be more widespread than previously considered. The non-linear extensions of additive partitioning methods introduced here therefore increases the versatility of partitioning methods. By separating the effect of species with intermediate functional traits from species with extreme functional traits on ecosystem functioning, they allow analysing complex biodiversity effects on ecosystem functioning. Non-linear extensions can therefore be an important tool to analyse biodiversity effects on ecosystem functioning time.

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5

Linking biodiversity effects on functional and compositional stability

Redrafted from:

Baert J.M., De Laender F., Sabbe K. and Janssen C.R. Biodiversity increases functional and compositional resistance, but decreases resilience in phytoplankton communities. *Ecology*. **2016**, 97, 3433-3440.

Abstract

There is now ample evidence that biodiversity stabilizes aggregated ecosystem functions (e.g. primary production) in changing environments. This stabilizing effect is driven by increased functional resistance (i.e. reduced changes in functions by environmental changes) rather than through increased functional resilience (i.e. rapid recovery following environmental changes) in primary producer systems. Although the temporal stability of these aggregated ecosystem functions directly results from the system's compositional dynamics, the mechanisms that link the stability of ecosystem functions to compositional dynamics have only been theoretically explored. In this chapter, it is demonstrated, using diatom communities, how biodiversity effects on compositional stability drive biodiversity effects on functional stability. In a microcosm experiment, communities spanning 5 richness levels (1, 2, 4, 6 and 8 species) were exposed to 3 concentrations of a chemical stressor (0, 25 and 250 $\mu\text{g L}^{-1}$ atrazine) for 4 weeks, after which all communities were transferred to atrazine-free medium for three more weeks. Slower compositional dynamics in more diverse systems explained positive effects of biodiversity on compositional and functional resistance, but negative biodiversity effects on functional and compositional resilience. Slower compositional dynamics reduced changes in evenness in more diverse communities. These results validate the theoretically proposed link between biodiversity effects functional and compositional stability in primary producer systems, and provide a mechanistic underpinning of biodiversity-stability relationships. Finally, it is discussed how the observed slower dynamics in more diverse systems, and their effect on evenness, can be expected to become increasingly important when stabilizing ecosystem functions against multiple fluctuating environmental stressors.

5.1. Introduction

Many ecosystem functions consist of the aggregate of the individual species' functional contributions (e.g. primary production, total biomass, nutrient retention). Hence, these functions inherently vary over time as species densities change in response to fluctuating environmental conditions. Spurred by the awareness of the ongoing global biodiversity decline, considerable research effort has been devoted to understanding if the temporal stability of ecosystem functions depends on the number of species in the system^{1–6}. Nowadays, there is ample empirical evidence that biodiversity generally stabilizes ecosystem functions^{1,7,8}. A recent meta-analysis revealed that this positive biodiversity-stability relationship is primarily driven by increased functional resistance (i.e. reduced changes in functions by environmental changes) rather than by increased resilience (i.e. rapid recovery following environmental changes) in more diverse systems⁹. Still, it remains poorly understood how diversity-dependent changes in resistance or resilience depend on the system's compositional dynamics. To date, however, potential biodiversity effects on compositional stability have only been theoretically explored^{10–12}.

Theoretical models identify several mechanisms by which biodiversity can stabilize ecosystem functions against environmental changes¹³. These mechanisms primarily rely on interspecific differences in environmental response, which stabilize ecosystem functions in a changing environment through the functional compensation between tolerant and sensitive species. Biodiversity is expected to increase the variability in environmental responses, which can promote the stabilization of ecosystem functions in two ways. First, a greater variability in environmental responses increases the likelihood that a system contains species that can thrive under given environmental conditions, and compensate for the functional loss of sensitive species to maintain ecosystem functions under these conditions. Hence, biodiversity is expected to increase the range of environmental conditions over which a system can maintain its functions (i.e. the insurance hypothesis^{14,15}). Second, a greater variability in environmental responses increases the likelihood of asynchronous species responses. Perfectly asynchronous fluctuations in species densities average each other out at the level of their aggregated functions. More asynchronous fluctuations thus lead to a better functional compensation between species, and reduces changes in the aggregated ecosystem functions. Hence, biodiversity is expected to reduce the temporal variability in ecosystem functions in fluctuating environments by increasing the number of asynchronous species responses (i.e. the portfolio effect¹⁰). Although functional compensation only requires interspecific differences in environmental response to occur, the strength of the effect is modulated by species interactions¹¹.

The number and strength of species interactions affect the extent and speed of compositional changes, and thus functional compensation^{16–19}. The strength of species interactions affects the asynchrony in environmental response between species. Negative interactions result in competitive release when environmental changes reduce the density of one of the species. This increases the asynchrony in species responses, and thus increases the extent of functional compensation. Positive interactions, in contrast, tend to synchronize species responses and reduce the extent of compensation^{11,20,21}.

In systems where competitive differences between species are limited, the increased number of species interactions can slow down compositional dynamics in more diverse systems^{12,16,19,22–24}. These slower compositional dynamics reduce the speed of functional compensation between species. Therefore, theoretical models predict that biodiversity should increase functional stability through increased compositional resistance in systems of ecologically similar species, like primary producer systems¹². By consequence, slower dynamics are expected to reduce functional resilience¹². Stronger competitive differences, in contrast, result in faster compositional changes^{25,26}. Hence, functional stability is expected to be driven by stronger compositional turnover and functional compensation between species in systems with asymmetrical competition^{11,20}.

The speed of compositional dynamics may also affect functional stability by changing the system's evenness. More even systems generally maintain ecosystem functions better in a changing environment by preventing low initial densities of species with favourable traits^{10,27,28}. Hence, the long-term preservation of evenness could positively contribute to a system's functional stability. Temporal fluctuations in evenness could be reduced by both slow community dynamics and strong functional compensation. In the latter case, alternating competitive superiority induced by environmental changes can prevent competitive exclusion (i.e. the storage effect^{29,30}) and increases compositional stability^{31,32}.

In this chapter, the theoretical prediction that slower compositional dynamics in more diverse communities drive the positive effect of diversity on compositional and functional stability in primary producer systems is tested. In a microcosm experiment, communities of marine diatoms (*Bacillariophyceae*), spanning 5 species richness levels (1,2,4,6 and 8 species), were exposed to 3 concentrations of the herbicide atrazine (0, 25 and 250 $\mu\text{g L}^{-1}$) for 4 weeks, after which communities were transferred to atrazine free medium to recover for 3 weeks. Biomass production and composition were compared between stressed and unstressed communities after 28 and 49 days, and tested if biodiversity increased functional and compositional resistance and decreased resilience because of slower composition

dynamics in more diverse systems. In addition, it was tested if diversity-dependent changes in the speed of community dynamics affect the temporal dynamics in evenness.

5.2. Materials and methods

5.2.1. Algal strains

Diatoms were isolated from a single phytoplankton sample collected near the Thornton bank (Southern bight of the North Sea) during the spring bloom in March 2013. Stock cultures were started from single cells according to the procedure described by Andersen (2005), and cultured in F/2 medium³⁴ based on artificial seawater (salinity $33\pm 1\text{‰}$; Instant Ocean®) supplemented with $30\text{ }\mu\text{g L}^{-1}$ Si. Stock cultures were kept in a climate room ($20\pm 1^\circ\text{C}$) at a 12-hour photoperiod ($35\pm 5\text{ }\mu\text{mol photons m}^{-2}\text{s}^{-1}$ light intensity; Lumilux® 18W cool white Osram). New cultures were inoculated weekly to sustain exponential growth. The photoperiod was prolonged to 16 hours two weeks before the start of the experiment.

5.2.2. Microcosm experiment

The experiment presented in chapter 2 was extended with 3 more weeks of exposure to atrazine-free medium. Communities were thus exposed to atrazine for 4 weeks after which all communities were transferred to atrazine-free medium for 3 more weeks. The atrazine concentrations (0, 25 and $250\text{ }\mu\text{g L}^{-1}$), representing a control, low stress and high stress treatment were selected based on preliminary tests.

Microcosms were established in triplicate for each treatment (351 microcosms in total). Communities were kept in sterilised 100ml glass Erlenmeyer flasks filled with 35ml F/2 medium at the appropriate atrazine (Sigma Aldrich) concentration. Species were inoculated at an equal proportion of the initial total biovolume ($10^7\text{ }\mu\text{m}^3\text{ mL}^{-1}$). Inoculating species from a single stock culture minimized the variability in cell viability between replicates and assemblages. Microcosms were cultured at $20\pm 1^\circ\text{C}$ and a $35\pm 5\text{ }\mu\text{mol photons m}^{-2}\text{ s}^{-1}$ 16-hour photoperiod. Each week, 80% of the growth medium was replaced by new medium spiked the appropriate atrazine concentrations to avoid nutrient limitation or stress reduction resulting from atrazine photolysis. Before removing growth medium, cells were left to settle and 1ml samples were taken to verify that the removed medium contained less than 1% of the total biomass. After 28 days, only atrazine-free medium was used for renewal. Prior to medium renewal, 1mL samples were taken from each microcosm to determine cell densities. Samples were fixed with formaldehyde (6% final concentration), and stored at 4°C in 24-well plates until analysis. Cell densities were determined using an inverse microscope and Whipple grid. Biomass production was calculated from the average cell volume of each

species, based on a sample of 50 cells³⁵. The absence of nutrient limitation and a constant atrazine exposure were ensured by weekly measuring nitrate, phosphate, and silicate concentrations spectrophotometrically (Aquamate®, Thermo Electron Corporation + Spectroquant® test kits, Merck Millipore) and atrazine concentrations by GC-MS (Thermo Quest Finnigan Trace DSQ coupled to Thermo Quest Trace 2000 series).

5.2.3. Data analysis

Functional resistance ($\Omega_{function}$) to atrazine was defined as the inversed absolute proportional change in function (i.e. biomass production) between control ($F_{control}$) and stressed communities (F_{stress}) after 28 days of atrazine exposure:

$$\Omega_{function} = \frac{F_{control,28}}{|F_{control,28} - F_{stress,28}|} \quad (1)$$

Values for functional resistance thus range from 1 when there is no resistance (i.e. $F_{stress}=0$) to infinity when there is no difference in function between the control condition and stress treatment. As species equilibrium densities differed by several orders of magnitude, biovolume, rather than density was hence selected on the basis on which to define species and ecosystem functioning (Appendix D Table S1). Compositional resistance ($\Omega_{composition}$) was defined as the Bray-Curtis dissimilarity index³⁶ of the species densities (N_i) between control and stressed communities:

$$\Omega_{composition} = BC_{28} = 1 - \frac{\sum_i |N_{i,control,28} - N_{i,stress,28}|}{\sum_i N_{i,control,28} + \sum_i N_{i,stress,28}} \quad (2)$$

Compositional resistance equals 1 if there are no differences in species densities between control and stress treatments and equals 0 when control and stress treatments have no species in common. Functional resilience ($\Delta_{function}$) was defined as the proportional change in deviation from the control communities between the end of atrazine exposure (day 28) and the end of the experiment (day 49):

$$\Delta_{function} = \frac{|F_{control,28} - F_{stress,28}|}{|F_{control,49} - F_{stress,49}|} \quad (3)$$

Hence, functional resilience is larger than 1 when functional differences between the control and stress treatment decrease between day 28 and 49, and less than 1 otherwise. Compositional resilience ($\Delta_{composition}$) was defined as the change in Bray-Curtis dissimilarity between day 28 and 49. Values are positive when control and stress communities become

more similar in composition between day 28 and 49, and are negative when compositions diverge.

$$\Delta_{composition} = BC_{49} - BC_{28} \quad (4)$$

The effect of species richness on biomass production, functional and compositional resistance and resilience, and Shannon-Wiener evenness was estimated using linear regression models. All models included Log_{10} species richness (continuous variable) and atrazine concentration (factor variable) as fixed effects. Time (factor variable) was included as a fixed effect in the linear regression model for productivity. Temporal autocorrelation of model residuals was assessed, but inclusion of an autocorrelation structure did not improve the model (ANOVA $p > 0.05$). Normality of model residuals was verified by the QQ-plot, and the linearity of fixed effects was evaluated by plotting model residuals against each fixed effect (See Appendix D Figure S7-11). Produced biomass, functional resistance and functional resilience needed to be log-transformed to improve the normality of the model residuals. Linear models were fitted using the lme4 package in R^{37,38}.

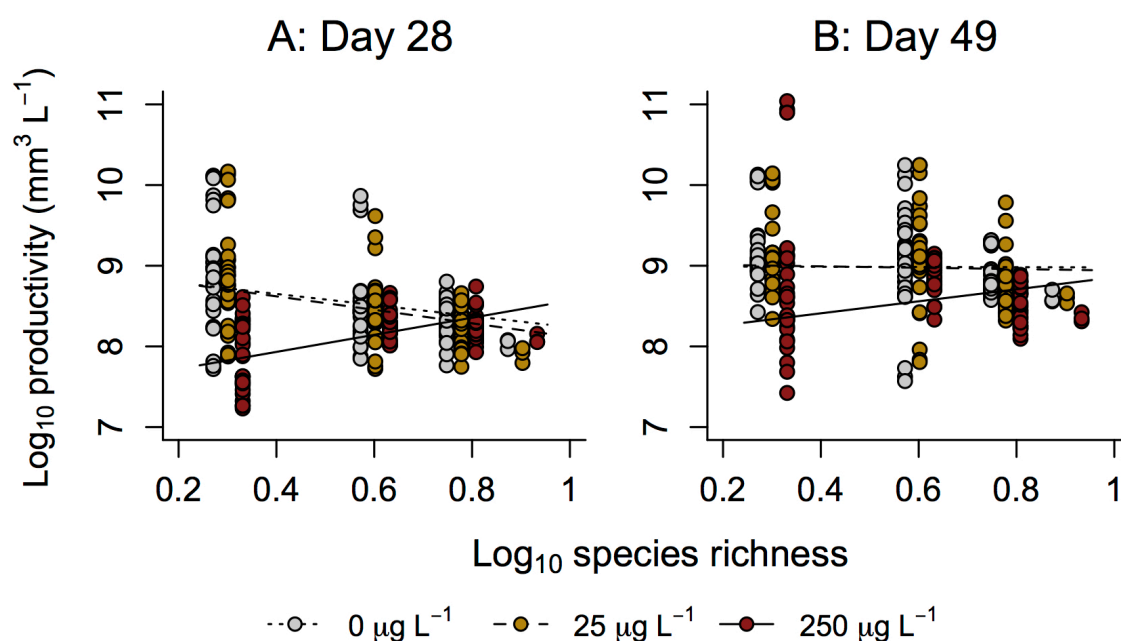


Figure 5.1: Biodiversity-ecosystem functioning relationships. BEF relationships after 4 weeks of exposure to atrazine (day 28) and at the end of the experiment (day 49). Regression coefficients are summarized in Table 5.1.

5.3. Results

Atrazine only affected biomass production and the biodiversity-productivity relationship in the high-stress treatment ($p < 0.001$; Table 5.1). Throughout the experiment, there was no significant difference in biomass production between low stress and control treatments (Table 5.1). After 28 days, atrazine significantly reduced biomass production in the high stress treatment. Biomass production was more resistant to high atrazine stress in more species rich communities ($p < 0.001$; Table 5.2, Figure 5.2A), which was associated with an increased resistance in composition ($p < 0.001$; Table 5.2, Figure 5.2C). This increased resistance in more species rich communities shifted the negative biodiversity-productivity relationship in control conditions to positive in the high-stress treatment (Figure 5.1A).

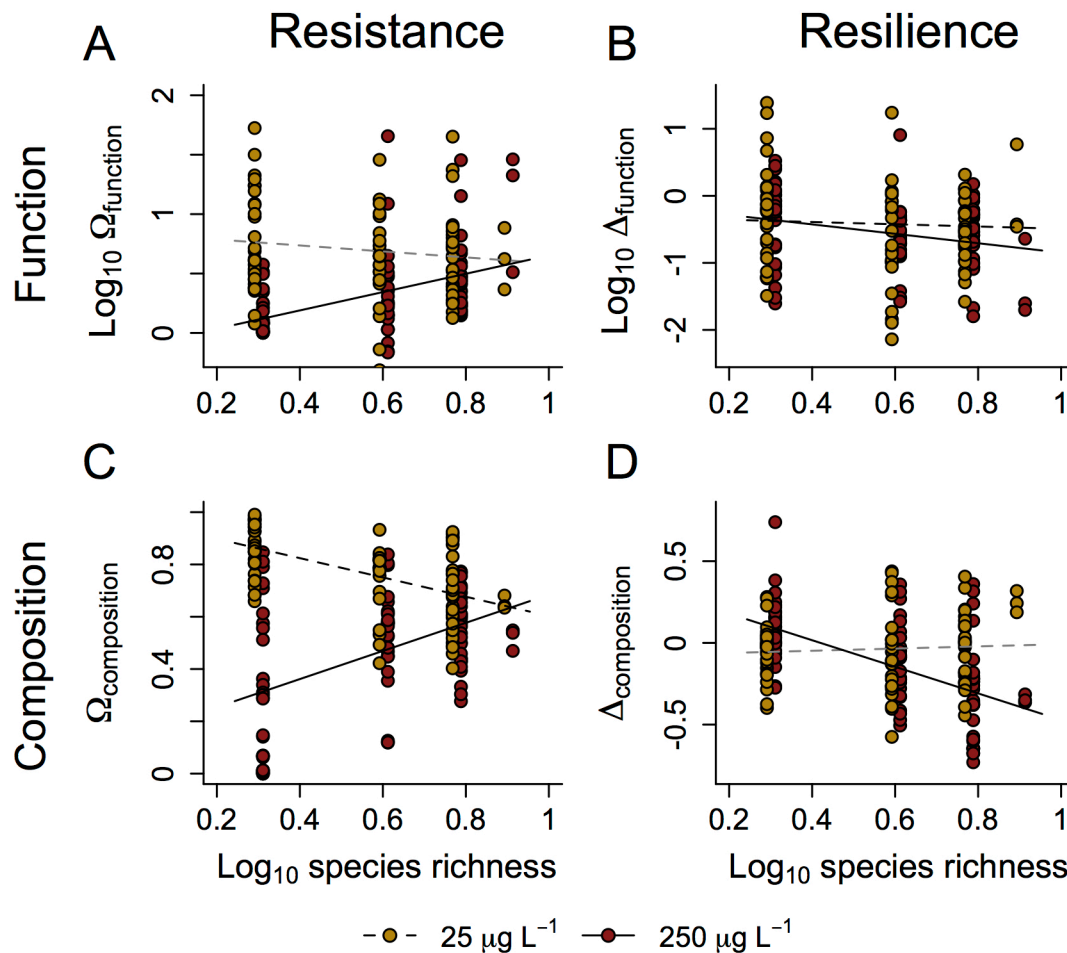


Figure 5.2: Biodiversity effects on functional and compositional resistance and resilience. Regression coefficients are summarized in Table 5.2. Note that measures of resistance and resilience are calculated in such way that higher values correspond to higher resistance and resilience.

Table 5.1: Biodiversity effects on productivity and evenness. Regression coefficients for the linear regression models estimating the effect of biodiversity on productivity and the change in evenness between the end of atrazine exposure and the end of the experiment.

	Productivity	ΔEvenness
Intercept	8.9254***	0.2029*
Log ₁₀ Diversity	-0.6868 ***	-0.7540***
25 µg L ⁻¹	0.0293	-0.1090
250 µg L ⁻¹	-1.4170***	-0.4394***
Log ₁₀ Diversity x 25 µg L ⁻¹	-0.1487	0.4266*
Log ₁₀ Diversity x 250 µg L ⁻¹	1.7464***	0.9757 ***
Day 49	0.0673	
Log ₁₀ Diversity x day 49	0.6740**	
25 µg L ⁻¹ x day 49	0.0029	
250 µg L ⁻¹ x day 49	0.7570***	
Log ₁₀ Diversity x 25 µg L ⁻¹ x day 49	0.0782	
Log ₁₀ Diversity x 250 µg L ⁻¹ x day 49	-1.2199***	

*p<0.05, **p<0.01 and ***p<0.001

At day 49, i.e. after 3 weeks of exposure to atrazine-free medium, differences in biomass production between high-stress and control communities decreased, but biomass production remained significantly lower in the high-stress treatment (p<0.001; Table 5.1, Figure 5.1B). Communities with lower species richness approached the control treatment faster in both function and composition (Table 5.2, Figure 5.2B and D).

In the high stress treatment, evenness increased between day 28 and 49 in the most species rich communities, but decreased in communities with low species richness (p<0.001; Figure 5.3, Table 5.1). In contrast, evenness decreased between day 28 and 49 in all communities in the control and low stress treatment (except for a slight increase in richness level two in the low stress treatment, p<0.05), and this decrease was more pronounced in the more diverse communities (p<0.001; Figure 5.3, Table 5.1).

Table 5.2: Biodiversity effects on functional and compositional resistance and resilience. Regression coefficients for linear regression models estimating the effect of biodiversity on functional and compositional resistance and resilience. “-” denotes that factors were not significant and removed in the optimal model.

	Resistance		Resilience	
	Function	Composition	Function	Composition
Intercept	0.8407***	0.0237	-0.2000	0.0730
Log ₁₀ Diversity	-0.2449	0.3742***	-0.5715*	-0.0659
250 µg L ⁻¹	-0.9595***	0.8300***	-	-0.4147***
Log ₁₀ Diversity : 250 µg L ⁻¹	1.0162***	-0.9123***	-	-0.8797***

*p<0.05, **p<0.01 and ***p<0.001

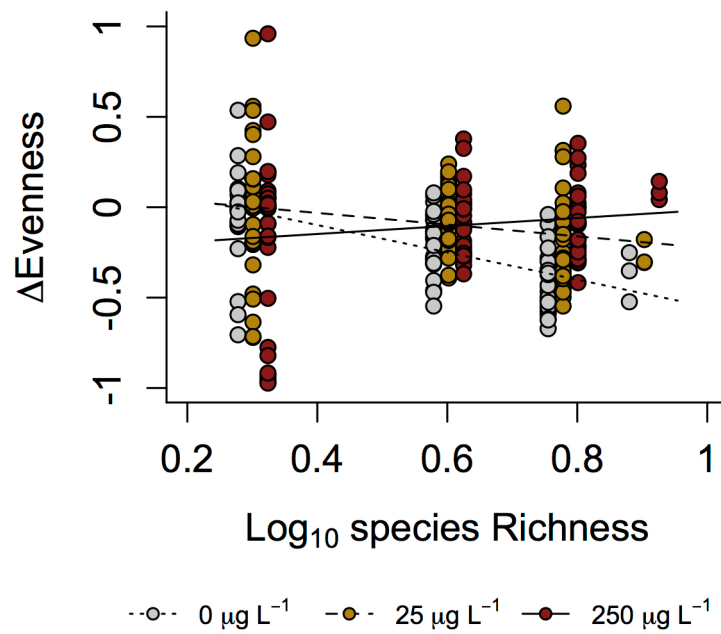


Figure 5.3: Biodiversity effects on evenness. Relationship between biodiversity and the change in evenness between the end of atrazine exposure (day 28) and the end of the experiment (day 49). Regression coefficients are summarized in Table 5.1.

5.4. Discussion

The results presented in this chapter demonstrate the tight link between functional and compositional stability in primary producer systems. In the microcosm study, biodiversity simultaneously increased compositional and functional resistance, but decreased functional and compositional resilience because of slower compositional dynamics in more diverse systems. These findings validate the theoretically proposed mechanistic underpinning of positive biodiversity-functional stability relationships¹², and support positive biodiversity effects on resistance as a main driver of functional stability in grassland systems⁹. Finally, the results presented here suggest that slower compositional dynamics in more diverse systems can additional stabilise ecosystem functions by maintaining evenness.

More diverse communities were less impacted by atrazine stress in the microcosm experiment (cf. the insurance hypothesis¹⁴; Figure 5.2A). However, this increased functional resistance did not involve large compositional changes to maintain productivity in stressed communities. Instead, community compositions were more similar between control and the high stress treatment in more diverse communities (Figure 5.2C). Hence, functional compensation did not involve extensive compensatory dynamics. Despite the occurrence in

many theoretical models, strong compensatory dynamics are not ubiquitous in real systems^{16,20,39,40}. Because the extent of species turnover depends on interspecific differences in response to environmental changes and the strength of species interactions^{10,11}, several factors could have contributed to the limited compositional turnover in the experiment. First, all species were severely impacted by the high stress treatment, reducing monocultures biomass by over 60% for 7 of the 8 species compared to the control treatment (Appendix D Table S1). This low interspecific variability in environmental response can limit the potential for compensatory dynamics⁴¹. Second, several dominant species in the control treatment were also among the most stress resistant (Appendix D Figures S1 and S3-7), which limited the occurrence of competitive release¹¹.

Productivity and composition were not only less affected by atrazine stress in more diverse communities, but also experienced less changes after the alleviation of atrazine stress (Figure 5.2B and C). Biodiversity thus increased functional and compositional resistance but decreased resilience in the microcosm experiment (Figure 5.2). This confirms the theoretical predictions of slower compositional dynamics in more diverse systems, which drive positive biodiversity effects on functional stability by increasing functional resistance through increased compositional resistance¹². In the microcosm experiment, slower compositional turnover in response to environmental changes caused more diverse communities to diverge even further from the control conditions between day 28 and 49 in the high-stress treatment (Figure 5.2C and D). Less diverse communities, in contrast, converged to the control treatment in both function and composition after stress alleviation (Figure 5.2C and D).

Compositional changes in the high stress treatment after stress alleviation were primarily driven by the recovery of species that are dominant in the control treatment, but were reduced to low numbers by atrazine (Appendix D Figure S2). Atrazine removal thus altered species competitive abilities, resulting in a compositional turnover between day 28 and 49 in the high stress treatment. This compositional turnover resulted in a status quo or a slight increase in evenness in the high stress treatment between day 28 and 49 (5.3). Because compositional dynamics were faster in less diverse communities, competitive replacement was more extensive. Although on average zero, changes in evenness spanned the whole -1 to 1 range for communities of 2. Changes in composition for these low diverse communities were thus ranged from a complete turnover in composition when competitive abilities changed after atrazine removal, to no changes when competitive abilities remained unchanged. More diverse communities showed far less fluctuations in composition, deviating less from the initial composition during exposure, and limited changes after stress alleviation (Appendix D Figure S3-6). In the low stress and control treatment, by contrast, species were

unaffected by atrazine removal (Appendix D Table S1). Hence species that became dominant during the first 4 weeks of the experiment, continued to increase in abundance between day 28 and 49 in the control and low stress treatment (Appendix D Figure S3-6), decreasing evenness (Figure 5.3). The decrease in evenness between day 28 and 49 was limited for the lowest diversity levels, suggesting that they attained equilibrium faster (Appendix D Figure S3). After stress alleviation on day 28, less diverse communities thus converged fast to the control communities in equilibrium, whereas more diverse communities diverged from the control treatment because both the high stress and control treatment continued to change between day 28 and 49. However, this divergence should only temporary if stress does not induce alternative stable states. If so, communities should converge to the control treatment if identical environmental conditions persist long enough^{42,43}.

The increased evenness in more diverse communities because of a slower compositional turnover in response to environmental changes suggests an additional positive biodiversity effect on functional stability. So far, empirical studies are mostly limited to a single environmental stressor⁴⁴, whereas natural systems are exposed to multiple selective environmental stressors^{45,46}. Species that stabilize ecosystem functions against one stressor can be sensitive to another stressor. Strong changes in species abundances in response to one stressor, may therefore hamper the system's ability to stabilize ecosystem functions against other stressors. Evenness increases a system's ability to respond to various environmental changes by preventing low densities of species with favourable traits^{10,27,28}. The long-term preservation of evenness can therefore be particularly important when stabilizing functions against multiple alternating environmental stressors. The results presented in this study thus emphasize the need for studies manipulating species richness under multiple fluctuating stressors to increase our understanding of the consequences of biodiversity changes for natural systems.

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6

Consequences of non-randomness in species sensitivities for ecosystem-level effects of environmental changes

Redrafted from: Baert J.M., De Laender F and Janssen C.R. The consequences of non-randomness in species-sensitivities in relation to their functional traits for ecosystem-level effects of chemicals. *Under Review*

Abstract

Estimating ecosystem-level effects from single-species bioassays is a main challenge for risk assessment. Most extrapolation procedures are based on the implicit assumption that species sensitivities are random in regard to their functional traits. Here, it is explored how non-randomness in species sensitivities affects how species-level and ecosystem level effects of chemical exposure correspond. The effect of a correlation between the trait value under control conditions and the sensitivity of the trait to chemical stress is studied for two traits (per capita growth rate and monoculture yield) under constant and temporary exposure. Theoretical model predictions are thereby validated against a 3-week microcosm experiment, in which 8 marine diatoms systems with different correlations between trait values and sensitivities were temporary (1 week) or constantly (3 weeks) exposed to two concentrations of the herbicide atrazine (100 and 250 $\mu\text{g L}^{-1}$). Negative correlations increased the reduction in ecosystem functioning (productivity) by atrazine for both traits. However, correlations in the per capita growth rate affected productivity only shortly following changes in environmental conditions, whereas correlations in the monoculture yield affected productivity throughout exposure. These results thus demonstrate that strong correlations between species sensitivities and functional traits indicate when scrutiny is needed for extrapolating species-level to ecosystem-level effects.

6.1. Introduction

Environmental risk assessment aims to derive environmental threshold concentrations for chemicals that protect the structure and function of ecosystems. Many risk assessment procedures worldwide however still rely on single-species bioassays^{1–5}. Hence the reliability of the ecosystem-level effects that are inferred from the species-level effects measured in bioassays, strongly depends on the assumptions made on how species-level and ecosystem-level effects are linked^{6–8}. Environmental risk assessment procedures generally need to balance pragmatism and environmental realism due to time or monetary constraints^{7,9}. Therefore, theoretically simple models, such as the cumulative species sensitivity distribution (SSD), have increasingly been used for both regulatory and scientific purposes since the 1990s^{10–12}. SSDs are obtained by fitting a statistical distribution, generally a lognormal or log-logistic distribution, to the single-species toxicity data^{10,12}. Environmental threshold concentrations are subsequently derived based on the fraction of species that is considered acceptable to be affected without putting the structure and functions of ecosystems at risk (e.g. 5% in EU legislation)^{1,2,4}. The SSD approach hence requires that the species from which it is derived are representative for all species in the system, and that a certain degree of functional redundancy between species exists so that ecosystem-level effects do not exceed species-level effects^{10,11,13}. A variety of statistical and ecological effects can cause violations of these assumptions, and consequently deviations between inferred and observed effects on ecosystem structure and function^{7,8,14–16}.

Ecosystem structure comprises the number and densities of species within the system. Changes in ecosystem structure by chemical exposure can arise through both direct effects on reproduction or survival rates, as well as through indirect effects by density changes in other species as a result of species interactions^{8,17,18}. Single-species bioassays thus allow inferring the concentration of the chemical at which species start to become affected, and changes in ecosystem structure thus start to arise. Still, the correct inference of direct species-level effects in the system requires that the set of species exposed in bioassays in lab conditions is a sufficiently large, random sample of the species present in the ecosystem^{6,13,19,20}. Otherwise, changes in ecosystem structure may start to occur at lower or higher chemical concentrations than expected. In addition, species-level effects observed in bioassays can also be unrepresentative because of differences in sensitivity between lab and field conditions^{21,22}. However, due to indirect effects through species interactions, effects on ecosystem structure can exceed the direct species-level effects measured in bioassays^{17,23,24}. The magnitude of indirect effects, and thus the overall change in ecosystem structure by chemicals, thereby depends on the type and strength of species interactions

within the system^{25,26}. Positive interactions (e.g. facilitation or mutualism) cause direct negative effects on one species to result in additional indirect negative effects on another species. Similarly, negative interactions (e.g. resource competition or predation) result in positive indirect effects.

In contrast to effects on ecosystem structure, chemical effects on ecosystem function do not necessarily exceed species-level effects^{7,23,24}. Many ecosystem functions comprise the sum of the individual species contributions (e.g. total biomass, productivity or nutrient retention). When functional reductions in one species are (partly) compensated by other species, this reduces the effect of chemicals on ecosystem functioning compared to their direct species-level effects^{23,27}. Whether, and to what extent, functional compensation occurs depends on the type of species interactions^{8,24,27} and the degree of functional redundancy between species¹⁸. Positive interactions invariably increase ecosystem-level effects compared to species-level effects, as reductions in one species will result in additional reductions in other species. Negative interactions, in contrast, can reduce ecosystem-level effects as decreases in one species are overcome by increases in other species. The extent by which loss in function in one species can be compensated for by other species thereby depends on the degree of functional redundancy between species²⁸. Depending on whether the replacing species are functionally less, equal or better than the affected species, ecosystem function can respectively decrease, remain unaffected or even increase¹⁸. An accurate prediction of ecosystem-level effects from species level effects thus requires limited indirect effects (Figure 6.1, dashed black line). Most concepts in environmental risk assessment are however based on the assumption of a certain degree of functional redundancy between species (Figure 6.1, yellow line)^{7,15}. Under this assumption, protecting ecosystem structure is also protective for ecosystem functioning. The SSD concept, for example, therefore generally assumes that avoiding effects of chemical on 95% of the species is generally considered sufficiently protective for the structure and function of ecosystems^{1,2,4,10,12}. SSDs are hence commonly derived from the species no observed effects concentrations (NOECs) for regulatory purposes, and the 5% percentile (i.e. the 5% hazardous concentration, HC₅) of the NOECs, multiplied with an assessment factor, set as an environmental threshold concentration^{1,2,4}.

There is now mounting evidence that ecosystems have indeed a certain degree of functional redundancy²⁴. Hence, most microcosm studies report an ecosystem-level NOEC that exceeds the species-level NOECs, and HC₅ values that are therefore protective for the system^{19,29–33}. However, effects on function can exceed effects on structure when functional redundancy between species is low. For example when keystone species or ecosystem

engineers are present, for which any loss of function will result in a disproportional effect on ecosystem functions^{7,34,35}. This is particularly important when keystone species rank among the most sensitive species in the system (Figure 6.1, red line).

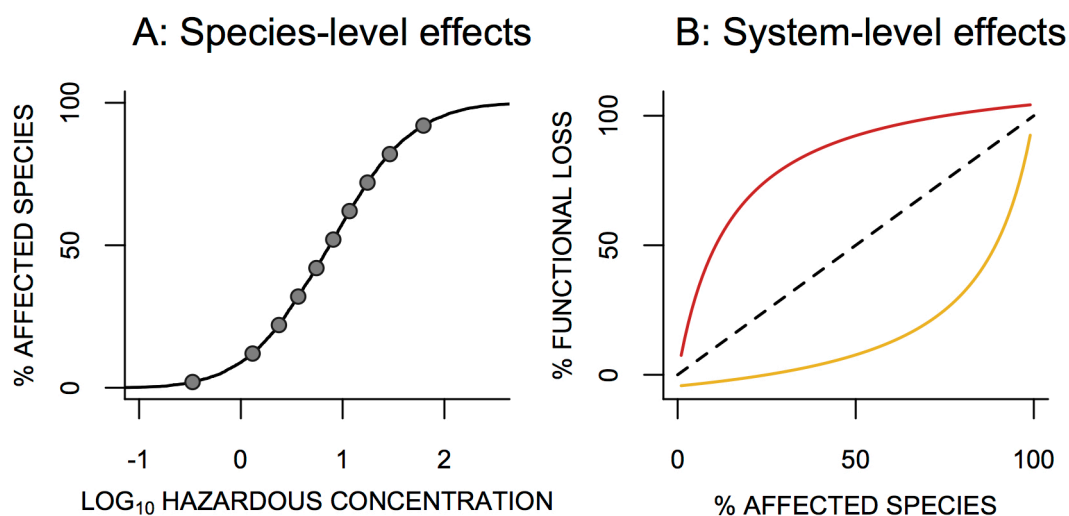


Figure 6.1: Extrapolating species-level effects to ecosystem-level effects. In legislation, the SSD (A) is often used to set environmental threshold values based on the fraction of species that is allowed to be put at risk. How these species-level effects correspond to ecosystem-level effects depend however on species interactions, driving indirect chemical effects B). In the absence of indirect effects, species-level effects correspond to ecosystem-level effects (dashed line). Functional compensation can cause ecosystem-level effects to be smaller (red line) than species-level effects, whereas low functional redundancy can cause ecosystem-level effects to exceed (yellow line) species-level effects.

Environmental risk assessment procedures, such as the SSD, thus not only assume that the species from which ecosystem level-effects are derived are a random sample of the species sensitivities in the system, but also implicitly assume that species sensitivities are randomly related to species functional traits^{7,36}. Ecological theory has provides important insights in how the type of species interactions and the degree of functional redundancy between species determines how effects on structure and function are linked. Including target or keystone species (i.e. non-random sampling) has been proposed as ways to account for non-randomness in species sensitivities^{10,12,32}. However, detailed knowledge of species interactions and functional redundancy is often unavailable at the ecosystem level^{8,37–39}. Identifying these systems where protecting ecosystem structure based on the species-level effects measured in bioassays is insufficient to preserve ecosystem functions is therefore an important objective for environmental risk assessment^{7,15}.

In this chapter, it is explored how correlations between the trait value under control conditions and the sensitivity of the trait to chemical stress affect how species-level effects of chemicals correspond to ecosystem-level effects. It is hypothesised that strong correlations between species sensitivities and functional traits should strongly affect the likelihood of functional compensation, and thus the effects of chemicals on ecosystem functioning. Chemical effects on two functional traits (the per capita growth rate and the monoculture yield) are thereby considered. Note that although both are measures of species fitness under given environmental conditions and are commonly used as endpoints of single-species bioassays⁶, the monoculture yields not a true functional trait, as it cannot be measured at the species level⁴⁰. However, for simplicity I will adhere to the term traits when referring to both endpoints. While effects on the per capita growth rate determines the speed at which species and the system can respond to chemical stress, changes in the monoculture yield relate to long term effects of chemicals⁴¹. First a community model introduced to demonstrate how correlations in both traits can be expected to alter the speed and extent of functional compensation, and thus the effect on ecosystem functioning for temporal and constant chemical exposure. Next these model predictions are validated against a 3-week microcosm experiment in which 8 communities of marine diatoms with different correlations between sensitivity and per capita growth rate and monoculture yield were exposed for one (temporary) or three weeks (constant) to two concentrations of the herbicide atrazine (100 and 250 µg L⁻¹).

6.2. Materials and methods

6.2.1. Community model

System-level effects of chemical stress were simulated using a generic Lotka-Volterra competition model for a system of n species:

$$\frac{dN_i}{dt} = \mu_i(c) N_i \left(1 - \frac{\sum_{j=1}^n \alpha_{ij} N_j}{K_i(c)} \right) \quad (1)$$

N_i is the biovolume density (mm³ L⁻¹), μ_i is the per capita growth rate (d⁻¹) and K_i is the carrying capacity (mm³ L⁻¹) of species i . The interaction strength between species pairs in the system is quantified by the parameters α_{ij} (-). Larger values of α_{ij} denote stronger competition between species i and j . Intraspecific interaction coefficients, $\alpha_{i,i}$, were set to 1. Hence growth rates and carrying capacities are identical between the community model (equation 1) and single species logistic growth curves (equation 4). Chemical stress was assumed to reduce both the per capita growth rate μ_i and equilibrium monoculture yield or carrying capacity, K_i . Log-logistic dose response relationships were used to simulate stress effects on both parameters:

$$\mu_i(c) = \frac{\mu_i(0)}{1 + \left(\frac{c}{EC_{50}}\right)^s} \quad (2)$$

$$K_i(c) = \frac{K_i(0)}{1 + \left(\frac{c}{EC_{50}}\right)^s} \quad (3)$$

Where c is the chemical concentration ($\mu\text{g L}^{-1}$), and $\mu_i(0)$ and $K_i(0)$ are the per capita growth rate and carrying capacity under control conditions respectively. The EC_{50} (g L^{-1}) is the concentration at which a 50% reduction occurs, and the parameter s (-) determines the steepness of the slope of the concentration-effect relationship. Per capita interaction strengths were assumed be unaffected by chemical exposure^{25,42}.

6.2.2. Model simulations

Two different scenarios of chemical exposure were simulated. Identical to the microcosm experiment, communities were first allowed to develop for one week before exposure to a chemical. In the first scenario, communities were temporarily exposed to a chemical for 1 week and left to recover in unstressed conditions for two more weeks. In the second scenario, communities were constantly exposed during 3 weeks. Simulations were run for 28 days in total for both scenarios.

A Monte-Carlo simulation procedure was used to quantify the effect of the correlation between species sensitivities (EC_{50}) and the functional trait value under unstressed conditions, $\mu_i(0)$ and $K_i(0)$, on system level-effects of chemical exposure. For each simulation run, model parameters were drawn from a proposed parameter distribution, covering a range of ecologically relevant scenarios. Carrying capacities under control conditions were sampled from a generic uniform distribution $U(1,100)$. Per capita growth rates under control conditions were sampled from the uniform distribution $U(0,1)$, corresponding to the range of growth rates observed in the microcosm experiment (Supplementary Table 1). Interactions strengths were restricted to the $U(0.75,1.25)$ range. This includes both strong competitive interactions that exceed the strength intraspecific completion ($\alpha > 1$) as well as weak competitive interactions ($\alpha < 1$). A larger parameter range, however, would result in too strong competitive differences causing many control treatments to become monocultures. Note that as negative interactions are essential for functional compensation, facilitative interactions ($\alpha < 0$) were not considered in the model²⁴. Species EC_{50} were drawn from a lognormal distribution $\log_{10} N(50,30)$, corresponding to the most commonly used statistical distribution for SSDs^{10,12}. The standard deviation was set at 30 to ensure a sufficiently large variation in species sensitivities. The slope parameter s was

sampled from the uniform distribution $U(1,5)$, allowing for both small and large intraspecific variability in stress tolerance⁴³. For simplicity, the same slope value was used for both stress effects on the growth rate and carrying capacity (equations 2 and 3). In analogy to the microcosm experiment, the number of species was set to 4. Systems were simulated under unstressed conditions and the two scenarios of chemical exposure for each Monte-Carlo run. Next, ecosystem-level effects on function were calculated as the percentage total biovolume lost compared to the control treatment. A total of 1000 simulations were run. Note that all simulations represent systems being sampled from the same SSD, differing only in their correlation between sensitivity and the per capita growth rate or carrying capacity, and the strength of species interactions. Using different, ecologically relevant, parameter distributions did not alter results (Appendix E Figure S1).

6.2.3. Algal strains

Eight species of marine diatoms (*Bacillariophyceae*) were isolated from a single phytoplankton sample taken in the Belgian part of the North Sea during the 2015 March spring bloom. Single cells were isolated with a micropipette, rinsed 3 times with growth medium and grown to monoclonal stock cultures based on the protocol by Andersen⁴⁴. F2 medium supplemented with Si at a 30 $\mu\text{g L}^{-1}$ final concentration was used as growth medium⁴⁵. Cultures were kept at $20\pm 1^\circ\text{C}$ and a 12h photoperiod at a $35\pm 5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ light intensity. New stock cultures were started weekly to keep stock cultures with the exponential growth phase.

6.2.4. Single-species toxicity tests

All species were exposed to 5 levels of atrazine (0, 50, 100, 250 and 250 $\mu\text{g L}^{-1}$), which comprise an environmental relevant range⁴⁶, for two weeks, using three replicates per treatment. This timespan largely exceeds the 72h used in standard alga growth inhibition test^{47,48}. However, division rates of the species used are typically within the 0.5-1 d^{-1} range (Appendix E Table S1). Hence, two weeks were required to measure atrazine effects on the per capita growth rate and the equilibrium monoculture yield or carrying capacity. Cultures were kept at the same temperature, photoperiod and light intensity as the stock cultures. Twice a week, 25 ml of the growth medium was replaced to avoid a decrease in the atrazine concentration through photolysis. Atrazine concentrations in the new growth medium were measured using a GC-MS (Thermo Quest Finnigan Trace DSQ coupled to Thermo Quest Trace 2000 series, Appendix E Table S2). Because diatoms settle at the bottom, the growth medium was removed from the upper part of the water column and contained less than 1% of the total biovolume. Cell densities were determined at day 4, 7, 9, 11 and 14 from 1 ml samples using a Whipple Grid. Biovolumes of each species were determined based on the

method proposed by Hillebrand et al.⁴⁹ The average volume of 50 cells was used for converting cell densities to biovolumes. Per capita growth rates and carrying capacities for each atrazine concentration were estimated by fitting a logistic growth curve to the cell density data.

$$\frac{dN_i}{dt} = \mu_i(c) N_i \left(1 - \frac{N_i}{K_i(c)}\right) \quad (4)$$

Next, a log-logistic dose response curve was fitted to both the per capita growth rates (equation 2) and carrying capacities (equation 3) to estimate the EC_{50} and slope (s) of the dose response curve for each species (Appendix E Figure S2, S3, and Table 1). All calculations were carried out in R⁵⁰ and parameters were estimated based on the least squares estimates.

6.2.5. Microcosm experiment

Eight communities of 4 species were randomly composed from the 8 stock cultures (Appendix E Table S3). Communities were allowed to develop for one week prior to the start of the experiment. Communities were exposed to the herbicide atrazine in two different exposure concentrations in a 3-week microcosm experiment: a constant exposure and a temporary exposure during the first week of the experiment. Species were exposed to 100 and 250 $\mu\text{g L}^{-1}$ atrazine. This corresponds to mild and severe stress, and ensures clear effects compared to the control treatment. In the temporary exposure, communities were exposed during the first week of the experiment, while in the constant exposure scenario species were exposed for 3 weeks. Communities were inoculated in 100 ml glass Erlenmeyer flasks filled with 35 ml of growth medium of the appropriate atrazine concentration and fitted with cellulose plugs. All 4 species were inoculated at an initial volume of $10^7 \mu\text{m}^3 \text{ ml}^{-1}$. To minimize variability between communities, species were inoculated from a single stock culture. Communities were established in three replicates for each exposure scenario. Cultures were kept under the same temperature and light conditions as the stock cultures. Twice a week, 25 ml of the growth medium was replaced to maintain atrazine concentrations and avoid nutrient limitation. Atrazine concentrations in the new growth medium were measured using a GC-MS (Thermo Quest Finnigan Trace DSQ coupled to Thermo Quest Trace 2000 series; Appendix E Table S4). At day 0, 7, 14 and 21, 1 ml samples were taken, fixated with formaldehyde at a 6% final concentration and stored at 4°C until analysis. Cell densities were determined using as Whipple grid and converted to biovolumes using the average volumes for each species identical to the single-species toxicity tests.

A linear mixed effect model was used to test if the % reduction in total biovolume compared to the control treatment over the course of the experiment depended on the correlation between species sensitivity and the per capita growth rate, $cor(\mu, EC_{50})$, or the carrying capacity, $cor(K, EC_{50})$, the exposure scenario (S, with 'constant' and 'temporary' as levels), atrazine concentration (C) and day (D). The exposure scenario and day were thereby included as factor variables. Including community composition as a random effect to account for the dependence of observations between exposure scenarios did not improve the model (ANOVA, $p=.82$), nor did fitting a temporal correlation structure to account for repeated measurements (ANOVA, $p=1$) as model residual remained uncorrelated after removing random effects and autocorrelation structures (Appendix E Figure S5). Up to three-way interactions were considered in the initial model:

$$\begin{aligned} \% \text{ loss} = & cor(\mu, EC_{50}) \times S \times D + cor(\mu, EC_{50}) \times S \times C + cor(\mu, EC_{50}) \times C \times D + cor(K, EC_{50}) \times S \times D \\ & + cor(K, EC_{50}) \times S \times C + cor(K, EC_{50}) \times C \times D + \varepsilon \end{aligned} \quad (5)$$

The optimal model structure was obtained by a backward selection. Normality and independence of model residuals was tested for the optimal model (Appendix 4 Figure S4 and S5)

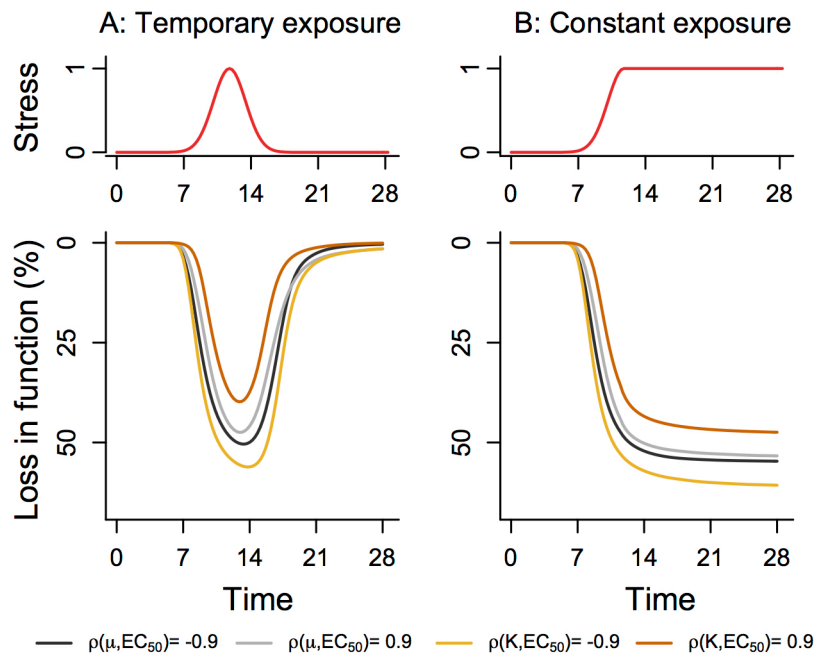


Figure 6.2: Ecosystem-level effects of environmental stress. Loss in total biovolume for different correlations between species sensitivity (expressed as the EC_{50}) and per capita growth rate (μ) or equilibrium monoculture yield (K) during temporary (A) and constant exposure (B) to environmental stress. Lines represent the average for 1000 Monte-Carlo simulations for systems with identical SSDs. Stress intensity was expressed as a normalized value.

6.3. Results

6.3.1. Community model

The correlation between species' sensitivity and the per capita growth rate of a species under control conditions affected the extent of ecosystem-level effects only during changes in environmental conditions (Figure 6.2). In contrast, the correlation between species' sensitivity and the equilibrium monoculture yield or carrying capacity under control conditions affected ecosystem-level effects throughout exposure. When systems have an equal SSD, the likelihood that function loss will occur following chemical exposure is higher when fast growing species are more sensitive, i.e. when the per capita growth rate and EC_{50} are negatively correlated (Figure 6.3A and C). This effect of the correlation between the per capita growth rate and EC_{50} is particularly strong at to intermediate chemical stress levels (expressed as the hazardous concentration, Figure 6.3A). At very low or high chemical stress levels, none or all of the species are respectively affected, which causes a reduction in the effect of the correlation. However, this effect of the correlation between species' sensitivity and the per capita growth rate disappears if chemical exposure persists long enough (Figure 6.2B and 6.3C). The effect of the correlation between species' sensitivity and the monoculture yield, in contrast, remains throughout chemical exposure (Figure 6.2B and 6.3D). For systems having an equal SSD, a more severe loss of ecosystem function by chemical exposure is thereby more likely to occur when the species sensitivity and the monoculture yield are negatively correlated, compared to systems where both are positively correlated (Figure 6.3B and D). Analogous the correlation between the EC_{50} and the per capita growth rate, the effects of correlation between the EC_{50} and monoculture yield is most pronounced at intermediate chemical stress levels (expressed as the hazardous concentration, Figure 6.3A).

6.3.2 Microcosm experiment

Temporary exposure to atrazine reduced the average productivity at day 7, i.e. after one week of exposure. Atrazine effects on productivity even increased at day 14, one week after exposure had ceased, but community productivity fully recovered at day 21 (Figure 6.4A, Table 6.1, Appendix E Table S5). Constant exposure to atrazine, in contrast, increasingly reduced the average productivity throughout the experiment (Figure 6.4B, Table 6.1, Appendix E Table S5). Changes in productivity by atrazine exposure depended on the correlation between species' sensitivity and both per capita growth and monoculture yield under unstressed conditions (Table 6.1, Appendix E Table S5). At the beginning of the experiment, the correlation between growth rate and sensitivity had a negative effect on the productivity as more sensitive species dominate the system under unstressed conditions

(Appendix E Figure S6). However, as predicted by the model, reductions in productivity by atrazine exposure were more severe during a temporary exposure when fast growing species were more tolerant (Table 6.1, Appendix E Table S5). This effect of a positive correlation between the per capita growth rate under unstressed conditions and sensitivity was positive after one week of atrazine exposure and increased at day 14. Although atrazine exposure had ceased at this point, effects on productivity were maximal at day 14 (Figure 6.4A). At day 21, the correlation between per capita growth rate and the monoculture yield capacity had again a negative effect. At this point, atrazine effects had disappeared (Figure 6.4A) and effects of the correlation between the per capita growth rate and sensitivity were similar to systems prior to atrazine exposure. Throughout the experiment, a positive correlation between the monoculture yield under control condition and its sensitivity to atrazine had a positive effect on productivity (Table 6.1, Appendix E Table S5).

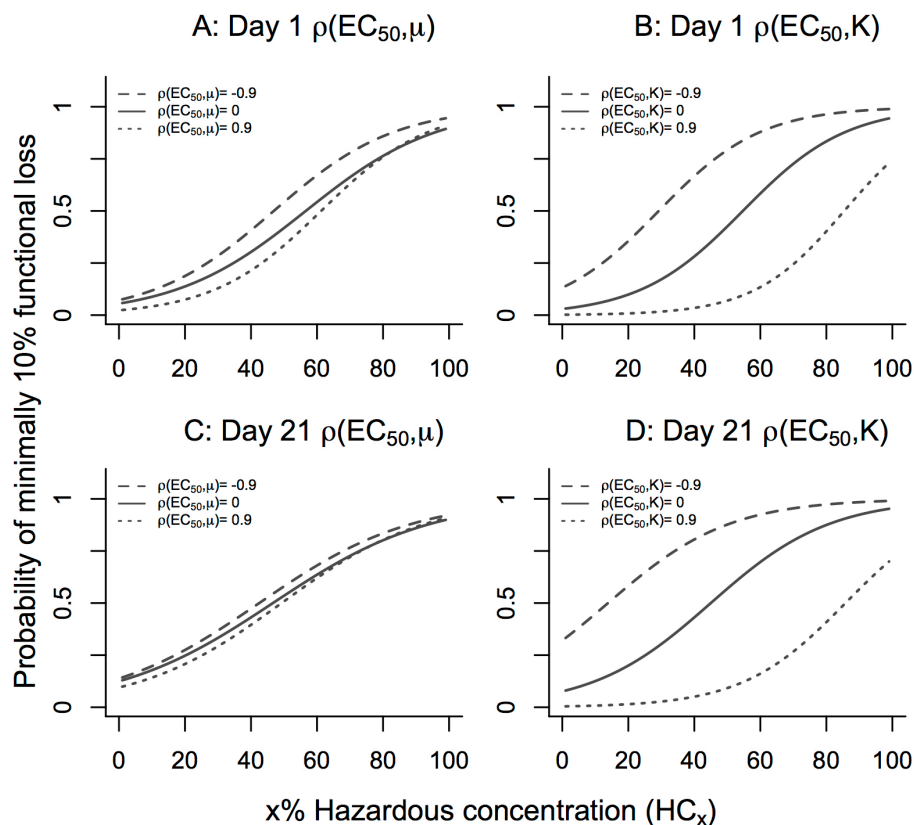


Figure 6.3: Probability of observing minimally 5% loss in ecosystem function. The environmental stress imposed by chemical is expressed as the hazardous concentration (HC_x), whereby x refers to the percentage of species in which direct effects occur. All simulations represent the constant exposure scenario. Upper panels represent the probability of observing effects for a given correlation between species sensitivity and the growth rate (A) or the carrying capacity (B) after 1 day of atrazine exposure. Lower panels represent the probability of observing effects for a given correlation between species sensitivity and the growth rate (C) or the carrying capacity (D) after 21 days of exposure. Curves were obtained by fitting a binomial regression model to model predictions.

In the constant exposure scenario, the correlation between the per capita growth rate and sensitivity did not alter the effect of atrazine on productivity (Table 6.1, Appendix E Table S5). Instead, differences in the effect of atrazine solely depended on the correlation between the carrying capacity and sensitivity. Atrazine effects on productivity were reduced when more productive species were more tolerant (Table 6.1, Appendix E Table S5). However, this effect was slightly less after one week of exposure. This was caused by the lack of data of the 250 $\mu\text{g L}^{-1}$ exposure treatment, where atrazine effects are more severe, at this day.

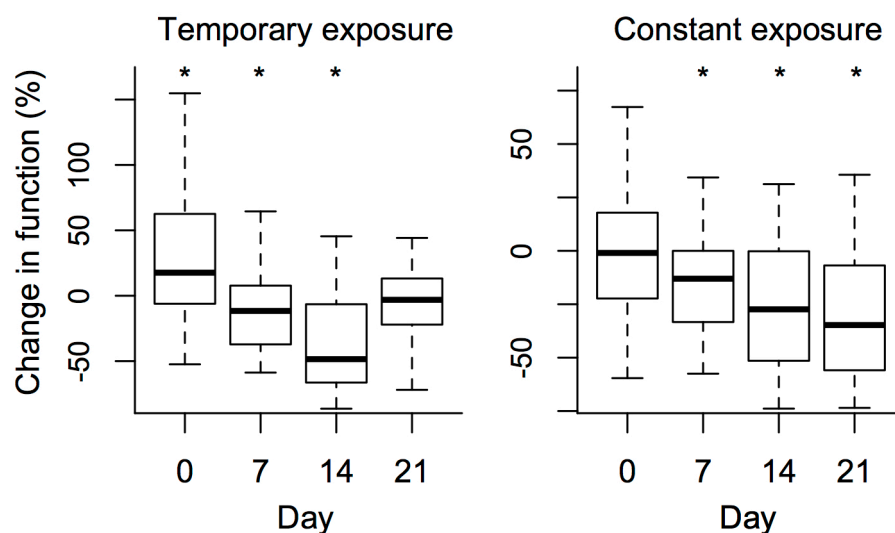


Figure 4: Boxplots for changes in productivity compared to the control treatment during (A) temporary and (B) constant exposure to atrazine. Asterisks indicate significant effects on ecosystem function. P-values were calculated from the t-statistics obtained from a linear mixed effect including only day and scenario as fixed effect, and community composition as a random effect. Boxplot whiskers correspond to maximal 1.5 times the interquartile range.

Table 6.1. Estimates of fixed effects of the linear mixed-effects model. Note that regression coefficients and p-values are expressed against day 0 and a constant exposure as a baseline.

Constant exposure				
	Day 0	Day 7	Day 14	Day 21
Intercept	44.44**	-60.43*	-48.96*	-64.15**
Cor(μ ,EC ₅₀)	NS	NS	NS	NS
Cor(K,EC ₅₀)	67.31*	-15.59*	67.31*	67.31*
Temporary exposure				
	Day 0	Day 7	Day 14	Day 21
Intercept	40.57**	-20.05*	-8.13*	36.79*
Cor(μ ,EC ₅₀)	-107.37***	15.75***	50.03***	-31.82***
Cor(K,EC ₅₀)	67.40*	129.09*	163.66*	146.75*

*p<0.05, **p<0.01 and ***p<0.001

6.4. Discussion

In this chapter, it is explored how correlations between functional trait values under unstressed conditions and their sensitivity to a chemical could affect ecosystem-level effects of chemical exposure. These correlations between species' sensitivity and functional traits can cause ecosystem-level effects of chemicals to differ strongly between systems with similar species sensitivity distributions, affecting the representativeness of species-level effects for ecosystem-level effects. Methods that infer ecosystem-level effects from single-species bioassays, such as the SSD approach, have often been criticized for ignoring potential effects of species interactions^{6,7,13,36,51}. Here, it is shown that the correlation between species sensitivities and functional traits can partly account for this lack of information (Table 6.1, Appendix E Table S5). In addition, inferring ecosystem-level effects requires measuring species-level effects that are relevant to both the ecosystem function and exposure scenario under assessment (Figures 6.2 and 6.3).

Environmental risk assessment generally requires the estimation of ecosystem-level effects from incomplete knowledge, as species-level toxicity data are often only available for a limited number of species. Reliable assessment of ecosystem-level effects thus strongly depends on how well the species-level effects in bioassays correspond to species-level and ecosystem-level effects under field conditions. The results presented here demonstrate how the probability that indirect effects on species interactions cause chemical effects on ecosystem function to exceed species-level effects can also be directly related to the non-randomness in species sensitivity (Figures 6.2 and 6.3). The correlation between species sensitivity and per capita growth rate alters chemical effects on ecosystem through the speed at which functional compensation can occur. When fast growing species are more sensitive, i.e. when the per capita growth rate and EC_{50} are negatively correlated, their replacement by tolerant species will be slow. This consequently results in stronger effects on ecosystem functioning following environmental changes (Figure 6.2A and 6.3A). The correlation between species sensitivity and the per capita growth rate however only affect the magnitude of effects following environmental changes. Hence, these effects are transient. The correlation between species' sensitivity and the equilibrium monoculture yield or carrying capacity, in contrast, determines the extent by which density reductions in sensitive species can be compensated for by tolerant species. When species' carrying capacities under unstressed conditions and sensitivities are negatively correlated, species with the highest functional abilities are most sensitive to chemical exposure. This increases the likelihood that reductions in sensitive species will be so severe that they cannot be fully compensated by tolerant species with lower functional abilities, causing species-level effects to result in larger

effects on ecosystem functioning (Figure 6.2B and 6.3D). Consequently, the effect of the correlation between species sensitivities and carrying capacities remains throughout chemical exposure.

These results thus stress the importance of using suitable endpoints in single-species bioassays. Different endpoints are regularly being used in single-species bioassays, measuring chemical effects on species growth, reproduction or survival, not all of them being representative for the ecosystem-level effects under assessment^{6,7,36}. When multiple endpoints are available for a species, the most sensitive endpoint is generally used into an SSD. This results in a combination of different endpoints for different species based on their taxonomic and trophic position, not all of them representative for the ecosystem function under consideration. For example, effects on the per capita growth rate are commonly used as an endpoint in bioassays⁴⁷. However, these reductions only affect the rate at which the system responds and are hence generally a bad predictors of changes in species equilibrium density and long-term effects⁵² (Figure 2B and 3C). Empirical studies have indeed reported a better correspondence between observed ecosystem level effects and those expected from the SSD in studies using a single, short-term exposure to a chemical^{53,54}, compared to studies using a chronic exposure^{30,31}. Still, current risk assessment routinely uses reductions in the per capita growth rate as an endpoint in algal toxicity tests^{47,48}.

The probability that effects on ecosystem functioning exceed effects on structure however decreases as biodiversity increases^{55,56}. More diverse systems have a greater chance that several species are functionally redundant, so that stress-tolerant species are able to (partly) compensate for the functional loss in sensitive species, and reduce ecosystem-level effects of chemicals^{23,28}. Moreover, as diversity increases the number of function response, this decreases the likelihood of strong correlations between sensitivities and functional traits in the system. Preserving ecosystem structure by preventing direct effects on species is therefore particularly important for low diverse systems.

The results presented here thus demonstrated that, when occurring, strong correlations could indicate when indirect chemical effects through species interactions can result in effects on ecosystem function that are equal to, or exceed effects on structure. Current risk assessment procedures still rely on the assumption that species interactions result in a certain degree of functional compensation between species so that protecting ecosystem structure suffices to also protect ecosystem functions^{10,36}. Therefore weighing the species sensitivities for their relative abundances and including target or keystone species (i.e. non-random sampling) have been proposed as ways to account for non-randomness in species

sensitivities^{10,12,32}. These methods thereby aim to lower threshold concentrations derived from the SSD to ensure their protectiveness for the structure and functions of ecosystems. The occurrence of strong correlations between species functional and functional traits can thus be a first indicator, based on the information gathered in bioassays, for when more scrutiny is needed when applying methods such as the SSD approach. Under these conditions, the use of an additional assessment factor is warranted.

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7

General discussion

7.1. From a general to a context-dependent approach

The field of biodiversity-ecosystem functioning research has been dominated by the search for a general bivariate relationship^{1–7}. Emerging in the 1980s from the growing awareness of an unprecedented global biodiversity decline, its main objective was to understand to what extent these biodiversity changes posed a threat to the many ecosystem functions on which society depends⁸. Meta-analyses have therefore often focussed on quantifying the relative empirical support for the several theoretically proposed biodiversity-ecosystem functioning or stability relationships^{9–11}. The hundreds of empirical studies conducted since the 1990s have now produced compelling evidence that biodiversity generally increases ecosystem functioning and ecosystem function stability^{5,7,11}. The occurrence of positive biodiversity effects is remarkably consistent across functional groups and ecosystem functions in terrestrial and aquatic systems, which has now led to a general consensus that ecosystems are indeed at risk by ongoing biodiversity losses^{7,12}. Still, the exact strength of biodiversity effects clearly differs between systems, and also negative biodiversity effects have frequently been reported^{11,13,14}. Due to the search for a general relationship, these differences in the consequences of biodiversity changes between systems have so far remained largely unexplained^{11,15}. Only recently, a number of studies started to compare biodiversity-ecosystem functioning relationships between ecosystems^{15,16} and different environmental conditions within a system^{15–22}. Differences in the shape of the BEF relationship thereby related to the environmental favourability^{15,16} or the types of species interactions in the system^{17–21}.

The notion of a strong-context dependence of biodiversity effects has led to a recent plea to adopt a more holistic approach in biodiversity research, integrating the classic frameworks of species coexistence and community dynamics into biodiversity theory^{6,23}. As a research field, ecology is prone to fragmentation. Contrary to for example physics or chemistry, ecology is not driven by a set of universal laws. Instead, *‘the great majority of ecology’s actual laws cannot simply be derived from first principles, building on the universal laws’*²⁴. This has often caused subdisciplines to emerge and diverge over time, driven by their own angle of approach and associated theories. Likewise, biodiversity effects on ecosystem functioning and ecosystem function stability have generally been treated separately over the past two decades^{3,5,7}. In the 1990s, major progress was already made in biodiversity research by revealing how empirically observed correlations between biodiversity and ecosystem functioning or ecosystem function stability could be explained from species interactions and fitness differences^{25–27}. This provided a mechanistic underpinning of biodiversity-ecosystem functioning relationships based on classic ecological theory on community composition and

compositional dynamics, explaining differences in strength of biodiversity effects between studies. To date, multivariate techniques, such as structural equation modelling, have been principally used to separate the effect of species richness from that of environmental factors on ecosystem functioning^{15,16}. Based on correlations, these data-driven methods do not provide the required mechanistic understanding of the environmental dependency of biodiversity effects. Moreover, ecologists have only recently tried to combine the evidence of biodiversity effects on ecosystem functioning and ecosystem function stability, although without finding any apparent correlation between the magnitude of both effects²⁸.

In this thesis, it is demonstrated how the environmental dependency of biodiversity effects on ecosystem functioning (**Chapters 2-4**) and biodiversity effects on ecosystem function stability (**Chapters 5-6**) are intimately linked by, and can be explained from classic ecological theory on compositional dynamics. Environmentally induced changes in species functional contributions are thereby expected to depend at least on the interspecific differences in environmental response and species interactions, driving direct and indirect effects on environmental changes²⁹⁻³². It is revealed how compositional dynamics based on these two elements can help to fill key gaps in biodiversity research, explaining differences in biodiversity effects between systems and between environmental conditions within a system. It is thereby demonstrated how a comprehensive mechanistic framework explaining the environmental dependence of biodiversity-ecosystem functioning relationships can be derived (**chapters 2-3**), complex biodiversity effects can arise (**chapter 4**), biodiversity effects on functional stability link to compositional stability (**chapter 5**) and consequences of environmental changes can be predicted (**chapter 6**).

7.2. An environment-dependent framework for BEF relationships

The framework presented in **chapters 2 and 3** reveals that environment-induced changes in biodiversity effects can be quantitatively explained from the classic ecological principles underlying compositional dynamics (Figures 2.2-2.4). Changes in species functional contributions driving changes in BEF-relationships were thereby demonstrated to depend on, and differ between systems because of differences in species interactions and species environmental responses (Figures 3.2-3.4). Changes in species complementarity³⁻¹⁷ and environmental favourability^{15,16} have already been identified as important drivers of changes in BEF relationships. The presented framework consequently provides for the first time, to my knowledge, a direct mechanistic underpinning for these drivers. Moreover, by focussing on species interactions and environmental responses, it links the theoretical frameworks

developed in the 1990s to explain biodiversity effects on ecosystem functioning and ecosystem function stability.

Despite being based on shared principles^{33–35}, both biodiversity effects have so far generally been treated separately^{3–5,7,28}. Theory on ecosystem functioning has thereby focussed on species interactions, driving complementarity effects, and interspecific differences in species functional traits, driving selection and dominance effects^{36,37}. Similarly, theory on ecosystem function stability is based on interspecific differences in how species' functional traits respond to environmental changes and species interactions^{33–35}. Only in 2013, a meta-analysis first compared how biodiversity effects on ecosystem functioning corresponded to biodiversity effects on ecosystem function stability. Although both biodiversity effects are generally positive, there was no apparent correlation between the magnitude of both effects^{28,38}. By linking biodiversity effects on ecosystem functioning and ecosystem function stability, the framework presented here however demonstrates that a variety of environmental responses in BEF relationships can arise. Moreover, the presented results explain how any correlation between biodiversity effects on ecosystem functioning and ecosystem function stability should not be a priori expected.

In 1999, Yachi and Loreau²⁷ already theoretically demonstrated that biodiversity provides a long-term insurance for ecosystem functions. Due to the positive effect of biodiversity on both ecosystem functioning and ecosystem function stability, biodiversity is expected to not only increase ecosystem functioning but also preserve these ecosystem functions over a wide range of environmental conditions (i.e. the insurance hypothesis)²⁷. The framework introduced in chapters 2 and 3 thus builds upon this insurance hypothesis. It demonstrates how environmental changes in BEF relationships can quantitatively be predicted from diversity-dependent changes in ecosystem functioning, i.e. biodiversity effects on ecosystem function stability (Fig. 2.3-4 and Fig. 3.2-4). The model presented in chapter 3 reveals how a general change in BEF relationships along an environmental gradient based on interspecific differences in environmental response, is modulated by a system-specific effect of species interactions (Fig. 3.2). Following the portfolio effect, biodiversity increases ecosystem function stability principally by the increased number of environmental responses, increasing the potential for functional compensation. The type and strength of species interactions, which determine the exact extent of functional compensation, is thereby predicted to have only a limited effect^{32,34,39–41}. Here, it is demonstrated that interspecific differences drive the general response of the slope of BEF relationships, but that the magnitude of environment-induced changes principally depends on the type and strength of species interactions (Figures 2.2 and 3.2). Biodiversity effects on ecosystem functioning can thus be positive,

neutral or absent depending on the interactions and environmental conditions. Biodiversity effects on ecosystem function stability, in contrast, refer to the system's behaviour over a range of environmental conditions. How the strength of biodiversity effects on ecosystem functioning and ecosystem function stability correlates should consequently strongly depend on the system and the environmental conditions under which biodiversity effects on ecosystem functioning are assessed, without any general correlation between the strength of both effects.

The framework explaining the environmental dependence of BEF relationships presented in chapters 2 and 3 thus provides an important step towards a more holistic approach in biodiversity research. It demonstrates that there is no general BEF relationship, but explains how observed differences in BEF relationships between systems and environmental conditions can be explained based on a single framework. This establishes that the consequences of biodiversity loss cannot be seen separately from the environmental conditions. Consequently, biodiversity effects on ecosystem functioning are inherently linked to biodiversity effects on ecosystem function stability in real-world ecosystems that are simultaneously experiencing biodiversity and environmental changes^{42–44}.

Environmental changes are predicted to be a main driver of biodiversity changes in the 21st century^{43,45}, which is expected to mainly affect ecosystem functioning by inducing species extinctions⁴⁶. However, environmental changes can also alter the densities and functioning of the remaining species, resulting in severe changes in ecosystem functioning in the absence of species losses⁴⁷. The framework presented here represents an important step forward in estimating and understanding the joint effect of projected biodiversity and environmental changes by reconciling the empirical evidence that is currently available from primary producer systems (Figures 3.3 and 3.4). The framework builds upon the assumption that environmental changes only affect species fitness, while keeping per capita interaction unaffected. In the diatom system presented in chapter 2, this assumption was supported by the data. Resource availability in the system was independent of biodiversity and the environmental conditions as algae species were strictly phototrophic and the aquatic environment is well mixed. However, this is a simplification for many real-world ecosystems, even for single-trophic level primary production systems⁴⁸. Positive feedback interactions between biodiversity and the environmental conditions are widespread, and known to alter per capita interactions⁴⁹. In algae systems this can for example occur through excretion of polysaccharides which contribute to biofilm formation or serve as an additional carbon resource availability for mixotrophic species⁵⁰, and of which the production depends on the environmental conditions⁵¹. Similar observations have been made in terrestrial primary

producer systems. Per capita interactions have been shown to become positive under drought conditions when biodiversity increases water availability^{18,52}. On higher trophic levels, phenotypic plasticity can be expected to have even higher consequence for per capita interactions as environmental changes, such as climate change, are known to induce behavioural changes^{54–56}. Despite these possible changes in per capita interactions in primary producer systems, such changes were not required to explain the intersystem differences in environmental response across 52 different systems (Figures 3.3 and 3.4). Thus although such changes will affect changes BEF relationships, model predictions suggest that their overall importance for explaining system-specific differences in primary producer systems is limited. In addition, model predictions reveal that, despite intersystem differences in species interactions, positive BEF relationships are likely to occur over a wide range of systems and environmental conditions, and endorse the current consensus that ecosystems worldwide are under threat by ongoing biodiversity changes. Moreover, the general response of BEF relationship revealed in chapter 3 demonstrates that expected upcoming environmental changes are likely to aggravate the consequences of biodiversity changes, and how this environmental effect depends on the properties of the species in the system.

7.3. Linking biodiversity effects to compositional dynamics

To date, most biodiversity experiments have directly manipulate species richness by assembling systems of different richness levels^{1,2,7}. The composition of these systems diverges from the initial composition over time due to stochastic or competitive processes whereby species can be reduced to low densities or even go extinct. Still, initial species richness and not observed biodiversity is principally used as a predictor variable when assessing biodiversity-ecosystem functioning relationships. This has led to an important debate on the validity of BEF-relationships in the 1990s due to concerns about the potential confounding effect of the number and identity of the sampled species when assembling the study systems^{57–60}. Now, there is mounting evidence that not only species richness, but also their relative abundances strongly affect ecosystem functioning and functional stability^{61,62}. This has now raised awareness of the need to incorporate coexistence theory and compositional dynamics more explicitly into biodiversity research^{15,23}.

7.3.1. Non-linear biodiversity effects

In **chapter 4** an extension to classic additive partitioning methods is introduced, allowing the quantification of complex, non-linear biodiversity effects resulting from strong compositional changes. Biodiversity effects on ecosystem functioning are defined based on species

deviations from the initial composition, and how these deviations depend on species' functional traits^{63,64}. Additive partitioning methods are thereby routinely used to calculate the strength of complementarity, dominance or selection effects. Its classic formulation is based on the average species deviation from the initial state, and the linear dependence on species' functional traits^{36,37}. More complex non-linear deviations from the initial composition in relation to species functional traits are however equally likely to arise by stochastic and competitive processes. The non-linear extension presented here thus allows for an increased understanding of how these changes in composition ultimately affect ecosystem functioning.

In biodiversity experiments, species often have similar initial functional contributions, which will rarely correspond to the equilibrium densities. Over time, competitive processes can induce larger average compositional changes. The effect of initial conditions thus wears off in these time series, as competition and environmental effects progressively determine the composition^{19,65}. Ecosystem functioning can thereby become increasingly driven by a particular (group of) species, causing non-linear deviations from the initial state that cannot be quantified using classic additive partitioning methods. So far, most experiments study vascular plant systems over the course of only a few years¹¹. Deviations from the initial conditions are thereby generally small, due to the limited time sexual reproduction, clonal growth or mortality. Long-term field data are now however becoming increasingly available and offers unprecedented opportunities to assess the consequences of biodiversity changes under changing environmental conditions^{16,66}. Strong deviations from species' initial contributions are thereby increasingly likely to occur. In chapter 4 it was demonstrated that these non-linear biodiversity effects can already occur in less than 10 generation in a grassland experiment, jeopardizing the validity of classic linear additive partitioning methods (Figures 4.2-5). Strong deviations from the initial conditions can result in outliers with severe leverage on the covariance used by these methods. Species with low functional contributions or low functional abilities under the given environmental conditions have hence often been left out of the analysis^{36,65}. The general formulation of additive partitioning methods introduced in chapter 4 allows circumventing this leverage problem by fitting the appropriate model structure to the data. The use and application of a 2nd order polynomial was thereby illustrated, but the general formulation also allows the use of other functions than polynomials if required.

Additive partitioning methods remain statistical tools, without any explicit ecological mechanistic underpinning. They are nevertheless essential for analysing biodiversity experiments. By quantifying complementarity, dominance or selection effects in ways that are interpretable and directly comparable between systems and environmental conditions,

they can provide a first step by indicating the potential underlying mechanisms^{36,65}. The non-linear extensions presented in chapter 4 are thus an essential refinement of the current, linear additive partitioning methods. They allow quantifying the effect of strong compositional changes by allocating effects on ecosystem function to (groups of) species. This can be particularly important for assessing biodiversity effects in systems where large interspecific differences in functional contributions can occur, such as long-term data series or under unfavourable environmental conditions that induce large fitness differences.

7.3.2. Compositional stability as a driver of ecosystem function stability

In **chapter 5** the theoretically proposed tight link between compositional and functional stability⁶⁷ is empirically demonstrated. Theoretical studies still dominate our view on the mechanisms driving biodiversity effects on ecosystem function stability. Similarly to biodiversity-ecosystem functioning relationships, the occurrence of biodiversity-ecosystem function stability relationships have mainly been studied based on correlations between species richness and stability, without assessing the underlying mechanisms⁷. Despite ecological theory placing great emphasis on compositional dynamics to explain biodiversity on ecosystem functioning, empirical support for biodiversity effects on compositional stability that underlie functional stability has therefore remained scarce^{32–34,41,66–68} and opposing findings between empirical and theoretical results have fuelled decades of debate on the underlying mechanisms^{66,67,69,70}. Here, empirical evidence is directly linked to theoretical predictions, establishing biodiversity that biodiversity has similar effects on functional and compositional stability in primary producer systems.

In the 1990s, introducing the concept of statistical averaging has been an essential step in resolving opposing empirical and theoretical results³³. It demonstrated that increased population-level fluctuations in more diverse systems, as theoretically demonstrated by May⁷¹, did not contradict the empirically observed greater ecosystem function stability in more diverse systems^{72,73}. Instead, the averaging of asynchronous population-level fluctuations was suggested to be a main mechanism driving positive biodiversity effects on ecosystem function stability^{33,71}. This has however led to a strong focus on interspecific differences in environmental responses as a main driver of these compensatory dynamics between species, whereby species interactions were mainly considered as a secondary factor, modulating the magnitude functional compensation^{32,34,35,40}.

The theoretical focus on compensatory dynamics as a driver of ecosystem function stability is now increasingly challenged by empirical results. Strong asynchronous fluctuations between species appear to be rare in most experiments^{41,68,74}. Instead, functional stability appears to mainly result from small compensations between a limited number of species

instead of large fluctuations in empirical systems⁴¹. Similarly, positive biodiversity effects on primary production stability in grassland systems have recently been demonstrated to be driven by an increased functional resistance, and not resilience¹⁶. These observations, however, do not oppose theoretical models. Instead, simple community models do predict a reduction in the speed of community dynamics in more diverse systems when interspecific differences in interactions are small⁶⁷. This causes an increased stability by compositional resistance and not compositional turnover.

The results presented in chapter 5 present, to my knowledge, the first empirical evidence of this theoretically predicted consistent biodiversity effect on functional and compositional stability (Figures 5.2.). Moreover, these results underscore the need for a further empirical validation of the mechanisms driving biodiversity effects. Theoretical studies indicate that the empirically observed positive biodiversity-ecosystem functioning relationships can be generated by various mechanisms⁴¹. The extent of compositional turnover in these models thereby depends on the interspecific differences in species interactions and environmental responses^{34,41}. The relative importance of species interactions compared to increased variety in species responses has therefore been debated on theoretical grounds^{32,34,35,40,41}. The extent and importance of compositional turnover or resistance for ecosystem function stability is therefore likely to differ strongly between systems. The empirical results presented here validate the theoretical predictions for systems of ecologically similar species, such as primary producer systems where compositional resistance is expected to underlie the observed functional resistance driving functional stability¹⁶. This demonstrates the need to move beyond the detection of compensatory dynamics, which is only one possible mechanism to underpin biodiversity-ecosystem function stability relationships^{41,66}. Instead, major progress is likely to be made from linking their occurrence and extent of compensatory dynamics to differences in the traits of the species present in the systems.

7.4. Estimating ecosystem-level effects of environmental changes

In **chapter 6** it is explored how estimates of the effects of environmental changes on ecosystem functions can be improved. Environmental and biodiversity changes are projected to be major drivers of changes in ecosystem function provisioning in the next century⁴⁶. Still, the magnitude of changes in ecosystem functioning, and the required conservation efforts to mitigate these changes, differ between systems^{75–77}. Accurate predictions of changes in the structure and function of systems by environmental changes thus require an in depth knowledge of the system⁴⁴, which is generally unavailable at a system-specific basis due to time, financial or methodological constraints. Hence, estimates of environmental effects are

generally based on imperfect knowledge of the systems. In chapter 6 it is demonstrated how ecosystem-level effects can be related to species level effects without explicitly taking species interactions into account. It is explained how non-randomness of species sensitivity to environmental changes in relation to their functional traits can help to identify systems that are at an increased risk by environmental changes.

Species-level effects are easily assessable. Single species bioassays are simple, resource and time efficient. Understanding how these species-level effects translate to ecosystem-level effects is therefore essential to improve estimates of consequences of environmental changes. Ecosystem level effects depend at least on the species sensitivities to environmental changes, that is the direct effect of environmental changes, and species interactions, which determine the strength of indirect effect by density changes in other species^{29,71}. Ecosystems consist of a myriad of possible interactions, of which the exact strength can be hard to measure^{78,53}. Species-level effects hence stand central in current environmental risk assessment procedures^{79,80}. Worldwide, legislations are currently based environmental quality standards that are derived from cumulative distribution in species sensitivities, allowing for effects in less than 5% of the species^{81–84}. Consequently, this assumption that effects in less than 5% of the species does not result in a noteworthy change in the structure and function of ecosystems inherently implies a certain degree of functional compensation between species^{79,80}.

In chapter 6 it is demonstrated that, depending on the type and strength of species interactions, strong changes in ecosystem functioning can already occur when species effects, and thus effects on ecosystem structure, are limited (Figures 6.2 and 6.3). The risk of strong effects on ecosystem functioning thereby depends how species-level effects correlate to species' functional traits (Figures 6.2 and 6.3). This determines the likelihood that functional compensation between species can occur. Classic ecological theory on community composition is thus essential to improve estimates of how environmental changes affect ecosystem functioning. In-depth knowledge of species-level effects and species interactions is generally unavailable for real-world ecosystems. However, these theoretical frameworks also help to elucidate how interspecific differences in ecosystem-level effects can be assessed from the correlation between in the sensitivity of species functional traits to environmental changes and the trait value under unstressed conditions. These correlations can easily be determined from bioassays, or a posteriori be determined from currently available species sensitivity, and can help flagging those systems at a potential increased risk by environmental changes.

7.5. General conclusion and outlook

Developing a mechanistic understanding of how biodiversity changes affects ecosystem functions is essential for assessing the consequences of the combined projected effects of environmental and biodiversity changes^{43,46}. Although bivariate relationships have played an essential role in raising awareness of the possible detrimental effects of a global biodiversity decline, these only provide limited insight^{1–7,85}. Over the past decades, multiple theoretical frameworks have been developed to explain these empirical relationships. However, not only are several of these theories poorly supported by empirical data^{41,68,74}, biodiversity effects on ecosystem functioning and ecosystem function stability have thereby also started to develop into two separate research fields^{6,7,28,38}. This has led to an inability to explain several observed patterns, such as the link between the magnitude of both effects^{28,38} or environment-induced changes in the biodiversity-ecosystem functioning relationship^{15,22}. In this thesis, it is demonstrated how the environmental dependency of biodiversity effects on ecosystem functioning (**Chapters 2-4**) and biodiversity effects on ecosystem function stability (**Chapters 5-6**) can be mechanistically explained and linked by two basic drivers of compositional dynamics and species coexistence: interspecific differences in interactions and environmental responses.

The introduced framework however only provides a first stepping-stone in developing a holistic theory on how biodiversity changes affect ecosystem functioning. The models and experiments presented here are based on primary producer systems, involving only one trophic level. Primary producer systems have played an important role in developing our insights into biodiversity effects, being simple and traceable, but have also biased our view on biodiversity-ecosystem functioning relationships. First, there is a literature bias towards terrestrial plant systems¹¹. In pelagic algal communities, for example, strong resource competition often tends to result in negative BEF relationships⁹. Due to the focus on the occurrence of positive BEF relationships, the prevalence of negative BEF relationships and their distribution across different systems remains poorly studied¹¹. Moreover, real ecosystems include multiple trophic levels, each with dozens of species, resulting in a myriad of possible interactions and complex feedback loops. Still, experiments involving multiple trophic levels have been scarce, and a conceptual framework extending complementarity, selection and dominance effects these systems is lacking^{12,13}. Moreover, the use of primary producer systems has resulted in a strong emphasis on primary production or total biomass as a proxy for a general ecosystem function^{7,11}. Not only are there hundreds of ecosystem functions on which society depends^{86,87}, many of them supported by the same ecosystem. This multidimensionality of biodiversity effects is only starting to emerge in biodiversity

research^{88,89}, as well as how classic biodiversity effects can be extended⁹⁰. Finally, biodiversity studies have been restricted to small spatial and temporal scales. Most biodiversity studies have been conducted in closed systems. Consequently, the focus has been on the effect of the initial species richness. Species could therefore subsequently go extinct, because of non-stable coexistence, or new species could immigrate into the system, requiring a constant manipulation of experimental systems to maintain the experimentally required number of species². Dispersal processes have therefore received little attention in biodiversity research. They are nevertheless an essential mechanism in global biodiversity changes^{43,44,91}. Although biodiversity decreases globally, local biodiversity changes are more complex. Both local increases and decreases occur, driven by natural or human-assisted dispersal^{92,93}, which can substantially alter biodiversity effects and ecosystem functions^{94,95}. Temporal scales, in addition, determine the extent by which phenotypic plasticity⁹⁶, and even (micro)evolution⁹⁷ can start to alter biodiversity effects on ecosystem functioning.

Developing a holistic framework on the combined effects of biodiversity and environmental changes will be a major challenge for the 21st century. Biodiversity research has already made part of this transition in the past two decades. The focus has already recently broadened from the net biodiversity changes^{1,2}, to a context-dependent approach^{15,22}, including how environmental changes can affect ecosystem function in the absence of biodiversity changes⁴⁷. This thesis thereby provides a first step in developing this mechanistic framework, explaining the context dependency in primary producer systems. Introducing complexity, multidimensionality and spatial structure and (micro)evolutionary processes are essential next steps that will challenge biodiversity research to become a more integrated research field by bringing the gaps between biodiversity research, community and ecosystem ecology^{6,23,98}.

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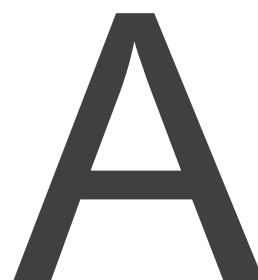
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Supporting information for chapter 2

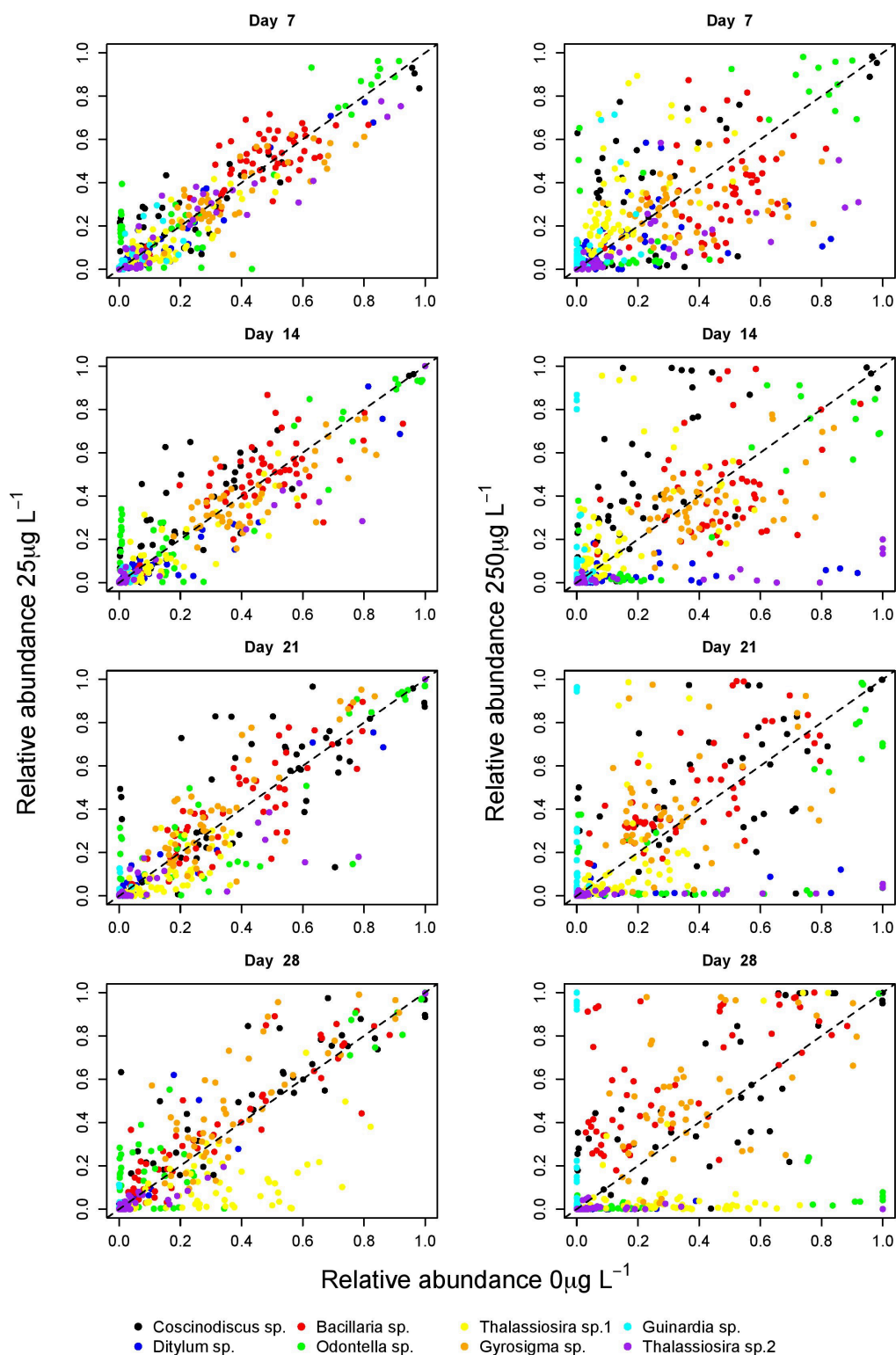


Figure S1: Atrazine induced changes in relative species abundances. Changes in relative species abundances compared to the control treatment in the 25 $\mu\text{g L}^{-1}$ (upper panels) and 250 $\mu\text{g L}^{-1}$ (lower panels) atrazine treatment. Note that low stress (upper panels) induces small compositional differences with relative abundances close to the 1:1 line. High stress (lower panels), in contrast, induces larger compositional changes. Sensitive species decrease in abundance, lying under above the 1:1 line, whereas tolerant species increase and lie above this line

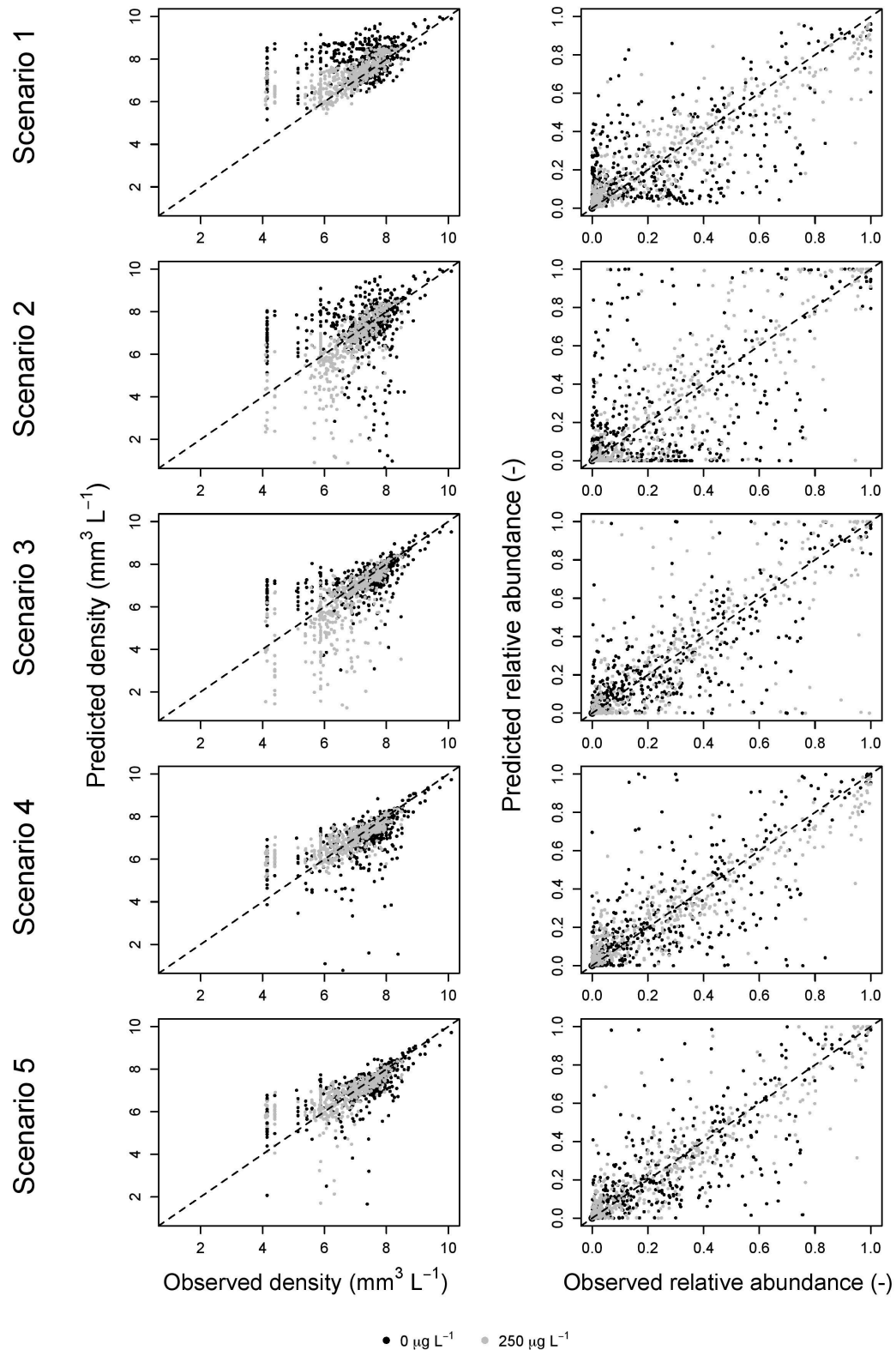


Figure S2: Community model performance under different scenarios of stress-induced effects on the per-capita strength of species interactions. Predicted species densities and relative abundances plotted against the observed values from the biodiversity experiment for the 5 scenarios. Model predictions correspond better to observations when deviations from the dotted 1:1 line (i.e. perfect prediction) are smaller.

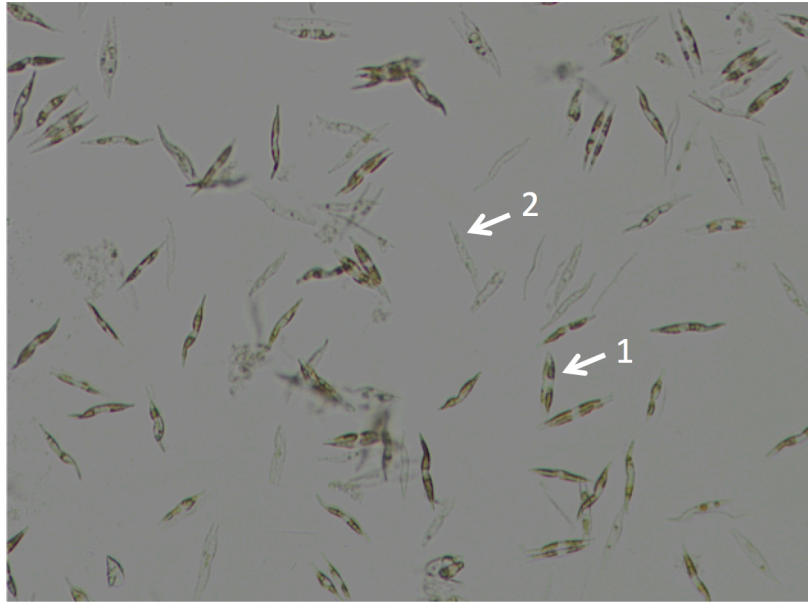


Figure S3: Assessment of cell mortality. Microscope image of a degrading diatom stock culture, illustrating the various stages ranging from living cells (1) to empty frustules (2). Note that this is heavily degraded stock culture to illustrate the different stages and does not represent any of the communities used in the experiment.

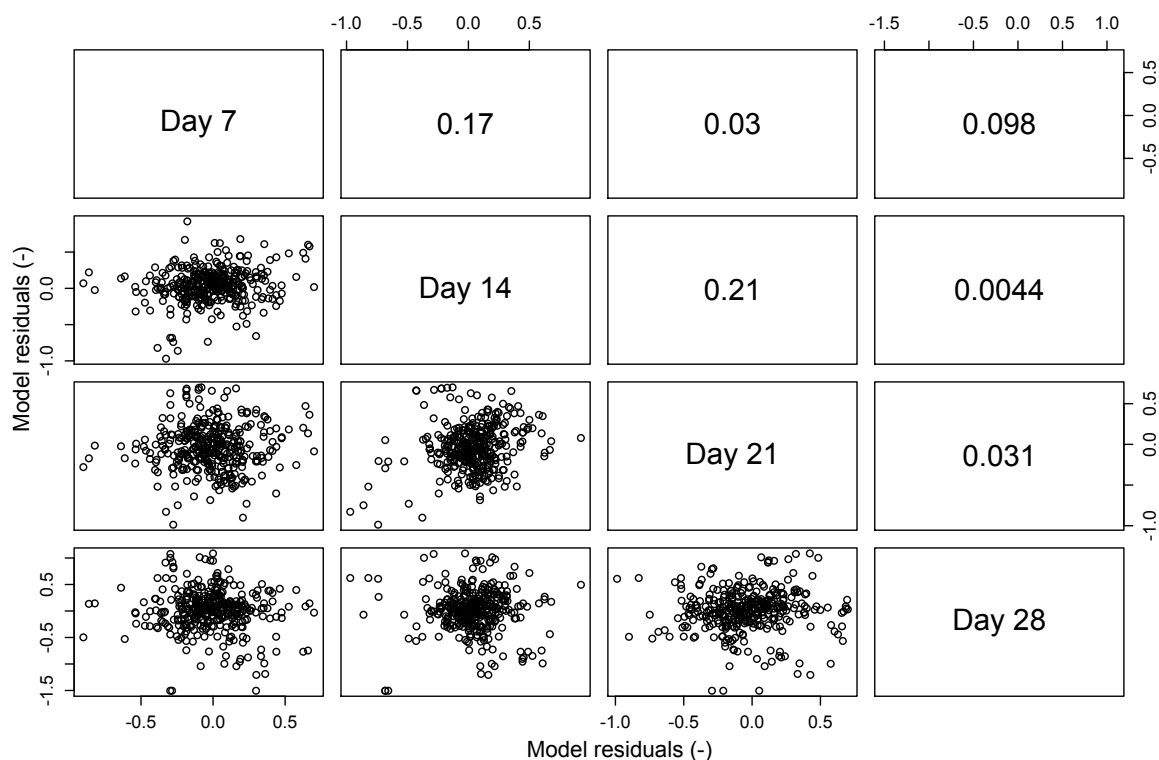


Figure S4: Linear mixed effects model residual correlation for \log_{10} biovolume. Pairs plot of the model residuals at each sampling day for the linear mixed effect model predicting the \log_{10} biovolume as a function of the \log_{10} diversity, atrazine concentration and time as fixed effects and species composition as a random effect (Table 2.1). Values in the upper triangle denote the correlation in model residuals between the different days.

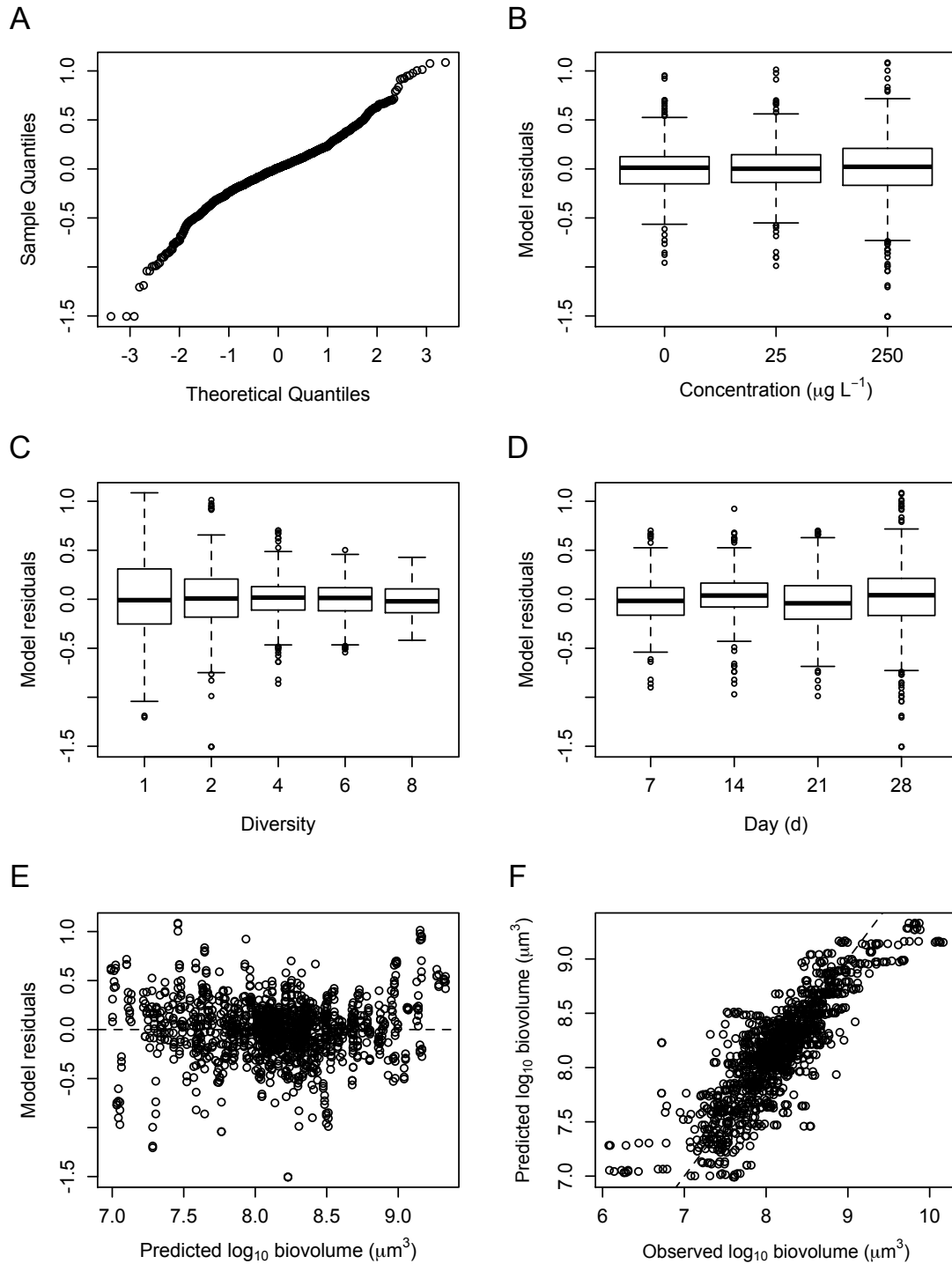


Figure S5: Linear mixed effect model residual diagnostics for the \log_{10} biovolume.

Linear mixed effects model predicting the \log_{10} biovolume as a function of \log_{10} diversity, atrazine concentration and time as fixed effects and species composition as a random effect (Table 2.1). Model residuals are plotted as Q-Q-plot (A), against the fixed effects (atrazine concentration, B; diversity, C; and day, D) and plotted against the predicted \log_{10} biovolume (E) to assess normality and homogeneity of model residuals. Model predictions are plotted against the observed \log_{10} biovolumes to assess model performance.

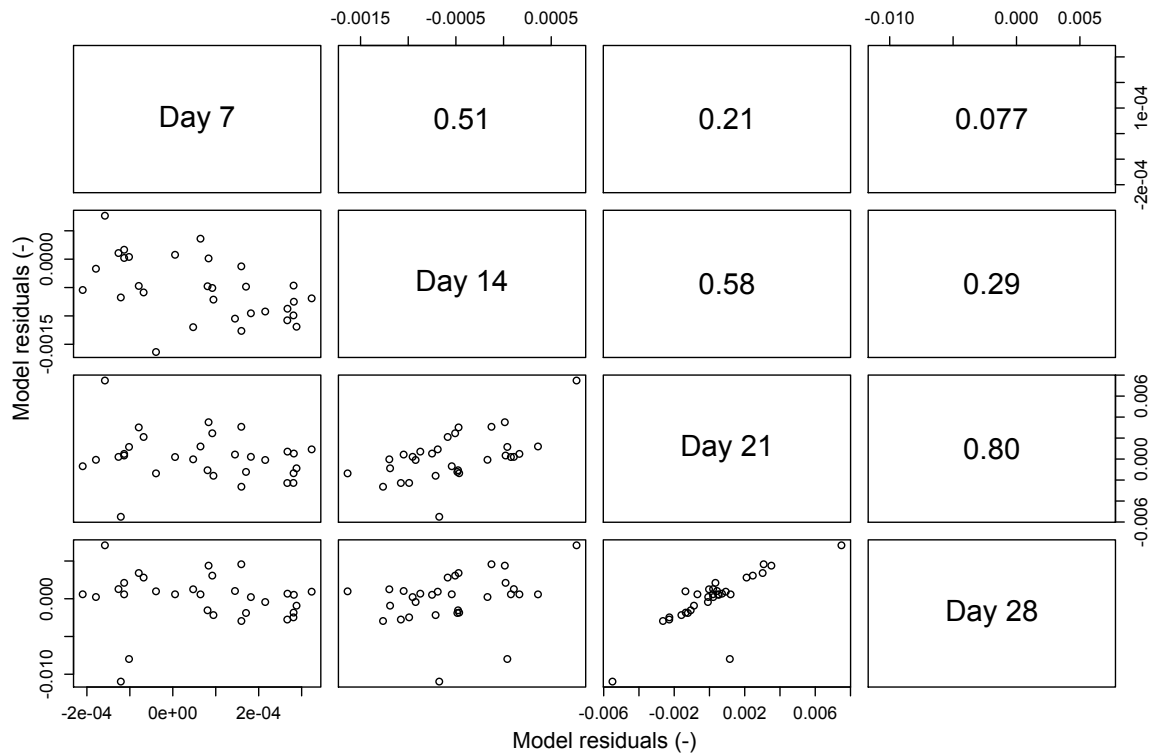


Figure S6: Linear mixed effects model 1 residual correlation for changes in dominance effects Pairs plot of the model residuals at each sampling day for the linear mixed effect model predicting the changes in dominance effects as a function of the \log_{10} diversity concentration and time as fixed effects and species composition as a random effect with temporal autocorrelation structure (Table 2.2, model1). Values in the upper triangle denote the correlation in model residuals between the different days.

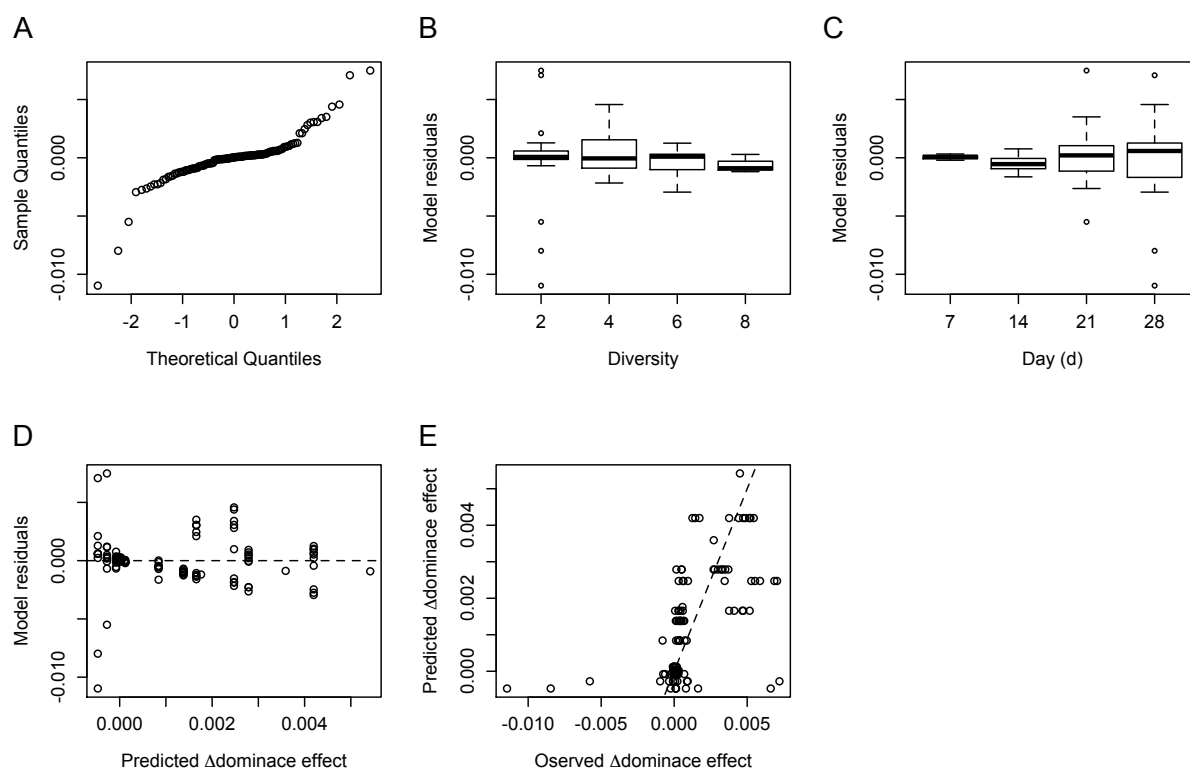


Figure S7: Model 1 residual diagnostics for changes in dominance effects. Linear mixed effects model predicting atrazine-induced changes in the dominance effect (Δ dominance effect) as a function of log10 diversity and time as fixed effects, community composition as random effects and a temporal autocorrelation structure (Table 2.2) Model residuals are plotted as QQ-plot (A), plotted against the fixed effects (Diversity, B and Day, C) and the predicted change in dominance effect (D) to assess normality and homogeneity of model residuals. Model predictions are plotted against the observed changes in dominance effects (E) to assess model performance.

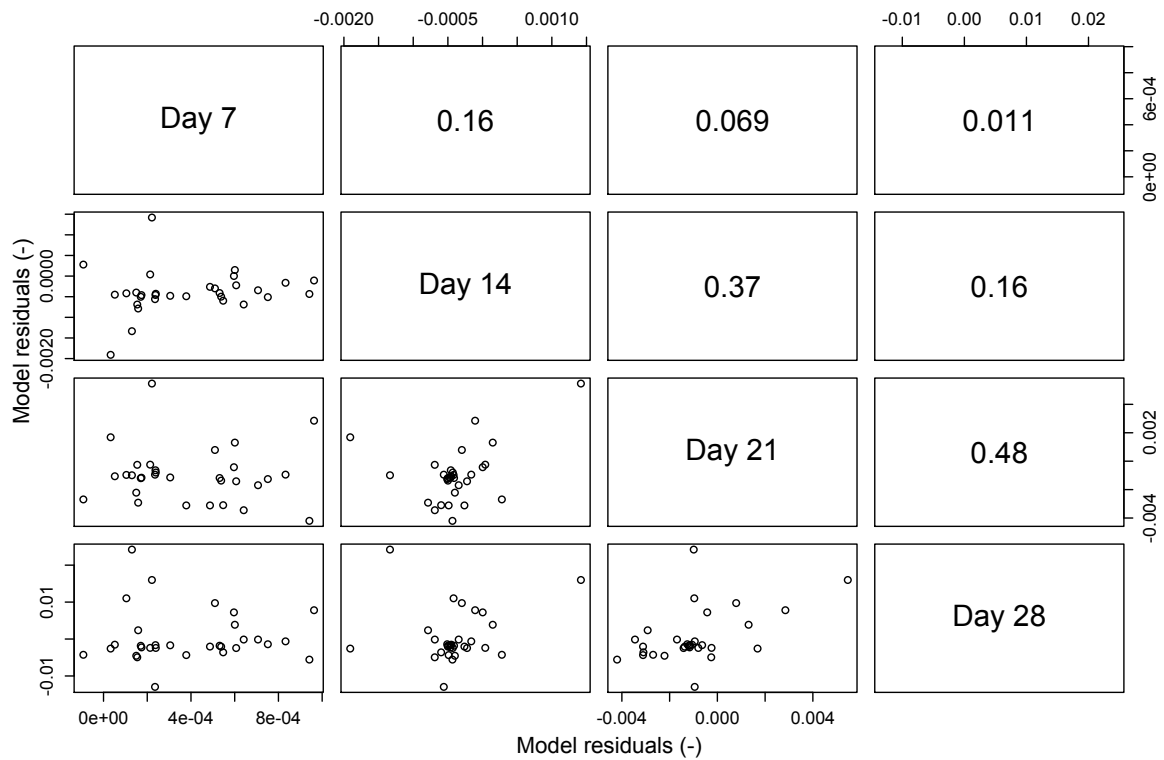


Figure S8: Linear mixed effects model 1 residual correlation for changes in trait-dependent complementarity effects Pairs plot of the model residuals at each sampling day for the linear mixed effect model predicting the changes in trait-dependent complementarity effects (Δ trait.-dep. comp. effect) as a function of the \log_{10} diversity concentration and time as fixed effects and species composition as a random effect with temporal autocorrelation structure (Table 2.2, model1). Values in the upper triangle denote the correlation in model residuals between the different days.

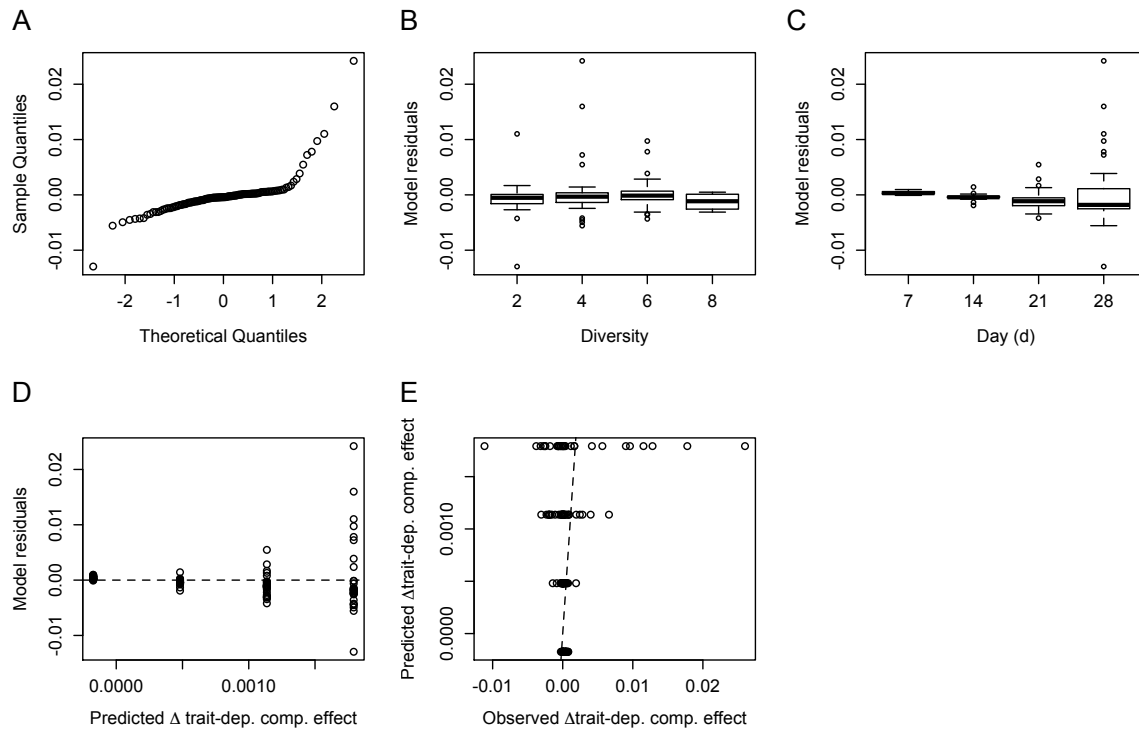


Figure S9: Model 1 residual diagnostics for changes in trait-dependent complementarity effects. Linear mixed effects model predicting atrazine-induced changes in the dominance effect (Δ trait-dep. comp. effect) as a function of log10 diversity and time as fixed effects, community composition as random effects and a temporal autocorrelation structure (Table 2.2) Model residuals are plotted as QQ-plot (A), plotted against the fixed effects (Diversity, B and Day, C) and the predicted change in dominance effect (D) to assess normality and homogeneity of model residuals. Model predictions are plotted against the observed changes in trait-dependent complementarity effects (E) to assess model performance.

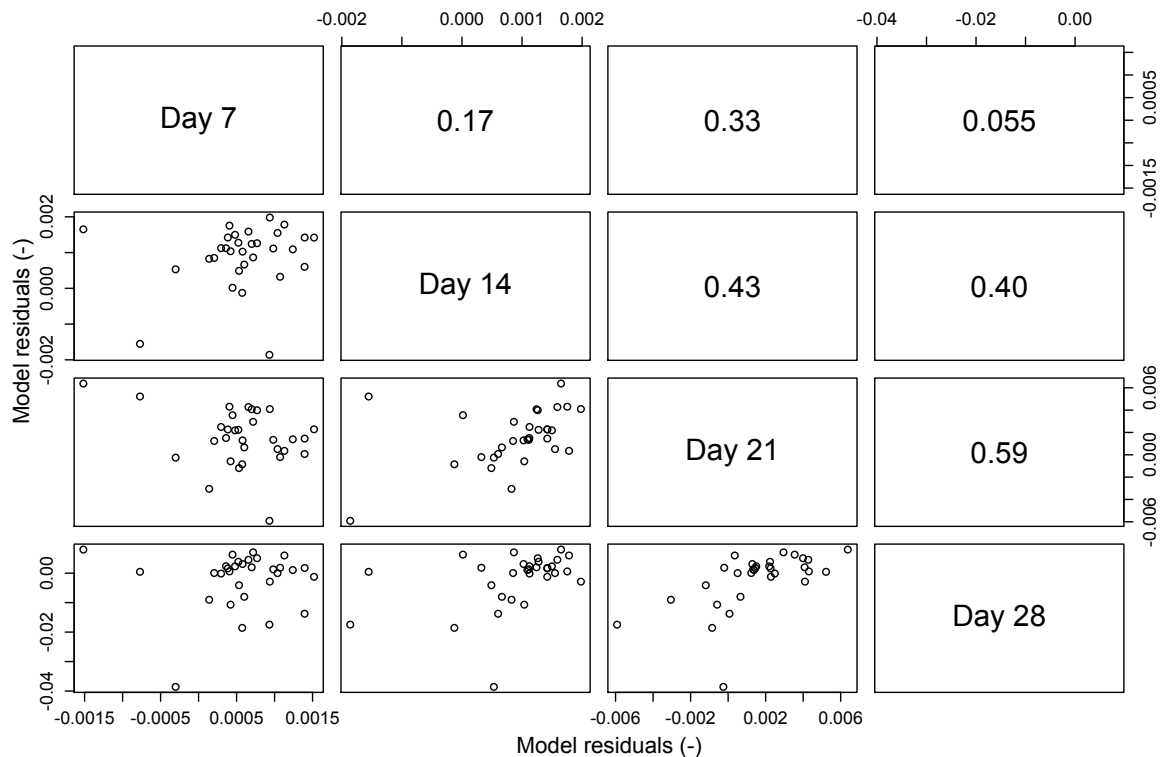


Figure S10: Linear mixed effects model 1 residual correlation for changes in trait-independent complementarity effects Pairs plot of the model residuals at each sampling day for the linear mixed effect model predicting the changes in trait-dependent complementarity effects (Δ trait.-indep. comp. effect) as a function of the \log_{10} diversity concentration and time as fixed effects and species composition as a random effect with temporal autocorrelation structure (Table 2.2, model1). Values in the upper triangle denote the correlation in model residuals between the different days.

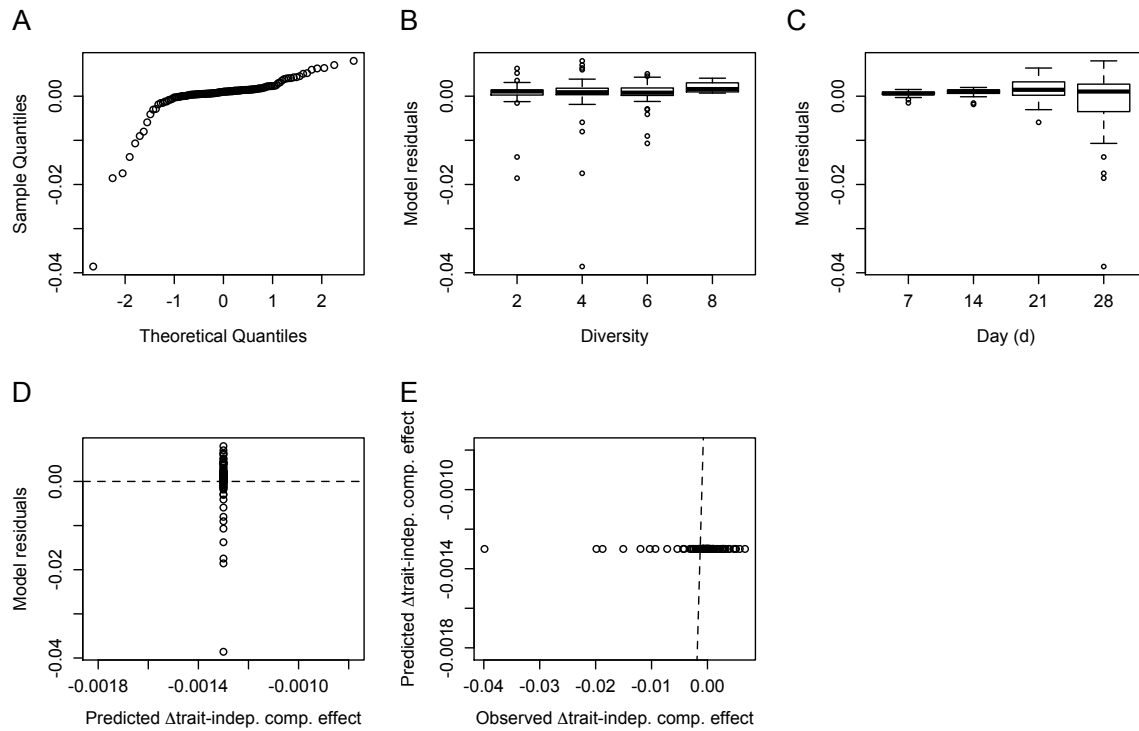


Figure S11: Model 1 residual diagnostics for changes in trait-independent complementarity effects. Linear mixed effects model predicting atrazine-induced changes in the dominance effect (Δ trait-indep. comp. effect) as a function of log₁₀ diversity and time as fixed effects, community composition as random effects and a temporal autocorrelation structure (Table 2.2) Model residuals are plotted as QQ-plot (A), plotted against the fixed effects (Diversity, B and Day, C) and the predicted change in dominance effect (D) to assess normality and homogeneity of model residuals. Model predictions are plotted against the observed changes in trait-independent complementarity effects (E) to assess model performance.

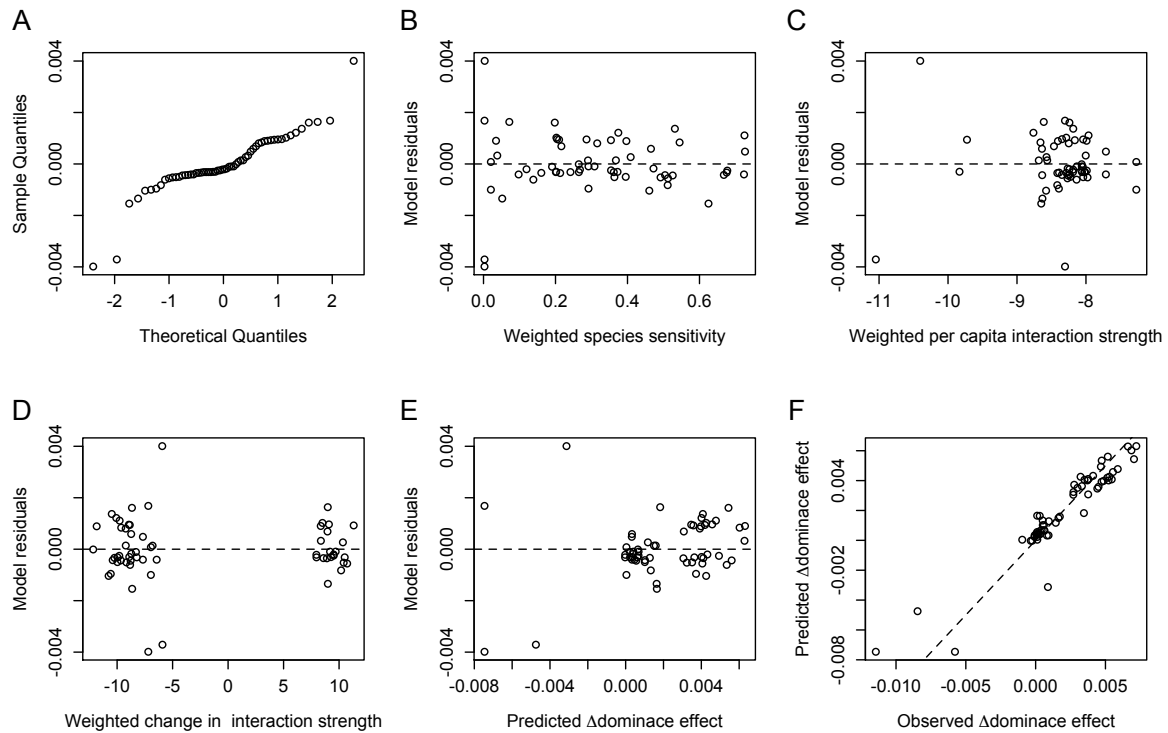


Figure S12: Model 2 residual diagnostics for changes in dominance effects. Optimized linear mixed effects model predicting atrazine-induced changes in the dominance effect (Δ dominance effect). Initial models included a function of the weighted mean species sensitivity to atrazine, per-capita interaction strengths and changes in per-capita interaction strengths as fixed effects and community composition as random effects (for optimal model structure, see Table 2.2) Model residuals are plotted as QQ-plot (A), plotted against the fixed effects (weighted mean species sensitivity, B; weighted mean per-capita interaction strength, C and weighted mean changes in the per-capita interaction strength, D) and the predicted change in dominance effect (E) to assess normality and homogeneity of model residuals. Model predictions are plotted against the observed changes in dominance effects (F) to assess model performance.

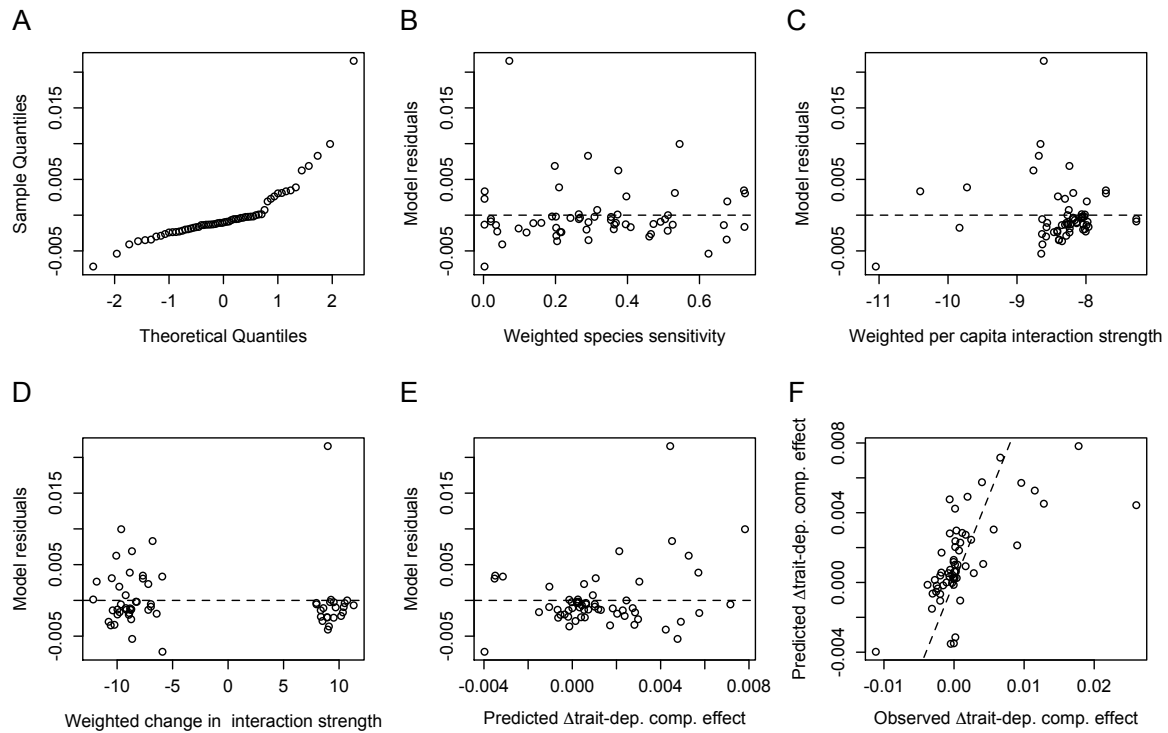


Figure S13: Model 2 residual diagnostics for changes in trait-dependent complementarity effects. Optimized linear mixed effects model predicting atrazine-induced changes in the trait-dependent complementarity effect (Δ trait-dep. comp. effect). Initial models included a function of the weighted mean species sensitivity to atrazine, per-capita interaction strengths and changes in per-capita interaction strengths as fixed effects and community composition as random effects (for optimal model structure, see Table 2.2) Model residuals are plotted as QQ-plot (A), plotted against the fixed effects (weighted mean species sensitivity, B; weighted mean per-capita interaction strength, C and weighted mean changes in the per-capita interaction strength, D) and the predicted change in dominance effect (E) to assess normality and homogeneity of model residuals. Model predictions are plotted against the observed changes in trait-dependent complementarity effects (F) to assess model performance.

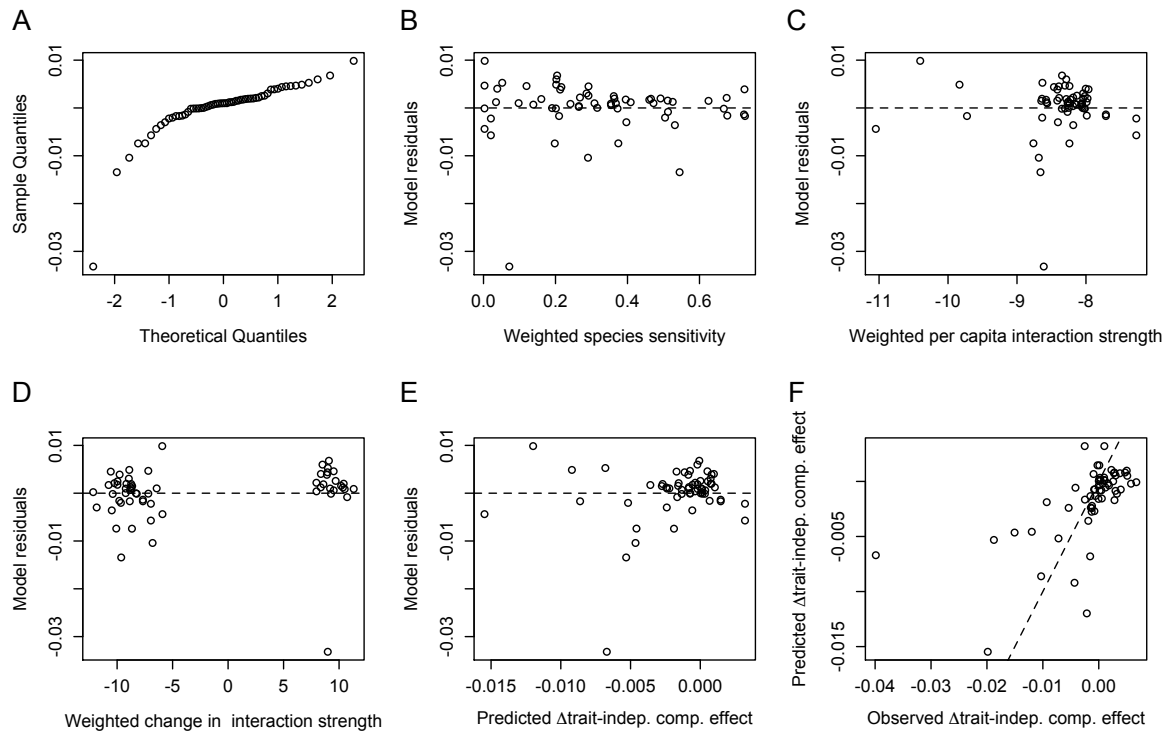


Figure S14: Model 2 residual diagnostics for changes in trait-independent complementarity effects. Optimized linear mixed effects model predicting atrazine-induced changes in the trait-independent complementarity effect (Δ trait-dep. comp. effect). Initial models included a function of the weighted mean species sensitivity to atrazine, per-capita interaction strengths and changes in per-capita interaction strengths as fixed effects and community composition as random effects (for optimal model structure, see Table 2.2) Model residuals are plotted as QQ-plot (A), plotted against the fixed effects (weighted mean species sensitivity, B; weighted mean per-capita interaction strength, C and weighted mean changes in the per-capita interaction strength, D) and the predicted change in dominance effect (E) to assess normality and homogeneity of model residuals. Model predictions are plotted against the observed changes in trait-independent complementarity effects (F) to assess model performance.

Table S1 Estimates for the fixed effects and p-values for the linear mixed models. The weighted average sensitivity to atrazine stress (M_{250}/M_0), the average strength of per capita interactions (A_0) and the average change in per capita interaction strength ($A_{250}-A_0$) and all pairwise interactions are included as fixed effects. Community composition was included as a random effect.

Dominance Effect				
	DF	t-value	Estimate (standard error)	p-value
Intercept	29	1.54	0.0216 (0.014)	0.14
M_{250}/M_0	24	-2.30	-0.0909 (0.039)	0.03
A_0	24	1.44	0.0024 (0.002)	0.16
$A_{250}-A_0$	24	0.25	0.0005 (0.002)	0.80
$M_{250}/M_0 \times A_0$	24	-2.31	-0.0111 (0.005)	0.03
$M_{250}/M_0 \times A_{250}-A_0$	24	-1.82	-0.0006 (0.0003)	0.08
$A_0 \times A_{250}-A_0$	24	0.15	0.00003 (0.00002)	0.89
Trait-dependent complementarity effect				
	DF	t-value	Estimate (standard error)	p-value
Intercept	29	-0.12	-0.0024 (0.02)	0.91
M_{250}/M_0	24	-3.07	-0.1723 (0.06)	0.005
A_0	24	-0.15	-0.0004 (0.002)	0.88
$A_{250}-A_0$	24	-0.84	-0.0024 (0.003)	0.41
$M_{250}/M_0 \times A_0$	24	-3.08	-0.0210 (0.007)	0.005
$M_{250}/M_0 \times A_{250}-A_0$	24	-0.62	-0.0003 (0.0005)	0.54
$A_0 \times A_{250}-A_0$	24	-0.86	-0.0003 (0.0003)	0.40
Trait-independent complementarity effect				
	DF	t-value	Estimate (standard error)	p-value
Intercept	29	2.63	0.069 (0.03)	0.01
M_{250}/M_0	24	1.09	0.076 (0.07)	0.29
A_0	24	2.71	0.009 (0.003)	0.01
$A_{250}-A_0$	24	1.51	0.005 (0.007)	0.14
$M_{250}/M_0 \times A_0$	24	1.07	0.009 (0.009)	0.30
$M_{250}/M_0 \times A_{250}-A_0$	24	0.58	0.0004 (0.0006)	0.57
$A_0 \times A_{250}-A_0$	24	1.53	0.0007 (0.0004)	0.14

Table S2: Estimated population parameters for the 8 species used in the experiment. V is the cell volume, K_0 and K_{250} are the carrying capacities, μ_0 and μ_{250} are the intrinsic growth rates at 0 and 250 $\mu\text{g L}^{-1}$ Atrazine, respectively

Code	Species	V (μm^3)	μ_0 (d^{-1})	μ_{250} (d^{-1})	K_0 (μm^3)	K_{250} (μm^3)
1	<i>Coscinodiscus sp.</i>	367008	0.33	0.22	948 839 324	314883704
2	<i>Ditylum sp.</i>	24757	0.27	0.01	862 505 671	4951400
3	<i>Bacillaria sp.</i>	6448	0.51	0.20	406 957 000	348711139
4	<i>Odontella sp.</i>	752767	0.24	0.01	10 583 784 289	55990944
5	<i>Thalassiosira sp.1</i>	25727	0.28	0.15	108 096 248	43707354
6	<i>Gyrosigma sp.</i>	3940	0.19	0.10	651 492 127	66671926
7	<i>Guinardia sp.</i>	14001	0.19	0.19	1 176 672 117	28473370
8	<i>Thalassiosira sp.2</i>	12556	0.43	0.01	629 079 021	15554781

Table S3: Community assemblages for diversity levels 2, 4 and 6. Numbers refer to the species code in supplementary table 1. Assemblages at diversity 1 and 8 are not given here since all possible combinations were used for these levels

Diversity = 2	Diversity = 4	Diversity = 6
1+3	1+2+4+8	1+2+3+4+5+7
2+4	2+4+5+6	1+2+4+5+6+7
7+8	3+4+5+8	1+3+4+5+6+7
1+7	2+3+4+5	1+2+3+5+6+7
3+5	1+2+3+6	1+2+3+5+6+8
5+6	3+5+7+8	2+3+5+6+7+8
4+6	2+3+4+7	1+3+4+5+6+8
3+8	1+5+6+8	2+3+4+5+6+7
2+5	1+3+5+6	2+4+5+6+7+8
1+3	1+2+4+7	3+4+5+6+7+8

B

Supporting information for chapter 3

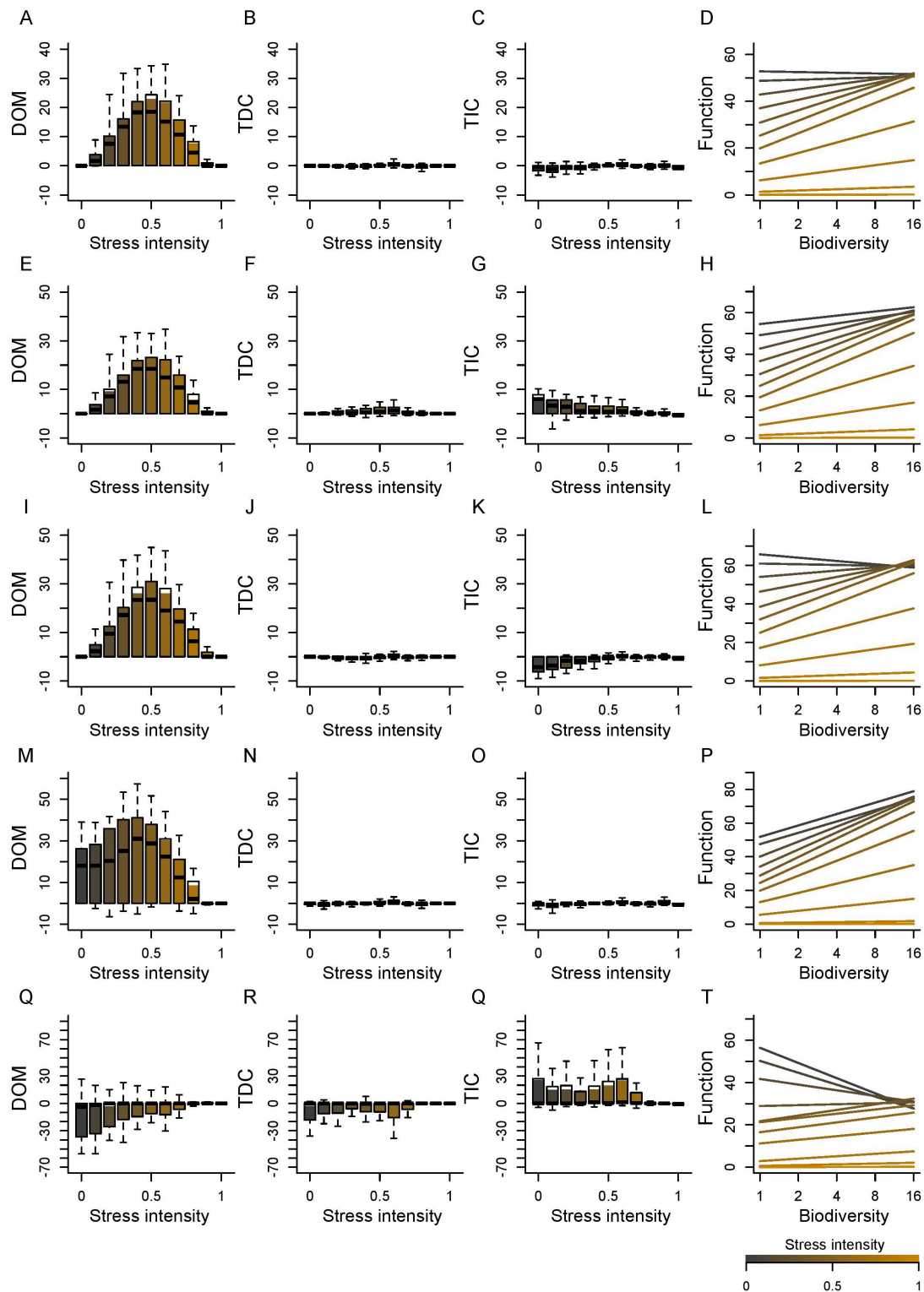


Figure S1. Simulated changes in the strength of biodiversity effects along stress gradients Changes in dominance (DOM; panels A, E, I, M and Q), trait-dependent complementarity (TDC; panels B, F, J, N and R) and trait-independent complementarity effects (TIC; panels C, G, K, O and S) and the biodiversity ecosystem function relationships (panels D, H, L, P and T) under 5 different scenarios: Neutral dynamics (A-D), positive complementarity effects (E-H), negative complementarity effects (I-L), positive dominance effects (M-P) and negative dominance effects (Q-T) under unstressed conditions.

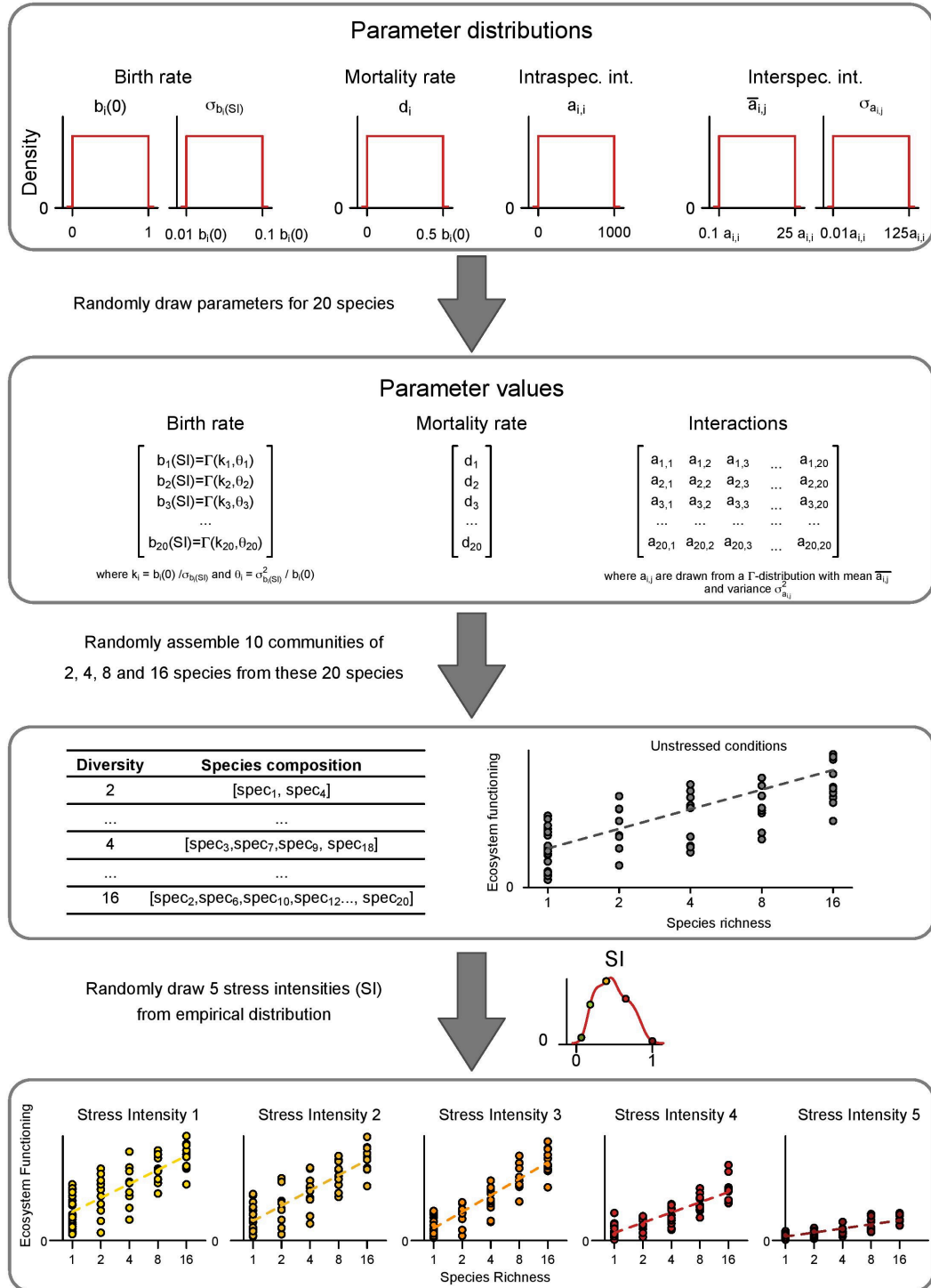


Figure S2. Flowchart of the modelling approach. For each model run first parameters are randomly drawn for 20 species from specified distributions defining the environmental response of the birth rate ($b_i(0)$ and $\sigma_{b_i(SI)}$), mortality rate (d_i), intraspecific ($a_{i,i}$) and interspecific ($a_{i,j}$) interactions. The interspecific interactions are thereby sampled from a gamma distribution of with mean value $\bar{a}_{i,j}$ and variance $\sigma_{a_{i,j}}^2$. Next, 10 communities consisting of 2, 4, 8 and 16 species are randomly assembled from this pool of 20 species. Finally 5 levels of stress intensity are drawn from the empirical distribution obtained from the meta-analysis and BEF relationships are simulated for control conditions and each level of environmental stress.

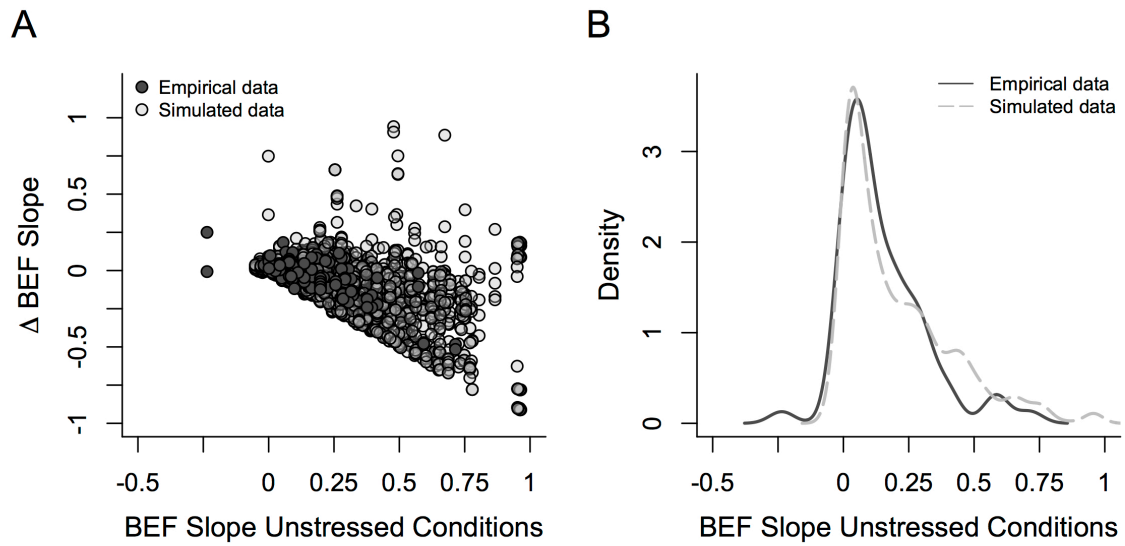


Figure S3. Comparison between empirical and simulated changes in BEF relationships. (A) Changes in slopes of the BEF relationship over an environmental plotted against the BEF slope under unstressed conditions for empirical data of 52 experiments and 1000 model simulations. (B) Probability density of empirical and simulated BEF relationships under unstressed conditions.

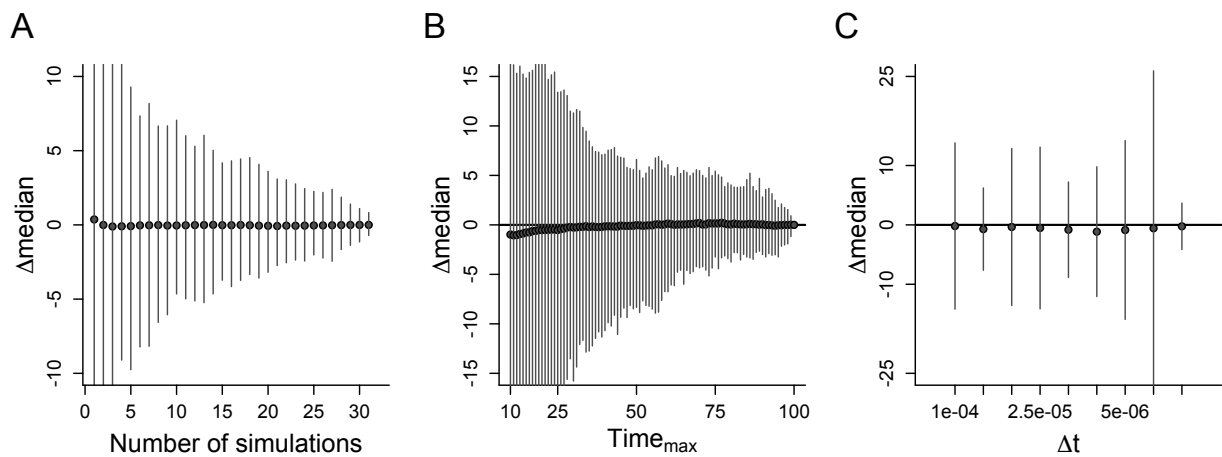


Figure S4. Sensitivity analysis of the runtime parameters. Deviation of the median estimated species density from the expected median species density (Δ median) as a function of the number times a community is simulated (A), the maximal simulation time (B) and the discrete time step (C). Figures are based on 25 randomly drawn biodiversity-ecosystem function relationships.

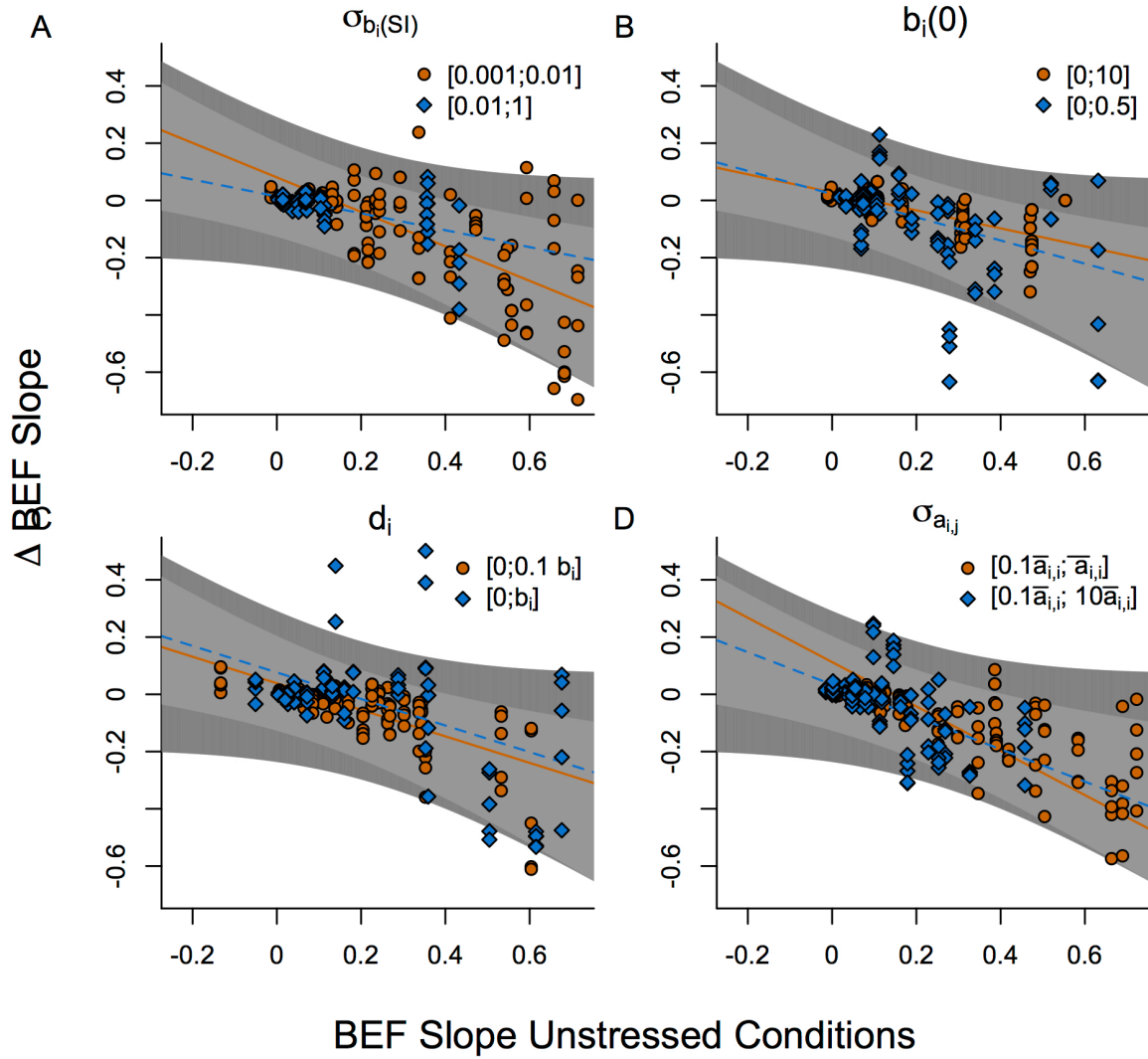


Figure S5. Sensitivity analysis of the parameter distributions Simulated changes in BEF relationships for different parameter distributions for (A) the width of species niches $\sigma_{b_i(SI)}$, (B) the mean birth rate $b_i(0)$, (C) the mortality rate d_i , (D) the variance in interspecific competition $\sigma_{a_{i,j}}$. Changes in 25 randomly drawn BEF relationships were simulated for each parameter distribution. Lines represent linear regressions between the slope of the BEF relationship under unstressed conditions and the change in slope along an environmental stress gradient. Shaded areas correspond to the prediction intervals of the empirical (light grey) and the theoretical linear regression model (dark grey) presented in Figure 3.3.

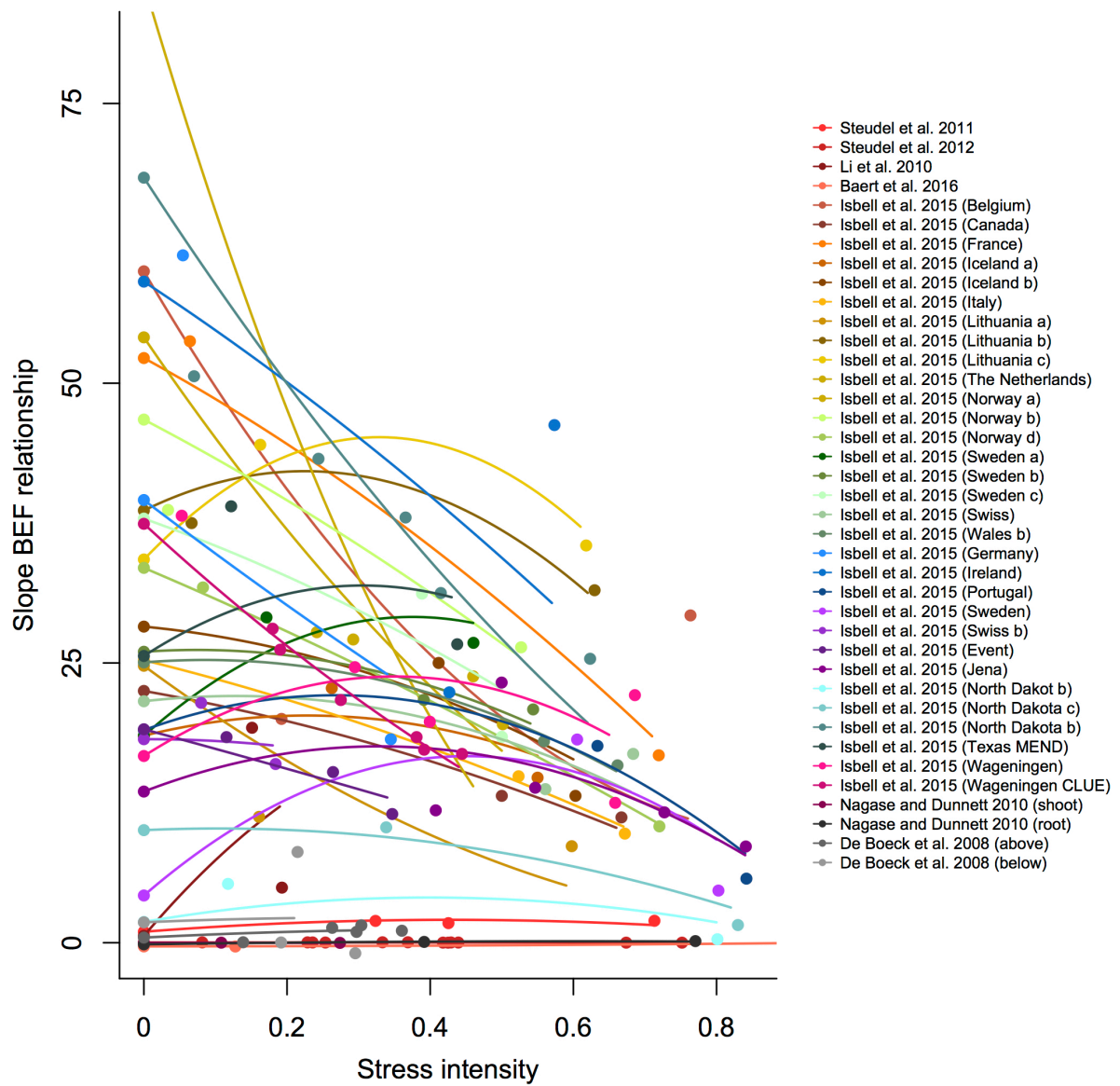


Figure S6. Changes in slopes of the BEF relationship for each of the 40 studies that included at least 3 environmental conditions. Observed slopes and regression lines are given for each study

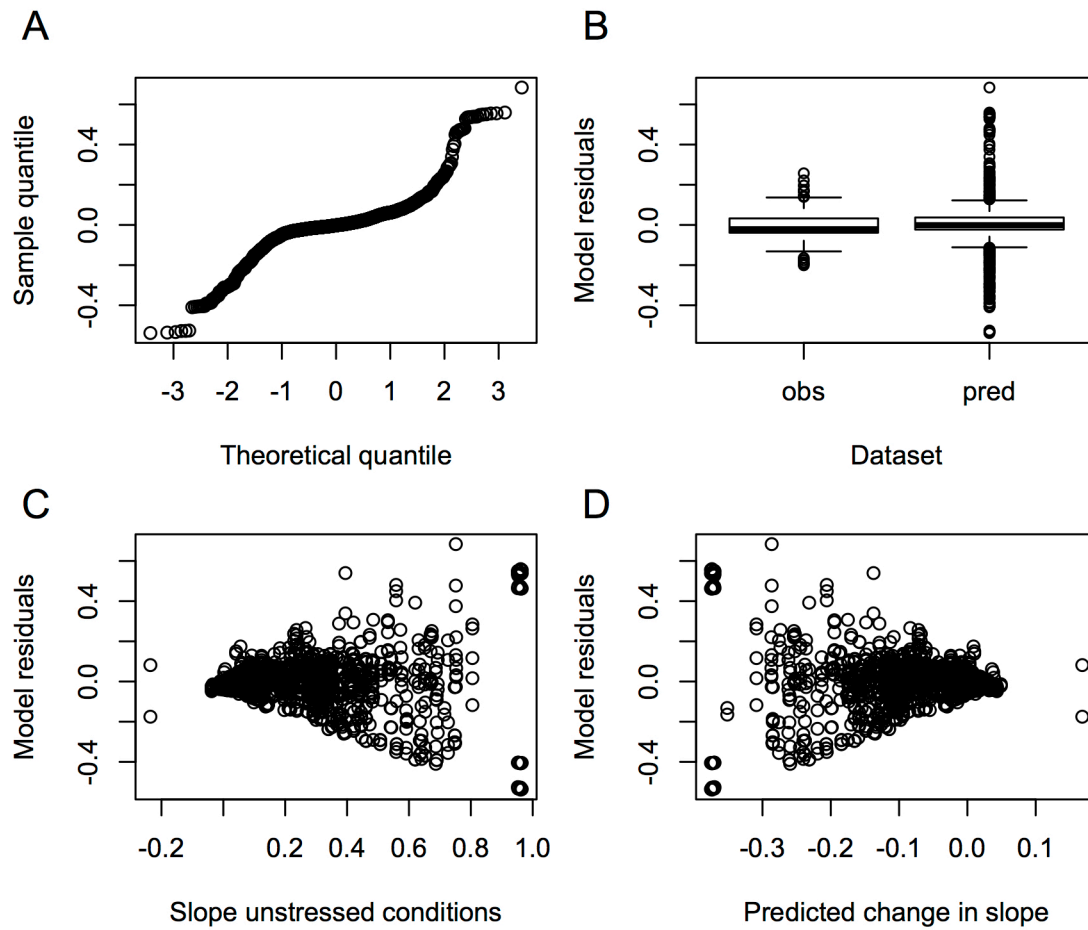


Fig S7. Residual diagnostics for the regression model between the slope under unstressed conditions and the average change in slope over stress gradient (Figure 3.3, Table 3.1) (A) QQ plot, (B) boxplot of model residuals for the meta-analysis (obs) and predicted changes (pred), (C) model residuals plotted against the slope under unstressed conditions and (D) model residuals plotted against the predicted average change in slope.

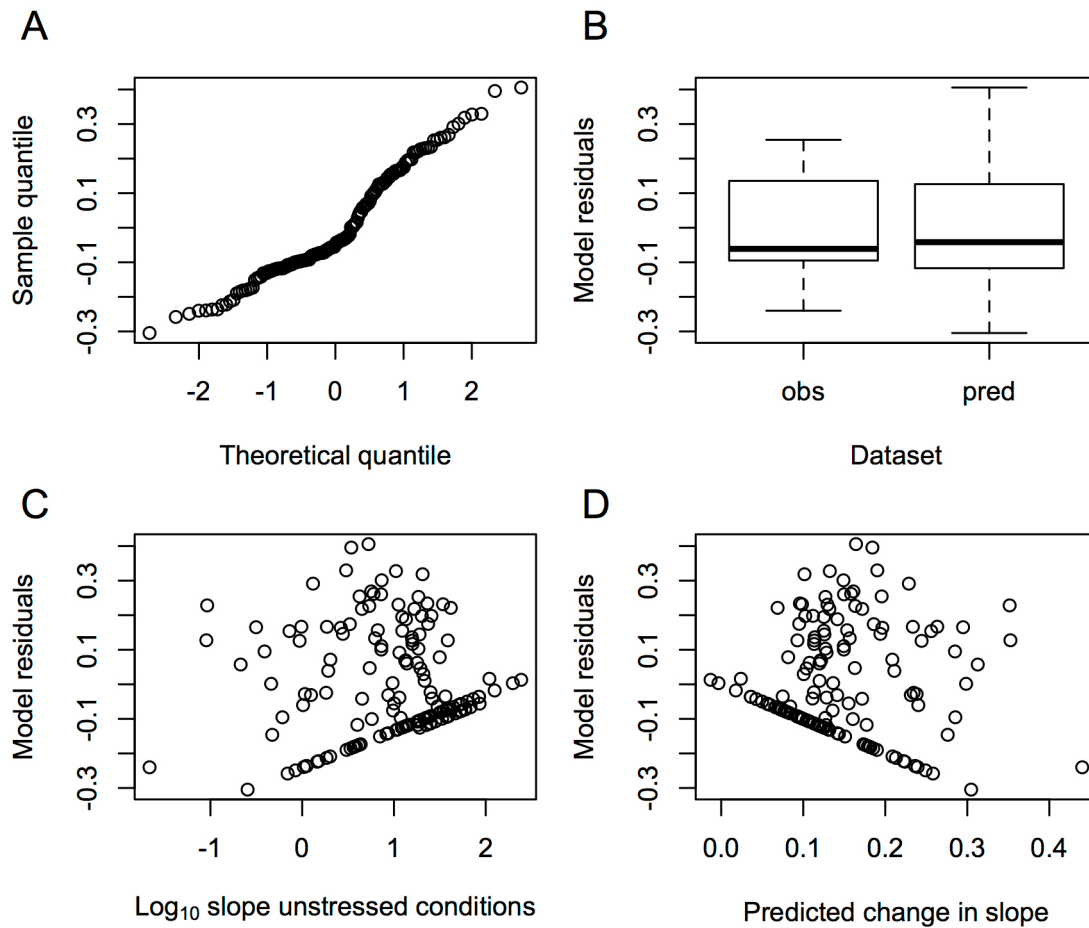


Fig S8. Residual diagnostics for the regression model between the slope under unstressed conditions and the stress level at which maximal biodiversity effects are attained (Fig 4, Table 1) (A) QQ plot, (B) boxplot of model residuals for the meta-analysis (obs) and predicted changes (pred), (C) model residuals plotted against the log₁₀ slope under unstressed conditions and (D) model residuals plotted against the predicted average change in slope.

Table S1. Overview of studies used in the meta-analysis

Study	System	Stressor	Stress levels	Origin Data
Steudel et al. 2011	Marshland vegetation	Drought	2	Table 1 Steudel et al. 2011
Steudel et al. 2011	Marshland vegetation	Salt	2	Table 1 Steudel et al. 2011
Steudel et al. 2011	Marshland vegetation	Shade	2	Table 1 Steudel et al. 2011
Steudel et al. 2012	Algae communities	Salt	7	Table 1 Steudel et al. 2012
Steudel et al. 2012	Algae communities	Temperature	7	Table 1 Steudel et al. 2012
Li et al. 2010	Algae communities	Cadmium	3	Figure 1c Li et al. 2010
Baert et al. 2016	Algae communities	Atrazine	3	Supplementary data Baert et al. 2016
Agrodiversity Belgium	Grassland	Drought	3	Extended data Isbell et al. 2015 Figure 5
Agrodiversity Canada	Grassland	Drought	3	Extended data Isbell et al. 2015 Figure 5
Agrodiversity France	Grassland	Drought	3	Extended data Isbell et al. 2015 Figure 5
Agrodiveristy Iceland a	Grassland	Drought	3	Extended data Isbell et al. 2015 Figure 5
Agrodiversity Iceland b	Grassland	Drought	3	Extended data Isbell et al. 2015 Figure 5
Agrodiveristy Italy	Grassland	Drought	3	Extended data Isbell et al. 2015 Figure 5
Agrodiversity Lithuania a	Grassland	Drought	3	Extended data Isbell et al. 2015 Figure 5
Agrodiversity Lithuania b	Grassland	Drought	3	Extended data Isbell et al. 2015 Figure 5
Agrodiversity Lithuania c	Grassland	Drought	3	Extended data Isbell et al. 2015 Figure 5
Agrodiveristy the Netherlands	Grassland	Drought	3	Extended data Isbell et al. 2015 Figure 5
Agrodiversity Norway a	Grassland	Drought	3	Extended data Isbell et al. 2015 Figure 5
Agrodiversity Norway b	Grassland	Drought	3	Extended data Isbell et al. 2015 Figure 5
Agrodiversity Norway c	Grassland	Drought	2	Extended data Isbell et al. 2015 Figure 5
Agrodiversity Norway d	Grassland	Drought	3	Extended data Isbell et al. 2015 Figure 5
Agrodiversity Sweden a	Grassland	Drought	3	Extended data Isbell et al. 2015 Figure 5
Agrodiversity Sweden d	Grassland	Drought	3	Extended data Isbell et al. 2015 Figure 5
Agrodiversity Sweden c	Grassland	Drought	3	Extended data Isbell et al. 2015 Figure 5
Agrodiveristy Switzerland	Grassland	Drought	3	Extended data Isbell et al. 2015 Figure 5
Agrodiversity Wales a	Grassland	Drought	2	Extended data Isbell et al. 2015 Figure 5
Agrodiversity Wales b	Grassland	Drought	3	Extended data Isbell et al. 2015 Figure 5
BIODEPT Germany	Grassland	Drought	3	Extended data Isbell et al. 2015 Figure 5

BIODEPT Greece	Grassland	Drought	2	Extended data Isbell et al. 2015	Figure 5
BIODEPT Ireland	Grassland	Drought	3	Extended data Isbell et al. 2015	Figure 5
BIODEPT Portugal	Grassland	Drought	3	Extended data Isbell et al. 2015	Figure 5
BIODEPT UK, Sheffield	Grassland	Drought	2	Extended data Isbell et al. 2015	Figure 5
BIODEPT UK, Silwood	Grassland	Drought	2	Extended data Isbell et al. 2015	Figure 5
BIODEPT Sweden	Grassland	Drought	3	Extended data Isbell et al. 2015	Figure 5
BIODEPT Switzerland	Grassland	Drought	3	Extended data Isbell et al. 2015	Figure 5
Event	Grassland	Drought	4	Extended data Isbell et al. 2015	Figure 5
Jena	Grassland	Drought	6	Extended data Isbell et al. 2015	Figure 5
North Dakota b	Grassland	Drought	3	Extended data Isbell et al. 2015	Figure 5
North Dakota b	Grassland	Drought	3	Extended data Isbell et al. 2015	Figure 5
Texas evenness	Grassland	Drought	6	Extended data Isbell et al. 2015	Figure 5
Texas MEND	Grassland	Drought	3	Extended data Isbell et al. 2015	Figure 5
Virginia	Grassland	Drought	4	Extended data Isbell et al. 2015	Figure 5
Wageningen Biodiversity	Grassland	Drought	6	Extended data Isbell et al. 2015	Figure 5
Wageningen CLUE	Grassland	Drought	7	Extended data Isbell et al. 2015	Figure 5
Hughes and Stachowicz 2004	Sea grass communities	Grazing	2	Figure 1B and C Hughes and Stachowicz 2004	
Nagase and Dunnet 2010 (shoot)	Grassland	Drought	3	Figure 2 Nagase and Dunnet 2010	
Nagase and Dunnet 2010 (root)	Grassland	Drought	3	Figure 3 Nagase and Dunnet 2010	
De Boeck et al. 2008	Grassland	Temperature	9	Figure 1 De Boeck et al. 2008	
Joshi et al. 2000	Grassland	Parasite	2	Figure 4 Joshi et al. 2000	
Dukes 2002	Grassland	Invasion	2	Figure 8 Dukes 2002	
Liiri et al. 2002	Arthropod communities	Drought	2	Figure 2 Liiri et al. 2002	
Mulder et al. 2001	Mosses	Drought	2	Figure 1 Mulder et al. 2001	
Richardson et al. 2009	Grassland	Drought	2	Figure 1 Richardson et al. 2009	
Lanta et al. 2012	Grassland	Drought	4	Figure 2 Lanta et al. 2012	

C

Supporting information for chapter 4

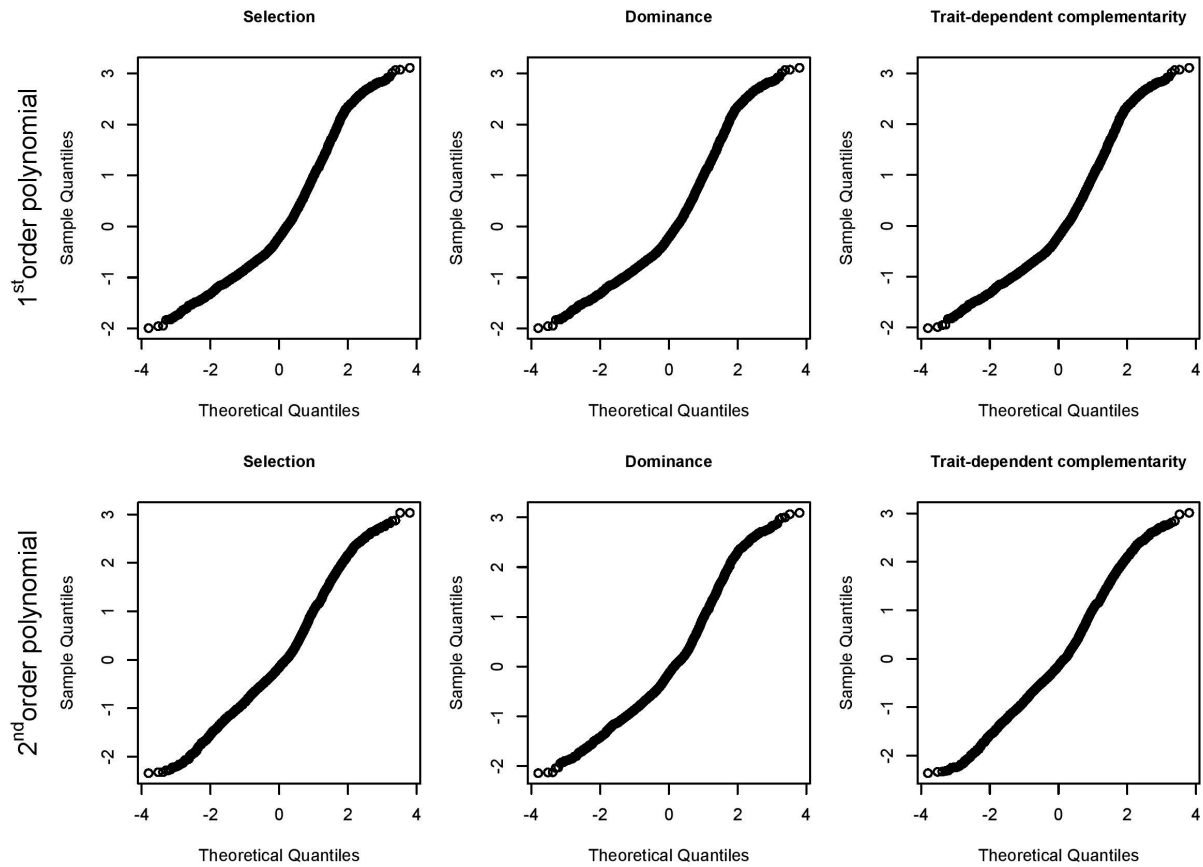


Figure S1. QQ-plot of normalised model residuals. Model residuals for selection, dominance and trait-dependent complementarity effects when assuming linear and second order relationships between species deviations from the null model and functional traits.

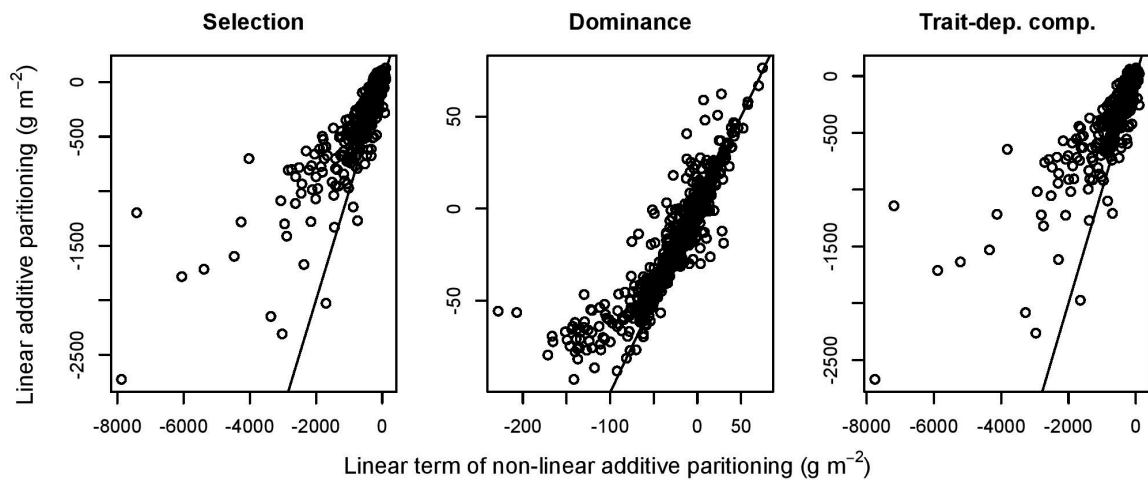


Figure S2. Comparison between biodiversity effects for linear additive partitioning and the linear term of non-linear additive partitioning methods based on 2nd order polynomials. Note that deviations from the 1:1 line hence represent 2nd order terms as first and second order terms sum to the biodiversity effects as calculated by linear additive partitioning.



Supporting information for chapter 5

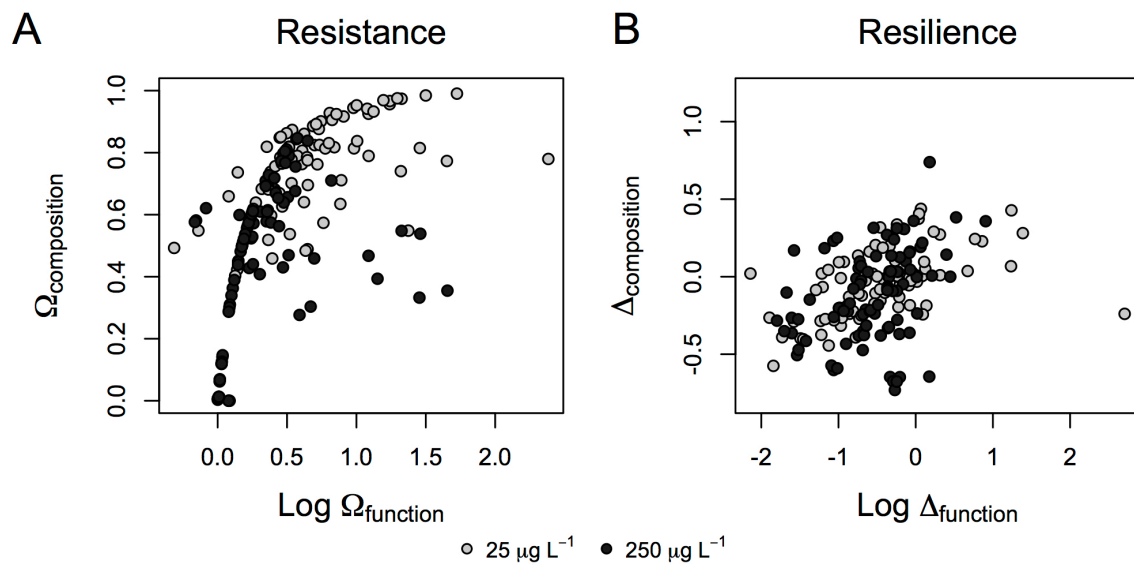


Figure S1. Functional resistance plotted against compositional stability (A) and functional resilience plotted against compositional resilience (B). Plotted for all communities at day 28 and 49 of the experiment

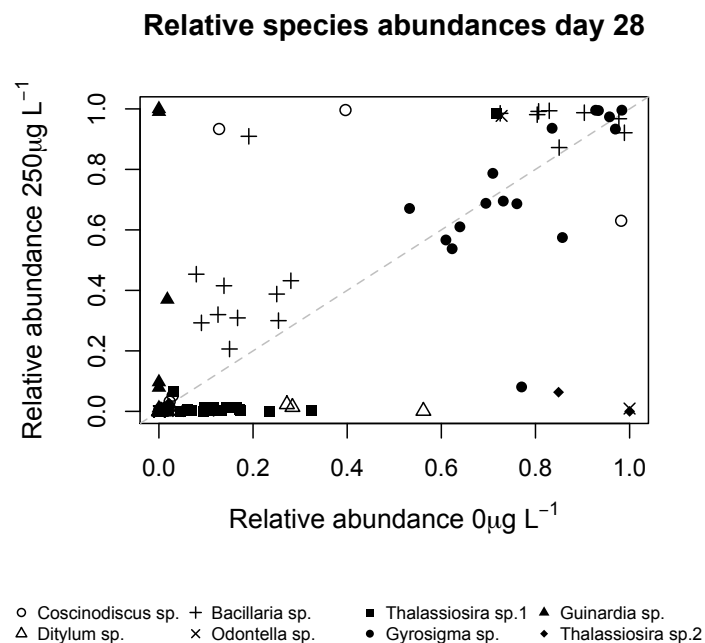


Figure S2: Relative species abundance in the high-stress treatment plotted against the relative abundance in the control treatment.

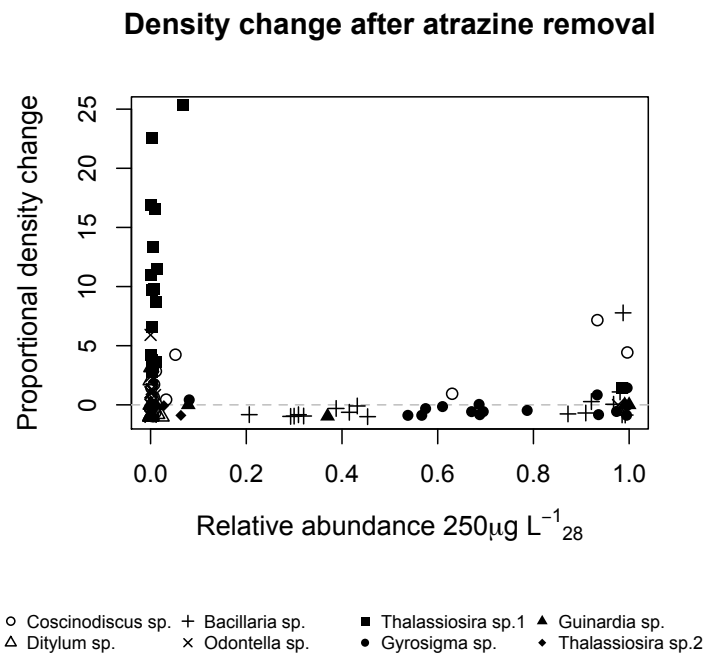


Figure S3: Proportional change in species densities in the high-stress treatment after atrazine removal. Plotted against the relative species abundance at the end of atrazine exposure (day 28)

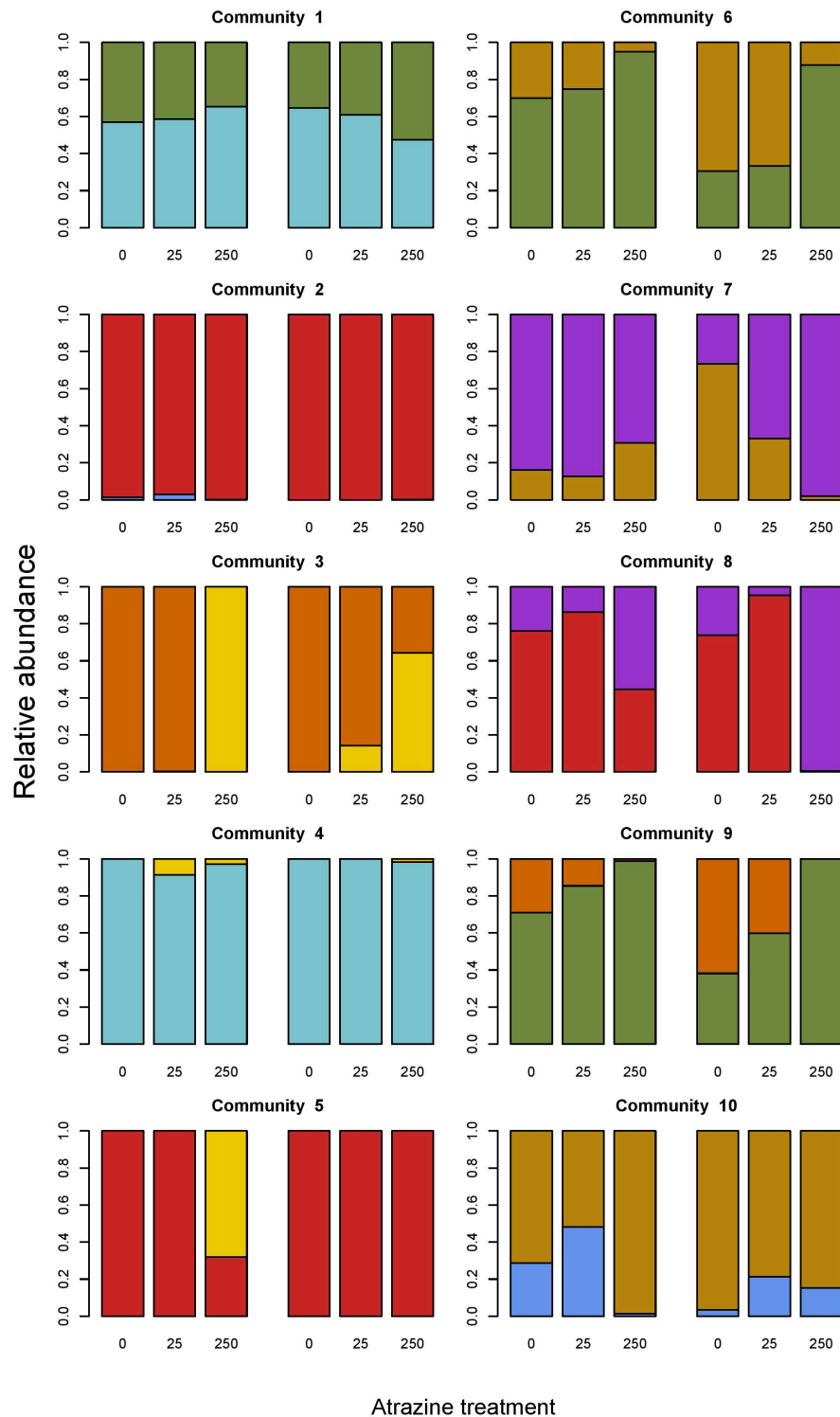


Figure S4: Species relative functional contribution to total community biomass for communities consisting of 2 species. For each community, the three left bars correspond to day 28, the three right bars to day 49. The sequence of communities corresponds to appendix D table 2.

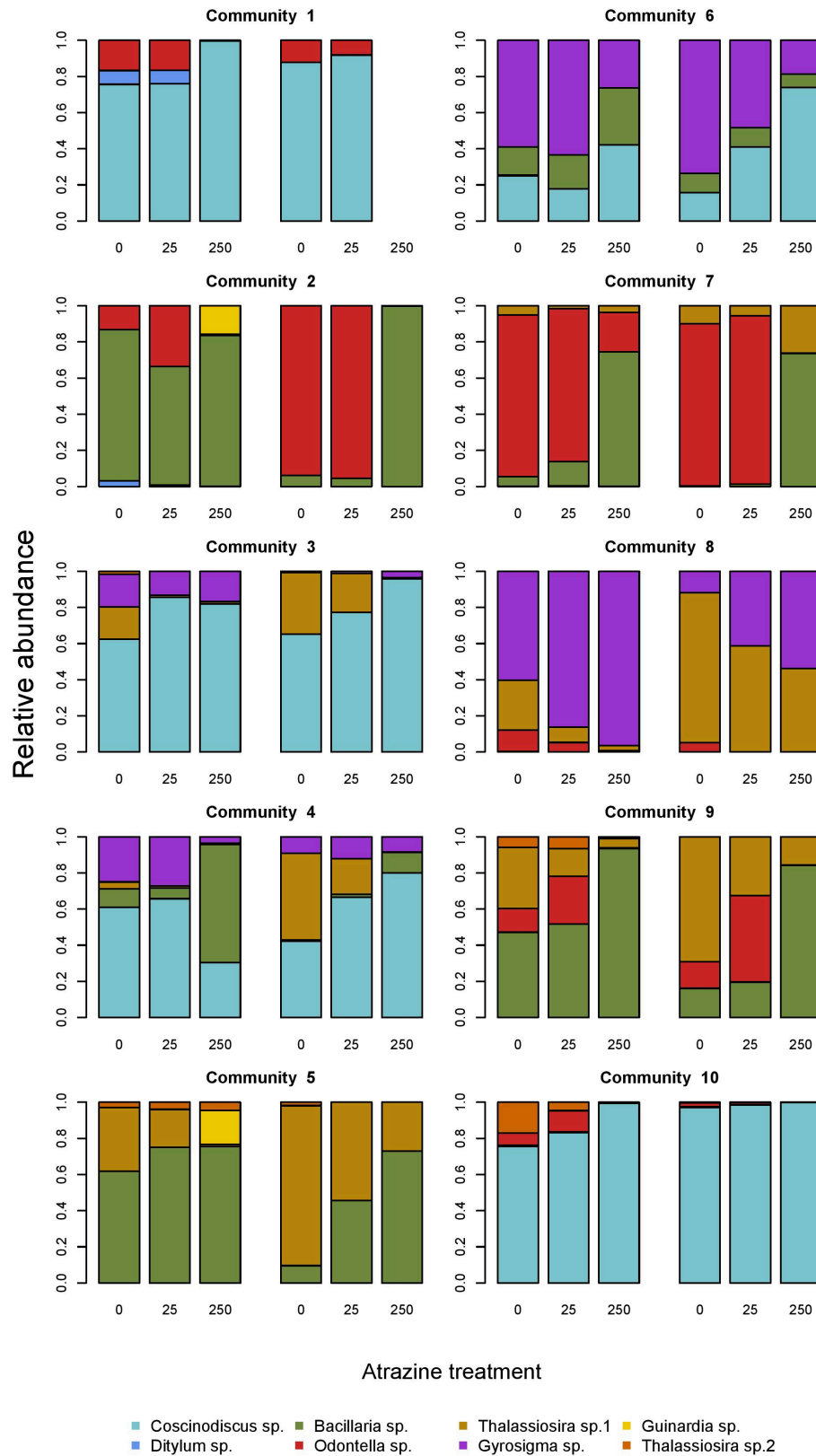


Figure S5: Species relative functional contribution to total community biomass for communities consisting of 4 species. For each community, the three left bars correspond to day 28, the three right bars to day 49. The sequence of communities corresponds to appendix D table 2.

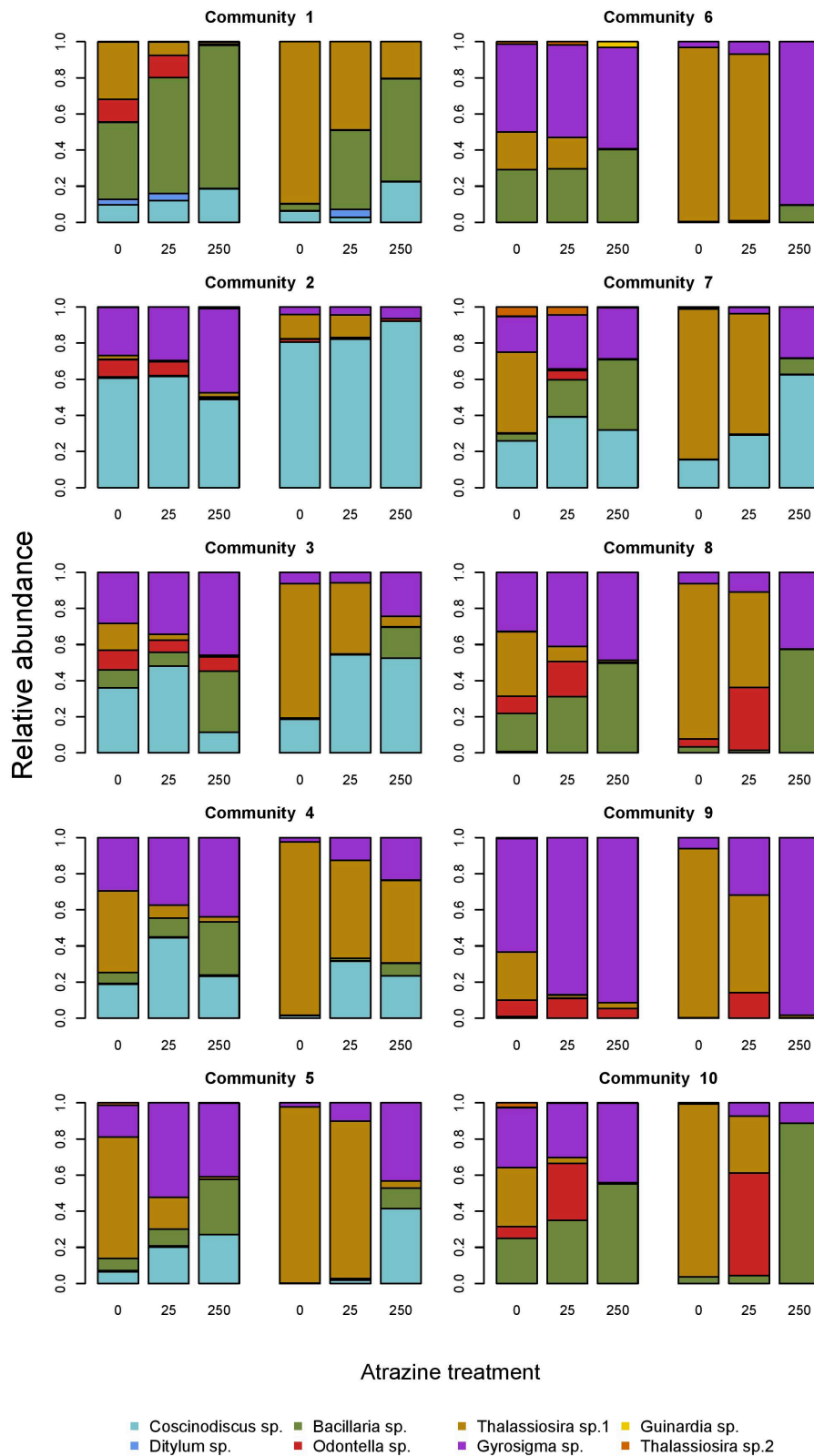


Figure S6: Species relative functional contribution to total community biomass for communities consisting of 6 species. For each community, the three left bars correspond to day 28, the three right bars to day 49. The sequence of communities corresponds to appendix D table 2.

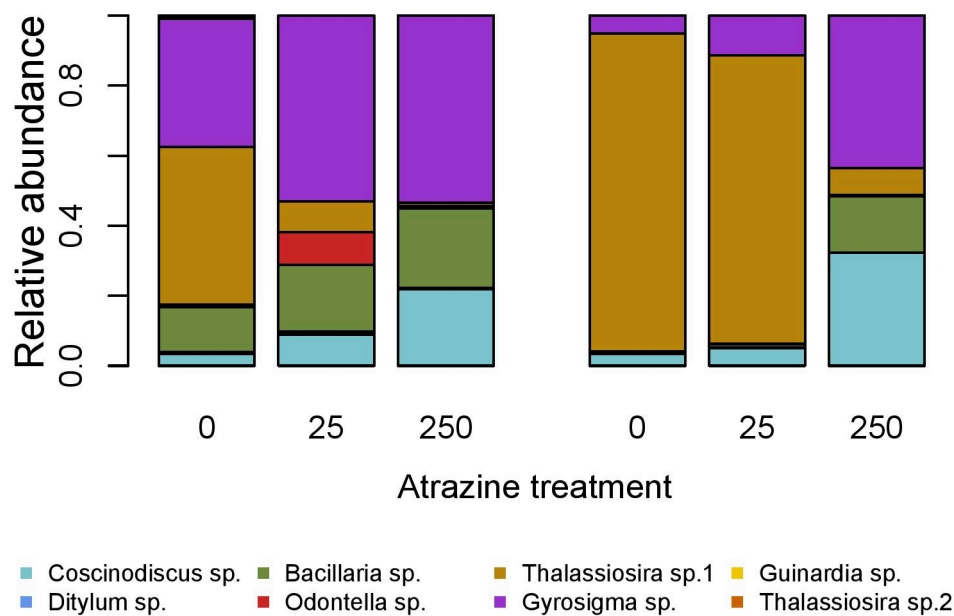


Figure S7: Species relative functional contribution to total community biomass for communities consisting of 8 species. For each community, the three left bars correspond to day 28, the three right bars to day 49. The sequence of communities corresponds to appendix D table 2.

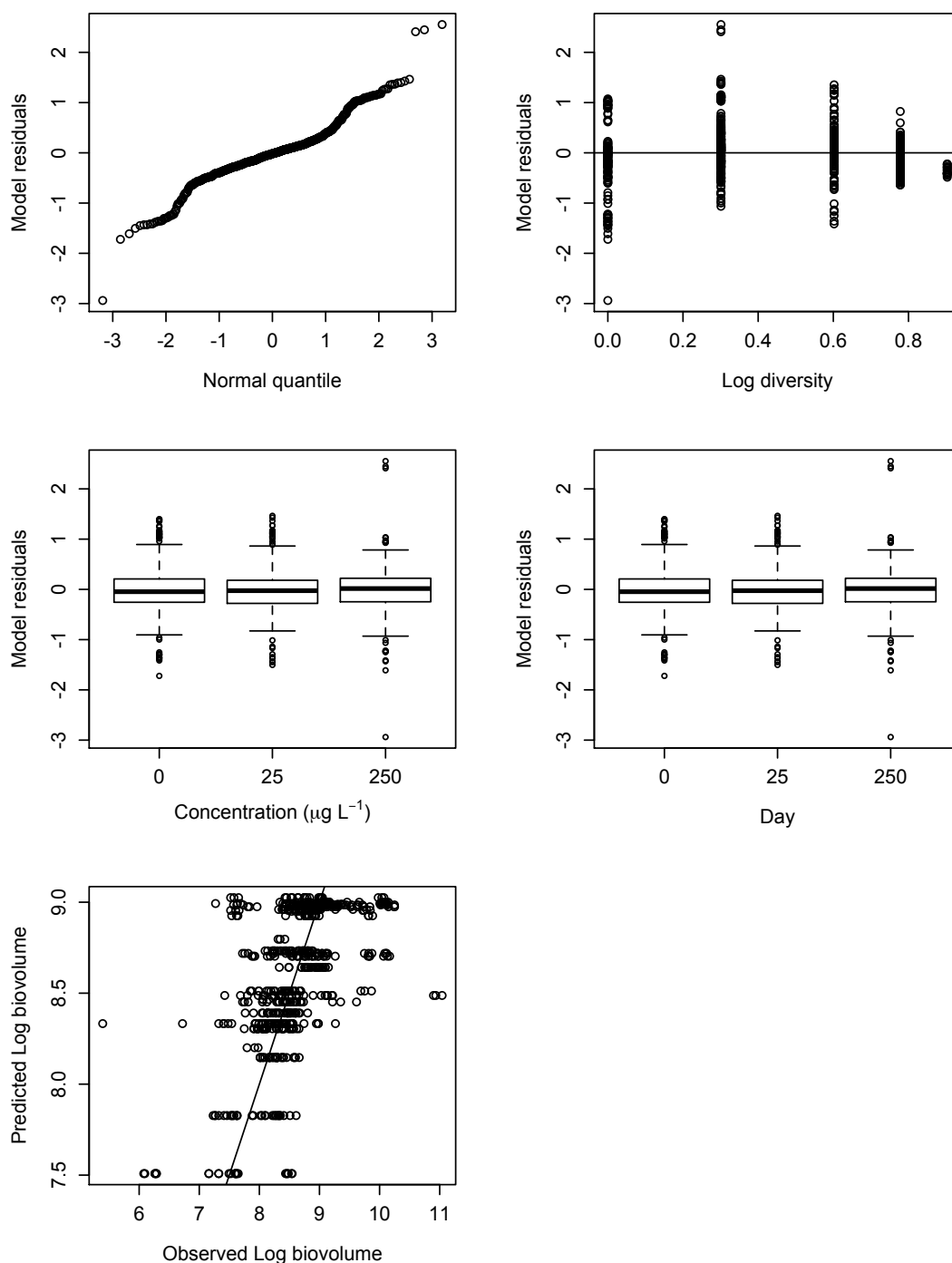


Figure S8: Residual diagnostics of the linear model predicting the log biovolume The model includes concentration, day and diversity and their pairwise interactions as predictor variables (Table 5.1, Figure 5.1).

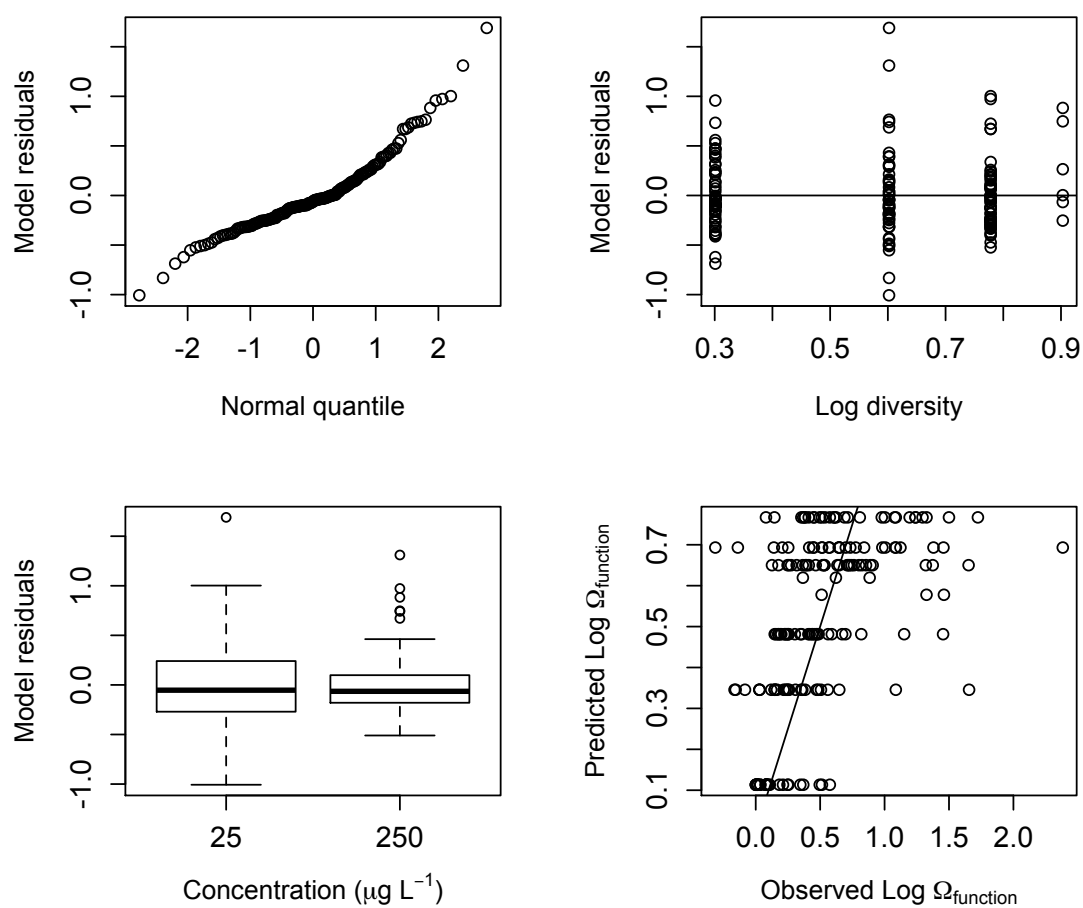


Figure S9: Residual diagnostics of the linear model predicting the log functional resistance. The model includes concentration, diversity and the interaction as predictor variables (Table 5.2, Figure 5.2).

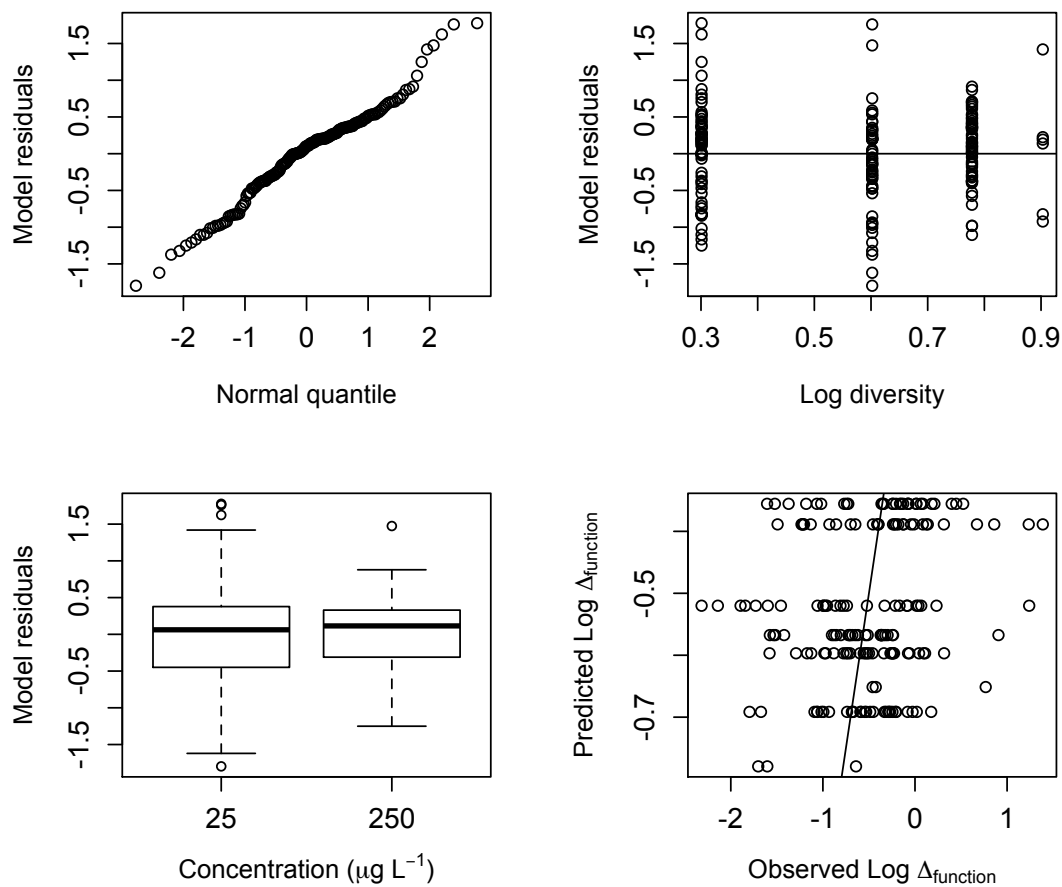


Figure S10: Residual diagnostics of the linear model predicting the log functional resilience. The model includes concentration, diversity and the interaction as predictor variables (Table 5.2, Figure 5.2).

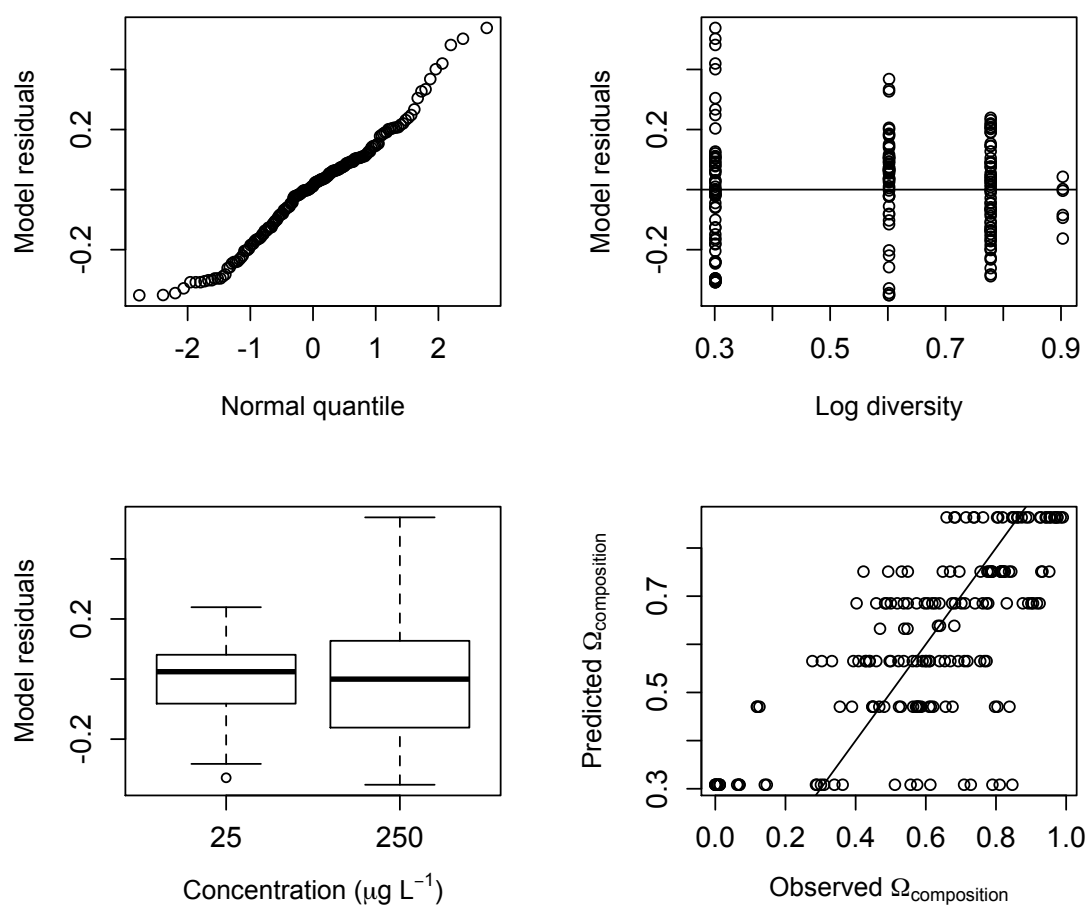


Figure S11: Residual diagnostics of the linear model predicting the compositional resistance. The model includes concentration, diversity and the interaction as predictor variables (Table 5.2, Figure 5.2).

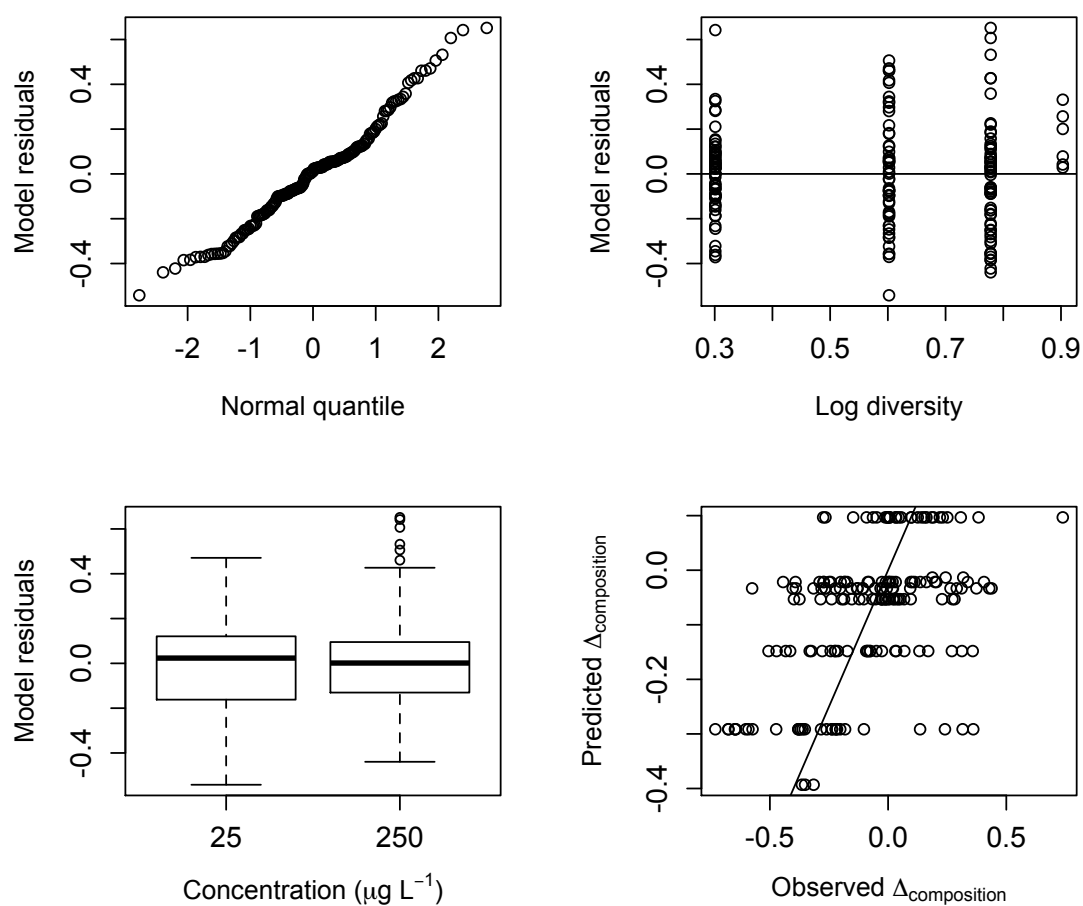


Figure S12: Residual diagnostics of the linear model predicting the compositional resilience. The model includes concentration, diversity and the interaction as predictor variables (Table 5.2, Figure 5.2).

Table S1: Summary of the volume, division rate and sensitivity to atrazine of the 8 species used in the experiment. Biomass reduction was calculated as the percentage reduction in the carrying capacity of the species compared to the control treatment.

Code	Species	V (μm^3)	Division rate (d^{-1})	Biomass reduction 25 $\mu\text{g L}^{-1}$ (%)	Biomass reduction 250 $\mu\text{g L}^{-1}$ (%)
1	<i>Coscinodiscus sp.</i>	367008	0.33	12	67
2	<i>Ditylum sp.</i>	24757	0.27	0	99
3	<i>Bacillaria sp.</i>	6448	0.51	5	14
4	<i>Odontella sp.</i>	752767	0.24	43	99
5	<i>Thalassiosira sp.1</i>	25727	0.28	29	60
6	<i>Gyrosigma sp.</i>	3940	0.19	0	90
7	<i>Guinardia sp.</i>	14001	0.19	48	98
8	<i>Thalassiosira sp.2</i>	12556	0.43	0	98

Table S2: Community assemblages for diversity levels 2, 4 and 6. Numbers refer to the species code in supplementary table 1. Assemblages at diversity 1 and 8 are not given here since all possible combinations were used for these levels

Diversity = 2	Diversity = 4	Diversity = 6
1+3	1+2+4+8	1+2+3+4+5+7
2+4	2+4+5+6	1+2+4+5+6+7
7+8	3+4+5+8	1+3+4+5+6+7
1+7	2+3+4+5	1+2+3+5+6+7
3+5	1+2+3+6	1+2+3+5+6+8
5+6	3+5+7+8	2+3+5+6+7+8
4+6	2+3+4+7	1+3+4+5+6+8
3+8	1+5+6+8	2+3+4+5+6+7
2+5	1+3+5+6	2+4+5+6+7+8
1+3	1+2+4+7	3+4+5+6+7+8

E

Supporting information for chapter 6

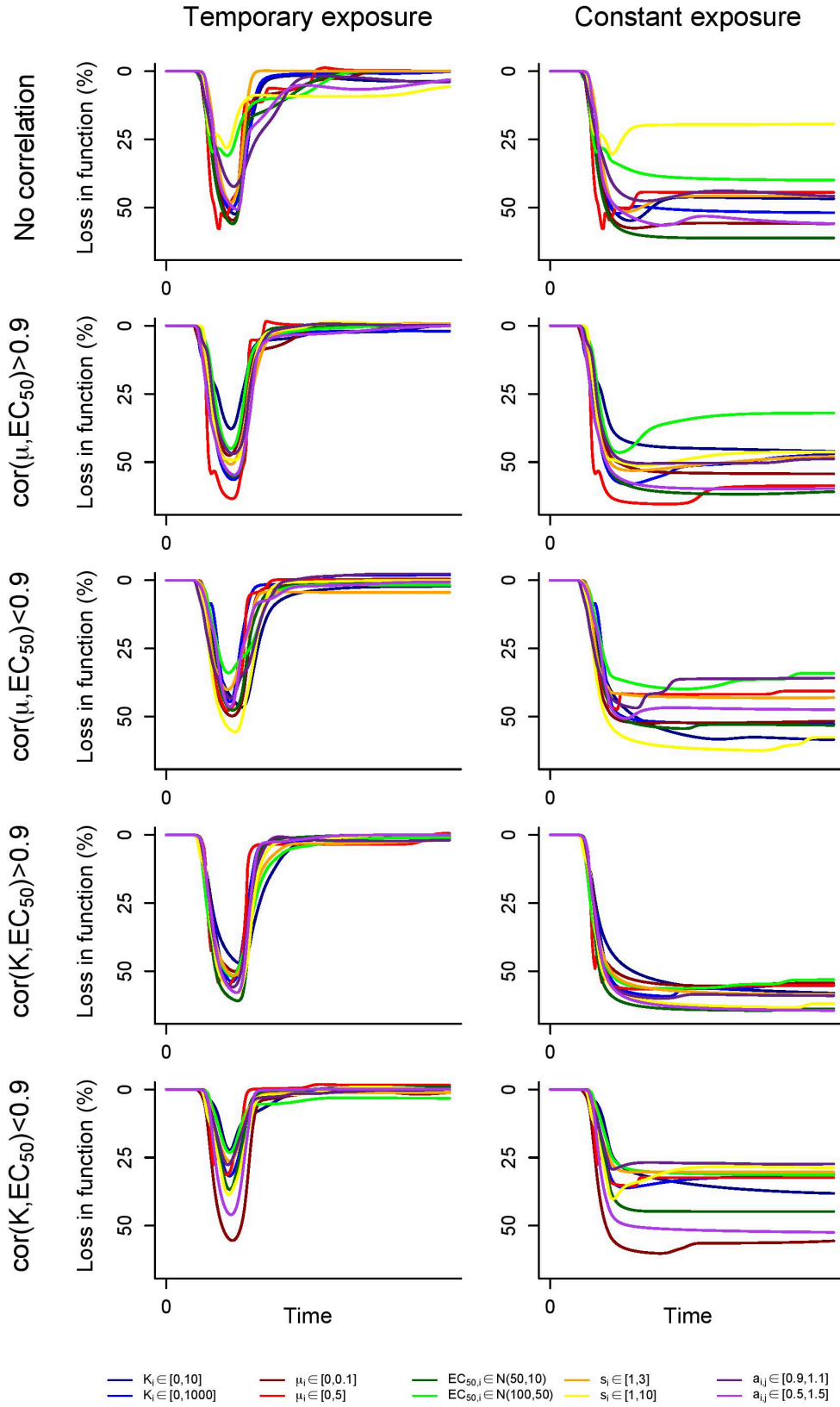


Figure S1: Model sensitivity analysis. Predicted average loss in ecosystem function under temporary (upper panels) and constant (lower panels) for 10 different parameter distributions.

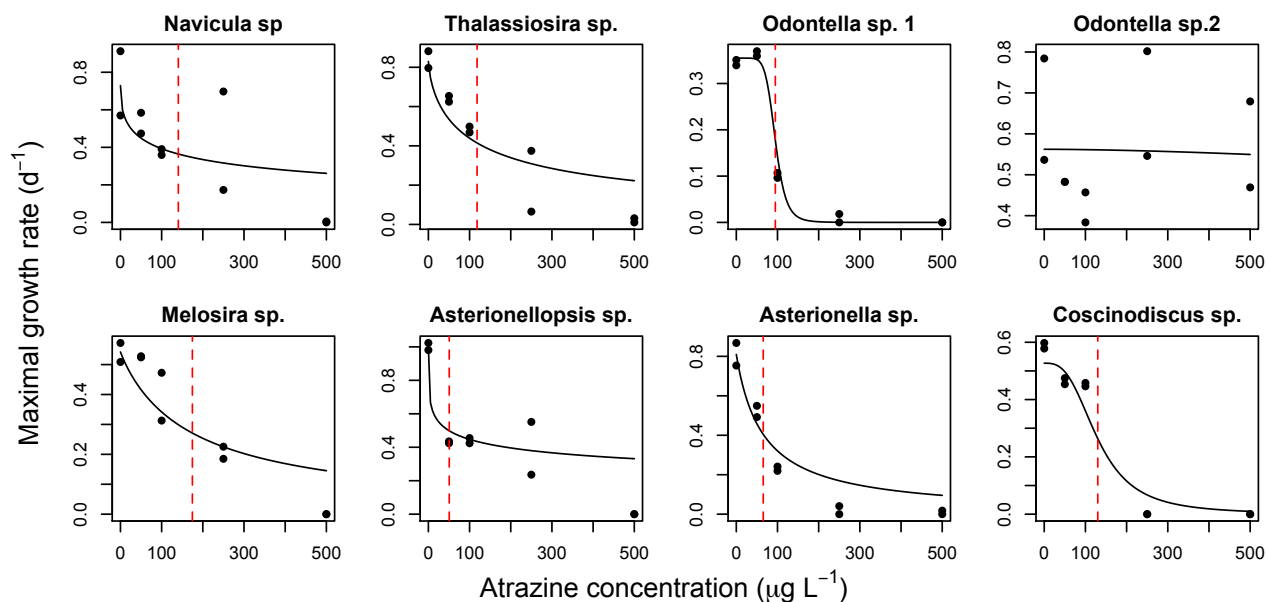


Figure S2: Atrazine dose-response curves for the per capita growth rate. Three-parameter log-logistic concentrations curves for the per-capita growth rate. Red lines indicate EC50 values.

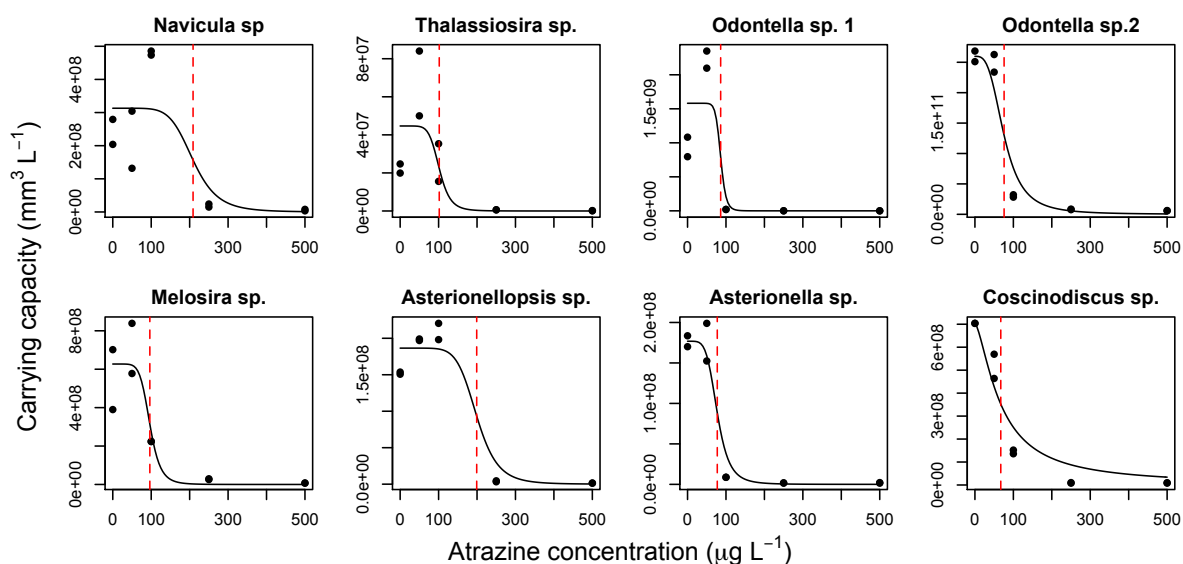


Figure S3: Atrazine dose-response curves for the carrying capacity. Three-parameter log-logistic concentrations curves for the carrying capacity. Red lines indicate EC50 values.

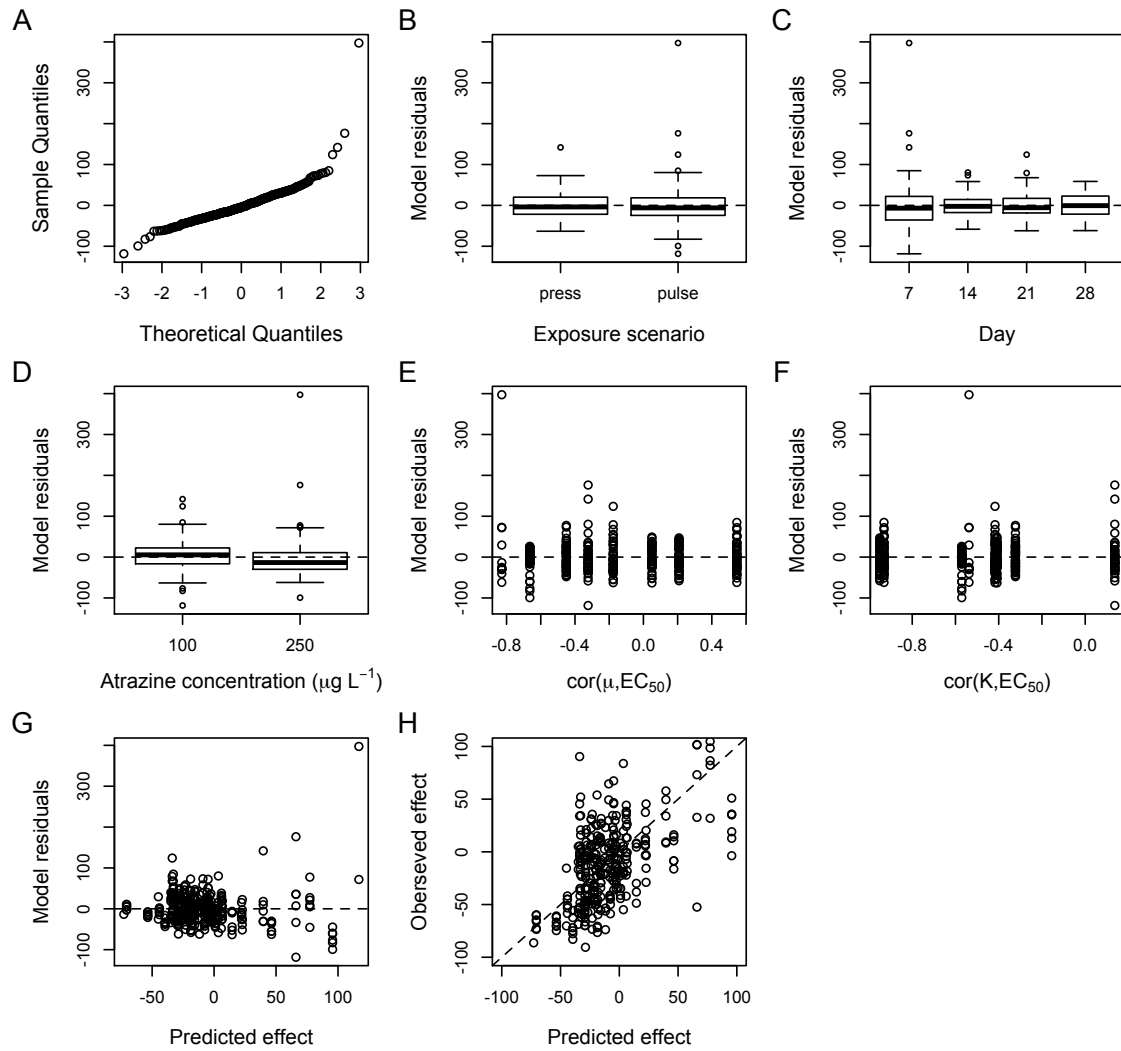


Figure S4: Residual diagnostics of the regression model. QQ plot of model residuals (A), model residuals plotted against the type of exposure (B), the sampling day (C), the atrazine concentration (D), the correlation between sensitivity and the per capita growth rate (E), the correlations between sensitivity and the carrying capacity (F), the predicted effect (G) and observed effects plotted against predicted effects (H).

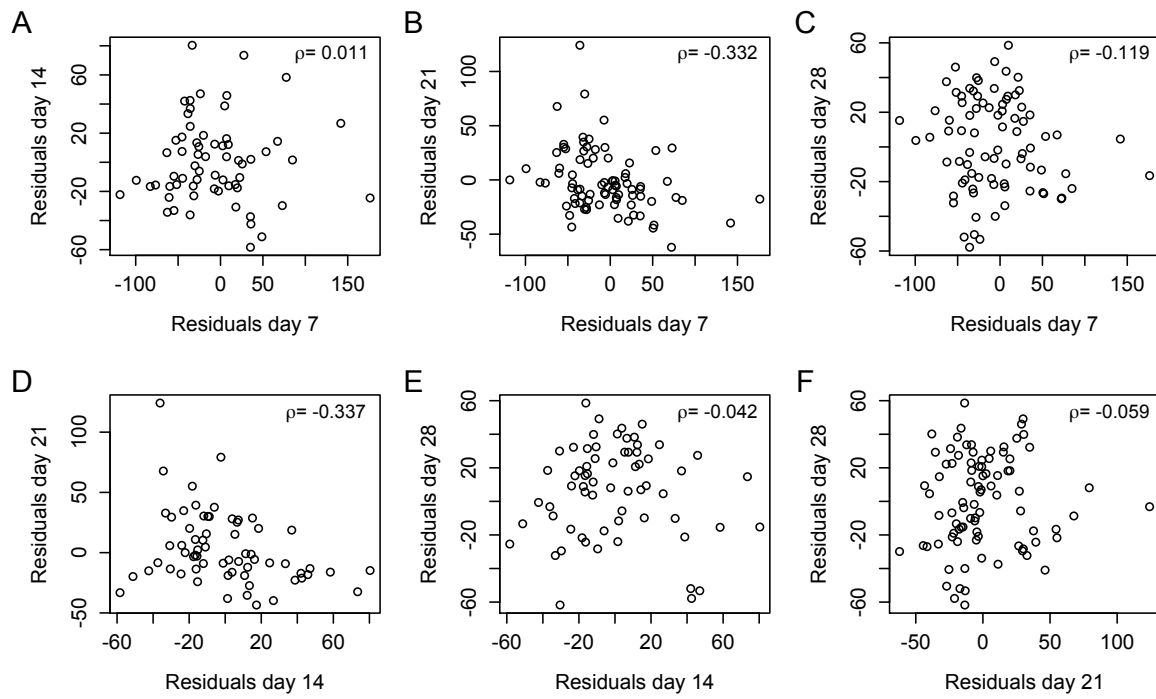


Figure S5: Temporal of the regression model residuals.

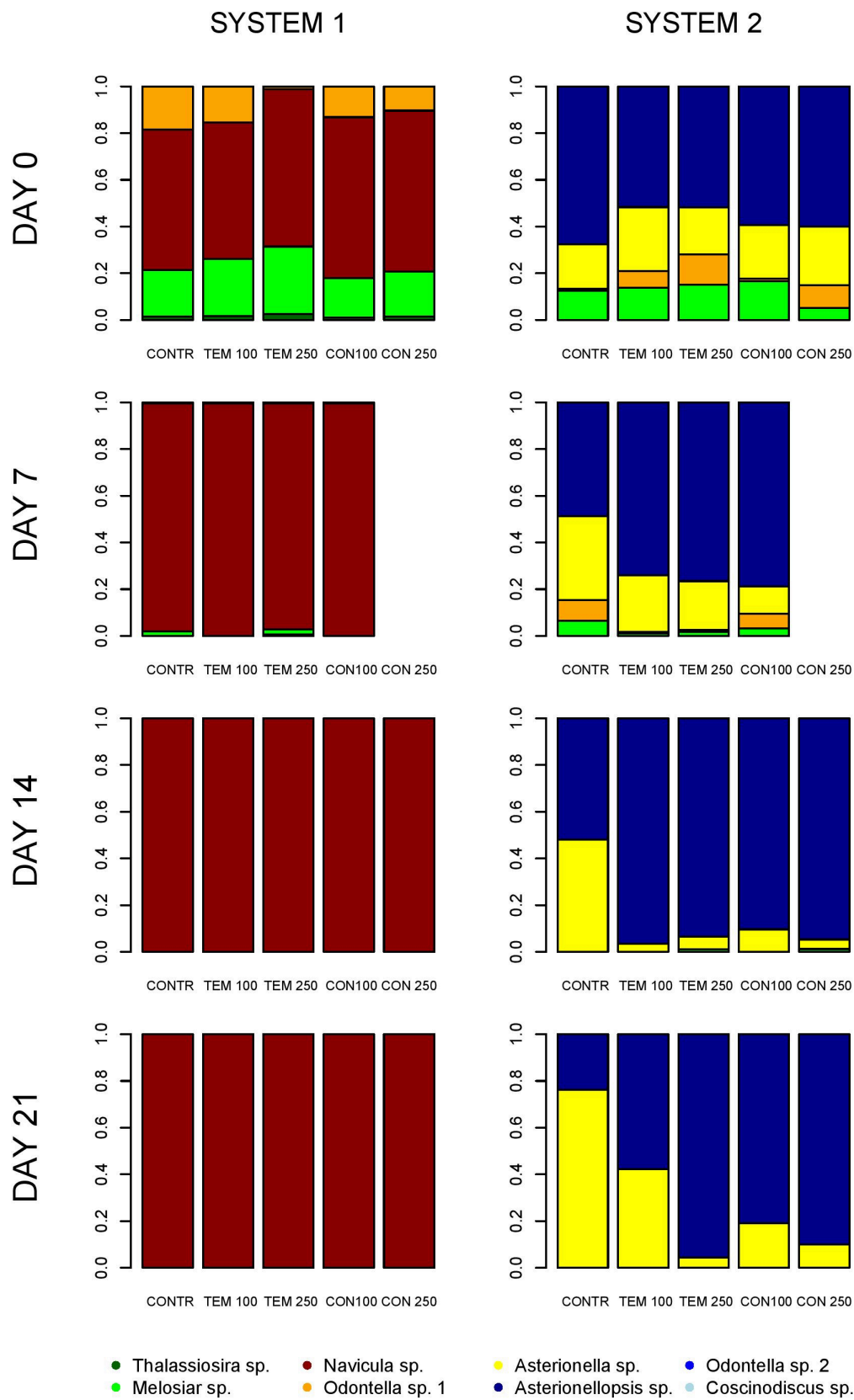


Figure S6: Composition of all 8 systems and each exposure scenario at day 0, 7, 14 and 21 of the experiment

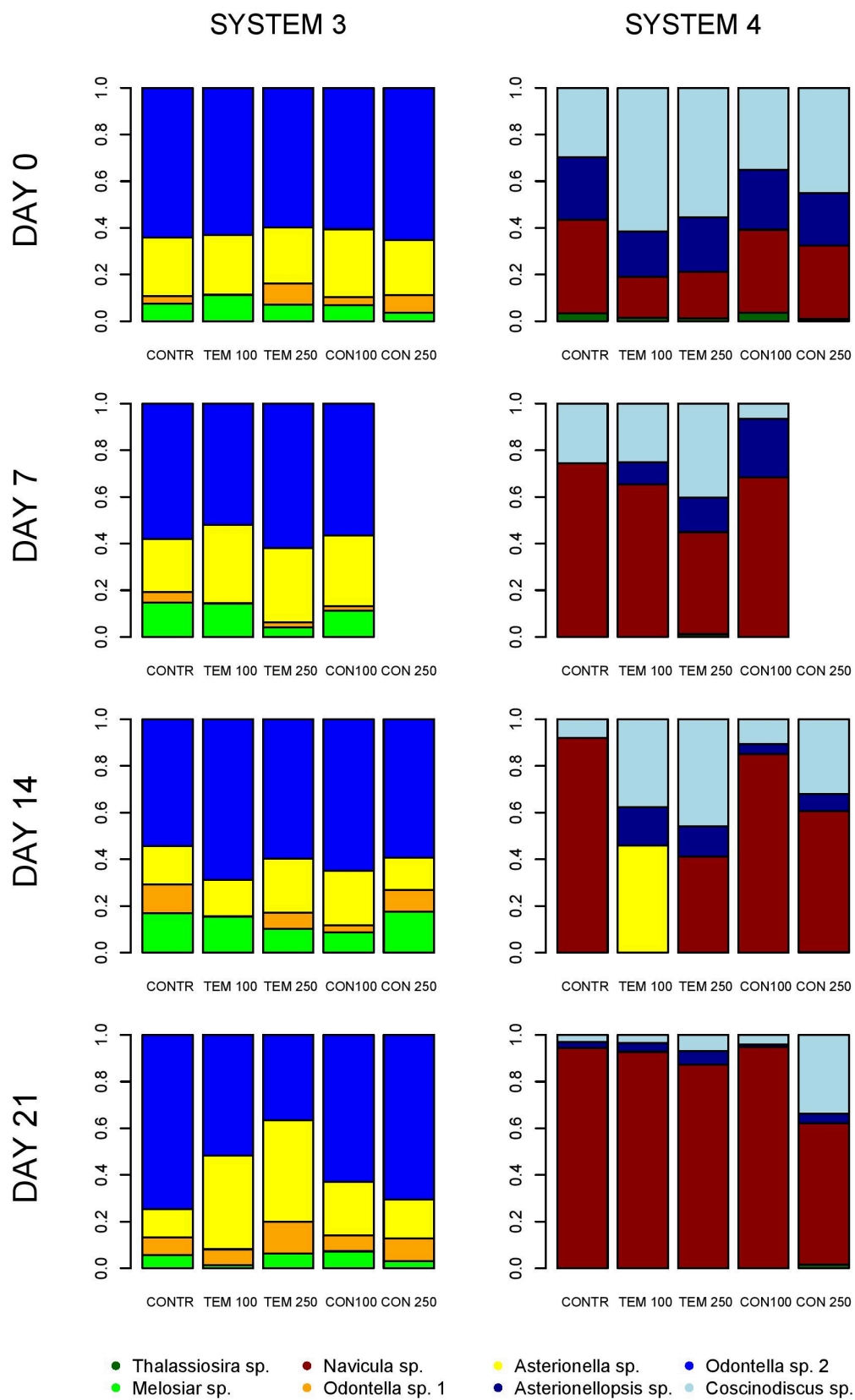


Figure S6: Composition of all 8 systems and each exposure scenario at day 0, 7, 14 and 21 of the experiment

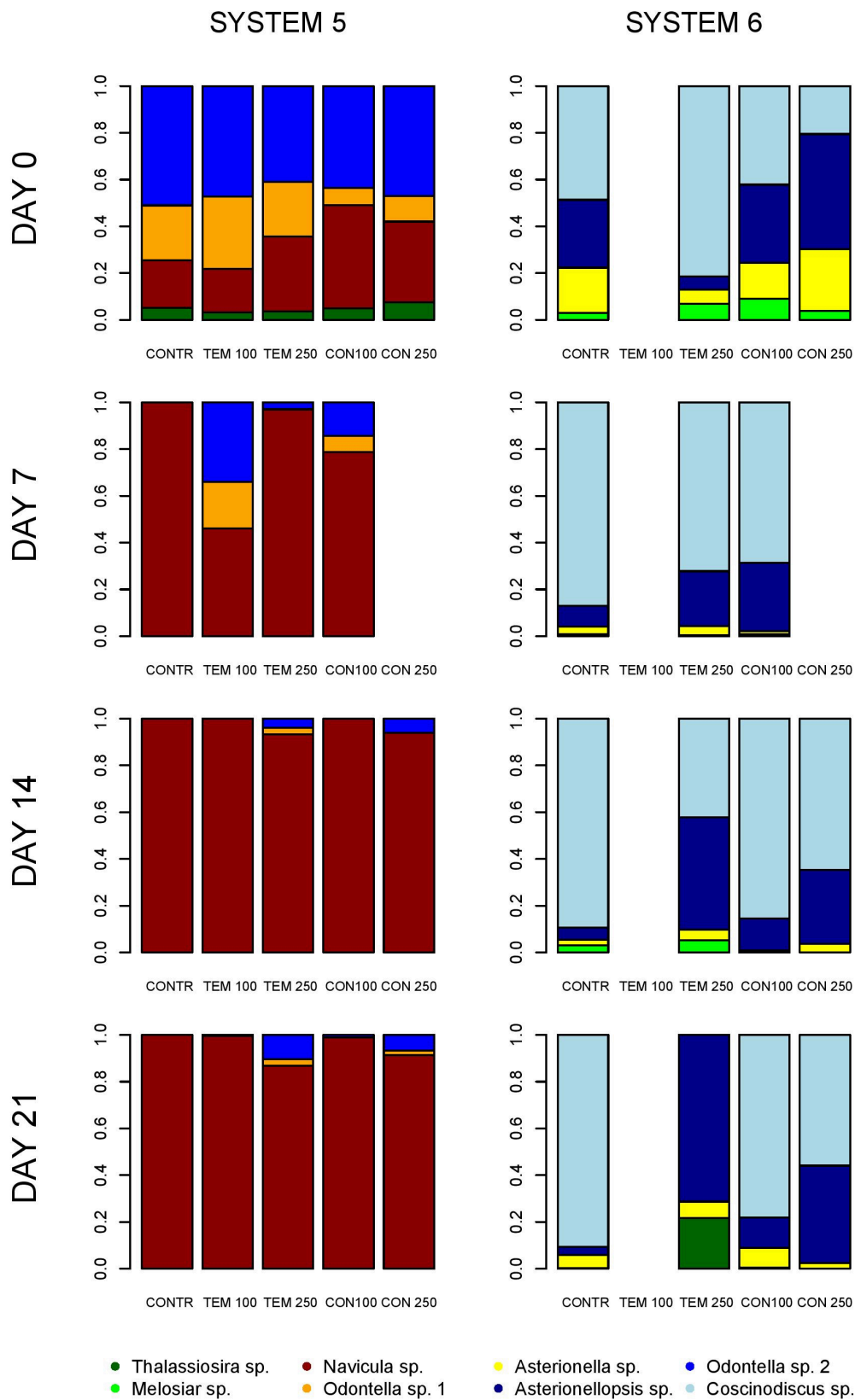


Figure S6: Composition of all 8 systems and each exposure scenario at day 0, 7, 14 and 21 of the experiment

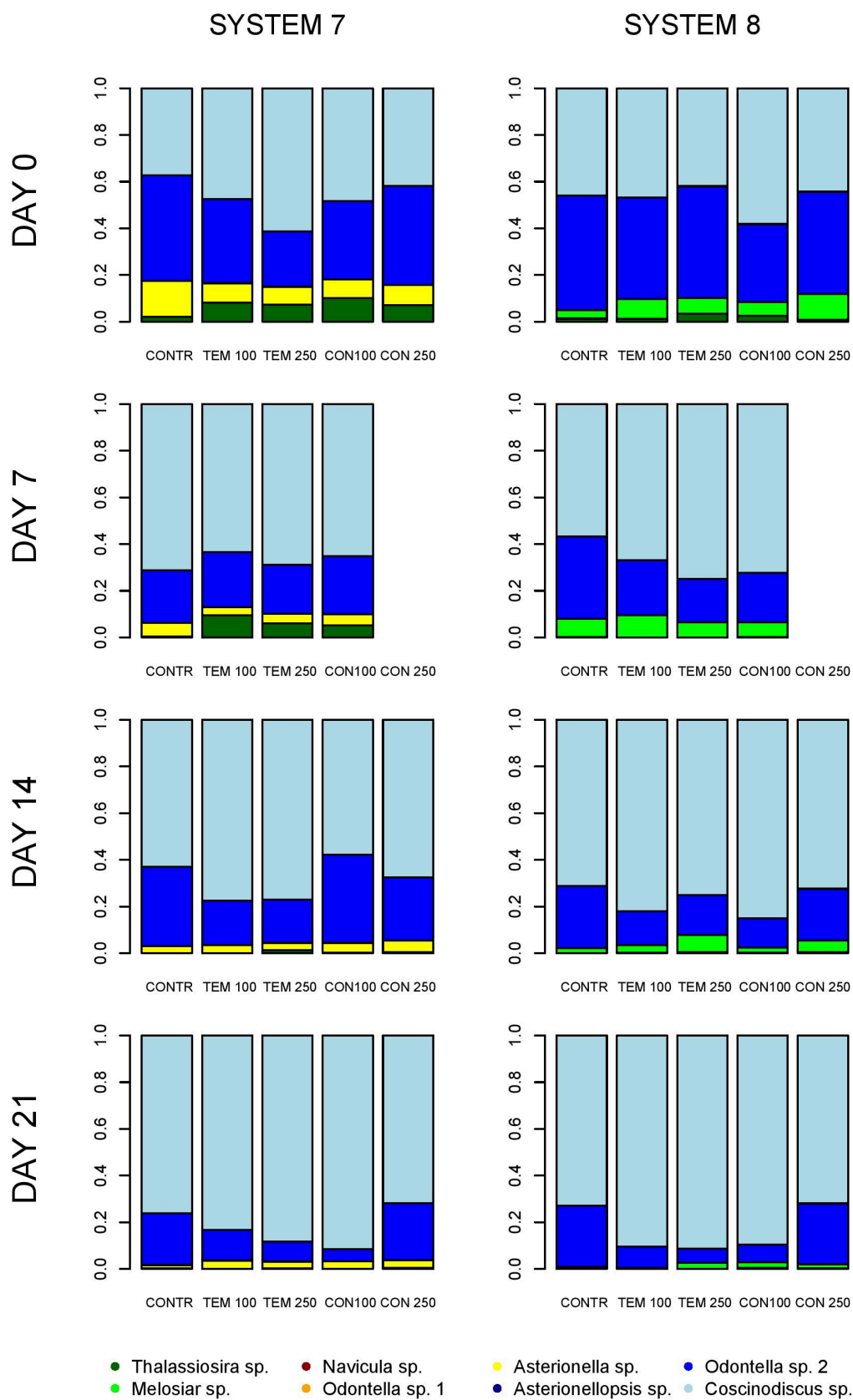


Figure S6: Composition of all 8 systems and each exposure scenario at day 0, 7, 14 and 21 of the experiment

Table S1: Demographic parameters and sensitivity to atrazine

	Maximal growth rate			Carrying capacity		
	$\mu(0)$ [d ⁻¹]	EC_{50} [$\mu\text{g L}^{-1}$]	s [-]	$K(0)$ [$\text{mm}^3 \text{L}^{-1}$]	EC_{50} [$\mu\text{g L}^{-1}$]	s [-]
<i>Melosira sp.</i>	0.73	140	1.05	313 025 313	209	15.1
<i>Navicula sp.</i>	0.83	118	1.60	44 663 605	102	16.6
<i>Thalassiosira sp.</i>	0.36	95	17.1	1 581 268 619	86	29.2
<i>Odontella sp. 1</i>	0.56	>500	3.83	259 776 100 996	76	7.35
<i>Odontella sp. 2</i>	0.54	175	2.20	626 976 642	96	16.3
<i>Asterionellopsis sp.</i>	1.00	51	0.70	186 494 436	199	16.9
<i>Asterionella sp.</i>	0.81	65	2.28	176 814 442	77	11.5
<i>Coscinodiscus sp.</i>	0.53	130	6.78	703 869 334	67	3.42

Table S2: Growth medium atrazine concentrations for single-species toxicity tests.

Nominal value ($\mu\text{g L}^{-1}$)	Measured value ($\mu\text{g L}^{-1}$)
50	62
100	114
250	254
500	503

Table S3: Community composition

	Community composition							
	1	2	3	4	5	6	7	8
<i>Melosira sp.</i>	X	X	X			X		X
<i>Navicula sp.</i>	X			X	X			
<i>Thalassiosira sp.</i>	X			X	X		X	X
<i>Odontella sp. 1</i>	X	X	X		X			
<i>Odontella sp. 2</i>			X		X		X	X
<i>Asterionellopsis sp.</i>		X		X		X		
<i>Asterionella sp.</i>		X	X			X	X	
<i>Coscinodiscus sp.</i>				X		X	X	X

Table S4: Growth medium atrazine concentrations for community toxicity tests.

Week	Nominal value ($\mu\text{g L}^{-1}$)	Measured value ($\mu\text{g L}^{-1}$)
1	100	121
1	250	293
2	100	128
2	250	309
3	100	103
3	250	290
4	100	122
4	250	212

Table S5: Full output linear mixed effects regression model

Variable	Estimate	Standard error	DF	t-statistic	p-value
Intercept	40.44	15.05	297	2.685	0.0077
Day 7	-60.43	25.60	297	-2.360	0.0189
Day 14	-48.96	20.92	297	-2.339	0.0199
Day 21	-64.15	20.92	297	-3.066	0.0024
Temp.exp	-9.48	20.92	297	-0.453	0.6509
Temp.exp x Day 7	35.04	33.12	297	1.058	0.2908
Temp.exp x Day 14	23.47	29.64	297	0.792	0.4291
Temp.exp x Day 21	59.20	29.64	297	1.997	0.0467
Cor(μ ,EC ₅₀)	30.07	19.67	5	1.53	0.1869
Cor(μ ,EC ₅₀) x Day 14	-16.87	33.61	297	-0.502	0.6160
Cor(μ ,EC ₅₀) x Day 21	1.17	27.44	297	0.043	0.9660
Cor(μ ,EC ₅₀) x Day 28	-28.39	27.44	297	-1.035	0.3016
Cor(μ ,EC ₅₀) x Temp.exp	-136.46	27.44	297	-4.972	<0.0001
Cor(μ ,EC ₅₀) x Day 7 x Temp.exp	139.46	43.90	297	3.177	0.0016
Cor(μ ,EC ₅₀) x Day 14 x Temp.exp	155.08	39.37	297	3.938	0.0001
Cor(μ ,EC ₅₀) x Day 21 x Temp.exp	168.34	39.37	297	4.275	<0.0001
Cor(K,EC ₅₀)	67.31	23.39	5	2.877	0.0347
Cor(K,EC ₅₀) x Day 7	-83.72	39.89	297	-2.099	0.0367
Cor(K,EC ₅₀) x Day 14	-44.48	32.58	297	-1.365	0.1732
Cor(K,EC ₅₀) x Day 21	-56.17	32.58	297	-1.724	0.0857
Cor(K,EC ₅₀) x Temp.exp	-60.10	32.58	297	-1.845	0.0660
Cor(K,EC ₅₀) x Day 14 x Temp.exp	101.18	51.72	297	1.956	0.0514
Cor(K,EC ₅₀) x Day 21x Temp.exp	109.36	46.31	297	2.361	0.0189
Cor(K,EC ₅₀) x Day 28 x Temp.exp	102.38	46.31	297	2.210	0.0278

Summary

The world population has increased by a tenfold since the industrial. Human activities have thereby progressively interfered with the environment, which have caused an unprecedented biodiversity decline in the past century. In the 1990s, increasing concerns about the consequences of this global biodiversity decline has led to several large-scale experiments to assess if biodiversity and ecosystem functions are interrelated. The hundreds of experiments that have since then been conducted have now provided compelling evidence that biodiversity indeed generally increases the functioning and functional stability of ecosystems. The further decline of global biodiversity that is forecasted for this century may hence jeopardize the provisioning of the many ecosystem functions on which society depends. However, it remains unclear to what extent ecosystem functions will be altered by biodiversity changes. Although biodiversity-ecosystem functioning relationships are generally positive, their exact shape does strongly differ between systems and environmental conditions. This provides a major challenge, as current theoretical frameworks are unable to explain this context-dependence of biodiversity-ecosystem functioning relationships. This thesis therefore incorporates the fundamental principles of compositional dynamics explicitly into biodiversity theory to develop a context-dependent framework that allows explaining differences in the consequences of biodiversity changes between systems and environmental conditions.

Chapter 1 revises the state of the art in biodiversity research. Both theoretical frameworks and empirical evidence of biodiversity effects on ecosystem functioning and functional stability are thereby summarized. Next, the currently unexplained, context-dependence of biodiversity-ecosystem functioning relationships is discussed. Finally, the rationale to develop a context-dependent framework by explicitly incorporating compositional dynamics is explained.

In **chapter 2** it is demonstrated how environmentally induced changes in the biodiversity-ecosystem functioning relationship can be quantitatively predicted. To this end, a marine Diatom microcosm experiment was used. First, biomass production was measured in communities of 1, 2, 4, 6 and 8 species, exposed to 3 concentrations of the herbicide atrazine. Atrazine altered the biodiversity-productivity relationship. A negative biodiversity-productivity relationship was observed under control conditions, whereas a positive relationship was observed under high atrazine stress. Next, a community model was used to demonstrate how changes in the biodiversity-productivity relationship, and underlying changes in community composition, species complementarity and dominance can be

predicted from the per-capita strength of species interactions and species tolerance to atrazine.

In **chapter 3** it is demonstrated that, in competitive systems, the biodiversity-ecosystem functioning relationship changes in a general, predictable way over an environmental stress gradient. First, a comprehensible community model is introduced to reveal that the slope of the BEF relationship is expected to change in a general, unimodal way along environmental stress gradients in competitive systems. It is demonstrated that this unimodal response, whereby maximal positive biodiversity effects occur at intermediate environmental stress, should invariably arise in when species respond differently to environmental changes. The exact shape of the unimodal response, that is the environmental stress level at which the maximal slope is attained, depends however on the type and strength of species interactions in the system. Systems with more positive/facilitative interactions are thereby predicted to experience maximal biodiversity effects at lower stress levels compared to systems with more negative/competitive interactions. Next, it is demonstrated that the model predictions strikingly concur with a meta-analysis of a global dataset of 52 experiments that manipulated the diversity of primary producers under at least 2 environmental conditions.

In **chapter 4** current additive partitioning methods are extended to quantify the complex biodiversity effects on ecosystem functioning that can arise over time. Species often have similar initial functional contributions, which are likely to change over time because of competitive differences. This can result in a non-linear relationship between species functional abilities and functional contributions to ecosystem functions. Current additive partitioning methods, however, assume a linear relationship. Deviations from linearity can hence strongly affect validity of these methods. In this chapter, a general additive partitioning method is derived that allows fitting an appropriate relationship between species functional abilities and contributions. The applicability and validity of this generalised method is illustrated based on the Cedar Creek biodiversity II experiment.

In **chapter 5** it is revealed how biodiversity effects on compositional stability can drive biodiversity effects on ecosystem function stability. Ecological theory generally focuses on functional compensation between species, resulting in a strong compositional turnover, as a mechanism to explain positive biodiversity effects on functional stability. However, theory also suggest that, in systems with more symmetrical interactions such as primary producer systems, increased functional stability should instead be driven by compositional stability. The experiment used in chapter 2 was therefore extended with 3 more weeks. Communities were thereby transferred to atrazine-free medium after 4 weeks of atrazine exposure. Confirming theoretical predictions, biodiversity indeed simultaneously increased functional

and compositional resistance to atrazine. However, these slower compositional dynamics in more diverse systems also caused them to recover more slowly after exposure had ceased.

In **chapter 6** it is discussed how non-randomness in species sensitivity to environmental changes could modulate the effect of environmental changes on ecosystem functioning. Due to monetary, logistic or ethical constraints ecosystem-level experiments are generally unfeasible. Ecosystem-level effects of environmental changes are hence generally estimated from species-level effects, which are easily obtainable from single-species bioassays. Here, a community model and Diatom microcosm experiment was presented to demonstrate how non-randomness between species sensitivities to environmental changes and their functional traits can affect the effect of environmental changes on ecosystem functioning. The focus is thereby on 2 species traits that can easily be measured in bioassays, the per capita growth rate and carrying capacity, and productivity as an ecosystem function. The correlation between species sensitivity and the growth rate appears to be particularly important during temporary changes in environmental conditions, as it determines the rate by which species can respond to these changes. Larger reductions in productivity thereby occur when the fast growing species are more sensitive to environmental changes. In contrast, the correlation between species sensitivity and the carrying capacity affects productivity during both temporary and permanent changes in environmental conditions. In both scenarios, effects on productivity increase when species with higher carrying capacities, and thus a higher ability to produce biomass, are more sensitive. Although ecosystem-level effects can to some extent be estimated from species-level effect – as assumed in contemporary risk assessment procedures – these results demonstrate that non-randomness in species sensitivity can result in a strong underestimation of ecosystem-level effects.

Chapter 7 summarizes the insights obtained in this thesis by integrating compositional dynamics explicitly into biodiversity theory. The developed context-dependent framework allows explaining differences in the biodiversity-ecosystem functioning relationships between systems and environmental conditions. The unimodal response along an environmental gradient thereby revealed that the consequences of predicted upcoming biodiversity loss are likely to increase by projected environmental changes such as climate change, ocean acidification or chemical pollutants. In addition, it is demonstrated that biodiversity effects on ecosystem functioning and ecosystem function stability, which have generally been treated separately, are inherently linked by the same underlying mechanisms. Although the results presented in this thesis help to deepen our understanding on how biodiversity affects ecosystem functions, there are still several major challenges ahead. Finally, I discuss how our current knowledge, which is largely based on simple primary producer systems, only

provides a first step towards a deeper understanding of biodiversity effects. Extending experiments to more complex systems, both multi trophic level and spatially explicit, will be the essential next step to help us understand and deal with the problems that we'll face in the 21st century.

Samenvatting

De exponentiële groei van de wereldbevolking sinds de industriële revolutie heeft ertoe geleid dat onze planeet steeds meer en meer onder druk is komen te staan door menselijke activiteiten. Dit veroorzaakte onder meer tot een ongezien wereldwijd verlies aan soorten in de voorbije eeuw. Begin de jaren 90 leidde de ongerustheid over de gevolgen van dit globale biodiversiteitsverlies tot groots opgezette experimenten om de relatie tussen biodiversiteit en werking van een ecosysteem na te gaan. De honderden experimenten die sindsdien zijn uitgevoerd tonen bijna eenduidig aan dat de werking en stabiliteit van een ecosysteem inderdaad verhoogt met toenemende biodiversiteit. Het is dus duidelijk dat de vele ecosysteemfuncties waar we voor ons dagdagelijks leven van afhankelijk zijn in de 21^e eeuw meer en meer onder druk zullen komen te staan door verder biodiversiteitsverlies. Echter, inschatten hoe groot de impact van biodiversiteitsverlies op de werking van een ecosysteem is, blijkt niet eenvoudig. Hoewel experimenten bijna eenduidig een positieve relatie aantonen, blijkt de sterkte van deze relatie sterk te verschillen tussen systemen en omgevingscondities. De theoretische denkkaders die ecologen de voorbije twee decennia gecreëerd hebben blijken bovendien niet in staat deze sterke context-afhankelijkheid van de relatie tussen biodiversiteit en de werking van een systeem te verklaren. In deze thesis worden daarom de basisprincipes van gemeenschapsdynamieken geïncorporeerd in het huidige theoretisch kader rond biodiversiteitseffecten op de werking van een ecosysteem. Hierbij wordt een contextafhankelijke kader gecreëerd dat beter toelaat verschillen in de gevolgen van biodiversiteitsverlies tussen omgevingscondities en systemen te verklaren.

In **hoofdstuk 1** worden de huidige inzichten rond hoe biodiversiteit de werking en stabiliteit van een ecosysteem kan beïnvloeden samengevat. Hierbij wordt zowel de experimenteel bekomen relaties tussen biodiversiteit en de werking of stabiliteit van een ecosysteem besproken, als ook de theoretische denkkaders die geschept werden om deze observaties te verklaren. Vervolgens wordt besproken hoe grote verschillen in biodiversiteitseffecten tussen systemen of omgevingscondities geobserveerd werden, welke tot op de dag van vandaag niet kwantitatief verklaard konden worden. Ten slotte wordt verklaard hoe in deze thesis dit ontbrekend een context-afhankelijk denkkader gecreëerd wordt op basis van de basisprincipes van gemeenschapsdynamieken in ecologie.

In **hoofdstuk 2** wordt aangetoond hoe veranderingen in de relatie tussen biodiversiteit en de werking van een ecosysteem ten gevolge van omgevingsveranderingen kwantitatief voorspeld kunnen worden. Hierbij werd gebruik gemaakt van een experiment met mariene Diatomeeën. Er werden gemeenschappen van 1, 2, 4, 6 en 8 soorten gemaakt die vervolgens werden blootgesteld aan 3 concentraties van het herbicide atrazine. De relatie

tussen diversiteit en productiviteit veranderde aanzienlijk tussen de verschillende omgevingscondities. In de afwezigheid van atrazine was deze relatie negatief, terwijl onder hoge concentraties van atrazine deze positief werd. Vervolgens werd aangetoond dat deze veranderingen in de biodiversiteit-productiviteit relatie, als ook de onderliggende veranderingen in de compositie, complementariteit en dominantie van de gemeenschappen door atrazine kwantitatief konden voorspeld worden door een model op basis van de sterkte van de interacties tussen de soorten en hun gevoeligheid voor atrazine.

In **hoofdstuk 3** wordt aangetoond dat de relatie tussen biodiversiteit en de werking van een ecosysteem op een algemene en voorspelbare manier verandert over een omgevings-gradiënt in competitieve systemen. Eerst wordt een eenvoudig gemeenschapsmodel gebruikt om aan te tonen hoe de helling van de relatie tussen biodiversiteit en de werking van een systeem op een algemene, unimodale manier verandert over een omgevings-gradiënt wanneer soorten op verschillende manieren reageren op omgevingsveranderingen. Een maximaal effect van biodiversiteitsveranderingen kan bijgevolg verwacht worden onder condities van intermediaire omgevingsstress. Echter, de intensiteit van omgevingsstress waarbij het effect van biodiversiteitsveranderingen maximaal wordt hangt hierbij af van het type van soortinteracties in het systeem. In systemen met meer positieve/faciliterende interacties is het effect van biodiversiteitsveranderingen maximaal bij lagere niveaus van omgevingsstress in vergelijking met systemen met meer negatieve/competitieve interacties. Vervolgens wordt aangetoond dat deze algemene unimodale response bevestigd wordt door een meta-analyse van 52 studies die biodiversiteit manipuleerden onder minstens 2 omgevingscondities.

In **hoofdstuk 4** worden bestaande additieve partitie methodes om biodiversiteitseffect op de werking van ecosystemen uitgebreid om meer complexe effecten die over tijd kunnen optreden correct te kwantificeren. Soorten hebben vaak gelijke initiële bijdragen in experimenten, welke over tijd sterk kunnen veranderen ten gevolge van competitie. De relatie tussen de functionele capaciteiten van soorten en hun functionele bijdrage tot ecosysteem functies, die wordt gebruikt voor het kwantificeren van biodiversiteitseffecten, kan hierdoor niet-lineair worden. Huidige partitie methodes gaan echter uit van een lineaire relatie, wat kan leiden tot een verkeerde kwantificatie van complementariteit- en dominantie-effecten op ecosysteem functies. De algemene, niet-lineaire partitie die geïntroduceerd wordt in hoofdstuk laat dus toe om een adequate relatie tussen de functionele capaciteiten van soorten en hun functionele bijdrage te fitten. Dit laat toe om op een eenvoudige manier complexe biodiversiteitseffecten te kwantificeren, wat geïllustreerd wordt aan de hand van het Cedar Creek biodiversity II experiment.

In **hoofdstuk 5** wordt aangetoond hoe gemeenschapsdynamieken aanleiding geven tot een verhoogde stabiliteit van ecosysteemfuncties in meer diverse systemen. Theoretisch wordt vaak de nadruk gelegd op vervanging tussen soorten, en dus sterke wijzigingen en de samenstelling van het systeem, als basis voor deze verhoogde stabiliteit. Echter, modellen suggereren dat in systemen waar soortinteracties vrij symmetrisch zijn, zoals primaire productie systemen, deze verhoogde stabiliteit gedreven wordt door sterke stabiliteit in gemeenschapscompositie. Het experiment uit hoofdstuk 2 werd hiervoor met 3 weken verlengd waarbij de gemeenschappen na 4 weken blootstelling aan atrazine werden getransfereerd naar groeimedium zonder atrazine. Zoals theoretisch voorspeld was productiviteit en gemeenschaps-composities inderdaad meer resistent aan atrazine blootstelling. Echter, meer diverse systemen keerden hierdoor echter minder snel terug naar de controle ten opzichte van minder diverse systemen.

In **hoofdstuk 6** wordt aangetoond hoe de effecten van omgevingsveranderingen op ecosysteemfuncties beter kunnen ingeschat worden op basis van informatie over soortengevoeligheid. Omwille van budgettaire, tijd, logistieke of ethische redenen zijn testen op ecosysteem niveau vaak niet voorhanden. Bijgevolg dienen ecosysteemeffecten vaak geschat te worden op basis van de gevoeligheden van de individuele soorten. In hoofdstuk 6 wordt daarom een gemeenschapsmodel en experiment met Diatomeeën gemeenschappen gebruikt om aan te tonen hoe correlaties tussen functionele capaciteiten van soorten en hun gevoeligheid een grote invloed kunnen hebben op effecten op ecosysteemfuncties. Hierbij wordt gefocust op 2 eigenschappen van soorten en hun gevoeligheid: de groeisnelheid en de draagkracht. De gevoeligheid van de groeisnelheid heeft hierbij vooral een effect op productiviteit gedurende korte, tijdelijke omgevingsveranderingen gezien het beïnvloed hoe snel soorten kunnen reageren. Grotere effecten treden hierbij op wanneer de snelst groeiende soorten het gevoeligst zijn aan omgevingsverandering. De gevoeligheid van de draagkracht, en dus de totale biomassa die kan geproduceerd worden, heeft een blijvend effect. Zowel bij tijdelijke als langdurige omgevingsveranderingen zijn de effecten het grootst wanneer de meest productieve soorten het gevoeligst zijn gezien dit de mogelijkheid voor functionele compensatie reduceert. Dit toont aan dat, hoewel effecten op ecosysteem functies in zekere mate kunnen voorspeld worden op basis van effecten op soortniveau (zoals aangenomen wordt in de huidige procedures voor ecologische risicoschatting), sterke correlaties voor zeer sterke onderschattingen van ecosysteem effecten kunnen leiden.

In **hoofdstuk 7** wordt samengevat hoe het integreren van de basisprincipes van gemeenschapsdynamieken in biodiversiteitsonderzoek een aantal belangrijke inzichten heeft opgeleverd. In deze thesis werd aangetoond dat dit essentieel is om een context-afhankelijk

theoretisch denkkader te ontwikkelen dat in staat is op verschillen in de relatie tussen biodiversiteit en de werking van een ecosysteem tussen systemen en omgevingscondities te kunnen verklaren. Er werd aangetoond hoe deze relatie op een algemene manier verandert en de gevolgen van biodiversiteitsverlies wellicht nog zullen toenemen ten gevolge van voorspelde omgevingsveranderingen zoals klimaatsverandering, oceaanverzuring of chemische verontreiniging. Ook werd aangetoond dat biodiversiteitseffecten op stabiliteit en functie, welke tot nog toe grotendeels afzonderlijk beschouwd werden, onlosmakelijk met elkaar verbonden zijn gezien ze door dezelfde onderliggende mechanismes gedreven worden. Hoewel deze thesis een belangrijke bijdrage heeft geleverd in het verder uitdiepen van onze kennis over biodiversiteitseffecten, is het ook duidelijk dat wel voor enorme uitdagingen blijven staan. Finaal wordt besproken hoe de huidige kennis, gebaseerd op eenvoudige primaire productie systemen, slechts een handvol stukjes van de puzzel omvat. Onderzoek naar meer complexe systemen, zowel multi-trofisch als spatiaal expliciet, zal hierbij een essentiële volgende stap zijn in het begrijpen en oplossen van de uitdagingen waar we in de 21^e eeuw mee geconfronteerd zullen worden.

Curriculum vitae

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